Robustness of organ size in *Arabidopsis*

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Cornell University

KITP: Morphogenesis in Animals and Plants: Search for Principles

August 16, 2019

Size is a critical characteristic

Organism size

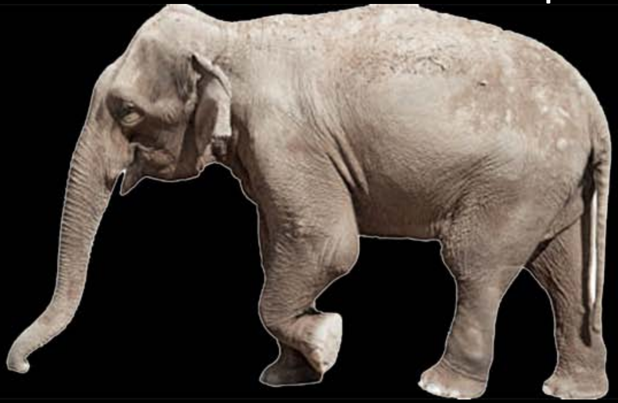
Giant sequoia

Wolffia

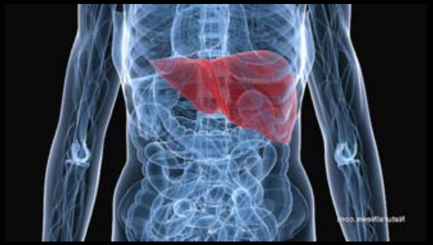
http://www.icr.org/article/6739/

mouse

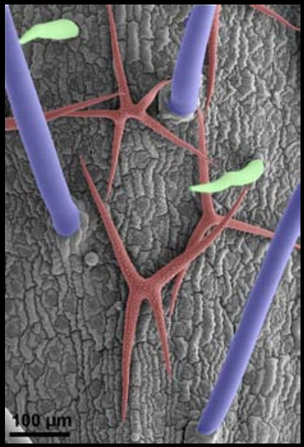
elephant



Organ size



Cell size



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

Arabidopsis **plants**

- 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-RCI2A

Tracking cell lineages with MorphoGraphX

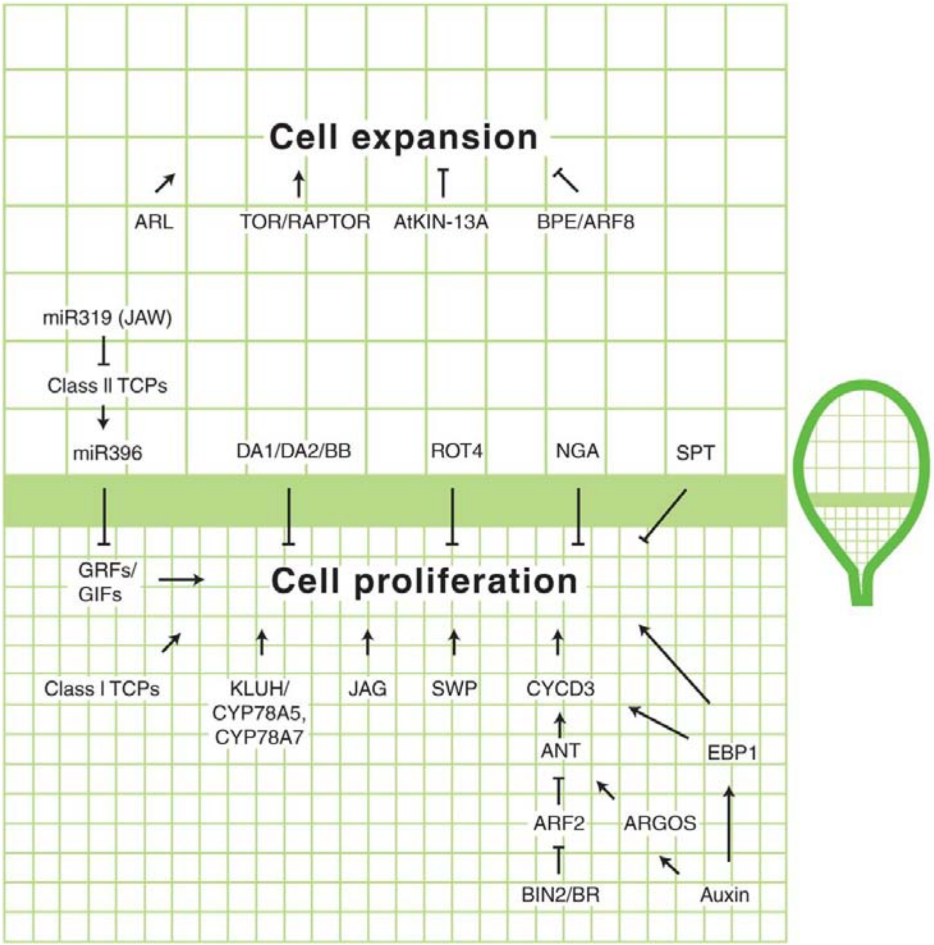
Gerardo Tauriello
Petros Koumoutsakos
Richard Smith

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!

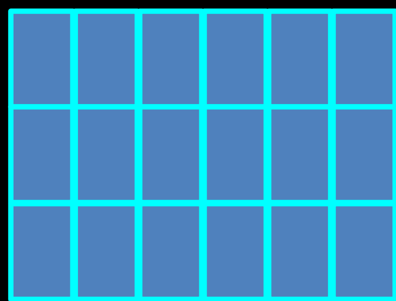
Traditional approach: isolate mutants with bigger or smaller organs



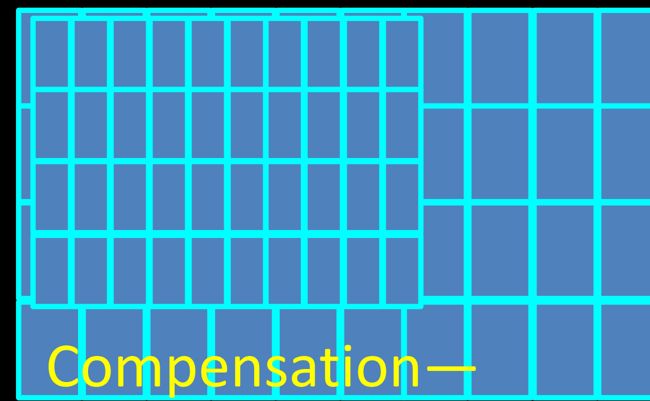
- Good parts list.
- Little conceptual understanding of the logic.
- No idea whether/if/how these sense size.

Czesnick H, Lenhard M. Size control in plants--lessons from leaves and flowers. *Cold Spring Harb Perspect Biol*. 2015;7(8):a019190. doi:10.1101/cshperspect.a019190.

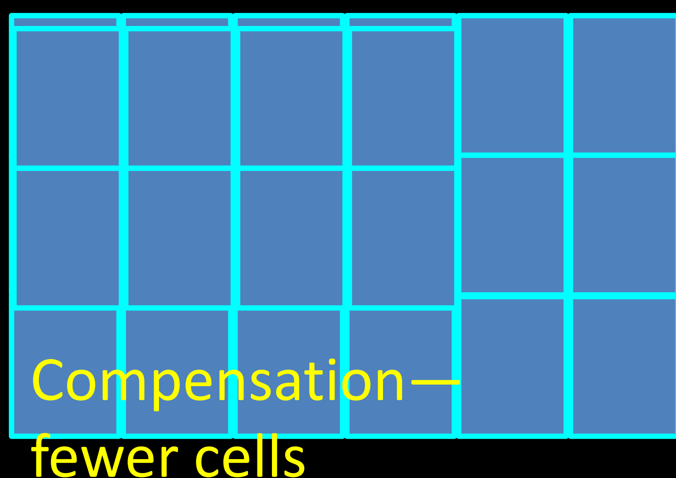
Does cell size or cell number control organ size?



Increased cell number



Increased cell size

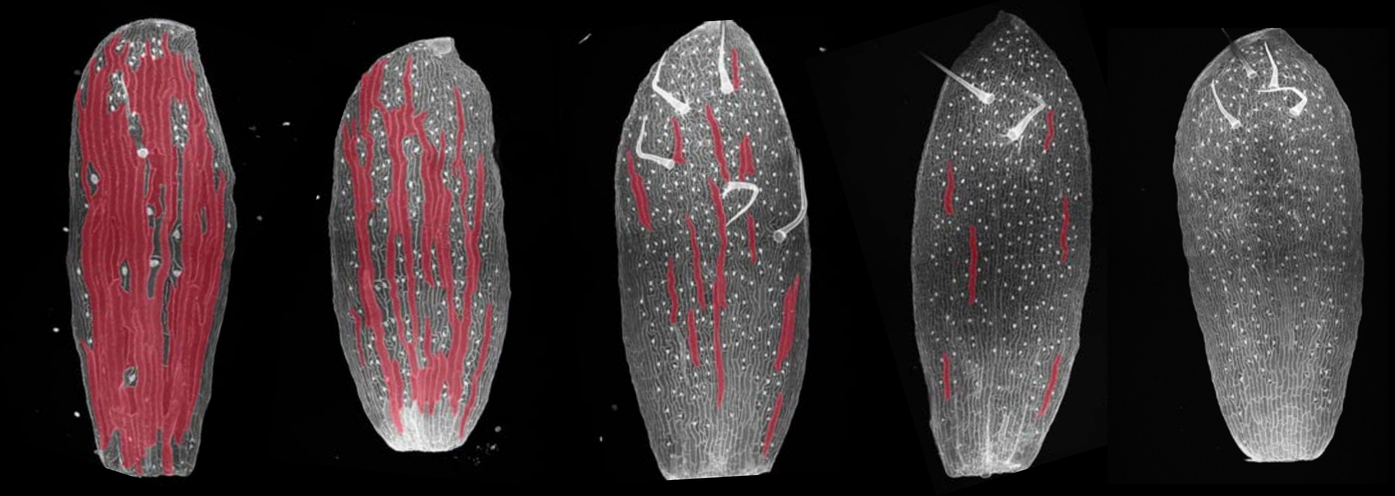


Points towards either: (1) existence of an organ “size” sensor

Or

(2) Control by growth somewhat separable from division

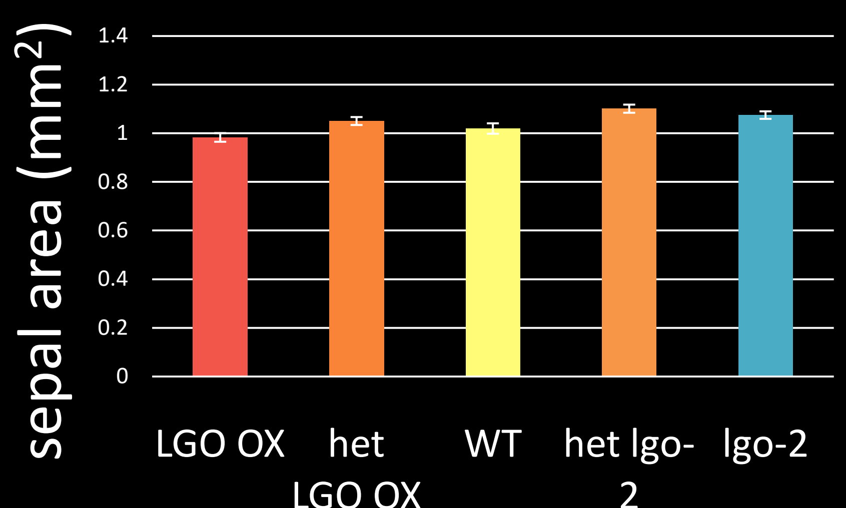
Cell size has little effect on organ size.



ML1::LGO   ML1::LGO/WT   WT   *lgo-2*/WT   *lgo-2*

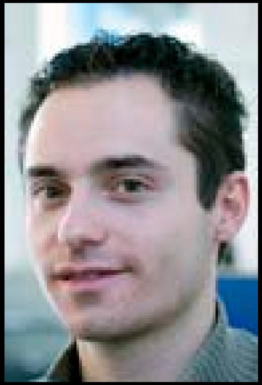
Giant cells false colored in red

average sepal area in LGO dosage series



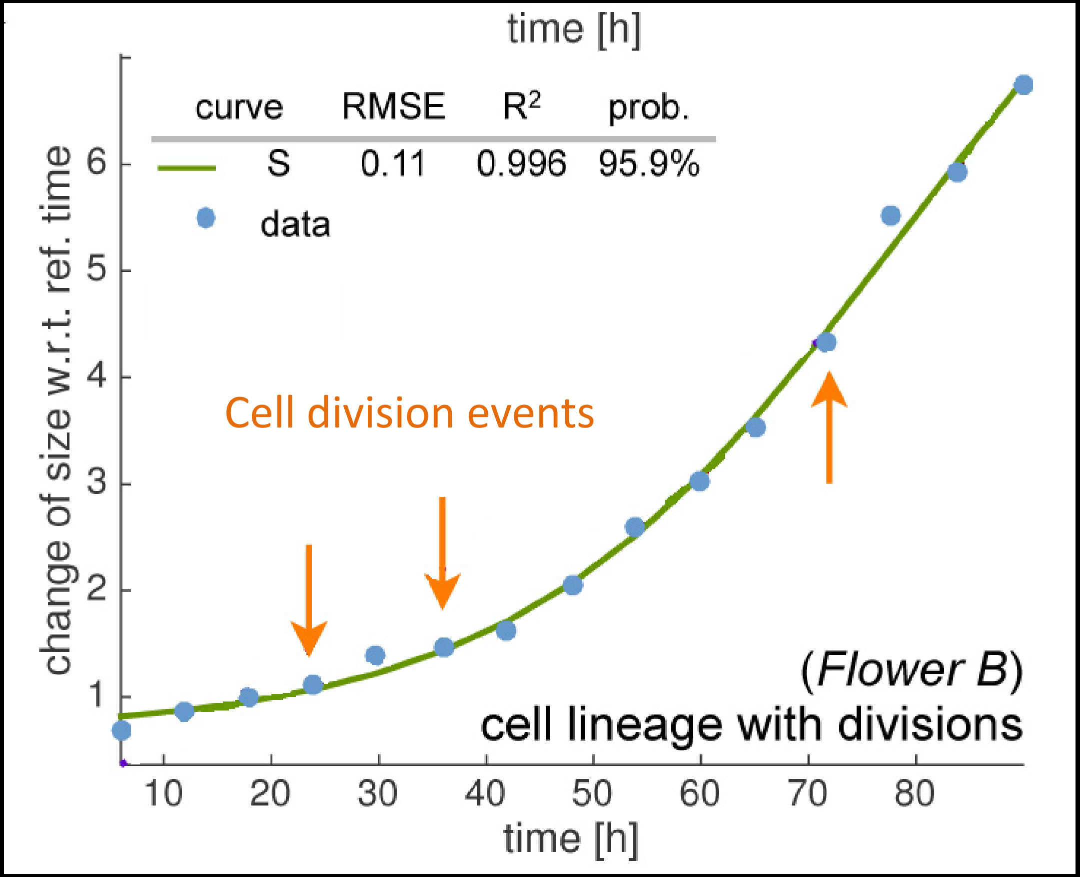
Robinson (2018) Plant Cell

Cell division has little effect on long term growth



Dr. Gerardo Tauriello
Petros Koumoutsakos
ETH Zurich

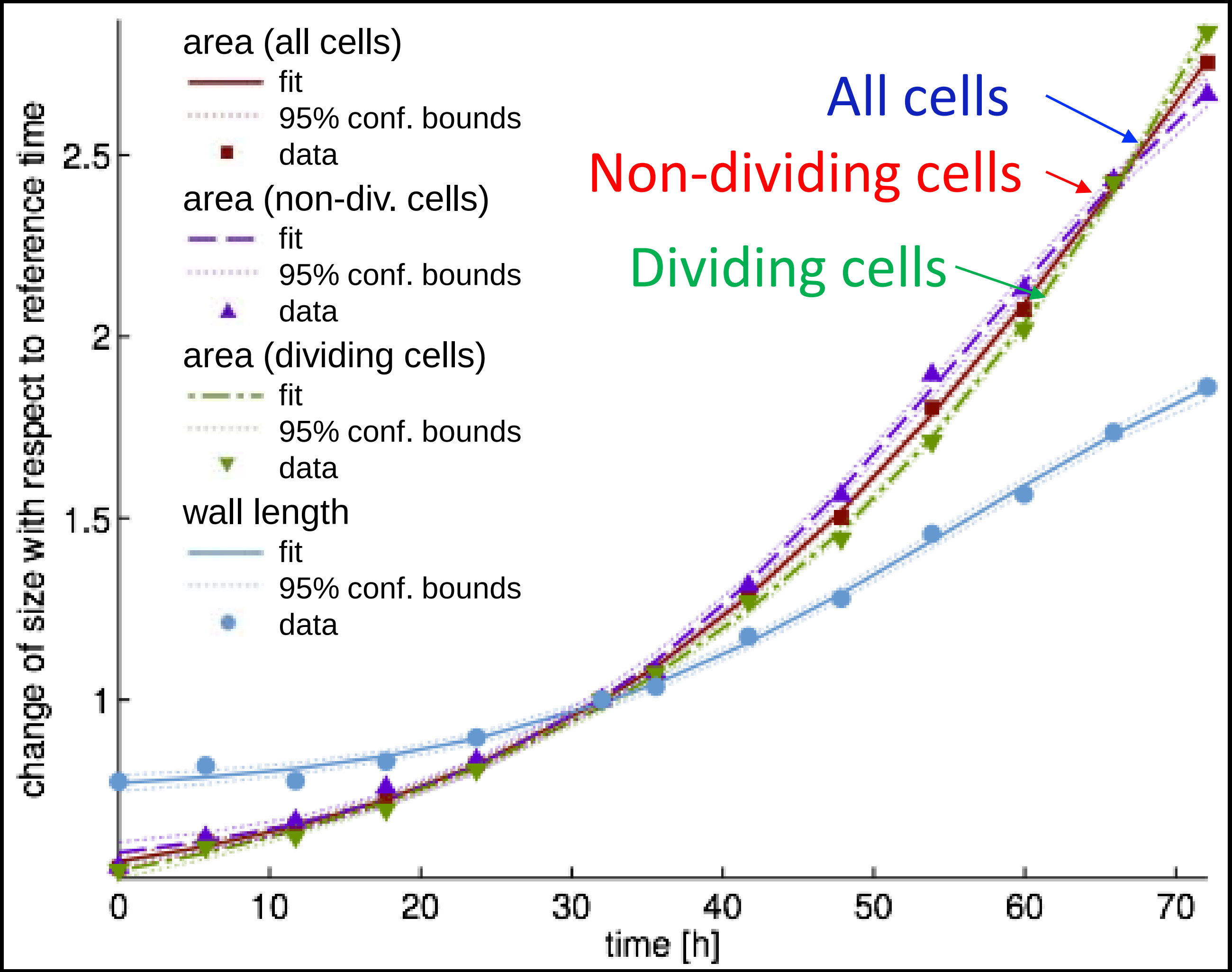
Dr. Heather Meyer



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

Tauriello et al., (2015) Plant Physiology

Cell division has little effect on growth



Tauriello et al., (2015) Plant Physiology

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.

Attempt 2: how do organs form with consistent sizes and shapes?

If there is an organ size sensor, it is probably involved in maintaining reproducibility, not setting the average size.

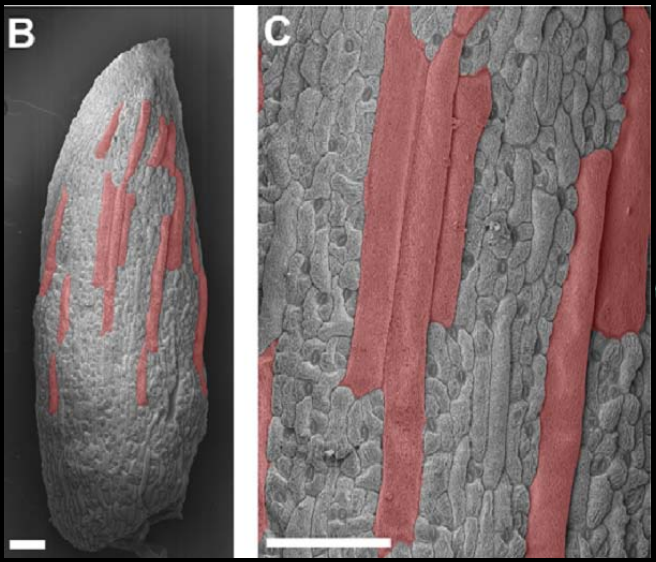
How do organs form with reproducible size and shape?

Sepals are the same size to enclose the bud

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

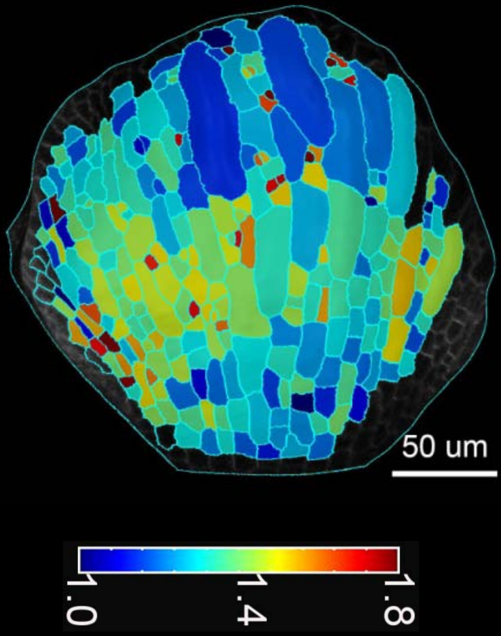
Arabidopsis sepal cells are highly variable

Variable cell size



Giant cells painted in red
Roeder, et al. 2010

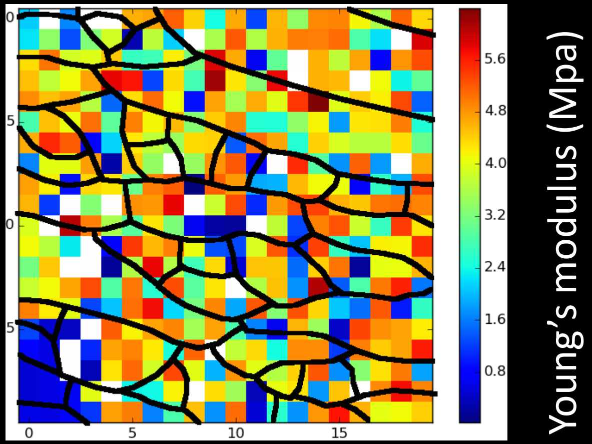
Variable growth rate



slow fast

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

Variable cell wall stiffness



Measured by atomic force microscopy (AFM)
Hong et al., 2016

Arezki Boudaoud
Mathilde Dumond

International interdisciplinary collaboration

First met Arezki Boudaoud at KITP Miniprogram: Morphodynamics of Plants, Animals and Beyond (2009)

HFSP

ENS (Lyon)
Arezki Boudaoud (PI)
Olivier Hamant (PI)
Mathilde Dumond
Nathan Hervieux

Cornell University (Ithaca)
Adrienne Roeder (PI)
Lilan Hong
Mingyuan Zhu

Max Planck Institute for Plant Breeding Research (Cologne)
Richard S. Smith (PI)
Anne-Lise Routier-Kierzkowska
Aleksandra Sapala
Hagen Reinhardt

Hokkaido University (Sapporo) now Stockholm University
Chun Biu Li (PI)
Satoru Tsugawa
Tamiki Komatsuzaki

Strategy: screening for mutants with variable sepal size or shape in the same flower

Wild type

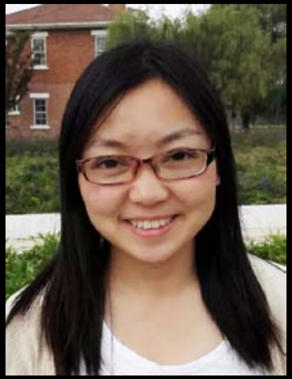
vos1

vos2

vos3

Shape variability

variable organ size and shape mutants



Dr. Lilan Hong

Stories: 3 short stories about variable organ mutants

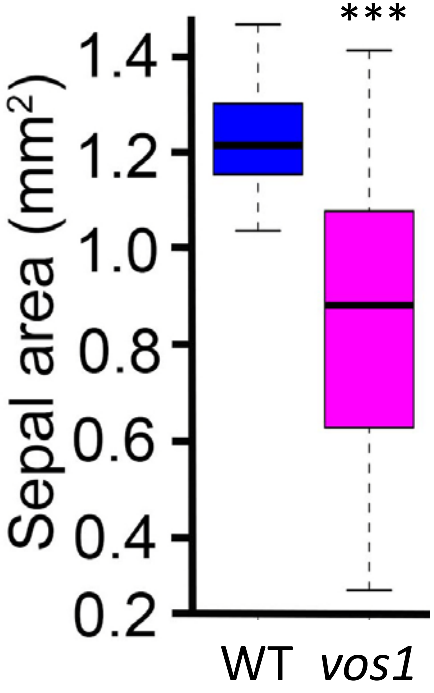
- ***variable organ size1*** (***vos1***) and spatial temporal averaging of noisy cell growth
- ***variable organ size 3*** (***vos3***) and coordination of growth on the front and back of the organ.
- ***variable organ size2*** (***vos2***) and synchrony of organ primordia initiation

A novel mutant has variable sepal size

WT *vos1* WT *vos1*

Hong et al. (2016) Dev Cell

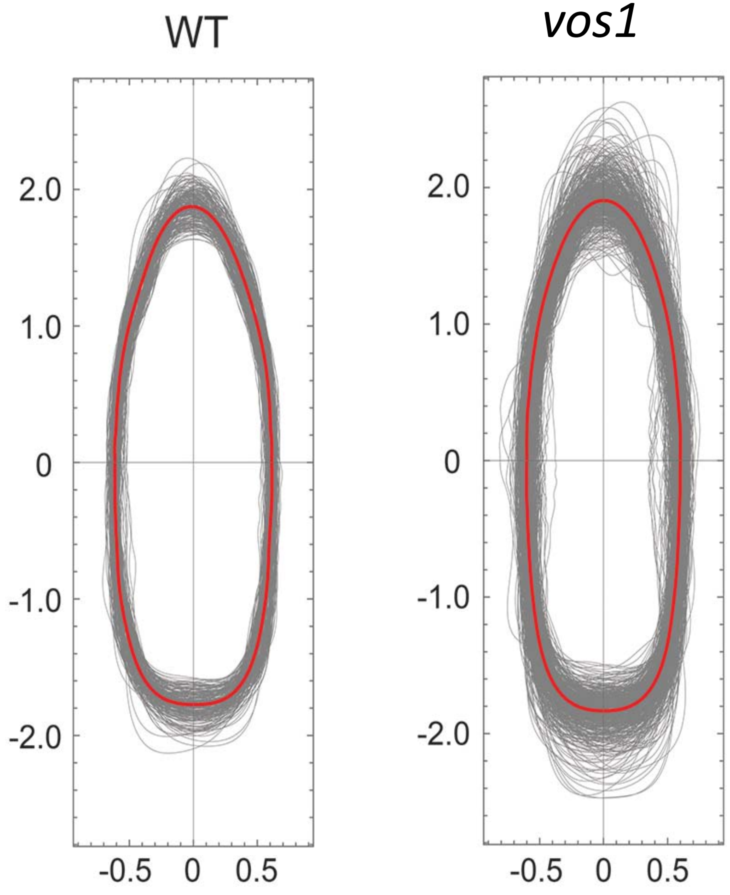
vos1 sepals have larger variance in area



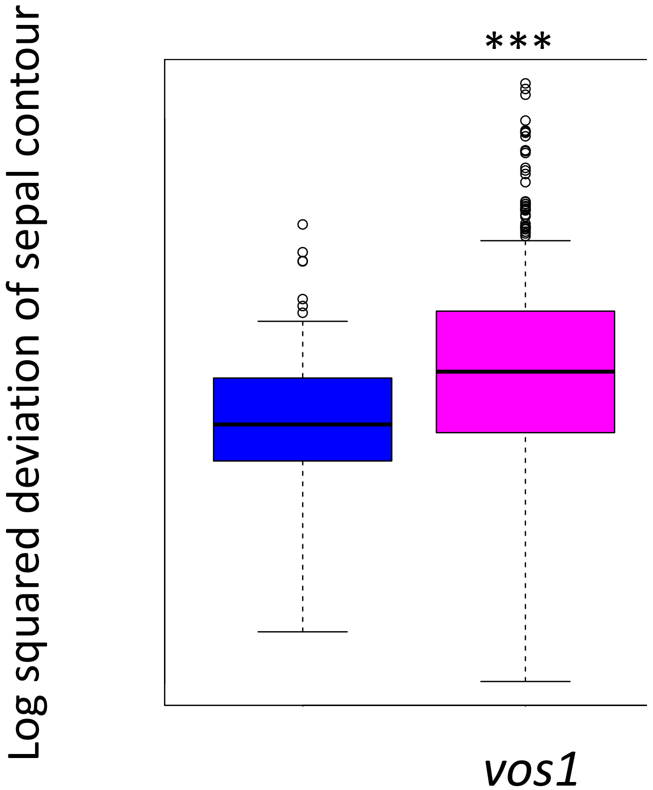
***: significantly different from WT, f test, P<0.001

Hong et al. (2016) Dev Cell

vos1 sepals have larger variance in shape



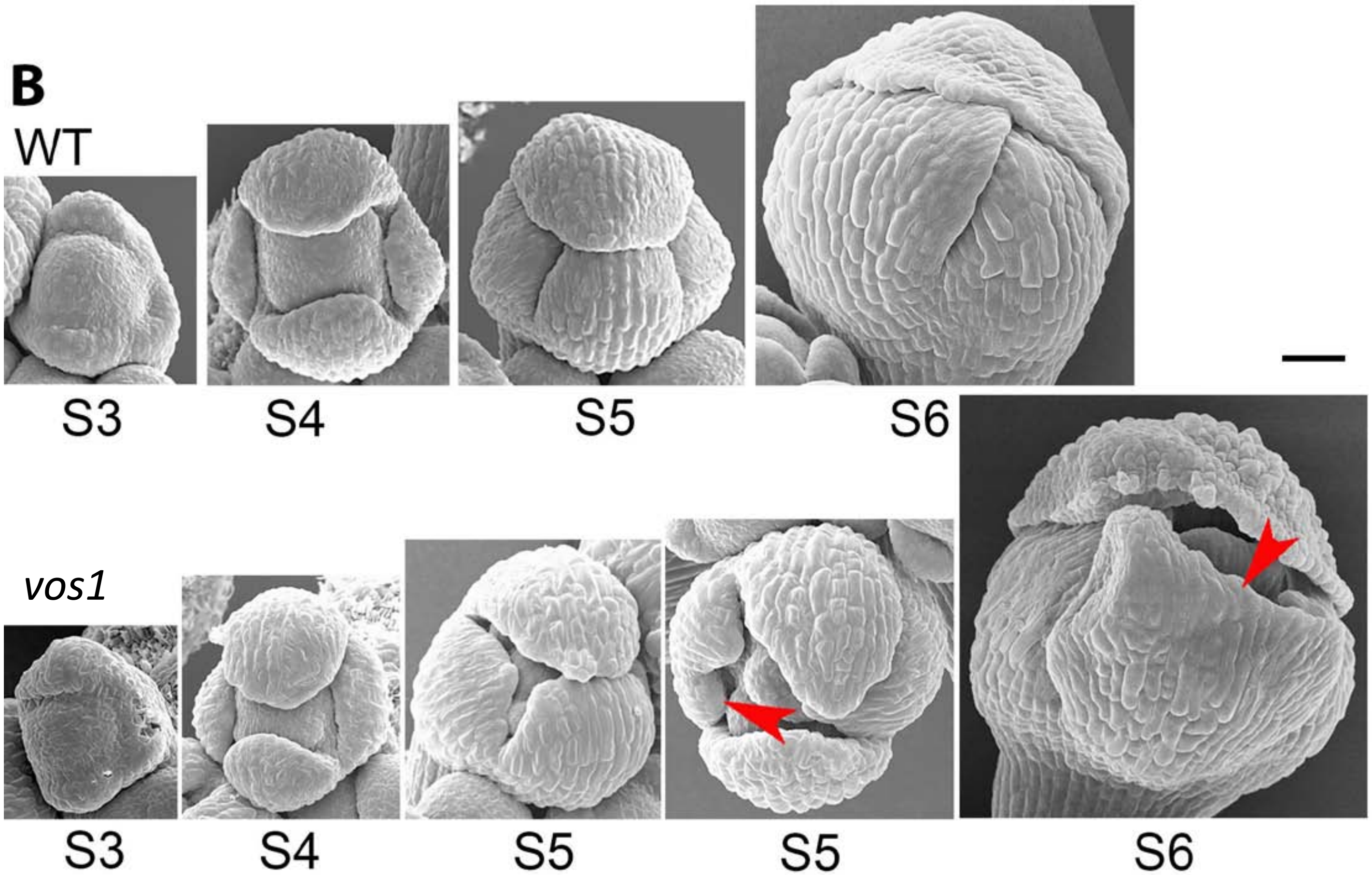
Sepal contours normalized by size



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

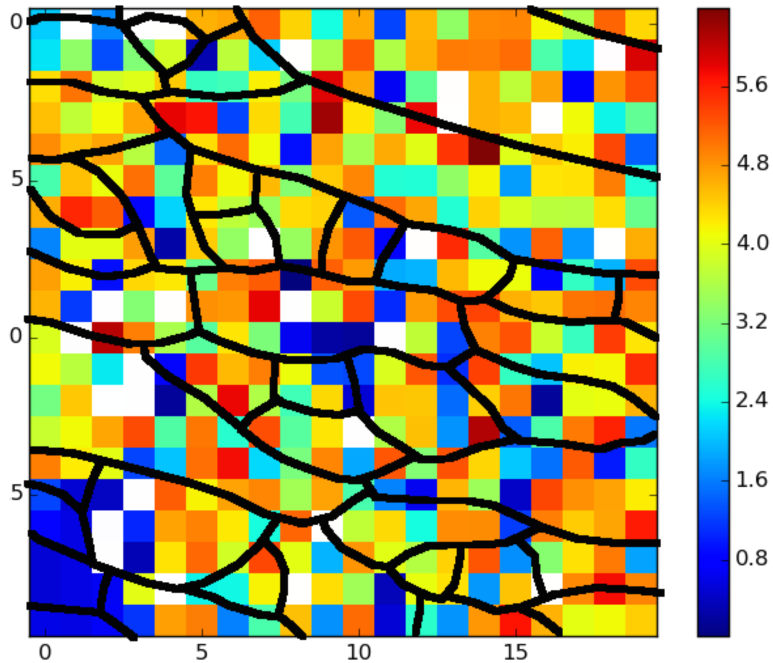
Hong et al. (2016) Dev Cell

vos1 sepal primordia form normally. Variability in sepal size arises during growth.

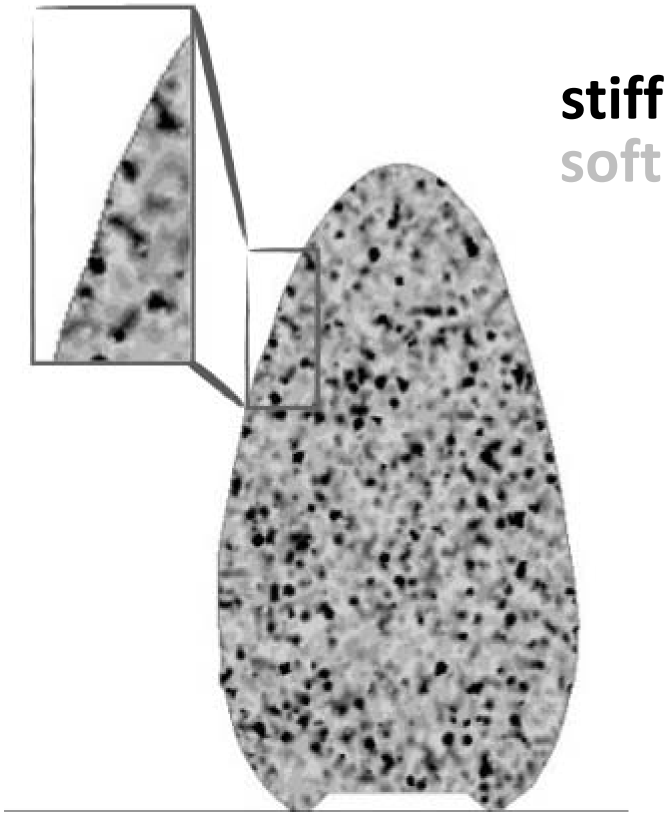


Hong et al. (2016) Dev Cell

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

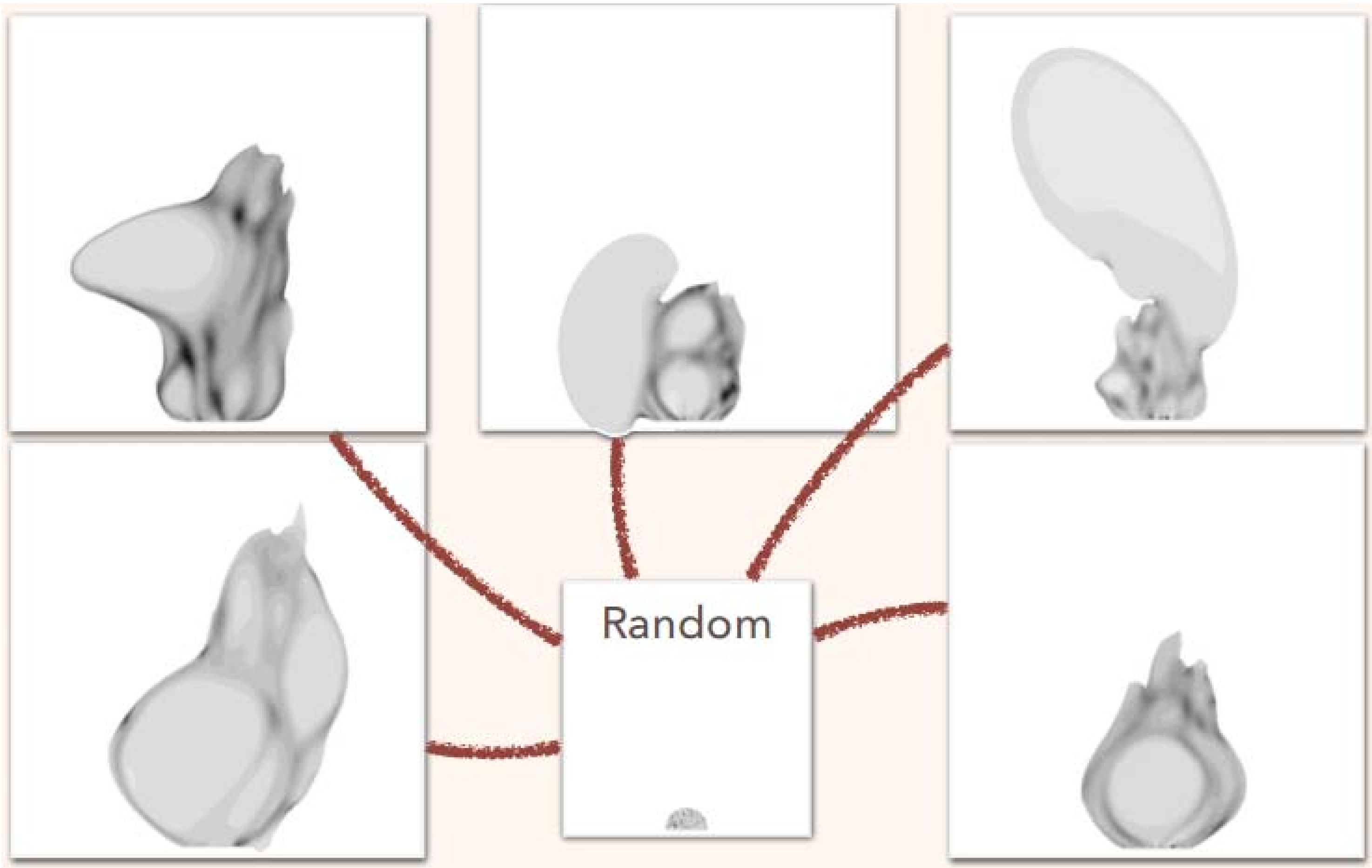
Hong et al. (2016) Dev Cell

Sepal models of variable stiffness in space

Hong et al. (2016) Dev Cell

Arezki Boudaoud & Mathilde Dumond

Variability in space leads to misshapen sepals



Hong et al. (2016) Dev Cell

Arezki Boudaoud & Mathilde Dumond

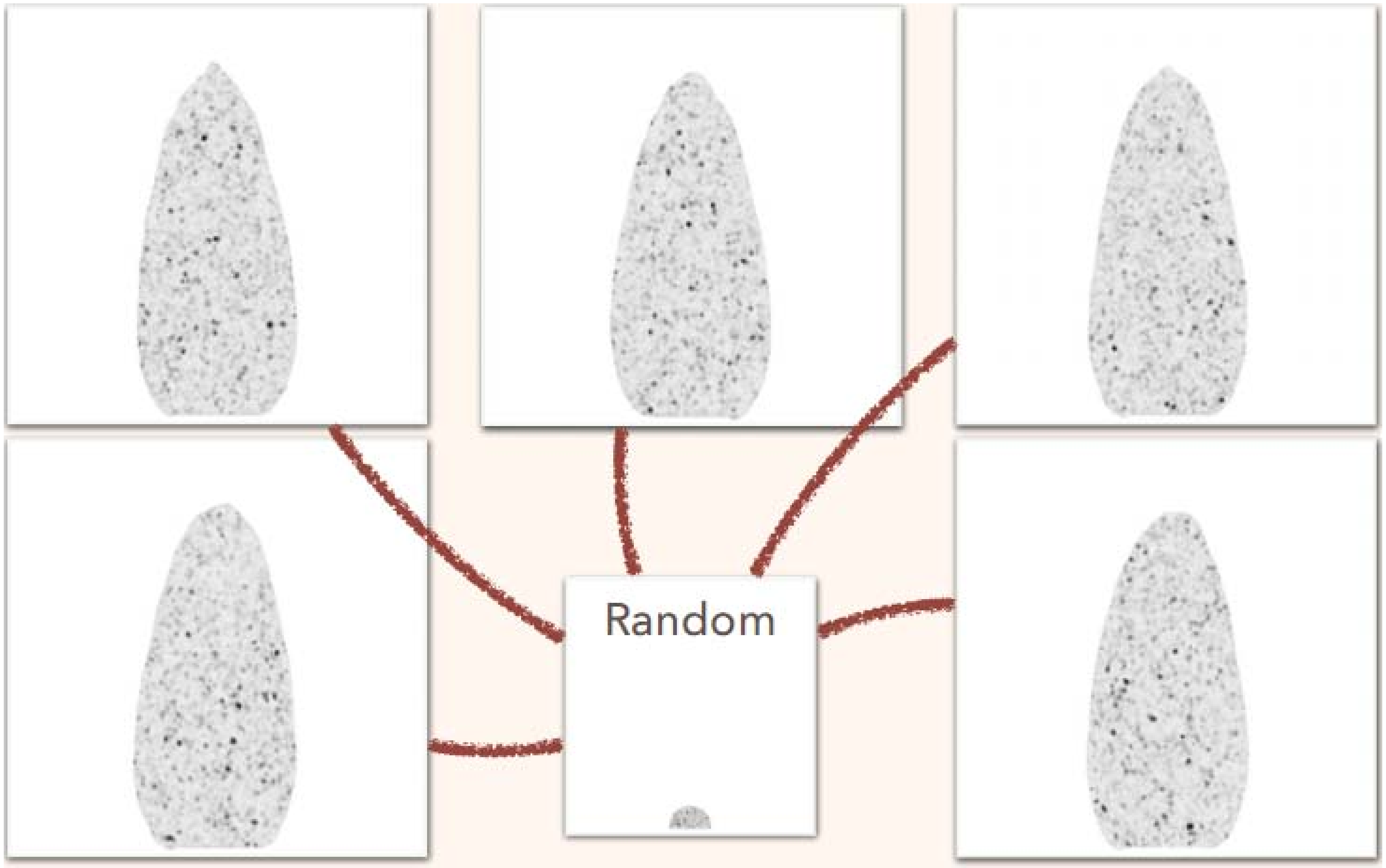
Sepal models of variable stiffness in space and time

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 → ? → Organ regularity

In a virtual sepal

Cellular variability in space

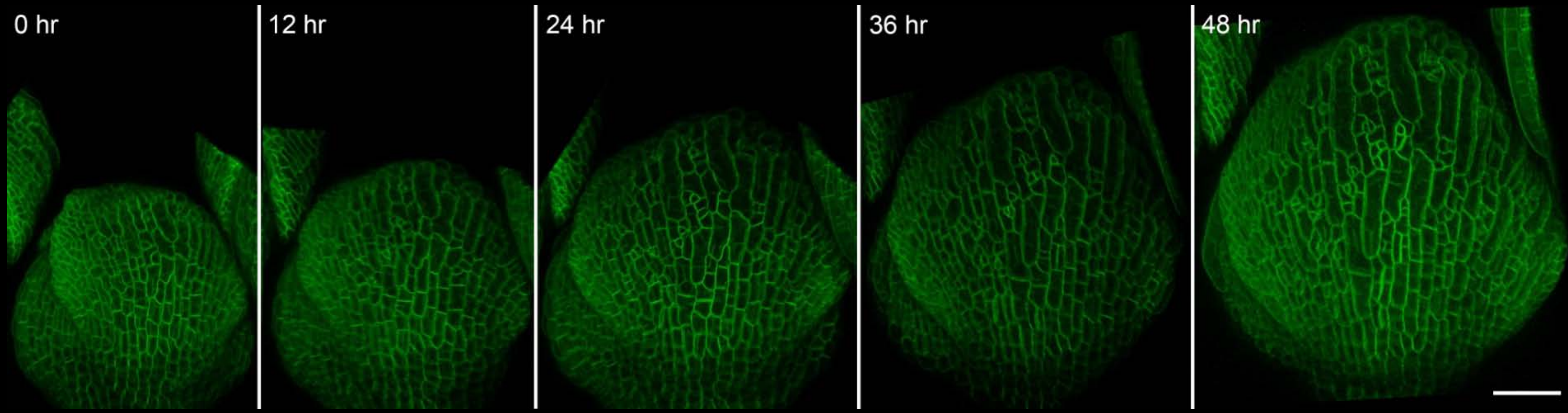
\+ → Regular organ morphology

Cellular variability in time

In a real sepal?

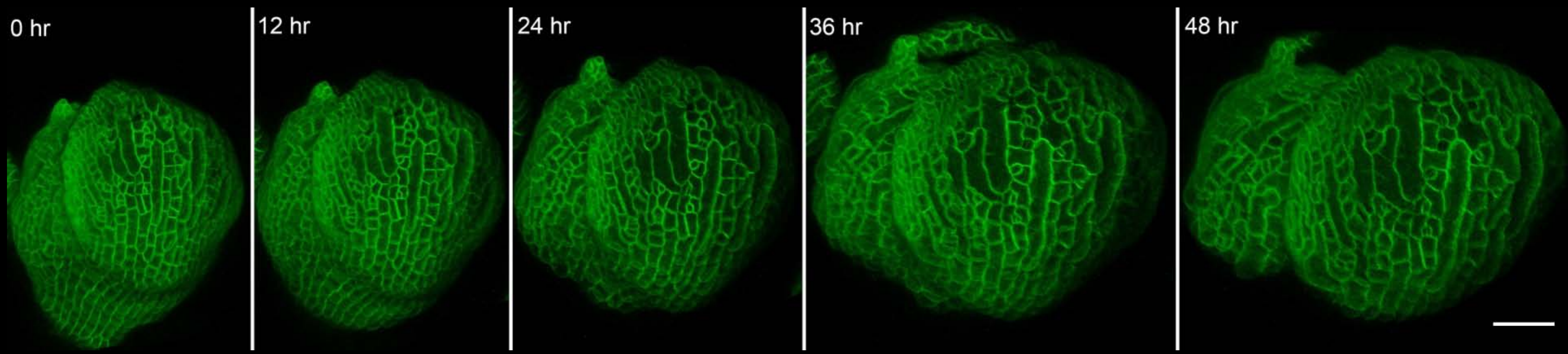
Live imaging of the sepals

Wild type



Stage 8-1 Stage 8-2 Stage 9-1 Stage 9-2 Stage 9-3

vos1

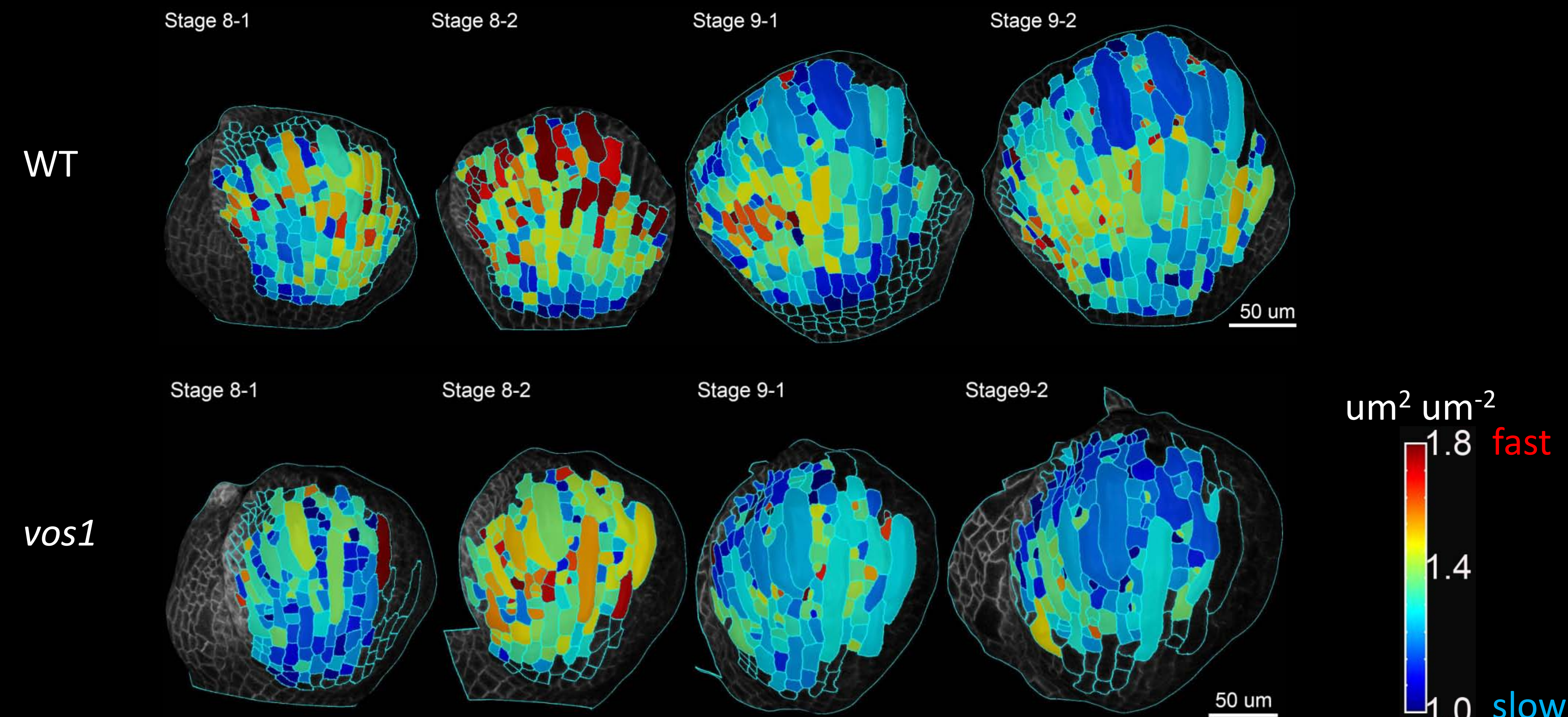


Stage 8-1 Stage 8-2 Stage 9-1 Stage 9-2 Stage 9-3

Hong et al. (2016) Dev Cell

Sepals have variable cellular growth

Areal growth rate

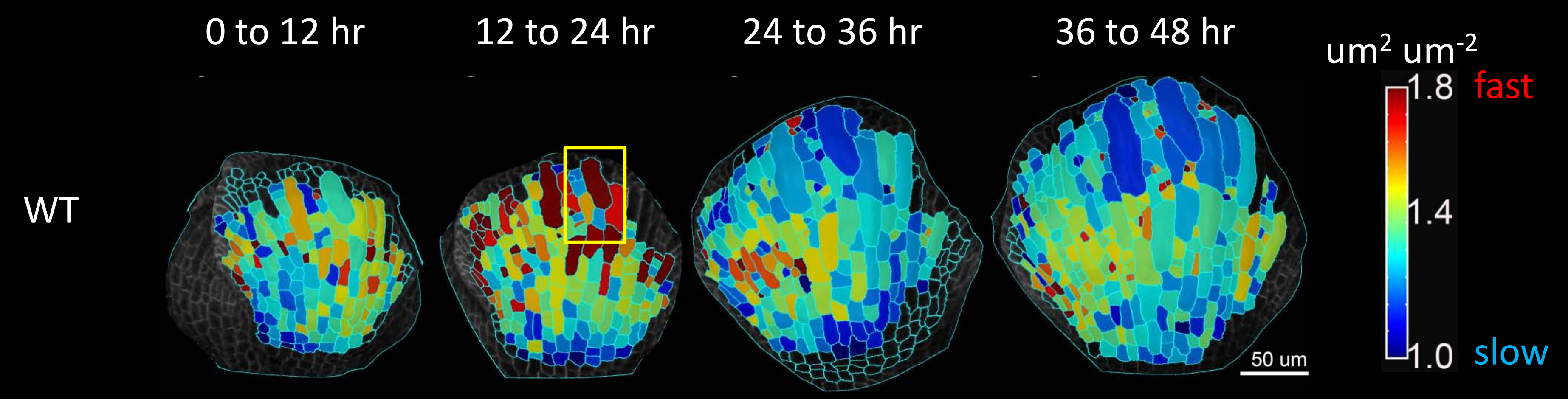


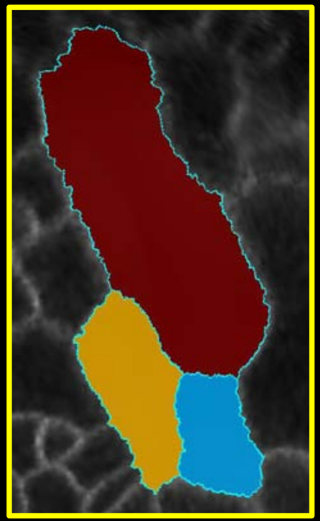
12 hr growth mapped on the beginning time point

Hong et al. (2016) Dev Cell

Sepals have cellular growth variability in space

Areal growth rate

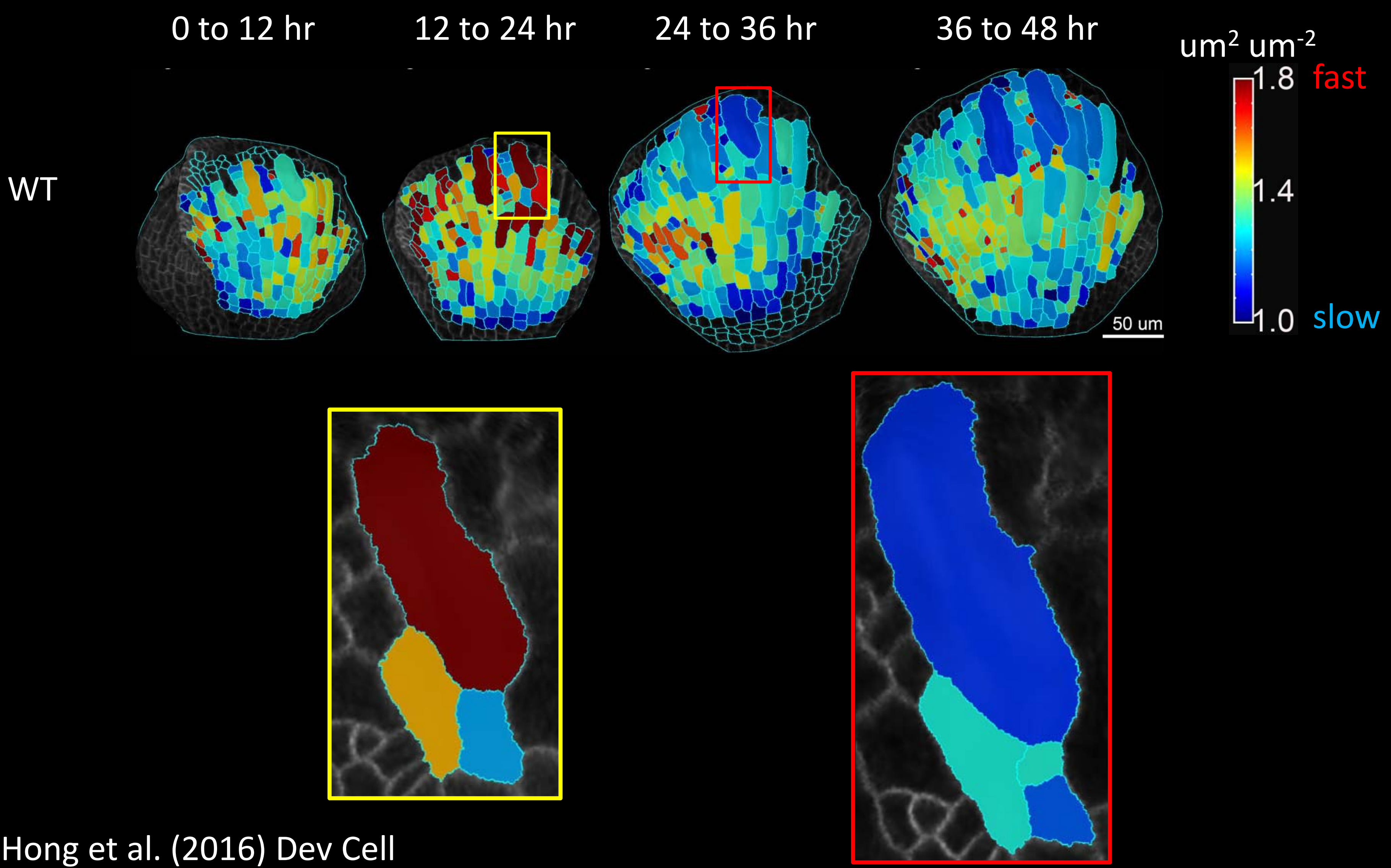




Hong et al. (2016) Dev Cell

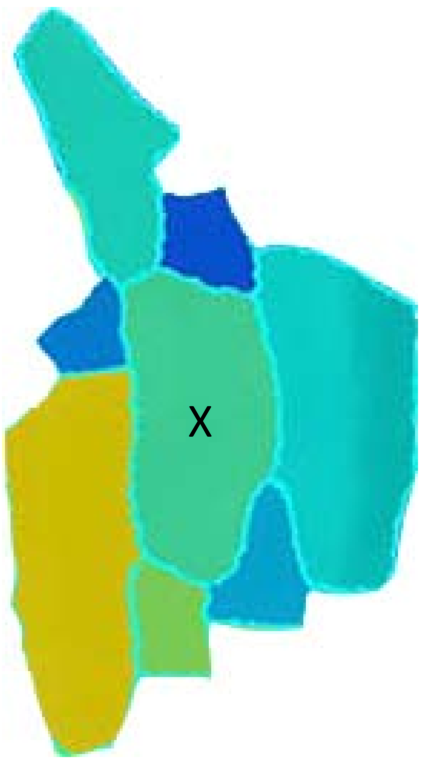
Sepals have cellular growth variability in time

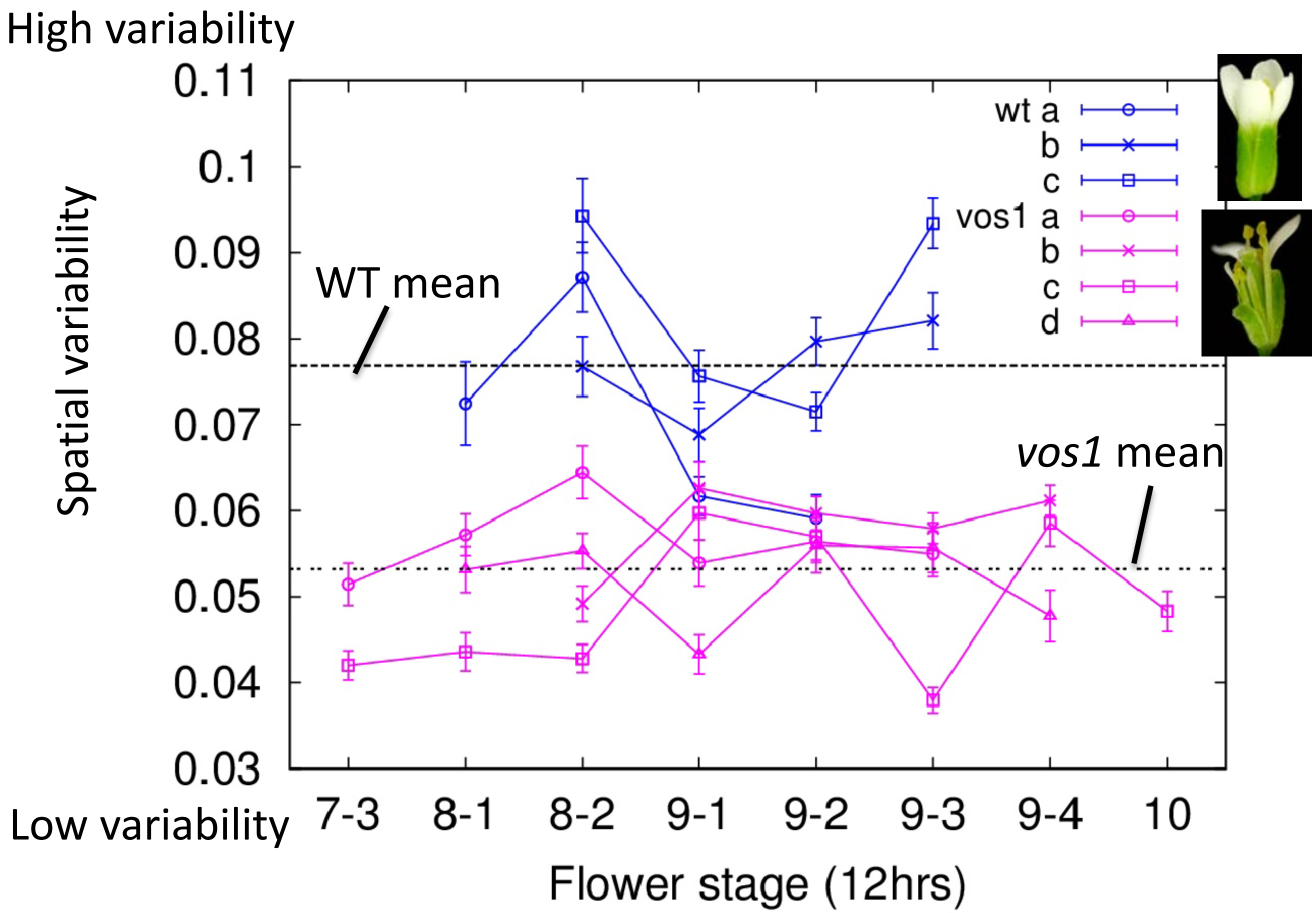
Areal growth rate



Hong et al. (2016) Dev Cell

Cell variability in space is reduced in *vos1*





Satoru Tsugawa
Hong et al. (2016) Dev Cell

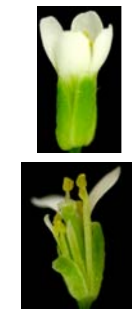
Error bars: SD.

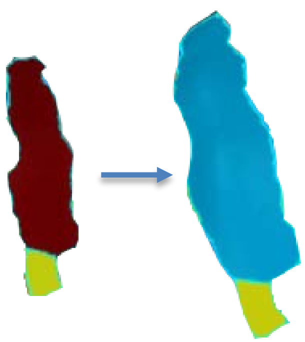
Cell growth variability in time is similar between WT and *vos1*

High variability

WT

vos1





Temporal variability

7-2

Low variability

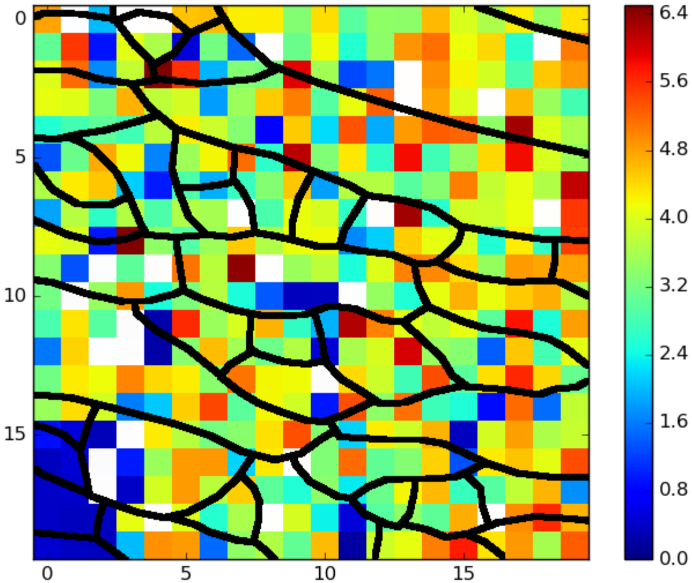
Satoru Tsugawa

Hong et al. (2016) Dev Cell

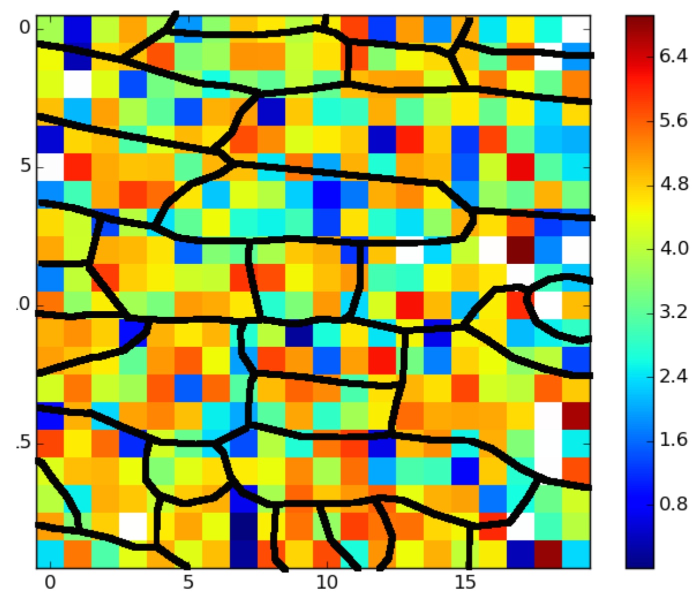
Error bars: SD.

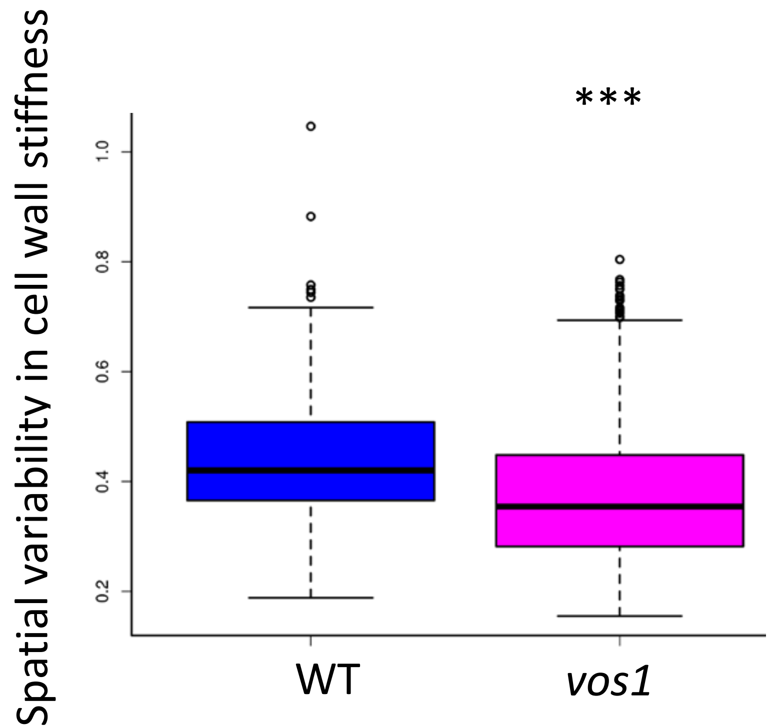
vos1 has low variability in space of cell wall stiffness

WT



vos1





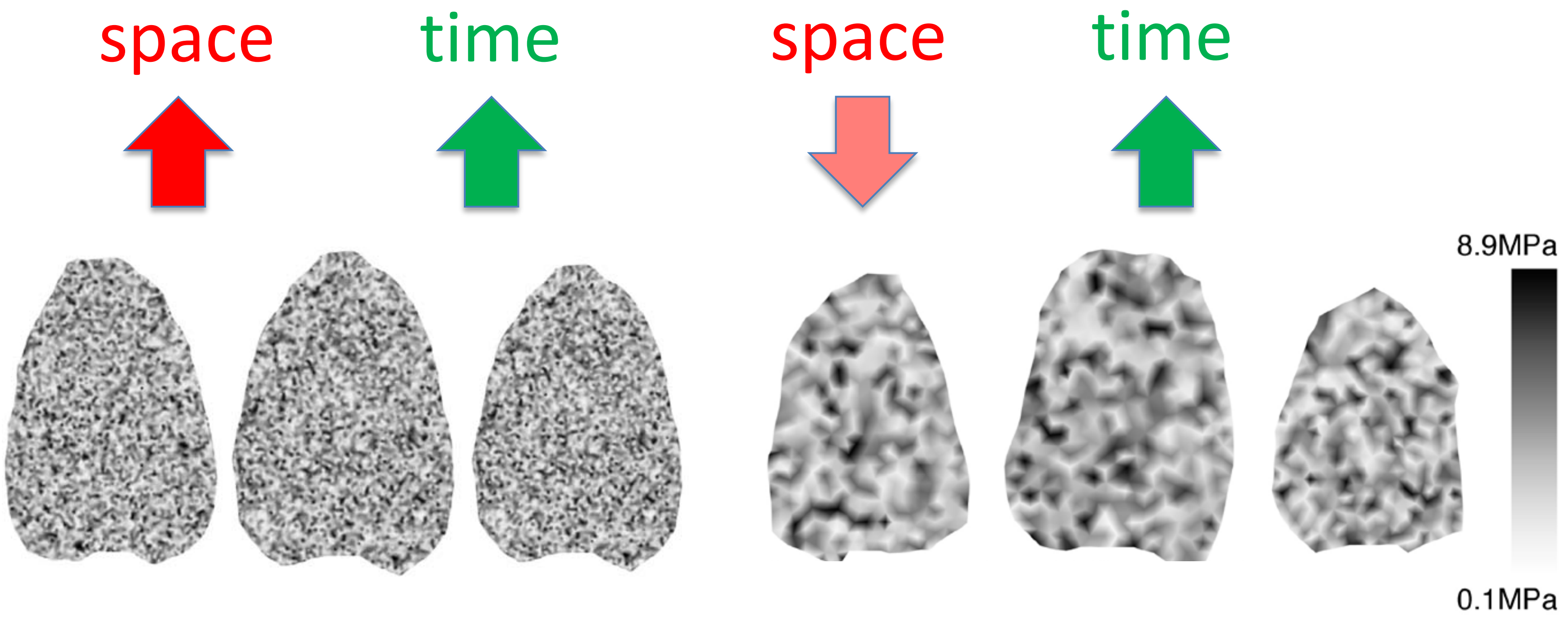
***: significantly different from WT, t test, P<0.001

Hong et al. (2016) Dev Cell

Can decreasing cell variability in space cause development of irregular sepals?

	Cell variability in			Sepal	
	space	time			
Wild type	↑	↑	→	regular	
vos1	↓	↑	- - ? - →	irregular	

Decreasing variability in space leads to irregular sepals in the model



Hong et al. (2016) Dev Cell

How does cell variability give rise to regular organ morphology?

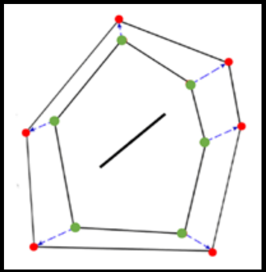
Cellular variability in space

+ ? → Regular organ morphology

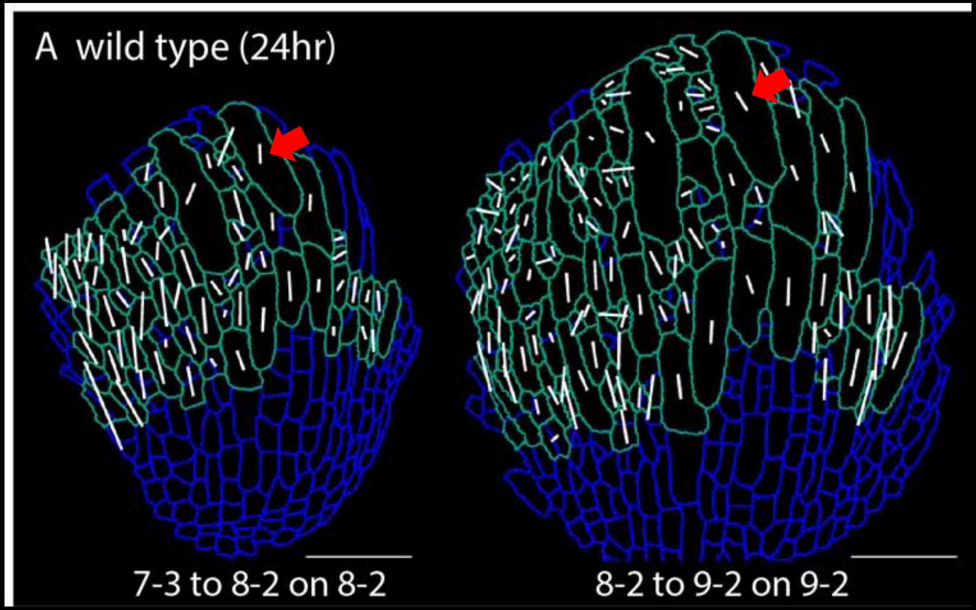
Cellular variability in time

Spatiotemporal averaging of cellular variability

Principal direction of growth (PDG)



0-24 hours | 24-48 hours | cumulative

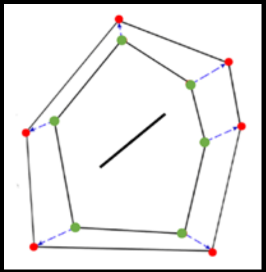


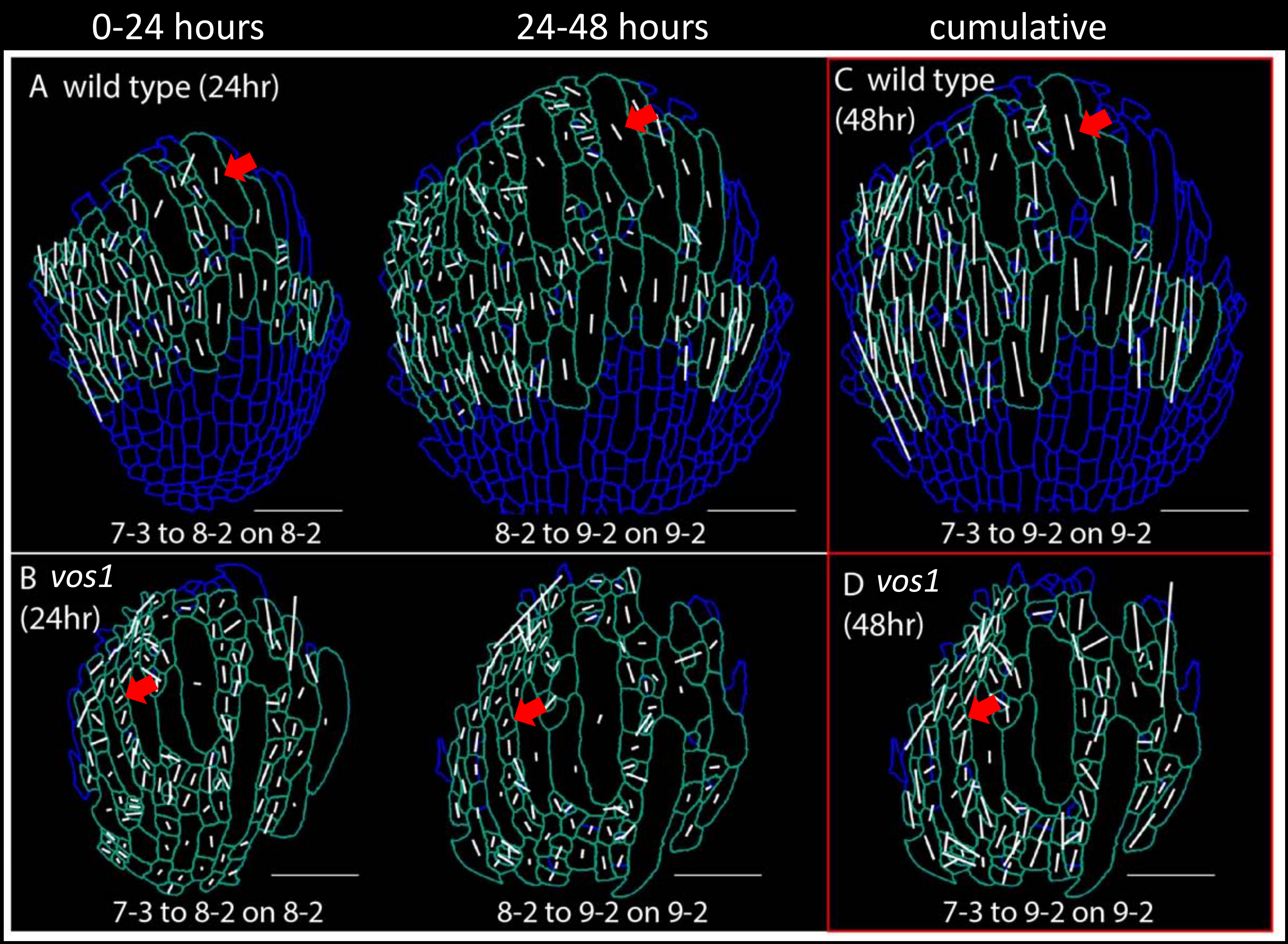
Cellular variability —spatiotemporal averaging→ **Organ regularity**

Hong et al. (2016) Dev Cell

Spatiotemporal averaging of cellular variability is defective in *vos1*

Principal direction of growth (PDG)

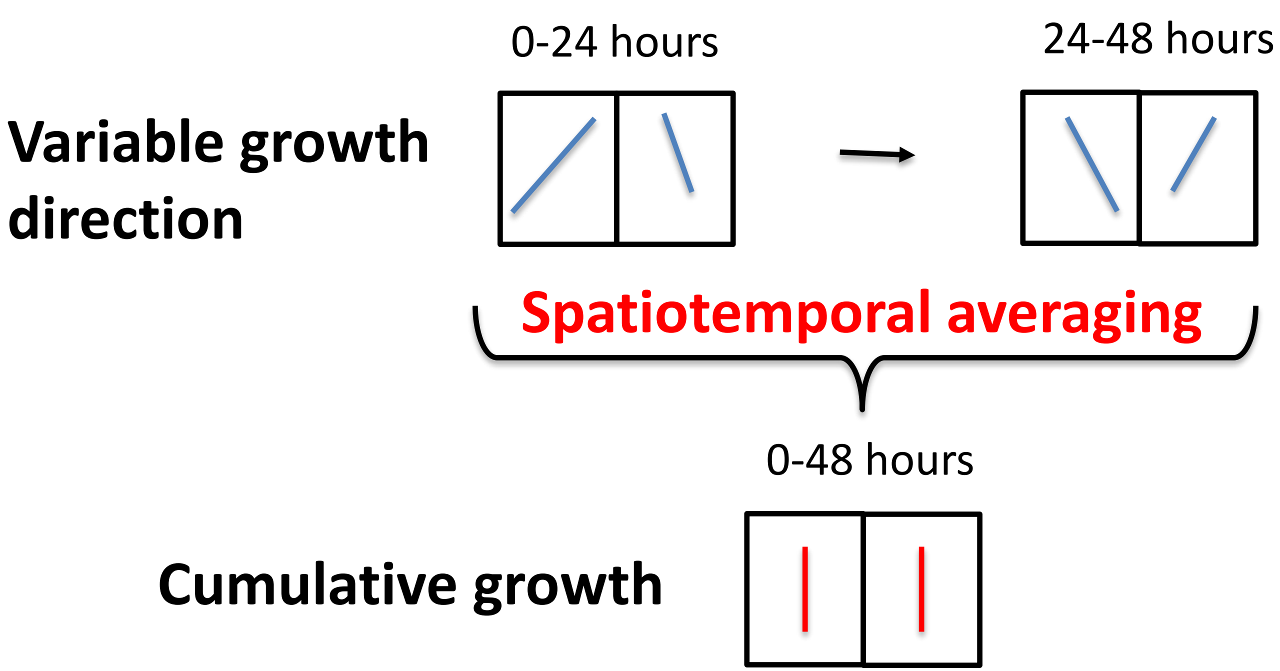




Hong et al. (2016) Dev Cell

Conclusion :
Variability produces regularity

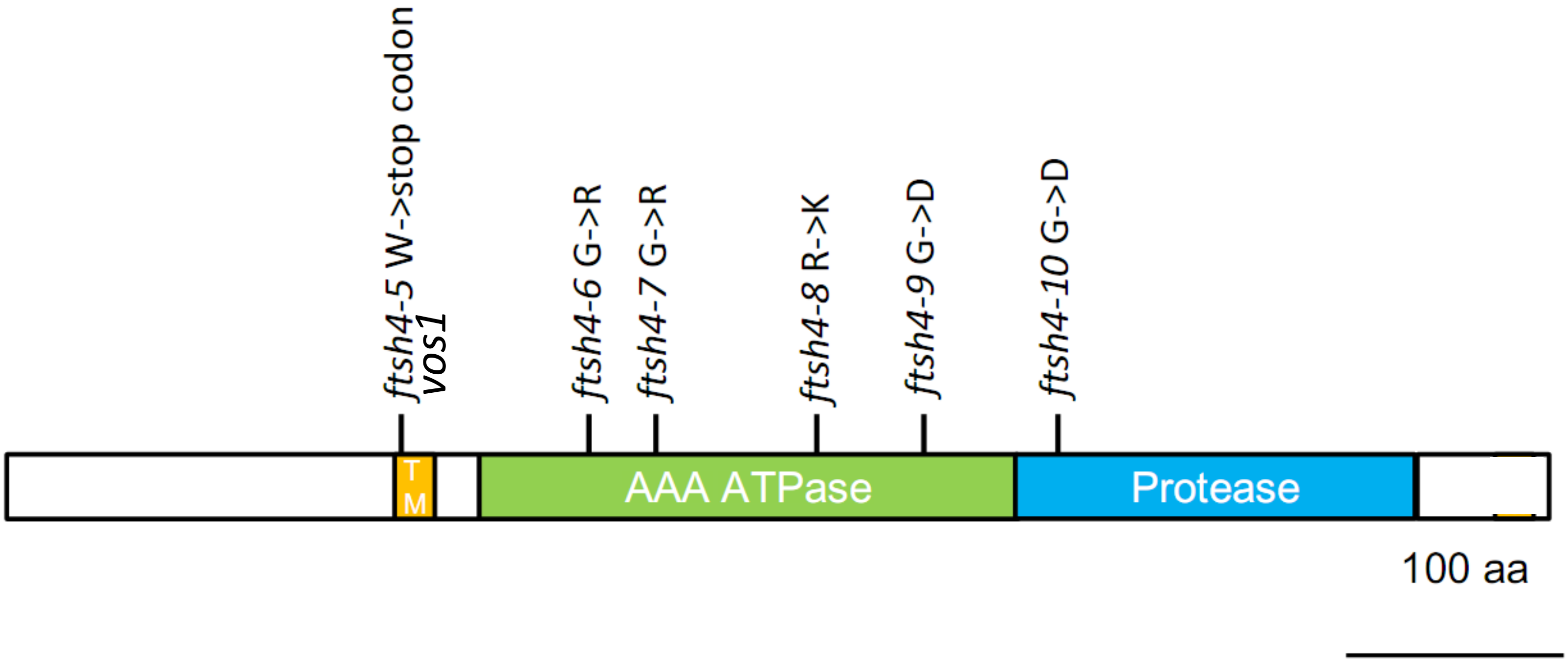
Cellular variability —spatiotemporal averaging→ **Organ regularity**

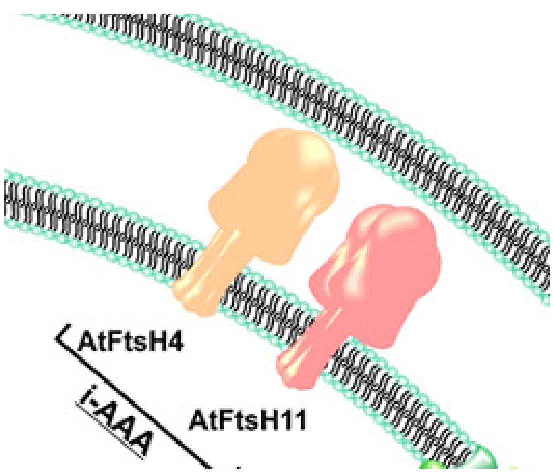


Hong et al. (2016) Dev Cell

Why is cellular variability reduced and spatiotemporal averaging blocked in *vos1* mutants?

Mutation of *FtsH4* causes the *vos1* phenotype





Mitochondrion

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

FtsH4 maintains protein quality in mitochondria.

Annu. Rev. Microbiol. 2005. 59:211–31

Hong et al. (2016) Dev Cell

Hypotheses

vos1 (FtsH4) mutation

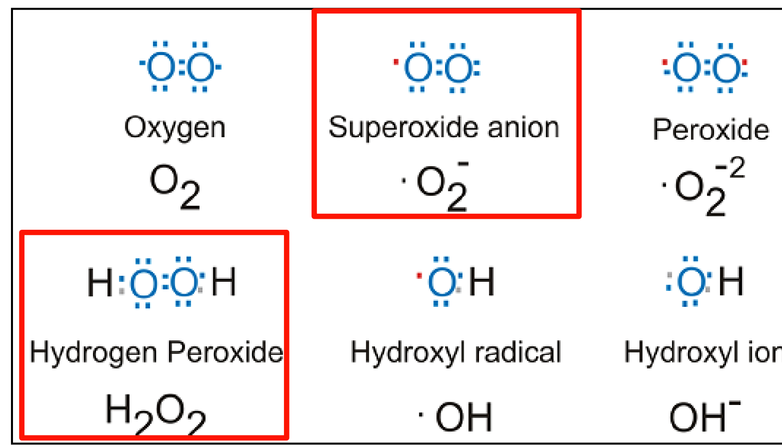
↓

Mitochondrial defects

↓

ROS ↑

ROS: reactive oxygen species



↓

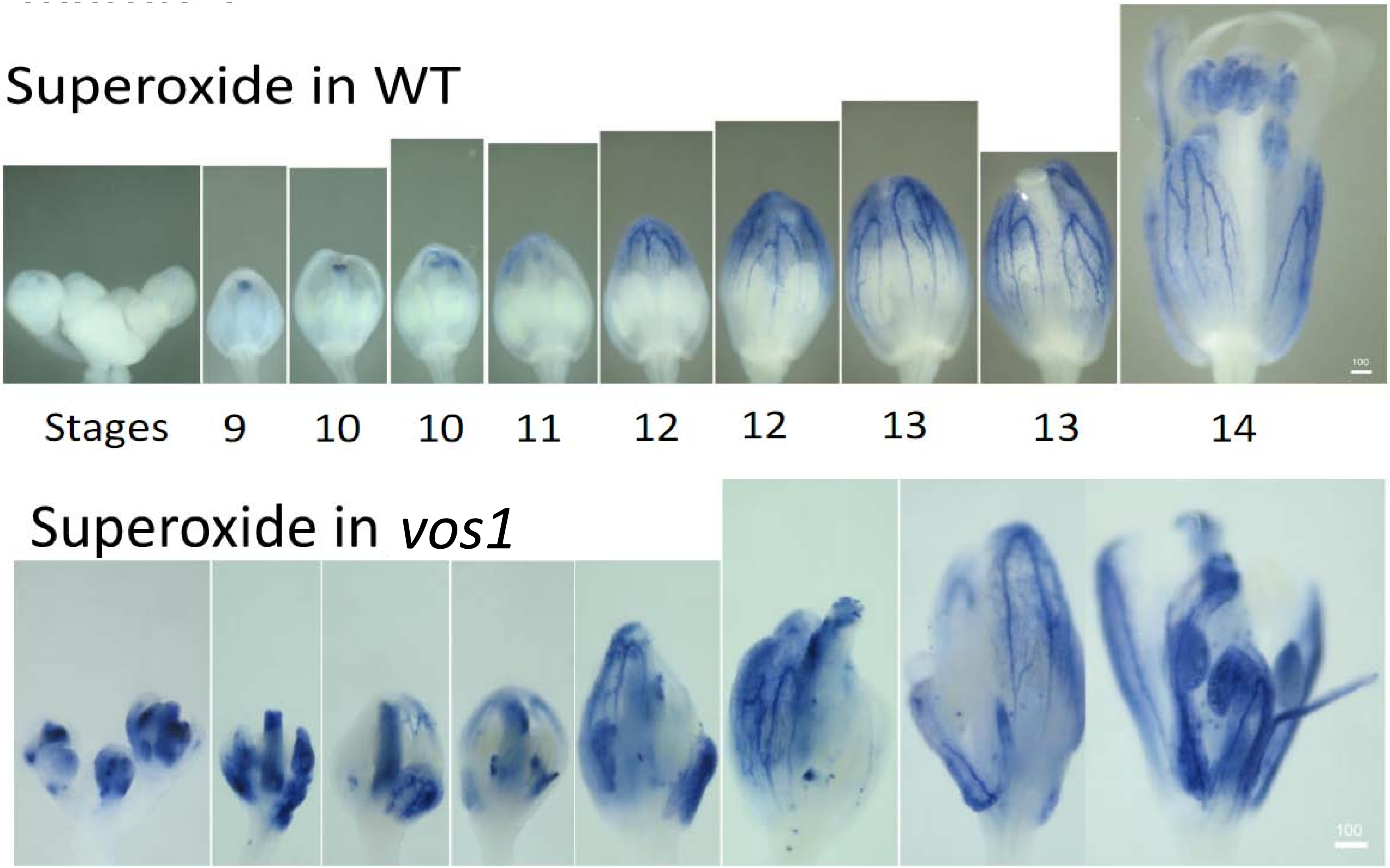
Reduced spatiotemporal averaging

↓

Variable sepal size and shape

Hong et al. (2016) Dev Cell

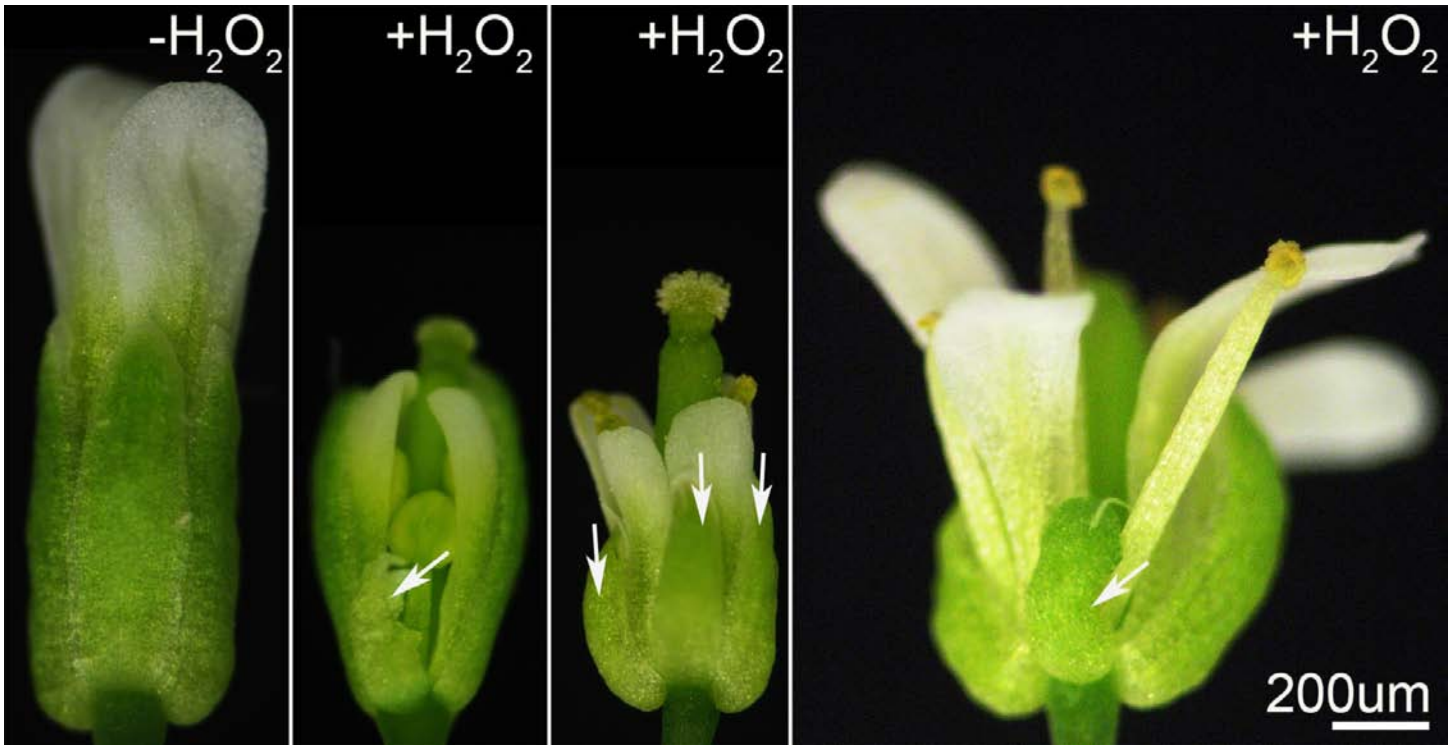
vos1 sepals have increased superoxide



NBT staining detects superoxide.

Hong et al. (2016) Dev Cell

H_2O_2 treatment of WT mimics *vos1* phenotype



Arrows show smaller sepals

Hong et al. (2016) Dev Cell

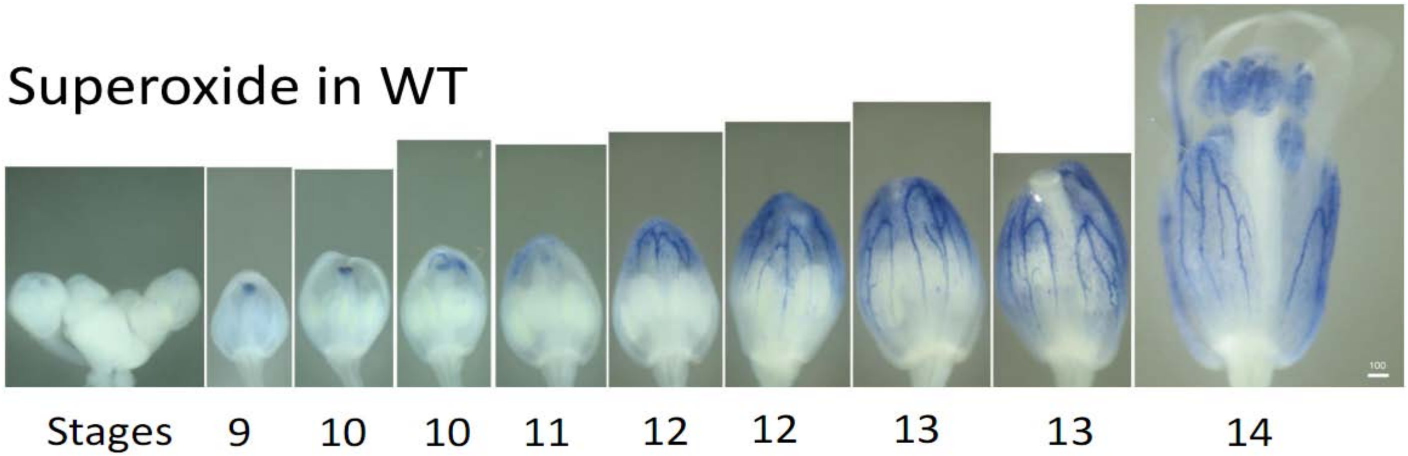
Decreasing the ROS level in *vos1* rescues the variable sepal size phenotype

WT *vos1* *vos1*+35S::*CAT2*

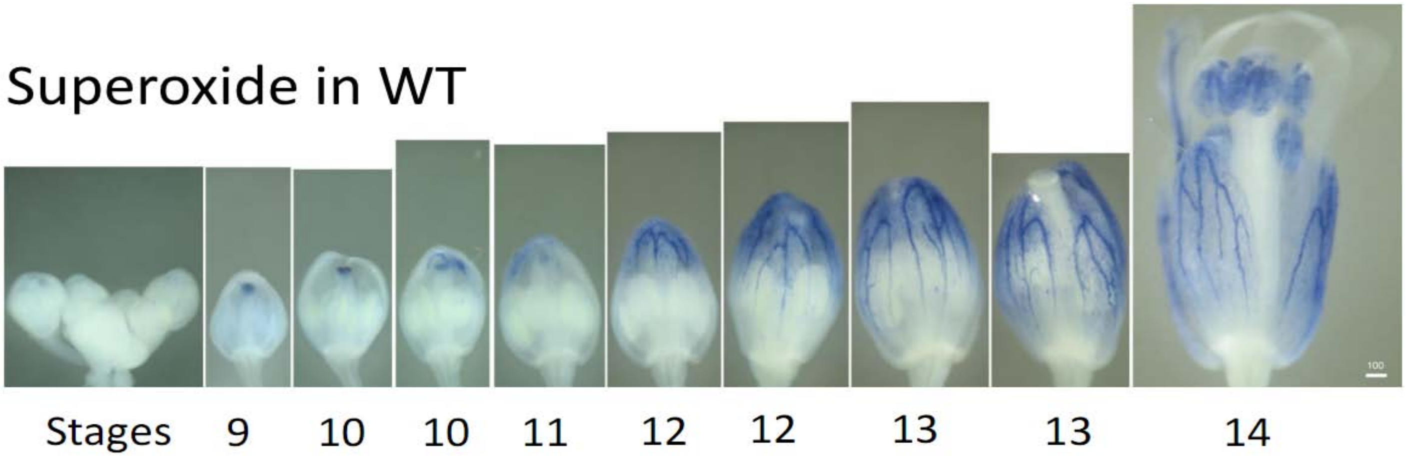
CAT2: CATALASE2, degrading H_2O_2

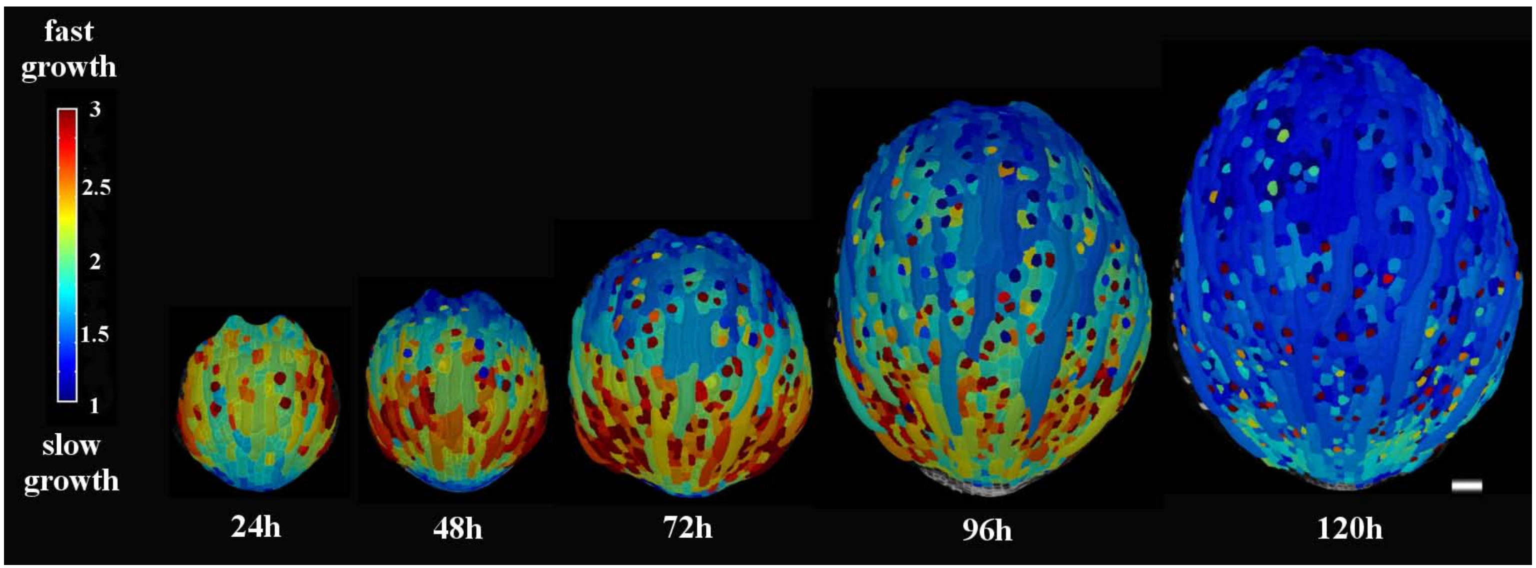
Hong et al. (2016) Dev Cell

Do ROS also regulate wild-type sepal growth?



ROS accumulation from the tip coincides with sepal maturation

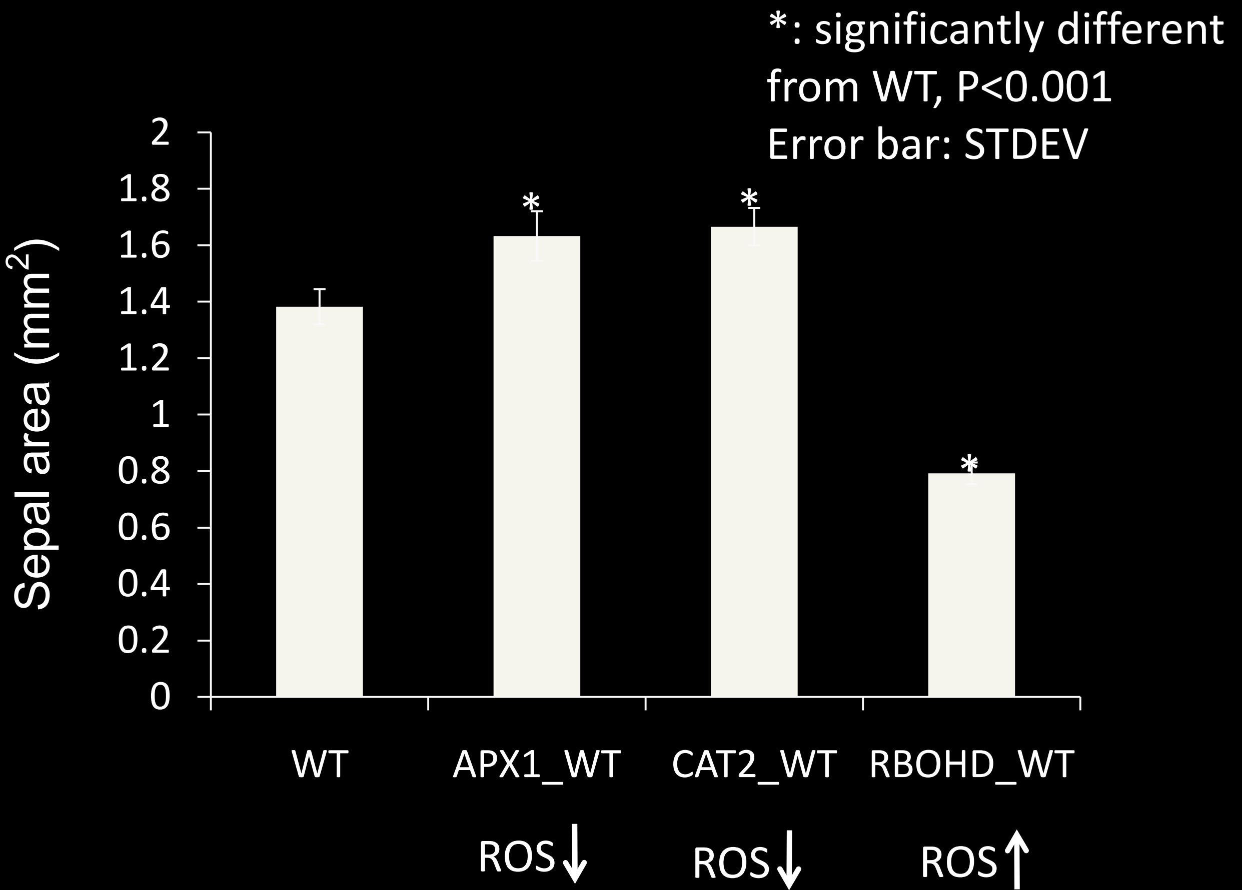




Hervieux et al. (2016) Current Biology

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

Hong et al. (2016) Dev Cell

Conclusion Part 1 :
Variability produces regularity

Cellular variability —spatiotemporal averaging→ **Organ regularity**

ROS inhibit spatiotemporal averaging.

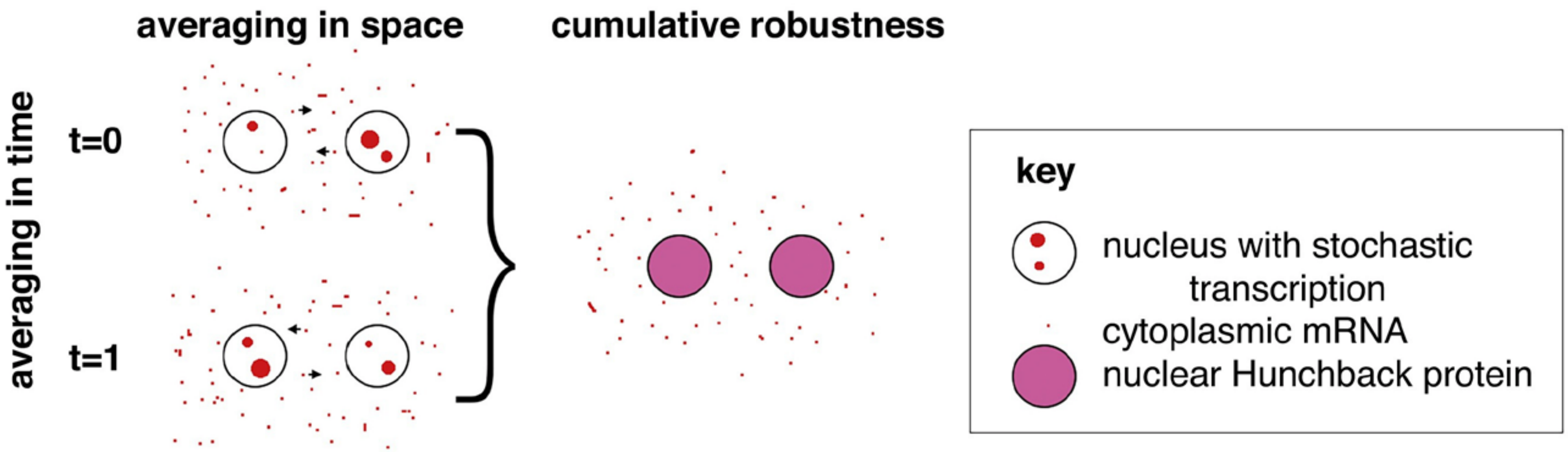
How?

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

What determines when ROS are produced?

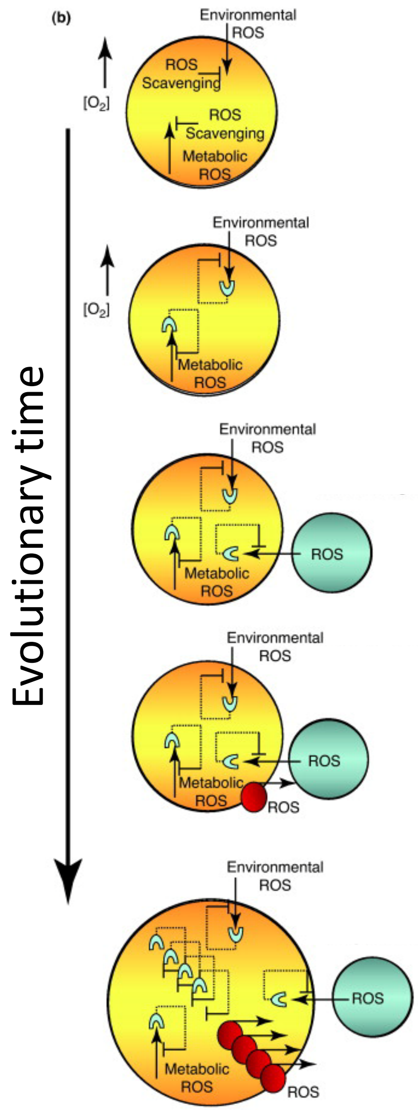
Is spatiotemporal averaging a general developmental principle for handling variability?

Spatiotemporal averaging of stochastic *Hunchback* expression in *Drosophila*



Based on Little et al., (2013) Cell

ROS as an ancestral signal???



TRENDS in Plant Science

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

Mittler R, Vanderauwera S, Suzuki N, et al. ROS signaling: the new wave? *Trends Plant Sci*. 2011;16(6):300-309. doi:10.1016/j.tplants.2011.03.007.

Stories: 3 short stories about variable organ mutants

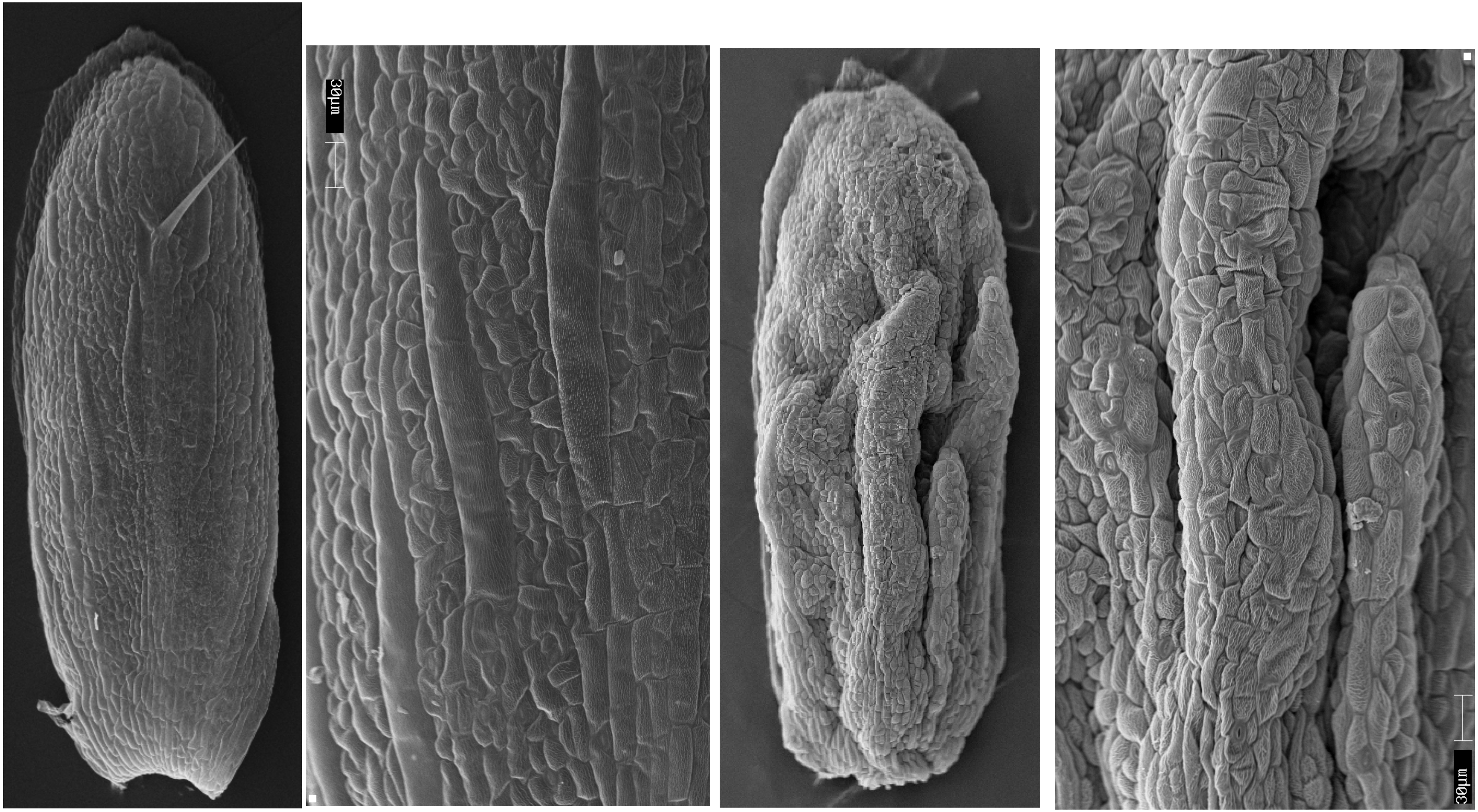
- ***variable organ size1*** (***vos1***) and spatial temporal averaging of noisy cell growth
- ***variable organ size 3*** (***vos3***) and coordination of growth on the front and back of the organ.
- ***variable organ size2*** (***vos2***) and synchrony of organ primordia initiation

The *vos3* mutation generates variability in 3D sepal shape

WT (Ler) *vos3*

Scale bars: 1 mm

vos3 has a ruffled sepal surface



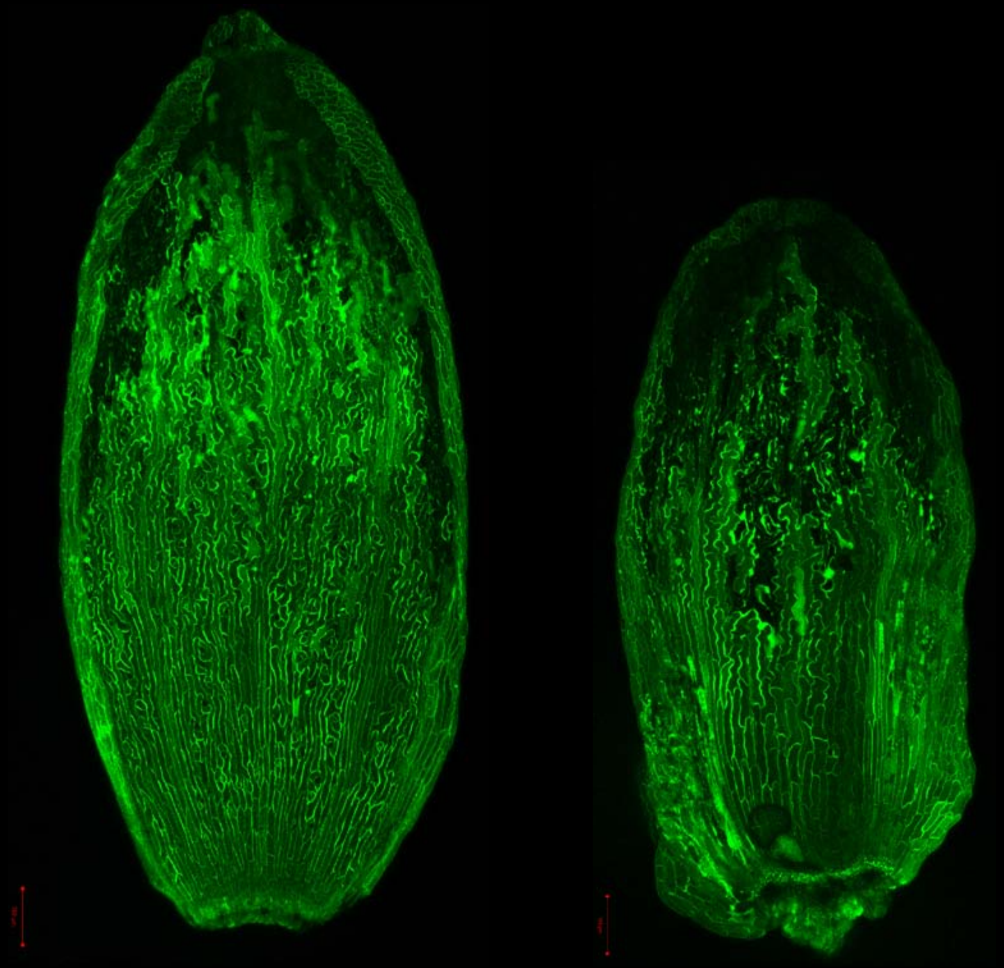
WT (Ler) *vos3*

SEM images; not with the same scale.

vos3 has a variably ruffled sepal surface

vos3

vos3 has a smooth inner sepal epidermis

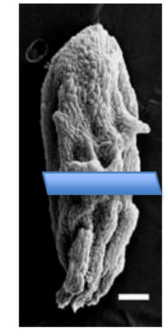


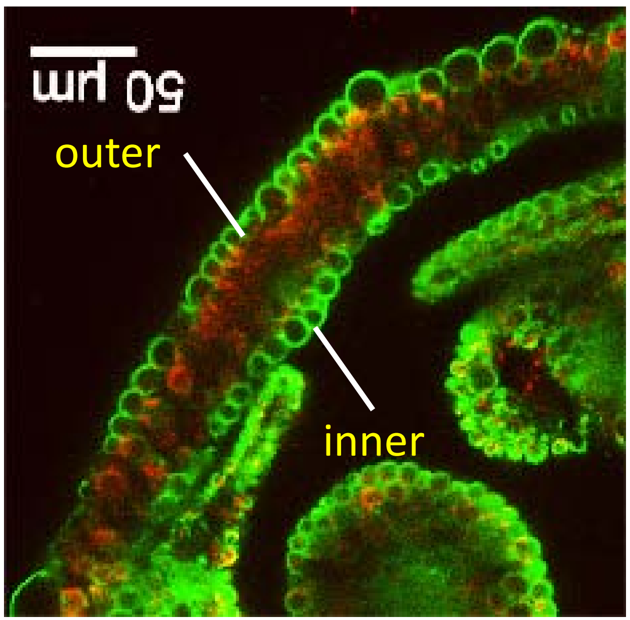
WT (Ler) *vos3*

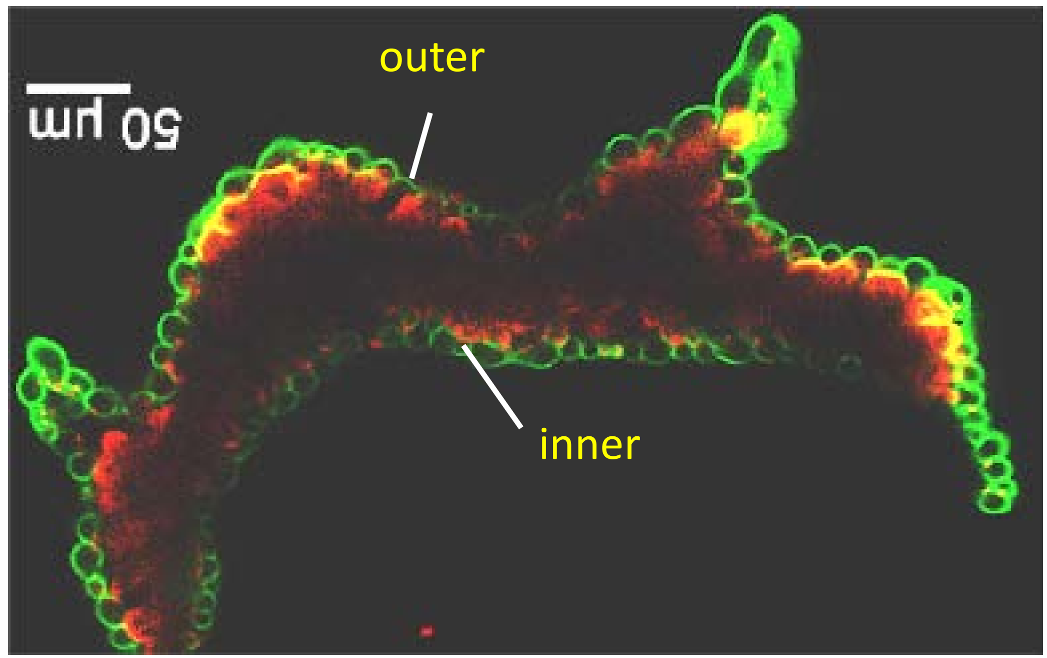
vos3 sepals have folds in the outer epidermis

WT

vos3







Chlorophyll

mCitrine-RCI2A: plasma membrane marker

Scale bars: 50 µm

vos3 forms up-curved leaves

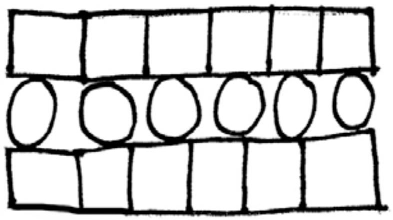
WT (Ler)

vos3

How do the folds arise on the *vos3* sepal?

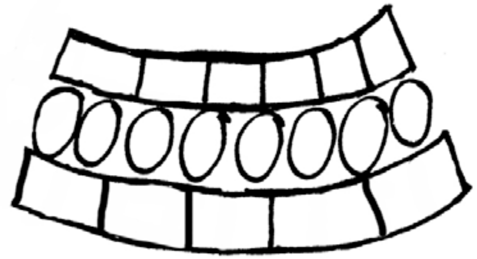
Proposal: an imbalance of growth disrupts the planar sepal growth

Top/
inner

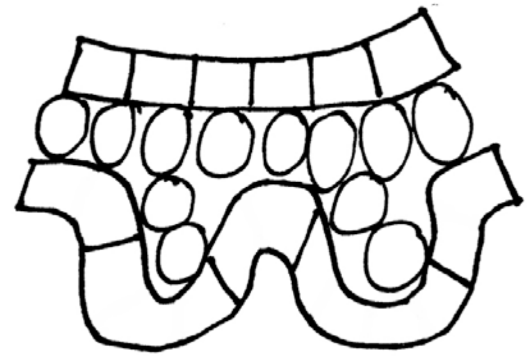


Bottom/
outer

WT leaves/sepals

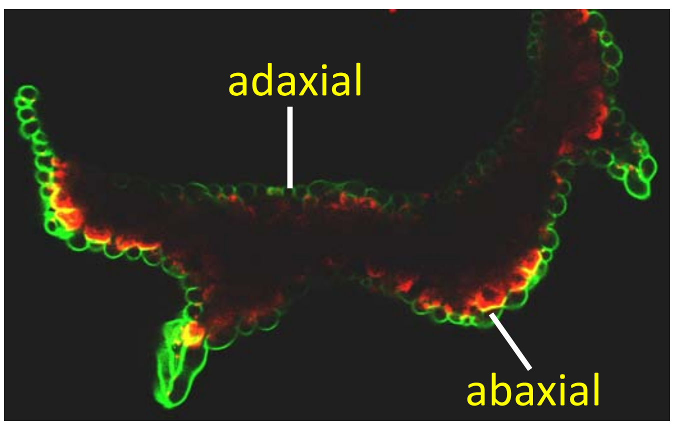


vos3 leaves

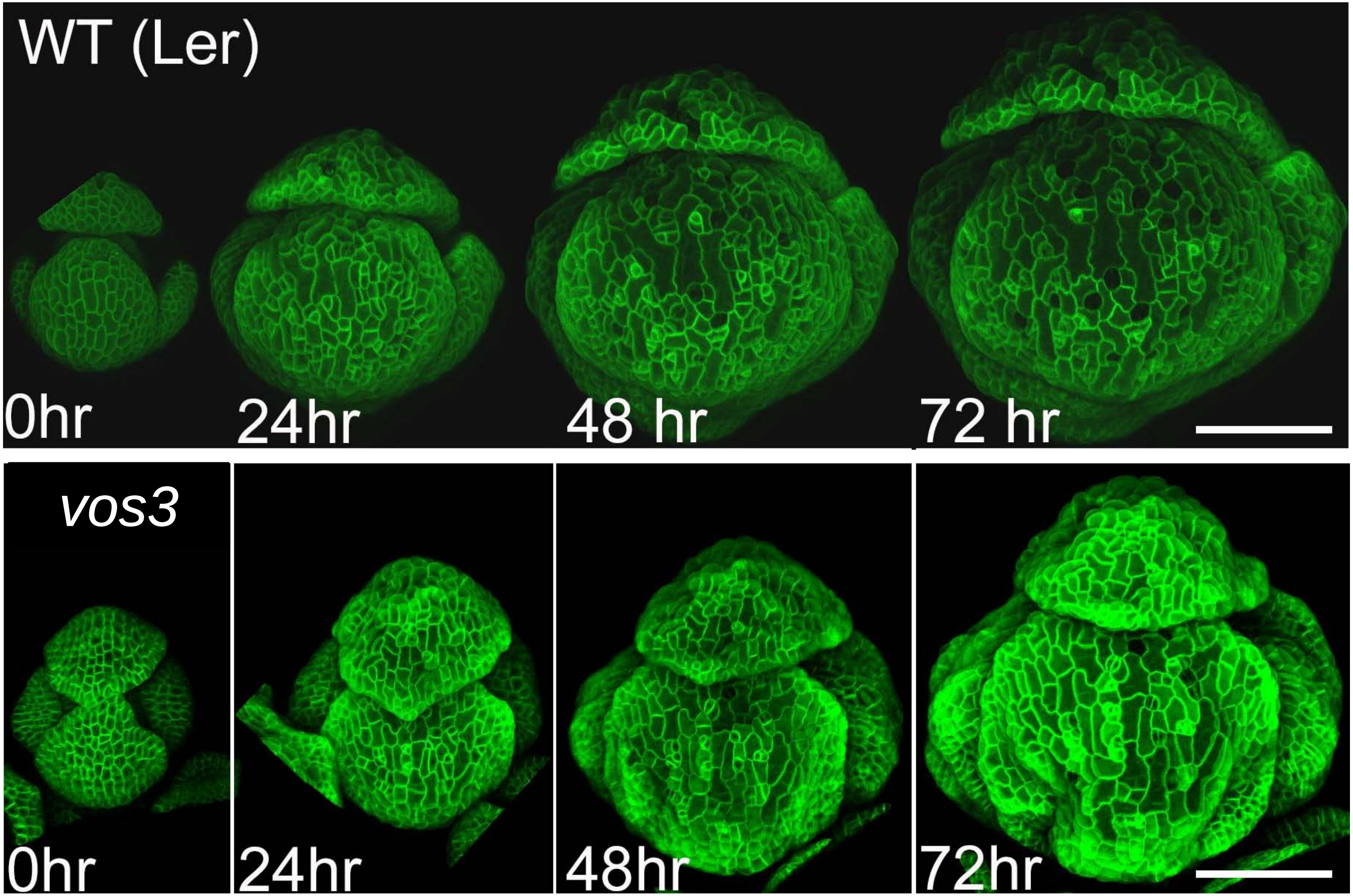


vos3 sepals

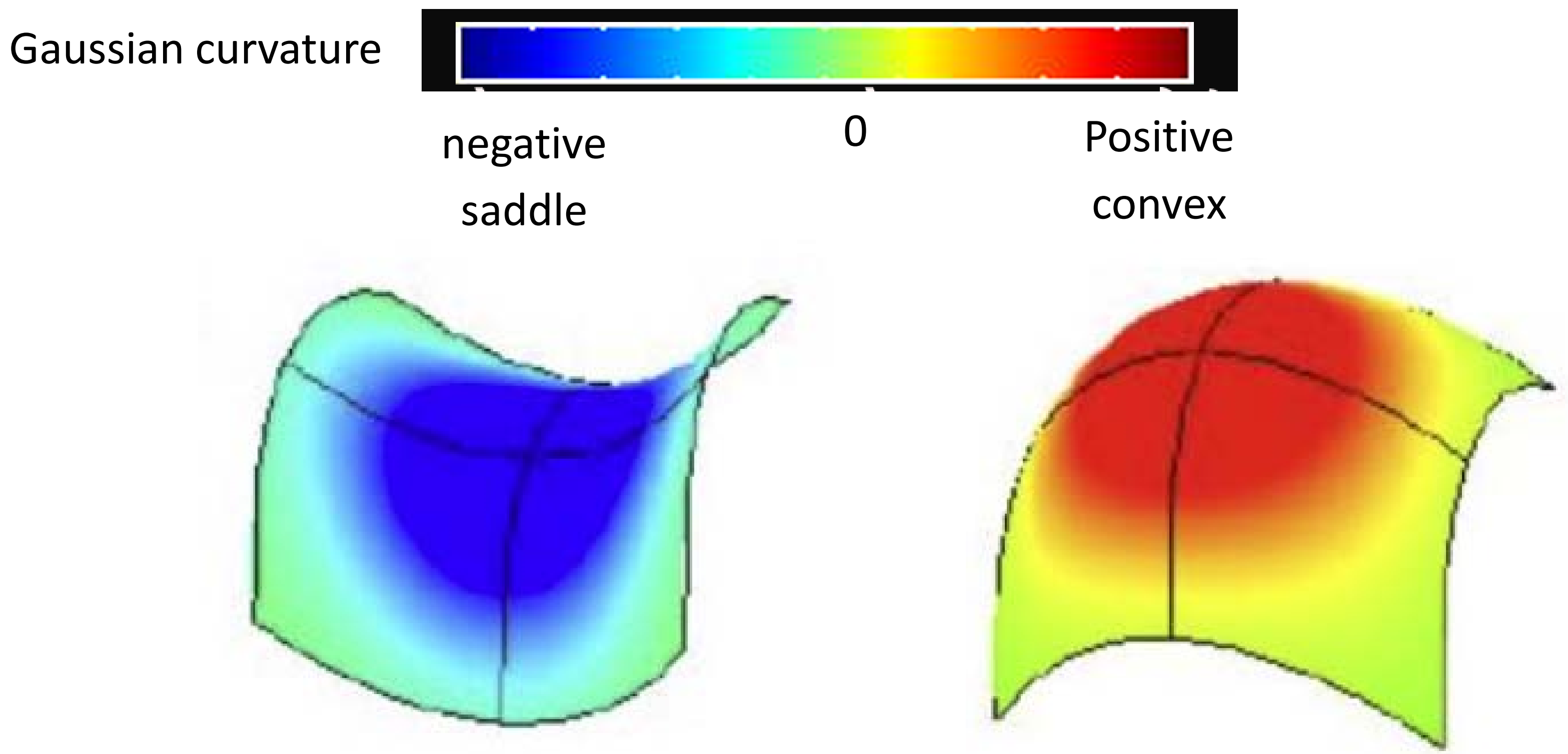
Hypotheses for fold generation: 1 Local outgrowth
2 Buckling



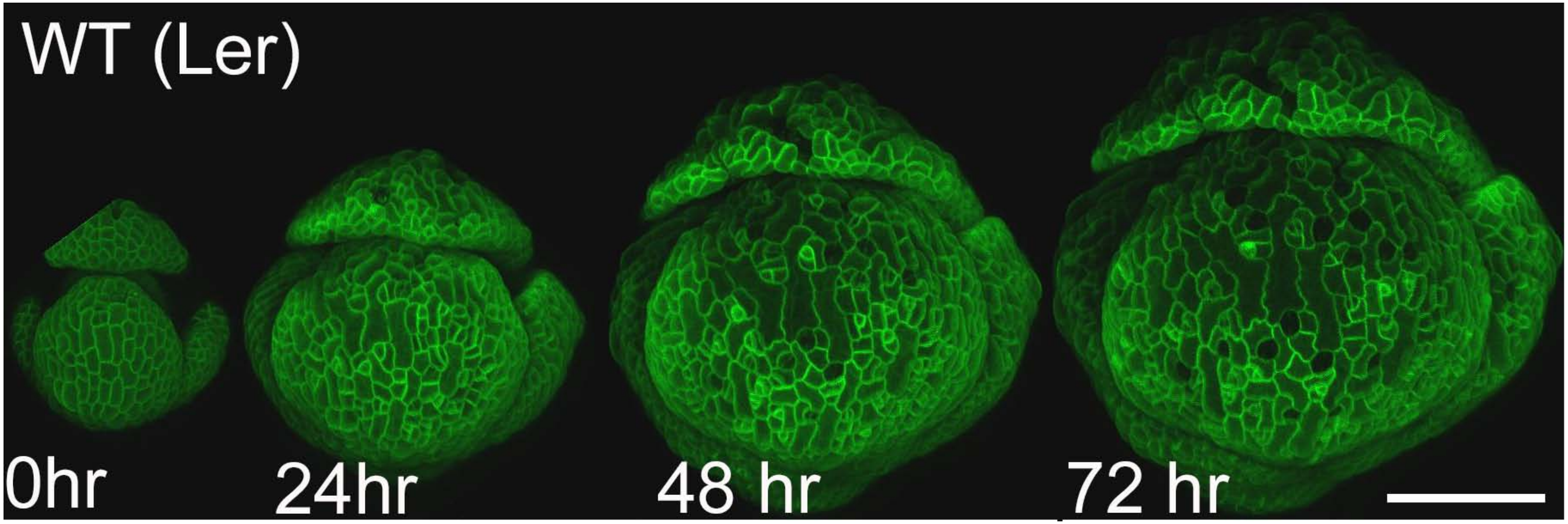
Live imaging to track the formation of the folds

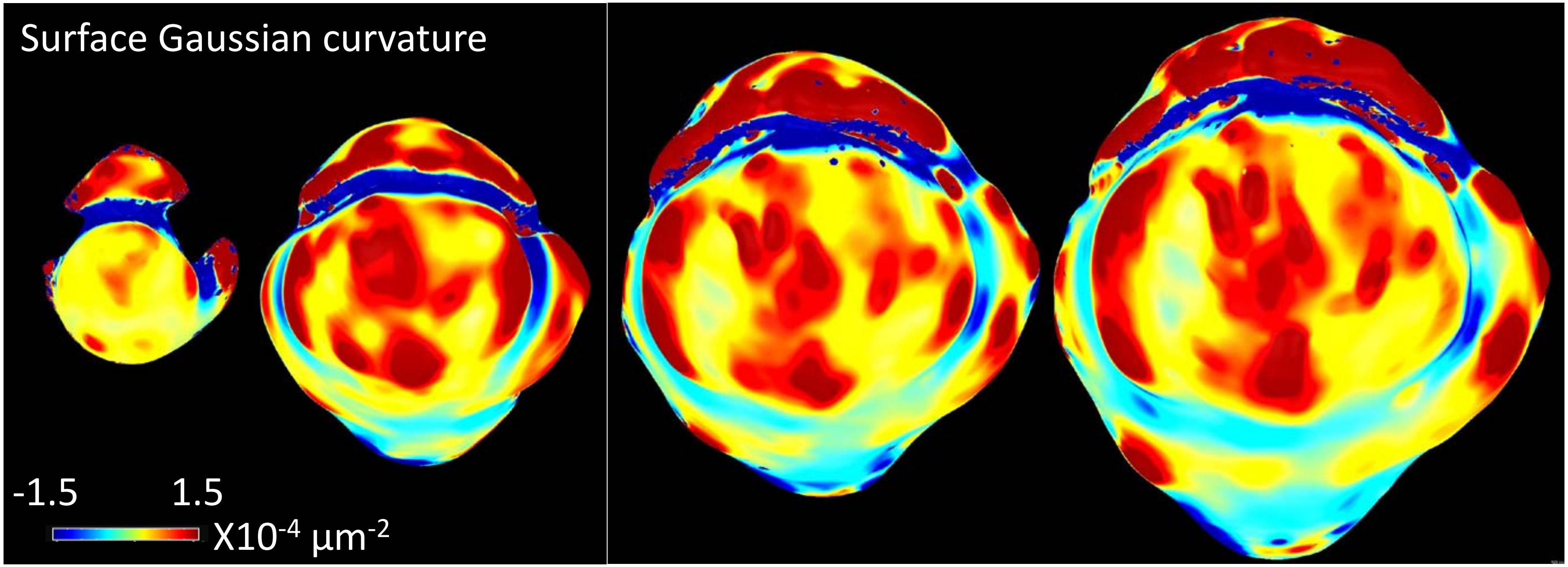


Gaussian curvature describes surface topology

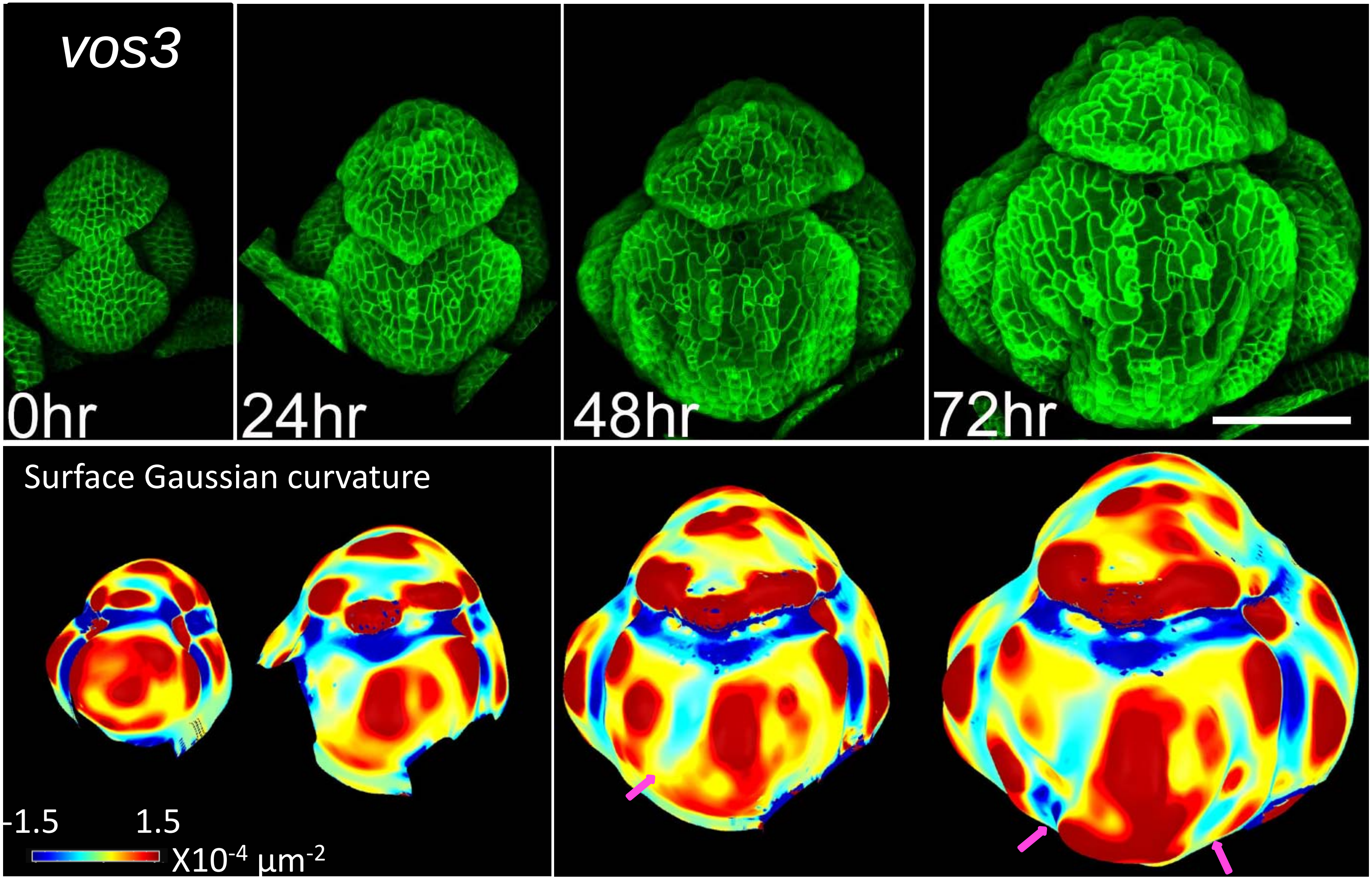


Live imaging to track the formation of the folds





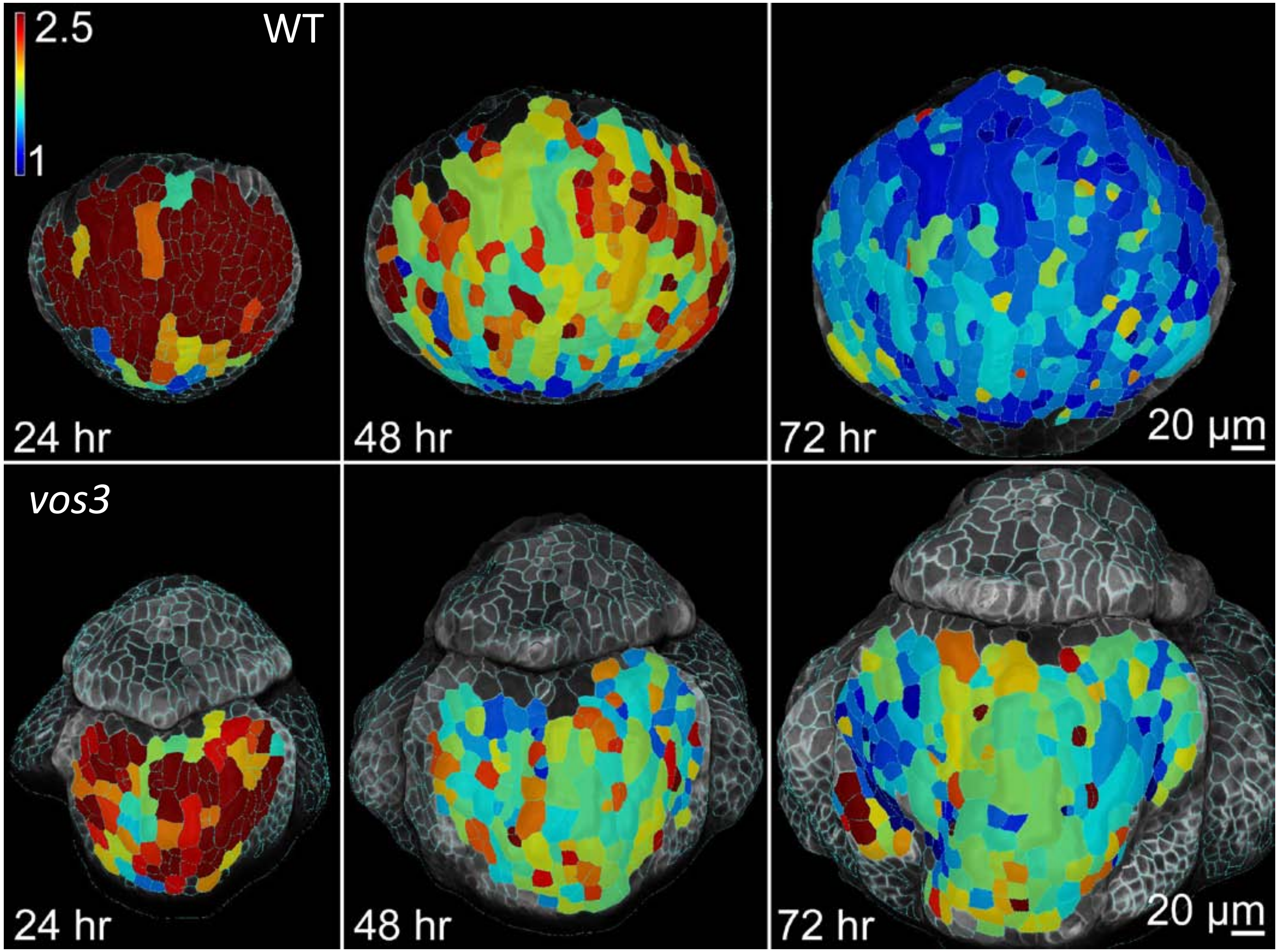
The folds starts gradually in sepal development



Arrows: creases on the epidermis

How is growth altered in *vos3* sepals to give rise to folds?

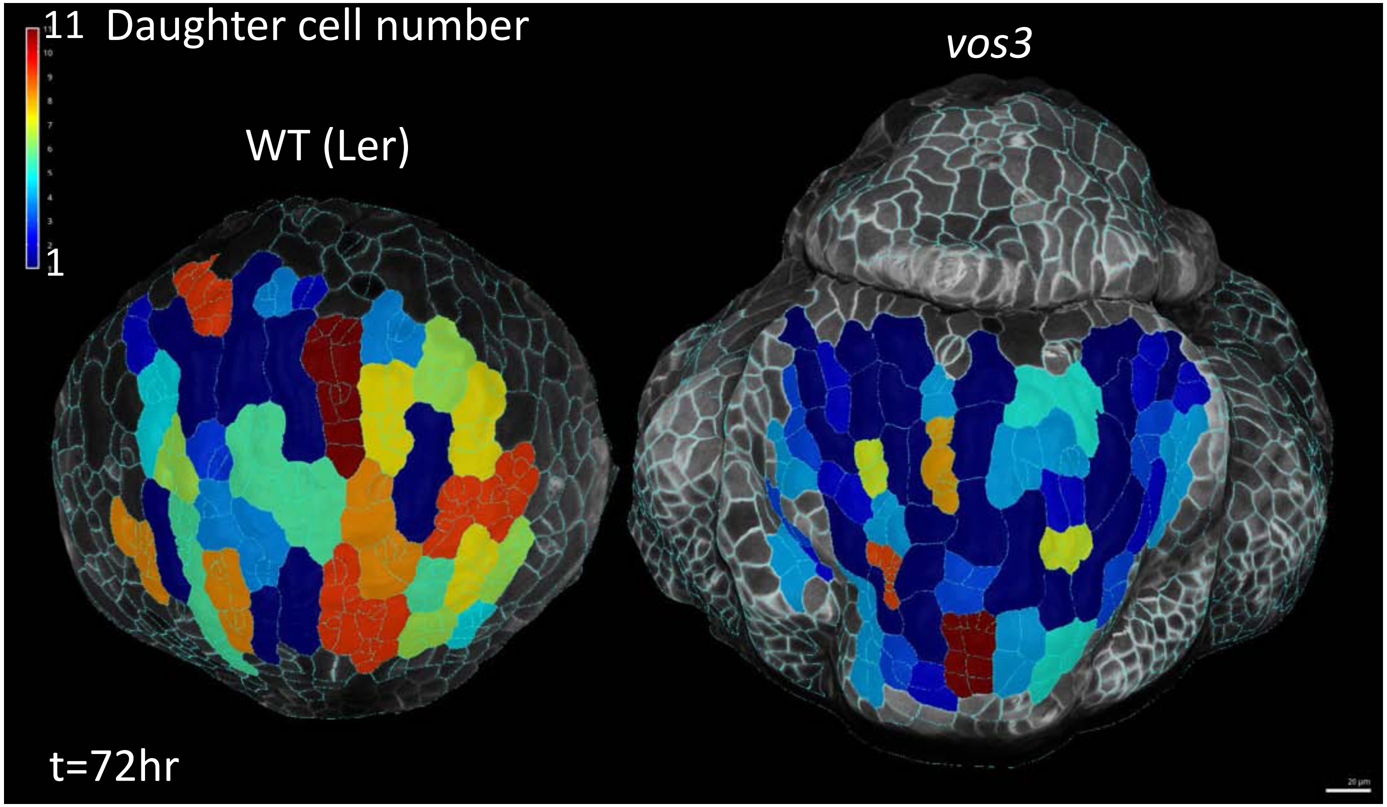
vos3 sepals maintain faster growth



Fast growth is not localized to lumps

Does increased cell division drive outgrowth?

vos3 sepals have a lower cell proliferation rate

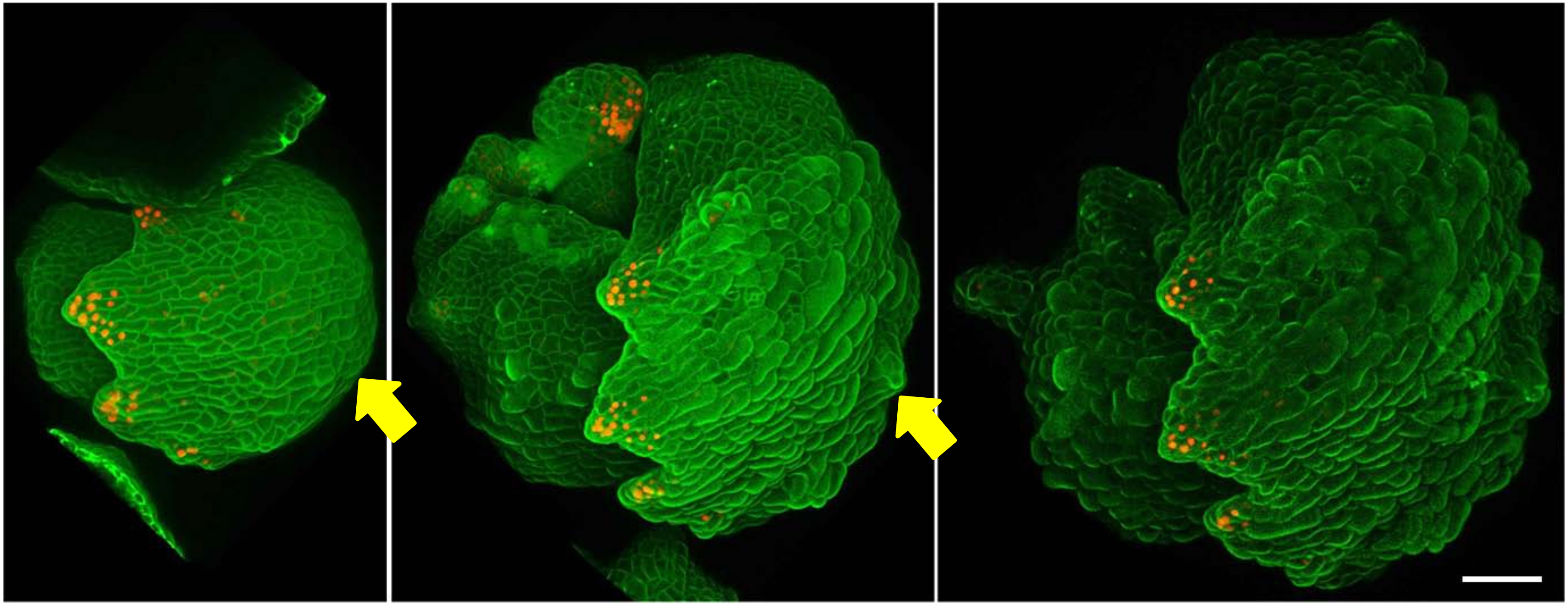


Cell division of t=0hr to t=72hr growth mapped on t=72hr

Auxin regulates outgrowth of primordia.

Does auxin signaling correlate with fold formation?

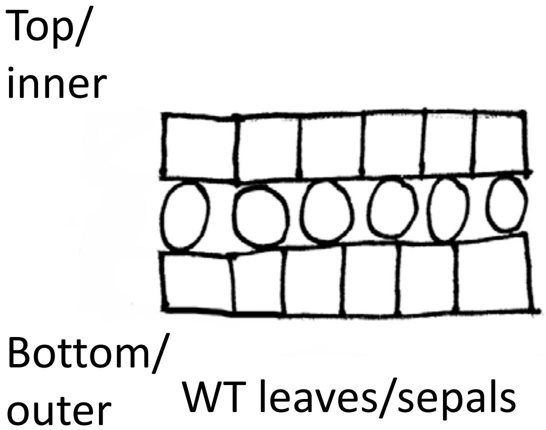
Auxin signaling does not cluster at the bump initiation sites, suggesting these are not primordia

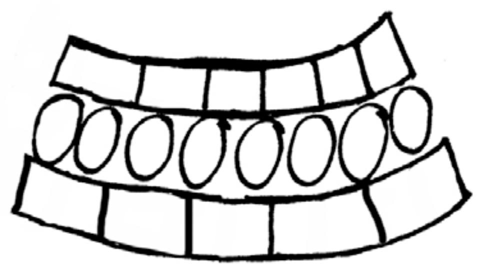


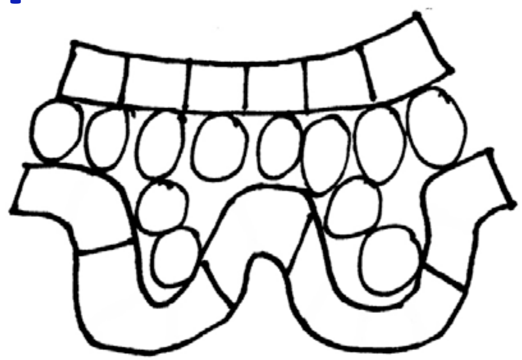
DR5::VENUS auxin response marker in *vos3*

Auxin accumulation precedes primordium formation

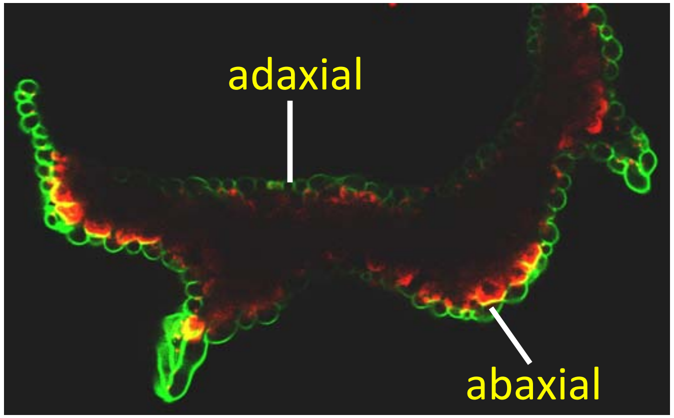
Proposal: an imbalance of growth disrupts the planar sepal growth



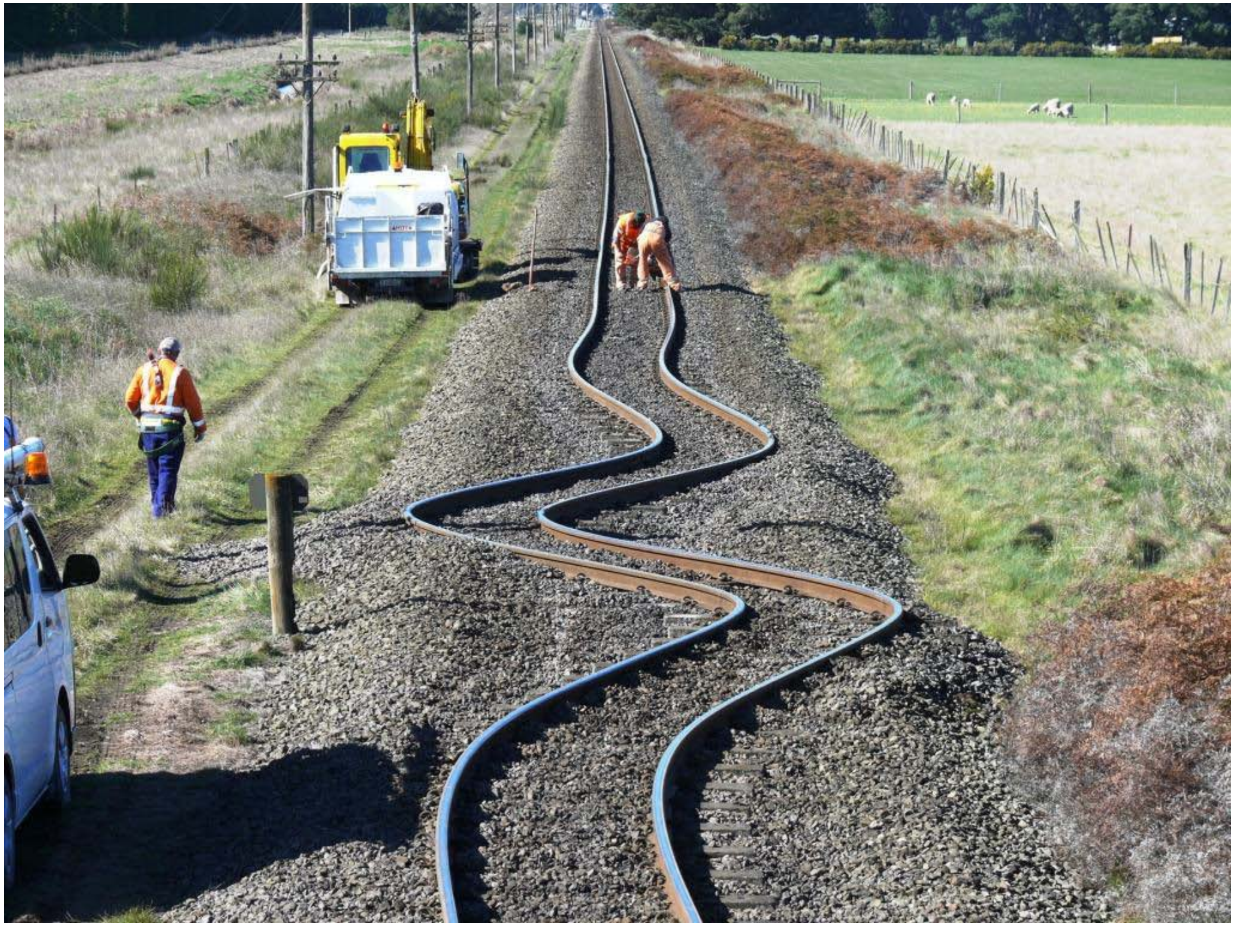




Hypotheses for fold generation: 1 Local outgrowth-not likely
2 Buckling



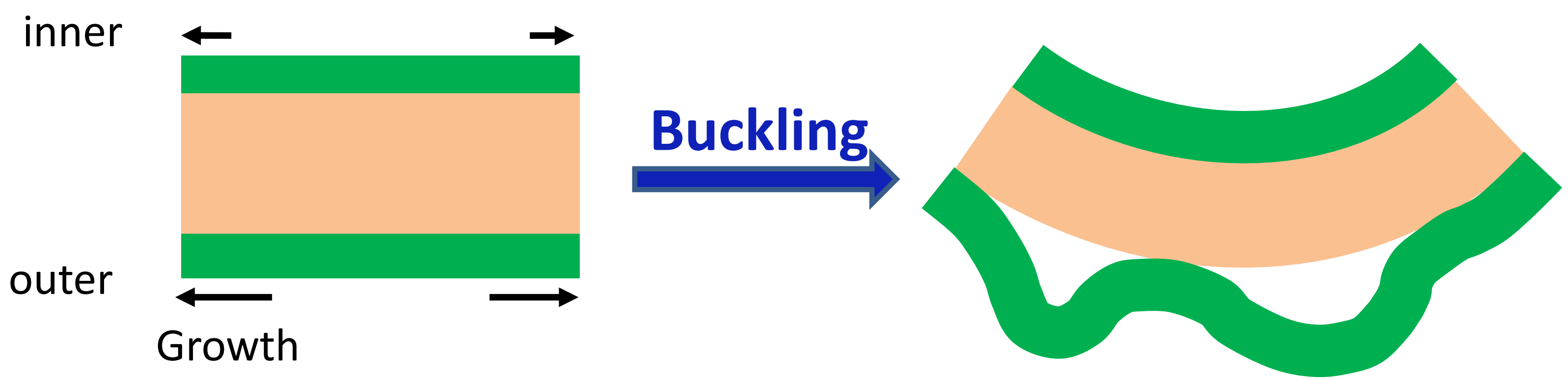
Buckling: "bend and give way under pressure or strain"



https://pwayblog.com/2015/11/04/buckling-prevention/

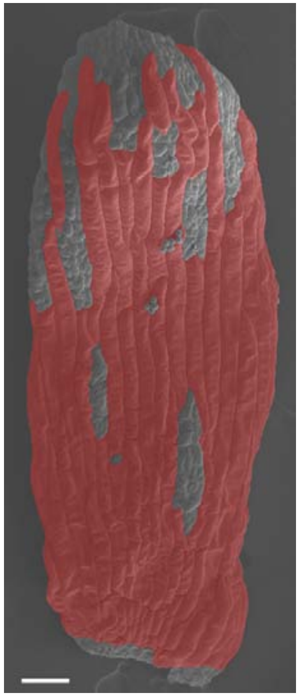
Buckling of a train track due to an earthquake in New Zealand, 2010

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?



ATML1::KRP1

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

Orienting growth on the outer epidermis suppresses lump formation

Roeder et al, Plos Biology, 2010

p*ATML1*::*KRP1*
Slower growth
on the abaxial epidermis

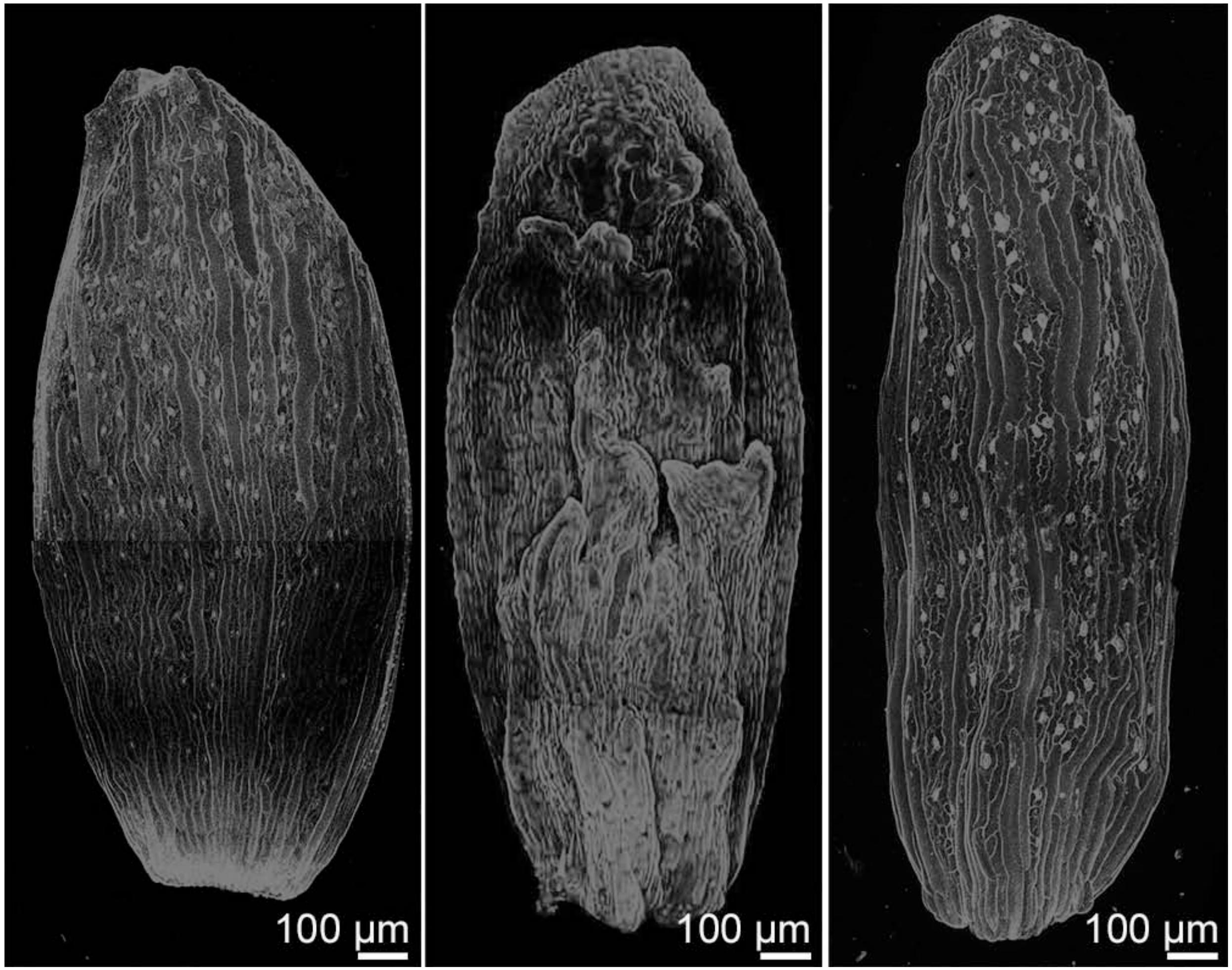
+

vos3
Arrows: lumps
on the sepal

→

vos3 p*ATML1*::*KRP1*
Slower growth
on the abaxial epidermis

Orienting growth on the outer epidermis suppresses outgrowth

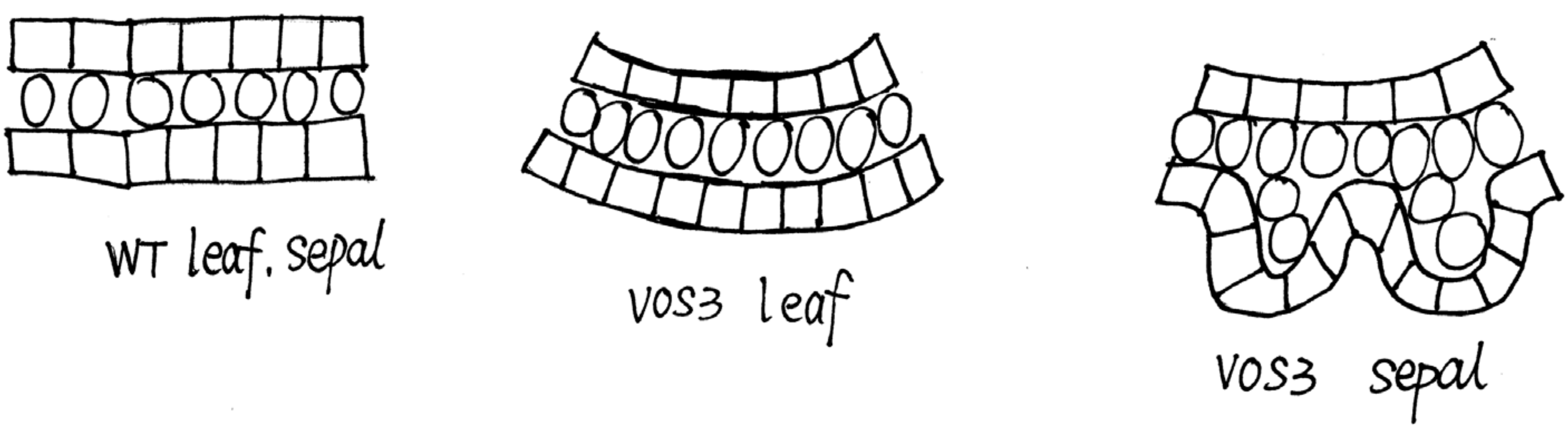


WT (L*er*)

vos3

vos3 / p*ATML1*::*KRP1*
Slower growth
on the abaxial epidermis

Proposal: an imbalance of growth disrupts the planar sepal growth

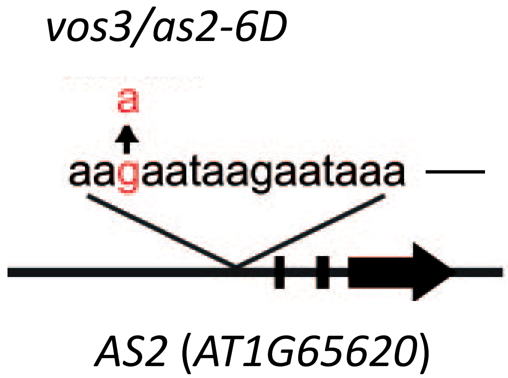


Hypotheses:

1. Initiation of primordia on the sepal epidermis – less likely
2. Buckling – possible (Further tests in progress)

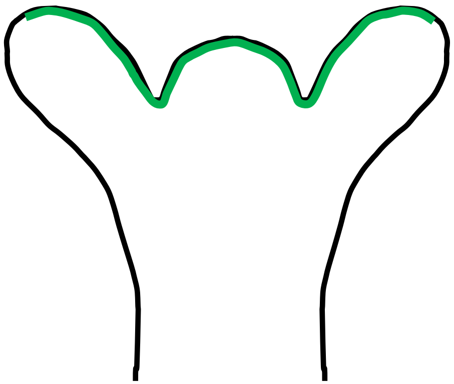
What gene is mutated in *vos3*?

vos3 has a mutation in the *ASYMMETRIC LEAVES2* (*AS2*) promoter



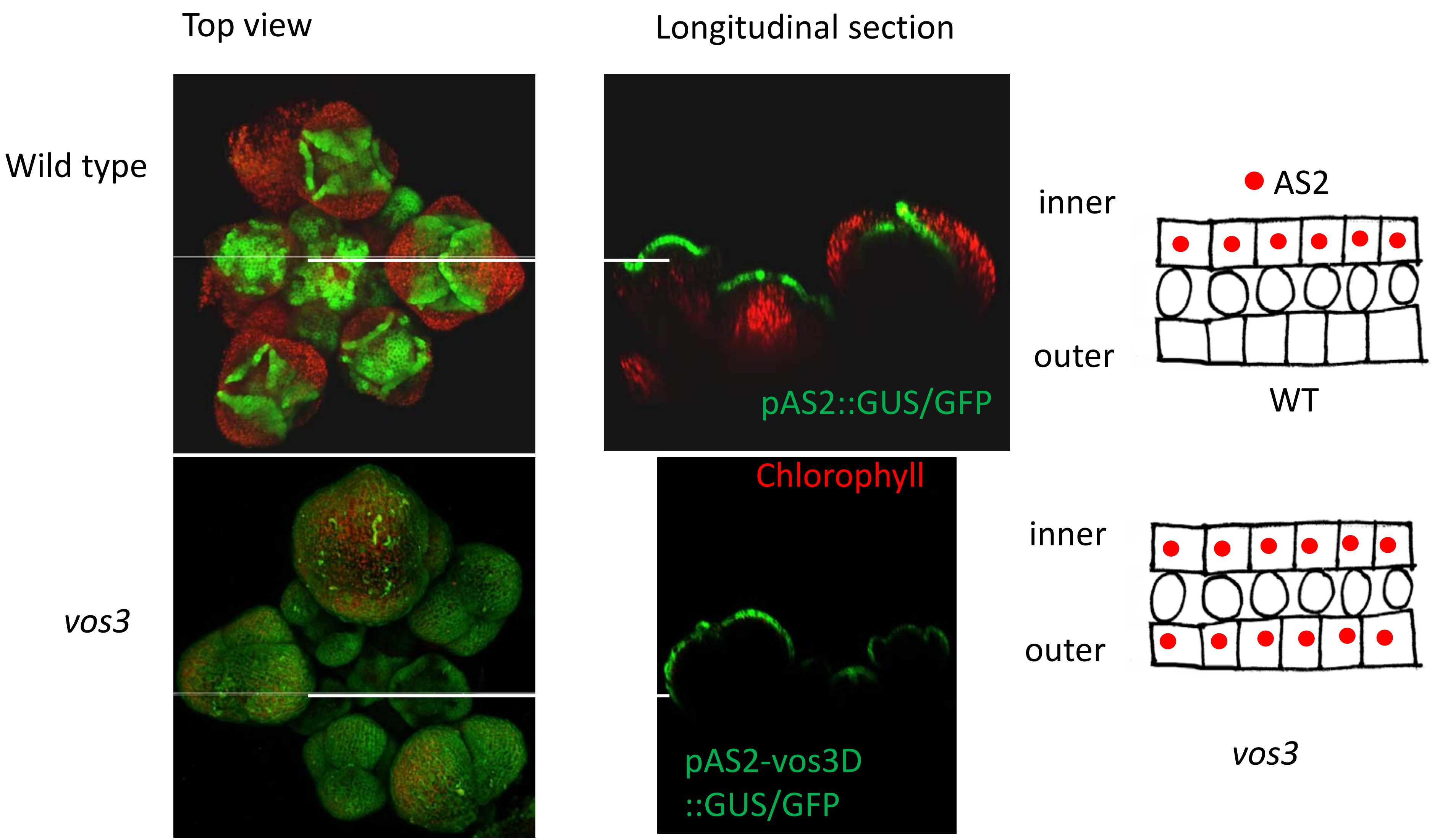
Wu et al, PNAS, 2008

WT *AS2* expression region

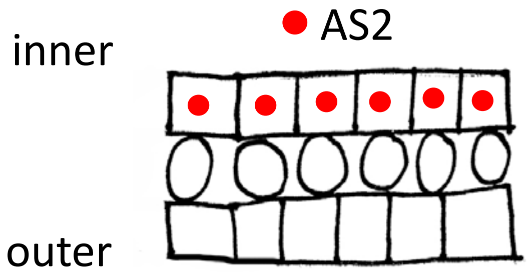


AS2: a transcription factor with a leucine zipper domain; thought to repress downstream genes.

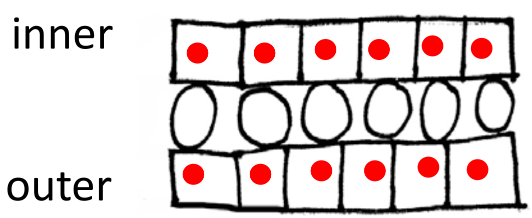
vos3 expands *AS2* expression to the outer sepal epidermis



Overexpressing *AS2* throughout the epidermis recapitulates the lumpy sepal phenotype



WT

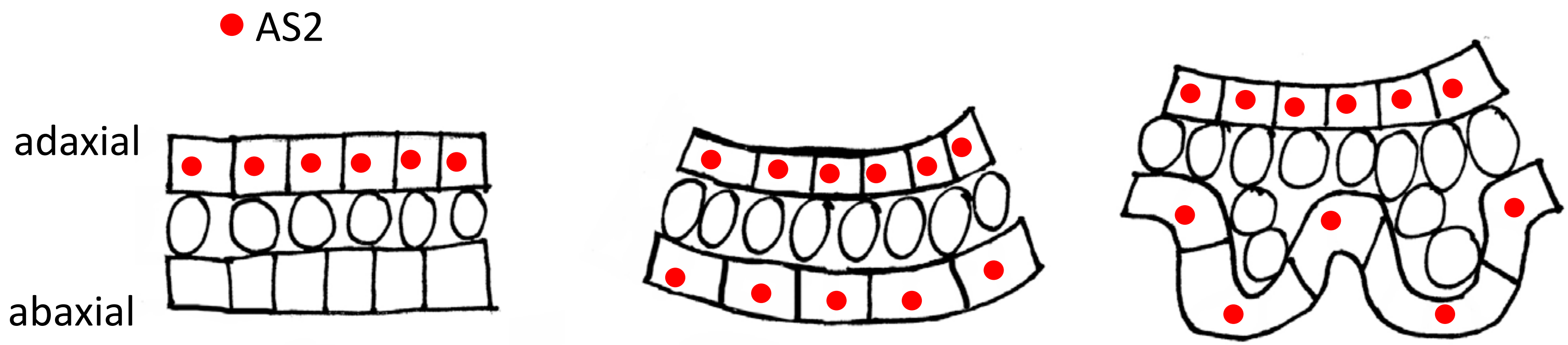


p*ATML1*::*AS2*

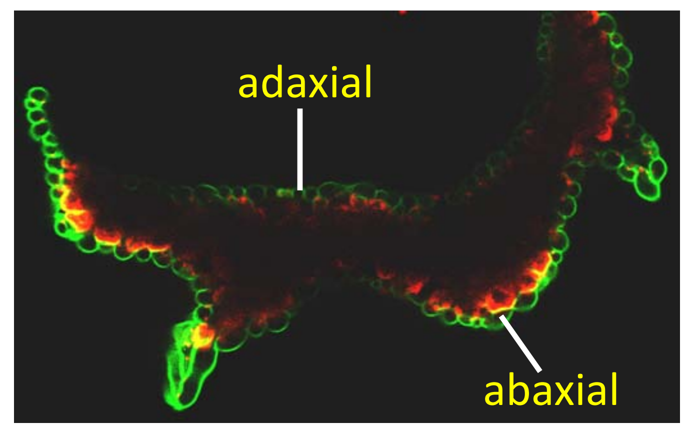
p*ATML1*::*AS2*

p*ATML1*::*AS2* expresses *AS2* on all the epidermis.

Developing a regular flattened laminar structure requires coordinated growth across the organ

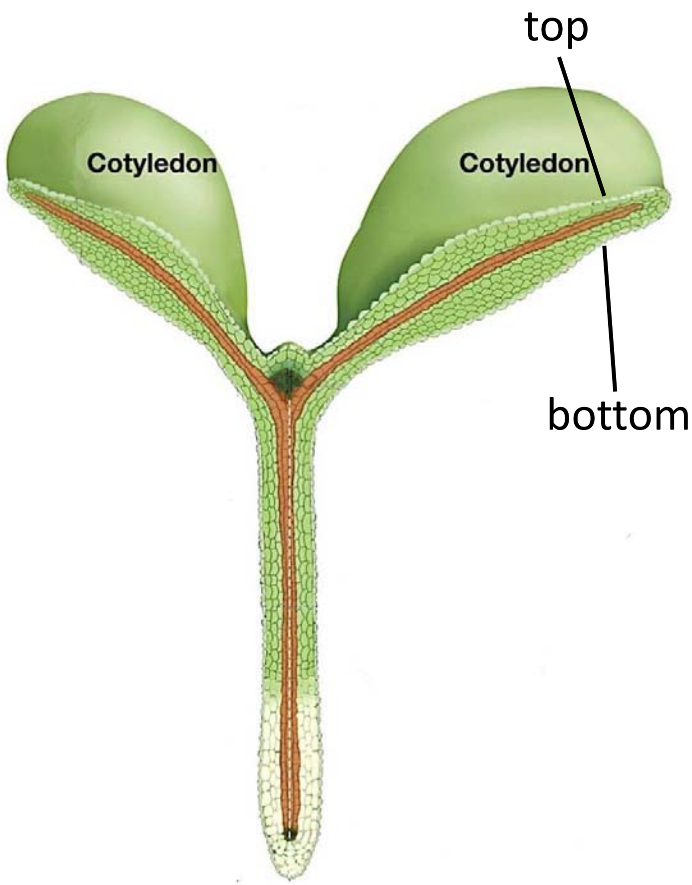


WT leaves/sepals | *as2-6D* leaves | *as2-6D* sepals

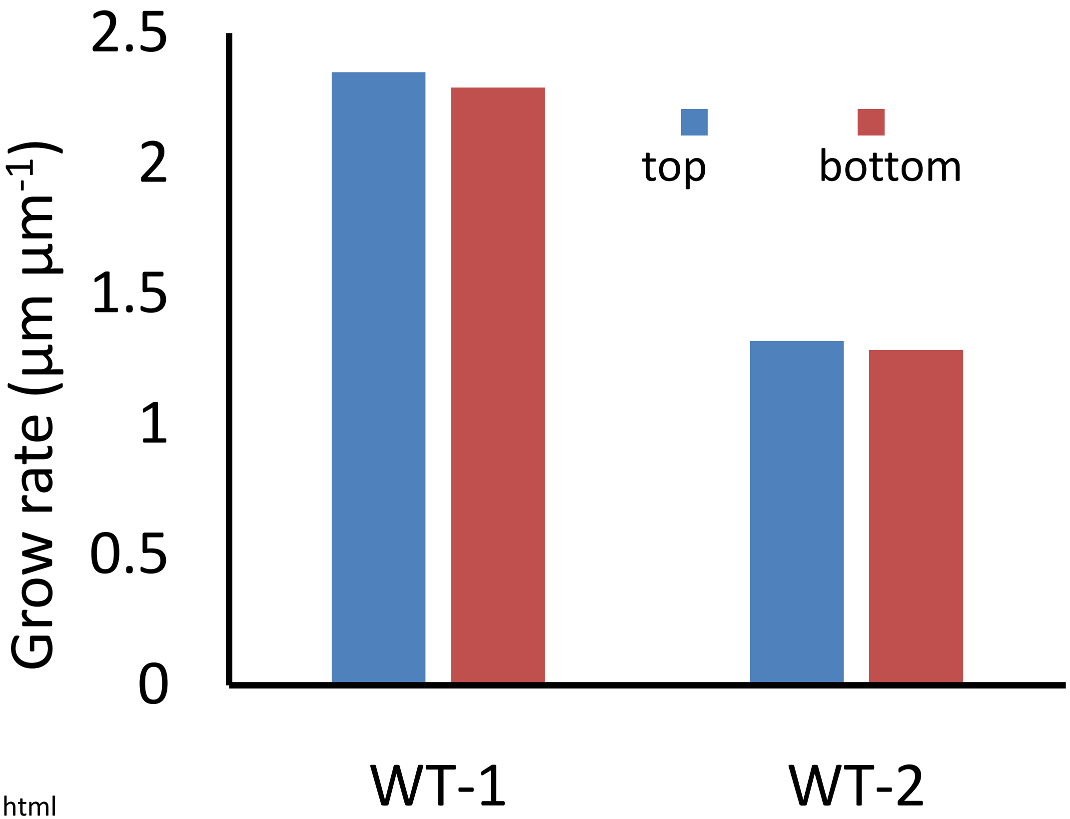


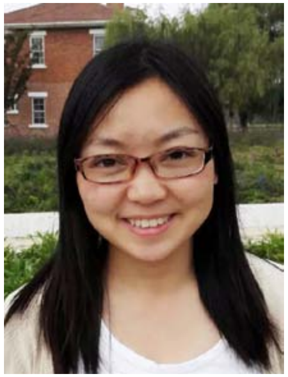
Is growth coordinated on the sides of the organ?

The *Arabidopsis* cotyledon has similar growth on both sides of the organ



http://crazywidow.info/26/1210031-plant-root-cell-diagram.html





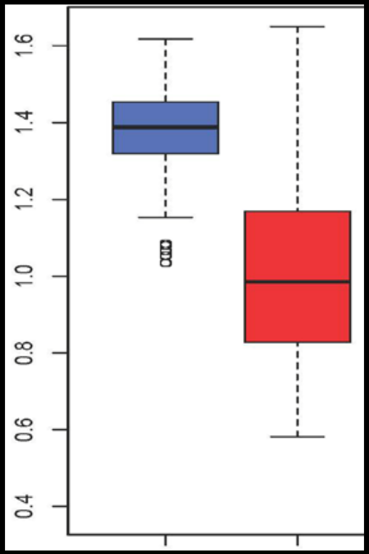
Dr. Lilan Hong

Stories: 3 short stories about variable organ mutants

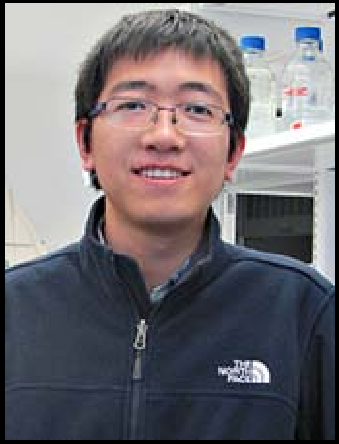
- ***variable organ size1*** (***vos1***) and spatial temporal averaging of noisy cell growth
- ***variable organ size 3*** (***vos3***) and coordination of growth on the front and back of the organ.
- ***variable organ size2*** (***vos2***) and synchrony of organ primordia initiation

Sepal size is variable in *vos2* mutants

WT *vos2* WT *vos2*

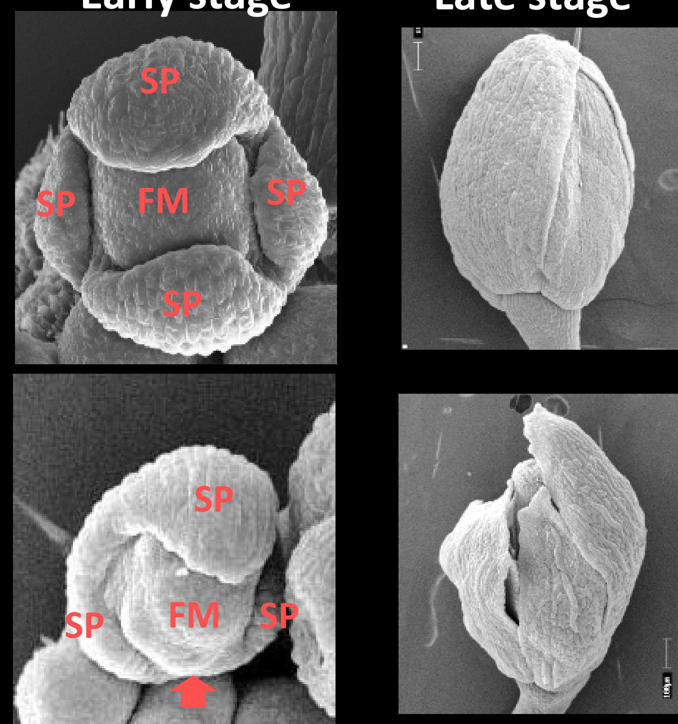


<u>v</u>ariable <u>o</u>rgan <u>s</u>ize and shape (vos)



Mingyuan Zhu

Sepal size variability arises early and continues through late stages



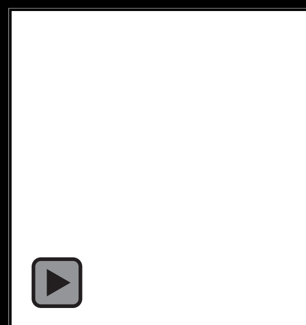
SP: Sepal primordium

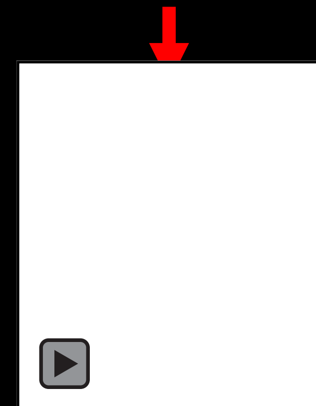
FM: Floral meristem

Arrow: delayed SP

Is sepal initiation delayed in *vos2* mutants?

Sepal initiation is delayed in *vos2*





35S::mCitrine-RCI2A

WT

vos2

The initiation of *vos2* sepals is delayed

35S::mCitrine-RCI2A

WT

vos2

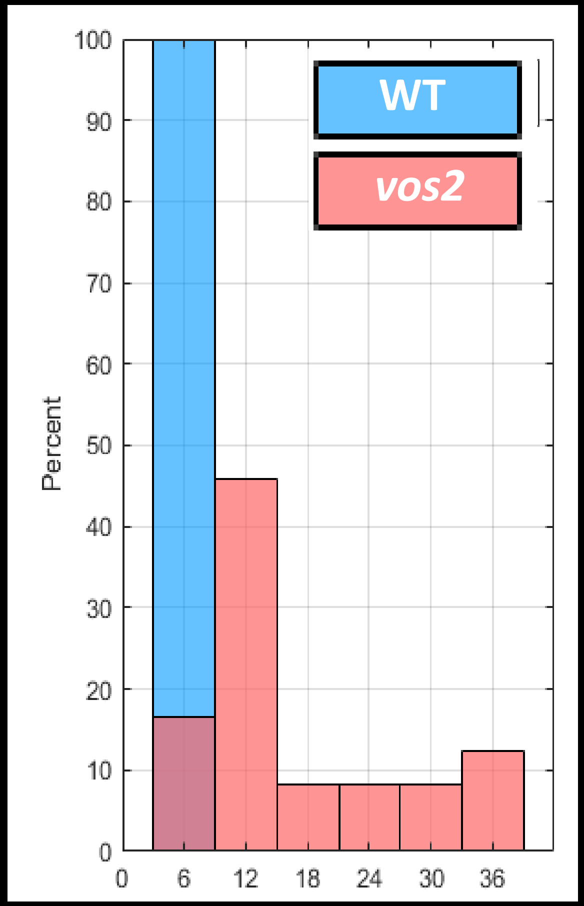
0hr

12hr

42hr

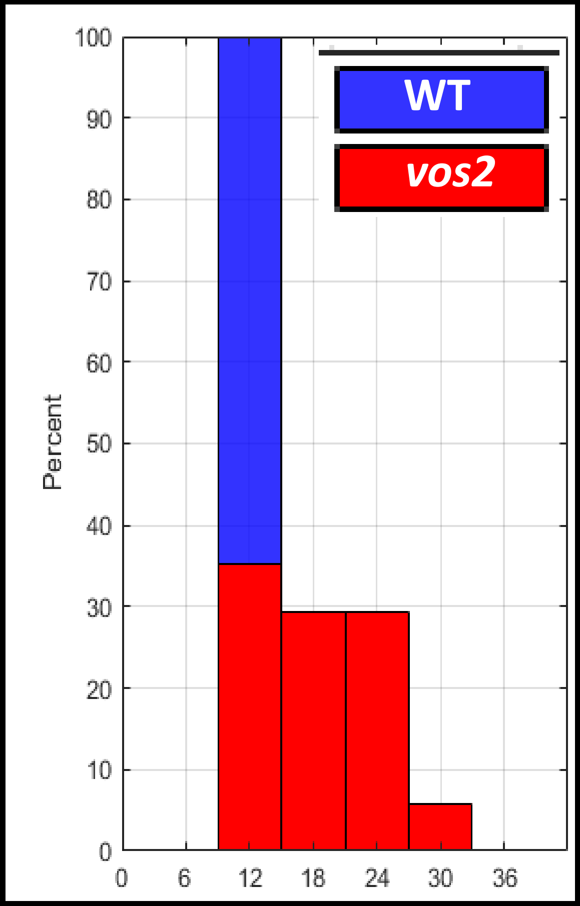
The initiation of *vos2* sepals is delayed and more variable

Timing: Outer -> Inner



Time (hr)

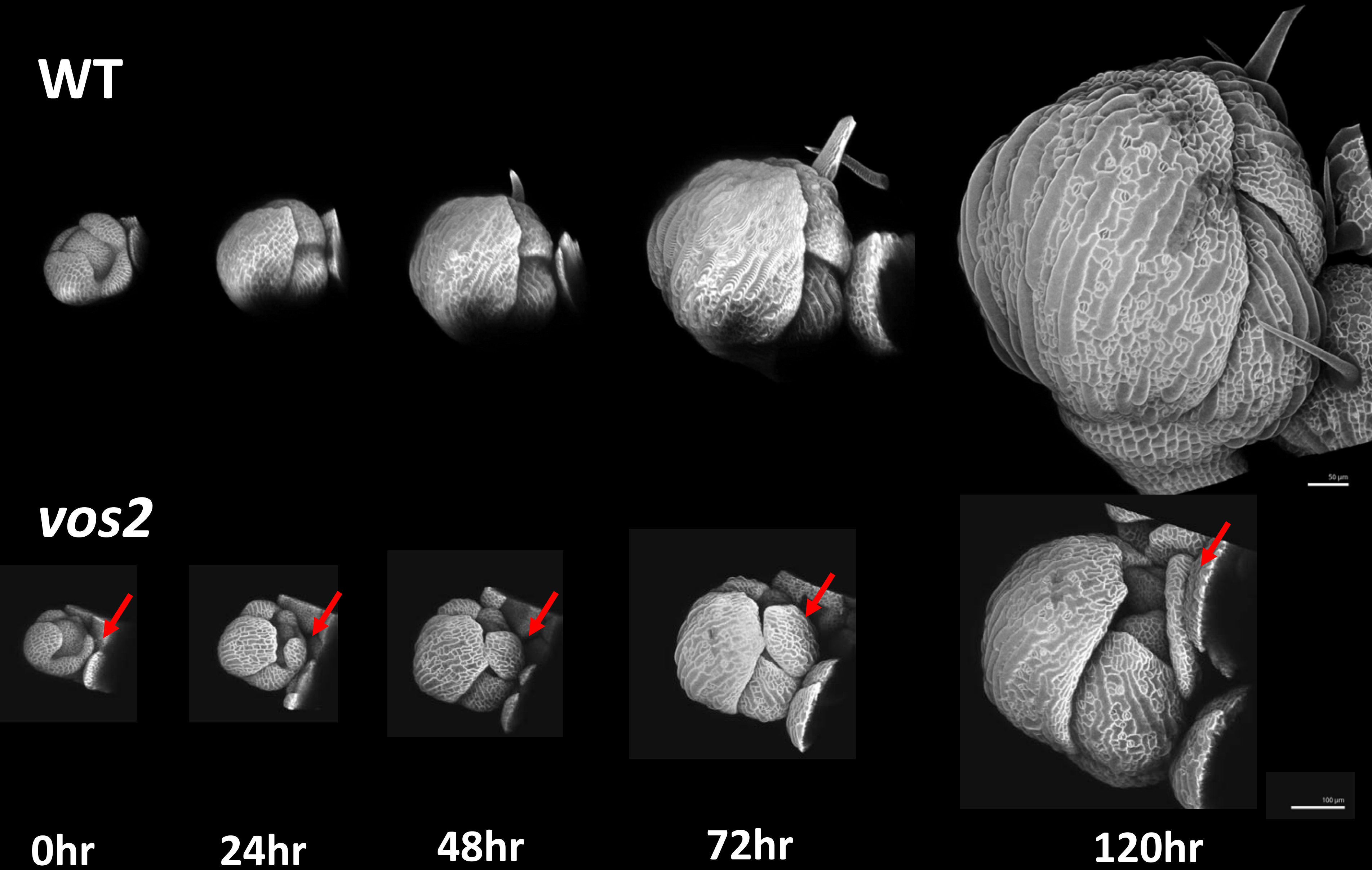
Timing: Outer -> lateral



Time (hr)

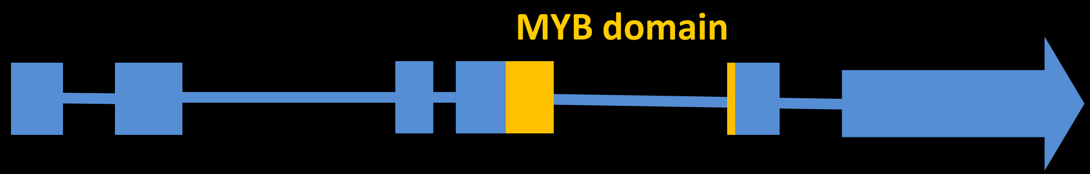
Does delayed initiation cause variable organ size?

Late initiation leads to smaller sepals

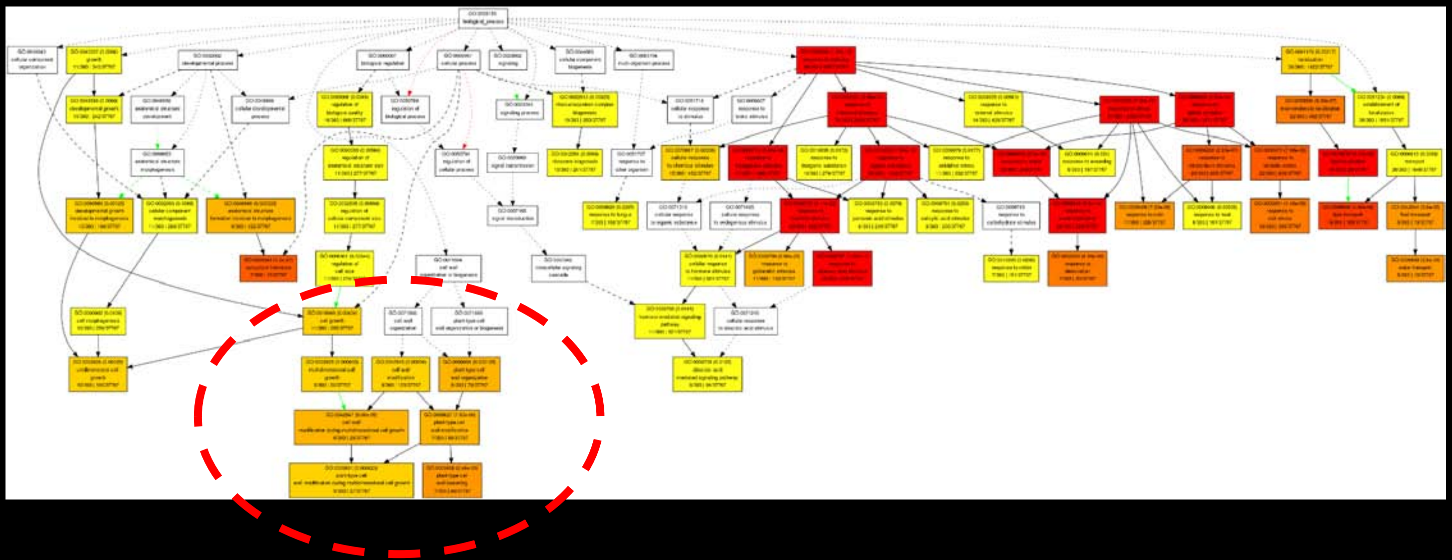


What gene is disrupted in the *vos2* mutant?

VOS2 encodes a MYB domain transcription factor



RNA-seq :
WT vs *vos2*

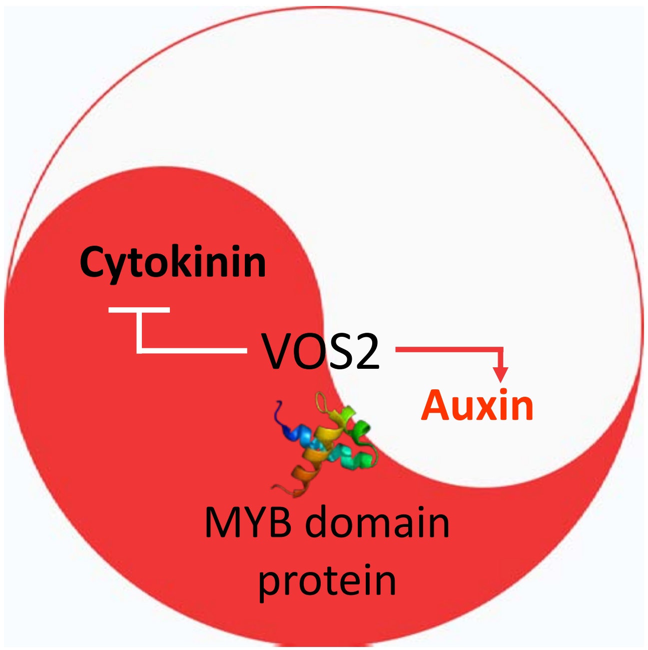


Cellular growth

cell wall modification

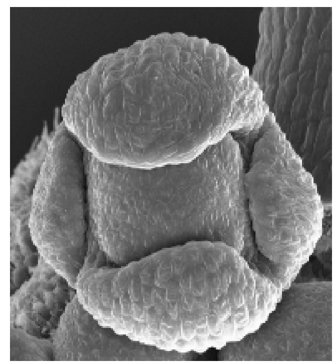
Response to stress or stimulus

Part 3 Conclusion:
Timing of organ primordium initiation is critical for robust organ size



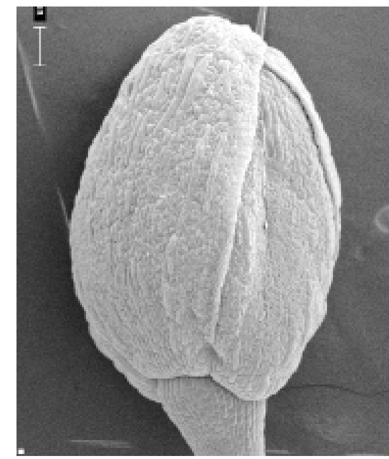
Hormone signaling maxima and minima (variation)

→



Synchronous primordium initiation and growth

→

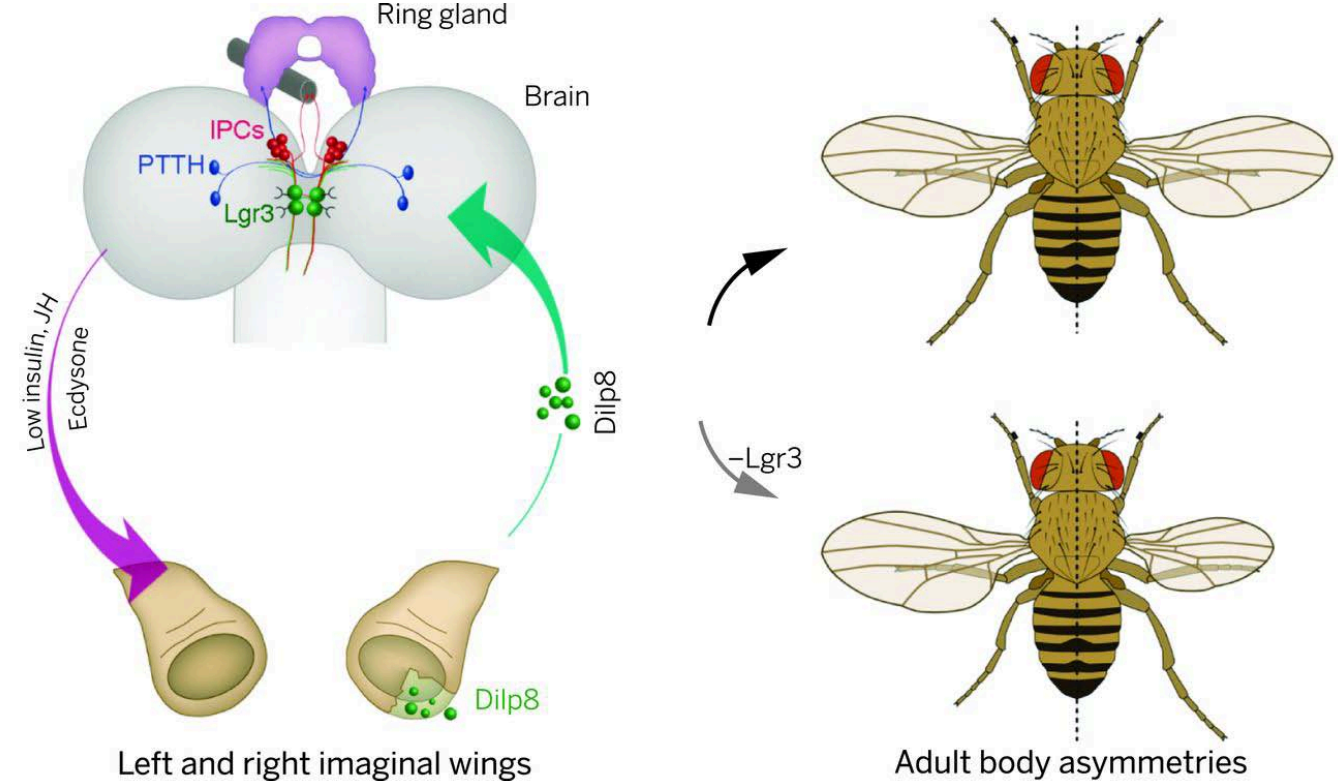


Organ regularity

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?



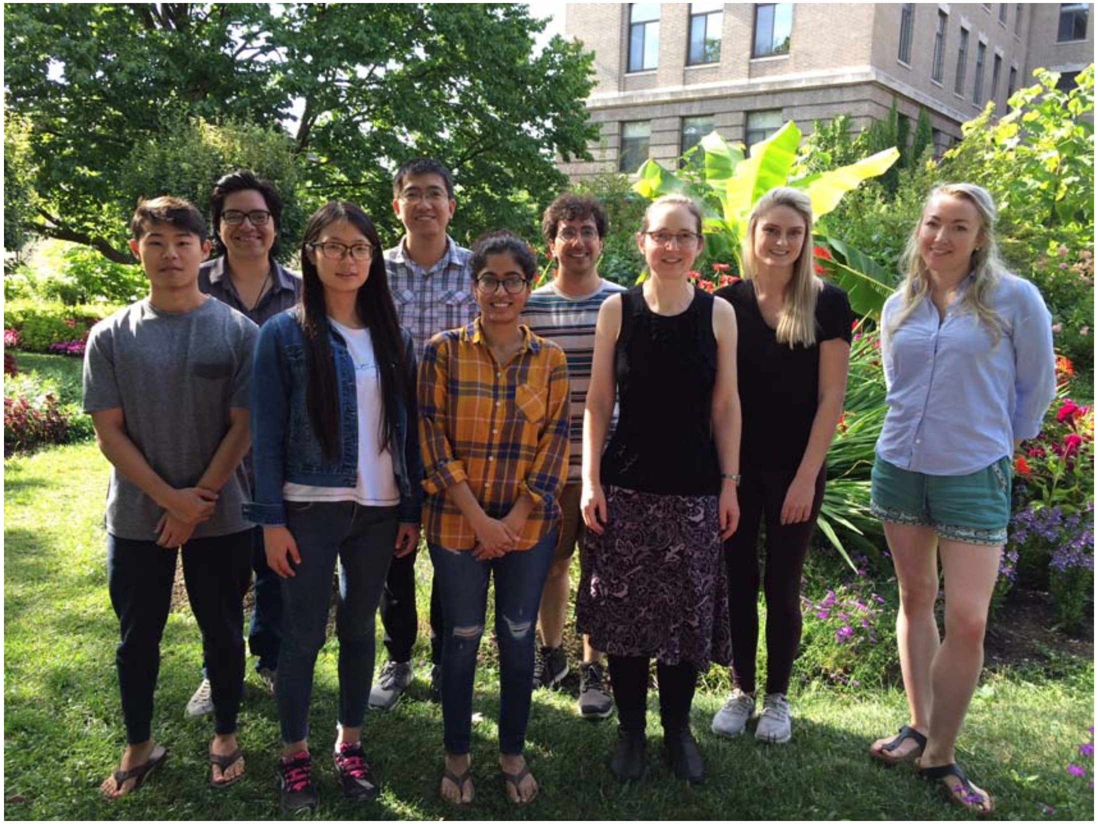
Diana M. Vallejo et al. Science 2015;350:aac6767

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



KITP

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Mingyuan Zhu
Vijaya Lakshmi Vadde
Joseph Cammarata
Kate Harline
Jessica McGory
Weiwei Chen
Heather Meyer
Dana Robinson

Collaborators on these projects:
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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

HFSP

NSF