Investigating Stem Cell Regulation Through the Novel 'Disking' Phenotype in *Arabidopsis thaliana*

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Abstract

Plants cannot reproduce or bear fruit without functional flower production, which comes from the production of stem cells. Some of these stem cells differentiate and produce flower tissue, among other key components. Understanding how plants control stem cell proliferation is integral in feeding our growing society. We study this pathway in the model plant, *Arabidopsis thaliana*. CLV and BAMs are a class of LRR receptor kinases that are known to regulate the growth and proliferation of stem cells.[1] CLE ligands perform this same function by binding to CLV receptors. In previous experiments, I discovered a disadvantageous 'disking' phenotype by knocking out some of these key receptors. To determine if CLE25 signals through BAM3, I generated a *clv1 bam1 cle25* mutant and brought it to the F3 generation. To determine if CLV3 signals through CLV1BAM1, I generated a *clv1bam1 clv3* mutant. This plant displayed a phenotype very similar to *clv3*, suggesting that CLE25 may signal through BAM3.

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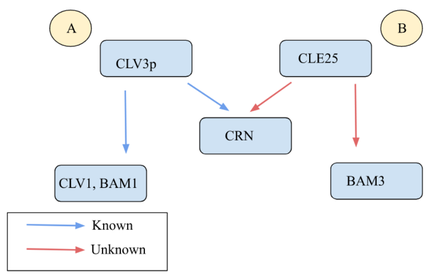
Stem Cells Are Regulated Through CLV and BAM Receptors

There are many receptors and proteins that plants use to perform regulatory activities. Among them are Leucine Rich Repeat (LRR) receptor kinases, like CLV and BAMs which are known to regulate the growth and proliferation of stem cells.[1] CLE ligands perform this same function by binding to CLV receptors. Removing these receptors shows what happens to the plant when that receptor's function is missing, typically producing a disadvantageous 'look' or phenotype. I discovered previously that the *clv1bam1/3* mutant produces a severely overproliferated meristem and forms a disk-like shape with its abundance of failed flowers. This phenotype has been seen before in the *cle25 clv3* mutant, which suggests that CLE25 may be signaling through BAM3. *clv1-15* and *clv3* also show the same phenotype. If *clv1-15 bam1* and *clv3* are similar genetically, as this suggests, then removing CLE25 from the *clv1-15 bam1* mutant should also produce a disk phenotype. This disk could further prove that CLE25 signals through BAM3, since their absence produces the same result in *clv1-15*. Further, if removing CLV3 from the c*lv1-15 bam1* mutant produces a phenotype similar to *clv3*, this could solidify the notion that CLV3 signals through CLV1 and BAM1.

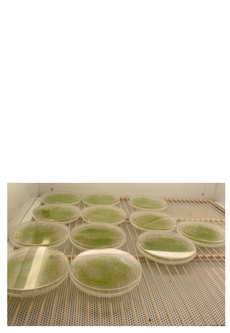


Hypothetical signaling pathway involved in stem cell regulation

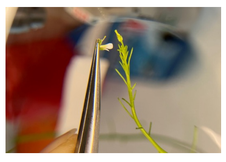
Wild-type Phenotype

Comparison of the disking phenotypes (top row) and the fasciated phenotypes (bottom row)
(cle-25-11 clv3-20 and *clv3* imaged by Dr. Amala John)[1]

Methods



Crossing Genotypes → Collecting seed and plating → Growing to maturity → DNA Analysis → Phenotyping → SEM Imaging

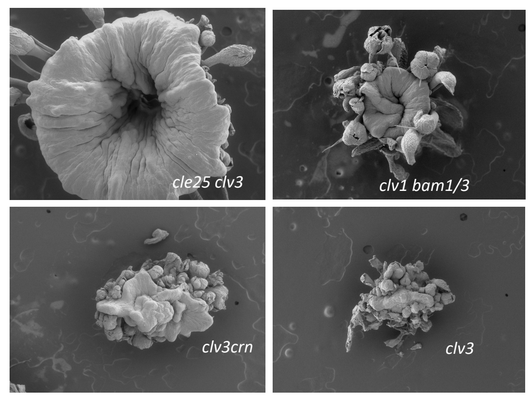


Retrieved from AIT Bioresources [4]

clv1 bam1 clv3 Phenocopies *clv3*

Support of Disking Phenotype

SEM imaging provided topographical analysis of the disk-like meristem that *cle25 clv3* and *clv1 bam1/3* produce. *clv3 crn* and *clv3* produce oblong meristems, depicted here as a reference.



SEM Comparison of cle25 clv3 and clv1 bam1/3 vs clv3 crn and clv3, imaged with help from Dr. Andrew Willoughby

clv1 bam1 clv3 Genotype

The *clv1 bam1 clv3* mutant displayed a fasciated, overproliferated phenotype with terminated flowers, very similar to the *clv3* mutant.

Phenotypical Comparison of clv1 bam1 clv3 and clv3. clv3 imaged by Dr. Amala John [1]

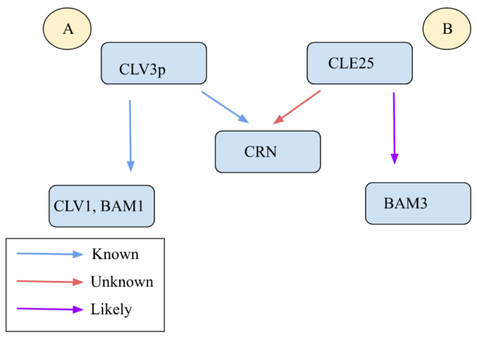
clv1 bam1 cle25 Genotype

Unfortunately, none of the F3 plants grown were mutant for all three genes, leaving it impossible to correlate the observable phenotype with this novel genotype.

CLE25 Likely Signals Through BAM3

This *clv1 bam1 clv3* and *clv3* phenocopy further solidifies the line of reasoning that CLV3 does signal through CLV1 and BAM1. Since the absence of CLV1 BAM1, CLV3, and CLV1 BAM1 CLV3 together, all produce the same fasciated phenotype.

Additionally, since the *clv1 bam1 clv3* mutant does not result in a disk, this provides evidence that CLE25 signals through BAM3. This is because The disk-like phenotype appeared in only *clv1-15 bam1/3 and cle25 clv3*. Since *clv3*, *clv1bam1,* and *clv1 bam1 clv3* are phenocopies, the only other genes involved in the *cle25 clv3* and *clv1bam1/3* phenocopy are likely signaling through each other.



New Hypothetical signaling pathway involved in stem cell regulation

Future Directions

To confirm this pathway, I will grow the *clv1bam1 cle25* genotype to the F4 generation and the *clv1bam1 clv3* genotype to the F3 generation.

I will then perform SEM analysis of the *clv1 bam1 clv3* meristem to confirm its shared phenotype with *clv3*.

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

4. Microbes&Plants@AIT. [AT_BioResources]. (2020, August 28). How plants acclimate in a #changing #climate by maintainung their energy homeostasis and increase their #stress #resistance? [Tweet]. Retrieved from https://twitter.com/AT_Bioresources/status/1299220091259834368.