

# Characterization of Alternative Polyadenylation & Gene Expression Profile in the *tcab1* Arabidopsis Mutant



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## 1 Abstract

Using the power of bioinformatics and next generation sequencing, we are using a molecular genetics, genomic, and transcriptomic approach to understand how the machinery inside the nucleus affects the development of Arabidopsis thaliana. The TELOMERASE CAJAL BODY PROTEIN 1 (TCAB1, known as WRAP53 $\beta$  in humans) is a WD40 domain containing protein that acts as a platform to facilitate protein and nuclear structure interactions that occur in the cell. Moreover, a nuclear structure closely related to these processes is the Cajal Body (CB). The CB takes part in modifications of different types of ribonucleoproteins (RNPs) involved in the maturation of the splicing apparatus, the survival of motor neuron (SMN) complex and the telomerase RNP machinery. Interestingly, it is known that TCAB1 plays an important role in the localization and trafficking of these complexes to the CB. Therefore, having a detrimental impact when in absence. My project focuses on the machinery of TCAB1 regarding to gene expression and alternative polyadenylation events through PAT-seq (PolyA tag followed by high-throughput sequencing). Specifically, we are interested in looking at the changes in gene regulation that TCAB1 affects directly and/or indirectly through its function.

## 4 TCAB1 Gene & Homologs

### Gene Structure

Arabidopsis TCAB1 / WRAP53 $\beta$

Gene structure and location of mutations on the TCAB1

### Protein Domains & Homologs

Human TCAB1 / WRAP53 $\beta$

Location of WD40 Domains on TCAB1 in *A.thaliana* and in its homologs.

## 5 Phenotypes

Wild Type Col	<i>tcab1-2</i> Mutants	Goal
Colin YFP	U2B' GFP	<i>tcab1-2</i> Coilin YFP × <i>tcab1-2</i> U2B GFP
		Screening -Antibiotic Media -Fluorescent Microscopy -Genotypic Ratios
		<i>tcab1-2</i> with Coilin YFP and U2B GFP

Confirmation of CB phenotype in two different protein markers known to be rich in CB.

CB phenotypes of *tcab1-2* nuclei. Overlay of GFP and YFP in *tcab1-2*

### Silique Phenotype Characterization

Many of the plants with the *tcab1-2* mutation developed a multiple silique phenotype. This phenotypes, if due to mutations in the TCAB1 gene, can lead us to question TCAB1's role in stem cells.

## 2 Hypothesis

Preliminary data of *tcab1-2* plants have shown developmental phenotypes in meristems in which supernumerary fruits arise from one meristem. Therefore, we expect that absence of the TCAB1 protein affects gene regulation in meristems, specifically in stem cells that play a role in fruit formation. Furthermore, we have found phenotypes that display disrupted formation of CBs leading us to assume that TCAB1 plays a role in CB form and function.

## 3 TCAB1 Functions & Cajal Bodies

A, B, D. Due to its WD40 Domains, TCAB1 acts as a platform for the assembly of protein and RNA complexes such as the ASMN complex, Telomerase, and various non-coding RNAs. C. CBs concentrate various factors in one place, one being TCAB1 and thus facilitating interactions.

TCAB1 / WRAP53 $\beta$ Function	Telomere Trafficking	Cajal body Structure	RNA Trafficking	SMN Trafficking
Cellular Processes	Telomere Elongation	DNA double-strand break repair	Ribonucleoprotein biogenesis	
Diseases	Dyskeratosis Congenita	Cancer	Spinal Muscular Atrophy	

TCAB1 is involved in maintenance of CBs, telomere elongation, and DNA repair. Dysfunction of this protein has been linked to premature aging, cancer, and neurodegeneration.

## 6 Methods

## 7 Future Work

### Alternative Polyadenylation Analysis of *A.thaliana*

Is the role of TCAB1 linked closely to the splicing complex? Does lack of TCAB1 function affect mRNA isoforms?

### Phenotypic Expression Ratio

How often is this phenotype expressed and what are its variations? Test weather the Expression ration is statistically significant.

### Specific PolyA Tag Sequencing of Specific Tissue

How does gene expression range from tissue to tissue? What is the role of TCAB1 in different tissues?

## References

Henriksson S, Farnebo M. On the road with WRAP53 $\beta$ : guardian of Cajal bodies and genome integrity. *Front. in Genetics*, 6 (2015).

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