Genetic Analysis of Cajal Bodies and Their Role in Telomere Biogenesis

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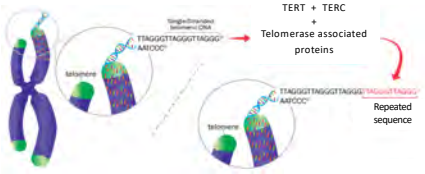
1 Abstract

The cell nucleus is composed of a number of distinct non-membranous sub compartments (nuclear bodies), which are formed by a unique set of proteins that carry out specific functions. Cajal bodies (CBs) are evolutionary conserved, dynamic nuclear structures central in the metabolism and assembly of different types of ribonucleoproteins (RNPs) such as those involved in telomere homeostasis, mRNA processing and gene silencing. To date Cajal body -like structures have been identified in vertebrates, yeasts, insects and plants. Since plants exhibit responses to their environment in the form of changes in nuclear organization and behavior, plants provide us with the means to identify factors that regulate and maintain nuclear structure during normal development and in response to environmental stress. Thus, we will take advantage of the powerful genetic tools available in the model plant *Arabidopsis thaliana* to understand the role(s) of the CB in the biogenesis of non-coding RNAs, with a specific focus on interaction(s) between CBs and the telomerase RNP at the organismal level. The main goal of my project is to identify the function of the telomerase RNA component 2 (TERT2) and its interaction with the CB in *Arabidopsis*. Specifically, I will characterize one mutation on the TERT2 locus. I will cross my plants with CB fluorescent markers such as coilin-YFP, and U2B"-GFP. I will compare the nuclear body phenotype of my mutants with other CB mutants that we have in the laboratory. Also, I will create double mutant plants to study the effect of this mutation in the genetic background of our CB mutants. Uniquely, *Arabidopsis* contains multiple telomerase RNAs (two) with different RNA and protein composition. Therefore, I will study the correlation between CB and telomere health using biochemistry, molecular biology, immunofluorescence and *in situ* hybridization techniques under stress, and normal conditions.

2 Telomeres

- **Telomerase**: (a revere transcriptase) enzyme made up of proteins and RNA subunits that adds the repeating sequence of TTTAGGG to the end of chromosomes to elongate it. (in humans the sequence is TTAGGG) (tert- catalytic subunit)
- **Telomerase RNA component or TERC**: Telomerase RNA Template. Single stranded RNA segment that serves as a template for a single strand of DNA (dyskerin- assistant protein found in cajal bodies)



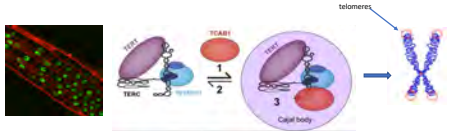
3 Cajal Bodies

Cajal bodies are sites for assembly and maturation of RNA/Protein complexes. Some of the most important are

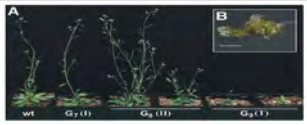
- Telomerase RNA component (TERC)

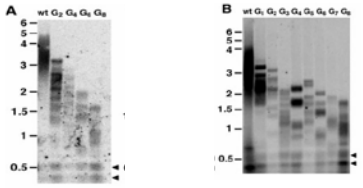
(Without the template to elongate telomeres we would assume they would diminish a lot quicker)

- Dyskerin is one of the proteins involved in the stabilization of TERC and is also located in Cajal Bodies



4 Telomerase

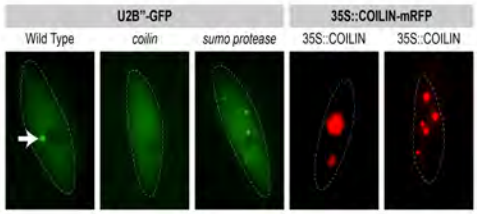




A) TRAP assay showing that plants with a homozygous disruption in the AtTERT gene lack telomerase.

B) TRF analysis of DNA extracted from AtTERT Arabidopsis mutants show degradation of the telomeres in successive generations. Telomere lengths were determined by hybridization with a (T3AG3) 4 prob.

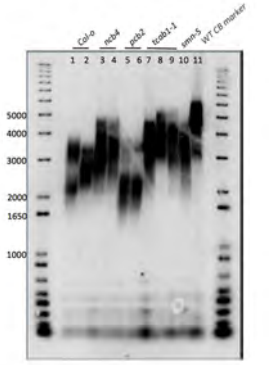
5 Cajal Body mutants



One cajal body	No cajal body	Poly cajal body	Over expression
(Wild Type)	(ncb)	(pcb)	

6 Gel results

- **ncb**: No cajal bodies. Their telomere length is approx. 5000kbs.
- **pcb**: Poly cajal bodies. By far the shortest telomers
- **tcab1-1**: average telomere length
- **WT**: Wild type. Longest telomeres. Just over 5000kb.



7 Techniques

Telomere length

- How much of the telomere is being lost each cell cycle?
- Does it lose the same amount each time?
- Does the telomere diminish the same amount under the same conditions every time?

Enzyme activity

- When is the enzyme activated?
- Is it activated constantly or in bursts?
- Is more enzyme activity good or bad for telomere length?

8 Analyzing Telomeres

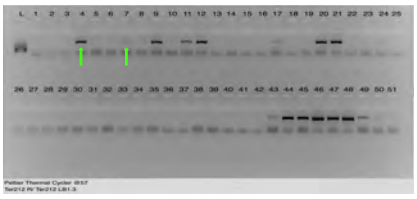
Telomere length
TRF: Telomere Restriction Fragment
(genomic DNA and telo probe)
PETRA: Primer extension telomere repeat amplification. Measurement of the length of the individual telomeres (PCR per Chromosome Arm)
Telomerase Activity
qTRAP: q Telomeric repeat amplification protocol (non-radioactive and quantitative)
TRAP: Telomeric repeat amplification protocol (radioactive and qualitative)
Fluorescent Imaging: *in situ* hybridization and chromosome fusions

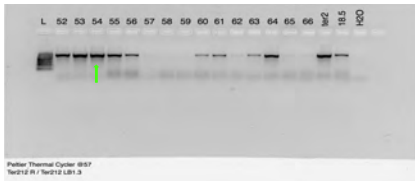
9 Gene

- All organisms have 1 TERT gene except Arabidopsis which has two TERT genes.
- Through evolution, the 1 gene turned into two because of gene duplication that is common in plants
- **Ter1**: (748 nucleotides) templating RNA for telomerase
- **Ter2**: (784 nucleotides) null mutation in TER2 increases telomerase activity, whereas overexpression of TER2 diminishes telomerase activity
- Share an approximate 220 nucleotide region

10 Current / Future work

| - examples of bands





F1 Ter212 x 18.5 (pcb) → F2 ter2/18.5 (heterozygous) → 9:3:3:1 → ter2/ter2 18.5/18.5 = Double mutant

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