Untangling How the Shelterin Complex Tangles DNA

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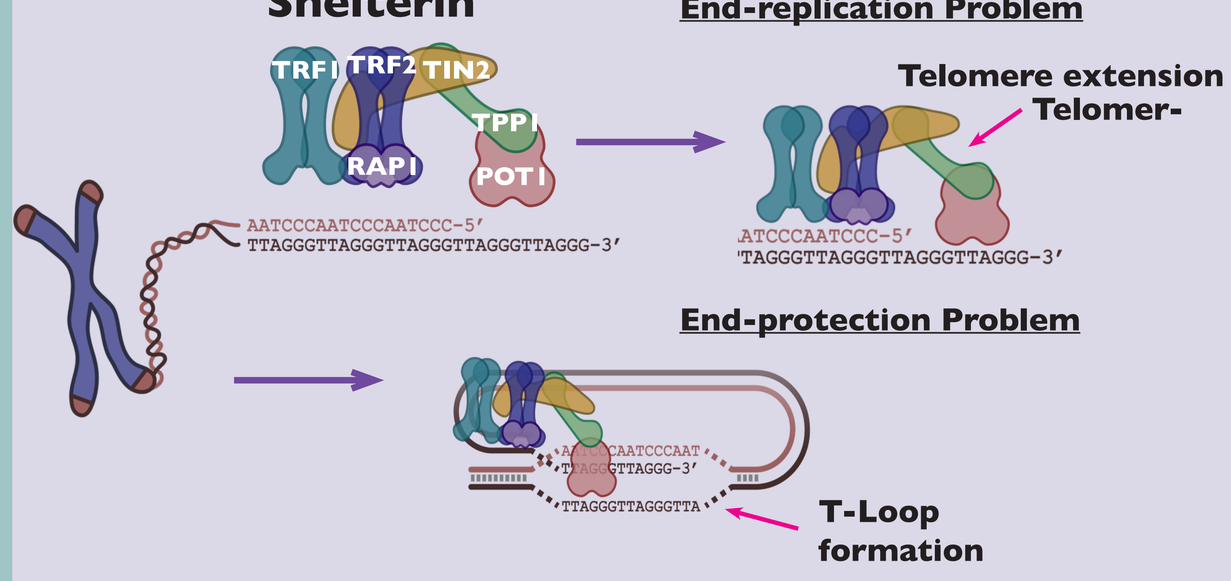
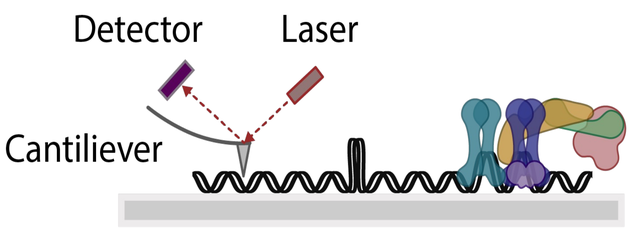
How is telomere function linked to changes in DNA structure?

- Telomeres are structures comprised of proteins and DNA containg the sequence 5' TTAGGG-3' to protect the ends of human chromosomes.
- It's inevitable that we lose some of our telomeric DNA each replication due to the end replication problem, and exposes cells to the DNA damage response, implicated in many cancers (16).
- To overcome the end-protection problem and maintain genomic instability, the shelterin complex of proteins binds to the telomeric end to protect it. It's known that TRF1 and TRF2 bind to dsDNA, which makes it particularly interesting in the field of DNA topology and how it affects the structure of DNA (18).
- This compelx of proteins is implicated in many diseases such as premature aging diseases and familiar cancer disposition and it has been shown that TRF2 functions by a wrapping function of the DNA around its component, allows for T-loop formation and inhibiting ATM activation and Non-Homologous End-Joining (19).

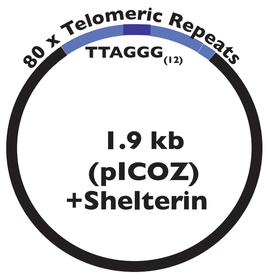
What is the molecular basis for the regulation of these two processes?



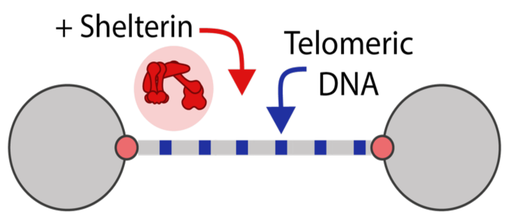
Direct visualization of Shelterin recruitment to telomeric repeats

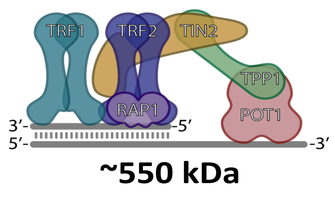


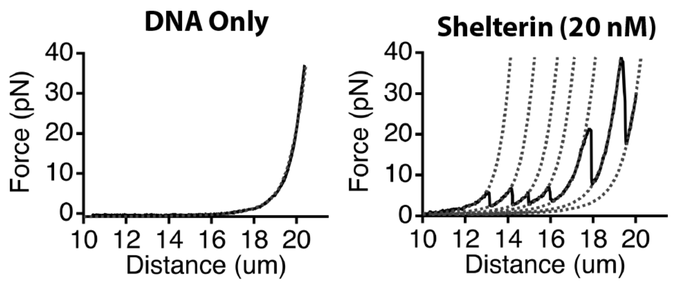
Immobilisation of DNA complexed with Shelterin using nickel chloride onto a mica surface and imaged in fluid.



Optical tweezers were used to measure the interaction between telomeric repeats.

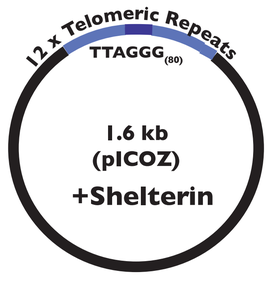


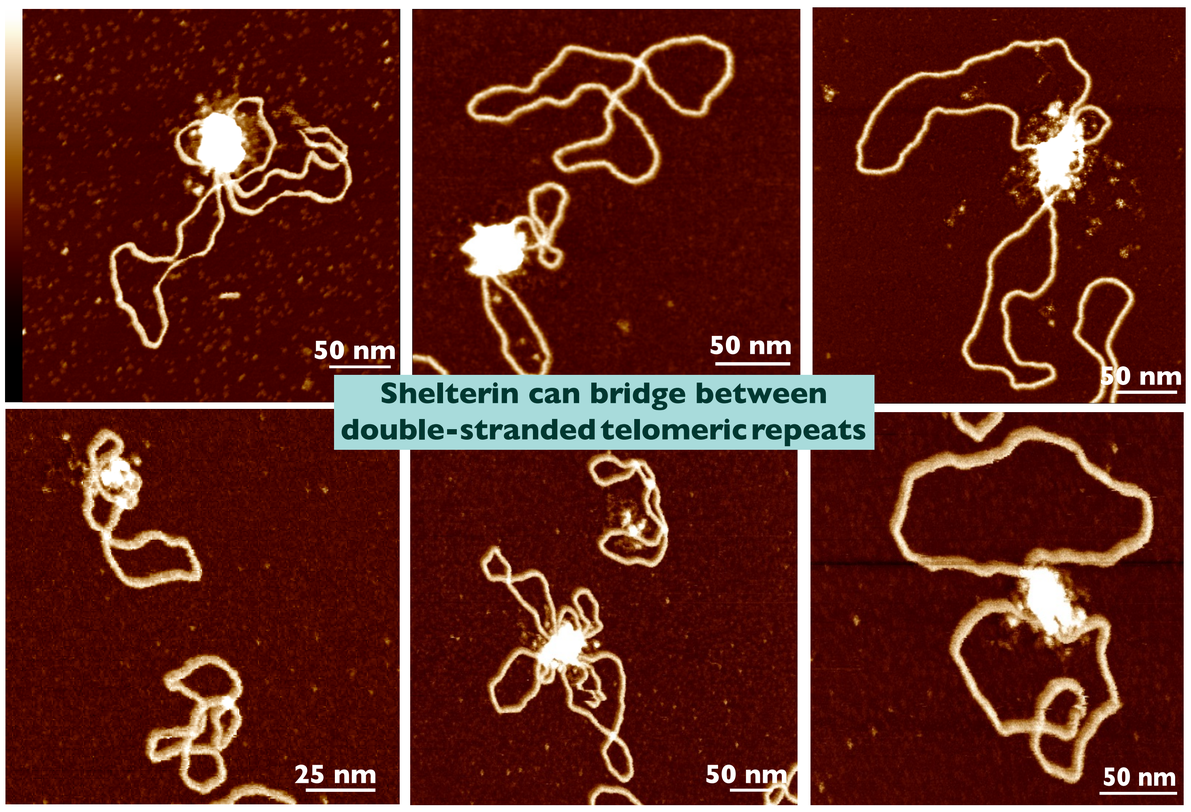




The addition of Shelterin to telomeric DNA causes "rips" in the force curve.

But what does this actually look like?

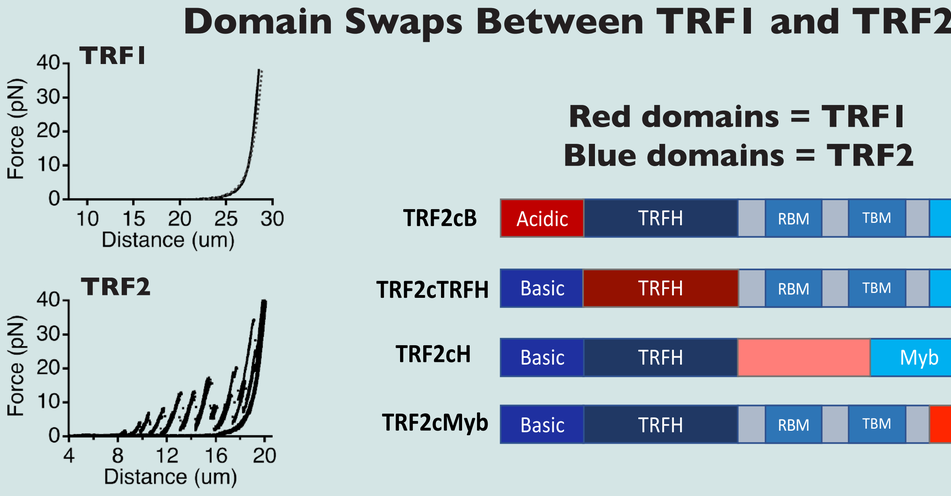




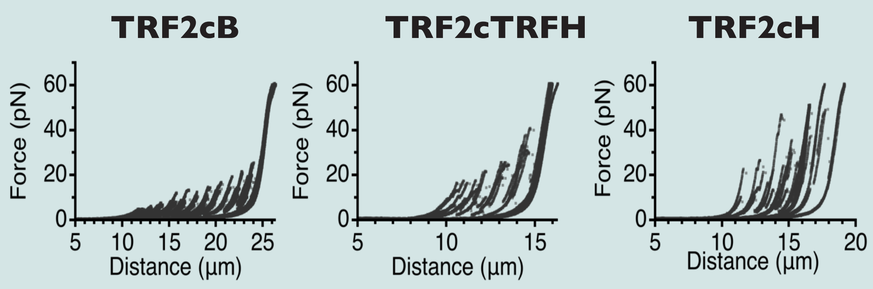
What mediates Shelterin bridging?

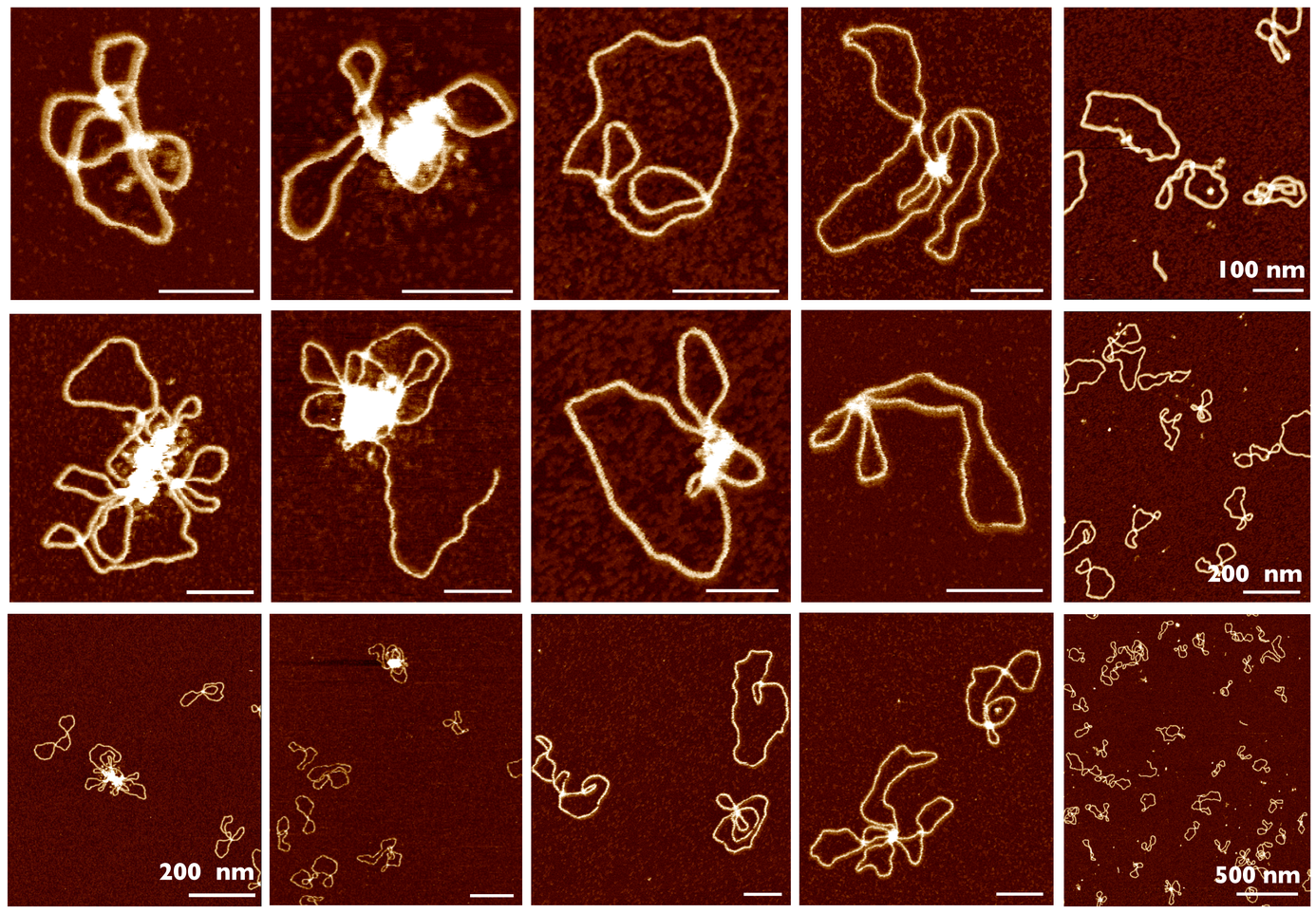
When examining the DNA binding proteins of the Shelterin complex, TRF1 and TRF2, it became apparent that TRF2 was responsible for the bridging action we see within the Shelterin complex since TRF1 seemed to produce a similar force curve to DNA alone. When looking at TRF1 with AFM, it didn't appear to bring together multiple plasmids, which we saw with TRF2 which mimicked what we saw with Shelterin.

Domain Swaps Between TRF1 and TRF2



TRF2 mutants were designed to deduce which domain of TRF2 was causing the bridging between telomeric repeats. TRF1 domains were introduced one by one to create these mutants. The mutants show difference from how TRF2 and TRF1 interact between telomeric repeats.





all scale bars 50 nm, unless stated otherwise unless stated otherwise

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