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# Differential Nuclear Localization Does Not Determine the Silencing Status of *Saccharomyces cerevisiae* Telomeres

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ABSTRACT

In *Saccharomyces cerevisiae*, genes near telomeres are transcriptionally repressed, a phenomenon termed telomere position effect (TPE). Yeast telomeres cluster near the nuclear periphery, as do foci of proteins essential for TPE: Rap1p, Sir2-4p, and yKu70p/yKu80p. However, it is not clear if localization of telomeres to the periphery actually contributes to TPE. We examined the localization patterns of two telomeres with different levels of TPE: truncated VII-L and native VI-R. For both telomeres, localization to the nuclear periphery or to the silencing foci was neither necessary nor sufficient for TPE. Moreover, there was no correlation between TPE levels and the extent of localization. Tethering the truncated VII-L telomere to the nuclear periphery resulted in a modest increase in TPE. However, tethering did not bypass the roles of yKu70p, Sir4p, or Esc1p in TPE. Using mutations in *RIF* genes that bypass the role of Ku in TPE, a correlation between the level of silencing and the number of Rap1p foci present in the nucleus was observed, suggesting that Sir protein levels at telomeres determine both the level of TPE and the number of foci.

SEVERAL lines of evidence suggest that the nuclear periphery is a region conducive to transcriptional silencing. The classic example is the human inactive X chromosome, which localizes to the nuclear periphery (Bourgeois *et al.* 1985). More recent work in mammalian cells shows that activated genes localize to the nuclear interior whereas repressed genes preferentially reside at the periphery (Kosak and Groudine 2004; Zink *et al.* 2004). Perhaps the most compelling experiment arguing that this peripheral localization has functional significance for gene repression comes from yeast, where tethering a weakened *HMR* silencer to the nuclear periphery increases silencing (Andrulis *et al.* 1998). This view of the nuclear periphery as a potential silencing subcompartment is complicated by recent evidence that the periphery also promotes gene expression (Ishii *et al.* 2002; Brickner and Walter 2004; Casolari *et al.* 2004; Menon *et al.* 2005; Schmid *et al.* 2006; Taddei *et al.* 2006).

Some or all telomeres are localized to the nuclear periphery in yeasts, flies, humans, and in the pathogenic protozoa *Trypanosoma brucei* and *Plasmodium falciparum*. In each of these organisms, genes near telomeres are transcriptionally silenced, a phenomenon termed telomere position effect (TPE; reviewed in Mondoux and Zakian 2005). In *Saccharomyces cerevisiae*, many of the proteins required for TPE, such as Rap1p, Sir2p, Sir3p, Sir4p, and the heterodimeric Ku complex colocalize in three to six foci at the nuclear periphery (Klein *et al.* 1992; Palladino *et al.* 1993; Gotta *et al.* 1996; Laroche *et al.* 1998). Deleting any one of the Sir proteins, the Sir4p-binding Ku proteins, or the C-terminal Sir-interaction domain of Rap1p eliminates TPE and disperses these foci (Aparicio *et al.* 1991; Hecht *et al.* 1995; Gotta *et al.* 1996; Boulton and Jackson 1998; Laroche *et al.* 1998).

Although Rap1p, Ku, and Sir2-4p are required for TPE at all telomeres, different telomeres have different TPE phenotypes. Most native telomeres have very low or no detectable silencing, but genes near the native VI-R telomere are silenced in almost all cells (Mondoux and Zakian 2007, accompanying article, this issue). TPE has been best studied at truncated telomeres, where the reporter gene is placed immediately adjacent to the telomeric tract with the concomitant deletion of the subtelomeric middle repetitive elements (subtelomeric elements, STE). Although all truncated telomeres show TPE, the level of TPE at different truncated ends also varies. For example, truncated telomere VII-L has a TPE level ~10-fold higher than truncated telomere V-R, even though both lack STEs (Gottschling *et al.* 1990). Although STEs contribute to the TPE phenotype of individual telomeres, even when the subtelomeric structure of the VII-L telomere is identical to that of the native VI-R telomere, the two telomeres have different TPE phenotypes (Mondoux and Zakian 2007).

Just as TPE levels vary from telomere to telomere, so do patterns of nuclear localization. By fluorescent *in situ*

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hybridization, ~70% of the subtelomeric Y′ sequences localize to the Rap1p foci (Gotta *et al.* 1996), suggesting that many, but not all, telomeres localize to these foci. Several individual telomeres have been visualized at the periphery by inserting a *lac* or *tet* operator array near a single telomere and fusing their respective repressors to fluorescent proteins (Tham *et al.* 2001; Hediger *et al.* 2002; Taddei *et al.* 2004; Bystricky *et al.* 2005). This type of analysis has shown that individual telomeres are at the periphery in some but not all cells. Furthermore, the fraction of telomeres localized to the periphery varies between telomeres and with position in the cell cycle. The genetic requirements for localization to the periphery also differ between telomeres. For example, the VI-R telomere requires the Ku complex for localization to the nuclear periphery (Hediger *et al.* 2002), whereas truncated VII-L (Tham *et al.* 2001), truncated VI-R (Hediger *et al.* 2002), and native XIV-L (Taddei *et al.* 2004) do not.

Telomere positioning at the nuclear periphery is clearly not sufficient for TPE, since yKu70p is required for TPE at truncated VII-L (Boulton and Jackson 1998), but VII-L remains at the periphery in its absence (Tham *et al.* 2001). Nonetheless, other than late in the cell cycle (Tham *et al.* 2001; Hediger *et al.* 2002), there is no known case where telomeres are away from the periphery and silenced. Thus, localization to the nuclear periphery may be necessary for TPE. Another model consistent with the current data is that the association of telomeres with the nuclear periphery promotes TPE by bringing telomeres into close proximity to the Rap1 foci, and that localization to the foci (rather than to the nuclear periphery) is important for TPE.

It is not known why different telomeres display different levels of TPE. Given that different telomeres have different patterns and mechanisms of localization to the nuclear periphery, one possible model is that nuclear localization determines the TPE levels of individual telomeres. Here, we tested whether peripheral localization or localization to the Rap1p foci contributes to TPE at two different telomeres, truncated VII-L and native VI-R. These two telomeres have different levels of TPE: in our strain background and growth conditions, truncated VII-L is silenced in ~15% of cells, whereas, in the same strain background, native VI-R has a very high level of TPE (~85% TPE; Mondoux and Zakian 2007). These two telomeres also have different genetic requirements for localization to the nuclear periphery, leading to the hypothesis that they might localize differently to the periphery or peripheral Rap1p foci, and that this differential localization might explain the difference in their TPE levels. However, here we report that both telomeres localized equally well to both the nuclear periphery and the Rap1p foci, and their localization was independent of their TPE status.

Because nuclear localization could not explain different levels of TPE between the two telomeres, we examined the relationship between the nuclear periphery, peripheral Rap1p foci, and TPE at the truncated VII-L telomere in more detail. We focused on the VII-L telomere because its inherently lower TPE level makes it possible to determine if genes or conditions increase (as well as decrease) its silencing behavior. Physically tethering the truncated VII-L telomere to the nuclear periphery increased the fraction of cells in which the telomere was at the periphery and resulted in a modest increase in TPE. Remarkably, tethering did not increase localization of the truncated VII-L telomere to the Rap1p foci. Finally, we examined the requirements for the formation of the foci of silencing proteins. The Ku complex is resident in these foci, and foci are dispersed in *yku*Δ cells (Laroche *et al.* 1998). However, Ku is not required for focus formation in certain genetic backgrounds, as deleting *RIF1* in a *yku70*Δ strain restores focus formation (Hediger *et al.* 2002). Deleting the *RIF* genes in *yku70*Δ cells also restores TPE (Mishra and Shore 1999). We find that the number of Rap1p foci per nucleus correlated with the level of TPE at truncated telomere VII-L, as deleting both *RIF1* and *RIF2* in a *yku70*Δ strain resulted in higher TPE levels and more foci per nucleus than *rif1*Δ *yku70*Δ alone. We hypothesize, therefore, that the level of Sir protein binding at the telomeres determines both TPE levels and Rap1p focus formation.

## MATERIALS AND METHODS

**Yeast strains and plasmids for TPE and visualization:** Both the strain with the truncated VII-L telomere (Tham *et al.* 2001) and the strain with "native" VI-R telomere used for visualization and TPE experiments were constructed in the YPH background (*ura3*-52 *lys2*-801 *ade2*-101 *trp1*-Δ63 *his3*-Δ200 *leu2*-Δ1; Sikorski and Hieter 1989). To construct the strain with the native VI-R telomere, the plasmid pAFS52-LacOp-ARS609 (Hediger *et al.* 2002) containing an 8-kb *lac* operator array (Robinett *et al.* 1996) was integrated at ARS609, placing the *lac* operator array ~15 kb from the VI-R telomere. A 2-μm plasmid expressing the C terminus of Sir4p (pCTC23; Chien *et al.* 1991) was introduced into the strain to reduce TPE. The *URA3* TPE reporter was introduced at the VI-R telomere in a manner analogous to the creation of native TPE reporter strains described in (Pryde and Louis 1999). To construct the VI-R TPE reporter, *URA3* was amplified from ADH4UCAIV, the same plasmid used to create the truncated VII-L telomere reporter (Gottschling *et al.* 1990), with primers 6RURAF5 (5′ atatagtatgctcacattttcttattgctgaatagttcttttttacgtttagctgggattcgg taatctccgagcagaag 3′) and 6RURAR4 (5′ atatagtatgctcacattttctt attgctgaatagttcttttttacgtttagctggggtgttgaagaaacatgaaattgcc 3′). Transformants were screened by Southern blotting and confirmed using pulsed-field gel electrophoresis. The resulting strain containing the native VI-R telomere was then restreaked several times on plates with rich media (YEPD) to lose the pCTC23 plasmid. LacI-GFP was introduced into the strain with plasmid pMAM6, which contains LacI-GFP under the control of the His promoter and was constructed from pWHTLacI (Tham *et al.* 2001) and pRS305 (Sikorski and Hieter 1989).

The truncated VII-L strain used in the tethering experiments contains upstream activation sequence (UAS) sites and

a *URA3* reporter gene adjacent to the truncated VII-L telomere in a W303 background strain (strain YDS 634; Chien *et al.* 1993) and has been modified to contain the LacO/LacI-GFP visualization system (Tham *et al.* 2001) and $Gal_{BD}$–Yif1p tethering system (Andrulis *et al.* 1998). All experiments using the $Gal_{BD}$–Yif1p tethering system were performed on at least two independent plasmid transformants.

*SIR4*, *YKU70*, *RIF1*, and *RIF2* were deleted in the tethering strain background using a PCR-mediated knockout that eliminated the complete open reading frame, replacing it with either a kanamycin- (Wach *et al.* 1994), hygromycin-, or nourseothricin-resistance cassette (Goldstein and McCusker 1999). *SAS2* and *ESC1* were deleted in these strain backgrounds by backcrossing several times to the mutants from the deletion strain collection (Research Genetics/Invitrogen, Carlsbad, CA). All deletion strains were verified by PCR and TPE phenotypes. The *MEC1* and *SIR1* mutant strains have been described previously (Goudsouzian *et al.* 2006; Mondoux and Zakian 2007).

**Immunofluorescence and microscopy:** The protocol for immunofluorescence was adapted from Tham *et al.* 2001. For visualization of a telomere and the nuclear envelope, the primary antibodies were mouse MAb414 (anti-p62; Covance, Berkeley, CA) and rabbit anti-GFP (Chemicon/Millipore, Temecula, CA). For visualization of a telomere and the Rap1p foci, the primary antibodies were rabbit anti-Rap1p (made by W.H. Tham using methods described in Conrad *et al.* 1990) and mouse anti-GFP (Chemicon/Millipore, Temecula, CA). Secondary antibodies were conjugated to Alexa 488 and Alexa 546 (1:100 in PBS/BSA; Molecular Probes, Eugene, OR).

Slides were imaged using a DeltaVision platform and a Nikon microscope with a 100× objective (Applied Precision, Issaquah, WA). Cells were optically sectioned into 18 0.15-μm slices and deconvolved using the DeltaVision restoration system. A mercury arc lamp was filtered at 546 nm and 488 nm to detect the AlexFluor fluorophores. For the Rap1p colocalization experiments, a telomere was scored as colocalized to a focus if there was any overlap between the two spots, *i.e.*, yellow staining (combination of the green telomere spot and red Rap1p spot). For nuclear envelope distance measurements, the diameter of the cell was measured at least twice using the DeltaVision software, and the larger diameter used to calculate the radius, which was then used to divide the area of the nuclear section into three equal zones (as in Hediger *et al.* 2002). The distance from the center of the telomere spot to the closest point of anti-p62 staining was then measured and the telomere's position assigned to zone I, II, or III. Calculation of telomere localization was based on at least three independent experiments with at least 50 nuclei counted per condition, per experiment. For all experiments, standard deviations were calculated and the significance of the data was assessed using the Student's *t*-test. When counting the number of foci per cell, at least 50 cells were counted for each mutant in two independent experiments.

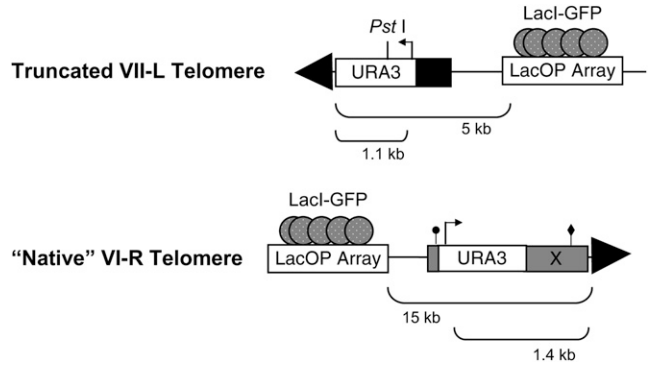


Figure 1.—Structure of the telomeric regions of the truncated VII-L and "native" VI-R telomeres used for localization and silencing analysis. The visualization system for the truncated VII-L telomere, which lacks subtelomeric structure, has been described previously (Tham *et al.* 2001). The binding of a LacI-GFP fusion protein to the lac operator array allows visualization of the position of the truncated VII-L telomere as a green spot. The *URA3* transcription start site on this telomere is ~1.1 kb from the start of the telomeric tract of $C_{1-3}A/TG_{1-3}$ DNA. The VI-R subtelomere contains a 380-bp "core-X" element (shaded) that contains an ARS consensus sequence (X-ACS; circle) and Abf1p binding site (diamond). The *URA3* gene is inserted within the X element in a manner designed to keep the X element largely intact. The lac operator array (Hediger *et al.* 2002) allows visualization of the position of the VI-R telomere.

## RESULTS

**Localization to the nuclear periphery is not correlated with silencing status:** The truncated VII-L and native VI-R telomeres have different levels of TPE (Gottschling *et al.* 1990; Mondoux and Zakian 2007) and different mechanisms for perinuclear localization (Tham *et al.* 2001; Hediger *et al.* 2002). To determine whether their different levels of TPE correlated with different patterns of nuclear localization, and whether localization to the nuclear periphery was necessary for TPE, we monitored nuclear localization and silencing at each telomere.

A strain for monitoring TPE (via a *URA3* reporter) and visualization (via the *lac* operator/LacI-GFP system) of the truncated VII-L telomere has been described previously (Tham *et al.* 2001; Figure 1). At telomere VI-R, *URA3* was integrated into the X element in a manner that largely retains its structure (Mondoux and Zakian 2007; Figure 1). The *lac* operator array was integrated ~15 kb from the VI-R telomere (Hediger *et al.* 2002; Figure 1), and LacI-GFP was introduced into the strain. Previous data (Tham *et al.* 2001) demonstrated that under all growth conditions, the fraction of cells in which the truncated VII-L telomere localizes to the nuclear periphery does not correlate with the fraction of cells in the population that exhibits TPE. Therefore, TPE levels at truncated VII-L do not correlate with nuclear position. However, it is possible that nuclear position correlates with TPE levels between telomeres. This model predicts that telomeres with the highest levels of TPE, such as the VI-R telomere, would be at the periphery more often than telomeres that have lower levels of TPE, like the truncated VII-L telomere.

To test this hypothesis, we examined the nuclear localization of the truncated VII-L and native VI-R telomeres. Cells were grown in medium lacking uracil, in which the reporter gene is expressed in essentially all cells (0% TPE) or in medium containing 5-fluoroorotic acid (5-FOA; Boeke *et al.* 1987), in which the *URA3*

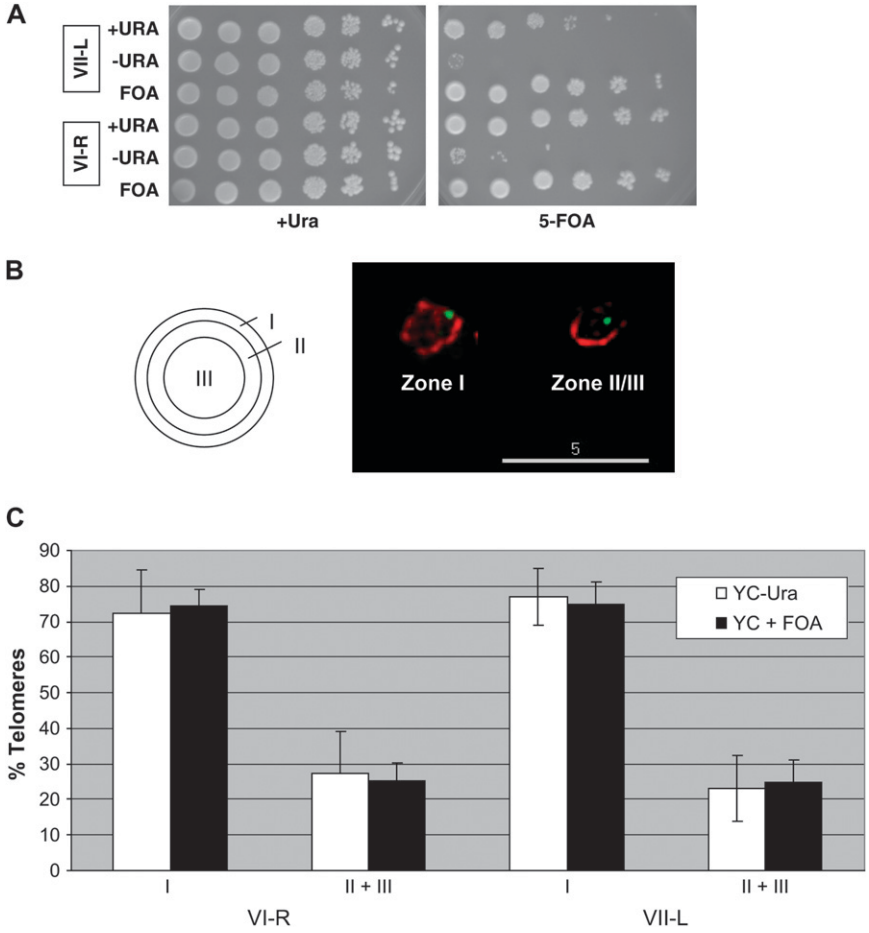


FIGURE 2.—The truncated VII-L and native VI-R telomeres localize to the nuclear periphery regardless of silencing state. (A) TPE assays. Strains were streaked onto plates that contain (+Ura) or lack (−Ura) uracil or onto plates containing 5-FOA (which selects against Ura$^+$ cells) at 30 ° for 3 days and then grown overnight in liquid media of the same type. Cells were diluted back and grown to an optical density of ~0.5, and 10-fold serial dilutions were spotted on plates containing uracil (+Ura) or 5-FOA and photographed after 3 days of growth. Although TPE is higher at the "native" VI-R telomere compared to the truncated VII-L telomere, silencing states in both strains can be manipulated via growth in −Ura (0% TPE) or 5-FOA (100% TPE) media. (B) Telomere localization. Fixed cells were imaged at 100× magnification for both the nuclear periphery (anti-p62) and the VII-L or VI-R telomere (anti-GFP) and two measurements were taken: the distance from the telomere to the periphery and the diameter of the nucleus. The radius was calculated and the nucleus divided into three zones of equal surface area as illustrated. Telomeres were scored as resident in zone I (peripheral, as in nucleus on left) or in zones II or III (nonperipheral, right). Bar, 5 μm. (C) Quantitation of localization. The truncated VII-L telomere and native VI-R telomere localize equally well to the periphery when grown in media lacking uracil (~0% TPE, white) or 5-FOA media (~100% TPE, black). Error bars (here and in other figures) represent standard deviations. There is no significant difference in peripheral localization between telomeres or between conditions by Student's *t*-test, which was used to determine significance here and elsewhere.

reporter gene is silenced in essentially all cells (~100% TPE; Figure 2A). We also grew cells in complete medium (YC + Ura) in which the telomeric *URA3* gene was repressed in only a subset of cells (~15% VII-L; ~85% VI-R). The truncated VII-L and native VI-R telomere reporter strains thus provide a system for examining a complete range of silencing states, from 0 to 100% silencing, at two different telomere ends with different subtelomeric structures and different mechanisms for nuclear localization.

Telomere position relative to the nuclear periphery was determined in formaldehyde-fixed cells using an antibody to a nuclear pore protein as a marker for the nuclear periphery and an antibody to GFP as a marker for the telomere. For each cell, both the distance between the telomere spot and the nuclear periphery and the diameter of the nucleus were measured. The area of the nuclear section was then calculated and divided into equal thirds, and the telomere was scored as localizing to zone I, II, or III, with zone I being defined as the peripheral zone (as in HEDIGER *et al.* 2002). Localization to the periphery did not correlate with subtelomeric structure, as the VII-L and VI-R localization patterns were identical. Localization of both telomeres to the peripheral zone I occurred in ~75% of cells, regardless of silencing status (Figure 2C). For example, in the FOA-grown cells, ~25% of the telomeres were silent but away from the periphery, and in the minus uracil-grown cells ~75% of the telomeres were at the periphery but expressed.

**Localization to Rap1p foci does not correlate with TPE levels:** Given that peripheral localization of telomeres did not correlate with TPE levels (Figure 2C), we reasoned that a subnuclear compartment, rather than the periphery itself, might promote silencing. The Rap1p foci, which contain the silencing proteins, are an obvious candidate for a subcompartment to which silent telomeres might be preferentially localized. In this model, a telomere would have a certain probability of being associated with the periphery regardless of transcriptional state, but when transcriptionally repressed and at the periphery, it would localize to a Rap1p focus (and vice versa). When the subtelomeric gene is expressed, the telomere could be at the

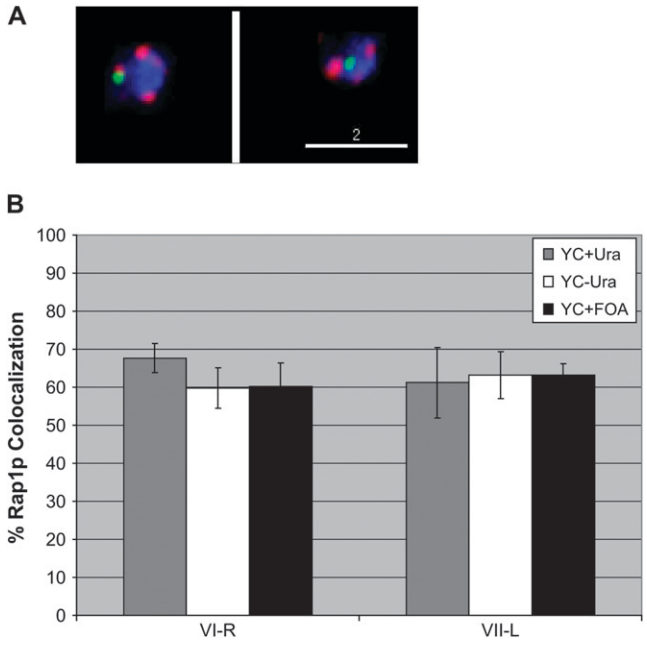


FIGURE 3.—Localization to the Rap1p foci does not correlate with TPE status. (A) Visualization of the Rap1 foci and telomere. Foci are visualized using an anti-Rap1p antibody (red) and telomeres are visualized via the LacO/LacI-GFP system and an anti-GFP antibody (green). Nuclear staining by DAPI is shown in blue. Bar, 2 μm. (B) Quantitation of colocalization of the truncated VII-L telomere and native VI-R telomere with the Rap1p foci. Telomeres were scored as colocalized if any portion of the telomere spot overlapped with a Rap1p spot (as in A, left). The truncated VII-L telomere and native VI-R telomere localize equally well to the Rap1p foci when grown in the presence of uracil (~15% TPE for VII-L and 85% TPE for VI-R, gray), in the absence of uracil (~0% TPE, white) or in the presence of 5-FOA (~100% TPE, black). There is no significant difference in localization to the silencing protein between telomeres or between conditions.

periphery or not, but, if at the periphery, it would be less likely to colocalize with a Rap1p focus than when it is repressed.

To test this model, we determined the frequency of localization of both the truncated VII-L and native VI-R telomeres to the silencing foci, using a Rap1p antibody to visualize the foci. Both the VII-L telomere and the VI-R telomere localized to a Rap1p focus in ~60% of the cells (Figure 3B). This localization pattern was identical for both telomeres in all silencing states, indicating that localization to the Rap1p foci did not correlate with subtelomeric structure or TPE levels. Thus, localization of telomeres to the foci is neither necessary nor sufficient for TPE.

**Tethering a telomere to the nuclear envelope modestly improves TPE:** If localization of telomeres to the nuclear periphery promotes TPE, physically tethering the truncated VII-L telomere to the nuclear envelope should improve its TPE phenotype. To tether the VII-L telomere to the nuclear periphery, four GAL-UAS sites were inserted between the telomere and the *URA3* reporter gene (CHIEN *et al.* 1993). A Gal DNA-binding domain–*Y*ip1 *i*nteracting *f*actor (Yif1p) fusion protein was expressed in these cells (ANDRULIS *et al.* 1998). When a weakened HMR locus is tethered to the periphery using this system, silencing was increased 10- to 100-fold compared to the control Gal binding domain alone (from silencing in <0.01% of cells to 0.1–1%; ANDRULIS *et al.* 1998).

To demonstrate that the truncated VII-L telomere was physically tethered to the nuclear envelope, we measured the distance between the telomere and the nuclear envelope in fixed cells in strains expressing the $Gal_{BD}$–Yif1p fusion protein and in strains expressing only the Gal DNA binding domain. The tethered strain had a significantly greater proportion of VII-L telomeres that were colocalized to the nuclear envelope (75%; Figure 4C) compared to the $Gal_{BD}$ strain (53%; $P < 0.04$). Thus, the truncated VII-L telomere can be physically tethered to the nuclear envelope via Yif1p.

Tethering increased TPE significantly, ~3.5-fold over the strain that expressed only the Gal binding domain ($P < 0.0005$; Figure 4B). However, despite this increase in TPE, the tethered VII-L telomere still had lower TPE than the VI-R telomere (~58% TPE *vs.* ~85% TPE). We tested whether the increased silencing at the tethered VII-L telomere was a result of increased localization to the Rap1p foci. The VII-L telomere colocalized with the Rap1p foci in ~50% of the cells in both the tethered strain and the empty vector strain in all silencing states (Figure 4D). Thus, the increase in TPE observed when the truncated VII-L telomere was tethered to the nuclear periphery was not due to a measurable increase in localization to the Rap1p foci.

**Tethering does not result in longer telomeres or bypass the requirement for yKu70p, Sir4p, or Esc1p in TPE:** Longer telomeres confer increased TPE because they have more binding sites for Rap1p, which can then recruit more Sir proteins to the telomere (KYRION *et al.* 1993). Thus, another possibility for the TPE increase between the tethered and untethered VII-L telomeres is that localization to the nuclear periphery results in telomere lengthening. We tested this possibility by examining the length of the tethered VII-L telomere by Southern blotting. Surprisingly, the tethered VII-L telomere was actually slightly shorter in the tethered strain (~75 bp; Figure 5), compared to $Gal_{BD}$ alone (Figure 5) or a no-plasmid control (data not shown). This telomere shortening was specific to the tethered telomere, as bulk telomere length was unchanged. The length of the tethered VII-L telomere was stable over ~75 generations, in contrast to the shortening phenotype observed when cells lack components of the telomerase holoenzyme (for example, *est3*Δ; Figure 5). These data demonstrate that increased telomere length does not explain the increase in TPE observed at the tethered telomere.

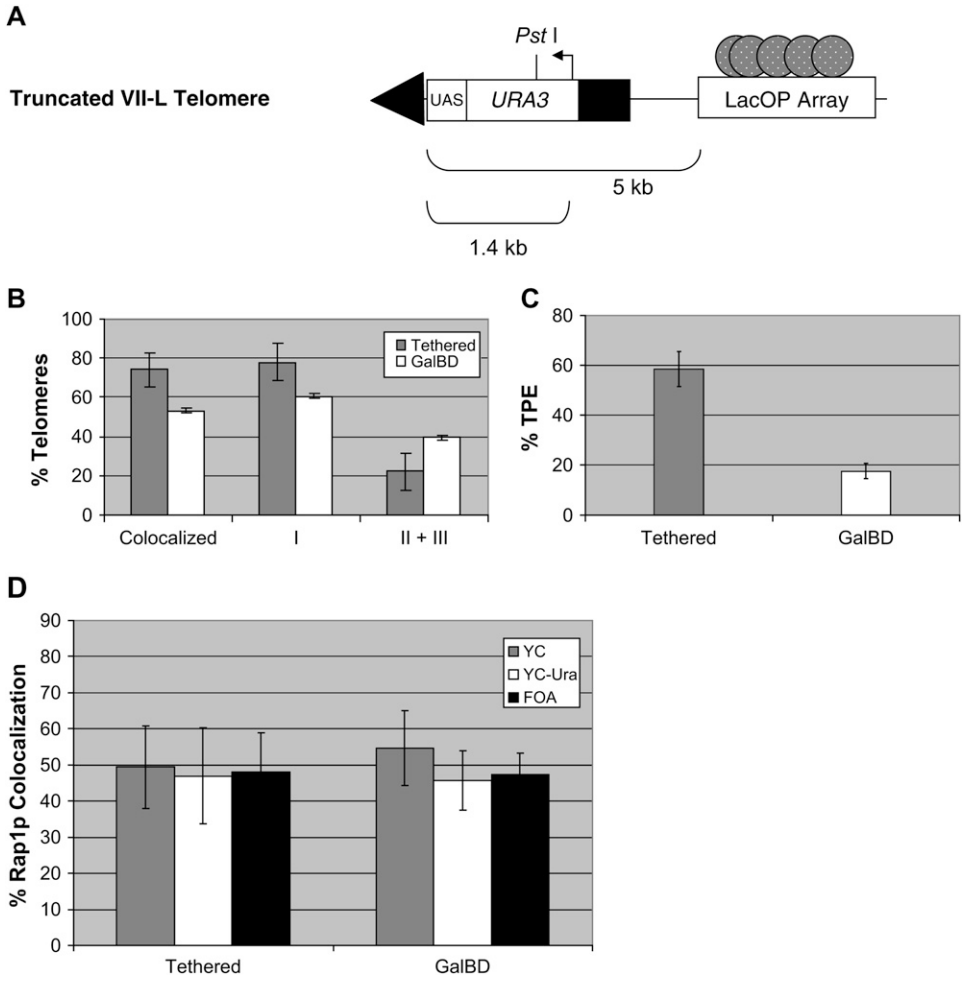


FIGURE 4.—Tethering the truncated VII-L telomere increases TPE but does not increase colocalization with the Rap1p foci. (A) Structure of the tethered VII-L telomere. Four UAS sites are immediately adjacent to the VII-L telomere (CHIEN *et al.* 1993) and can bind $Gal_{BD}$ or $Gal_{BD}$–Yif1p (tethered strain; ANDRULIS *et al.* 1998). The *lac* op/LacI-GFP system was introduced at the same telomere for visualization (THAM *et al.* 2001). (B) As in Figure 3, YC-grown cells were imaged for both the nuclear periphery (anti-p62) and the VII-L telomere (anti-GFP) and the nucleus divided into three zones of equal surface area. VII-L telomeres were scored as overlapping with the nuclear envelope (colocalized) or resident in zone I (peripheral), II, or III (nonperipheral). The truncated VII-L telomere was significantly tethered to the nuclear envelope in the presence of $Gal_{BD}$–Yif1p compared to the strain expressing $Gal_{BD}$ alone ($P < 0.04$). (C) Quantitation of TPE. As in Figure 2, the level of TPE was calculated by colony counting. TPE at the tethered truncated VII-L telomere is significantly higher than TPE at the truncated VII-L telomere in the strain expressing $Gal_{BD}$ alone ($P < 0.0005$). (D) Quantitation of colocalization between the tethered or $Gal_{BD}$ truncated VII-L telomere and the Rap1p foci. As in Figure 3, telomeres were scored as colocalized with the Rap1p foci if any portion of the telomere spot overlapped with a Rap1p spot. Localization is the same across all silencing states and tethering states. All experiments using the tethering system were repeated with at least two independent transformants.

Since TPE was increased at the tethered VII-L telomere despite its shorter length, it seemed possible that this increase was the result of a bypass of factors normally required for TPE. Both the Sir proteins and Ku complex are brought to the telomere at least in part by interactions with Rap1p (MORETTI *et al.* 1994; TSUKAMOTO *et al.* 1997; MORETTI and SHORE 2001). Deletion of either the Ku complex (BOULTON and JACKSON 1996, 1998) or Sir4p (APARICIO *et al.* 1991) abolish TPE at the truncated VII-L telomere. Sir4p also binds to Esc1p, an inner nuclear membrane protein (ANDRULIS *et al.* 2002). Together, the Ku complex, Sir4p, and Esc1p function in redundant peripheral localization pathways (HEDIGER *et al.* 2002; TADDEI *et al.* 2004). We tested whether tethering the truncated VII-L telomere to the nuclear periphery bypassed the role of these proteins in TPE by comparing TPE levels in the mutant and wild-type versions of the tethered and empty vector strains. In the absence of *YKU70* or *SIR4*, TPE was abolished at both the tethered telomere and the telomere bound to $Gal_{BD}$ alone (Figure 6). Therefore, tethering a telomere to the nuclear periphery does not increase TPE by bypassing the requirement for either the Ku or Sir proteins in TPE. The increase in *HM* silencing observed by tethering is also Sir4p dependent (ANDRULIS *et al.* 1998).

Unlike the deletion of *YKU70* or *SIR4*, deletion of *ESC1* decreases, but does not abolish, TPE at the truncated VII-L telomere (ANDRULIS *et al.* 2002). If tethering bypassed the requirement for Esc1p in TPE, we would expect TPE levels to be the same in the tethered wild-type strain and the tethered *esc1*Δ strain. In agreement with previous results, TPE at the truncated VII-L telomere was decreased in the absence of Esc1p (Figure 6). In addition, although TPE increased when the telomere was tethered, the tethered wild-type strain had higher levels of TPE than the tethered *esc1*Δ strain (Figure 6), indicating that tethering also does not bypass the role of Esc1p in TPE.

**The presence of the Rap1p foci correlates with the potential for TPE:** We found no evidence for a cor-

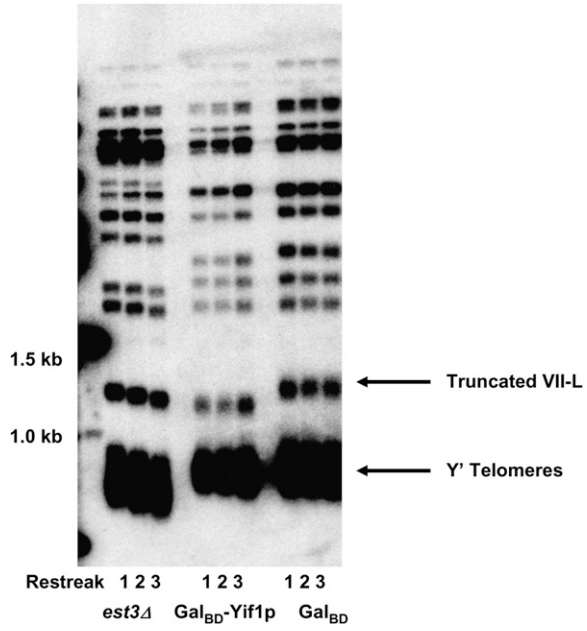


FIGURE 5.—The truncated VII-L telomere is stably shorter when tethered to the nuclear envelope. Genomic DNA was digested with *Pst*I (cuts *URA3* at truncated VII-L, see Figure1) and *Xho*I (cuts in subtelomeric Y′ DNA), run on Southern blot and probed for telomere sequence. Although the lengths of the Y′ telomeres are unchanged in the tethered (center lanes) and empty vector (right lanes) strains, the tethered VII-L telomere is ~75 bp shorter than the VII-L telomere bound to Gal$_{BD}$ alone. This telomere length is stable over successive restreaks (~25 cell divisions each, indicated 1–3), unlike the *est3*Δ control, which lacks telomerase and thus gets shorter with each restreak (LENDVAY *et al.* 1996).

relation between a telomere's localization to the Rap1p foci and its level of TPE (Figures 3 and 4). However, the model that telomere clustering in the Rap1p foci creates a high local concentration of silencing factors that facilitates TPE is still attractive as, to date, no mutation or condition has been reported that supports TPE in the absence of the foci. We therefore tested whether eliminating other proteins that affect telomeres would disrupt the Rap1p foci yet maintain TPE. Sir1p, Mec1p, and Sas2p are required for wild-type levels of TPE at some telomeres and under some conditions (REIFSNYDER *et al.* 1996; PRYDE and LOUIS 1999; CRAVEN and PETES 2000; MONDOUX and ZAKIAN 2007). Using a Rap1p antibody to visualize the foci (as in Figure 3), we observed that Rap1p foci formed in *sir1*Δ, *mec1*Δ, and *sas2*Δ cells (data not shown). The Rap1p foci are known to disperse upon DNA damage (MCAINSH *et al.* 1999). Therefore, we also examined whether foci would form in *xrs2*Δ and *tel1*Δ cells, both of which maintain wild-type or slightly reduced TPE (RUNGE and ZAKIAN 1996; BOULTON and JACKSON 1998). Rap1p foci were present in both *xrs2*Δ and *tel1*Δ strains (data not shown).

**The number of silencing foci correlates with TPE levels:** Unlike the Sir4p-binding proteins Sir1p and Esc1p, the Sir4p-binding Ku complex is absolutely required for TPE in wild-type cells (BOULTON and JACKSON 1998), and its deletion disperses the Rap1p foci (LAROCHE *et al.* 1998). Like the Sir proteins, Rif1p and Rif2p bind to the carboxyl terminus of Rap1p, and this binding is mutually exclusive with binding of the Sir complex (MORETTI *et al.* 1994; WOTTON and SHORE 1997). *rif1*Δ *rif2*Δ strains have very long telomeres (MORETTI *et al.* 1994; WOTTON and SHORE 1997), and this lengthening is telomerase dependent (TENG *et al.* 2000). Thus, when Rif proteins are absent, more Sir proteins can bind to telomeres both because telomeres are longer and because the Sir complex no longer competes with Rifs for binding to telomere-bound Rap1p. Due to higher Sir binding, *rif* cells have increased levels of TPE (KYRION *et al.* 1993). Silencing can be restored to wild-type levels at the truncated VII-L telomere even in a *yku70*Δ mutant by deleting both the *RIF1* and *RIF2* genes. Moreover, a low level of TPE (~0.1%) is seen when *RIF1* alone is deleted in a *yku70*Δ strain (MISHRA and SHORE 1999).

In agreement with previous results (MISHRA and SHORE 1999), the deletion of *RIF1*or both *RIF1* and *RIF2* restored TPE in a *yku70*Δ strain (Figure 7A). The TPE level in *yku70*Δ *rif1*Δ cells was low (~0.1%), while TPE in the *yku70*Δ *rif1*Δ *rif2*Δ strain was close to wild type (~8% in *yku70*Δ *rif1*Δ *rif2*Δ *vs.* ~23% in wild type, $P < 0.05$; Figure 7B). As reported previously, the Rap1p foci were dispersed in *yku70*Δ cells (LAROCHE *et al.* 1998; Figure 7C) and restored in *yku70*Δ *rif1*Δ cells (HEDIGER *et al.* 2002; Figure 7C). As expected, foci were also restored in a *yku70*Δ *rif1*Δ *rif2*Δ strain (Figure 7C), indicating that Ku is not essential for the formation of Rap1p foci in this background. In addition, the number of Rap1p foci per nucleus correlated with the level of TPE. In wild-type haploid cells, most nuclei contain three to six Rap1p foci (KLEIN *et al.* 1992), a result confirmed here (Figure 7D). TPE in the *yku70*Δ *rif1*Δ *rif2*Δ strain was reduced threefold compared to the wild-type strain (Figures 7, B and C). The number of foci per nucleus in this strain was also significantly different from wild type ($P < 4.4 \times 10^{-8}$). Although most of the nuclei had three to six foci, no nuclei were observed with more than six foci (0% *vs.* 13% in wild type), and more nuclei were observed with fewer than three foci (28% *vs.* 14% in wild type). TPE in the *yku70*Δ *rif1*Δ mutant was reduced >100-fold compared to wild type (Figure 7A), and most of the nuclei in this mutant contained only one to four foci (Figure 7D). Moreover, no *yku70*Δ *rif1*Δ nuclei had more than six foci (0% *vs.* 13%), and almost half had fewer than three foci (46% *vs.* 14% in wild type). This distribution was significantly different from wild type ($P < 3.2 \times 10^{-16}$).

## DISCUSSION

We investigated whether there are differences in the nuclear localization of the truncated VII-L and native

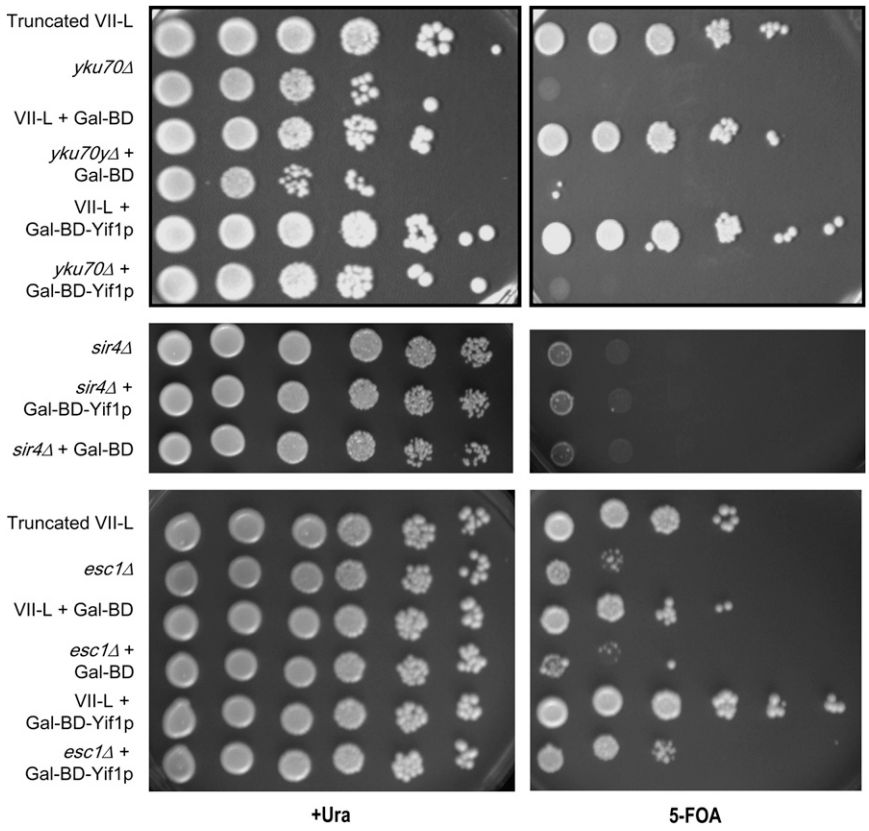


FIGURE 6.—Tethering does not bypass the function of the telomere localization factors yKu70, Esc1p, or Sir4p in TPE. As in Figure 2, 10-fold serial dilutions of strains lacking factors known to be involved in telomere localization and silencing were plated onto +Ura and 5-FOA plates to observe TPE levels. In the mutant strains, TPE was eliminated (*sir4*Δ, *yku70*Δ) or decreased (*esc1*Δ) at both the tethered and $Gal_{BD}$ truncated VII-L telomeres. The level of TPE in the mutant tethered strains (+$Gal_{BD}$–Yif1p) was reduced compared to TPE in the wild-type tethered strain, indicating that tethering does not bypass the functions of these genes in TPE.

VI-R telomeres that could explain their different TPE phenotypes. However, both telomeres localized equally well to the nuclear periphery in both silent and expressed states (Figure 2C). Likewise, both telomeres localized equally well to the Rap1p foci, and this localization was also independent of transcriptional state (Figure 3B). Even when tethered to the nuclear periphery, the truncated VII-L telomere was not associated more often with Rap1p foci, and its silencing level was still lower than that of the native VI-R telomere (Figure 4). Likewise, silencing of the *HMR* locus does not require its localization to the nuclear periphery, as a plasmid-borne *HMR* locus is silent and away from the nuclear envelope in a y*ku*Δ *esc1*Δ strain (GARTENBERG *et al.* 2004). However, *HM* silencing occurs in both y*ku*Δ and y*ku*Δ *esc1*Δ cells in which there are no Rap1p foci (LAROCHE *et al.* 1998; MAILLET *et al.* 2001; GARTENBERG *et al.* 2004) while neither strain is competent for TPE (BOULTON and JACKSON 1998; GARTENBERG *et al.* 2004). We find no correlation between telomere localization and TPE levels even in cells competent for TPE with intact Rap1p foci. Our data seem to rule out models in which telomere placement at the periphery serves as a mechanism to maintain transcriptional repression by bringing telomeres in close proximity to silencing proteins. In addition, our data argue against models in which placement near the nuclear periphery promotes TPE by other mechanisms. For example, our data do not support a model in which association with the nuclear periphery constrains telomere mobility in a manner that makes it more difficult for RNA polymerase to transcribe through a telomere-linked gene.

Although the Rap1p foci did not constitute a nuclear subcompartment that enhanced silencing via colocalization with telomeres, the presence of the Rap1p foci correlated with the potential for silencing. Thus, a possible model is that the integrity of the Rap1p foci is required for TPE. The foci could act as heterochromatin-assembly "factories," and a telomere's localization to a focus might be necessary but not sufficient to establish, but not to maintain, TPE. Alternatively, localization with a focus might be necessary for maintenance as well, but the amount of time required for the telomere to "visit" the focus might be too brief to distinguish in our assay.

Our favored model for the restoration of the foci of silencing proteins in the *yku70*Δ *rif*Δ mutants is that, as is the case for TPE, it is not the presence of the Ku complex or Rap1p at the telomere *per se*, but the amount of Sir2, Sir3, and Sir4 protein they can recruit to the telomere that is responsible for the formation of the foci. Ku was not required for focus formation in *rif* strains that could recruit sufficient levels of Sir proteins to telomeres via a Ku-independent pathway (Figure 7C; HEDIGER *et al.* 2002). Likewise, Ku is not needed for TPE in these strains (Figure 7A; MISHRA and SHORE 1999). In the model we propose, the loss of Ku or deletion of the Rap1p C terminus disperses the foci (HECHT *et al.* 1995; LAROCHE *et al.* 1998) because of the concomitant loss of

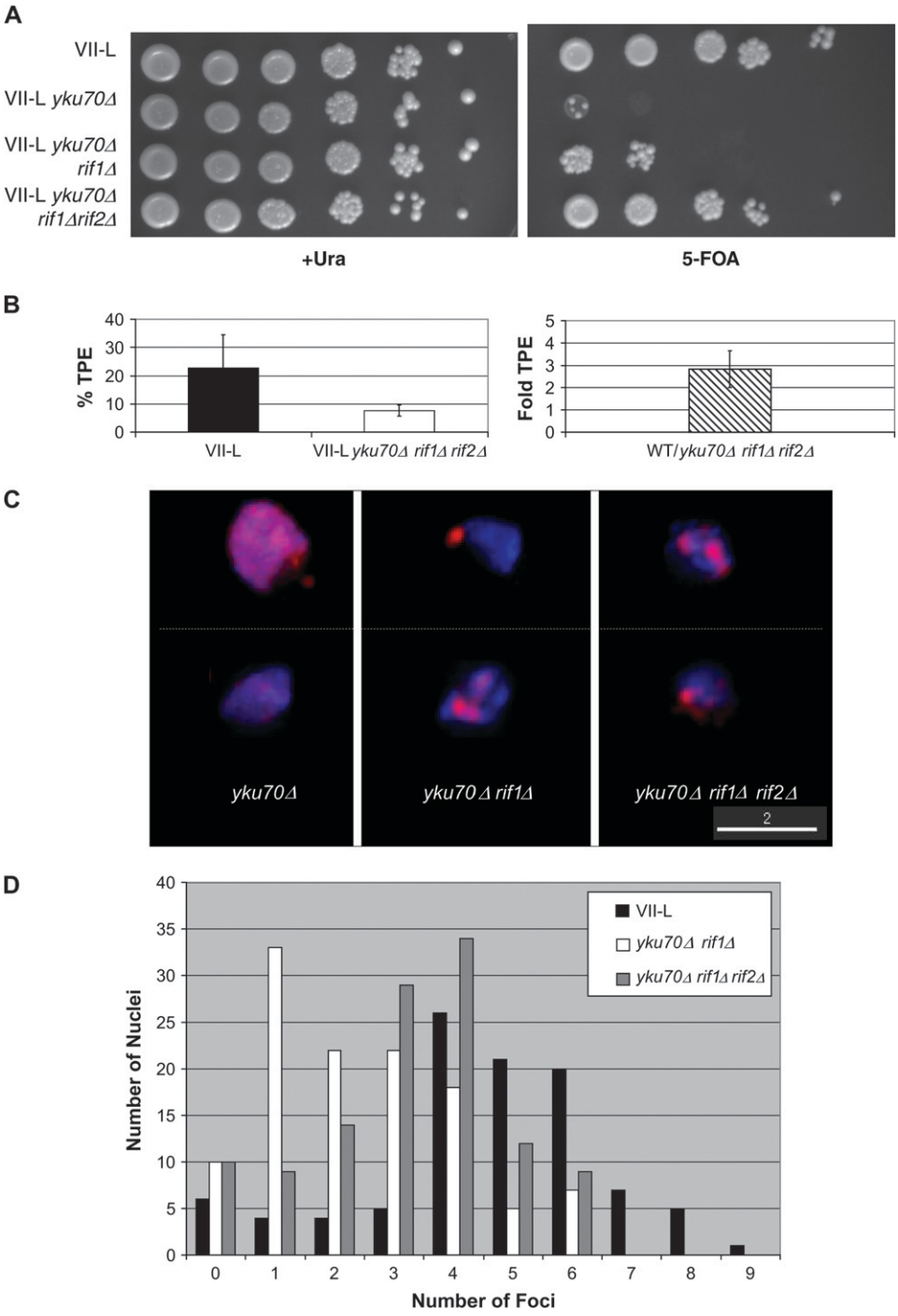


FIGURE 7.—Rap1p focus formation correlates with TPE levels and does not require the Ku complex. (A) TPE assays were performed as in Figure 2. Deleting *YKU70* eliminates TPE at truncated VII-L; deleting *RIF1* restores a low level of silencing, and deleting *RIF1* and *RIF2* restores a near wild-type level of TPE to *yku70*Δ cells (as in MISHRA and SHORE 1999). (B) Quantitation of TPE. TPE is approximately threefold lower in the triple mutant *yku70*Δ *rif1*Δ *rif2*Δ compared to wild type ($P < 0.05$). (C) Immunofluoresence of fixed cells in which the Rap1p foci were visualized via anti-Rap1p (red) and the DNA visualized via DAPI stain (blue). 100× images were taken on the DeltaVision microscope, deconvolved, and shown as flattened stacks. Bar, 2 μm. Rap1p foci and TPE are eliminated in the *yku70*Δ mutant (as in LAROCHE *et al.* 1998), and Rap1p is diffuse throughout the nucleus. In *yku70*Δ *rif1*Δ (HEDIGER *et al.* 2002) or *yku70*Δ *rif1*Δ *rif2*Δ cells, both the potential for TPE and the presence of the Rap1p foci are restored, although the number of foci present in each nucleus varies (two representative nuclei are shown for each mutant). (D) Distribution of Rap1p foci in TPE restoration mutants. In wild-type cells, most nuclei have 3–6 Rap1p foci. This distribution is significantly different from that in the *yku70*Δ *rif1*Δ *rif2*Δ mutant, which exhibits a threefold decrease in TPE compared to wild type. In this mutant, there are fewer nuclei with more than six foci and more nuclei with fewer than three foci ($P < 4.4 \times 10^{-8}$). In the *yku70*Δ *rif1*Δ cells, in which TPE is decreased ~100-fold compared to wild type, only half the nuclei have 3–6 Rap1p foci and the rest have fewer than three foci. This distribution is also different from that in wild-type cells ($P < 3.2 \times 10^{-16}$). When counting the number of foci per cell, at least 50 cells were counted for each mutant in two independent experiments.

Sir proteins at the telomere. Deleting the Rif proteins in the *yku70*Δ background restores the foci because of the concomitant increase in Sir proteins at the telomere. Therefore, the presence of the foci themselves need not be required for TPE. Rather, the conditions that create the foci (*i.e.*, the level of Sir-protein binding to telomeres) are the same conditions that permit TPE. This model would predict that no mutations could bypass the requirement for the Sir proteins in either TPE or focus formation, and no such mutations have been identified. Our finding that TPE levels correlated with the number of Rap1p foci per nucleus is also consistent with the idea that more Sir binding at the telomere contributes to both focus formation and TPE levels.

Despite the lack of correlation between the nuclear periphery and silencing status in wild-type strains,

tethering the truncated VII-L telomere to the nuclear periphery increased TPE (Figure 4C). However, the tethered VII-L telomere was still silenced in fewer cells (58%) than the untethered native VI-R telomere (85%). Likewise, tethering a weakened *HMR* locus to the periphery increases its silencing level, but its new level is still much lower than that for a wild type, untethered *HMR* locus (Andrulis *et al.* 1998). Improved silencing of the tethered VII-L telomere was not due to bypassing the functions of three proteins, yKu70p, Sir4p, and Esc1p, that affect both TPE and nuclear localization (Figure 6). In addition, this increase in TPE was not due to a measurable increase in localization to Rap1p foci (Figure 4D), a surprising result given that Rap1p foci are also at the nuclear periphery, or to an increase in telomere length (Figure 5).

The data presented here show that localization to the nuclear periphery or to Rap1p foci does not correlate with level of TPE. Furthermore, localization to the nuclear periphery or to the Rap1p foci was neither necessary nor sufficient for silencing of either the VI-R or the VII-L telomere (Figures 2 and 3). Telomeres can be away from the periphery and be silent, suggesting that localization is not important for silencing. However, these data do not rule out a model in which an individual telomere must visit the periphery to establish or maintain TPE. Neither subtelomeric sequence (Mondoux and Zakian 2007) nor, as shown here, nuclear localization can explain the difference in TPE levels between the native VI-R and the truncated VII-L telomeres. These results do not exclude the possibility that *trans* mechanisms other than nuclear localization, like higher order chromosome structure or dynamics, could play a role in determining the TPE phenotypes of individual telomeres. There could also be *cis* influences on TPE, for example, proximal sequences that promote or repress TPE, or transcriptional activity from nearby genes. The VI-R telomere has a high level of TPE, which may be due in part to the repression of its most proximal gene, *YFR057w* (Wyrick *et al.* 1999; Vega-Palas *et al.* 2000). Nucleosome spacing could also influence preferential recruitment of the Sir proteins to particular telomeres, as it influences directional silencing at the silent mating-type loci (Zou *et al.* 2006).

Localization of telomeres to the periphery may be important for a telomere function other than silencing. Peripheral localization of telomeres does not determine their late replication (Hiraga *et al.* 2006), but it could influence recombination, as the Ku complex regulates telomere–telomere recombination as a maintenance mechanism in the absence of telomerase (Tsai *et al.* 2002). Furthermore, double-strand breaks in the XI-L subtelomeric region cannot be repaired if its localization to the periphery is eliminated via disruption of the Nup84 complex (Therizols *et al.* 2006). In mammalian cells, localization away from the periphery correlates with large-scale compaction at the IgH locus (Kosak *et al.* 2002), and telomere localization could also be linked to changes in higher-order chromosome structure. When the truncated VII-L telomere was tethered to the nuclear envelope, it was shorter than the telomere bound to the Gal DNA binding domain alone (Figure 6). Therefore, the nuclear localization of telomeres may play a role in length regulation either because the telomeres themselves must leave the periphery in order to be lengthened by telomerase or because active telomerase is not available in particular nuclear subcompartments.

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## LITERATURE CITED

Andrulis, E. D., A. M. Neiman, D. C. Zappulla and R. Sternglanz, 1998 Perinuclear localization of chromatin facilitates transcriptional silencing. Nature **394:** 592–595.

Andrulis, E. D., D. C. Zappulla, A. Ansari, S. Perrod, C. V. Laiosa *et al.*, 2002 Esc1, a nuclear periphery protein required for Sir4-based plasmid anchoring and partitioning. Mol. Cell. Biol. **22:** 8292–8301.

Aparicio, O. M., B. L. Billington and D. E. Gottschling, 1991 Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. Cell **66:** 1279–1287.

Boeke, J. D., J. Trueheart, G. Natsoulis and G. R. Fink, 1987 5-Flouroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154:** 164–175.

Boulton, S. J., and S. P. Jackson, 1996 Identification of a *Saccharomyces cerevisiae* Ku80 homologue: roles in DNA double strand break rejoining and in telomeric maintenance. Nucleic Acids Res. **24:** 4639–4648.

Boulton, S. J., and S. P. Jackson, 1998 Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. EMBO J. **17:** 1819–1828.

Bourgeois, C. A., F. Laquerriere, D. Hemon, J. Hubert and M. Bouteille, 1985 New data on the in-situ position of the inactive X chromosome in the interphase nucleus of human fibroblasts. Hum. Genet. **69:** 122–129.

Brickner, J. H., and P. Walter, 2004 Gene recruitment of the activated INO1 locus to the nuclear membrane. PLoS Biol. **2:** e342.

Bystricky, K., T. Laroche, G. van Houwe, M. Blaszczyk and S. M. Gasser, 2005 Chromosome looping in yeast: telomere pairing and coordinated movement reflect anchoring efficiency and territorial organization. J. Cell Biol. **168:** 375–387.

Casolari, J. M., C. R. Brown, S. Komili, J. West, H. Hieronymus *et al.*, 2004 Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. Cell **117:** 427–439.

Chien, C. T., P. L. Bartel, R. Sternglanz and S. Fields, 1991 The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. Proc. Natl. Acad. Sci. USA **88:** 9578–9582.

Chien, C. T., S. Buck, R. Sternglanz and D. Shore, 1993 Targeting of SIR1 protein establishes transcriptional silencing at *HM* loci and telomeres in yeast. Cell **75:** 531–541.

Conrad, M. N., J. H. Wright, A. J. Wolf and V. A. Zakian, 1990 RAP1 protein interacts with yeast telomeres *in vivo*: overproduction alters telomere structure and decreases chromosome stability. Cell **63:** 739–750.

Craven, R. J., and T. D. Petes, 2000 Involvement of the checkpoint protein Mec1p in silencing of gene expression at telomeres in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **20:** 2378–2384.

Gartenberg, M. R., F. R. Neumann, T. Laroche, M. Blaszczyk and S. M. Gasser, 2004 Sir-mediated repression can occur independently of chromosomal and subnuclear contexts. Cell **119:** 955–967.

Goldstein, A. L., and J. H. McCusker, 1999 Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast **15:** 1541–1553.

Gotta, M., T. Laroche, A. Formenton, L. Maillet, H. Scherthan *et al.*, 1996 The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae*. J. Cell Biol. **134:** 1349–1363.

Gottschling, D. E., O. M. Aparicio, B. L. Billington and V. A. Zakian, 1990 Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. Cell **63:** 751–762.

Goudsouzian, L., C. Tuzon and V. A. Zakian, 2006 *S. cerevisiae* Tel1p and Mre11p are required for normal levels of Est1p and Est2p telomere association. Mol. Cell **24:** 603–610.

Hecht, A., T. Laroche, S. Strahl-Bolsinger, S. M. Gasser and M. Grunstein, 1995 Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell **80:** 583–592.

Hediger, F., F. R. Neumann, G. Van Houwe, K. Dubrana and S. M. Gasser, 2002 Live imaging of telomeres: yKu and Sir proteins define redundant telomere-anchoring pathways in yeast. Curr. Biol. **12:** 2076–2089.

Hiraga, S., E. D. Robertson and A. D. Donaldson, 2006 The Ctf18 RFC-like complex positions yeast telomeres but does not specify their replication time. EMBO J. **25:** 1505–1514.

Ishii, K., G. Arib, C. Lin, G. Van Houwe and U. K. Laemmli, 2002 Chromatin boundaries in budding yeast: the nuclear pore connection. Cell **109:** 551–562.

Klein, F., T. Laroche, M. E. Cardenas, J. F. Hofmann, D. Schweizer *et al.*, 1992 Localization of RAP1 and topoisomerase II in nucleic and meiotic chromosomes of yeast. J. Cell Biol. **117:** 935–948.

Kosak, S. T., and M. Groudine, 2004 Gene order and dynamic domains. Science **306:** 644–647.

Kosak, S. T., J. A. Skok, K. L. Medina, R. Riblet, M. M. Le Beau *et al.*, 2002 Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. Science **296:** 158–162.

Kyrion, G., K. Liu, C. Liu and A. J. Lustig, 1993 RAP1 and telomere structure regulate telomere position effects in *Saccharomyces cerevisiae*. Genes Dev. **7:** 1146–1159.

Laroche, T., S. G. Martin, M. Gotta, H. C. Gorham, F. E. Pryde *et al.*, 1998 Mutation of yeast Ku genes disrupts the subnuclear organization of telomeres. Curr. Biol. **8:** 653–656.

Lendvay, T. S., D. K. Morris, J. Sah, B. Balasubramanian and V. Lundblad, 1996 Senescence mutants of *Saccharomyces cerevisiae* with a defect in telomere replication identify three additional *EST* genes. Genetics **144:** 1399–1412.

Maillet, L., F. Gaden, V. Brevet, G. Fourel, S. G. Martin *et al.*, 2001 Ku-deficient yeast strains exhibit alternative states of silencing competence. EMBO Rep. **2:** 203–210.

McAinsh, A. D., S. Scott-Drew, J. A. Murray and S. P. Jackson, 1999 DNA damage triggers disruption of telomeric silencing and Mec1p- dependent relocation of Sir3p. Curr. Biol. **9:** 963–966.

Menon, B. B., N. J. Sarma, S. Pasula, S. J. Deminoff, K. A. Willis *et al.*, 2005 Reverse recruitment: the Nup84 nuclear pore subcomplex mediates Rap1/Gcr1/Gcr2 transcriptional activation. Proc. Natl. Acad. Sci. USA **102:** 5749–5754.

Mishra, K., and D. Shore, 1999 Yeast Ku protein plays a direct role in telomeric silencing and counteracts inhibition by rif proteins. Curr. Biol. **9:** 1123–1126.

Mondoux, M. A., and V. A. Zakian, 2005 Telomere position effect: silencing near the end, pp. 261–316 in *Telomeres*, Ed. 2, edited by T. de Lange, V. Lundblad and E. H. Blackburn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Mondoux, M. A., and V. A. Zakian, 2007 Subtelomeric elements influence but do not determine silencing levels at *Saccharomyces cerevisiae* telomeres. Genetics. **177:** 2541–2546.

Moretti, P., K. Freeman, L. Coodly and D. Shore, 1994 Evidence that a complex of SIR proteins interacts with the silencer and telomere-binding protein RAP1. Genes Dev. **8:** 2257–2269.

Moretti, P., and D. Shore, 2001 Multiple interactions in Sir protein recruitment by Rap1p at silencers and telomeres in yeast. Mol. Cell. Biol. **21:** 8082–8094.

Palladino, F., T. Laroche, E. Gilson, A. Axelrod, L. Pillus *et al.*, 1993 SIR3 and SIR4 proteins are required for the positioning and integrity of yeast telomeres. Cell **75:** 543–555.

Pryde, F. E., and E. J. Louis, 1999 Limitations of silencing at native yeast telomeres. EMBO J. **18:** 2538–2550.

Reifsnyder, C., J. Lowell, A. Clarke and L. Pillus, 1996 Yeast *SAS* silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. Nat. Genet. **14:** 42–49.

Robinett, C. C., A. Straight, G. Li, C. Willhelm, G. Sudlow *et al.*, 1996 In vivo localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition. J. Cell Biol. **135:** 1685–1700.

Runge, K. W., and V. A. Zakian, 1996 *TEL2*, an essential gene required for telomere length regulation and telomere position effect in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:** 3094–3105.

Schmid, M., G. Arib, C. Laemmli, J. Nishikawa, T. Durussel *et al.*, 2006 Nup-PI: the nucleopore-promoter interaction of genes in yeast. Mol. Cell **21:** 379–391.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

Taddei, A., F. Hediger, F. R. Neumann, C. Bauer and S. M. Gasser, 2004 Separation of silencing from perinuclear anchoring functions in yeast Ku80, Sir4 and Esc1 proteins. EMBO J. **23:** 1301–1312.

Taddei, A., G. Van Houwe, F. Hediger, V. Kalck, F. Cubizolles *et al.*, 2006 Nuclear pore association confers optimal expression levels for an inducible yeast gene. Nature **441:** 774–778.

Teng, S.-C., J. Chang, B. McCowan and V. A. Zakian, 2000 Telomerase-independent lengthening of yeast telomeres occurs by an abrupt Rad50p-dependent, Rif-inhibited recombinational process. Mol. Cell **6:** 947–952.

Tham, W. H., J. S. Wyithe, P. K. Ferrigno, P. A. Silver and V. A. Zakian, 2001 Localization of yeast telomeres to the nuclear periphery is separable from transcriptional repression and telomere stability functions. Mol. Cell **8:** 189–199.

Therizols, P., C. Fairhead, G. G. Cabal, A. Genovesio, J. C. Olivo-Marin *et al.*, 2006 Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. J. Cell Biol. **172:** 189–199.

Tsai, Y. L., S. F. Tseng, S. H. Chang, C. C. Lin and S. C. Teng, 2002 Involvement of replicative polymerases, Tel1p, Mec1p, Cdc13p, and the Ku complex in telomere-telomere recombination. Mol. Cell. Biol. **22:** 5679–5687.

Tsukamoto, Y., J. Kato and H. Ikeda, 1997 Silencing factors participate in DNA repair and recombination in *Saccharomyces cerevisiae*. Nature **388:** 900–903.

Vega-Palas, M. A., E. Martin-Figueroa and F. J. Florencio, 2000 Telomeric silencing of a natural subtelomeric gene. Mol. Gen. Genet. **263:** 287–291.

Wach, A., A. Brachat, R. Pöhlmann and P. Philippsen, 1994 New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast **10:** 1793–1808.

Wotton, D., and D. Shore, 1997 A novel Rap1p-interacting factor, Rif2p, cooperates with Rif1p to regulate telomere length in *Saccharomyces cerevisiae*. Genes Dev. **11:** 748–760.

Wyrick, J. J., F. C. Holstege, E. G. Jennings, H. C. Causton, D. Shore *et al.*, 1999 Chromosomal landscape of nucleosome-dependent gene expression and silencing in yeast. Nature **402:** 418–421.

Zink, D., M. D. Amaral, A. Englmann, S. Lang, L. A. Clarke *et al.*, 2004 Transcription-dependent spatial arrangements of CFTR and adjacent genes in human cell nuclei. J. Cell Biol. **166:** 815–825.

Zou, Y., Q. Yu and X. Bi, 2006 Asymmetric positioning of nucleosomes and directional establishment of transcriptionally silent chromatin by *Saccharomyces cerevisiae* silencers. Mol. Cell. Biol. **26:** 7806–7819.

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