Robustness of organ size in *Arabidopsis*

Adrienne Roeder
Cornell University

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KITP: Morphogenesis in Animals and Plants: Search for Principles
Size is a critical characteristic

Organism size
- Giant sequoia
- Wolffia
- Mouse
- Elephant

Organ size
- Liver
- Flower

Cell size
- Cell under microscope
Size “is the material that evolution largely works on.”
But the field is still mostly in the dark

– Peter Lawrence Science 2013

Arabidopsis thaliana

Arabidopsis lyrata
The *Arabidopsis* sepal is a good model for studying organ size

- Relatively invariant to environmental fluctuations
- Four sepals allow comparison within a single flower
- Accessible for imaging and manipulation
Watching sepal growth at cellular scale using live imaging

Nuclei: H2B-YFP
Plasma membrane: mCitrine-RC12A
Tracking cell lineages with MorphoGraphX

Gerardo Tauriello
Petros Koumoutsakos
Richard Smith
How is organ size controlled?

• How does an organ stop growing when it reaches the right size?
  – Does an organ measure its size?

Spoiler alert: Still open questions!
Traditional approach: isolate mutants with bigger or smaller organs

- Good parts list.
- Little conceptual understanding of the logic.
- No idea whether/if/how these sense size.

Does cell size or cell number control organ size?

Increased cell size

Increased cell number

Compensation—smaller cell size

Points towards either: (1) existence of an organ “size” sensor

Or

(2) Control by growth somewhat separable from division
Cell size has little effect on organ size.

Giant cells false colored in red

average sepal area in LGO dosage series

Robinson (2018) Plant Cell
Cell division has little effect on long term growth

Collaboration emerged from KITP Miniprogram: Morphodynamics of Plants, Animals and Beyond (2009)

Cell division has little effect on growth

How is organ size controlled?

• How does an organ stop growing when it reaches the right size?
  – Does an organ measure its size?

• Not counting cell number or cell size.
If there is an organ size sensor, it is probably involved in maintaining reproducibility, not setting the average size.

Attempt 2: how do organs form with consistent sizes and shapes?
How do organs form with reproducible size and shape?

A person’s arms are the same length with a precision of 0.2%

Sepals are the same size to enclose the bud.
Arabidopsis sepal cells are highly variable

Variable cell size
Giant cells painted in red
Roeder, et al. 2010

Variable growth rate
Areal growth rate ($10^{-3}$ um$^2$ um$^{-2}$)
Hong et al., 2016

Variable cell wall stiffness
Young's modulus (Mpa)
Measured by atomic force microscopy (AFM)
Hong et al., 2016

- stiff
- soft

Arezki Boudaoud
Mathilde Dumond
International interdisciplinary collaboration

First met Arezki Boudaoud at KITP Miniprogram: Morphodynamics of Plants, Animals and Beyond (2009)
Strategy: screening for mutants with variable sepal size or shape in the same flower

Wild type

\textit{vos1}

\textit{vos2}

\textit{vos3}

Shape variability

\underline{variable organ size and shape mutants}

Dr. Lilan Hong
Stories: 3 short stories about variable organ mutants

• *variable organ size*1 (*vos*1) and spatial temporal averaging of noisy cell growth

• *variable organ size* 3 (*vos*3) and coordination of growth on the front and back of the organ.

• *variable organ size*2 (*vos*2) and synchrony of organ primordia initiation
A novel mutant has variable sepal size

Hong et al. (2016) Dev Cell
vos1 sepals have larger variance in area

***: significantly different from WT, f test, P<0.001

Hong et al. (2016) Dev Cell
vos1 sepals have larger variance in shape

Sepal contours normalized by size

Log squared deviation of sepal contour

***: significantly different from WT, t test, P<0.001

Hong et al. (2016) Dev Cell
vos1 sepal primordia form normally. Variability in sepal size arises during growth.

Hong et al. (2016) Dev Cell
How does cellular variability result in regular organs?

Measured by atomic force microscopy (AFM)

A continuous mechanical sepal model with spatial stiffness variability

Arezki Boudaoud & Mathilde Dumond

Hong et al. (2016) Dev Cell
Mechanical properties are spatially variable.

Arezki Boudaoud & Mathilde Dumond

Sepal models of variable stiffness in space

Hong et al. (2016) Dev Cell

Arezki Boudaoud & Mathilde Dumond
Variability in space leads to misshapen sepals
Sepal models of variable stiffness in space and time

Mechanical properties are spatially and temporally variable

Hong et al. (2016) Dev Cell

Arezki Boudaoud & Mathilde Dumond
Variability in space and time leads to regular sepals

Hong et al. (2016) Dev Cell

Arezki Boudaoud & Mathilde Dumond
Cellular variability in space

In a virtual sepal

Cellular variability in **space**

**+**

Regular organ morphology

Cellular variability in **time**

In a real sepal?
Live imaging of the sepals

Wild type

0 hr | 12 hr | 24 hr | 36 hr | 48 hr
Stage 8-1 | Stage 8-2 | Stage 9-1 | Stage 9-2 | Stage 9-3

vos1

0 hr | 12 hr | 24 hr | 36 hr | 48 hr
Stage 8-1 | Stage 8-2 | Stage 9-1 | Stage 9-2 | Stage 9-3

Hong et al. (2016) Dev Cell
Sepals have variable cellular growth

Areal growth rate

WT

vos1

12 hr growth mapped on the beginning time point

Hong et al. (2016) Dev Cell
Sepals have cellular growth variability in space

Areal growth rate

0 to 12 hr | 12 to 24 hr | 24 to 36 hr | 36 to 48 hr

WT

Hong et al. (2016) Dev Cell
Sepals have cellular growth variability in time

Areal growth rate

0 to 12 hr  12 to 24 hr  24 to 36 hr  36 to 48 hr

Hong et al. (2016) Dev Cell
Cell variability in space is reduced in vos1

Satoru Tsugawa
Hong et al. (2016) Dev Cell

Error bars: SD.
Cell growth variability in time is similar between WT and vos1.
vos1 has low variability in space of cell wall stiffness

Hong et al. (2016) Dev Cell
Can decreasing cell variability in space cause development of irregular sepals?

Wild type

vos1

Cell variability in

space

time

Sepal

regular

irregular
Decreasing variability in space leads to irregular sepals in the model.
How does cell variability give rise to regular organ morphology?

Cellular variability in **space**

+ ?

→  Regular organ morphology

Cellular variability in **time**
Spatiotemporal averaging of cellular variability

Principal direction of growth (PDG)

Hong et al. (2016) Dev Cell

Cellular variability $\xrightarrow{\text{spatiotemporal averaging}}$ Organ regularity
Spatiotemporal averaging of cellular variability is defective in *vos1*

<table>
<thead>
<tr>
<th></th>
<th>0-24 hours</th>
<th>24-48 hours</th>
<th>cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> wild type (24hr)</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>B</strong> vos1 (24hr)</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>C</strong> wild type (48hr)</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>D</strong> vos1 (48hr)</td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Principal direction of growth (PDG)

Hong et al. (2016) Dev Cell
Conclusion:
Variability produces regularity

Cellular variability \[ \xrightarrow{\text{spatiotemporal averaging}} \] Organ regularity

Variable growth direction

0-24 hours

\[ \overset{\text{Spatiotemporal averaging}}{\longrightarrow} \]

24-48 hours

Cumulative growth

0-48 hours

Hong et al. (2016) Dev Cell
Why is cellular variability reduced and spatiotemporal averaging blocked in vos1 mutants?
Mutation of *FtsH4* causes the *vos1* phenotype

FtsH4 is a mitochondrial localized protein that has an AAA-ATPase domain and a protease domain. FtsH4 maintains protein quality in mitochondria.


Hong et al. (2016) Dev Cell
Hypotheses

vos1 (FtsH4) mutation

Mitochondrial defects

ROS: reactive oxygen species

ROS ↑

Reduced spatiotemporal averaging

Variable sepal size and shape

Hong et al. (2016) Dev Cell
**vos1** sepals have increased superoxide

Superoxide in WT

Superoxide in **vos1**

NBT staining detects superoxide.

Hong et al. (2016) Dev Cell
**H₂O₂ treatment of WT mimics vos1 phenotype**

Arrows show smaller sepals

Hong et al. (2016) Dev Cell
Decreasing the ROS level in vos1 rescues the variable sepal size phenotype

WT  vos1  vos1+35S::CAT2

CAT2: CATALASE2, degrading H₂O₂

Hong et al. (2016) Dev Cell
Do ROS also regulate wild-type sepal growth?
ROS accumulation from the tip coincides with sepal maturation

Superoxide in WT

Stages 9 10 10 11 12 12 13 13 14

Hervieux et al. (2016) Current Biology
Hypothesis: if ROS act as a signal terminating growth in wild type, reducing ROS should increase sepal size.
ROS limit WT sepal growth

CAT2: 35S:AtCAT2; AtCAT2, CATALASE2
APX1: 35S:AtAPX; AtAPX1, ASCORBATE PEROXIDASE1
RBOHD: 35S:AtRBOHD; AtRBOHD, NADPH oxidase

*: significantly different from WT, P<0.001
Error bar: STDEV

Hong et al. (2016) Dev Cell
Conclusion Part 1:
Variability produces regularity

Cellular variability → spatiotemporal averaging → Organ regularity

ROS inhibit spatiotemporal averaging.

How?

ROS are a major signal inducing maturation of the sepals and termination of growth.

What determines when ROS are produced?
Is spatiotemporal averaging a general developmental principle for handling variability?
Spatiotemporal averaging of stochastic Hunchback expression in Drosophila

Based on Little et al., (2013) Cell
ROS as an ancestral signal???

Constitutive buffering of ROS for survival

Simple signal transduction pathway to tune ROS scavenging

ROS from interaction with other organisms/cell-to-cell communication requires additional signaling pathways

Active ROS production as an advantage in cell-to-cell communication/defense against other organisms

Current - multiple ROS production and Scavenging pathways integrated into a signal transduction network

Stories: 3 short stories about variable organ mutants

• **variable organ size 1** \( (vos_1) \) and spatial temporal averaging of noisy cell growth

• **variable organ size 3** \( (vos_3) \) and coordination of growth on the front and back of the organ.

• **variable organ size 2** \( (vos_2) \) and synchrony of organ primordia initiation
The *vos3* mutation generates variability in 3D sepal shape

WT (Ler)  

*vos3*

Scale bars: 1 mm
vos3 has a ruffled sepal surface

WT (Ler)  vos3

SEM images; not with the same scale.
vos3 has a variably ruffled sepal surface
vos3 has a smooth inner sepal epidermis
vos3 sepals have folds in the outer epidermis

Chlorophyll
mCitrine-RCI2A: plasma membrane marker

Scale bars: 50 µm
vos3 forms up-curved leaves
How do the folds arise on the vos3 sepal?
Proposal: an imbalance of growth disrupts the planar sepal growth

Hypotheses for fold generation: 1 Local outgrowth 2 Buckling
Live imaging to track the formation of the folds

WT (Ler)

0hr  24hr  48 hr  72 hr

vos3

0hr  24hr  48hr  72hr
Gaussian curvature describes surface topology.

Gaussian curvature

- Negative saddle
- 0
- Positive convex
Live imaging to track the formation of the folds

WT (Ler)

Surface Gaussian curvature

Surface Gaussian curvature

-1.5  1.5  $\times 10^{-4}$ $\mu$m$^{-2}$
The folds start gradually in sepal development.

Surface Gaussian curvature

Arrows: creases on the epidermis
How is growth altered in *vos3* sepals to give rise to folds?
vos3 sepals maintain faster growth

Fast growth is not localized to lumps
Does increased cell division drive outgrowth?
vos3 sepals have a lower cell proliferation rate

Cell division of t=0hr to t=72hr growth mapped on t=72hr
Auxin regulates outgrowth of primordia.

Does auxin signaling correlate with fold formation?
Auxin signaling does not cluster at the bump initiation sites, suggesting these are not primordia.

DR5::VENUS auxin response marker in vos3

Auxin accumulation precedes primordium formation.
Proposal: an imbalance of growth disrupts the planar sepal growth

Hypotheses for fold generation: 1 Local outgrowth-not likely 2 Buckling
Buckling: “bend and give way under pressure or strain”


Buckling of a train track due to an earthquake in New Zealand, 2010
Modeling suggests buckling may occur when

- The outer epidermis grows at faster than the adaxial epidermis.
- The outer epidermis is softer than the adaxial epidermis.
Will orienting and slowing growth restore a smooth sepal surface?

Test by crossing in ATML1::KRP1 which inhibits cell division and promotes giant cell formation.

ATML1::KRP1
Orienting growth on the outer epidermis suppresses lump formation

Roeder et al, Plos Biology, 2010

pATML1::KRP1
Slower growth on the abaxial epidermis

vos3
Arrows: lumps on the sepal

vos3 pATML1::KRP1
Slower growth on the abaxial epidermis
Orienting growth on the outer epidermis suppresses outgrowth.

WT (Ler)  

vos3  

vos3 / pATML1::KRP1  

Slower growth on the abaxial epidermis.
Proposal: an imbalance of growth disrupts the planar sepal growth

Hypotheses:
1. Initiation of primordia on the sepal epidermis – less likely
2. Buckling – possible (Further tests in progress)
What gene is mutated in vos3?
**vos3 has a mutation in the ASYMMETRIC LEAVES2 (AS2) promoter**

**vos3/as2-6D**

Repressor binding site

**WT AS2 expression region**

**AS2 (AT1G65620)**

AS2: a transcription factor with a leucine zipper domain; thought to repress downstream genes.
vos3 expands AS2 expression to the outer sepal epidermis
Overexpressing AS2 throughout the epidermis recapitulates the lumpy sepal phenotype

pATML1::AS2 expresses AS2 on all the epidermis.
Developing a regular flattened laminar structure requires coordinated growth across the organ.

WT leaves/sepals

as2-6D leaves

as2-6D sepals

AD2
Is growth coordinated on the sides of the organ?
The *Arabidopsis* cotyledon has similar growth on both sides of the organ.
Stories: 3 short stories about variable organ mutants

• *variable organ size*1 (*vos*1) and spatial temporal averaging of noisy cell growth

• *variable organ size* 3 (*vos*3) and coordination of growth on the front and back of the organ.

• *variable organ size*2 (*vos*2) and synchrony of organ primordia initiation
Sepal size is variable in vos2 mutants

Variable organ size and shape (vos)

Mingyuan Zhu
Sepal size variability arises early and continues through late stages.

WT

vos2

**Early stage**

**Late stage**

SP: Sepal primordium

FM: Floral meristem

Arrow: delayed SP
Is sepal initiation delayed in vos2 mutants?
Sepal initiation is delayed in vos2

WT

vos2

35S::mCitrine-RC12A
The initiation of vos2 sepals is delayed.
The initiation of vos2 sepals is delayed and more variable
Does delayed initiation cause variable organ size?
Late initiation leads to smaller sepals

WT

vos2

0hr  24hr  48hr  72hr  120hr
What gene is disrupted in the vos2 mutant?
VOS2 encodes a MYB domain transcription factor

RNA-seq: WT vs vos2

Cellular growth  
cell wall modification  
Response to stress or stimulus
Part 3 Conclusion: Timing of organ primordium initiation is critical for robust organ size.
Surprise: Organ initiation is not supposed to matter in organ size

How does an organ stop growing when it reaches the right size?

Does an organ measure its size?

Diana M. Vallejo et al. Science 2015;350:aac6767
How is organ size controlled?

- Spatiotemporal averaging of noisy cell growth produces regular size and shape organs.
- ROS is important for stopping growth in sepals.
- Surprisingly, timing of initiation of organ primordia contributes to size control.
- Coordinating growth across the organ is important for shape.

Still really don’t know how size is controlled

=> Need new approaches
Roeder lab:
Lilan Hong
--New faculty member
Zhejiang University
Mingyuan Zhu
Vijaya Lakshmi Vadde
Joseph Cammarata
Kate Harline
Jessica McGory
Weiwei Chen
Heather Meyer
Dana Robinson

Collaborators on these projects:
Arezki Boudaoud, Mathilde Dumond,
Simone Bovio, Vincent Mirabet
Olivier Hamant, Nathan Hervieux
Richard Smith, Anne-Lise Routier-Kierzkowska,
Aleksandra Sapala
Chun Biu Li, Tamiki Komatsuzki, Satoru Tsugawa
Gerardo Tauriello, Petros Koumoutsakos