ELS1, a Nucleolar SUMO Protease that regulates nuclear structure

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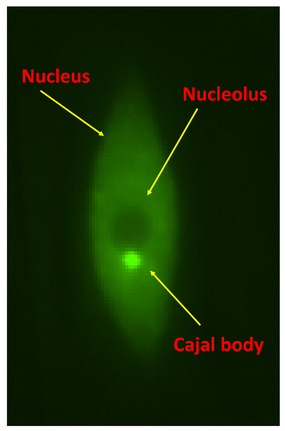
Abstract

The main goal of my research is to understand the basic biology of the cell nucleus, using *Arabidopsis thaliana* as a model system. The cell nucleus is a double membrane-bound organelle found in eukaryotic cells that contains the majority of the cells genetic material. Within the cell nucleus is the nucleolus, where ribosomal subunit synthesis takes place. Also within the nucleus is a structure called Cajal body (CB), which is a nuclear subdomain involved in the modification of different small ribonucleoproteins (RNPs).

I will be studying a protease of the Ubiquitin-Like Protease (ULP) family. This group of proteases is conserved in evolution and regulates the amount of active and inactive SUMO (Small Ubiquitin-Like Modifier) in the cell nucleus. SUMO is small protein that covalently binds and detaches from target proteins within the cells to modify their function to regulate development and environmental responses in plants. My research involves characterizing ELS1 (ESD4 LIKE SUMO PROTEASE), a SUMO protease in *Arabidopsis.* I am interested in the localization of this protease in the cell its function in the nucleus and in the SUMO pathway. ULP proteases are involved in two key steps in the SUMOylation pathway. They can regulate the amount of free SUMO from inactive to active (ULP1) and reverse the SUMO reaction by deconjugating SUMO from the targeted protein (ULP2). Previously, I genotyped and amplified the DNA of several crosses to identify mutations in the *ELS1* gene. After distinguishing between the mutants, I used live microscopy to analyze the nucleus of these plants. I will use fluorescent markers GPF and YFP to study Cajal bodies and the nucleolus. This will determine whether ELS1 is functioning as a ULP1 or ULP2 protease. As a lab, our studies will help understand the role of ELS1 in the SUMOylation pathway and in the maintenance of nuclear architecture.

Nuclear Domains

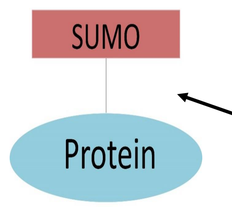
- The nucleus is a membrane bound structure that contains the cells genetic information and is found in all eukaryotic cells.
- Within the nucleus lies the nucleolus, the site for ribosomal subunit synthesis. DNA transcribes to ribosomal RNA (rRNA), which is then translated to proteins making up ribosomal subunits.
- Another structure found in the nucleus is the Cajal body (CB). This nuclear subdomain is involved in the production of many different kinds of small ribonucleoproteins (RNPs). The number of CBs in the nucleus vary among organisms. For example, wild type *Arabidopsis* plants contain one CB.



Microscopy image of *Arabidopsis* wild type cell nucleus, nucleolus and a single Cajal body.

SUMO

- **S**mall **U**biquitin-related **Mo**difier is a protein modification that coordinates gene expression, necessary for the development and environmental responses of animals and plants.
- SUMO interacts with other proteins via an isopeptide bond within cells to modify their function.



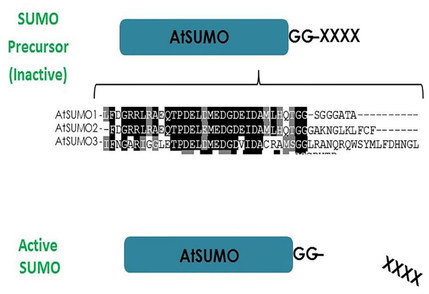
The figure shows the isopeptide bond between the C-terminal glycine residue of SUMO and a lysine residue, an amino acid known as the SUMO acceptor on the target protein

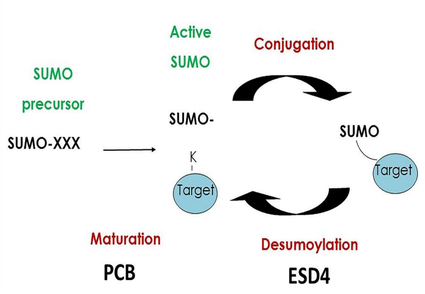
Before SUMO can interact with other proteins, it needs to be activated. When active, the C-terminal glycine is exposed so it may bind to the lysine sides of targeted proteins for modification. SUMO protease is an important factor in SUMO activation.

SUMOylation Pathway

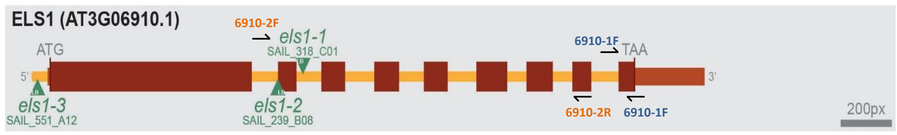
- Ubiquitin-Like Protease's (ULPs) regulate the amount of active and inactive SUMO in the cell.
- Two Ubiquitin like Protease are involved in two key steps in the process of SUMOylation Pathway PCB (Maturation process) and ESD4 (DeSUMOylation process)

Goal: Identify the role of ELS1 Protease in SUMOylation Pathway.





ELS1 a Protease of the ULP Family



ELS1 gene structure. Exons are represented in red and Introns are represented in yellow. Green triangles show where T-DNA insertions (mutations) are located on the gene. Yellow and Blue represent two different sets of primers that I will use for RT-PCR.

Results

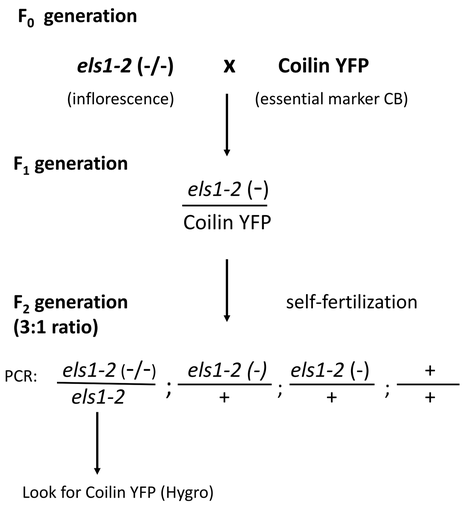


Fig. 1. The figure shows the crossing scheme of *els1-2* Mutant

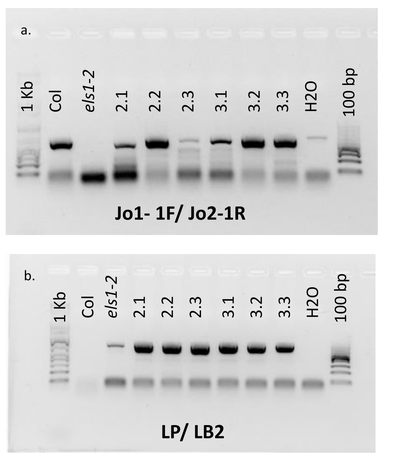


Fig. 2. PCR analysis of *els1-2* T-DNA mutant. Two sets of different primers were used in both gels. Results show all plants are heterozygous.

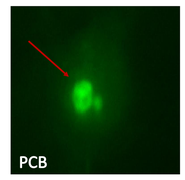
Closely Related Ubiquitin-Like Protease's Localized

Goal:

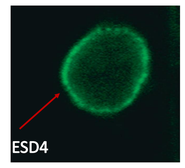
1. Identify where Els1 protease is located in the cell nucleus and its role in the cell
2. Identify what the cajal phenotype look like

Phylogenetic tree shows 3 closely related SUMO proteases in *Arabidopsis*.

Subcellular Localization



PCB Protein Localization in the nucleus



ESD4 (Early in Short Days 4) Protein Localization in the nuclear envelope

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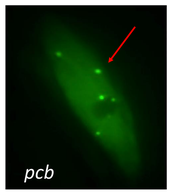
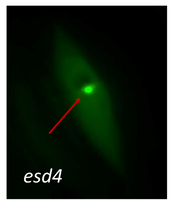
Goal 1
ELS1 Protein is localized where in the nucleus?

CB phenotype



pcb Mutant shows many Cajal bodies



esd4 Mutant shows a single Cajal body

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Goal 2
els1 mutants affect CB structure?

Future Work

- I collected seeds from crosses and plated them in Basta/Hygro plates. Waiting for Results (F_2). Results may show what Cajal phenotype looks like.
- Other crosses 18.5, 8.2, GFP-PCB and GFP-NO (rescue the phenotype of *els1-2* mutant)
- Experiment with RNA /Developmental RT-PCR to determine where *ELS1* is expressed in the cell, the concentration and tissue localization.

Acknowledgements

Research reported in this publication was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of National Institutes of Health under grant number P20GM103451. New Mexico Alliance for Minority Participation (New Mexico AMP), Plant Education Experience in Research (PEER), MariJo DeAguero, Dr. Mario Izaguirre-Sierra.