Defective Repair of Uracil Causes Telomere Defects in Mouse Hematopoietic Cells *

Haritha Vallabhaneni1, Fang Zhou1, Robert W. Maul2, Jaya Sarkar1, Jinhu Yin 1, Ming Lei3, Lea Harrington4, Patricia J. Gearhart2, and Yie Liu1

1Laboratory of Molecular Gerontology, 2Laboratory of Molecular Biology and Immunology, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224
3National Center for Protein Science Shanghai, State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, China
4Department of Medicine, Institute for Research in Immunology and Cancer, University of Montréal, Montréal, Québec H3C 3J7, Canada

*Running title: Uracil misincorporation disrupts telomere maintenance

To whom correspondence should be addressed: Y. L. or P. J. G. liuyie@mail.nih.gov or gearhartp@mail.nih.gov. Phone: 410-558-8419; Fax: 410-558-8157

Key words: uracil DNA glycosylase; uracil misincorporation; base excision repair; telomeres; telomerase.

Background: Telomeres may be susceptible to uracil misincorporation, which is removed by uracil DNA glycosylase (UNG).

Results: UNG deficiency increases uracil in telomeres and causes alterations in multiple aspects of telomere maintenance in proliferating mouse hematopoietic cells.

Conclusion: Accumulation of uracil interferes with telomere maintenance.

Significance: UNG-initiated base excision repair is necessary for the preservation of telomere integrity.

ABSTRACT

Uracil in the genome can result from misincorporation of dUTP instead of dTTP during DNA synthesis, and is primarily removed by uracil DNA glycosylase (UNG) during base excision repair. Telomeres contain long arrays of TTAGGG repeats and may be susceptible to uracil misincorporation. Using model telomeric DNA substrates, we showed that the position and number of uracil substitutions of thymine in telomeric DNA decreased recognition by the telomere single-strand binding protein, POT1. In primary mouse hematopoietic cells, uracil was detectable at telomeres, and UNG deficiency further increased uracil loads and led to abnormal telomere shortening. In UNG-deficient cells, the frequencies of sister chromatid exchange and fragility in telomeres also significantly increased in the absence of telomerase. Thus, accumulation of uracil and/or UNG deficiency interferes with telomere maintenance, thereby underscoring the necessity of UNG-initiated base excision repair for the preservation of telomere integrity.

Telomeres are nucleoprotein structures at the ends of all linear eukaryotic chromosomes. In mammals, telomeres are composed of the shelterin protein complex and a double-stranded tract of short tandem repeats of TTAGGG that ends in a single-stranded 3' overhang on the G-strand (1). Telomeres cap and protect chromosome ends from eliciting a DNA damage response and illegitimate recombination events (2). A critical aspect of telomere maintenance is telomere length homeostasis. Telomere length...
is regulated by telomerase, the shelterin components POT1, TRF1 and TRF2 that directly bind telomere DNA, and molecular processes such as telomere recombination and replication. Telomerase is a ribonucleoprotein complex that adds telomere repeats to the chromosome ends (1), and is regulated by POT1 and its heterodimeric partner TPP1 (3). TRF1 and TRF2 are negative regulators of telomere length (4-7). In addition, oxidized bases in the telomere repeat sequence disrupt telomere length (8-10). However, it is unknown if uracil misincorporation impacts the affinity of telomerase and telomere binding proteins to telomeric DNA, and affects the length, recombination, and replication of telomeres.

Uracil can arise in DNA by misincorporation of dUTP instead of dTTP opposite adenine during DNA synthesis, or by deamination of cytosine to uracil opposite guanine (11). Deamination can occur either spontaneously throughout the genome or by the enzyme activation-induced deaminase in the immunoglobulin loci. Thus, uracil through misincorporation produces U:A base pairs, which are not mutagenic since uracil resembles thymine during replication, whereas uracil through deamination yields U:G base pairs, which are highly mutagenic by producing C:G transitions after replication. Uracil in DNA is primarily recognized and excised by the enzyme UNG during base excision repair. UNG-deficient mice have significantly elevated steady-state levels of genomic uracil but low mutation frequencies (12). These data indicate that the major role for UNG in mice is to remove uracil from misincorporated dUTP, and suggest that misincorporation is the predominant pathway for introducing uracil into mouse genomic DNA. To evaluate competition between oligonucleotides with or without uracil, 5 pM \(^{32}\)P-labeled oligonucleotides were mixed with different concentrations of unlabeled competing oligonucleotides and 50 pM of human POT1 protein in a buffer or 2 µl of \textit{in vitro} translated mouse POT1a or POT1b protein lysate at room temperature for 25 min. Free and protein-bound \(^{32}\)P-labeled oligonucleotides were separated on a 4%
polyacrylamide native gel and quantified by Phosphor-Imager analysis.

**Telomerase Direct Primer Extension Assay** - Telomerase extension was determined as described previously (17), except glycogen (2 μg) was added to each sample instead of linear polyacrylamide as a carrier prior to DNA recovery. Single-stranded substrates (Midland Certified) were assayed using recombinant human telomerase captured on anti-Flag resin and washed with 0.3-0.6 M K-glutamate (17). After telomerase extension, products were resolved on a 10% w/v denaturing acrylamide gel. Quantification of individual radiolabeled species within each lane was carried out using ImageJ64. Individual measurements were assessed for each primer and, where appropriate, normalized to input primer amount and a control primer, d(TTAGGG)2, that was 5’-end-labeled with γ-32P-ATP using polynucleotide kinase (NEB) and added into the reactions prior to product extraction. Statistical analysis was carried out with at least two separate tests: two-way comparisons (e.g. comparing the sum of all repeats added to a dUTP-substituted primer compared to its corresponding unsubstituted primer) were assessed using an unpaired students’ t-test with Welch’s correction, and multiple comparisons (e.g. individual repeat signal intensities and the sum of all repeats, compared between multiple primers simultaneously) were assessed using 1-way ANOVA with Tukey post-test (Prism 5.0, GraphPad Inc.). When the null hypothesis could not be rejected using both tests, the results were cited as not significantly different (p>0.05).

**Detection of Uracil in Mouse Hematopoietic Cells** – 1 μg of genomic DNA from Ung+/+ and Ung−/− bone marrow cells was incubated with (treated) or without (mock) 10 nM UNG (provided by Dr. J. Stivers, Johns Hopkins University) in a buffer (50 mM HEPES pH 7.5, 20 mM KCl, 4 mM EDTA, and 2 mM dithiothreitol) for 30 min at 37°C. 5 ng APE1 (provided by Dr. D. Wilson III, NIA/NIH) and 10 mM MgCl2 were then added to the reaction and further incubated at 37°C for 1 hr. qPCR analysis was performed on 10 ng DNA using telomere (18) or Gapdh primers (19) using Power SYBR Green master mix (Life Technologies). Relative amplification was calculated by standard ΔCt method. Sensitivity of mock- or UNG-treated samples was calculated against input DNA (2− (ΔCt (mock) − ΔCt (input DNA))).

**RESULTS**

**Uracil in Telomere DNA Interferes with Binding of POT1**

Rogue bases at telomeric DNA may alter the affinity of telomere-associated proteins. Shelterin proteins TRF1, TRF2, and POT1 directly bind to telomere DNA, in order to regulate telomeres (2,3). Here, we employed EMSA to investigate how uracil in telomeric DNA may impact recognition and binding of innate telomere binding shelterin.
proteins. We focused on testing telomeric DNA substrates in which one or more thymines were replaced by uracil.

POT1 binds the single-stranded (ss) telomeric 3’-overhang in diverse eukaryotic chromosomes (22) and regulates telomere length (3). TPP1 facilitates the recruitment of POT1 to telomeres and enhances POT1 binding to ssDNA (15,23-26). First, we examined binding of the human POT1/TPP1 protein complex to a 32P-labeled ss telomere oligonucleotide [GGT1T2AGGGT3T4AG; underlined is the minimal sequence required for efficient POT1 binding (27)] in the presence of increasing amounts of unlabeled telomere oligonucleotides that contained no uracil (unmodified) or one or more thymines replaced by uracil (modified). Competition between the 32P-labeled oligonucleotide and excess of unlabeled oligonucleotide would lead to a decrease in the amount of POT1/TPP1-bound 32P-labeled oligonucleotide complex in EMSA. This decrease is directly proportional to the binding affinity of POT1/TPP1 to the respective unlabeled competing oligonucleotide. Compared to the unmodified competing oligonucleotide (Fig. 1A, lanes 2-6), modified competing oligonucleotides that contained uracil within the POT1 binding site (Fig. 1A, lanes 7-21) showed diminished affinity for POT1/TPP1, as evident from increased retention of the protein-oligonucleotide complex. Modified competing oligonucleotides with uracil at T1 and T2 positions displayed lower affinity for POT1/TPP1, than those with uracil at positions T3 and T4 positions (Fig. 1A, lanes 12-16 and lanes 7-11 respectively). A similar observation was also reported when thymine was replaced with adenine (14). This can plausibly be attributed to the fact that although POT1 binds to ss DNA in an extended conformation, where both oligonucleotide/oligosaccharide binding folds contact DNA, it is the N-terminal fold that makes more extensive contacts with the first six nucleotides at the 5’-end of the ss DNA (27). Significantly, modified competing oligonucleotides harboring uracil at all four thymines in the POT1 binding sequence (Fig. 1A, lanes 17-21) exhibited weaker POT1/TPP1 binding than those that had uracil at only two positions (Fig. 1A, lanes 7-16). As a control, modified competing oligonucleotides with uracil outside the POT1 binding sequence had no effect on binding (Fig. 1B).

To extend the binding studies of human POT1 in mice, we performed similar experiments with mouse POT1 proteins. Mice have two isoforms of POT1: POT1a and POT1b (14,28), and both share similar DNA binding properties in vitro (14,29). Human POT1 protein combines the functions of two mouse POT1 proteins (29) and also has very similar sequence specificity and DNA affinity as mouse POT1 (30). Similar to human POT1, mouse POT1a and POT1b form heterodimers with TPP1 that can be tethered to double-stranded telomeric DNA by TIN2 (30-32). We examined the binding of mouse POT1a and POT1b to the telomere oligonucleotides with or without uracil. Modified competing oligonucleotides harboring uracil at all four thymines in the POT1 binding sequence exhibited weaker POT1a and POT1b binding (Fig. 1C). Thus, the presence of uracil in place of thymine within the core POT1 binding site in telomeric DNA significantly diminished the binding affinity of both human and mouse POT1, which depended on the position and number of uracil replacements.

We then examined binding of the two other bona fide telomeric proteins TRF1 and TRF2 to a telomere repeat-containing oligonucleotide duplex without or with uracil replaced by thymine. The binding of human TRF1 and TRF2 to the duplex oligonucleotides with uracil replacing a single T in the TTAGGG sequence was comparable to that without uracil (data not shown). In
order to determine if increased uracil load in the telomere repeats affected the binding of TRF1 or TRF2, all the thymines in the telomere repeat region of the duplex were replaced with uracil (Fig. 2A). Despite this high uracil content, the affinity of both TRF1 and TRF2 to the uracil-modified duplex remained the same as that of the unmodified duplex (Fig. 2B and 2C). Collectively, this in vitro analysis demonstrated that uracil in the telomeric substrate had deleterious effects on the binding affinity of POT1, but had no significant impact on binding of TRF1 or TRF2.

**Limited Tracts of Uracil in Telomeric DNA Do Not Affect Overall Telomerase Extension**

Telomerase recognizes and extends ss DNA primers by virtue of limited primer-template base pairing between telomeric DNA and the telomerase RNA, and by a DNA-binding ‘anchor’ site within telomerase reverse transcriptase itself (33,34). These and other properties of the enzyme enable it to tolerate substitution of dTTP in the primer without significant impairment of binding or extension (17,35-38). We tested whether uracil substitution would affect the ability of recombinant human telomerase to utilize substrates with extensive or limited complementarity to the telomerase RNA template (Fig. 3). We immuno-purified recombinant FLAG-human telomerase reverse transcriptase (hTERT) and human telomerase RNA after reconstitution in rabbit reticulocyte lysate (17) and assayed the ability to extend the primer TT (5’-AGGGTTAGGGTTAGGGTT-3’) or the same sequence in which the first, second, or third TT ‘registers’ were replaced by dUTP (UU1, UU2, UU3, respectively) (Fig. 3A). Quantification of the results showed no significant difference in primer utilization, by comparing the intensity of specific repeat sequences, or the sum of all repeats added, between TT and UU1, UU2 or UU3 (Fig. 3A, C). We also tested the impact of dUTP substitution on a largely non-telomeric primer, GTT (5’-AATCCGTCGAGCAGAGTT-3’) containing dUTP substitutions at the penultimate TTP (GUT), last TTP (GTU) or both terminal TTP residues (GUU). Again, there was no significant difference in primer utilization by telomerase when comparing the sum of all repeats added, or when comparing the intensity of individual repeats (Fig. 3B, C). Thus, limited substitution of TTP with uracil did not significantly affect the overall primer extension activity of telomerase in vitro.

**Uracil Accumulation Due to Defective Removal Disrupts Telomere Length Homeostasis in Ung−/− Hematopoietic Cells**

Our in vitro data support the hypothesis that uracil in telomeric DNA substrates diminishes the affinity of POT1. However, it is not known how the accumulation of uracil affects telomeres in vivo. In order to investigate the potential effects of accumulation of uracil at telomeres, we utilized mouse models deficient in the enzyme UNG that removes misincorporated uracil. Though Ung−/− deficient mice show enhanced uracil in the genome (12), accumulation of uracil at telomeres has not been investigated. In order to measure uracil at telomeres, genomic DNA from Ung+/+ and Ung−/− bone marrow cells were examined by a quantitative PCR method (19). First, genomic DNA was treated in vitro with mock or UNG enzyme. The resulting abasic sites were then nicked by addition of APE1. Thus, samples with higher levels of uracil would have decreased intact DNA. To quantify the levels of intact DNA, qualitative PCR analysis was carried out using telomere specific primers (18). Compared to input DNA, the mock-treated Ung+/+ and Ung−/− DNA showed no decrease in amplification. However, after UNG treatment, Ung−/− DNA had a 56% decrease in PCR amplification, suggesting the presence of uracil in telomeres (Figure 4A).
Furthermore, \textit{Ung}⁻/⁻ DNA had an additional 23% decrease in amplification efficiency, suggesting that UNG removes uracil at telomeres and in its absence, more uracil accumulates. As a control, uracil content was measured at the \textit{Gapdh} locus (Fig. 4B). Unlike telomeres, the \textit{Gapdh} locus is not sensitive to UNG treatment in either \textit{Ung}⁺/⁺ or \textit{Ung}⁻/⁻ DNA. Collectively, these results suggest that telomeres are prone to uracil accumulation and that decreased repair due to lack of UNG exacerbates this phenomenon.

Next, we rationalized that the increased uracil content in telomeres in \textit{Ung}⁻/⁻ mice may disrupt telomere length homeostasis. Mounting evidence supports the notion that telomere dysfunction primarily affects the hematopoietic lineage in both humans and mice (39,40). We therefore examined telomere length in primary bone marrow cells from \textit{Ung}⁻/⁻ mice by Q-FISH (Fig. 5, A-C). \textit{Ung}⁻/⁻ mice exhibited a significant, albeit variable, increase in telomere length compared to \textit{Ung}⁺/⁺ mice. Wild-type telomere lengths were occasionally observed in \textit{Ung}⁻/⁻ mice (data not shown). Because uracil in telomeric DNA had no effect on telomerase activity \textit{in vitro} (Fig. 3), we further delved into the involvement of telomerase in telomere lengthening in mice deficient for both UNG and telomerase. We generated \textit{Ung}⁺/⁺\textit{Tert}⁺/⁺ and \textit{Ung}⁻/⁻\textit{Tert}⁻/⁻ mice by crossing \textit{Ung}⁻/⁻ mice with a strain lacking \textit{TERT} (13). Consistent with the \textit{in vitro} data, the majority of \textit{Ung}⁻/⁻\textit{Tert}⁻/⁻ mice continued to show significantly longer telomeres as compared to their \textit{Ung}⁺/⁺\textit{Tert}⁺/⁺ counterparts (Fig. 5D and E and data not shown). Thus, UNG deficiency and, in turn, defective uracil removal can lead to lengthening of telomeres, even in the absence of telomerase.

**Telomerase Deficiency Exacerbates Telomere Recombination and Fragility in the Absence of UNG**

Accumulation of oxidative base lesions or deficiency of the glycosylases that remove them results in an increase in telomere sister chromatid exchange (T-SCE) in mice (9,10). Thus, we inquired if defective removal of misincorporated uracil would impact T-SCE in \textit{Ung}⁻/⁻ mice. We examined the frequency of T-SCEs in primary bone marrow cells from \textit{Ung}⁺/⁺ and \textit{Ung}⁻/⁻ mice by CO-FISH (Fig. 6A). T-SCEs in \textit{Ung}⁻/⁻ mouse cells were comparable to \textit{Ung}⁺/⁺ mouse cells (Fig. 6B). Because T-SCEs have been reported to be prevalent in telomerase-deficient mouse cells (41-44), we tested T-SCEs in primary bone marrow cells from \textit{Ung}⁺/⁺\textit{Tert}⁺/⁺ and \textit{Ung}⁻/⁻\textit{Tert}⁻/⁻ mice. There was a significant increase in T-SCEs in \textit{Ung}⁻/⁻\textit{Tert}⁻/⁻ cells, compared to \textit{Ung}⁺/⁺\textit{Tert}⁺/⁺ cells (Fig. 6C).

Common fragile sites represent specific chromosomal regions that are sensitive to replication stress, such as treatment with low levels of the DNA polymerase inhibitor aphidicolin and display breaks or gaps in metaphase chromosomes (45). Telomeres are aphidicolin-induced fragile sites, showing aberrant discontinuous multiple telomere signals, namely fragile telomeres (46). Though the underlying cause for the appearance of these structures is still unknown, conditions that cause incomplete DNA replication or stalled replication forks commonly result in telomere fragility (46-49). UNG is known to localize to replication foci and play an important role in DNA replication (12,50). Furthermore, accumulation of oxidative base lesions results in telomere fragility (10). These reports led us to inquire if uracil misincorporation or UNG deficiency would lead to defective telomere replication, manifesting in telomere fragility. For this purpose, we performed telomere-FISH on primary mouse bone marrow cells from \textit{Ung}⁺/⁺ and \textit{Ung}⁻/⁻ mice (Fig. 6D). Although the frequency of fragile telomeres was comparable in \textit{Ung}⁺/⁺ and \textit{Ung}⁻/⁻ cells (Fig. 6E), UNG ablation caused a significant
increase in fragile telomeres in the telomerase null background (Fig. 6F), similar to our observation for T-SCE events. Taken together, these results support the idea that telomerase deficiency exacerbates aberrant telomere homologous recombination and debilitates telomere replication in the absence of uracil removal by UNG.

**DISCUSSION**
UNG deficiency is associated with B-cell lymphomas and pathological changes in lymphoid organs in mice and humans (51,52). Mounting evidence supports the notion that telomere dysfunction primarily affects the hematopoietic lineage in both human and mice, suggesting the importance of telomere maintenance in highly proliferating cells (39,40). Owing to the long arrays of TTAGGG repeats, telomeres might be susceptible to misincorporation of uracil. The finding that uracil is dramatically increased in telomeres from wild type mice, compared to the Gapdh control locus, underscores the susceptibility of DNA ends to instability. Uracil misincorporation could occur more frequently because telomerase has low fidelity (53) and may incorporate dUTP more readily than DNA polymerases delta and epsilon that replicate Gapdh genes. In addition, DNA ends may be protected by shelterin proteins which would limit access to the UNG enzyme and prevent removal of uracil.

Inappropriately long telomeres may have serious consequences on cells, for instance by resulting in stalled replication forks or the formation of secondary structures that might perturb normal cell cycle progression or by promoting tumorigenesis (54-59). Overall, telomere integrity depends on the maintenance of length equilibrium, replication, and homologous recombination, all of which could be affected by base alterations such as uracil misincorporation in telomeric DNA. Telomere length is regulated by a complex set of factors including the shelterin complex and telomerase. The shelterin proteins TRF1, TRF2 and POT1, when bound to telomeric DNA substrate, are negative regulators of telomere length (4-7,60). Unlike oxidative base lesions of guanine (61), uracil replacement of thymine in telomeric DNA substrates did not affect binding of TRF1 and TRF2 in vitro, although POT1 binding was significantly reduced. This is consistent with the structural reports that the specificity of TRF1 and TRF2 is primarily accredited to their direct contacts with the cluster of G’s in the telomeric DNA repeats (62), while the specific conformation imposed on the ssDNA by POT1 precludes its binding to uracil (27,63).

Recent studies show that mutations in POT1 predispose families to cutaneous melanoma and abnormal telomere shortening (57,59). Somatic POT1 mutations also lead to telomere shortening and favor the acquisition of the malignant features of chronic lymphocytic leukemia (56). Further, one of the two mouse POT1 proteins, POT1a, is required for maintaining telomere length homeostasis, with its conditional deletion leading to telomere over-lengthening (64). Our data show a correlation between binding of POT1 proteins to uracil substrates in vitro and abnormal telomere length maintenance in Ung<sup>-/-</sup> mice. The addition of telomere repeats by telomerase requires its access to the terminal residues of the 3' single-stranded overhang; however, POT1 is believed to coat the entire 3' overhang (27), thereby preventing access of telomerase and acting as a negative regulator of telomere length (3). When uracil replaces thymine at the extreme 3' end of telomeric and non-telomeric substrates in vitro, the overall extension activity by telomerase is unaffected, in agreement with our in vivo observations. However, uracil in telomeric DNA weakens the binding affinity of POT1 in vitro. This might reduce the amount of bound POT1 and thus increase the accessibility of telomerase to the 3' overhang,
which could potentially contribute to the telomere lengthening phenotype we observed. However, our in vivo data suggests that telomere lengthening could be independent of telomerase. Thus, there may be another route for telomere lengthening in UNG-deficient mouse cells. In 8-oxoguanine DNA glycosylase null strains of budding yeast, Rad52, a key protein of the homologous recombination pathway, is involved in telomere lengthening in some of the clones (8). Here, we have shown that ablation of UNG in a telomerase-deficient background not only leads to telomere lengthening but also to an increase in T-SCEs in mice. It is possible that uracil in telomeric DNA enhances homologous recombination at telomeres, leading to telomere lengthening in Ung−/− Tert−/− mice.

UNG plays an important role in DNA replication (12,50); however its deficiency alone did not have a significant impact on telomere fragility or recombination in proliferating hematopoietic cells. The level of uracil accumulated in vivo in UNG-deficient cells might not be substantial enough to result in detectable levels of either fragile telomeres or T-SCEs. Also, the nature and type of base lesions or alterations may differentially influence telomere phenotypes. For instance, it has been shown that TRF1 is required for efficient telomere replication and suppression of telomere fragility (46). Aberrant binding of TRF1 to telomeric DNA containing oxidized bases (61) may contribute to increased telomere fragility, as observed in 8-oxoguanine or endonuclease III-like protein 1 DNA glycosylase null hematopoietic cells (10). Conversely, uracil does not directly impair the binding of TRF1 to telomeric substrates. In our studies, telomerase deficiency elevated fragile telomeres and T-SCEs in Ung−/− mouse cells, suggesting a possible cooperative role of telomerase and UNG-sponsored base excision repair in maintaining telomere integrity in mice. Telomere replication and telomerase-mediated extension are believed to be coupled processes (65,66). Since UNG has been implicated in uracil removal during and after DNA replication (12,50,67), there may be a window of time when both telomerase and UNG collaborate to ensure maintenance of telomere integrity during telomere replication.

In summary, we have demonstrated that enhanced uracil substitutions of thymine in telomeric DNA result in alterations/aberrations in multiple aspects of telomere maintenance in proliferating mouse hematopoietic cells, especially in the absence of telomerase. Since reduced base excision repair capacity has been associated with aging and UNG mutations with hyper IgM syndrome in humans (51,68), our findings in mice could hold true for humans as well.

REFERENCES

1. Blackburn, E. H. (2001) Switching and signaling at the telomere. Cell 106, 661-673
2. de Lange, T. (2005) Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev 19, 2100-2110
3. Lue, N. F., Yu, E. Y., and Lei, M. (2013) A popular engagement at the ends. Nat Struct Mol Biol 20, 10-12
4. van Steensel, B., and de Lange, T. (1997) Control of telomere length by the human telomeric protein TRF1. Nature 385, 740-743
5. Smogorzewska, A., van Steensel, B., Bianchi, A., Oelmann, S., Schaefer, M. R., Schnapp, G., and de Lange, T. (2000) Control of human telomere length by TRF1 and TRF2. *Mol Cell Biol* **20**, 1659-1668

6. Ancelin, K., Brunori, M., Bauwens, S., Koering, C. E., Brun, C., Ricoul, M., Pommier, J. P., Sabatier, L., and Gilson, E. (2002) Targeting assay to study the cis functions of human telomeric proteins: evidence for inhibition of telomerase by TRF1 and for activation of telomere degradation by TRF2. *Mol Cell Biol* **22**, 3474-3487

7. Wan, B., Yin, J., Horvath, K., Sarkar, J., Chen, Y., Wu, J., Wan, K., Lu, J., Gu, P., Yu, E. Y., Lue, N. F., Chang, S., Liu, Y., and Lei, M. (2013) SLX4 assembles a telomere maintenance toolkit by bridging multiple endonucleases with telomeres. *Cell Rep* **4**, 861-869

8. Lu, J., and Liu, Y. (2010) Deletion of Ogg1 DNA glycosylase results in telomere base damage and length alteration in yeast. *EMBO J* **29**, 398-409

9. Wang, Z., Rhee, D. B., Lu, J., Bohr, C. T., Zhou, F., Vallabhaneni, H., de Souza-Pinto, N. C., and Liu, Y. (2010) Characterization of oxidative guanine damage and repair in mammalian telomeres. *PLoS Genet* **6**, e1000951

10. Vallabhaneni, H., O'Callaghan, N., Sidorova, J., and Liu, Y. (2013) Defective repair of oxidative base lesions by the DNA glycosylase Nth1 associates with multiple telomere defects. *PLoS Genet* **9**, e1003639

11. Krokan, H. E., Drablos, F., and Slupphaug, G. (2002) Uracil in DNA—occurrence, consequences and repair. *Oncogene* **21**, 8935-8948

12. Nilsen, H., Rosewell, I., Robins, P., Skjelbred, C. F., Andersen, S., Slupphaug, G., Daly, G., Krokan, H. E., Lindahl, T., and Barnes, D. E. (2000) Uracil-DNA glycosylase (UNG)-deficient mice reveal a primary role of the enzyme during DNA replication. *Mol Cell* **5**, 1059-1065

13. Liu, Y., Snow, B. E., Hande, M. P., Yeung, D., Erdmann, N. J., Wakeham, A., Itie, A., Siderovski, D. P., Lansdorp, P. M., Robinson, M. O., and Harrington, L. (2000) The telomerase reverse transcriptase is limiting and necessary for telomerase function in vivo. *Curr Biol* **10**, 1459-1462

14. He, H., Multani, A. S., Cosme-Blanco, W., Tahara, H., Ma, J., Pathak, S., Deng, Y., and Chang, S. (2006) POT1b protects telomeres from end-to-end chromosomal fusions and aberrant homologous recombination. *EMBO J* **25**, 5180-5190

15. Wang, F., Podell, E. R., Zaug, A. J., Yang, Y. T., Baciu, P., Cech, T. R., and Lei, M. (2007) The POT1-TPP1 telomere complex is a telomerase processivity factor. *Nature* **445**, 506-510

16. Gomez, M., Wu, J., Schreiber, V., Dunlap, J., Dantzer, F., Wang, Y., and Liu, Y. (2006) PARP1 is a TRF2-associated poly(ADP-ribose)polymerase and protects eroded telomeres. *Mol Biol Cell* **17**, 1686-1696

17. Oulton, R., and Harrington, L. (2004) A human telomerase-associated nuclease. *Mol Biol Cell* **15**, 3244-3256

18. Cawthon, R. M. (2002) Telomere measurement by quantitative PCR. *Nucleic Acids Res* **30**, e47

19. Maul, R. W., Saribasak, H., Martomo, S. A., McClure, R. L., Yang, W., Vaisman, A., Gramlich, H. S., Schatz, D. G., Woodgate, R., Wilson, D. M., 3rd, and Gearhart, P. J. (2011) Uracil residues dependent on the deaminase AID in immunoglobulin gene variable and switch regions. *Nat Immunol* **12**, 70-76
20. Bailey, S. M., Goodwin, E. H., and Cornforth, M. N. (2004) Strand-specific fluorescence in situ hybridization: the CO-FISH family. *Cytogenet Genome Res* **107**, 14-17

21. Rhee, D. B., Wang, Y., Mizesko, M., Zhou, F., Haneline, L., and Liu, Y. (2010) FANCC suppresses short telomere-initiated telomere sister chromatid exchange. *Hum Mol Genet* **19**, 879-887

22. Baumann, P., and Cech, T. R. (2001) Pot1, the putative telomere end-binding protein in fission yeast and humans. *Science* **292**, 1171-1175

23. Xin, H. W., Liu, D., Wan, M., Safari, A., Kim, H., Sun, W., O'Connor, M. S., and Zhou, S. Y. (2007) TPP1 is a homologue of ciliate TEBP-beta and interacts with POT1 to recruit telomerase. *Nature* **445**, 559-562

24. Ye, J. Z. S., Hockemeyer, D., Krutchinsky, A. N., Loayza, D., Hooper, S. M., Chait, B. T., and de Lange, T. (2004) POT1-interacting protein PIP1: a telomere length regulator that recruits POT1 to the TIN2/TRF1 complex. *Genes Dev* **18**, 1649-1654

25. Liu, D., Safari, A., O'Connor, M. S., Chan, D. W., Laegeler, A., Qin, J., and Songyang, Z. (2004) PTOP interacts with POT1 and regulates its localization to telomeres. *Nat Cell Biol* **6**, 673-680

26. Houghtaling, B. R., Cuttonaro, L., Chang, W., and Smith, S. (2004) A dynamic molecular link between the telomere length regulator TRF1 and the chromosome end protector TRF2. *Curr Biol* **14**, 1621-1631

27. Lei, M., Podell, E. R., and Cech, T. R. (2004) Structure of human POT1 bound to telomeric single-stranded DNA provides a model for chromosome end-protection. *Nat Struct Mol Biol* **11**, 1223-1229

28. Hockemeyer, D., Daniels, J. P., Takai, H., and de Lange, T. (2006) Recent expansion of the telomeric complex in rodents: Two distinct POT1 proteins protect mouse telomeres. *Cell* **126**, 63-77

29. Palm, W., Hockemeyer, D., Kibe, T., and de Lange, T. (2009) Functional dissection of human and mouse POT1 proteins. *Mol Cell Biol* **29**, 471-482

30. Takai, K. K., Kibe, T., Donigian, J. R., Frescas, D., and de Lange, T. (2011) Telomere protection by TPP1/POT1 requires tethering to TIN2. *Mol Cell* **44**, 647-659

31. Frescas, D., and de Lange, T. (2014) TRF2-tethered TIN2 can mediate telomere protection by TPP1/POT1. *Mol Cell Biol* **34**, 1349-1362

32. Frescas, D., and de Lange, T. (2014) Binding of TPP1 to TIN2 is required for POT1a,b-mediated telomere protection. *J Biol Chem*, 10.1074/jbc.M114.592592

33. Skordalakes, E., and Lue, N. F. (2012) *TERT Structure, Function, and Molecular Mechanisms, in Telomerase:Chemistry, Biology and Clinical Application*. John Wiley & Sons, Inc., Hoboken, NJ, USA. doi: 10.1002/9781118268667.ch3

34. Tzfati, Y., and Chen, J. J.-L. (2012) *Telomerase RNA: Structure, Function, and Molecular Mechanisms, in Telomerase:Chemistry, Biology and Clinical Application*, John Wiley & Sons, Inc., Hoboken, NJ, USA. doi: 10.1002/9781118268667.ch2

35. Collins, K., and Greider, C. W. (1995) Utilization of ribonucleotides and RNA primers by Tetrahymena telomerase. *EMBO J* **14**, 5422-5432

36. Harrington, L., Hull, C., Crittenden, J., and Greider, C. (1995) Gel shift and UV cross-linking analysis of Tetrahymena telomerase. *J Biol Chem* **270**, 8893-8901

37. Hammond, P. W., and Cech, T. R. (1997) dGTP-dependent processivity and possible template switching of euplotes telomerase. *Nucleic Acids Res* **25**, 3698-3704
38. Huard, S., and Autuxier, C. (2004) Human telomerase catalyzes nucleolytic primer cleavage. *Nucleic Acids Res* 32, 2171-2180
39. Liu, Y., and Harrington, L. (2012) *Murine models of dysfunctional telomeres and telomerase*, in *Telomerase: Chemistry, Biology and Clinical Application*, John Wiley & Sons, Inc. doi: 10.1002/9781118268667.ch9
40. Armanios, M. (2013) Telomeres and age-related disease: how telomere biology informs clinical paradigms. *J Clin Invest* 123, 996-1002
41. Wang, Y., Erdmann, N., Giannone, R. J., Wu, J., Gomez, M., and Liu, Y. (2005) An increase in telomere sister chromatid exchange in murine embryonic stem cells possessing critically shortened telomeres. *Proc Natl Acad Sci U S A* 102, 10256-10260
42. Wang, Y., Giannone, R. J., and Liu, Y. (2005) Telomere sister chromatid exchange in telomerase deficient murine cells. *Cell Cycle* 4, 1320-1322
43. Morrish, T. A., and Greider, C. W. (2009) Short telomeres initiate telomere recombination in primary and tumor cells. *PLoS Genet* 5, e1000357
44. Wang, F., Yin, Y., Ye, X., Liu, K., Zhu, H., Wang, L., Chiourea, M., Okuka, M., Ji, G., Dan, J., Zuo, B., Li, M., Zhang, Q., Liu, N., Chen, L., Pan, X., Gagos, S., Keefe, D. L., and Liu, L. (2012) Molecular insights into the heterogeneity of telomere reprogramming in induced pluripotent stem cells. *Cell Res* 22, 757-768
45. Glover, T. W., Berger, C., Coyle, J., and Echo, B. (1984) DNA polymerase alpha inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. *Hum Genet* 67, 136-142
46. Sfeir, A., Kosiyatrankul, S. T., Hockemeyer, D., MacRae, S. L., Karlseder, J., Schildkraut, C. L., and de Lange, T. (2009) Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication. *Cell* 138, 90-103
47. McNees, C. J., Tejera, A. M., Martinez, P., Murga, M., Mulero, F., Fernandez-Capetillo, O., and Blasco, M. A. (2010) ATR suppresses telomere fragility and recombination but is dispensable for elongation of short telomeres by telomerase. *J Cell Biol* 188, 639-652
48. Gu, P., Min, J. N., Wang, Y., Huang, C., Peng, T., Chai, W., and Chang, S. (2012) CTC1 deletion results in defective telomere replication, leading to catastrophic telomere loss and stem cell exhaustion. *EMBO J* 31, 2309-2321
49. Badie, S., Escandell, J. M., Bouwman, P., Carlos, A. R., Thanasoula, M., Gallardo, M. M., Suram, A., Jaco, I., Benitez, J., Herbig, U., Blasco, M. A., Jonkers, J., and Tarsounas, M. (2010) BRCA2 acts as a RAD51 loader to facilitate telomere replication and capping. *Nat Struct Mol Biol* 17, 1461-1469
50. Otterlei, M., Warbrick, E., Nagelhus, T. A., Haug, T., Slupphaug, G., Akbari, M., Aas, P. A., Steinsbekk, K., Bakke, O., and Krokan, H. E. (1999) Post-replicative base excision repair in replication foci. *EMBO J* 18, 3834-3844
51. Imai, K., Slupphaug, G., Lee, W.-I., Revy, P., Nonoyama, S., Catalan, N., Yel, L., Forveille, M., Kavli, B., Krokan, H. E., Ochs, H. D., Fischer, A., and Durandy, A. (2003) Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. *Nat Immunol* 4, 1023-1028
52. Nilsestuen, L. O., Stamp, G., Andersen, S., Hrivnak, G., Krokan, H. E., Lindahl, T., and Barnes, D. E. (2003) Gene-targeted mice lacking the Ung uracil-DNA glycosylase develop B-cell lymphomas. *Oncogene* 22, 5381-5386
53. Drosopoulos, W. C., and Prasad, V. R. (2007) The active site residue Valine 867 in human telomerase reverse transcriptase influences nucleotide incorporation and fidelity. *Nucleic Acids Res* **35**, 1155-1168

54. Zheng, Y. L., Zhang, F., Sun, B., Du, J., Sun, C., Yuan, J., Wang, Y., Tao, L., Kota, K., Liu, X., Schlegel, R., and Yang, Q. (2014) Telomerase enzymatic component hTERT shortens long telomeres in human cells. *Cell Cycle* **13**, 1765-1776

55. Taboski, M. A., Sealey, D. C., Dorrens, J., Tayade, C., Betts, D. H., and Harrington, L. (2012) Long telomeres bypass the requirement for telomere maintenance in human tumorigenesis. *Cell Rep* **1**, 91-98

56. Ramsay, A. J., Quesada, V., Foronda, M., Conde, L., Martinez-Trillos, A., Villamor, N., Rodriguez, D., Kwarcia, A., Garabaya, C., Gallardo, M., Lopez-Guerra, M., Lopez-Guillermo, A., Puente, X. S., Blasco, M. A., Campo, E., and Lopez-Otin, C. (2013) POT1 mutations cause telomere dysfunction in chronic lymphocytic leukemia. *Nat Genet* **45**, 526-530

57. Robles-Espinoza, C. D., Harland, M., Ramsay, A. J., Aoude, L. G., Quesada, V., Ding, Z., Pooley, K. A., Pritchard, A. L., Tiffen, J. C., Petljak, M., Palmer, J. M., Symmons, J., Johansson, P., Stark, M. S., Gartside, M. G., Snowden, H., Montgomery, G. W., Martin, N. G., Liu, J. Z., Choi, J., Makowski, M., Brown, K. M., Dunning, A. M., Keane, T. M., Lopez-Otin, C., Gruis, N. A., Hayward, N. K., Bishop, D. T., Newton-Bishop, J. A., and Adams, D. J. (2014) POT1 loss-of-function variants predispose to familial melanoma. *Nat Genet* **46**, 478-481

58. Seow, W. J., Cawthon, R. M., Purdie, M. P., Hu, W., Gao, Y. T., Huang, W. Y., Weinstein, S. J., Ji, B. T., Virtamo, J., Hosgood, H. D., 3rd, Bassig, B. A., Shu, X. O., Cai, Q., Xiang, Y. B., Min, S., Chow, W. H., Berndt, S. I., Kim, C., Lim, U., Albanes, D., Caporaso, N. E., Chanock, S., Zheng, W., Rothman, N., and Lan, Q. (2014) Telomere length in white blood cell DNA and lung cancer: a pooled analysis of three prospective cohorts. *Cancer Res* **74**, 4090-4098

59. Shi, J., Yang, X. R., Ballew, B., Rotunno, M., Calista, D., Fargnoli, M. C., Ghiorzo, P., Bressac-de Paillerets, B., Nagore, E., Avril, M. F., Caporaso, N. E., McMaster, M. L., Cullen, M., Wang, Z., Zhang, X., Group, N. D. C. S. W., Laboratory, N. D. C. G. R., French Familial Melanoma Study, G., Bruno, W., Pastorino, L., Queirolo, P., Banuls-Roca, J., Garcia-Casado, Z., Vaysse, A., Mohamdi, H., Riazalhosseini, Y., Foglio, M., Jouenne, F., Hua, X., Hyland, P. L., Yin, J., Vallabhaneni, H., Chai, W., Minghetti, P., Pellegrini, C., Ravichandran, S., Eggermont, A., Lathrop, M., Peris, K., Scarra, G. B., Landi, G., Savage, S. A., Sampson, J. N., He, J., Yeager, M., Goldin, L. R., Demenais, F., Chanock, S. J., Tucker, M. A., Goldstein, A. M., Liu, Y., and Landi, M. T. (2014) Rare missense variants in POT1 predispose to familial cutaneous malignant melanoma. *Nat Genet* **46**, 482-486

60. Loayza, D., and De Lange, T. (2003) POT1 as a terminal transducer of TRF1 telomere length control. *Nature* **423**, 1013-1018

61. Opresko, P. L., Fan, J., Danzy, S., Wilson, D. M., 3rd, and Bohr, V. A. (2005) Oxidative damage in telomeric DNA disruptions recognized by TRF1 and TRF2. *Nucleic Acids Res* **33**, 1230-1239

62. Court, R., Chapman, L., Fairall, L., and Rhodes, D. (2005) How the human telomeric proteins TRF1 and TRF2 recognize telomeric DNA: a view from high-resolution crystal structures. *EMBO Rep* **6**, 39-45
63. Lei, M., Podell, E. R., Baumann, P., and Cech, T. R. (2003) DNA self-recognition in the structure of Pot1 bound to telomeric single-stranded DNA. Nature 426, 198-203
64. Wu, L., Multani, A. S., He, H., Cosme-Blanco, W., Deng, Y., Deng, J. M., Bachilo, O., Pathak, S., Tahara, H., Bailey, S. M., Deng, Y., Behringer, R. R., and Chang, S. (2006) Pot1 deficiency initiates DNA damage checkpoint activation and aberrant homologous recombination at telomeres. Cell 126, 49-62
65. Marcand, S., Brevet, V., Mann, C., and Gilson, E. (2000) Cell cycle restriction of telomere elongation. Curr Biol 10, 487-490
66. Zhao, Y., Sfeir, A. J., Zou, Y., Buseman, C. M., Chow, T. T., Shay, J. W., and Wright, W. E. (2009) Telomere Extension Occurs at Most Chromosome Ends and Is Uncoupled from Fill-In in Human Cancer Cells. Cell 138, 463-475
67. Kavli, B., Sundheim, O., Akbari, M., Otterlei, M., Nilsen, H., Skorpen, F., Aas, P. A., Hagen, L., Krokan, H. E., and Slupphaug, G. (2002) HUNG2 is the major repair enzyme for removal of uracil from U : A matches, U : G mismatches, and U in single-stranded DNA, with hSMUG1 as a broad specificity backup. J Biol Chem 277, 39926-39936
68. Maynard, S., Schurman, S. H., Harboe, C., de Souza-Pinto, N. C., and Bohr, V. A. (2009) Base excision repair of oxidative DNA damage and association with cancer and aging. Carcinogenesis 30, 2-10

FOOTNOTES

H. Vallabhaneni and F. Zhou contributed equally to this work

We sincerely thank Drs. Vilhelm Bohr, David Wilson, Robert Brosh, Michael Seidman, Samuel Wilson, Roger Woodgate, and Hilde Nilsen for constructive suggestions during this study. The work was supported entirely by the Intramural Research Program of the National Institutes of Health, National Institute on Aging. The authors declare no competing financial interests.

The abbreviations used are: UNG, uracil DNA glycosylase; EMSA, electrophoretic mobility shift assay; TRAP, telomeric repeat amplification protocol; CO-FISH, chromosome orientation fluorescence in situ hybridization; TERT, telomerase reverse transcriptase; T-SCE, telomere sister chromatid exchange; ss, single-stranded.

FIGURE LEGENDS

FIGURE 1. Affinity of POT1 to uracil-containing telomere substrates. A, representative EMSA showing the affinity of purified human POT1/TPP1 to telomere oligonucleotides with or without uracil. The oligonucleotide GGT1T2AGGGT3T4AG (underlined is the POT1 binding sequence; number indicates the position of thymine in the oligonucleotide) was 32P-labeled, incubated with purified POT1/TPP1 in the absence (lane 1) or presence of increasing concentrations (5-fold molar excess) of various unlabeled competing oligonucleotides [shown at the top; thymine at T1, T2 and/ or T3, T4 positions was replaced by uracil (red)]. B, the experiment was conducted as in (A) except for the oligonucleotide GGTTAGGGTTAGGGT5T6, with thymine at T5 and T6 positions replaced by uracil. C, the experiment was conducted as in (A) except that the lysates containing in vitro translated mouse POT1a and POT1b were used.
Corresponding graphs show the fraction of POT1-bound $^{32}$P-labeled oligonucleotides plotted against fold change of molar excess of competing oligonucleotides. POT1-bound $^{32}$P-labeled oligonucleotide was calculated as a fraction of total radioactivity in each lane. Error bars: standard deviation (SD) from three independent experiments.

FIGURE 2. **Affinity of TRF1 and TRF2 to uracil-containing telomere duplex substrates.** A, sequence of the unmodified and uracil-containing oligonucleotides used in the EMSA assay (the complementary strand is not shown). The unmodified oligonucleotide was $^{32}$P-labeled, incubated with purified TRF1 (B) or TRF2 (C) in the presence of increasing amounts (5-fold molar excess) of unlabeled competing oligonucleotides with or without uracil. Free and TRF1- or TRF2-bound $^{32}$P-labeled unmodified oligonucleotides are indicated. Corresponding graphs show the fraction of TRF1- or TRF2-bound $^{32}$P-labeled oligonucleotide plotted against fold change of molar excess of competing oligonucleotides. TRF1- or TRF2-bound $^{32}$P-labeled oligonucleotide was calculated as a fraction of total radioactivity in each lane. SD from three independent experiments.

FIGURE 3. **Utilization of deoxyuracil-substituted oligonucleotide substrates by telomerase in vitro.** A, the ss substrate 5'-AGGG TTAGGGTTAGGGTT-3' (TT) or dUTP substitutions at the 1$^{st}$, 2$^{nd}$, or 3$^{rd}$ d(TT) positions (UU1, UU2, UU3, corresponding to each underlined TT in the primer sequence above), or B, the substrate 5'-AATCCGTCGAGCAGAGTT-3' (GTT) or dUTP substitutions within the same primer sequence at the penultimate dTTP (GUT), 3' terminal dTTP (GTU) or both terminal dTTPs (GUU), were assayed using recombinant human telomerase purified from rabbit reticulocyte lysate (17). Inverted triangles indicate incubation with 50, 100 and 200 pmol primer, respectively. RN: pretreatment with RNase A prior to addition of telomerase extract. 3'-TT or 3'-UU3 indicates primers 3'-end labeled with $^{32}$P-dGTP and terminal deoxynucleotidyl transferase for 2 minutes (to generate a radiolabeled product at n+1). GG-1, GG-2, GG-3 indicate the $^{32}$P-labelled dGTP residues in each telomeric repeat added by telomerase. C, upper panel, primer extension of TT, UU3, GTT or GUU (200 pmol, n=3); middle panel, loading control $^{32}$P-d(TTAGGG)$_2$; lower panel, quantification of average signal intensity of each repeat (GG-1, GG-2, GG-3, GG-4, GG-5, and GG-6, or the sum of repeats GG-1 to GG-6), normalized to the average signal intensity of the control primer $^{32}$P-d(TTAGGG)$_2$. $^{32}$P-TT, 5'-endlabeled TT primer (the position of the primer is shown, and the lane containing this image was cropped). Error bars indicate SD of at least three independent experiments. Statistical analysis failed to establish a significant difference among all the comparisons (see Methods).

FIGURE 4. **Detection of uracil at telomeres in Ung$^{+/+}$ and Ung$^{-/-}$ bone marrow cells.** PCR amplification efficiency at the telomeric (A) and Gapdh (B) loci. Mock- or UNG-treated genomic DNA from Ung$^{+/+}$ and Ung$^{-/-}$ cells was nicked by APE1 in vitro. Quantitative PCR was carried out using telomere- or Gapdh- specific primers. PCR amplification efficiency in the mock-treated samples was normalized to input DNA. Error bars indicate SD of 5 independent experiments. P-values were calculated using a Student’s t-test. a.u. arbitrary units.

FIGURE 5. **Telomere length in primary Ung$^{+/+}$ and Ung$^{-/-}$ bone marrow cells by Q-FISH analysis.** A, representative metaphase spreads of Ung$^{+/+}$ and Ung$^{-/-}$ cells showing DAPI staining (blue) and telomere fluorescence signals (red) by telomere-FISH using a Cy3-labeled telomere probe. Note the greater intensity of telomere staining in Ung$^{-/-}$ cells. B, representative jitter plots
showing complete distribution of telomere signals from individual \( Ung^{+/+} \) and \( Ung^{-/-} \) littermates. Two individual mice of each genotype are shown; a total of 6 mice per genotype were examined. C, a representative histogram from \( Ung^{+/+} \) and \( Ung^{-/-} \) mice showing relative frequency of telomeres with different signal intensities. D, representative jitter plots of telomere signals from \( Ung^{+/+} Tert^{-/-} \) and \( Ung^{-/-} Tert^{-/-} \) littermates. Two individual mice of each genotype are shown; a total of 6 mice per genotype were examined. E, a representative histogram from \( Ung^{+/+} Tert^{-/-} \) and \( Ung^{-/-} Tert^{-/-} \) mice showing relative frequency of telomeres with different signal intensities. Yellow bars represent mean telomere signal intensity. P-values were calculated using a Student’s \( t \)-test. a.u. arbitrary units.

FIGURE 6. T-SCEs and fragile telomeres in \( Ung^{+/+} \) and \( Ung^{-/-} \) primary bone marrow cells. A, an example of a metaphase showing T-SCE by CO-FISH. The arrow and inset show telomeres labeled with G and C probes to distinguish both strands. B and C, quantitation of T-SCEs in mouse cells with indicated genotypes. D, examples of fragile telomeres with arrows and inset depicting multiple signals at a telomere chromatid by telomere-FISH using a Cy3-labeled telomere probe. E-F, quantitation of fragile telomeres in mouse cells with indicated genotypes. A total of 3 mice per genotype were examined for each condition. At least 50 metaphases per sample were counted. Error bars indicate SD. P-values were calculated using a Student’s \( t \)-test.
Fig. 1

A) Competing oligo

B) 5'GGTTAGGGTTAGGGT$_{6}$' 5'GGTTAGGGTTAGGG

C) 5'GGT$_{2}$AGG$_{2}$T$_{4}$AG3' 5'GGUAGGGUAG3'

Fold 0 5 25 125 625 3125

Fraction of labeled oligo bound

Fold of competing cold oligo

POT1/TPP1-bound oligo

Free oligo

POT1a-bound oligo

Free oligo

POT1b-bound oligo

Free oligo

Fold 0 5 25 125 625 3125

Fraction of labeled oligo bound

Fold of competing cold oligo

POT1a-WT cold

Pot1a-U mut cold

Fold 0 5 25 125 625 3125

Fraction of labeled oligo bound

Fold of competing cold oligo

Pot1b-WT cold

Pot1b-U mut cold
A

| Competing oligo | Unmodified | Modified |
|-----------------|------------|----------|
| 5'- GTGGATCCGTACTTAGGTTAGGGACACGAATTCTGA-3' | 5'- GTGGATCCGTACUUAGGGUGAGGACACGAATTCTGA-3' |

B

| Fold | Unmodified | Modified |
|------|------------|----------|
| 0    |            |          |
| 5    |            |          |
| 25   |            |          |
| 125  |            |          |
| 625  |            |          |
| 3125 |            |          |

C

| TRF2-bound oligo | Free oligo |
|------------------|------------|
| TRF2-bound oligo | Free oligo |

Fig. 2

A. Unmodified and modified oligo sequences.

B. Graph showing the fraction of labeled oligo bound at different folds of competing cold oligo.

C. Graph showing the fraction of labeled oligo bound at different folds of competing cold oligo.
Fig. 4

A. Telomere

- **Mock**
- **Treated**

Relative amplification (a.u.)

- **Ung**
- **Ung**

|                | Ung+/+ | Ung-/- |
|----------------|--------|--------|
| **Mock**       | [1.00] | [0.98] |
| **Treated**    | [1.02] | [0.96] |

P-values:
- P = 0.009
- P = 0.002
- P = 0.0006

B. Gapdh

- **Mock**
- **Treated**

Relative amplification (a.u.)

|                | Ung+/+ | Ung-/- |
|----------------|--------|--------|
| **Mock**       | [1.00] | [1.00] |
| **Treated**    | [1.00] | [1.00] |

P-value:
- P > 0.05
Fig. 5

A. DAPI + telomere

B. Ung\textsuperscript{+/+} vs. Ung\textsuperscript{--}.

C. Ung\textsuperscript{+/+} vs. Ung\textsuperscript{--}.

D. Ung\textsuperscript{+/+} vs. Ung\textsuperscript{--}/Tert\textsuperscript{--}.

P<0.0001
Defective Repair of Uracil Causes Telomere Defects in Mouse Hematopoietic Cells
Haritha Vallabhaneni, Fang Zhou, Robert W. Maul, Jaya Sarkar, Jinhu Yin, Ming Lei, Lea Harrington, Patricia J. Gearhart and Yie Liu

J. Biol. Chem. published online January 8, 2015

Access the most updated version of this article at doi: 10.1074/jbc.M114.607101

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts