Tankyrase promotes telomere elongation in human cells

Susan Smith* and Titia de Lange

Human telomeres are maintained by telomerase, a reverse transcriptase that adds telomeric repeats to chromosome ends [1,2]. In human tumors and immortalized cells, telomeres are often maintained at a constant length setting [3,4], indicating that telomerase-mediated telomere elongation is tightly regulated. Tankyrase, a telomeric poly(ADP-ribose) polymerase (PARP) [5], was identified through its interaction with TRF1 [6], a negative regulator of telomere extension by telomerase [7]. Tankyrase-mediated ADP-ribosylation inhibits binding of TRF1 to telomeric repeats *in vitro* [5], suggesting that tankyrase might regulate TRF1 and therefore control telomere dynamics *in vivo*. Here, we present evidence that tankyrase acts as a positive regulator of telomere elongation *in vivo*, apparently by inhibiting TRF1. Overexpression of tankyrase in the nucleus diminished the level of unmodified TRF1 in immunoblots and led to reduced immunofluorescence of TRF1 at interphase telomeres. Long-term overexpression of tankyrase in telomerase-positive human cells resulted in a gradual and progressive elongation of telomeres. A PARP-deficient form of tankyrase failed to affect TRF1 and did not alter telomere length dynamics, consistent with ADP-ribosylation of TRF1 as the main cause of altered telomere homeostasis. Our results indicate that tankyrase can induce telomere elongation in human cells. We propose that tankyrase-mediated ADP-ribosylation of TRF1 opens the telomeric complex, allowing access to telomerase.

Address: The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA.

*Present address: Skirball Institute of Biomolecular Medicine, New York University School of Medicine, 540 First Avenue, New York, New York 10016, USA.

Correspondence: Titia de Lange
E-mail: delange@rockvax.rockefeller.edu

Received: **12 July 2000**
Revised: **14 August 2000**
Accepted: **15 August 2000**

Published: **6 October 2000**

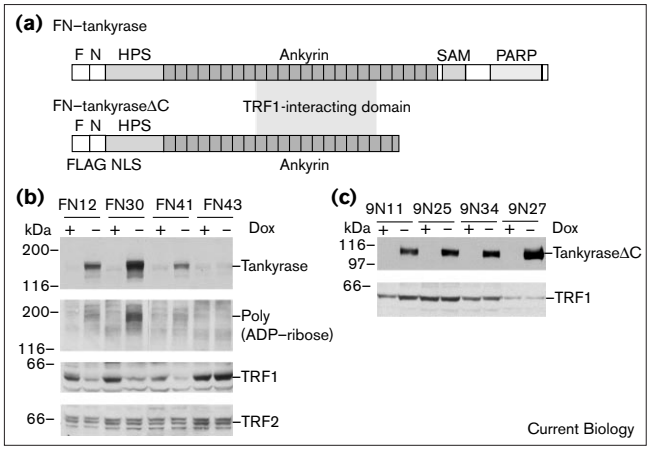
Current Biology 2000, 10:1299–1302

0960-9822/00/$ – see front matter

Results and discussion

To determine whether tankyrase influenced telomere dynamics *in vivo*, we generated a version of tankyrase that would be expected to exert its major effect in the nucleus.

Figure 1

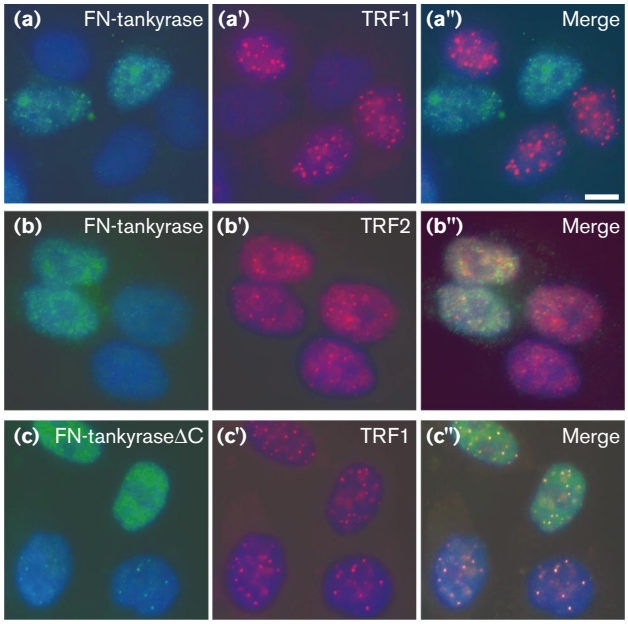


Tetracyclin-regulated expression of tankyrase proteins in HTC75 cells. **(a)** Tankyrase constructs. F, FLAG epitope tag; N, NLS from SV40; HPS, homopolymeric tracts of histidine, proline and serine. **(b,c)** Immunoblot analysis of inducible tankyrase expression in HTC75 cells. (b) Four independent clonal cell lines, three expressing FN–tankyrase (FN12, FN30, FN41) and, as a control, a fourth line that does not express full-length FN–tankyrase (FN43). (c) Four independent clonal cell lines expressing FN–tankyraseΔC (9N11, 9N25, 9N27, 9N34). Whole cell extracts from cultures grown in the presence (uninduced) or absence (induced) of doxycyclin (dox) for 8 population doublings (PDs) were probed with (b) anti-tankyrase antibody 465, anti-poly(ADP-ribose) antibody 10H, anti-TRF1 antibody 371, or anti-TRF2 antibody 647, or (c) anti-FLAG antibody M2, or anti-TRF1 antibody 371. The 9N27 lanes contain 50% less protein than the other cell lines.

Previous experiments indicated that, while a small fraction of tankyrase localized to human telomeres in metaphase spreads [5] and interphase cells [8], a substantial fraction of the protein was found at other subcellular sites: nuclear-pore complexes in interphase and centrosomes during mitosis [8]. Tankyrase does not contain a nuclear localization signal (NLS) and transfected tankyrase is excluded from the nucleus unless it is co-transfected with TRF1 [8]. Therefore, we added an NLS to its amino terminus, resulting in a protein that accumulated in the nucleus (FN–tankyrase; Figure 1a; see below). In parallel, we generated a similarly tagged version of tankyrase with a carboxy-terminal deletion that included the PARP and sterile-alpha-module (SAM) domains, but retained the TRF1-interaction domain (FN–tankyraseΔC; Figure 1a). Both forms of tankyrase were stably expressed and detectable in immunoblots (Figure 1b,c).

The effect of full-length and truncated tankyrase on the accumulation of TRF1 on telomeres was determined by transient transfection of HeLa1.2.11 cells, which afford sensitive detection of telomeric proteins because of their

Figure 2



Tankyrase releases TRF1 from telomeres in a reaction that depends on its PARP domain. Indirect immunofluorescence analysis of HeLa cells transiently transfected with **(a,b)** FN–tankyrase or **(c)** FN–tankyraseΔC. Cells were dually stained with (a–c) anti-FLAG antibody M2 (green) and (a′,c′) anti-TRF1 antibody 371 (red) or (b′) anti-TRF2 antibody 508 (red). (a″–c″) Superimposition of the red and green images. DNA was stained with 4,6-diamidino-2-phenylindole (DAPI, blue). The scale bar represents 5 μm.

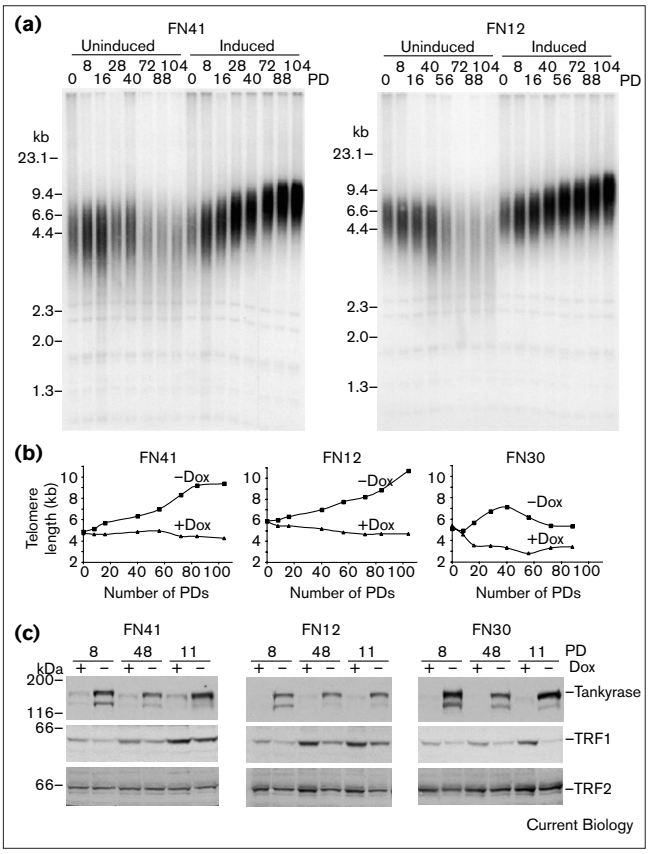
long telomeres [9]. Indirect immunofluorescence to detect the FN–tankyrase in transfected cells showed that the protein was predominantly nuclear (Figure 2a). Dual labeling of the same cells with anti-TRF1 antibody showed that, in the tankyrase-overexpressing cells, TRF1 no longer displayed a punctate pattern, suggesting that in transfected cells TRF1 no longer accumulated at telomeres. We did not detect non-telomeric TRF1 in association with the overexpressed tankyrase protein. Non-telomeric TRF1 might be dispersed throughout the nucleus and therefore difficult to detect, or the released protein might be unstable. Additionally, the anti-TRF1 antibody might not react with poly(ADP-ribosyl)ated TRF1 (see below). Finally, we cannot rule out the possibility that poly(ADP-ribosyl)ated TRF1 remains on telomeres, but is not detected by TRF1 antibodies because of the modification. This is unlikely, however, as poly(ADP-ribosyl)ation of TRF1 *in vitro* inhibits its binding to telomeric DNA.

Overexpression of FN–tankyrase had no effect on the staining pattern of the related telomeric-repeat-binding protein TRF2 [10,11] (Figure 2b), consistent with the specificity of tankyrase for TRF1 *in vitro* [5]. Comparison of the localization patterns of FN–tankyrase and TRF2 indicated that they did not colocalize, suggesting that FN–tankyrase did not reside at telomeres. Lack of telomeric accumulation for tankyrase would be expected if TRF1, its binding partner, had been released from telomeres in transfected cells or was inhibited from binding to telomeres. A similar loss of TRF1 from telomeres was noted in HTC75 cell lines induced to express FN–tankyrase (see below; data not shown). These observations indicated that tankyrase can modulate the accumulation of TRF1, but not TRF2, on telomeres *in vivo*.

The release of TRF1 from telomeres in response to overexpression of tankyrase is likely to be due to the PARP activity of the enzyme, as ADP-ribosylation of TRF1 inhibits its ability to form a stable complex with telomeric DNA *in vitro* [5]. In contrast to the full-length protein, expression of FN–tankyraseΔC had no detectable effect on the distribution of TRF1 (Figure 2c). In fact, FN–tankyraseΔC accumulated at telomeres, as evidenced by its colocalization with TRF1. Inspection of cells expressing different amounts of the transfected protein suggested that FN–tankyraseΔC localized to telomeres in a saturable fashion; at low levels of expression, FN–tankyraseΔC localized exclusively to telomeres, whereas at higher expression levels the protein was also detectable throughout the nucleoplasm. These observations are consistent with FN–tankyraseΔC binding to and ultimately saturating the TRF1-binding sites at telomeres. FN–tankyraseΔC (unlike FN–tankyrase) remained on telomeres, probably because it was unable to ADP-ribosylate and therefore release TRF1 and itself from telomeres. A similar colocalization for TRF1 and FN–tankyraseΔC to telomeres was noted in HTC75 cell lines induced to express FN–tankyraseΔC (see below; data not shown).

To examine the long-term effect of tankyrase on TRF1 and telomere length homeostasis, we expressed tankyrase stably in HTC75 cells. HTC75 is a telomerase-positive human fibrosarcoma cell line that maintains telomeres at a stable length setting [7]. This cell line contains the tetracycline-controlled gene expression system, allowing controlled analysis of changes in telomere length in response to exogenous genes. Immunoblot analysis with anti-tankyrase antibodies indicated doxycyclin-controlled expression of FN–tankyrase in three stable clonal HTC75 cell lines, FN12, FN30 and FN41 (Figure 1b). Probing with an anti-poly(ADP-ribose) antibody revealed a diffuse signal that coincided with induction of FN–tankyrase, but migrated more slowly, consistent with the presence of poly(ADP-ribosyl)ated FN–tankyrase [5] resulting from automodification. The induction of FN–tankyrase was accompanied by a reduction in TRF1 levels. We presume that this reduction is due to the generation of poly(ADP-ribosyl)ated TRF1. We did not, however, detect modified forms of TRF1 by immunoblot analysis. The modified forms of

Figure 3



FN–tankyrase induces telomere lengthening. **(a)** Southern blot analysis of *HinfI*/*Rsa*I-digested genomic DNA from FN41 and FN12, which express FN–tankyrase. Cell lines were grown for 104 PDs in the presence (uninduced) or absence (induced) of doxycyclin and DNA samples were analyzed at the indicated PDs. Blots were probed with a TTAGGG-repeat probe to detect telomeric restriction fragments. **(b)** Graphical presentation of telomere length changes in FN41, FN12 and FN30. Plots represent the mean telomere length values derived from Southern blot analysis of cultures grown in the presence (uninduced; triangles) or absence (induced; squares) of doxycyclin. **(c)** Immunoblot analysis of whole cell extracts from cell lines (FN41, FN12 and FN30) grown in the presence (uninduced) or absence (induced) of doxycyclin for 8, 48 or 112 PDs. Blots were probed with anti-tankyrase antibody 465, anti-TRF1 antibody 371, or anti-TRF2 antibody 647.

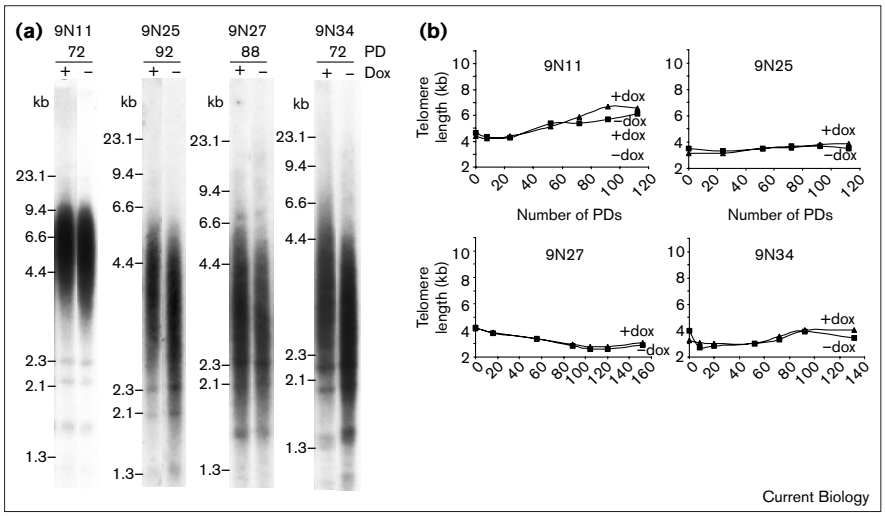
TRF1 might not react well with anti-TRF1 antibodies or they might be unstable. Lack of detection of TRF1 could also result from the heterogeneous migration of poly(ADP-ribosyl)ated forms [5], which might dilute the signal below the level of detection. In contrast to TRF1, the levels of TRF2 were unchanged by expression of FN–tankyrase, consistent with the previous finding that TRF1, but not TRF2, is ADP-ribosylated *in vitro* [5].

The reduction in TRF1 levels was likely to be due to ADP-ribosylation of TRF1 by the overexpressed FN–tankyrase. To test this directly, we generated HTC75 cells expressing the PARP-deficient form of FN–tankyrase, FN–tankyraseΔC (Figure 1a). Immunoblot analysis with anti-FLAG antibody indicated doxycyclin-controlled expression of FN–tankyraseΔC in four stable clonal cell lines, 9N11, 9N25, 9N27 and 9N34 (Figure 1c). In contrast to the full-length protein, induction of FN–tankyraseΔC had no detectable effect on the steady state level of TRF1 detected in immunoblots (Figure 1c).

To assess the effect of tankyrase expression on telomere dynamics, the HTC75 cell lines were grown for over 100 PDs and the length of telomeric restriction fragments was determined by standard genomic blotting. Previous studies indicated that control HTC75 cell lines, transfected with the empty vector, showed no change in telomere length when grown for over 100 PDs in the presence or absence of doxycyclin [7]. In contrast, the three cell lines expressing FN–tankyrase showed a gradual and progressive increase in telomere length that was dependent on doxycyclin (Figure 3a,b). The calculated increase in telomere length in the absence of doxycyclin was 3.5 kb for FN41 at PD 72, 4.8 kb for FN12 at PD 104, and 2.0 kb for FN30 at PD 40. Telomeres showed progressive elongation at similar rates of 48 bp (FN41), 46 bp (FN12), or 50 bp (FN30) per PD. In the presence of doxycyclin, telomere length remained relatively constant, but did become more heterogeneous, particularly in later PDs. Although the three cell lines showed similar rates of elongation, each displayed a unique pattern of elongation, stabilization and, in one case, shortening. Immunoblot analysis at PD 48 and 112 indicated that each of the cell lines continued to express high levels of the transgene and maintained a reduced level of TRF1 (Figure 3c). The observed shortening and/or stabilization of telomere length that occurred in the presence of transgene expression suggests the presence of an additional telomere length regulation mechanism that is not directly affected by the tankyrase expression. This additional mechanism could depend on TRF2, which is also a regulator of telomere maintenance [9] but is not affected by tankyrase. It is not unlikely that each subclone of the HTC75 cell line varies somewhat with regard to such additional pathways. Minor variations in telomere length dynamics have also been noted in an analysis of TRF1-mediated length control [7].

The gradual elongation of telomeres in the cell lines expressing FN–tankyrase is similar to the telomere elongation induced by a dominant-negative allele of TRF1 [7] and is consistent with perturbation of the telomerase-mediated telomere maintenance pathway. Expression of FN–tankyrase and reduction of TRF1 levels was not accompanied by a notable change in telomerase activity as detected in cell extracts using the TRAP assay (data not shown), suggesting that tankyrase, like TRF1, does not modulate telomere dynamics by a major direct effect on telomerase activity detectable with the TRAP assay.

Figure 4



FN–tankyraseΔC does not induce telomere lengthening. **(a)** Southern blot analysis of *Hinf*I/*Rsa*I-digested genomic DNA from four clonal cell lines (9N11, 9N25, 9N27 and 9N34) expressing FN–tankyraseΔC. Cell lines were grown in the presence (uninduced) or absence (induced) of doxycyclin and DNA samples were analyzed at the indicated PDs. Blots were probed with a TTAGGG-repeat probe to detect telomeric restriction fragments. **(b)** Graphical presentation of telomere length changes in clonal lines 9N11, 9N25, 9N27 and 9N34. Plots represent the mean telomere length values derived from Southern blot analysis of cultures grown in the presence (uninduced; triangles) or absence (induced; squares) of doxycyclin.

Telomere length was next assessed in HTC75 clones expressing FN–tankyraseΔC. In contrast to cell lines expressing the full-length protein, four cell lines expressing FN–tankyraseΔC showed no doxycyclin-dependent telomere elongation (Figure 4a,b). Although telomere length fluctuated, telomere length changes were not correlated with induction of the truncated tankyrase allele. These results suggested that the carboxy-terminal PARP domain was required for the telomere elongation induced by FN–tankyrase. However, direct confirmation for the role of this domain will require analysis of point mutations in the PARP active site. Nonetheless, in contrast to the FN–tankyrase cell lines, TRF1 was not detectably modified in the FN–tankyraseΔC lines (Figure 1c) or released from telomeres (Figure 2c) and the telomeres did not elongate (Figure 4a,b). These data are consistent with the idea that tankyrase-induced telomere elongation is dependent on ADP-ribosylation and concomitant release of TRF1 from telomeres.

The data suggest that tankyrase can function as a positive regulator of telomere length *in vivo*. It will be important to determine whether tankyrase is required for telomerase-mediated telomere maintenance, particularly in human tumor cells. Recent studies indicate that inhibition of telomerase can limit growth of human cancer cells [12,13]. Thus, tankyrase, which can be inhibited by small molecules [5], holds promise as a target for anti-cancer therapies.

Supplementary material

Supplementary material including additional methodological detail is available at http://current-biology.com/supmat/supmatin.htm.

Acknowledgements

We thank Grigoriy Shostak for technical assistance, Tom Meier for comments on the manuscript, and members of the de Lange lab for helpful discussions. S.S. was a Leukemia and Lymphoma Society Special Fellow. This work was supported by a grant to T.d.L. from the NCI (CA76027) and a grant to S.S. from the Edward Mallinckrodt, Jr. Foundation. T.d.L. is a recipient of the Burroughs Wellcome Fund Scholar Award in Toxicology.

References

1. Nugent CI, Lundblad V: **The telomerase reverse transcriptase: components and regulation.** *Genes Dev* 1998, **12:**1073-1085.
2. Greider CW, Blackburn EH: **Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts.** *Cell* 1985, **43:**405-413.
3. Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S: **Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity.** *EMBO J* 1992, **11:**1921-1929.
4. Counter CM, Botelho FM, Wang P, Harley CB, Bacchetti S: **Stabilization of short telomeres and telomerase activity accompany immortalization of Epstein-Barr virus-transformed human B lymphocytes.** *J Virol* 1994, **68:**3410-3414.
5. Smith S, Giriat I, Schmitt A, de Lange T: **Tankyrase, a poly(ADP-ribose) polymerase at human telomeres.** *Science* 1998, **282:**1484-1487.
6. Chong L, van Steensel B, Broccoli D, Erdjument-Bromage H, Hanish J, Tempst P, de Lange T: **A human telomeric protein.** *Science* 1995, **270:**1663-1667.
7. van Steensel B, de Lange T: **Control of telomere length by the human telomeric protein TRF1.** *Nature* 1997, **385:**740-743.
8. Smith S, de Lange T: **Cell cycle dependent localization of the telomeric PARP, tankyrase, to nuclear pore complexes and centrosomes.** *J Cell Sci* 1999, **112:**3649-3656.
9. Smogorzewska A, van Steensel B, Bianchi A, Oelmann S, Schaefer MR, Schnapp G, de Lange T: **Control of human telomere length by TRF1 and TRF2.** *Mol Cell Biol* 2000, **20:**1659-1668.
10. Broccoli D, Smogorzewska A, Chong L, de Lange T: **Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2.** *Nat Genet* 1997, **17:**231-235.
11. Bilaud T, Brun C, Ancelin K, Koering CE, Laroche T, Gilson E: **Telomeric localization of TRF2, a novel human telobox protein.** *Nat Genet* 1997, **17:**236-239.
12. Hahn WC, Stewart SA, Brooks MW, York SG, Eaton E, Kurachi A, *et al.*: **Inhibition of telomerase limits the growth of human cancer cells.** *Nat Med* 1999, **5:**1164-1170.
13. Zhang X, Mar V, Zhou W, Harrington L, Robinson MO: **Telomere shortening and apoptosis in telomerase-inhibited human tumor cells.** *Genes Dev* 1999, **13:**2388-2399.