Elucidating Receptor-Ligand Interactions that Regulate Stem Cell Proliferation in *Arabidopsis thaliana*

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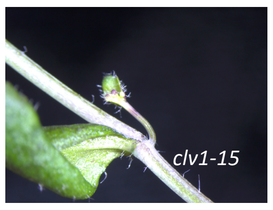
Abstract

Plants cannot reproduce without robust flower production, a process that originates from the division of stem cells. In most vascular plants, stem cells are located in a niche in the Shoot Apical Meristem (SAM). Some of these stem cells renew themselves while others differentiate to become flowers. In *Arabidopsis thaliana*, the Leucine Rich Repeat (LRR) receptor kinases CLV1 and their homologs the BAMs, together with their CLE peptide ligands, are known to regulate the growth and proliferation of stem cells in the mersitem.[1] To determine how CLAVATA receptors interact with various CLE peptides to regulate stem cell proliferation, I grew various combinations of receptor mutants and observed their phenotypes. My results showed that when plants had to express BAM3 ectopically to compensate for the loss of other receptors, as in the *clv1bam1* mutant, a disadvantageous 'PITting' phenotype occurred. This phenotype did not occur in mutants with ectopic BAM1 and BAM2, demonstrating that these receptors can compensate for a lack of both the CLAVATA signaling pathway and the BAM3 receptor. Therefore, there is strong evidence to suggest that ectopic BAM3 expression in *clv1-15* is what is driving the PIT phenotype. Additionally, the *clv1bam1/3* mutant displayed an unusual 'disk' phenotype, only seen before in *cle25 clv3*. This phenotypic similarity could indicate that CLE25 could be signaling through BAM3 in the *clv1-15 bam1* mutant. Further, *clv1-15 bam1* phenocopies the *clv3* mutant, suggesting that CLV3 might usually signal through CLV1 and BAM1. To investigate these results further, I will be generating a *clv1-15 bam1 cle25* mutant as well as a *clv3 clv1-15 bam1* mutant. These genetic experiments will test whether CLE25 signals through BAM3 and whether CLV3 signals through CLV1 BAM1.

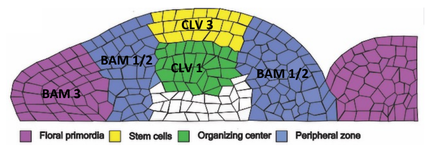
CLV and BAM Receptors are Necessary for Stem Cell Regulation



Floral Primordia outgrowth and stem cell production is regulated by the CLAVATA and BAM signaling pathway.[2] In the single *CLAVATA* mutant, *clv1-15,* the primary inflorescence of the plant will halt the formation of new lateral organs, which is considered Primary Inflorescence Termination or *PIT*.

However, a complete loss of CLV and its BAMS as in the *clv1 bam1/2/3* mutant produces a severely overproliferated Shoot Apical Meristem (SAM) as well as greatly decreased flower production.[3] The failure of BAMs to compensate for the absence of CLV in *clv1-15* could be due to the ectopic expression of the BAMs observed in the center of the Shoot Apical Meristem or SAM. I will be investigating which of these BAM receptors are interacting with which CLV receptor kinase to regulate stem cell proliferation and floral primordia outgrowth.

Depiction of a Shoot Apical Meristem and the respective locations of each receptor

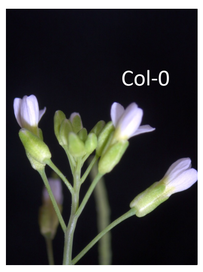


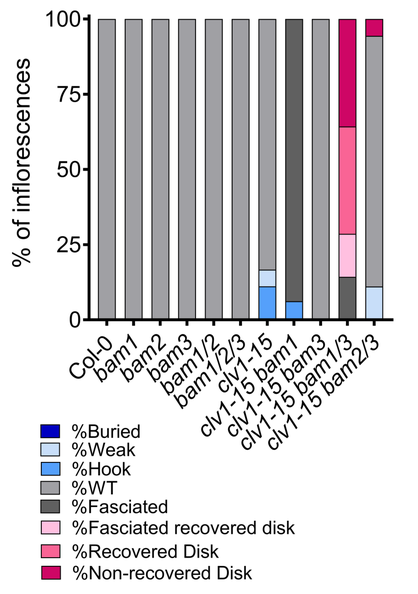
Methods

Seed Plating → Two-day stratification at 4°C → 1 Week in 24-hour light Chamber at 24°C → Seeds Transferred to soil → 2 months in 16°C growth chamber → Phenotyping

BAM 3 Drives 'PIT'

Does BAM receptor activity drive PIT in clv1-15 mutants?



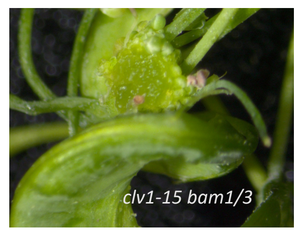


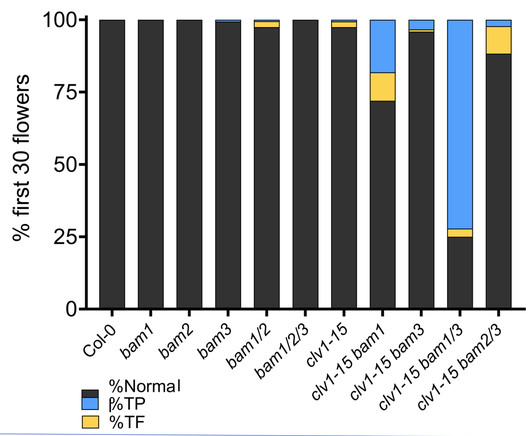
The *clv1-15 bam3* genotype was 100% wildtype, indicating that ectopic BAM3 may be driving PIT.

BAM3 May Signal Through CLE25

The *clv1-15 bam1/3* genotype showed a "disk" inflorescence phenotype, where the stem cells overproliferate and form a disk shape, as well as an abundance of terminated primordia. This has been observed previously in the *cle25 clv3* mutant. This suggests that CLE25 could be signaling through BAM3.

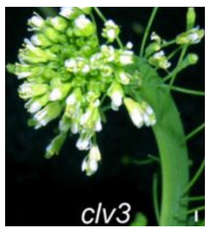
To investigate this further, I will be crossing a CLE25 reporter into the *clv1bam1* mutant to see if CLE25 is expressed ectopically in floral primordia as it is in *clv3*. I will also generate a *clv1 bam1 cle25* mutant and see if it forms a disk like *cle25 clv3*

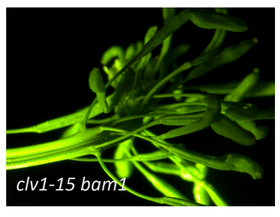




Future Directions

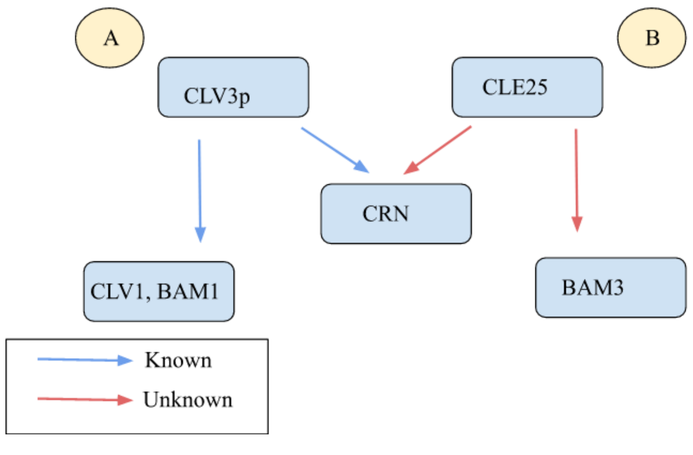
The c*lv-15 bam1* mutant phenotypically looks the same as the *clv3* mutant, indicating that it's possible that CLV3 is signaling through CLV1 and BAM1 for floral primordia growth.





To test this hypothesis, it would be productive to grow a *clv3 clv1-15 bam1* triple mutant in a future experiment to identify more concretely what receptors CLV3 is signaling through.

This, along with the possible CLE25/BAM3 pathway points to a new framework in plant stem cell regulation, depicted by the hypothesized signaling cascade in the figure at right.



References

1. John, A., Smith, E. S., Jones, D. S., Soyars, C. L., & Nimchuk, Z. L. (2022, May 28). *A network of clavata receptors buffers auxin-dependent meristem maintenance*. bioRxiv. Retrieved November 28, 2022, from https://www.biorxiv.org/content/10.1101/2022.05.27.493758v1.full 2. Jones, D. S., John, A., VanDerMolen, K. R., & Nimchuk, Z. L. (2021, January 11). *Clavata signaling ensures reproductive development in plants across thermal environments*. ScienceDirect. Retrieved November 29, 2022, from https://www.sciencedirect.com/science/article/pii/S0960982220315128 3. Nimchuk, Z. L. (2017, March 29). *CLAVATA1 controls distinct signaling outputs*. PLOSGenetics. Retrieved November 26, 2022, from https://journals.plos.org/plosgenetics/article?id=10.1371%2Fjournal.pgen.1006681#sec008