

Telomerase maturation and Cajal Bodies



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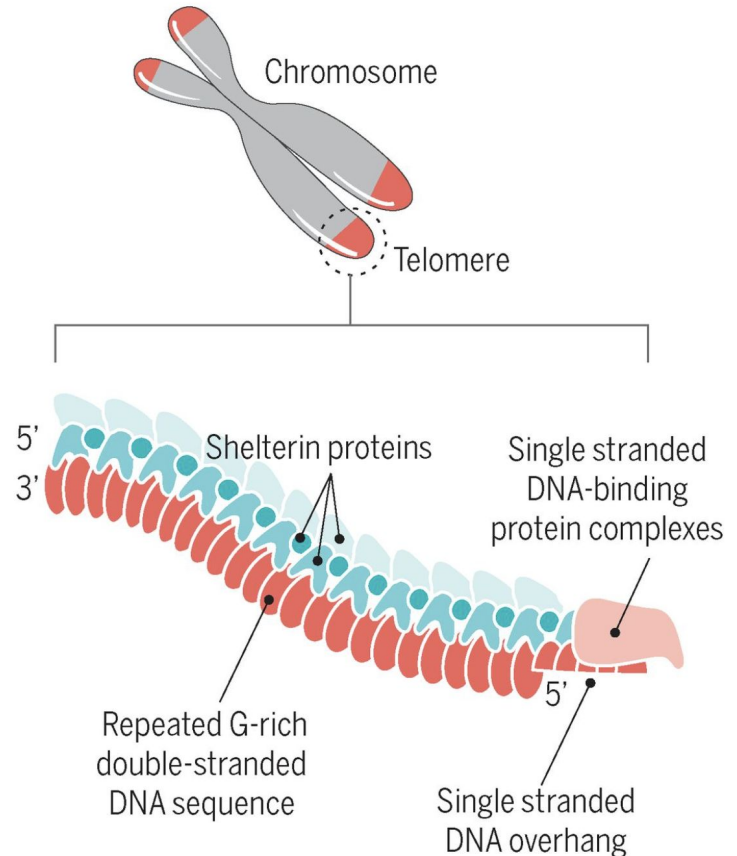
Human telomerase: biogenesis, trafficking, recruitment, and activation

- I° Biogenesis of human telomerase
- II° Telomerase localization to CBs
- III° Telomerase recruitment to telomeres
- IV° Telomerase activation and telomere length regulation

Introduction:

ABOUT HUMAN TELOMERE & TELOMERASE

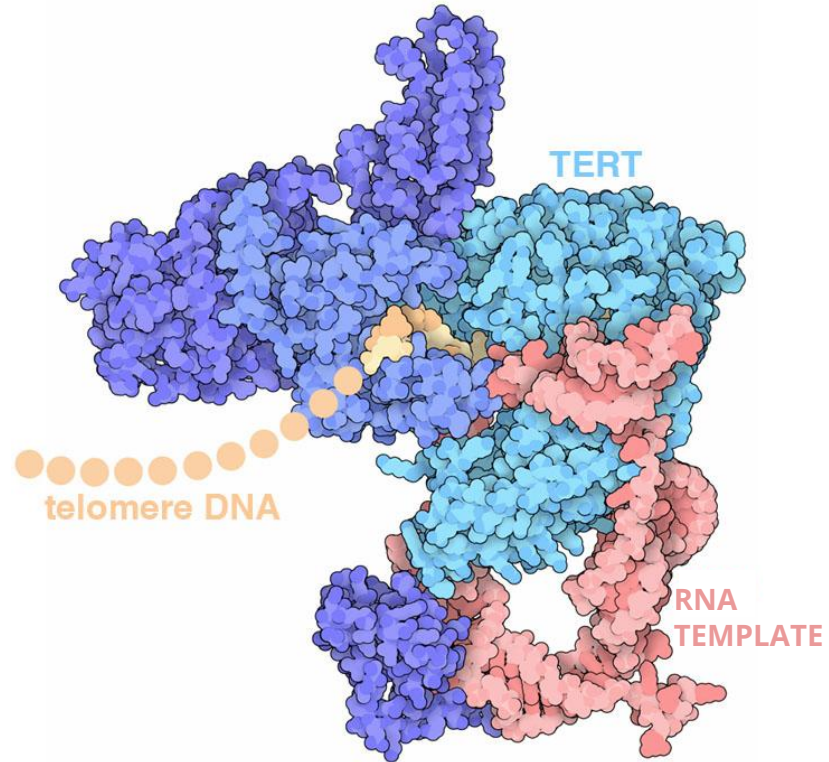
- ❑ Human chromosomes are **capped by telomeres**
- ❑ Telomeres: variable numbers of a **repetitive sequence**
- ❑ **Six-protein shelterin complex** stabilized telomeres
- ❑ **Hayflick limit**



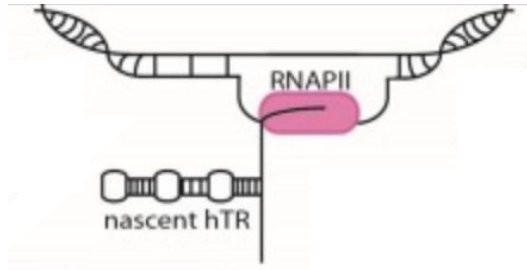
Introduction:

ABOUT TELOMERASE

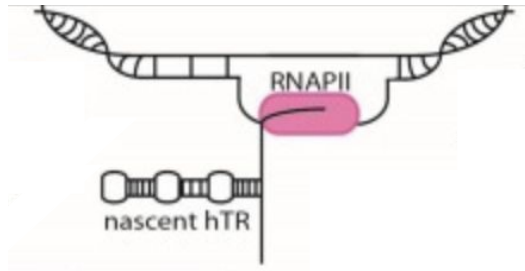
- ❑ Catalyzes the **extension of telomeric DNA**
- ❑ Contains a **reverse transcriptase (TERT)**
- ❑ **Promoter mutations** in hTERT lead to oncogenesis
- ❑ Aplastic anemia or dyskeratosis congenita



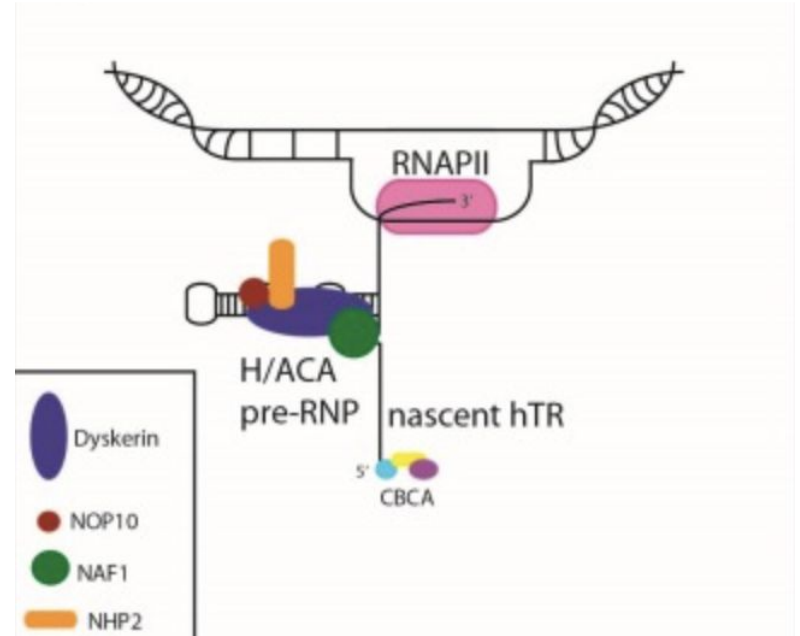
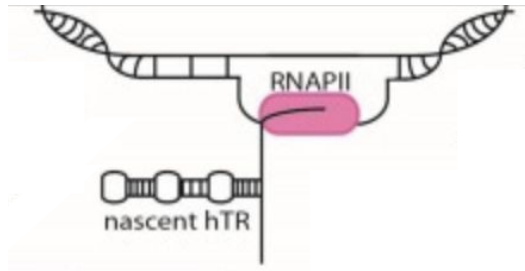
Biogenesis of human telomerase: Telomerase RNA (TR) synthesis and maturation



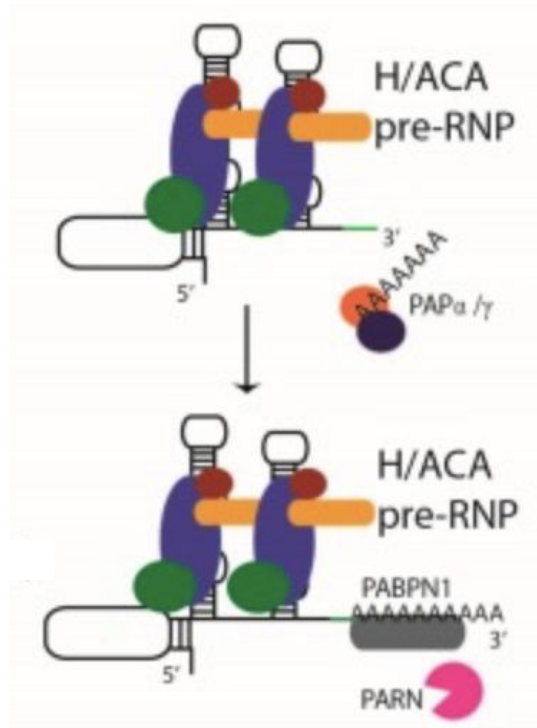
Biogenesis of human telomerase: Telomerase RNA (TR) synthesis and maturation



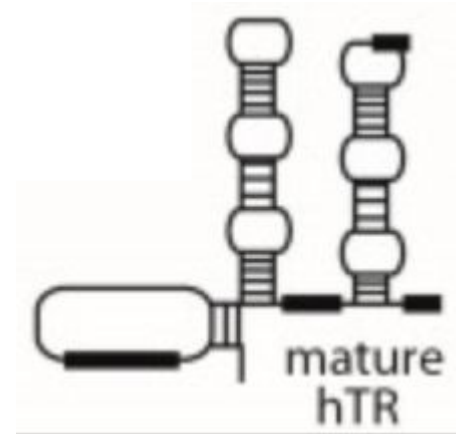
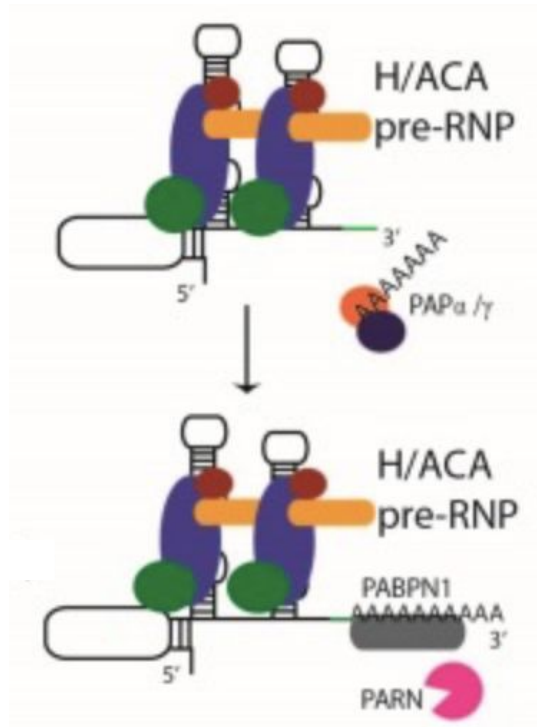
Biogenesis of human telomerase: Telomerase RNA (TR) synthesis and maturation



Biogenesis of human telomerase: Telomerase RNA (TR) synthesis and maturation



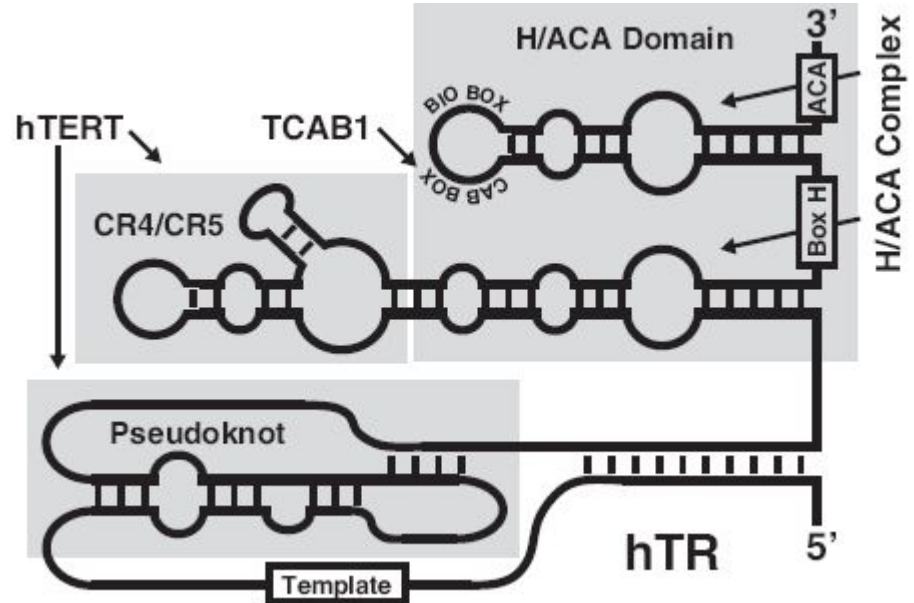
Biogenesis of human telomerase: Telomerase RNA (TR) synthesis and maturation



Biogenesis of human telomerase: Telomerase RNA (TR) synthesis and maturation

ABOUT FUNCTIONAL FEATURES IN TR

- ❑ The **template** for reverse transcription
- ❑ The **pseudoknot domain**
- ❑ A **stem-loop** that interacts with TERT (CR4/CR5)
- ❑ A **3' element** required for RNA stability, trafficking and activity..

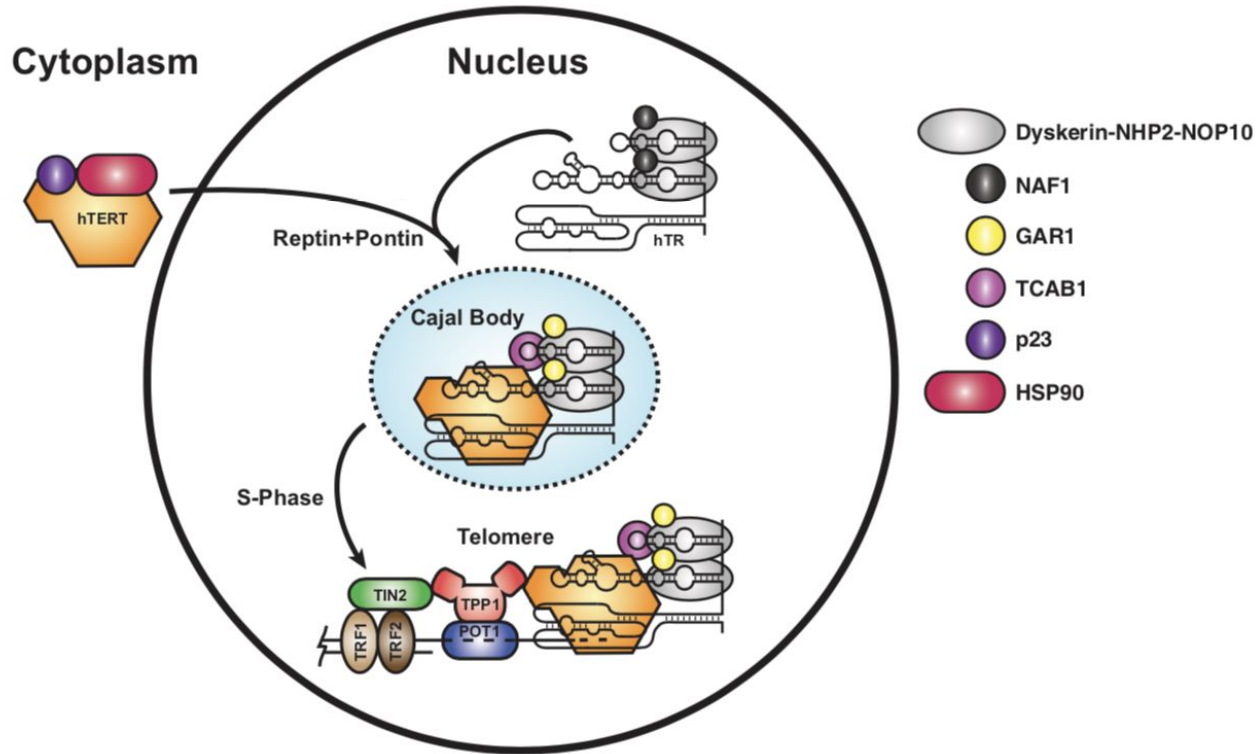


Biogenesis of human telomerase: hTERT

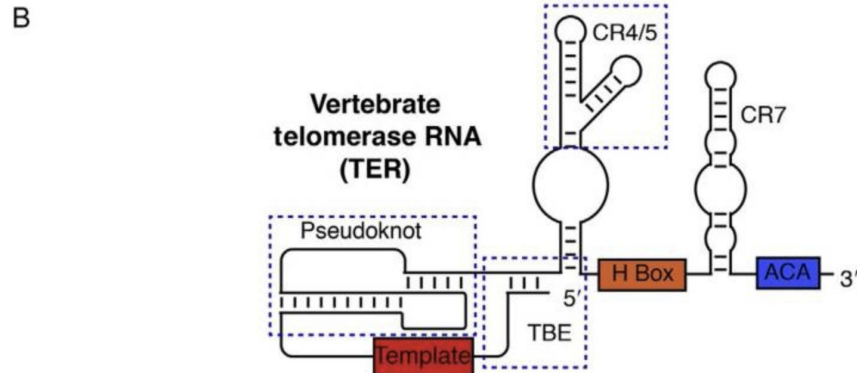
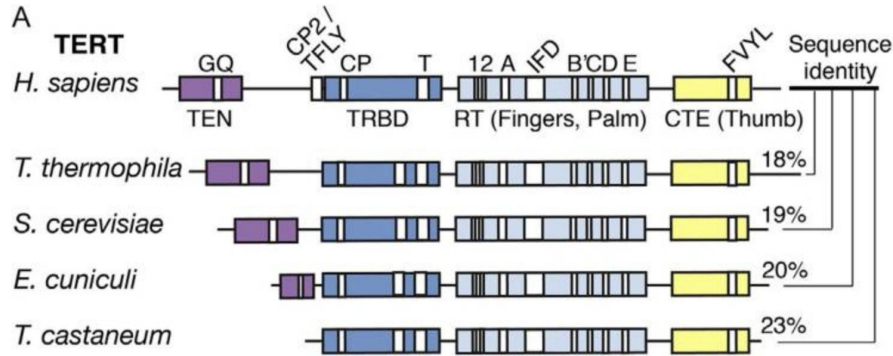


- ❑ The telomerase N-terminal (TEN) domain **participates in catalysis and drives telomerase localization** to telomeres
- ❑ The TR-binding domain (TRBD) **interacts with hTR**
- ❑ The reverse transcriptase (RT) and C- terminal extension (CTE) **form the catalytic core of telomerase**

Biogenesis of human telomerase: telomerase assembly



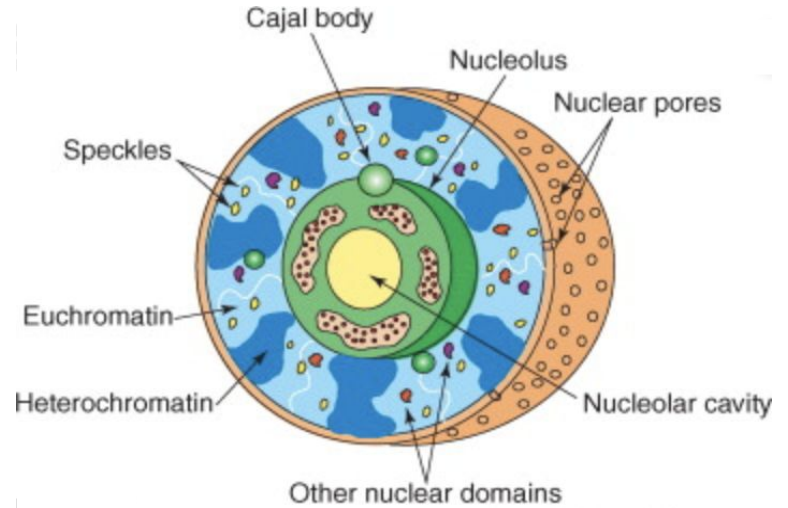
Biogenesis of human telomerase: structural organization of the telomerase RNP



Telomerase localization to Cajal Body (CB):

ABOUT CAJAL BODY FUNCTION

- ❑ Contribution to the biogenesis of :
 - small **nuclear** RNPs (snRNPs)
 - small **nucleolar** RNPs (snoRNPs)
- ❑ Contain **small Cajal body-specific RNPs** (scaRNPs)
- ❑ Contain **RNP of telomerase**
- ❑ **Assembly point** for the incorporation of hTERT into the nascent RNA complex



Telomerase localization to Cajal Body (CB):

ABOUT CAJAL BODY FUNCTION

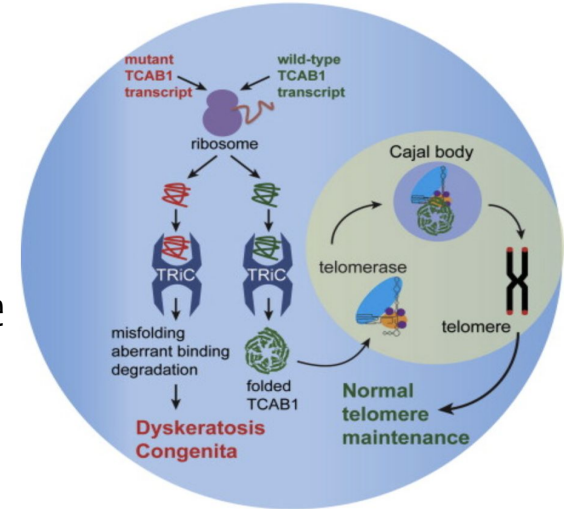
- ❑ Contribution to the biogenesis of :
 - ❑ small **nuclear** RNPs (snRNPs) → Pre-mRNA splicing
 - ❑ small **nucleolar** RNPs (snoRNPs) → rRNA maturation
- ❑ Contain **small Cajal body-specific RNPs** (scaRNPs) → mRNA processing
- ❑ Contain **RNP of telomerase**
- ❑ **Assembly point** for the incorporation of hTERT into the nascent RNA complex

Telomerase localization to Cajal Body (CB):

Proteostatic Control of Telomeras Function through TRIC-Mediated Folding of TCAB1-
Cell, Volume 159, Issue 6, 4 December 2014, Pages 1251-1252

ABOUT TCAB1 FUNCTION IN CAJAL BODY

- ❑ Autosomal recessive TCAB1 mutation :
 - ❑ mutation **do not disrupt interaction** with hTR
 - ❑ TCAB1 is **not required** for the enzymatic activity of telomerase
- ❑ Elimination of Cajal Body:
 - ❑ Rescue by over-expression of telomerase
 - ❑ Telomerase **do not localize** to telomere with TCAB1 loss

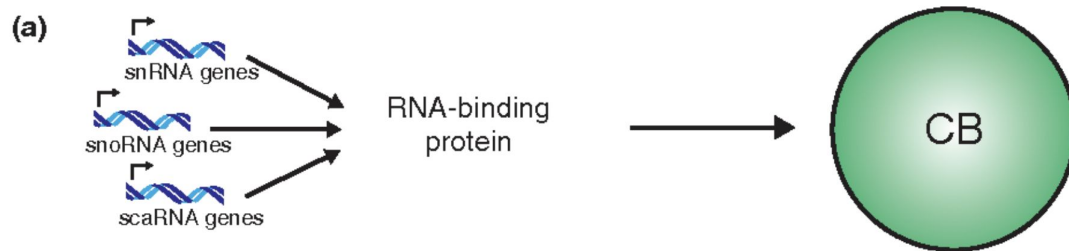


➔ CB and TCAB1 make a **major contribution** to telomerase trafficking and recruitment to telomere

An important component of Cajal Body: Coilin

ABOUT CAJAL BODY FORMATION

- 2 models:
 - CB self-assembly via **RNA binding protein**
 - CB self-assembly via **Coilin**

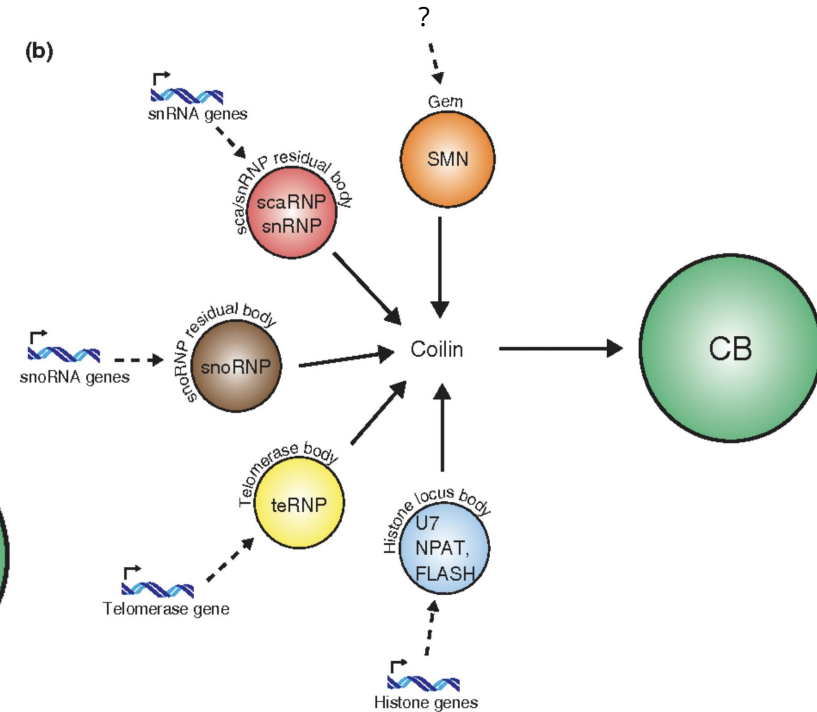
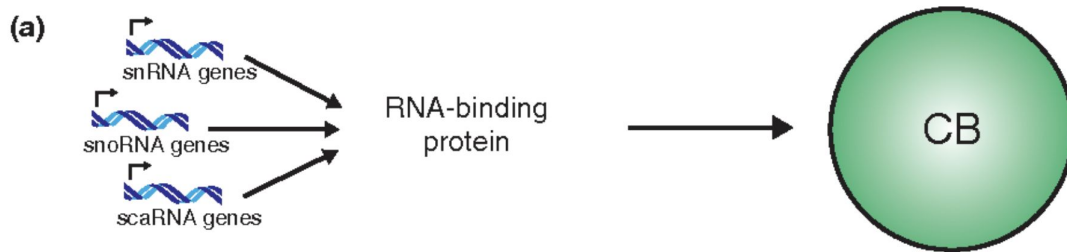


An important component of Cajal Body: Coilin

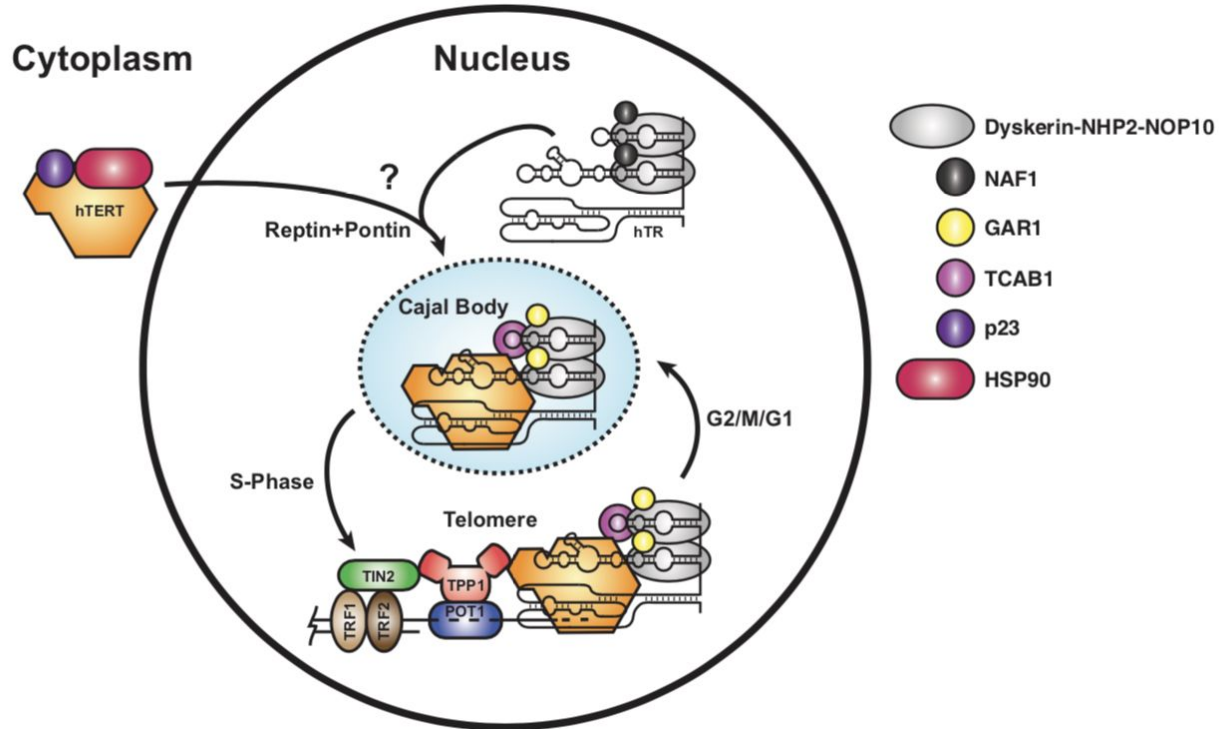
ABOUT CAJAL BODY FORMATION

□ 2 models:

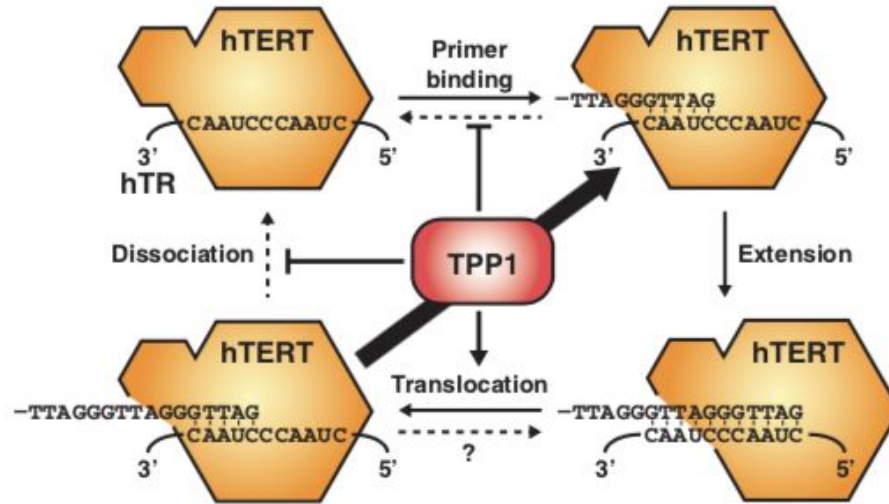
- CB self-assembly via **RNA binding protein**
- CB self-assembly via **Coilin**



Telomerase recruitment to telomeres:



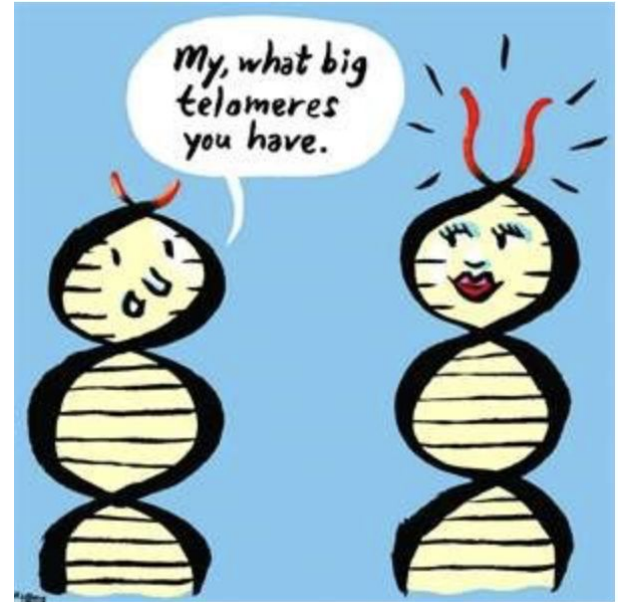
Telomerase activation and telomere length regulation:



- TPP1 controls the telomerase catalytic cycle and its modulation

To conclude:

- ❑ The telomerase is **assembled and recruited to the telomere**
- ❑ The **telomere length maintenance** is a key process in normal human development
- ❑ Inappropriate telomere lengthening is a **hallmark of cancer**



An activity switch in
human telomerase
based on RNA
conformation and
shaped by TCAB1

I° Introduction

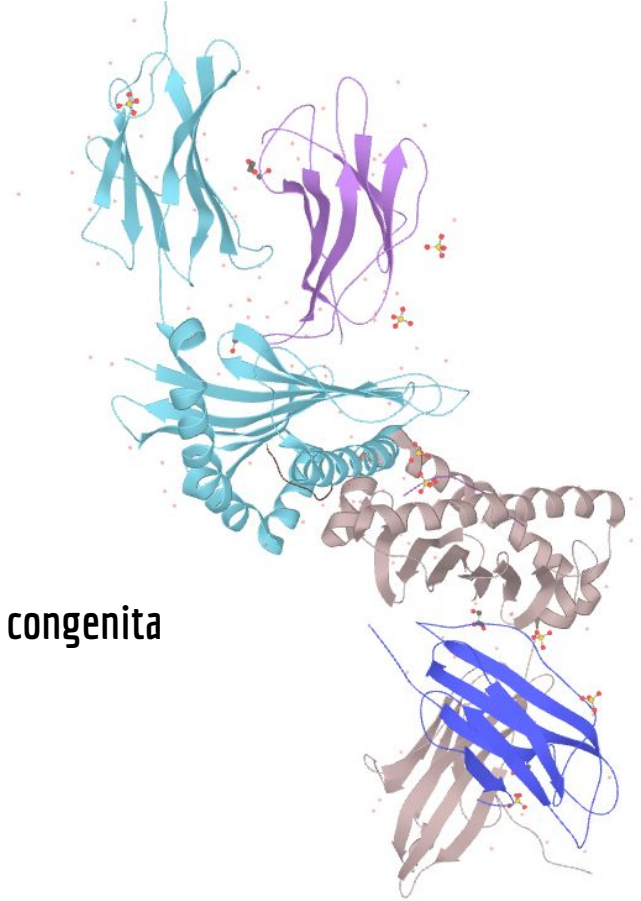
II° Results & discussion

III° Conclusion

Introduction:

ABOUT TELOMERASE

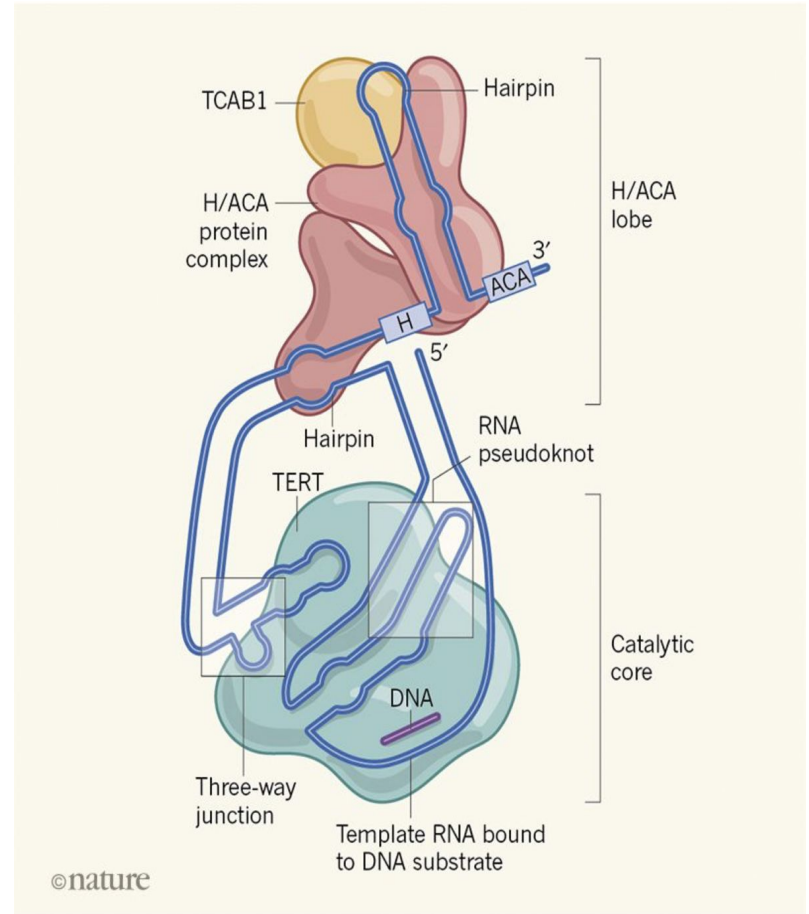
- ❑ Localize in the **nucleus**
- ❑ Multiple subunit **ribonucleoprotein** : maintain the telomere
- ❑ Inactivating mutations in telomerase genes causes **dyskeratosis congenita**
- ❑ Upregulation is central to **carcinogenesis**



Introduction:

ABOUT TELOMERASE RIBONUCLEOPROTEIN COMPLEX

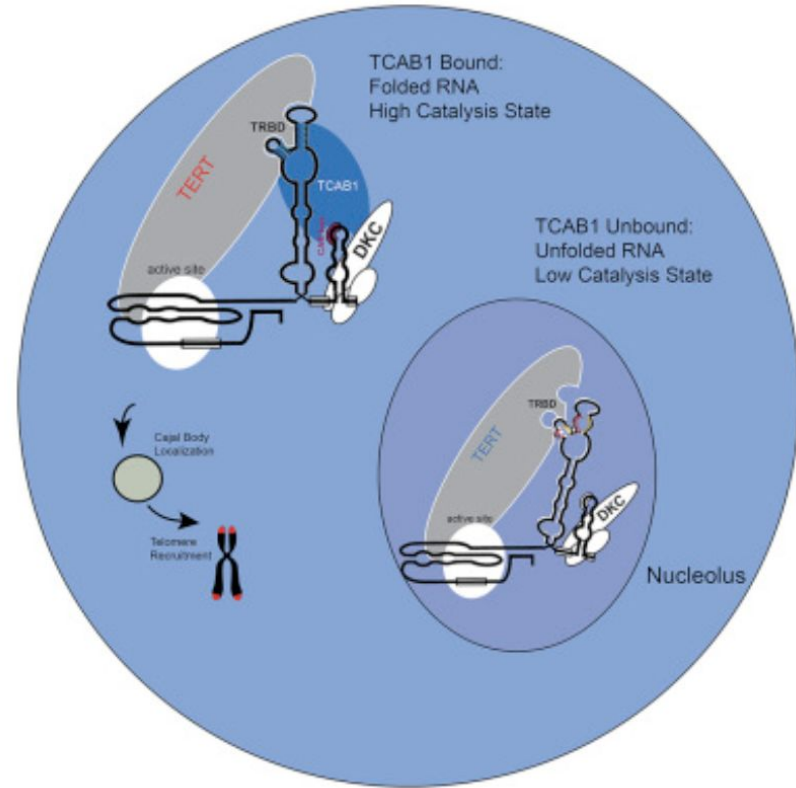
- ❑ Pseudoknot/template (PK/T) domain: **catalysis & fold** into triple helical structure
- ❑ Three-way junction element (CR4/5) domain
- ❑ P6.1 helix : **critical role in catalysis and TERT association**
- ❑ Mutations in P6.1 : **reduction of TERT binding and telomerase catalytic activity**



Introduction:

ABOUT HUMAN TELOMERASE'S STUDIES

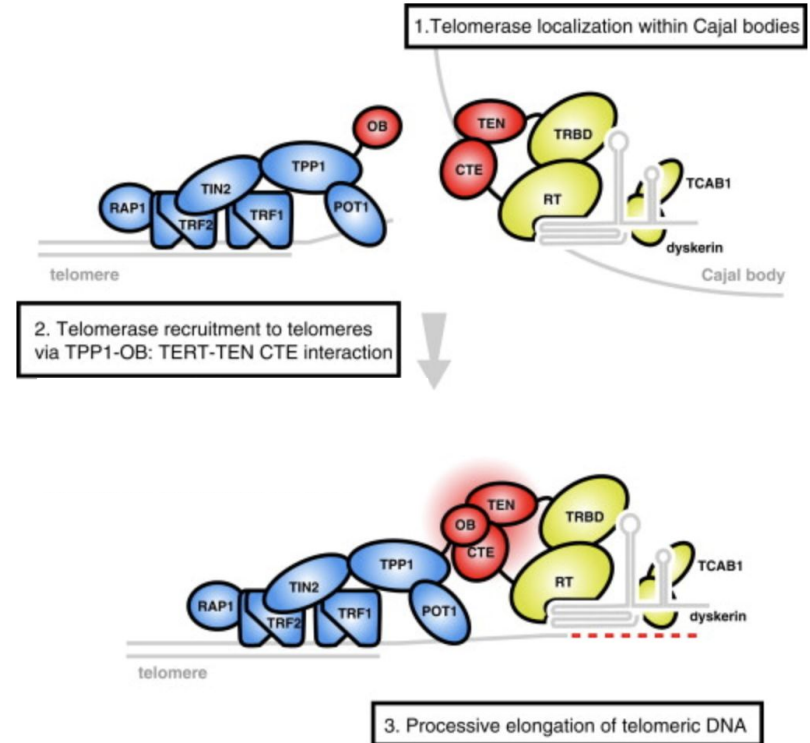
- ❑ CR4/5 serves as an **activation domain**
- ❑ **Engagement of CR4/5** by TRBD of TERT
- ❑ P6.1 and P6 RNA helix **form a clamp**
- ❑ CR4/5 facilitates **telomerase enzymatic activity**



Introduction:

ABOUT THE RECRUITMENT OF TELOMERASE ENZYME

- ❑ Interaction between **TEN domain** and **OB fold domain**
- ❑ Importance of **dyskerin core complex** and **TCAB1**
- ❑ 2 essential functions of **TCAB1** binding to **scaRNA** domain
 - Enhancing recruitment to **telomere**
 - Facilitating proper **localization of telomerase**

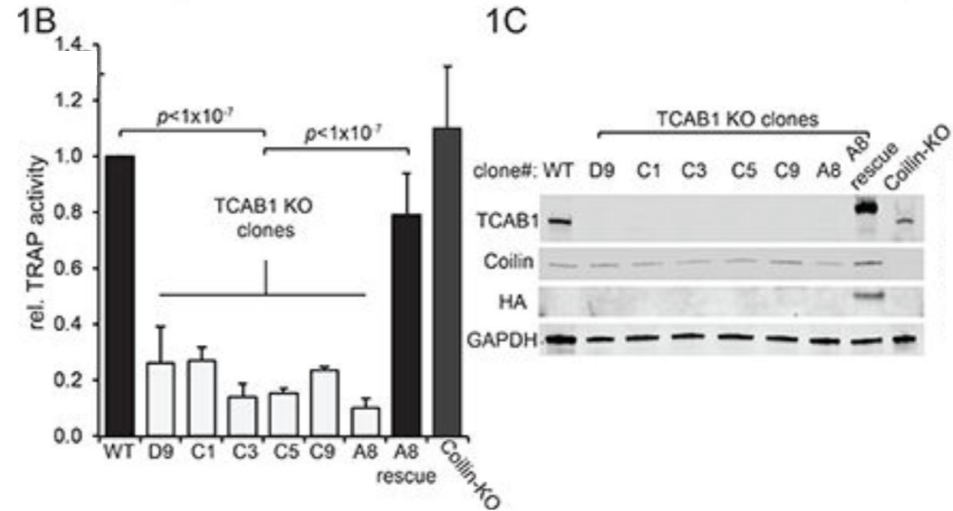
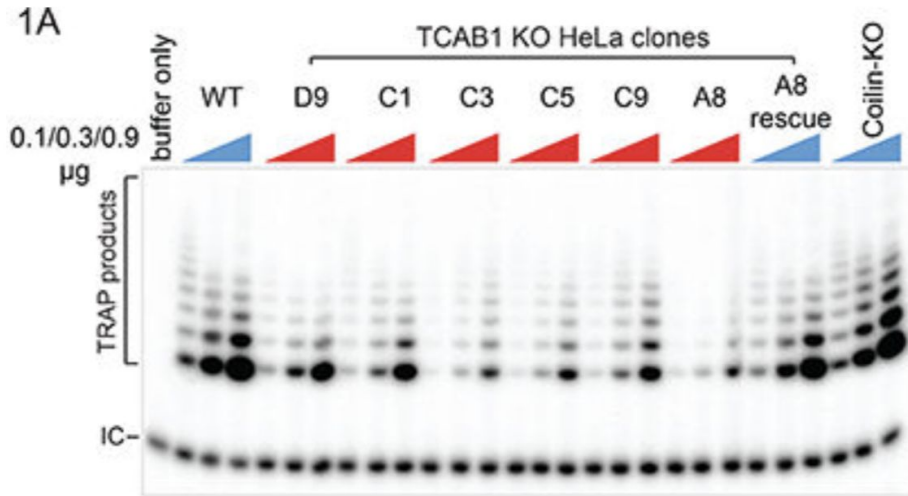


TPP1 OB-Fold Domain Controls Telomere Maintenance by Recruiting Telomerase to Chromosome Ends - August 3, 2012 - Cell Journal

To understand the **molecular function of TCAB1 and the CAB box** of the scaRNA domain in telomerase function, they inactivated TCAB1 :

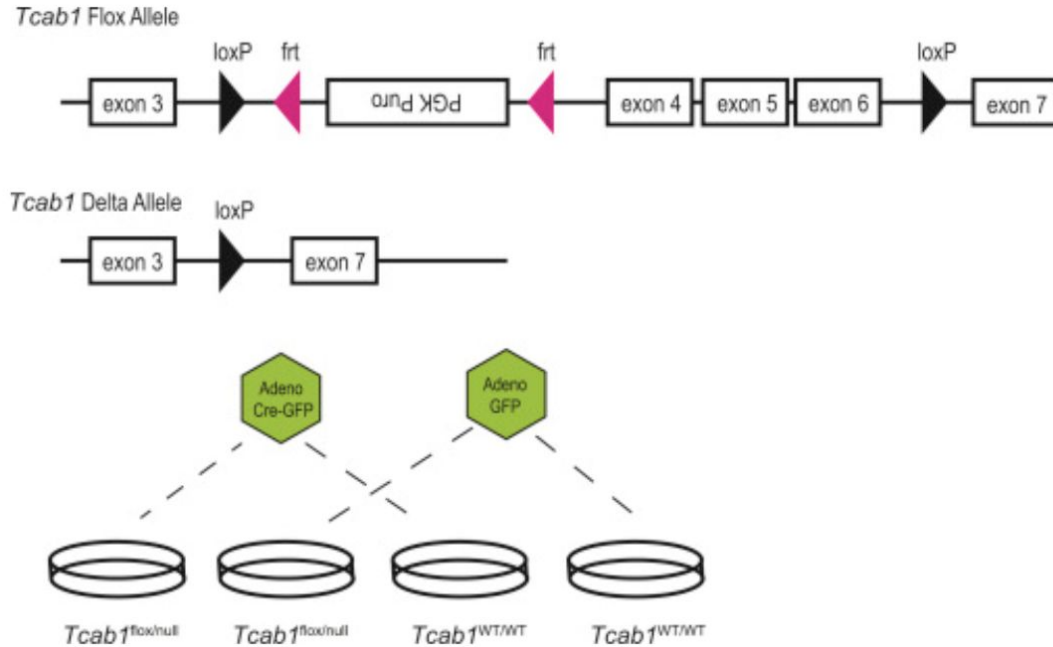
- ❑ in human cancer cells,
- ❑ in human embryonic stem cells (ESCs)
- ❑ in mouse embryo fibroblasts (MEFs)

Results: TCAB1 loss compromises telomerase activity in human cells and in mouse cells

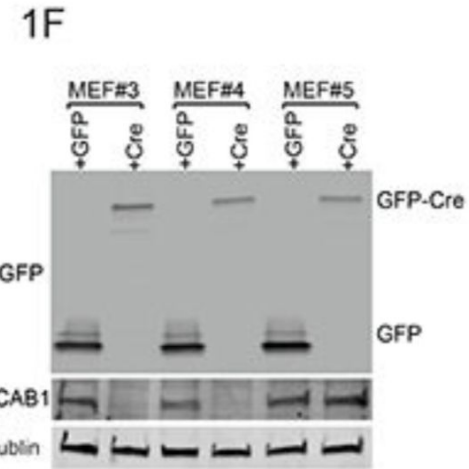
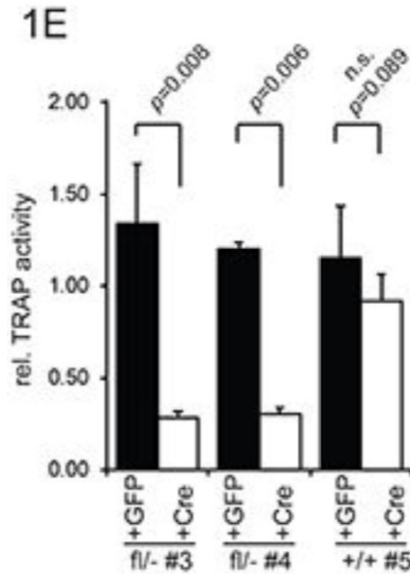
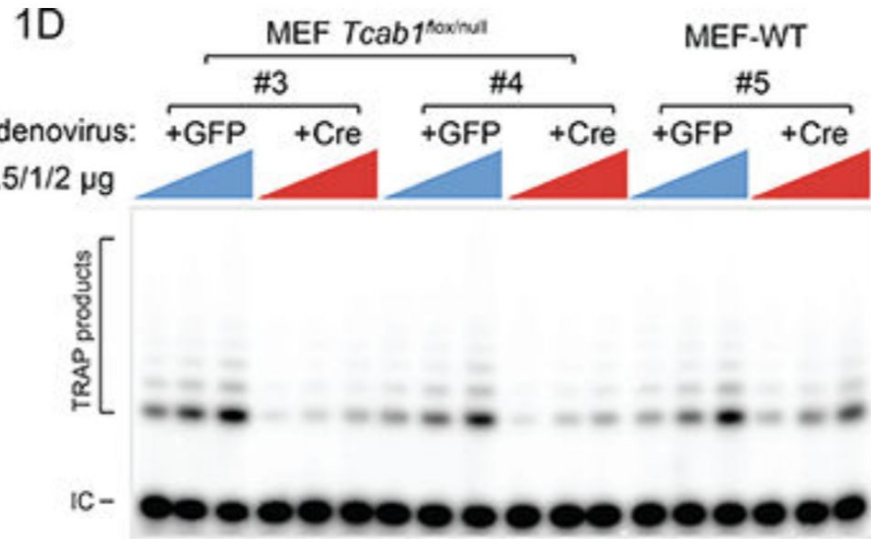


Inactivation of TCAB1 but not coilin impairs telomerase activity in human cancer cells

Results: TCAB1 loss compromises telomerase activity in human cells and in mouse cells



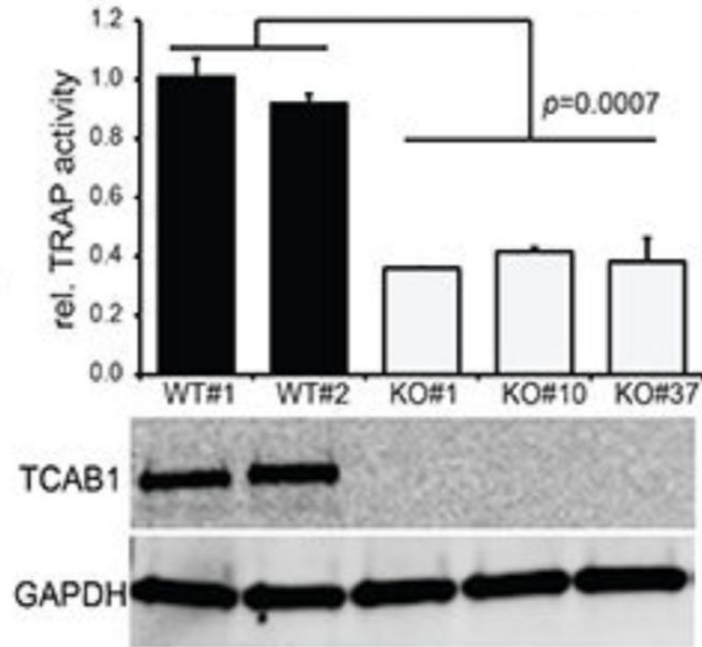
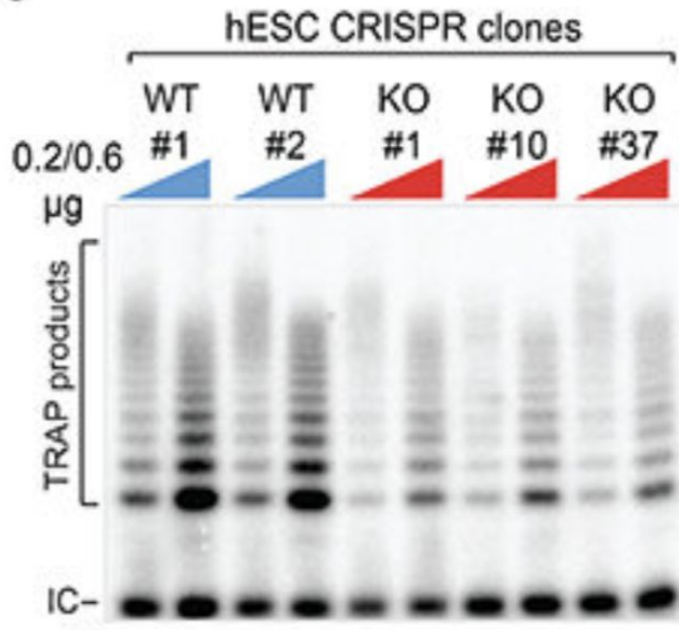
Results: TCAB1 loss compromises telomerase activity in human cells and in mouse cells



Inactivation of TCAB1 impairs telomerase activity in primary mouse cells

Results: TCAB1 loss compromises telomerase activity in human cells and in mouse cells

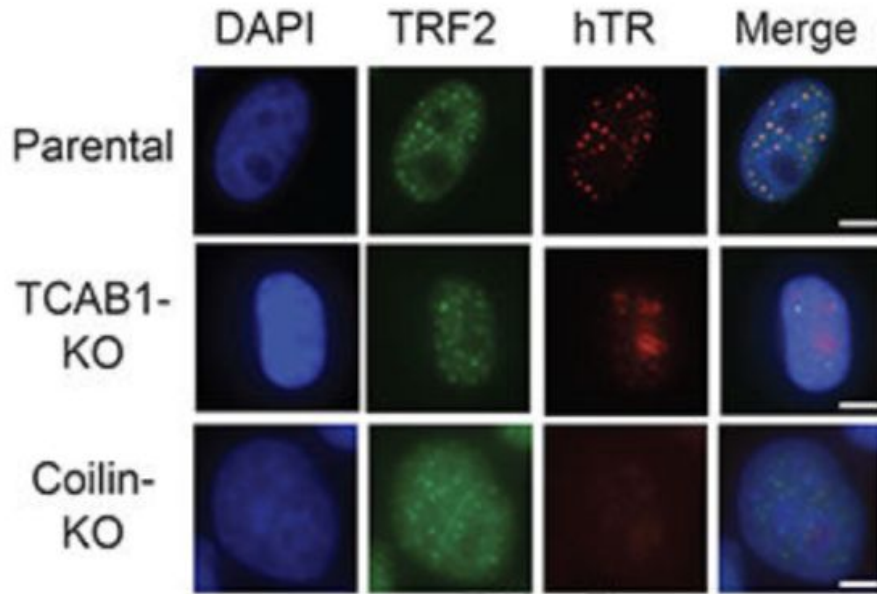
1G



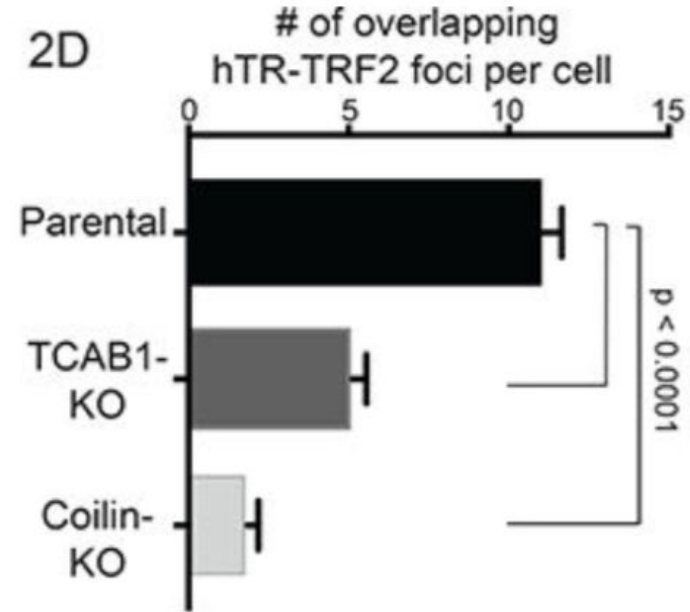
TCAB1 have a conserved role in supporting telomerase enzymatic function

Results: TCAB1 loss impairs trafficking, recruitment and telomerase maturation

2A

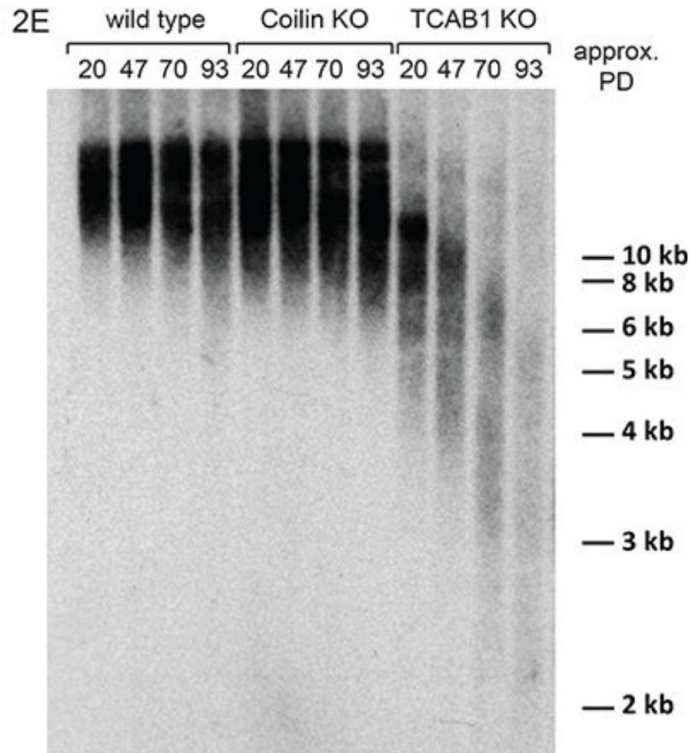


2D



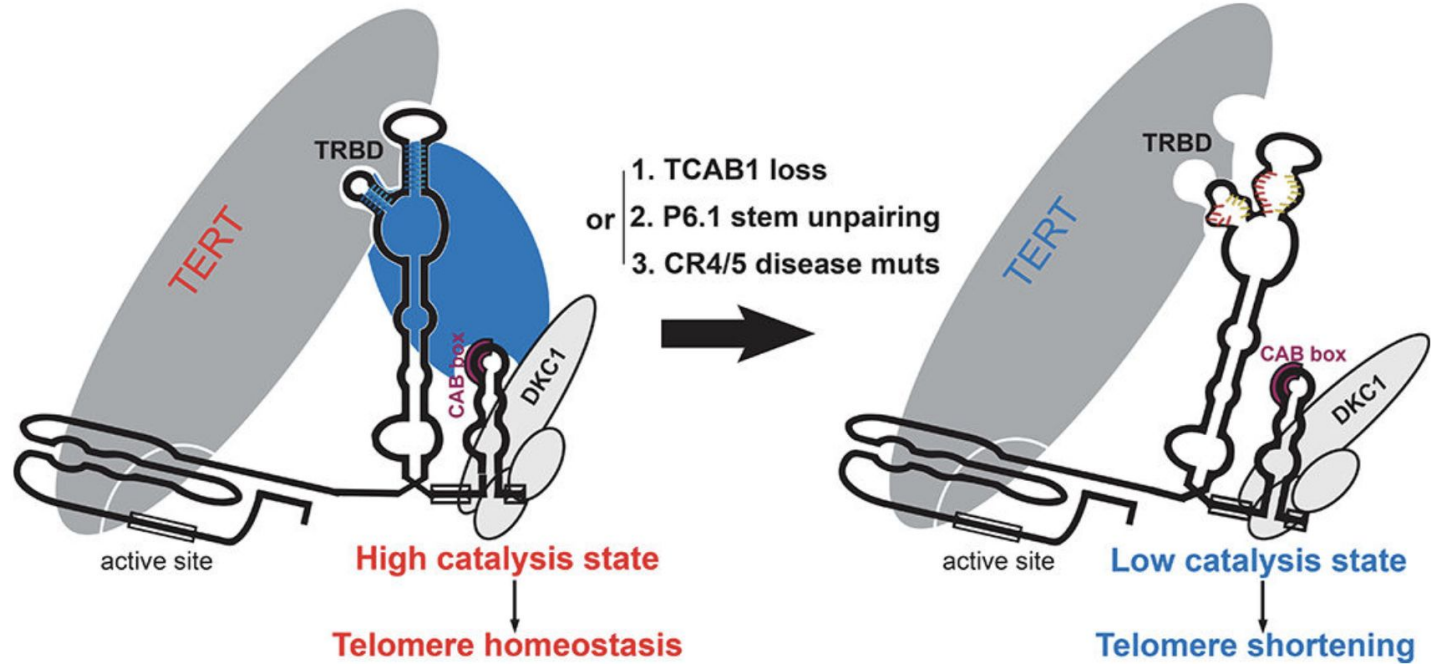
Loss of TCAB1 caused a quantitative reduction in recruitment of telomerase to telomere

Results: TCAB1 loss impairs trafficking, recruitment and telomerase maturation



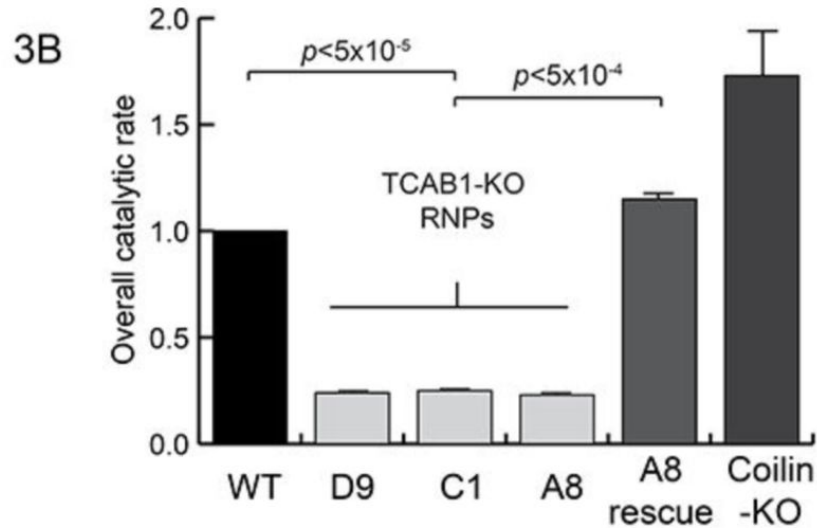
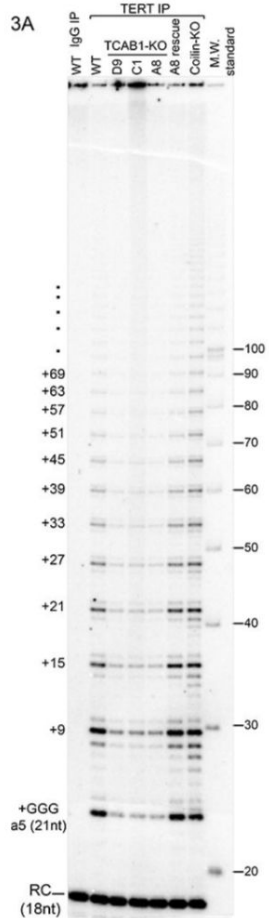
- TCAB1 in telomerase function does not necessitate a fully assembled Cajal Body
- TCAB1 loss impairs:
 - telomerase catalytic function,
 - disrupts telomerase localization to Cajal bodies
 - provokes progressive telomere shortening

Discussion: activity switch within telomerase based on RNA conformation and controlled by TCAB1



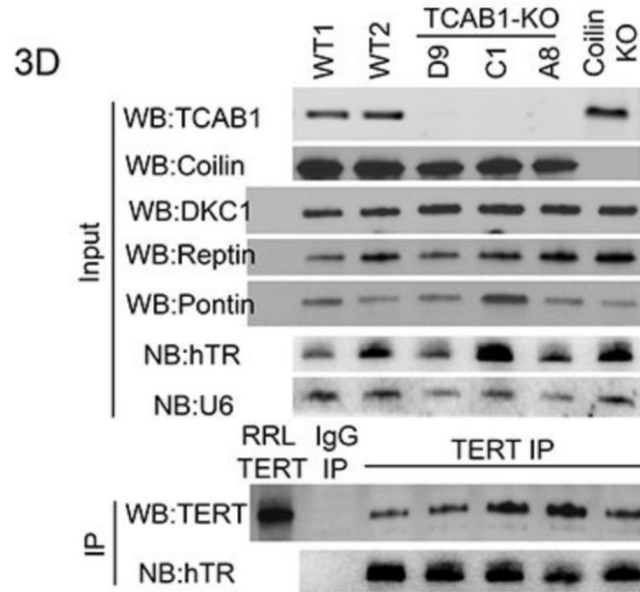
Telomerase can exist in **two catalytic states** based on altered CR4/5 conformation and TCAB1 toggle this **RNA activity switch** to control telomerase catalytic function

Results: Preserved assembly of the TERT-hTR catalytic core in cells lacking TCAB1



TCAB1 loss causes a reduced rate of enzymatic activity

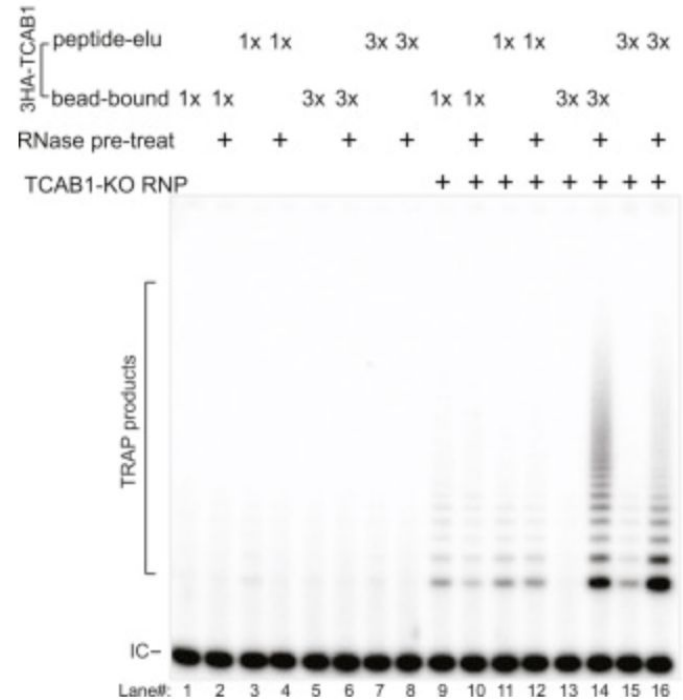
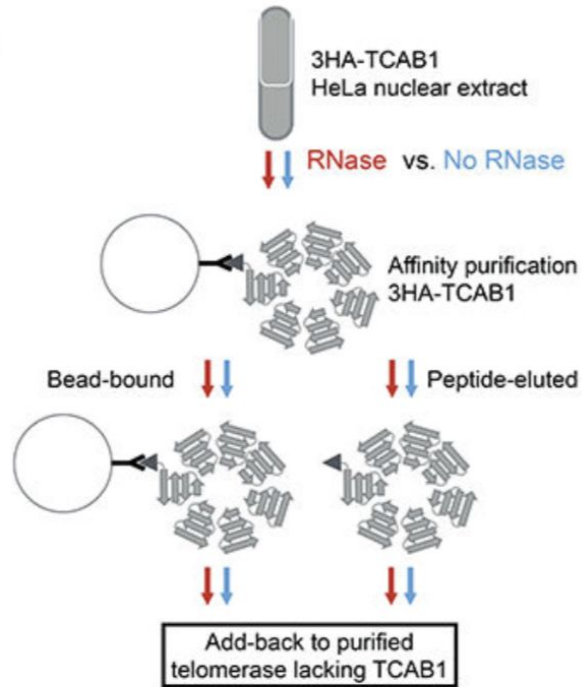
Results: Assembly of the TERT-hTR catalytic core in cells lacking TCAB1



TCAB1 loss did not affect the assembly of the catalytic core

Results: TCAB1 stimulates telomerase activity through an interaction with the CAB box

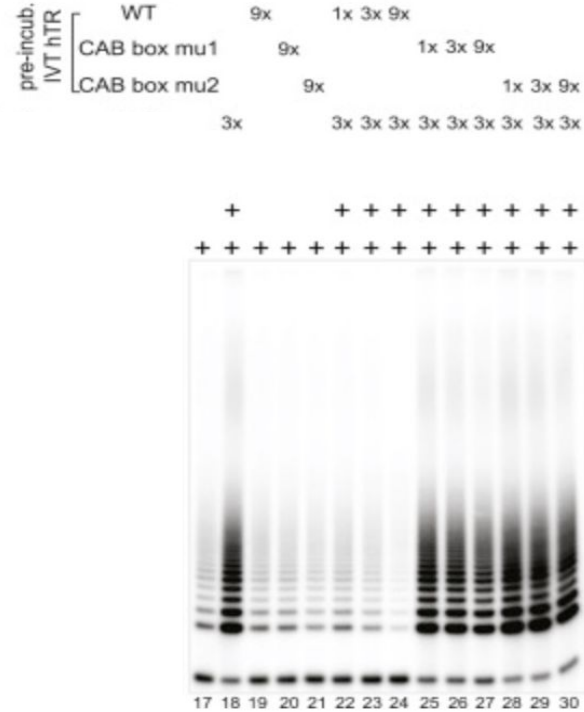
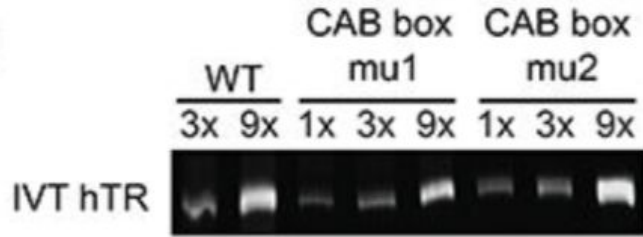
4A



RNA binding site on TCAB1 needs to be vacant to bind hTR and stimulate telomerase activity

Results: TCAB1 stimulates telomerase activity through an interaction with the CAB box

4C

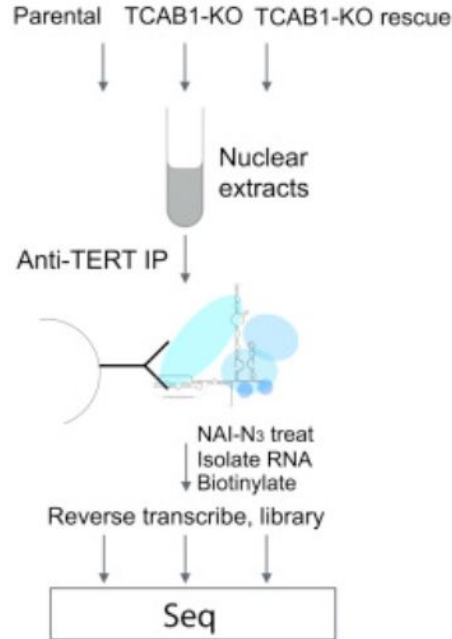


TCAB1 promotes telomerase catalytic function through direct binding of hTR after assembly of the enzyme catalytic core

Discussion: a required role for the scaRNA domain in either enzyme assembly or catalytic activation

- ❑ TCAB1 contains a **WD40 repeat domain** providing diverse surfaces for interactions with proteins and nucleic acids
- ❑ the TCAB1-CAB box interaction also **controls catalytic activity**
- ❑ For TCAB1, the **WD40 domain is required for binding the hTR CAB box and the CR4/5 domain**

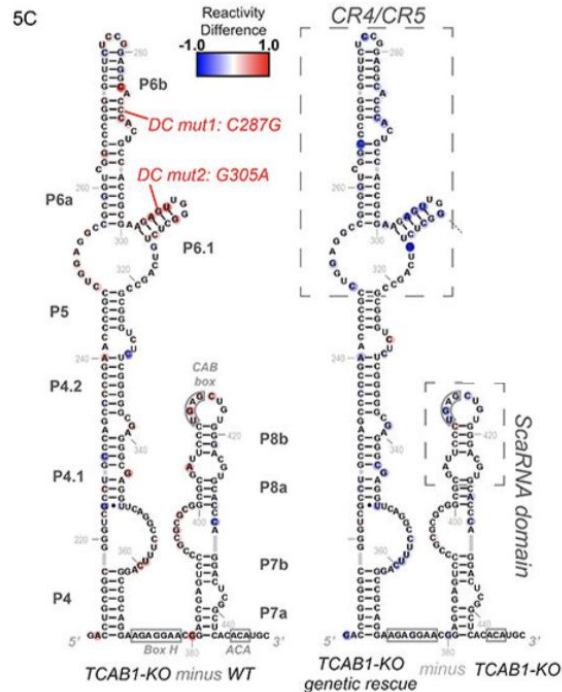
Results: TCAB1 drives regional RNA folding in an hTR domain critical for catalysis



ABOUT icSHAPE

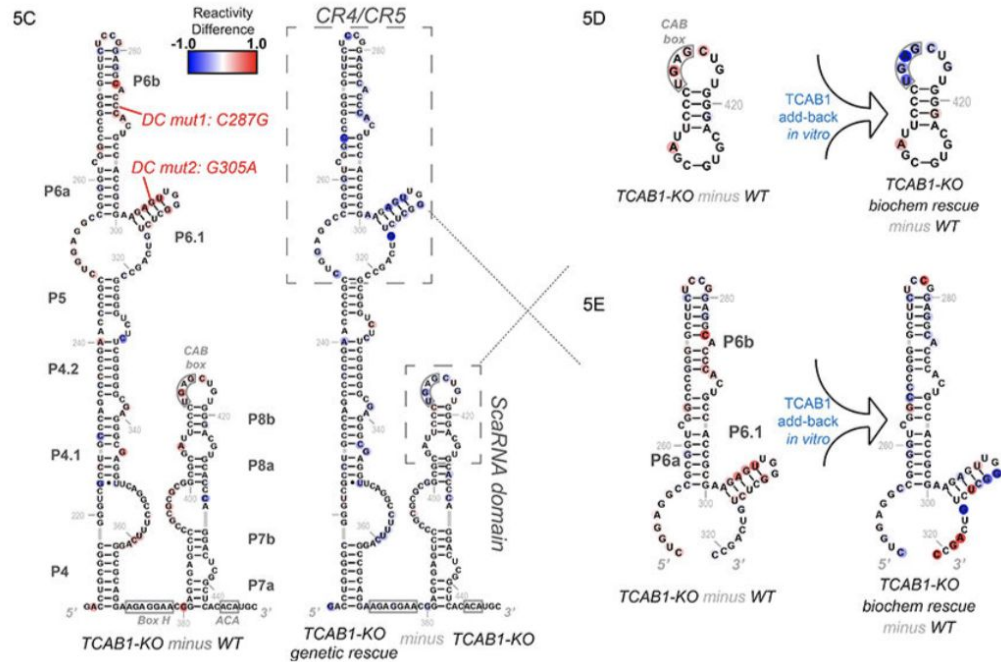
- ❑ Immobilized complex with **NAI-N3**
- ❑ Isolation of modified RNA sequences with **Trizol reagent**
- ❑ Biotinylation
- ❑ Reverse transcription, library and sequencing

Results: TCAB1 drives regional RNA folding in an hTR domain critical for catalysis



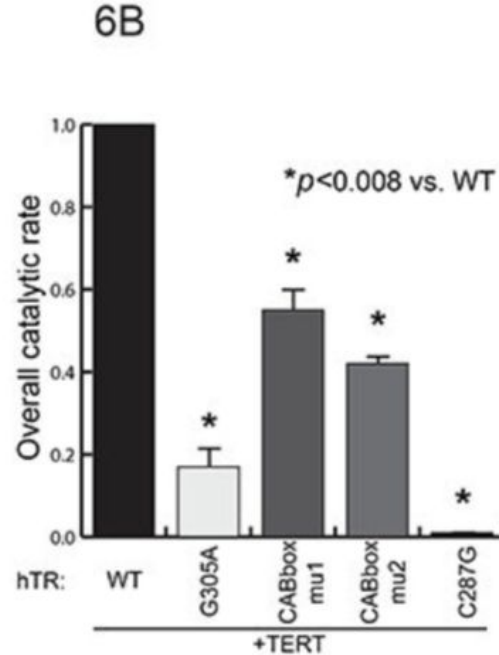
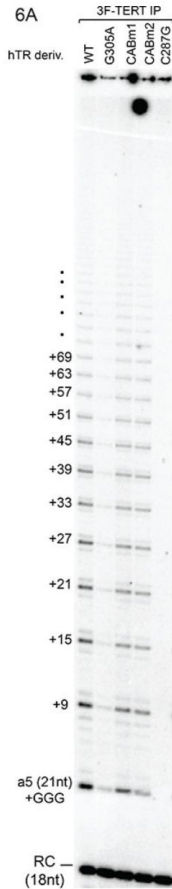
The CAB box loop represent the TCAB1 binding site on hTR

Results: TCAB1 drives regional RNA folding in an hTR domain critical for catalysis



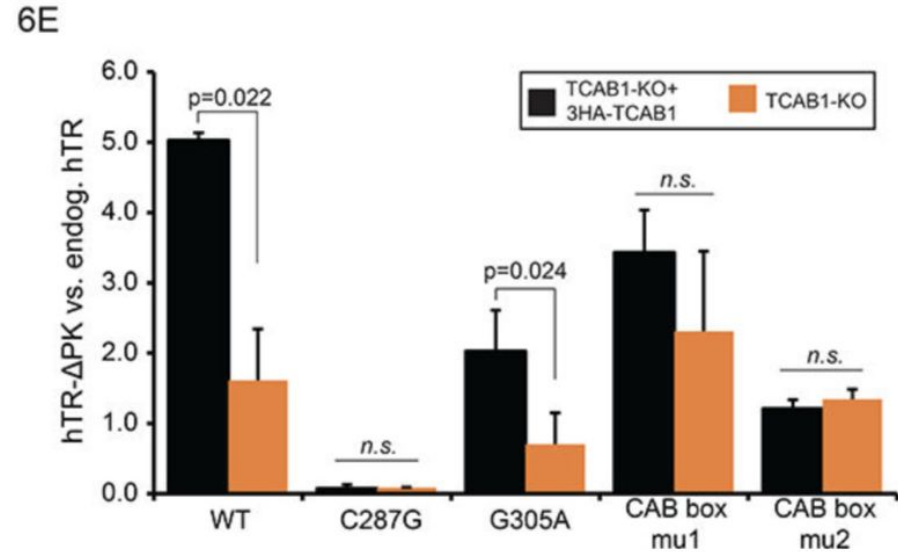
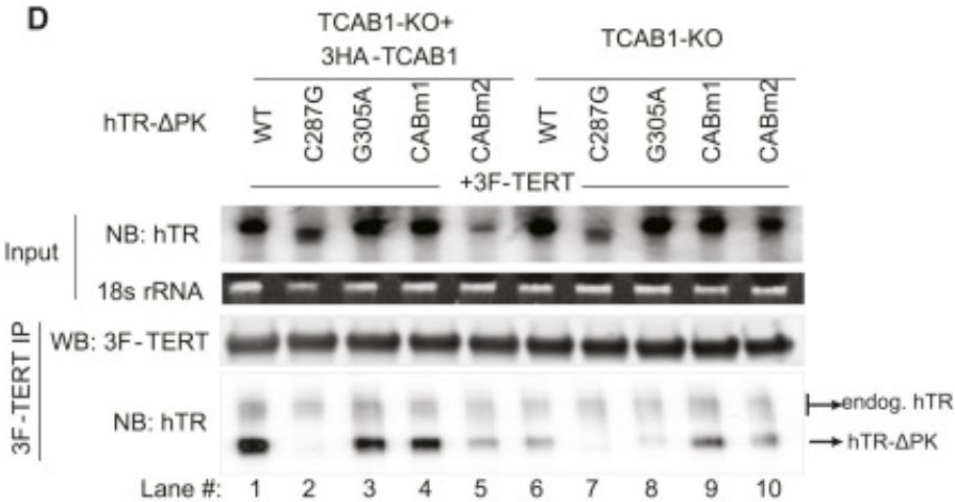
Loss of TCAB1 leads to unfolding in the hTR CR4/5 domain and recombinant TCAB1 promotes refolding of this domain

Results: Impaired hTR CR4/5 - TERT association in patient-derived hTR mutants



Mutations in hTR CR4/5 (P6.1 P6b) and disruption of TCAB1 functions operate in impairing catalysis

Results: Impaired hTR CR4/5 - TERT association in TCAB1 knockout cells and in patient-derived hTR mutants



G305A mutation in hTR impairs TERT binding and TCAB1 loss further disrupts TERT association

Discussion: impaired catalytic function and unfolding of CR4/5

- ❑ TCAB1 loss affects **Cajal Bodies Localization, telomere recruitment** and **CR4/5 folding**
- ❑ CR4/5 unfolding is the **primary defect** in the steps of telomerase assembly
- ❑ Recruitment of telomerase to telomeres depends upon **TERT-TEN domain** and **TTP1 interaction**



To conclude:

- ❑ **Telomerase catalytic activity is reduced** in the absence of TCAB1
- ❑ TCAB1 directly **stimulates catalytic activity** of the assembled telomerase complex
- ❑ **TCAB1 controls folding of CR4/5**, a distal domain in the telomerase RNA
- ❑ An **RNA activity switch** in the telomerase toggled by TCAB1 **controls telomerase activity**

What could we do after?



WRAP53 promotes cancer cell survival and is a potential target for cancer therapy

[S Mahmoudi](#), [S Henriksson](#), [L Farnebo](#), [K Roberg](#) & [M Farnebo](#)



The diagnosis and treatment of dyskeratosis congenita: a review

[M Soledad Fernández García](#)^{1,2} and [Julie Teruya-Feldstein](#)¹



TCAB1: a potential target for diagnosis and therapy of head and neck carcinomas

[Chong-kui Sun](#),^{#1} [Xiao-bo Luo](#),^{#1} [Ya-ping Gou](#),¹ [Ling Hu](#),¹ [Kun Wang](#),¹ [Chao Li](#),² [Zhen-ting Xiang](#),¹ [Ping Zhang](#),¹ [Xiang-li Kong](#),¹ [Chao-liang Zhang](#),¹ [Qin Yang](#),^{1,3} [Jing Li](#),¹ [Li-ying Xiao](#),^{✉1} [Yan Li](#),^{✉1} and [Qian-ming Chen](#)¹

THANKS FOR
YOUR
ATTENTION!

