400 cps per part per million of total Pb. The ion probe analyses reproduced the TIMS value within <0.1% standard errors (2σ, $n = 35$). The mass fractionation for SIMS analysis was <0.15% per atomic mass unit and was smaller than the in-run precision. Comparison of TIMS and SIMS Pb isotope data for basaltic glass, feldspar, chalcopyrite, and zircon indicates that instrumental mass fractionation is generally smaller than the precision of the analyses [see also G. D. Layne and N. Shimizu, in *Secondary Ion Mass Spectrometry SIMS XI*, G. Gillen, R. Lareau, J. Bennett, F Stevie, Eds. (Wiley, New York, 1997), pp. 63–65.

14. Y. Nakamura and M. Tatsumoto, *Geochim. Cosmochim. Acta* **52**, 2909 (1988); C. Chauvel, W. McDonough, G. Guille, R. Maury, R. Duncan, *Chem. Geol.* **139**, 125 (1997); C. Hemond, C. W. Devey, C. Chauvel, *ibid.* **115**, 7 (1994).
15. B. B. Hannan and D. W. Graham, *Science* **272**, 991 (1996).
16. J. M. Eiler *et al.*, *Geochim. Cosmochim. Acta* **61**, 2281 (1997).
17. S.-s. Sun and W. F. McDonough, in *Magmatism in the Oceanic Basins*, A. D. Saunders and M. J. Norry, Eds. (Blackwell Scientific, London, 1989), pp. 313–345.
18. C. Chauvel, A. W. Hofmann, P. Vidal, *Earth Planet. Sci. Lett.* **110**, 99 (1992); J. C. Lassiter, E. H. Hauri, H. G. Barsczus, *Eos* (*Spring Suppl.*) **79**, S378 (1998).
19. We dedicate this paper to the memory of Rafael and Niva Katzman. We thank M. O. Garcia for the Loihi samples that we used as standards during this work and W. Lee for discussion. This work was supported by NSF grants EAR-9219958 and EAR-9804891 (S.R.H.), EAR-9628749 (N.S.), and EAR-9413985 and EAR-9725351 (E.H.H).

5 August 1998; accepted 22 October 1998

Tankyrase, a Poly(ADP-Ribose) Polymerase at Human Telomeres

Susan Smith, Izabela Giriat, Anja Schmitt,* Titia de Lange†

Tankyrase, a protein with homology to ankyrins and to the catalytic domain of poly(adenosine diphosphate–ribose) polymerase (PARP), was identified and localized to human telomeres. Tankyrase binds to the telomeric protein TRF1 (telomeric repeat binding factor–1), a negative regulator of telomere length maintenance. Like ankyrins, tankyrase contains 24 ankyrin repeats in a domain responsible for its interaction with TRF1. Recombinant tankyrase was found to have PARP activity in vitro, with both TRF1 and tankyrase functioning as acceptors for adenosine diphosphate (ADP)–ribosylation. ADP-ribosylation of TRF1 diminished its ability to bind to telomeric DNA in vitro, suggesting that telomere function in human cells is regulated by poly(ADP-ribosyl)ation.

Human telomere function requires two telomere-specific DNA binding proteins, TRF1 and TRF2 (*1, 2*). TRF2 protects chromosome ends (*3*), and TRF1 regulates telomere length (*4*). Overexpression of TRF1 in a telomerase-expressing cell line leads to progressive telomere shortening, whereas inhibition of TRF1 increases telomere length (*4*). TRF1 does not control the expression of telomerase itself but is thought to act in cis by inhibiting telomerase at telomere termini.

To identify additional telomere-associated proteins, we used a yeast two-hybrid screen with human TRF1 as bait (*5, 6*). This screen yielded two overlapping partial cDNAs (TR1L-4 and TR1L-12) (Fig. 1A). A full-length testis cDNA isolated with TR1L-4 encoded an open reading frame of 1327 amino acids, predicting a protein of 142 kD (Fig. 1A) (*7*). The central domain of this protein contains 24 ankyrin (ANK) repeats, a 33–amino acid motif that mediates protein-protein interactions (*8*), and its COOH-terminal region has homology to the catalytic domain of PARP, a highly conserved nuclear enzyme found in most eukaryotes (*9*). We therefore named the protein tankyrase (TRF1-interacting, ankyrin-related ADP- ribose polymerase).

The tankyrase-interacting domain in TRF1 was identified by two-hybrid analysis (*6*) with TR1L-12 (Fig. 1A). The tankyrase fragment, consisting of 10 ANK repeats, interacted with full-length TRF1 but not with a TRF1 mutant lacking the NH_2-terminal acidic domain of TRF1 (Fig. 1B). Consistent with this observation, significant interaction occurred with the isolated NH_2-terminal 68 amino acids of TRF1, which encompass the acidic domain (Fig. 1B). These results indicate that the acidic domain of TRF1 is necessary and sufficient for interaction with tankyrase. This domain is absent from TRF2, and a two-hybrid analysis (*6*) indicated that tankyrase does not interact with TRF2.

Three observations suggested that tankyrase was a member of the ankyrin family, a group of structural proteins that link integral membrane proteins to the underlying cytoskeleton (*10*). First, tankyrase, like all ankyrins, contained 24 copies of the ANK motif, whereas other ANK repeat–containing proteins typically have 4 to 8 repeats. Second, the ANK repeats in tankyrase and the ankyrins shared characteristic sequence features, such as the presence of a hydrophobic amino acid at position 3 and an Asn or a Asp at position 29 (Fig. 2A). Third, the fifth ANK copy in tankyrase was notably shorter than all others, a feature also observed in ankyrins. Apart from the ANK repeat domain, however, there was no detectable homology between tankyrase and ankyrins. The ankyrin domain of tankyrase is flanked at the NH_2-terminus by a region carrying homopolymeric His, Ser, and Pro tracts and at the COOH-terminus by a sterile alpha module (SAM) motif (Fig. 2B), which is postulated to function in protein-protein interaction (*11*).

The most striking feature of tankyrase is the homology to PARP. In response to DNA dam-

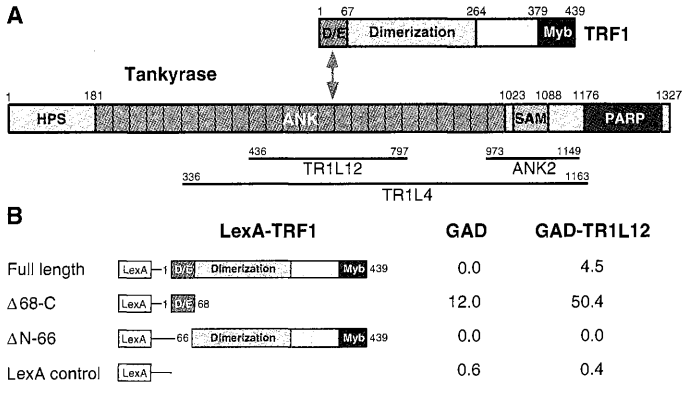

Fig. 1. Domain structure of tankyrase and two-hybrid interaction with TRF1. (**A**) Schematic representation of the structure of tankyrase and TRF1. Lines below the schematic indicate inserts of the two-hybrid plasmids (TR1L-4 and TR1L-12) and a plasmid used to generate recombinant protein for antibody production (ANK2). HPS, region containing homopolymeric runs of His, Pro, and Ser; ANK, ankyrin-related domain; SAM, homology to the sterile alpha motif; PARP, homology to the catalytic domain of PARP; Myb, Myb-type DNA binding motif; D/E, acidic domain rich in Glu and Asp. (**B**) Two-hybrid assay for the tankyrase interaction domain in TRF1. β-Galactosidase concentrations (Miller units; average of three independent transformations) were measured for strains expressing the indicated fusion proteins (*6*). GAD, GAL4 activation domain.

The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.

*Present address: European Molecular Biology Laboratory–Heidelberg, Meyerhofstrasse 1, D-69117, Heidelberg, Germany.

†To whom correspondence should be addressed. E-mail: delange@rockvax.rockefeller.edu

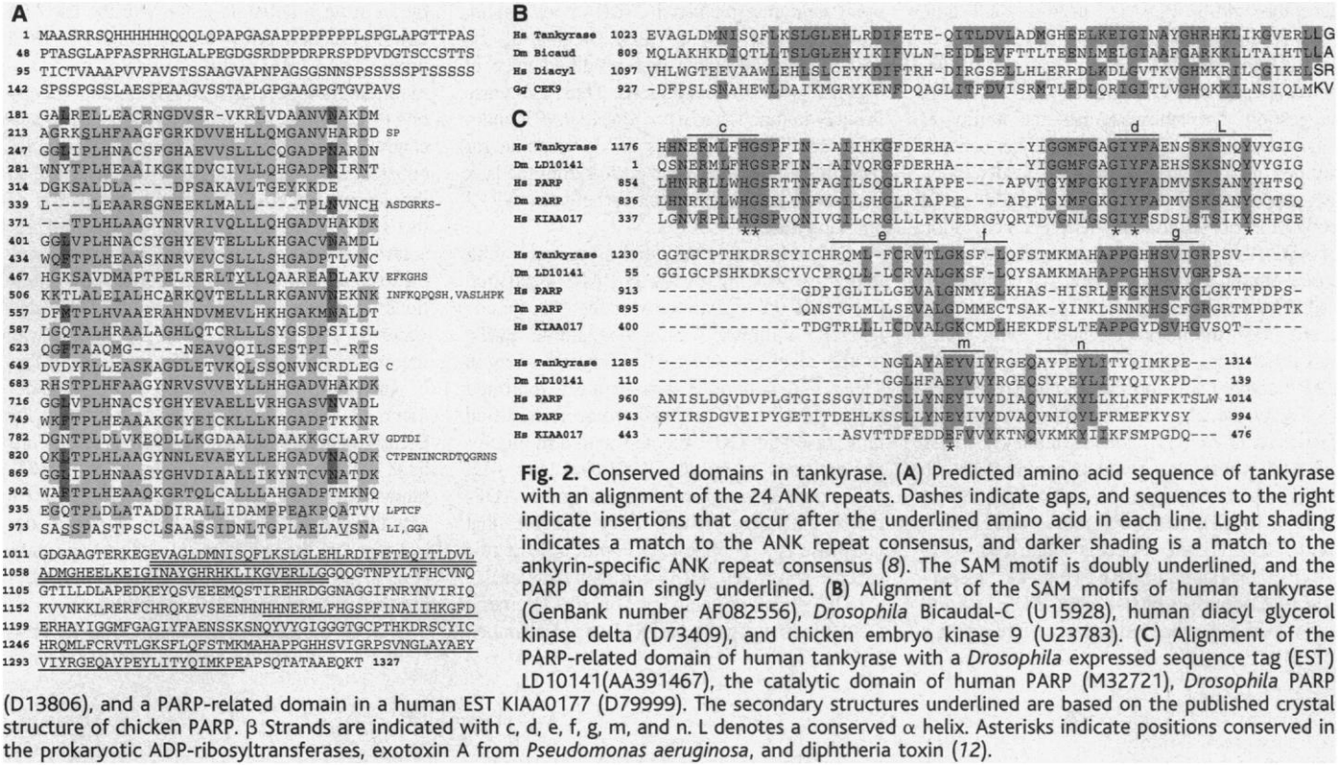

Fig. 2. Conserved domains in tankyrase. (**A**) Predicted amino acid sequence of tankyrase with an alignment of the 24 ANK repeats. Dashes indicate gaps, and sequences to the right indicate insertions that occur after the underlined amino acid in each line. Light shading indicates a match to the ANK repeat consensus, and darker shading is a match to the ankyrin-specific ANK repeat consensus (*8*). The SAM motif is doubly underlined, and the PARP domain singly underlined. (**B**) Alignment of the SAM motifs of human tankyrase (GenBank number AF082556), *Drosophila* Bicaudal-C (U15928), human diacyl glycerol kinase delta (D73409), and chicken embryo kinase 9 (U23783). (**C**) Alignment of the PARP-related domain of human tankyrase with a *Drosophila* expressed sequence tag (EST) LD10141(AA391467), the catalytic domain of human PARP (M32721), *Drosophila* PARP (D13806), and a PARP-related domain in a human EST KIAA0177 (D79999). The secondary structures underlined are based on the published crystal structure of chicken PARP. β Strands are indicated with c, d, e, f, g, m, and n. L denotes a conserved α helix. Asterisks indicate positions conserved in the prokaryotic ADP-ribosyltransferases, exotoxin A from *Pseudomonas aeruginosa*, and diphtheria toxin (*12*).

age, PARP catalyzes the formation of poly(ADP-ribose) onto a protein acceptor using nicotinamide adenine dinucleotide (NAD^+) as a substrate (*9*). The catalytic domain of PARP consists of secondary structure units (multiple β strands and one α helix) (Fig. 2C) that form a cavity known as the NAD^+-binding fold, a tertiary structure that is also present in all ADP-ribosylating toxins (*12*). Tankyrase has 28 to 30% amino acid identity with the catalytic domains of human and *Drosophila* PARP (Fig. 2C), including all critical amino acids implicated in NAD^+ binding and catalysis. Other conserved aspects of the previously defined PARPs such as their automodification and DNA binding domains (*9*) are not represented in tankyrase, indicating that tankyrase is not just a PARP isoform but a substantially different protein.

Northern (RNA) blot analysis revealed that multiple tankyrase mRNAs (*13*) were ubiquitously expressed in human tissues, with the highest amounts detectable in testis (Fig. 3A). TRF1 and TRF2 transcripts show a similar ubiquitous expression pattern (*1, 2*). A single protein of ~142 kD was detected by tankyrase immunoblot analysis of HeLa cells and rat testis, and this protein comigrated with the in vitro translation product of tankyrase cDNA (Fig. 3B) (*14*). A survey of mammalian cell lines suggested that tankyrase protein is ubiquitously expressed (*15*), consistent with the RNA data.

Because TRF1 is predominantly associated with telomeres in human cells, including the telomeres of mitotic chromosomes, we used indirect immunofluorescence analysis of metaphase chromosomes to determine whether TRF1 positions tankyrase at chromosome ends. Metaphase spreads were dually probed with anti-tankyrase and antiserum to TRF1 (*16*). The results revealed that, like TRF1, tankyrase is located at or near the physical ends of metaphase chromosomes (Fig. 3C). Most of the tankyrase protein colocalized with TRF1, as evidenced by the merge of the two signals. These data suggest that tankyrase is a component of the human telomeric complex.

To investigate whether tankyrase has PARP activity, we tested baculovirus-derived recombinant protein in an assay that measures the addition of radiolabeled ADP-ribose to protein

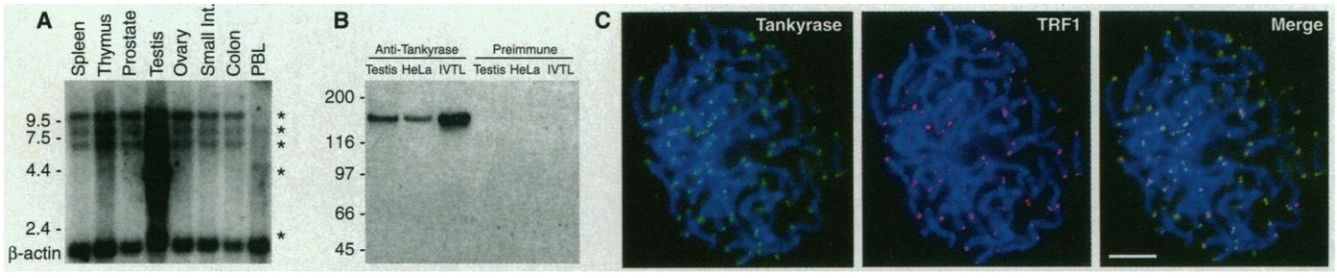

Fig. 3. Expression and localization of tankyrase. (**A**) Northern blot of polyadenylated RNAs from human tissues (Clontech) probed with a tankyrase cDNA (TR1L-4) (*13*). Asterisks indicate tankyrase transcripts. The blot was rehybridized with a β-actin probe, and a double exposure of both signals is shown. Molecular size markers are indicated on the left in kilobases. (**B**) Immunoblot of the following protein samples: salt-extracted nuclear pellet from rat testis (Testis), whole-cell lysates from HeLa cells (HeLa), and products of a coupled in vitro transcription-translation (IVTL) of full-length tankyrase cDNA, probed with the indicated antibodies (*14*). Molecular size markers are indicated on the left in kilodaltons. (**C**) Colocalization of tankyrase and TRF1 at telomeres. Indirect immunofluorescence analysis of swollen, formaldehyde-fixed metaphase spreads from HeLa cells stained with anti-tankyrase (green) and anti-TRF1 (red) (*16*). "Merge" represents superimposition of the red and green images. DAPI staining of DNA is shown in blue. Scale bar, 5 μm.

acceptors with [^{32}P]NAD$^+$ used as a substrate (*17*). Incubation of tankyrase in the presence of 1.3 μM radiolabeled NAD$^+$ produced ^{32}P-labeled species that comigrated with tankyrase, suggesting that tankyrase has the ability to ADP-ribosylate itself (Fig. 4A). Higher concentrations of NAD$^+$ (0.04 to 1 mM) yielded much larger products, likely reflecting the addition of poly(ADP-ribose) to tankyrase. The generation of ADP-ribosylated tankyrase depended on the concentration of tankyrase (Fig. 4A), was eliminated by heat inactivation of the enzyme, and could be immunoprecipitated with anti-tankyrase (Fig. 4B) (*18*), indicating that the PARP activity was intrinsic to tankyrase.

Tankyrase also has the ability to modify TRF1. At low NAD$^+$ concentration (1.3 μM), the ADP-ribosylated products comigrated with TRF1, whereas at higher NAD$^+$ concentrations (0.04 to 1 mM), the slower and variable mobility of the labeled products suggested poly(ADP-ribosyl)ation of TRF1 (Fig. 4A). Inspection of Coomassie blue–stained SDS gels did not reveal a larger molecular weight species upon tankyrase-mediated TRF1 modification, indicating that only a small fraction of the TRF1 in the reactions was modified even at high tankyrase concentrations. Thus, tankyrase is likely to function as a processive PARP under these conditions. TRF2 is not a substrate for modification in vitro, as expected from the lack of protein-protein interaction between TRF2 and tankyrase.

To confirm that the labeling reaction with tankyrase was analogous to PARP-catalyzed poly(ADP-ribosyl)ation, we added the specific PARP inhibitor 3-aminobenzamide (3AB) to the reactions (*19*). Modification of both TRF1 and tankyrase was strongly inhibited by 3AB (Fig. 4C). Furthermore, modified tankyrase and TRF1 reacted with a monoclonal antibody to poly(ADP-ribose) (Fig. 4D) (*17*), consistent with their carrying ADP-ribose polymers. These data indicate that tankyrase is a genuine PARP with at least two specific substrates, TRF1 and tankyrase itself.

The effect of tankyrase on the telomeric DNA binding activity of TRF1 was determined by an in vitro gel-shift assay with the use of a double-stranded array of [TTAGGG]$_{12}$ as a probe (*20*). TRF1 binds to DNA as a homodimer, and several such dimers can occupy one [TTAGGG]$_{12}$ molecule at high TRF1 concentrations (*6*) (Fig. 4E). When TRF1 was incubated with baculovirus-derived tankyrase in the absence of NAD$^+$, a slight stimulation of the TRF1 DNA binding activity occurred, resulting in the formation of higher order complexes, especially at high tankyrase concentrations. However, this stimulation of TRF1 also occurred with total insect cell protein and was therefore unlikely to represent a specific effect of tankyrase. A similar nonspecific enhancement of TRF1 was previously reported for β-casein and several other proteins (*1*). In contrast, when NAD$^+$ was included in the TRF1-tankyrase mixtures, a reduction of the TRF1 activity resulted (Fig. 4E). This effect was dependent on the addition of active tankyrase (Fig. 4E), consistent with ADP-ribosylation being the cause of the TRF1 inhibition.

The identification of a telomeric PARP rais-

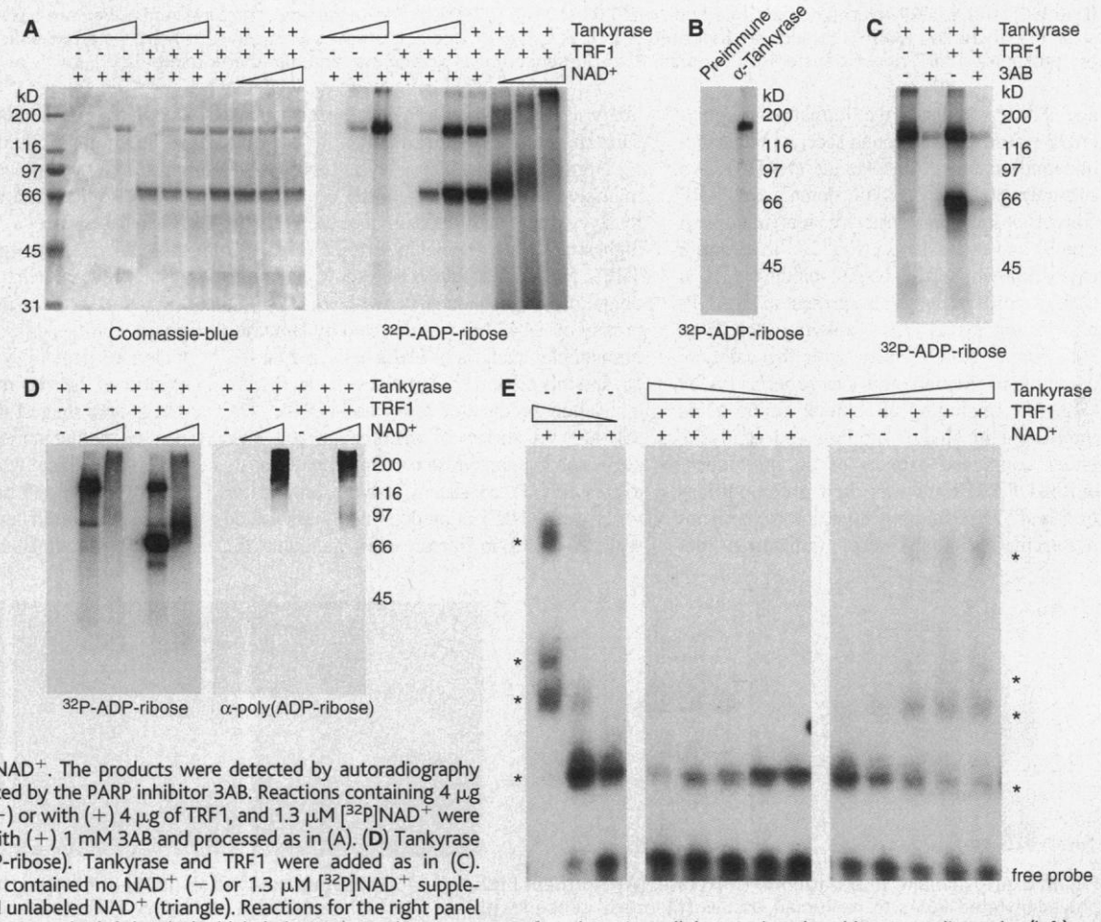

Fig. 4. Tankyrase is a PARP that inhibits TRF1 in vitro. (**A**) Tankyrase ADP-ribosylates itself and TRF1. Tankyrase was allowed to modify itself and TRF1 in the presence of [^{32}P]NAD$^+$, and the products were analyzed by Coomassie blue staining (left) and autoradiography (right) of SDS-PAGE gels (*17*). Reactions contained the proteins indicated above the lanes at the following amounts: TRF1 at 4 μg (+) and tankyrase at 4 μg (+) or at a range of 0, 0.8, and 4 μg (triangle). All reactions contained 1.3 μM [^{32}P]NAD$^+$ (+), and three reactions were also supplemented with increasing amounts of unlabeled NAD$^+$ (0.04, 0.2, and 1 mM, triangle). (**B**) ADP-ribosylation activity is intrinsic to tankyrase. Tankyrase was immunoprecipitated with preimmune serum or anti-tankyrase (α-Tankyrase) as indicated and incubated in a PARP assay with [^{32}P]NAD$^+$. The products were detected by autoradiography (*18*). (**C**) Tankyrase is inhibited by the PARP inhibitor 3AB. Reactions containing 4 μg of tankyrase (+), without (−) or with (+) 4 μg of TRF1, and 1.3 μM [^{32}P]NAD$^+$ were incubated without (−) or with (+) 1 mM 3AB and processed as in (A). (**D**) Tankyrase products contain poly(ADP-ribose). Tankyrase and TRF1 were added as in (C). Reactions for the left panel contained no NAD$^+$ (−) or 1.3 μM [^{32}P]NAD$^+$ supplemented with 1 μM or 1 mM unlabeled NAD$^+$ (triangle). Reactions for the right panel were identical to the reactions on the left but lacked labeled NAD$^+$. Products were transferred to nitrocellulose and analyzed by autoradiography (left) or immunoblotted with monoclonal antibody 10H to poly(ADP-ribose) (*17*) (right). (**E**) Tankyrase inhibition of TRF1. A gel-shift assay for the TTAGGG repeat–binding activity of TRF1 was performed with a duplex [TTAGGG]$_{12}$ DNA as a probe. Binding reactions contained the components indicated above the lanes. Tankyrase concentration was varied from 200 to 2.5 ng per 20-μl incubation in threefold dilution steps (triangle). TRF1 was either present at 13 ng (+) or varied from 120 to 13 ng in threefold dilution steps (triangle). NAD$^+$ was at absent (−) or present at 0.2 mM (+). The asterisks indicate the position of TRF1-containing complexes as determined by antibody super-shift experiments.

es the possibility that the function of human telomeres is regulated by this type of protein modification. Because ADP-ribosylation usually inhibits protein activity (*21*), we favor the view that tankyrase is a negative regulator of another factor acting at telomeres. Although the in vivo targets of tankyrase remain to be established, TRF1 is a strong candidate, because it is a substrate for tankyrase in vitro and ADP-ribosylation inhibits the ability of TRF1 to bind to telomeric DNA. However, the PARP activity of tankyrase could also be directed at other telomere-associated factors, including telomerase, and ADP-ribosylation might enhance rather than inhibit the activity of the target protein (*22*). In vivo functional analysis will be required to determine whether tankyrase acts positively or negatively in the regulation of telomere length. PARPs have previously been implicated in the cellular response to DNA damage (*9*). The presence of a PARP activity at telomeres may also hint at a role for tankyrase in the protection of telomeres from inappropriate DNA damage processing activities.

References and Notes

1. L. Chong *et al.*, *Science* **270**, 1663 (1995).
2. D. Broccoli, A. Smogorzewska, L. Chong, T. de Lange, *Nature Genet.* **17**, 231 (1997); S. Smith and T. de Lange, *Trends Genet.* **13**, 21 (1997); T. Bilaud *et al.*, *Nature Genet.* **17**, 236 (1997).
3. B. van Steensel *et al.*, *Cell* **92**, 401 (1998).
4. B. van Steensel and T. de Lange, *Nature* **385**, 740 (1997).
5. Full-length human TRF1 fused to LexA (*6*) was used as a bait for the screening of 1×10^7 LD40 transformants of a human fetal liver two-hybrid library (Clontech, Palo Alto, CA) created in pGad10. Of 13 positives, 12 contained an identical 2.4-kb insert (TR1L-4) and one had a 1-kb insert (TR1L-12), which was contained within TR1L-4. DNA sequence analysis indicated that TR1L-4 was a partial cDNA.
6. A. Bianchi *et al.*, *EMBO J.* **16**, 1785 (1997).
7. A full-length tankyrase cDNA TT20 containing a 4134–nucleotide (nt) insert was isolated as follows. First, two overlapping cDNAs, 32 and 21, encompassing 8901 nt encoding amino acids 235 to 1327 were isolated from a HeLa cDNA library that had been probed with a polymerase chain reaction product (representing amino acids 973 to 1163) made from TR1L-4. The 5′ end sequence was extended by rapid amplification of cDNA ends (RACE) and then used to screen a human testis library (Stratagene, La Jolla, CA). Analysis of two other testis library isolates, TT7 and TT9 (GenBank accession numbers AF082558 and AF082559), indicated that they were similar to TT20 along their length, but each had an insertion of ~100 nt [TT7, insertion after amino acid 640 (in ANK repeat 14), and TT9, insertion after amino acid 881 (in ANK repeat 21)]. Both insertions contained stop codons resulting in truncated proteins, as confirmed by in vitro translation. It is not known if these truncated proteins are expressed in vivo.
8. P. Bork, *Proteins* **17**, 363 (1993); P. Michaely and V. Bennett, *Trends Cell Biol.* **2**, 127 (1992); L. L. Peters and S. E. Lux, *Semin. Hematol.* **30**, 85 (1993).
9. G. de Murcia and J. M. de Murcia, *Trends Biochem. Sci.* **19**, 172 (1994); P. A. Jeggo, *Curr. Biol.* **8**, R49 (1998); T. Lindahl, S. Satoh, G. G. Poirier, A. Klungland, *Trends Biochem. Sci.* **20**, 405 (1995).
10. V. Bennett, *J. Biol. Chem.* **267**, 8703 (1992).
11. C. P. Ponting, *Protein Sci.* **4**, 1928 (1995); J. Schultz, C. P. Ponting, K. Hofmann, P. Bork, *ibid.* **6**, 249 (1997).
12. M. Domenighini, C. Magagnoli, M. Pizza, R. Rappuoli, *Mol. Microbiol.* **14**, 41 (1994); A. Ruf *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 7481 (1996).
13. The tankyrase cDNA TR1L-4 hybridized to three mRNAs of ~6, 8, and 10 kb with the same ubiquitous expression pattern. Testis express two additional transcripts, a 2.5-kb species and an abundant 4.5-kb species. The 4.2-kb TT20 tankyrase cDNA isolated from the testis library is large enough to represent the 4.5-kb transcript, suggesting that this cDNA is nearly full length. The larger transcripts present in most tissues may be due to longer 3′ untranslated regions (UTRs) because sequence analysis of a tankyrase cDNA from a HeLa cell library revealed the same sequence as the full-length 4.5-kb cDNA, but with an additional 5 kb of 3′ UTR. When the most 3′ 1 kb of this cDNA was used as a probe in a Northern blot, it hybridized exclusively to the largest (10-kb) transcript.
14. For immunoblots, HeLa cells were suspended directly in Laemmli loading buffer and loaded at ~50,000 cells per lane. Crude nuclei were isolated from rat testis (after hypotonic lysis), extracted with 0.4 M KCl, pelleted, and suspended in Laemmli buffer. In vitro–translated tankyrase was generated with a coupled transcription-translation reticulocyte lysate system (Promega). A 1-μg amount of TT20 was incubated with T3 RNA polymerase under standard conditions, and 10% of the reaction was loaded per lane. Protein samples were fractionated on SDS-polyacrylamide gels, transferred to nitrocellulose, and blocked in 5% milk in phosphate-buffered saline (PBS) containing 0.1% Tween-20. Antibody incubations were in 1% milk in PBS containing 0.1% Tween-20. Blots were first incubated with rabbit antibody to tankyrase (4 μg/ml) or rabbit preimmune serum (1:500) and then with horseradish peroxidase–conjugated donkey antibody to rabbit immunoglobulin G (IgG) (Amersham) (1:2500). Bound antibody was detected by enhanced chemiluminescence (Amersham). For generation of antibody to tankyrase (anti-tankyrase), the Ank2 fragment representing amino acids 973 to 1149 of tankyrase was fused to vector pET-22b(+) (Novagen) and expressed in *Escherichia coli*. The protein was isolated in inclusion bodies and used to immunize one rabbit. The resulting immune serum, rabbit anti-tankyrase 465, was affinity purified against Ank2 protein coupled to CnBr-activated Sepharose (Sigma).
15. Tankyrase protein was detected by protein immunoblot analysis in the following human cell lines: 293, transformed embryonic kidney cells; IMR90 and WI38, primary lung fibroblasts; WI38 VA13/2RA, immortalized lung fibroblasts; GM847, SV-40–immortalized fibroblasts; Daudi and Raji, lymphoma; HT1080, fibrosarcoma; and MCF7, breast adenocarcinoma. Several of these cell lines expressed only the larger set of tankyrase mRNAs (6 to 10 kb), indicating that the 142-kD polypeptide can be expressed from one of these transcripts.
16. For chromosome spreads, HeLa cells were treated with colcemide (0.5 μg/ml, 60 min), harvested by trypsinization, hypotonically swollen in 10 mM tris-HCl (pH 7.4), 10 mM NaCl, and 5 mM $MgCl_2$, and sedimented onto cover slips for 15 s at 3000 rpm in a Sorvall RT6000B centrifuge. Chromosomes were swollen for 15 min in 25% PBS, fixed in 3.7% formaldehyde in 25% PBS for 10 min, and permeabilized with 0.5% NP-40 in 25% PBS for 10 min. Samples were blocked with 1% bovine serum albumin (BSA) in PBS, and then incubated with rabbit anti-tankyrase (1 μg/ml) and a mouse polyclonal serum to full-length baculovirus-derived TRF1 (1:10,000). Primary antibodies were detected with fluorescein isothiocyanate (FITC)–conjugated donkey antibody to rabbit IgG and tetramethyl rhodamine isothiocyanate (TRITC)–conjugated donkey antibody to mouse IgG (1:100) (Jackson Laboratories). DNA was stained with 4,6-diamino-2-phenylindole (DAPI) (0.2 μg/ml). Images were obtained with a Zeiss Axioplan 2 microscope with a Photometrics charge-coupled device camera and then processed and merged with Adobe Photoshop. Immunolocalization analysis of cycling HeLa cells indicates additional subcellular locations for tankyrase (S. Smith and T. de Lange, in preparation).
17. PARP activity assays were done with baculovirus-derived tankyrase essentially as described [F. Simonin, O. Poch, M. Delarue, G. de Murcia, *J. Biol. Chem.* **268**, 8529 (1993)], but without addition of DNA. To make baculovirus-derived protein, we generated an NH_2-terminally $(His)_6$-tagged version of human tankyrase in the expression vector pFastBac HTb (Gibco BRL) and used this construct to generate a recombinant plasmid in DH10Bac *E. coli*. The recombinant DNA was used to transfect SF21 insect cells, and recombinant virus was isolated and amplified. Protein was purified as described for baculovirus-derived TRF1 (*6*). Samples containing tankyrase (0 to 4 μg) and TRF1 (0 or 4 μg) (*6*) were incubated for 30 min at 25°C in assay buffer (0.1 ml) containing 50 mM tris-HCl (pH 8.0), 4 mM $MgCl_2$, 0.2 mM dithiothreitol (DTT), 1.3 μM [^{32}P]NAD^+ (4 μCi), and various concentrations of unlabeled NAD^+ (0 to 1 mM). Reactions were stopped by addition of 20% trichloroacetic acid (TCA). Acid-insoluble proteins were collected by centrifugation, rinsed in 5% TCA, suspended in Laemmli loading buffer, and fractionated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were visualized by Coomassie-blue stain and autoradiography. For immunoblot analysis, reactions were performed the same way except that [^{32}P]NAD^+ was omitted. Samples were immunoblotted (*14*) and probed with 10H, a mouse monoclonal antibody to poly(ADP-ribose) (1:250) [H. Kawamitsu *et al.*, *Biochemistry* **23**, 3771 (1984)] followed by horseradish peroxidase–conjugated sheep antibody to mouse IgG (Amersham).
18. For immunoprecipitation analysis, 80 μg of tankyrase in 1 ml of buffer D [20 mM Hepes (pH 7.9), 100 mM KCL, 20% glycerol, 0.2 mM EDTA, 0.2 mM EGTA, 1 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 0.1% NP-40, 0.1% Triton X-100, and 1 mg BSA per ml] was precleared by incubation with an irrelevant rabbit serum at room temperature for 1 hour, followed by addition of protein G Sepharose (Pharmacia). Nonspecific antibody complexes and protein aggregates were removed by centrifugation, and the supernatant was used for immunoprecipitation analysis. A 0.5-ml sample of supernatant was incubated with 2 μg of anti-tankyrase or 2 μg of preimmune IgG from the same rabbit [purified by affinity chromotography on protein G Sepharose (Pharmacia)] for 1 hour at room temperature. Antigen-antibody complexes were collected on protein G beads and washed three times with buffer D and twice with 50 mM tris-HCl (pH 8.0). The beads were then assayed for PARP activity by addition of 20 μl containing 50 mM tris-HCl (pH 8.0), 4 mM $MgCl_2$, 0.2 mM DTT, and 1.3 uM [^{32}P]NAD^+ (0.8 μCi). The reactions were incubated and processed as described (*17*).
19. M. R. Purnell and W. J. Whish, *Biochem. J.* **185**, 775 (1980).
20. Gel-shift assays were performed with an end-labeled 142-bp Hind III–Asp^{718} fragment from plasmid pTH12 [Z. Zhong, L. Shiue, S. Kaplin, T. de Lange, *Mol. Cell. Biol.* **12**, 4834 (1992)] containing 12 tandem TTAGGG repeats. Baculovirus-derived TRF1 (13 to 120 ng) (*6*) was incubated for 30 min at room temperature in a 20-μl reaction containing 20 mM Hepes-KOH (pH 7.9), 100 mM KCl, 0.5 mM DTT, 5% glycerol, 0.1% NP-40, 100 ng of sheared *E. coli* DNA, 100 ng of β-casein, and 1 ng of labeled probe. In some cases, reactions were supplemented with NAD^+ (0.2 mM) and baculovirus-derived human tankyrase (2.5 to 200 ng). Samples were fractionated on a 0.7% agarose gel run in 0.1× TBE (8.9 mM tris-base, 8.9 mM boric acid, and 0.2 mM EDTA) at 130 volts for 1 hour at room temperature. Gels were dried onto Whatman DE81 paper and autoradiographed.
21. Reviewed in F. R. Althaus and C. Richter, *Mol. Biol. Biochem. Biophys.* **37**, 1 (1987); S. L. Oei *et al.*, *Biochemistry* **37**, 1465 (1998).
22. F. Rusceti *et al.*, *J. Biol. Chem.* **273**, 14461 (1998).

23. We thank an anonymous reviewer, T. Meier, and members of the de Lange laboratory for comments on this manuscript; J. Lue for technical assistance; and G. de Murcia for a gift of 10H antibody. T.d.L. is a recipient of the Burroughs Wellcome Fund Scholar Award in Toxicology. S.S. is a Leukemia Society of America Special Fellow. A.S. was a fellow of the German National Science Foundation. Supported by grants from the NIH (GM49046 and CA76027), the Rita Allen Foundation, and the Sandoz Foundation.

23 September 1998; accepted 22 October 1998