The core-enclosing helix in yeast telomerase RNA is essential for binding to the TERT catalytic protein subunit and for telomerase activity *in vivo* and *in vitro*

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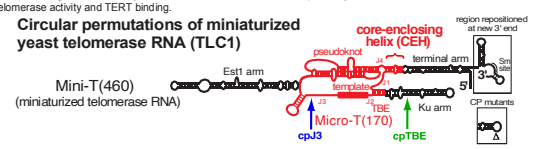
Introduction

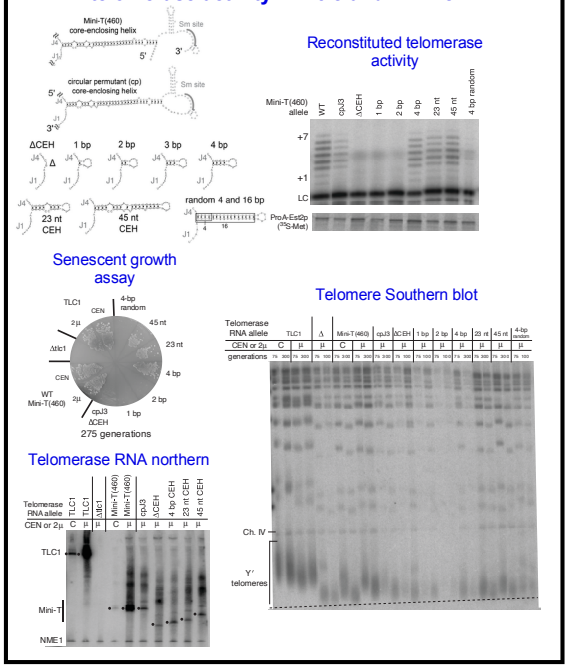
The telomerase RNP countervails the chromosome end-replication problem, completing genome replication to prevent cellular senescence. Increased telomerase function is linked to 90% of cancers, while reductions are associated with premature aging and telomere syndromes that tend to cause organ failure. At its core, the telomerase RNP is composed of a reverse transcriptase (TERT) and a long noncoding RNA. Although the majority of the 1157-nt *Saccharomyces cerevisiae* telomerase RNA, TLC1, is rapidly evolving, the central catalytic core is highly conserved, even sharing features with humans. Consistent with the importance of the conserved structural elements, TLC1 can be pared down to "Mini-T" RNAs one-third the size of wild-type that maintain short, stable telomeres *in vivo*. We have previously shown that TLC1 RNA and its functional miniaturized derivatives can be circularly permuted, with the 5' and 3' ends shifted to many new locations, but not within junctions J1, J2, or J4 in the central catalytic core. These findings defined the Area of Required Connectivity (ARC). In sharp contrast, the RNA backbone can be broken 3' of the template with retention of robust *in-vitro* and *in-vivo* function.

We hypothesize that the Area of Required Connectivity in the catalytic core of yeast telomerase RNA contains multiple binding sites for TERT and that the core-enclosing helix (CEH) is one of the elements needed for this critical interaction. To test this, we used circularly permuted, miniaturized telomerase RNAs, cpJ3 and cpTBE, that have the RNA's ends relocated to locations outside of the essential Area of Required Connectivity. We showed previously that moving the RNA ends to these positions still allows telomerase activity *in vitro* and *in vivo* (Mefford *et al., EMBO* 2013). Moving the ends away from the CEH thus allowed us to precisely evaluate how CEH structure relates to telomerase activity and TERT binding.

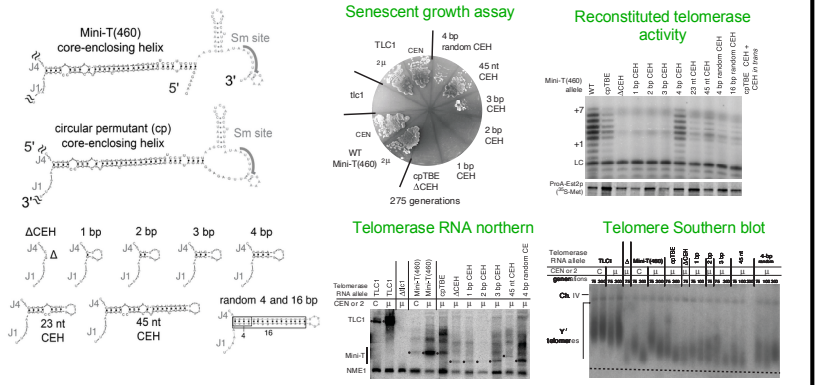
Circular permutations of miniaturized yeast telomerase RNA (TLC1)



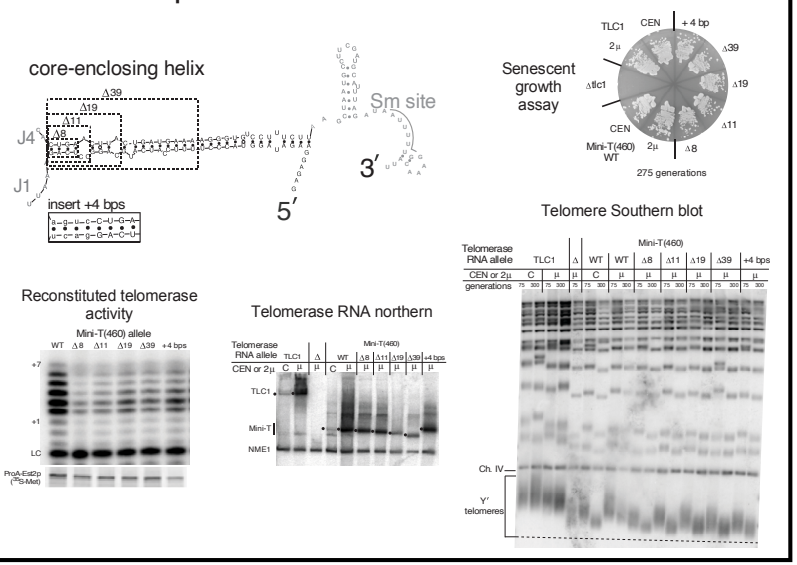
A short core-enclosing helix (CEH) is sufficient for telomerase activity *in vitro* and *in vivo*



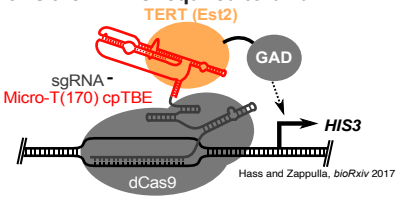
A 4-bp CEH is also sufficient for telomerase function in Mini-T(460) cpTBE



The sequence of the CEH is not essential for function



New CRISPR-based "CARRY two-hybrid" assay shows the CEH is required to bind TERT



sgRNA-Micro-T	GAD-Est2	+ histidine	– histidine
–	–	10^0 10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} dilution of cells	10^0 10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6}
WT	+		
cpTBE	+		
cpTBE ΔCEH	+		
cpTBE 1-bp CEH	+		
cpTBE 2-bp CEH	+		
cpTBE 3-bp CEH	+		
cpTBE 4-bp CEH	+		
cpTBE random 4-bp CEH	+		
cpTBE 23-nt CEH	+		

Results and Conclusions

With telomerase RNA ends relocated to multiple alternative locations (cpJ3 and cpTBE), we observed that 4 base pairs are necessary for the essential CEH element at the core of yeast telomerase to be active *in vitro* and maintain yeast telomeres *in vivo*, whereas ΔCEH, 1-bp, and 2-bp alleles were catalytically dead and senesced.

Using the new CRISPR-dCas9-based "CARRY two-hybrid" assay to assess binding of the circularly permuted Mini-T RNAs to TERT showed that the 4-bp CEH RNA bound to TERT, but the shorter-CEH constructs did not. This is consistent with the telomerase activity and *in-vivo* genetic complementation results.

We conclude that a major reason why the CEH is essential in yeast telomerase RNA is because it is needed to bind TERT to form the core RNP enzyme. Although the 8 nucleotides that form this 4-bp stem at the base of the CEH are nearly invariant among *Saccharomyces species*, our results with sequence-randomized and truncated-CEH alleles strongly suggest that this binding interaction with TERT is dictated more by secondary than primary structure.

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