CRISPR/Cas9 Yields Mutants in the Moss SUMO System

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Introduction

Background:

Small ubiquitin-related modifier (SUMO) is a post-translational modification that enables organisms to respond to and manage environmental stress by rapidly altering protein function, stability, and localization. The SUMO system is outlined below.

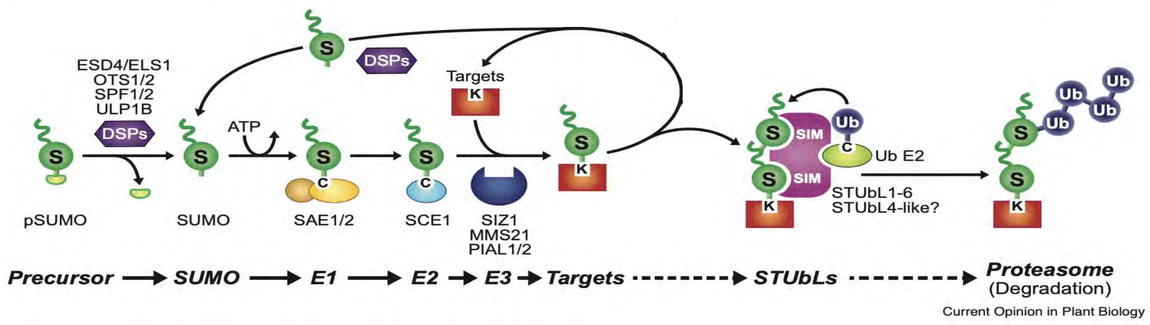
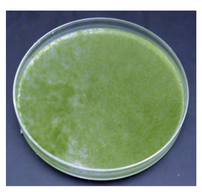
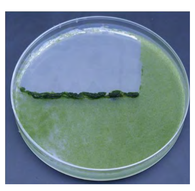


Figure 1: SUMOylation cycle. Adapted from Augustine and Vierstra, (2018) Curr. Opin. Plant Biol. 45: 143-154.





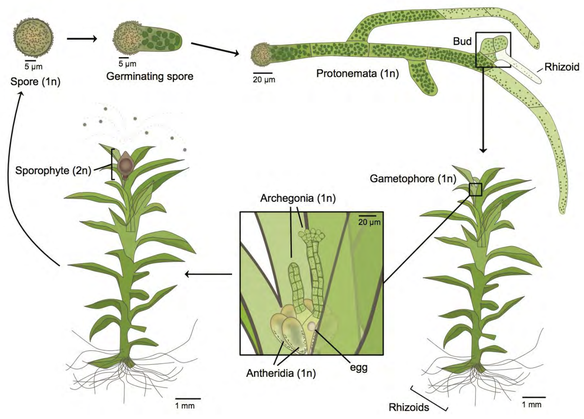


Figure 2: Moss culturing and life cycle. The moss, *Physcomitrium patens*, is a powerful model organism because it is haploid, can regenerate from a single cell, and has a simple life cycle. Image from Wu et al., (2018) Biophys. Rev. 10:1683–1693.

Purpose:

Generate mutant moss lines to genetically dissect the role of the SUMO system in plant development and how it shields moss from stress.

Methods

Cloning and Transformation:

PIAL1, SUMO1a, SUMO1-v-a/b, and STUBL5 genes targeted by:

1. Protospacer ligation into entry plasmid
2. Protospacer recombination into destination plasmid containing Cas9
3. Protoplasts generation by digesting moss cell wall
4. CRISPR/Cas9 plasmid transformation into protoplasts
5. Cells rebuild cell wall and then selection for plasmid uptake
6. Cells expression of CRISPR/Cas9 thereby editing the target locus.

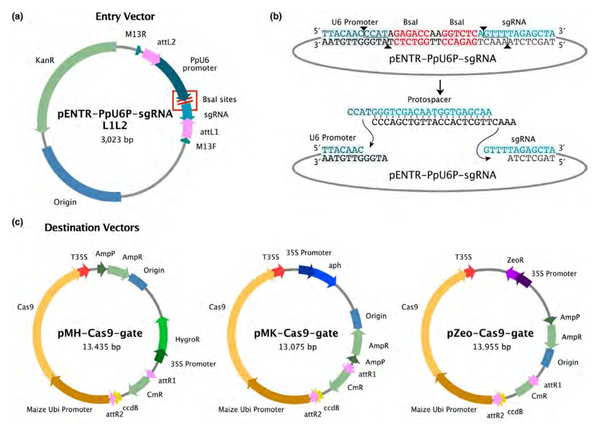


Figure 3: CRISPR/Cas9 Cloning Constructs. From Mallett et al., (2019) Plant Direct. 3:doi:10.1002/pld3.168.

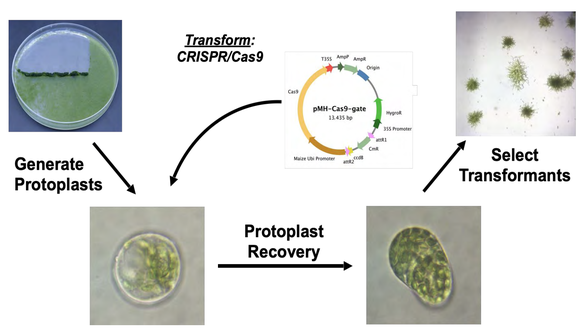


Figure 4: Moss transformation protocol.

Screening for Mutants:

- DNA is extracted from selected moss lines
- PCR amplification of region flanking CRISPR cut sites
- Sanger sequencing using nested primers to sequence the altered locus

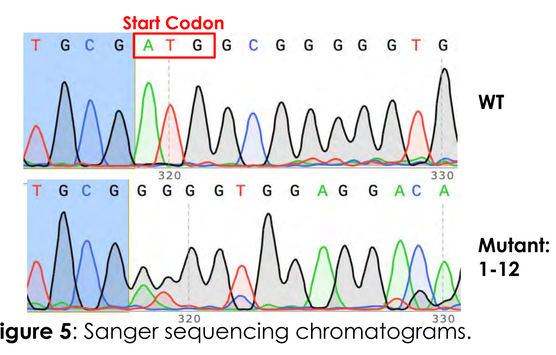


Figure 5: Sanger sequencing chromatograms.

Results

Sanger Sequencing:

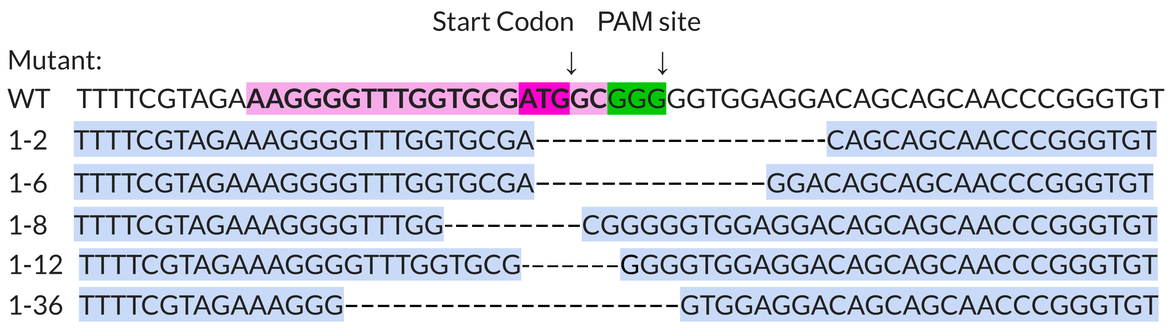


Figure 6: SUMO1a mutant DNA sequence alignment reveal loss of the start codon.

Immunoblot Analyses:

- SUMO1a mutants show effective knockout of SUMO1a protein.
- SUMOylation is induced by heat stress in moss.
- Most SUMO1a mutants maintain the ability to induce SUMOylation upon heat stress.

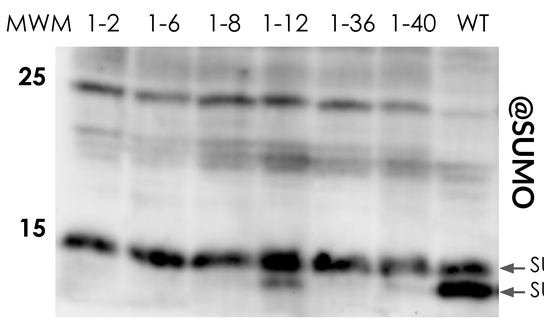


Figure 7: Successful knockout of SUMO1a in mutants 1-2, 1-6, 1-8, 1-36, 1-40. Mutant 1-12 most likely has two moss lines, one with SUMO1a knocked out and one without.

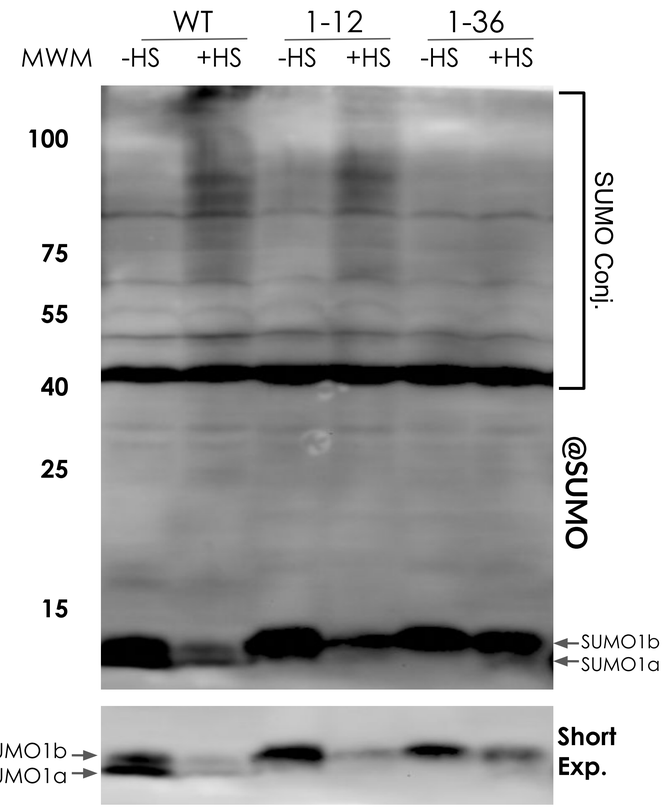


Figure 8: Moss kept at 25°C (-HS) or heat-stressed (+HS) at 38°C for 30 minutes. Increase in SUMOylation is seen clearly in mutant 1-12.

Conclusion

- Mutants were generated and identified in SUMO1a, SUMO-v-a/b, and STUBL5 that will enable genetic analysis of the importance of these genes in plant development and stress defense.

Future Work:

- Transform PIAL1 CRISPR/Cas9 constructs into moss and screen for knockout lines.
- Screen for phenotypes in mutant lines subjected under normal growth and various stress treatments

Acknowledgments and References

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- Augustine, Robert C., and Richard D. Vierstra. “SUMOylation: Re-Wiring the Plant Nucleus during Stress and Development.” Current Opinion in Plant Biology, vol. 45, Oct. 2018, pp. 143–54.
- Mallett, D. R., Chang, M., Cheng, X., & Bezanilla, M. (2019). Efficient and modular CRISPR-Cas9 vector system for *Physcomitrella patens*. *Plant direct*, *3*(9), e00168. https://doi.org/10.1002/pld3.168
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