A contradictory GLABRA3 allele helps define gene interactions controlling trichome development in *Arabidopsis*

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**Coordinating Cell Fate with Cell Position**

- Why is it important?
  - Some structures need to be separated (leaves, trichomes)
  - Some structures need to be connected (vascular system)
  - All essential for efficient and proper plant function
Why Study Trichomes?

- Highly endoreduplicated, unicellular epidermal cells
- Fairly easily observed
- Non-random distribution
  - Undergo 'lateral specification'
  - Spacing Pattern
- Cell fate specification, pattern formation, and cellular differentiation

Oppenheimer Lab Image Library, University of Florida
http://plaza.ufl.edu/oppenhe/oppenlab/pictures.html

Molecular Model for Trichome Initiation in Arabidopsis

- Three genes involved in promoting trichome initiation
  - GLABROUS1 (GL1)
    - R2R3-Myb-type transcription factor
  - TRANSPARENT TESTA GLABRA1 (TTG1)
    - WD-40 repeats
  - GLABRA3 (GL3)/ENHANCER OF GLABRA3 (EGL3)
    - bHLH type transcription factor

- Gene involved in repressing trichome promotion
  - TRIPTYRON (TRY)
    - Single repeat R3 Myb

Amplification of stochastic variation

Trichome initiation
Esch et al., found a highly pleiotropic trichome phenotype in EMS-treated Columbia

- Multiple phenotypes
- Varied in degree of maturation
- Mostly lacked papillae
- Fewer trichomes, with an increased amount of clustering

**SHAPSHIFTER (SST) Mutant**
**SST Crosses**

- **F1 sst/sst and Col (WT/WT)**
  - Fewer trichomes
  - More branches
  - Not as extreme as in sst/sst

- **Segregation of F2**
  - 3:1 Wild type:sst phenotype
    - Recessive mutation
    - Single locus mutation

- **Segregation of F3**
  - Supported F2

**Mapping SST**

- **F2 of Col sst and Ler cross**
  - PhyC, CiW9, and Ciw10 marker primers
    - Between PhyC and CiW9 in chromosome 5
  - Simple Sequence Length Polymorphism (SSLP) marker primers were made using the InDel polymorphism collection (Celeron)
    - Mapped two clones, one of which contained GL3
**gl3-sst?**

**Morphological Evidence**

- sst crossed to gl3-1
  - Less branched trichomes with larger diameter than gl3-1
  - No wild type plants

**Molecular Evidence**

- Sequencing of sst at the GL3 locus
  - C → T in codon 78
    - Converts Leu → Phe

- Transgenic expression of MYB5::GFP-GL3
  - Rescued sst and gl3-1 phenotype
More characteristics of gl3-sst

- Mutant trichomes have a dramatic increase in cell expansion in early stages of development
- Allows for a larger surface area for branch initiation

- Used DAPI-staining to look at DNA content
  - Columbia trichomes contain 16-64C (x=32)
  - Much more DNA in gl3-sst

- Larger cells… More DNA?

- Used N7 GFP fusion protein
  - Columbia trichomes single sphere
  - gl3-sst interconnected lobes
More DNA… More endoreduplication?

- Used Lac Operator/Repressor-GFP to visualize interphase chromatin
  - Chromosomally integrated Lac operator array
  - 3SS::GFP-Lac 1 transgene

Wild Type  

-gl3-sst

Extra Endoreduplication

- Suggests that chromatids of endoreduplication chromosomes are tightly associated
  - Possibly polytene
  - Suggests endoreduplication without karyokinesis
Cytoskelton Abnormalities?

- Large size and failure to undergo karyokinesis suggests a hindrance to microtubule formation
  - Wild type plants treated with microtubule destabilization agents (i.e., oryzalin) have swollen trichomes at initiation
  - Wild type plants treated with antimicrotubule drugs result in disruption of karyokinesis, and are similar to nuclei of \( gl3-sst \)

More Characteristics of \( gl3-sst \)

- General lack of papillae
- Used SEM EDAX to determine elemental composition of trichomes
  - Wild type trichomes contain C, O, Mg, Ca, with P in papillae
  - \( gl3-sst \) lacked Mg and P, and when no papillae were present no Ca
**gl3-sst** trichomes are not maturing

- Papillae formation is one of the final steps to trichome maturation
  - Results suggest that Mg, P and Ca deposition in the trichome cell wall plays an important role in trichome maturation

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**gl3-sst** is a functional allele

- **gl3-1/gl3-1 egl3/egl3** are glabrous
- **gl3-sst/gl3-sst** was crossed to **egl3/egl3**
  - F2 generation resulted in wild type and **gl3-sst** trichomes
  - F3 generation analyzed
    - Population of plants exhibiting only **gl3-sst**-like trichomes was analyzed for genotype
    - Were found to be homozygous for both **gl3-sst** and **egl3**
The \textit{gl3-sst} mutation appears in a region that codes for the protein domain that mediates interactions with GL1 and TRY. To confirm that this mutation affects protein-protein interactions the authors utilized the yeast two-hybrid system.

\begin{itemize}
\item GL3 and GL3-SST were fused to the activation domain (AD) of GAL4.
\item GL1, TRY and TTG1 were fused to the binding domain (BD) of GAL4.
\end{itemize}
Complex yeast two-hybrid assays

- It has been proposed that TRY and GL1 compete for the same binding site on GL3
  - Originally observed by overexpression of CPC causing a lack of trichomes
    - TRY encodes a CPC-like protein (i.e., homologous)
- Employed the pBridge system
  - Assays competitive interaction between proteins

Competitive Interaction Assay

- TRY fused to GAL4 BD
- Free TRY expressed through a Methionine repressible promoter (free = no AD or BD)
- GL3 and GL3-SST on AD
Localization of GL3, GL1 and TRY

Importantly, these localization experiments were in mutant backgrounds

- MYB5::GFP-GL3 rescued gl3-1
- ATML1::GFP-GL1 rescued gl1
- TRY::GFP-TRY rescued try
  - Clustered trichomes
  - Extra branched trichomes

Control Plants showed no GFP expression
Enhanced TRY expression

- *gl3-sst* has clustered trichomes at approximately the frequency as *try* (~15%)
  - *MYB5::GFP-TRY* in *try*
    - Can rescue *try*
  - *MYB5::GFP-TRY* in *gl3-sst*
    - Number of leaf trichomes was reduced
    - Reduced number of clustered trichomes to 1.5%

Support of the Threshold Model

- Comes in two ways
  - Fewer trichomes
    - Increased amount of trichome inhibition
  - More trichome clusters
    - Trichome inhibition is relaxed

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Given the interaction between GL1 and TTG1 with gl3-sst (4-fold and 2-fold less, respectively, yeast two-hybrid assays), fewer cells are reaching the critical concentration of the activation complex, resulting in less trichomes. Inhibition of clustering signal is supported by the TYP expression analysis.

References

