EMBO reports

scientific report

Subtelomeric repetitive elements determine TERRA regulation by Rap1/Rif and Rap1/Sir complexes in yeast

Nahid Iglesias[1†], Sophie Redon[1], Verena Pfeiffer[1], Martina Dees[2], Joachim Lingner[1+] & Brian Luke[1,2++]

[1]Swiss Institute for Experimental Cancer Research, School of Life Sciences, Frontiers in Genetics National Center of Competence in Research, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, and [2]Zentrum für Molekulare Biologie der Universität Heidelberg, DKFZ-ZMBH Allianz, Heidelberg, Germany

Telomeric repeat-containing RNA (TERRA) has been implicated in the control of heterochromatin and telomerase. We demonstrate that yeast TERRA is regulated by telomere-binding proteins in a chromosome-end-specific manner that is dependent on subtelomeric repetitive DNA elements. At telomeres that contain only X-elements, the Rap1 carboxy-terminal domain recruits the Sir2/3/4 and Rif1/2 complexes to repress transcription in addition to promoting Rat1-nuclease-dependent TERRA degradation. At telomeres that contain Y′ elements, however, Rap1 represses TERRA through recruitment of Rif1 and Rif2. Our work emphasizes the importance of subtelomeric DNA in the control of telomeric protein composition and telomere transcription.

Keywords: non-coding RNA; Rap1; telomere; TERRA

EMBO *reports* (2011) **12**, 587–593. doi:10.1038/embor.2011.73

INTRODUCTION

Telomeres are essential heterochromatic structures at chromosome ends that ensure genome stability, mediate chromosome positioning and regulate the lifespan of cells that lack telomerase. In *Saccharomyces cerevisiae,* telomeric DNA is bound by the repressor/activator protein Rap1. Rap1 is essential for telomere-length regulation, chromosome-end protection and silencing of subtelomeric inserted reporter genes (Kyrion *et al*, 1993; Shore, 1994; Marcand *et al*, 1997; Pardo & Marcand, 2005). Rap1 elicits many of its functions through the recruitment of Sir proteins (Sir2, 3 and 4) and Rif proteins (Rif1 and 2) by its carboxy-terminal domain (Hardy *et al*, 1992; Moretti *et al*, 1994; Cockell *et al*, 1995; Wotton & Shore, 1997). The histone deacetylase Sir2 functions with Sir3 and Sir4 to promote silencing at telomeres (Rine & Herskowitz, 1987; Aparicio *et al*, 1991), whereas Rif1 and Rif2 regulate telomere length by mediating the Rap1-counting mechanism for telomerase control (Hardy *et al*, 1992; Shore, 1994; Wotton & Shore, 1997). In addition to telomeric DNA and its associated proteins, yeast telomeres contain the non-coding telomeric repeat-containing RNA (TERRA), which is transcribed by the RNA polymerase II (RNAPII; Luke *et al*, 2008).

TERRA is conserved from yeast to humans (Luke & Lingner, 2009) and localized at telomeres in mammalian cells (Azzalin *et al*, 2007; Schoeftner & Blasco, 2008; Zhang *et al*, 2009), indicating that it might have an important telomeric function. Precise TERRA regulation promotes genomic stability, as both depletion and accumulation of TERRA have been reported to cause telomeric abnormalities (Azzalin *et al*, 2007; Deng *et al*, 2009). Moreover, several findings indicate that TERRA might regulate telomerase (reviewed by Luke & Lingner, 2009). For example, TERRA-mimicking RNA oligonucleotides inhibit telomerase activity *in vitro* (Schoeftner & Blasco, 2008; Redon *et al*, 2010). When the function of the yeast nuclear 5′–3′ exonuclease Rat1 is reduced, TERRA accumulates and telomeres shorten because telomerase-mediated elongation is impaired (Luke *et al*, 2008). Finally, forced telomere transcription (by using the Gal 1,10-promoter) leads to shortening of the transcribed telomere *in cis* (Sandell *et al*, 1994).

Although the function of TERRA is unknown, aspects of its regulation have been characterized. In human cells, the nonsense-mediated RNA decay machinery negatively regulates TERRA at chromosome ends (Azzalin *et al*, 2007; Chawla & Azzalin, 2008), whereas the DNA methyltransferases DNMT3b and DNMT1 methylate TERRA promoters within CpG islands and downregulate its expression (Nergadze *et al*, 2009). The MLL histone methyltransferase promotes TERRA transcription, probably through a p53-dependent mechanism (Caslini *et al*, 2009).

[1]Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Frontiers in Genetics National Center of Competence in Research, Ecole Polytechnique Fédérale de Lausanne (EPFL), Station 19, Lausanne 1015, Switzerland
[2]Zentrum für Molekulare Biologie der Universität Heidelberg, DKFZ-ZMBH Allianz, Im Neuenheimer Feld 282, Heidelberg 69120, Germany
[†]Present address: Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115-5730, USA
[+]Corresponding author. Tel: +41 21 693 0721; Fax: +41 21 693 0720; E-mail: joachim.lingner@epfl.ch
[++]Corresponding author. Tel: +49 6221 54 6897; Fax: +49 6221 54 5894; E-mail: b.luke@zmbh.uni-heidelberg.de

Received 27 February 2011; revised 23 March 2011; accepted 30 March 2011; published online 28 April 2011

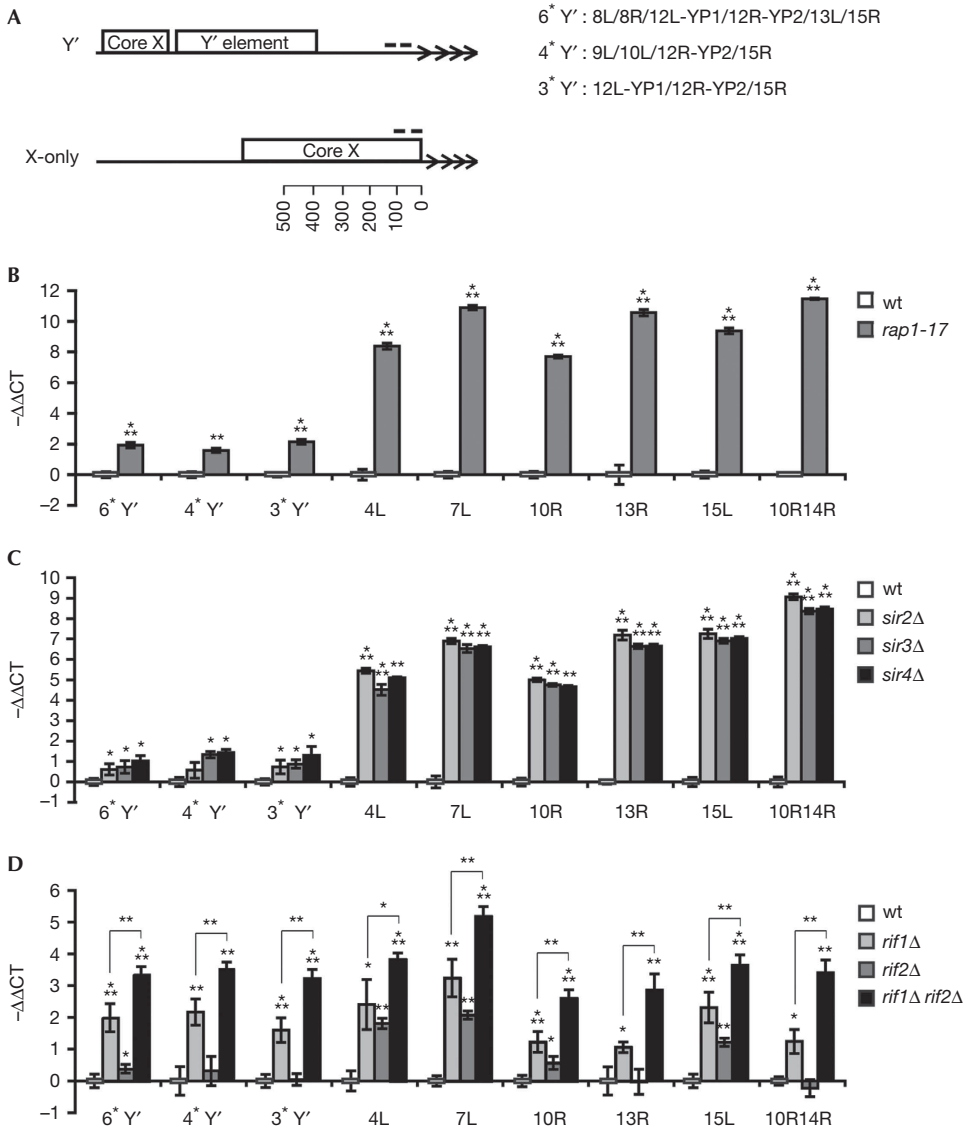

Fig 1 | Regulation of TERRA by Rap1 complexes. (**A**) Representation of the Y′ and X-only telomeres indicating the positions of the primers (black bars) used in qRT–PCR analysis. Arrows represent the telomeric tract. Distances to the telomeric tract (bases) are indicated below. (**B**) Rap1 negatively regulates TERRA. qRT–PCR analysis of Y′ and X-only TERRA in wt and *rap1-17* cells. Average values of three independent biological replicates normalized against actin with standard deviation are shown as fold change over wt. The wt value is arbitrarily set to 1. Statistical analyses were calculated using Student's *t*-test (*$P<0.05$, **$P<0.01$ and ***$P<0.001$). (**C**) Sir2, 3 and 4 are negative regulators of TERRA at X-only telomeres. qRT–PCR analysis of Y′ and X-only TERRA in indicated cells. Average values were normalized and expressed as in (**B**). (**D**) Rif1 and Rif2 affect both Y′ and X-only TERRA levels. qRT–PCR analysis of Y′ and X-only TERRA in indicated cells. Average values were normalized and expressed as in (**B**). qRT–PCR, quantitative real-time PCR; TERRA, Telomeric repeat-containing RNA; wt, wild type.

In yeast, degradation of TERRA is regulated by the 5′–3′ exonuclease Rat1, whereas the poly(A) polymerase Pap1 contributes to its stability (Luke *et al*, 2008).

Here, we demonstrate that TERRA regulation relies on the presence or absence of Y′ elements, which are repetitive sequences found in approximately 50% of the subtelomeric regions (Chan & Tye, 1983; Walmsley *et al*, 1984; Louis, 1995). At telomeres containing Y′ elements, Rap1, in conjunction with its interacting partners Rif1 and Rif2, regulates TERRA. Conversely, at telomeres that only contain the so-called X-element, Rap1 promotes both TERRA repression through Rap1 binding to the SIR (2/3/4) complex and Rat1-mediated TERRA degradation. Therefore, subtelomeric repetitive elements determine distinct Rap1-mediated regulatory pathways for TERRA transcription and degradation.

RESULTS AND DISCUSSION

Differential regulation of TERRA at Y′ and X-only telomeres

We developed reverse transcription coupled to quantitative real-time PCR protocols to measure TERRA derived from Y′-containing

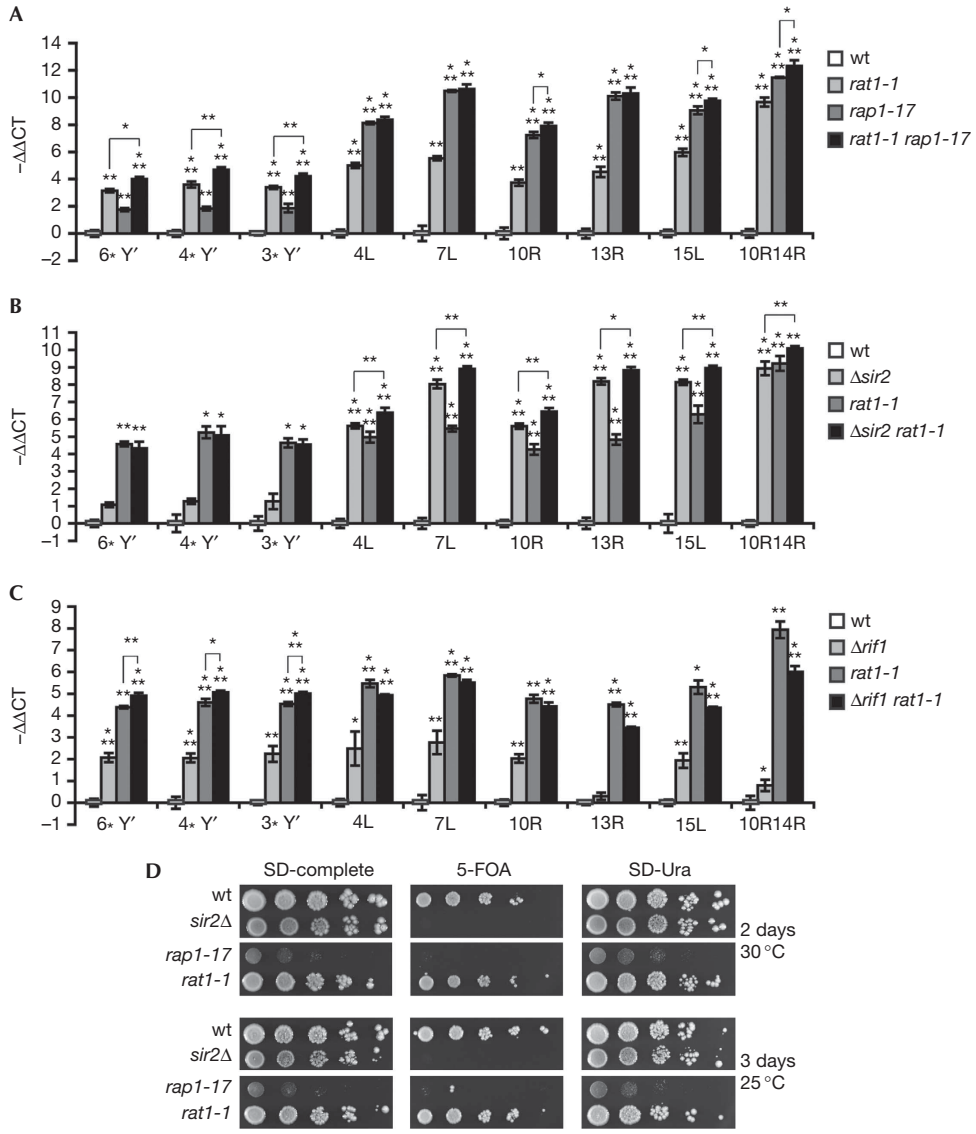

Fig 2 | Y′ and X-only telomeres are differentially regulated. (**A**) qRT–PCR analysis of Y′ and X-only TERRA in wt, *rat1-1*, *rap1-17* and *rat1-1/rap1-17* cells. Average values of three independent biological replicates normalized against actin with standard deviation are shown as fold change over wt. The wt value is arbitrarily set to 1. Statistical analyses were calculated using Student's *t*-test (*$P<0.05$, **$P<0.01$ and ***$P<0.001$). (**B**) qRT–PCR analysis of Y′ and X-only TERRA in the indicated strains showing that Sir2 and Rat1 negatively regulate TERRA from X-only telomeres through different pathways. Average values were normalized and expressed as in (**A**). (**C**) qRT–PCR analysis of Y′ and X-only TERRA in the indicated strains showing that Rif1 and Rat1 negatively regulate Y′ TERRA through different pathways. Average values were normalized and expressed as in (**A**). (**D**) A *URA3* cassette was integrated at the subtelomeric region of telomere 7L in wt, *rap1-17*, *rat1-1* and *sir2*Δ strains. The strains were grown overnight at 25 °C in YPD and spotted onto the indicated plates in 10-fold serial dilutions. Plates were incubated at either 30 °C or 25 °C and were photographed following 2–3 days of incubation. qRT–PCR, quantitative real-time PCR; TERRA, Telomeric repeat-containing RNA; wt, wild type; YPD, yeast extract, peptone and dextrose.

and Y′-lacking chromosome ends. For Y′ telomeres, three sets of primer pairs measure TERRA from different subsets of chromosomes that contain the highly conserved Y′ elements in their subtelomeres (Fig 1A). Conversely, primer pairs with single chromosome-end specificity could be designed to measure TERRA transcribed from several chromosome ends harbouring only an X-element (X-only, from telomere 4L, 7L, 10R, 13R, 15L or 10R14R; Fig 1). As Rap1 binds directly to the double-stranded telomere repeats (Berman *et al*, 1986; Shore & Nasmyth, 1987) and functions as either an activator or a repressor of transcription (Shore, 1994), we sought to determine whether Rap1 is a regulator of TERRA transcription. TERRA levels were analysed in the C-terminally truncated *rap1-17* mutant (Δ663–827 aa) in which both Sir2/3/4 and Rif1/2 can no longer be recruited to chromosome ends (Shore, 1994). We found that TERRA levels were upregulated at all telomeres, with a much greater effect (more than

100-fold) at X-only-containing telomeres (Fig 1B). To determine whether the Sir2/3/4 complex or Rif1 and Rif2 were responsible for TERRA repression, we prepared RNA from *sir2/3/4* deletion mutants and from *rif1*Δ and *rif2*Δ deletion mutants. We found that the *sir2*Δ, *sir3*Δ and *sir4*Δ mutants strongly derepressed TERRA at X-only-containing telomeres, with weaker effects at Y′ telomeres (Fig 1C). By contrast, the *rif1*Δ mutant affected TERRA levels at all telomeres (Fig 1D). The *rif2*Δ mutation derepressed TERRA only slightly; however, when combined with *rif1*Δ, TERRA derepression was stronger than that in the single mutants at all tested telomeres (Fig 1D). Overall, the analysis suggests that TERRA repression at X-only telomeres is mostly mediated by Sir2, Sir3 and Sir4, whereas Rif1 and Rif2, individually, only contribute slightly to TERRA repression at both X and Y′ telomeres. At Y′ telomeres, Sir proteins have a smaller role and TERRA repression is mostly mediated by Rif1 and, to a lesser extent, by Rif2. Thus, the transcriptional control of Y′ TERRA is similar to that of experimentally inserted subtelomeric reporter genes at Y′ telomeres, which do not rely on Sir2/3/4 (Pryde & Louis, 1999). Furthermore, it has been demonstrated by chromatin immunoprecipitation that the Sir3 protein does not localize to Y′ telomeres (Zhu & Gustafsson, 2009). Rif1 and Rif2 might be regulating Y′ TERRA by transcription or through its rate of turnover.

Rap1 controls many TERRA regulatory pathways

Rap1-mediated regulation of TERRA might involve control of TERRA transcription and/or degradation. The *rat1-1* 5′–3′ exonuclease mutant was previously found to stabilize TERRA (Luke *et al*, 2008). To detect a possible collaboration between Rap1 complexes and Rat1, we combined the *rat1-1* mutation with *rap1-17*, *sir2*Δ or *rif1*Δ mutants (Fig 2). Although TERRA levels in *rap1-17/rat1-1* double mutants were slightly higher than those in the respective single mutants at all Y′ telomeres, at some X-only telomeres there was no additivity. Therefore, *rap1-17* and *rat1-1* are epistatic, to an extent, indicating that a portion of TERRA repression through the C-terminus of Rap1 might be due to the promotion of TERRA degradation through Rat1, in addition to exerting a Rat1-independent role for the repression of TERRA (Fig 2A). When testing *sir2*Δ and *rif1*Δ in combination with *rat1-1*, we again observed differences between Y′ and X-only telomeres. The combination of *rat1-1* with *sir2*Δ led to a slight increase in TERRA levels at X-only telomeres, compared with the respective single mutants (Fig 2B). A different image was obtained when testing *rif1*Δ with *rat1-1* (Fig 2C). The *rat1-1* mutation was epistatic with *rif1*Δ at X-only telomeres but not at Y′ telomeres, in which the effects were additive. Together, the results support a model in which Sir2 promotes TERRA repression at X-only telomeres in a Rat1-independent manner. Indeed, *rat1-1* mutants do not share the silencing defects of *sir2*Δ mutants (Fig 2D), which supports the notion that these proteins perform independent functions at telomeres. Rif1 also represses TERRA independently of Rat1; however, unlike *sir2*Δ mutants, the additive effect is only seen at Y′ telomeres. Therefore, Rif1 and Sir2 probably, repress TERRA at Y′ and X-only telomeres, respectively, through transcriptional repression and not through the promotion of RNA degradation. At X-only telomeres, however, Rif1 might also promote TERRA turnover, as Rif1 and Rat1 function in the same genetic pathway in terms of TERRA repression.

Table 1|Half-life of the indicated RNAs in the indicated strains

Strains	Half-life (min)‡							
	6*Y′		4*Y′		3*Y′		ACT1	
	n1	n2	n1	n2	n1	n2	n1	n2
rpb1-1	18	18	15	13	13	14	30	30
rpb1-1 rap1-17	45	53	44	39	39	32	23	22
*rpb1-1 rif1*Δ	13	12	10	8	14	14	36	27

‡Determined by the equation $t_{1/2} = 0.693/k$, where k is the rate constant for RNA decay.

Table 2|Half-life of non-Y′ TERRAs and ACT1 in *rpc40 rpb1-1*

RNAs	Half-life (min)*	
	n1	n2
4 L	14	16
7 L	21	27
10R	12	11
13R	23	33
15 L	14	15
10R14R	13	18
ACT1	27	34

*Determined by the equation $t_{1/2} = 0.693/k$, where k is the rate constant for RNA decay.

The *rap1-17* mutation increases the half-life of TERRA

The above genetic interactions suggested that Rap1 promotes TERRA degradation, at least partly, through Rat1. Consistent with the conclusion that TERRA is a RNAPII transcript (Luke *et al*, 2008), we observed that a common mutation of both the RNAPI and III subunit *rpc40* did not affect TERRA half-lives (supplementary Fig S1 online). Therefore, we tested this hypothesis by combining the *rap1-17* mutation with a temperature-sensitive allele of the RNAPII subunit, *rpb1-1*. In this strain, we were able to turn off transcription by inactivating RNAPII after a shift from permissive (25 °C) to non-permissive temperature (39 °C). We analysed TERRA stemming from different subsets of Y′ telomeres and found that, following RNAPII inactivation, Y′ TERRA was degraded with a half-life of 13–18 min (Table 1; supplementary Fig S2A online). We also followed the messenger RNA half-life from actin (ACT1) for comparison (Table 1; supplementary Fig S2A online). TERRA half-lives were also determined for transcripts from X-only telomeres, yielding a similar value of 12–28 min (Table 2; supplementary Fig S3 online). When RNAPII was inactivated in a *rap1-17* background, the half-life of Y′-derived TERRA increased approximately threefold from 13–18 to 35–49 min, whereas ACT1 RNA degradation rates were not increased in this mutant (Table 1; supplementary Fig S2B online). Deletion of *RIF1* did not result in an increase in TERRA half-life at Y′ telomeres, consistent with our epistasis analysis of TERRA levels (Table 1; supplementary Fig S2C online; Fig 2C). We were unable to measure TERRA half-life in *sir2*Δ *rpb1-1* strains, as the *sir2* deletion suppressed *rpb1-1* for unknown reasons (data not shown). It should be noted that in some of the above experiments

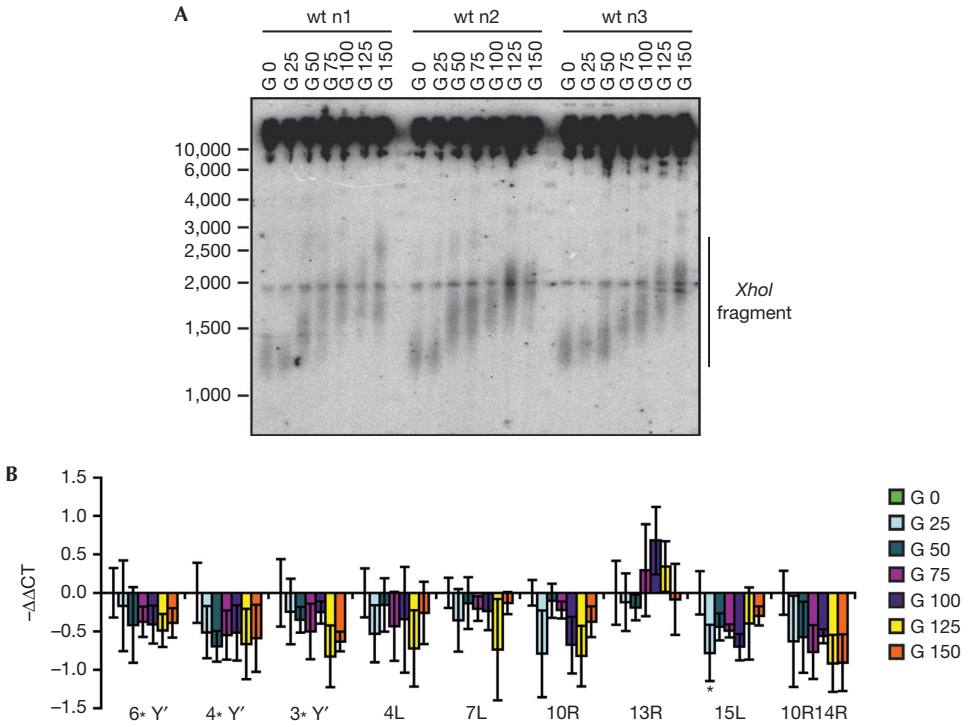

Fig 3 | Telomere length does not effect TERRA levels. (**A**) Telomere elongation was obtained by tethering telomerase to telomeres, using the Cdc13–Est2 fusion protein. Southern blot analysis of Y′ telomeres from three strains expressing a Cdc13–Est2 fusion protein on plasmid, propagated for 25, 50, 75, 100, 125 and 150 generations (G) at 30 °C, after which the plasmid was shuffled out from the cells. Genomic DNA was digested by *Xho*I. (**B**) qRT–PCR analysis of Y′ and X-only TERRA in the same cells as in (**A**). Average values of three independent biological replicates normalized against actin with standard deviation are shown as fold change over wt. The wt value is arbitrarily set to 1. Statistical analyses were calculated using Student's *t*-test (*$P<0.05$). qRT–PCR, quantitative real-time PCR; TERRA, Telomeric repeat-containing RNA; wt, wild type.

an *rpc40/rpb1-1* double mutant was used to determine TERRA half-life, as we noticed that either RNAPI or III can transcribe X-only TERRA, to a small extent, after RNAPII was inactivated over prolonged periods; however, this was not the case in the *rap1-17* mutants. In summary, the threefold increase of TERRA half-life in the *rap1-17* mutant, and the several 100-fold increase of total TERRA (Fig 1B), is consistent with the notion that Rap1 is repressing TERRA through several pathways.

No effect of telomere length on TERRA levels

The *rap1-17* mutant has several phenotypes, including aberrantly long telomeres as well as loss of silencing in the subtelomeres (Shore, 1994). Similarly, *rif1*Δ and *rif2*Δ strains have long telomeres, whereas *sir*Δ strains have less pronounced changes in telomere length (Palladino *et al*, 1993; Askree *et al*, 2004; Gatbonton *et al*, 2006). To test whether telomere lengthening affects TERRA levels, we over-elongated telomeres by tethering telomerase to telomeres on expression of a Cdc13–Est2 fusion protein (Evans & Lundblad, 1999; Fig 3A). However, telomere over-elongation did not show marked differences in TERRA levels (Fig 3B). If anything, TERRA levels decreased slightly. Consistently, we did not observe changes in RNAPII occupancy at telomeres after telomere lengthening, when assessed by chromatin immunoprecipitation (supplementary Fig S4A online). When we assessed TERRA levels during telomere shortening in *tel1*Δ cells, we observed a slight (less than twofold) increase in TERRA levels (supplementary Fig S4B online). Overall, TERRA levels do not correlate positively with telomere length in *S. cerevisiae*.

In summary, we have shown that TERRA is negatively regulated at the level of transcription and degradation by Rap1, which recruits several factors to different telomeres in a differential manner, depending on whether they have a Y′ element. The telomere-binding protein Rap1 seems to be crucial for all levels of TERRA regulation, with the most pronounced effects observed at X-only telomeres. At Y′ telomeres, Rap1 regulates TERRA both by the promotion of degradation and through a degradation-independent mechanism that is dependent on Rif1 and Rif2. As TERRA half-life is not increased by *rif1*Δ and as the effects of the *rat1-1* mutation on Y′ TERRA were exacerbated in the *rat1-1/rif1*Δ double mutant, it seems likely that Rif1 represses transcription (Figs 2C, 4) at Y′ telomeres. At X-only telomeres, Rap1 promotes telomeric repression through both Sir2/3/4 and Rif1/2 complexes (Fig 4). However, the genetic interactions place Rap1 in the Rat1-mediated 5′–3′ RNA degradation pathway, suggesting that, in addition to repressing telomere transcription through recruitment of Sir proteins, Rap1 also promotes Rat1-mediated TERRA turnover. As Rap1 is a highly conserved protein throughout evolution (Li *et al*, 2000; Chen *et al*, 2011), these findings in *S. cerevisiae* might provide a model for TERRA regulation throughout the eukaryotic domain.

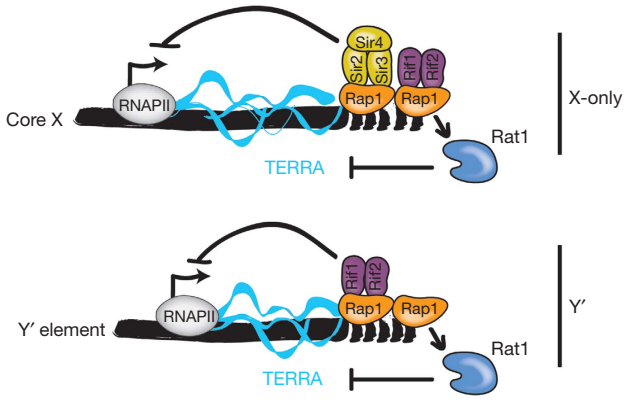

Fig 4 | A model for Rap1-negative regulation of TERRA at X-only telomeres and Y′ telomeres. Upper panel: Rap1 associated with telomeric repeats (dashed line) affects TERRA levels at X-only telomeres through both Rat1-mediated degradation (probably through Rif1 and Rif2) and transcriptional silencing through the Sir2/3/4 complex. Lower panel: at Y′-containing telomeres, TERRA is regulated by Rap1 through the Rif1 and Rif2 proteins (degradation independent), as well as by the nuclear 5′–3′ exonuclease Rat1. RNAPII, RNA polymerase II; TERRA, Telomeric repeat-containing RNA.

METHODS

Strain constructions. All yeast strains used in this study were derived from the BY4741 background and are listed in supplementary Table S1 online.

Spotting assay. Yeast cultures grown to stationary phase were diluted to 1×10^7 cells/ml (or 5×10^6 cells/ml for 5-fluoroorotic acid plates); 10-fold dilutions were spotted on the indicated selective plates and grown at different temperatures for 3 days (see figure legends).

RNA preparation for quantitative real-time PCR. For RNA isolation, cells were grown at 30 °C in 15 ml of medium until OD_{600} (optical density measured at 600 nm wavelength) reached 0.6–0.8. For RNA half-life experiments, cells were grown in YPD (yeast extract, peptone and dextrose) media at 25 °C until early log phase, centrifuged and resuspended in 50-ml Falcon tubes, rapidly shifted to 39 °C by adding the same volume of YPD preheated to 53 °C and incubated at 39 °C in a water bath. Samples of 2 ml were taken at indicated times. We found that three consecutive DNase I treatments using the RNase-Free DNase Set (QIAGEN) were required to completely remove telomeric DNA from total RNA. The first DNase I treatment was performed in solution, as recommended by the supplier. DNase I-treated RNA was precipitated, digested a second time on column using the RNeasy kit (QIAGEN) as recommended by the supplier, purified, and the third DNase treatment was again performed on the column. The reverse transcription was performed with 3 μg of RNA, using the SuperScript III Reverse Transcriptase (Invitrogen). TERRA was reverse-transcribed using the CA oligonucleotide (reverse telomeric) as follows: 3 μg RNA, 769 μM of each of the four deoxyribonucleotide triphosphates, 10 μM CA oligonucleotide and 2 μM ACT1 R oligonucleotide in a final reaction volume of 13 μl was incubated for 1 min at 90 °C, followed by a fast cooling step during 1 min to 55 °C. In all, 4 μl of 5 × First-Strand Reverse RT buffer, 1 μl of 0.1 M dithiothreitol, 1 μl of 40 U/μl RNasin Plus RNase inhibitor (Promega) and 1 μl of SuperScript III RT (200 units/μl) were added when the reaction was at 55 °C and further incubated at 55 °C for 60 min and 70 °C for 15 min. Reverse transcription of mRNA with oligo$(dT)_{15}$ was performed as described above, except that 500-ng oligo$(dT)_{15}$ was used; the mixture was first incubated for 5 min at 65 °C and then chilled on ice. The RT reaction was performed at 50 °C. For qPCR, the cDNA was diluted 2.5 times in H_2O. A volume of 2 μl was quantified in a final volume of 20 μl by real-time PCR with the Power SYBR Green PCR Master mix (Applied Biosystems) using an Applied Biosystems 7900 HT Fast Real-Time PCR System. Primers were used at a final concentration of 0.2–0.6 μM. The reactions were incubated for 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. All primer sequences and final concentrations used, as well as the TERRA that they amplified, are listed in supplementary Table S2 online. The TERRA identity in amplified products was verified using TOPO cloning (Invitrogen), followed by sequencing of at least 6–10 sequences per strain. Statistical significance was determined using a two-tailed Student's *t*-test.

Supplementary information is available at EMBO *reports* online (http://www.emboreports.org).

ACKNOWLEDGEMENTS

We thank C. Boone, V. Lundblad, S. Marcand, R. Young, E. Louis, V. Zakian, J. Berman and D. Shore for reagents. We also thank A. Porro for critical reading of the manuscript. V.P. is supported by an EMBO postdoctoral fellowship. Work in J.L.'s laboratory is supported by the Swiss National Science Foundation, the European Community's Seventh Framework Programme FP7/2007-2011 (grant agreement number 200950) and a European Research Council advanced investigator grant (grant agreement number 232812). Work in B.L.'s lab is supported by the Netzwerk Alterns-Forschung, which is funded from the Ministerium für Wissenschaft, Forschung und Kunst Baden-Württemberg.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Aparicio OM, Billington BL, Gottschling DE (1991) Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. *Cell* **66:** 1279–1287

Askree SH, Yehuda T, Smolikov S, Gurevich R, Hawk J, Coker C, Krauskopf A, Kupiec M, McEachern MJ (2004) A genome-wide screen for *Saccharomyces cerevisiae* deletion mutants that affect telomere length. *Proc Natl Acad Sci USA* **101:** 8658–8663

Azzalin CM, Reichenbach P, Khoriauli L, Giulotto E, Lingner J (2007) Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends. *Science* **318:** 798–801

Berman J, Tachibana CY, Tye BK (1986) Identification of a telomere-binding activity from yeast. *Proc Natl Acad Sci USA* **83:** 3713–3717

Caslini C, Connelly JA, Serna A, Broccoli D, Hess JL (2009) MLL associates with telomeres and regulates telomeric repeat-containing RNA transcription. *Mol Cell Biol* **16:** 4519–4526

Chan CS, Tye BK (1983) A family of *Saccharomyces cerevisiae* repetitive autonomously replicating sequences that have very similar genomic environments. *J Mol Biol* **168:** 505–523

Chawla R, Azzalin CM (2008) The telomeric transcriptome and SMG proteins at the crossroads. *Cytogenet Genome Res* **122:** 194–201

Chen Y *et al* (2011) A conserved motif within RAP1 has diversified roles in telomere protection and regulation in different organisms. *Nat Struct Mol Biol* **18:** 213–221

Cockell M, Palladino F, Laroche T, Kyrion G, Liu C, Lustig AJ, Gasser SM (1995) The carboxy termini of Sir4 and Rap1 affect Sir3 localization: evidence for a multicomponent complex required for yeast telomeric silencing. *J Cell Biol* **129:** 909–924

Deng Z, Norseen J, Wiedmer A, Riethman H, Lieberman PM (2009) TERRA RNA binding to TRF2 facilitates heterochromatin formation and ORC recruitment at telomeres. *Mol Cell* **35:** 403–413

Evans SK, Lundblad V (1999) Est1 and Cdc13 as comediators of telomerase access. *Science* **286:** 117–120

Gatbonton T, Imbesi M, Nelson M, Akey JM, Ruderfer DM, Kruglyak L, Simon JA, Bedalov A (2006) Telomere length as a quantitative trait: genome-wide survey and genetic mapping of telomere length-control genes in yeast. *PLoS Genet* **2:** e35

Hardy CF, Sussel L, Shore D (1992) A RAP1-interacting protein involved in transcriptional silencing and telomere length regulation. *Genes Dev* **6:** 801–814

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

Li B, Oestreich S, de Lange T (2000) Identification of human Rap1: implications for telomere evolution. *Cell* **101:** 471–483

Louis EJ (1995) The chromosome ends of *Saccharomyces cerevisiae*. *Yeast* **11:** 1553–1573

Luke B, Lingner J (2009) TERRA: telomeric repeat-containing RNA. *EMBO J* **28:** 2503–2510

Luke B, Panza A, Redon S, Iglesias N, Li Z, Lingner J (2008) The Rat1p 5′–3′ exonuclease degrades telomeric repeat-containing RNA and promotes telomere elongation in *Saccharomyces cerevisiae*. *Mol Cell* **32:** 465–477

Marcand S, Gilson E, Shore D (1997) A protein-counting mechanism for telomere length regulation in yeast. *Science* **275:** 986–990

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

Nergadze SG, Farnung BO, Wischnewski H, Khoriauli L, Vitelli V, Chawla R, Giulotto E, Azzalin CM (2009) CpG-island promoters drive transcription of human telomeres. *RNA* **15:** 2186–2194

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

Pardo B, Marcand S (2005) Rap1 prevents telomere fusions by nonhomologous end joining. *EMBO J* **24:** 3117–3127

Pryde FE, Louis EJ (1999) Limitations of silencing at native yeast telomeres. *EMBO J* **18:** 2538–2550

Redon S, Reichenbach P, Lingner J (2010) The noncoding RNA TERRA is a natural ligand and direct inhibitor of human telomerase. *Nucleic Acids Res* **38:** 5797–5806

Rine J, Herskowitz I (1987) Four genes responsible for a position effect on expression from HML and HMR in *Saccharomyces cerevisiae*. *Genetics* **116:** 9–22

Sandell LL, Gottschling DE, Zakian VA (1994) Transcription of a yeast telomere alleviates telomere position effect without affecting chromosome stability. *Proc Natl Acad Sci USA* **91:** 12061–12065

Schoeftner S, Blasco MA (2008) Developmentally regulated transcription of mammalian telomeres by DNA-dependent RNA polymerase II. *Nat Cell Biol* **10:** 228–236

Shore D (1994) RAP1: a protean regulator in yeast. *Trends Genet* **10:** 408–412

Shore D, Nasmyth K (1987) Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. *Cell* **51:** 721–732

Walmsley RW, Chan CS, Tye BK, Petes TD (1984) Unusual DNA sequences associated with the ends of yeast chromosomes. *Nature* **310:** 157–160

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

Zhang L, Ogawa Y, Ahn J, Namekawa S, Silva S, Lee JT (2009) Telomeric RNAs mark sex chromosomes in stem cells. *Genetics* **182:** 685–698

Zhu X, Gustafsson CM (2009) Distinct differences in chromatin structure at subtelomeric X and Y′ elements in budding yeast. *PLoS ONE* **4:** e6363