G-quadruplex DNA and the Regulation of Human Telomere Accessibility

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Introduction

Q: What are telomeres?
A: *Telomeres are structures which cap the ends of chromosomes (Fig. 1a, 1b).*

Q: What do telomeres do?
A: *They prevent DNA degradation and maintain genome integrity.*

Q: How are telomeres linked to cancer?
A: *In healthy cells, telomeres shorten over time, leading to cellular aging. However, cancer cells deregulate this behavior and can extend the telomeres indefinitely, which makes them immortal!*

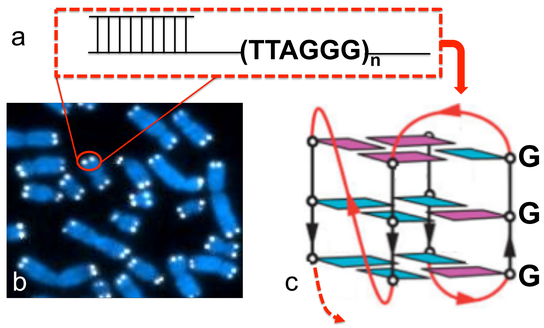


Figure 1. *(a) Schematic representation of the telomere. Note the single-stranded overhang comprised of G-rich TTAGGG repeats. Adapted from Yildiz Lab. (b) Telomeres (white) cap the ends of chromosomes. (c) G-quadruplex. Parallel, planar interactions stabilize the structure. Adapted from Phan AT et al. (2007).*

Q: What is a G-quadruplex (GQ)?
A: *A quartet of guanine-rich, single stranded DNA regions folded into a hill-like structure (Figure 1c).*

Q: How are GQs and telomeres related?
A: *Human telomeres are composed of G-rich, repeats—TTAGGG—which, when single-stranded, form stable GQs (Figure 1a, 1c).*

Aims

- To study the *dynamics* and *folding* of G-quadruplex forming, telomeric DNA
- To investigate the effect of quadruplex formation on the binding properties of telomeres

Methods

Förster Resonance Energy Transfer (FRET):
The *efficiency* of energy transfer between light-absorbing molecules is *directly related to the distance* between them. As two dyes become closer, the FRET efficiency, *E*, increases (Fig. 2). *E* can be easily monitored, which allows nanoscale dynamics to be measured.

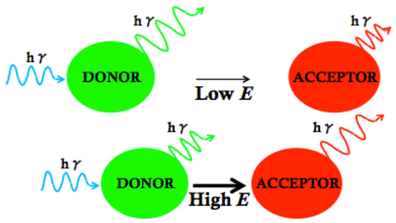
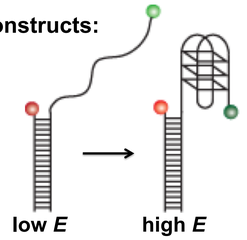


Figure 2. *Larger separation between dyes leads to lower FRET efficiency, whereas proximal dyes have more energy transfer. The donor passes energy to the acceptor molecule.*

Design of Telomere DNA Constructs:

- Dyes were placed at the *base* and *end* of the telomeric, G-quadruplex forming region
- High *E* indicates folding into GQ structure



Reaction Condition Selection:
To find conditions that would ensure stable GQ formation during experiments, FRET efficiency was monitored during salt titrations (Fig. 3).

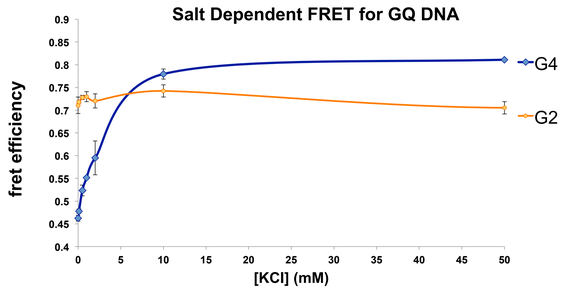
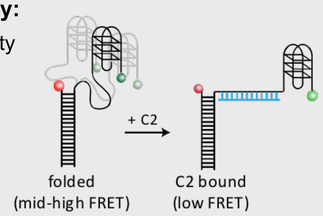


Figure 3. *Salt titration revealed $(TTAGGG)_4$–denoted G4—reliably formed GQ structures in 25mM KCl. Note that G2 is unaffected by salt concentration because the single-stranded region is too short to form a quadruplex.*

C2 Accessibility Assay:

- Determine accessibility by adding G2 complement (C2)
- *If C2 can bind*, FRET efficiency **plummets**!
- Simulates protein binding



Results

Single Molecule, Ensemble, and Atomic Force Microscopy C2 Accessibility Assays:

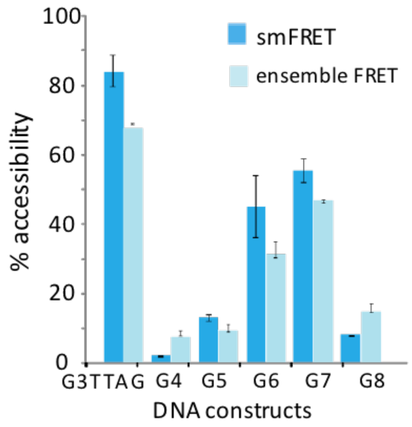


Figure 4. *Single molecule FRET (smFRET) agreement with bulk solution FRET measurements. Due to its inability to fold into a GQ, G3TTAG is easily accessed by C2. However,* ***constructs which can fold the entire overhang into GQs (G4, G8) are mostly inaccessible.*** *Generally, $(TTAGGG)_{4n}$ overhangs disallow binding.*

$$\%\ \text{accessibility}_{DNA} = \frac{\left|\sum_{\Delta<0} \text{FRET}_{(DNA+\ C2)} - \text{FRET}_{DNA}\right|}{\sum_{\Delta<0} \text{FRET}_{DNA}} \times 100\%$$

$$\text{where } \Delta = \text{FRET}_{(DNA+\ C2)} - \text{FRET}_{DNA}$$

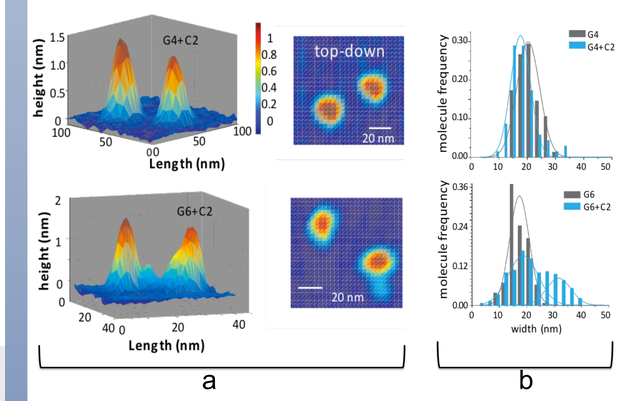


Figure 5. *(a) Atomic force microscopy images show unchanged G4 constructs after C2 addition (upper).* ***G6, however, has enough unfolded overhang to allow C2 to bind****, which results in a shallow handle near the peak (lower). (b) Notable shift in quadruplex population toward greater width, suggesting C2 binding.*

Accessibility to Telomerase:

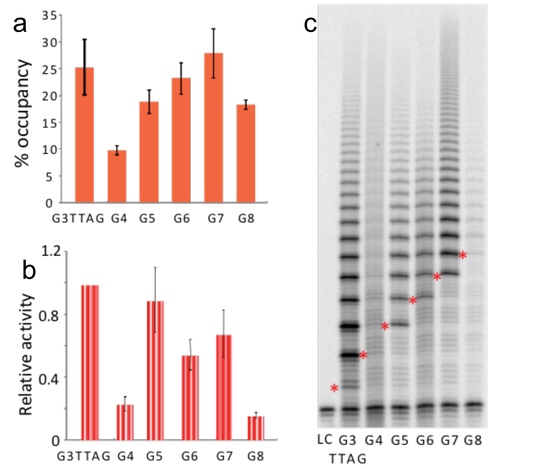


Figure 6. *(a) Amount of telomerase able to occupy each GQ construct. (b) Relative ability of telomerase to extend each telomeric region. (c) Functional gel assay showing DNA extension from telomerase. Note the G4 and G8 lanes showing little extension.*

Conclusions

- **Accessibility** is regulated by the *number of overhang (TTAGGG) repeats* and *protein footprint size*
- **Quartets** of (TTAGGG) *dramatically* reduce protein binding
- **Telomerase activity** is linked to G-quadruplex formation and stability

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