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INTRODUCTION

Mutations in several components of telomerase have been identified in telomere syndromes. One of the disease-associated mutations occurs in TCAB1, a telomerase RNP component essential for telomere elongation. Absence of TCAB1 is associated with mislocalization of TR. Here we show that TCAB1 mediates telomere elongation by promoting telomerase RNP assembly. **Our work reveals a potential mechanism for the role of TCAB1 in the assembly of the telomerase RNP utilizing several strategies.**

RESULTS

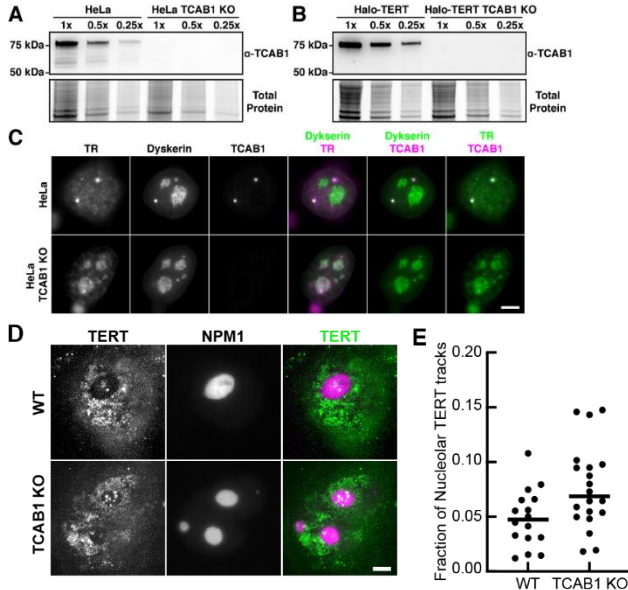


Figure 1. TR is mislocalized to nucleoli in TCAB1 knock-out cells. (A-B) Western blot of TCAB1 protein in TCAB1 knock-out cell lines. (C) Immunofluorescence coupled to fluorescence in-situ hybridization of TR. (D-E) Live cell single molecule imaging of TERT and quantification of nucleolar TERT in WT vs. TCAB1 KO cells.

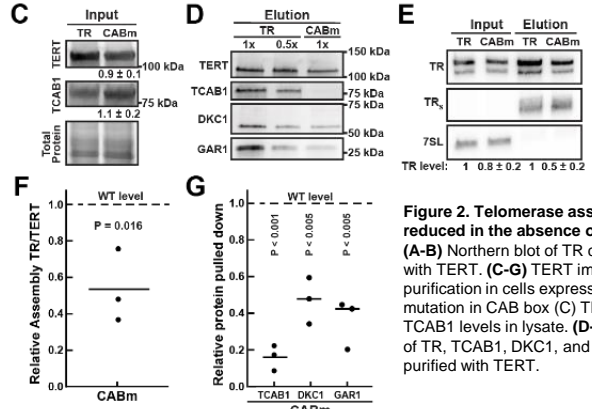
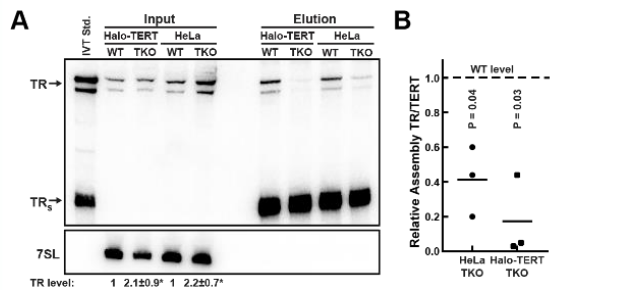


Figure 2. Telomerase assembly is reduced in the absence of TCAB1. (A-B) Northern blot of TR co-purified with TERT. (C-G) TERT immunopurification in cells expressing TR mutation in CAB box (C) TERT and TCAB1 levels in lysate. (D-G) levels of TR, TCAB1, DKC1, and GAR1 co-purified with TERT.

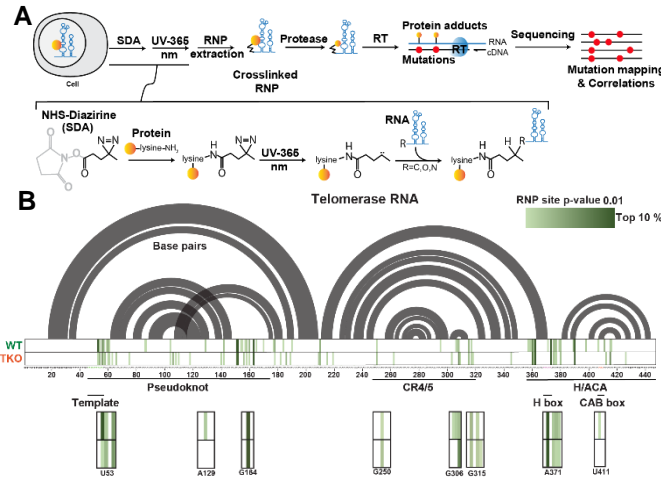


Figure 3. Revealing telomerase RNP via ribonucleoprotein mutational profiling. (A) Schematic representation of the RNP-Map (B) RNP Map crosslinking in telomerase RNA.

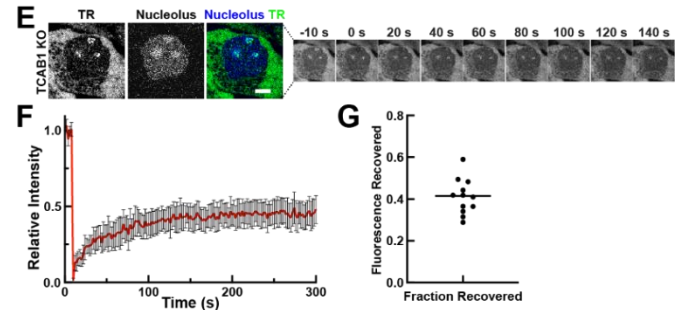
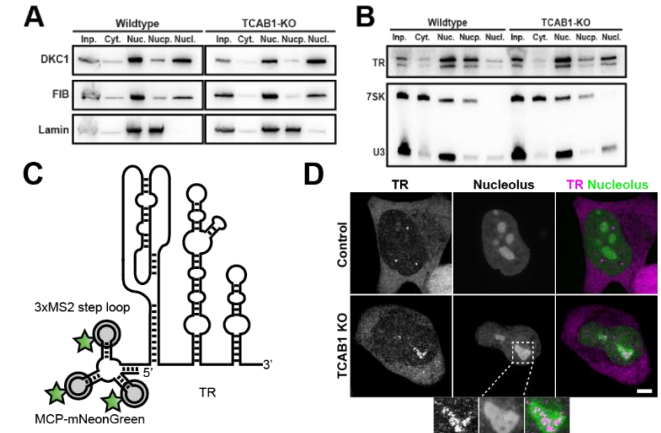


Figure 4. TR is largely sequestered in the nucleoli in the absence of TCAB1. (A-B) Western and northern blots of cellular fractionates. (C-D) Live cell microscopy of MS2-tagged TR. (E-G) Fluorescence recovery after photobleaching of MS2-TR signal in nucleoli in TCAB1 knock-out cells.

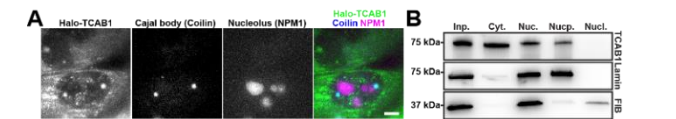


Figure 5. TCAB1 largely does not enter the nucleolus. (A) Live cell microscopy of HaloTagged TCAB1. (B) Untagged TCAB1 in cellular compartments.

Summary

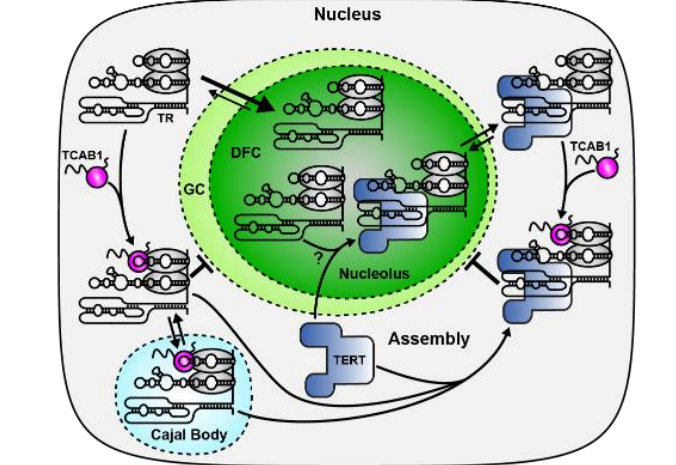


Figure 6. Model of the role of TCAB1 in telomerase assembly and potential locations where assembly may occur.

Acknowledgements

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References

Klump BM, Perez GI, Adams-Boone K, et al. TCAB1 prevents nucleolar accumulation of the telomerase RNA to promote telomerase assembly. Under review. doi:10.1101/2021.05.27.445986