Asian Journal of Andrology (2010) 12: 779–782

www.nature.com/aja

Research Highlight

For cancers there is more to life than a longer G-strand

Jeremy D. Henson[1, 2]

[1]*Children's Medical Research Institute, Sydney, NSW 2154, Australia*
[2]*University of Sydney, NSW 2154, Australia*

Asian Journal of Andrology (2010) 12: 779–782. doi: 10.1038/aja.2010.103; published online 13 September 2010.

Three recent studies from the Wright [1], Chai [2] and de Lange [3] labs have elucidated previously neglected aspects of cancer cell telomere maintenance. Because inhibiting telomere maintenance can selectively kill cancer cells without harming normal cells [4, 5], this new understanding of the mechanism and dynamics involved may provide useful new strategies for treating cancer. Until recently, research had focused on how cancer cells elongate the telomeric G-strand. However, during every cell division the telomeric C-strand also needs to be elongated and the appropriate end-structure recreated to maintain telomere length and function.

Telomeres are specialized structures that prevent the recognition of our chromosome ends as broken DNA. Loss of telomere function can trigger a DNA damage response (DDR) resulting in cellular senescence or apoptosis [6]. Telomeres consist of repetitive DNA containing a G-strand, with the sequence $[TTAGGG]_n$, and a C-strand, $[AATCCC]_n$. The G-strand overhangs the C-strand, providing a stretch of single-stranded DNA. This allows the telomere to be folded into a t-loop structure (Figure 1), which combined with specialized telomere binding proteins allows the telomere to avoid detection as a DNA end [6]. Telomeres shorten with every cell division and eventually become too short to avoid triggering a DDR. Cancer cells need to replace the lost telomeric sequence. The telomeric G-strand can be lengthened by telomerase or ALT (Alternative Lengthening of Telomeres) using an RNA or DNA template, respectively [4, 5].

Most malignant cancers depend on telomerase or ALT for their continued growth and survival [4, 5]. Prostate cancer is a good example. Telomerase activity has been demonstrated in 79% of prostate cancers (359 tumours from 11 studies) [4, 7] and anti-telomerase therapy is being trialled clinically in prostate cancer [4]. Because normal cells do not depend on telomerase (or ALT), anti-telomerase therapies have had minimal side effects and no adverse effects on bone marrow stem cells have been found [4]. This demonstrates the high specificity of anti-telomere maintenance therapy for cancer cells. Androgen ablation therapy may also work in part through down regulating telomerase expression [4] or disrupting telomeric structure [8]. Unfortunately, solely inhibiting telomerase mediated elongation of the telomeric G-strand cannot be used as a single agent because a sustained period is required for the telomeres to shorten enough to start eradicating tumour cells [9]. Targeting telomerase alone would also not be effective if the cancer activated ALT. ALT has not been investigated in prostate cancer, but has been found in 5%–15% of most carcinomas tested so far [5] and a characteristic marker of ALT has been found in 10% of prostate cancers [5, 10].

Strategies to use telomere maintenance as a target to treat cancer could be greatly improved by an understanding of how human cells regenerate the correct end structure after replication, how the C-strand is elongated, and how both of these are coordinated with telomere

Correspondence to: Dr Jeremy D Henson
Children's Medical Research Institute, 214 Hawkesbury Rd., Westmead NSW 2154, Australia.
Fax: +61-2-9687-2120
E-mail: jhenson@cmri.org.au

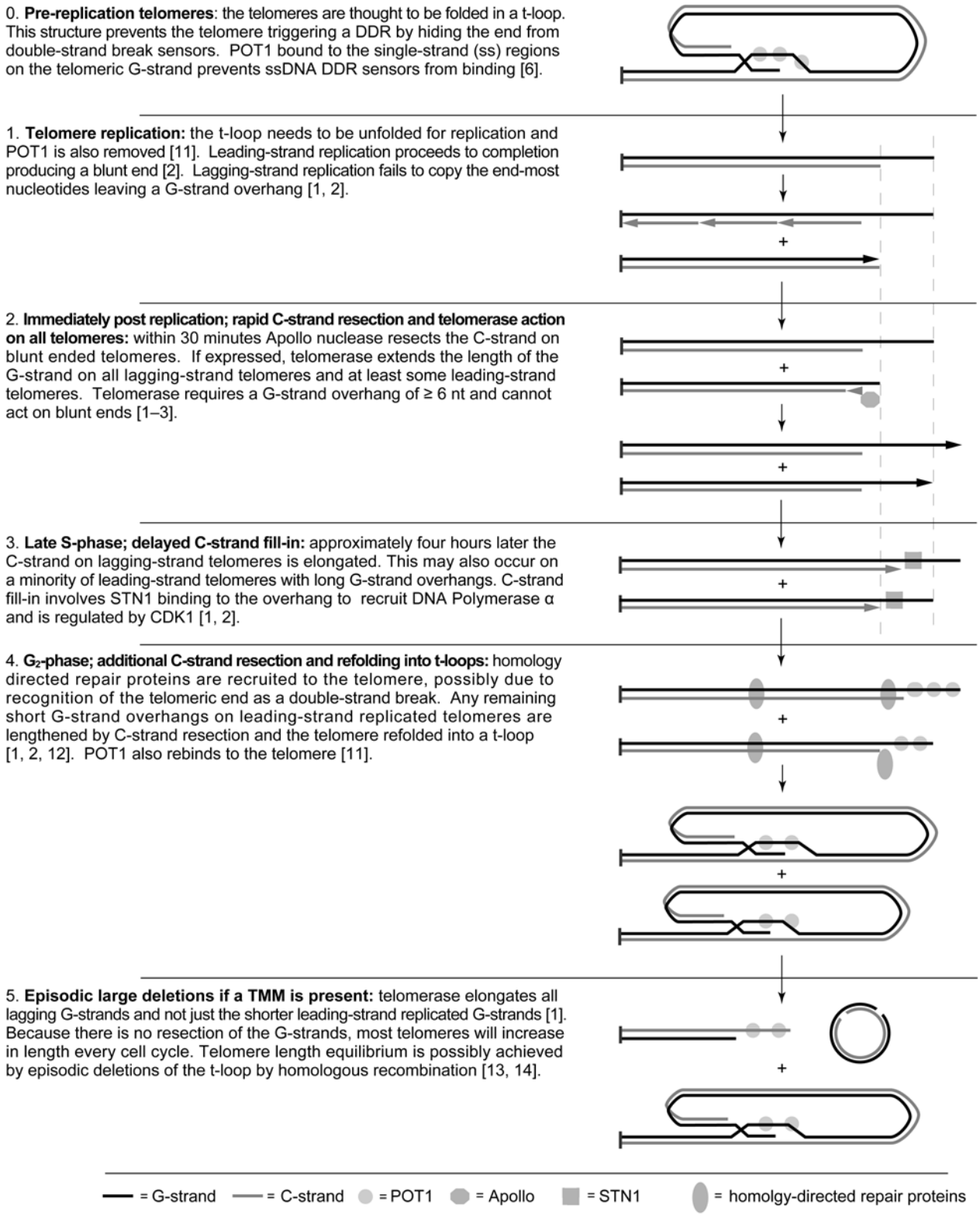

Figure 1. Telomere end-structure maintenance. DDR, DNA damage response.

length maintenance. To investigate this, Zhao *et al.* [1] and Dai *et al.* [2] separated leading- and lagging-strand replicated telomeres using similar systems, but then used different techniques for measuring the G-strand overhang lengths. These studies were performed in cell cycle synchronized and telomerase$^+$ human cell lines. Telomerase did not appear essential for the general overhang length dynamics [2]. Wu *et al.* [3] further contributed by using knock-out mouse models to study the role of Apollo nuclease. The consensus of these studies is summarized in Figure 1.

After replication, recreation of the telomeric G-strand overhang of ~60 nucleotide (nt) [1] proceeded differently for leading- and lagging-strand replicated telomeres. Leading-strand replication synthesized a new G-strand to the end of the C-strand template [2], creating a blunt end. The daughter G-strand was shorter than the parental G-strand because the template was shorter (by 60 nt). Apollo immediately resected the C-strand to provide the overhang that telomerase requires. Telomerase then elongated the G-strand on some telomeres until it overhung the C-strand by > 70 nt [1–3]. However, many leading telomeres may not have been appreciably elongated [2]. In late S-phase, the leading telomeres with longer overhangs had their C-strands elongated to reduce the overhang length back to 60 nt [1, 2]. The leading telomeres with shorter overhangs remained stable until G_2-phase when their overhangs were returned towards 60 nt by C-strand resection [2]. Lagging-strand replication is unable to synthesize new C-strand at the very end of the G-strand template. The resulting G-strand overhang on every lagging telomere was immediately extended by telomerase to an average total length of >100 nt [1, 2]. Four hours later (in late S-phase) C-strand elongation reduced the overhangs back to ~60 nt long [1, 2]. For both leading and lagging strands, C-strand elongation appeared to require STN1 binding to the overhang to recruit DNA polymerase α [2].

Both resection and elongation of the C-strand are required to regenerate the correct telomere end-structure, which is vital for telomere function. If either is inhibited a DDR is generated at the telomeres [2, 3], resulting in either senescence or apoptosis [2, 15]. Both C-strand resection and elongation are also vital for telomere length maintenance and this provides two new telomere maintenance targets for killing cancer cells.

C-strand elongation is required after G-strand elongation by telomerase or ALT, otherwise C-strand ends will shorten as a result of resection and incomplete replication on leading- and lagging-strand telomeres, respectively. C-strand length will also limit leading-strand replication of the G-strand in the next cell cycle. Since C-strand fill-in is delayed by 4 h from telomerase elongation of the G-strand it may be a separately regulated independent target [2]. Inhibiting both telomerase catalytic activity and C-strand fill-in could shorten the lag time for telomerase inhibitors to start working. If it overlaps with the process of C-strand elongation in ALT, targeting C-strand fill-in may target both telomere length maintenance mechanisms (TMMs) with the one therapy. This would have the added advantage of neutralizing the possibility of the cancer becoming resistant by activating the alternate TMM if only one is targeted. In yeast, C-strand fill-in also regulates telomerase activity [16]. Therefore, further understanding of this process could provide multiple strategies for TMM targeted anti-cancer therapies.

Both telomerase and ALT require an overhang on the G-strand in order to elongate it [2, 5]. Inhibiting the C-strand resection by Apollo nuclease, could inhibit one or both TMMs. This is supported by a study that associated a dominant-negative allele of Apollo (that prevented C-strand resection) with a severe form of dyskeratosis congenita [3, 15]. Dyskeratosis congenita is a disease caused by defective telomere maintenance and manifests several human generations after onset with premature ageing, bone marrow failure and immunodeficiency [17]. Thus these studies on telomere end-structure maintenance have provided new insights for increasing the effectiveness of current anti-TMM strategies and suggest new strategies that may target both TMMs at once.

References

1 Zhao Y, Sfeir AJ, Zou Y, Buseman CM, Chow TT, *et al*. Telomere extension occurs at most chromosome ends and is uncoupled from fill-in in human cancer cells. Cell 2009; 138: 463–75.
2 Dai X, Huang C, Bhusari A, Sampathi S, Schubert K, *et al*. Molecular steps of G-overhang generation at human telomeres and its function in chromosome end protection. EMBO

J 2010; 29: 2788–801.
3 Wu P, van Overbeek M, Rooney S, de Lange T. Apollo contributes to G overhang maintenance and protects leading-end telomeres. Mol Cell Mol Cell 2010 Jul 7. [Epub ahead of print].
4 Marian CO, Shay JW. Prostate tumor-initiating cells: a new target for telomerase inhibition therapy? Biochim Biophys Acta 2009; 1792: 289–96. Epub 2009 Mar 2.
5 Henson JD, Reddel RR. Assaying and investigating Alternative Lengthening of Telomeres activity in human cells and cancers. FEBS Lett 2010 Jun 11. [Epub ahead of print]
6 de Lange T. How telomeres solve the end-protection problem. Science 2009; 326: 948–52.
7 Meeker AK. Telomeres and telomerase in prostatic intraepithelial neoplasia and prostate cancer biology. Urol Oncol 2006; 24: 122–30.
8 Kim SH, Richardson M, Chinnakannu K, Bai VU, Menon M, *et al*. Androgen receptor interacts with telomeric proteins in prostate cancer cells. J Biol Chem 2010; 285: 10472–6.
9 Marian CO, Wright WE, Shay JW. The effects of telomerase inhibition on prostate tumor-initiating cells. Int J Cancer 2010; 127: 321–31.
10 Fordyce CA, Heaphy CM, Joste NE, Smith AY, Hunt WC, *et al*. Association between cancer-free survival and telomere DNA content in prostate tumors. J Urol 2005; 173: 610–4.
11 Verdun RE, Crabbe L, Haggblom C, Karlseder J. Functional human telomeres are recognized as DNA damage in G2 of the cell cycle. Mol Cell 2005; 20: 551–61.
12 Verdun RE, Karlseder J. The DNA damage machinery and homologous recombination pathway act consecutively to protect human telomeres. Cell 2006; 127: 709–20.
13 Pickett HA, Cesare AJ, Johnston RL, Neumann AA, Reddel RR. Control of telomere length by a trimming mechanism that involves generation of t-circles. EMBO J 2009; 28: 799–809. Epub 2009 Feb 12.
14 Wang RC, Smogorzewska A, de Lange T. Homologous recombination generates T-loop-sized deletions at human telomeres. Cell 2004; 119: 355–68.
15 Touzot F, Callebaut I, Soulier J, Gaillard L, Azerrad C, *et al*. Function of Apollo (SNM1B) at telomere highlighted by a splice variant identified in a patient with Hoyeraal-Hreidarsson syndrome. Proc Natl Acad Sci USA 2010; 107: 10097–102.
16 Shore D, Bianchi A. Telomere length regulation: coupling DNA end processing to feedback regulation of telomerase. EMBO J 2009; 28: 2309–22.
17 Gu B, Bessler M, Mason PJ. Dyskerin, telomerase and the DNA damage response. Cell Cycle 2009; 8: 6–10.