

Joseph A. Baur · Jerry W. Shay · Woodring E. Wright

Spontaneous reactivation of a silent telomeric transgene in a human cell line

Received: 26 September 2003 / Revised: 26 November 2003 / Accepted: 2 December 2003 / Published online: 20 January 2004
© Springer-Verlag 2004

Abstract Subtelomeric reporter genes in human cells are silenced in a telomere length-dependent manner. Here we show that a subtelomeric reporter gene is expressed in only a subpopulation of cells within a clone and that this heterogeneity is generated by switching between expression states. We observed frequent reversion from the silenced state back to active expression. This process was more prominent for subtelomeric transgenes; however, we also observed cases of spontaneous reversion in control clones bearing the reporter at an internal site. We additionally show that treatment of these cells with 5-bromodeoxyuridine results in strong activation of the transgene. Although similar findings have been reported previously in mouse cells, this is, to our knowledge, the first direct observation of ongoing fluctuations in transcription in clonal populations of human cells. Our results suggest that this mechanism, as opposed to progressive silencing or a delayed fixing of expression states, accounts for the variegation in expression observed for subtelomeric transgenes in human cells. These data imply that telomere shortening during human aging could lead to stochastic activation of subtelomeric genes.

Abbreviations *BrdU*: 5-Bromodeoxyuridine · *CMV*: Cytomegalovirus · *DsRed2*: *Discosoma sp.* red fluorescent protein · *FACS*: Fluorescence activated cell sorting · *hTERT*: Human telomerase reverse transcriptase · *hTPE*: Human telomere position effect · *IRES*: Internal ribosome entry site · *TPE*: Telomere position effect · *TRF*: Terminal restriction fragment (analysis) · *TSA*: Trichostatin A

Introduction

Silencing near telomeres, termed telomere position effect (TPE), was first observed in *Drosophila* (Hazelrigg et al. 1984) and has since been studied extensively in *Saccharomyces cerevisiae* and other lower organisms (Gottschling et al. 1990; Tham and Zakian 2002). It is characterized by the semi-stable repression of subtelomeric reporter genes in a manner that is proportional to both the length of the telomere tract (Kyrion et al. 1993) and the proximity of the reporter to the telomere (Renauld et al. 1993). Telomere position effect was recently reported to affect an endogenous *S. cerevisiae* gene of unknown function (Vega-Palas et al. 2000). Although the involvement of telomeric silencing has been suggested in the regulation of surface antigen expression for several parasites (Horn and Cross 1995; Tham and Zakian 2002), a biologically relevant role for TPE has yet to be demonstrated.

One of the hallmarks of TPE in yeast is the spontaneous switching of a telomeric gene from an active to a silent state and vice versa. Our initial studies using a luciferase reporter to demonstrate silencing near human telomeres (human telomere position effect, or hTPE) (Baur et al. 2001) did not allow analysis at the single-cell level. We and others (Koering et al. 2002) have now confirmed the existence of human hTPE using fluorescent reporter genes in order to detect single cells. While the fraction of expressing cells varies widely in internal control clones, cell lines bearing a telomeric reporter consistently show expression in only a few percent of the cells. Such a pattern could conceivably be generated by several mechanisms, including slow progressive silencing, fixing of expression states at some point after the first few divisions (for example the chromatin structure at the integration site might take time to stabilize), or stochastic switching between expression states. While progressive silencing has been observed for transgenes in mammalian cells (Walters et al. 1996), available data on transgene expression at yeast telomeres (Gottschling et al. 1990) and at other variegating loci within mammalian cells

Communicated by V. Zakian

J. A. Baur · J. W. Shay · W. E. Wright (✉)
Department of Cell Biology,
University of Texas Southwestern Medical Center,
Dallas, TX 75390-9039, USA
e-mail: Woodring.Wright@UTSouthwestern.edu

(Feng et al. 1999; Ronai et al. 1999) favor the third hypothesis. In the only published report examining TPE in human cells at the single-cell level, however, switching between expression states was not detected (Koering et al. 2002), raising the possibility that variegation in these cells is generated through one of the other two mechanisms. Here we show that in HeLa cells spontaneous reactivation of the transgene can be detected in initially negative subclones, demonstrating that heterogeneity is in fact generated through an ongoing stochastic process of switching between expression states.

Materials and methods

Generation of clones

The construction of luciferase-expressing clones has been described previously (Baur et al. 2001). To generate DsRed-expressing clones, the *AflIII/BfrI* fragment (containing the gene encoding the DsRed1 protein) from pDsRed1-N1 (Clontech, Palo Alto, Calif.) was blunt ligated into the *SmaI/HpaI* backbone from pSX-neo1.6T₂AG₃ (Hanish et al. 1994) such that the Cytomegalovirus (CMV) promoter was placed at the base of the telomere repeats. Next, a blunted fragment containing an internal ribosome entry site (IRES) and the blasticidin resistance gene was ligated into the *HpaI* site in the same orientation as the DsRed1 protein. When DsRed2 became available, the new coding region was inserted by exchanging the DsRed fragment defined by *SalI/NotI* (this required a partial digest with *NotI*) and the resulting vector was designated pSX2. The control vector lacking repeats was generated by blunt ligation after excision of the *ClaI/SacII* fragment. Vectors were linearized with *ClaI/PvuI* (with repeats) or *PvuI* alone (without repeats) and transfected into HeLa cells using FuGENE 6 transfection reagent (Roche, Basel, Switzerland) according to the manufacturer's instructions. Stable clones were obtained by ring-cloning after selection in 1 μ g/ml blasticidin for 1 week. Individual clones were isolated by placing a glass ring over them (sealed with vacuum grease) and transferred to separate dishes by standard trypsinization methods.

Analysis of clones

Cells were observed primarily on a Zeiss Axiovert 100M inverted microscope attached to a MacIntosh G4 computer using Openlab imaging software. Scanning was performed on a FACScan (Becton Dickinson, San Jose, Calif.).

Initial subcloning

Cells were plated on several 10 cm dishes at a density of 300 cells/dish. The next day, well-isolated subclones were selected and photographed. Bright-field and fluorescent images were collected at regular intervals in order to follow changes in the expression pattern as each clone grew.

Subcloning into 96-well plates

Single cells were sorted into each well of a 96-well plate using a MoFlo high-speed cell sorter (Cytomation, Fort Collins, Colo.) and a FACStar Plus (Becton Dickinson, San Jose, Calif.). Sorting into 96-well plates was an automated feature of the cell sorters. Medium (100 μ l) was placed in each well prior to sorting and cells were selected by size only (to avoid fragments and doublets). Wells containing cells were identified by microscopy the following day (2–4 cell stage) and the remainder of each well was checked

carefully to ensure that no other cells were present. Cells were observed every 2–3 days thereafter for at least 3 weeks.

Retroviral infections

The amphotrophic retroviral packaging cell line PA317 (Miller 1990) was infected using supernatants from PE501 cells that had been transiently transfected with retroviral plasmid DNA. Following selection, supernatants were harvested from PA317 cells, purified by passage through a 0.45 μ m sterile filter, and stored at –80°C for later use. Infection of target cells was carried out by 8–16 h exposure to supernatant diluted 1:2 in regular medium with 4 μ g/ml (final) polybrene (Sigma, St. Louis, Mo.). Cells were then allowed to recover for 12–24 h before selection.

Terminal restriction fragment (TRF) analysis

Cells were suspended in 100 mM NaCl, 100 mM EDTA, and 10 mM TRIS, pH 8 at 20,000 cells/ μ l. Genomic DNA was extracted by bringing the final concentrations of Triton X-100 and proteinase K up to 1% and 2 mg/ml, respectively, and incubating for 12 h at 55°C, followed by inactivation of proteinase K at 70°C for 30 min. Samples were then dialyzed overnight against TE, pH 8. After dialysis, 1 μ g DNA was digested with a mixture of six restriction enzymes (*AluI*, *CfoI*, *HaeI*, *HinfI*, *MspI*, and *RsaI*) with 4 bp target sites and run on a 0.7% agarose gel overnight at 70 V. The gel was denatured for 20 min in 0.5 M NaOH and 1.5 M NaCl, rinsed 10 min in water, dried 1 h at 55°C, neutralized for 15 min in 1.5 M NaCl and 0.5 M TRIS, pH 8, and probed with ³²P-labeled (T₂AG₃)₄. After washing in 2 \times SSC for 15 min and 0.1 \times SSC with 0.1% SDS twice for 10 min, the gel was exposed to a Phosphor screen and analyzed using a Storm 860 PhosphorImager (Molecular Dynamics/Amersham, Piscataway, N.J.).

5-Bromodeoxyuridine treatment

Cells were treated with 50 μ M 5-bromodeoxyuridine (BrdU) for 2–5 days in regular medium. A small decrease in growth rate was noted; however, toxicity was dramatically reduced as compared with trichostatin A (TSA) treatment.

Results

In order to detect switching in human cells, we used a linearized plasmid containing telomere repeats (Hanish et al. 1994) to construct a series of clones bearing the fluorescent reporter DsRed2 either next to a newly formed telomere, or randomly integrated into the genome (Fig. 1A). Clones were verified by Southern blot hybridization and those containing multiple copies of the DsRed2 construct were discarded. While the number of cells expressing the reporter varied from 0 to nearly 100% in our internal controls (random integration), all of the telomeric clones recovered in our initial experiment expressed the DsRed2 protein in only a few percent of the cells (Fig. 1C), consistent with the findings of Koering et al. (2002). The fact that expression near human telomeres can be increased by the histone deacetylase inhibitor TSA (Baur et al. 2001; Koering et al. 2002), but not by the demethylating agent 5-azacytidine (Koering et al. 2002), suggests that a methylation-independent process causes the majority of the silencing in these cells.

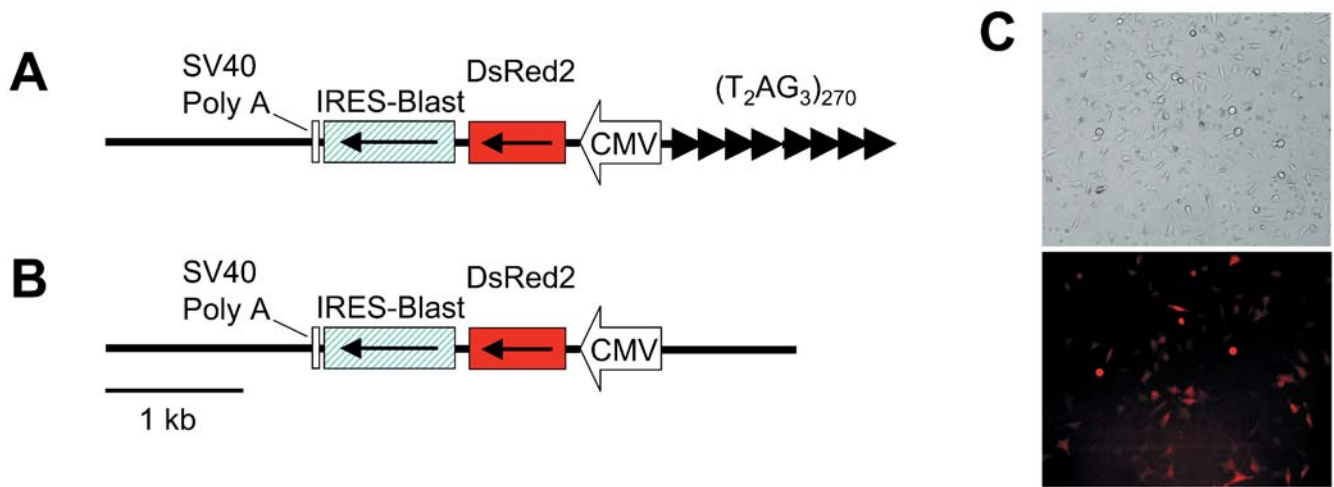


Fig. 1 A, B Structure of the constructs used in the generation of clones. Both vectors were transfected into HeLa cells in linear form using FuGENE 6 transfection reagent (Roche, Basel, Switzerland). In the chromosome truncation vector (**A**), the CMV promoter was located approximately 80 nucleotides from the base of the T_2AG_3 repeats. The fluorescent protein DsRed2 was transcribed away from the telomere and an internal ribosome entry site (*IRES*) was used to

allow expression of the blasticidin resistance gene (*Blast*) from the same transcript. Transcription was terminated using the SV40 polyadenylation signal (*SV40 Poly A*). In the vector used to generate internal (control) integrations (**B**), the T_2AG_3 repeats were replaced with an additional 1 kb of plasmid sequences. **C** Bright field and fluorescent images of a representative clone bearing a telomeric DsRed2 reporter

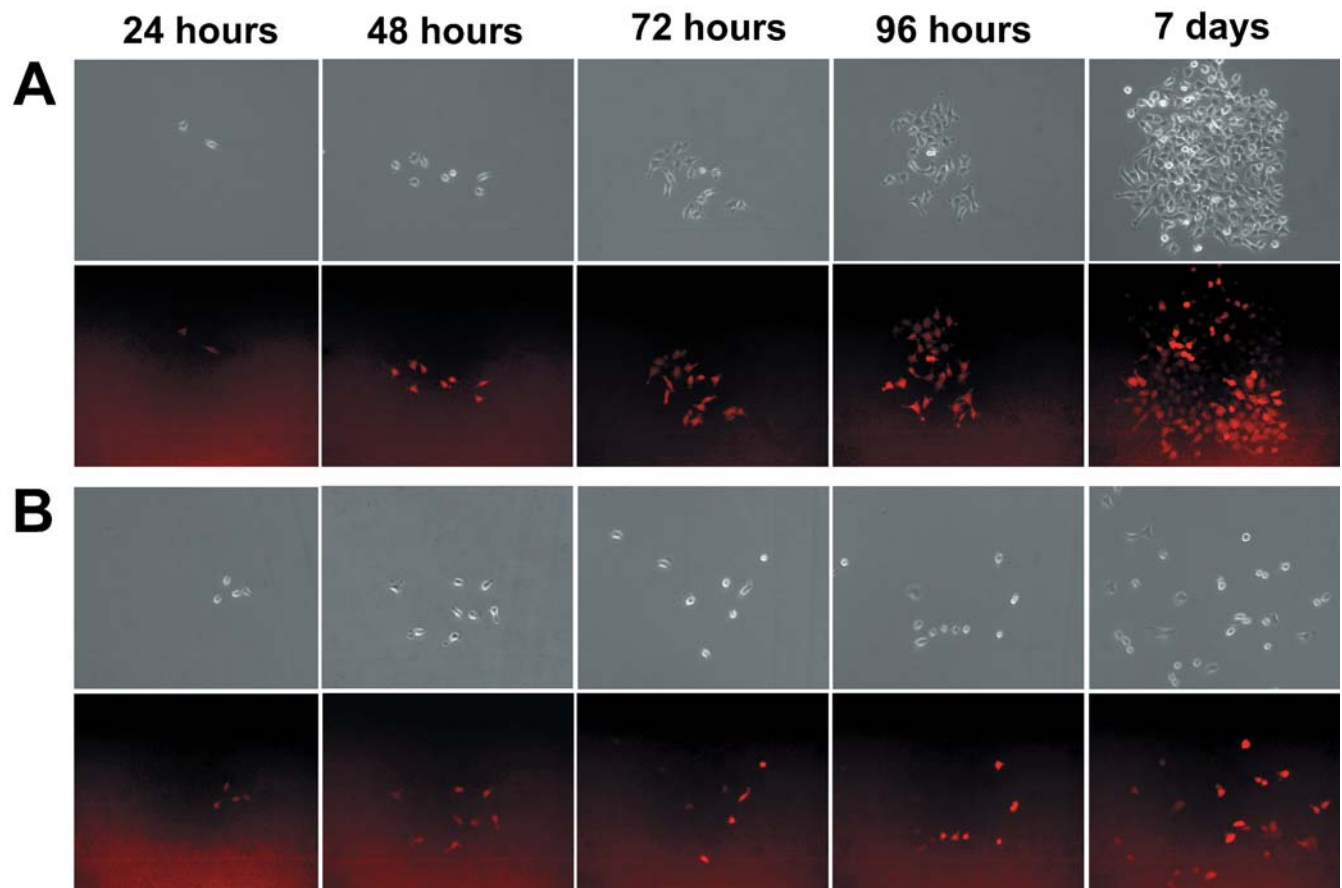


Fig. 2A, B Development of heterogeneity within subclones bearing a telomeric reporter. Paired bright field and fluorescent images are shown for two different subclones at five time points. While all

cells initially express a uniform level of the DsRed2 protein (24 and 48 h time points), significant differences become apparent by 72 h (**B**) or 96 h (**A**), and both show obvious heterogeneity by 1 week

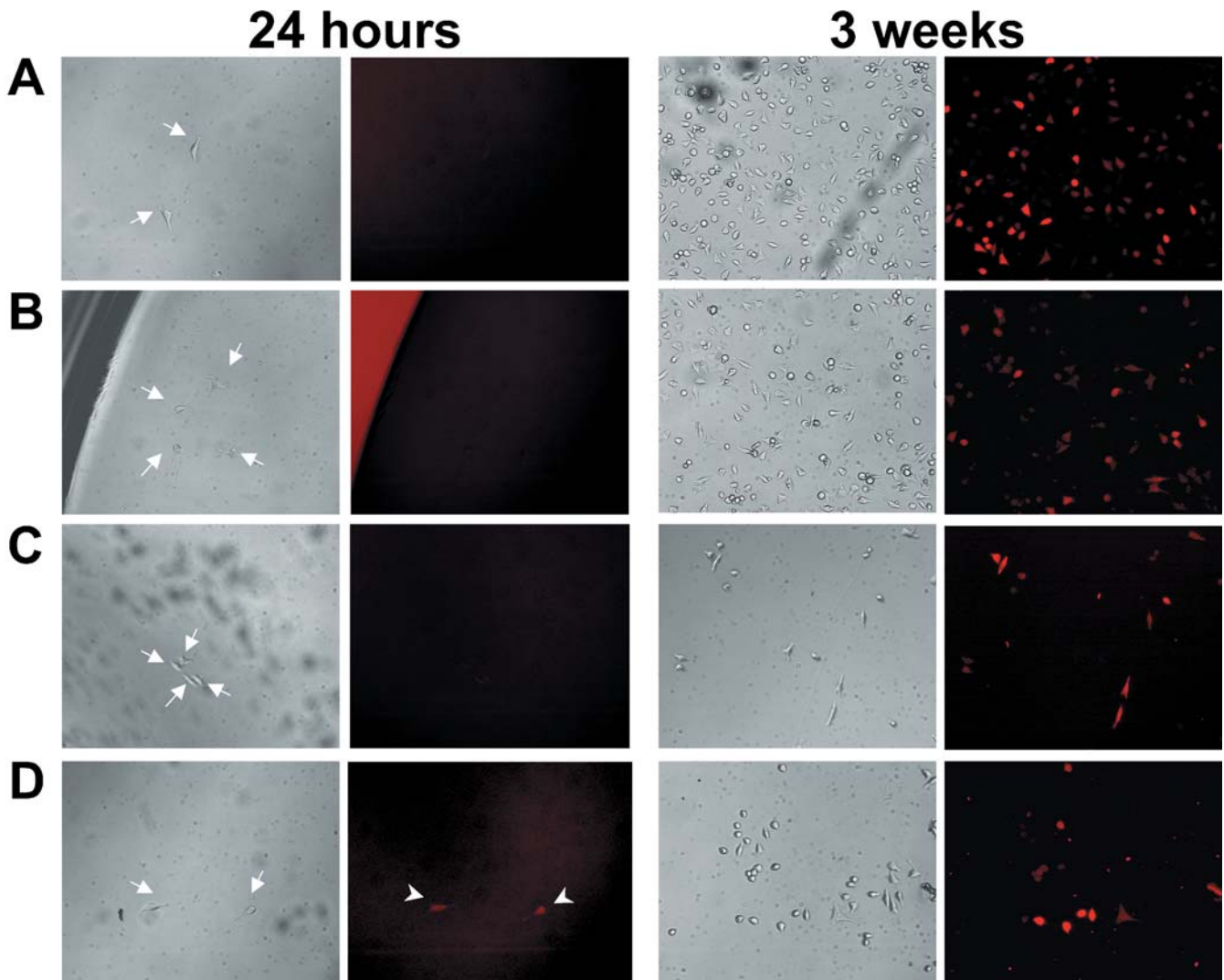


Fig. 3A–D Spontaneous reversal of silencing in cells bearing a telomeric reporter gene. Each row represents a different subclone derived from a single parental clone in which the gene for DsRed2 fluorescent protein had been placed next to a newly formed telomere. Subclones were initially negative (A–C) or very weak (D) for DsRed2 expression but by 3 weeks after subcloning most

had developed sporadic expression in a small fraction of cells, resembling the pattern of expression in the parental cell line. *Arrows* indicate the positions of cells in the original bright field images. *Arrowheads* indicate weakly positive cells in the fluorescent image of subclone D

A previous attempt to demonstrate switching in human cells using fluorescent activated cell (FAC) sorted populations was not successful (Koering et al. 2002). Because FAC sorting cannot produce a 100% pure population of positive or negative cells, we chose to examine the progeny of single positive or negative cells by fluorescence microscopy. The presence of cells of opposite phenotype in a growing subclone would clearly indicate that a switching event had occurred. We subcloned cells bearing a single telomeric insertion of the DsRed2 construct and followed their growth and expression of the transgene (Fig. 2). In each case, cells that were initially positive gave rise to mixed populations within one week. Positive cells arising from initially negative cells were observed only rarely during this first experiment and the possibility that these rare cells were

the result of contamination by floating cells could not be ruled out.

To demonstrate more clearly spontaneous reactivation of the transgene, we subcloned the progeny of a single cell into 96-well plates to eliminate the possibility of cross-contamination by floating cells. Growth of each subclone was then followed by fluorescence microscopy for an extended period of time. Each subclone was first observed at the 1- to 4-cell stage and was grown for approximately 20 population doublings. In 18 of 19 subclones that were initially negative, DsRed2-expressing cells were detected within 1 to 2 weeks (Fig. 3A–C), with 11 of the subclones becoming similar in appearance to the parent clone by week 3. Subclones that were initially weakly positive gave both strongly positive and completely negative cells within 1 to 2 weeks (Fig. 3D). Our

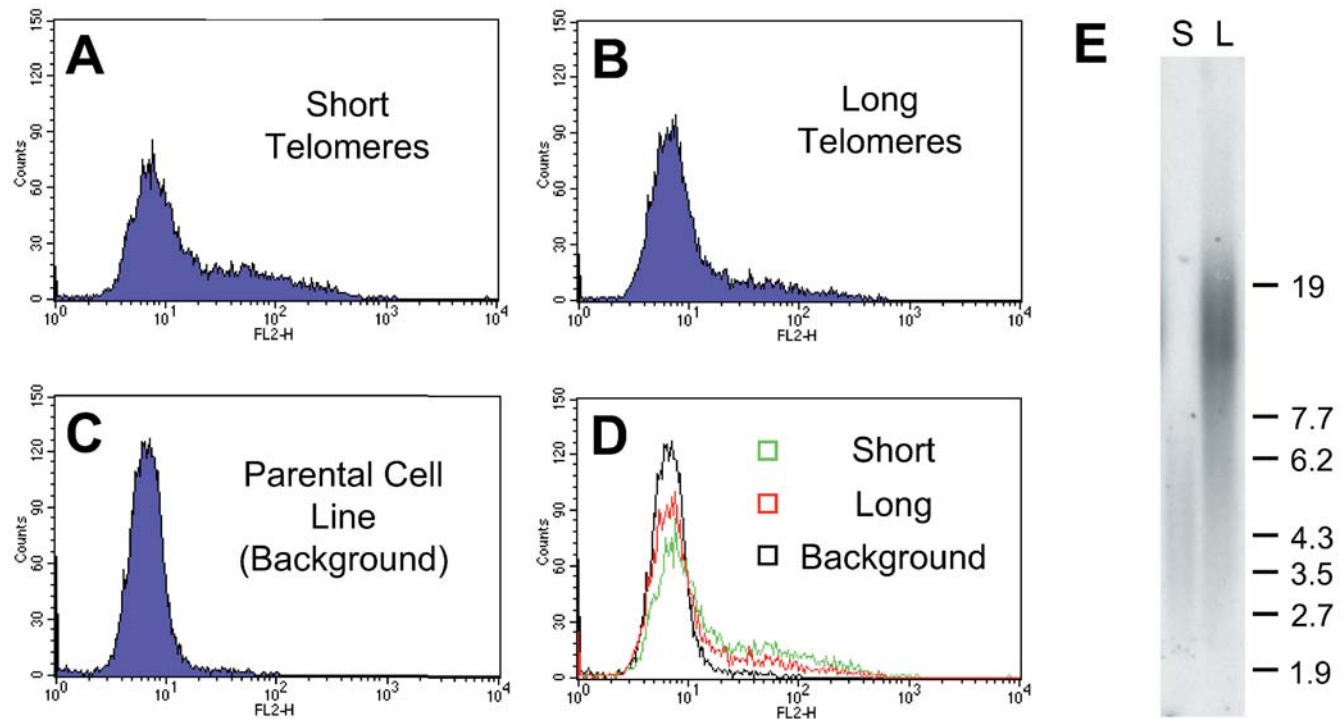


Fig. 4A–D Elongation of telomeres by hTERT overexpression decreases expression of DsRed2 in telomeric clones. Clones were infected with an empty vector (pBabe) or a retrovirus encoding the telomerase catalytic component (hTERT). Fluorescence-activated cell sorting (FACS) analysis is shown for a cell line bearing a telomeric DsRed2 reporter before telomere elongation (**A**) or after telomere elongation (**B**). FACS analysis of the parental cell line,

which does not express DsRed2, is shown in **C**, and the three histograms are overlaid in **D**. **E** Terminal restriction fragment (TRF) analysis of the clones with long (*L*) and short (*S*) telomeres. Genomic DNA was digested with a mixture of six restriction enzymes, separated on a 0.7% agarose gel, and probed with an oligonucleotide complementary to telomere repeats. Markers shown are λ *Sty* fragments (in kilobases)

data suggest that TPE in these cells resembles the variegation observed at yeast telomeres (Gottschling et al. 1990) and in the cells of transgenic mice (Rakyan et al. 2002), where all-or-none patterns of switching predominate over gradual changes in gene expression (Robertson et al. 1995; Ronai et al. 1999). The fact that Koering et al. (2002) did not observe fluctuations in expression at human telomeres likely indicates that cell type, the site of chromosome truncation, and/or the direction of transcription can influence the rate of switching.

We previously demonstrated, using a luciferase reporter, that telomere elongation enhances the silencing of telomeric transgenes (Baur et al. 2001). Elongation of telomeres in cells bearing a telomeric DsRed2 reporter also leads to a reduction in transgene expression (Fig. 4). The reduction in reporter expression is manifested primarily as a decrease in the fraction of positive cells, as opposed to intensity per cell, consistent with previous reports in human cells (Walters et al. 1995, 1996). This suggests that telomere elongation enhances silencing through effects on the heritability of the two states, as opposed to reducing the level of expression in the active state.

5-Bromodeoxyuridine has been shown to suppress position-effect variegation at internal loci in HeLa cells by a mechanism that remains poorly understood (Suzuki

et al. 2001). Treating clones bearing a telomeric reporter gene with BrdU resulted in a loss of variegation and an enhancement of transgene expression. Relief of silencing for a telomeric DsRed2 reporter was observed after a 72 h incubation in medium containing 50 μ M BrdU (Fig. 5A). Silencing was also relieved in low-expressing internal controls and in cells bearing a luciferase reporter at the telomere (Fig. 5B). It has previously been reported that silencing near telomeres can be relieved using the histone deacetylase inhibitor TSA (Baur et al. 2001; Koering et al. 2002); however, BrdU may be a preferable alternative in the future since it is significantly less toxic at the effective dose (data not shown). The fact that BrdU suppresses position-effect variegation of transgenes at both internal and telomeric loci suggests a common mechanism of repression.

As early as 1972, Lin and Riggs were able to show that incorporation of BrdU into DNA increases the binding affinities of certain proteins, including histone H1 (Lin and Riggs 1972; Lin et al. 1976). Since histone H1 binds to AT-rich regions of DNA that mediate scaffold/matrix attachment and are known to have dramatic effects on neighboring promoters (Blasquez et al. 1989; Stief et al. 1989; Klehr et al. 1991), it was hypothesized that BrdU might affect binding of DNA to the nuclear matrix (Suzuki et al. 2001). Indeed, BrdU has recently been

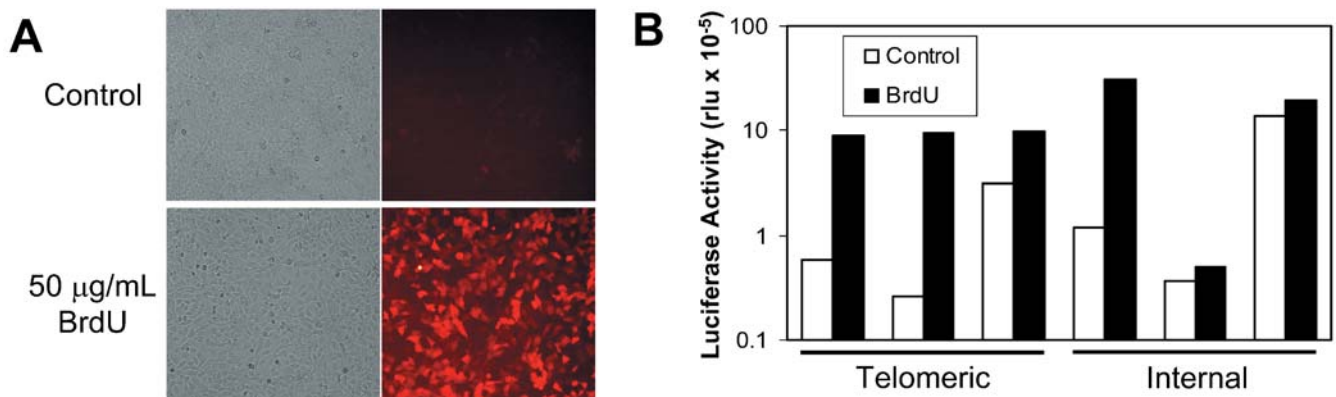


Fig. 5 Loss of telomeric silencing in the presence of 5-bromodeoxyuridine (*BrdU*). Cells bearing a telomeric reporter were grown in regular medium (*Control*) or medium supplemented with 50 $\mu\text{g/ml}$ BrdU for 72 h before analysis. **A** Expression of DsRed2 was dramatically upregulated in the presence of BrdU and toxicity was minimal compared with that observed when the cells were treated with trichostatin A (TSA). As with TSA (Baur et al. 2001), BrdU also affected silencing in low-expressing internal control clones

(data not shown). **B** Silencing of a luciferase reporter was relieved after BrdU treatment in both telomeric and internal clones. Representative data are presented showing that expression after treatment is higher and more uniform between clones. Occasional resistant clones were noted among the internal controls (*middle*) and may represent cases in which methylation or rearrangement of the gene has occurred

shown to enhance binding of scaffold/matrix attachment regions to the nuclear matrix, and, moreover, the effects of BrdU are potentiated by competitive inhibitors of histone H1 binding (Suzuki et al. 2001; Ogino et al. 2002). These findings are especially intriguing in the light of the fact that human telomeres are attached to the nuclear matrix via their TTAGGG repeats (de Lange 1992). Elucidation of the role played by the nuclear matrix in hTPE will be an interesting area for future studies.

Discussion

We have shown in human cells that a clonal population derived from a cell bearing a telomeric reporter gene can acquire a variegated phenotype through a stochastic process of switching between expression states. Taken together with previous studies of transgene expression in mammalian cells (Walters et al. 1995, 1996; Dorer 1997; Feng et al. 1999; Ronai et al. 1999), our data suggest that many repressive loci within the human genome may be similar in this respect. The fact that expression at both telomeric and internal loci is enhanced by treatment with either TSA or BrdU is also suggestive of a common silencing mechanism.

At present, TPE is distinguished from other repressive effects in human cells only by its dependence on telomere length and its relative strength (tenfold lower expression on average relative to internal loci) (Baur et al. 2001). We observed 100% penetrance of variegation within the first 2 weeks of clonal growth in telomeric clones as opposed to ~50% in internal controls (our unpublished results), although at later time points many of the internal controls that were initially uniform in expression did become more heterogeneous. Some suggestive evidence has been presented that heterochromatin protein 1 (HP1) may be

specifically involved in telomeric silencing (Koering et al. 2002). Overexpression of HP1 was previously shown to enhance variegation at centromeric loci while suppressing variegation at non-centromeric loci (Festenstein et al. 1999). This suggests that variegation may occur by at least two distinct mechanisms in mammalian cells despite the similar outward appearances of various mammalian position effects. It will be interesting in the future to see whether or not a specific set of proteins is involved in telomeric silencing.

A change in the expression of endogenous human genes regulated by telomere length has not yet been demonstrated. The present results suggest that if such regulation occurs, it will likely be manifested primarily by an “all-or-none” change in expression in a small fraction of cells rather than a quantitative increase in expression per cell. However, detailed studies in yeast have revealed regulatory elements at endogenous telomeres that produce marked differences in transgene behavior as compared with truncated chromosome ends (Fourel et al. 1999; Pryde and Louis 1999). Whether or not endogenous human telomeres possess elements that might alter or block TPE remains to be seen.

It has been suggested that the high frequency of duplications and rearrangements in subtelomeric regions may facilitate “rapid adaptive evolution” (Mefford and Trask 2002). This hypothesis is supported by the finding that a block of subtelomeric sequence containing three olfactory receptor genes is duplicated polymorphically on at least 14 different chromosome ends in humans but is single copy in non-human primates (Trask et al. 1998). Telomere position effect may contribute to this process through silencing of subtelomeric genes and the production of a variegated phenotype, allowing the majority of cells to progress through potentially harmful genetic intermediates while continuously sampling the products

of rearranged genes. A similar role has been proposed for prion proteins in the generation of genetic diversity (True and Lindquist 2000). Collapse of the silent domains near human telomeres (i.e. loss of silencing due to telomere shortening) might be predicted to trigger inappropriate expression of a host of rearranged subtelomeric genes as part of the aging process. It is also possible that the telomere shortening accompanying cell division might provide a method for timing changes that occur over many years in long-lived organisms. It is difficult to imagine how conventional timing mechanisms (biochemical oscillations, lunar cycles of changes, changing gradients during early development) could be adapted to count decades. Telomere position effect-regulated changes in gene expression produced by slow but ongoing cell turnover could provide such a mechanism. Clearly further study is required to elucidate the role (if any) of TPE in human gene regulation, aging, and disease.

Acknowledgements This work was supported by Department of Defense grant BC000422 (J.A.B.) and National Institutes of Health grant AG07792 (W.E.W. and J.W.S.)

References

- Baur JA, Zou Y, Shay JW, Wright WE (2001) Telomere position effect in human cells. *Science* 292:2075–2077
- Blasquez VC, Xu M, Moses SC, Garrard WT (1989) Immunoglobulin kappa gene expression after stable integration. I. Role of the intronic MAR and enhancer in plasmacytoma cells. *J Biol Chem* 264:21183–21189
- de Lange T (1992) Human telomeres are attached to the nuclear matrix. *EMBO J* 11:717–724
- Dorer DR (1997) Do transgene arrays form heterochromatin in vertebrates? *Transgenic Res* 6:3–10
- Feng YQ, Alami R, Bouhassira EE (1999) Enhancer-dependent transcriptional oscillations in mouse erythroleukemia cells. *Mol Cell Biol* 19:4907–4917
- Festenstein R, Sharghi-Namini S, Fox M, Roderick K, Tolaini M, Norton T, Saveliev A, Kiuoussis D, Singh P (1999) Heterochromatin protein 1 modifies mammalian PEV in a dose- and chromosomal-context-dependent manner. *Nat Genet* 23:457–461
- Fourel G, Revardel E, Koering CE, Gilson E (1999) Cohabitation of insulators and silencing elements in yeast subtelomeric regions. *EMBO J* 18:2522–2537
- Gottschling DE, Aparicio OM, Billington BL, Zakian VA (1990) Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. *Cell* 63:751–762
- Hanish JP, Yanowitz JL, de Lange T (1994) Stringent sequence requirements for the formation of human telomeres. *Proc Natl Acad Sci U S A* 91:8861–8865
- Hazelrigg T, Levis R, Rubin GM (1984) Transformation of white locus DNA in drosophila: dosage compensation, zeste interaction, and position effects. *Cell* 36:469–481
- Horn D, Cross GA (1995) A developmentally regulated position effect at a telomeric locus in *Trypanosoma brucei*. *Cell* 83:555–561
- Klehr D, Maass K, Bode J (1991) Scaffold-attached regions from the human interferon beta domain can be used to enhance the stable expression of genes under the control of various promoters. *Biochemistry* 30:1264–1270
- Koering CE, Pollice A, Zibella MP, Bauwens S, Puisieux A, Brunori M, Brun C, Martins L, Sabatier L, Pulitzer JF, Gilson E (2002) Human telomeric position effect is determined by chromosomal context and telomeric chromatin integrity. *EMBO Rep* 3:1055–1061
- Kyrion G, Liu K, Liu C, Lustig AJ (1993) RAP1 and telomere structure regulate telomere position effects in *Saccharomyces cerevisiae*. *Genes Dev* 7:1146–1159
- Lin SY, Riggs AD (1972) Lac operator analogues: bromodeoxyuridine substitution in the lac operator affects the rate of dissociation of the lac repressor. *Proc Natl Acad Sci U S A* 69:2574–2576
- Lin S, Lin D, Riggs AD (1976) Histones bind more tightly to bromodeoxyuridine-substituted DNA than to normal DNA. *Nucleic Acids Res* 3:2183–2191
- Mefford HC, Trask BJ (2002) The complex structure and dynamic evolution of human subtelomeres. *Nat Rev Genet* 3:91–102
- Miller AD (1990) Retrovirus packaging cells. *Hum Gene Ther* 1:5–14
- Ogino H, Fujii M, Satou W, Suzuki T, Michishita E, Ayusawa D (2002) Binding of 5-bromouracil-containing S/MAR DNA to the nuclear matrix. *DNA Res* 9:25–29
- Pryde FE, Louis EJ (1999) Limitations of silencing at native yeast telomeres. *EMBO J* 18:2538–2550
- Rakyan VK, Blewitt ME, Druker R, Preis JI, Whitelaw E (2002) Metastable epialleles in mammals. *Trends Genet* 18:348–351
- Renaud H, Aparicio OM, Zierath PD, Billington BL, Chhablani SK, Gottschling DE (1993) Silent domains are assembled continuously from the telomere and are defined by promoter distance and strength, and by SIR3 dosage. *Genes Dev* 7:1133–1145
- Robertson G, Garrick D, Wu W, Kearns M, Martin D, Whitelaw E (1995) Position-dependent variegation of globin transgene expression in mice. *Proc Natl Acad Sci U S A* 92:5371–5375
- Ronai D, Berru M, Shulman MJ (1999) Variegated expression of the endogenous immunoglobulin heavy-chain gene in the absence of the intronic locus control region. *Mol Cell Biol* 19:7031–7040
- Stief A, Winter DM, Stratling WH, Sippel AE (1989) A nuclear DNA attachment element mediates elevated and position-independent gene activity. *Nature* 341:343–345
- Suzuki T, Yaginuma M, Oishi T, Michishita E, Ogino H, Fujii M, Ayusawa D (2001) 5-Bromodeoxyuridine suppresses position effect variegation of transgenes in HeLa cells. *Exp Cell Res* 266:53–63
- Tham WH, Zakian VA (2002) Transcriptional silencing at *Saccharomyces* telomeres: implications for other organisms. *Oncogene* 21:512–521
- Trask BJ, Friedman C, Martin-Gallardo A, Rowen L, Akinbami C, Blankenship J, Collins C, Giorgi D, Iadonato S, Johnson F, Kuo WL, Massa H, Morrish T, Naylor S, Nguyen OT, Rouquier S, Smith T, Wong DJ, Youngblom J, van den Engh G (1998) Members of the olfactory receptor gene family are contained in large blocks of DNA duplicated polymorphically near the ends of human chromosomes. *Hum Mol Genet* 7:13–26
- True HL, Lindquist SL (2000) A yeast prion provides a mechanism for genetic variation and phenotypic diversity. *Nature* 407:477–483
- Vega-Palas MA, Martin-Figueroa E, Florencio FJ (2000) Telomeric silencing of a natural subtelomeric gene. *Mol Gen Genet* 263:287–291
- Walters MC, Fiering S, Eidemiller J, Magis W, Groudine M, Martin DI (1995) Enhancers increase the probability but not the level of gene expression. *Proc Natl Acad Sci U S A* 92:7125–7129
- Walters MC, Magis W, Fiering S, Eidemiller J, Scalzo D, Groudine M, Martin DI (1996) Transcriptional enhancers act in cis to suppress position-effect variegation. *Genes Dev* 10:185–195