SIZE CONTROL IN PLANTS

RIKEN PSC

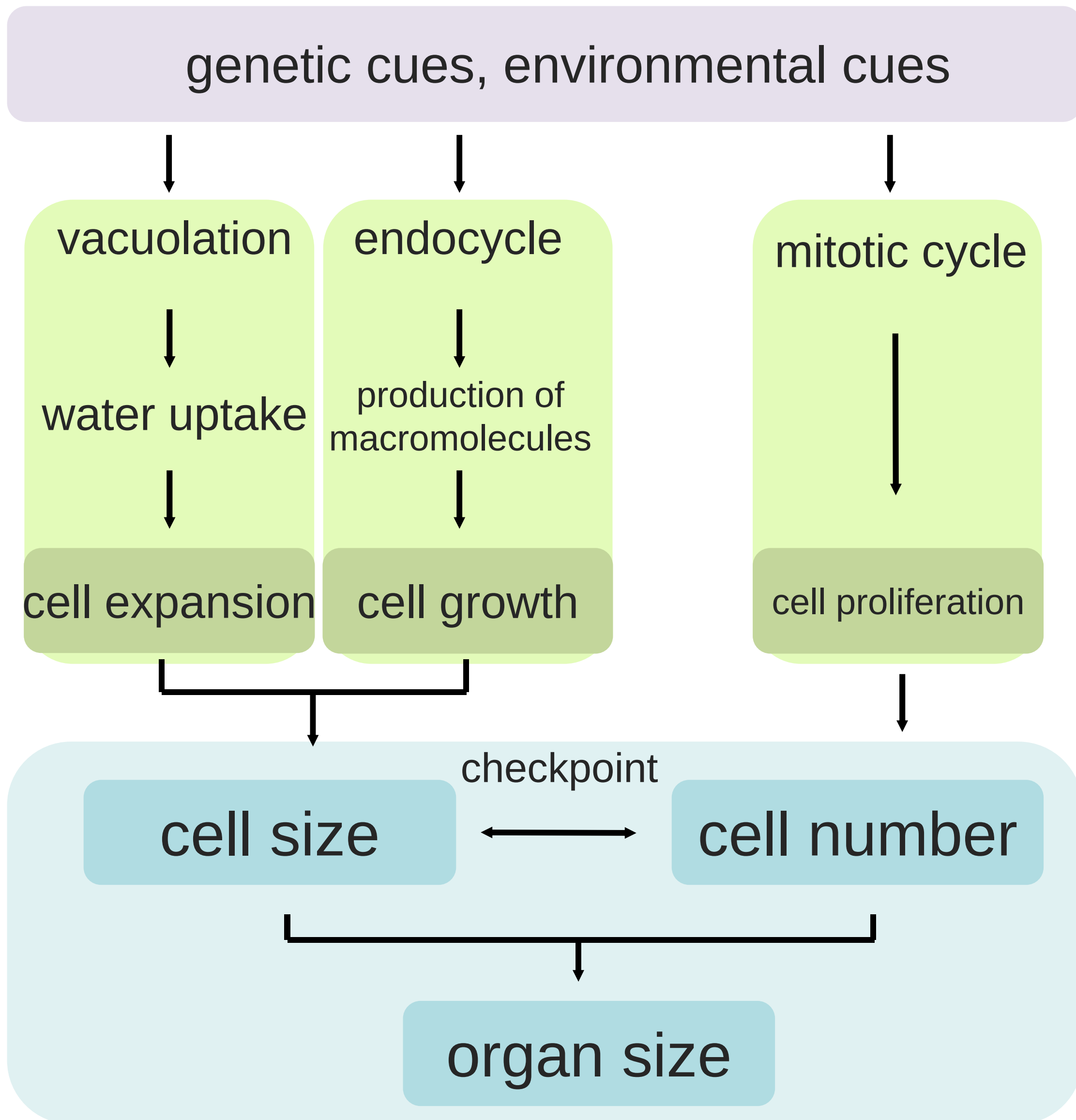
Keiko Sugimoto

Keith Roberts at the north Norfolk coast

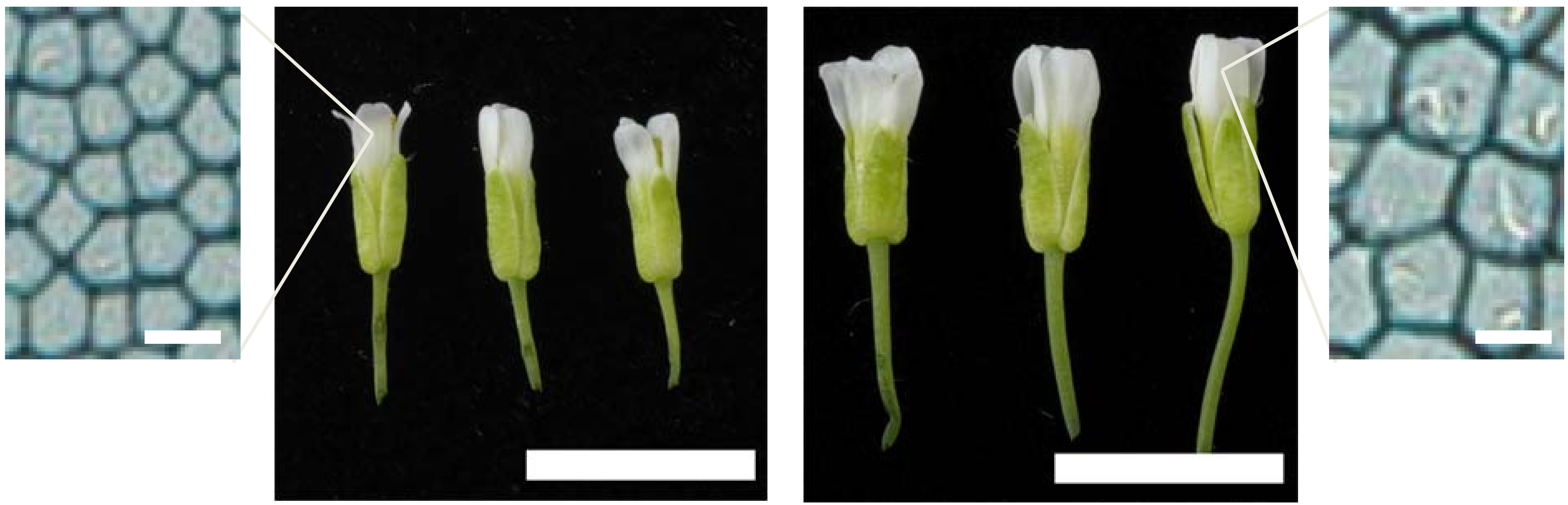
SIZE CONTROL IN PLANTS

1. Genetic dissection of cell-size control in plants.
2. Molecular characterisation of the link between cell size and ploidy.
3. Genetic dissection of organ-size control in plants.

How do plants control cell/organ size?



Cell size is correlated with ploidy.

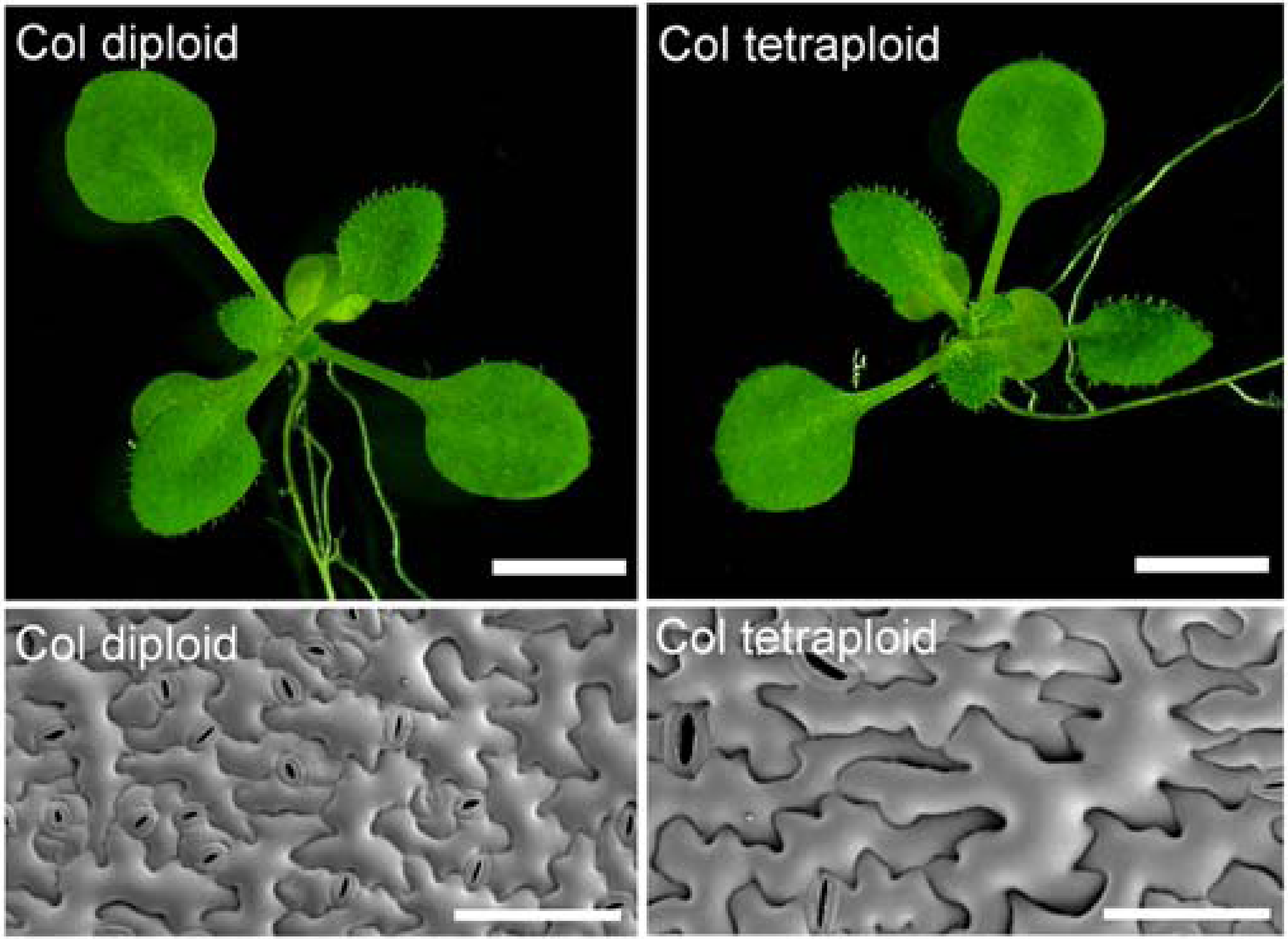


Diploid (2C) Tetraploid (4C)

Yield has depended on polyploidisation events.

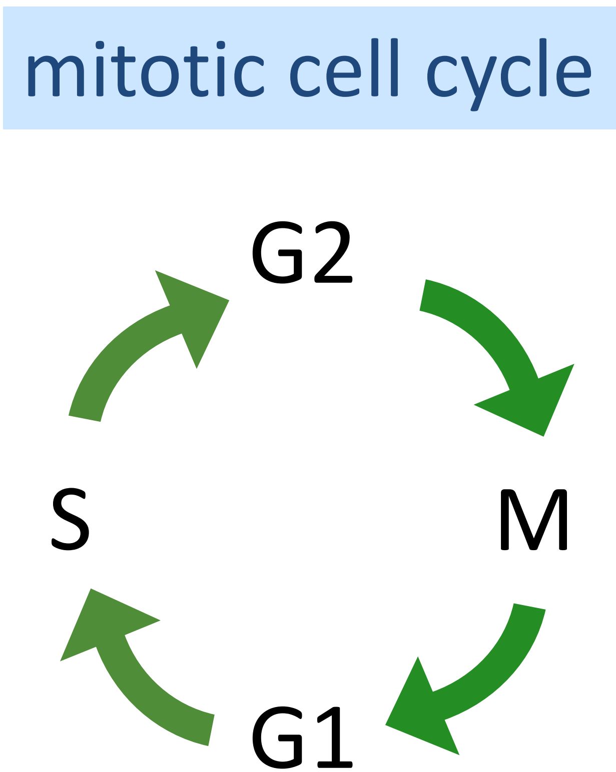
Examples: wheat, sugar beet, potato, coffee, banana, cotton, alfalfa

Cell size is correlated with ploidy.

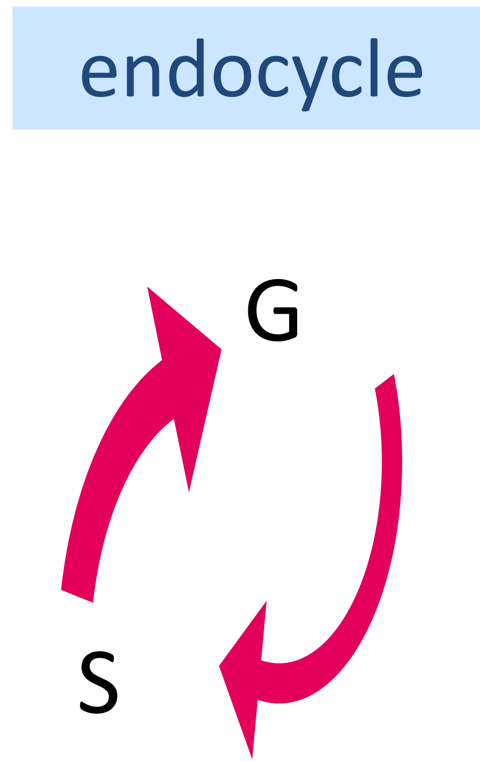


but not necessarily with organ size…

Cells increase ploidy levels through endoreduplication.



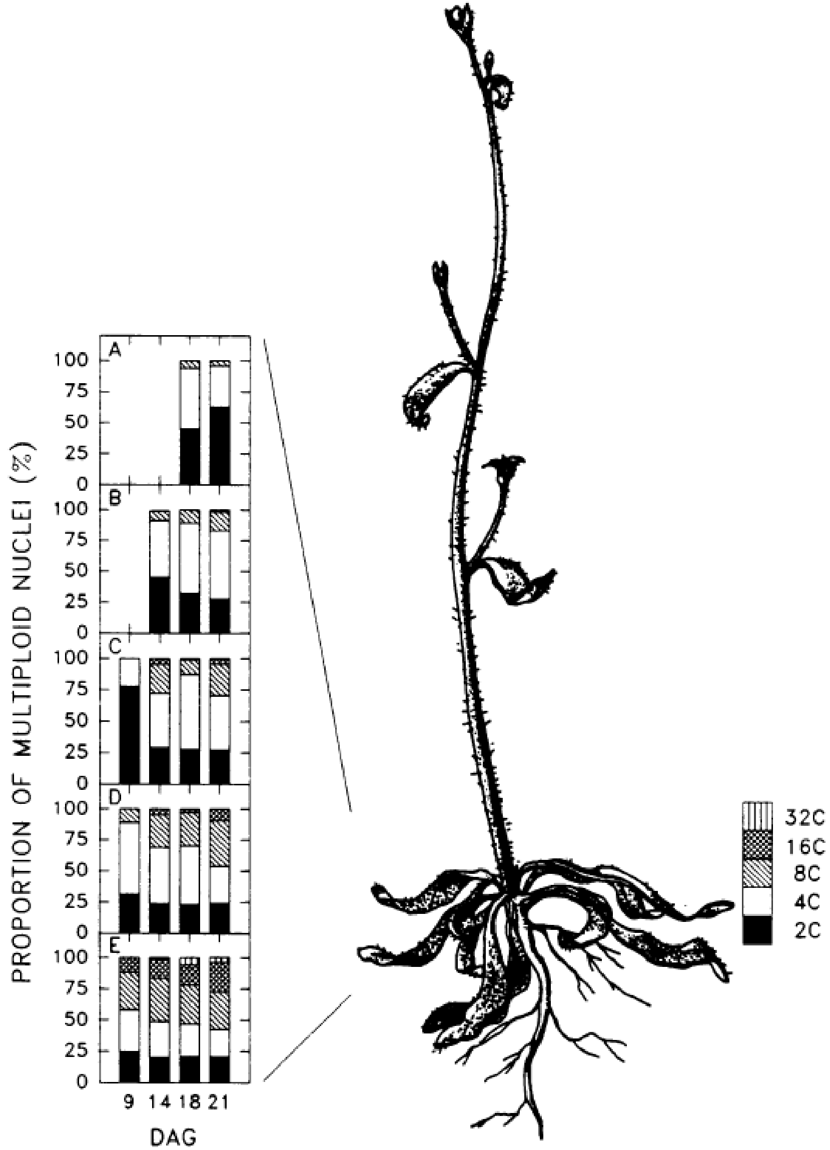
Ploidy level: 2C, 4C



Ploidy level: 2C, 4C, 8C, …, >32C

Many cell types endoreduplicate in *Arabidopsis*.

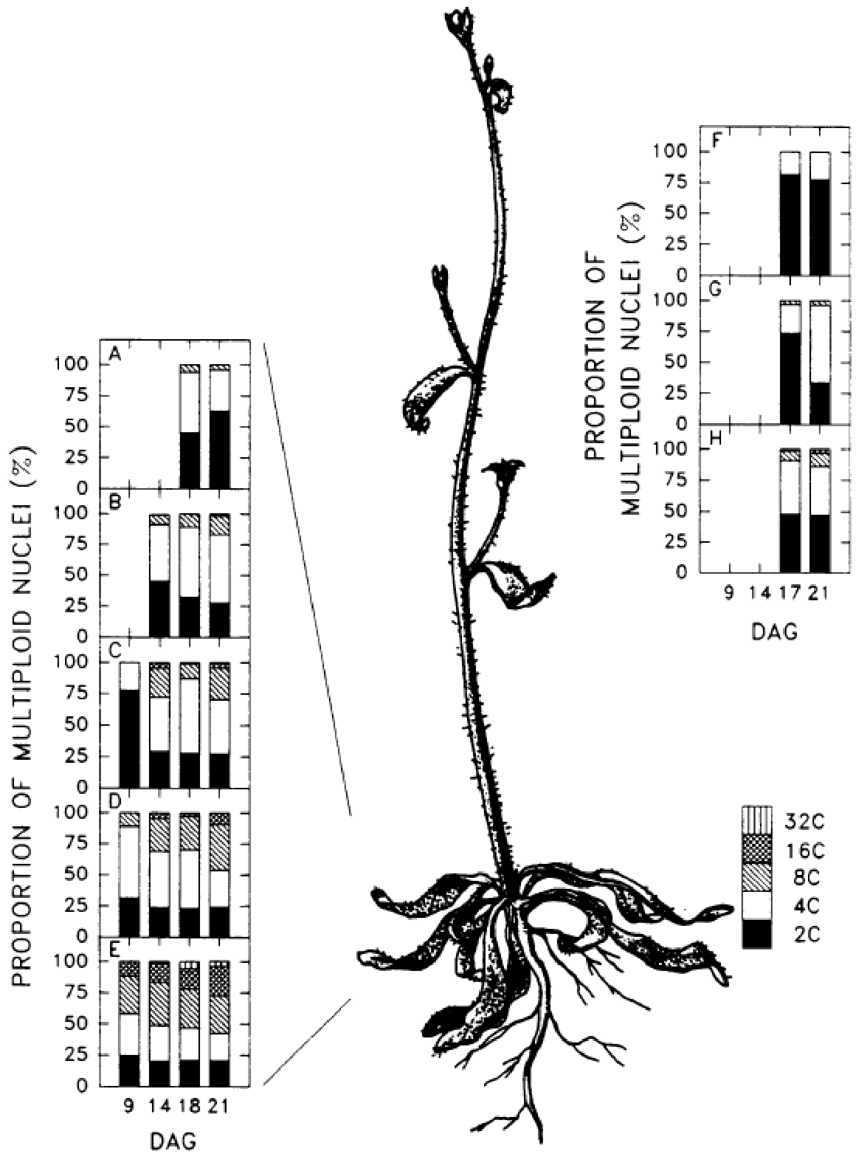
True leaves and cotyledons



Galbraith et al (Plant Phys 1991)

Many cell types endoreduplicate in *Arabidopsis*.

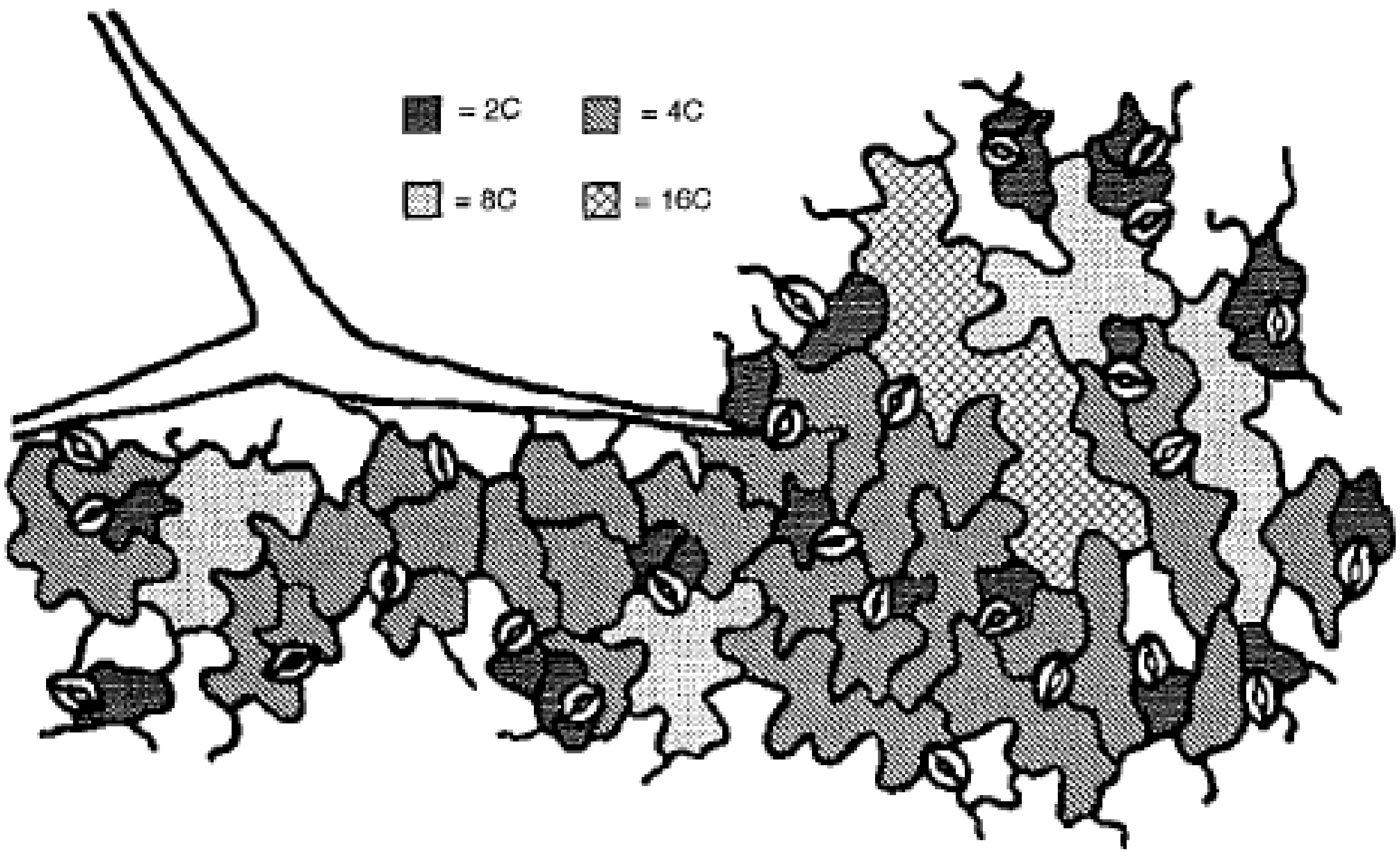
True leaves and cotyledons



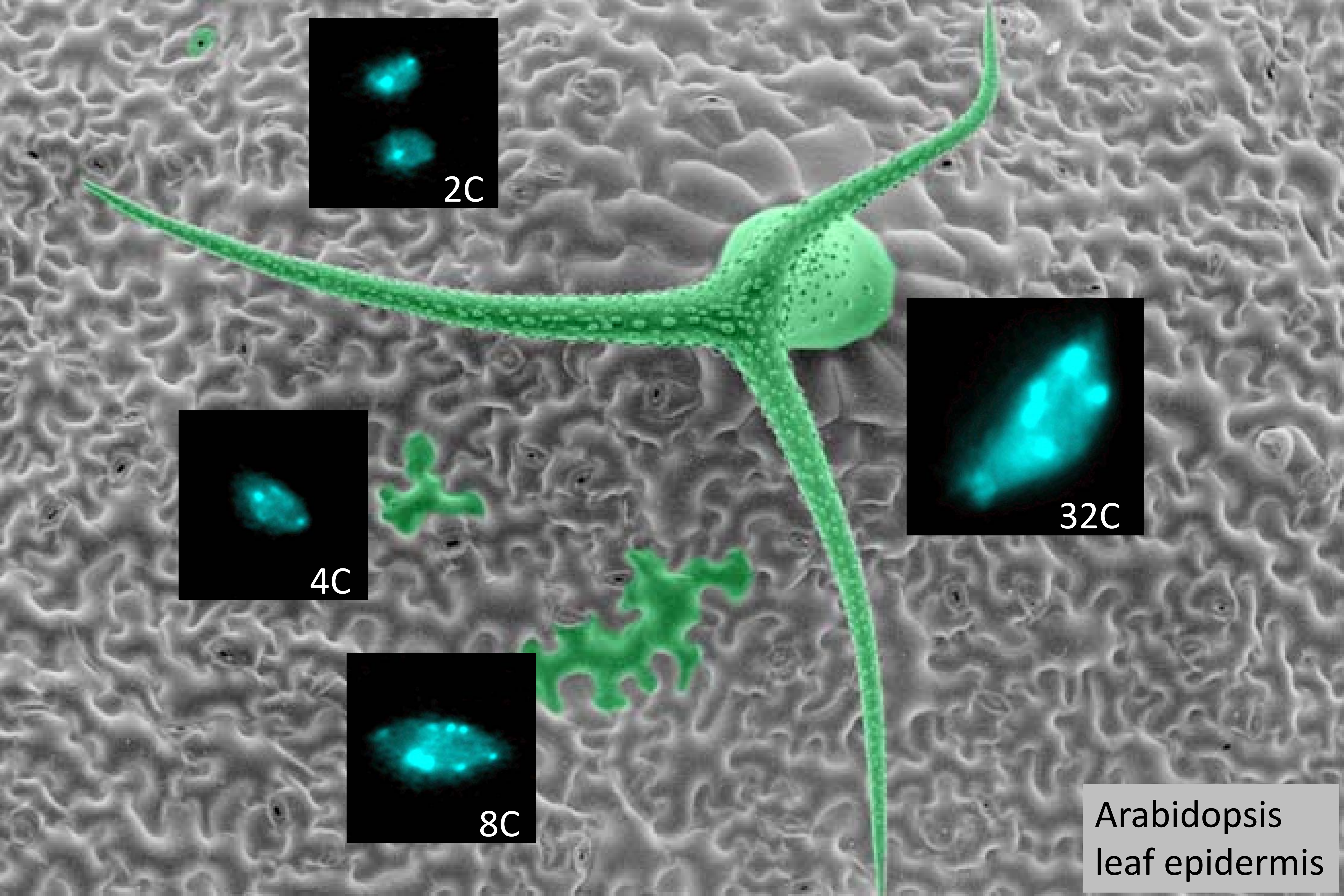
flowers

Galbraith et al (Plant Phys 1991)

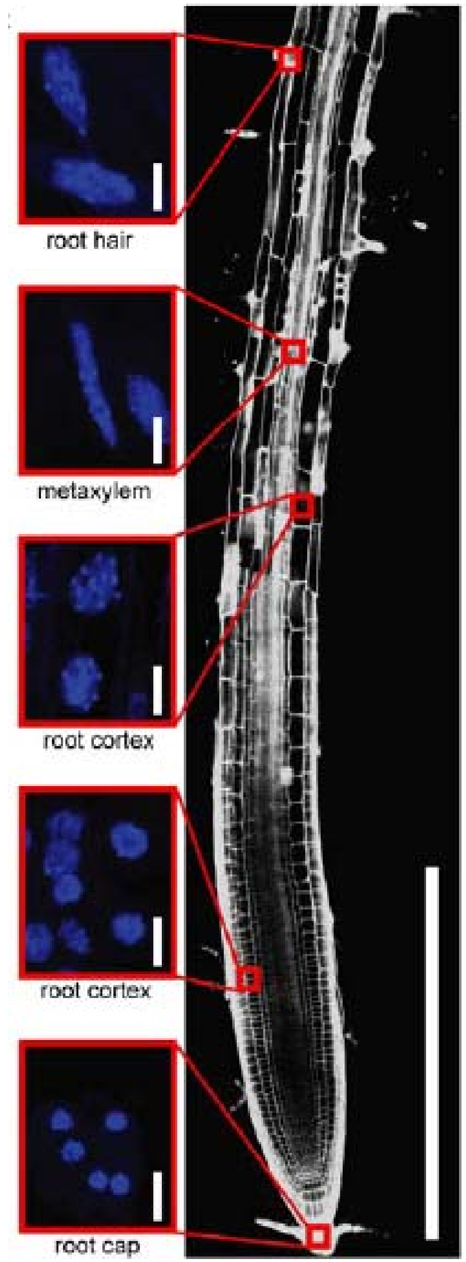
Arabidopsis leaf cells endoreduplicate up to 16C.



Melaragno (Plant Cell 1993)


2C
4C
8C
32C
Arabidopsis
leaf epidermis

Arabidopsis root cells endoreduplicate up to 16C.

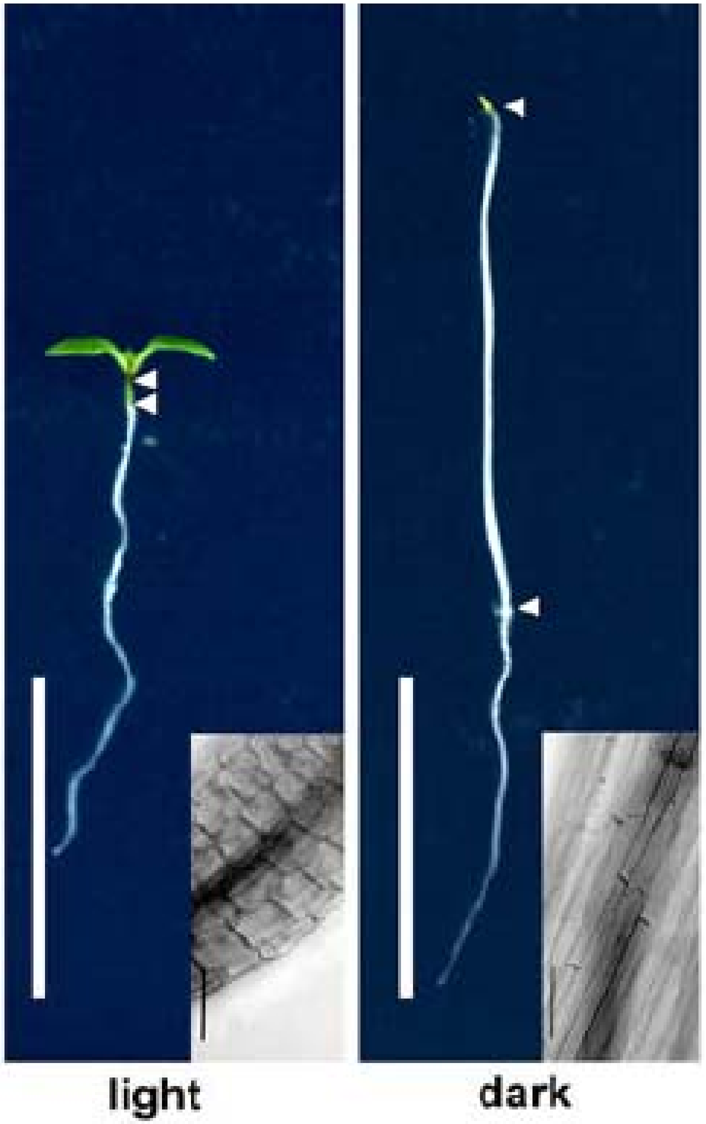


cell expansion /
cell differentiation

cell proliferation

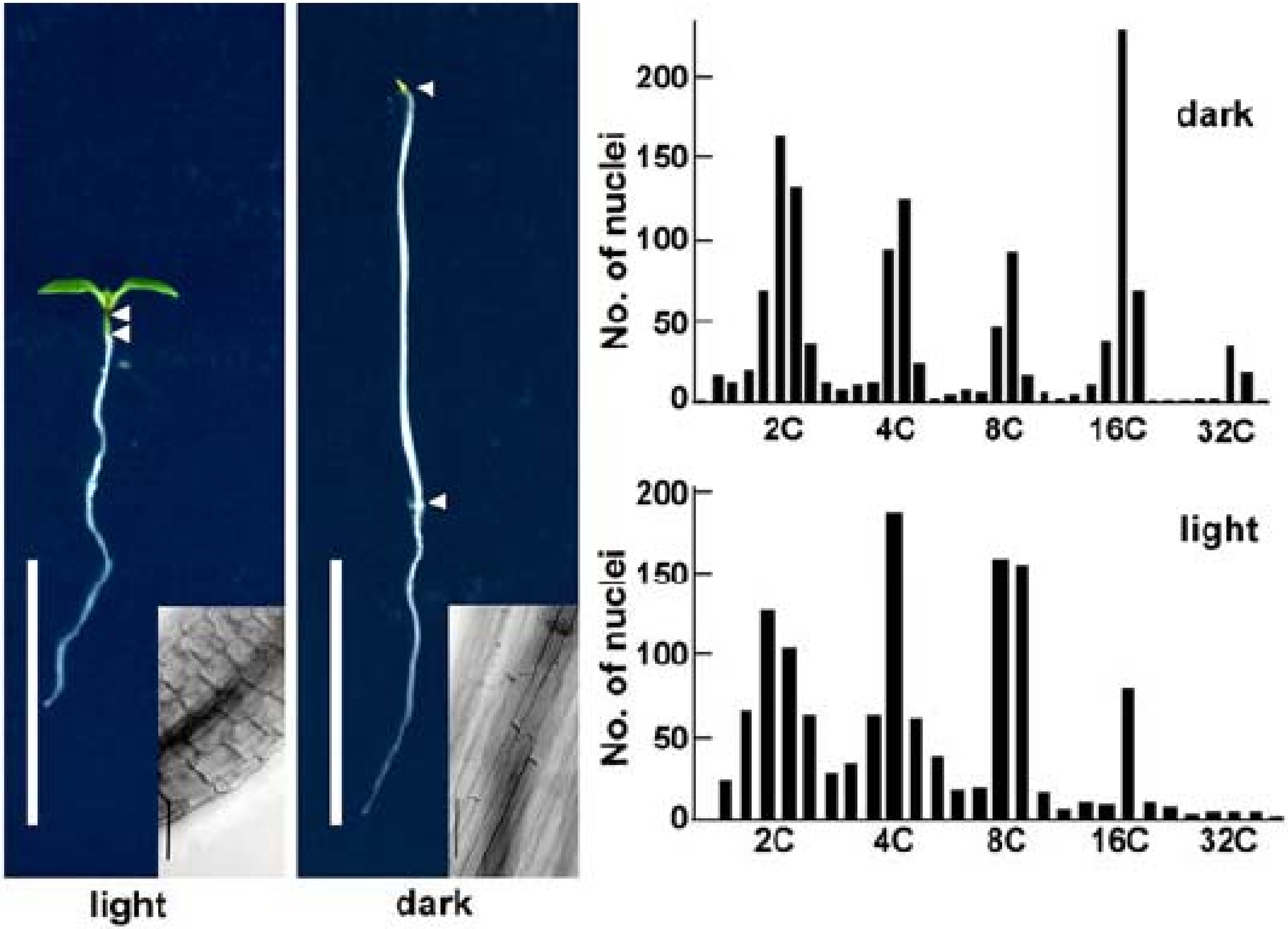
Arabidopsis root cells

Cell size is correlated with ploidy.



Arabidopsis hypocotyl cells

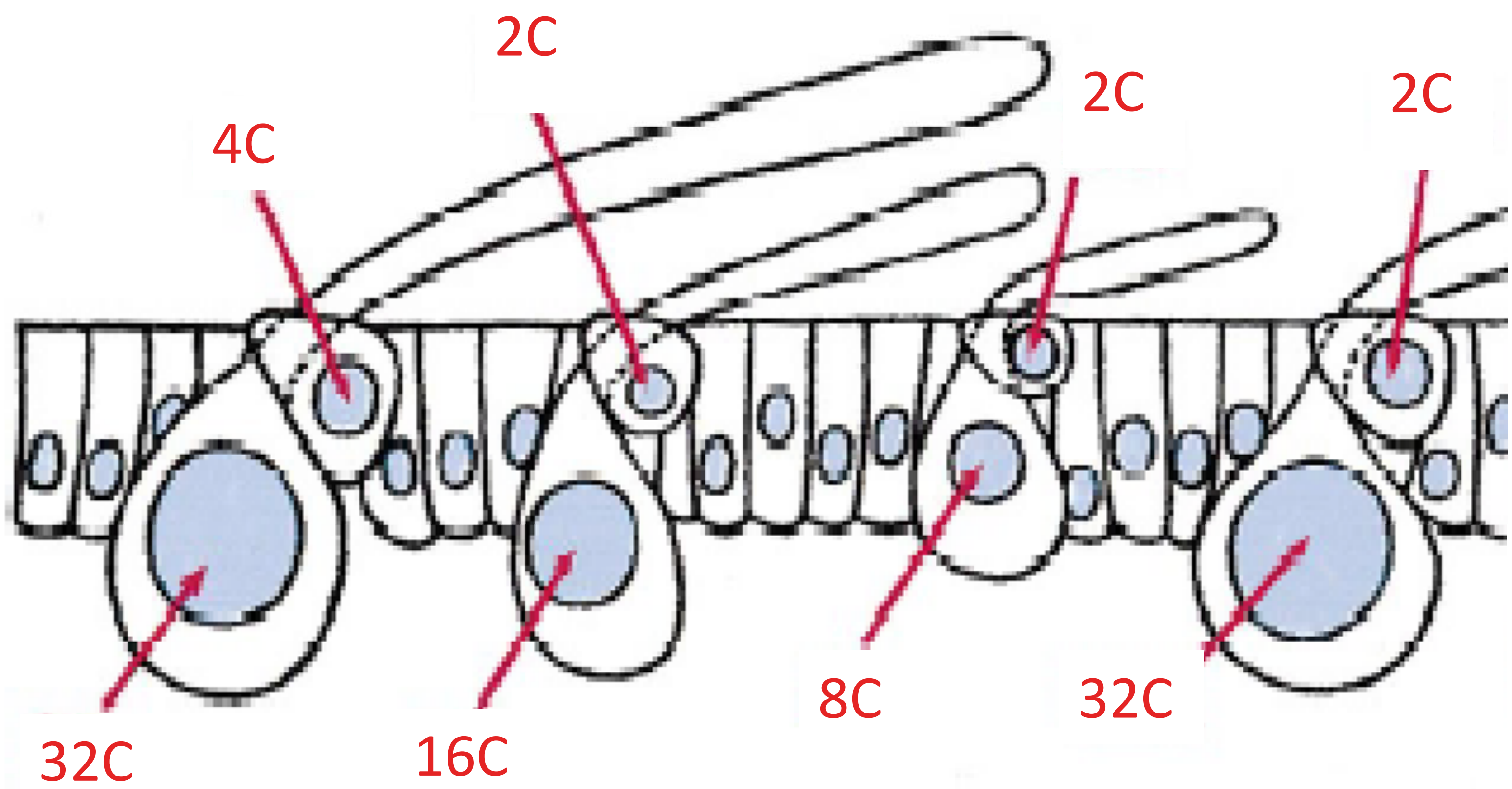
Cell size is correlated with ploidy.



Arabidopsis hypocotyl cells

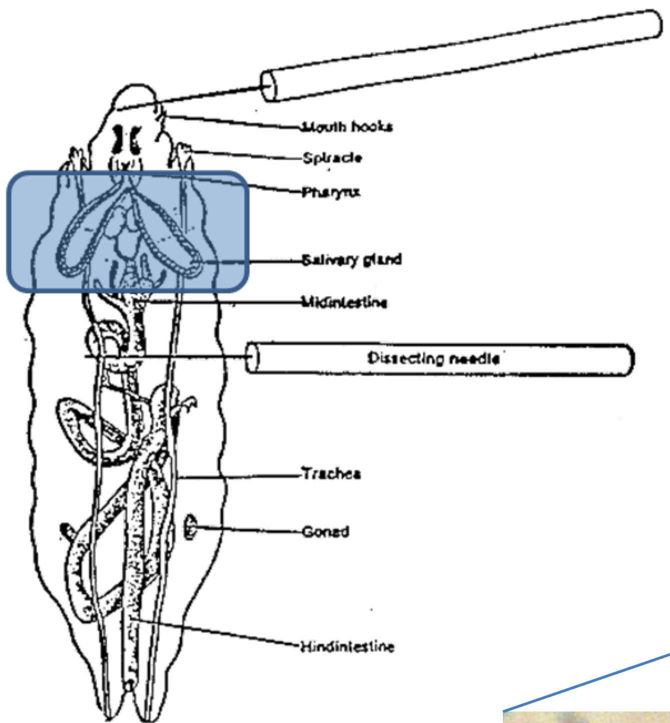
Cell size is correlated with ploidy.

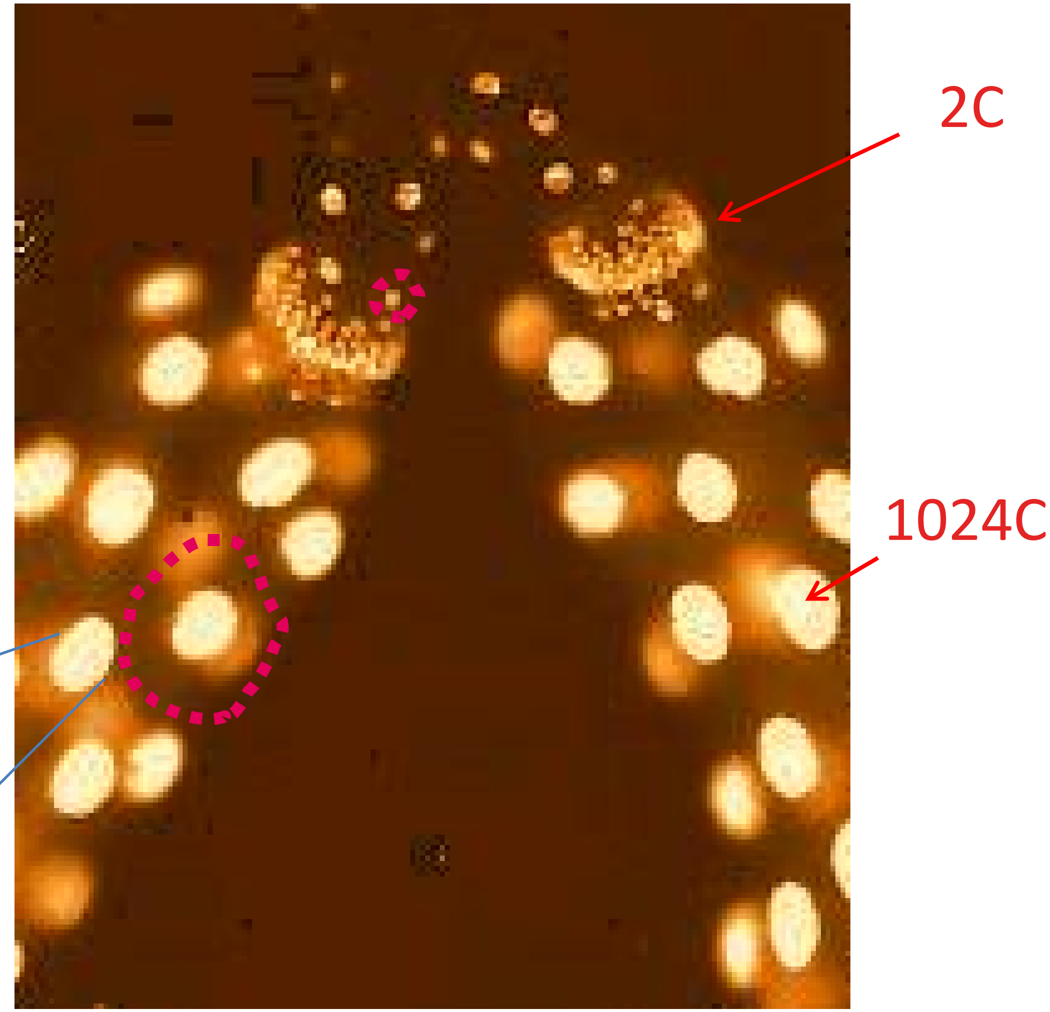
- Moth wing epithelium -



Cell size is correlated with ploidy.

- Drosophila salivary glands -

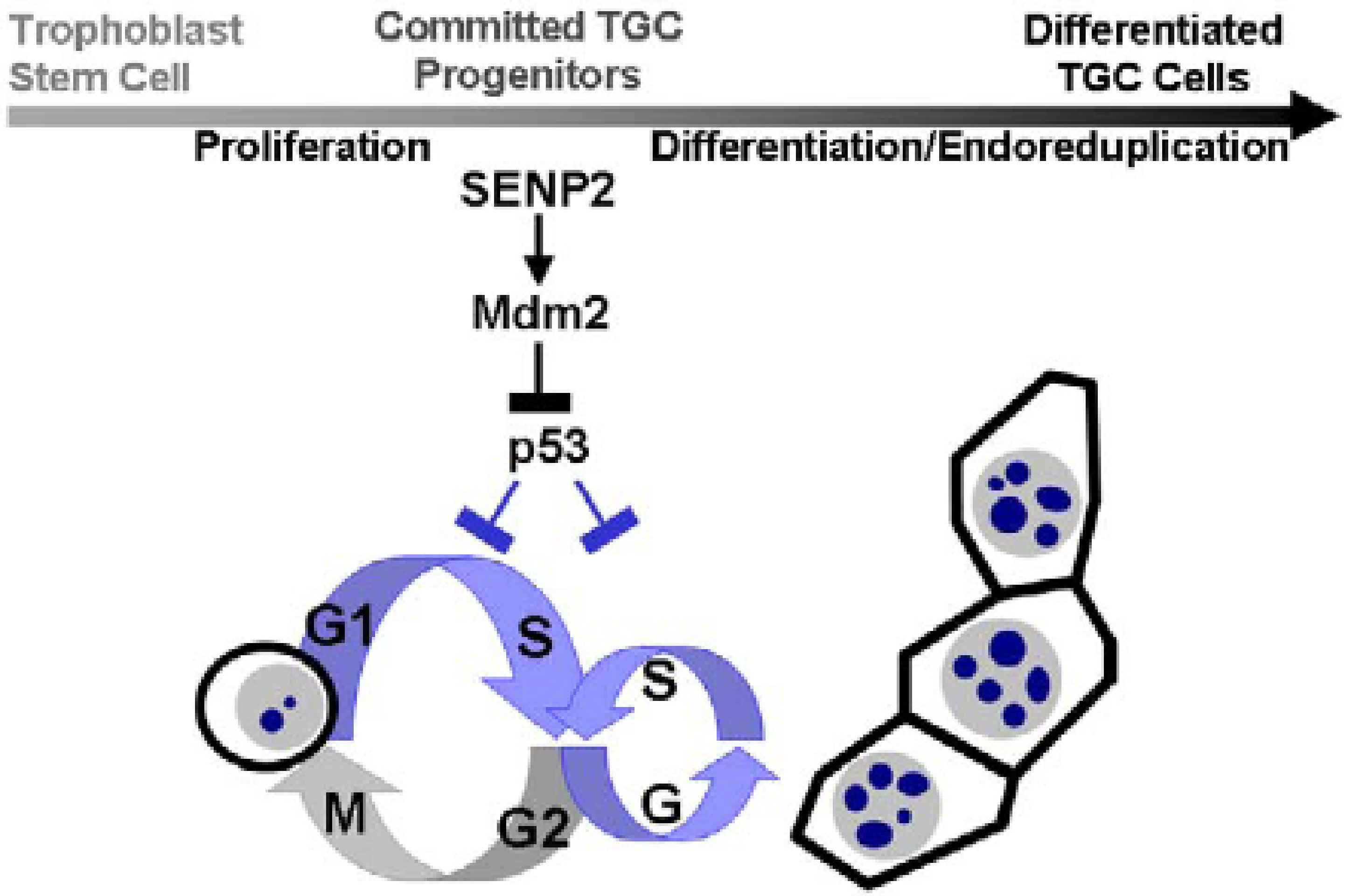




polytene chromosomes

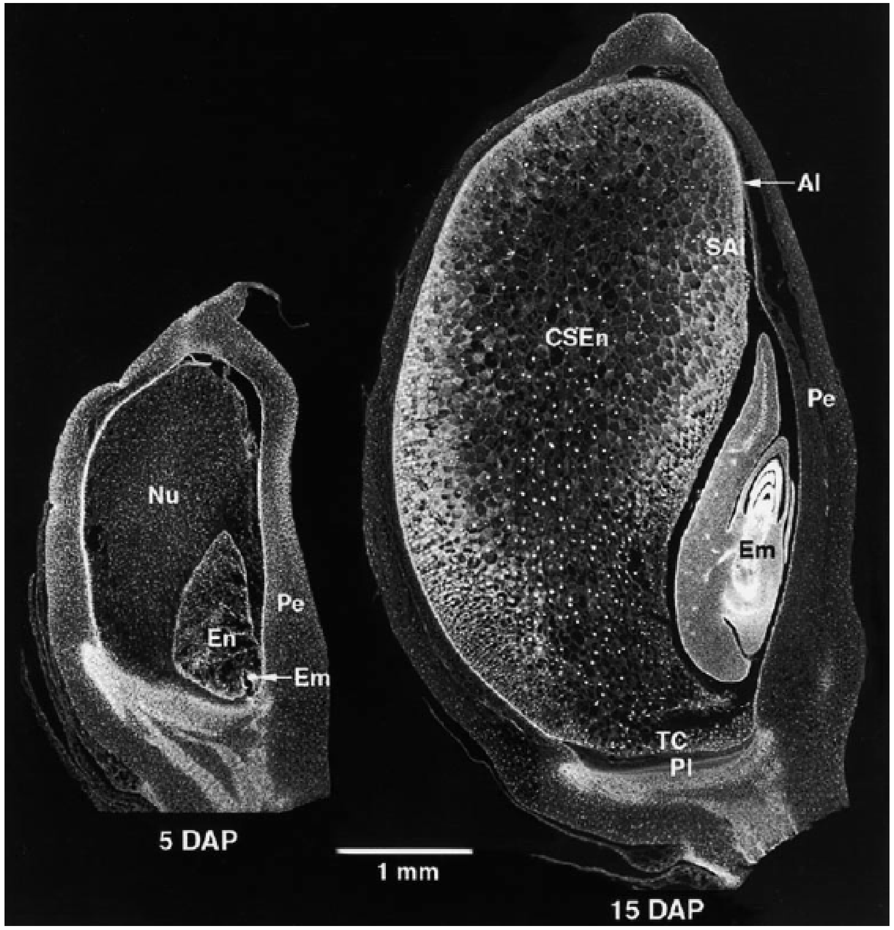
Cell size is correlated with ploidy.

- Mouse trophoblast giant cell (TGC) in placenta development-



application potential: endoreduplication in plants

■ endosperm development (e.g. maize)

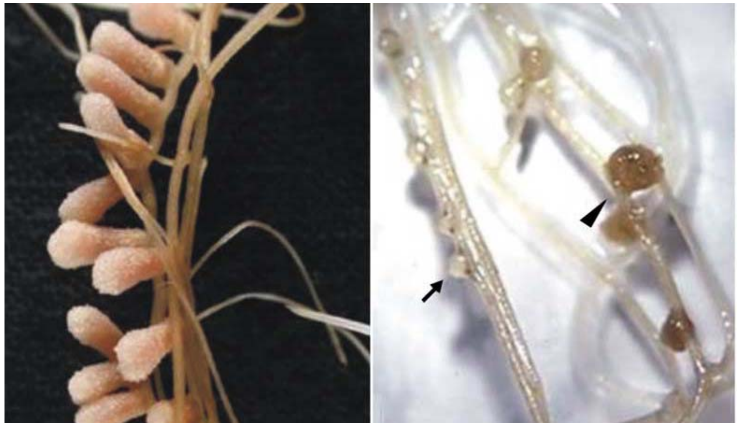


(Larkins 2001)

■ fruit development (e.g. tomato)

(Tanksley 2004)

■ symbiosis (e.g. medicago)

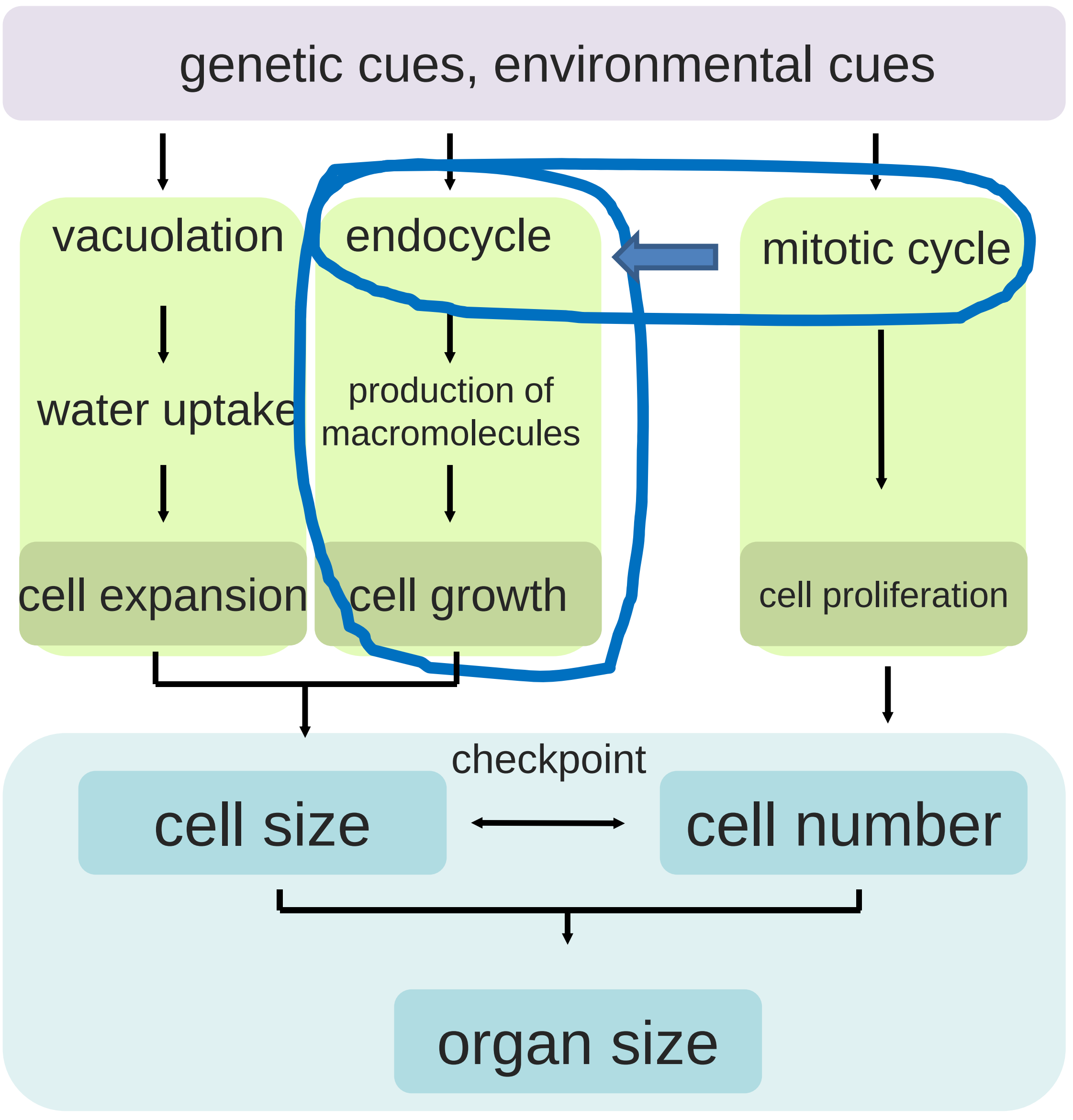


(Kondorosi 2004)

General questions

- How do cells switch from the mitotic cell cycle to the endocycle?
- How do genetic and environmental cues influence the endocycle transition?
- Does endocycling utilise the same cell cycle machinery as the mitotic cycle?
- How does an increase in ploidy through endoreduplication link with cell differentiation/cell expansion?

How do plants control cell/organ size?



Our genetic approach: size mutants in *Arabidopsis*

1. *hyp6,7/rhl1-3/bin3-5*

   --- Christian Breuer, Nicola Stacey

2. *high ploidy 1-4 (hpy1-4)*

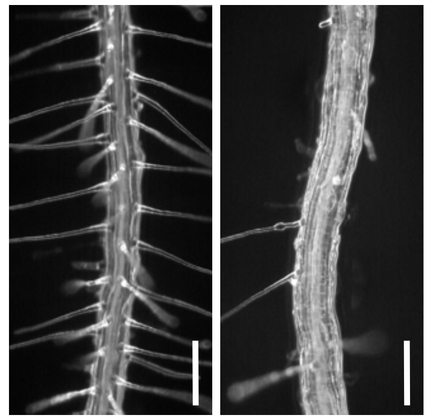
   --- Takashi Ishida, Sumire Fujiwara

3. *full-length cDNA over-expression (FOX) lines*

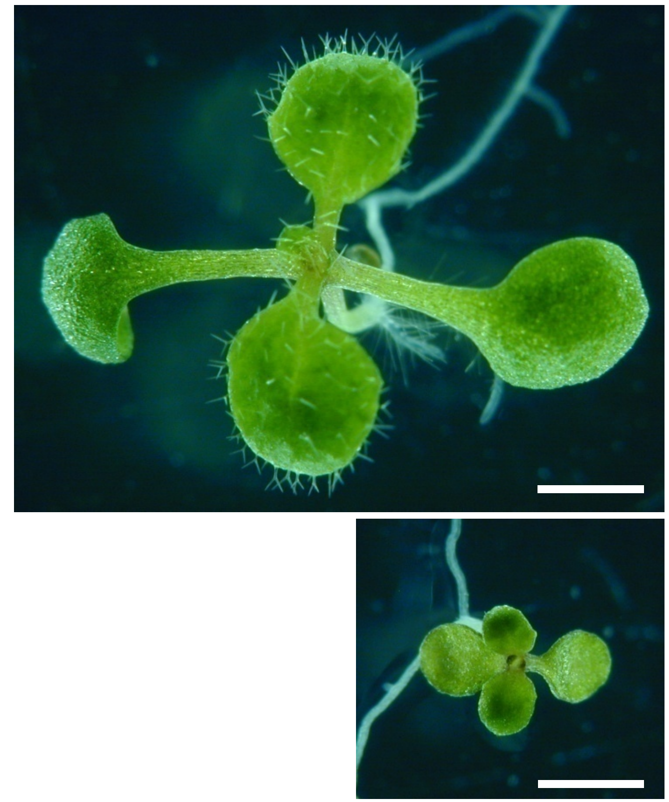
   --- Christian Breuer, Ayako Kawamura

hyp/rhl/bin display similar dwarf phenotypes.

hypocotyl 6 (hyp6)
hypocotyl 7 (hyp7)



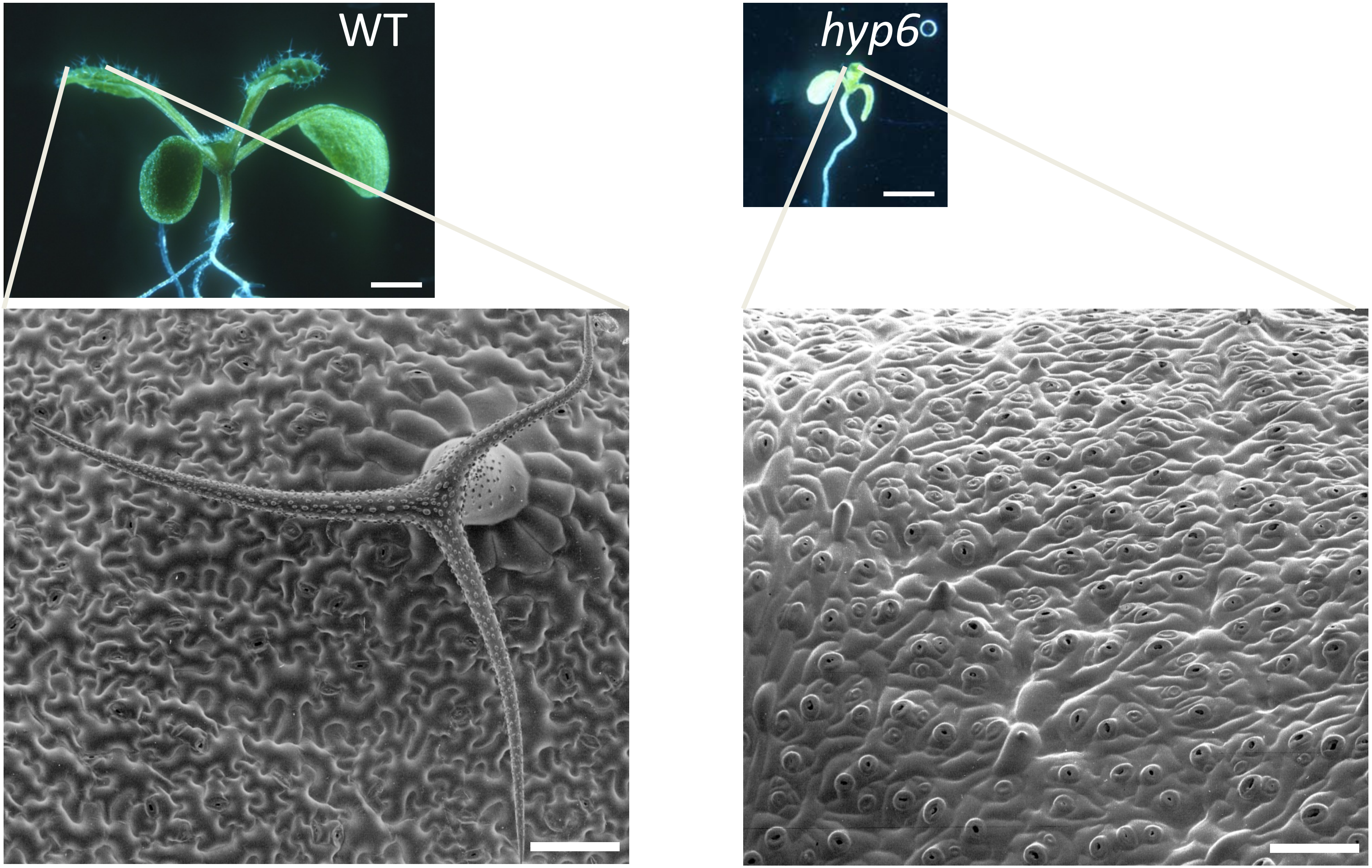
root hairless 1 (rhl1)
root hairless 2 (rhl2)
root hairless 3 (rhl3)



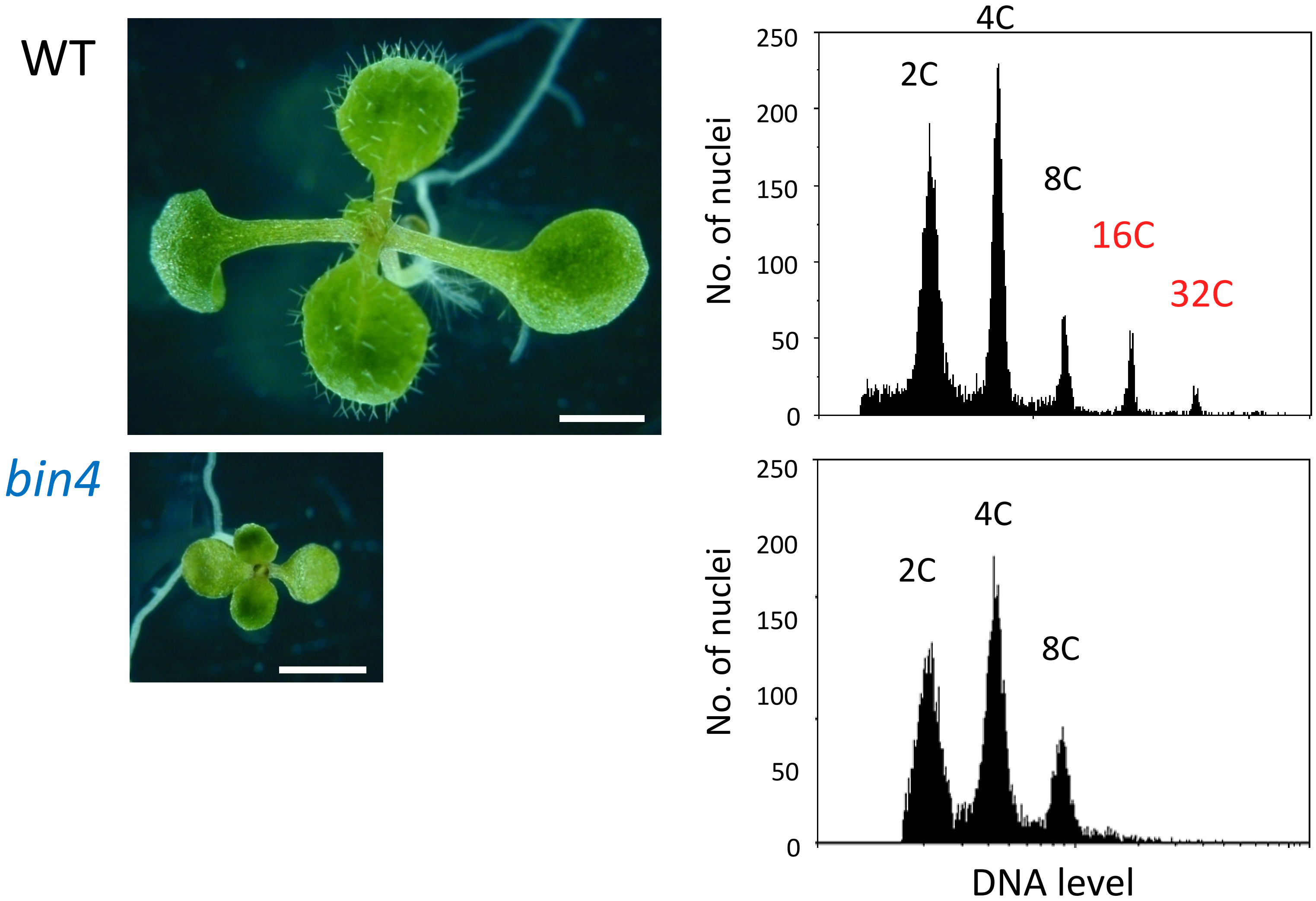
brassinosteroid insensitive 3 (bin3)
brassinosteroid insensitive 4 (bin4)
brassinosteroid insensitive 5 (bin5)

In collaboration with Herman Hofte, Joanne Chory
(Curr Bio 2002, PNAS 2005, Plant Cell 2007)

hyp/rhl/bin have reduced cell size phenotypes.

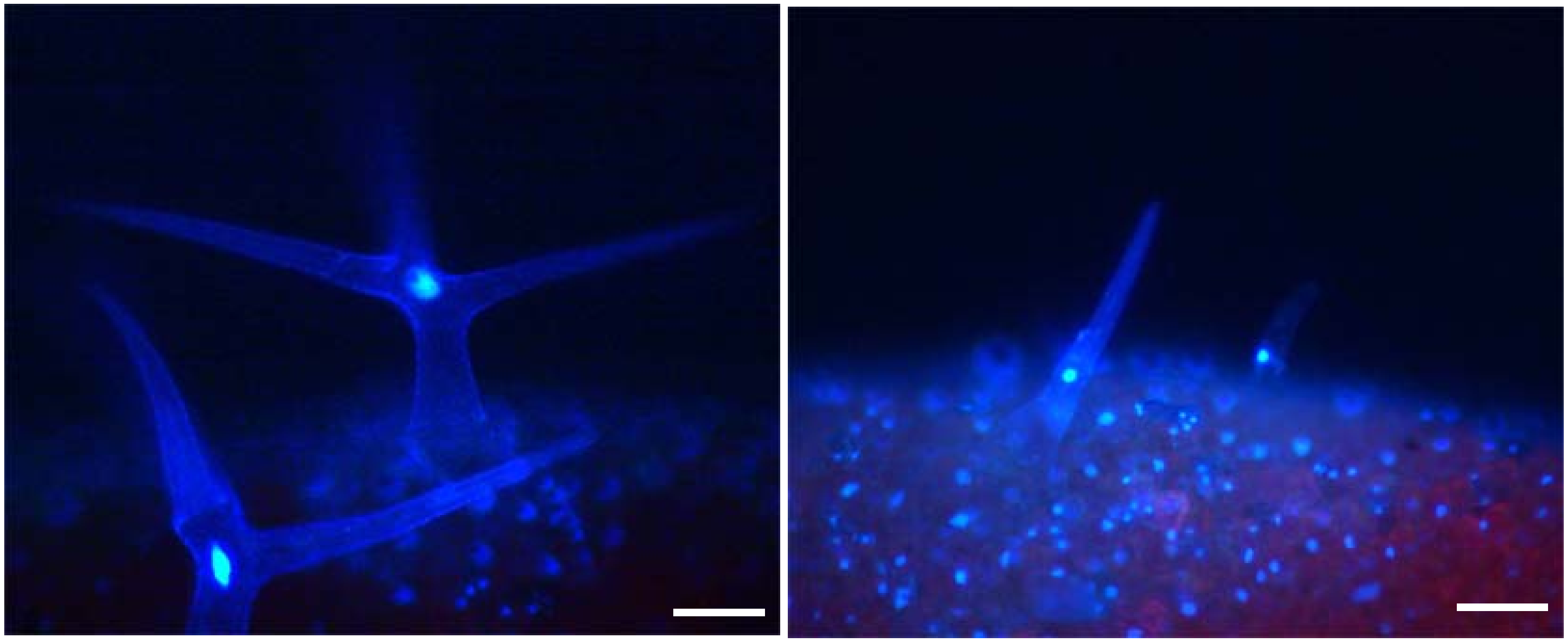


hyp/rhl/bin are defective in endoreduplication.



hyp/rhl/bin are defective in endoreduplication.

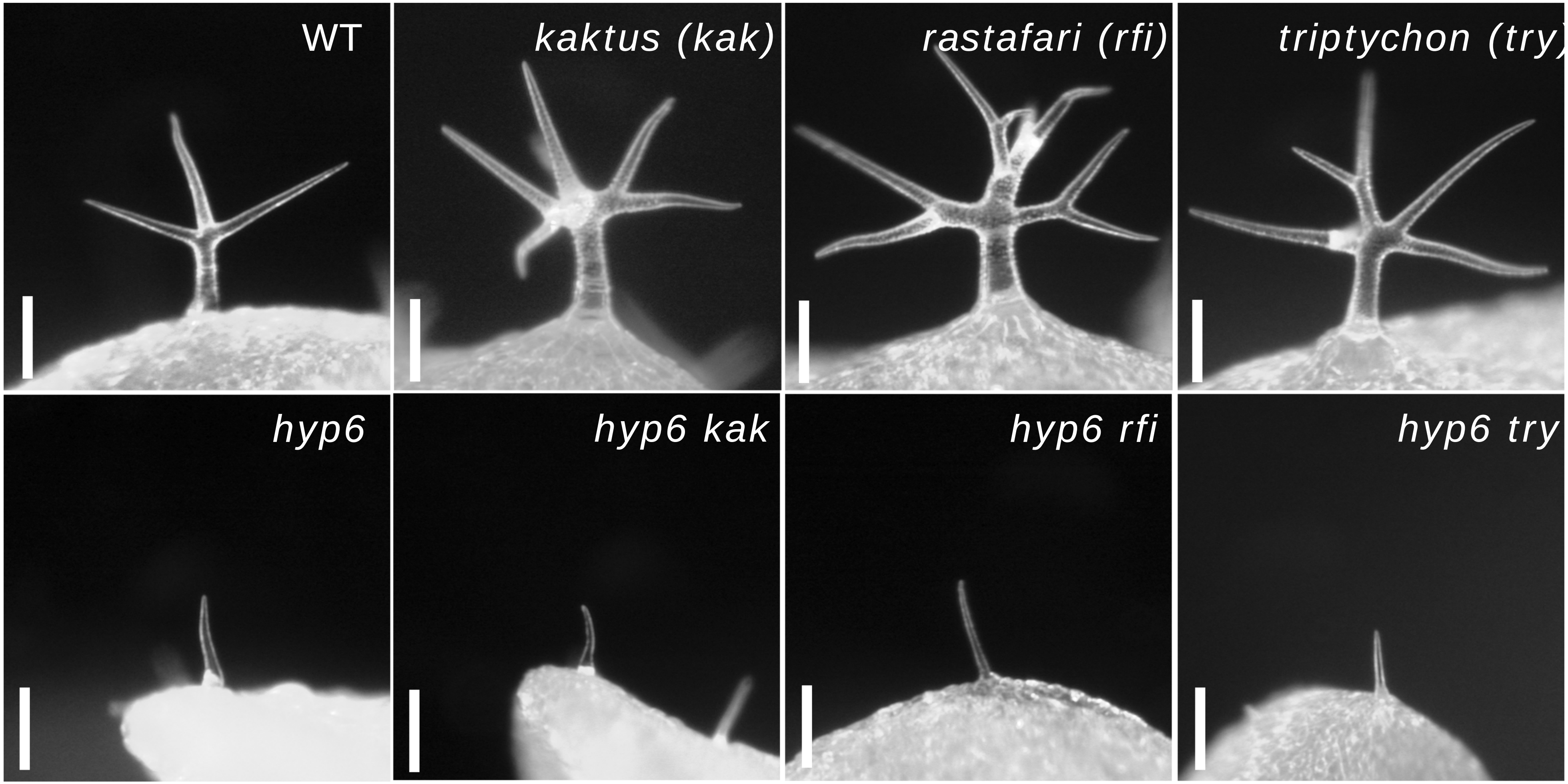
WT *hyp6*



Ploidy level < 32C Ploidy level < 8C

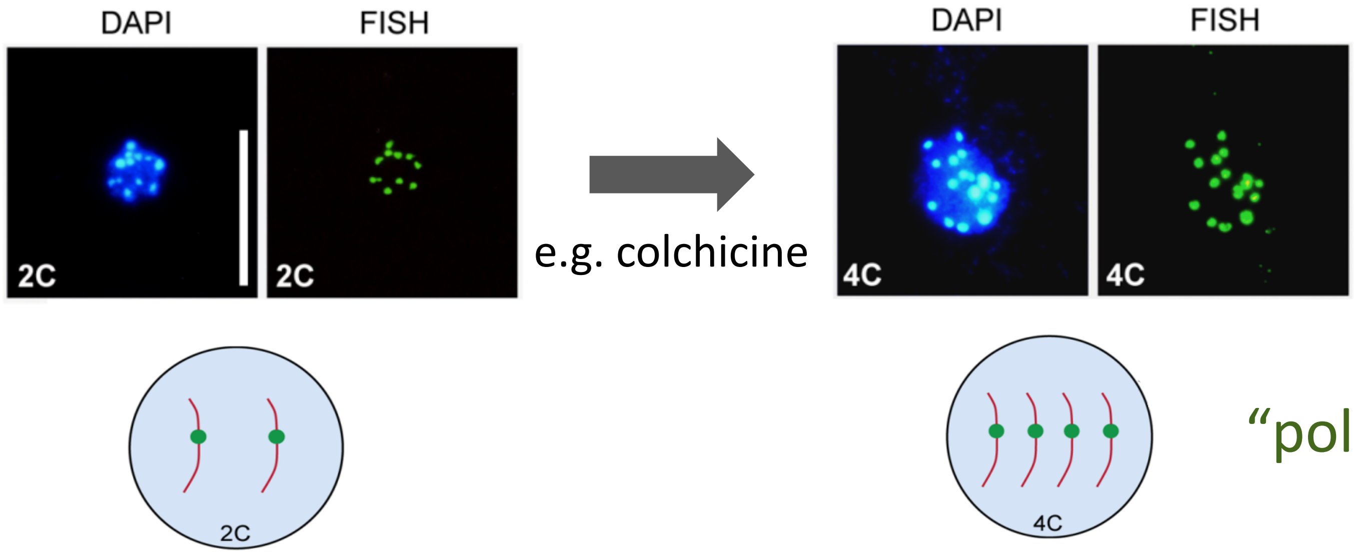
DAPI-stained nuclei in trichomes

HYP/RHL/BIN are required for endoreduplication.



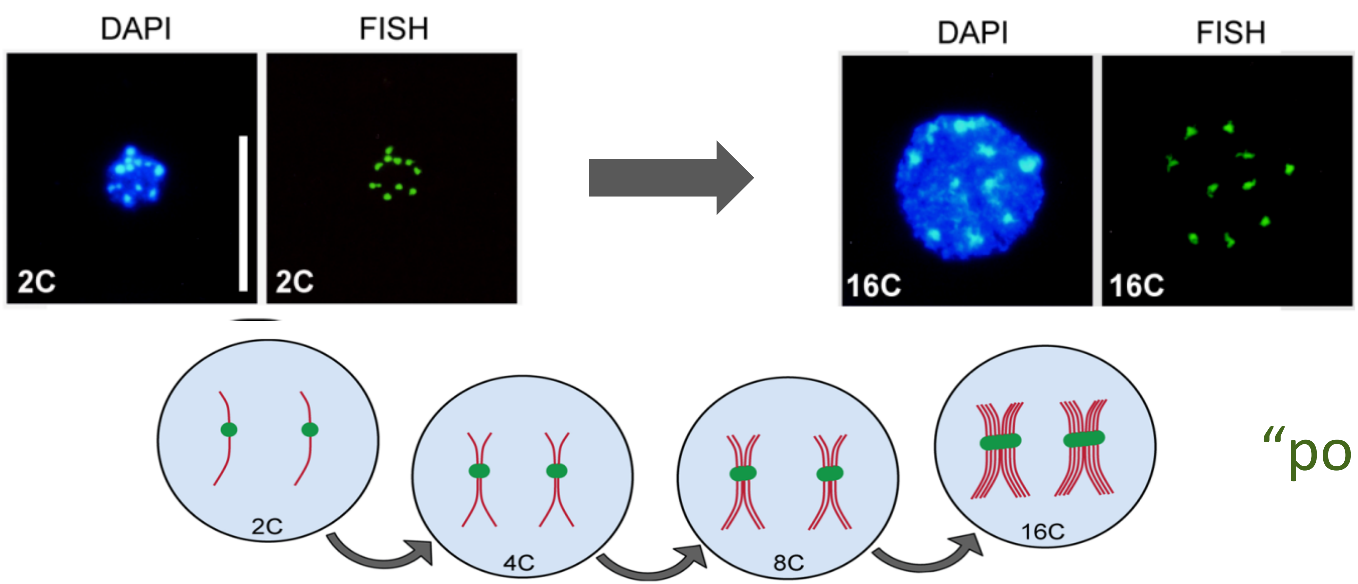
Two distinct cellular mechanisms to increase ploidy

Polyploidisation



"polyploid"

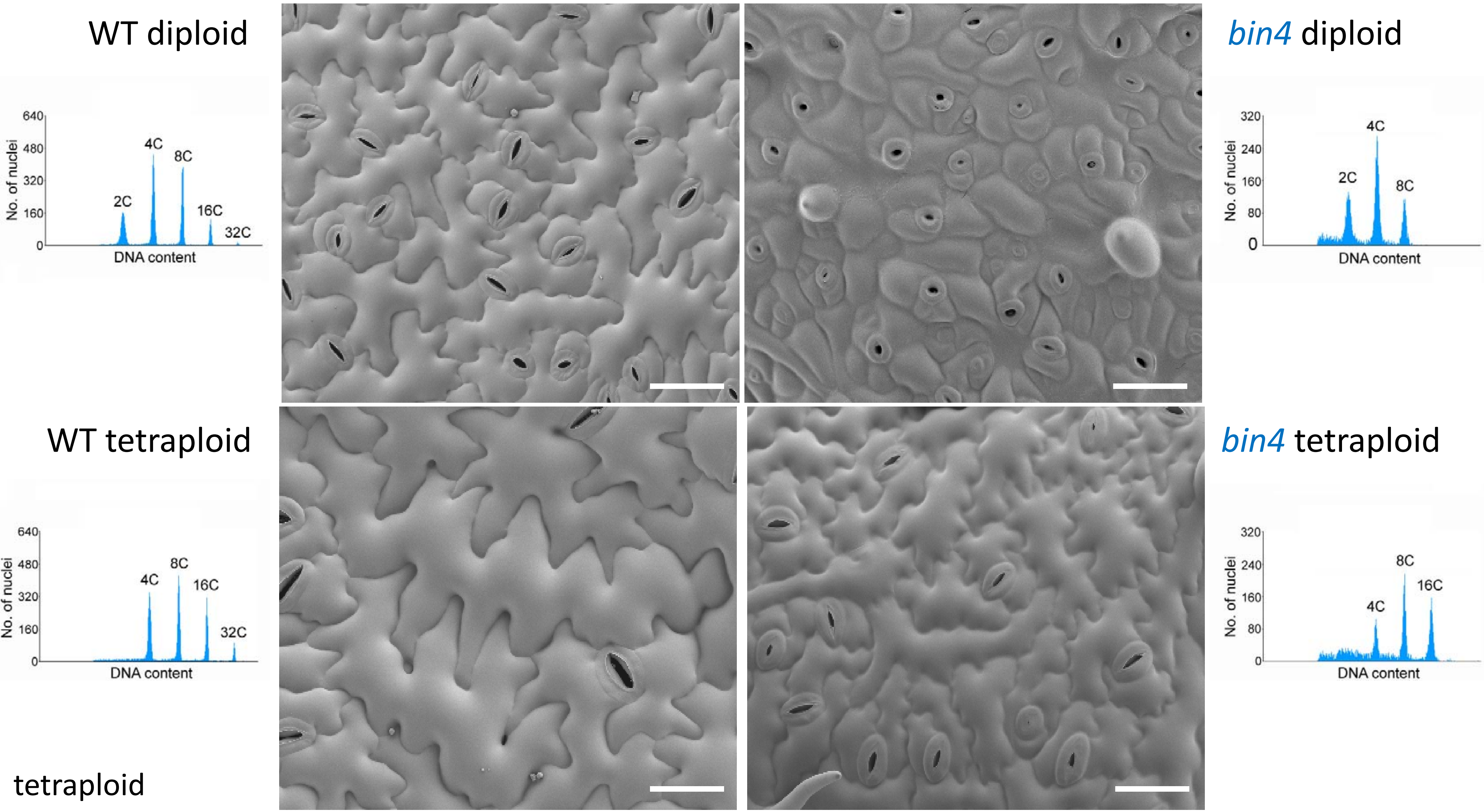
Endocycle



"polytene"

In collaboration with Yoshitaka Azumi

Bypassing endocycle defects by colchicine treatment can partially rescue the cell size phenotype in *hyp/rhl/bin*.



tetraploid
from Hirokazu Tsukaya

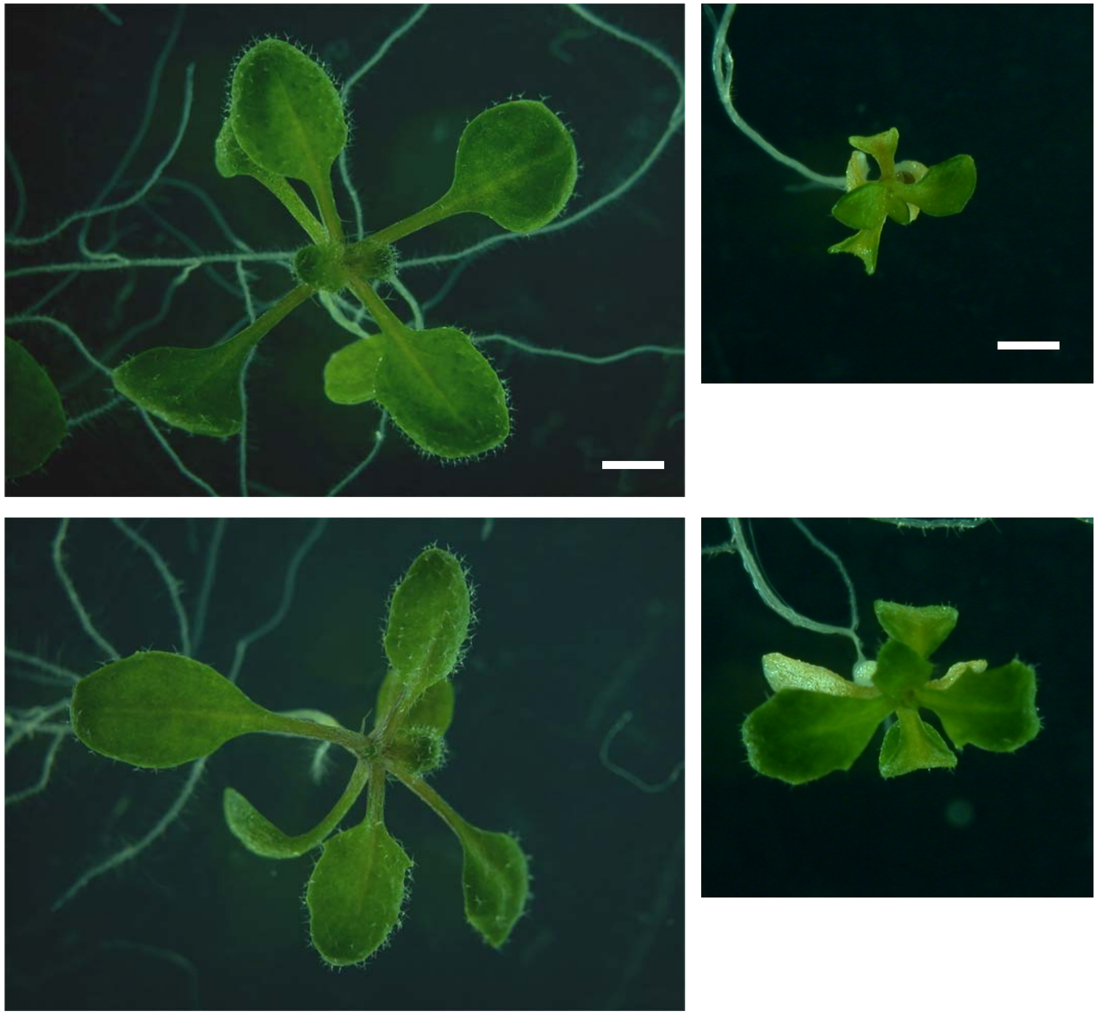
Raising nuclear DNA content can partially rescue the organ size phenotype in *hyp/rhl/bin*.

WT diploid

bin4 diploid

WT tetraploid

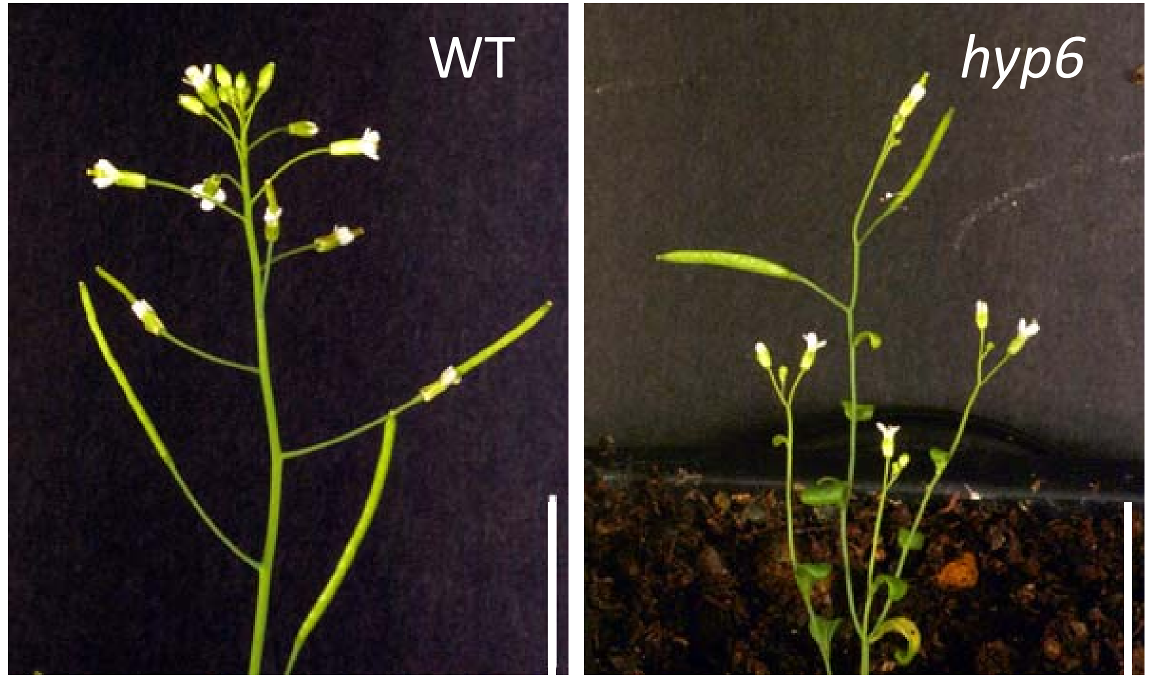
bin4 tetraploid



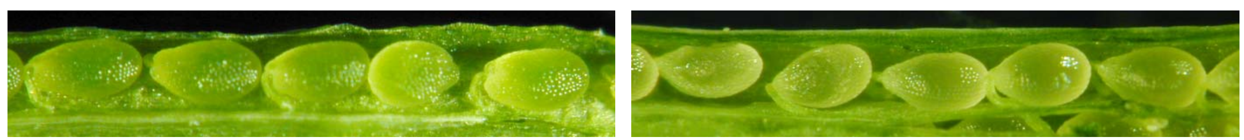
hyp/rhl/bin are dwarfs but have nearly normal flowers.

30-day-old plants

Flowers and siliques



Seeds



WT *hyp6*

HYP/RHL/BIN are all topoisomerase VI subunits.

ROOT HAIRLESS 2 (RHL2)
BRASSINOSTEROID INSENSITIVE 5 (BIN5) = TOP6A

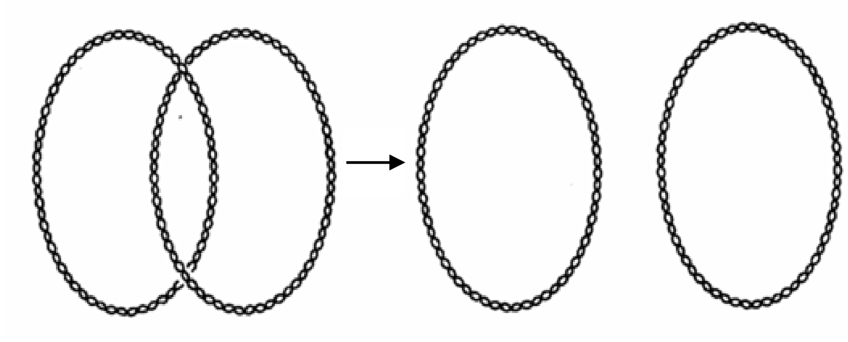
HYPOCOTYL 6 (HYP6)
ROOT HAIRLESS 3 (RHL3) = TOP6B
BRASSINOSTEROID INSENSITIVE 3 (BIN3)

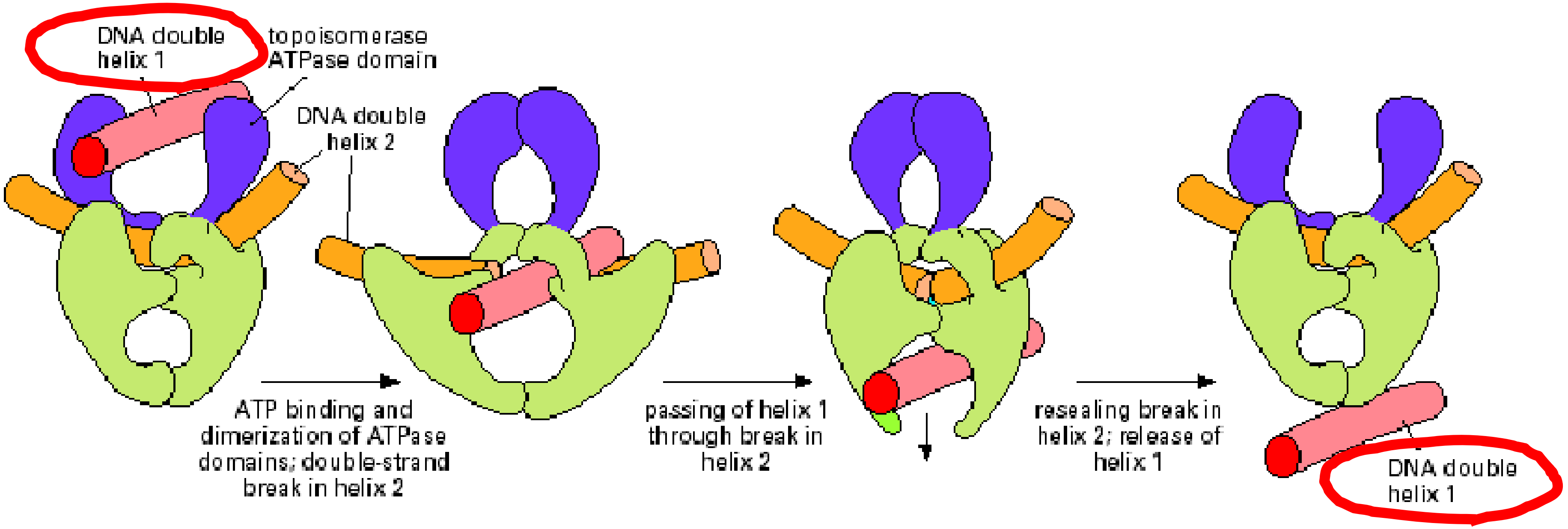
HYPOCOTYL 7 (HYP7) = New TOP6 subunit
ROOT HAIRLESS 1 (RHL1)

BRASSINOSTEROID INSENSITIVE 4 (BIN4) = New TOP6 subunit

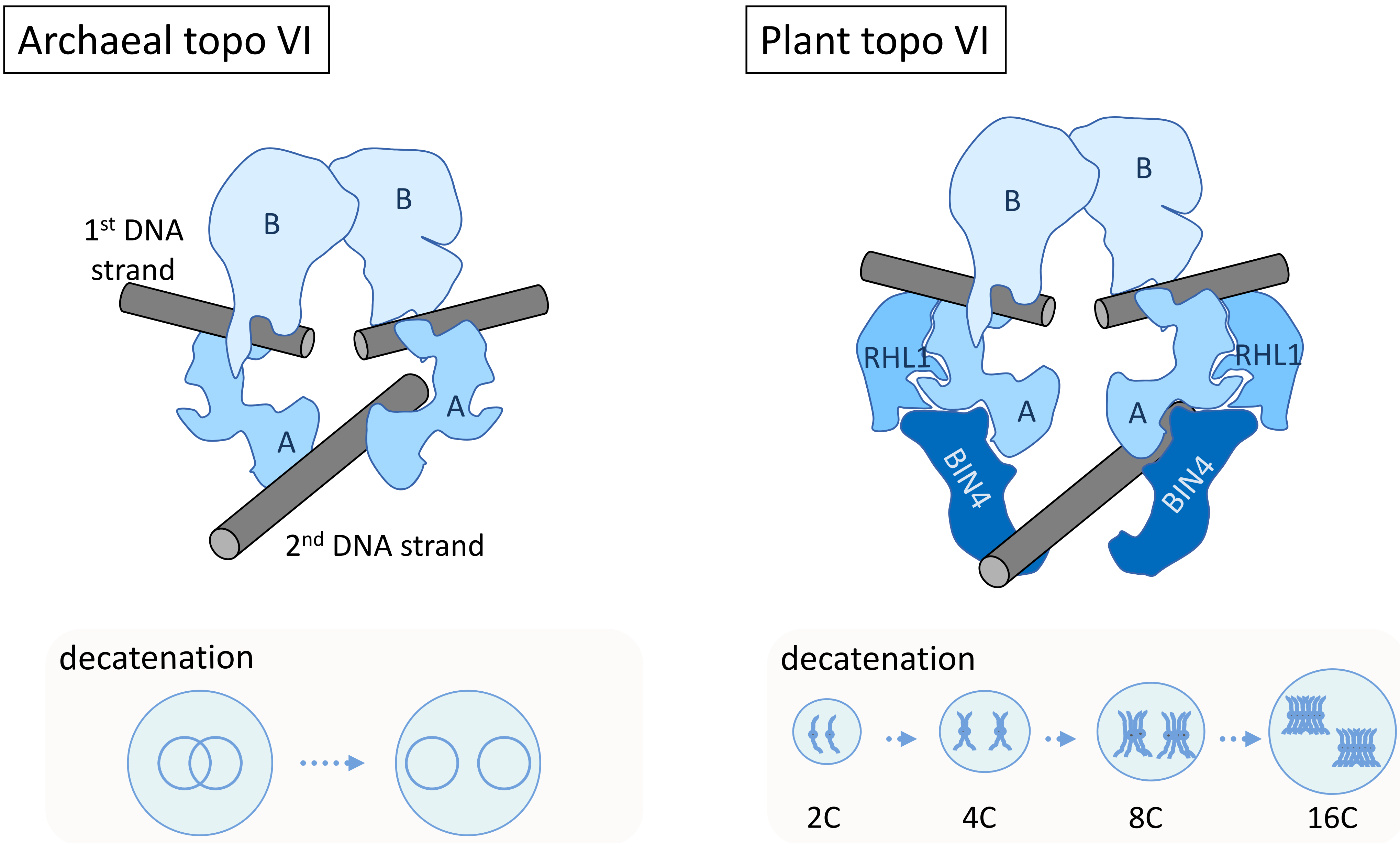
(Curr Bio 2002, PNAS 2005, Plant Cell 2007)

Predicted role of DNA topoisomerase VI: decatenation

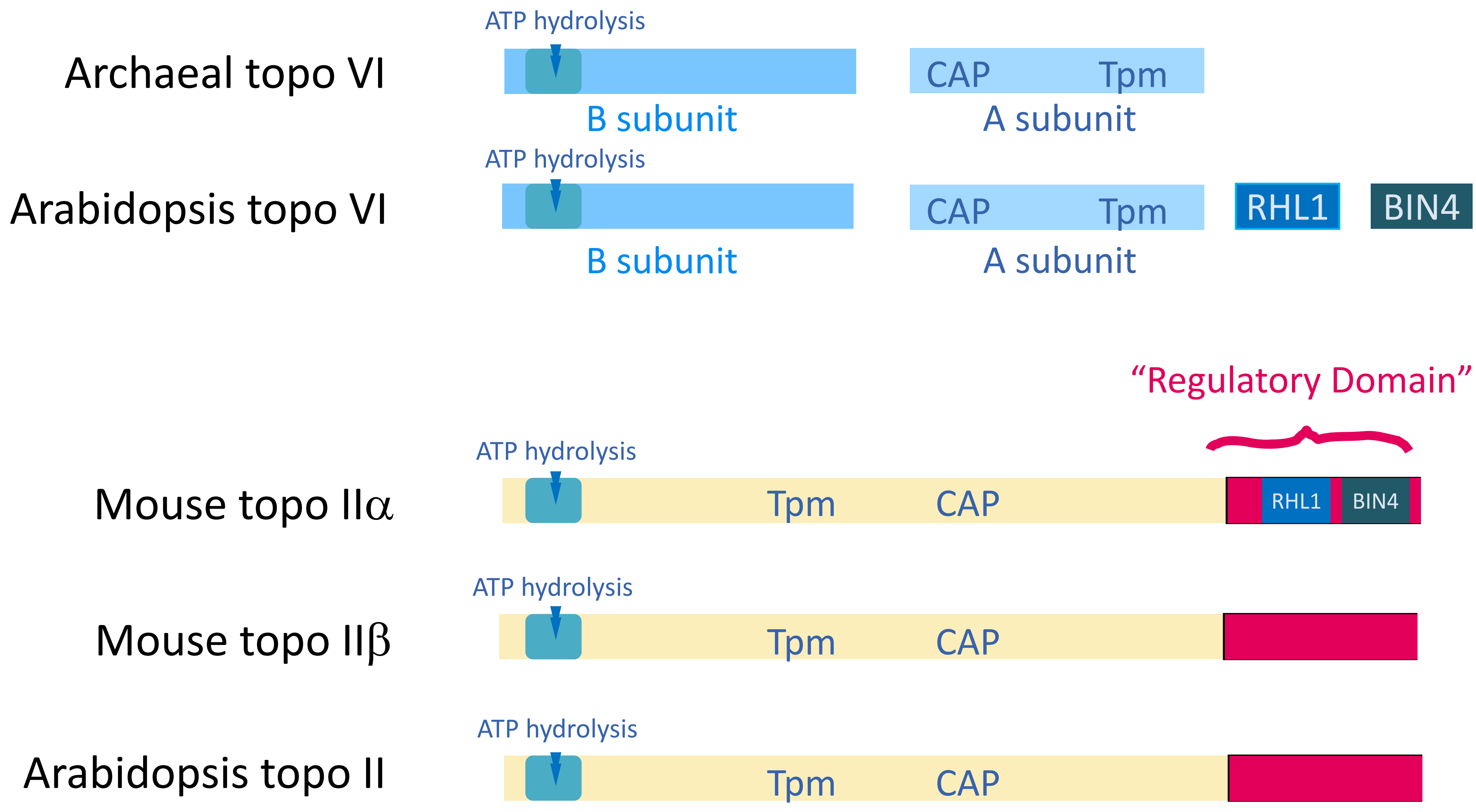




Predicted model of the plant topo VI complex



RHL1 and BIN4 have weak homology to the C-terminus of mammalian topo IIα.



TOPO II is required for the mitotic cell cycle but not for the endocycle.

Light-grown

control

100 mM etoposide

Dark-grown

control

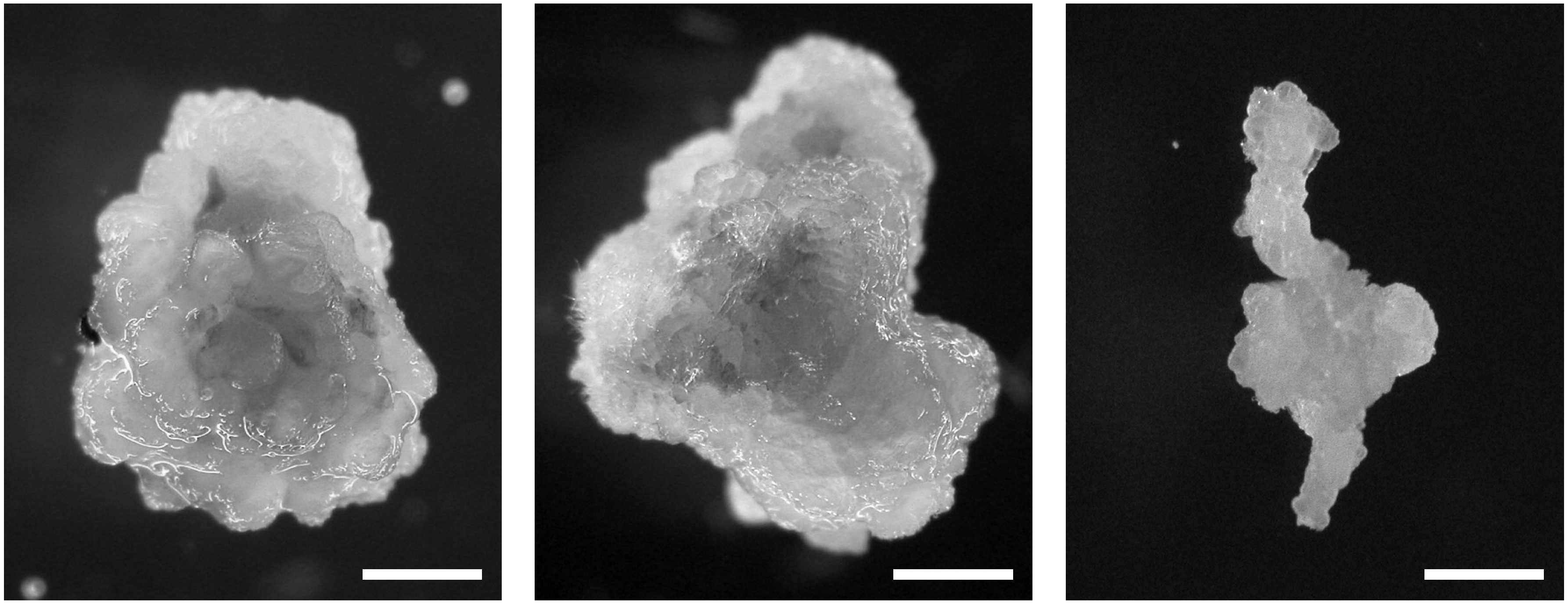
100 mM etoposide

Topo II is involved in the mitotic cell cycle.

WT
control

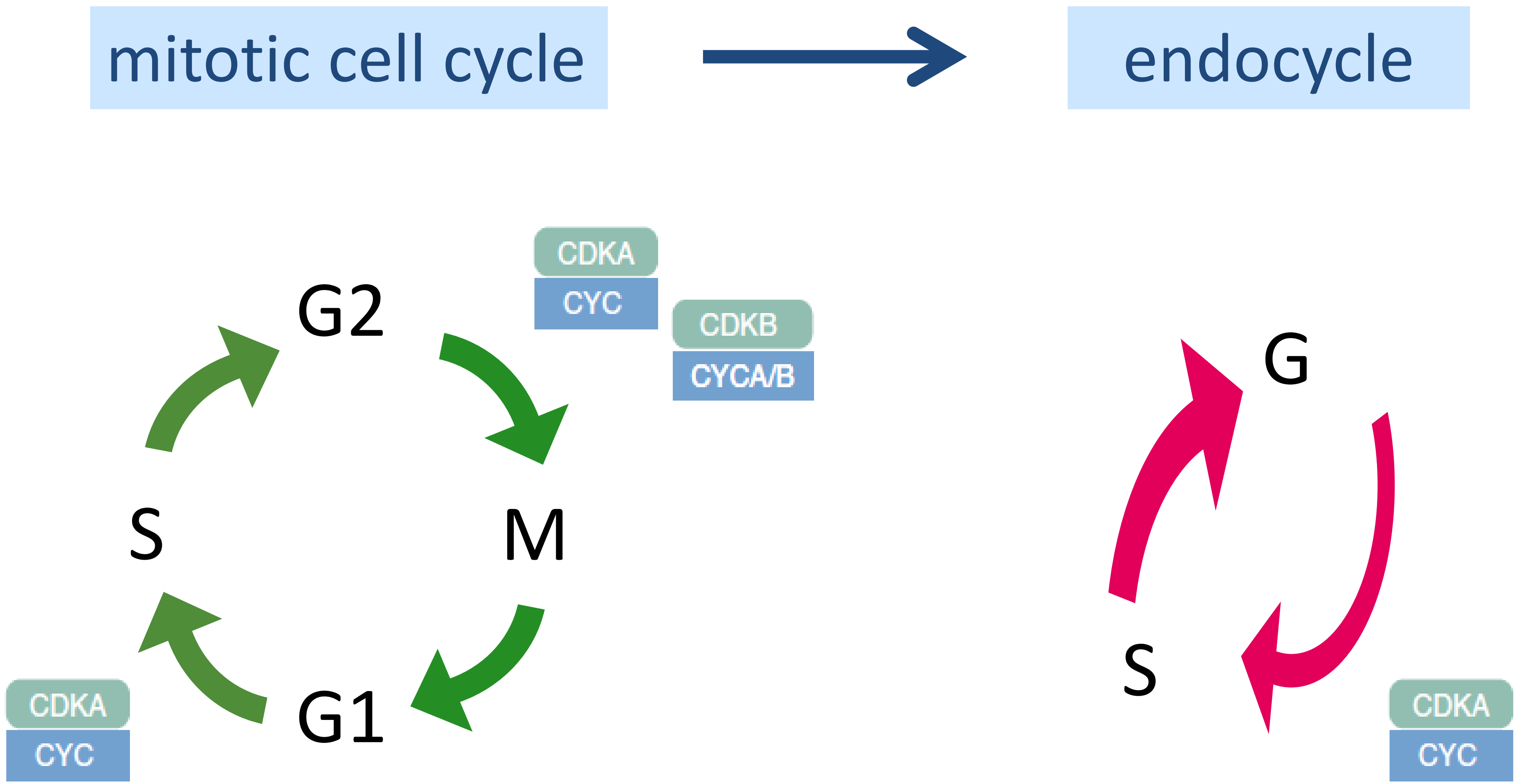
hyp6

WT
100 mM etoposide

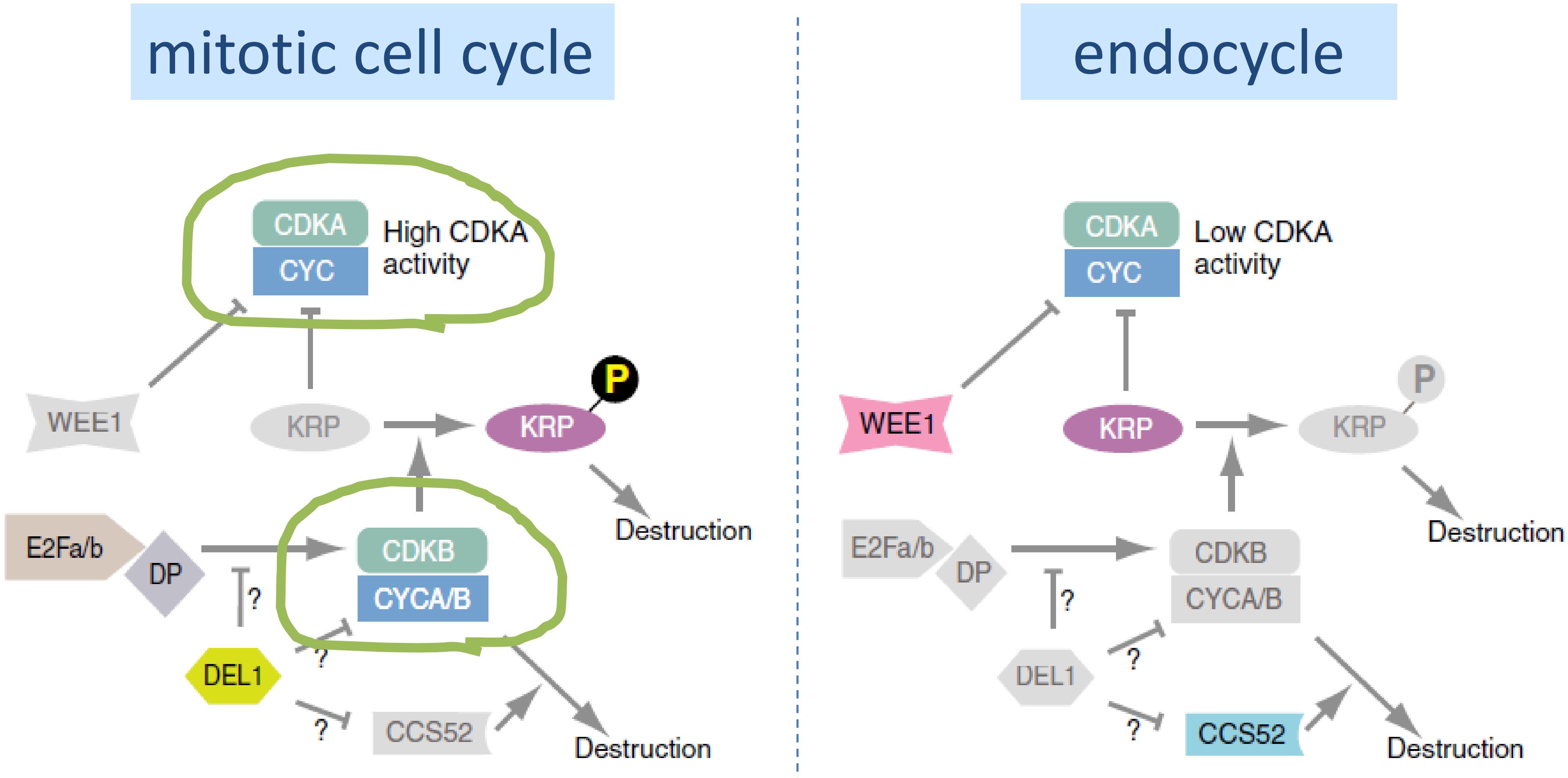


4-week-old callus induced from roots

How do cells switch from the mitotic cell cycle to the endocycle?

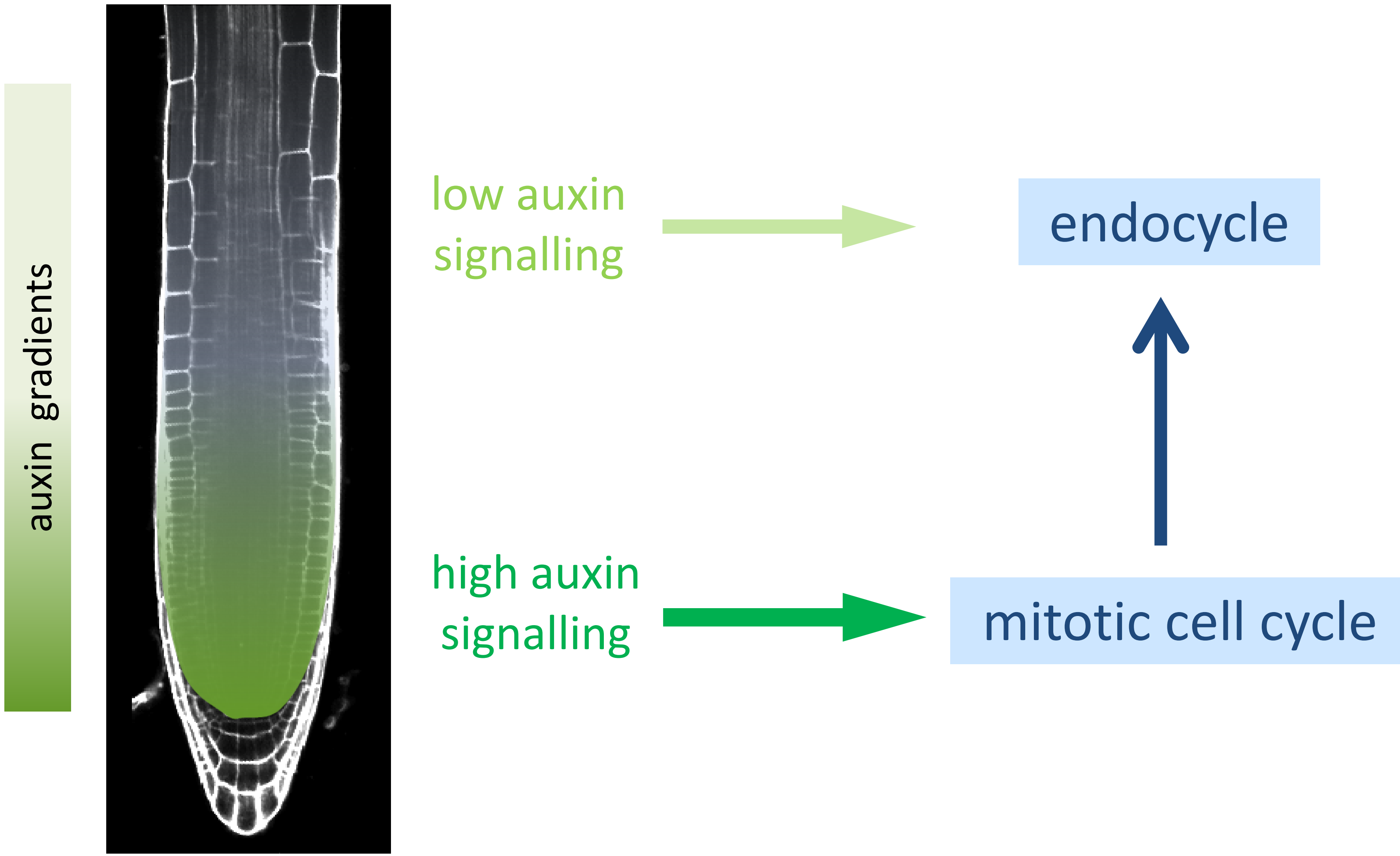


How do cells switch from the mitotic cell cycle to the endocycle?



Inze and De Veylder (2006)

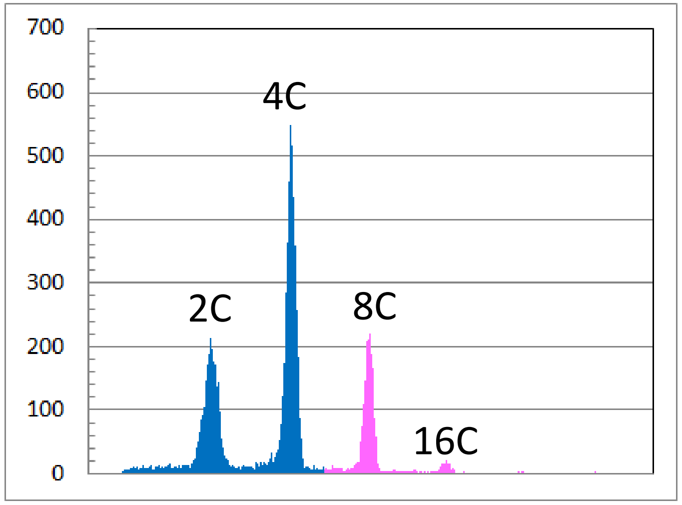
Auxin gradients control the transition from the mitotic cell cycle to the endocycle in the meristem.



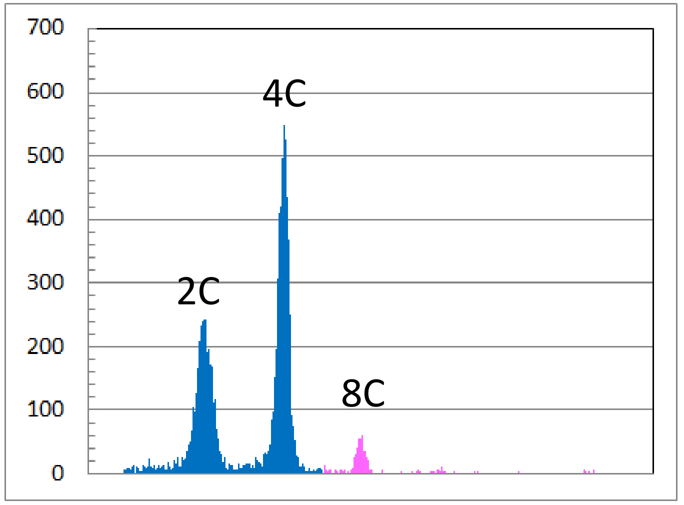
(Ishida et al, Development in press)

Exogenously applied auxin blocks the endocycle progression.

Control



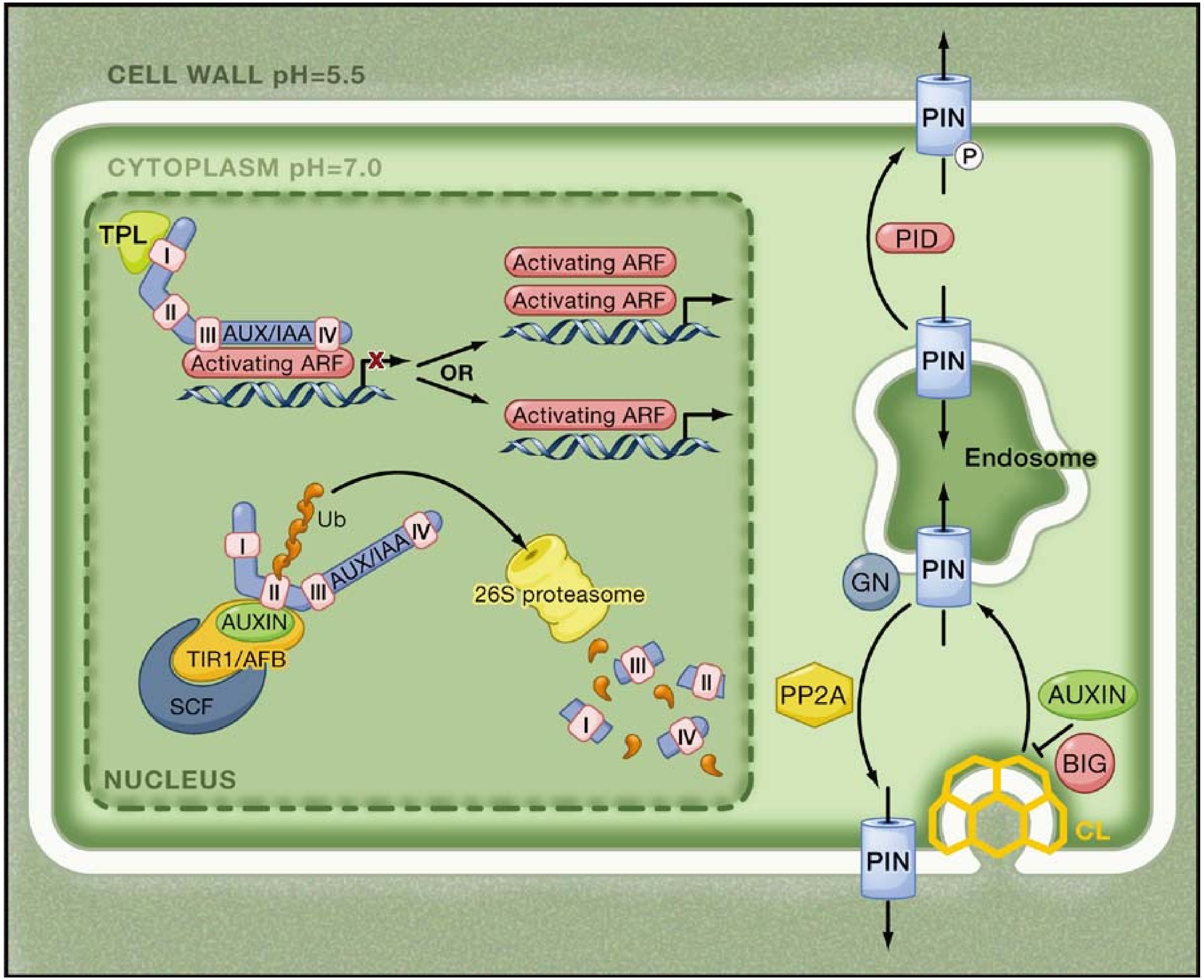
＋ NAA 1uM



14-day-old 1st true leaves

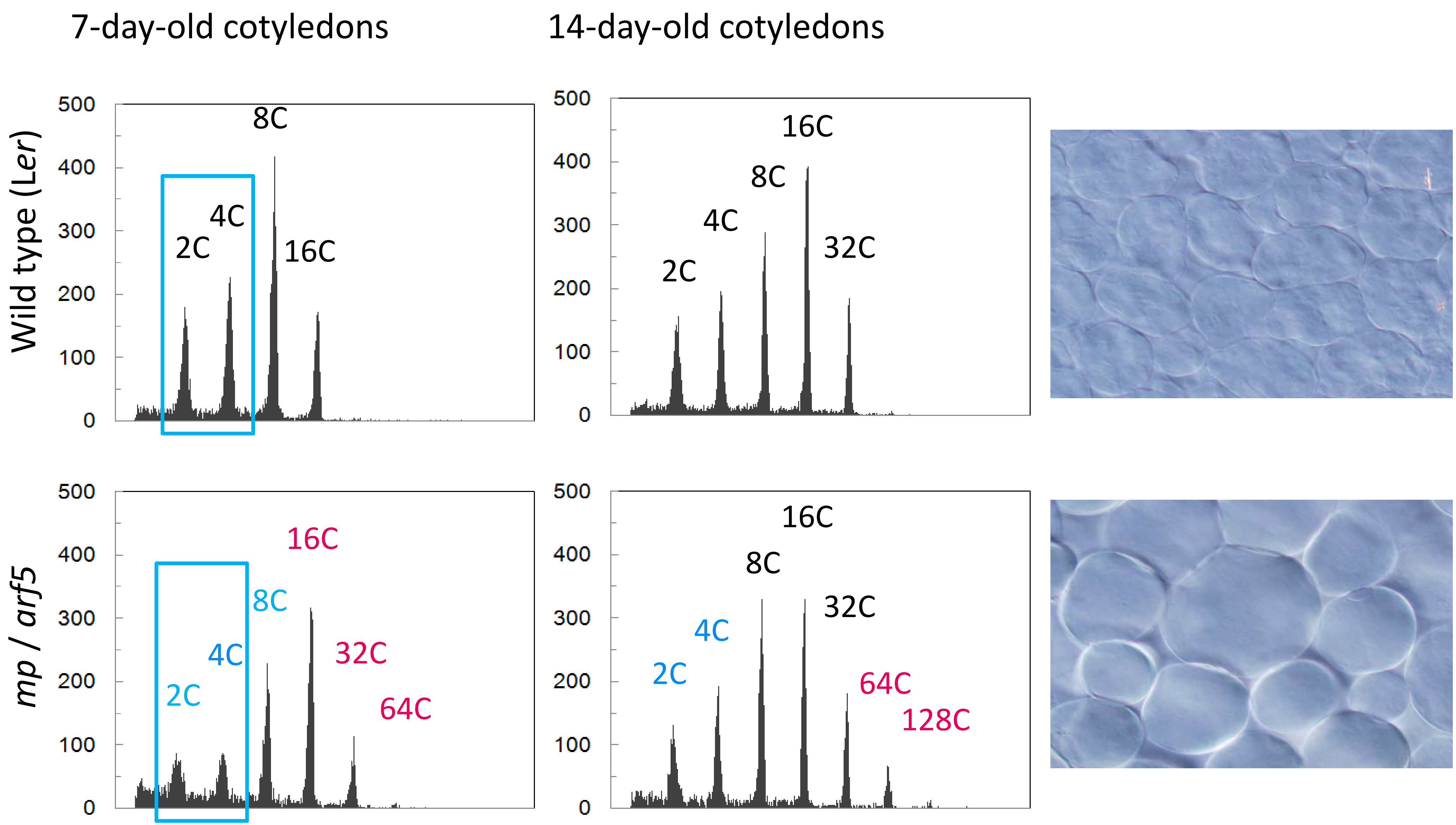
Takashi Ishida

Cellular auxin signalling is mediated by the TIR-AUX/IAA-ARF pathway.



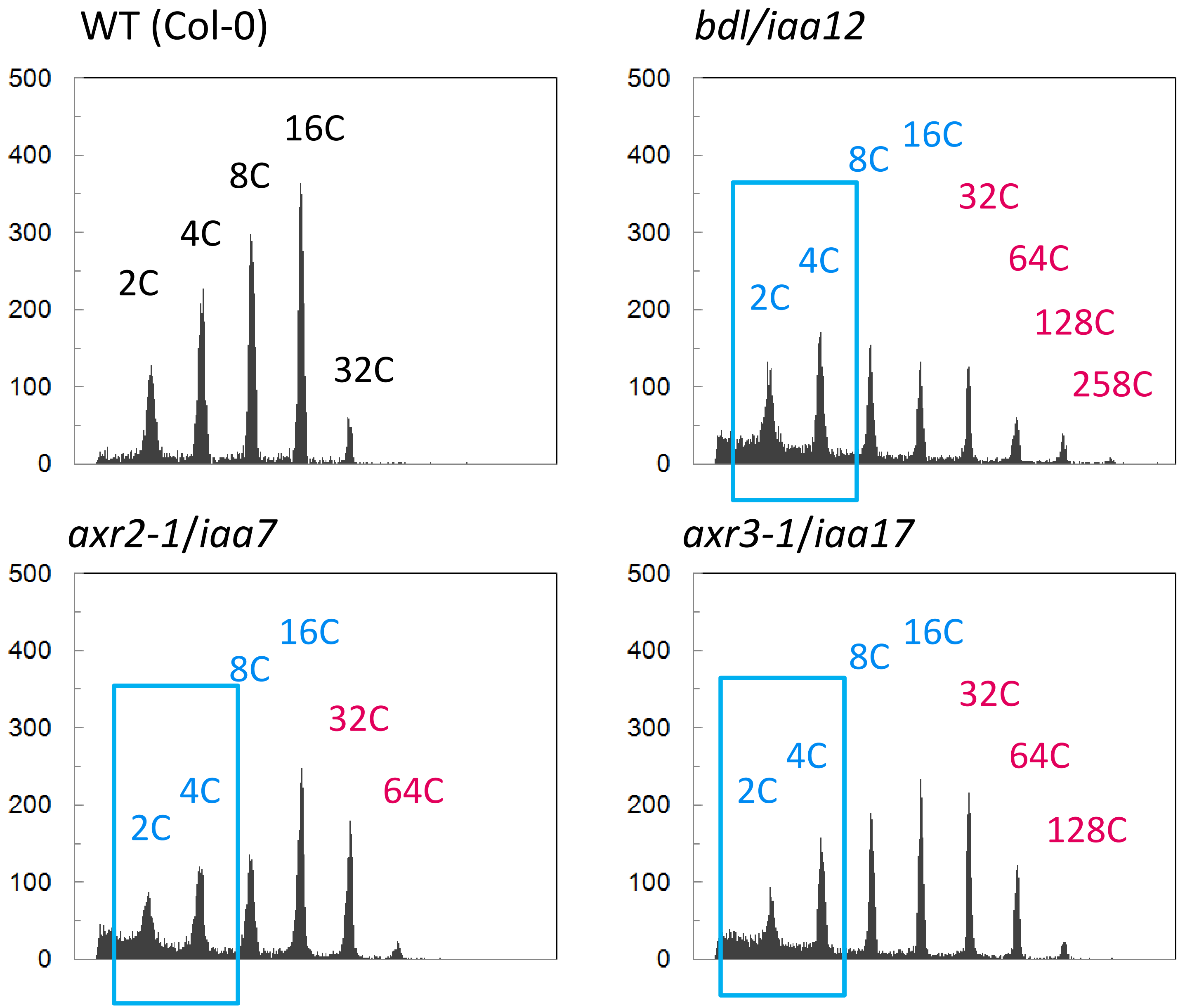
(Vanneste and Friml 2009)

Reduced levels of auxin signalling promote the endocycle.



Takashi Ishida

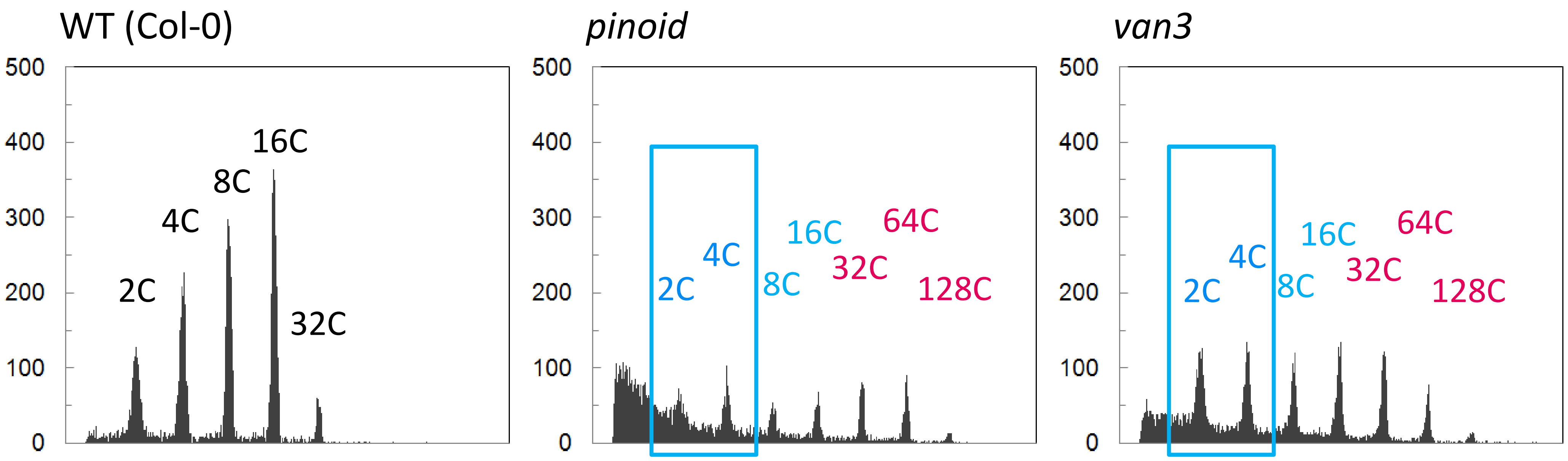
Reduced levels of auxin signalling promote the endocycle.



14-day-old cotyledons

Takashi Ishida

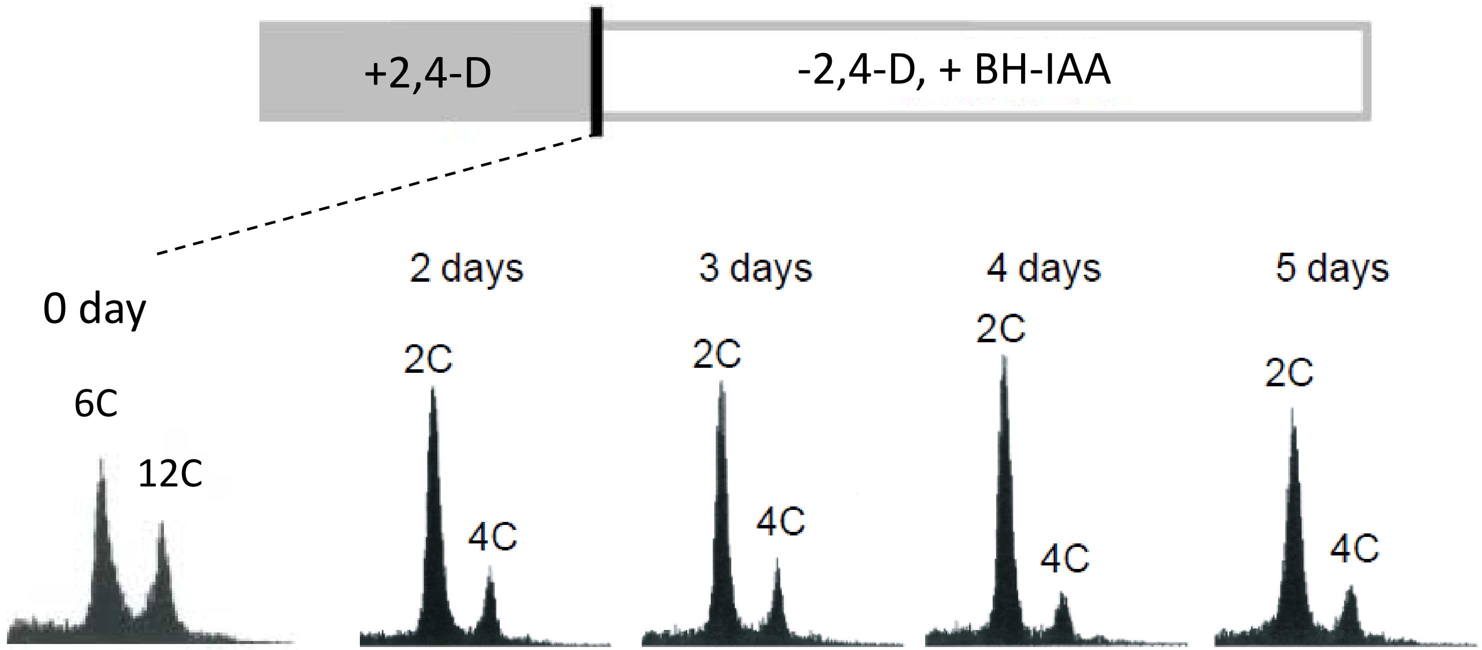
Reduced levels of auxin transport promote the endocycle.



14-day-old cotyledons

Takashi Ishida

Blocking auxin signalling with an auxin antagonist BH-IAA converts mitotic cells into endocyling cells.

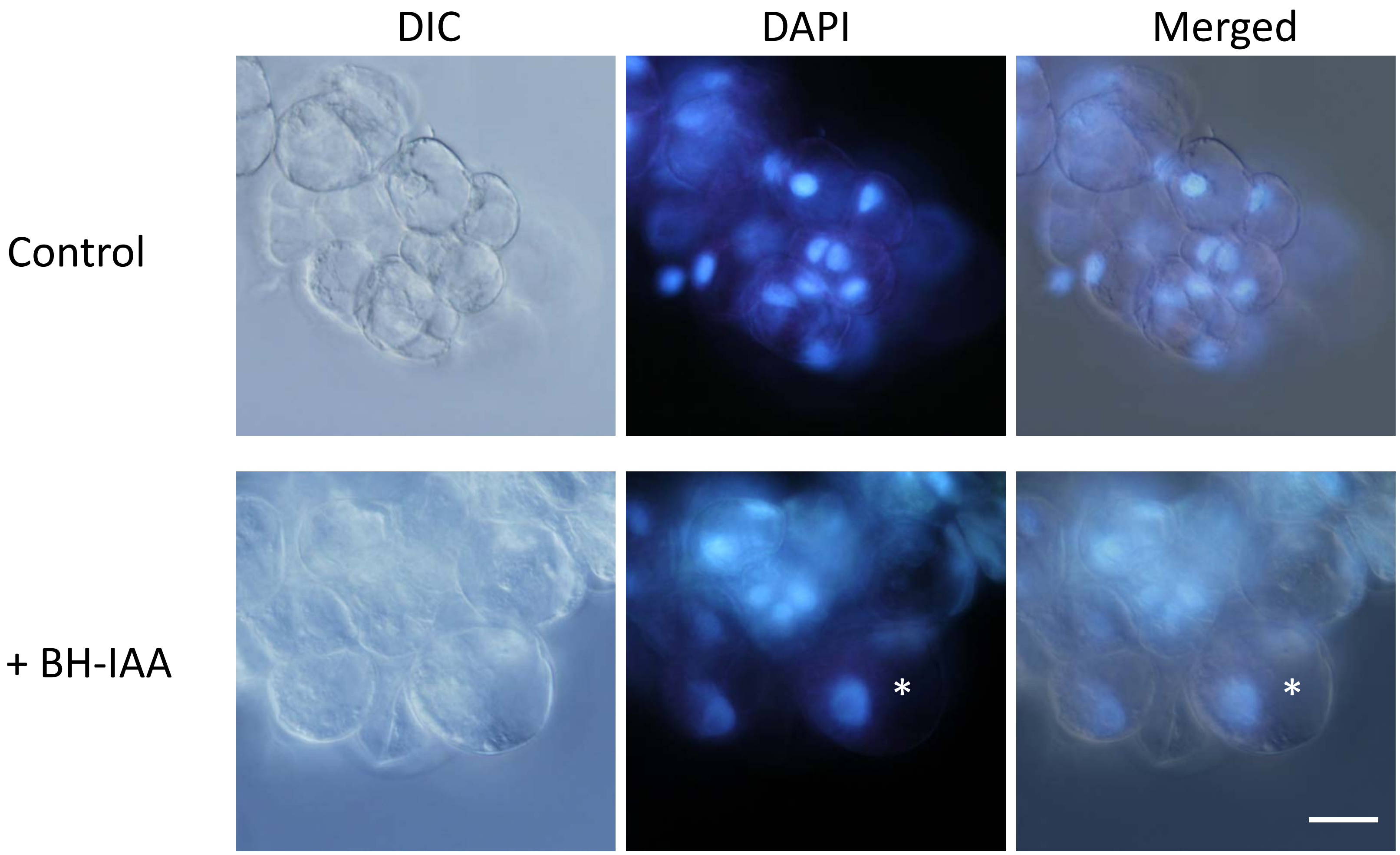


DMSO

Arabidopsis MM2d culture cells
PEO-IAA from Kenichiro Hayashi

In collaboration with Masaaki Umeda (NAIST)

Blocking auxin signalling with an auxin antagonist BH-IAA converts mitotic cells into endocyling cells.

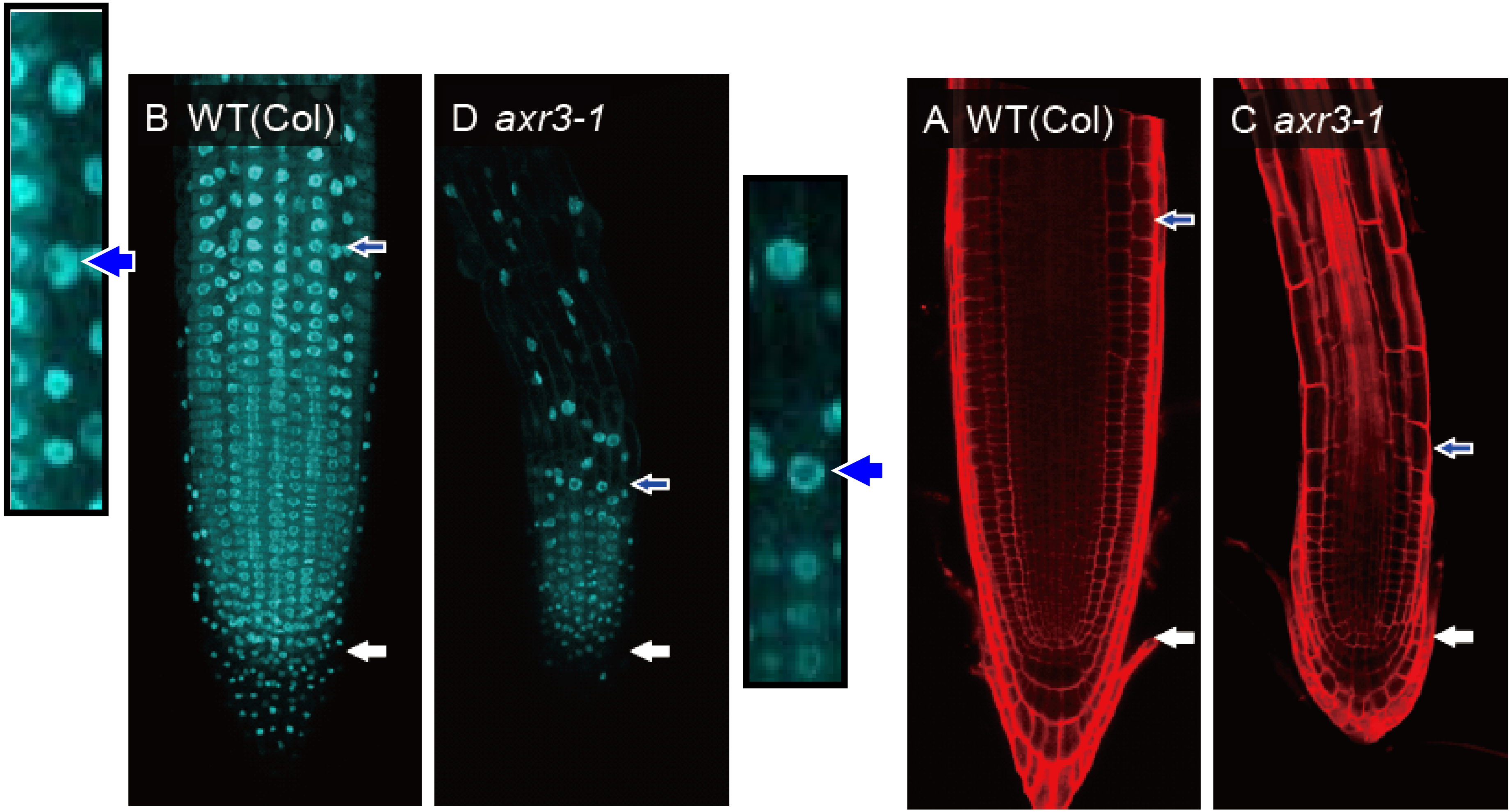


* >24C nuclei (13%, n=240)

MM2d culture cells, 5 days after BH-IAA treatment

Masaaki Umeda (NAIST)

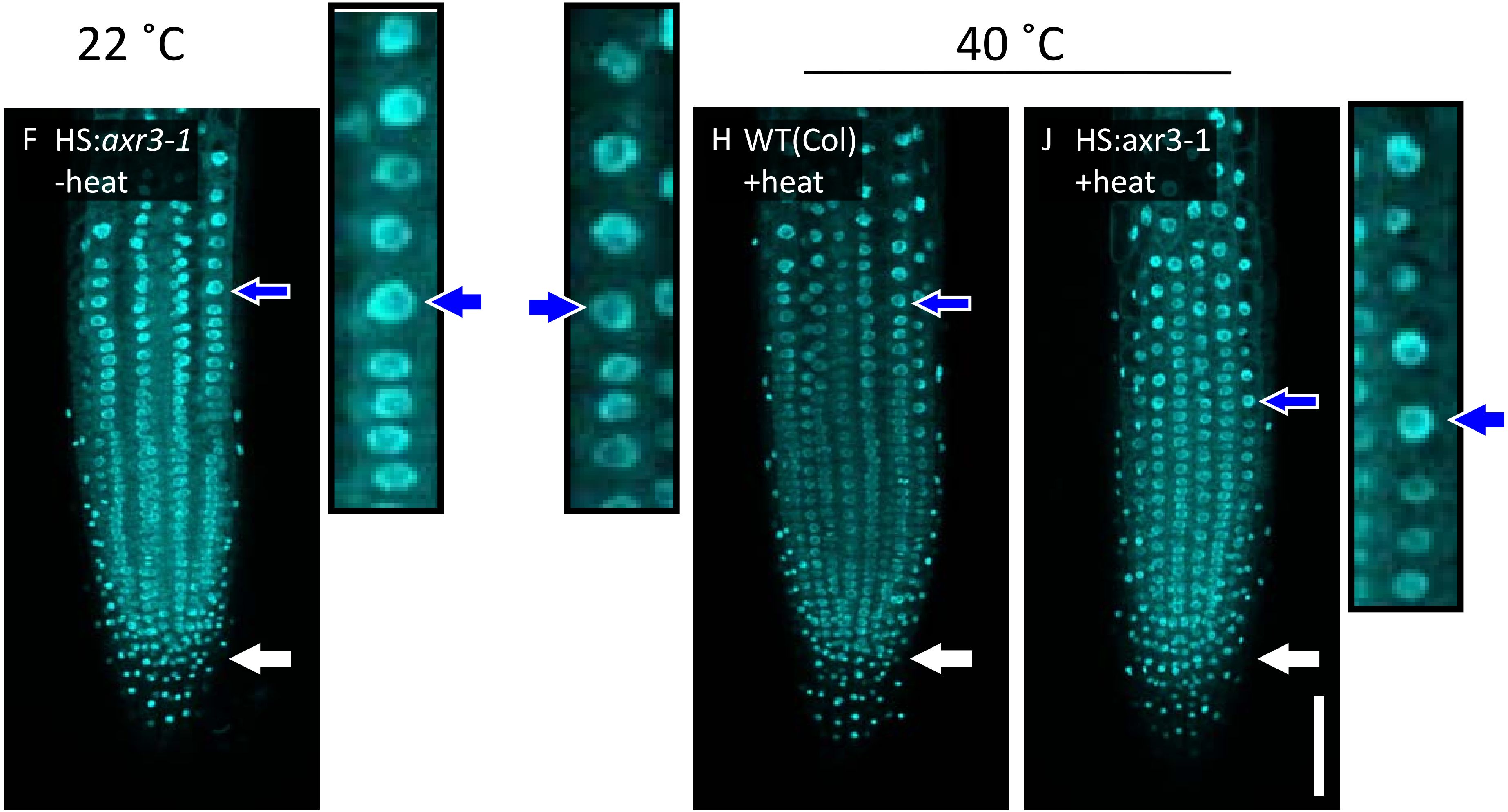
The *axr3-1* mutations result in an early onset of endocyling and accompanying cell expansion in the root meristem.



6-day-old roots, nuclei visualised by DAPI staining

Takashi Ishida

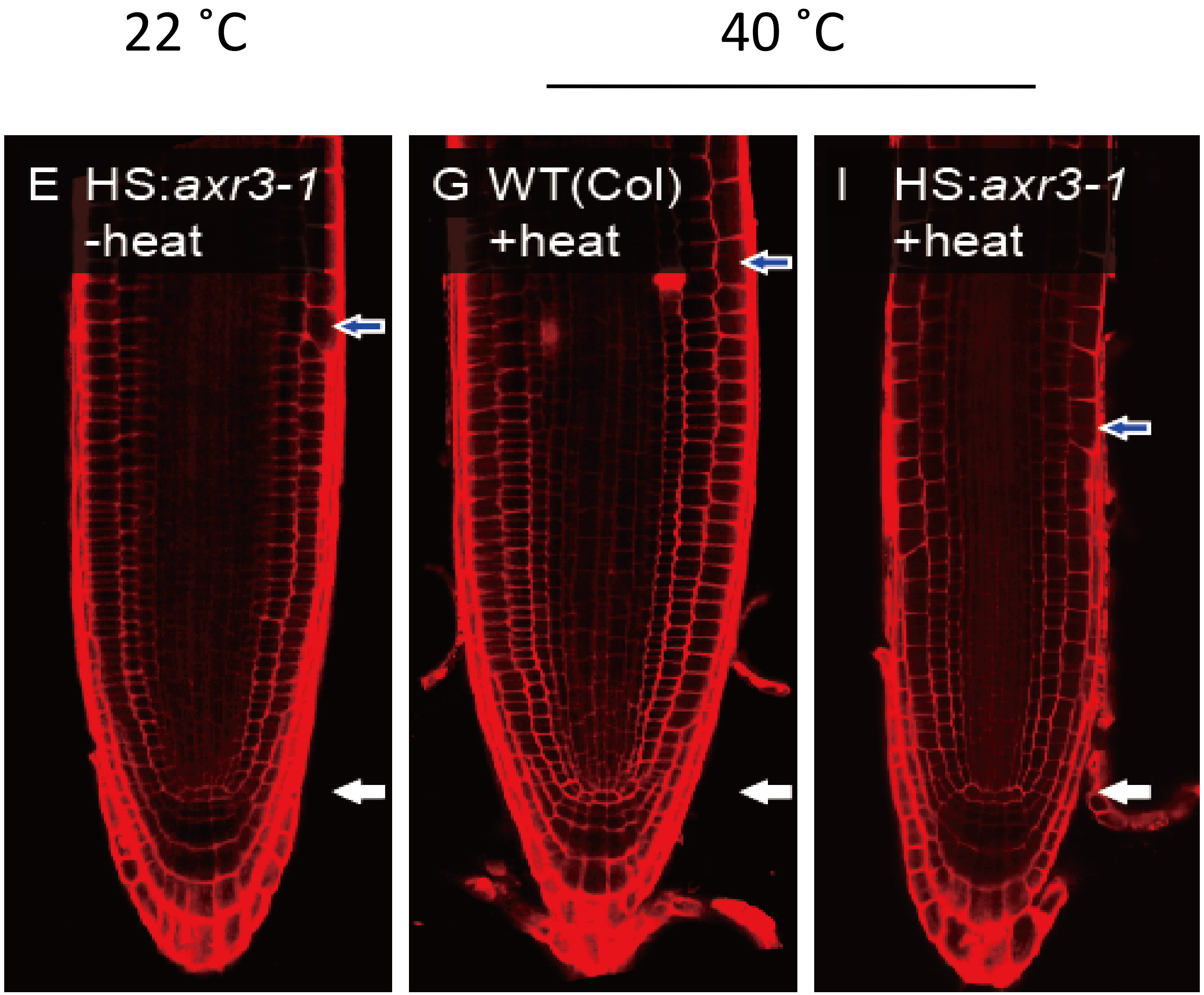
Transient reduction of auxin signalling induces early endocycling.



DAPI-stained nuclei in 6-day-old roots

Takashi Ishida

Transient reduction of auxin signalling induces early cell expansion.

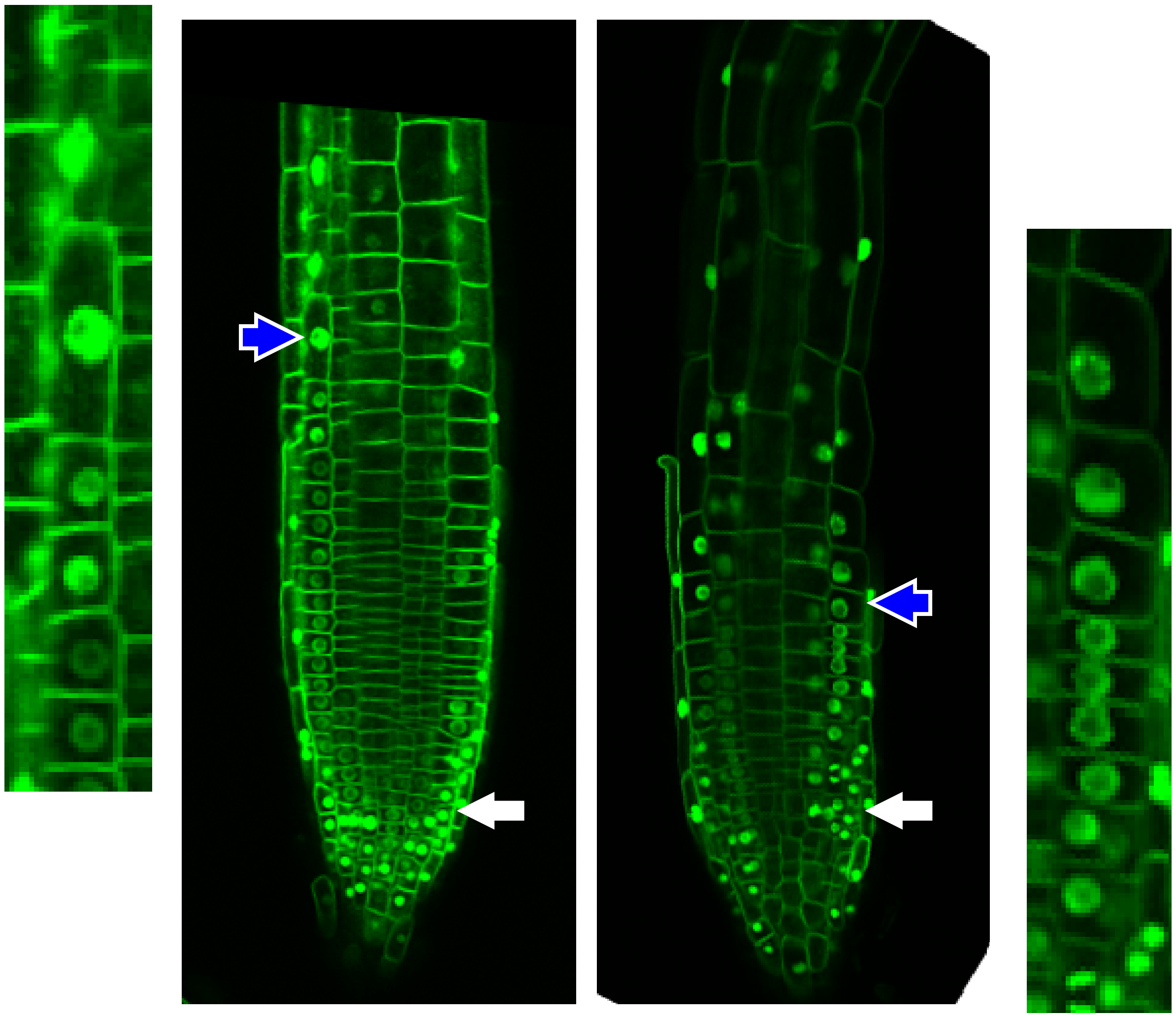


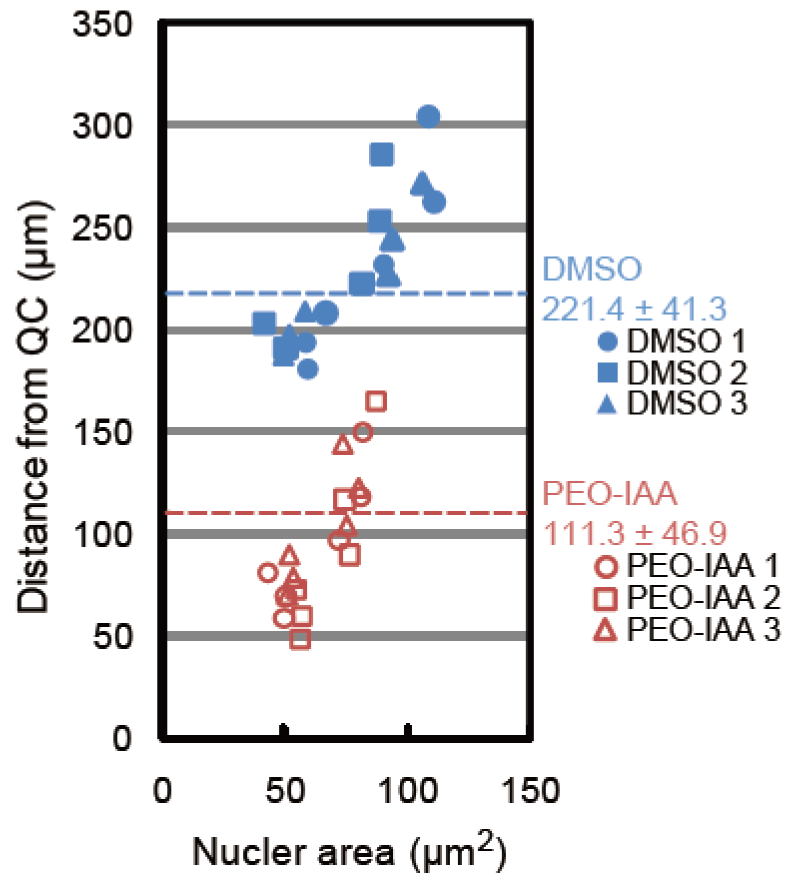
6-day-old roots

Takashi Ishida

Auxin antagonists promote an early onset of endocycling.

DMSO + PEO-IAA 10μM



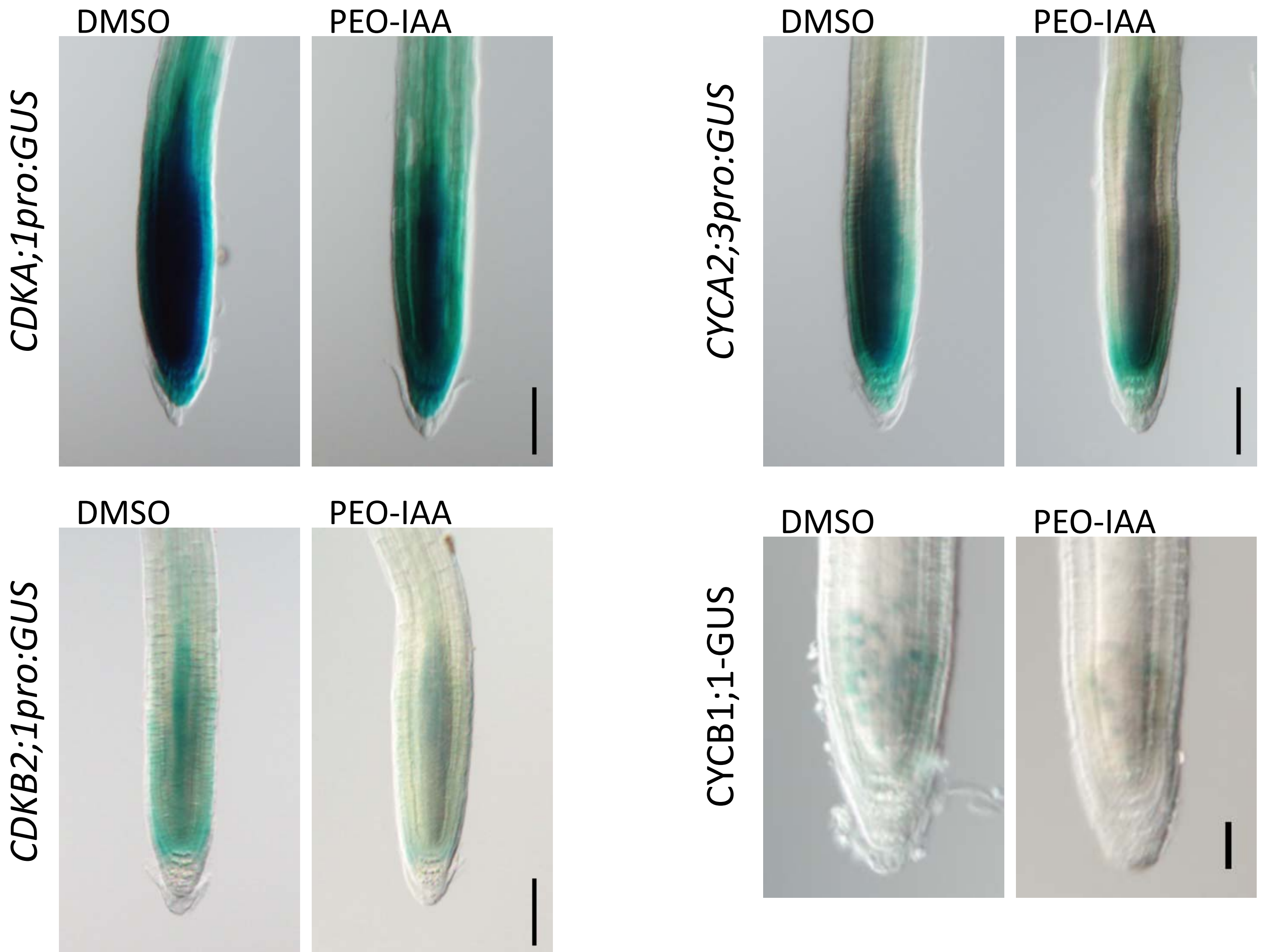


6-day-old roots, nuclei visualised by Histone2B-YFP

PEO-IAA from Kenichiro Hayashi

Takashi Ishida

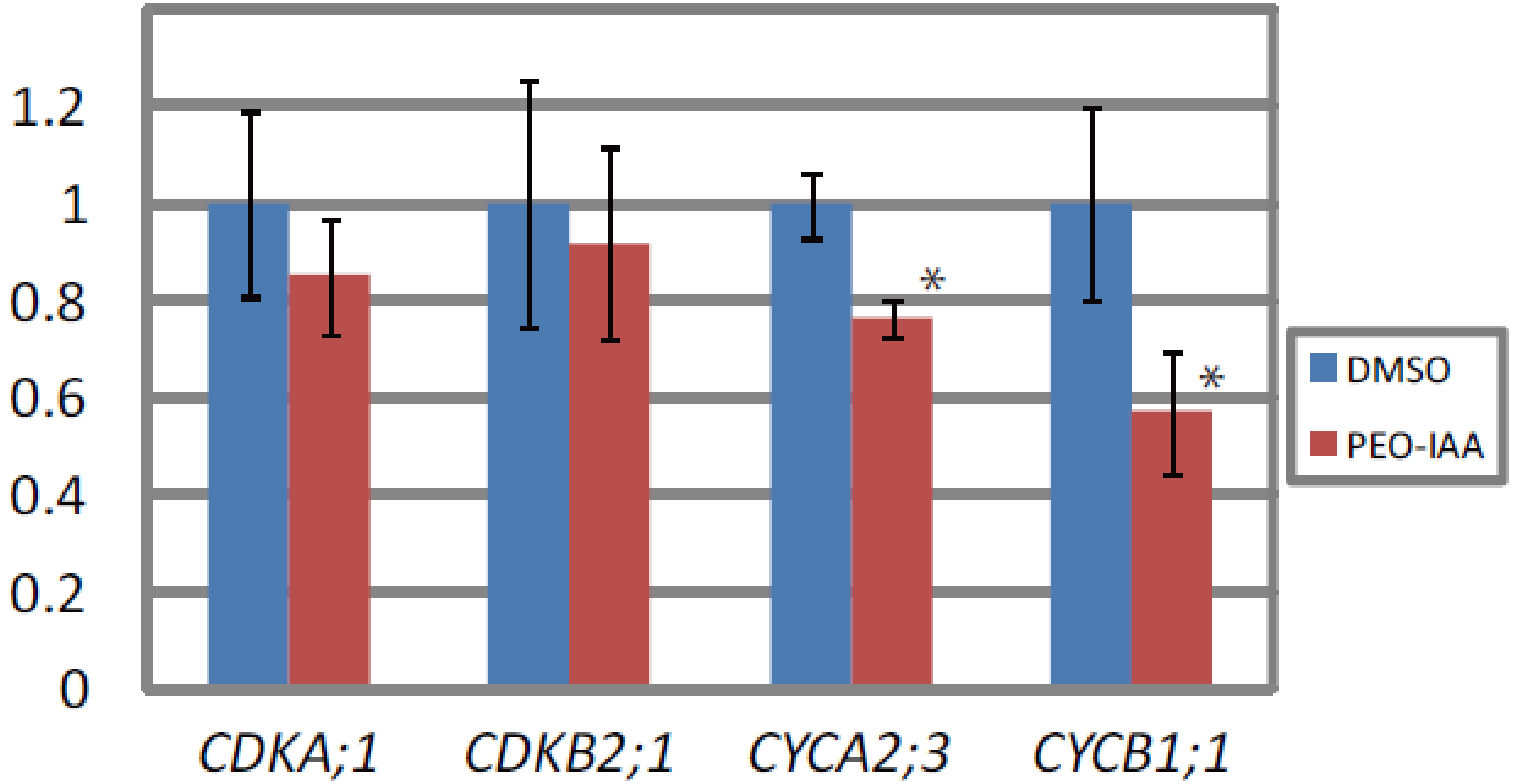
Reduced levels of auxin signalling rapidly block the expression of cell cycle genes.



6-day-old roots treated with 20mM PEO-IAA for 3hr

In collaboration with Masaaki Umeda (NAIST)

Reduced levels of auxin signalling rapidly block the expression of cell cycle genes.

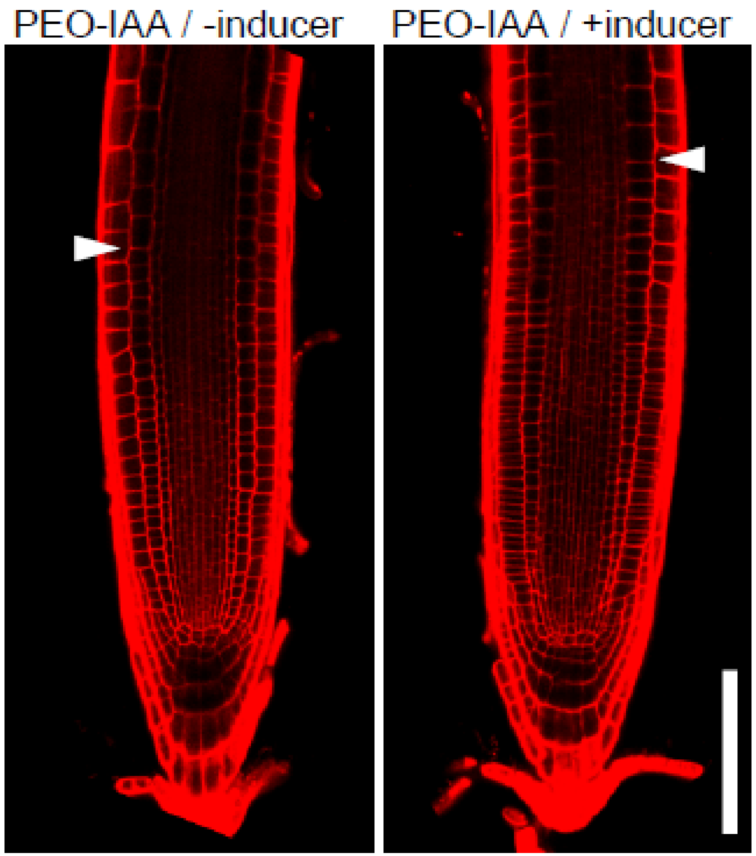


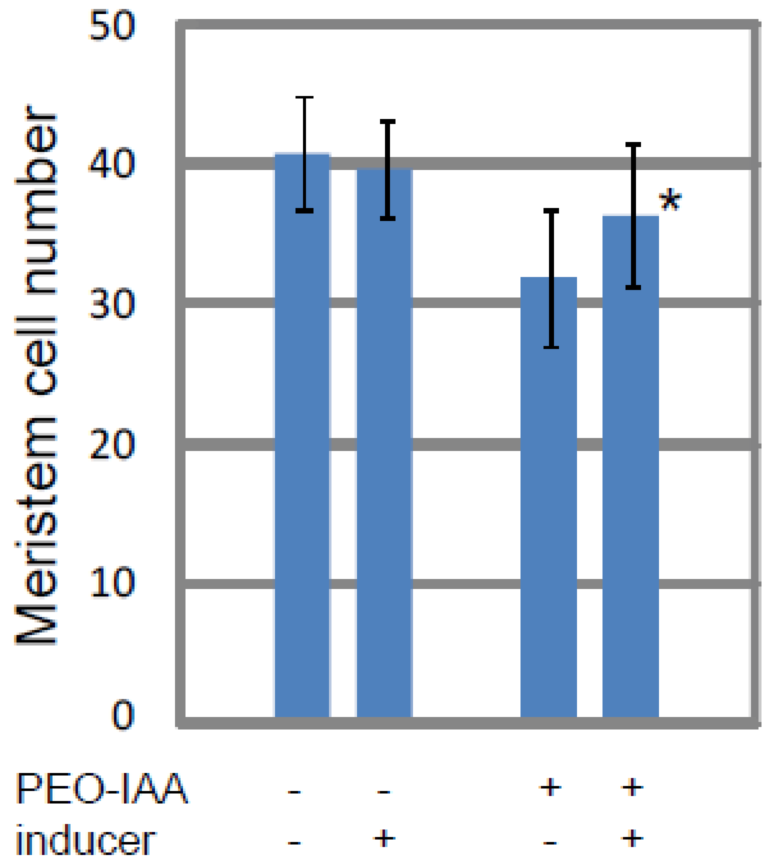
(* P > 0.05)

6-day-old roots treated with 20mM PEO-IAA for 3hr

Takashi Ishida

The expression of CYCA2;3 partially suppresses PEO-IAA-induced cell differentiation.



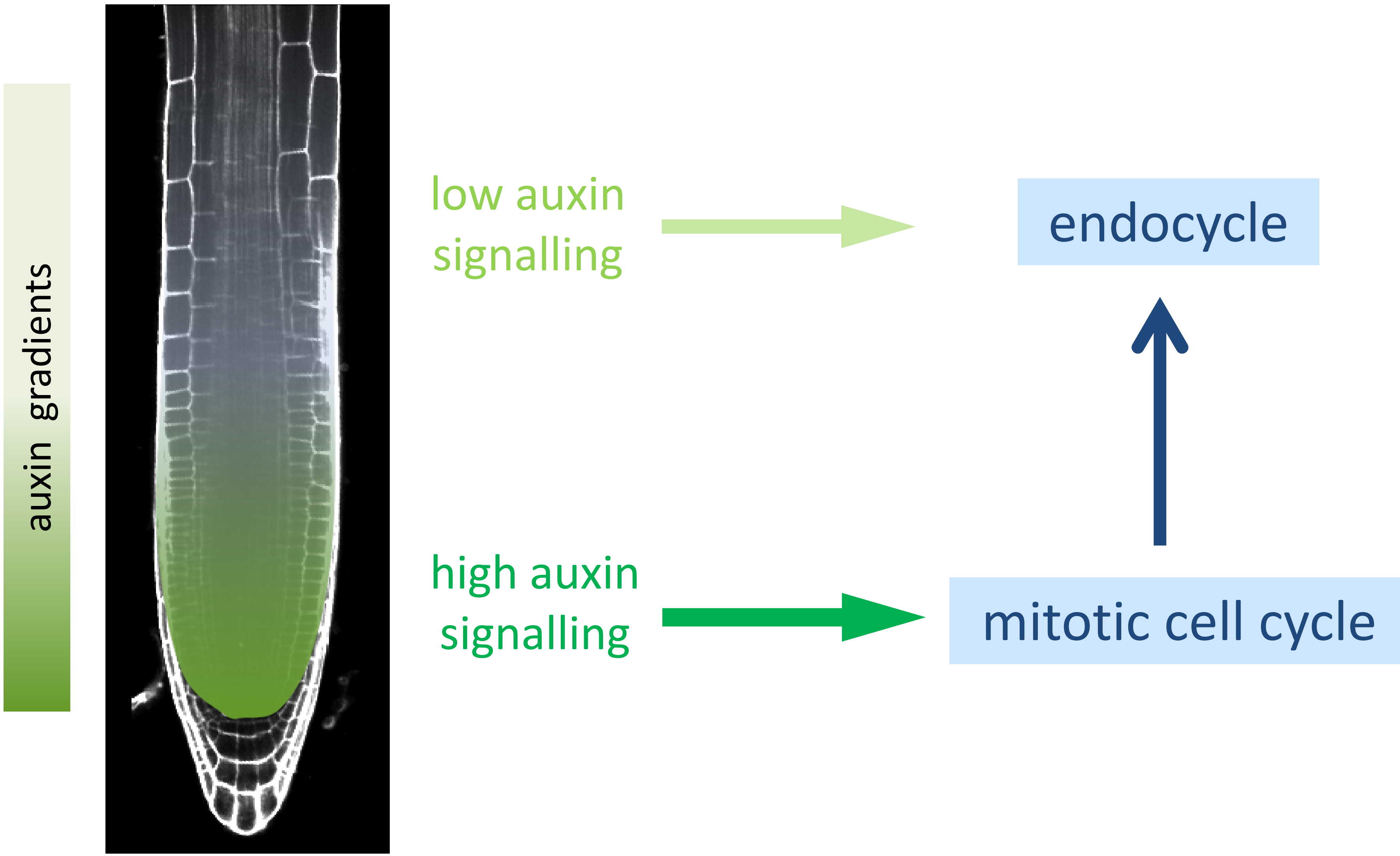


(* P > 0.05)

6-day-old roots; 1-day-treatment with β-estradiol (inducer)

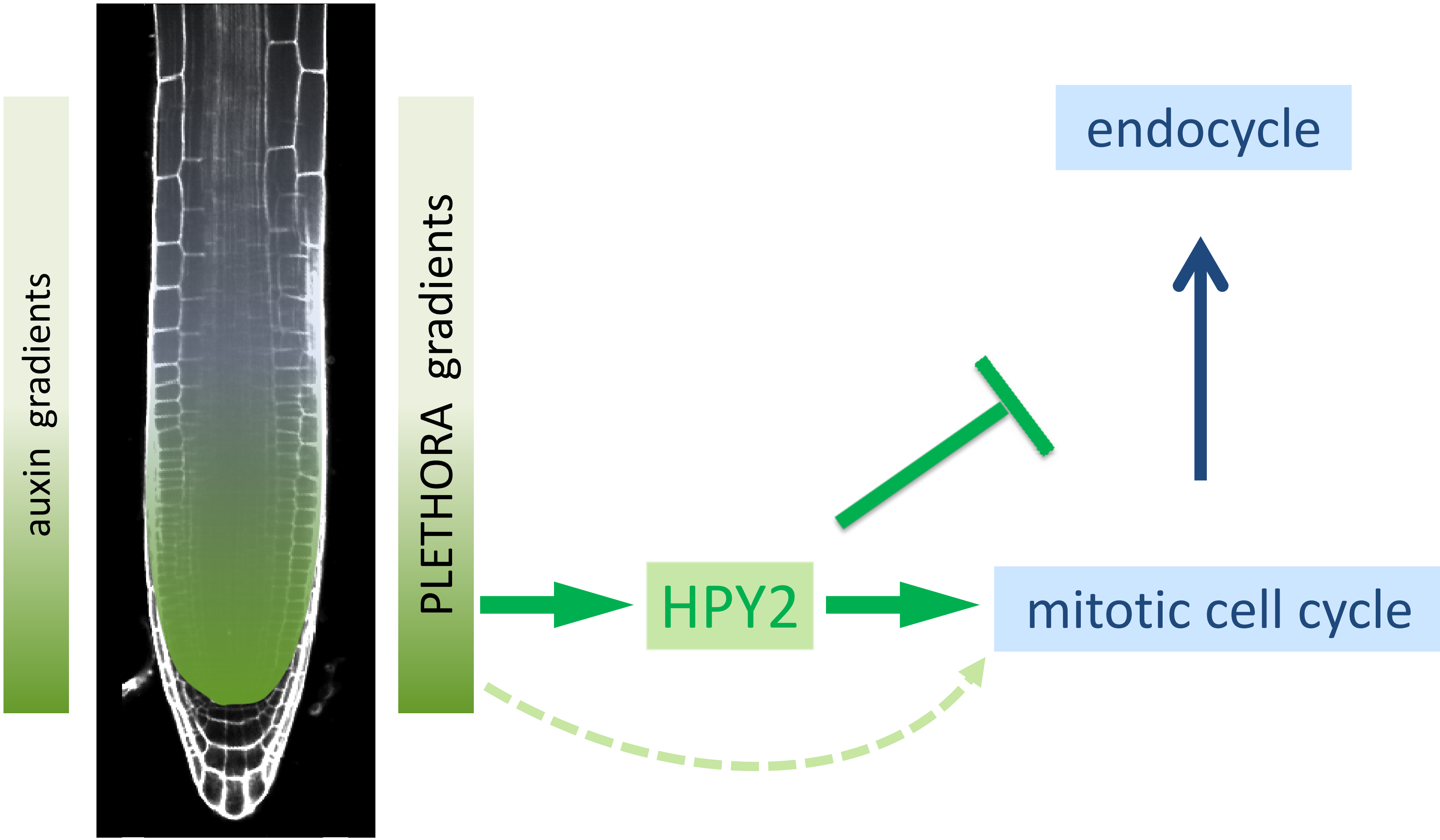
Takashi Ishida

Auxin gradients control the transition from the mitotic cell cycle to the endocycle in the meristem.



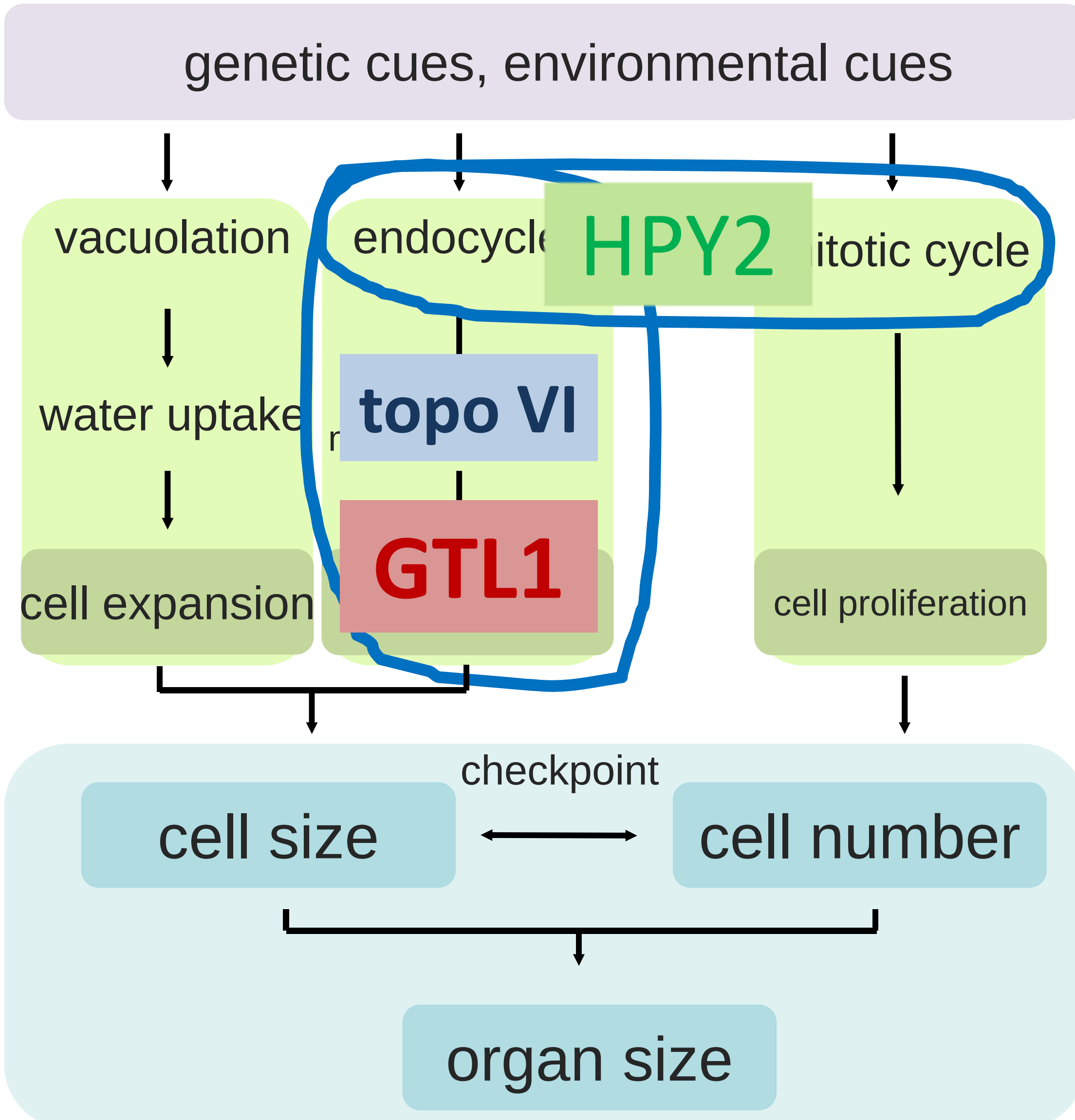
(Ishida et al, Development in press)

HPY2 acts downstream of PLT to promote the mitotic cycle and to repress the endocycle transition.



(Ishida et al, Plant Cell 2009)

How do HPY2 and GTL1 contribute to the size control?



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