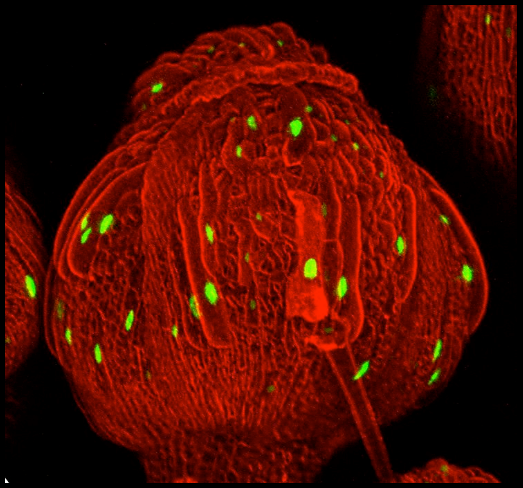
Imaging and modeling the patterning of *Arabidopsis* sepal cells



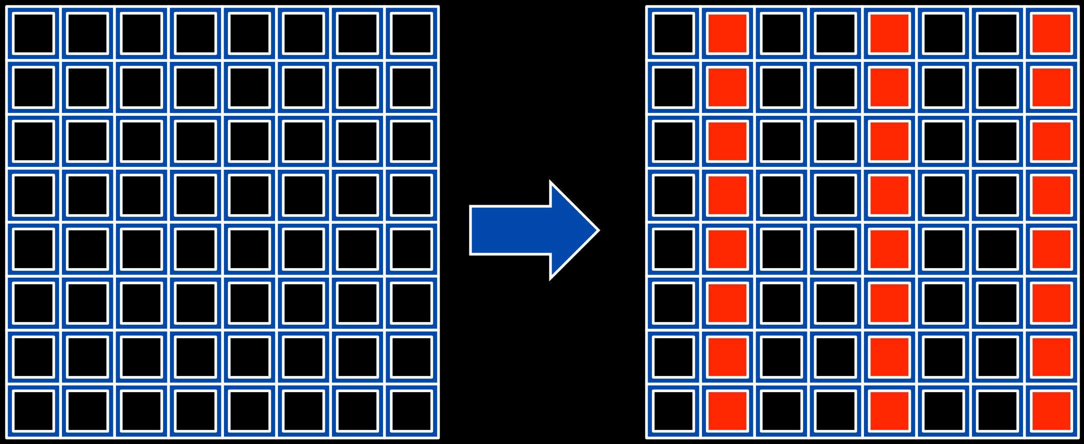
Adrienne Roeder

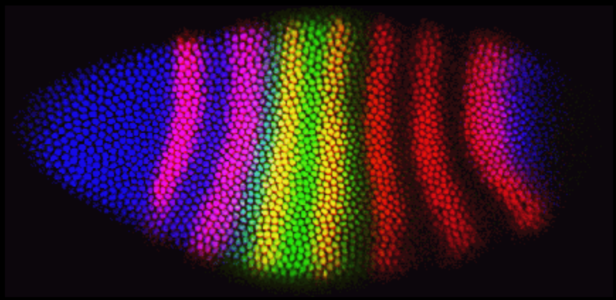
California Institute of Technology

KITP September 10, 2009

How are patterns formed de novo from a field of identical cells?

Intercellular signaling and cell fate specification





http://superfly.ucsd.edu/%7Edavek/intro.html

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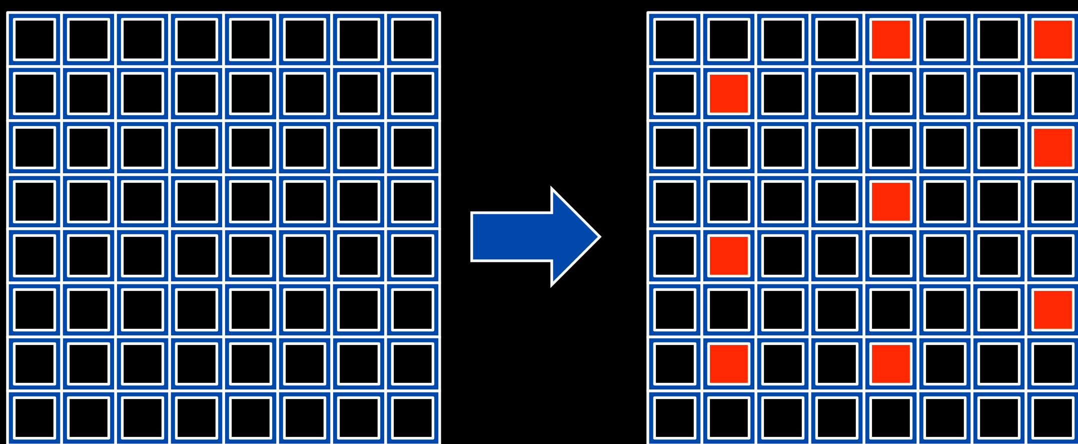
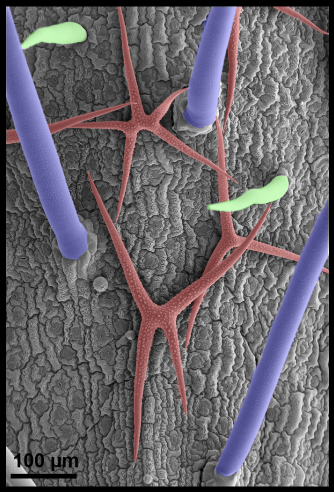
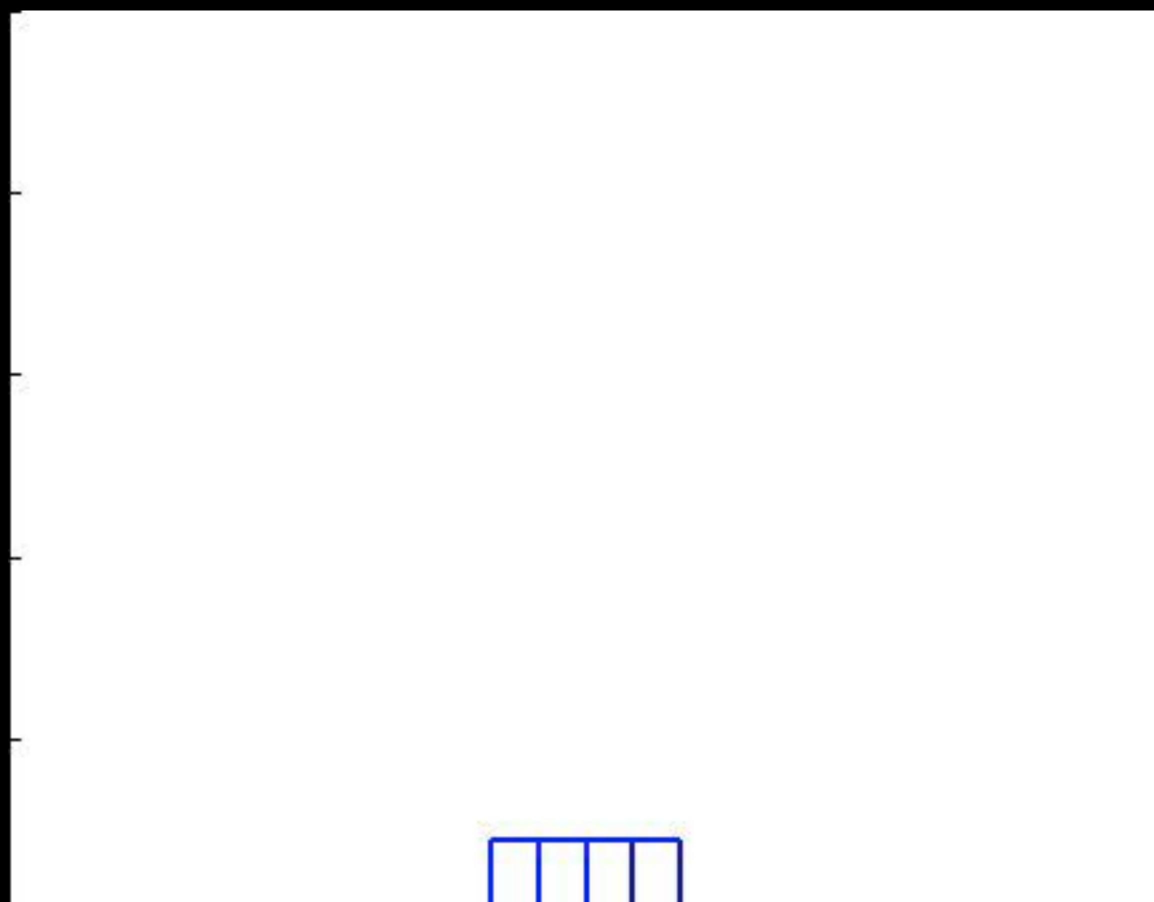
Intercellular signaling and cell fate specification





Patterning occurs while cells are growing and dividing

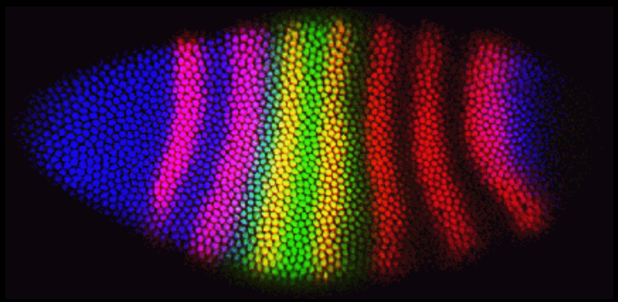


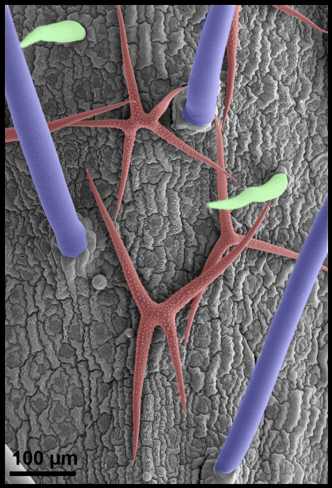
How are fate and division integrated to achieve patterning

Signaling
fate specification

cell division
growth

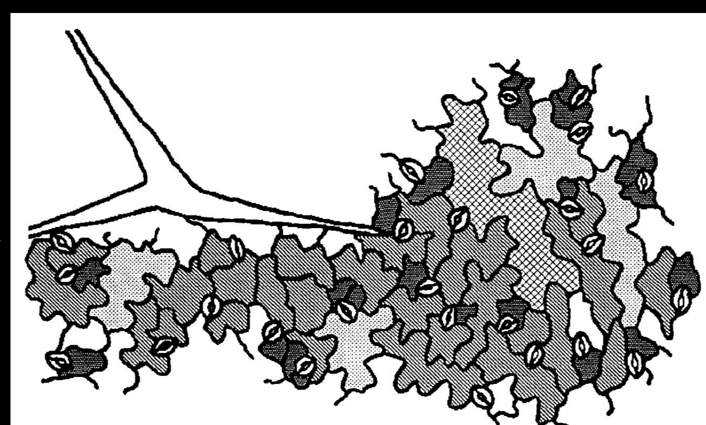
pattern





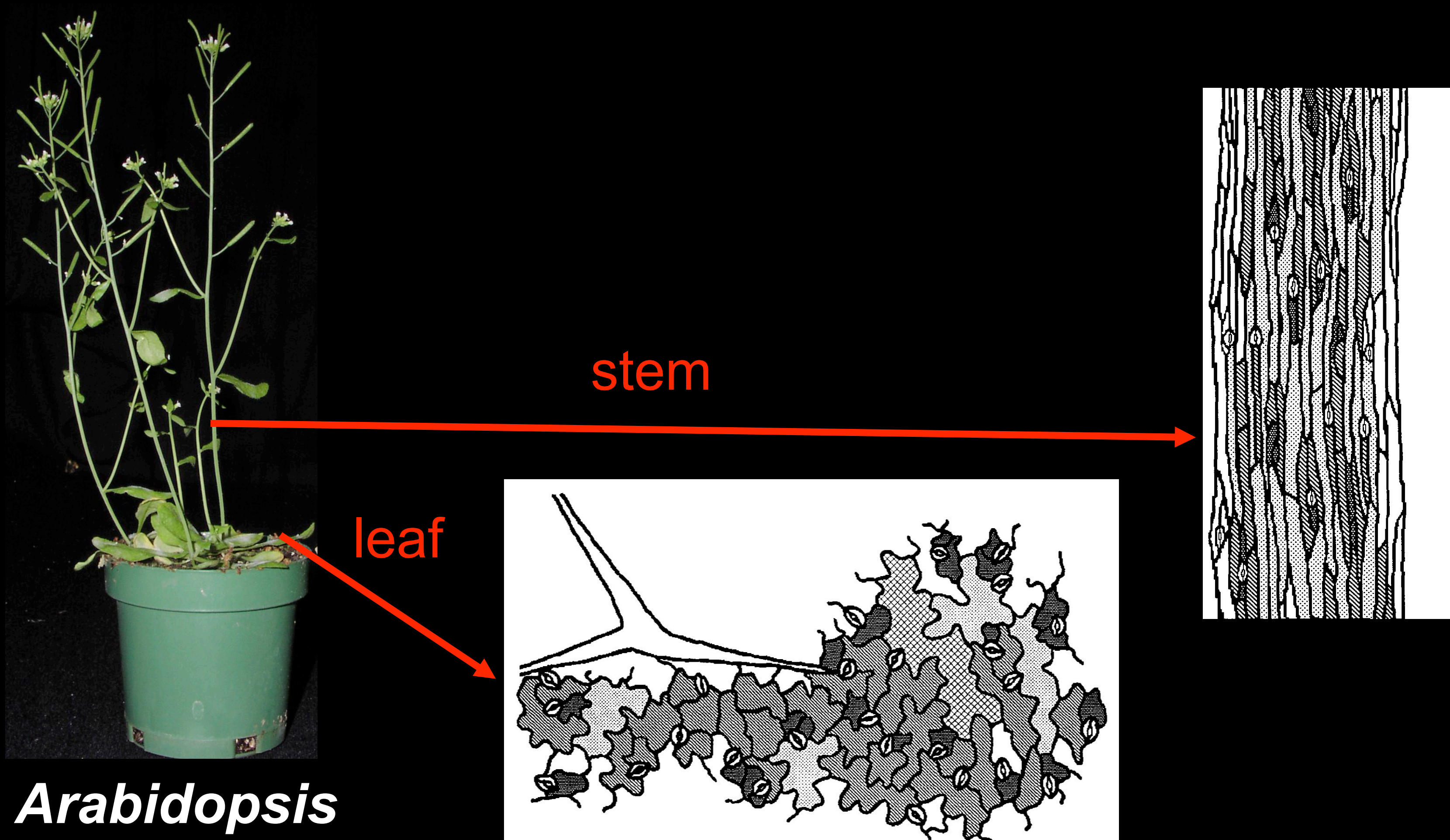
Epidermal cells have a range of sizes

***Arabidopsis* plants**



(Melaragno et al., 1993)

Epidermal cells have a range of sizes



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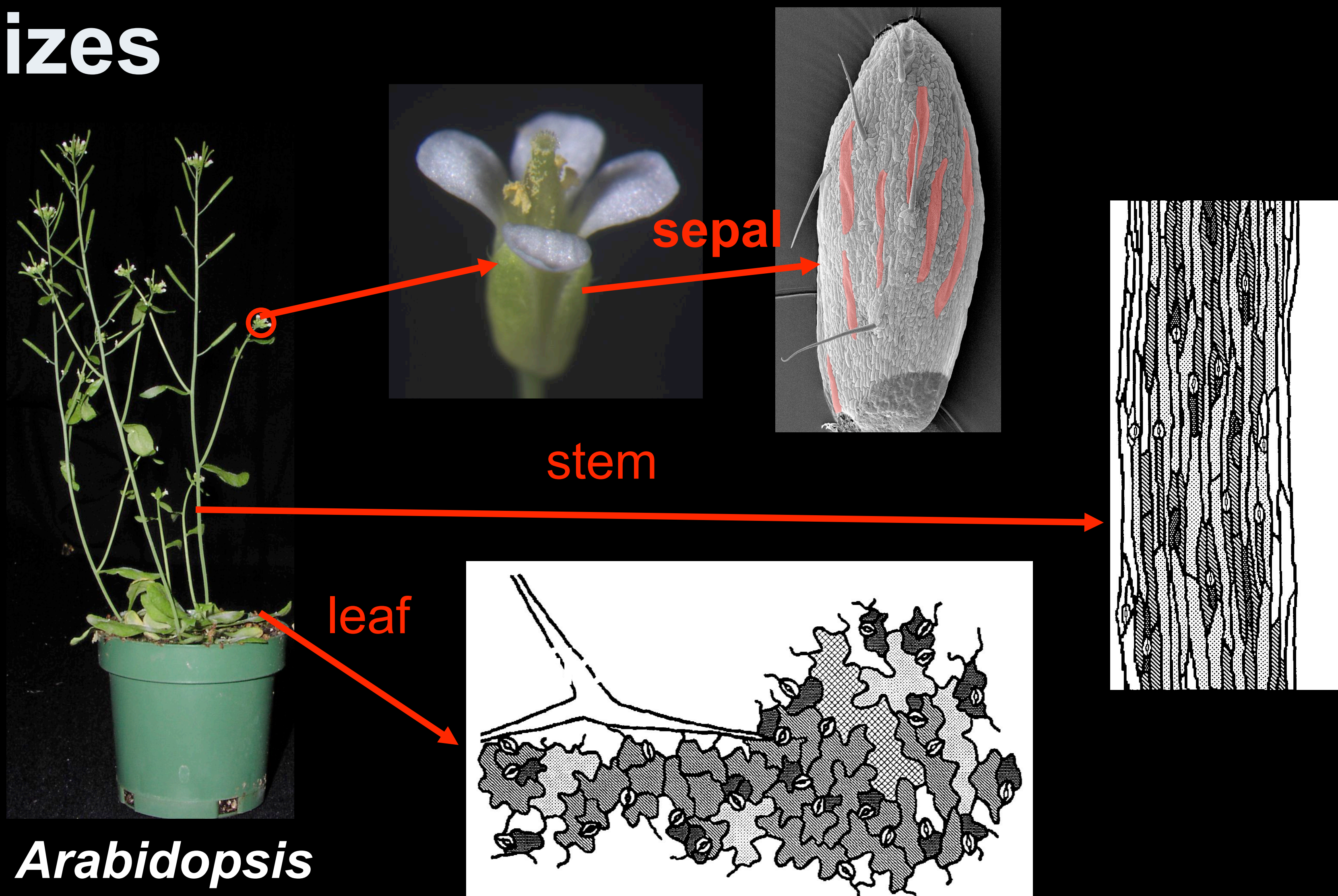
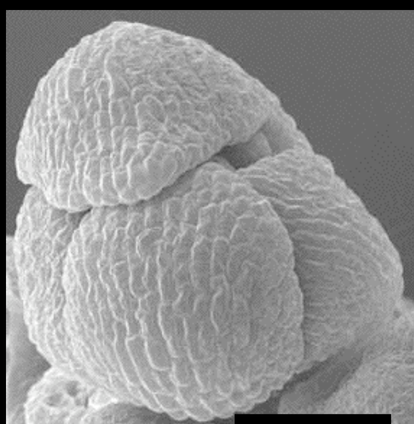


***Arabidopsis* plants**

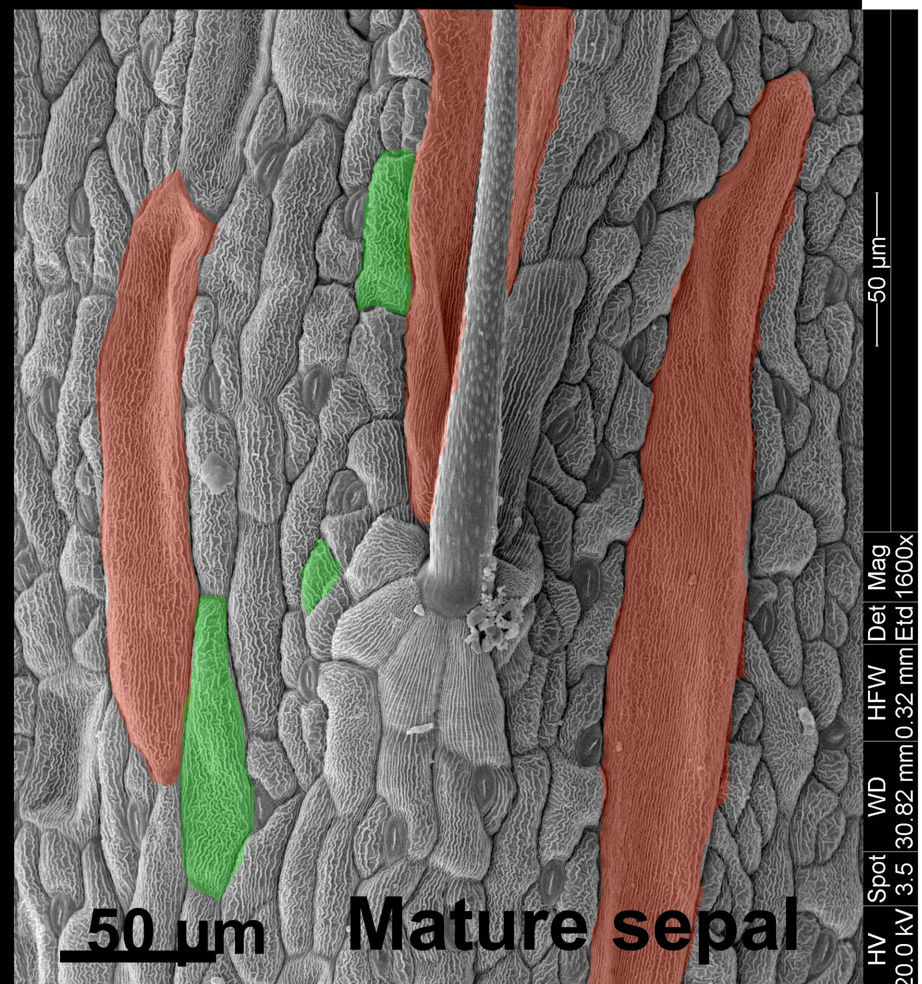
(Melaragno et al., 1993)

How are cell sizes in the sepal patterned?



Immature sepal

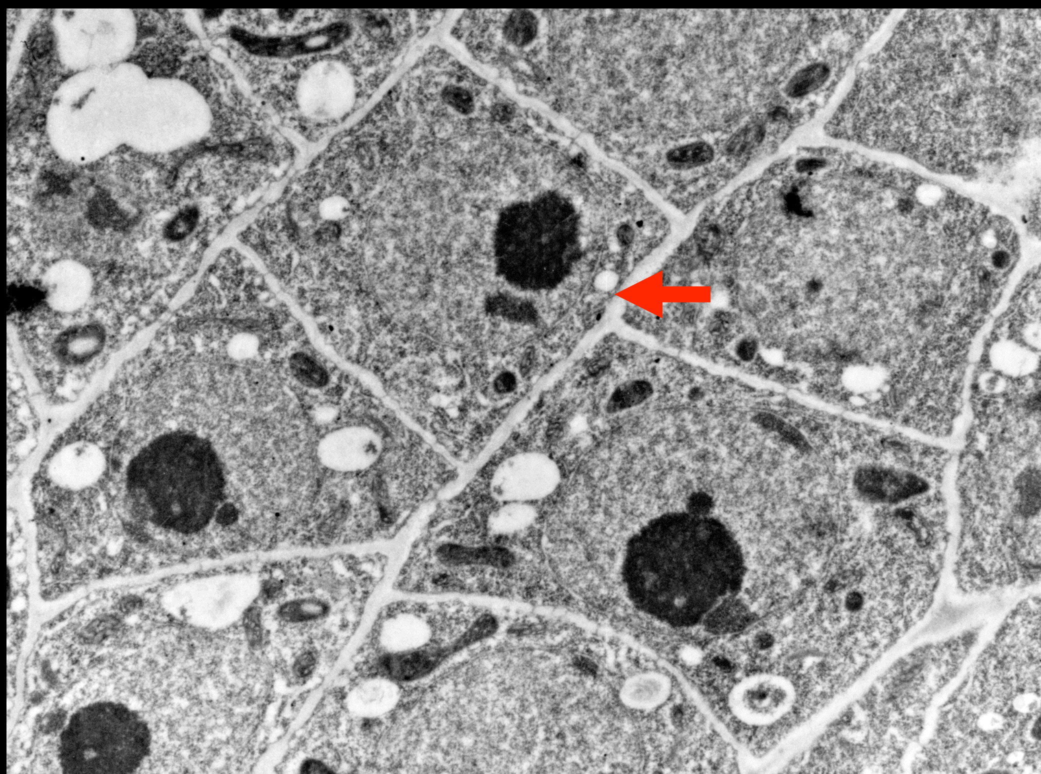
?



Hypotheses for the generation of cells size diversity

- Do larger cells expand faster than smaller cells?
- Do larger cells stop dividing earlier than smaller cells but continue to expand?

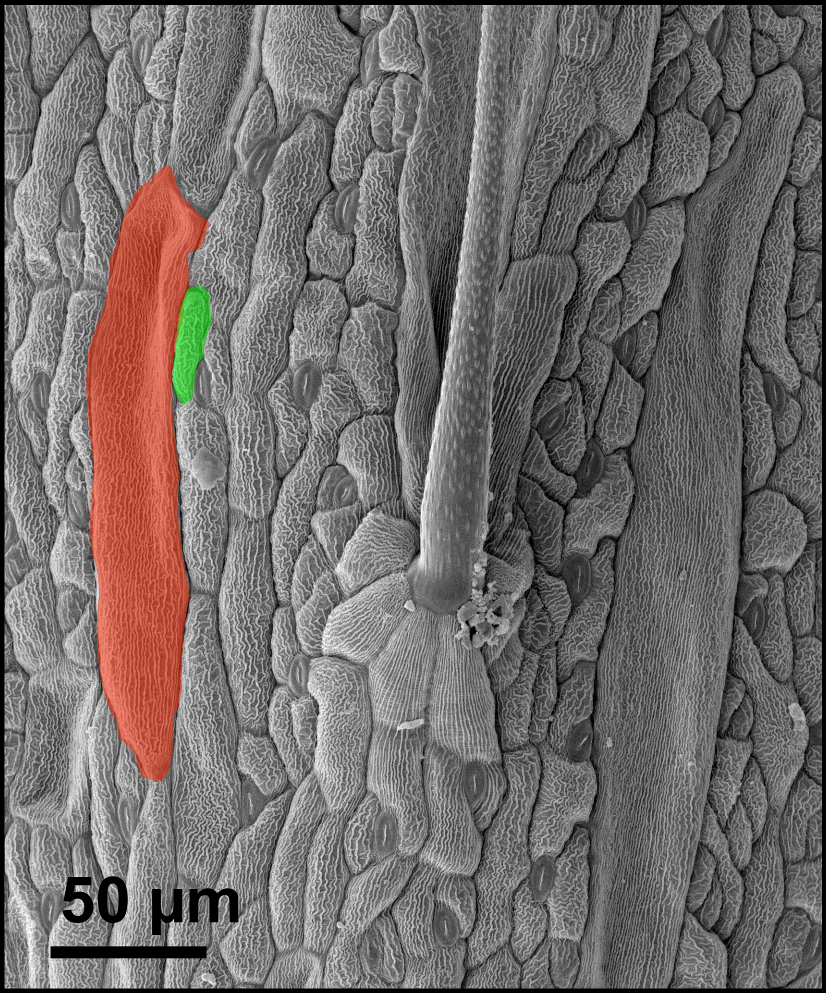
Plant cell walls constrain growth



1. No migration
2. No slipping
3. Growth tightly coordinated

Picture from Elliot Meyerowitz

Small cells and large cells must grow at the same rate



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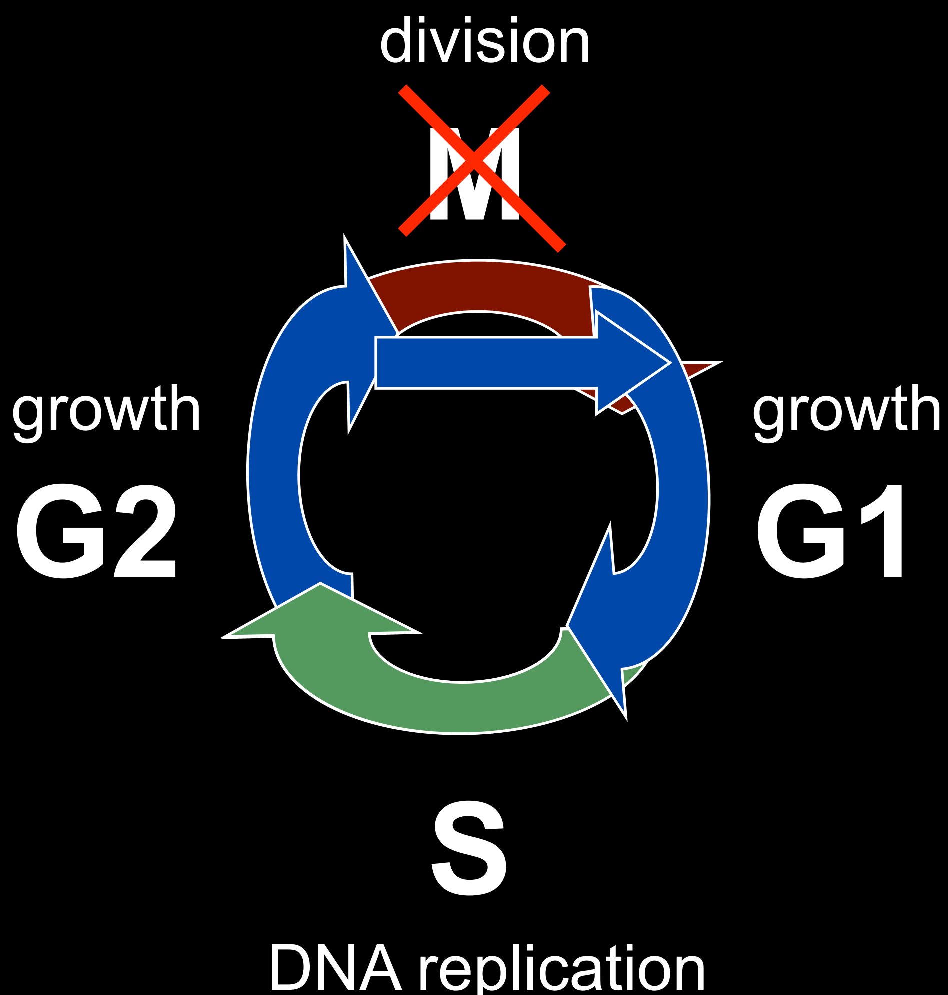
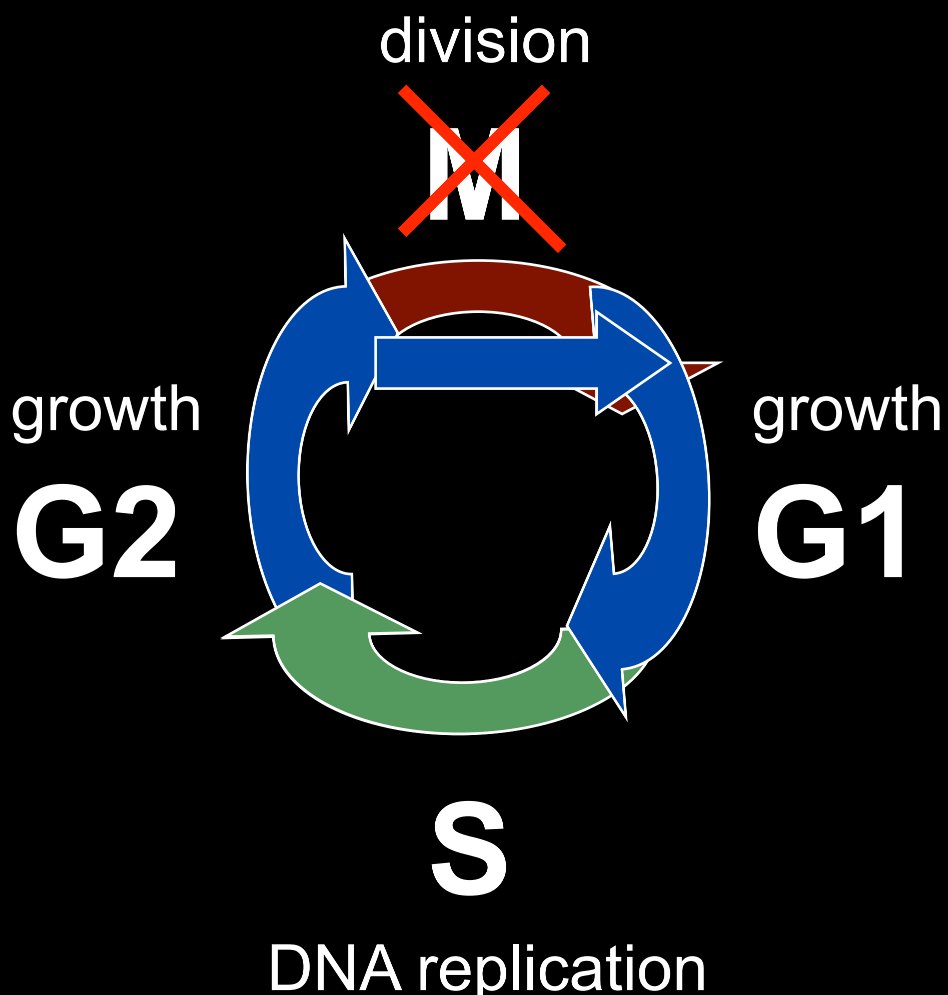
Endoreduplication: DNA replication without division

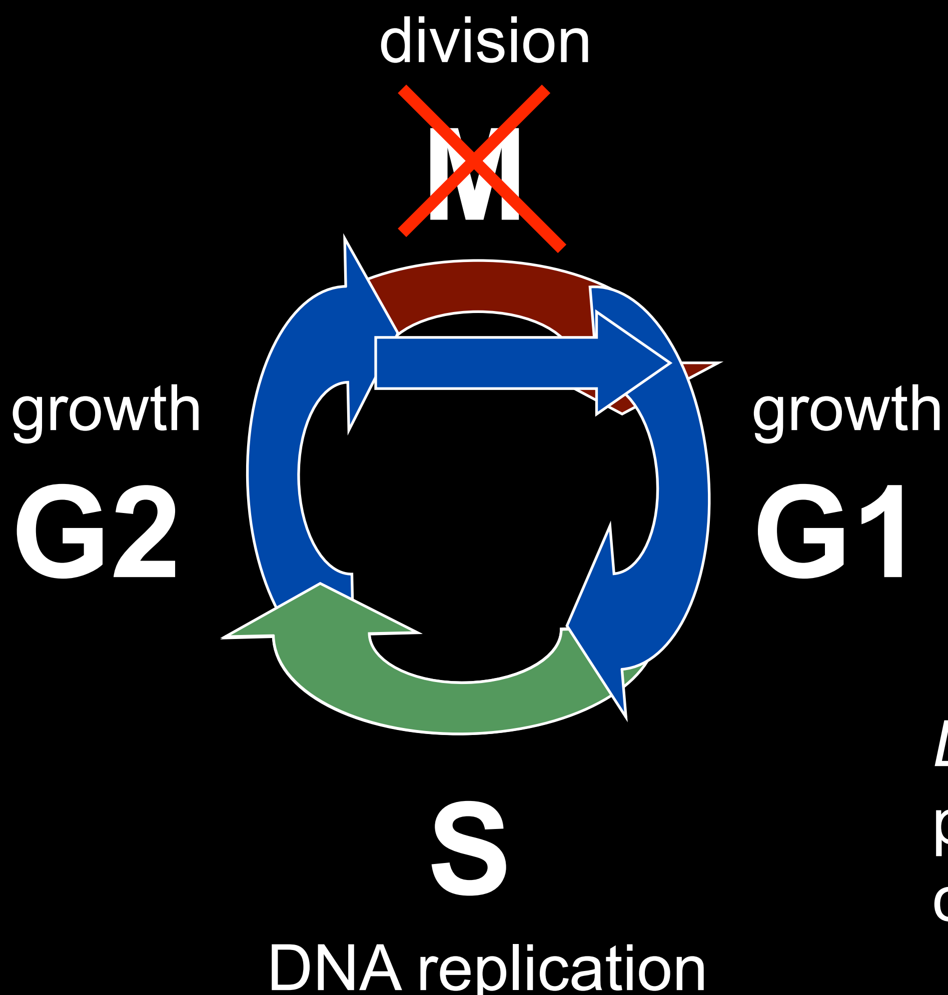


Endoreduplication:
DNA replication without division



Found in:
Plants,
Insects,
Mammals:
megakaryocytes

Endoreduplication: DNA replication without division



Found in:
Plants,
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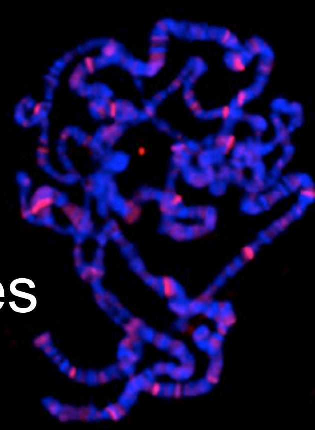
Drosophila polytene chromosomes



http://www.fhcrc.org/science/shared_resources/imaging/image_gallery/polytenes_2.jpg

Questions for today:

1. How does endoreduplication create the cell size pattern in the epidermis?
2. Does endoreduplication increase growth of the organ?
3. How does patterning extend to the whole organ?
4. What causes variability in cell sizes?

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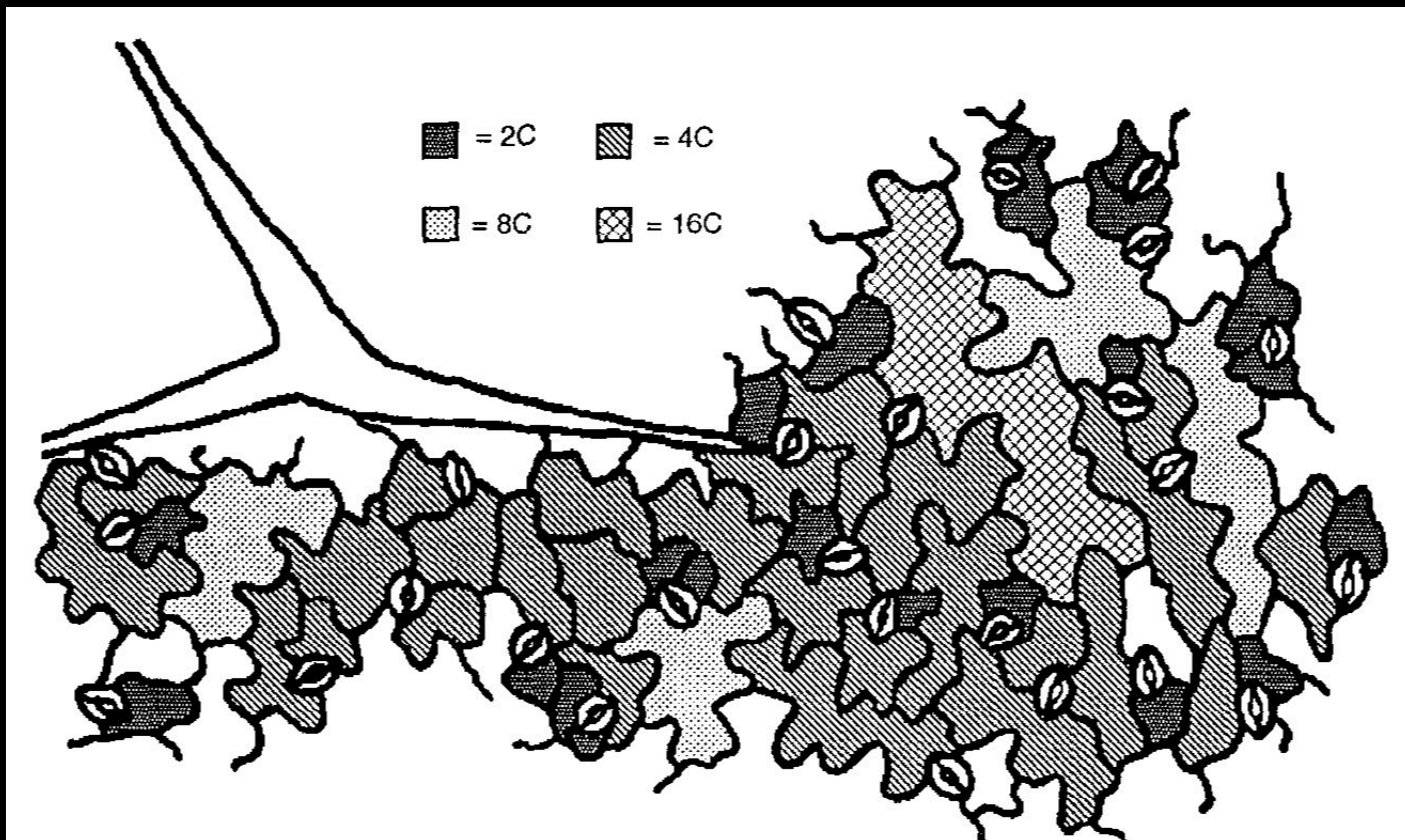
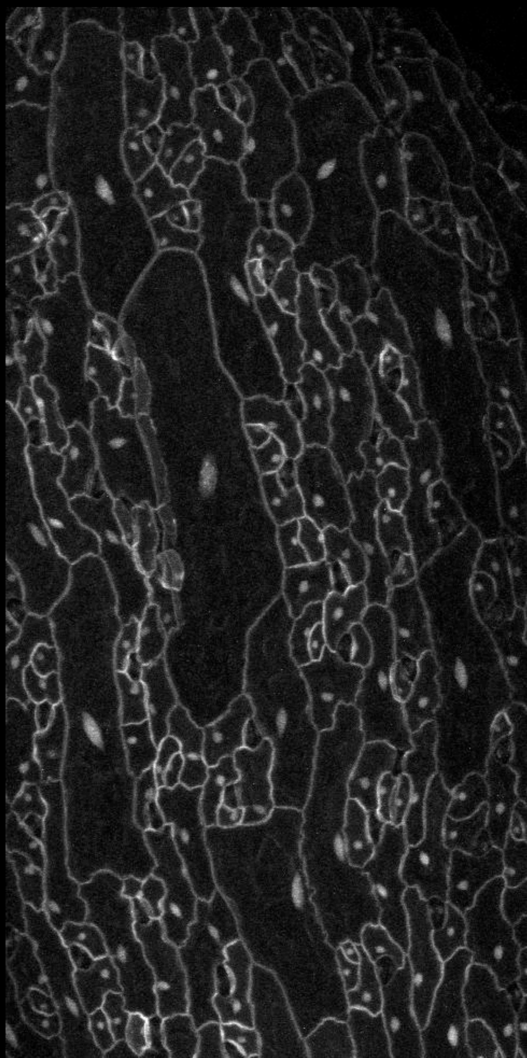
Cell size is correlated with DNA content



(Melaragno et al., 1993)

Giant cells have an increased DNA content



DNA

from epidermal cell

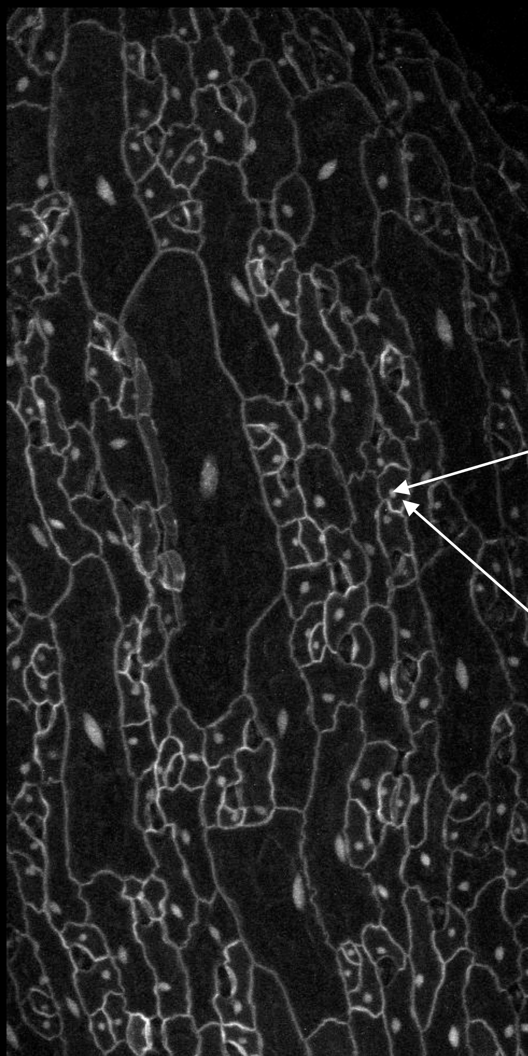
ATML1::H2B-mYFP

plasma membrane

of epidermal cells

ATML1::mCitrine-PIP2;2

Giant cells have an increased DNA content



Small diploid cell

DNA

from epidermal cell

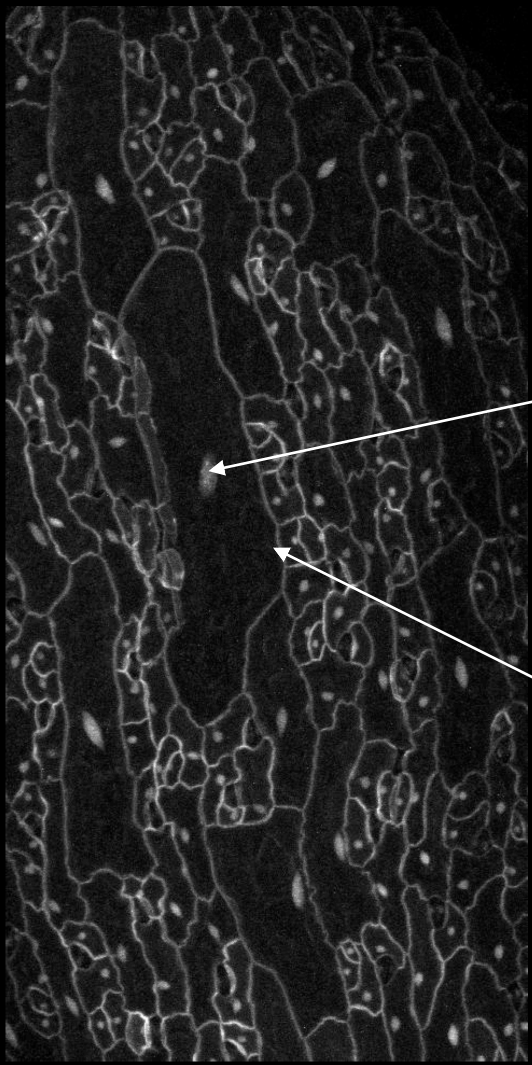
ATML1::H2B-mYFP

plasma membrane

of epidermal cells

ATML1::mCitrine-PIP2;2

Giant cells have an increased DNA content



Giant cell

DNA

from epidermal cell

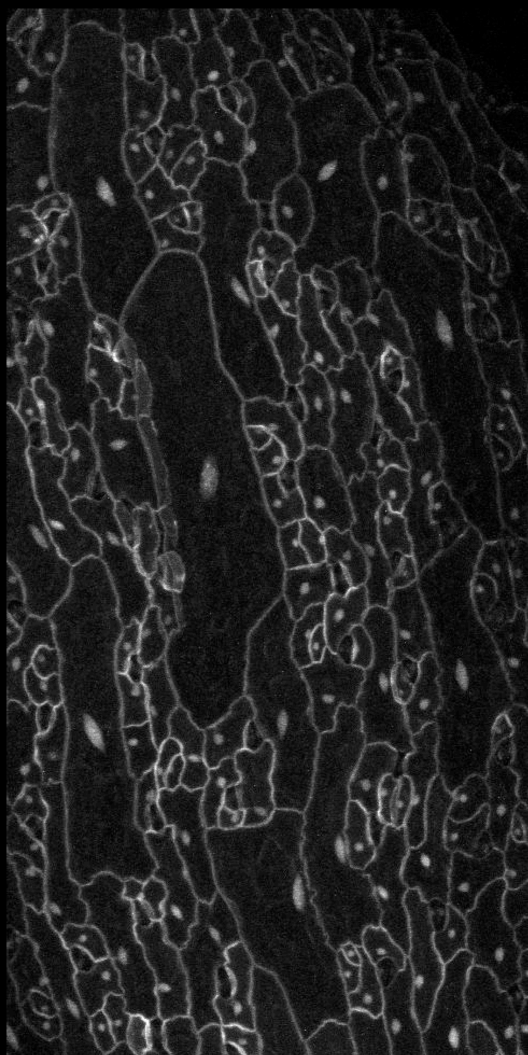
ATML1::H2B-mYFP

plasma membrane

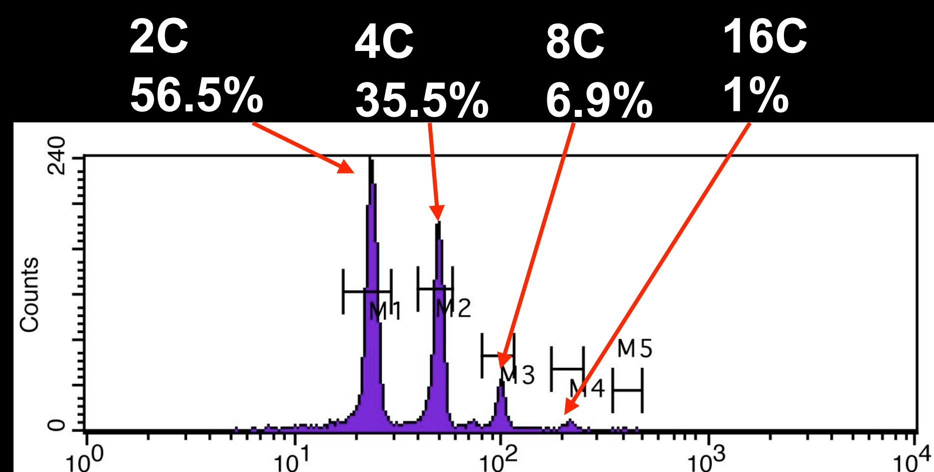
of epidermal cells

ATML1::mCitrine-PIP2;2

Giant cells endoreduplicate to 16C



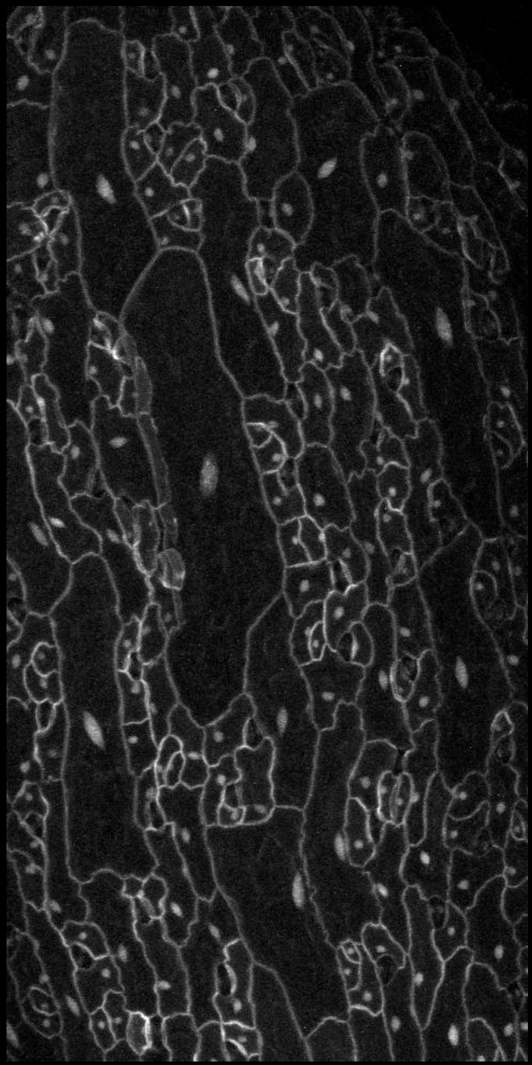
DNA content of epidermal nuclei

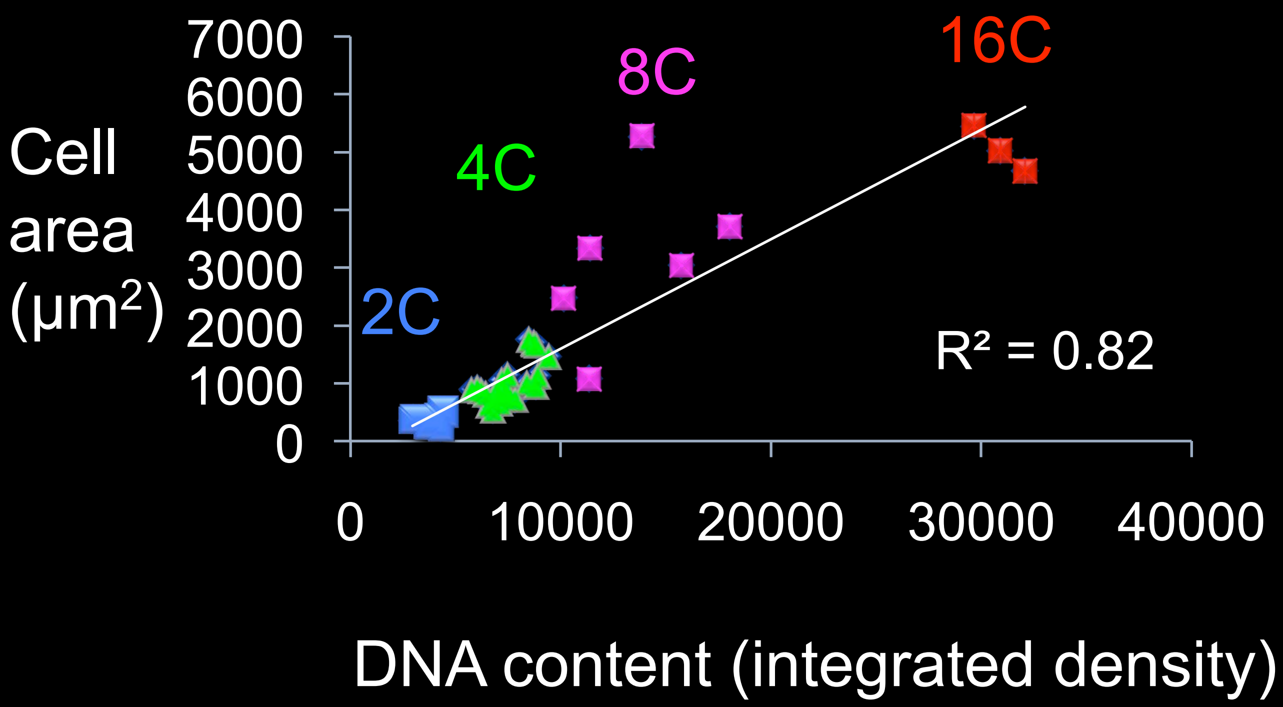


DNA content

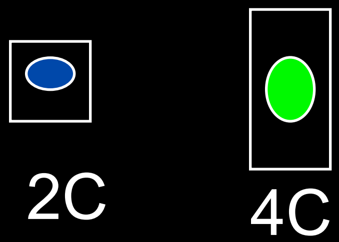
2C = two copies of the chromosomes

Size correlates about 80% with DNA content





Nuclear : Cytoplasm ratio is a constant



How does endoreduplication create the cell size pattern?

In plants, once a cell endoreduplicates, it almost never divides.

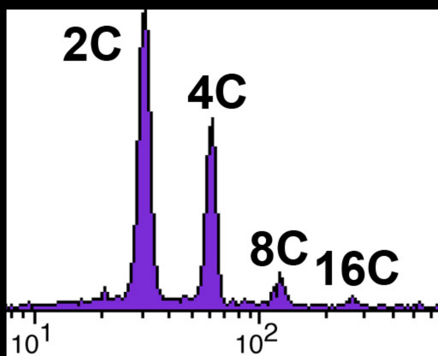
Collaboration with Computable Plant Group to create a model

Alex Cunha

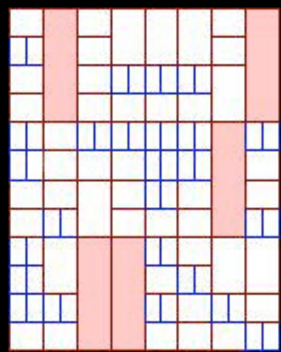
Vijay Chickarmane

Computational morphodynamics

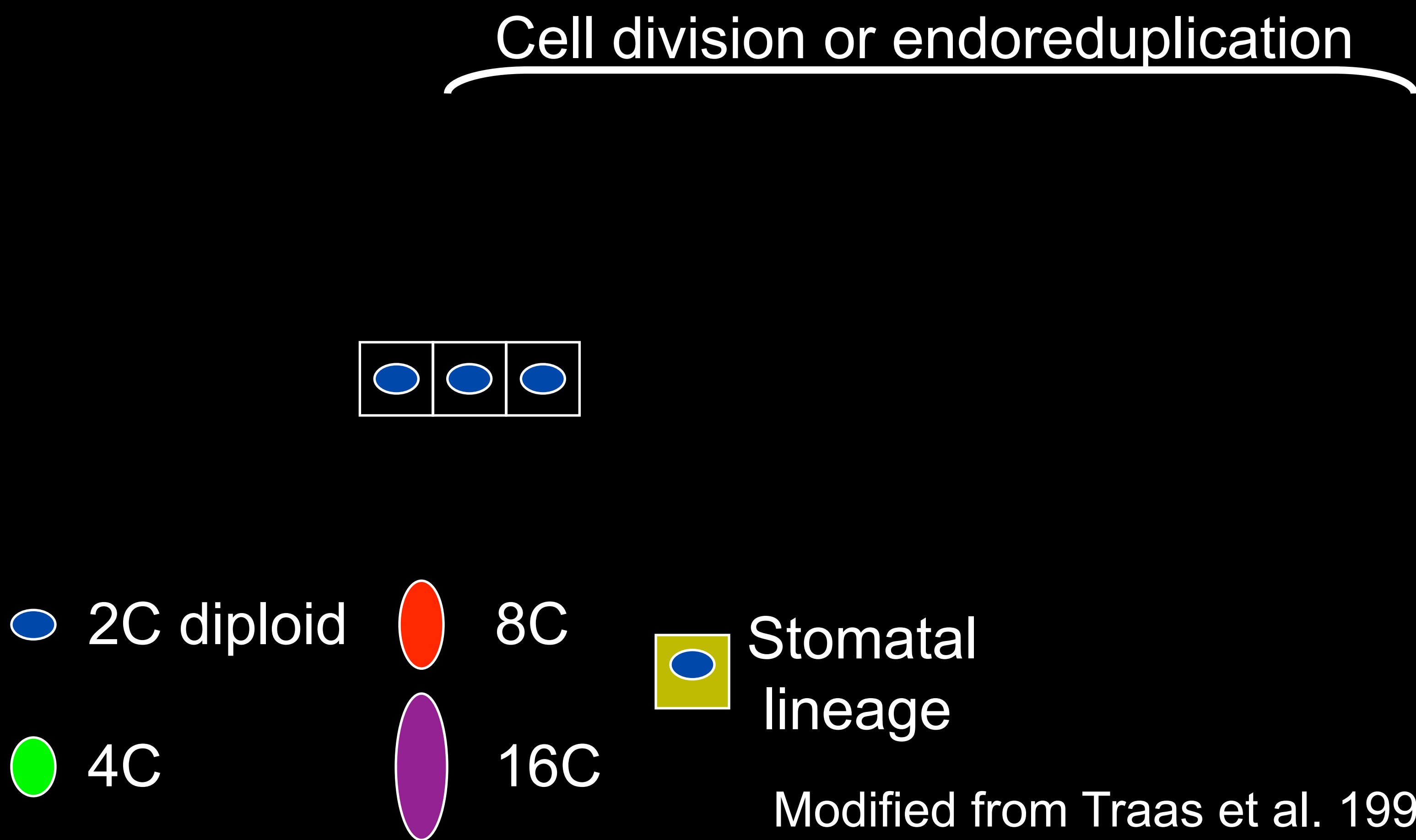
experiments



modeling



Hypothesis: Cell size diversity generated by timing of endoreduplication



Modified from Traas et al. 1998

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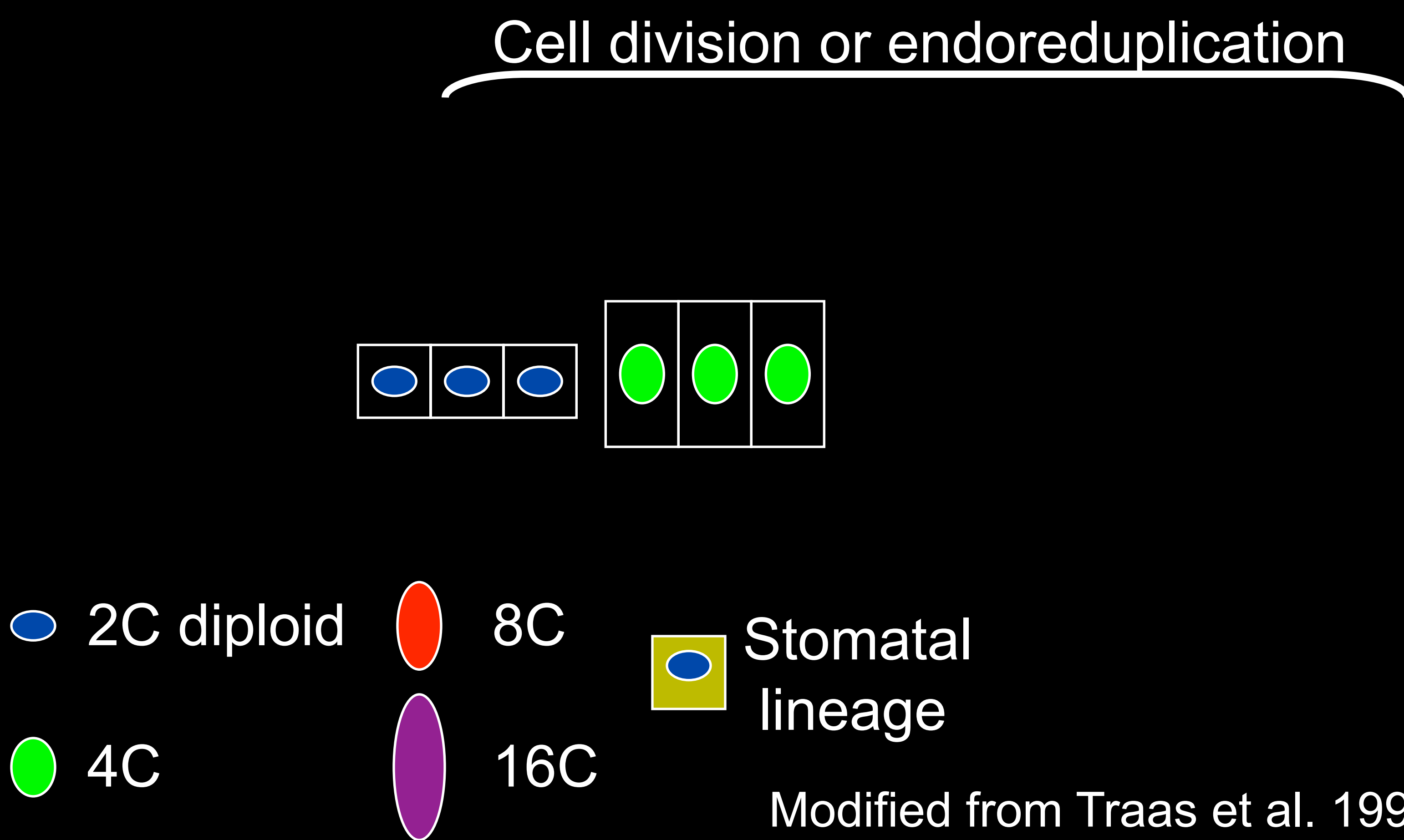

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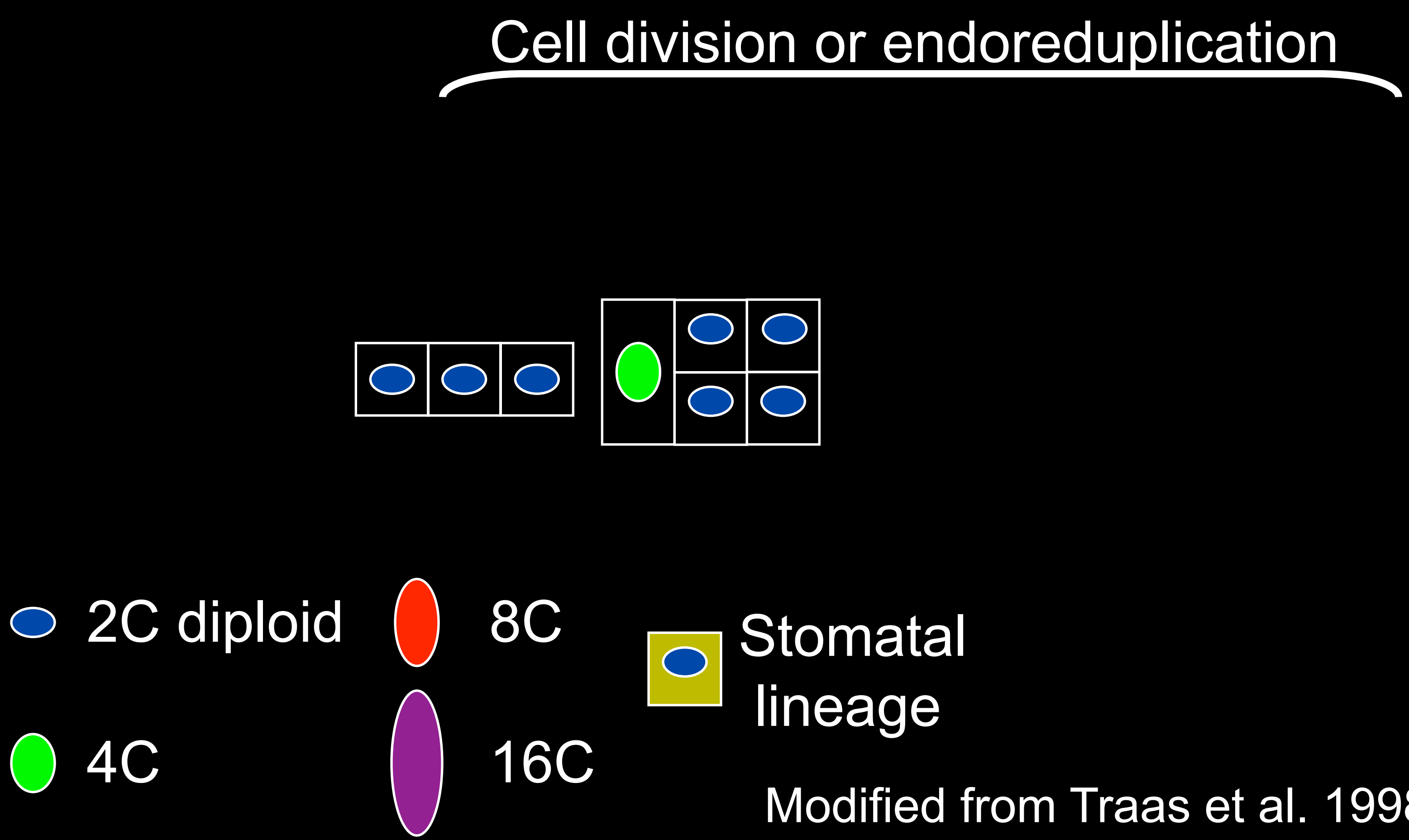

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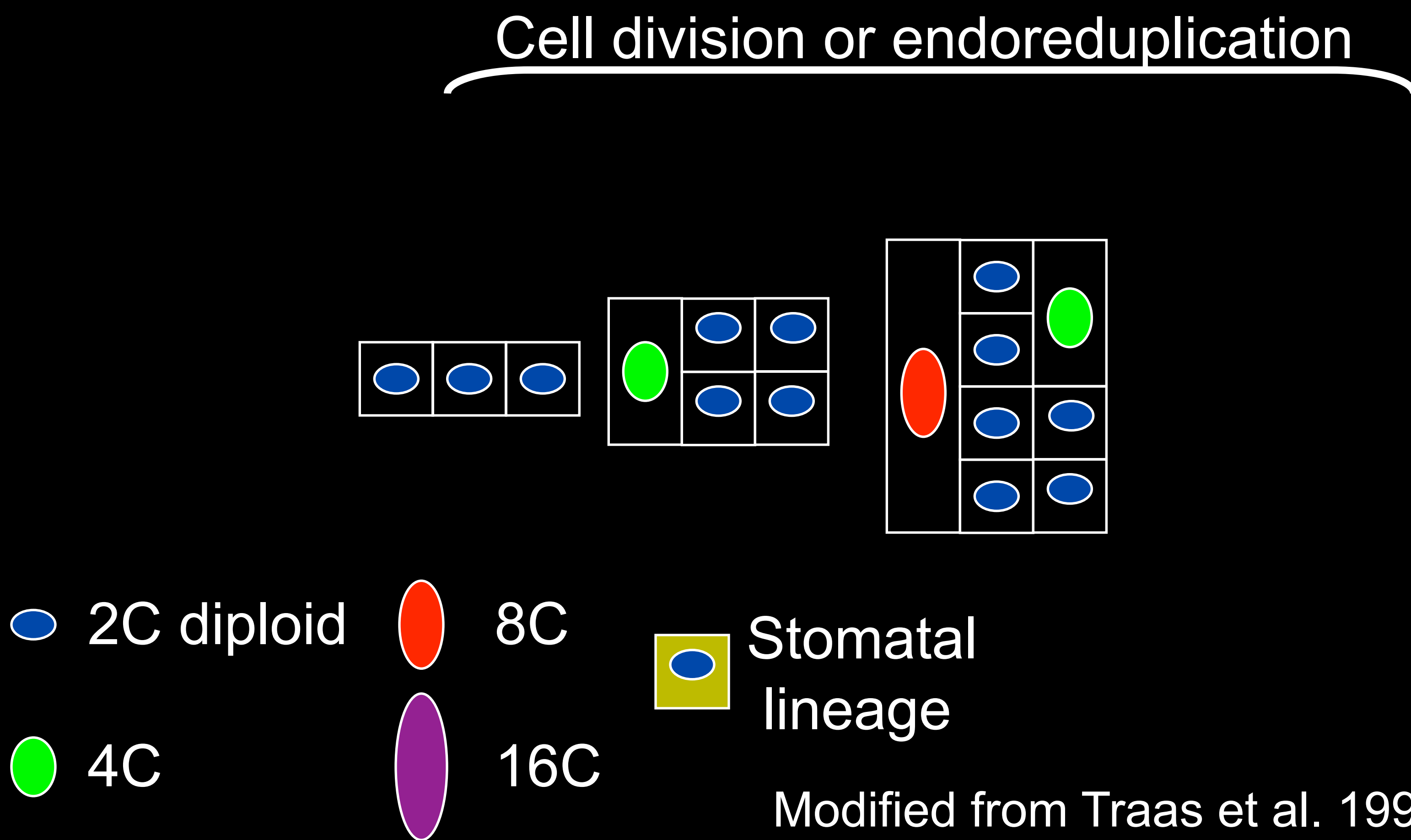

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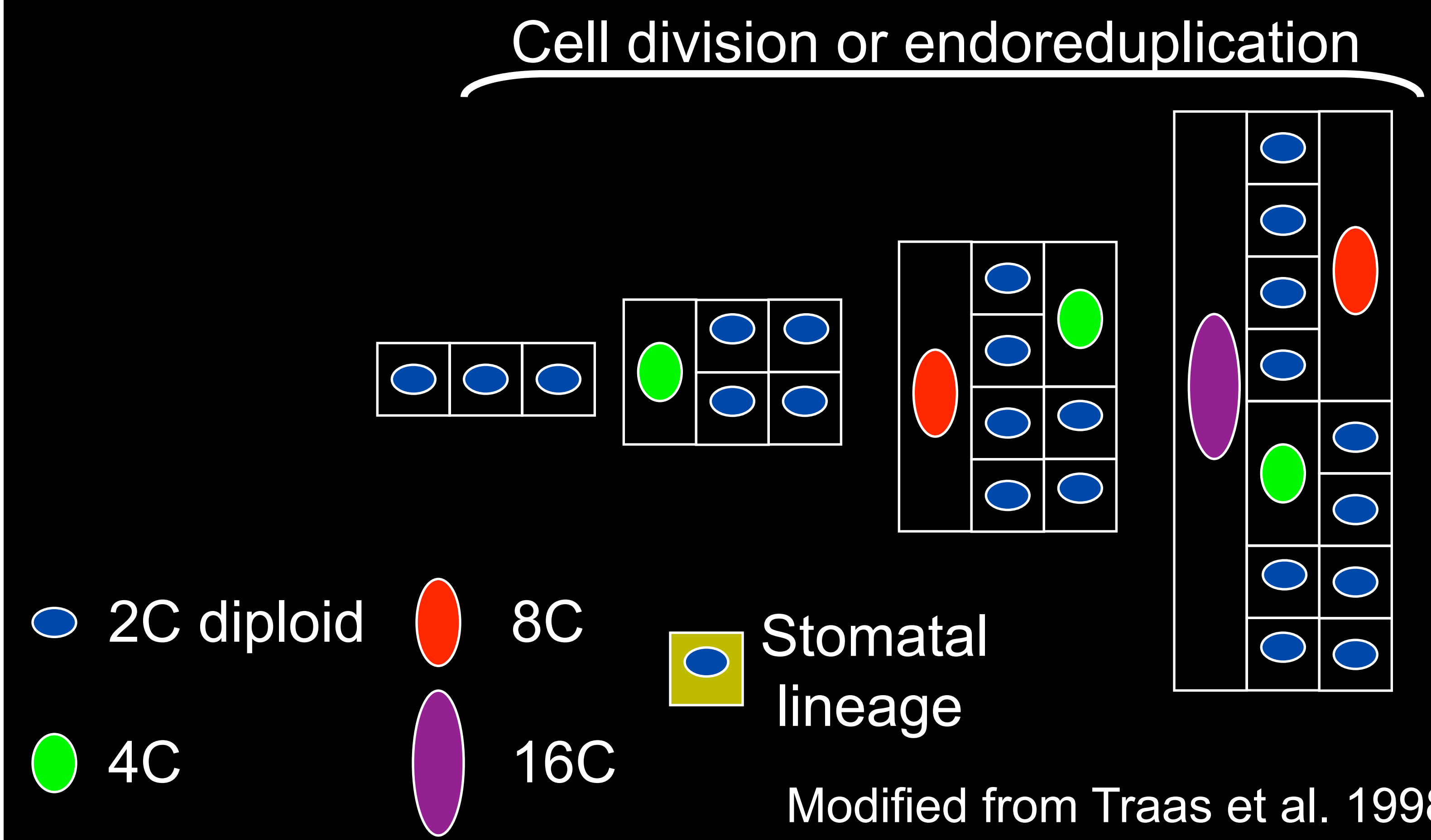

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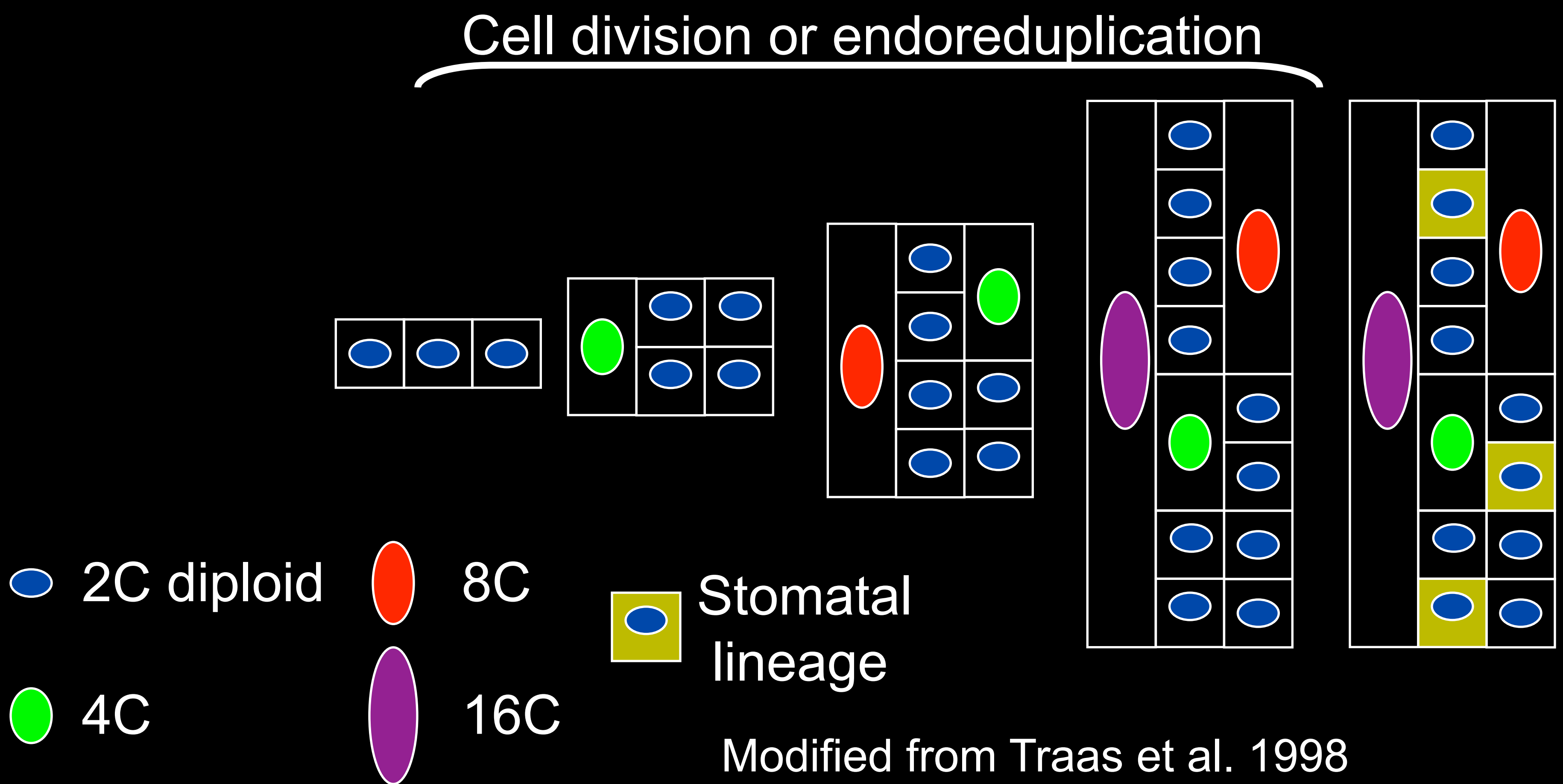

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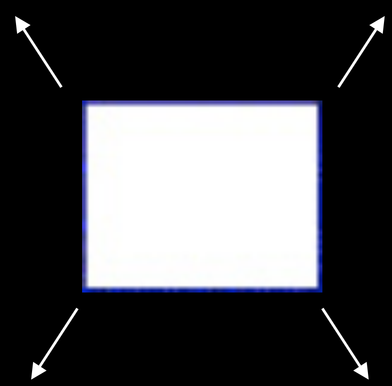
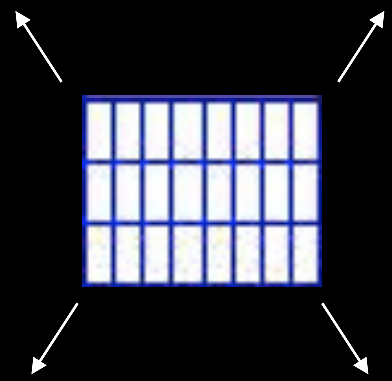
Modeling the sepal as a uniformly growing template



Template:
sepal epidermis = expanding rectangle

Modeling the sepal as a uniformly growing template



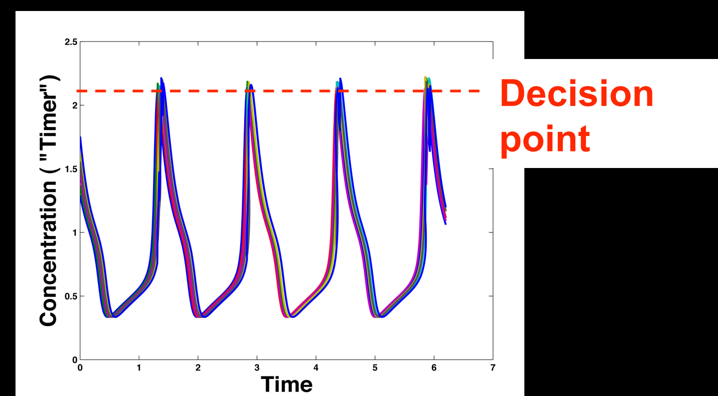
Template:
sepal epidermis = expanding rectangle

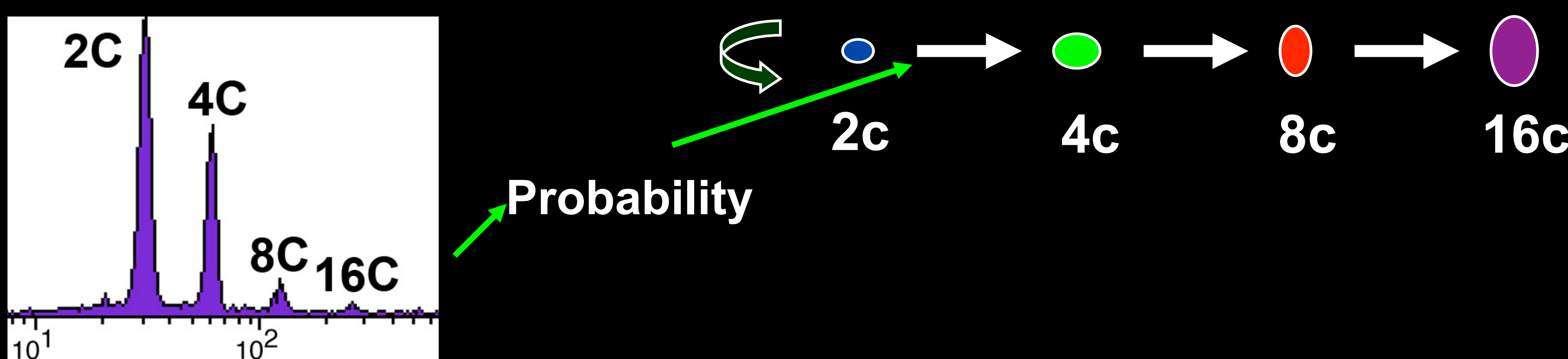
Cells grow with the template.

Computational modeling: Cells divide or endoreduplicate at the decision point

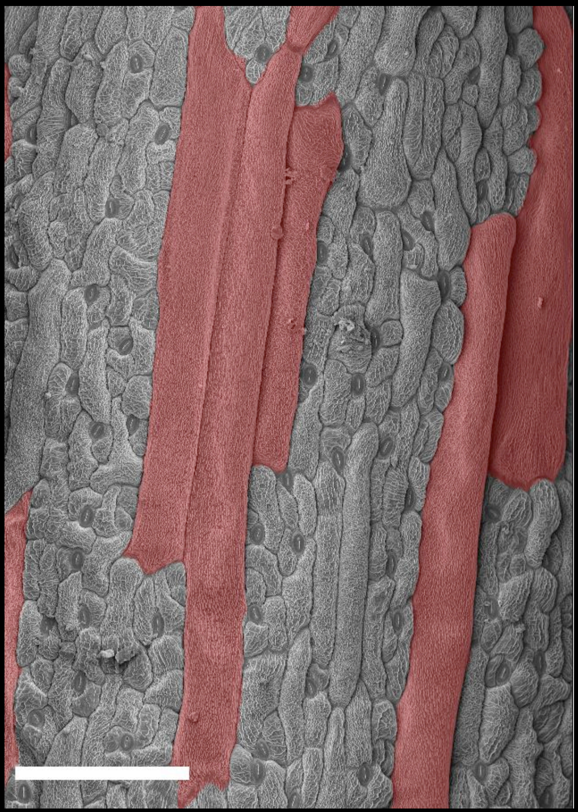
1. 2C cell at decision point => divide OR endoreduplicate with a given probability.

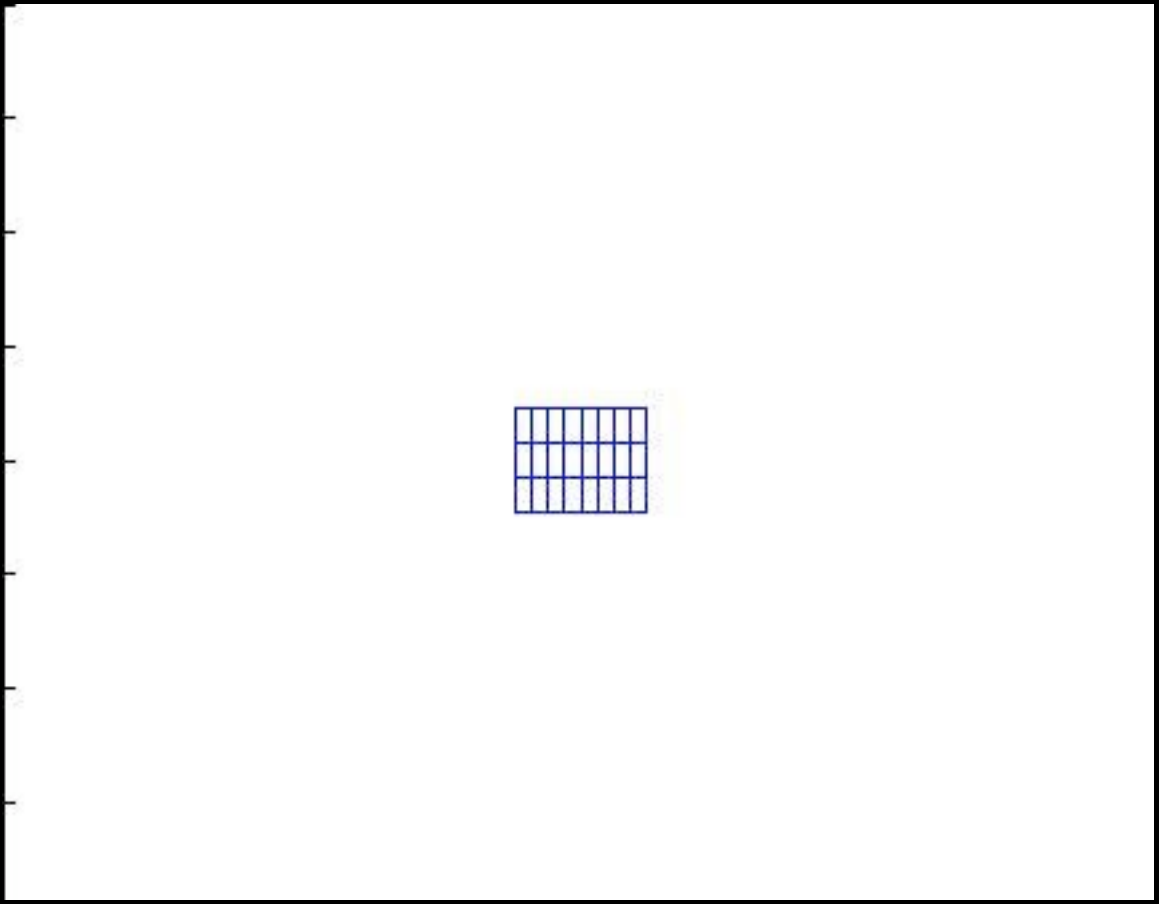
2. Endoreduplicated cell at decision point => endoreduplicate.





Model recapitulates wild type giant cells and small cells





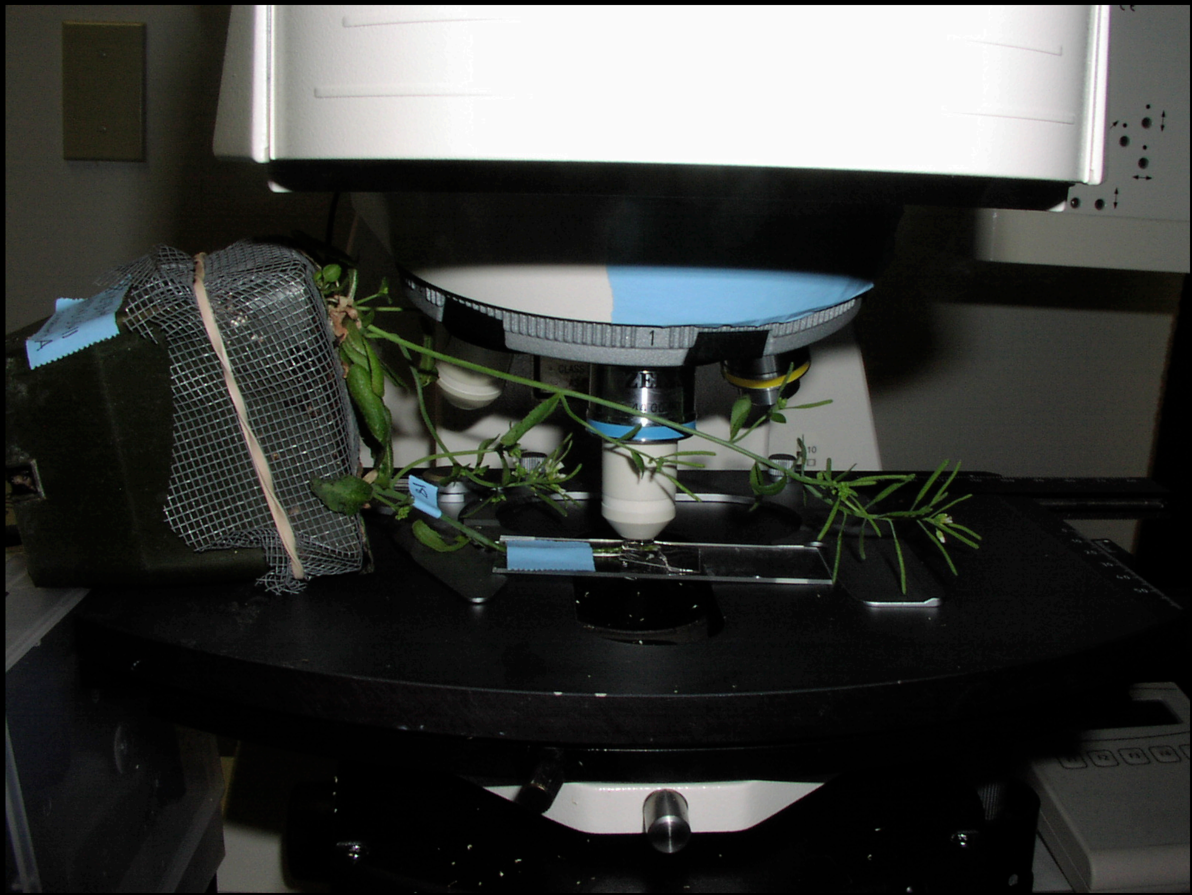
How does endoreduplication create the cell size distribution?

in silico: **The time at which a cell endoreduplicates can create a pattern of diverse sizes.**

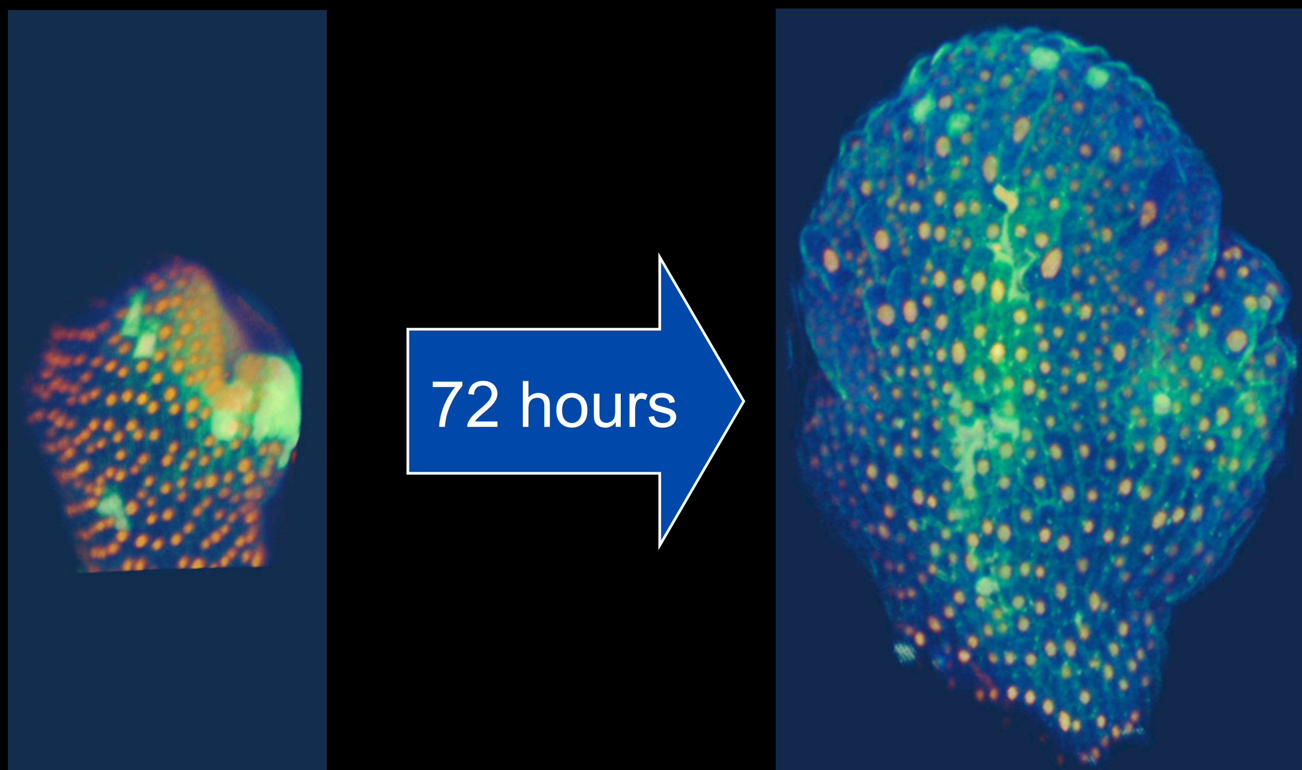
in vivo: **?**

test with live imaging

Testing the hypothesis by imaging living sepals



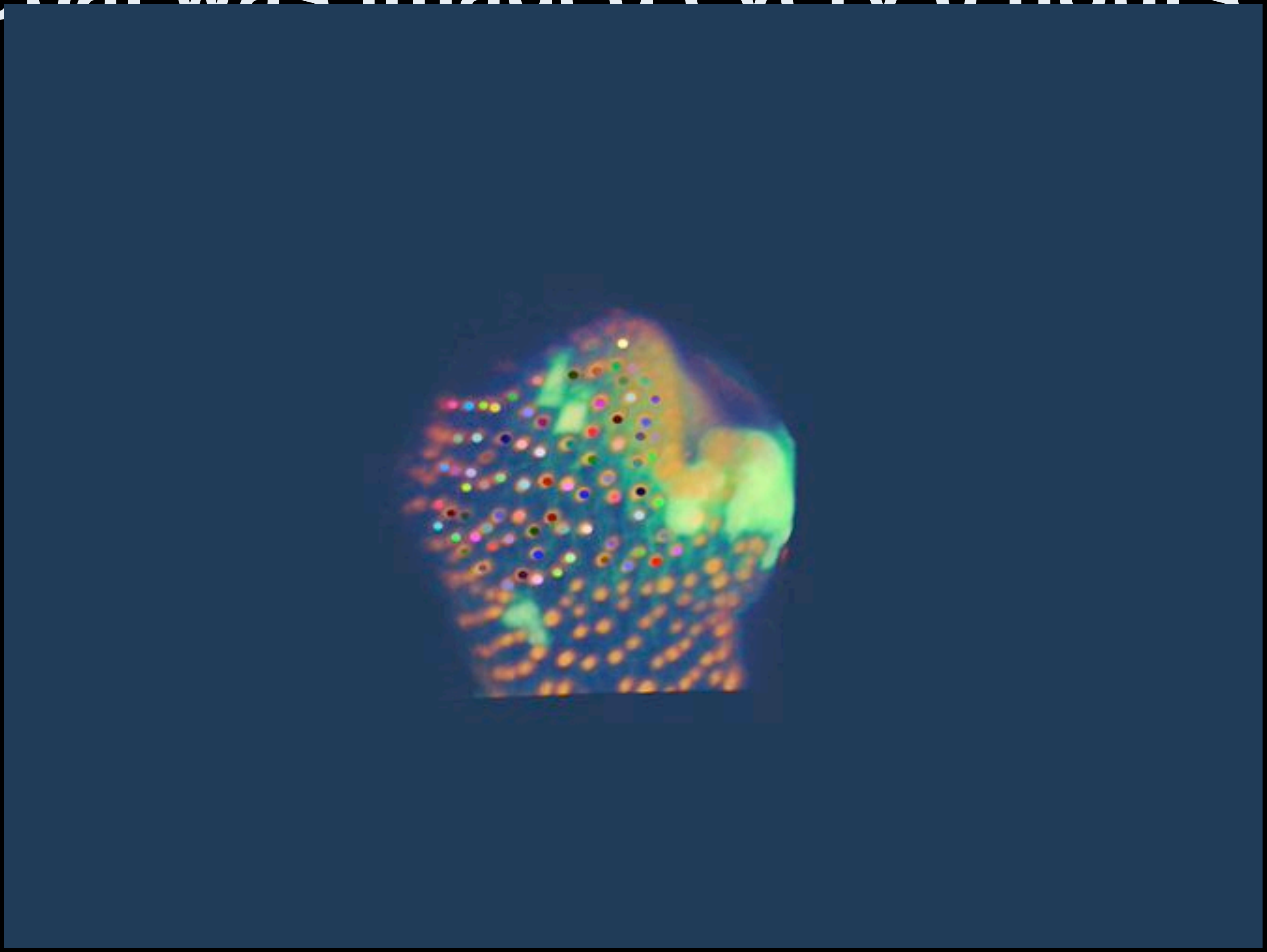
Sepal was imaged ever 6 hours for 3 days



Epidermal cell DNA
ATML1::Histone2B-mYFP

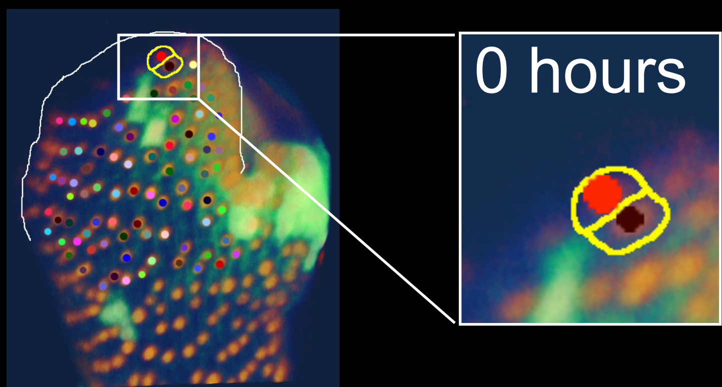
Cell wall
Propidium iodide

Sepal was imaged every 6 hours



Do the giant cells endoreduplicate while the small cells divide?

Giant cells endoreduplicate while small cells divide



12

24

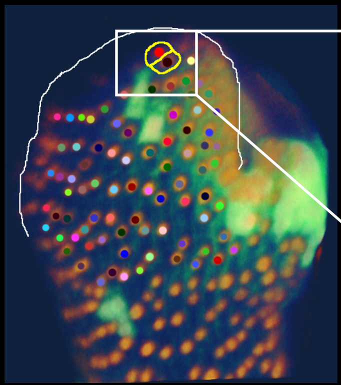
36

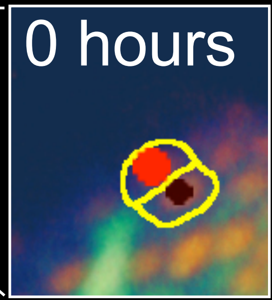
48

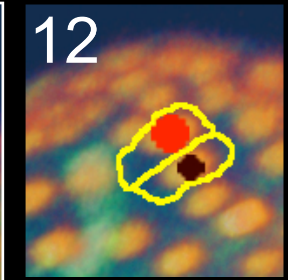
60

72

Giant cells endoreduplicate while small cells divide







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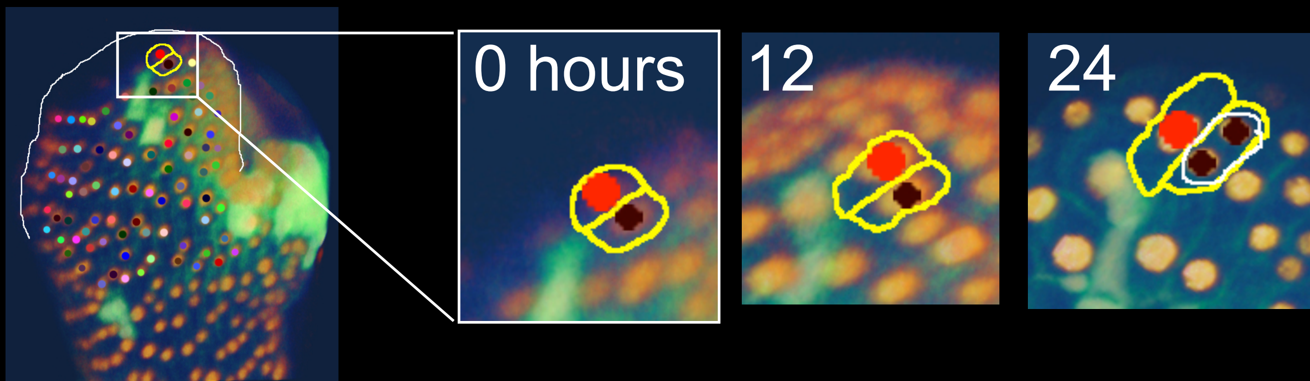

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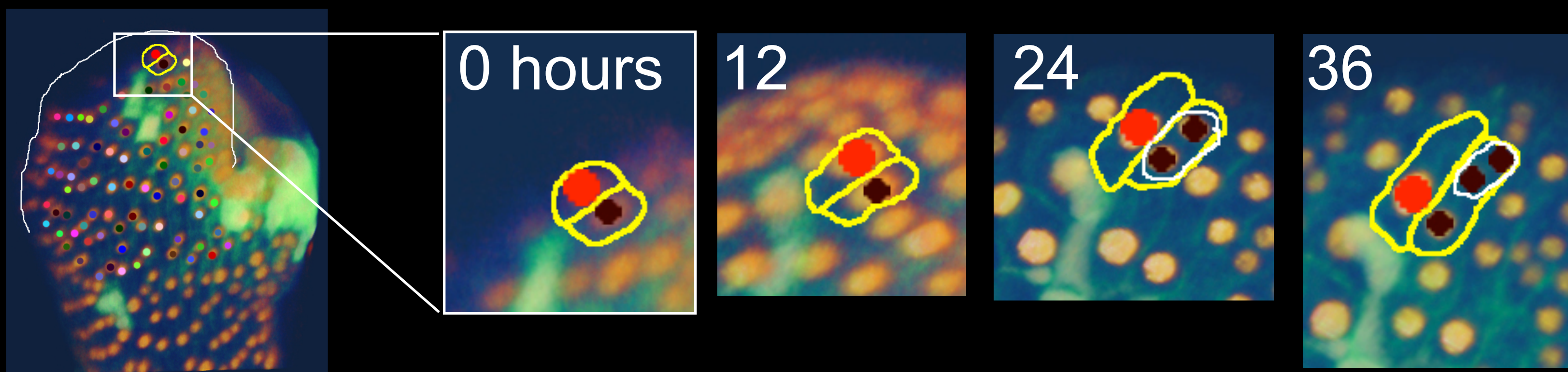

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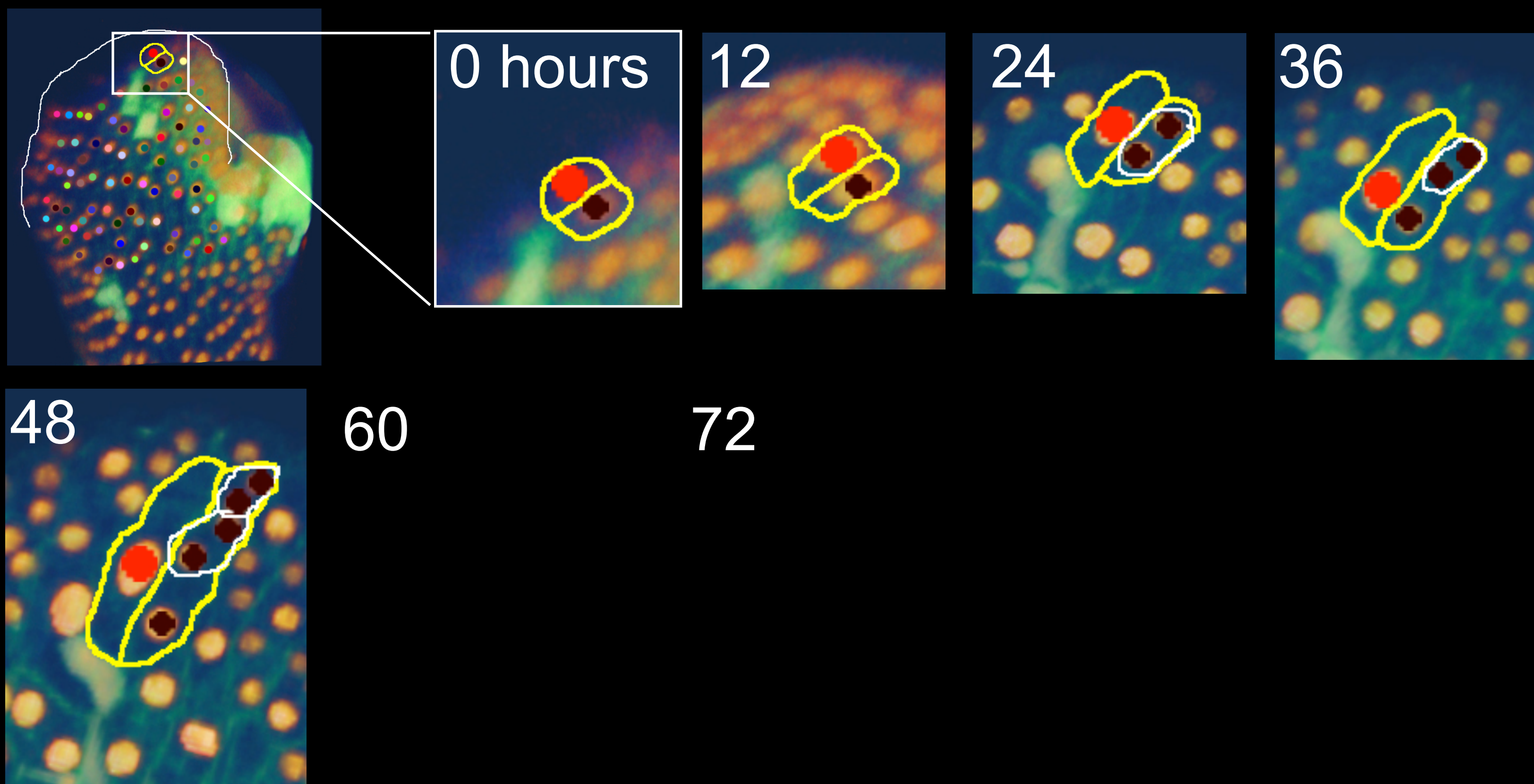
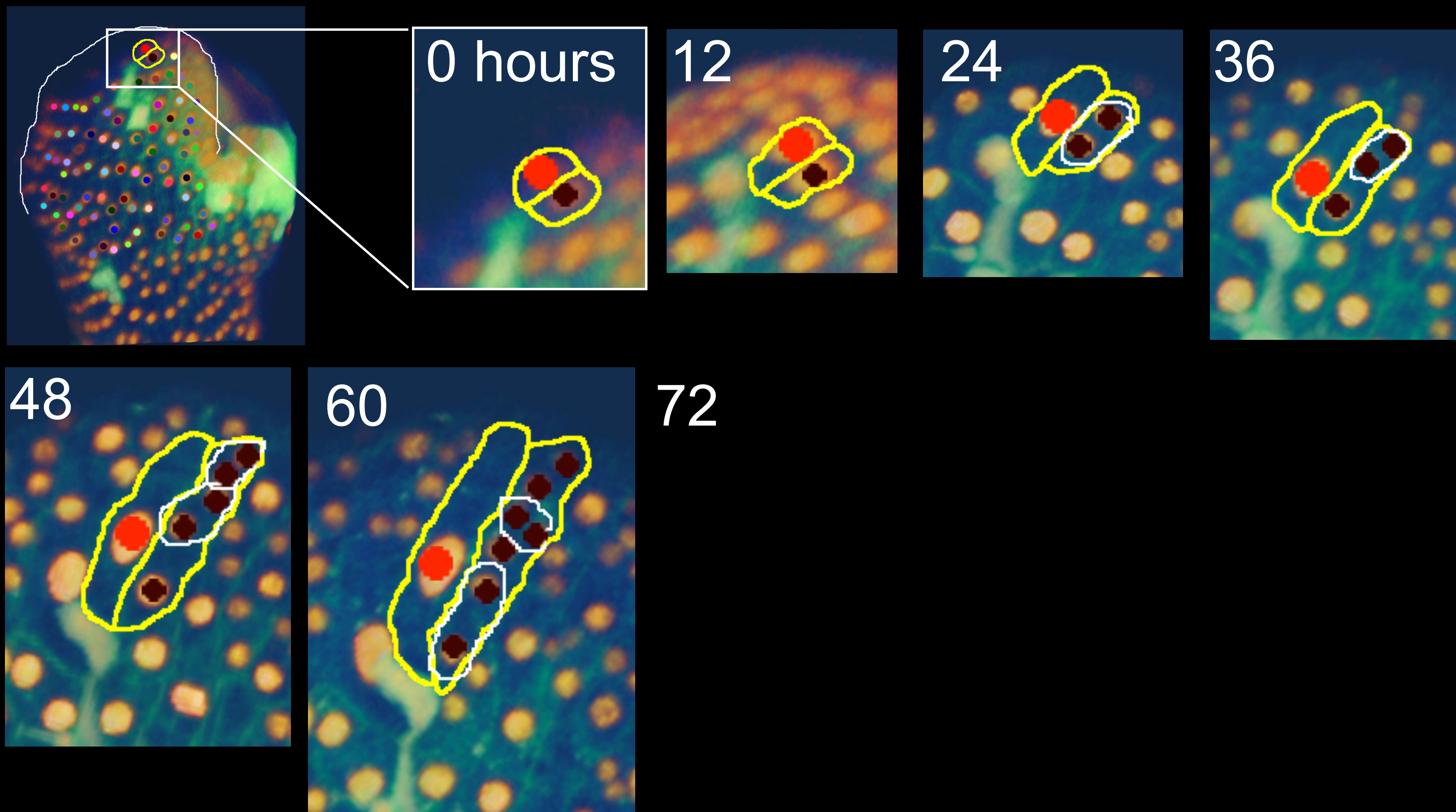


Giant cells endoreduplicate while small cells divide



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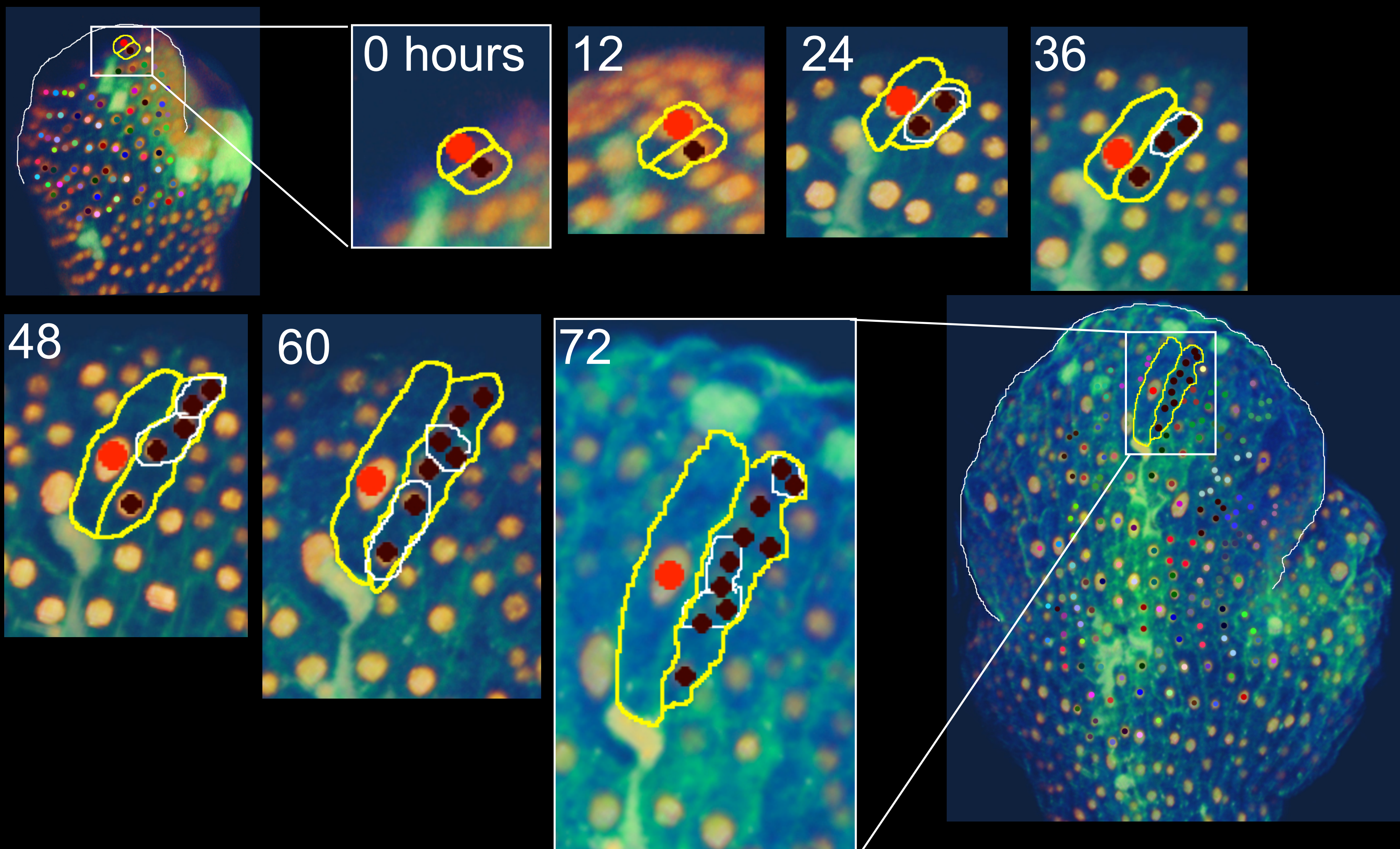
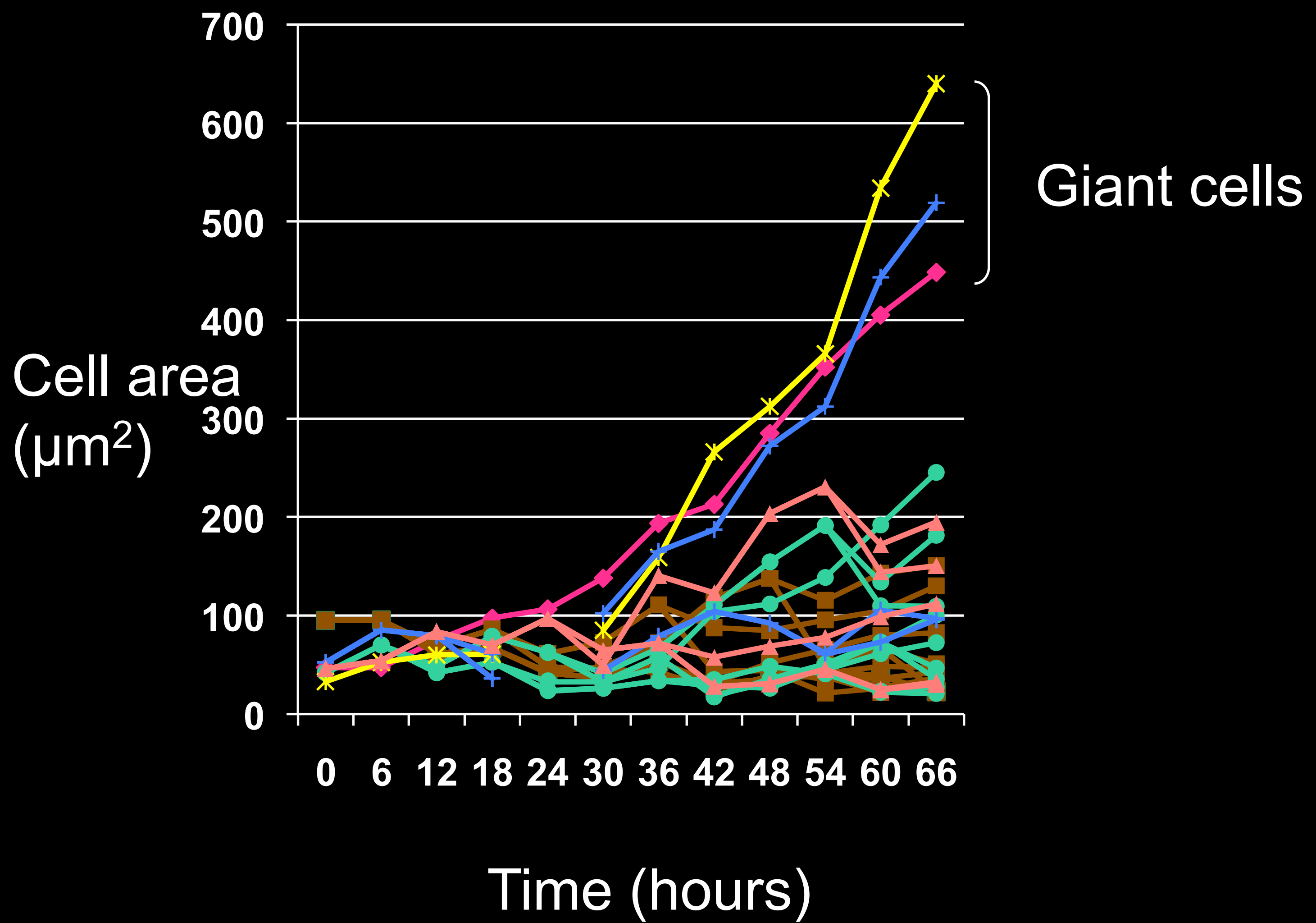


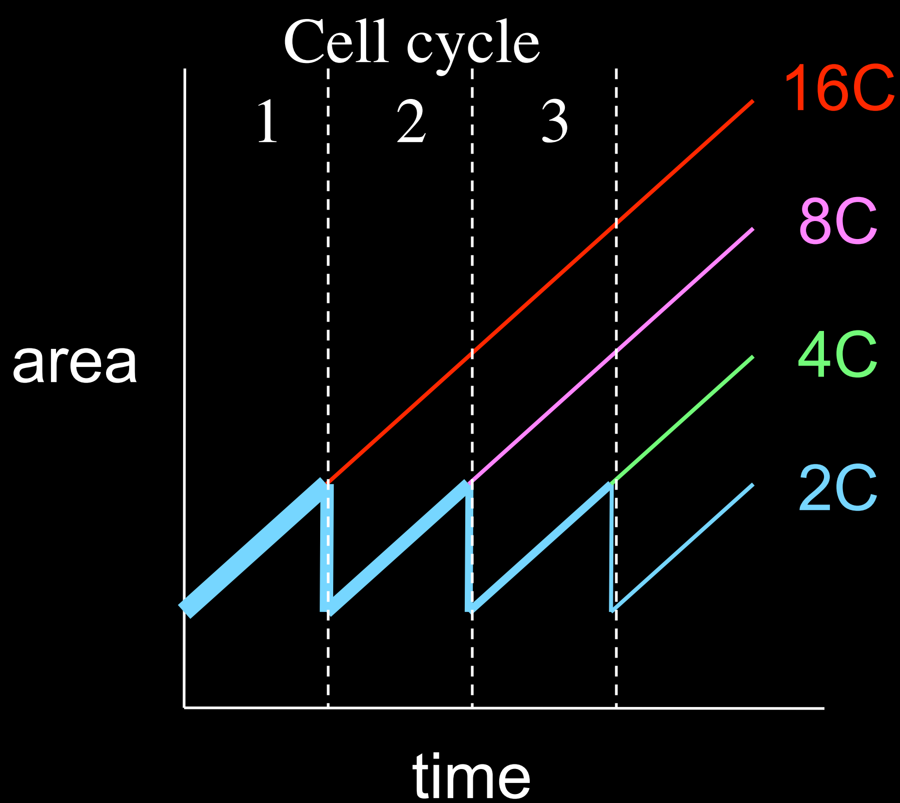
Change in cell area over time



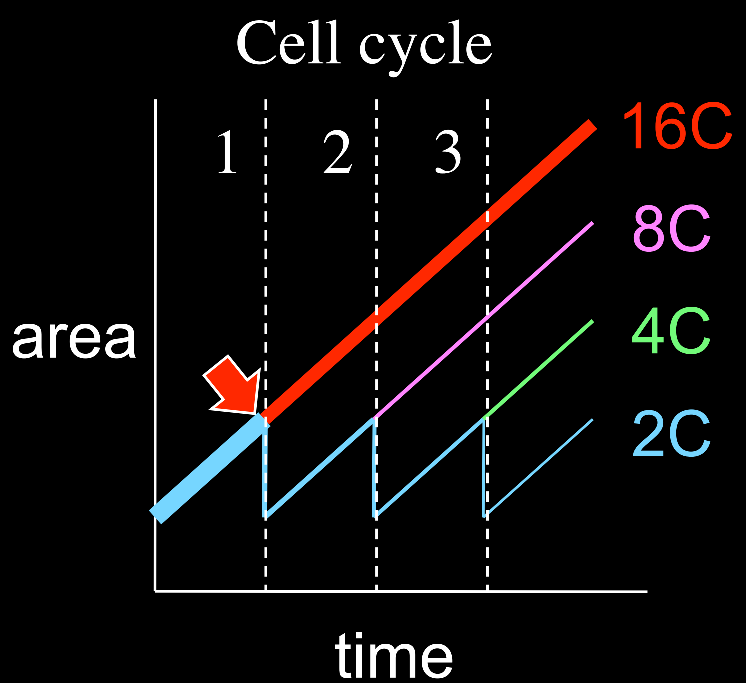
Summary:

Early endoreduplication ➡ **Giant cell**

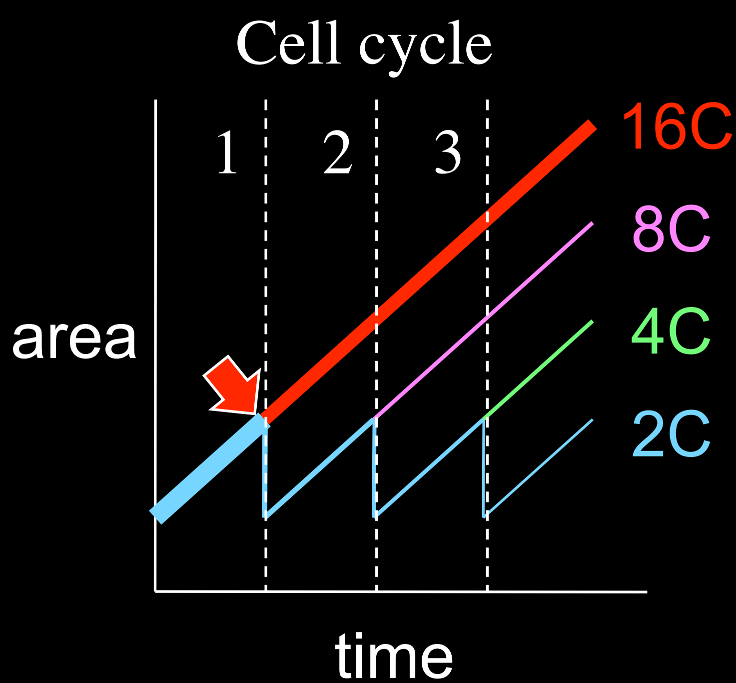
Late / no endoreduplication ➡ **Small cell**

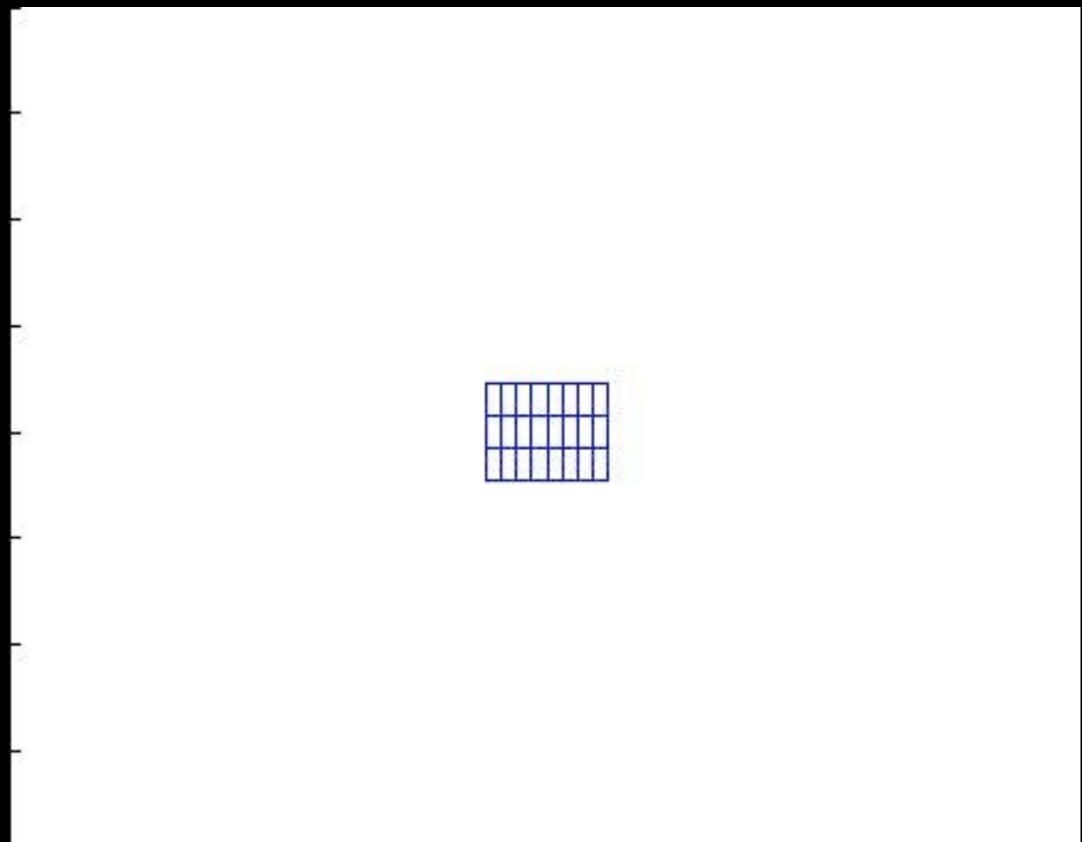


Model prediction: extra giant cells

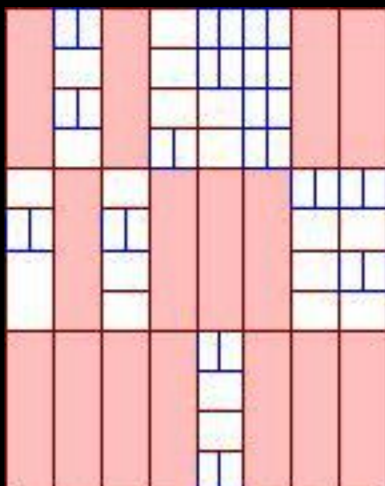


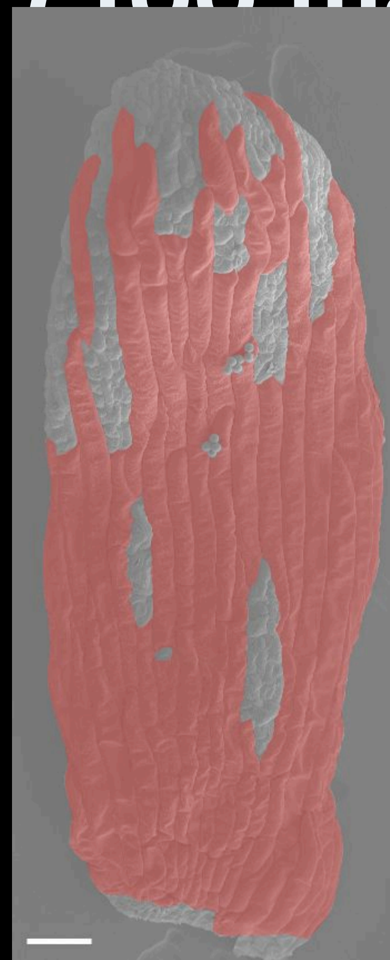
Model prediction: extra giant cells

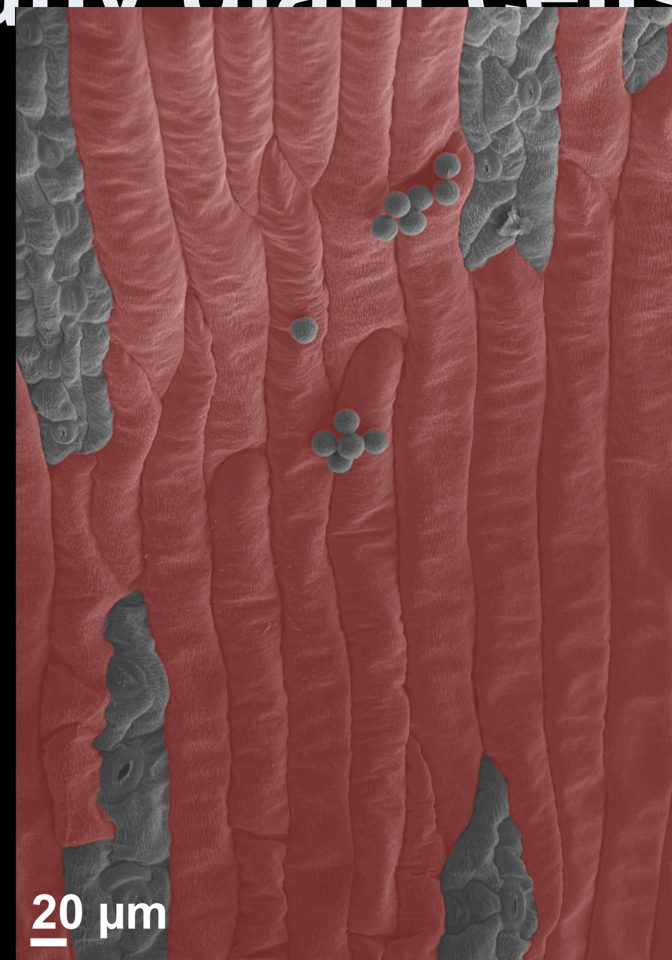




ATML1::KRP1 too many giant cells







ATML1::KRP1

Ectopic expression of a cell cycle inhibitor throughout the epidermis

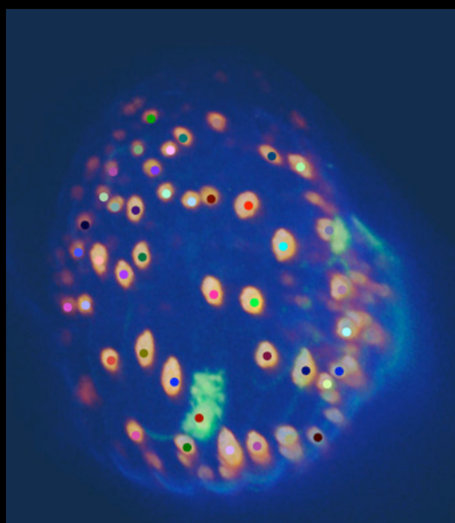
Bemis and Torii, 2007

How is a cell size distribution created?

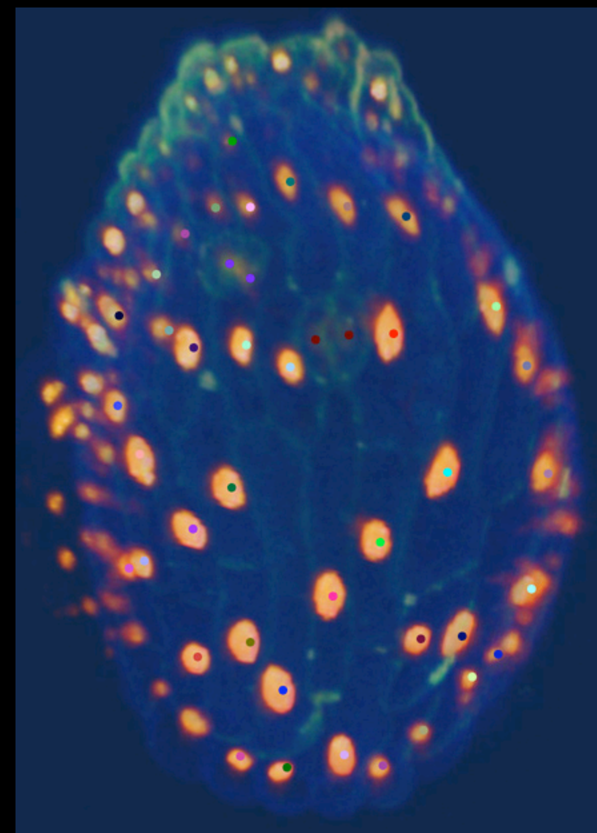
in silico:
Extra early endoreduplication ➡ *ATML1::KRP1* phenotype

in vivo: ?
test with live imaging

Live imaging of *ATML1::KRP1* sepal



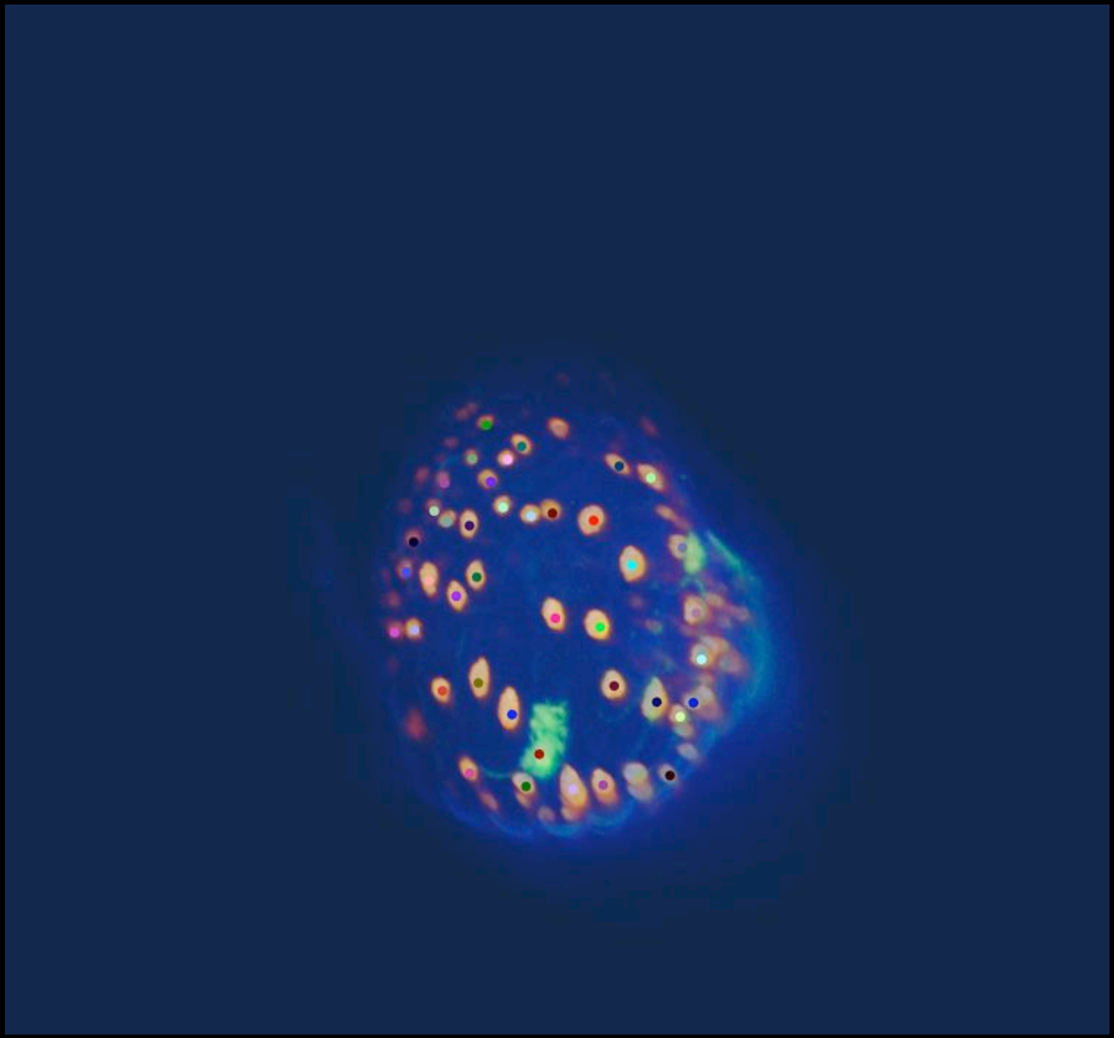
60 hours



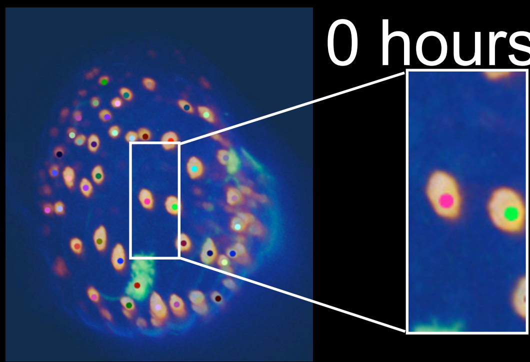
Epidermal cell DNA
ATML1::HISTONE2B-mYFP

Cell wall/dead cells
Propidium iodide

ATML1::KRP1 sepal cells endoreduplicate



ATML1::KRP1 cells endoreduplicate instead of dividing



0 hours

12

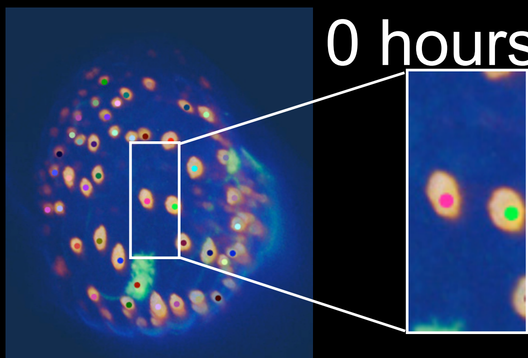
24

36

48

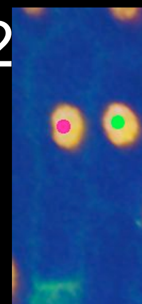
60

ATML1::KRP1 cells endoreduplicate instead of dividing



0 hours

12



24

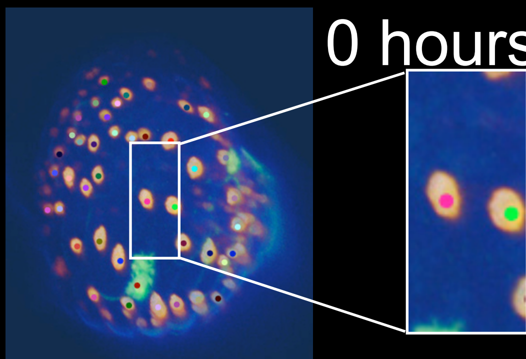
36

48

60

ATML1::KRP1 cells endoreduplicate instead of dividing

0 hours



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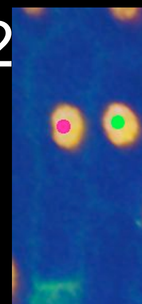
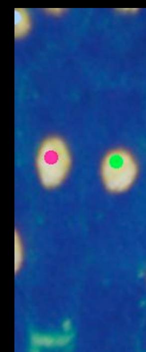


24

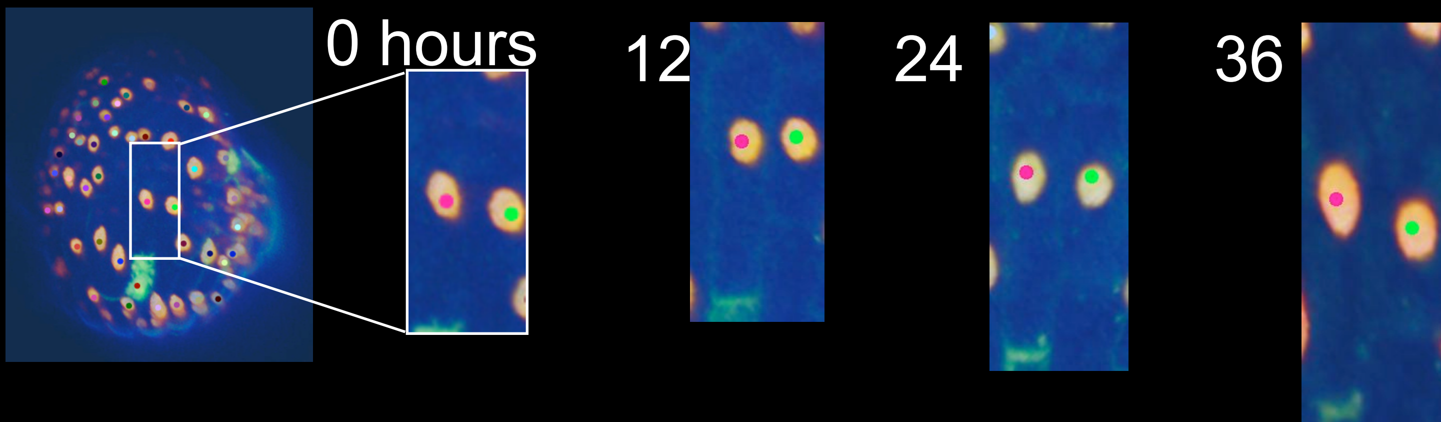


36

48

60

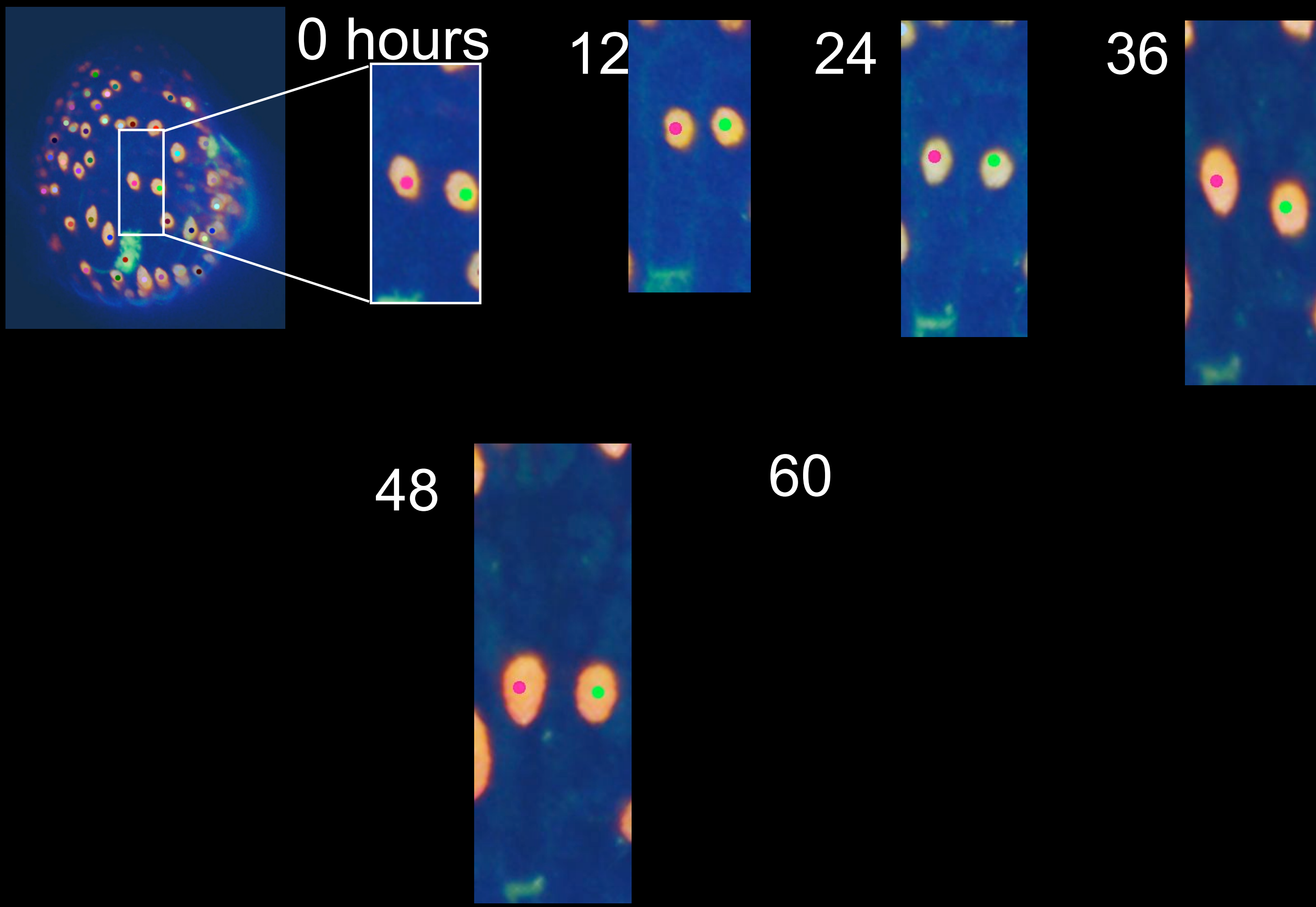
ATML1::KRP1 cells endoreduplicate instead of dividing



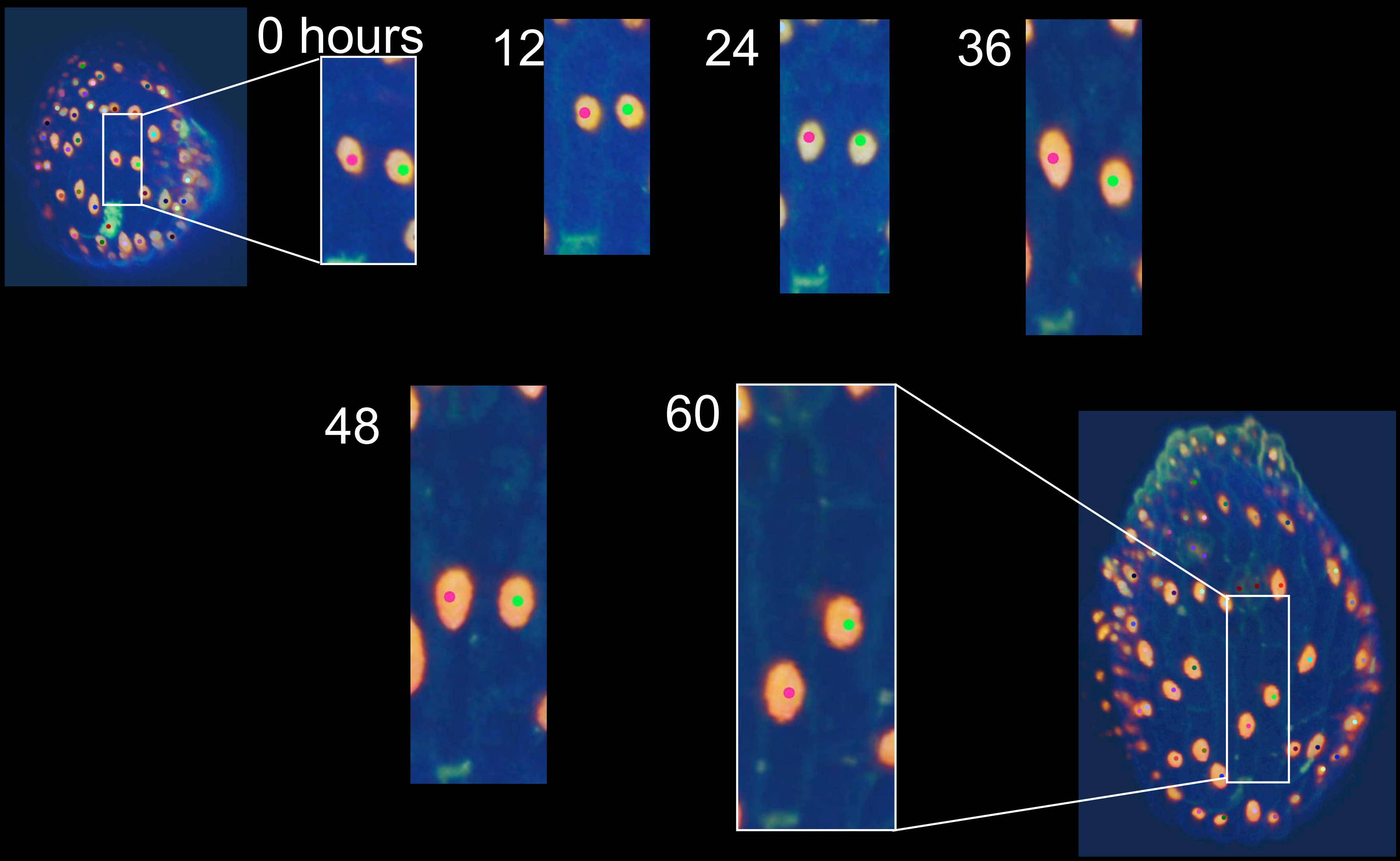
48

60

ATML1::KRP1 cells endoreduplicate instead of dividing

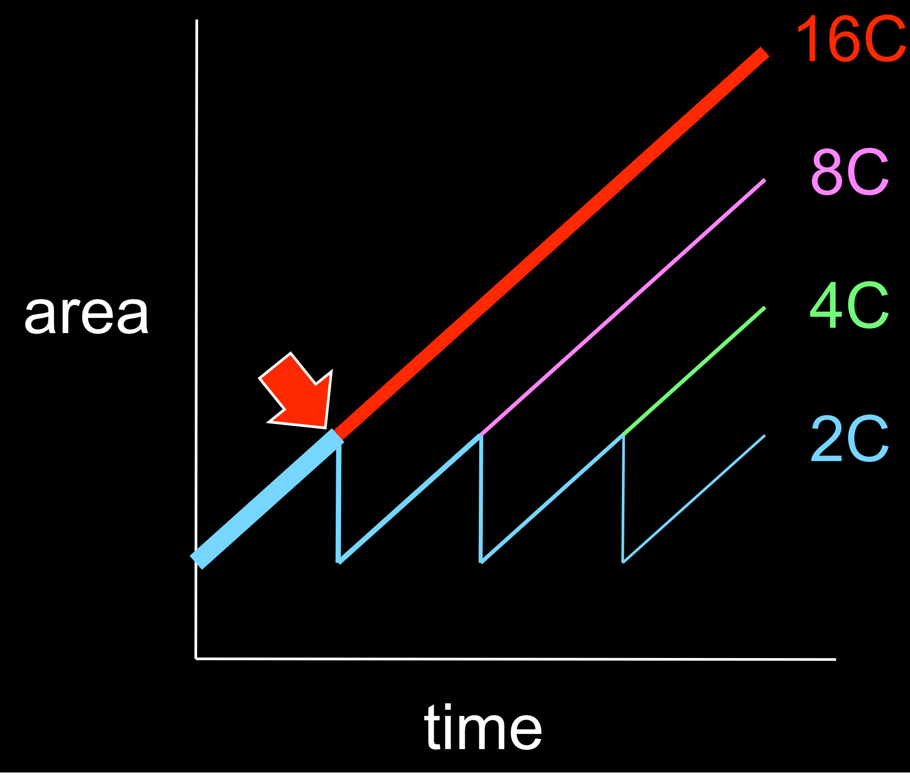


ATML1::KRP1 cells endoreduplicate instead of dividing

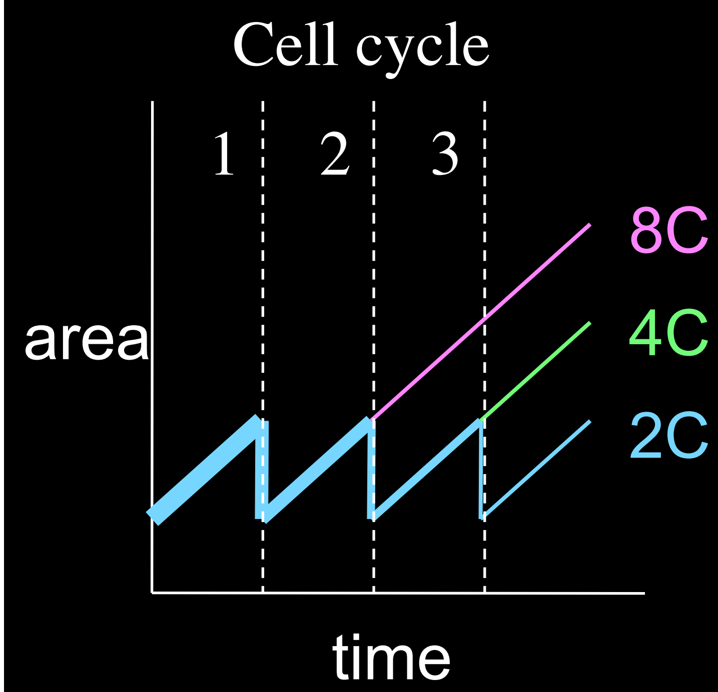


Summary:

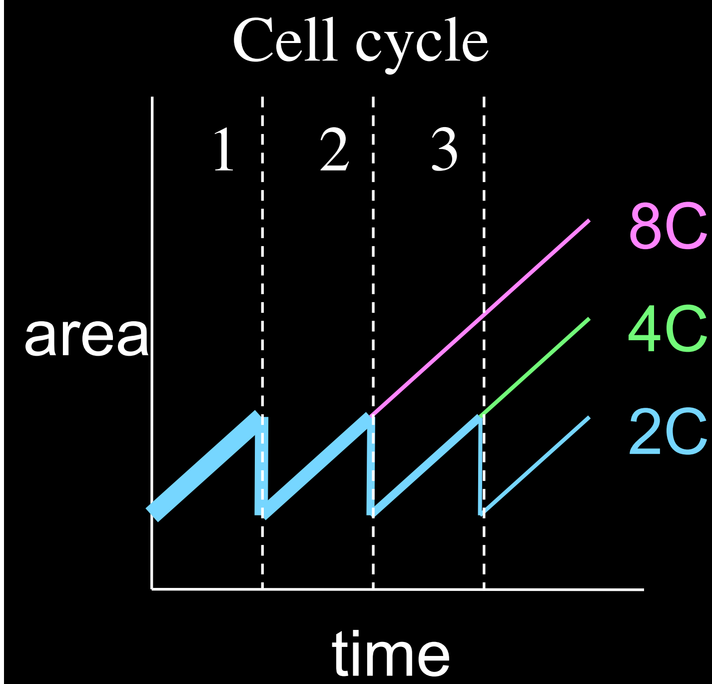
Overexpression of the cell cycle inhibitor KRP1 increases the probability that cells will enter endoreduplication early and become enlarged.

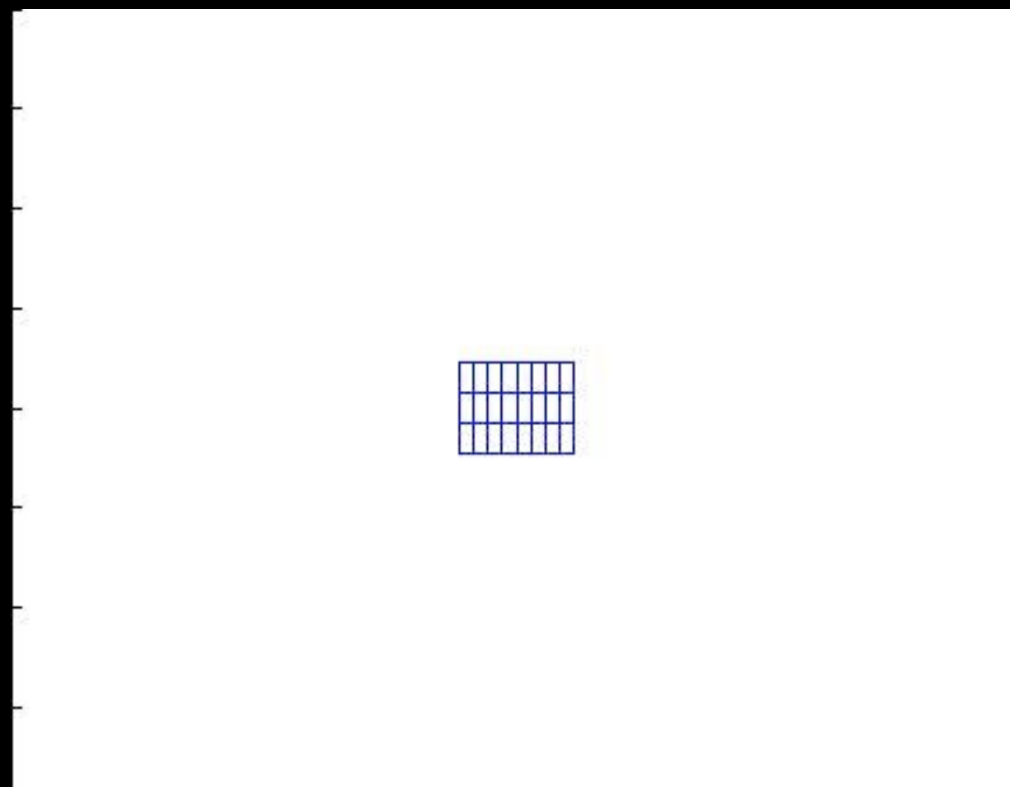


Model Prediction:
No giant cells

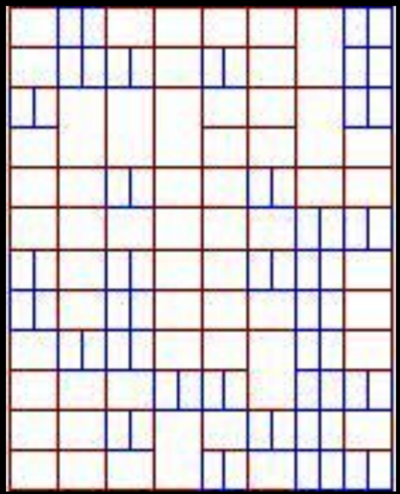


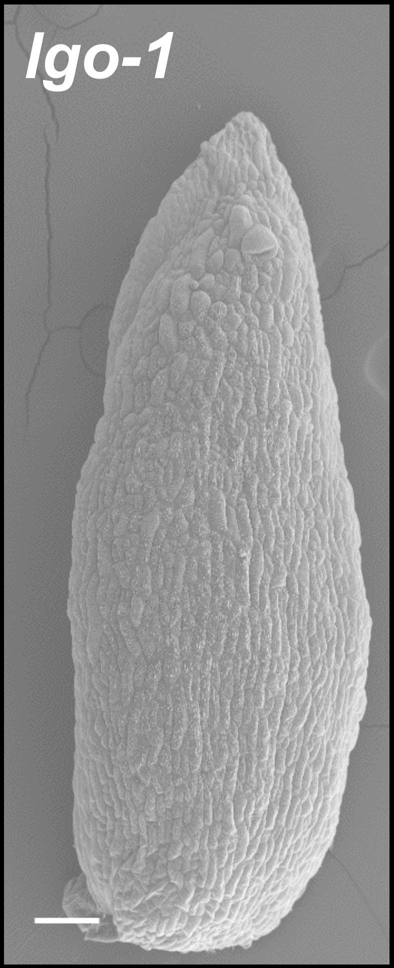
Model Prediction:
No giant cells

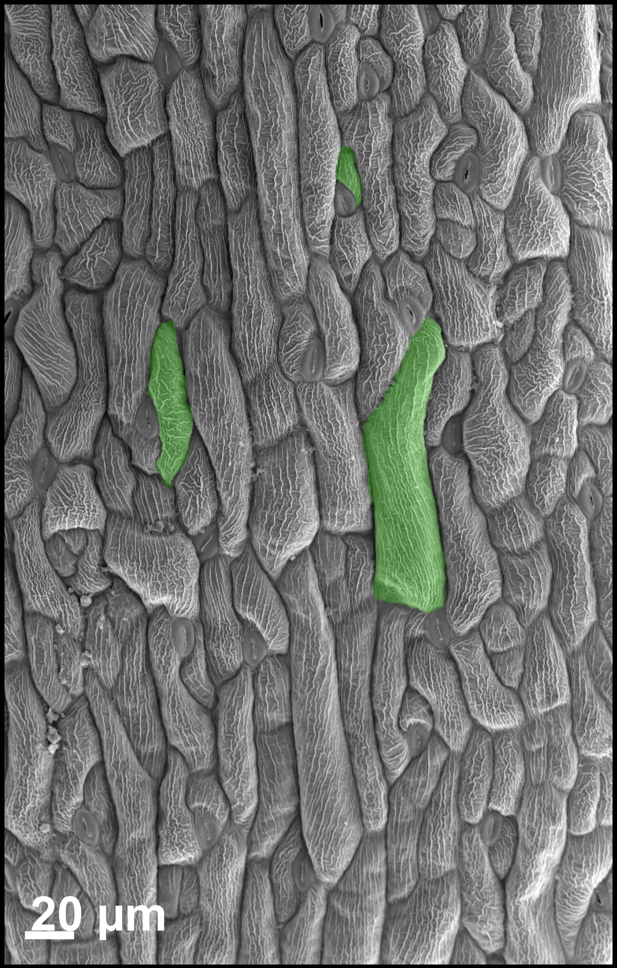




loss of giant cells from organs (*lgo*) mutant







lgo (loss of giant cells from organs)

Mutation in a cell cycle inhibitor in the SIAMESE family

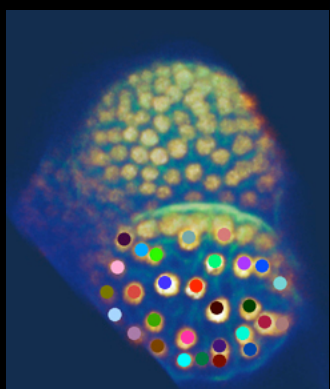
How is a cell size distribution created?

in silico:
No early endoreduplication →
lgo phenotype

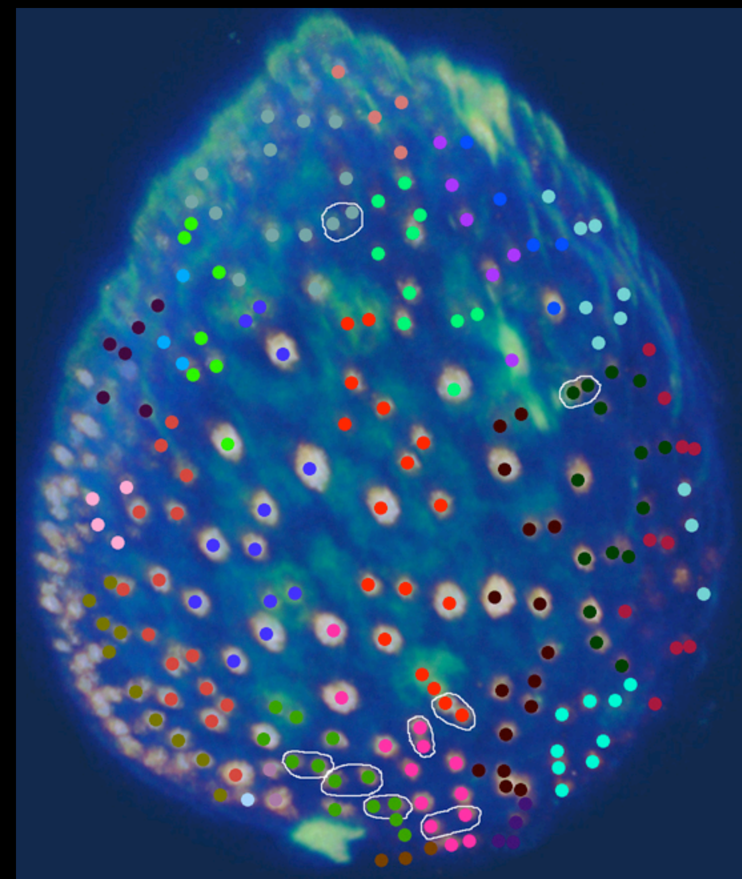
↓

in vivo: ?
test with live imaging

Live imaging of *lgo-1* sepal



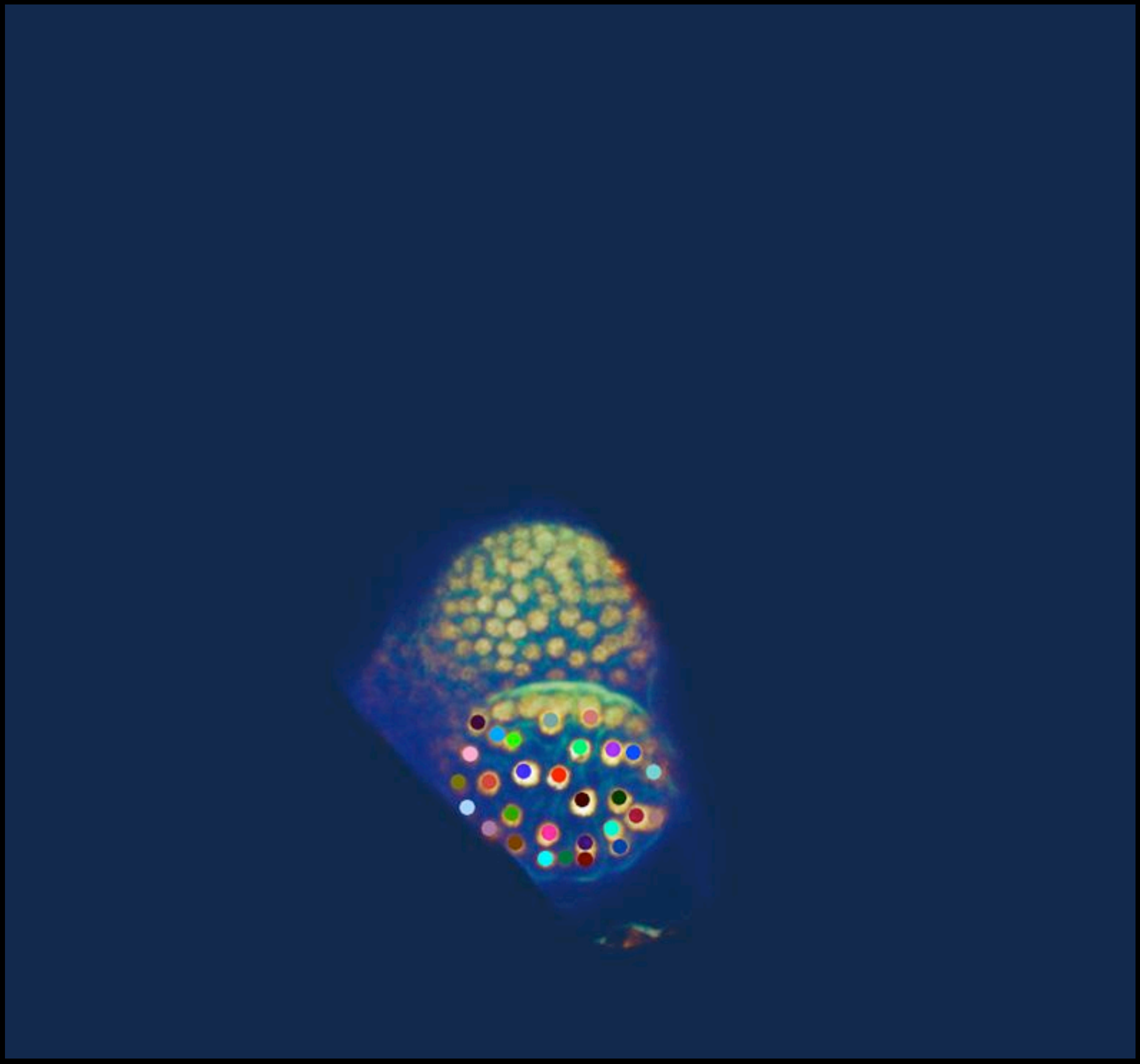
72 hours



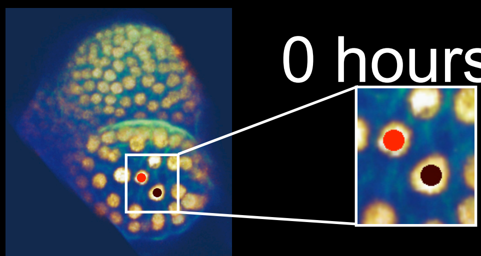
Epidermal cell DNA
ATML1::HISTONE2B-mYFP

Cell wall/dead cells
Propidium iodide

lgo cells divide instead of endoreduplicating



lgo cells divide instead of endoreduplicating



0 hours

12

24

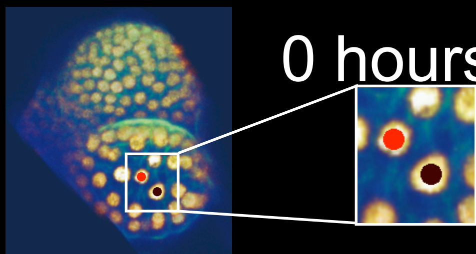
36

48

72

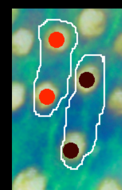
60

lgo cells divide instead of endoreduplicating



0 hours

12



24

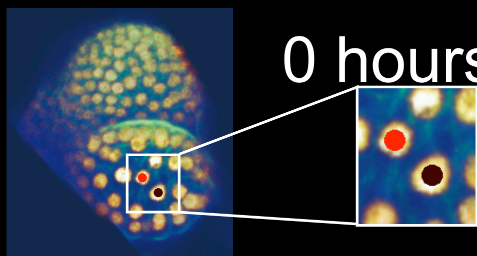
36

48

72

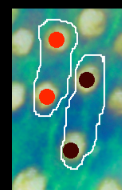
60

lgo cells divide instead of endoreduplicating

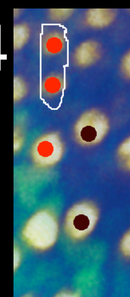


0 hours

12



24



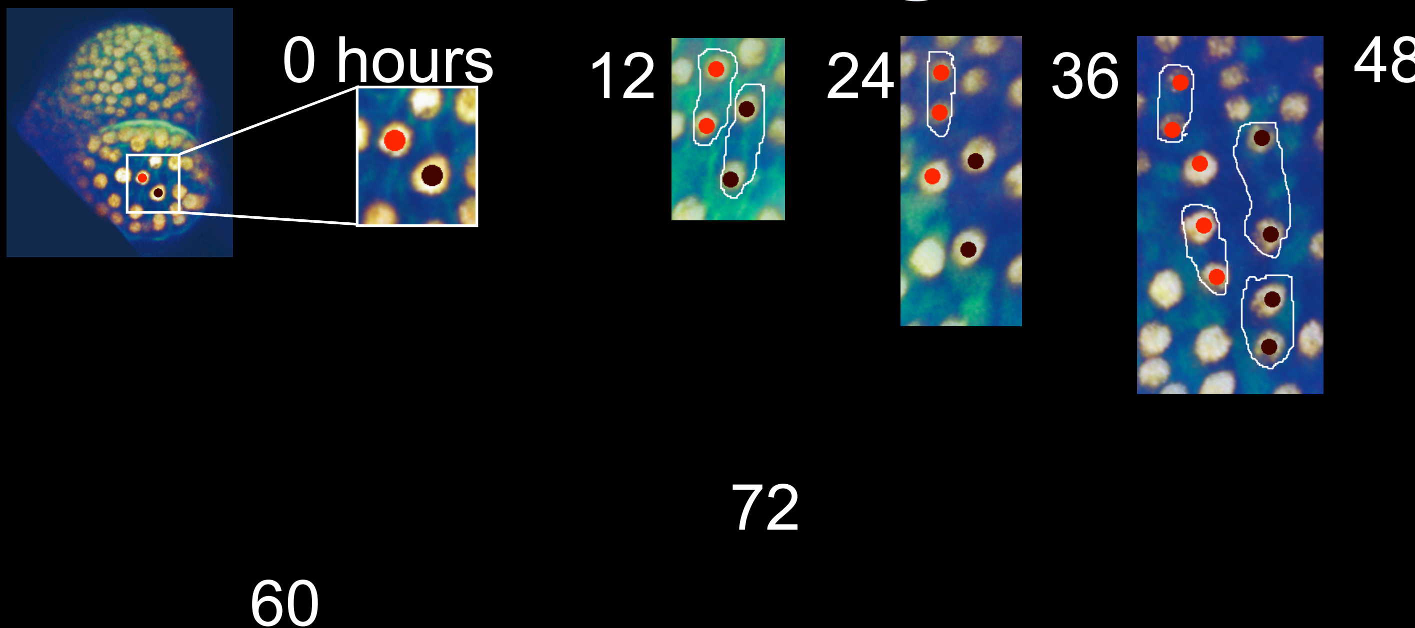
36

48

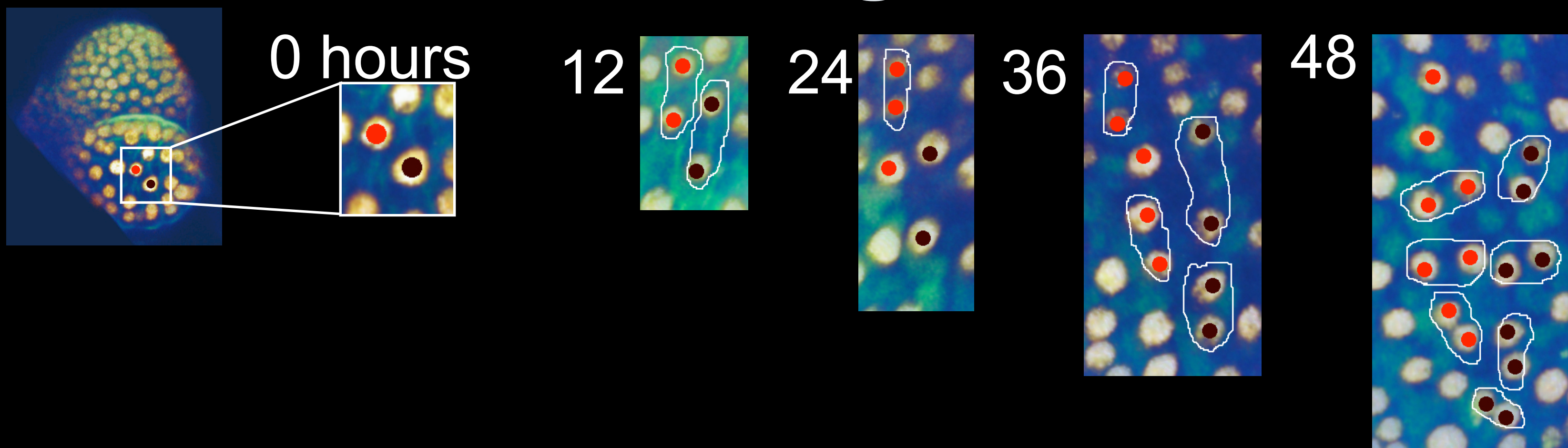
72

60

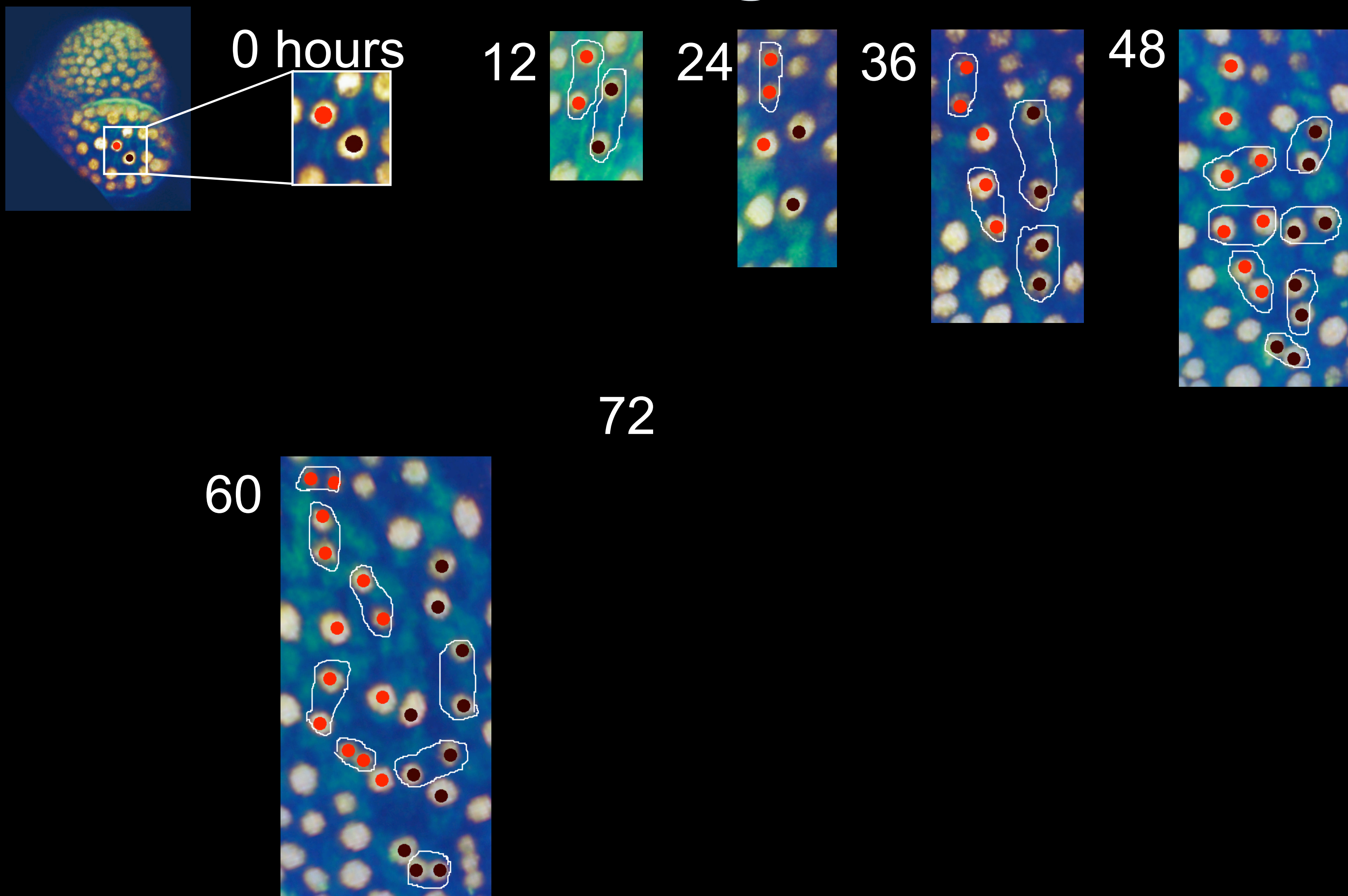
lgo cells divide instead of endoreduplicating



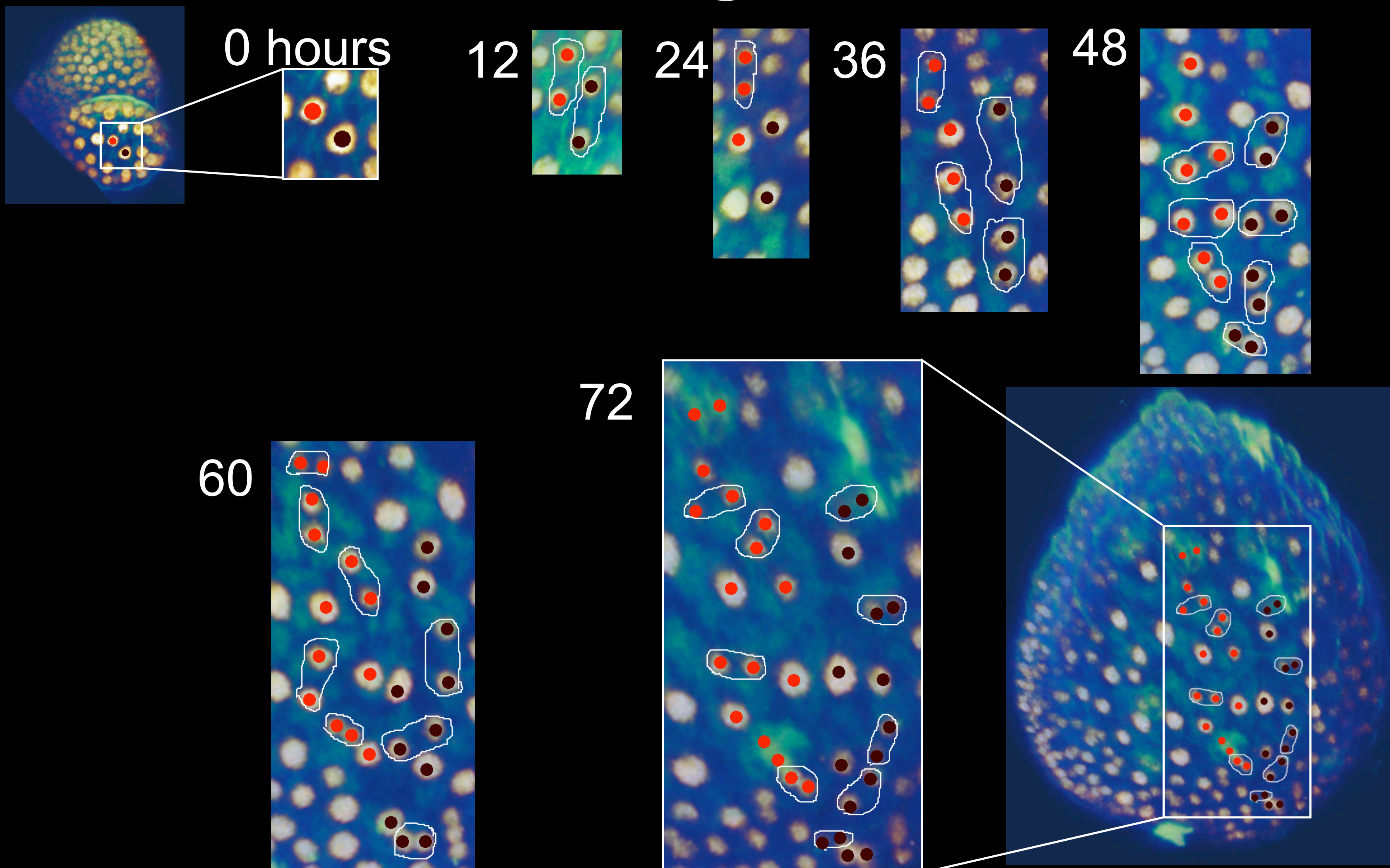
lgo cells divide instead of endoreduplicating



lgo cells divide instead of endoreduplicating



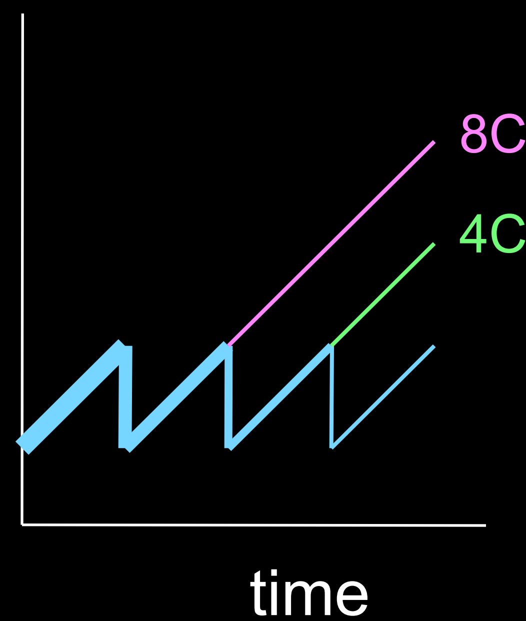
lgo cells divide instead of endoreduplicating



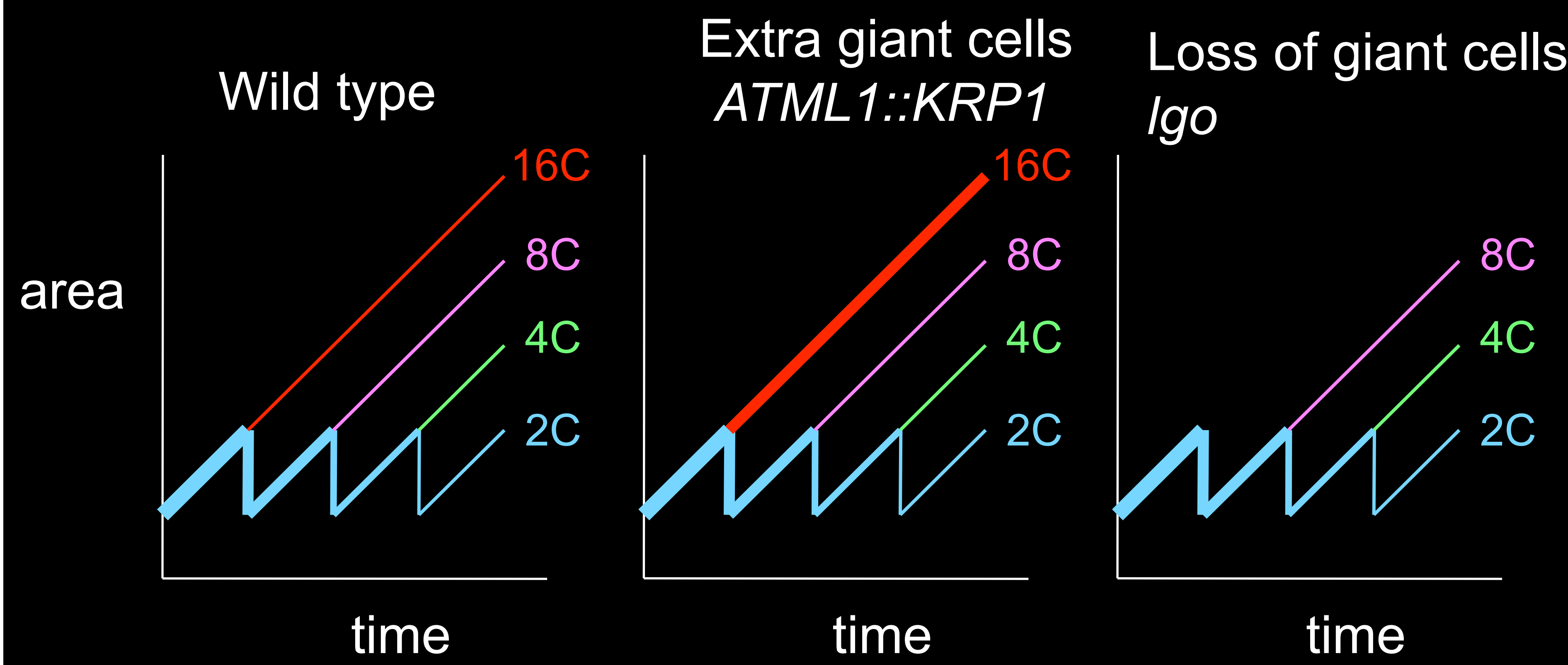
Summary:

Loss of the putative cell cycle inhibitor LGO in the *lgo* mutant decreases the probability of entering endoreduplication early.

Consequently, those cells continue to divide creating more smaller cells.



Cell cycle inhibitors change the timing of endoreduplicaiton and the resulting cell size.



Questions for today:

1. How does endoreduplication create the cell size pattern in the epidermis?
2. Does endoreduplication increase growth of the organ?
3. How does patterning extend to the whole organ?
4. What causes variability in cell sizes?

Does the level of endoreduplication in the epidermis affect growth?

Literature: Purpose of endoreduplication is faster growth.

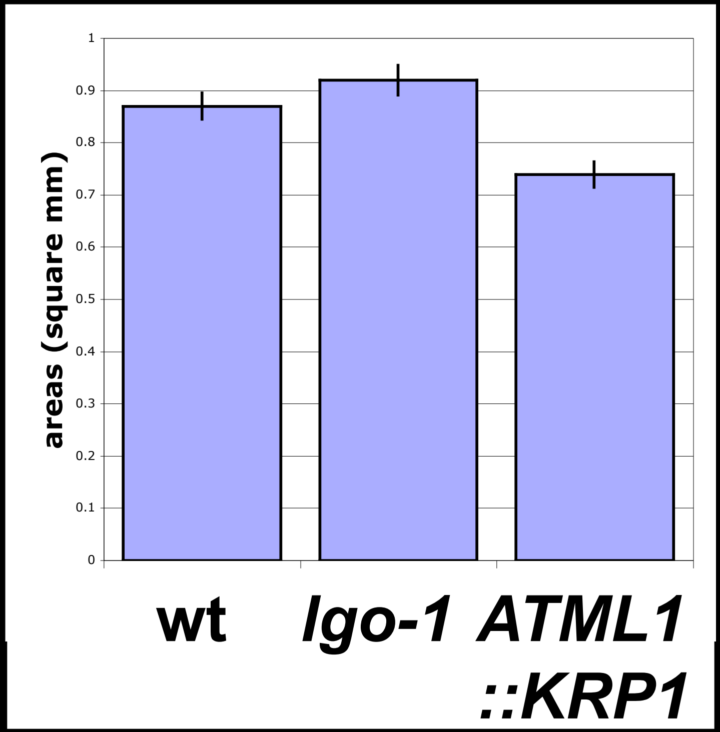
Assumption of the model: Endoreduplicating cells grow at the same rate as dividing cells.

Overall growth of the plants is not affected

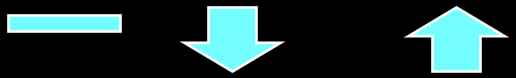
	Wild type	*lgo-1*	*ATML1::KRP1*
endoreduplication	—	↓	↑

Alex Cunha's sepal segmentation measures size

lgo-1 and *ATML1::KRP1* do not dramatically change mature sepal size

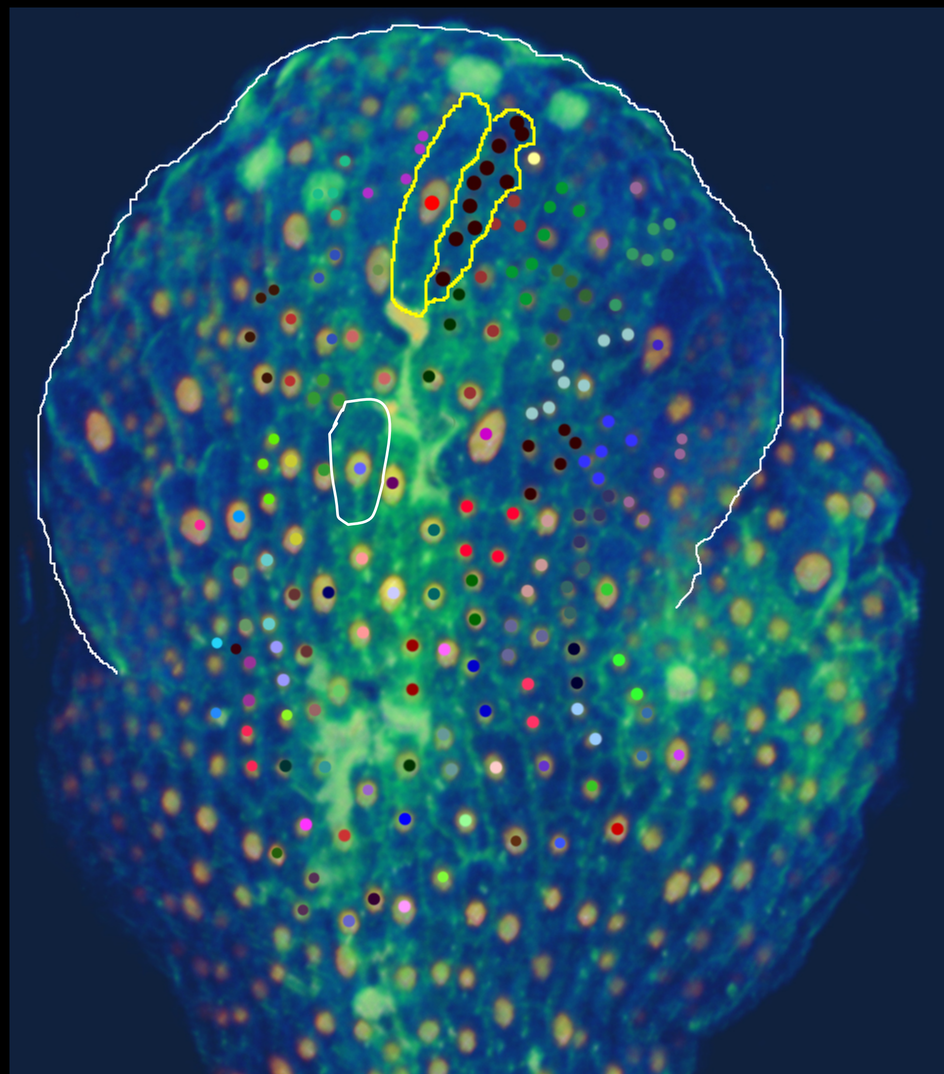


endoreduplication



Error bars are 95% confidence interval on the mean

Growth is uniform locally regardless of division or endoreduplication



Wild type
72 hours

Summary

Endoreduplication in the epidermis does not promote growth.

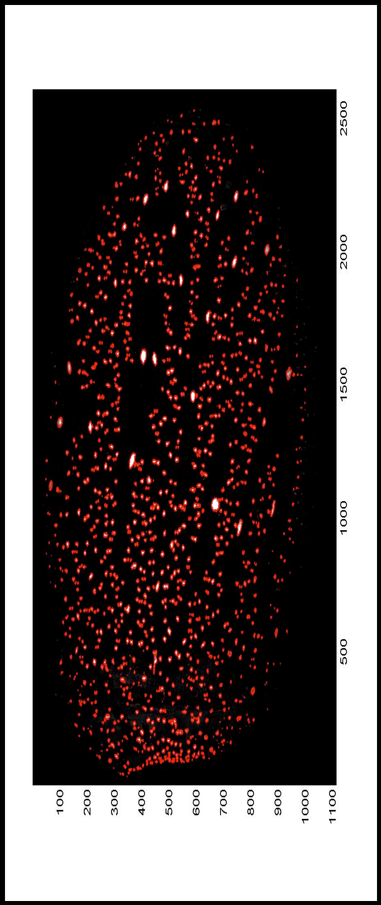
Growth is uniform locally, but not globally within the sepal epidermis.

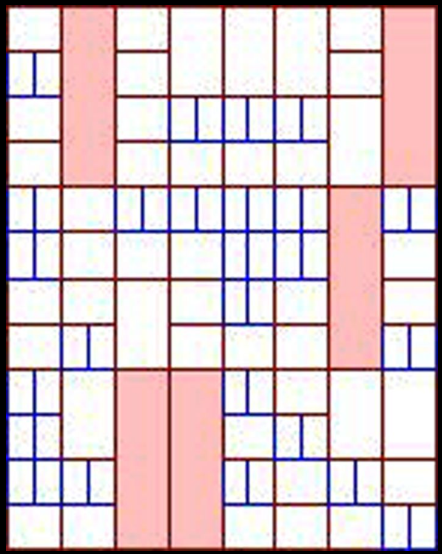
Uniform growth is a reasonable assumption for the model.

Questions for today:

1. How does endoreduplication create the cell size pattern in the epidermis?
2. Does endoreduplication increase growth of the organ?
3. How does patterning extend to the whole organ?
4. What causes variability in cell sizes?

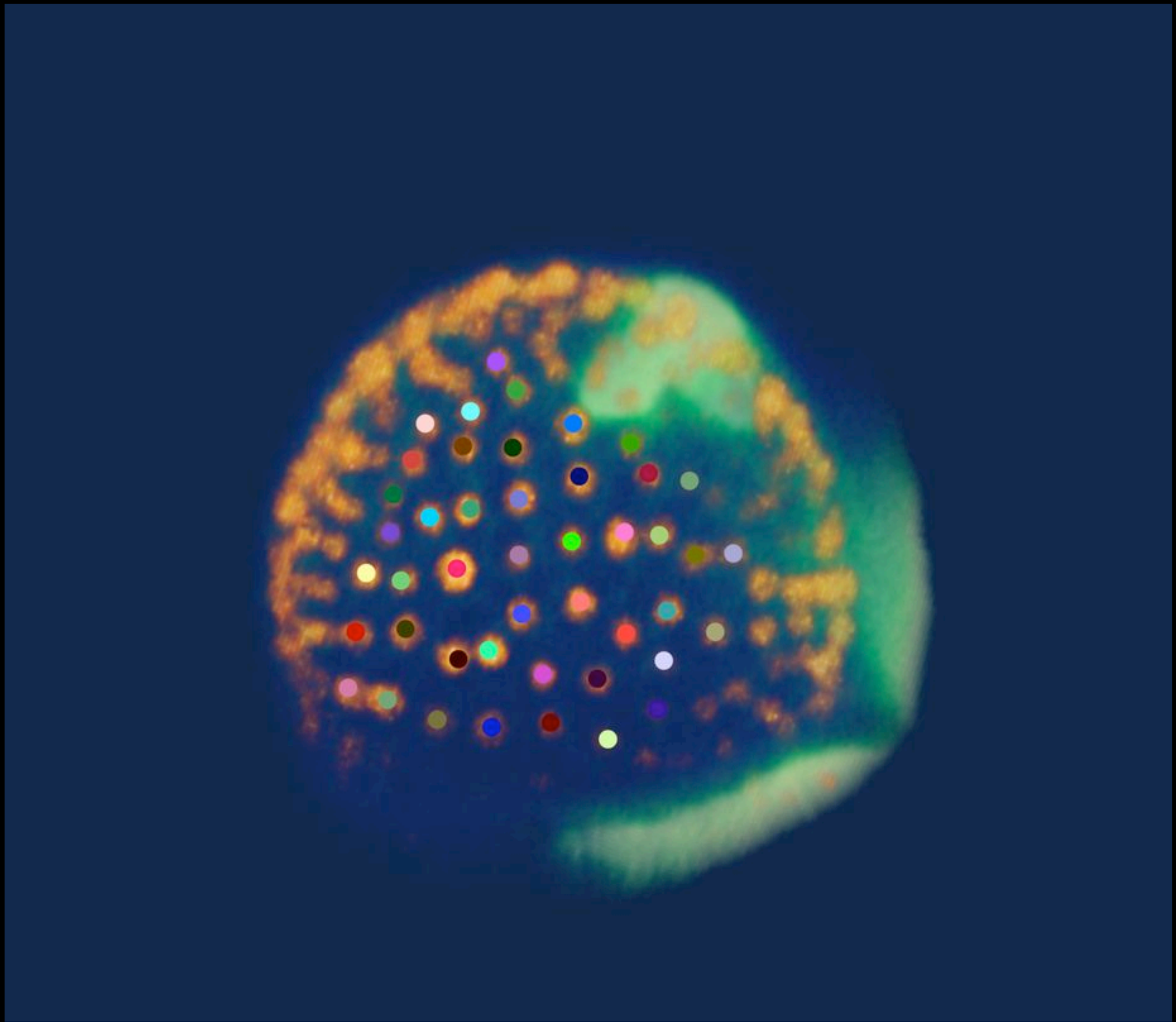
Problem: Sepal epidermis contains ~1600 cells, model ~100



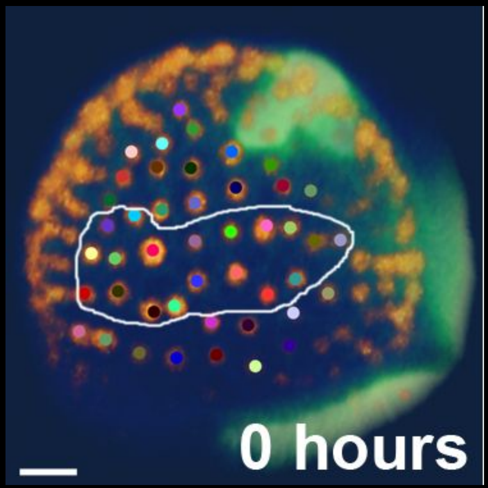


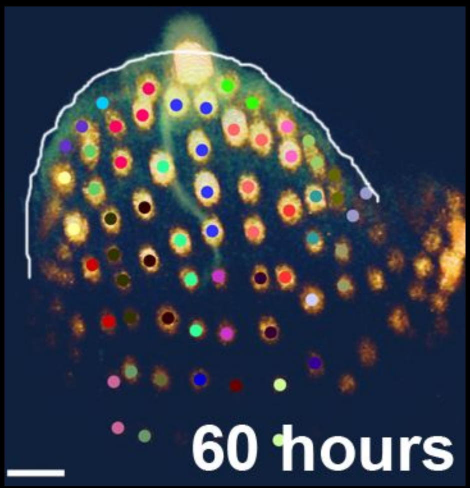
Nuclear Segmentation—Boguslaw Obara UCSB
Tigran Bacarian UCI

What should the sepal template be?



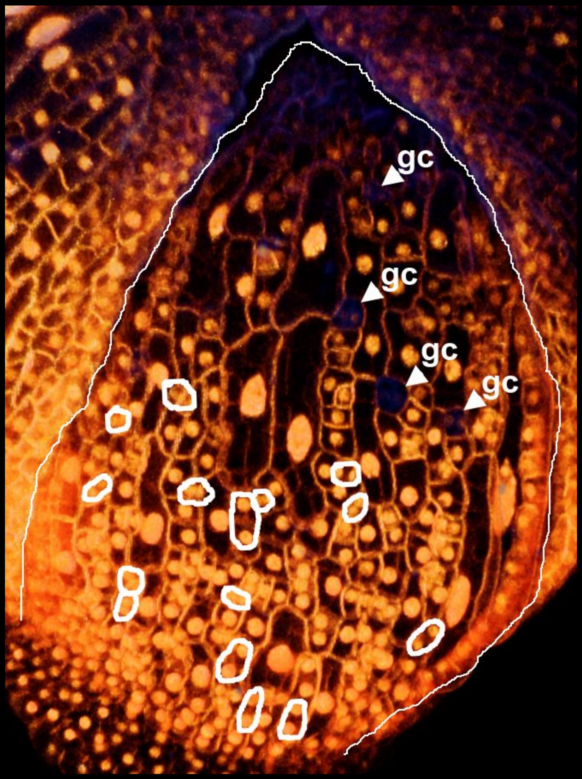
Sepal template is 8 cells wide

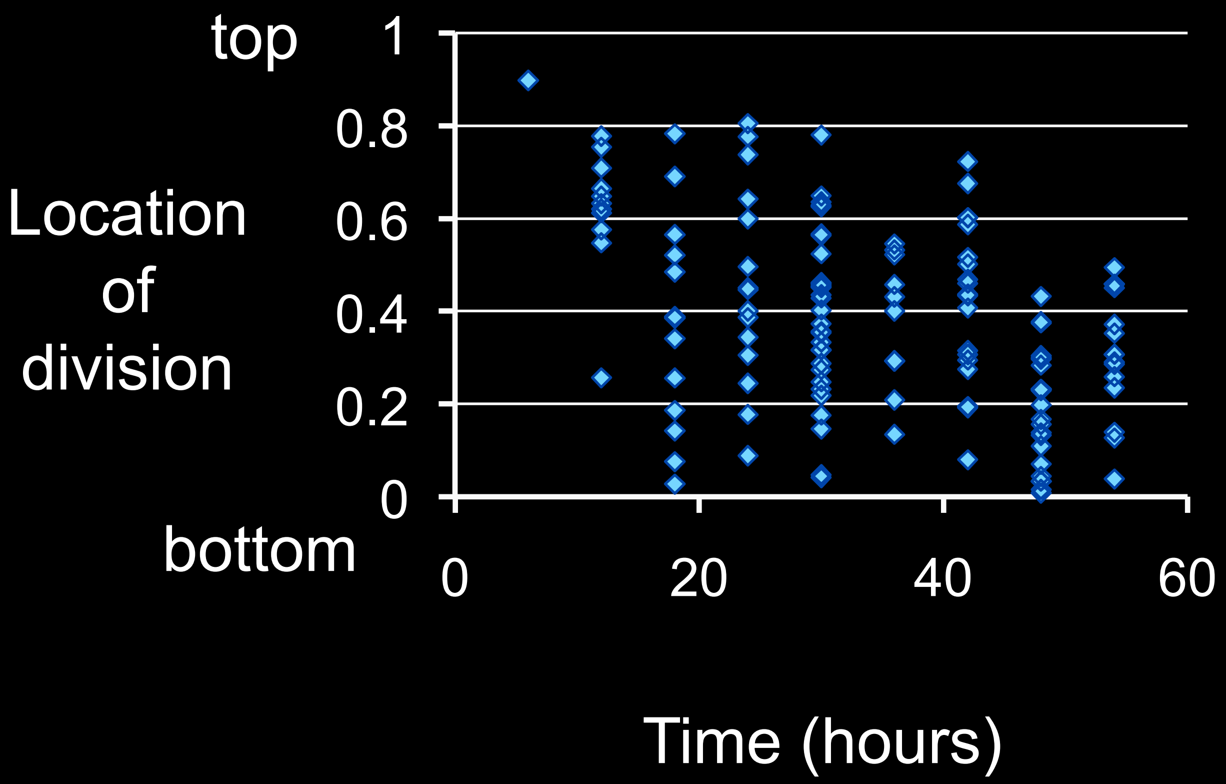




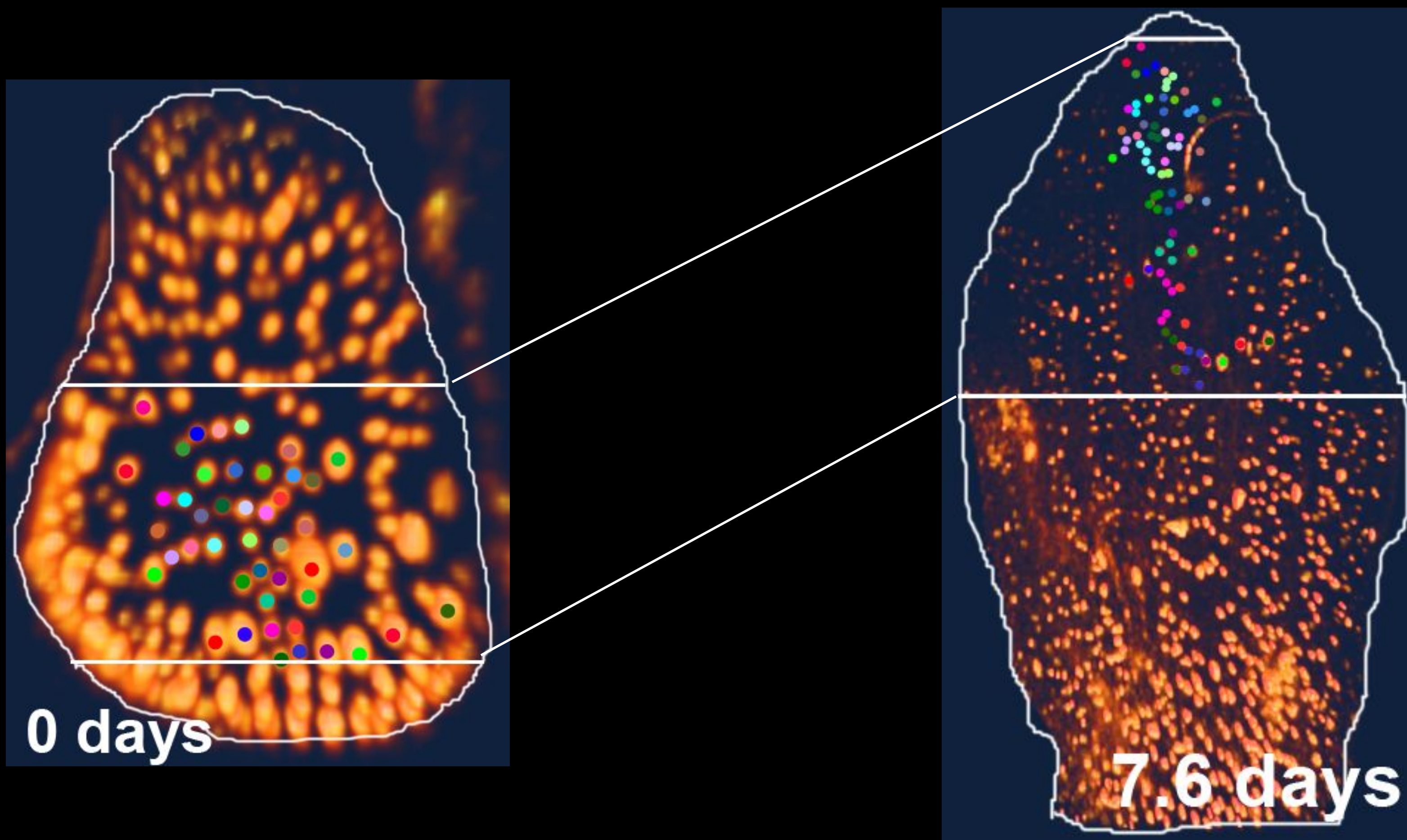
New model template

Basipetal wave in termination of cell division





The bottom of the sepal generates more cells



Intercalary growth model

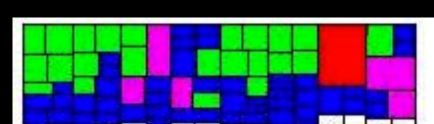
key

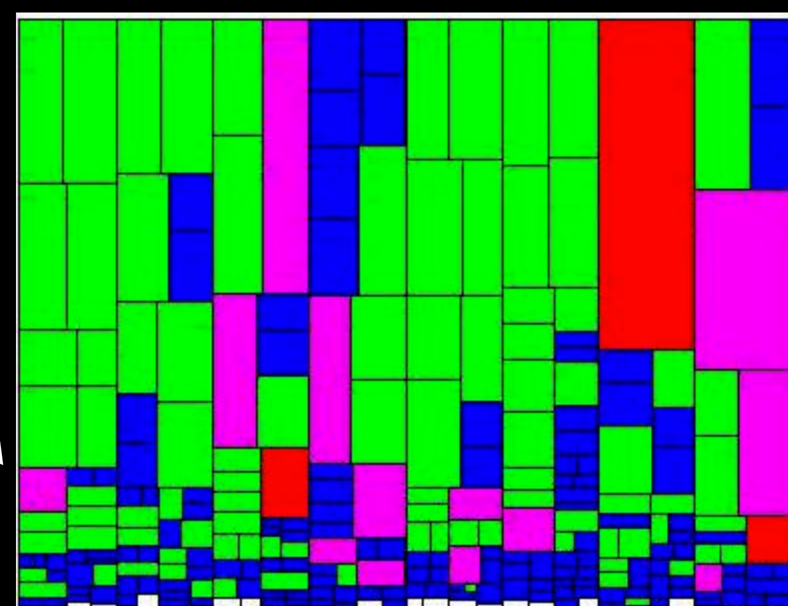
16C
8C
4C
2C

Cells terminate after
3 patterning cell cycles

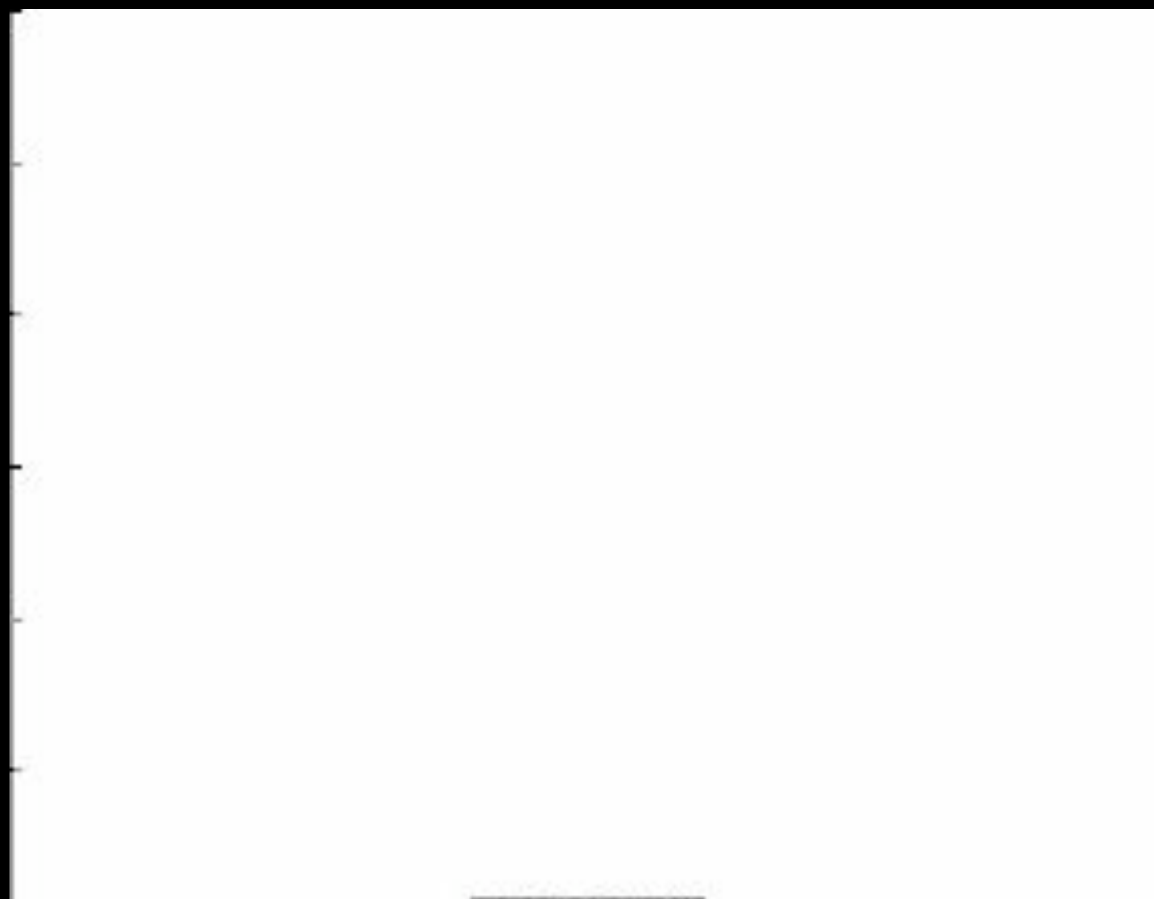
Cells enter patterning
divisions

Generative layer





Intercalary growth model



Summary:

Repeating the patterning process as new cells are created can reproduce the whole sepal epidermis.

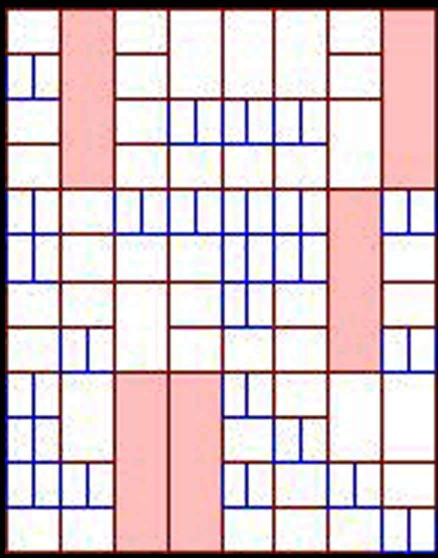
Questions for today:

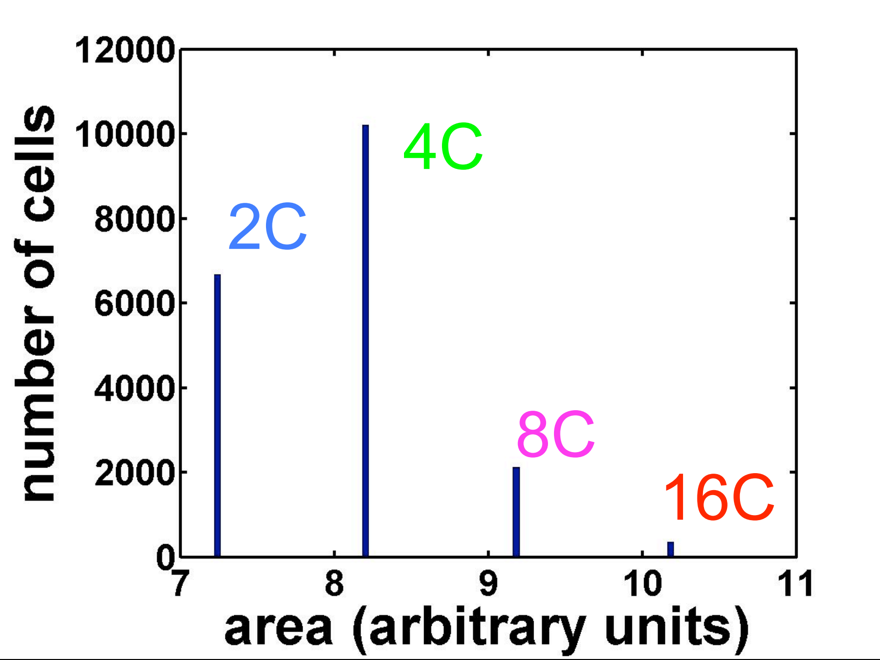
1. How does endoreduplication create the cell size pattern in the epidermis?
2. Does endoreduplication increase growth of the organ?
3. How does patterning extend to the whole organ?
4. What causes variability in cell sizes?

Further testing the model:

Do the cell size distributions *in silico* and *in vivo* match?

in silico cell size distribution has 4 sizes

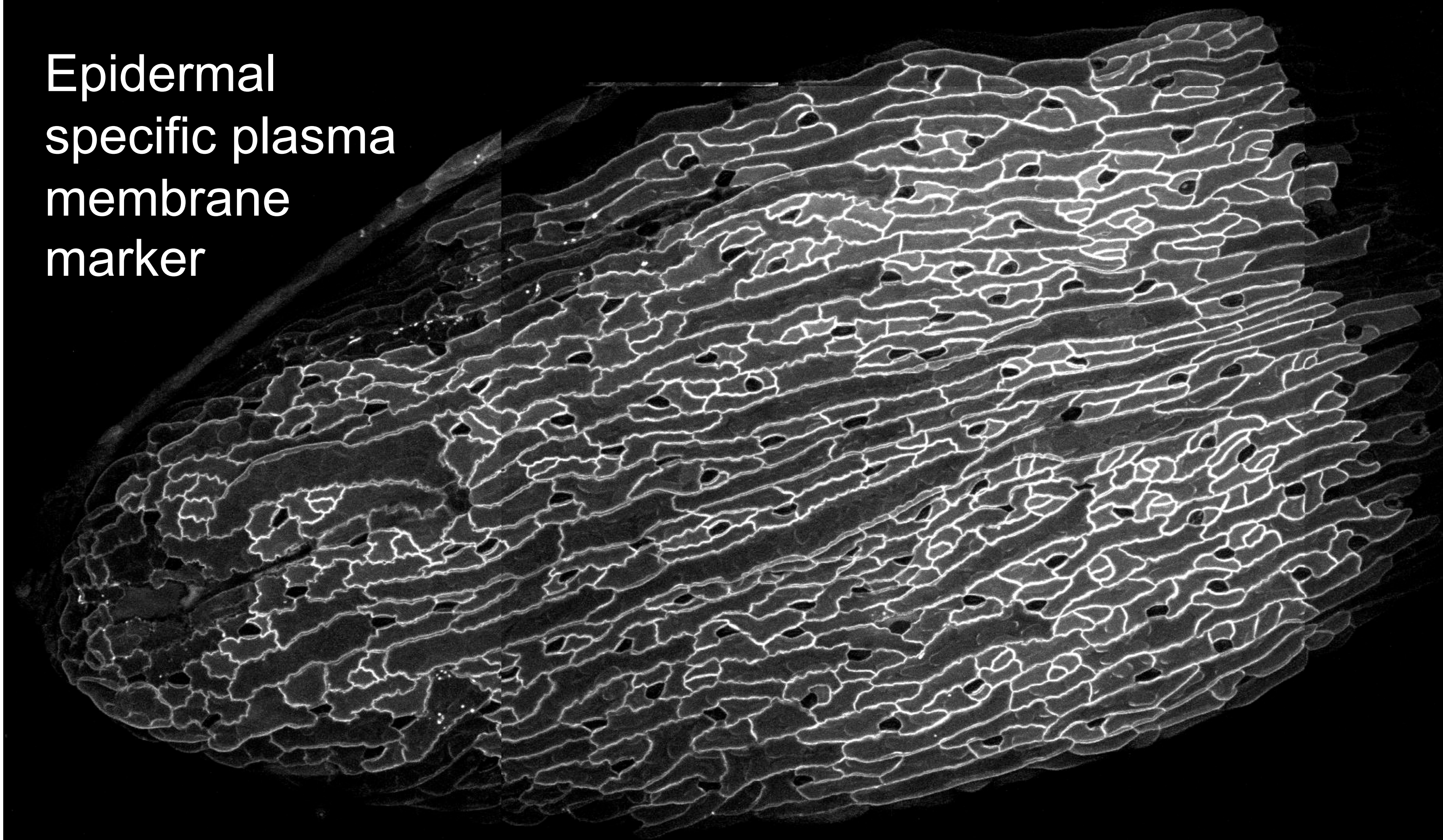




Does this match the real sepal?

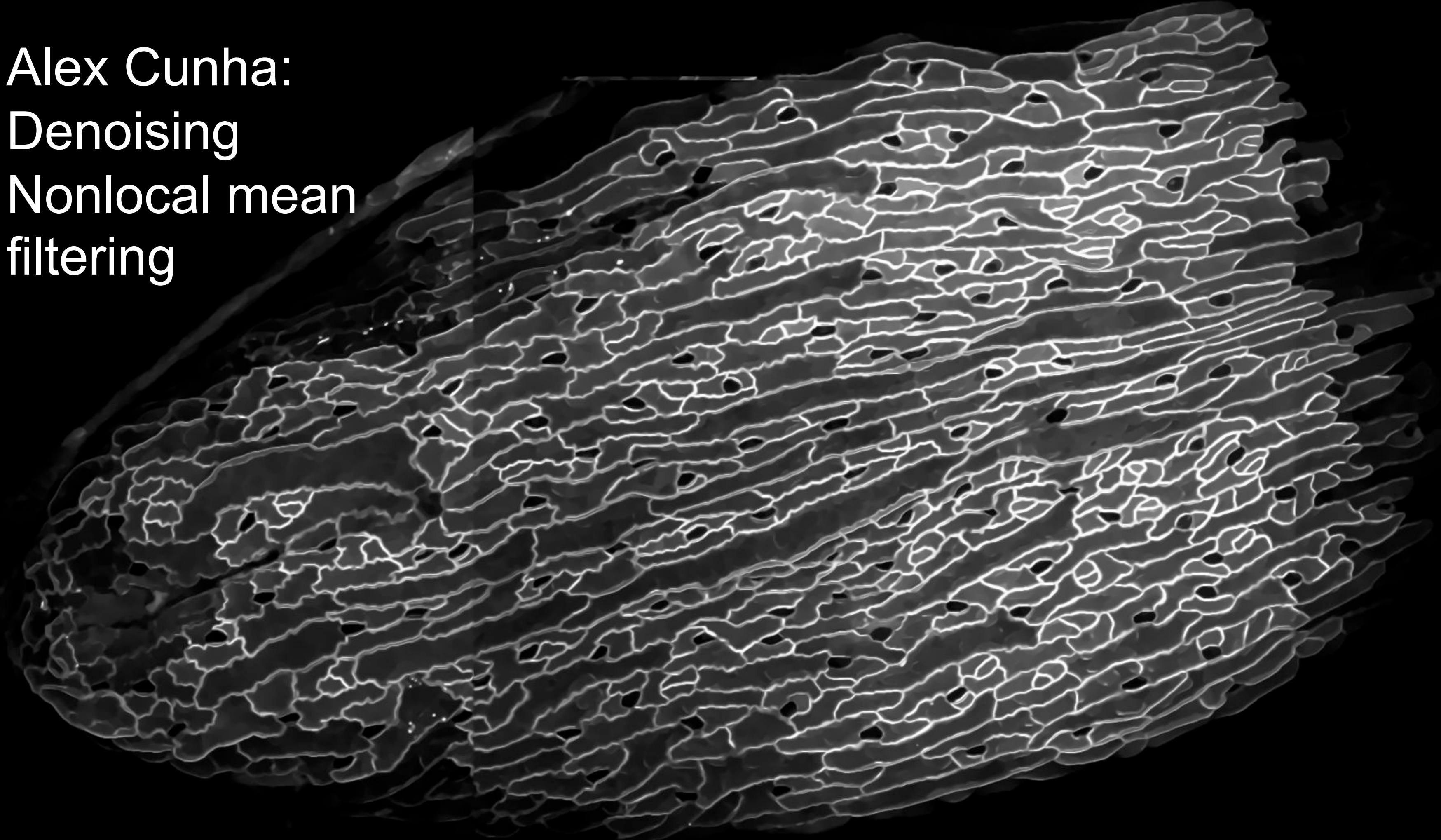
Semi-automated segmentation to quantify the cell size distribution

Epidermal
specific plasma
membrane
marker



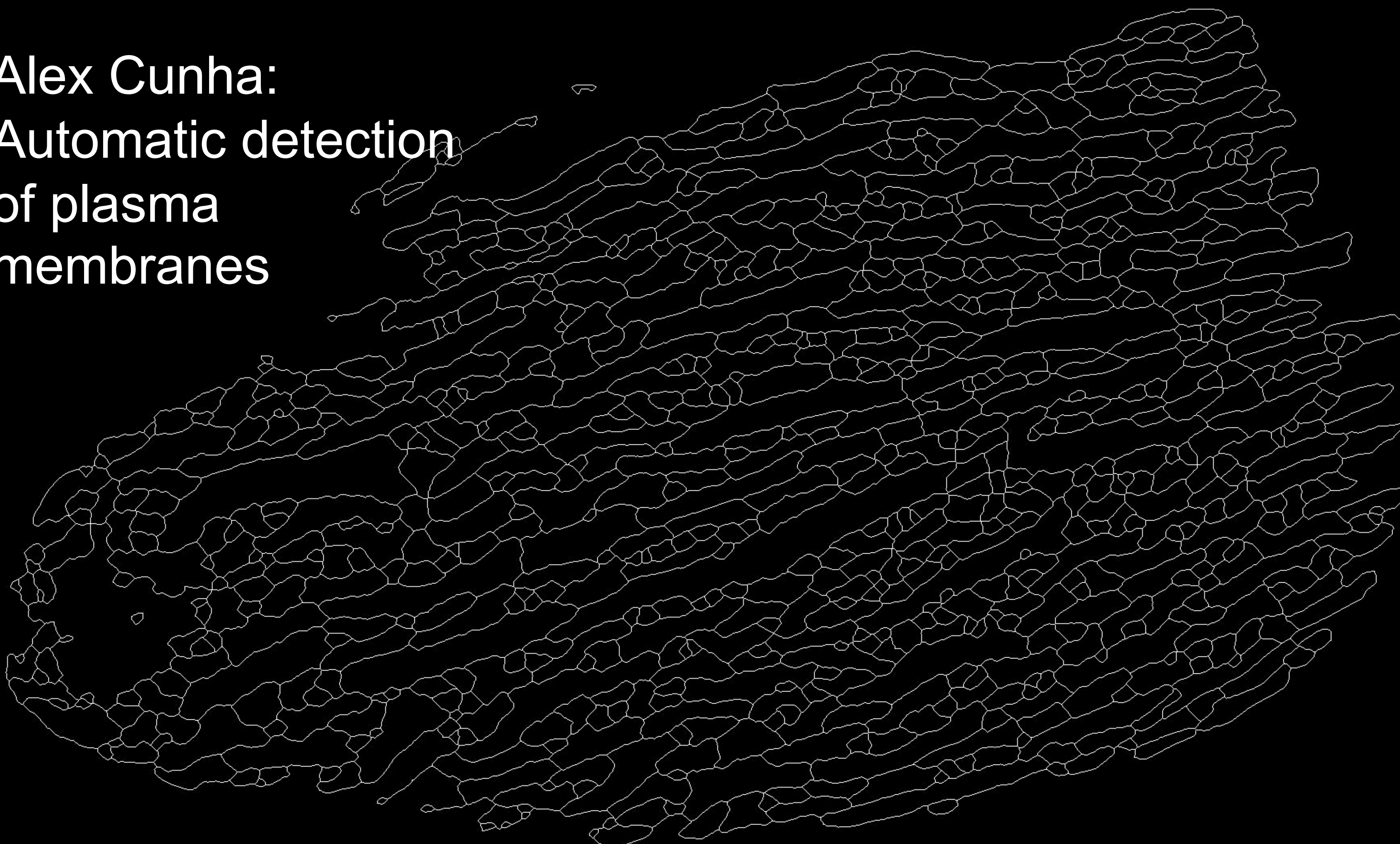
Semi-automated segmentation to quantify the cell size distribution

Alex Cunha:
Denoising
Nonlocal mean
filtering



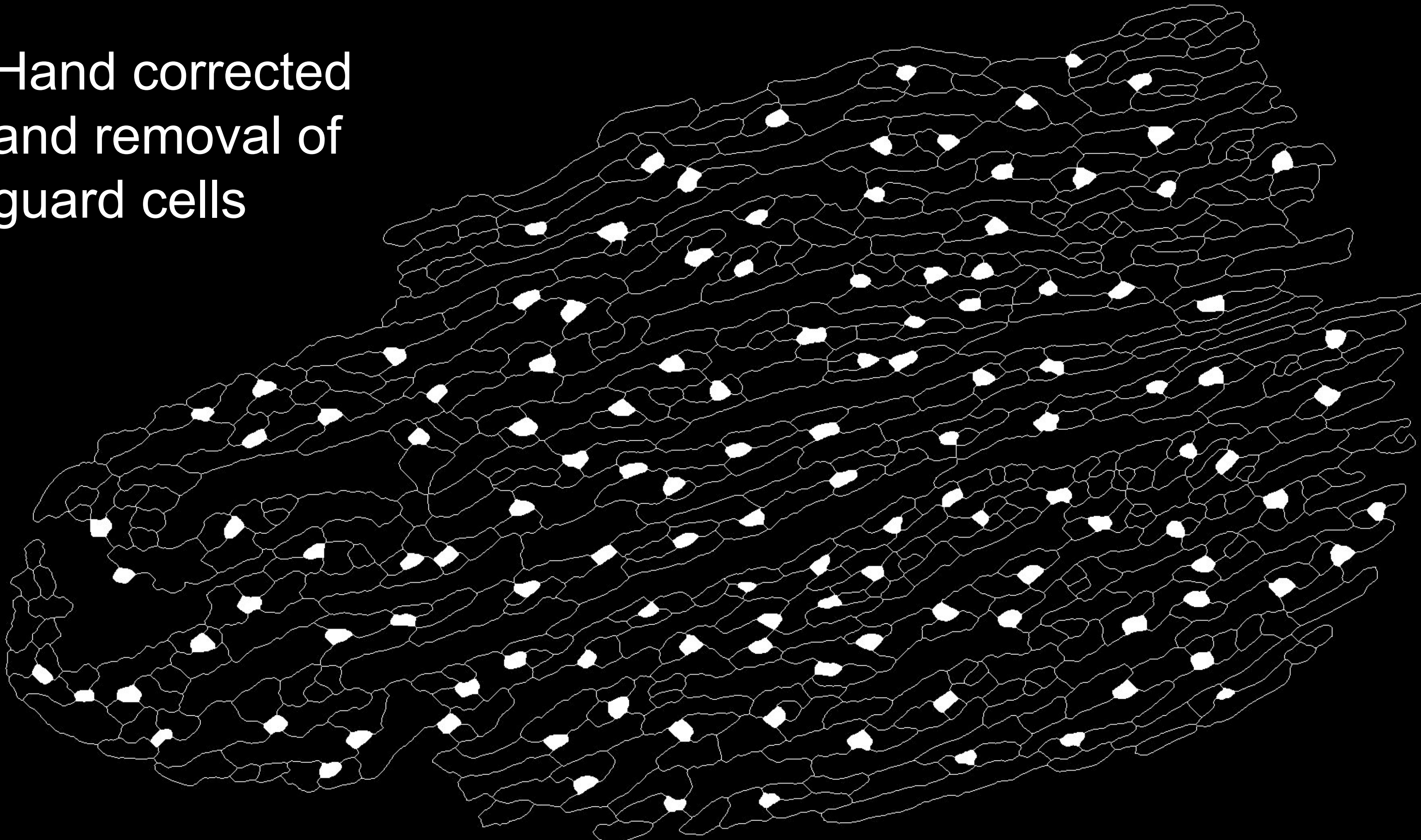
Semi-automated segmentation to quantify the cell size distribution

Alex Cunha:
Automatic detection of plasma membranes



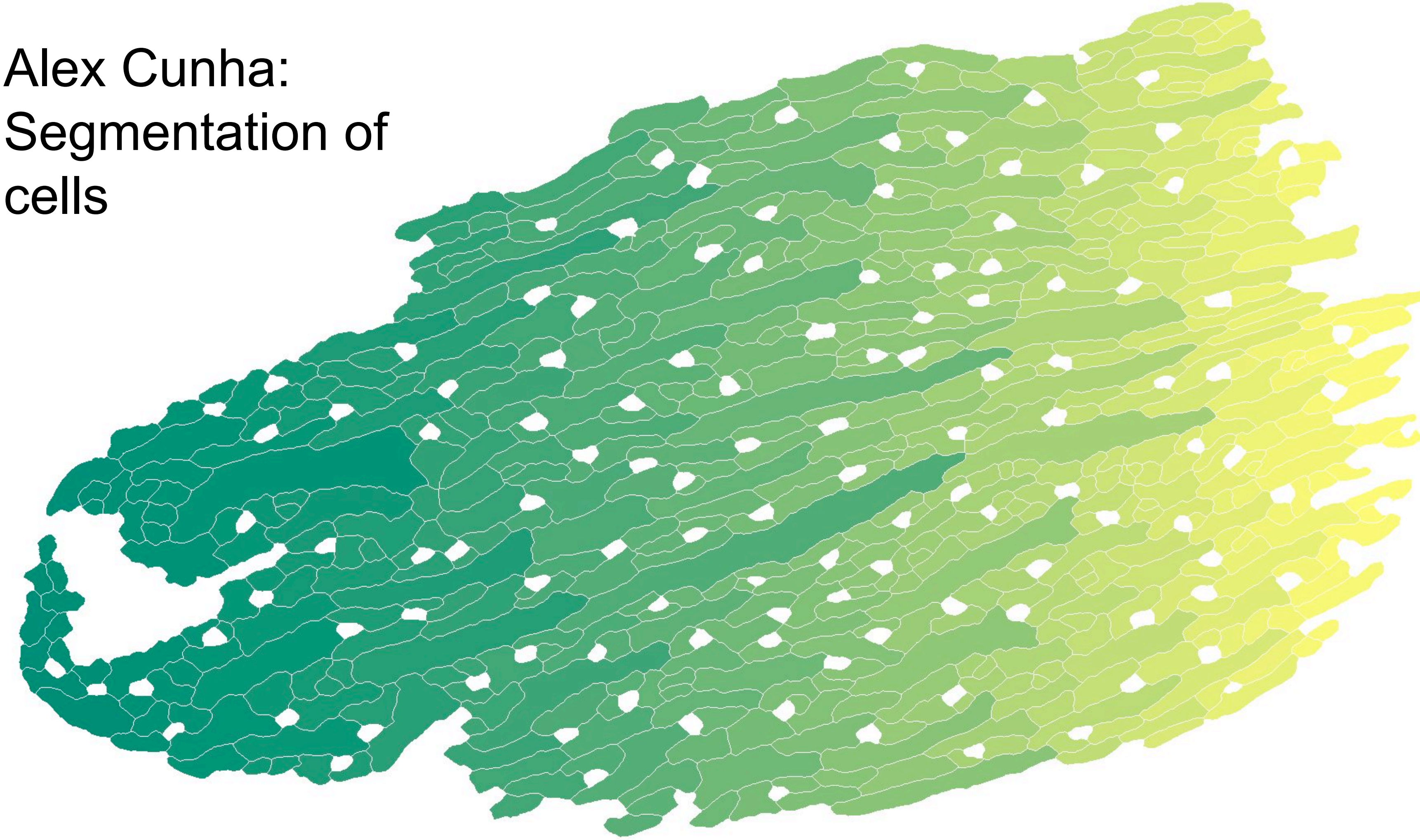
Semi-automated segmentation to quantify the cell size distribution

Hand corrected and removal of guard cells

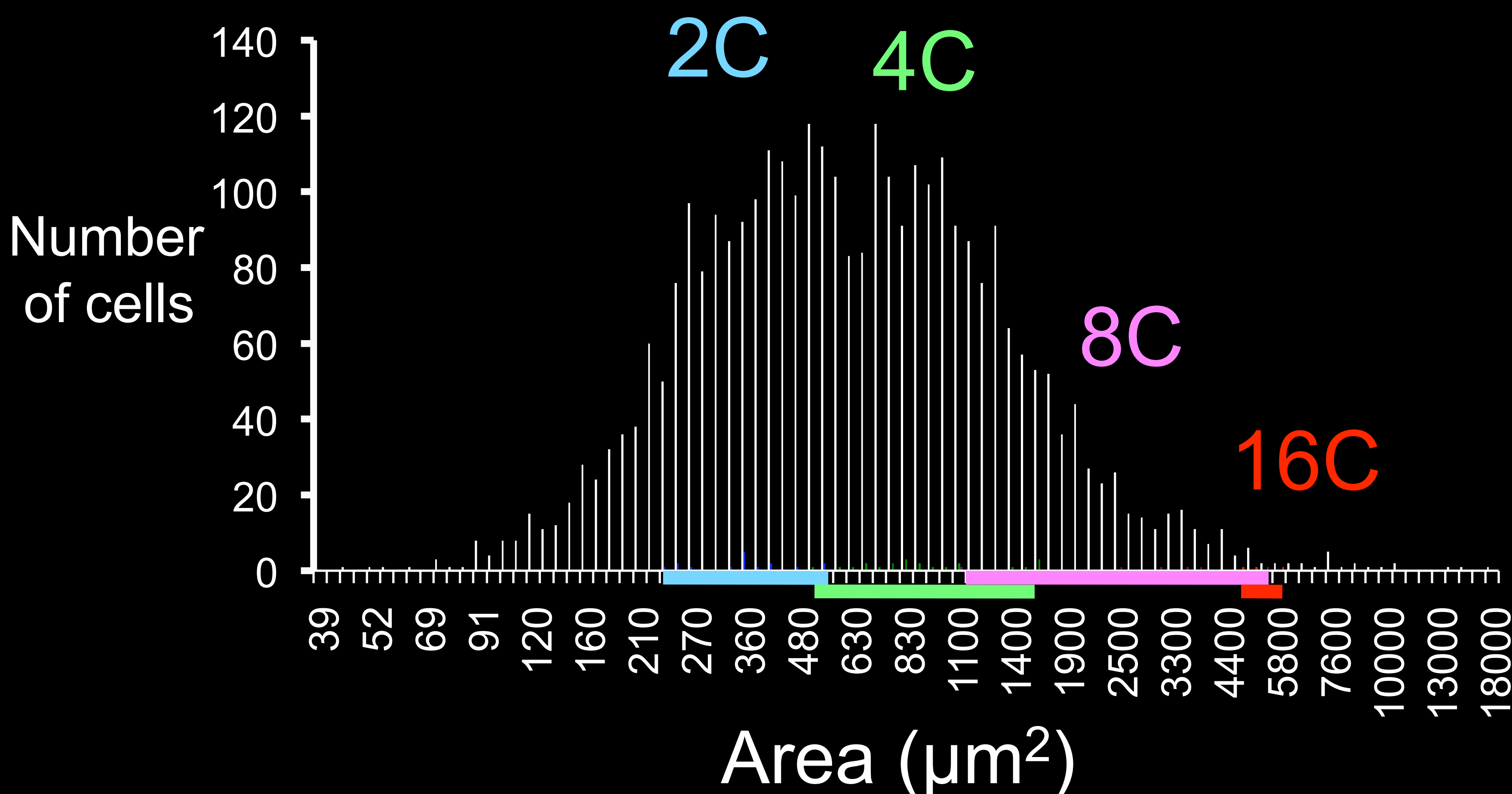


Semi-automated segmentation to quantify the cell size distribution

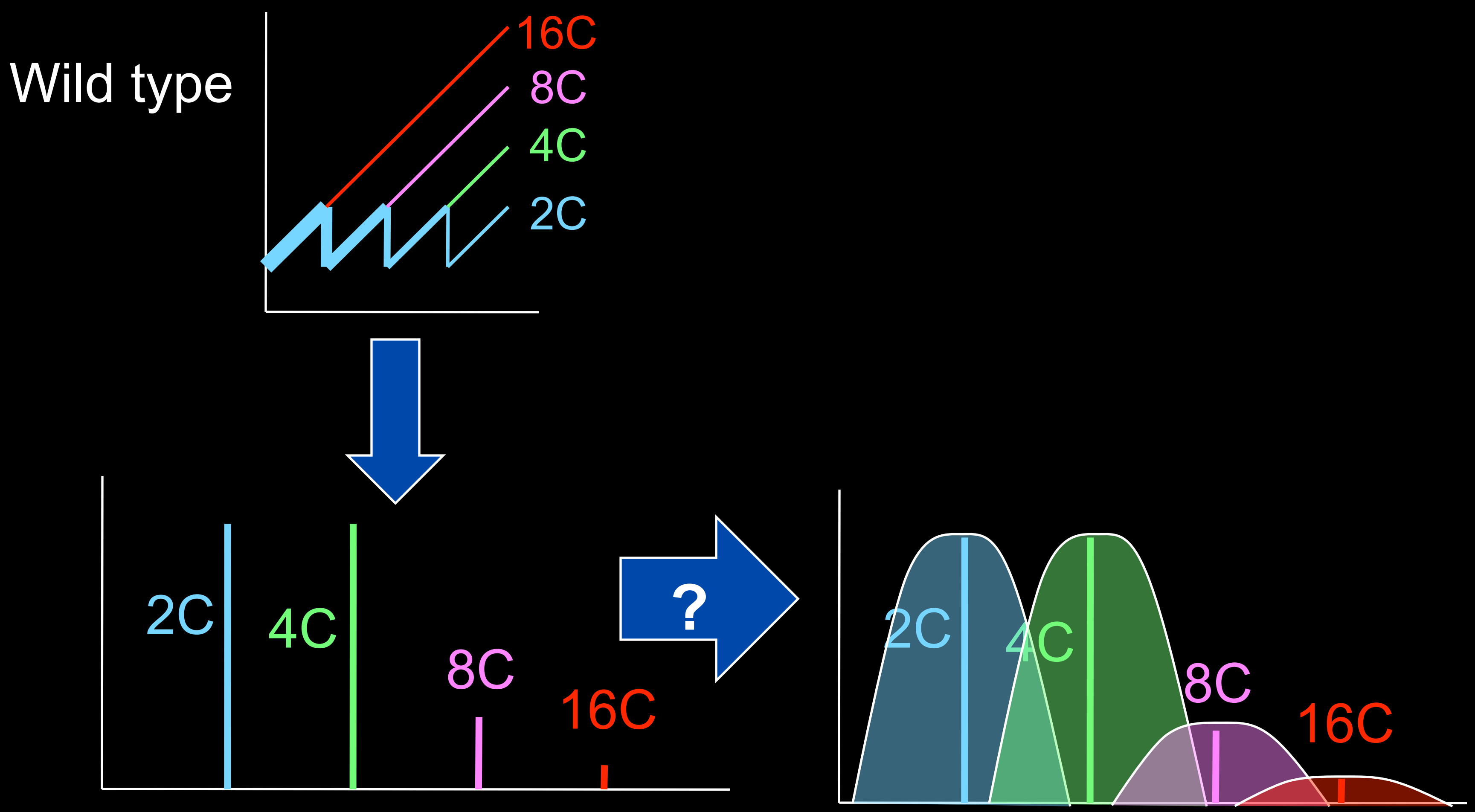
Alex Cunha:
Segmentation of cells



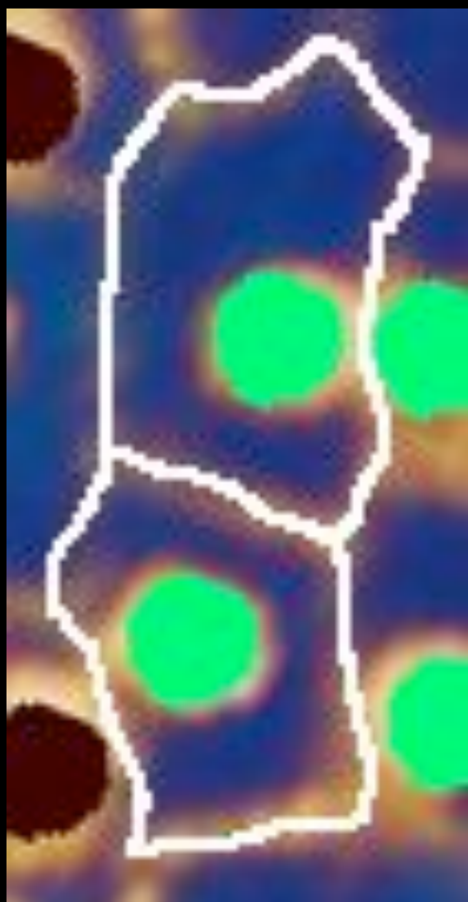
Cell areas for each DNA content overlap



What creates the variability in cell sizes?



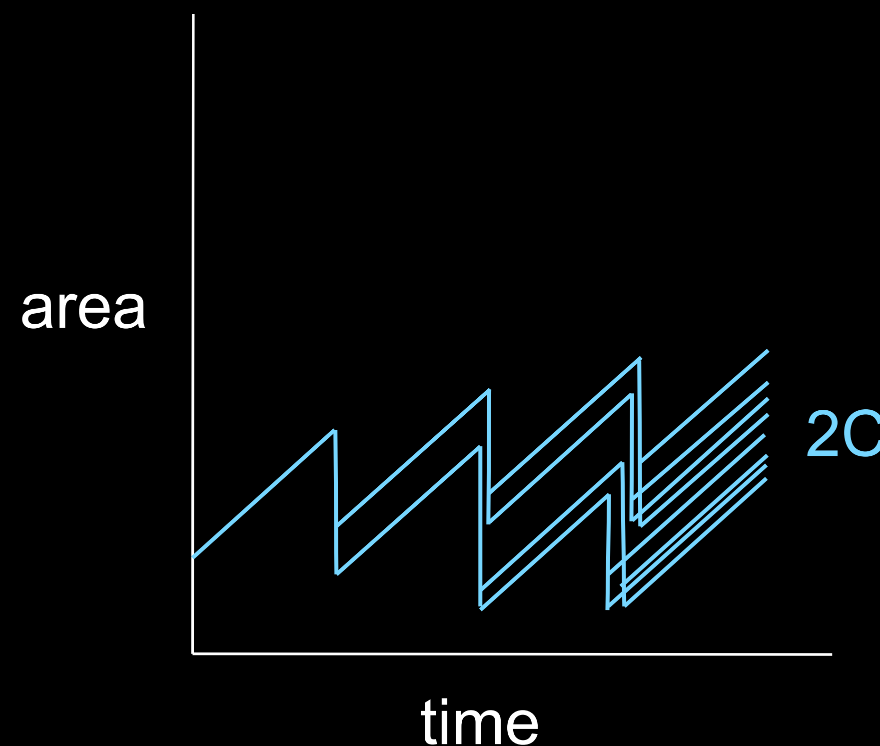
Divisions are unequal



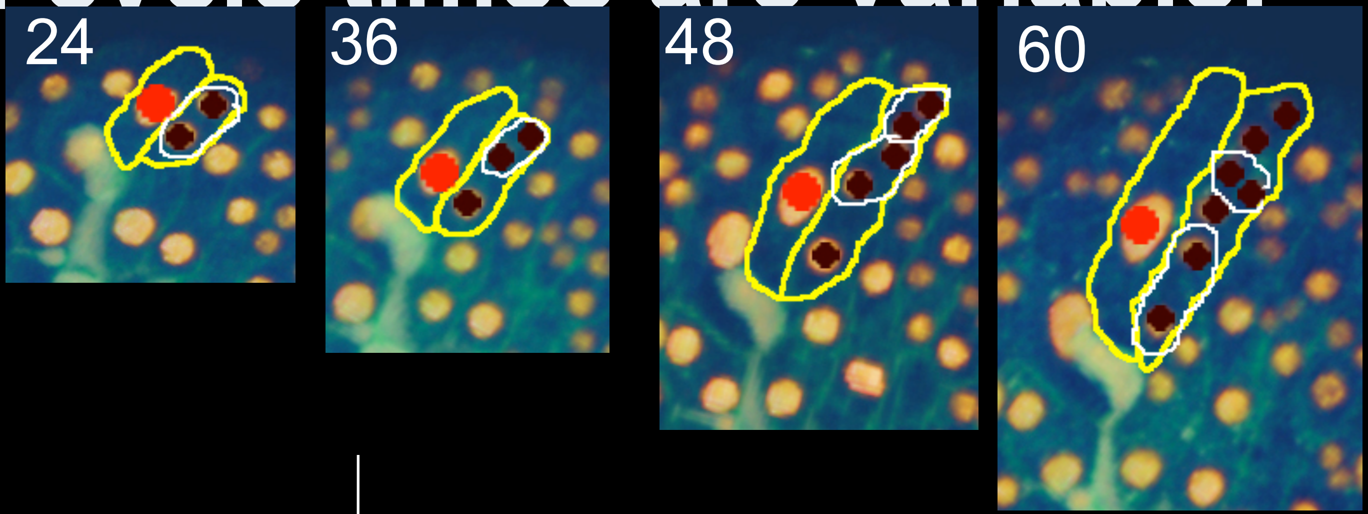
58%

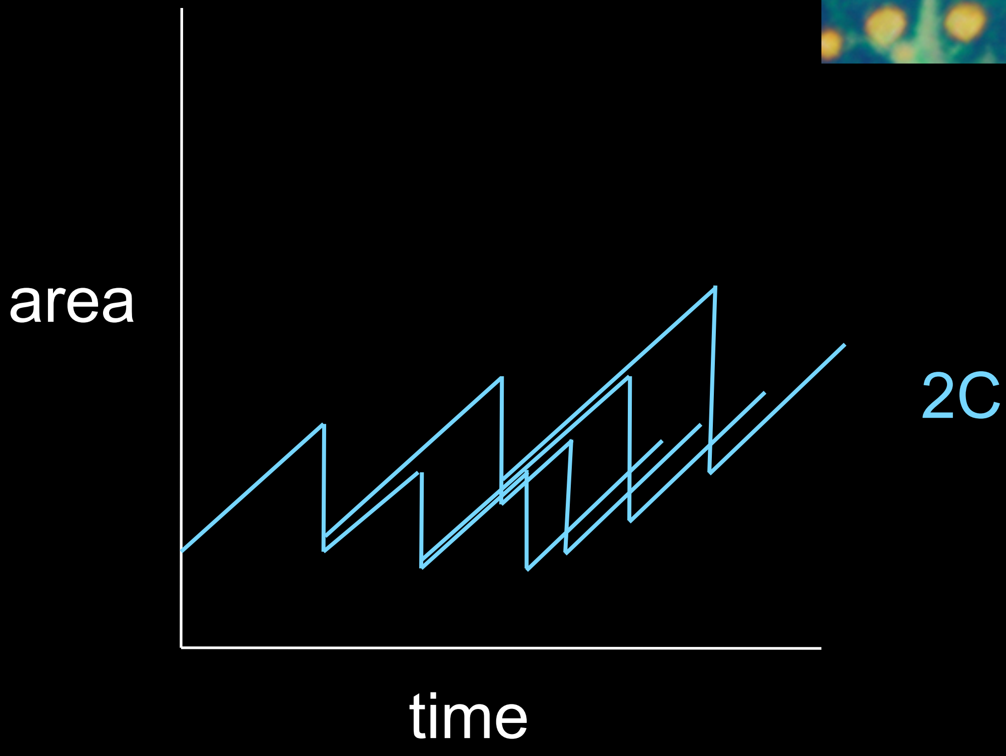
42%

Daughter cells after division

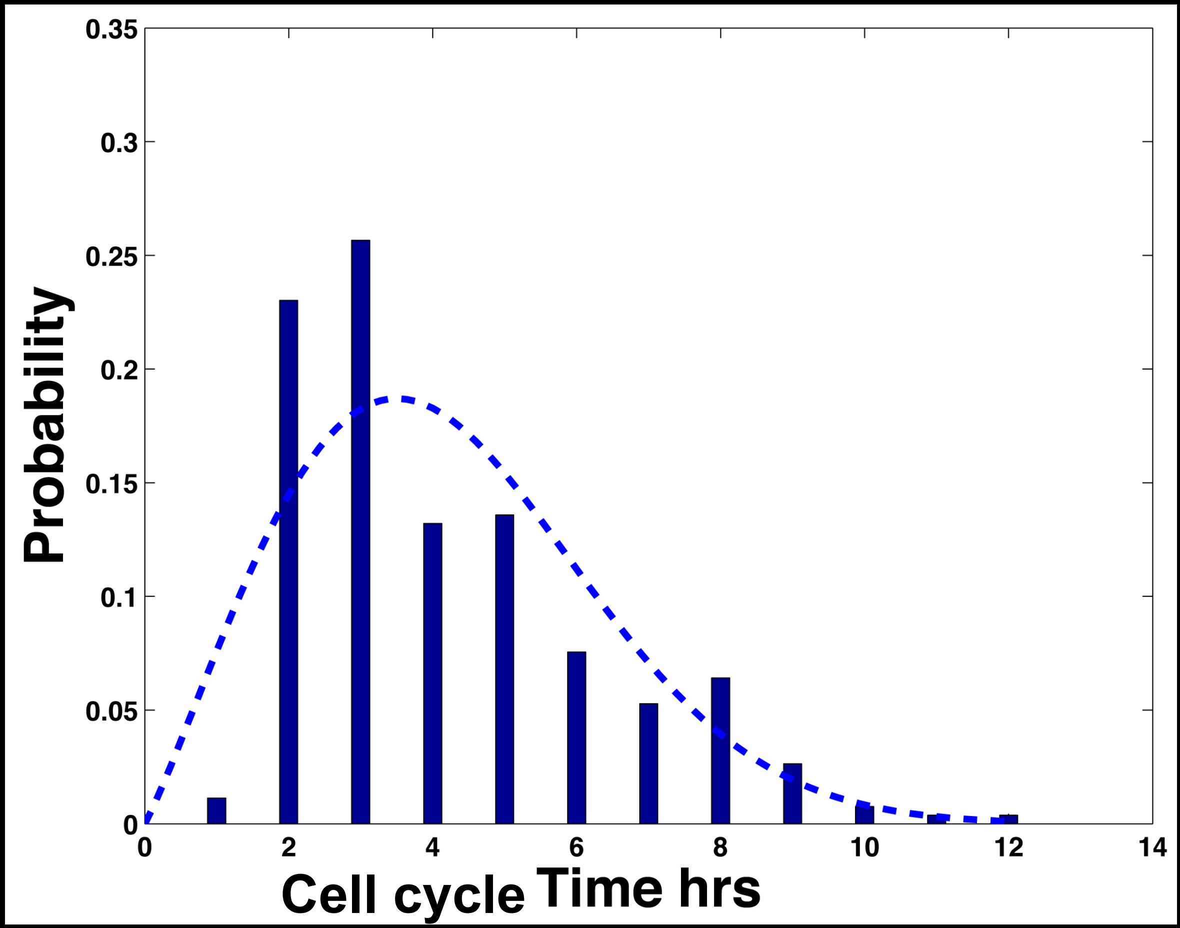


Cell cycle times are variable.

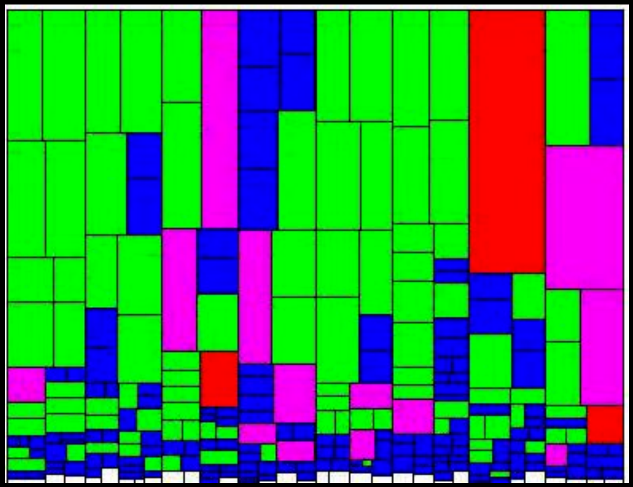




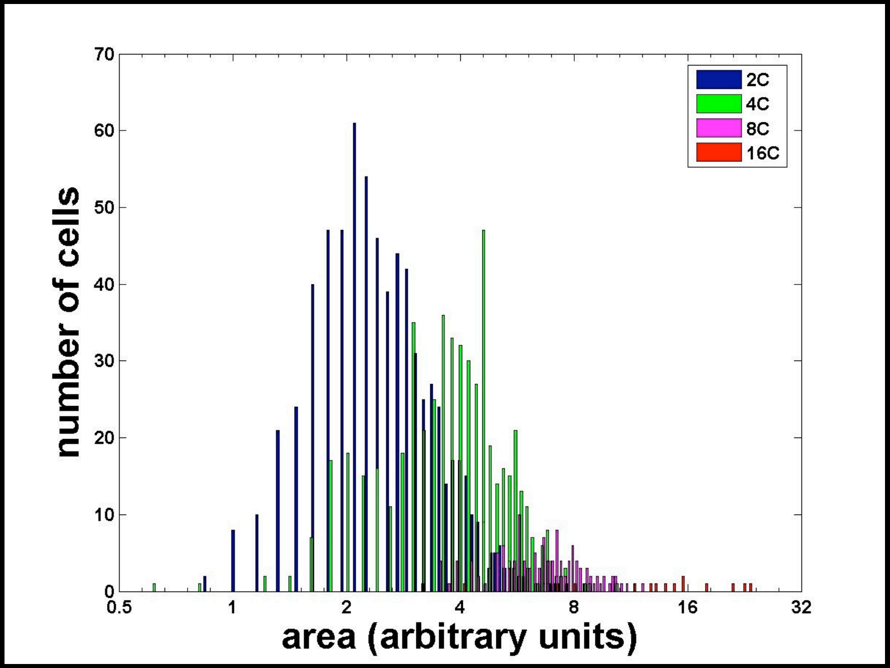
Cell cycle time distribution in wild type



New model with unequal divisions and asynchronous cell cycles

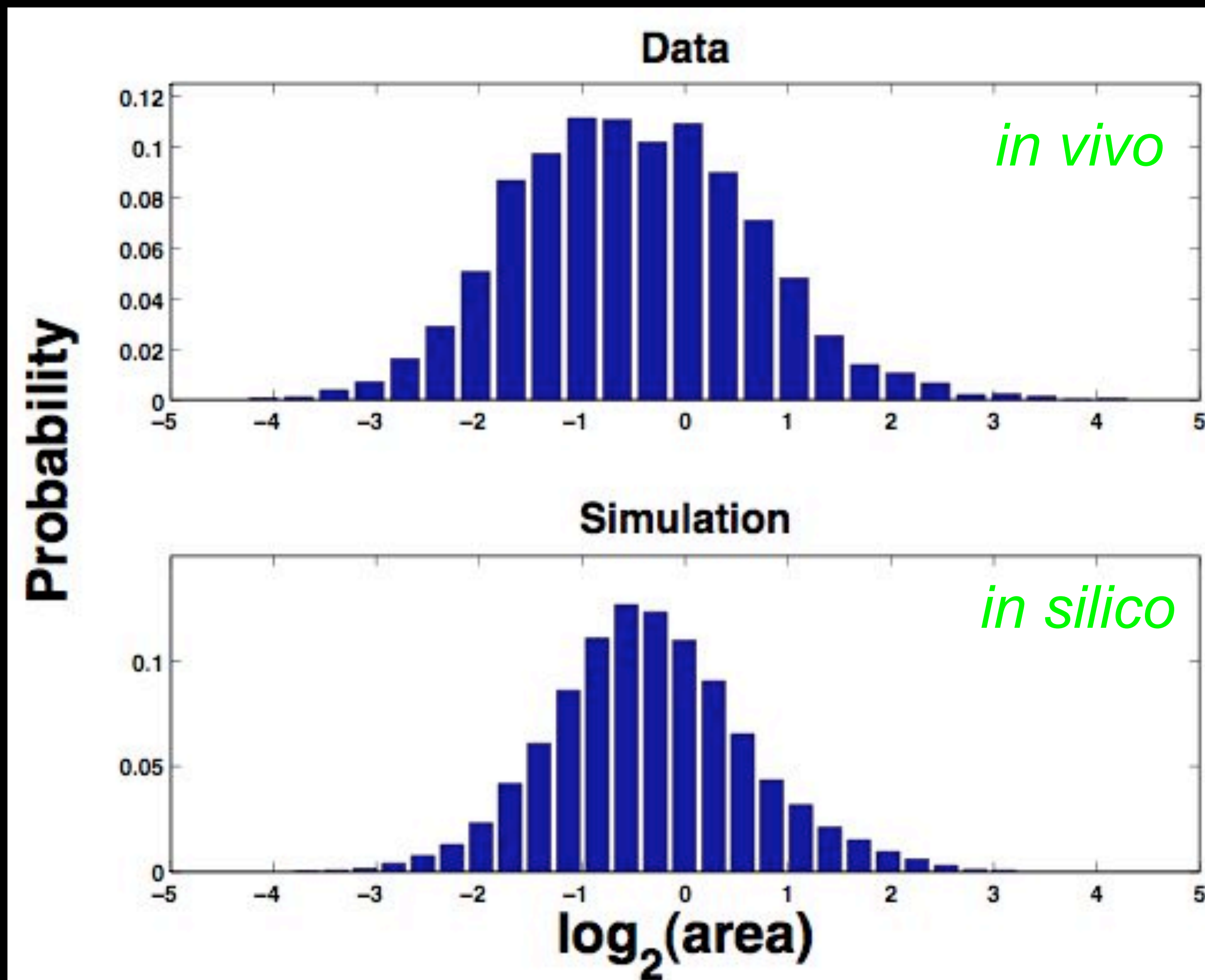


in silico

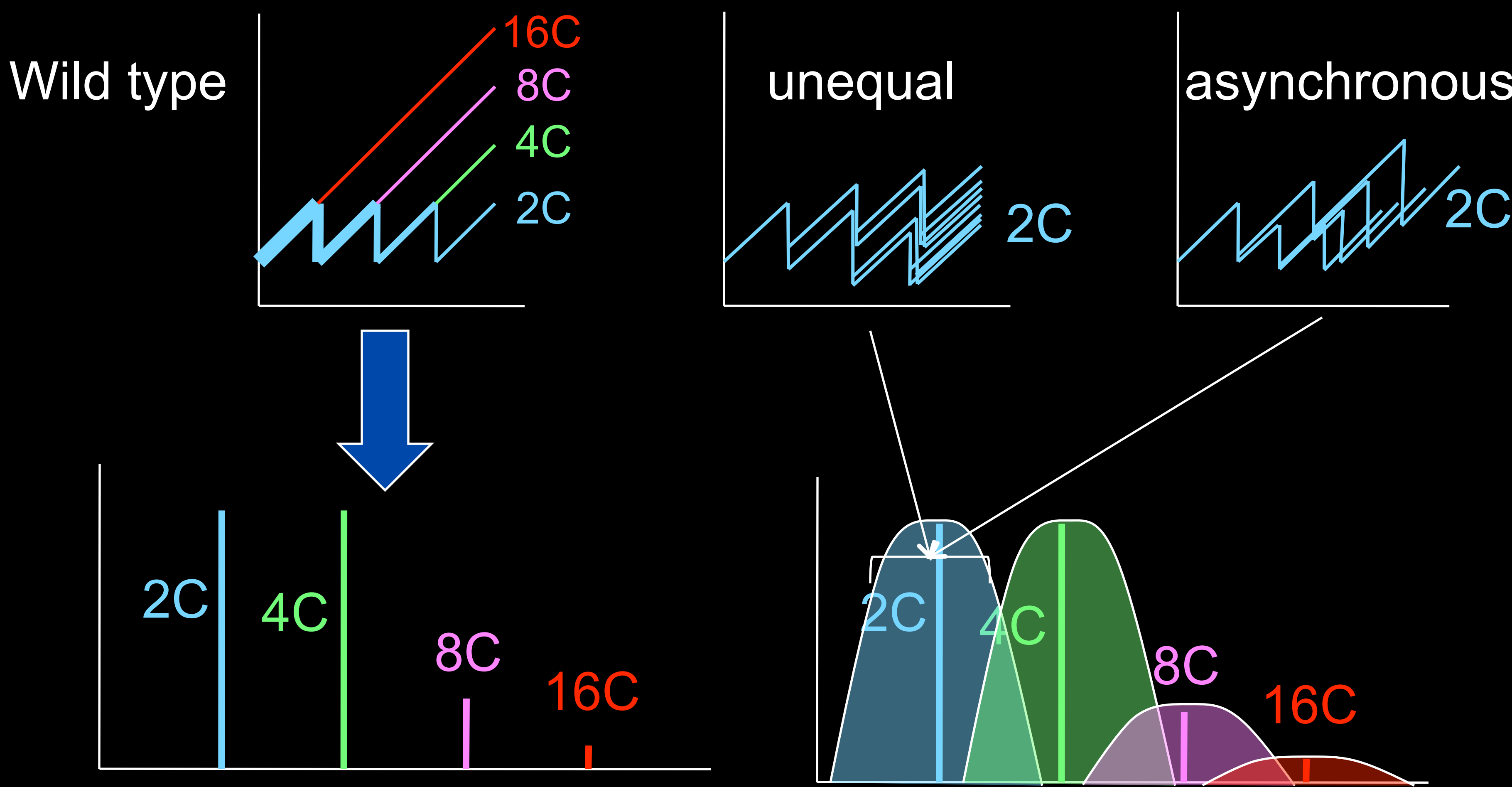


2C 4C 8C 16C

New model cell size pattern matches that of the in vivo sepal



Unequal and asynchronous cell divisions => variability



Take home points:

1. The **stochastic timing** of entry into endoreduplication is the major determinant of the relative **cell size pattern**.

2. Loss/gain of **cell cycle inhibitors** can change the timing of endoreduplication and the resulting **cell size pattern**.

3. Endoreduplication does not increase organ growth.

4. Variability in **cell cycle time** and **unequal divisions** creates the **variability** in cell size around the mean size established by endoreduplication.

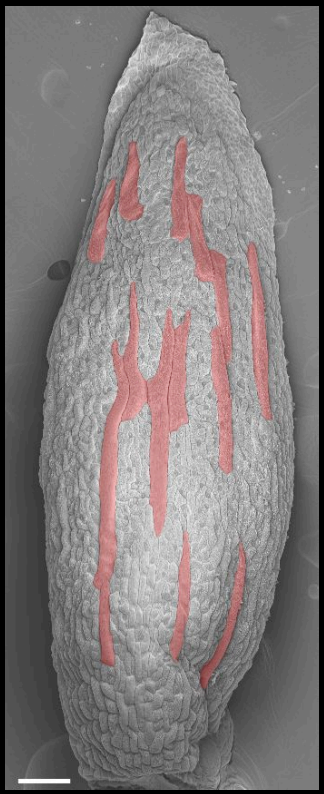
Conclusions:

fate specification  cell cycle

cell size pattern

Stochastic regulation of **cell division** without any need for chemical messages sent between the cells, can explain the cell size distribution.

Physical communication through shared cell walls must underlie their ability to grow at the same rates as their neighbors.



Future directions:

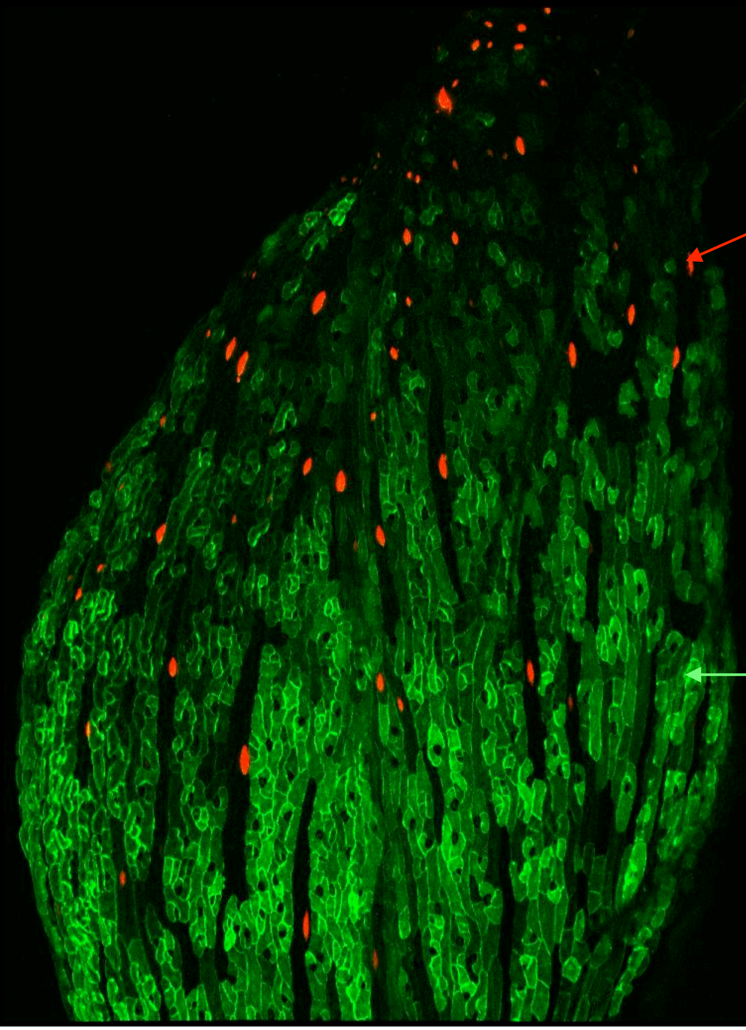
1. Cell size and cell fate
2. Spatial distribution of giant cells
3. Purpose of giant cells

Are giant cells a distinct cell type?

or

Are giant cells merely an extreme in a range of sepal epidermal cell sizes?

Giant cells and small cells have distinct patterns of gene expression



Giant cell specific enhancer element: nuclear localization

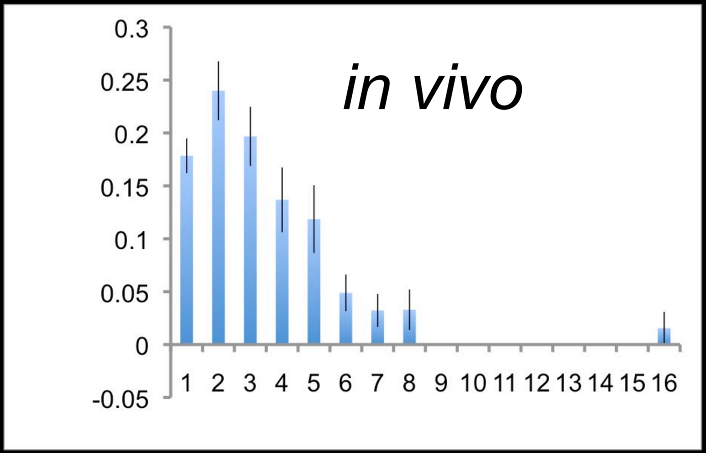
Small cell enhancer element: ER localization

Is the spatial location of giant cells random?

Is the spatial location of giant cells random?

Giant cells often form in clusters.

Percent of cells

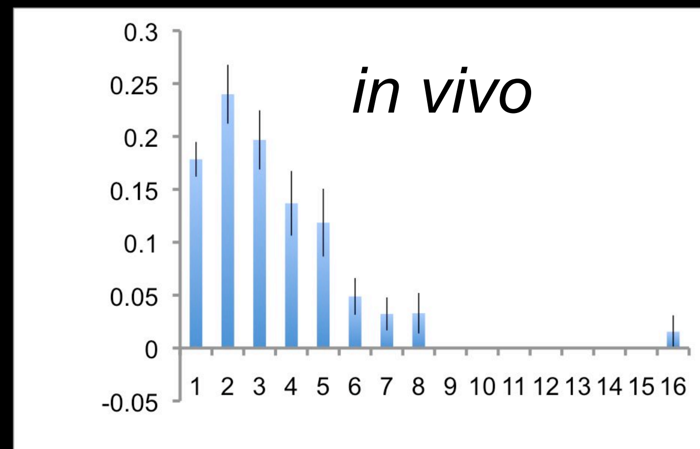


Number of cells in cluster

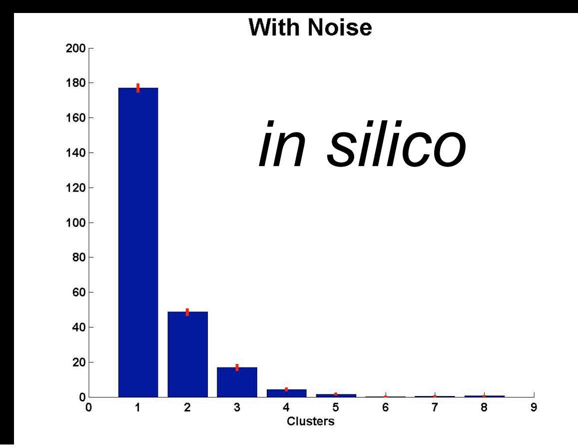
Is the spatial location of giant cells random?

Giant cells often form in clusters.

Percent of cells

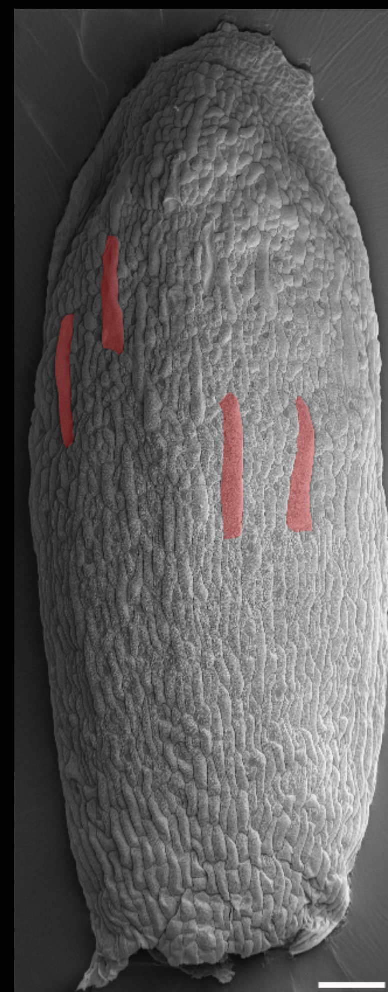


Number of cells in cluster



Hypothesis: intercellular signaling controls giant cell spacing

The ACR4 receptor kinase promotes giant cell formation.



acr4 mutant

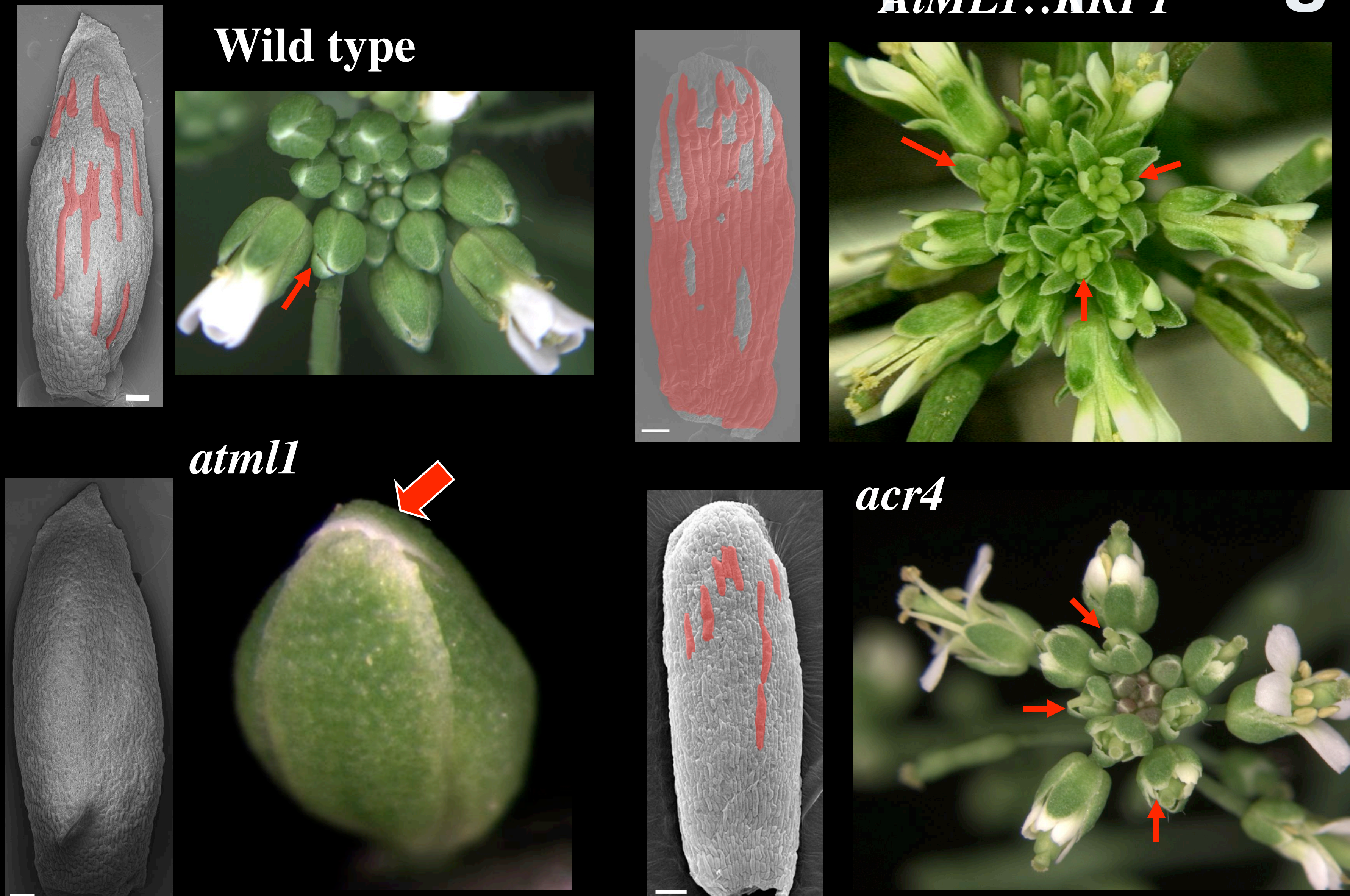
Purpose of giant cells?

1. Defense against insects.

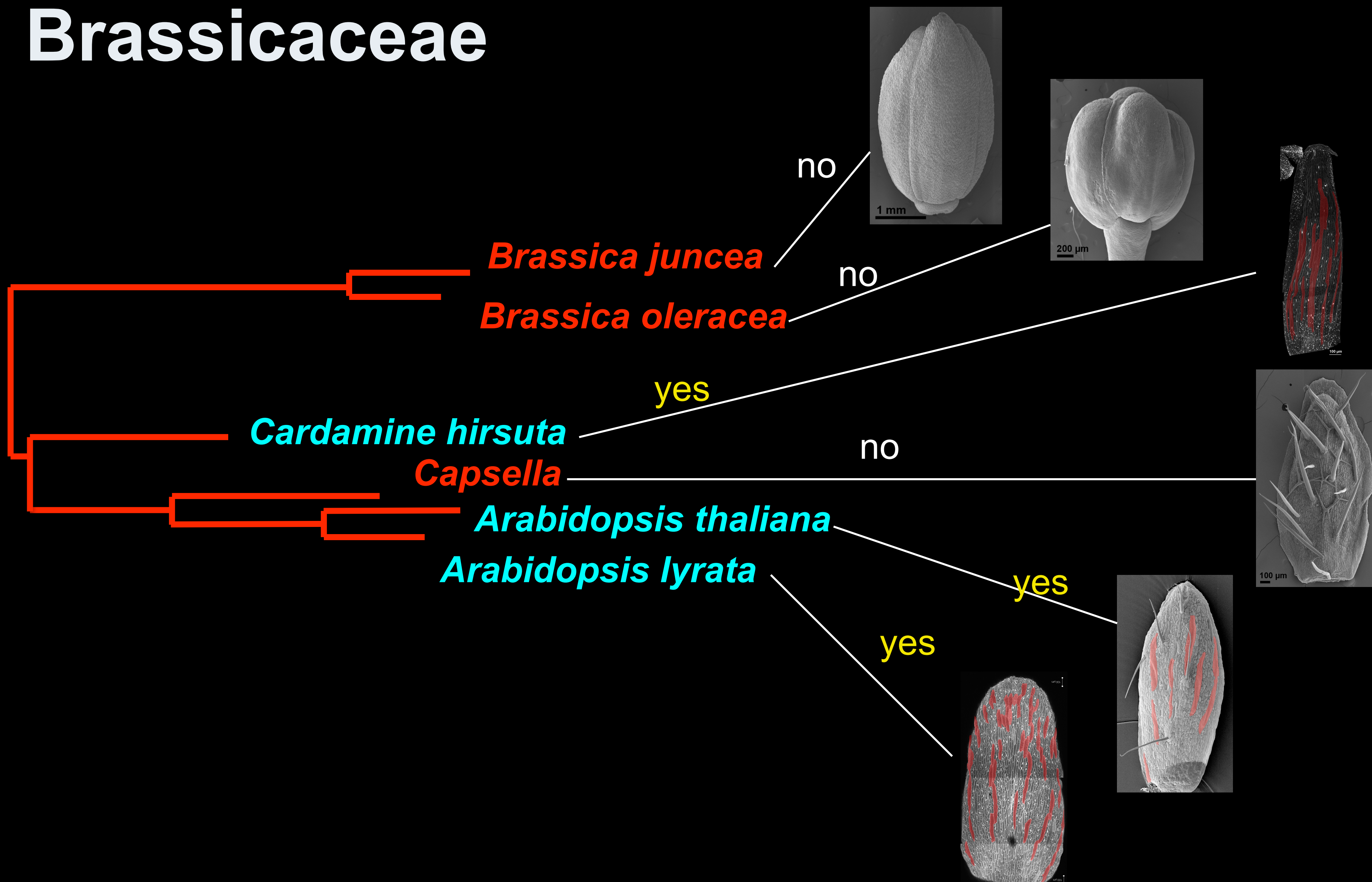
2. Mechanics of sepal curvature.

Wild-type flowers opening

Cell size influences sepal opening



Giant cells are not conserved in Brassicaceae



Tree adapted from Yang et al. (1999) Molecular Phylogenetics and Evolution 13, 455–462.

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