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

Jeremy D. Henson

Children’s Medical Research Institute, Sydney, NSW 2154, Australia
University of Sydney, NSW 2154, Australia

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]. 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...
0. **Pre-replication telomeres**: the telomeres are thought to be folded in a t-loop. This structure prevents the telomere triggering a DDR by hiding the end from double-strand break sensors. POT1 bound to the single-strand (ss) regions on the telomeric G-strand prevents ssDNA DDR sensors from binding [6].

1. **Telomere replication**: the t-loop needs to be unfolded for replication and POT1 is also removed [11]. Leading-strand replication proceeds to completion producing a blunt end [2]. Lagging-strand replication fails to copy the end-most nucleotides leaving a G-strand overhang [1, 2].

2. **Immediately post-replication; rapid C-strand resection and telomerase action on all telomeres**: within 30 minutes Apollo nuclease resects the C-strand on blunt ended telomeres. If expressed, telomerase extends the length of the G-strand on all lagging-strand telomeres and at least some leading-strand telomeres. Telomerase requires a G-strand overhang of ≥ 6 nt and cannot act on blunt ends [1–3].

3. **Late S-phase; delayed C-strand fill-in**: approximately four hours later the C-strand on lagging-strand telomeres is elongated. This may also occur on a minority of leading-strand telomeres with long G-strand overhangs. C-strand fill-in involves STN1 binding to the overhang to recruit DNA Polymerase α and is regulated by CDK1 [1, 2].

4. **G-phase; additional C-strand resection and refolding into t-loops**: homology directed repair proteins are recruited to the telomere, possibly due to recognition of the telomeric end as a double-strand break. Any remaining short G-strand overhangs on leading-strand replicated telomeres are lengthened by C-strand resection and the telomere refolded into a t-loop [1, 2, 12]. POT1 also rebinds to the telomere [11].

5. **Episodic large deletions if a TMM is present**: telomerase elongates all lagging G-strands and not just the shorter leading-strand replicated G-strands [1]. Because there is no resection of the G-strands, most telomeres will increase in length every cell cycle. Telomere length equilibrium is possibly achieved by episodic deletions of the t-loop by homologous recombination [13, 14].

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**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 G2-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


