Human Molecular Genetics, 2005, Vol. 14, Review Issue 2 ***R191–R196***
doi:10.1093/hmg/ddi266

The first molecular details of ALT in human tumor cells

Alessandra Muntoni and Roger R. Reddel*

Cancer Research Unit, Children's Medical Research Institute, 214 Hawkesbury Road, Westmead, Sydney, New South Wales 2145, Australia

Received June 15, 2005; Revised and Accepted July 14, 2005

The activation of a telomere maintenance mechanism (TMM) is indispensable for cellular immortalization, a hallmark of human cancer. Although most human cancers use telomerase as their TMM, some use an alternative lengthening of telomeres (ALT) mechanism. The latter especially include specific subtypes of soft tissue sarcomas where ALT occurs most often in tumors with complex karyotypes, astrocytic brain tumors and osteosarcomas. The prognostic significance of ALT varies according to the type of tumor. Some ALT cells have atypical features, suggesting the possibility that there is more than one ALT mechanism. ALT cells are characterized by instability at a specific minisatellite locus (although they are stable at microsatellite loci) and by high rates of telomeric recombinational exchange. We propose a revised model whereby unequal telomeric exchange and asymmetrical chromosome segregation could result in telomere length maintenance in a cell population. In at least some ALT cells, telomere maintenance requires the integrity of the MRN (MRE11-RAD50-NBS1) recombination complex and is efficiently repressed by its sequestration. Microsatellite instability (MSI) often results in disruption of MRN, so ALT may usually be incompatible with MSI. We suggest that ALT in human tumors is a dysregulated version of an aspect of normal mammalian telomere homeostasis, which may be a vestige of the TMM used by ancient eukaryotes. Understanding the molecular basis of ALT has important implications for the diagnosis and treatment of tumors that use this TMM.

INTRODUCTION

Telomeres are specialized structures at the end of chromosomes, and in human cells they contain repetitive $(TTAGGG)_n$ DNA. In most human somatic cells, the telomeres lose ~50–150 bp per cell division. When the telomere length declines below a certain threshold, an irreversible growth arrest state named replicative senescence is triggered. Cellular senescence may prevent the chromosomal instability that accompanies extreme telomere shortening, and the limitation it imposes on proliferative potential is also thought to suppress tumorigenesis (1). Many cancers contain cells that have escaped from this limitation by activating a telomere maintenance mechanism (TMM). Most commonly, this is achieved by the activation of telomerase (2), but some cancers maintain telomere lengths by one or more mechanisms that do not involve telomerase [alternative lengthening of telomeres (ALT)] (3). Here, we review recent developments in understanding the molecular details of ALT and its significance in human cancer.

ALT REQUIRES A FUNCTIONAL MRN COMPLEX

It has been known for some time that the ALT mechanism most likely involves recombination-mediated DNA replication. *Saccharomyces cerevisiae* cells that survive in the absence of telomerase require a functional RAD52 gene (4), which encodes a protein required for DNA recombination. The class of survivors (called type II) with a telomere phenotype that most closely resembles human ALT cells is also dependent on RAD50 (5), a component of a protein complex that is involved in recombination. Individual telomeres in human ALT cells undergo steady telomere attrition upon which sudden lengthening and shortening events are superimposed

*To whom correspondence should be addressed. Tel: +61 296872800; Fax: +61 296872120; Email: rreddel@cmri.usyd.edu.au

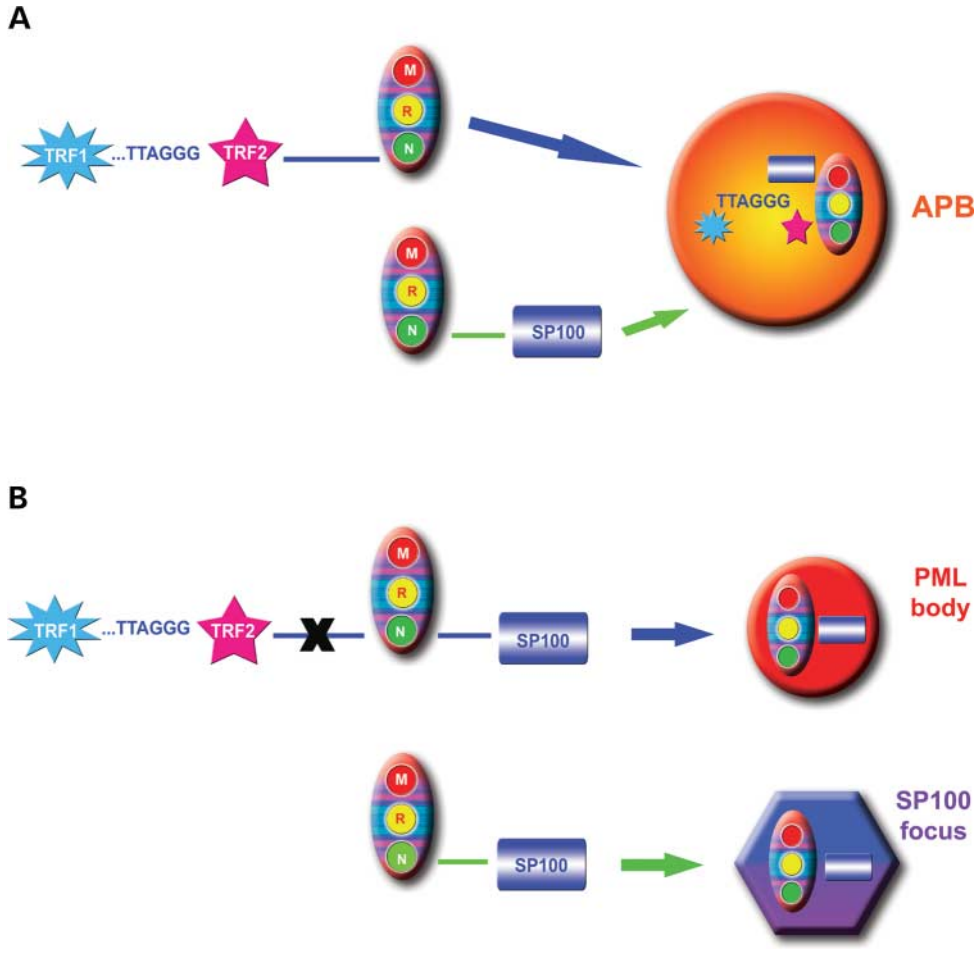

Figure 1. Proposed model for suppression of ALT caused by over-expression of SP100. (**A**) Telomeric DNA bound to TRF1 and TRF2 is recruited to PML bodies via interaction of TRF2 with the MRN complex (blue arrow). PML bodies containing telomeric DNA and associated proteins are referred to as APBs. The MRN complex also interacts with SP100 (green arrows), a constitutive component of PML bodies. (**B**) When SP100 is over-expressed, it forms additional subnuclear aggregates that do not contain PML protein and sequesters MRN. This prevents accumulation of telomeric materials in PML bodies, i.e. suppresses formation of APBs.

in a manner that is suggestive of recombination (6). One of the hallmarks of the ALT mechanism is the presence of ALT-associated PML bodies (APBs), which are subnuclear structures where PML protein colocalizes with telomeric DNA, telomere-binding proteins and several proteins involved in DNA synthesis and recombination (7). The latter include the MRE11/RAD50/NBS1 (MRN) complex (8,9). APBs appear as soon as ALT is activated (7) and disappear when ALT is repressed in somatic cell hybrids (10). Functional evidence for the involvement of recombination in the ALT mechanism was provided by showing that DNA sequences are copied from telomere to telomere in ALT cells (11). These observations are all consistent with ALT being a recombination-mediated DNA replication mechanism.

The first molecular details of ALT in human cells are now beginning to emerge. It had previously been found that impairment of NBS1 function inhibited formation of APBs (8). Recently, it was found that over-expression of SP100 (a protein which is a constitutive component of PML bodies) in ALT cell lines is able to sequester the MRN complex and inhibit the formation of APBs (12) (Fig. 1). This caused suppression of the ALT mechanism, as demonstrated by disappearance of the rapid telomere length changes typical of ALT and by steady telomere shortening at the rate seen in TMM-negative normal cells (12). There is no evidence that SP100 is normally involved in regulating ALT, but this study established the first functional link between a protein complex and ALT, thus providing empirical tools for its disruption.

INSTABILITY OF A SPECIFIC MINISATELLITE LOCUS (BUT NOT MICROSATELLITE LOCI) OCCURS IN ALT

Sister chromatid exchange (SCE) was not found to be increased at interstitial sites in ALT cells (13), and the overall frequency of homologous recombination was found to be similar in ALT and telomerase-positive cell lines (14). The recombination-mediated DNA replication that occurs in ALT cells thus does not appear to be accompanied by a global increase in recombination. It was therefore surprising that a specific minisatellite (MS32) was found to be highly

unstable in a subset of ALT cell lines, but not in telomerase-positive or mortal cultures (15). High mutation rates at this particular locus were also seen in a subset of ALT-positive soft tissue sarcomas, indicating that minisatellite instability occurs *in vivo* and is not a culture artifact. However, the association between minisatellite instability and ALT is not a generalized phenomenon: four subterminal minisatellites analyzed in this study were not unstable in ALT tumors, despite being highly recombinogenic in the germline (15). The reasons for the association of ALT with increased exchange rates at a specific chromosomal site remain to be clarified.

In contrast, microsatellite instability (MSI) has not been observed in ALT cells (16). MSI arises from disruption of the mismatch repair (MMR) mechanism. Disruption of proteins involved in MMR led to increased proliferation in telomerase-null yeast strains prior to emergence of survivors (17) and to increased frequency of aberrant homologous recombination in human cells (18). In human cells, however, MMR-deficiency may often be incompatible with ALT because MSI usually results in disruption of the MRN complex (19).

ALT IS CHARACTERIZED BY INCREASED RATES OF TELOMERIC RECOMBINATION

It has been found recently that ALT cells undergo post-replicative telomeric exchanges with higher frequency than telomerase-positive cells (13,14). In addition, as mentioned earlier, SCEs were not increased at interstitial sites in ALT cells, in agreement with a report describing similar frequencies of homologous recombination in ALT and telomerase-positive cell lines (14). It could be argued that telomeres of ALT cells might simply have a higher probability of recombining because of their considerable length, but this seems not to be the case, as telomerase-positive cells with very long telomeres showed no significant increase in telomere exchanges when compared with mortal cells (13). Hence, telomeric recombination seems to be genuinely associated with the ALT mechanism. One possible explanation is that activation of ALT results in recruitment of proteins required for SCE at telomeres. If so, repression of ALT should result in normalization of the telomeric SCE rates. Alternatively, telomeric recombination might be an upstream event in a pathway of molecular changes that are necessary but not sufficient for activation of ALT. These hypotheses need to be tested.

It needs to be noted that, although the telomeric exchanges are often referred to as telomeric SCEs (t-SCEs), the chromosome-orientation FISH (CO-FISH) technique used to detect them cannot determine whether the exchanges involved sister chromatids or whether they involved other telomeres or even extrachromosomal telomeric DNA. It is also possible that there is more than one source of telomeric exchanges. In addition to exchanges that are related to ALT, there may also be telomeric SCEs that result from DNA damage: telomeres are vulnerable to breakage by specific forms of DNA damage (20) and SCEs are involved in the repair of double-strand breaks (21).

It has recently been proposed that unequal SCE at telomeres might be responsible for the rapid telomere elongation and shortening events observed in ALT (22). Before mitosis, homologous recombination occurs between symmetrically paired chromosomes or sister chromatids and leads to exchanges of equal amounts of DNA. However, telomeres are repetitive sequences, and sister chromatids can find sites of homology anywhere along their length. If alignment between the two telomeric strands occurs asymmetrically, homologous recombination results in a net gain of telomeric sequences in one chromatid and a net loss in the other (Fig. 2). In this case, the daughter cell that inherits chromosomes with almost depleted telomeres would soon senesce and be lost from the population, but the one inheriting chromosomes with elongated telomeres would have an increased proliferative potential. If sufficient numbers of t-SCEs occurred, a cell population could survive by lengthening telomeres in some cells in this way and sacrificing the cells in which telomeres were depleted. Furthermore, if this process is repeated whenever telomeres become excessively shortened, the population would effectively become immortal.

To be effective, however, we propose that this mechanism would need to be coupled with unequal segregation of chromosomes at mitosis, leading all those with lengthened telomeres to the same daughter cell. It is claimed that asymmetrical segregation of DNA strands occurs in adult stem cells (23), and it is possible that this process may be regulated by telomeres: hematopoietic stem cells can be divided into distinct subpopulations with extremely different telomere lengths (0.1–33 kb) (24). It would be interesting to speculate that the short-telomere stem cell progenies are bound to differentiate, whereas the long-telomere clones are retained in the progenitor compartment, thus keeping the self-renewal potential of the population intact. It is conceivable that telomerase-negative cells could adapt this mechanism in order to bypass the limits on proliferative potential, but no supporting experimental evidence is available yet.

THE ROLE OF EXTRACHROMOSOMAL TELOMERIC REPEATS IN ALT CELLS

ALT cells contain abundant extrachromosomal telomeric repeat (ECTR) DNA, in linear, t-loop and circular forms (7,25–28). Extrachromosomal t-loops and circles might be generated by abortive telomeric exchanges or by inappropriate repair of the Holliday junction structure formed by the t-loop at the insertion of the single-stranded telomere terminus into duplex telomeric DNA (Fig. 3). In this regard, it is interesting to note that circular ECTR can also occur in non-ALT cells when the function of TRF2 is disrupted (28). TRF2 is required for the establishment of t-loops (29a) and presumably is involved in protecting the Holliday junction structure from repair (28). APBs appear to contain both linear and circular forms of telomeric DNA (A. Muntoni, C. Fasching and R. Reddel, unpublished data). Linear ECTR might participate in ALT telomere elongation either as a template for recombination-mediated replication or by an end-joining reaction, but it is mostly short (up to a few kilobases) and is unlikely to account for the rapid gains of telomeric sequences observed in ALT cells. Extrachromosomal t-loops and circles can be quite large (27), and could possibly participate in ALT

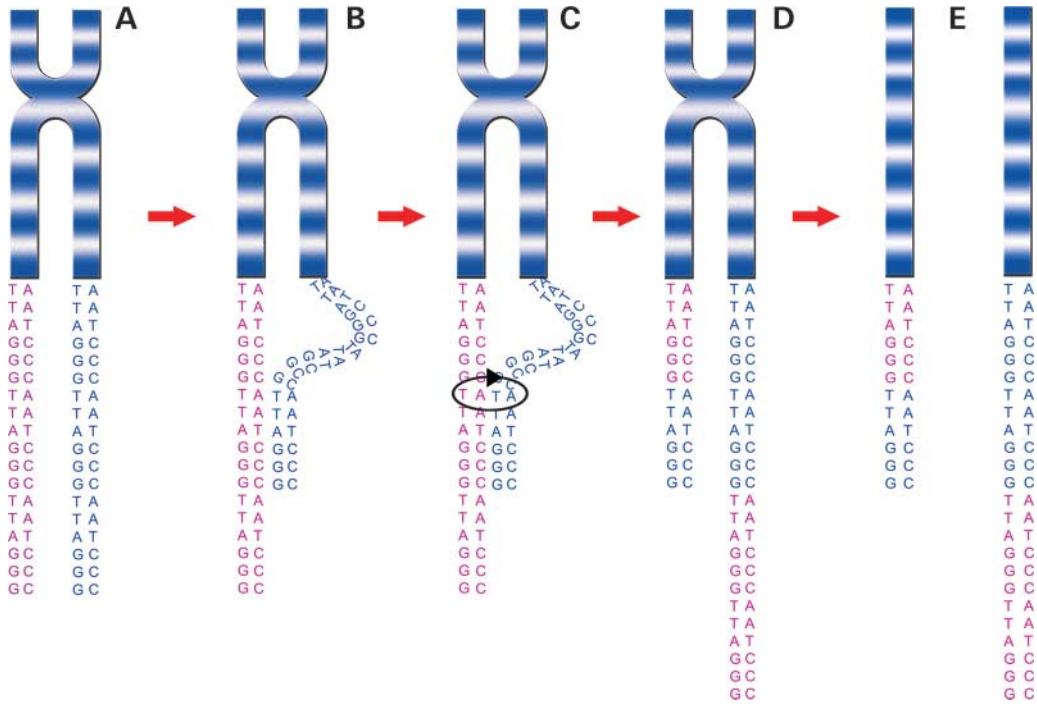

Figure 2. Graphical representation of the mechanism by which unequal t-SCE generates daughter cells with different telomere lengths and hence different lifespan potential. Post-replicative chromosomes have telomeres of essentially equal length (**A**). If the sister chromatids align their telomeres asymmetrically (**B**) and exchange telomeric sequences (**C**), this will result in two sister chromatids (**D**) and ultimately two daughter cells (**E**) with different telomere lengths. The daughter cell with shorter telomeres will soon senesce and be lost from the population, whereas the one with longer telomeres will have an extended proliferative potential.

by end-joining and/or as copy templates in a roll-and-spread mechanism (29b).

FEATURES OF ALT *IN VIVO*

Observational and clinical studies on ALT positive tumors might help fill in the gaps in our understanding of the ALT mechanism. ALT is most commonly activated in tumors of neuroepithelial origin (astrocytomas) or mesenchymal origin, including osteosarcomas, and soft tissue sarcomas (30). The reason for this is unknown, but we speculate that some mesenchymal and neuroepithelial cells repress telomerase more tightly than epithelial cells and therefore have a higher relative probability of activating ALT during tumorigenesis. In sarcomas, ALT is more frequently activated in the subtypes with complex karyotypes, which are evidence of chromosomal instability (31,32). It could be argued that the ALT mechanism is, in part, the cause of this instability, because the critically short telomeres found in ALT cells are prone to end-to-end fusions, anaphase bridges, break–fusion–break events and ultimately severe chromosomal rearrangements. Not all soft tissue sarcomas associated with complex karyotypes are ALT-positive (30), however, clearly showing that other factors contribute to chromosomal instability.

The relationship between tumor TMM and patient outcome is the subject of ongoing study. Observations in model systems suggested that ALT tumors may be relatively benign, although they may be lethal in humans *in vivo* (reviewed in 33). In osteosarcomas, the absence of both telomerase activity and ALT is associated with a longer survival (34). In contrast, ALT activity is associated with longer survival of patients with high-grade astrocytomas (30,35) and is a better prognostic indicator than age (35). Therefore, the prognostic significance of ALT seems to vary among tumor types. Possibly, different tumor types activate ALT through different genetic pathways, and it may be these pathways rather than ALT itself that are responsible for different patient outcomes. In addition, ALT tumors may be less likely to metastasize than telomerase-positive tumors, although this does not seem to confer improved survival (30).

WILL ALT SURVIVORS EMERGE IN TUMORS TREATED WITH TELOMERASE INHIBITORS?

In view of the imminent use of telomerase inhibitors as anticancer treatments, it will be important to determine whether inhibition of telomerase can select for cancer cells that activate the ALT mechanism. One study attempted to address this question by using a dominant negative TERT to inhibit telomerase activity in colon cancer cells. A clone was identified, which underwent episodic telomere lengthening, but lacked the characteristic features of ALT cells and eventually reactivated telomerase (36). An explanation for the inability to activate ALT may be that the colon cancer cell line used in this experiment was MMR-deficient and, as described earlier, such lines very frequently have disruptions in one or

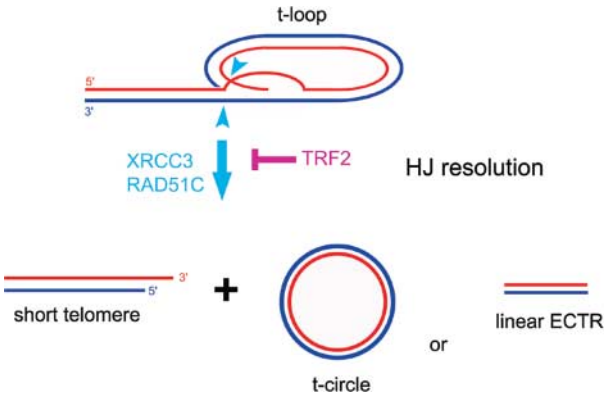

Figure 3. Proposed model for generation of linear and circular ECTR DNA [adapted from Wang *et al.* (28)]. Telomeric DNA is organized in a t-loop structure (42). If the t-loop is inappropriately processed as a Holliday junction, both strands will be cleaved at the point of G-overhang invasion in the displacement loop. This mechanism will generate a shorter telomere along with a telomeric circle or a fragment of linear ECTR DNA. Holliday junction resolution at telomeres involves the activity of XRCC3 (28) and might involve RAD51C (43). TRF2 is speculated to inhibit telomeric homologous recombination by protecting the t-loop from being inappropriately resolved as a Holliday junction (28).

more members of the MRN complex (19) which is required for ALT (12).

In another study, telomerase-positive immortal human fibroblasts showed sustained proliferation in culture after spontaneously inactivating telomerase (37). These cells did not express APBs or a telomere length pattern typical of ALT, and they reactivated telomerase upon treatment with the demethylating agent 5-aza-2′-deoxycytidine.

IS THERE MORE THAN ONE ALT MECHANISM?

Some telomerase-negative human tumors lack all of the known features of ALT activity, which raises the question whether there may be more than one ALT mechanism. A hint that this might be the case was provided by two recent studies of a telomerase-negative immortalized cell line, AG11395, which does not form APBs (38,39). *S. cerevisiae* cells are able to survive in the absence of telomerase either by activating a TMM similar to ALT (type II survivors) or by amplifying telomere sequences along with the subtelomeric Y′ repeat element (type I survivors) (4,5). Mouse embryonic stem cells that survive in the absence of telomerase underwent amplification of both telomeric and non-telomeric sequences (40). The AG11395 cell line contains amplified SV40 sequences within its telomeres, but in many other regards they are similar to ALT cells, exhibiting telomere dynamics (38) and ECTR content (39) typical of ALT. Instead of APBs, they contain nuclear aggregates of telomeric DNA and other proteins involved in recombination, including the MRN complex, but without PML. It remains unclear whether the TMM used by these cells corresponds to that described in type I telomerase-null yeast survivors or is instead a variant of the known ALT mechanism. If there is more than one ALT mechanism in human tumors, it will be important to develop assays that distinguish them and to determine the implications for cancer therapy and patient outcome.

IS ALT AN ATAVISTIC TMM?

It has recently been proposed that linear chromosomes and t-loops co-evolved in early eukaryotes (41). T-loops potentially can help solve the two biological problems presented by linear chromosomes: they are double-strand breaks which need to be protected from being recognized as such and they are incompletely replicated. It has been suggested that t-loops solve the first of these problems by hiding the telomere end and the second by providing a ready-made recombination intermediate that allows the telomere to use itself as a template for recombination-mediated DNA replication, and thus synthesis of new telomeric DNA to compensate for the loss that occurs during each cell cycle. Furthermore, it was proposed that later in eukaryotic evolution this TMM was suppressed and supplanted by telomerase (41), a specialized reverse transcriptase that uses an integral RNA template molecule to synthesize telomeric DNA. In this view, the ALT mechanism found in human tumors represents a throwback to the earliest eukaryotic TMM. Some types of tumors, however, very frequently activate ALT (e.g. 77% of malignant fibrous histiocytomas) (30), so it seems more likely that they are utilizing a dysregulated form of a modern TMM rather than a long-suppressed ancient mechanism. We suggest that an ALT-like mechanism is a part of normal mammalian telomere biology and that its normal regulation is lost in ALT-positive tumors. This would be analogous to the situation with telomerase, which is important for normal biology and becomes regulated abnormally in many human tumors.

ACKNOWLEDGEMENTS

The authors wish to thank L. Colgin, C. Fasching, J. Henson, W.-Q. Jiang and H. Pickett for critically reviewing the manuscript and A. Neumann and E. Collins for advice. A.M. is supported by a National Health and Medical Research Council of Australia Peter Doherty fellowship (ID 321714). Work in the authors' laboratory is supported by the National Health and Medical Research Council of Australia. Funding to pay the Open Access publication charges for this article was provided by the Children's Medical Research Institute.

Conflict of Interest statement. None declared.

REFERENCES

1. Hanahan, D. and Weinberg, R.A. (2000) The hallmarks of cancer. *Cell*, **100**, 57–70.
2. Shay, J.W. and Bacchetti, S. (1997) A survey of telomerase activity in human cancer. *Eur. J. Cancer*, **33**, 787–791.
3. Bryan, T.M. and Reddel, R.R. (1997) Telomere dynamics and telomerase activity in *in vitro* immortalised human cells. *Eur. J. Cancer*, **33**, 767–773.
4. Lundblad, V. and Blackburn, E.H. (1993) An alternative pathway for yeast telomere maintenance rescues *est1*$^-$ senescence. *Cell*, **73**, 347–360.
5. Teng, S.C. and Zakian, V.A. (1999) Telomere–telomere recombination is an efficient bypass pathway for telomere maintenance in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **19**, 8083–8093.

6. Murnane, J.P., Sabatier, L., Marder, B.A. and Morgan, W.F. (1994) Telomere dynamics in an immortal human cell line. *EMBO J.*, **13**, 4953–4962.
7. Yeager, T.R., Neumann, A.A., Englezou, A., Huschtscha, L.I., Noble, J.R. and Reddel, R.R. (1999) Telomerase-negative immortalized human cells contain a novel type of promyelocytic leukemia (PML) body. *Cancer Res.*, **59**, 4175–4179.
8. Wu, G., Lee, W.H. and Chen, P.L. (2000) NBS1 and TRF1 colocalize at promyelocytic leukemia bodies during late S/G_2 phases in immortalized telomerase-negative cells: implication of NBS1 in alternative lengthening of telomeres. *J. Biol. Chem.*, **275**, 30618–30622.
9. Zhu, X.D., Kuster, B., Mann, M., Petrini, J.H. and de Lange, T. (2000) Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. *Nat. Genet.*, **25**, 347–352.
10. Perrem, K., Bryan, T.M., Englezou, A., Hackl, T., Moy, E.L. and Reddel, R.R. (1999) Repression of an alternative mechanism for lengthening of telomeres in somatic cell hybrids. *Oncogene*, **18**, 3383–3390.
11. Dunham, M.A., Neumann, A.A., Fasching, C.L. and Reddel, R.R. (2000) Telomere maintenance by recombination in human cells. *Nat. Genet.*, **26**, 447–450.
12. Jiang, W.Q., Zhong, Z.H., Henson, J.D., Neumann, A.A., Chang, A.C. and Reddel, R.R. (2005) Suppression of alternative lengthening of telomeres by Sp100-mediated sequestration of MRE11/RAD50/NBS1 complex. *Mol. Cell. Biol.*, **25**, 2708–2721.
13. Londono-Vallejo, J.A., Der-Sarkissian, H., Cazes, L., Bacchetti, S. and Reddel, R.R. (2004) Alternative lengthening of telomeres is characterized by high rates of telomeric exchange. *Cancer Res.*, **64**, 2324–2327.
14. Bechter, O.E., Zou, Y., Shay, J.W. and Wright, W.E. (2003) Homologous recombination in human telomerase-positive and ALT cells occurs with the same frequency. *EMBO Rep.*, **4**, 1138–1143.
15. Jeyapalan, J.N., Varley, H., Foxon, J.L., Pollock, R.E., Jeffreys, A.J., Henson, J.D., Reddel, R.R. and Royle, N.J. (2005) Activation of the ALT pathway for telomere maintenance can affect other sequences in the human genome. *Hum. Mol. Genet.*, **14**, 1785–1794.
16. Tsutsui, T., Kumakura, S., Tamura, Y., Tsutsui, T.W., Sekiguchi, M., Higuchi, T. and Barrett, J.C. (2003) Immortal, telomerase-negative cell lines derived from a Li-Fraumeni syndrome patient exhibit telomere length variability and chromosomal and minisatellite instabilities. *Carcinogenesis*, **24**, 953–965.
17. Rizki, A. and Lundblad, V. (2001) Defects in mismatch repair promote telomerase-independent proliferation. *Nature*, **411**, 713–716.
18. Elliott, B. and Jasin, M. (2001) Repair of double-strand breaks by homologous recombination in mismatch repair-defective mammalian cells. *Mol. Cell. Biol.*, **21**, 2671–2682.
19. Giannini, G., Ristori, E., Cerignoli, F., Rinaldi, C., Zani, M., Viel, A., Ottini, L., Crescenzi, M., Martinotti, S., Bignami, M. *et al.* (2002) Human MRE11 is inactivated in mismatch repair-deficient cancers. *EMBO Rep.*, **3**, 248–254.
20. Petersen, S., Saretzki, G. and Von Zglinicki, T. (1998) Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. *Exp. Cell Res.*, **239**, 152–160.
21. Johnson, R.D. and Jasin, M. (2000) Sister chromatid gene conversion is a prominent double-strand break repair pathway in mammalian cells. *EMBO J.*, **19**, 3398–3407.
22. Bailey, S.M., Brenneman, M.A. and Goodwin, E.H. (2004) Frequent recombination in telomeric DNA may extend the proliferative life of telomerase-negative cells. *Nucleic Acids Res.*, **32**, 3743–3751.
23. Merok, J.R., Lansita, J.A., Tunstead, J.R. and Sherley, J.L. (2002) Cosegregation of chromosomes containing immortal DNA strands in cells that cycle with asymmetric stem cell kinetics. *Cancer Res.*, **62**, 6791–6795.
24. Garcia-Escarp, M., Martinez-Munoz, V., Barquinero, J., Sales-Pardo, I., Domingo, J.C., Marin, P. and Petriz, J. (2005) A rare fraction of human hematopoietic stem cells with large telomeres. *Cell Tissue Res.*, **319**, 405–412.
25. Ogino, H., Nakabayashi, K., Suzuki, M., Takahashi, E.I., Fujii, M., Suzuki, T. and Ayusawa, D. (1998) Release of telomeric DNA from chromosomes in immortal human cells lacking telomerase activity. *Biochem. Biophys. Res. Commun.*, **248**, 223–227.
26. Tokutake, Y., Matsumoto, T., Watanabe, T., Maeda, S., Tahara, H., Sakamoto, S., Niida, H., Sugimoto, M., Ide, T. and Furuichi, Y. (1998) Extra-chromosome telomere repeat DNA in telomerase-negative immortalized cell lines. *Biochem. Biophys. Res. Commun.*, **247**, 765–772.
27. Cesare, A.J. and Griffith, J.D. (2004) Telomeric DNA in ALT cells is characterized by free telomeric circles and heterogeneous T-loops. *Mol. Cell. Biol.*, **24**, 9948–9957.
28. Wang, R.C., Smogorzewska, A. and de Lange, T. (2004) Homologous recombination generates T-loop-sized deletions at human telomeres. *Cell*, **119**, 355–368.
29a. Stansel, R.M., de Lange, T. and Griffith, J.D. (2001) T-loop assembly *in vitro* involves binding of TRF2 near the 3′ telomeric overhang. *EMBO J.*, **20**, 5532–5540.
29b. Natarajan, S. and McEachern, M.J. (2002) Recombinational telomere elongation promoted by DNA circles. *Mol. Cell. Biol.*, **22**, 4512–4521.
30. Henson, J.D., Hannay, J.A., McCarthy, S.W., Royds, J.A., Yeager, T.R., Robinson, R.A., Wharton, S.B., Jellinek, D.A., Arbuckle, S.M., Yoo, J. *et al.* (2005) A robust assay for alternative lengthening of telomeres (ALT) in tumors demonstrates the significance of ALT in sarcomas and astrocytomas. *Clin. Cancer Res.*, **11**, 217–225.
31. Ulaner, G.A., Hoffman, A.R., Otero, J., Huang, H.Y., Zhao, Z., Mazumdar, M., Gorlick, R., Meyers, P., Healey, J.H. and Ladanyi, M. (2004) Divergent patterns of telomere maintenance mechanisms among human sarcomas: sharply contrasting prevalence of the alternative lengthening of telomeres mechanism in Ewing's sarcomas and osteosarcomas. *Genes Chromosomes Cancer*, **41**, 155–162.
32. Montgomery, E., Argani, P., Hicks, J.L., DeMarzo, A.M. and Meeker, A.K. (2004) Telomere lengths of translocation-associated and nontranslocation-associated sarcomas differ dramatically. *Am. J. Pathol.*, **164**, 1523–1529.
33. Reddel, R.R. and Bryan, T.M. (2003) Alternative lengthening of telomeres: dangerous road less travelled. *Lancet*, **361**, 1840–1841.
34. Ulaner, G.A., Huang, H.Y., Otero, J., Zhao, Z., Ben-Porat, L., Satagopan, J.M., Gorlick, R., Meyers, P., Healey, J.H., Huvos, A.G., Hoffman, A.R. and Ladanyi, M. (2003) Absence of a telomere maintenance mechanism as a favorable prognostic factor in patients with osteosarcoma. *Cancer Res.*, **63**, 1759–1763.
35. Hakin-Smith, V., Jellinek, D.A., Levy, D., Carroll, T., Teo, M., Timperley, W.R., McKay, M.J., Reddel, R.R. and Royds, J.A. (2003) Alternative lengthening of telomeres and survival in patients with glioblastoma multiforme. *Lancet*, **361**, 836–838.
36. Bechter, O.E., Zou, Y., Walker, W., Wright, W.E. and Shay, J.W. (2004) Telomeric recombination in mismatch repair deficient human colon cancer cells after telomerase inhibition. *Cancer Res.*, **64**, 3444–3451.
37. Kumakura, S., Tsutsui, T.W., Yagisawa, J., Barrett, J.C. and Tsutsui, T. (2005) Reversible conversion of immortal human cells from telomerase-positive to telomerase-negative cells. *Cancer Res.*, **65**, 2778–2786.
38. Fasching, C.L., Bower, K. and Reddel, R.R. (2005) Telomerase-independent telomere length maintenance in the absence of ALT-associated PML bodies. *Cancer Res.*, **65**, 2722–2729.
39. Marciniak, R.A., Cavazos, D., Montellano, R., Chen, Q., Guarente, L. and Johnson, F.B. (2005) A novel telomere structure in human alternative lengthening of telomeres cell line. *Cancer Res.*, **65**, 2730–2737.
40. Niida, H., Shinkai, Y., Hande, M.P., Matsumoto, T., Takehara, S., Tachibana, M., Oshimura, M., Lansdorp, P.M. and Furuichi, Y. (2000) Telomere maintenance in telomerase-deficient mouse embryonic stem cells: characterization of an amplified telomeric DNA. *Mol. Cell. Biol.*, **20**, 4115–4127.
41. de Lange, T. (2004) Opinion: T-loops and the origin of telomeres. *Nat. Rev. Mol. Cell Biol.*, **5**, 323–329.
42. Griffith, J.D., Comeau, L., Rosenfield, S., Stansel, R.M., Bianchi, A., Moss, H., de Lange, T. (1999) Mammalian telomeres end in a large duplex loop. *Cell*, **97**, 503–514.
43. Liu, Y., Masson, J.Y., Shah, R., O'Regan, P. and West, S.C. (2004) RAD51C is required for Holliday junction processing in mammalian cells. *Science*, **303**, 243–246.