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# Characterization of 5'-Flanking Regions of Various Human Telomere Maintenance Factor-Encoding Genes

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## 1. Introduction

Telomeres are the unique nucleoprotein complex structures located at the end of linear eukaryotic chromosomes (Blackburn, 2000; de Lange, 2006). They are composed of TTAGGG repeats that are typically 10 kb at birth and gradually shorten with cell divisions (de Lange, 2006). Telomerase is composed of the protein subunit TERT and the RNA subunit TERC (TR). It elongates the telomere by adding telomeric repeats (Greider & Blackburn, 1987). The 50 to 300 nucleotides from the terminal end of the telomeres are single stranded 3'-protluded G-overhang structures which make the t-loop configuration (de Lange, 2006; Griffith et al., 1999). Mammalian telomeres are included in heterochoromatin and attached to the nuclear matrix (Oberdoerffer & Sinclair, 2007; Gonzalez-Suarez & Gonzalo, 2008). Telomere shortening causes instability of the ends of chromosomes to lead to replicative senescence (O'Sullivan & Karlseder, 2010; Lundblad & Szostak, 1989). Therefore, the ends of telomeres should be protected from damaging or cellular activities. The t-loop structures are regulated by shelterin protein factors, TRF1, TRF2, Rap1, TIN2, TPP1, POT1 (Gilson & Geli, 2007; O'Sullivan & Karlseder, 2010), and Rec Q DNA helicases, WRN and BLM (Chu & Hickson, 2009). TRF1 and TRF2, which bind to duplex telomeric DNA and retain shelterin on the telomere repeats, were shown to interact with various functional proteins (Giannone et al., 2010). Molecular structural analysis of Rap1 revealed that its mechanism of action involves interaction with TRF2 and Taz1 proteins (Chen et al., 2011). A recent study showed that depletion of TPP1 and its partner TIN2 causes a loss of telomerase recruitment to telomeres (Abreu et al., 2010). POT1 is an important regulator of telomerase length, in stimulating the RecQ helicases WRN and BLM (Opresko et al., 2005). Tankyrase-1 (TANK1), which is classified as a poly(ADP-ribose) polymerase family protein, is also known to regulate telomere homeostasis by modifying TRF1 (Smith et al., 1998; Schreiber et al., 2006). Dyskerin, which is encoded by the *DKC1* gene, is a key auxiliary protein that is contained in a Cajal body with TERT (Cohen et al., 2007). Defects in the shelterin components and telomerase are thought to down-regulate telomere structure

and length (O'Sullivan & Karlseder, 2010). The shelterin proteins also play important roles in protecting chromosomal ends from being recognized by DNA damage response (DDR) machinery (O'Sullivan & Karlseder, 2010). Although the biological significance of the shelterin complex proteins has been studied, the molecular mechanisms that regulate expression of those genes encoding telomere associated proteins is less well-characterized. We hypothesized that expressions of those telomere-associated protein-encoding genes are regulated by a similar mechanism. In order to analyze these promoter activities promptly, we isolated 200 to 300-bp of the 5'-upstream regions of these telomere regulatory protein-encoding genes and applied them to a multiple transfection assay system (Uchiumi et al., 2010a). Previously, we have observed that *WRN* and *TERT* promoter activities were up-regulated by 2-deoxy-D-glucose (2DG) and *trans*-resveratrol (Rsv) in accordance with the activation of telomerase (Zhou et al., 2009; Uchiumi et al., 2011). A potent inhibitor of glucose metabolism, 2DG is thought to mimic glucose deprivation *in vivo* such that it is mimetic of caloric restriction (CR) (Roth et al., 2001). Resveratrol (Rsv), which is a polyphenol contained in grape skins and red wine, activates sirtuin-mediated deacetylation (Stefani et al., 2007; Knutson & Leeuwenburgh, 2008). We report here that most of the promoters of the shelterin protein-encoding genes positively responded to the CR mimetic agents, 2DG and Rsv. These results suggest that telomerase and telomere maintenance factors are simultaneously regulated at the initiation of the transcription.

## 2. Materials and methods

### 2.1 Chemicals

The reagents 2-deoxy-D-glucose (2DG) and *trans*-resveratrol (Rsv) were purchased from Wako Chemicals (Tokyo, Japan) and Cayman Chemicals (Ann Arbor, MI), respectively.

### 2.2 Cells and cell culture

HeLa-S3 cells (Zhou et al., 2009) were cultured in Dulbecco's modified eagle (DME) medium supplemented with heat-inactivated 10% fetal calf serum (FCS) (Sanko-Pure Chemical, Tokyo, Japan), 2 mM L-glutamine (Invitrogen, CA, USA), penicillin (100 IU/mL) (MEIJI SEIKA, Tokyo, Japan), and streptomycin (100 µg/mL) (MEIJI SEIKA).

### 2.3 Construction of Luc-reporter plasmids

Luc reporter plasmids carrying promoter regions for the human *TERT* and *TERC* genes have been constructed and designated as pGL4-TERT, and pGL4-TERC, respectively (Zhou et al., 2009; Uchiumi et al., 2010a). Extraction of DNA from HeLa-S3 cells, and subsequent PCR for the promoter regions of interest were performed as described previously (Uchiumi et al., 2010a; Zhou et al., 2009). Primer-sets were designed against human genomic sequences from the Cross-Ref NCBI-data base (http://www.ncbi.nlm.nih.gov/sites/gquery/) for the 5'-flanking regions of the genes of interest (Table. 1). PrimeStar Taq polymerase (Takara, Kyoto, Japan) was used for all amplifications.

Amplification conditions consisted of: 30 cycles of 98˚C for 10 sec, 55˚C for 5 sec, and 72˚C for 30 sec. PCR products were digested with *Kpn*I and *Xho*I and then separated on 0.9% agarose gels.

After electrophoresis, DNA bands of the correct length were recovered from the gel with Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) and subcloned

into the *Kpn*I-*Xho*I site of the pGL4-basic vector (pGL4[luc 2.10]) (Promega). The resultant cloned plasmids were designated pGL4-DKC1, pGL4-POT1, pGL4-RAP1, pGL4-TANK1, pGL4-TANK2, pGL4-TIN2, pGL4-TPP1, pGL4-TRF1, and pGL4-TRF2. Clone sequences were confirmed using a DNA Sequencing System (Applied Biosystems, Foster City, CA) with Rv (5'-TAGCAAAATAGGCTGTCCCC-3' and GL (5'-CTTTATGTTTTTGGCGTCTT-CC-3') primers purchased from Operon Biotechnologies (Tokyo, Japan).

| Name | Sequence |
|---|---|
| hDysk-7065 | 5'-TCGGTACCGTGAGCCCAGGCGCAGGCGC-3' |
| AhDysk-7414 | 5'-ATCTCGAGGGAACGACCGCAGACTCCC-3' |
| hPOT-1509 | 5'-TCGGTACCTGAGAACTGAATATTGCTGTG-3' |
| AhPOT-1164 | 5'-ATCTCGAGAATATCATCTTACCAAAGAC-3' |
| hRAP1-5667 | 5'-TCGGTACCTCGCGGCGCTTCCCAGCCC-3' |
| AhRAP1-5970 | 5'-ATCTCGAGCTGTCACCGCAGACGCCTC-3' |
| hTANK1-8541 | 5'-TCGGTACCGACTGAAAGTGAGAAATGC-3' |
| AhTANK1-8860 | 5'-ATCTCGAGAGCGACGCGACGCCGCCATC-3' |
| hTANK2-4227 | 5'-TCGGTACCAGGAGAAAGGGATGTGGAAG-3' |
| AhTANK2-4519 | 5'-ATCTCGAGGCGGCGCGAAGGGTTTGTGG-3' |
| hTIN2-8835 | 5'-TCGGTACCGCAGGCTCCGCGAAGAAAGC-3' |
| AhTIN2-8508 | 5'-ATCTCGAGTGGAGAAGCTGACCGTCTC-3' |
| hTPP1-8283 | 5'-TCGGTACCTCGACGATGCTATCGGGAC-3' |
| AhTPP1-7995 | 5'-ATCTCGAGCGTGATGACGCAAGAGCGGA-3' |
| hTRF1-1070 | 5'-TCGGTACCTCCTCCTATCCTAATCTCGC-3' |
| AhTRF1-1371 | 5'-ATCTCGAGGAAACATCCTCCGCCATGTT-3' |
| hTRF2-9454 | 5'-TCGGTACCGATCCCGGCCTGTTTTTCAG-3' |
| AhTRF2-9170 | 5'-ATCTCGAGCGGGGCCCGCCGTCCCGGC-3' |

Table 1. Primers used for amplifying 5'-upstream region of various human telomere-associated genes

### 2.4 Transient transfection assay

Transient transfection of Luc-reporter plasmids was performed using multi-well culture plates that had been prepared and treated with DNA/DEAE-dextran (Uchiumi et al., 2010a). After 4 h of transfection, 2DG or Rsv was added to the culture medium (Zhou et al., 2009; Uchiumi et al., 2011). After a further incubation (19 to 24 h), cells were collected and lysed with 40 μL of 1 x Cell culture lysis reagent, mixed, and stored at -80°C. Luc assays were performed according to the manufacturer's instructions (Promega). In brief, Luc assay reagent (40 μL) was added to 10 μL of protein sample and mixed briefly. Immediately after mixing, chemiluminescence was measured for 7.5 sec with a Minilumat LB9506 luminometer (Berthold, Bad Wildbad, Germany). Protein assays were performed with the Luc sample (2.5 μL) and Protein Assay Reagent (Bio-Rad Lab., Hercules, CA, USA).

## 3. Results

### 3.1 Isolation of 5'-flanking regions of human telomere-associated protein-encoding genes

Previously, we isolated and characterized 5'-flanking regions of the human *TERT* and *TERC* genes (Zhou et al., 2009; Uchiumi et al., 2010a). In this study, those of different human telomere-associated protein-encoding genes were obtained by PCR and inserted into the MCS of the pGL4-basic (pGL4[luc 2.10]) vector. Putative transcription-factor binding elements were found by TF-SEARCH analysis. As summarized in Fig. 1, c-Ets/Elk1, Sp1/GC-box, CREB, OCT, p300, SRY, GATA, E2F, NF-κB/c-Rel, CCAAT-box, and other motifs are located within 300-bp from the 5'-upstream region of the cDNAs. Although all of these telomere-associated protein factors are commonly involved in the maintenance of telomeres, a rigid rule in the order of the *cis*-elements could not be found in their core promoter regions. However, one or more Sp1/GC-box elements are located in 5'-upstream regions of the *DKC1*, *RAP1*, *TANK1*, *TIN2*, *TPP1*, *TRF1*, *TRF2*, *TERT*, and *TERC* genes, but not in the *POT1* and *TANK2* genes. Similar to the 5'-flanking region of the *WRN* gene, all of the isolated DNA fragments have no obvious TATA-box like sequences except the 5'-flanking region of the *TERC* gene (Uchiumi et al., 2010a).

### 3.2 Effect of Rsv on the promoter activities of 5'-flanking regions of the shelterin-encoding genes

The natural compound Rsv is known to have life-span promoting properties in yeast and metazoans by affecting the insulin-signaling cascade (Fröjdö et al., 2008). In order to examine the effect of Rsv on the isolated 5'-upstream regions of the shelterin encoding genes, Luc assays were performed. Luc expression plasmids which contained 5'-flanking regions of various telomere maintenance factor-encoding genes were transfected into HeLa-S3 cells by the DEAE-dextran based multiple transfection method (Uchiumi et al., 2010a). Luc activities of reporter plasmid-transfected cells were normalized to that of the pGL4-PIF1 transfected cells, because PIF1 has been suggested to have a negative effect on telomere elongation in yeast cells (Schulz & Zakian, 1994), and it has been shown that the change in the PIF1 promoter activity is largely unaffected after treatment with Rsv (Uchiumi et al., 2011). As shown in Table 2, treatment with Rsv (10 μM) for 24 h augmented Luc activities from the cells transfected with Luc reporter plasmids. Apparent positive responses to the Rsv treatment of the 5'-flanking regions of the *TERT* and *TERC* genes were observed, consistent with the activation of telomerase by Rsv in HeLa-S3 cells (Uchiumi et al., 2011). Although no obvious GC-box like elements are found in the 300-bp 5'-upstream regions of the *POT1* and *TANK2* genes (Fig. 1), Luc activities of these plasmid-transfected cells increased 2.53- and 1.69-fold, respectively, by Rsv treatment.

### 3.3 Effect of 2DG on the promoter activities of 5'-flanking regions of the shelterin-encoding genes

2DG is known to affect life span by its CR mimetic effect on various species (Roth et al., 2001). We previously observed that treatment with 2DG induces telomerase activity along with transcriptional activation of the *TERT* and *WRN* genes in HeLa-S3 cells (Zhou et al., 2009). Therefore, we examined the effect of 2DG on the promoter activities of shelterin-encoding genes. Although most of the Luc activities of cells transfected with shelterin promoter-Luc expression constructs were diminished by 2DG, the treatment induced

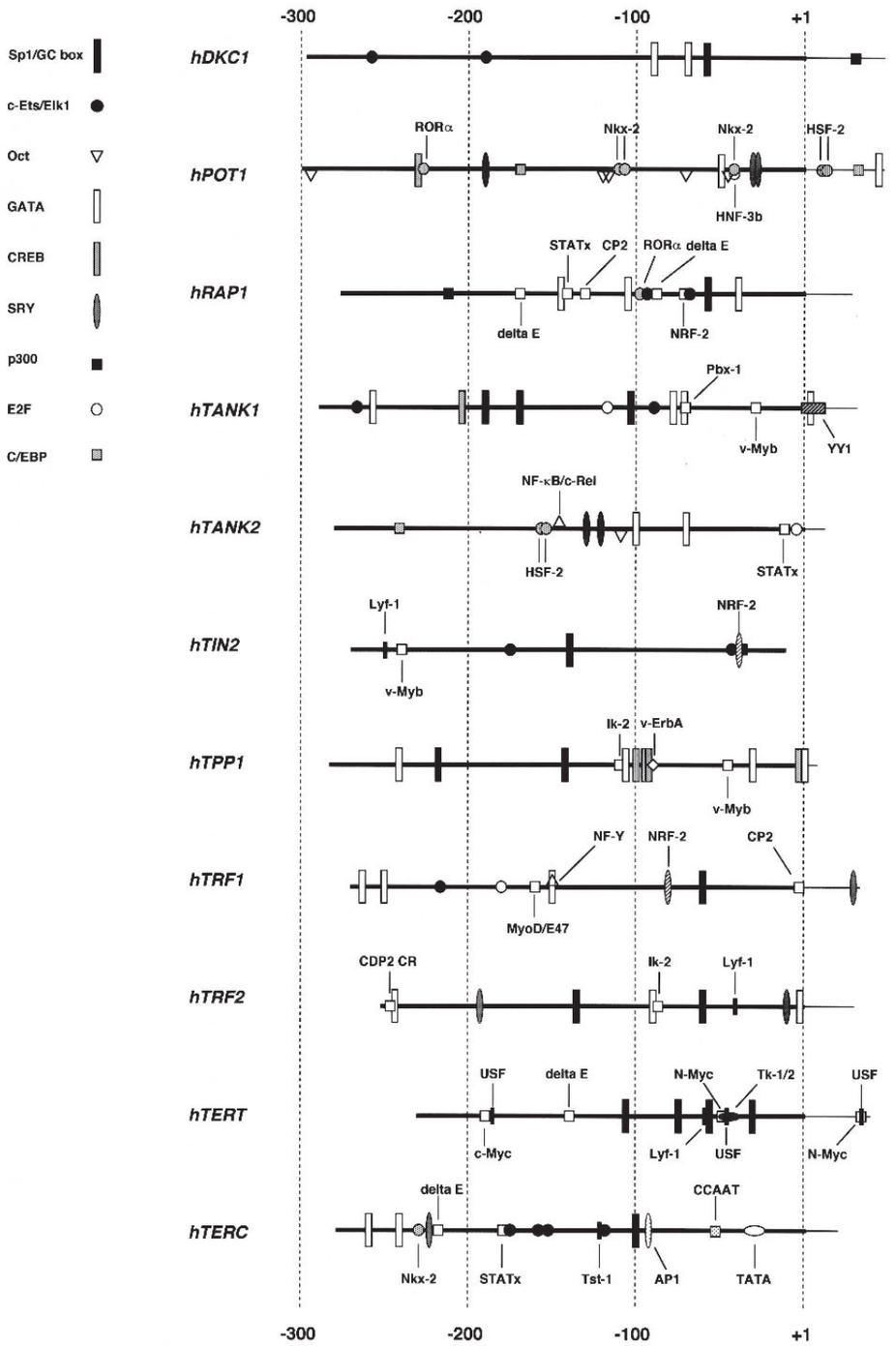



Fig. 1. Promoter regions of the human genes encoding telomere-associated proteins or shelterin protein factors. PCR-amplified 5'-flanking regions of these genes, which were inserted upstream of the *Luciferase* gene of the pGL4-basic vector (pGL4[luc 2.10]), are shown. Transcription start sites (or 5'-end of cDNAs) are designated +1. The TF-SEARCH program (http://www.cbrc.jp/research/db/TFSEARCH.html) was performed and putative transcription-factor binding-elements (score > 85) are shown schematically.

| Reporter | Rsv (10 μM) | Relative Luc activity | Fold |
|---|---|---|---|
| pGL4-PIF1 | - | 1.000 ± 0.033 | 1.00 |
| pGL4-PIF1 | + | 1.000 ± 0.088 | |
| pGL4-RTEL | - | 2.170 ± 0.119 | 1.23 |
| pGL4-RTEL | + | 2.667 ± 0.326 | |
| pGL4-DKC1 | - | 1.271 ± 0.117 | 1.88 |
| pGL4-DKC1 | + | 2.390 ± 0.325** | |
| pGL4-POT1 | - | 0.018 ± 0.003 | 2.53 |
| pGL4-POT1 | + | 0.045 ± 0.008** | |
| pGL4-RAP1 | - | 0.746 ± 0.023 | 1.84 |
| pGL4-RAP1 | + | 1.372 ± 0.164* | |
| pGL4-TANK1 | - | 0.069 ± 0.023 | 1.54 |
| pGL4-TANK1 | + | 0.107 ± 0.014 | |
| pGL4-TANK2 | - | 0.0059 ± 0.00155 | 1.69 |
| pGL4-TANK2 | + | 0.0099 ± 0.00230 | |
| pGL4-TIN2 | - | 0.128 ± 0.022 | 1.48 |
| pGL4-TIN2 | + | 0.190 ± 0.013 | |
| pGL4-TPP1 | - | 0.463 ± 0.032 | 1.54 |
| pGL4-TPP1 | + | 0.714 ± 0.115* | |
| pGL4-TRF1 | - | 0.648 ± 0.078 | 1.83 |
| pGL4-TRF1 | + | 1.189 ± 0.104*** | |
| pGL4-TRF2 | - | 0.139 ± 0.005 | 1.61 |
| pGL4-TRF2 | + | 0.224 ± 0.013*** | |
| pGL4-TERT | - | 0.794 ± 0.042 | 1.93 |
| pGL4-TERT | + | 1.532 ± 0.081*** | |
| pGL4-TERC | - | 0.557 ± 0.142 | 1.97 |
| pGL4-TERC | + | 1.096 ± 0.067* | |

Table 2. Effect of Resveratrol (Rsv) on promoter activities of telomere-associated genes in HeLa-S3 cells Various reporter plasmids were introduced into HeLa-S3 cells by multiple DEAE-dextran method transfections. After 4 h of transfection, the culture medium was discarded and changed to Rsv-containing or non-containing medium. Cells were harvested after 24 h of treatment, then Luc assays were performed. Relative values represent Luc activities compared with that of the pGL4-PIF1 transfected cells. Results show means ± S.D. from three independent samples (N=3). Significance of differences between control and Rsv treated cells were analyzed by Student's *t*-test (*$p<0.05$, **$p<0.01$, ***$p<0.005$).

relatively positive values compared to that of the pGL4-PIF1-transfected cells (Table 3). Similar to the response to Rsv (Table 2), the *TERT* and *TERC* promoters were activated by the 2DG treatment. The increase in relative promoter activity (compared with that of the pGL4-PIF1-transfected cells) after 2DG (8 mM) treatment was significant for the *RTEL*, *DKC1*, *POT1*, *RAP1*, *TANK1*, *TIN2*, *TPP1*, and *TRF1* promoters (Table 3). These results suggest that the CR mimetic compound 2DG affects the balance of gene expression to protect telomeres.

| Reporter | Relative Luc activity | | |
|---|---|---|---|
| | 2DG (mM) | 4 | 8 |
| pGL4-PIF1 | - | 1.000 ± 0.206 | 1.000 ± 0.141 |
| pGL4-PIF1 | + | 1.000 ± 0.230 | 1.000 ± 0.148 |
| pGL4-RTEL | - | 1.800 ± 0.802 | 2.550 ± 0.648 |
| pGL4-RTEL | + | 4.011 ± 0.917 | 6.651 ± 1.958* |
| pGL4-DKC1 | - | 1.136 ± 0.111 | 2.560 ± 0.265 |
| pGL4-DKC1 | + | 8.767 ± 4.556 | 6.698 ± 0.921*** |
| pGL4-POT1 | - | 0.030 ± 0.008 | 0.027 ± 0.010 |
| pGL4-POT1 | + | 0.139 ± 0.065 | 0.102 ± 0.015*** |
| pGL4-RAP1 | - | 0.993 ± 0.247 | 2.201 ± 0.236 |
| pGL4-RAP1 | + | 5.456 ± 1.411* | 4.977 ± 0.749*** |
| pGL4-TANK1 | - | 0.047 ± 0.012 | 0.106 ± 0.012 |
| pGL4-TANK1 | + | 0.272 ± 0.110 | 0.567 ± 0.150* |
| pGL4-TANK2 | - | 0.012 ± 0.003 | 0.006 ± 0.002 |
| pGL4-TANK2 | + | 0.066 ± 0.011*** | 0.033 ± 0.032 |
| pGL4-TIN2 | - | 0.130 ± 0.038 | 0.213 ± 0.023 |
| pGL4-TIN2 | + | 0.686 ± 0.273 | 0.474 ± 0.093** |
| pGL4-TPP1 | - | 0.604 ± 0.151 | 0.751 ± 0.099 |
| pGL4-TPP1 | + | 3.211 ± 0.237*** | 5.721 ± 1.302* |
| pGL4-TRF1 | - | 0.853 ± 0.131 | 1.355 ± 0.279 |
| pGL4-TRF1 | + | 2.178 ± 0.408** | 3.442 ± 0.567** |
| pGL4-TRF2 | - | 0.173 ± 0.073 | 0.232 ± 0.022 |
| pGL4-TRF2 | + | 0.378 ± 0.036* | 0.693 ± 0.244 |
| pGL4-TERT | - | 0.586 ± 0.094 | 1.707 ± 0.316 |
| pGL4-TERT | + | 1.844 ± 0.498* | 3.456 ± 0.963* |
| pGL4-TERC | - | 0.651 ± 0.120 | 0.897 ± 0.119 |
| pGL4-TERC | + | 1.878 ± 0.426** | 2.516 ± 0.507** |

Table 3. Effect of 2-deoxy-D-glucose (2DG) on promoter activities of telomere-associated genes in HeLa-S3 cells Various reporter plasmids were introduced into HeLa-S3 cells by multiple DEAE-dextran method transfections. After 4 h of transfection, the culture medium was discarded and changed to 2DG-containing (4 and 8 mM) or non-containing medium. Cells were harvested after 24 h (4 mM) or 19 h (8 mM) of the 2DG treatment, then Luc assays were performed. Relative values represent Luc activities compared with that of the pGL4-PIF1 transfected cells. Results show means ± S.D. from three independent samples (N=3). Significance of differences between control and 2DG treated cells were analyzed by Student's *t*-test (*$p<0.05$, **$p<0.01$, ***$p<0.005$).

## 4. Discussion

### 4.1 The promoter regions of the shelterin-encoding genes coordinately respond to CR mimetic drugs

In the present study, 5'-flanking regions of different human telomere-associated protein factor-encoding genes were isolated, and these Luc reporter plasmids were used for transient transfection assays. The shelterin- or telomere-associated protein-encoding genes, including *TERT*, *TERC*, *DKC1*, and double-stranded break repair protein-encoding genes,

such as *ATM* and *ATR*, are conserved among human, mouse and yeast (Stern & Bryan, 2008). Given that these telomere-associated proteins are localized to the telomere t-loop to protect the specific structure, and appear to act in co-operation with each other (O'Sullivan & Karlseder, 2010), their gene expression should be regulated synchronously when the telomeric region needs to be protected. Aging or cellular senescence are thought to be controlled by a genomic maintenance regulatory system (Vieg, 2007). Our hypothesis is that aging or longevity affecting reagents might have an effect on the expression of the telomere-associating protein-encoding genes. The results (Tables 2 and 3) indicate that promoter activities of the shelterin-encoding genes are simultaneously up-regulated by Rsv and 2DG in HeLa-S3 cells when they are compared with *PIF1* promoter activity. Previously, we have reported that multiple GC-boxes are commonly located in the human *TERT* and *WRN* promoter regions and that might play a role in the positive response to Rsv and 2DG in HeLa-S3 cells (Uchiumi et al., 2010c). Although there are no canonical roles of transcription factor binding elements or their order in these promoter regions, Sp1 binding elements or GC-boxes are found in all of them except 5'-upstream of the *POT1* and *TANK2* genes (Fig. 1). Therefore, GC-box binding factors may up-regulate this telomere-associated gene expression. However, there are no GC-box like motifs in the 300-bp up-stream regions of the *POT1* and *TANK2* genes, which are relatively AT-rich and contain Oct-1 binding sites. This observation suggests that POU family proteins might also be involved in the positive regulation of these genes. Apparent up-regulation of promoter activities by Rsv and 2DG treatment was observed in the cells transfected with the Luc reporter plasmids containing 200-bp 5'-upstream regions of the *TERT* and *TERC* genes (Tables 2 and 3). It is noteworthy that the duplicated GGAA-motifs are found in both promoter regions (Uchiumi et al., 2010a, Uchiumi et al., 2011b), suggesting that the GGAA-motif binding factors, including Ets family proteins, might be involved in the positive response to the aging or longevity affecting signals.

Previously, we observed elevation of the human *WRN* promoter activity in accordance with activation of telomerase after Rsv and 2DG treatment of HeLa-S3 cells (Uchiumi et al., 2011; Zhou et al., 2009). 2DG suppresses glucose metabolism to establish a limit for the usage or uptake of glucose into cells (Roth et al., 2001). On the other hand, Rsv is known to activate sirtuin family protein deacetylases (Kaeberlein, 2010). It is thought that both Rsv and 2DG are CR mimetic drugs (Stefani et al., 2007; Roth et al., 2001), and that CR can extend the mean and maximum life spans of numerous organisms (Carvallini et al., 2008; Roth et al., 2001). The present study suggests that induction of telomerase activity in concert with up-regulation of the telomere-associated protein- or shelterin-encoding gene expression may play a role in regulating the aging process through the telomere maintainance system.

### 4.2 A possible role for telomere maintenance system in aging/senescence regulation

Aging or senescence is a complicated biological process involving various regulatory factors (Campisi & d'Adda di Fagagna, 2007; Kuningas et al., 2008; Sanz & Stefanatos, 2008). Aging could be explained by a mitochondrial free radical theory (Benz & Yau, 2008). On the other hand, cellular senescence could be caused by DNA damage or the associated signals on chromosomes (Vieg, 2007). It is well known that cellular senescence is correlated with the cell growth arrest (Campisi & d'Adda di Fagagna, 2007). DNA damage signals activate ATM or ATR, and then phosphrylate p53 to induce transcription of the *CDKN1A* gene that encodes cyclin-dependent kinase inhibitor 1A (p21). These sequentially occurring events arrest the cell cycle at G1-phase (Meek, 2009). Repair of DNA damage will occur at this stage, unless the cell has initiated apoptosis. Thus, aging is thought to be controlled through both reactive oxygen

species (ROS) generated by mitochondria and damages to DNA including telomeric regions of the chromosomes (Sahin & DePinho, 2010). Recently, it was shown that telomere dysfunction causes activation of p53 which directly represses PGC-1α and PGC-1β, leading to mitochondrial compromise (Sahin et al., 2011). Moreover, an experiment to reactivate telomerase in telomerase-deficient mice ameliorated DNA damage signaling and reversed neurodegeneration (Jaskelioff et al., 2011). These lines of evidence suggest that telomeres exert signals to affect mitochondria along with DNA repair systems. The concept that telomere length-associated signaling stimulates mitochondrial function might have combined the mitochondrial free radical theory with the molecular mechanism of chromosomal maintenance system against DNA damaging stresses. Rsv has been shown to have effect activation of PGC-1α to improve mitochondrial function in mouse brown adipose tissue and muscle (Lagouge et al., 2006). The present study indicates that shelterin protein-encoding gene promoters are simultaneously activated by Rsv treatment. Thereby, accumulation of shelterin proteins might lead to stabilization of telomeric regions of the chromosome and activation of PGC-1α.

### 4.3 Hormesis, the beneficial effects from low doses of toxic stresses, might be a determinant of longevity

The deficiencies in RecQ DNA helicases, including WRN and BLM, are known to cause premature aging (Chu & Hickson, 2009). In the present study, we have observed that CR mimetic drug treatment activates promoters of the shelterin protein-encoding genes, suggesting that CR evokes functions of the telomere maintenance machinery. Hormesis is a phenomenon that generally refers to the beneficial effects from low level toxic or other harmful damage, such as irradiation, heat shock, or food restriction (Schumacher, 2009). High doses of 2DG and Rsv have harmful or toxic effects on cells, leading to cell death or apoptosis (Lin et al., 2003; Cosan, et al., 2010). In contrast, relatively low doses of these CR mimetic reagents, as used in the present study, have effects similar to hormesis. Therefore, resistance to stresses eventually provoked by prolonged low doses of CR mimetic reagents could promote the longevity of organisms. Thus, the results obtained in the present study are consistent with the concept of hormesis.

### 4.4 Molecules that are involved in the regulation of the aging process

From studies of life spans of the *C. elegans*, it has been suggested that the insulin/IGF-1 pathway influences aging (Kenyon, 2010). In this signaling system, DAF-16 (FoxO transcription factor) plays a role in activating genes that act to extend life span (van der Horst & Burgering, 2007). AMP-activated protein kinase (AMPK), which is known to extend the life span of nematodes (Apfeld et al., 2004), phosphorylates FoxO, PGC-1α, and CREB to induce various genes encoding mitochondrial and oxidative metabolism regulating factors (Cantó & Auwerx, 2010). The other biologically important function of AMPK is that it blocks the mTOR (mammalian target of rapamycin) pathway (Cantó & Auwerx, 2010). A recent study suggested that mTOR is a prime target in the genetic control of aging to determine life span and aging in yeast, worms, flies, and mice (Zoncu et al., 2011). The mTOR pathway accelerates growth by regulating signals downstream of insulin/IGF-1 receptors (Zoncu et al., 2011). Activation of mTOR is thought to speed up aging in adulthood, and reduced mTOR signaling would have the opposite effect, acting downstream of dietary restriction. Thus the anti-aging effect could be expected by mTOR inhibition, such as dietary restriction, rapamycin, introduction of the AMPK expression vector, and genetic inactivation of mTOR by techniques such as RNA interference.

## 5. Conclusions and future perspectives

It would be advantageous for cells to estimate the state of chromosomes just by monitoring telomeric regions. Monitoring the somatic genes, including promoter, exon, intron or other regions that harbors genetic information, would not work for that purpose, because single or multiple mutations might be lethal to the cell. Thus, microsatellite regions, including telomeres, would be suitable for a DNA damage monitoring system. Recently, it was shown that telomere length regulates mitochondrial function by activating PGC-1α (Sahin et al., 2011). This effect is the same as Rsv treatment (Lagouge et al., 2006). The CR mimetic drugs may have a common role in strengthening telomere maintenance. In the present study, we performed a multiple transfection experiment, which showed that shelterin protein-encoding gene promoters simultaneously respond to CR mimetic drugs in HeLa-S3 cells. Given that anti-aging drugs induce or activate the DNA repair system, especially by maintenance of telomeres, this multiple transfection system has demonstrable potential to contribute to the evaluation and development of such drugs.

## 6. Acknowledgments

The authors are grateful to Ryosuke Akiyama for outstanding technical assistance. This work was supported in part by a Research Fellowship from the Research Center for RNA Science, RIST, Tokyo University of Science, Tokyo, Japan.

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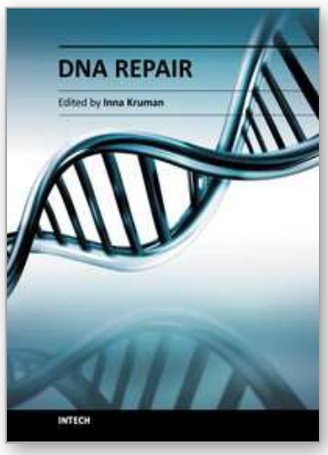
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**DNA Repair**

Edited by Dr. Inna Kruman

ISBN 978-953-307-697-3
Hard cover, 636 pages
**Publisher** InTech
**Published online** 07, November, 2011
**Published in print edition** November, 2011

The book consists of 31 chapters, divided into six parts. Each chapter is written by one or several experts in the corresponding area. The scope of the book varies from the DNA damage response and DNA repair mechanisms to evolutionary aspects of DNA repair, providing a snapshot of current understanding of the DNA repair processes. A collection of articles presented by active and laboratory-based investigators provides a clear understanding of the recent advances in the field of DNA repair.

**How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Fumiaki Uchiumi, Takahiro Oyama, Kensuke Ozaki and Sei-ichi Tanuma (2011). Characterization of 5'-Flanking Regions of Various Human Telomere Maintenance Factor-Encoding Genes, DNA Repair, Dr. Inna Kruman (Ed.), ISBN: 978-953-307-697-3, InTech, Available from: http://www.intechopen.com/books/dna-repair/characterization-of-5-flanking-regions-of-various-human-telomere-maintenance-factor-encoding-genes

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