Human telomere RNA structure and function

Yan Xu

Division of Chemistry, Department of Medical Sciences, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki, 889-1692, Japan

Correspondence: Yan Xu
E-mail: xuyan@med.miyazaki-u.ac.jp
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A recent finding demonstrated that telomere DNA is transcribed into telomeric repeat-containing RNA (referred to as TERRA) in mammalian cells. The existence of TERRA RNA may reveal a new level of regulation and protection of chromosome ends that could promote valuable insight into fundamental biological processes such as cancer and aging. Revealing the structure and function of telomere RNA will be essential for understanding telomere biology and telomere-related diseases. In fact, others and we have shown by NMR and X-ray crystallography that human telomere RNA forms G-quadruplex structures. We found that human telomere RNA forms a G-quadruplex dimer in the living cells by employing a light-switching probe. Recently, we also demonstrated that telomere RNA G-quadruplex structures play an important role in providing a protective structure for telomere ends. This review highlights the structures and topologies for telomere RNA G-quadruplex and recent efforts in the understanding of telomere RNA function; outlines the future challenges in the field.

Keywords: Human telomere; telomere RNA structure; telomere RNA function; G-quadruplex

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Human telomere RNA structure

A recent finding demonstrated that telomere DNA is transcribed into telomeric repeat-containing RNA (referred to as TERRA) in mammalian cells [1, 2]. TERRA molecules were detected in different human and rodent cell lines and contained mainly UUAGGG repeats of heterogeneous length. The existence of TERRA RNA may reveal a new level of regulation and protection of chromosome ends that could facilitate valuable insights into fundamental biological processes such as cancer and aging [1, 2]. Revealing the structure and function of telomere RNA will be essential for understanding telomere biology and telomere-related diseases. In fact, others and we have shown by NMR and X-ray crystallography that human telomere RNA forms G-quadruplex structures [3-5]. We examined the conformation of the 12-mer human telomere RNA sequence r(UAGGGUUAGGGU) in the presence of Na⁺ by multi-method approaches including circular dichroism (CD), NMR, and gel electrophoresis. We have demonstrated that the telomere RNA forms a parallel dimeric G-quadruplex (Figure 1a) [3]. The telomere RNA G-quadruplex was found to induce a strong RNase resistance for UUAGGG repeats telomere RNA [3]. Phan et al. demonstrated a same folding topology for the 12-mer human telomeric RNA sequence in K⁺ solution (Figure 1b) [4]. Neidle et al. reported a
K\(^+\)-stabilized crystal structure of r(UAGGGUUAGGGU), indicating a parallel G-quadruplex \[^5\]. Recently, we reported the structural features of human telomere RNA r(UAGGGU) in the presence of K\(^+\) and Na\(^+\) \[^6\]. It has been demonstrated that a novel U-tetrad is formed at the 3’ end of a parallel human telomeric RNA G-quadruplex (Figure 1c). The U-tetrad dramatically stabilizes the human telomeric RNA G-quadruplex structure, adding considerably to our understanding of the diversity of RNA G-quadruplex architectures. A human 24-mer telomere RNA sequence r(UUAGGG)\(_4\) was also suggested by CD spectroscopy to form a parallel G-quadruplex \[^3-5\].

To investigate whether TERRA RNA G-quadruplexes exist in living cells, we designed and synthesized a light-switching pyrene probe. The pyrene probe switching its fluorescence from monomer to excimer emission was used to detect G-quadruplex structure. Using this probe, we found that human TERRA RNA forms a parallel G-quadruplex structure in living cells (Figure 1d) \[^7\]. When the pyrene-labeled probe is free in solution without G-quadruplex formation, the pyrene molecules are spatially separated and only the monomer emission peaks are observed. Formation of G-quadruplex brings the pyrene molecules at the 5’ and/or 3’ ends into close proximity, allowing the formation of an excimer. The excimer possesses broad red-shifted emission, in contrast with the pyrene monomer. The change in emission color serves as a way to rapidly probe the G-quadruplex structure in living cells.

This discovery of telomere RNA raises the crucial question of how telomeric RNA is specifically associated with telomeric DNA in terms of chromosome-end regulation and protection. The protective functions of telomeres are thought to be due to telomere-specific DNA conformations. In humans, telomeric DNA consists of a duplex region,
composed of TTAGGG repeats, ending in a shorter G-rich single-stranded overhang [8-13]. Previous studies have suggested that human telomere DNA may exist in multiple states such as G-quadruplex or T-loop [14-21]. For example, we along with two other groups determined the topology of human telomeric G-quadruplex in K+ solution [17-19]. Our recent studies also demonstrated that telomeric-overhang DNA forms a higher-order DNA structure containing consecutive G-quadruplexes [22]. To investigate the possible association between telomere RNA and telomere DNA, we designed a click reaction in which only the DNA-RNA G-quadruplex could undergo an azide-alkyne cycloaddition, even in the presence of the corresponding DNA-DNA or RNA-RNA dimeric G-quadruplex. The click reaction can trap a particular species or produce a snapshot of the various inter-converting structures that are present in a

![Figure 2](http://www.smartsctech.com/index.php/tt)
complex solution. Using the click chemistry, we successfully found that a 12-mer human telomere RNA and 12-mer human telomere DNA sequence can form a DNA-RNA hybrid type G-quadruplex structure (Figure 3) [23].

Human telomere RNA function

TERRA RNA has been shown to directly pair with the template region of hTR as a telomerase ligand and natural direct inhibitor of human telomerase [24]. Lingner et al. proposed three possible modes for telomerase sequestration by TERRA. The released TERRA from the telomere may bind and inhibit telomere-proximal telomerase molecules. Telomere DNA bound TERRA may also bind and sequester telomerase and prevent its access to the telomere. Moreover, telomeric DNA-RNA G-quadruplexes should avoid the exposure of homologous single-stranded terminal DNA. Consistent with higher-order telomeric DNA G-quadruplex structures, the telomeric DNA-RNA G-quadruplex and TERRA RNA G-quadruplexes may be formed at the terminus of this superhelix structure to provide a protective effect (Figure 3). Human telomeric RNA inhibits chromosome end fusions and has an effect on the inhibition of cellular senescence [25]. The higher-order G-quadruplex structure should avoid the exposure of homologous single-stranded terminal DNA because it blocks the access of a key DNA damage regulator(s) to the single stranded DNA. The consecutive formation of RNA G-quadruplexes by long
TERRA RNA is consistent with a recent result that TERRA molecules have an approximate length of 200 bases [26].

Recent studies indicated that siRNA-mediated depletion of telomeric RNA caused an increase in telomere dysfunction-induced foci and aberrations in metaphase telomeres [27-28]. Conversely, increased levels of telomeric RNA observed to be associated with thermal shock, may help protect telomeres against stress-induced damage [29]. Some proteins have been suggested to be associated with telomeric RNA [29], such as TRF2, which was found to recruit telomeric RNA to telomeric DNA [27]. Telomeric RNA also promotes POT1 binding to telomeric ssDNA by removing hnRNP-A1, suggesting that the accumulation of TERRA helps to complete the RPA-to-POT1 switch on telomeric ssDNA and promotes telomere capping [30].

The finding of telomere RNA molecules opens new doors to better understanding of the essential biological role of telomeres. There is a clear need to revisit the structural and functional mechanisms of telomeres accompanying telomere RNA participation. Telomere RNA, a newly emerging player, might have clinical relevance in the treatment of cancer, as the RNA molecule may contribute to the telomeric alterations accompanying malignant transformation [2]. Thus, telomere RNA G-quadruplex structures should be a valuable target for anticancer agents directed against telomeres. To stabilize telomere RNA G-quadruplex structures by small molecules can lead to telomerase inhibition. This would transform telomere RNA into a potential target for anticancer agents directed against telomeres. Furthermore, telomere DNA, telomere RNA, and related-proteins may form TDRP bodies (telomeric DNA, RNA and proteins referred to as TDRP), similar to P bodies in mRNA surveillance and mRNA decay, RNA-mediated silencing and translational control. To form the higher-order telomere structures is believed to achieve the “capping” function of telomere [31]. Recent studies suggest that telomeres can promote telomere capping by inducing POT1 protein binding to single-strand telomere DNA. Therefore, the targeting of telomere maintenance will be an exciting prospect in future cancer strategies [32].

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Conflicting interests

The authors have declared that no conflict of interests exist.

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