Published in final edited form as:

DNA Repair (Amst). 2011 January 2; 10(1): 34–44. doi:10.1016/j.dnarep.2010.09.008.

Factors that influence telomeric oxidative base damage and repair by DNA glycosylase OGG1

David B. Rhee, Avik Ghosh, Jian Lu, Vilhelm A. Bohr, and Yie Liu*

Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, Maryland, United States

Abstract

Telomeres are nucleoprotein complexes at the ends of linear chromosomes in eukaryotes, and are essential in preventing chromosome termini from being recognized as broken DNA ends. Telomere shortening has been linked to cellular senescence and human aging, with oxidative stress as a major contributing factor. 7, 8-dihydro-8-oxoguanine (8-oxodG) is one of the most abundant oxidative guanine lesions, and 8-oxoguanine DNA Glycosylase (OGG1) is involved in its removal. In this study, we examined if telomeric DNA is particularly susceptible to oxidative base damage and if telomere-specific factors affect the incision of oxidized guanines by OGG1. We demonstrated that telomeric TTAGGG repeats were more prone to oxidative base damage and repaired less efficiently than non-telomeric TG repeats *in vivo*. We also showed that the 8-oxodG-incision activity of OGG1 is similar in telomeric and non-telomeric double-stranded substrates. In addition, telomere repeat binding factors TRF1 and TRF2 do not impair OGG1 incision activity. Yet, 8-oxodG in some telomere structures (*e.g.*, fork-opening, 3'-overhang, and D-loop) were less effectively excised by OGG1, depending upon its position in these substrates. Collectively, our data indicate that the sequence context of telomere repeats and certain telomere configurations may contribute to telomere vulnerability to oxidative DNA damage processing.

1. Introduction

All eukaryotic linear chromosomes consist of nucleoprotein complexes called telomeres. Telomeres are composed of extended tracts of short G-rich tandem repeat sequences, 5'-TTAGGG-3', and a short terminal single-stranded 3'-overhang in humans and mice. Functional telomeres prevent chromosome termini from being recognized as broken DNA ends. On the other hand, dysfunctional telomeres, as a consequence of loss of telomere repeats or loss of protection by telomere-associated proteins, can trigger a DNA damage response and subsequently lead to genomic instability, cell proliferation defects, and cell death [1].

All functional telomeres are coated with telomere-specific binding proteins (*e.g.*, TRF1 and TRF2) and their associated proteins, collectively known as the shelterin protein complex [2].

© Published by Elsevier B.V.

*Address correspondence to: Yie Liu, 251 Bayview Drive, Suite 100, Room 06B121, Baltimore, MD 21224, United States. Phone: +1-410-558-8419. Fax: +1-410-558-8157. liuyie@mail.nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest statement

The authors declare that there are no conflicts of interest.

With the aid of shelterin proteins, telomeres normally exist in a loop structure (also known as T-loop) with a 3'-single-stranded overhang invading telomeric double-stranded DNA [3]. Disruption of T-loop and exposure of the 3'-overhang destabilize telomeres since capped telomeres normally inhibit undesirable DNA recombination and damage response [4,5]. Evidence indicates that shelterin proteins also regulate telomerase extension and efficient telomere replication [6-10].

Genomic DNA is continuously exposed to DNA damaging agents through both endogenous (e.g., reactive oxygen species, ROS, as by-products of normal metabolism) and exogenous (e.g., physical and chemical agents such as γ - or UVA-irradiation) means. ROS can generate oxidative DNA lesions including oxidized bases and single-strand-breaks (SSBs) [11]. 7, 8-dihydro-8-oxoguanine (8-oxodG) is one of the most abundant and widely studied oxidative base lesions. The primary repair pathway responsible for removing such oxidative lesions is the base excision repair (BER) pathway. Briefly, 8-oxoguanine DNA Glycosylase (OGG1) initiates the BER pathway by removing the damaged guanine base, followed by incision, repair synthesis, and ligation by various members in the BER pathway to complete the repair process [12-14]. OGG1 glycosylase activity on 8-oxodG modified substrates has been extensively studied. For instance, OGG1 can remove 8-oxodG opposite of cytosine efficiently [15]. OGG1's incision is affected by flanking sequences with preference for 5'-(C/G)me-FapyC-3' [16], opposing abasic sites or SSBs towards the 3' of the catalytic pocket [17-19], and the position of 8-oxodG in fork substrates [19].

Telomere DNA contains runs of triple guanine-containing repeats that are unique with respect to oxidative reactivity [20]. In addition, telomeric DNA shows a preferential binding to Fe^{2+} , resulting in local structure rearrangements [21]. These factors may contribute to oxidative DNA damage in telomeres. Indeed, oxidative stress has been shown to induce oxidative DNA guanine damage in telomeric DNA *in vitro* and *in vivo* [22-27]. The consequence of oxidative guanine DNA damage has been investigated previously. The presence of 8-oxodG in telomeric nucleotides disrupts telomerase activity [28] and also inhibits the binding of TRF1 and TRF2 [29] *in vitro*. Our recent studies imply that oxidative base damage affects telomere length homeostasis, recombination, and DNA breakage repair. We also demonstrated that OGG1 is required for repairing oxidized bases in telomeres in budding yeast and mice [26,27]. However, it is unclear if telomere DNA is more prone to oxidative base damage when compared to non-telomere DNA *in vivo*.

Several mechanisms may contribute to a steady state level of oxidative base lesions in telomeres. It is possible that telomeric DNA repeats may favor base oxidation. In addition, oxidative base damage and repair may be influenced by telomere-specific factors (e.g., telomere special structures or telomere repeat binding proteins). In this study, we examined if telomeric DNA repeats are more prone to oxidative base damage *in vivo*, if oxidative base damage is less efficiently repaired in telomeres, and if telomere-specific factors can affect OGG1 base incision activity in telomeres. Our data suggest that triplet guanines in telomeric repeats contribute to the preferential accumulation of oxidative base damage in telomeres and the repair of oxidative base lesions in some telomere configurations can be problematic.

2. Materials and methods

2.1. Cell culture, induction of oxidative DNA damage, and genomic DNA isolation

U2OS osteosarcoma cells were maintained in DMEM (Dulbecco's modified Eagle's Medium) growth medium supplemented with 10% fetal bovine serum (FBS) at 37°C in a 5% CO_2 atmosphere. To induce oxidative DNA damage *in vivo*, cells were treated with 1 mM of menadione (Sigma Aldrich) in serum-free DMEM growth medium for 30 min, washed with PBS and incubated for additional 6 hours in serum-free DMEM growth

medium before being collected [30]. DNA isolation was performed as previously described [26,27,31]. Briefly, freshly minced mouse kidney (C57BL/6 genetic background) or U2OS cell pellets were incubated with DNA lysis buffer (10 mM Tris, pH 7.5, 400 mM NaCl, 1 mM EDTA, 0.5 % SDS, and 0.1mg/ml proteinase-K) overnight at 37°C. DNA was isolated using standard high salt extraction (6M NaCl with vigorous shaking, followed by ethanol precipitation), treated with 100 µg/ml of RNase A (for 3 hours, followed by ethanol precipitation), and re-suspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.2). For *in vitro* oxidative DNA damage induction, isolated mouse kidney DNA was treated with various concentrations of H₂O₂ and Cu²⁺, followed by ethanol precipitation.

2.2. Measurement of oxidative base lesions

Identification of oxidative base lesions in DNA was performed as described with minor modifications [31]. In brief, 2 µg of DNA was treated with 10 units each of *Hind*III and *Rsa*I restriction enzymes (New England Biolabs) at 37°C for 3 hours. Enzymes were inactivated by heating at 60°C for 20 minutes. Samples were treated with 8 units of *E. coli* formamidopyrimidine-DNA glycosylase (Fpg) (New England Biolabs) at 37°C for 1 hour. Duplicate samples were treated with a mock buffer. Fpg was inactivated by heating at 60°C for 15 minutes. Genomic single-stranded DNA fragments treated with Fpg or mock buffer were separated on 0.8 % alkaline agarose gel and detected by Southern blot using ³²P-labeled (AATCCC)₄ or (CA)₁₂ probes and visualized by autoradiography. ImageQuant software was applied in quantifying DNA cleavage in Fpg-treated and untreated samples. The mean length (ML) was calculated as a center of mass and expressed in kilobase. $ML = \sum (MW_i \times ODi) / \sum (ODi)$. MW_i is the length of the DNA at each row, and ODi is the densitometer output at each row. The frequency of Fpg-sensitive lesions/10⁶ was calculated as $(ML^{-Fpg} / ML^{+Fpg} - 1) / ML^{-Fpg} \times 10^6$.

2.3. Enzyme and purified proteins

Recombinant human 8-oxoguanine DNA glycosylase, OGG1 (α isoform, New England Biolabs) was used in the oligonucleotide incision assay. Recombinant histidine-tagged human TRF1 and TRF2 proteins were purified using a baculovirus/insect cell expression system as described previously [29].

2.4. Oligonucleotides

Oligonucleotides used in this study are shown in Table S1 (Midland Certified, Midland TX). To construct duplex substrates, 8-oxoguanine modified oligonucleotides were first 5' end labeled using [γ -³²P] ATP (3000 Ci/mmol) with T4 polynucleotide kinase (New England Biolabs) and annealed to the template oligonucleotides in a 1:2 molar ratio. Alternatively, 8-oxoguanine modified oligonucleotides were 3' end labeled using [α -³²P] ddATP (3000 Ci/mmol) with terminal deoxynucleotidyl transferase (New England Biolabs) and annealed to the template oligonucleotides in a 1:2 molar ratio. Annealing reactions were incubated at 95°C for 5 minutes and cooled to room temperature for 30 minutes. The D-loop substrate was prepared as described [32]. Briefly, labeling the 5' end of oligonucleotides (C3/C4) was conducted in the presence of [γ -³²P] ATP (3000 Ci/mmol) and T4 polynucleotide kinase. Labeled oligonucleotides (C3/C4) were incubated with the complementary strand (C1) at 95°C for 5 minutes and cooled stepwise (1.2°C/min) to 60°C, followed by addition of oligonucleotides (C2) and incubated at 60°C for 1 hour, which was cooled stepwise (1.2°C/min) to 25°C.

2.5. Incision Assays

OGG1 incision assays were performed in a reaction mixture (10 µl) containing 1x NEB #2 buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl₂, 1 mM DTT and 100 µg/ml BSA).

The reactions were incubated at 37°C for 15 min and terminated by addition of equal volume of 2x formamide loading buffer (95% (v/v) formamide, 20 mM EDTA, 0.1% (w/v) bromophenol blue and xylene cyanol). Alternatively, reactions were incubated at 37°C for the time points indicated and terminated by adding equal volume of 2x formamide loading buffer. Substrates were allowed to denature at 95°C for 10 min, followed by incubation in ice for 2 min. Samples were resolved by electrophoresis on a 15% Polyacrylamide/7 M urea gel (National Diagnostic Inc.). Gels were visualized by Phosphor-Imager and analyzed using the ImageQuant™ software (GE Bioscience). The percentage of incision was calculated as the amount of radioactivity present in the product band relative to the total radioactivity. The incision rates were examined primarily among the substrates under conditions where less than 30% of the substrates have been incised. For shelterin protein's effect on hOGG1's incision activity, incision assays were conducted in a reaction mixture (10 µl) containing 1x Tel buffer (20 mM HEPES, pH 7.9, 150 mM KCl, 1 mM MgCl₂, 0.1 mM EDTA, 0.5 mM DTT, 5% glycerol, 0.1% NP-40 and 100 µg/ml BSA). Purified TRF1 was first added to the reaction mixture and incubated at room temperature for 20 minutes, followed by addition of hOGG1 and further incubation at 37°C for 15 minutes. 500 µg/µl of tRNA (Roche) was added to each reaction prior to adding TRF1 to neutralize the non-specific exonuclease activity.

3. Results

3.1. Differential oxidative base damage and repair in telomeric and non-telomeric regions

Evidence indicates that oxidative stress induces oxidative guanine damage preferentially in short oligonucleotides with telomere repeats *in vitro* [22-25]. However, telomere DNA is masked in the telomere-specific structures and coated by the telomere-specific proteins. These telomere-specific factors may hinder or facilitate oxidative base damage as well as base repair in telomeres, which can affect the steady-state level of oxidative base lesions in telomeres *in vivo*. It is thus unclear if telomeric DNA is indeed prone to oxidative base damage *in vivo*. We examined and compared the level of base oxidation in telomeric (TTAGGG) and minisatellite (TG) regions of genomic DNA isolated from mouse kidney by measuring Fpg-sensitive sites. Fpg excises oxidative purine lesions such as 8-oxodG and resulting abasic sites are further processed to single-strand-breaks via AP-lyase activity of Fpg [33]. The extent of DNA fragmentation caused by Fpg reflects the frequency of oxidative base lesions, which appear as smaller molecular weight fragments after Fpg treatment (Figure S1, left panel). The mean values of DNA fragments in Fpg-treated (+) and mock-treated (-) samples were applied to estimate the frequencies of oxidative base lesions in telomeric and TG minisatellite regions [31] (Figure S2, top panel). In mouse kidney DNA, more Fpg-sensitive sites were found in telomeric than in minisatellite DNA (approximately 45.36 ± 7.4 and 20.76 ± 1.36 sites/10⁶ bp in telomeres and minisatellites, respectively) (Table 1). Similarly, a human cell line, U2OS, displayed more Fpg-sensitive lesions in telomeric TTAGGG repeats compared to TG minisatellites (approximately 5.39 ± 0.67 and 3.76 ± 1.11 sites/10⁶ bp in telomeres and minisatellites, respectively) (Figure S1, right panel, Figure S3, top panel, Table 2). Although both types of repeats harbor similar contents of guanines (50%), the fact that more oxidative base lesions were detected in telomeric DNA suggests that either triple guanines of telomeric DNA repeats or simply the repeat sequences themselves are prone to oxidation. In addition, more base damages in telomeres could reflect that OGG1-initiated BER repair may be less effective in telomeres.

To examine if telomeric DNA repeat sequences themselves are more sensitive to oxidative stress, mouse kidney DNA was treated with increasing amounts of H₂O₂ and Cu²⁺ *in vitro* [26] and the level of oxidized bases (*i.e.*, Fpg-sensitive sites) was examined for both telomeric and minisatellite regions of genomic DNA. Higher doses of H₂O₂ and Cu²⁺ treatment caused Fpg-dependent increase of shorter telomere DNA fragments, whereas TG

minisatellite DNA fragments displayed a moderate shortening under the same treatment conditions (Figure S1, left panel, Figure S2, middle and lower panels). Accordingly, H₂O₂ and Cu²⁺ treatment increased the frequencies of Fpg-sensitive sites in telomeric DNA fragments significantly more than in TG minisatellite DNA fragments (approximately 303.04 ± 53.41 and 81.57 ± 11.88 sites/ 10^6 bp at high exposure in telomeres and TG minisatellites, respectively) (Table 1). Because this test was performed on naked DNA without the presence of other factors (e.g. TRF1 or TRF2), this observation suggests that telomere repeat sequences (possibly triple guanines or telomeric DNA repeats as a whole) can contribute to preferential base damage in telomeric DNA.

To determine if there was differential oxidative base damage repair in telomeric DNA and TG minisatellites, U2OS cells were treated briefly with 1 mM of menadione *in vivo* to induce high incidences of oxidative DNA damage and allowed to recover for 6 hours according to a previously described protocol [30]. The level of oxidative DNA lesions was examined for both telomeric and TG minisatellite regions. The starting frequencies of Fpg-sensitive sites in telomeric DNA fragments and TG minisatellites were comparable immediately after the menadione treatment (approximately 7.72 ± 1.75 and 9.56 ± 6.22 respectively). However, after 6 hours of recovery time, the Fpg-sensitive sites were undetectable in TG minisatellites while even higher frequencies of Fpg-sensitive sites were detected in telomeric DNA fragments (not detected and approximately 14.25 ± 1.21 sites/ 10^6 bp in minisatellites and telomeres, respectively) (Figure S1, right panel, Figure S3, middle and lower panels, Table 2). These data suggest that OGG1-initiated BER may be less effective in telomeres *in vivo* and oxidative DNA damage can therefore accumulate in telomeres over time.

3.2. The incision activity of α -hOGG1 is comparable in telomeric and non-telomeric double-stranded substrates

Telomere special factors (e.g., telomeric DNA repeat sequences, telomere special structures, or telomere binding proteins) may affect the repair of oxidative base damage in telomeres and contribute to differential repair of oxidative base damage in telomeric and non-telomeric regions. To examine if triple guanines of telomeric DNA repeats or repeat sequences themselves affect oxidative base repair, we employed an *in vitro* α -hOGG1-incision assay. The α -hOGG1 enzyme is primarily responsible for removing 8-oxodG in the nucleus of human cells and its incision activity was examined on 8-oxodG containing double-stranded oligonucleotide substrates, with telomeric or non-telomeric DNA repeats harboring either one or triple guanines. Specifically, in telomeric-duplex-1 (TD1), the middle guanine of one of the telomere repeats was modified with 8-oxoguanine, whereas in non-telomeric-duplex-1 (NTD1) and non-telomeric-duplex-2 (NTD2), 8-oxoguanine modification was surrounded by non-telomeric sequences (Table S1, Figure 1). Notably, NTD1 had two guanines flanking the 8-oxodG to mimic triple guanines of telomeric DNA, whereas NTD2 was flanked by cytosine and adenine to mimic single 8-oxodG condition. These duplexes (0.5 nM) were incubated with serial dilutions (16, 32, 64, 128, or 256 nM) of α -hOGG1 and the percentage of incision product was measured and compared between telomeric and non-telomeric DNA repeats containing oligonucleotide substrates. 16 nM (lowest concentration of enzyme) of α -hOGG1 was able to efficiently excise the substrates and the percentages of excised products increased with higher enzyme concentrations (Figure 2). The percentage of excised products from telomeric duplex TD1 (Lanes 1-6) were equivalent to non-telomeric duplex NTD1 (Lanes 7-12), which suggests that α -hOGG1 can excise 8-oxodG from a triple guanine repeat (G8G, 8 refers to 8-oxodG) of a non-telomeric or telomeric duplex with similar efficiency. Moreover, the percentage of excised products was comparable between telomeric substrate TD1 and non-telomeric substrate (NTD2) with a single guanine (Lanes 13-18).

Taken together, these studies suggest that triple guanines of telomeric DNA repeats and repeat sequences themselves do not impact OGG1 incision activity.

3.3. α -hOGG1 is able to excise more than one 8-oxodG base lesion in telomeric triplet guanines

Our data suggest that a single 8-oxodG in telomeric substrates can be excised efficiently by α -hOGG1 *in vitro*. As telomeric DNA has GGG tracts separated by TTA bridges, hole migration through this strand could lead to damage at multiple guanines of a triple G section [24,25,34]. It is unclear whether multiple 8-oxodG base lesions in telomeric DNA substrates affect α -hOGG1 incision. The incision activity of α -hOGG1 was examined on telomeric double-stranded oligonucleotide substrates with either single or triple 8-oxodG base modifications. Specifically, all three guanines in one of the telomeric repeats was replaced by 8-oxodG in telomeric-duplex-2 (TD2) as opposed to one guanine in telomeric duplex TD1 (Table S1, Figure 1). α -hOGG1 was incubated with 0.5 nM of TD1 or TD2 duplexes in serial dilutions (16, 32, 64, 128, or 256 nM) and the percentage of incision product was measured between the two substrates. Our data indicate that the percentage of incision products was comparable between single and triple 8-oxodG containing substrates (Figure 3), which suggest that the number of 8-oxodG within GGG of telomeric DNA repeats do not impact α -hOGG1 incision activity *in vitro*.

Interestingly, the incision products of the triple 8-oxodG containing substrate TD2 appeared more diffuse compared to that of the single 8-oxodG substrate (Figure 3, lanes 6 and 12). This suggests that more than one of triple 8-oxodG was being excised, but it was unclear if there was a preferential incision of 8-oxodG at different positions of the telomeric triple G repeat. Previous studies indicate that guanines in a telomeric DNA substrate are not equally susceptible to oxidative damage and that oxidative stress can induce base damage preferentially at 5' or at the middle G in GGG of telomeric DNA repeats [24,25]. Thus, we examined if there was preferential incision of 8-oxodG at the 5', the middle or the 3' position in GGG of telomeric DNA repeats by α -hOGG1. To better visualize the incision products, the TD2 substrate was either 5' labeled with γ - ^{32}P -dATP or 3' labeled with α - ^{32}P -ddATP. To observe incision of 8-oxodG in telomeric GGG, the TD2 substrate was incubated with a fixed amount (8 nM) of α -hOGG1 enzyme. The reactions were stopped and collected at different time points (15 sec, 30 sec, 1 min, 2 min, 4 min, and 8 min) and the incision products were separated to obtain a 1 bp difference. Three individual excised products were observed in 5'- and 3'-labeled substrates at the indicated positions (Figure 4, A and B). With 5'-end labeling, primarily the middle 8-oxodG and secondarily the 8-oxodG on the 5'-site were excised efficiently; however, fewer 8-oxodG at the 3'-site was removed (Figure 4C). Similar results were observed with the 3' labeled substrate (Figure 4D). These incision products likely represent the first excision of 8-oxoG from the 8-oxoG triplet, since 8-oxoG flanked by an abasic site can be poorly excised by OGG1 [17-19]. Taken together, these results suggest that there is preferential incision of 8-oxodG by α -hOGG1 at different positions in the telomeric DNA repeat.

3.4. An 8-oxodG near ssDNA/dsDNA junction reduces α -hOGG1 incision activity

Mammalian telomeres have unique structural features such as the 3'-overhang. The single-stranded 3'-overhang of the G-strand displaces the upstream G-strand and then base pairs with the C-strand creating a displacement loop or D-loop, which creates a circular structure known as T-loop [3,4]. This telomeric structure may regulate the access or the function of BER proteins at telomeres. We examined the α -hOGG1 incision activity on 8-oxodG containing telomeric double-stranded oligonucleotide substrates with or without a 3'-overhang, namely telomeric-overhang-1 (TOH1) and telomeric-duplex-4 (TD4). TOH1 has 39-mer duplex region followed by an 18 bp 3'-overhang. The duplex region contains an 8-

oxodG lesion 12 bp away from the opening of the single-stranded 3'-overhang (Table S1, Figure 1). α -hOGG1 was incubated with 0.5 nM of substrates in serial dilutions and the percentage of incision products were measured between two substrates. When TOH1 was incubated with 16 nM enzyme, the incision product was visible and increased up to 60% with 256 nM enzyme (Figure S4, lanes 2-7). The TD4 substrate showed a similar trend upon the same enzymatic treatment (Figure S4, lanes 9-14). This result suggests that α -hOGG1 can efficiently excise a guanine lesion that is distant from 3'-overhang.

The junction between the single-stranded 3'-overhang and the duplex DNA appears to be essential in preserving the integrity of 3'-overhang, hence the formation of T-loop [35,36]. The repair of oxidative guanine lesions at the junction sites may be critical for telomere capping. We examined if 8-oxodG modification at the ssDNA/dsDNA junction could be removed efficiently by α -hOGG1 using TOH2 and TD5. TOH2 has 8-oxodG modification one base pair away from the first base of 3'-overhang. TD5 has the same sequence as TOH2 except with complimentary strand extended the 3'-overhang region to complete the duplex. Interestingly, the α -hOGG1 incision activity was greatly reduced on the overhang substrate TOH2. 16 nM of α -hOGG1 was able to initiate the incision (~30% product) on the duplex substrate TD5, whereas 64 nM of enzyme was required to initiate product formation (less than 5% product) from the overhang substrate TOH2 (Figure 5A, lanes 2-6 and 8-12). The difference in the percentage of incision products was as high as ~15-fold.

Since the position of the 8-oxodG near ssDNA/dsDNA junction may dictate α -hOGG1 incision activity, we examined if 8-oxodG in a telomeric replication fork context can be incised efficiently by α -hOGG1. Two 8-oxodG containing telomeric fork substrates, TF1 and TF2, were constructed for this purpose. TF1 contains 8-oxodG 12 bp away from the fork region (resembles an unreplicated region), while TF2 has 8-oxodG next to the fork ssDNA/dsDNA junction (Table S1, Figure 1). α -hOGG1 was incubated in serial dilutions with 0.5 nM substrate and the incision activity was compared between telomeric fork substrates and its corresponding double-stranded telomeric substrates (TF1 & TD4, and TF2 & TD5, respectively). By using substrates TF1 and TD4, the incision products were visible after addition of 16 nM enzyme and increased at similar rates in both substrates with increasing concentration of enzyme (Figure S5, lanes 1-7 and 8-14). However, α -hOGG1 was unable to excise 8-oxodG efficiently from TF2, another fork substrate in which the lesion is next to the fork region. While a large excess (256 nM) of enzyme was required to generate only about 20% incision product using the telomeric fork TF2 (Figure 5B, lanes 1-7), 32 nM of the enzyme was able to generate more than ~30% incision product from a similar telomeric duplex substrate (TD5) (Figure 5B, lanes 8-14). The difference in percentage of incision products was as high as ~16-fold.

Together, these results imply that α -hOGG1 can efficiently excise a guanine lesion that is distant from ssDNA/dsDNA junctions (i.e. 3'-overhang/double stranded telomeres and telomere replication forks), but its ability to excise a telomeric 8-oxodG lesion next to ssDNA/dsDNA junctions is severely compromised.

3.5. Telomeric D-loop formation does not impair α -hOGG1 incision activity

To further examine the effects of telomeric structures on oxidative damage repair at telomeres, we examined α -hOGG1 incision activity on *in vitro* assembled D-loop structures DL1 & DL2 (Table S1, Figure S6). These substrates consist of a bubble structure with two 30 bp duplex arms separated by a 33 nt ssDNA melted region, one strand of which is annealed to an invading ssDNA. The melted region and the invading ssDNA carry four telomeric repeats, such that the DNA substrate mimics a telomeric D-loop. The invading telomeric strand contains a 8-oxodG lesion in place of the middle guanine of the first (DL1) or the last (DL2) of four telomeric repeats [32]. α -hOGG1 was incubated in serial dilutions

with 0.5 nM of substrates and the incision activity was compared between one of the D-loop substrates (DL1) and the double-stranded telomeric substrate TD1. α -hOGG1 excised the lesion from the *in vitro* assembled D-loop substrate DL1 slightly more efficiently than the duplex substrate TD1 using higher concentrations (>64 nM) of α -hOGG1; however, the incision activity on *in vitro* assembled D-loop substrate DL1 was comparable to the duplex substrate TD1 under conditions where less than 30% of the substrates have been incised (Figure 6A). We also tested the effect of the position of the 8-oxodG lesion on α -hOGG1 incision activity on the damaged telomeric D-loop substrate. Similar to the observation on double-stranded oligonucleotides with 3'-overhang, 8-oxodG positioned closer to the 5' site (DL2) was excised slightly more efficiently than the one close to 3'-end (DL1) (Figure 6B). Collectively, these results suggest that a telomeric D-loop structure does not impair α -hOGG1 incision activity and that the position of the 8-oxodG lesion may dictate the enzymatic efficiency on oxidatively damaged telomeric D-loops *in vitro*.

3.7. Telomere binding proteins do not impede α -hOGG1 incision activity on telomeric duplex substrates

Shelterin protein complex masks the ends of linear chromosomes from DNA repair at telomeres [4] and protects TTAGGG repeats [37,38]. Additionally, over-expression of shelterin protein TRF2 can lead to inefficient SSB repair at telomeres [39]. We hypothesized that shelterin proteins such as TRF1 and TRF2 would affect α -hOGG1 incision activity when bound to telomeres. To evaluate the effect of shelterin proteins on α -hOGG1 incision activity, we examined activity on a double-stranded telomeric oligonucleotide substrate bound by either TRF1 or TRF2. To ensure that TRF1 is bound to the substrate with 8-oxodG modification in one of telomeric repeats, EMSA was performed using the same experimental conditions as for α -hOGG1 incision assay. Under these experimental conditions, purified TRF1 approximately had 40% reduction in its binding to telomeric substrate TD1 in comparison to the control substrate, yet it was detectable at TD1 (data not shown and [29]). TRF1 alone did not excise the substrate (Figure 7, lanes 9-13). When the substrate TD1 was pre-incubated with 16 nM purified TRF1 prior to α -hOGG1 (4 nM) treatment, the incision activity did not change compared to α -hOGG1 treatment alone (Figure 7, lanes 2 and 3), although 1.6 fold increase in incision activity was observed in large excess (256 nM) of TRF1 (Figure 7, lanes 3-8). To examine if this minor stimulation was dependent on TRF1's ability to bind to oligonucleotide substrates containing telomeric DNA repeats, the same assay was performed on oligonucleotide substrates containing non-telomeric DNA repeats. Interestingly, we found that a large excess of TRF1 can also weakly enhance α -hOGG1 glycosylase activity on non-telomeric substrates NTD1 and NTD2 (data not shown), even though EMSA showed no binding of TRF1 to these substrates (data not shown). These data indicate that TRF1 binding does not have a significant effect on the activity of α -hOGG1. We also tested if TRF2 had any effect on α -hOGG1 incision activity. Under the same experimental condition, TRF2 did not enhance α -hOGG1 incision activity (Figure 8). Together, our results suggest that α -hOGG1 is able to proficiently excise 8-oxodG containing oligonucleotide substrates bound by telomere binding proteins *in vitro*.

4. Discussion

Due to its low oxidation potential, guanine succumbs to oxidation more than other DNA nucleobases [40-42]. Additionally, studies indicate that 5' guanine of stacked GG and GGG sequences oxidize more easily than a lone guanine in DNA and that the 5' guanine of GGG oxidizes more easily than the 5' guanine of GG [40,43,44]. Telomere tracks consisting of GGG repeats may greatly increase this oxidation potential when compared to the other repeats with single G. In fact, human cells with long telomeres show increased sensitivity to hydrogen peroxide, but not to etoposide and bleomycin, supporting that telomeres are

particularly vulnerable to oxidative damage [45]. Here, we provide evidence that telomeric DNA is more prone to oxidative base damage than minisatellite TG repeats *in vivo*. Furthermore, *in vitro* exposure to oxidants on naked DNA displayed more Fpg-sensitive lesions in telomeres than minisatellite TG repeats. These observations suggest that the nature of TTAGGG in telomeres could be the primary driving force behind the preferential accumulation of oxidative base damage.

Alternatively, unique telomere factors *i.e.* telomere-specific binding proteins, repeat sequences, and telomere-specific structures may influence base oxidation and/or repair at telomeres and in turn promote accumulation of oxidative base damage in telomeres. The facts that there is preferential base damage in telomere- versus non-telomeric G-rich DNA and that UV-induced damage at telomeres was more pronounced compared to other genomic regions [31] suggest that DNA damage repair at telomeres is less effective than in non-telomeric regions. In this study, we demonstrated that acute oxidant exposure causes high incidences of Fpg-sensitive sites in TG minisatellite and telomeric DNA fragments. However, Fpg-sensitive sites were nearly undetectable in TG minisatellites, whereas these lesions persist at a higher level in telomeres after 6 hours of recovery time. These observations suggest that repair of oxidative DNA damage may be less effective in telomeres *in vivo*, which could be influenced by unique telomere factors.

α -hOGG1 is the primary glycosylase that removes 8-oxodG and me-Fapy with comparable efficiencies [16]. Here, we evaluated telomere special factors (*i.e.*, telomeric DNA repeat sequences, telomeric structures or telomere binding proteins) in influencing telomeric 8-oxodG removal by α -hOGG1. Initially, we investigated the impact of telomere repeat sequences in the α -hOGG1 incision activity. The sequence-dependent repair efficiency of α -hOGG1 has been reported [16]. Although the study was conducted using me-Fapy as the substrate, it showed that α -hOGG1 incision activity on me-Fapy was affected by the flanking sequences, with preference for 5'-(C/G)me-FapyC-3'. Nevertheless, α -hOGG1 incision activities on the telomeric and non-telomeric sequence contexts were similar.

Previous studies demonstrate that oxidant exposure increases 8-oxodG content and more than one guanine in GGG of telomeric DNA are oxidized [24,25,34]. In this study, we demonstrated that substrates containing single and triple 8-oxodG lesions were excised equally by α -hOGG1, showing no preference for either substrate. In addition, α -hOGG1 appears to favor incising middle and 5' 8-oxodG in GGG of telomeric DNA. In agreement with our findings, a previous report showed that α -hOGG1 preferred to excise 5' me-Fapy on guanine doublets [16]. It is possible that this preference could be a structurally conserved mechanism since oxidative damage induces 8-oxodG preferentially at the 5' site of 5'-GGG-3' sequence [24,25]. It has been proposed that optimal DNA twisting plays an important role in lesion recognition [46]. Flipping middle 8-oxodG by OGG1 may be preferred since DNA torsional rigidity near the 8-oxodG greatly affects the α -hOGG1 recognition and incision activity [46,47]. α -hOGG1's preference in excising 5' - and middle 8-oxodG in GGG of telomeric DNA repeats may also explain the frequent appearance of SSBs at 5' guanine in the telomere repeats after oxidative stress [24,25].

One of the unique structural features that shape telomeres is the 3'-overhang, also known as the G-rich overhang. It is important in T-loop formation and plays a vital part in the initiation process [4]. Here, we demonstrated that α -hOGG1 was unable to efficiently excise 8-oxodG near a 3' overhang opening. Previous studies have shown that an opposing abasic site greatly reduced α -hOGG1 incision activity [17], which was influenced by the DNA torsional rigidity. An optimal DNA twisting plays an important part in lesion recognition by OGG1 [46], therefore OGG1 may not efficiently recognize 8-oxodG lesions close to dsDNA/ssDNA junctions where the degree of DNA twisting may be perturbed by ssDNA.

In addition, a SSB, 3' of the catalytic pocket hinders α -hOGG1 glycosylase activity [18,19]. An 8-oxodG near a 3' overhang completely lacks the complementary strand, and it is therefore possible that α -hOGG1 is compromised significantly in repairing oxidative base damages in the presence of a strand end near its active site pocket. If these base lesions are not repaired, these damaged bases may pose a problem since 8-oxodG perturbs TRF1 and TRF2 from binding to telomeric DNA repeats [29] and the junction between the telomeric duplex and the 3' opening plays an important role in telomere length maintenance. For instance, TRF2 is implicated in T-loop formation by binding to the junction site [48,49]. Disruption of TRF2 binding to the junction region can lead to truncation of 3' overhang, telomere uncapping, and telomere fusion mediated by nonhomologous end-joining [35,36,50].

Efficient repair prior to DNA replication is critical in preventing 8-oxodG mutagenesis [42]. In this study, we showed that 8-oxodG at the telomeric fork opening led to inefficient α -hOGG1 excision activity, possibly due to the mechanisms mentioned above. Oxidative guanine damage, 8-oxodG, disrupts binding of shelterin proteins [29], which has been shown to be involved for proper replication at telomeres [8,9]. It is possible that oxidative DNA damage can have undesired consequences during replication if not readily repaired. Furthermore, adenine will be incorporated incorrectly if 8-oxodG is not repaired promptly [42], which can impede the binding of TRF1 and TRF2 to telomeres, consequently affecting their regulation on telomere length and telomere capping [29].

T-loop protects the ends of linear chromosome from being recognized as broken DNA [4]. The D-loop, as a result of T-loop formation, is a structure formed when the tip of G-rich strand binds to C-rich strand via strand invasion [4]. Our data showed that 8-oxodG in a D-loop can be excised by α -hOGG1 with differential efficiency, depending upon its position. Similar to the observation on 8-oxodG containing oligonucleotides with a 3' overhang, embedded 8-oxodG in the D-loop was excised less effectively by α -hOGG1 when it was close to the terminal end. Although α -hOGG1 is not active on single-stranded substrates, it may excise 8-oxodG when 3'-overhang is formed in a D-loop with double-stranded telomere DNA. This incision activity may play an important part in telomere length maintenance since 8-oxodG at single telomeric nucleotides disrupts telomerase activity [28].

Shelterin caps the ends of chromosomes to prevent spurious DNA repair. A previous study has shown that over-expression of TRF2 leads to inefficient SSB repair at telomeres [39]. It is possible that TRF1 and TRF2 may hinder the access of BER proteins to repair base lesions. However, α -hOGG1 can efficiently excise 8-oxodG in the telomere oligonucleotides pre-bound with TRF1 or TRF2, suggesting that shelterin does not inhibit the BER function in telomeres. Although TRF1 in large excess weakly enhances OGG1 incision activity, such high concentration of TRF1 is unlikely to be present at the site of 8-oxoG base lesions *in vivo*, especially 8-oxoG is known to perturb binding of TRF1 to telomere substrate [29]. Thus this weak stimulation may not be biologically relevant *in vivo*.

In this study, we demonstrate that telomeres are prone to the accumulation of oxidative guanine lesions and 8-oxodG removal by OGG1 could be affected by its position in different telomere configurations (*e.g.*, fork-opening, 3'-overhang, and D-loop). Telomere shortening has been linked to human aging, bone marrow failure syndromes, and cancer development [51], and oxidative DNA damage is a contributing factor [52]. We recently demonstrated that telomere shortening occurs in *Ogg1* deficient mice experiencing high degrees of oxidative stress [27]. Telomere base damage associated telomere attrition may thus contribute to premature aging and influence cancer formation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Shakima St Clair for technical assistance, Mary Pfeiffer for editing manuscript, and Drs. Jason Aulds, Brian Berquist, Robert Brosh, Huseyin Saribasak, and David Wilson for comments. This work was supported by funds from the Intramural Research Program of the NIA, National Institutes of Health. DBR was supported by UT-ORNL Graduate School of Genome Science and Technology. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abbreviation

8-oxodG	7,8-dihydro-8-oxogaunine
OGG1	8-oxoguanine DNA glycosylase
α-hOGG1	human 8-oxoguanine DNA glycosylase alpha isoform
BER	Base excision repair
TRF1	Telomere repeat-binding factor 1
TRF2	Telomere repeat-binding factor 2
ROS	Reactive oxygen species
me-Fapy	Methylated-formamidinopyrimidine
Fpg	Formamidinopyrimidine DNA glycosylase
TD	Telomere-duplex substrate
NTD	Non-telomeric-duplex substrate
DL	D-loop substrate
TF	Telomeric-fork substrate
TOH	Telomeric 3' overhang substrate
APE1	Apurinic endonuclease 1
SSB	Single-strand-break
nt	nucleotide

References

1. Longhese MP. DNA damage response at functional and dysfunctional telomeres. *Genes Dev.* 2008; 22:125–140. [PubMed: 18198332]
2. Palm W, de Lange T. How shelterin protects mammalian telomeres. *Annual review of genetics.* 2008; 42:301–334.
3. Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, de Lange T. Mammalian telomeres end in a large duplex loop. *Cell.* 1999; 97:503–514. [PubMed: 10338214]
4. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev.* 2005; 19:2100–2110. [PubMed: 16166375]
5. d'Adda di Fagagna F, Teo SH, Jackson SP. Functional links between telomeres and proteins of the DNA-damage response. *Genes Dev.* 2004; 18:1781–1799. [PubMed: 15289453]
6. Wang F, Podell ER, Zaug AJ, Yang Y, Baciu P, Cech TR, Lei M. The POT1-TPP1 telomere complex is a telomerase processivity factor. *Nature.* 2007; 445:506–510. [PubMed: 17237768]

7. Shore D, Bianchi A. Telomere length regulation: coupling DNA end processing to feedback regulation of telomerase. *Embo J*. 2009; 23:23.
8. Tatsumi Y, Ezura K, Yoshida K, Yugawa T, Narisawa-Saito M, Kiyono T, Ohta S, Obuse C, Fujita M. Involvement of human ORC and TRF2 in pre-replication complex assembly at telomeres. *Genes Cells*. 2008; 13:1045–1059. [PubMed: 18761675]
9. Sfeir A, Kosiyatrakul ST, Hockemeyer D, MacRae SL, Karlseder J, Schildkraut CL, de Lange T. Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication. *Cell*. 2009; 138:90–103. [PubMed: 19596237]
10. Ye J, Lenain C, Bauwens S, Rizzo A, Saint-Leger A, Poulet A, Benarroch D, Magdinier F, Morere J, Amiard S, Verhoeven E, Britton S, Calsou P, Salles B, Bizard A, Nadal M, Salvati E, Sabatier L, Wu Y, Biroccio A, Londono-Vallejo A, Giraud-Panis MJ, Gilson E. TRF2 and apollo cooperate with topoisomerase 2alpha to protect human telomeres from replicative damage. *Cell*. 142:230–242. [PubMed: 20655466]
11. Dianov GL, Parsons JL. Co-ordination of DNA single strand break repair. *DNA Repair (Amst)*. 2007; 6:454–460. [PubMed: 17123872]
12. Memisoglu A, Samson L. Base excision repair in yeast and mammals. *Mutat Res*. 2000; 451:39–51. [PubMed: 10915864]
13. Dodson ML, Lloyd RS. Mechanistic comparisons among base excision repair glycosylases. *Free radical biology & medicine*. 2002; 32:678–682. [PubMed: 11937293]
14. Robertson AB, Klungland A, Rognes T, Leiros I. DNA repair in mammalian cells: Base excision repair: the long and short of it. *Cell Mol Life Sci*. 2009; 66:981–993. [PubMed: 19153658]
15. Tchou J, Kasai H, Shibutani S, Chung MH, Laval J, Grollman AP, Nishimura S. 8-oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proceedings of the National Academy of Sciences of the United States of America*. 1991; 88:4690–4694. [PubMed: 2052552]
16. Asagoshi K, Yamada T, Terato H, Ohya Y, Monden Y, Arai T, Nishimura S, Aburatani H, Lindahl T, Ide H. Distinct repair activities of human 7,8-dihydro-8-oxoguanine DNA glycosylase and formamidopyrimidine DNA glycosylase for formamidopyrimidine and 7,8-dihydro-8-oxoguanine. *J Biol Chem*. 2000; 275:4956–4964. [PubMed: 10671534]
17. Barone F, Dogliotti E, Cellai L, Giordano C, Bjoras M, Mazzei F. Influence of DNA torsional rigidity on excision of 7,8-dihydro-8-oxo-2'-deoxyguanosine in the presence of opposing abasic sites by human OGG1 protein. *Nucleic acids research*. 2003; 31:1897–1903. [PubMed: 12655006]
18. Rogacheva MV, Saparbaev MK, Afanasov IM, Kuznetsova SA. Two sequential phosphates 3' adjacent to the 8-oxoguanosine are crucial for lesion excision by E. coli Fpg protein and human 8-oxoguanine-DNA glycosylase. *Biochimie*. 2005; 87:1079–1088. [PubMed: 15979229]
19. Parsons JL, Zharkov DO, Dianov GL. NEIL1 excises 3' end proximal oxidative DNA lesions resistant to cleavage by NTH1 and OGG1. *Nucleic acids research*. 2005; 33:4849–4856. [PubMed: 16129732]
20. Hirakawa K, Aoshima M, Hiraku Y, Kawanishi S. Photohydrolysis of methotrexate produces pteridine, which induces poly-G-specific DNA damage through photoinduced electron transfer. *Photochemistry and photobiology*. 2002; 76:467–472. [PubMed: 12462639]
21. Rai P, Wemmer DE, Linn S. Preferential binding and structural distortion by Fe²⁺ at RGGG-containing DNA sequences correlates with enhanced oxidative cleavage at such sequences. *Nucleic acids research*. 2005; 33:497–510. [PubMed: 15659581]
22. Kawanishi S, Oikawa S. Mechanism of telomere shortening by oxidative stress. *Ann N Y Acad Sci*. 2004; 1019:278–284. [PubMed: 15247029]
23. Henle ES, Han Z, Tang N, Rai P, Luo Y, Linn S. Sequence-specific DNA cleavage by Fe²⁺-mediated fenton reactions has possible biological implications. *J Biol Chem*. 1999; 274:962–971. [PubMed: 9873038]
24. Kawanishi S, Oikawa S, Murata M, Tsukitome H, Saito I. Site-specific oxidation at GG and GGG sequences in double-stranded DNA by benzoyl peroxide as a tumor promoter. *Biochemistry*. 1999; 38:16733–16739. [PubMed: 10606504]
25. Oikawa S, Kawanishi S. Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. *FEBS Lett*. 1999; 453:365–368. [PubMed: 10405177]

26. Lu J, Liu Y. Deletion of Ogg1 DNA glycosylase results in telomere base damage and length alteration in yeast. *Embo J*. 2009; 29:398–409. [PubMed: 19942858]
27. Wang Z, Rhee DB, Lu J, Bohr CT, Zhou F, Vallabhaneni H, de Souza-Pinto NC, Liu Y. Characterization of oxidative guanine damage and repair in mammalian telomeres *PLoS genetics*. 2010 In press.
28. Szalai VA, Singer MJ, Thorp HH. Site-specific probing of oxidative reactivity and telomerase function using 7,8-dihydro-8-oxoguanine in telomeric DNA. *J Am Chem Soc*. 2002; 124:1625–1631. [PubMed: 11853436]
29. Opresko PL, Fan J, Danzy S, Wilson DM 3rd, Bohr VA. Oxidative damage in telomeric DNA disrupts recognition by TRF1 and TRF2. *Nucleic acids research*. 2005; 33:1230–1239. [PubMed: 15731343]
30. de Souza-Pinto NC, Maynard S, Hashiguchi K, Hu J, Muftuoglu M, Bohr V. The Recombination Protein Rad52 Cooperates with the Excision Repair Protein Ogg1 for the Repair of Oxidative Lesions in Mammalian Cells. *Molecular and cellular biology*. 2009
31. Kruk PA, Rampino NJ, Bohr VA. DNA damage and repair in telomeres: relation to aging. *Proceedings of the National Academy of Sciences of the United States of America*. 1995; 92:258–262. [PubMed: 7816828]
32. Ghosh A, Rossi ML, Aulds J, Croteau D, Bohr VA. Telomeric D-loops containing 8-oxo-2'-deoxyguanosine are preferred substrates for Werner and Bloom syndrome helicases and are bound by POT1. *J Biol Chem*. 2009; 284:31074–31084. [PubMed: 19734539]
33. Klungland A, Bjelland S. Oxidative damage to purines in DNA: role of mammalian Ogg1. *DNA Repair (Amst)*. 2007; 6:481–488. [PubMed: 17127104]
34. Giese B, Amaudrut J, Kohler AK, Spormann M, Wessely S. Direct observation of hole transfer through DNA by hopping between adenine bases and by tunnelling. *Nature*. 2001; 412:318–320. [PubMed: 11460159]
35. Yang Q, Zheng YL, Harris CC. POT1 and TRF2 cooperate to maintain telomeric integrity. *Molecular and cellular biology*. 2005; 25:1070–1080. [PubMed: 15657433]
36. Zhu XD, Niedernhofer L, Kuster B, Mann M, Hoeijmakers JH, de Lange T. ERCC1/XPF removes the 3' overhang from uncapped telomeres and represses formation of telomeric DNA-containing double minute chromosomes. *Mol Cell*. 2003; 12:1489–1498. [PubMed: 14690602]
37. Konig P, Fairall L, Rhodes D. Sequence-specific DNA recognition by the myb-like domain of the human telomere binding protein TRF1: a model for the protein-DNA complex. *Nucleic acids research*. 1998; 26:1731–1740. [PubMed: 9512546]
38. Bianchi A, Stansel RM, Fairall L, Griffith JD, Rhodes D, de Lange T. TRF1 binds a bipartite telomeric site with extreme spatial flexibility. *Embo J*. 1999; 18:5735–5744. [PubMed: 10523316]
39. Richter T, Saretzki G, Nelson G, Melcher M, Olijslagers S, von Zglinicki T. TRF2 overexpression diminishes repair of telomeric single-strand breaks and accelerates telomere shortening in human fibroblasts. *Mech Ageing Dev*. 2007; 128:340–345. [PubMed: 17395247]
40. Saito I, Takayama M, Sugiyama H, Nakatani K, Tsuchida A, Yamamoto M. Photoinduced DNA Cleavage via Electron Transfer: Demonstration That Guanine Residues Located 5' to Guanine Are the Most Electron-Donating Sites. *Journal of the American Chemical Society*. 1995; 117:6406–6407.
41. Sugiyama H, Saito I. Theoretical studies of GG-specific photocleavage of DNA via electron transfer: significant lowering of ionization potential and 5'-localization of HOMO of stacked GG bases in B-form DNA. *Journal of the American Chemical Society*. 1996; 118:7063–7068.
42. Neeley WL, Essigmann JM. Mechanisms of formation, genotoxicity, and mutation of guanine oxidation products. *Chem Res Toxicol*. 2006; 19:491–505. [PubMed: 16608160]
43. Saito I, Nakamura I, Nakatani K. Mapping of the Hot Spots for DNA Damage by One-Electron Oxidation: Efficacy of GG Doublets and GGG Triplets as a Trap in Long-Range Hole Migration. *J Am Chem Soc*. 1998; 120:12686–12687.
44. Hall DB, Holmlin RE, Barton JK. Oxidative DNA damage through long-range electron transfer. *Nature*. 1996; 382:731–735. [PubMed: 8751447]

45. Rubio MA, Davalos AR, Campisi J. Telomere length mediates the effects of telomerase on the cellular response to genotoxic stress. *Experimental cell research*. 2004; 298:17–27. [PubMed: 15242758]
46. Sidorenko VS, Zharkov DO. Correlated cleavage of damaged DNA by bacterial and human 8-oxoguanine-DNA glycosylases. *Biochemistry*. 2008; 47:8970–8976. [PubMed: 18672903]
47. Barone F, Lankas F, Spackova N, Sponer J, Karran P, Bignami M, Mazzei F. Structural and dynamic effects of single 7-hydro-8-oxoguanine bases located in a frameshift target DNA sequence. *Biophys Chem*. 2005; 118:31–41. [PubMed: 16039038]
48. Stansel RM, de Lange T, Griffith JD. T-loop assembly in vitro involves binding of TRF2 near the 3' telomeric overhang. *Embo J*. 2001; 20:5532–5540. [PubMed: 11574485]
49. Court R, Chapman L, Fairall L, Rhodes D. How the human telomeric proteins TRF1 and TRF2 recognize telomeric DNA: a view from high-resolution crystal structures. *EMBO Rep*. 2005; 6:39–45. [PubMed: 15608617]
50. Deng Y, Guo X, Ferguson DO, Chang S. Multiple roles for MRE11 at uncapped telomeres. *Nature*. 2009; 460:914–918. [PubMed: 19633651]
51. Sahin E, Depinho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature*. 464:520–528. [PubMed: 20336134]
52. von Zglinicki T. Telomeres: influencing the rate of aging. *Ann N Y Acad Sci*. 1998; 854:318–327. [PubMed: 9928440]

Designation	Top + Bottom	Nucleotide Sequence
Non-Telomeric-Duplex-1 (NTD1)	A1 + A2	5'-GTGGATCCGTACTCT X CTACGTGTCAACACGAATTCGA-3' 3'-CACCTAGGCATGAGACCCATGCACAGTTGTGCTTAAGCT-5'
Non-Telomeric-Duplex-2 (NTD2)	A3 + A4	5'-GTGGATCCGTACTCT X ATACGTGTCAACACGAATTCGA-3' 3'-CACCTAGGCATGAGAGCTATGCACAGTTGTGCTTAAGCT-5'
Telomeric-Duplex-1 (TD1)	B1 + B6	5'-GTGGATCCGTACTTAC X CTTAGGGTTAACACGAATTCGA-3' 3'-CACCTAGGCATGAATCCCAATCCCAATTGTGCTTAAGCT-5'
Telomeric-Duplex-2 (TD2)	B2 + B6	5'-GTGGATCCGTACTTAC X CTTAGGGTTAACACGAATTCGA-3' 3'-CACCTAGGCATGAATCCCAATCCCAATTGTGCTTAAGCT-5'
Telomeric-Duplex-3 (TD3)	B3 + B7	5'-GTGGATCCGTACACACGAATTCGATGCTTAGGGTTAC X -3' 3'-CACCTAGGCATGTGTGCTTAAGCTACGAATCCCAATCC-5'
Telomeric-Duplex-4 (TD4)	B4 + B8	5'-GTGGATCCGTACTTAC X CTTAGGGTTAACACGAATTCGATCTGTGTCTGTGTCTG-3' 3'-CACCTAGGCATGAATCCCAATCCCAATTGTGCTTAAGCTAGACACACACACACAC-5'
Telomeric-Duplex-5 (TD5)	B5 + B9	5'-GTGGATCCGTACACACGAATTCGATGCTTAGGGTTAC X CTTATCTGTGTCTGTCT-3' 3'-CACCTAGGCATGTGTGCTTAAGCTACGAATCCCAATCCCAATAGACACACACACAGA-5'
Telomeric-3'-Overhang-1 (TOH1)	B4 + B6	5'-GTGGATCCGTACTTAC X CTTAGGGTTAACACGAATTCGATCTGTGTCTGTGTCTG-3' 3'-CACCTAGGCATGAATCCCAATCCCAATTGTGCTTAAGCT-5'
Telomeric-3'-Overhang-2 (TOH2)	B5 + B7	5'-GTGGATCCGTACACACGAATTCGATGCTTAGGGTTAC X CTTATCTGTGTCTGTCT-3' 3'-CACCTAGGCATGTGTGCTTAAGCTACGAATCCCAATCCC-5'
Telomeric-Fork-1 (TF1)	B4 + B10	5'-GTGGATCCGTACTTAC X CTTAGGGTTAACACGAATTCGATCTGTGTCTGTGTCTG-3' 3'-CACCTAGGCATGAATCCCAATCCCAATTGTGCTTAAGCTTTTTTTTTTTTTTTTTTT-5'
Telomeric-Fork-2 (TF2)	B5 + B11	5'-GTGGATCCGTACACACGAATTCGATGCTTAGGGTTAC X CTTATCTGTGTCTGTCT-3' 3'-CACCTAGGCATGTGTGCTTAAGCTACGAATCCCAATCCCTCTTTTTTTTTTTTTTT-5'

Figure 1.

Double-stranded oligonucleotide substrates. Oligonucleotides modified with 8-oxodG were either 5' end-labeled or 3' end-labeled and annealed to the corresponding complementary strands. The bold X denotes the 8-oxodG modifications.

Figure 2.

-hOGG1 incision activity is comparable between telomeric and non-telomeric DNA repeats containing double-stranded substrates. Substrates (0.5 nM) were incubated alone (lanes 1, 7, and 13) or together with increasing concentrations of OGG1 (16, 32, 64, 128, or 256 nM, lanes 2-6, 8-12, and 14-18). No significant difference in incision rates was observed among these substrates under conditions where less than 30% of the substrates have been incised. Similar incision rates were observed among the substrates with higher concentrations of OGG1. The percentage cut was plotted against -hOGG1 concentrations. Values and error bars represent the mean and SE from three independent experiments.

Figure 3.

-hOGG1 incision activity is comparable between single and triple 8-oxodG containing double-stranded telomeric substrates. Substrates (0.5 nM) were incubated alone (lanes 1 and 7) or together with increasing concentrations of -hOGG1 (16, 32, 64, 128, or 256 nM, lanes 2-6 and 8-12). The incision products of triple 8-oxodG containing substrate was more diffused than single 8-oxodG containing substrate, indicated by three bars as opposed to two bars (arrows). No significant difference in incision rates was observed between the two substrates under conditions where less than 30% of the substrates have been incised. Similar incision rates were observed between the substrates with higher concentrations of OGG1. The percentage cut was plotted against -hOGG1 concentrations. Values and error bars represent the mean and SE from three independent experiments.

Figure 4.

-hOGG1 prefers to excise middle 8-oxodG base lesion in telomeric DNA repeats. Substrates (0.5 nM) were incubated with fixed concentration of -hOGG1 (8 nM) and collected at different time points (15 sec, 30 sec, 1 min, 2 min, 4 min, and 8 min, lanes 1-6 and 7-12). The middle 8-oxodG in telomeric GGG was excised primarily in both 5' labeled (A and C) substrate and 3' labeled (B and D) substrates. Arrows – The position of excised 8-oxodG. The peak -hOGG1 incision activity was plotted against time points. Values and error bars represent the mean and SE from three independent experiments.

Figure 5.

The position of 8-oxodG base lesion affects hOGG1 incision activity on telomeric substrates with 3' overhang and fork-opening. (A) Substrates (0.5 nM) with a 3'-overhang were incubated alone (lanes 1 and 7) or together with increasing concentrations of OGG1 (16, 32, 64, 128, or 256 nM, lanes 2-6 and 8-12). (B) Substrates (0.5 nM) with a fork-opening were incubated alone (lanes 1 and 8) or together with increasing concentrations of hOGG1 (8, 16, 32, 64, 128, or 256 nM, lanes 2-7 and 9-14). 8-oxodG modification near the opening of 3'-overhang or the fork-opening significantly reduced hOGG1's incision activity under conditions where less than 30% of the substrates have been incised. Similar observation was detected between the substrates with higher concentrations of OGG1. The percentage cut was plotted against hOGG1. Values and error bars represent the mean and SE from three independent experiments.

Figure 6.

-hOGG1 incision activity is influenced by position of 8-oxodG base lesion on D-loop substrates. Substrates (0.5 nM) were incubated alone (lanes 1 and 7) or together with increasing concentrations of OGG1 (16, 32, 64, 128, or 256 nM, lanes 2-6 and 8-12). (A) Comparable incision rates were observed between the substrates under conditions where less than 30% of the substrates have been incised, although at higher concentrations, -hOGG1 incision activity appeared to be slightly more efficient in D-loop substrate. (B) The D-loop substrate with 8-oxodG modification closer to 5' end was excised slightly more efficiently than the D-loop substrate with 8-oxodG modification closer to 3' end under conditions where less than 30% of the substrates have been incised. Similar observation was detected between the substrates with higher concentrations of OGG1. The percentage cut was plotted against -hOGG1 concentrations. Values and error bars represent the mean and SE from three independent experiments.

Figure 7.

The shelterin protein TRF1 does not greatly affect α-hOGG1 incision activity. Substrate (0.2 nM) was incubated alone (lane 1) or together with fixed amount of α-hOGG1 (4 nM, lanes 2-8) and increasing concentrations of TRF1 (20.4, 40.8, 81.5, 163, or 326 nM, lanes 4-8 and 9-13). The percentage of incision products was plotted against TRF1 concentrations. Values and error bars represent the mean and SE from three independent experiments.

Figure 8.

The shelterin protein TRF2 does not stimulate -hOGG1 incision activity. (A) Substrate (0.2 nM) was incubated together with fixed amount of -hOGG1 (1.6 nM, lanes 2-6)(256 nM, lanes 8-12) and increasing concentrations of TRF1 (163 nM and 326 nM, lanes 2-3 and 8-9) or TRF2 (180 nM, 360 nM, and 720 nM, lanes 4-6 and 10-12). (B) The percentage of incision products was plotted against TRF1 or TRF2 concentrations.

Table 1

The frequencies of Fpg-sensitive sites/10⁶ bp in telomeric (TTAGGG) and minisatellite (TG) regions of mouse kidney.

	Telomeric DNA	Minisatellite DNA
<u>Endogenous</u> ^a	45.36 ± 7.4 ^c	20.76 ± 1.36 ^c
<u>In vitro treatment</u> ^b		
6 mM H ₂ O ₂ + 2μM Cu ²⁺	137.88 ± 17.71 ^c	68.69 ± 31.74 ^c
18 mM H ₂ O ₂ + 8μM Cu ²⁺	303.04 ± 53.41 ^c	81.57 ± 11.88 ^c

^aRepresenting Fpg sensitive sites *in vivo*.

^bThe mouse kidney DNA was exposed to H₂O₂ and Cu²⁺ in testing tubes and examined for Fpg sensitive sites. The *in vitro* treatment was performed on naked DNA without the presence of other factors (e.g. such as telomere repeat binding factors), the results therefore represent the contribution of DNA sequences to oxidative damage.

^cBecause of semi-quantitative nature of the method, the frequency does not represent the absolute values of base lesions in these genomic regions. The values were derived from three independent experiments.

Table 2

The frequencies of Fpg-sensitive sites/ 10^6 bp in telomeric (TTAGGG) and minisatellite (TG) regions of U2OS cells.

	Telomeric DNA	Minisatellite DNA
<u>Endogenous</u> ^a	5.39 ± 0.67^c	3.76 ± 1.11^c
<u>In vivo treatment</u> ^b		
0 hr	7.72 ± 1.75^c	9.56 ± 6.22^c
6 hr	14.25 ± 1.21^c	Not detectable ^c

^aRepresenting Fpg sensitive sites *in vivo*.

^bU2OS cells were exposed to menadione in culture and examined for Fpg sensitive sites at 0 and 6 hours of recovery time.

^cBecause of semi-quantitative nature of the method, the frequency does not represent the absolute values of base lesions in these genomic regions. The values were derived from three independent experiments.