The folding complexity of TERRA G-quadruplexes unveiled at the single-molecule level

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Guanine-rich sequences, which can be found in telomeres and the regulatory regions of many genes in the human genome, have the potential to self-associate into flat guanine quartets, giving rise to four-stranded arrangements known as G-quadruplexes. The presence of these sequences in vivo, along with the fact that their unique conformation provides selective recognition sites for small molecules, have enabled G-quadruplexes as important drug-design targets for the treatment of various human disorders, including cancer.

We and others previously studied single-molecule mechanical folding and unfolding processes of long human telomeric RNA (TERRA) [1,2]. Here, we report on the unfolding dynamics of short TERRA molecules by optical tweezers. Specifically, molecules with five repeats of the telomeric sequence GGGUUA, thus, capable of assembling into a maximum of one G-quadruplex. We find that the inherent capacity of single-stranded RNA to self-interact and fold into a condensate blocks the formation of the G-quadruplex with a significant probability. The unfolding dynamics observed in TERRA molecules with more telomeric repeats or with more random extra single-stranded RNA confirm this stochastic bistability with coherent conformational probability. In contrast, DNA analogue molecules did not show this condensation blockage. Therefore, the strong conformational competition exhibited by RNA telomeric sequences confers on these short molecules a unique bistable folding complexity that is not found in proteins, which normally fold into a unique conformation for fixed external conditions. This knowlegde is important to understand G-quadruplex-binding drugs and the mechanical activity of the telomerase.

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[2] Yangyuoru, PM; Zhang ,AY; Shi, Z; Koirala, D.; Balasubramanian, S; Mao, H. ChemBioChem 2013, 14, 1931-1935.