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# An epidermis-driven mechanism positions and scales stem cell niches in plants

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How molecular patterning scales to organ size is highly debated in developmental biology. We explore this question for the characteristic gene expression domains of the plant stem cell niche residing in the shoot apical meristem. We show that a combination of signals originating from the epidermal cell layer can correctly pattern the key gene expression domains and notably leads to adaptive scaling of these domains to the size of the tissue. Using live imaging, we experimentally confirm this prediction. The identified mechanism is also sufficient to explain de novo stem cell niches in emerging flowers. Our findings suggest that the deformation of the tissue transposes meristem geometry into an inductive scaling and positional input for the apical plant stem cell niche.

## INTRODUCTION

Development requires coordination between molecular patterning and morphogenesis (

<sup>1</sup>

,

<sup>2</sup>

). Several studies suggest how morphogens can

drive gene patterning, growth, and sh

ape generation in different systems

(

<sup>3</sup>

,

<sup>4</sup>

) and how mechanical constraints might feedback on growth (

<sup>5</sup>

,

<sup>6</sup>

).

Although adaptation to size is ofte

nseen in developmental tissues (

<sup>1</sup>

,

<sup>7</sup>

),

how tissue geometry and size feedback on gene expression domains is

less understood. Using a combination of computational and experimental approaches, we address this question in the shoot apical meristem (SAM), the stem cell niche source of all aboveground plant organs (8).

Considered as a main orchestrator of the SAM activity (9), WUSCHEL (WUS) is expressed in a central domain, a few cell layers below the apex of the SAM. It encodes a transcription factor, moving between cells, and is involved in perpetuating stem cell activity and keeping differentiating cells at bay (11).

At the very tip of the SAM, the stem cells express the CLAVATA3 peptide (CLV3) that diffuses in the tissue and down-regulates WUS, closing the canonical CLV3/WUS feedback loop (13).

), thought to be the main factor maintaining SAM homeostasis. On the flanks of the meristem, WUS also represses the differentiation program including the KANADII (KAN1) marker (12).

Spacial aspects of WUS maintenance via cytokinin signaling have recently been elucidated: LONELY GUY (LOG) proteins, which catalyze cytokinin biosynthesis, are expressed in the epidermis (L1), and ARABIDOPSIS HISTIDINE KINASE (AHK) cytokinin receptors are expressed inside the tissue, a few cell layers away from the L1 (15).

(Fig. 1A). Along with previously published data (17), this implies that the epidermis may provide a major positional cue for SAM maintenance.

### RESULTS AND DISCUSSION

To investigate this hypothesis, we propose an integrated quantitative description of the processes regulating the SAM homeostasis where the L1 is central to the patterning of the stem cell regulatory genes (Fig. 1B). The spatial model integrates the SAM regulatory interactions described in a system of differential equations, using Hill formalism for transcription together with mass action and passive diffusion-like transport (Supplementary Materials) (11).

It includes the CLV3/WUS feedback loop and the repression of KAN1 by WUS. In particular, the L1 produces four signals modeled as diffusive molecules. Of those, cytokinin acts as an activator of WUS by binding the AHK receptors. Given the expression pattern of the AHKs (Fig. 1A), we hypothesized a repressive L1 signal, keeping the AHKs away from the epidermis (termed AHK-). Cytokinin and AHK- form an incoherent feed-forward motif regulating WUS

(  
20  
) (Fig. 1, A and B). Two additional signals produced by  
the epidermis also activate  
CLV3  
(  
11  
,  
12  
,  
19  
) and  
KAN1  
(  
12  
).  
Different representations of the meristematic tissue are used  
throughout this study. Two abstract tissue templates, one 2D and  
one 3D, are composed of overlapping spherical cells constrained in  
a parabolic shape (Fig. 1C and fig. S1). A realistic tissue template  
obtained from segmented confocal data (  
20  
) is also used and includes  
cell volumes and cell contact surfaces (Fig. 1D). For all these tem-  
plates, the bottom layer of cells implements a specific boundary  
condition (referred to as sink), abstr  
acting the diffusion of molecules  
outside of the meristem and into the  
tissues below (stem and vascula-  
ture), where molecules are removed  
from simulations based on their  
diffusion rate. As such, the faster a molecule diffuses in the meristematic  
tissue, the faster it is carried out of the system at the bottom boundary.  
In contrast, epidermal  
cells have a nonflux boundary to the outside of  
the tissue.  
Model parameters are optimized to obtain the wild-type expression  
domains of  
WUS  
,  
CLV3  
, and  
KAN1  
using a dedicated strategy based  
on the Covariance Matrix Adaptat  
ion Evolution Strategy (CMA-ES)  
algorithm (  
21  
) (Supplementary Materials and fig. S2). At equilibrium,  
the model achieves a correct representation of the expression domains  
showing that the epidermis-driv  
en model is sufficient for SAM  
patterning. This result is robust in the different representations of the  
meristematic tissue (Fig. 1, C and D). The model is also able to qual-  
itatively reproduce a large set of experimentally observed perturba-  
tions including perturbations of the  
CLV3/WUS  
feedback loop and  
perturbations of cytokinin signaling (Supplementary Materials and  
fig. S3).  
Several mutants of  
*Arabidopsis thaliana*  
result in meristematic  
tissue defects (  
12  
,  
22  
—  
24  
) ; an example is the  
*clavata*  
phenotype,  
causing plants with markedly enlarged and flattened meristems.  
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m

m-thick transversal sections cutting through the center of the meristem were extracted and further projected following maximum intensity.

When necessary, images were rotated so that the meristem would be displayed vertically. The images were then cropped to contain only the SAMs. Finally, each image was converted to png format and analyzed using the python PIL library ([www.pythonware.com/products/pil/](http://www.pythonware.com/products/pil/)).

In the following, we consider a reference frame with an origin at the bottom left corner of each image where discrete

x

and

y

values

correspond to pixel positions in the image. To measure the shape of a meristem, for each row of pixels, we extracted the topmost pixel registering FM4-64 fluorescence (for each

x

: maximum

y

with red

channel value >50). This results in a collection of

x

,

y

coordinates

outlying the shape of the meristem. The parameters of a second-degree polynomial (describing a parabola) were then fit to the extracted coordinates using the numpy.polyfit function. The parameter

a

of a

parabola described by

$ax^2$

+

$bx$

+

c

= 0 controls how narrow the domain contained within the parabola is (where

$ax^2$

+

$bx$

+

c

<0).

In the case of the parabolas outlying the meristem shape, the higher

the value of the

a

parameter, the flatter the meristem. This value was

computed for each meristem. The parabolas and

a

parameters are

displayed on the meristem images in figs. S8 to S19.

Thesameimageswereusedtoquantifytheshapeoftheexpression

domains of the

pWUS

>>

GFP

and

pCLV3

>>

GFP

constructs. We

chose to measure the elongation of the domains along the axis of the reference frame. To do so, we find the maximal distance between two pixels registering GFP fluorescence (green channel value >50) and having the same

y

coordinate, giving us the longest horizontal axis of

the GFP expression domain. We repeat the procedure using the same

x

coordinate and find the longest vertical axis. As a measure of the

GFP domain elongation, we use the ratio of the longest vertical axis over the longest horizontal axis. Thus, the higher the value, the more the domain is horizontally elongated. Axis and elongation values are displayed on their corresponding meristem images in figs. S8 to S19. Figure 3A and fig. S7A show a correlation between the shape of the meristematic tissue and the shape of the expression domains of both

WUS  
and  
CLV3

. The flatter the SAM, the more horizontally elongated the domains of expression are. Figure S21 shows the correlation between the size of the meristems from Fig. 2 and the shape of the meristems. We observe a strong correlation between the shape of the tissue and its size, with the larger meristems also being the flatter ones. Note that the number of meristems plotted in fig. S21 may differ from the number of meristems displayed in figs. S8 to S19, because only the meristems that could also be processed for Fig. 2 are displayed. Regression analysis is performed as described in the previous section. To assess if the L1 model can capture this effect, we generated a set of new 2D templates. The optimization template was first scaled by a factor and then scaled horizontally again by the same factor. This generated a set of smaller and narrower tissue templates and a set of larger and flatter ones. The factors displayed in fig. S20 are 0.9, 0.95, 1, 1.05, 1.1, 1.15, and 1.2. As exemplified, the larger and flatter meristems show elongated domains of expression for both

WUS  
and  
CLV3

, where as the domain tends to narrow down for the smaller and narrower meristems. The data obtained for all parameter sets optimized for the 2D template are shown in Fig. 3C and fig. S7C.

Primordium growth (Fig. 4 and movie S1)

Cells are allowed to grow by increasing the cell radii and are allowed to divide into two daughter cells when a threshold size is attained. A set of spring forces between cell centers moves cells apart when overlapping and back inside the lid when pushed out of it. Cells falling below the lower boundary of the lid are removed from the simulation (

12  
)

As the simulation runs, a sphere moves from the inside of the parabola, exiting laterally, and stopping when its center reaches the parabola. As the sphere moves, the lid is updated to comprise the parabola and the part of the sphere outside of the parabola. This results in an increase of the space allocated to cells. The growth simulation is performed using the organism software (<http://dev.thep.lu.se/organism>).

For 200 time points evenly spaced over the length of the simulation, the cell positions and radii are recorded and the cells belonging to the boundaries (L1 and Sink) are found, allowing us to find the equilibrium of the L1 model as previously described. The final movie is obtained by assembling the equilibrium variables of the model for each time point.

It is worth noting that the growth and cell division algorithm used to generate the movie is noisy, likely more than what could be expected from the actual meristematic tissue. This generates large unrealistic fluctuations of the expression domains of

WUS  
and  
CLV3

as the simulation runs. Keeping such an algorithm, the fluctuations could however be reduced by extending the model to add additional feedback loops known to be present in the system, such as the negative feedback of cytokinin of its signaling mediated by type B Arabidopsis response regulators. Another plausible approach is to trade some of the expression domain pattern quality for more robustness of the patterns by adding additional components to the cost functions used in the optimization of the parameters.

WUS  
and  
CLV3

live imaging (Fig. 4)

pWUS::GFP-ER  
and  
pCLV3::dsRED-N7  
plants were grown under

conditions described in the Realistic template section. For the live imaging, plants were transferred immediately after bolting to square boxes containing lukewarm MS media with 1% agar and allowed to solidify. The plants were then stained with FM4-64 and imaged with a Zeiss LSM 780 confocal microscope.

#### 1D simulations (Fig. 5)

To illustrate the scaling effect driven by the incoherent feed-forward loop controlling

WUS

expression, simulations were performed on a set of 1D tissue templates (ranging from 5 to 14 cells), each having an L1 cell at one extremity and a Sink cell at the other. The simplified model comprises the activation of

WUS

mediated by cytokinin and its repression mediated by AHK4.

The 10-cell template was chosen to manually fit the parameters of the system to obtain a central

WUS

domain. The chosen parameters are

$\nu$

$w$

$\frac{1}{4}$

1

:

7

$k$

$L_c$

$w$

$\frac{1}{4}$

0

:

75

$n$

$L_c$

$w$

$\frac{1}{4}$

10

$k$

$L_a$

$w$

$\frac{1}{4}$

0

:

2

$n$

$L_a$

$w$

$\frac{1}{4}$

10

$g$

$w$

$\frac{1}{4}$

1

:

7

$p$

$L_c$

$\frac{1}{4}$

15

$g$

$L_c$

$\frac{1}{4}$

1

$D$

$L_c$

$\frac{1}{4}$

100

$p$

$L_a$

$\frac{1}{4}$

2

$g$

$L_a$

$\frac{1}{4}$

1

$D$

$L_a$

$\frac{1}{4}$

1

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## SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/2/1/e1500989/DC1>

Fig. S1. Templates.

Fig. S2. Optimization strategy.

Fig. S3. The model is able to represent a large collection of perturbations.

Fig. S4.

pCLV3::WUS

.

Fig. S5.

WUS

domain size variation.

Fig. S6.

CLV3

domain size variation.

Fig. S7.

pCLV3

>>

GFP

meristem shapes and expression domains.

Fig. S8. clasp1

pWUS

>>

GFP

meristems, grown in long days.

Fig. S9. clasp1

pWUS

>>

GFP

meristems, grown in long days followed by short days.

Fig. S10. Col.0

pWUS

>>

GFP

meristems, grown in long days.

Fig. S11. Col.0

pWUS

>>

GFP

meristems, grown in long days followed by short days.

Fig. S12. WS-4

pWUS

>>

GFP

meristems, grown in long days.

Fig. S13. WS-4

pWUS

>>

GFP

meristems, grown in long days followed by short days.

Fig. S14. clasp1

pCLV3

>>

GFP

meristems, grown in long days.

Fig. S15. clasp1

pCLV3

>>

GFP

meristems, grown in long days followed by short days.

Fig. S16. Col.0

pCLV3

>>

GFP

meristems, grown in long days.

Fig. S17. Col.0

pCLV3

>>

GFP

meristems, grown in long days followed by short days.

Fig. S18. WS-4

pCLV3

>>

GFP

meristems, grown in long days.

Fig. S19. WS-4

pCLV3

>>

GFP

meristems, grown in long days followed by short days.

Fig. S20. Example of expression domain variations upon tissue shape changes.

Fig. S21. Meristem size measure versus shape measure.

Fig. S22. Long-range and short-range signals.

Fig. S23.

WUS

sensitivity analysis.

Fig. S24.

CLV3

sensitivity analysis.

Table S1. Example parameter sets.

Movie S1. Primordium growth.

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J.G. designed the study, performed modeling, collected and analyzed data, and prepared the manuscript. B.L. and P.T. equally contributed in collecting and analyzing data. C.S., Y.R., and A.S. collected and analyzed data. C.S. commented on the manuscript. O.H. and E.M.M. contributed in designing the study and commented on the manuscript. H.J. designed the study and prepared the manuscript.

**Competing interests:**

The authors declare that they have no competing interests.

**Data and materials availability:**

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the corresponding author.

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