CDB Part IB
Plant Development

Lecture 4:

Patterning of shoot growth

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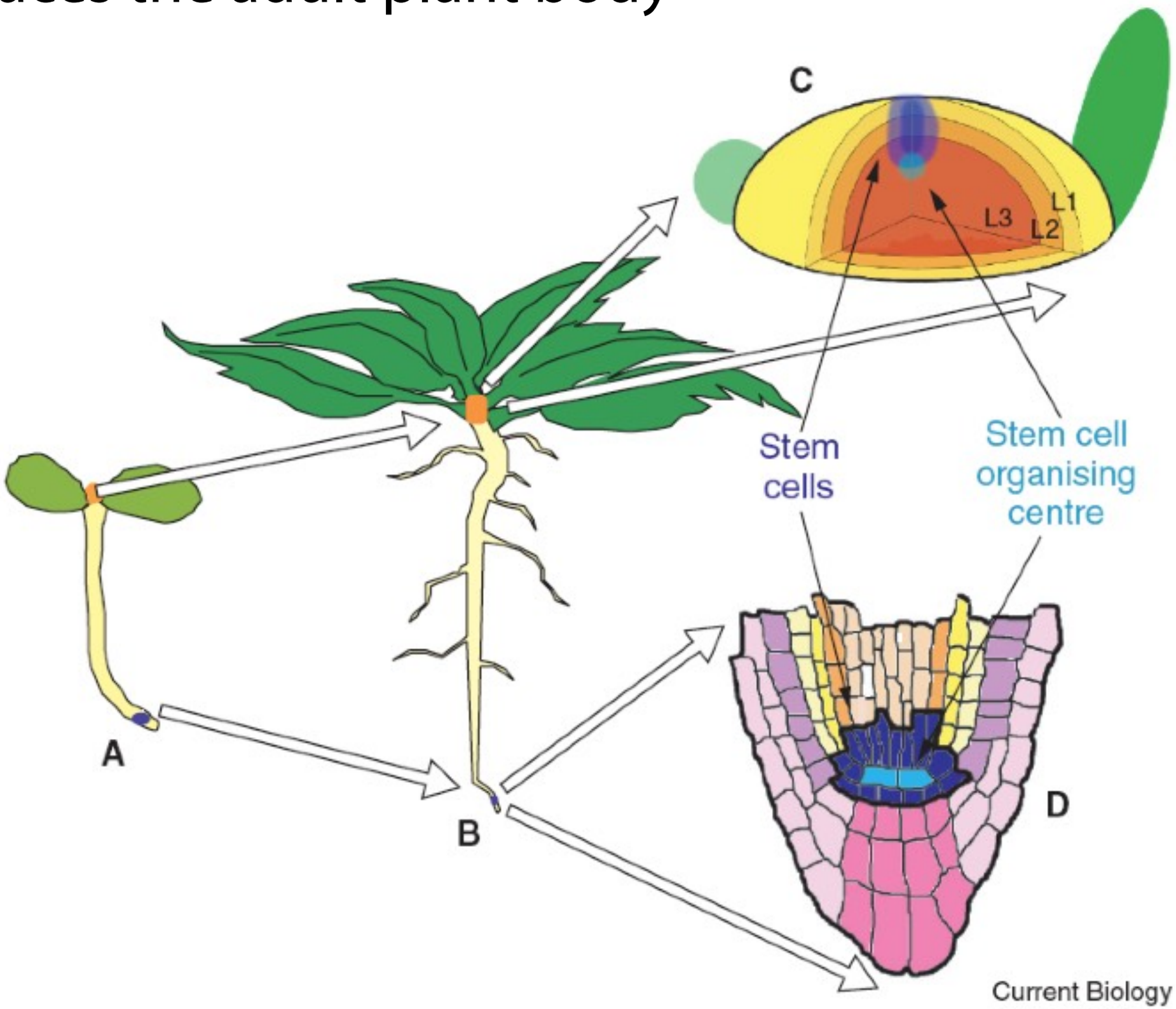
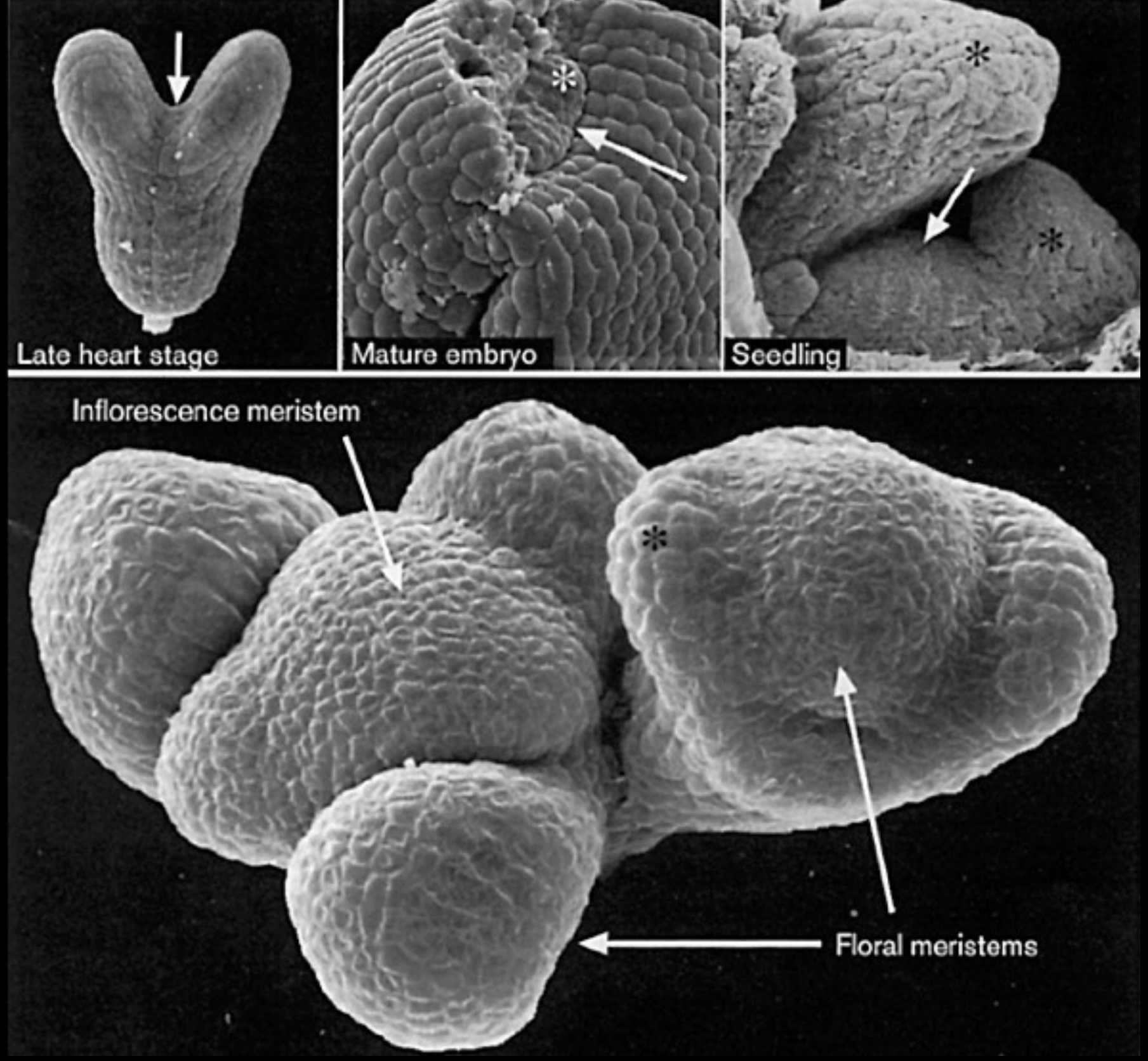
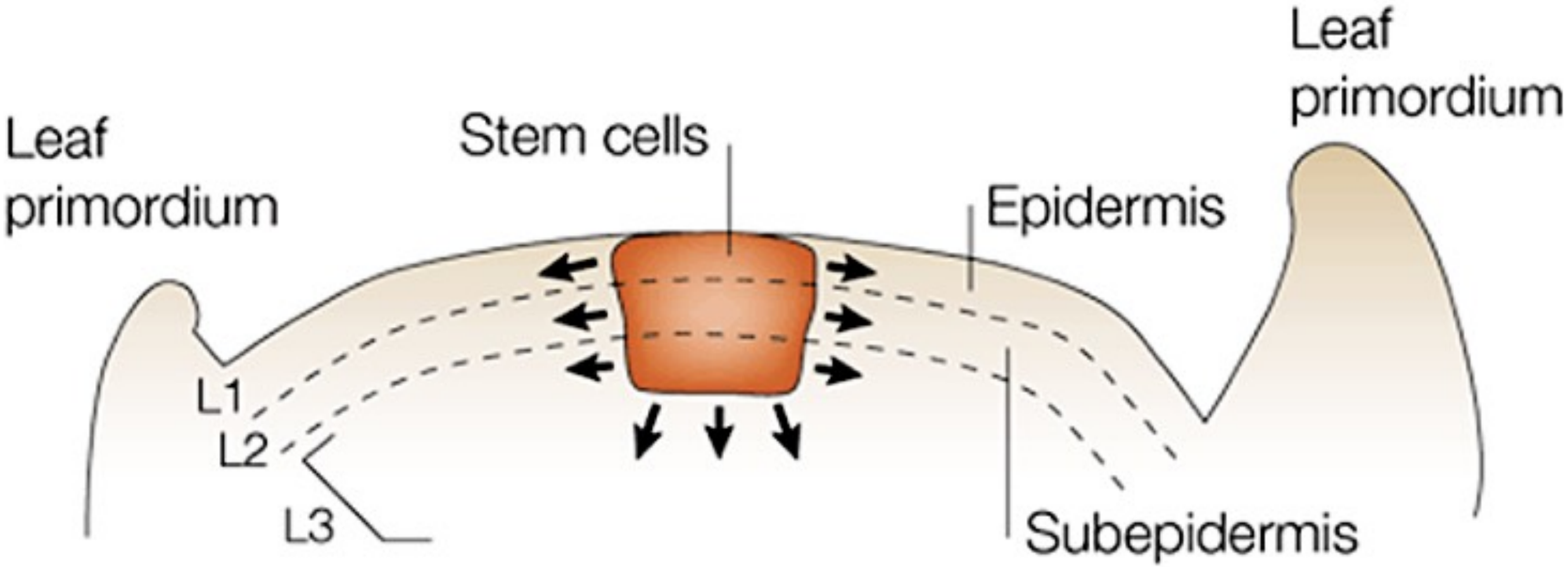
Continued growth of shoot and root meristems produces the adult plant body



Arabidopsis apical meristems

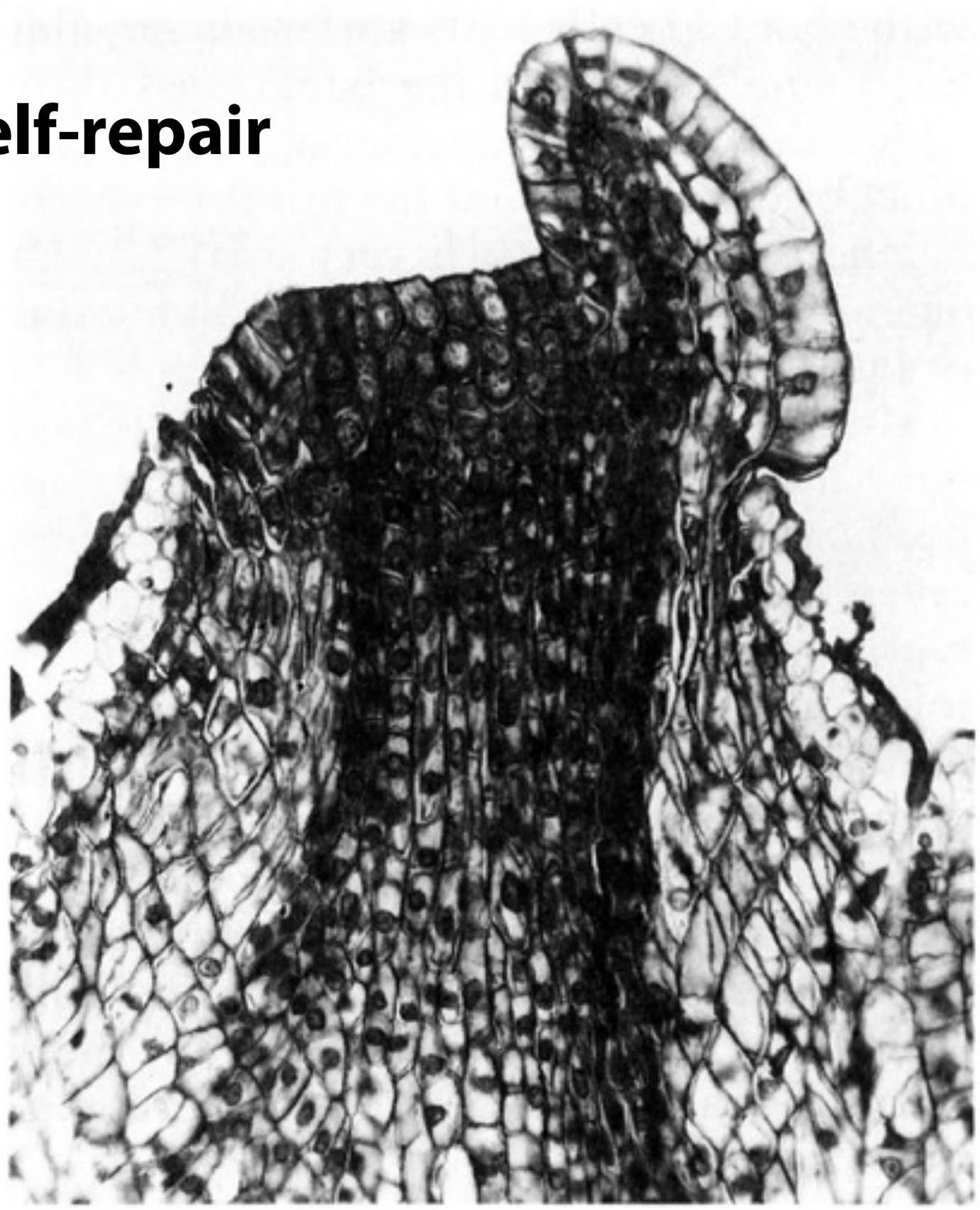


The shoot meristem is branched and indeterminate, capable of producing lateral primordia at the flanks of the meristem.



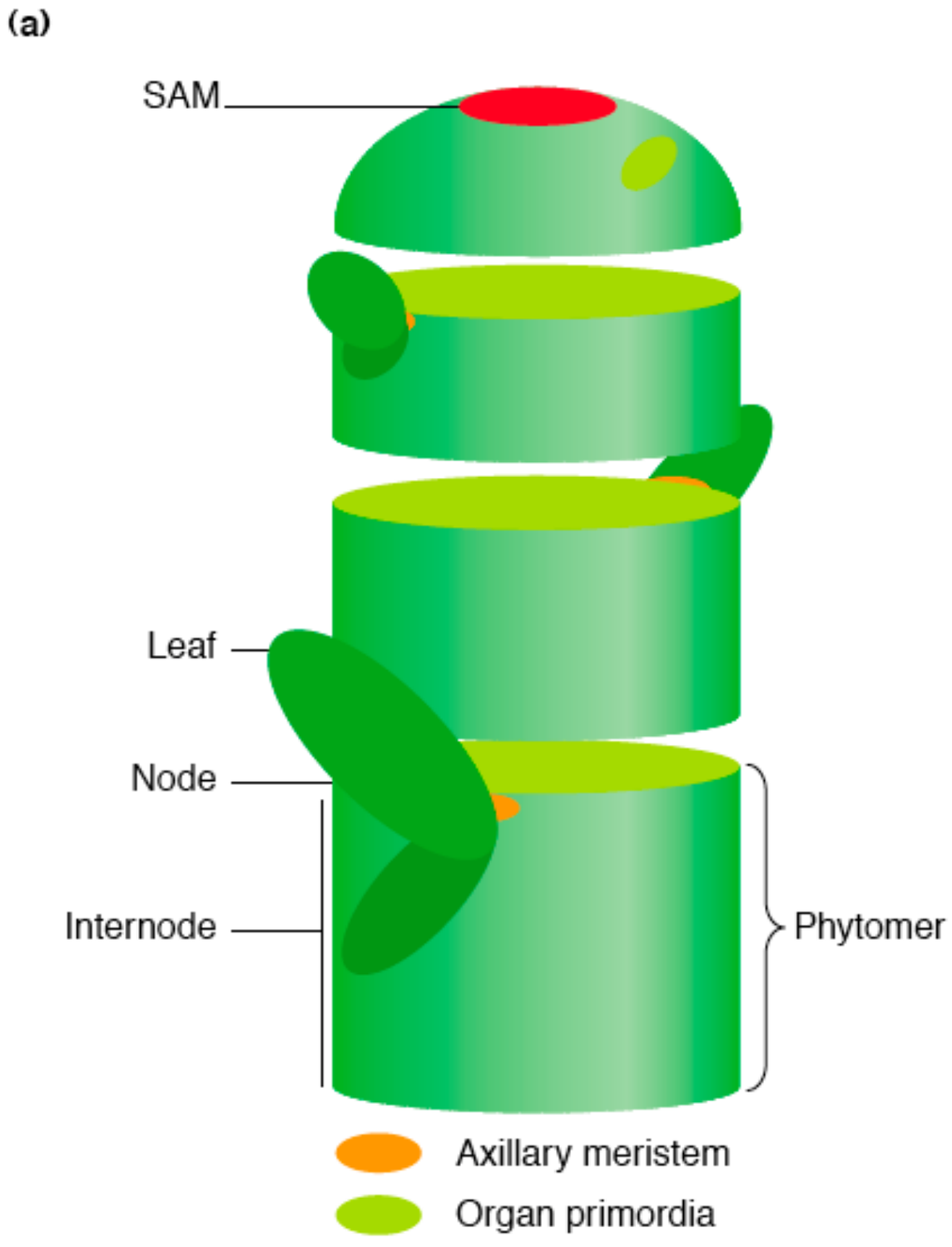
A meristem is self-organising and renews itself, maintaining a balance between cell proliferation and differentiation

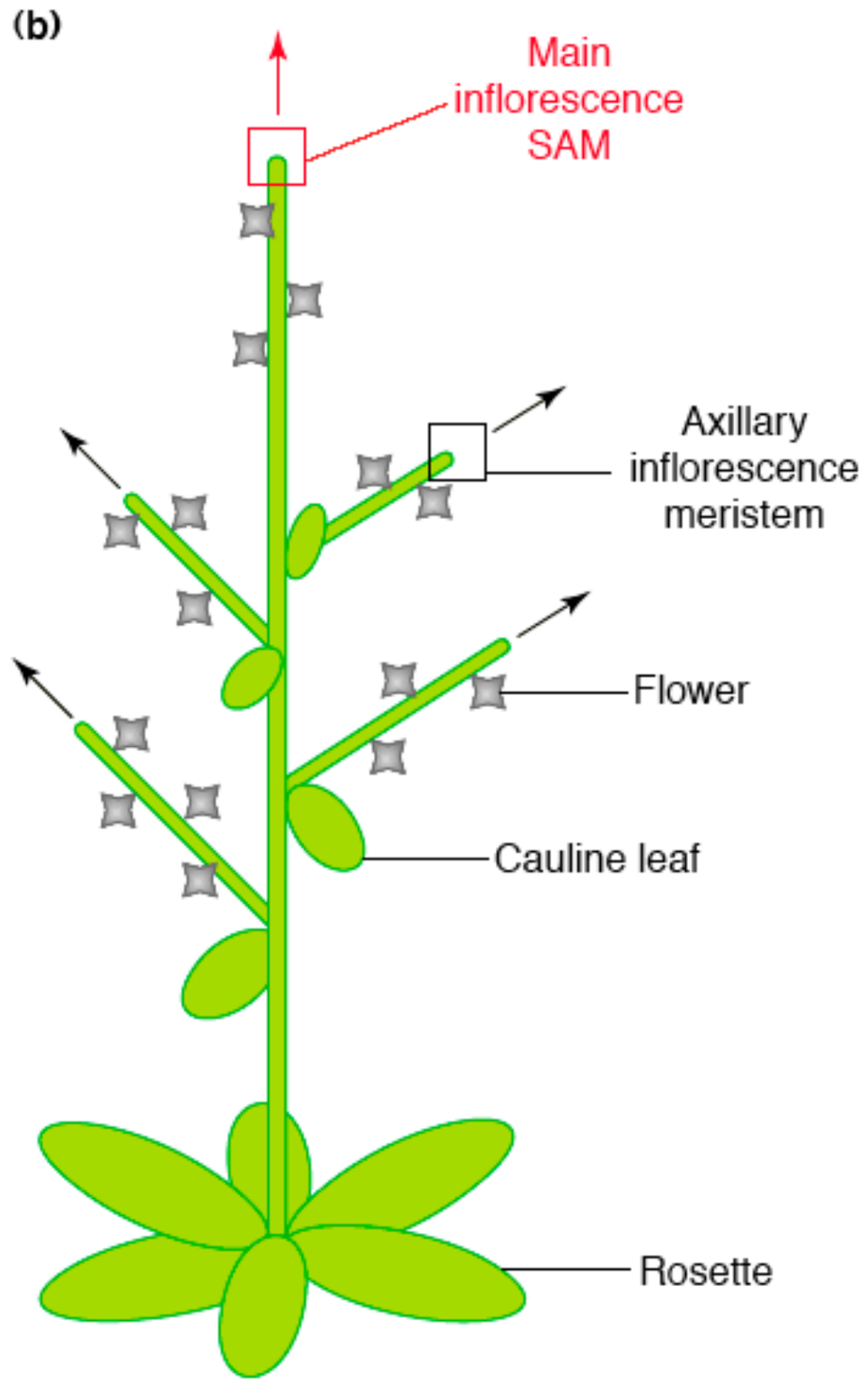
…capable of self-repair



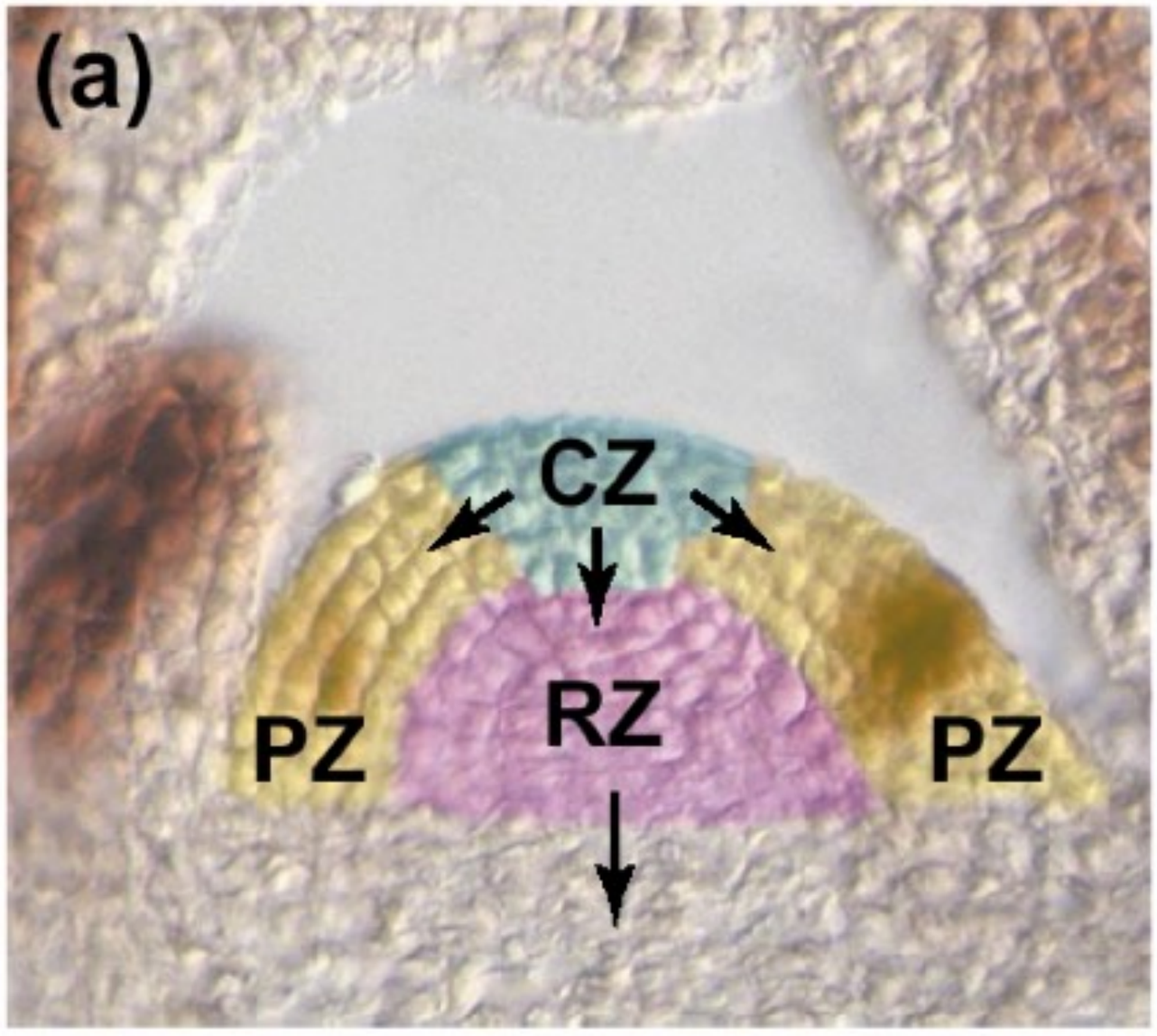
Sussex, Brookhaven Symp. Biol. 16:1-12 (1964)

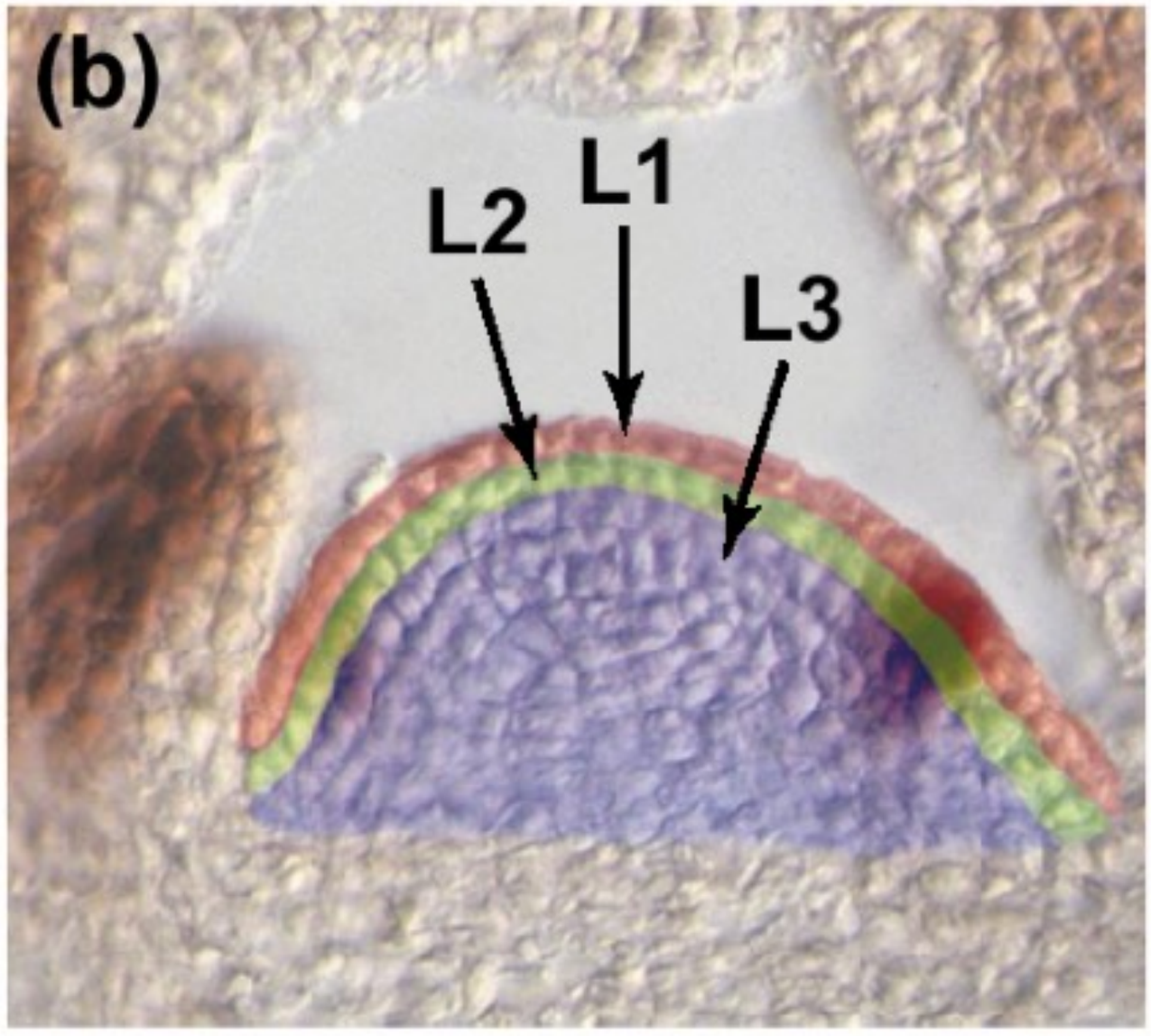
Modular growth and production of lateral organs





The Arabidopsis shoot meristem is divided into functionally distinct zones





Central zone = undifferentiated cells
Peripheral zone = formation of new lateral organs
Rib zone = formation of new stem

The meristem contains three different layers of cells, L1, L2 & L3. These generally maintain distinct lineages

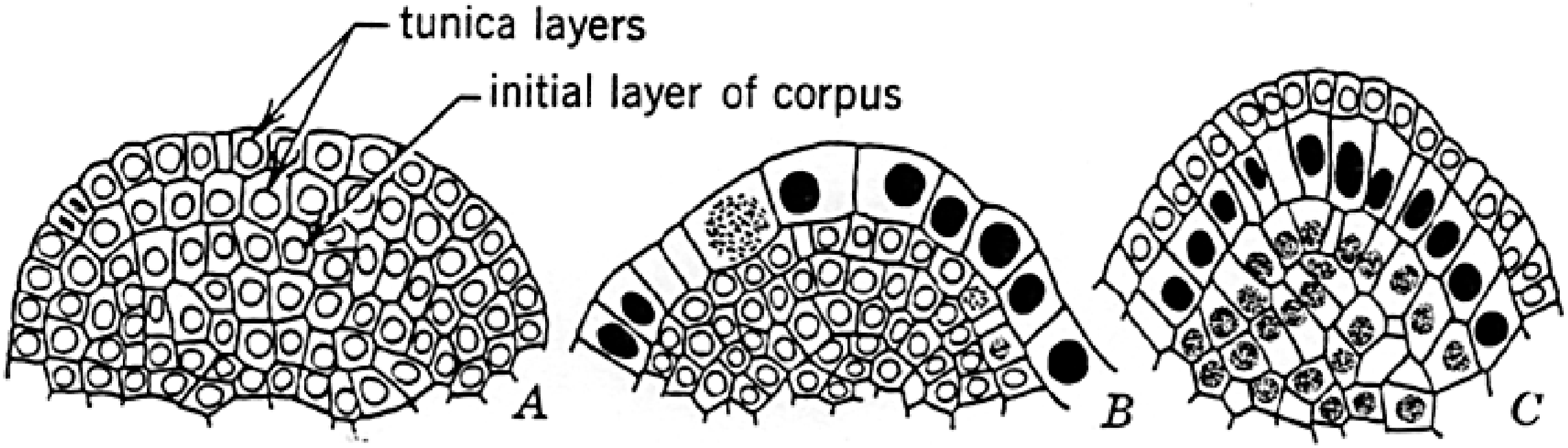
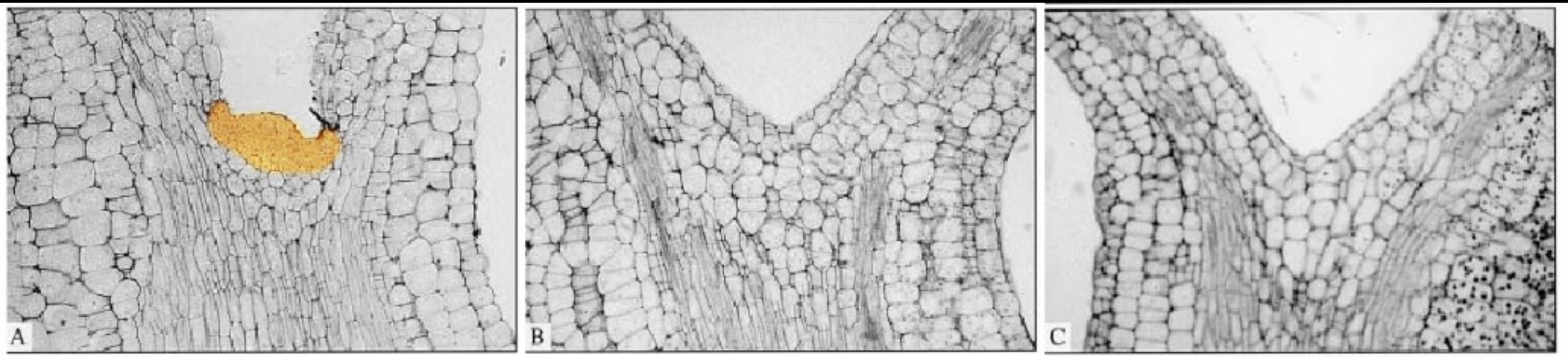


FIG. 5.2. Shoot apices of *Datura* from a diploid plant (*A*) and from several periclinal cytochimeras. Chromosomal combinations are indicated by values given below each drawing.

SHOOT MERISTEMLESS (STM) and WUSCHEL (WUS) are homeodomain genes that are required for formation and maintenance of the shoot apical meristem in Arabidopsis.

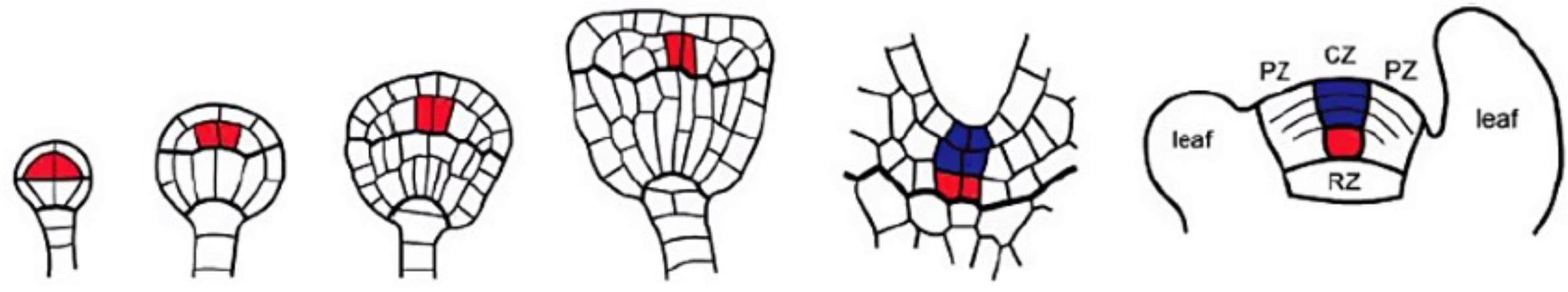


wt

shootmeristemless (stm)

wuschel (wus)

WUSCHEL expression



16-cell | early globular | late globular | transition | late heart | seedling shoot meristem

F

C

ANT::WUS

C

Ectopic expression of WUSCHEL induces stem cell proliferation

WUS and STM initiate and maintain meristem growth

- but how is the size of the apical meristem constrained?

the growth of the shoot meristem is negatively regulated by….

the CLAVATA gene pathway

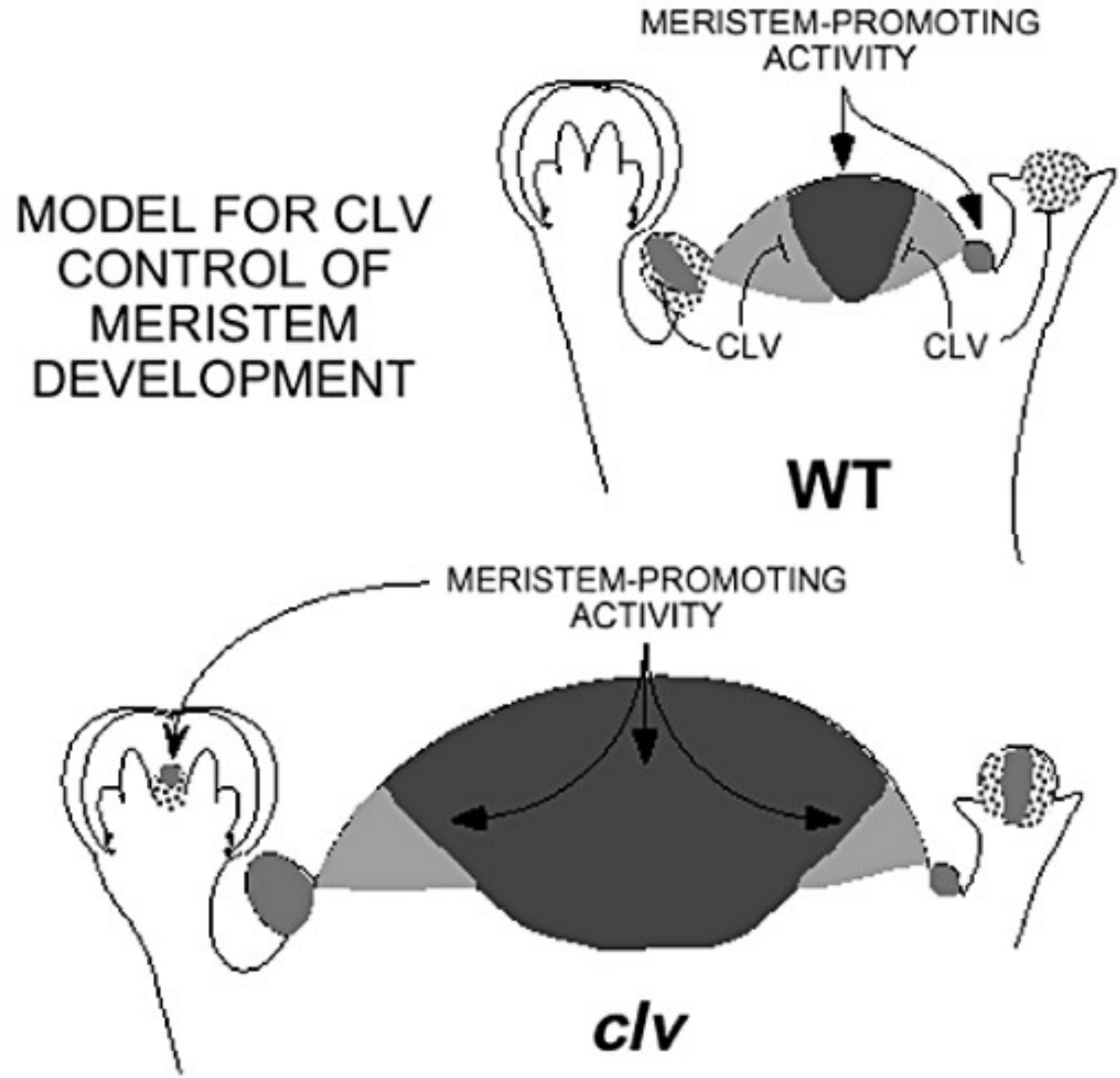
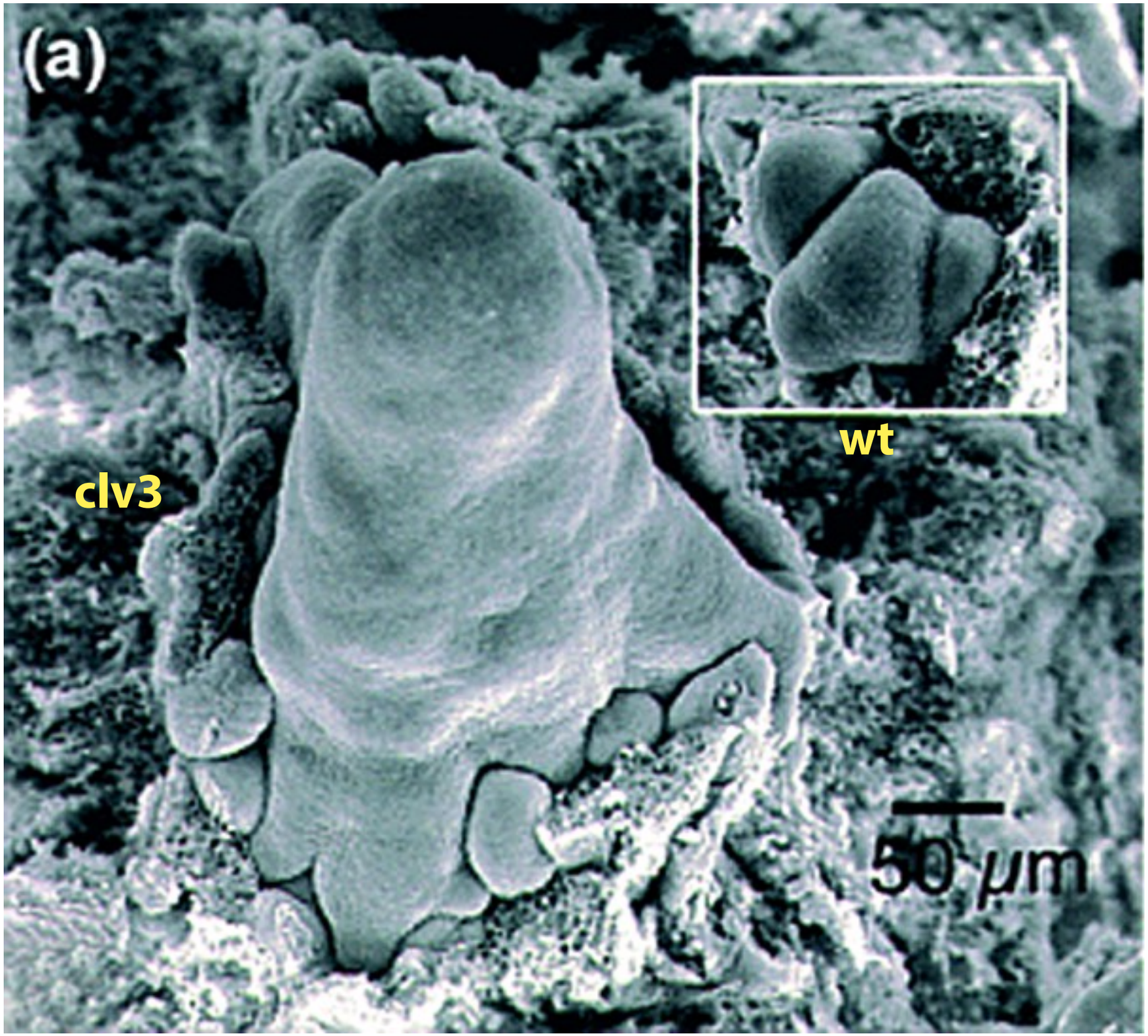


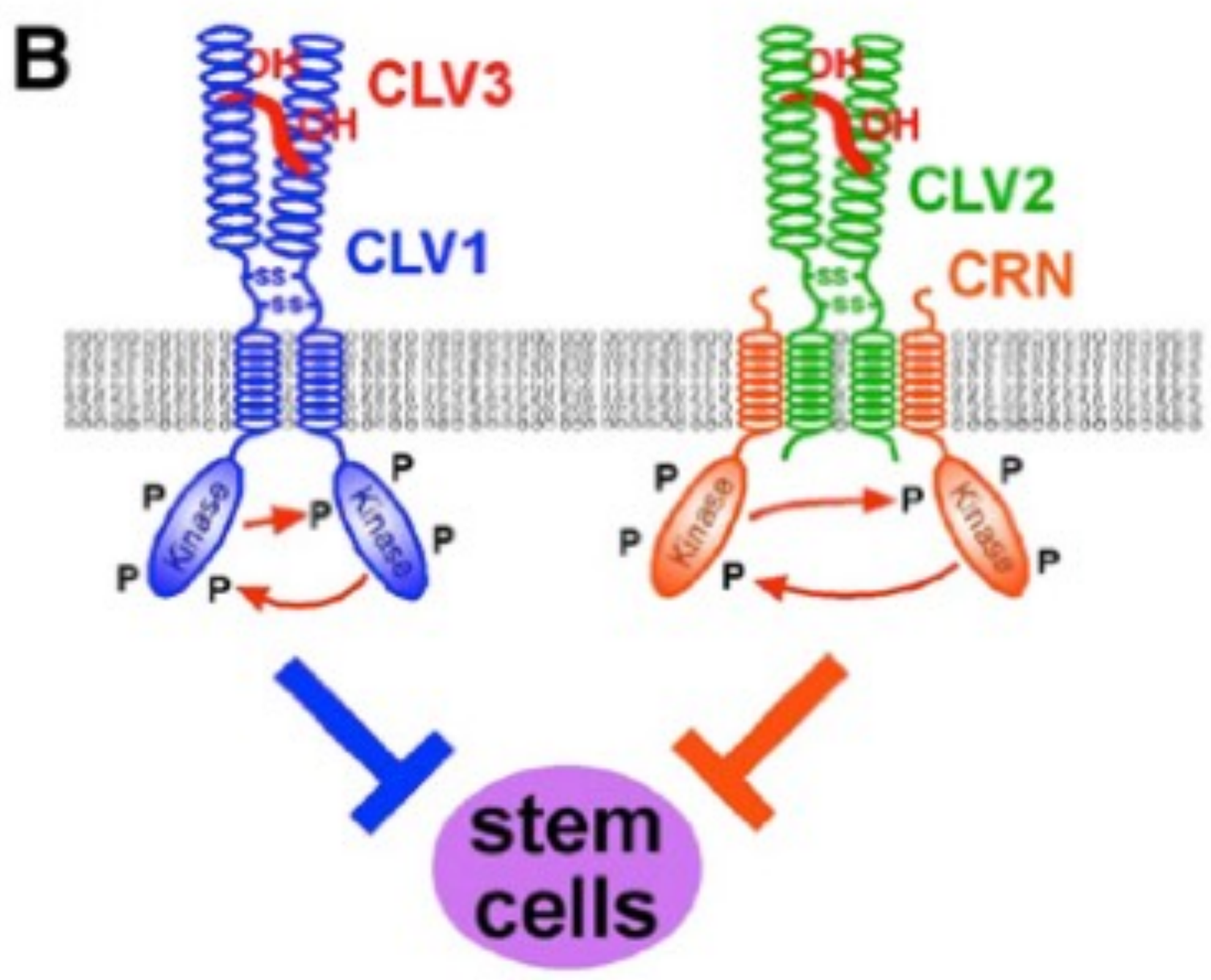
Fig. 9. Model for *CLV* action. The model postulates that there is a meristem-promoting activity (MPA) in shoot meristems and young flowers that maintains cells in a proliferative, undifferentiated state.

Mutations in the genes, *clavata1*, *clavata2*, *coryne* and *clavata3*, produce similar phenotypes - enlarged meristems.



These genes function in the same regulatory pathway.

Clavata1, *Clavata2 and Coryne* are membrane-localised receptor proteins expressed in cells deep in the central zone of the meristem



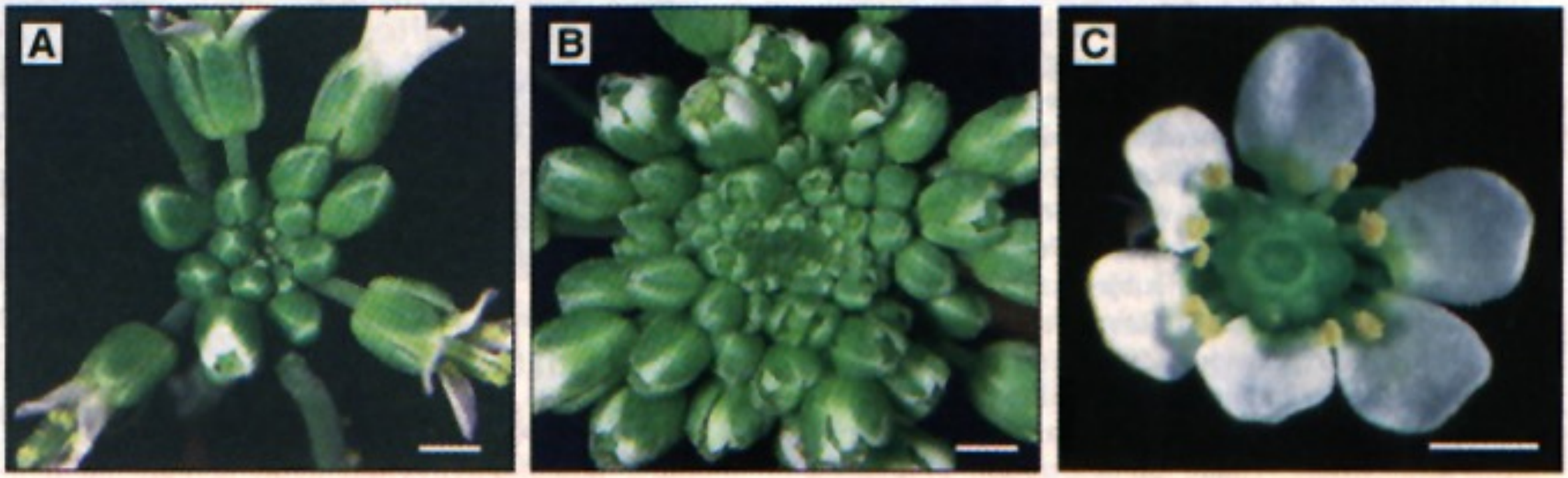


Fig. 1. *clv3* shoot and flower phenotypes. (**A**) Wild-type inflorescence meristem. (**B**) *clv3-2* inflorescence meristems undergo fasciation, growing as a ring or line rather than a point. (**C**) *clv3-2* mutant flowers contain extra organs of all types, particularly stamens and carpels. Bars, 1 mm.

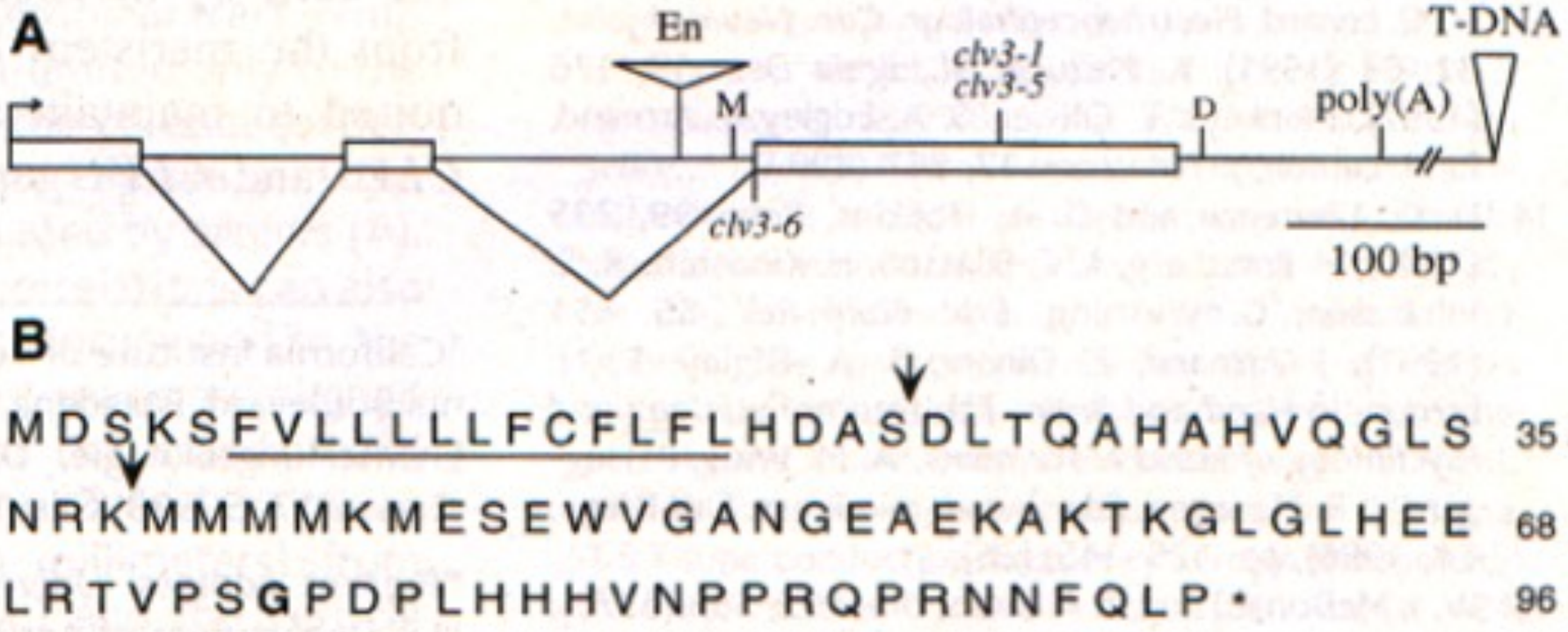
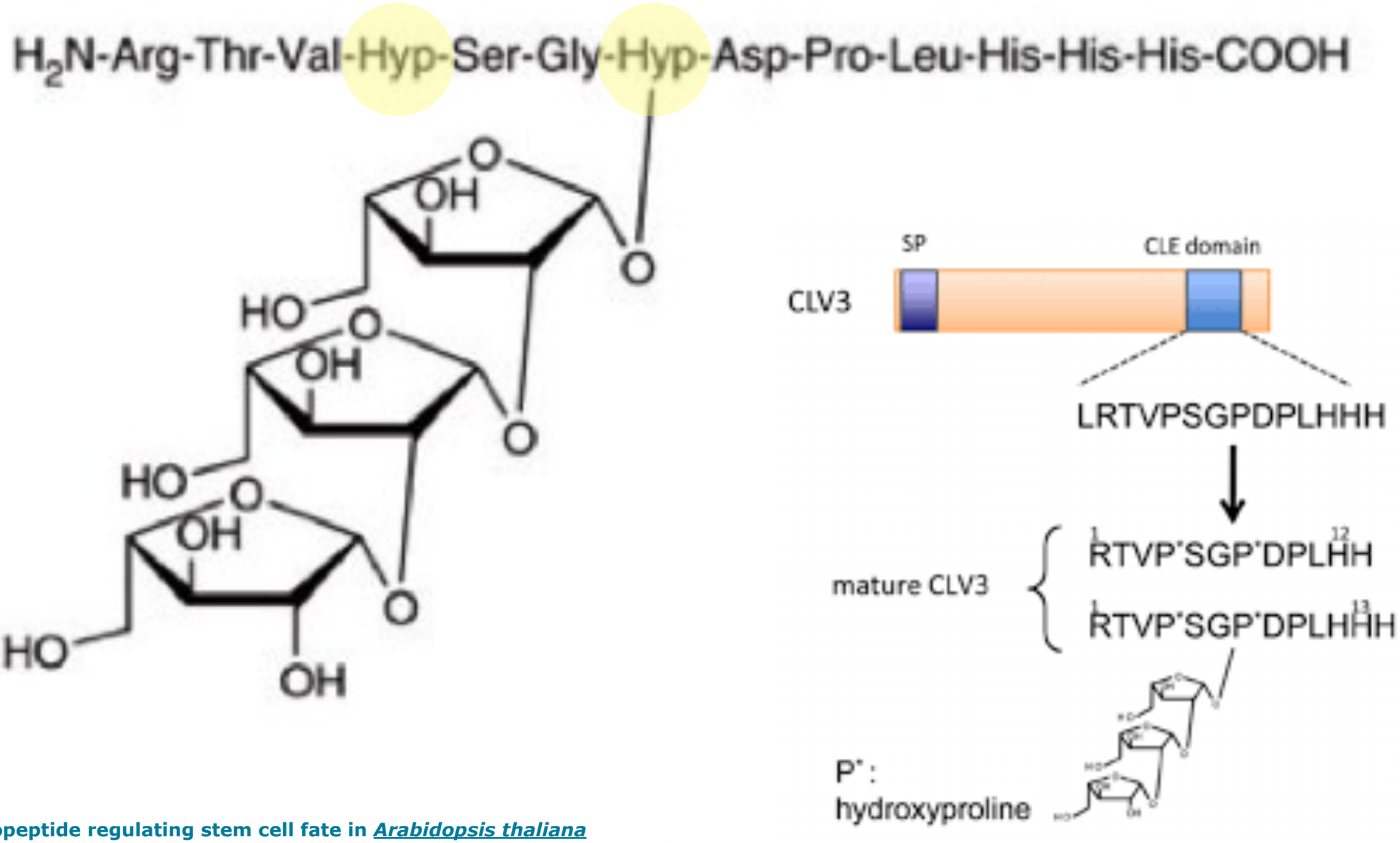
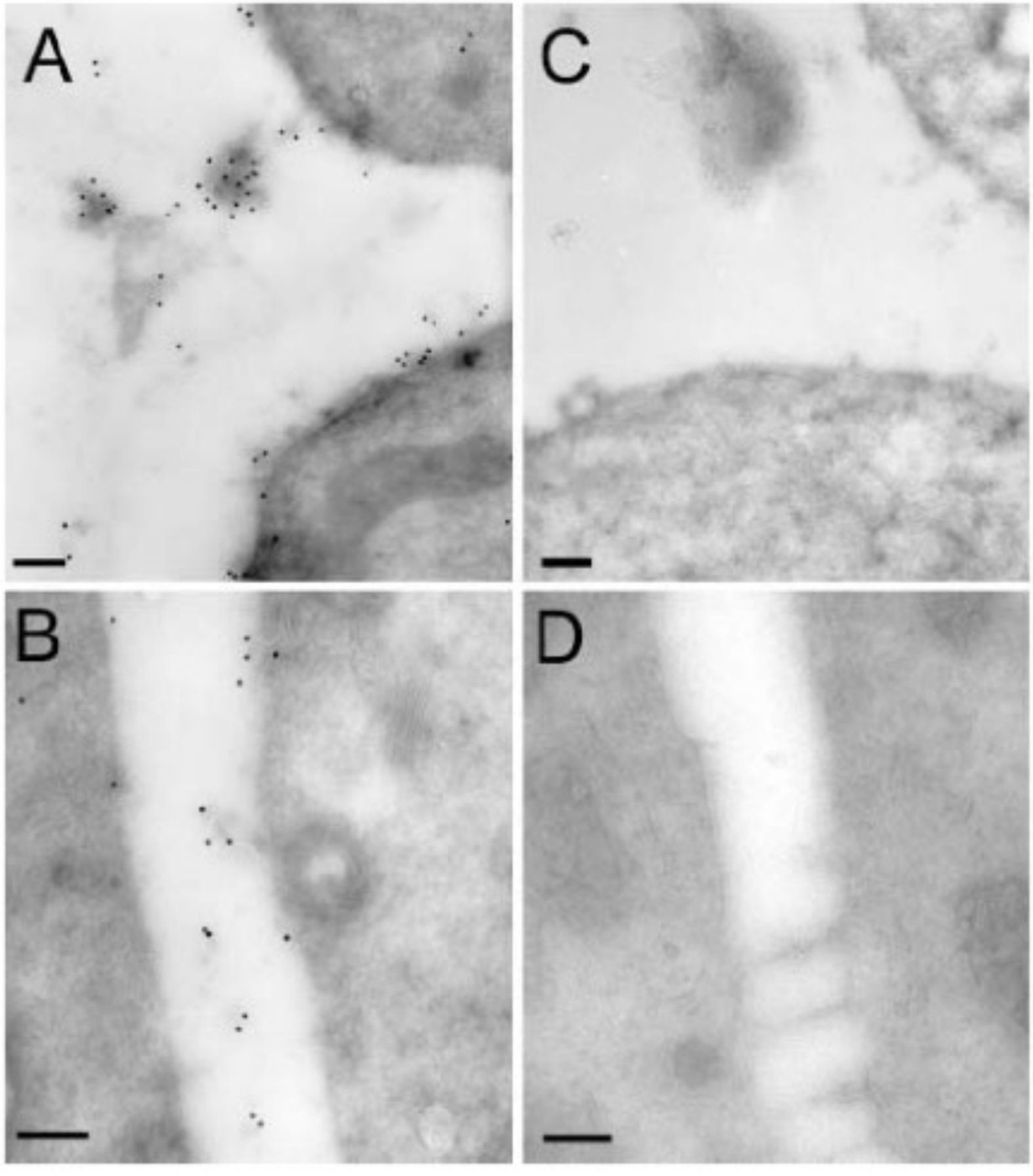


Fig. 2. *CLV3* genomic region and peptide sequence. (**A**) The *CLV3* genomic region. The translation start site is denoted by the arrow and the exons by boxes. The relative positions of the *clv3* mutations are shown. Restriction sites: M, Mfe I; D, Dra I. The genom-

Clavata3 forms a 13 amino acid arabinosylated glycopeptide

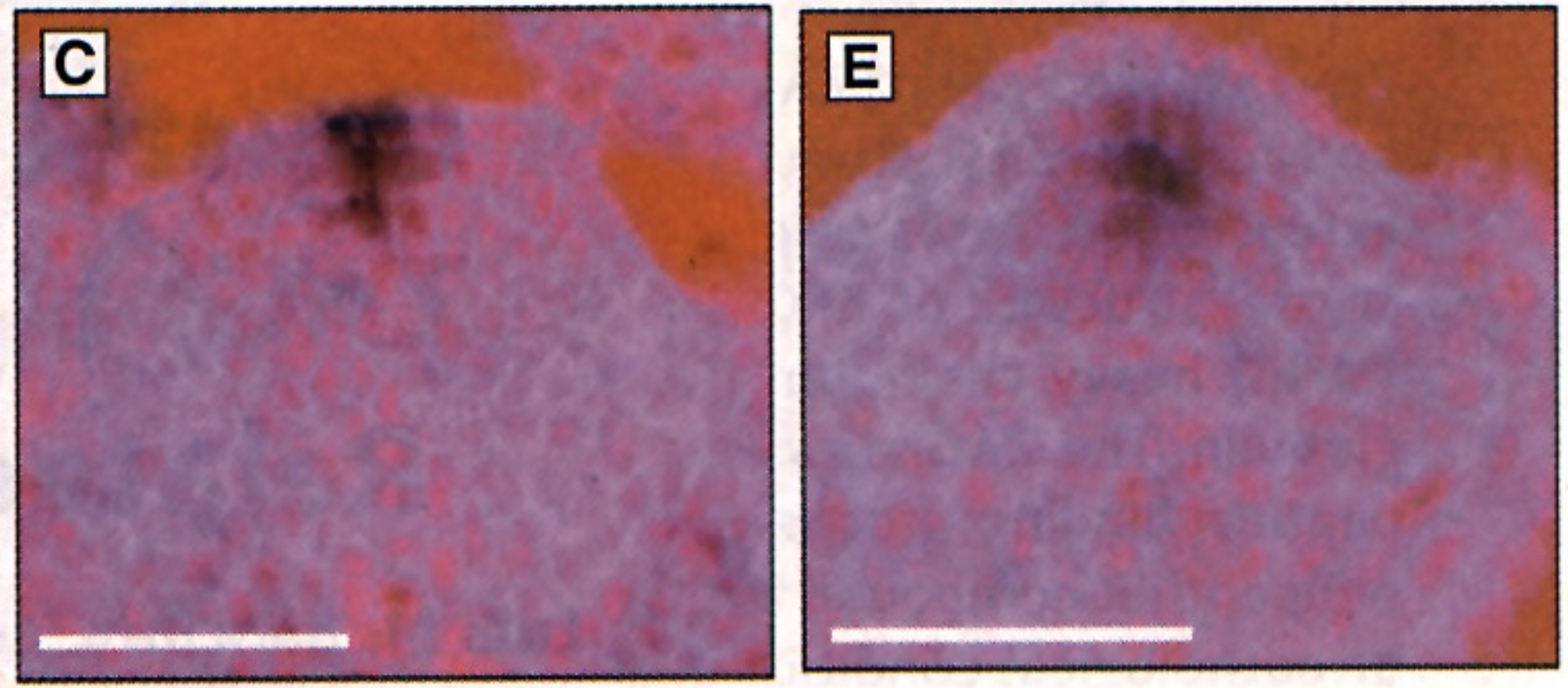


A glycopeptide regulating stem cell fate in *Arabidopsis thaliana*
Kentaro Ohyama, Hidefumi Shinohara, Mari Ogawa-Ohnishi & Yoshikatsu Matsubayashi
Nature Chemical Biology **5**, 578 - 580 (2009)
doi:10.1038/nchembio.182

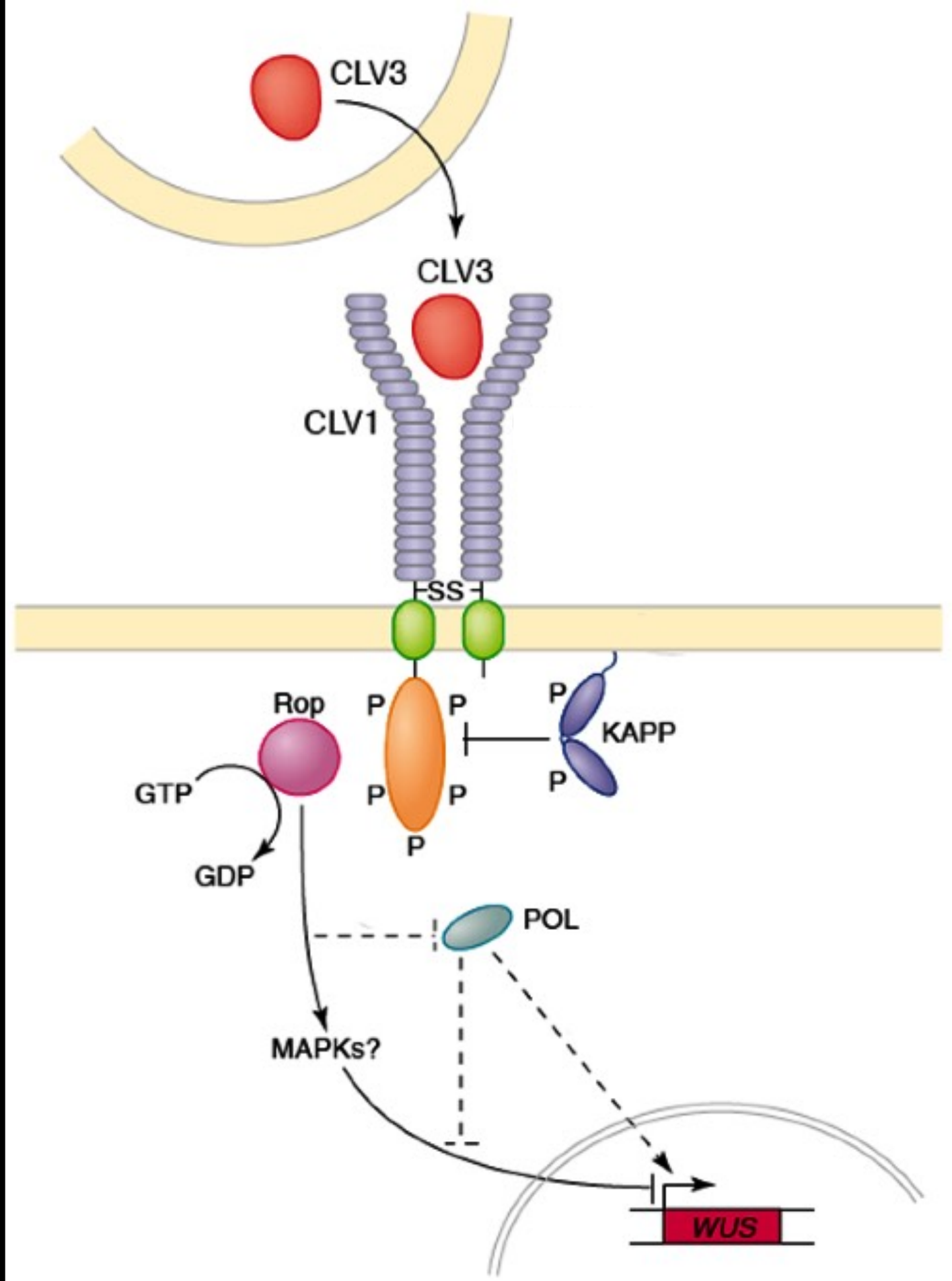


CLV3 Is Localized in the Extracellular Space.

C*lavata3* encodes a secreted peptide expressed in cells towards the apical surface of the meristem, and is a ligand for the CLAVATA1/ CLAVATA2/CORYNE receptors

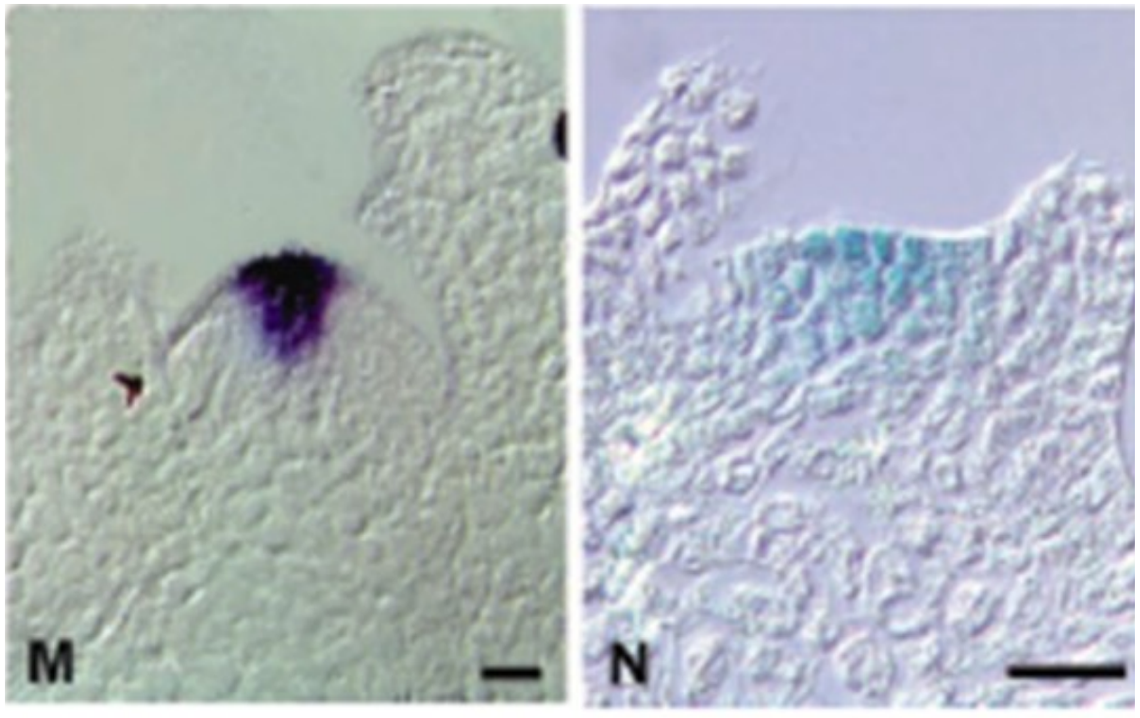


CLAVATA3 acts as a signal across the meristem, repressing WUSCHEL activity in the central zone of the shoot meristem.



CLV and WUS form a feedback loop

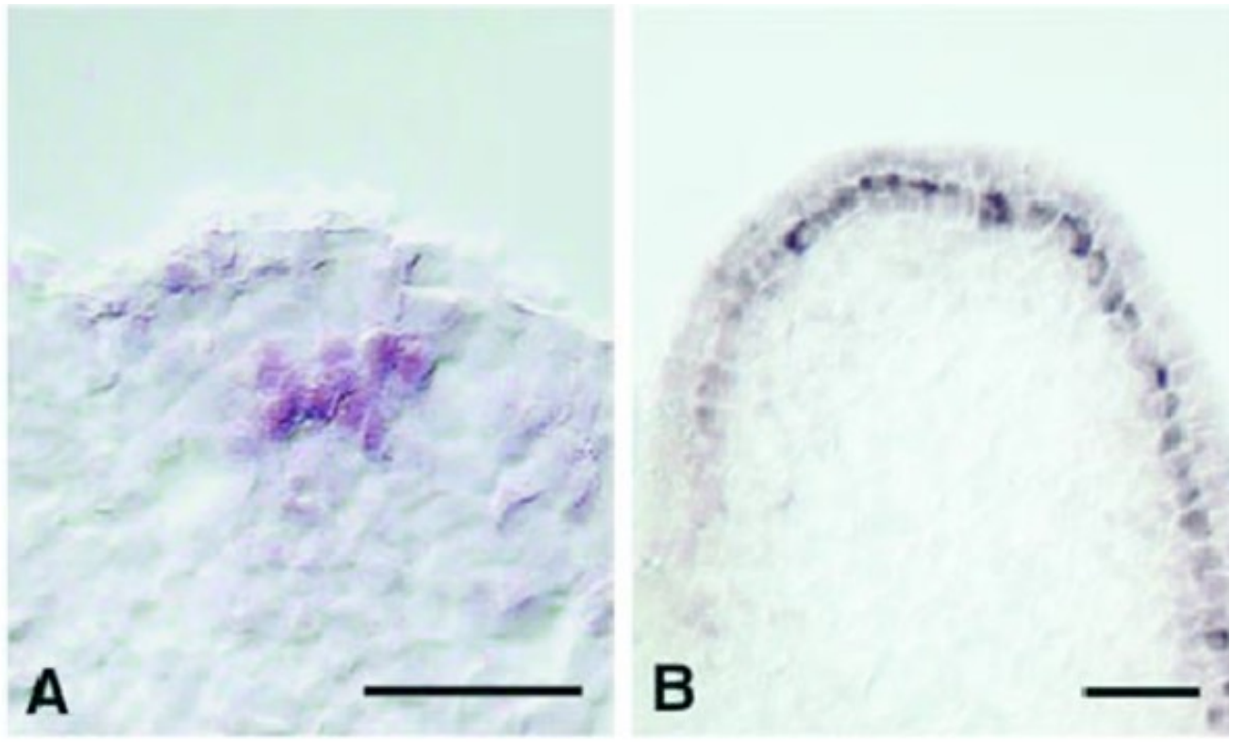
WUS promotes CLV expression



CLV3 in wild-type

CLV3 in *wus*

CLV prevents expansion of WUS domain

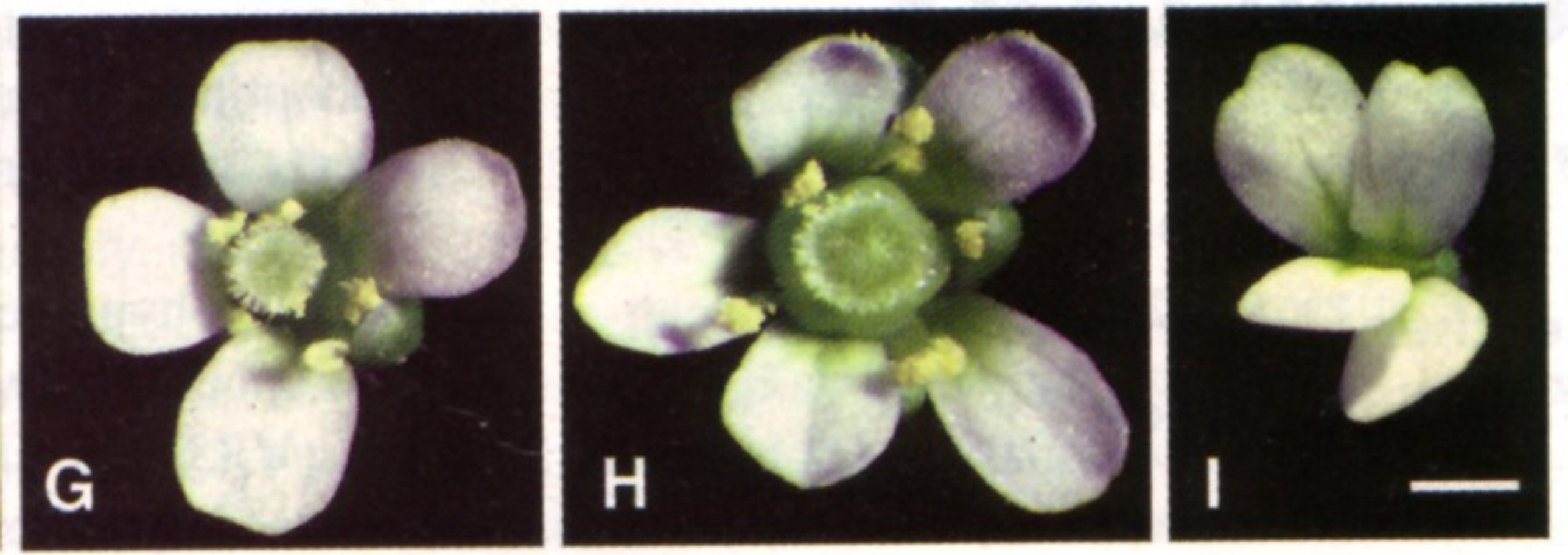


WUS in wild-type

WUS in *clv*

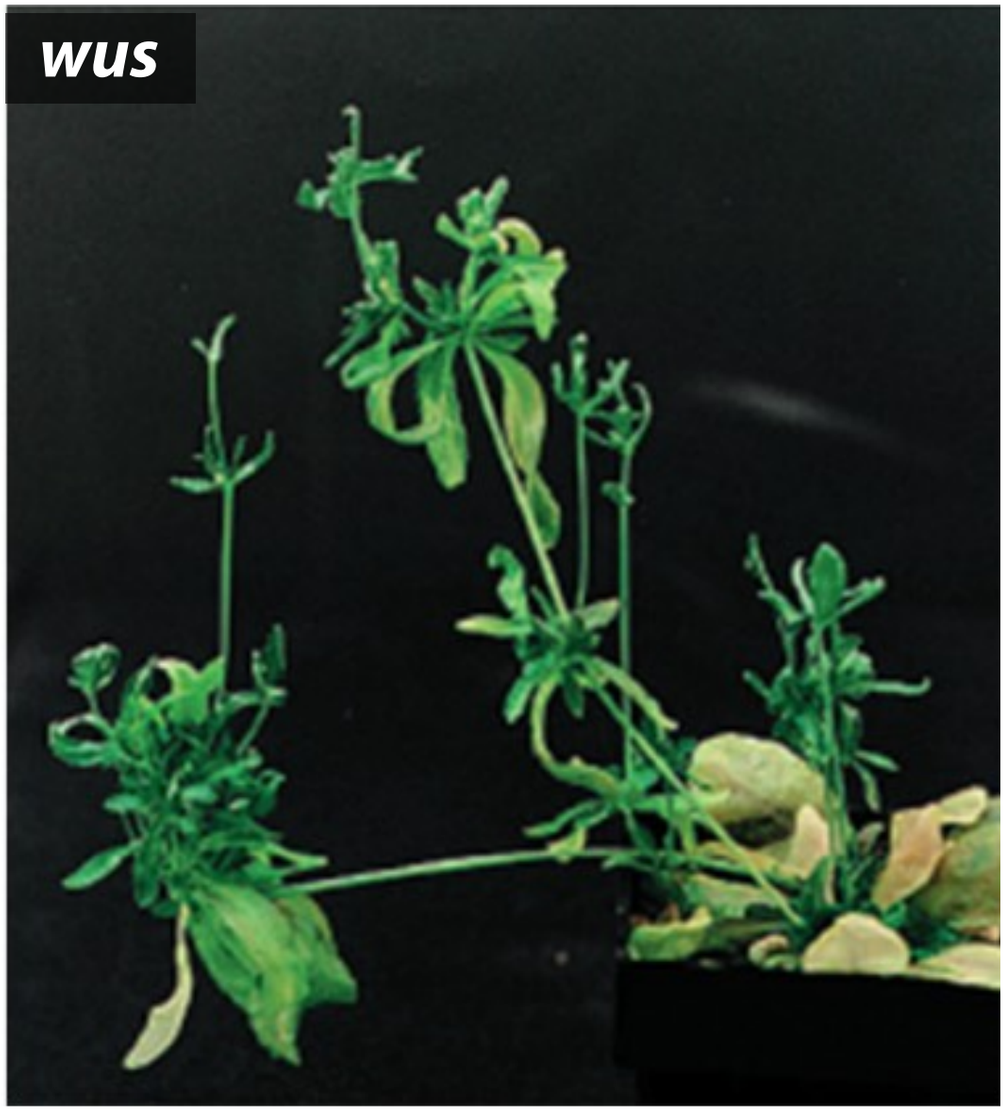
Brand et al (2000) Science 289, 617-619

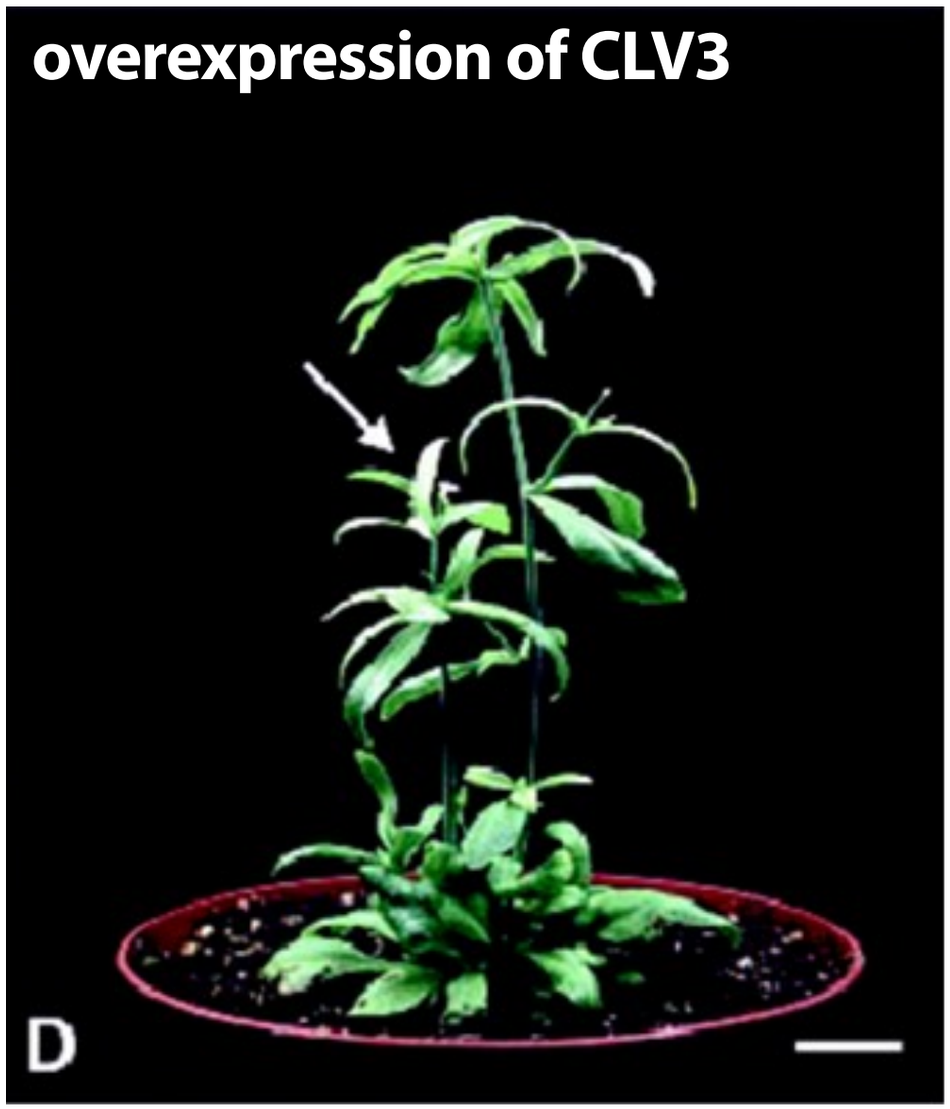
wt *clv3-2* *35S:clv3*



Clavata 3 gene function is required to maintain meristem size, and sufficient to alter meristem size - Like Wuschel, but in opposition

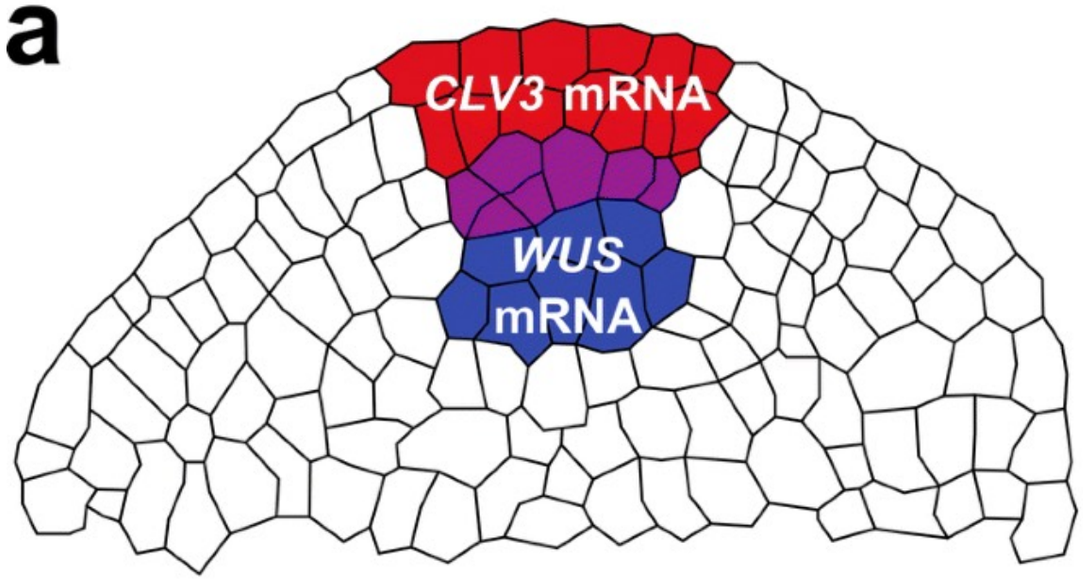
CLV and WUS form a negative feedback loop

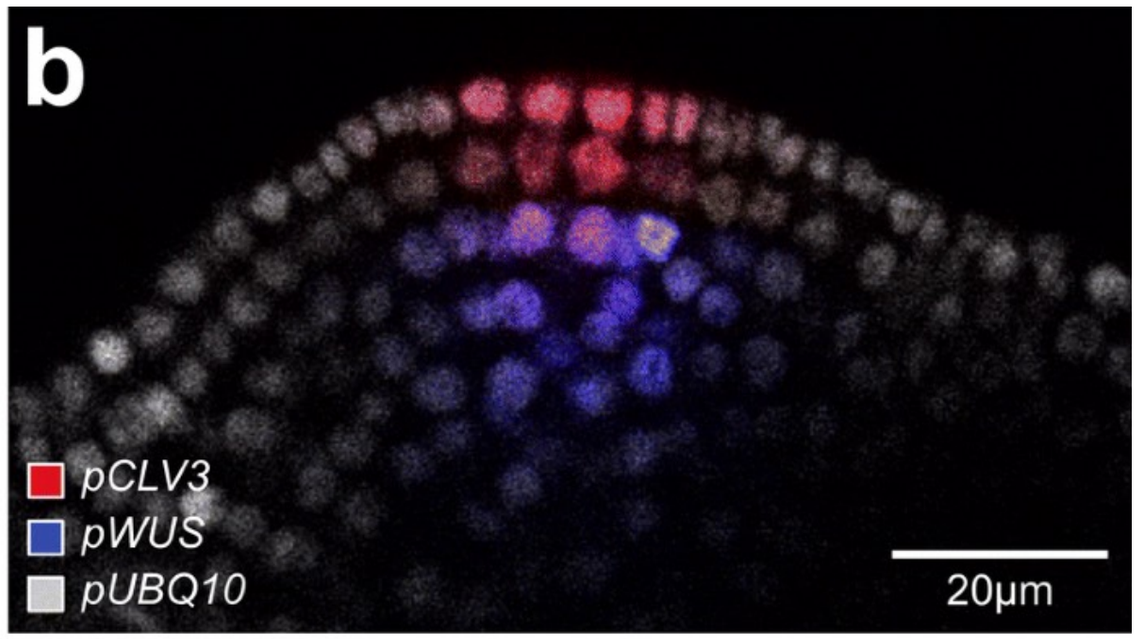


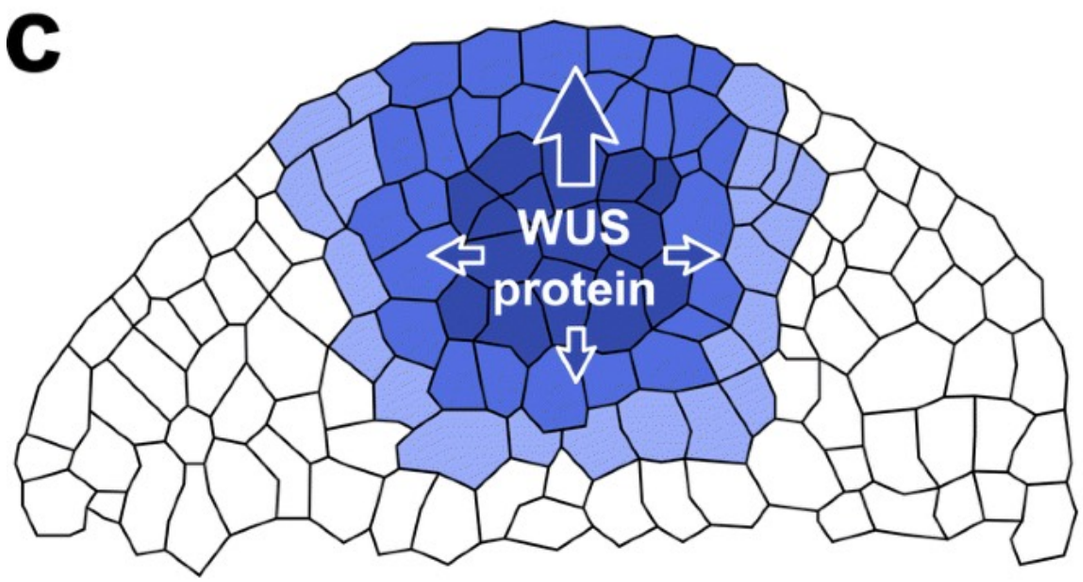


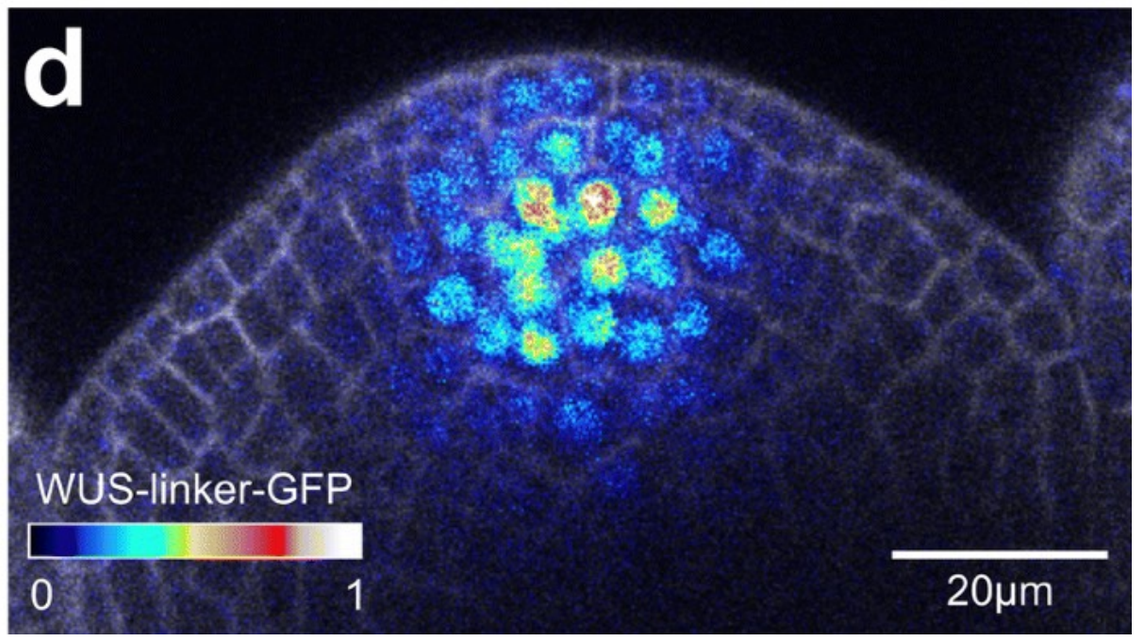
Brand et al (2000) Science 289, 617-619

The Wuschel protein directly activates the Clavata3 promoter, and is capable of moving to surrounding cells in the meristem





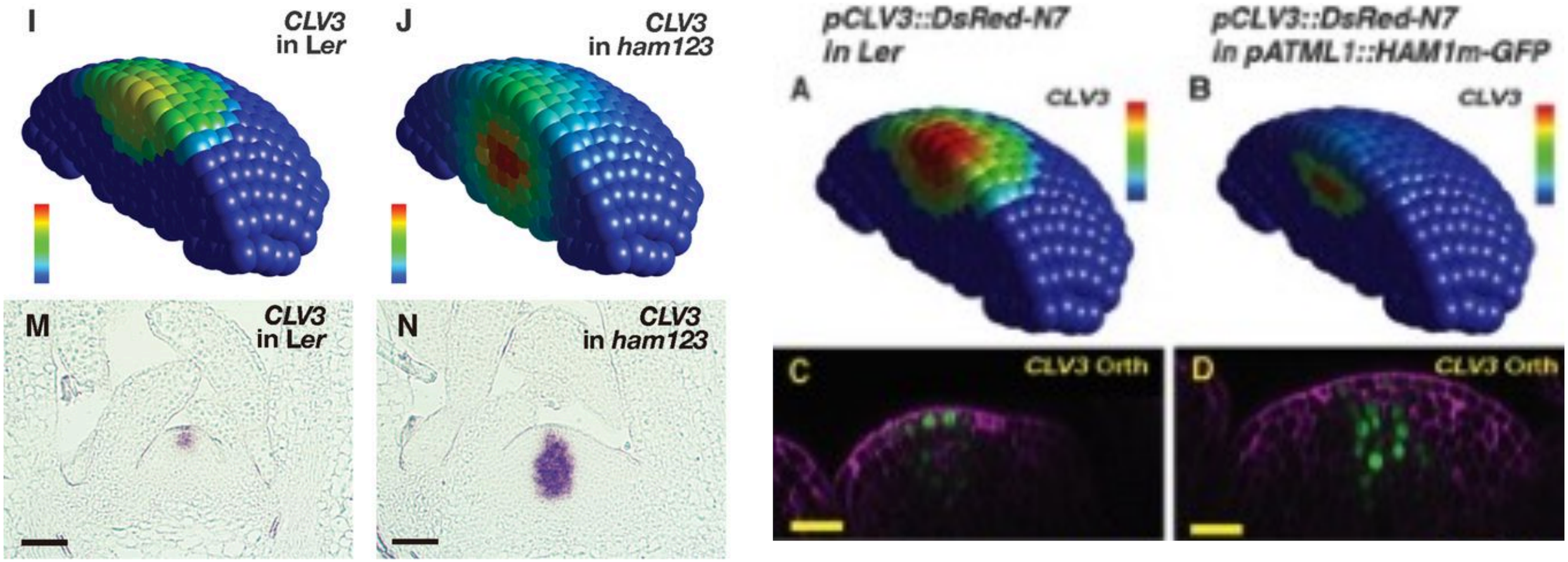




Localisation of key stem cell regulators in the SAM. **a** Schematic representation of the *CLV3* (red) and *WUS* (blue) mRNA expression domains. Note the overlap in the L3 (purple). **b** Confocal slice through the center of a *pCLV3* (red), *pWUS* (blue), *pUBQ10* (gray) triple reporter SAM. **c** Schematic representation of WUS protein localisation (intensity coded in blue). **d** Confocal slice through the center of a *pWUS::WUS-linker-GFP* rescue SAM. GFP was colour coded on a linear scale

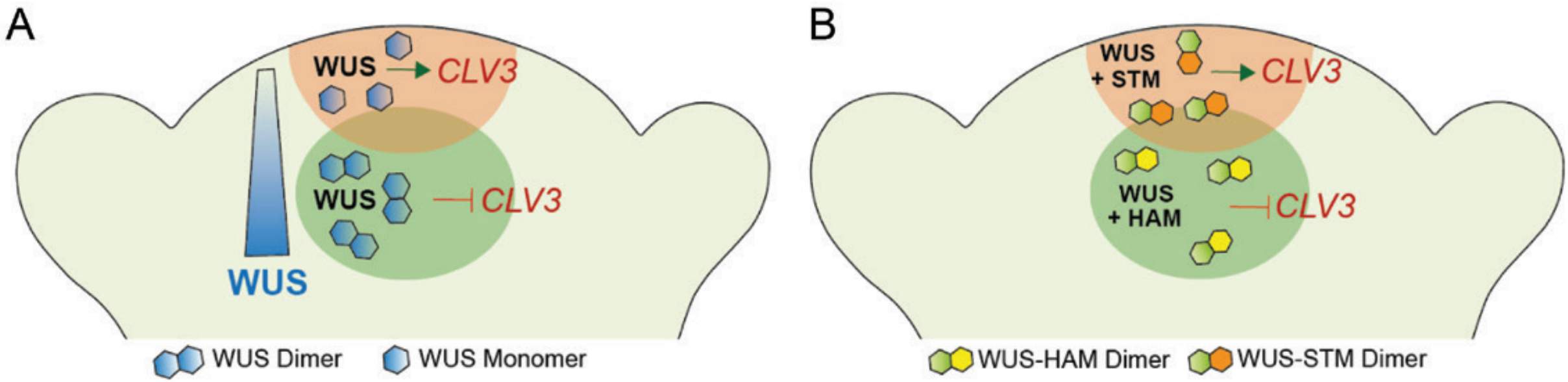
Fuchs and Lohmann, 2020

Other transcription factors like HAIRY MERISTEM (HAM) repress CLV3 expression

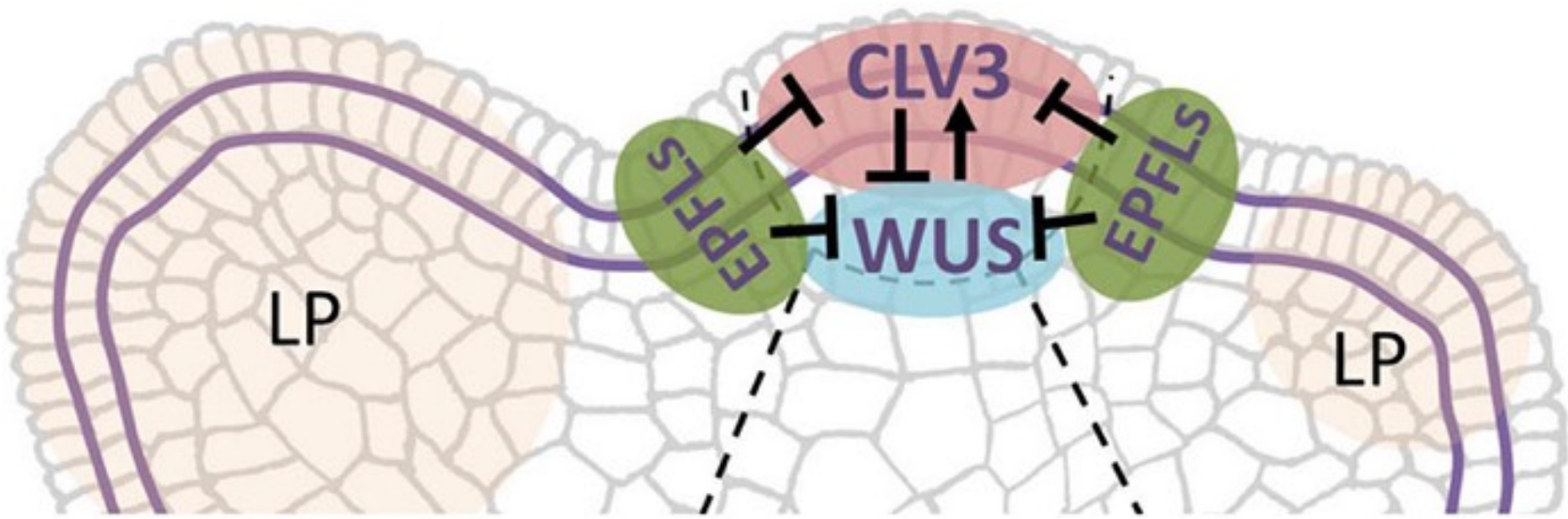


Zhou et al. (2015) Nature 517, 377; Zhou et al. (2018) Science 361, 502

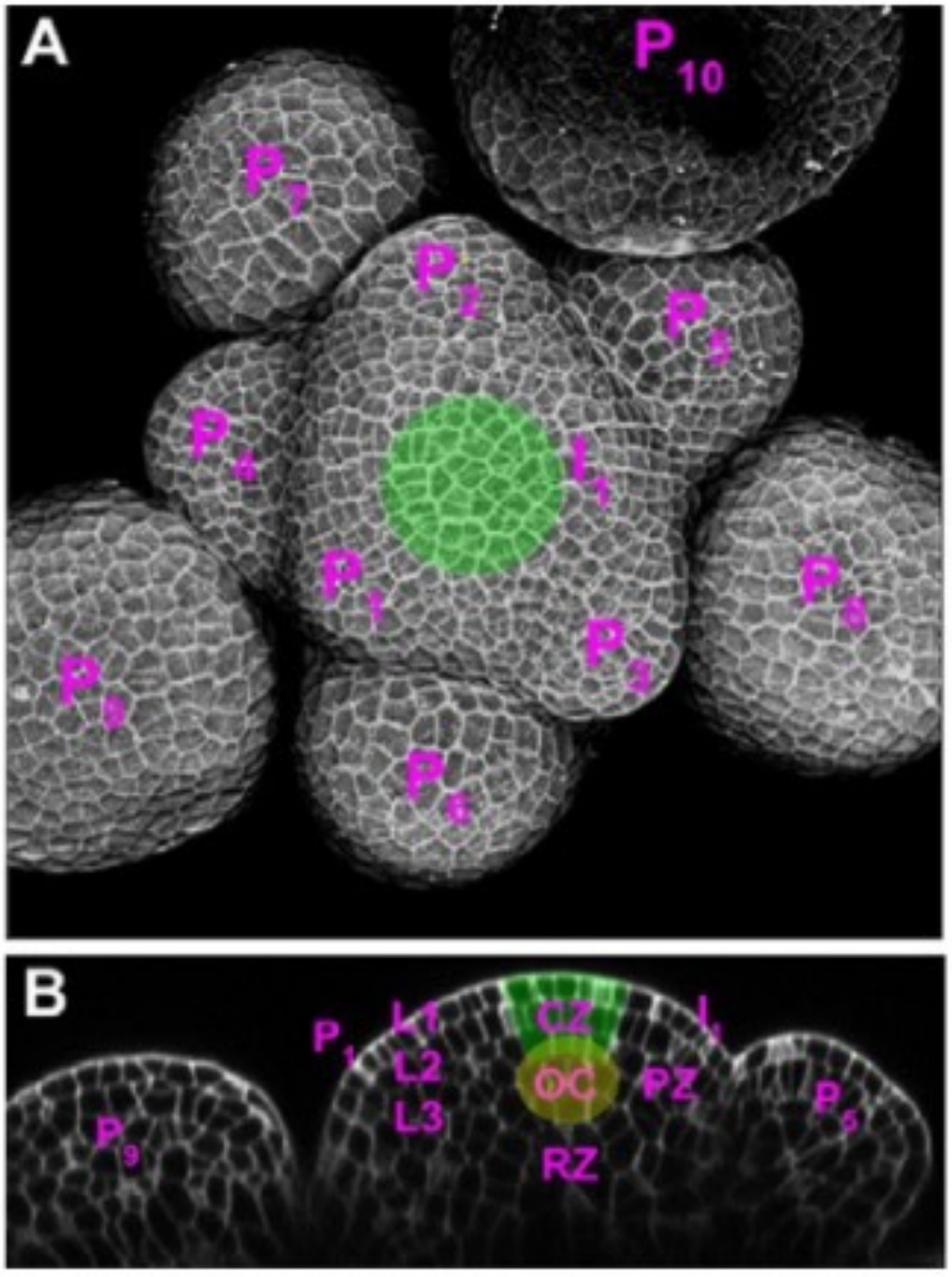
The Wus-Clv interaction domain is constrained by genetic interactions

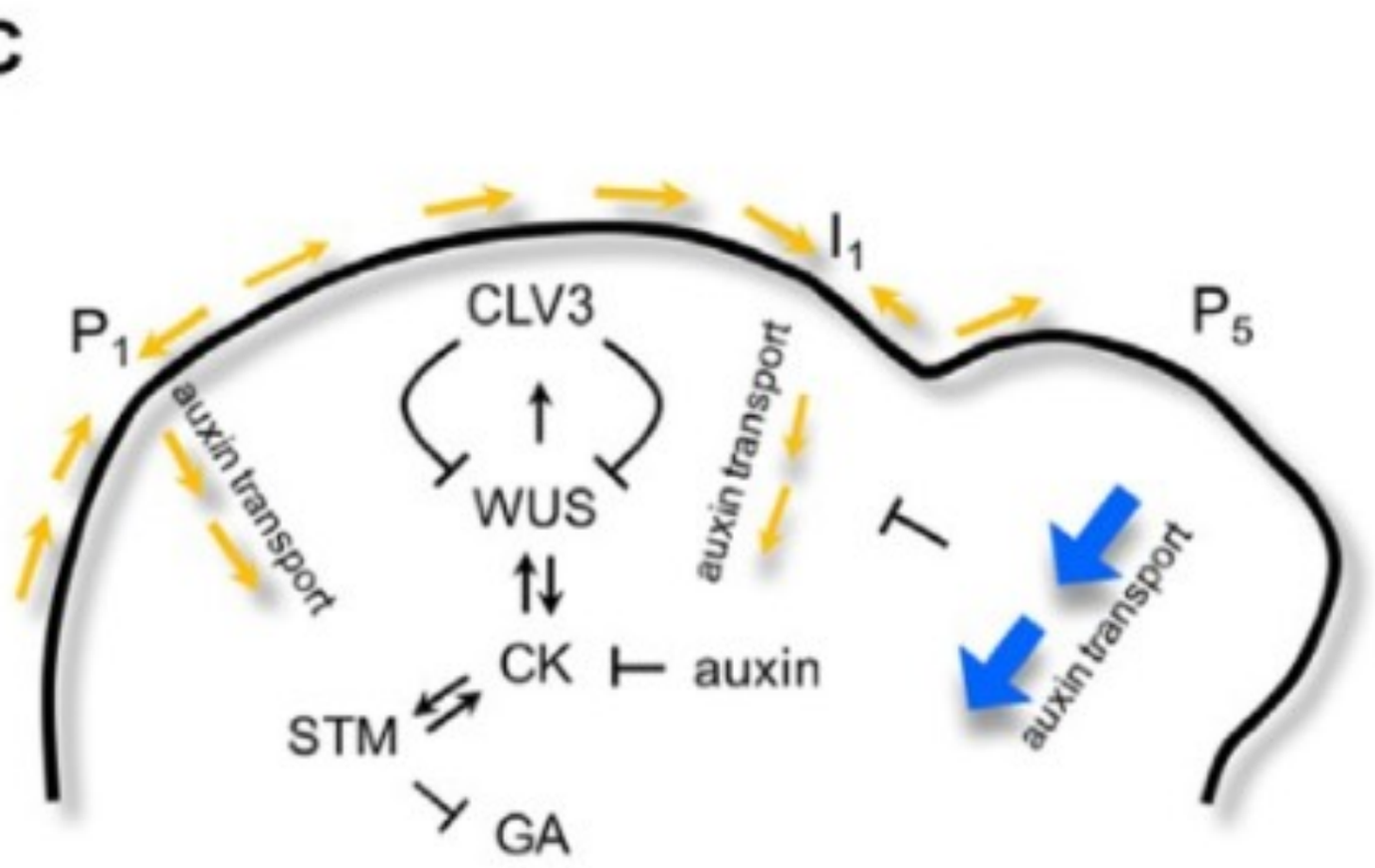


1. Regulatory activities are modulated by protein dimerisation



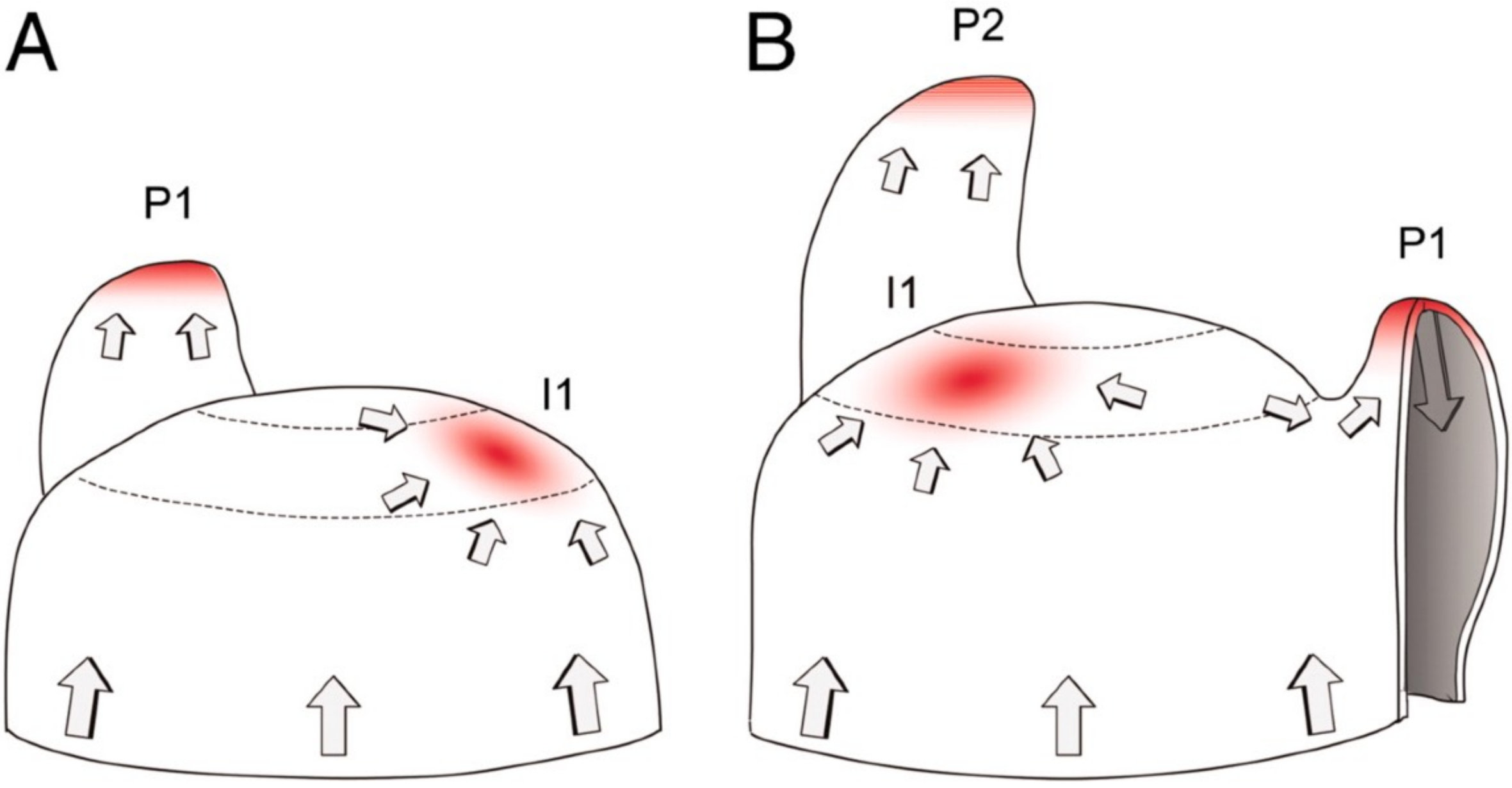
2. EPFL (Epidermal Patterning Factor-like) peptide-Erecta receptor interactions inhibit lateral spread



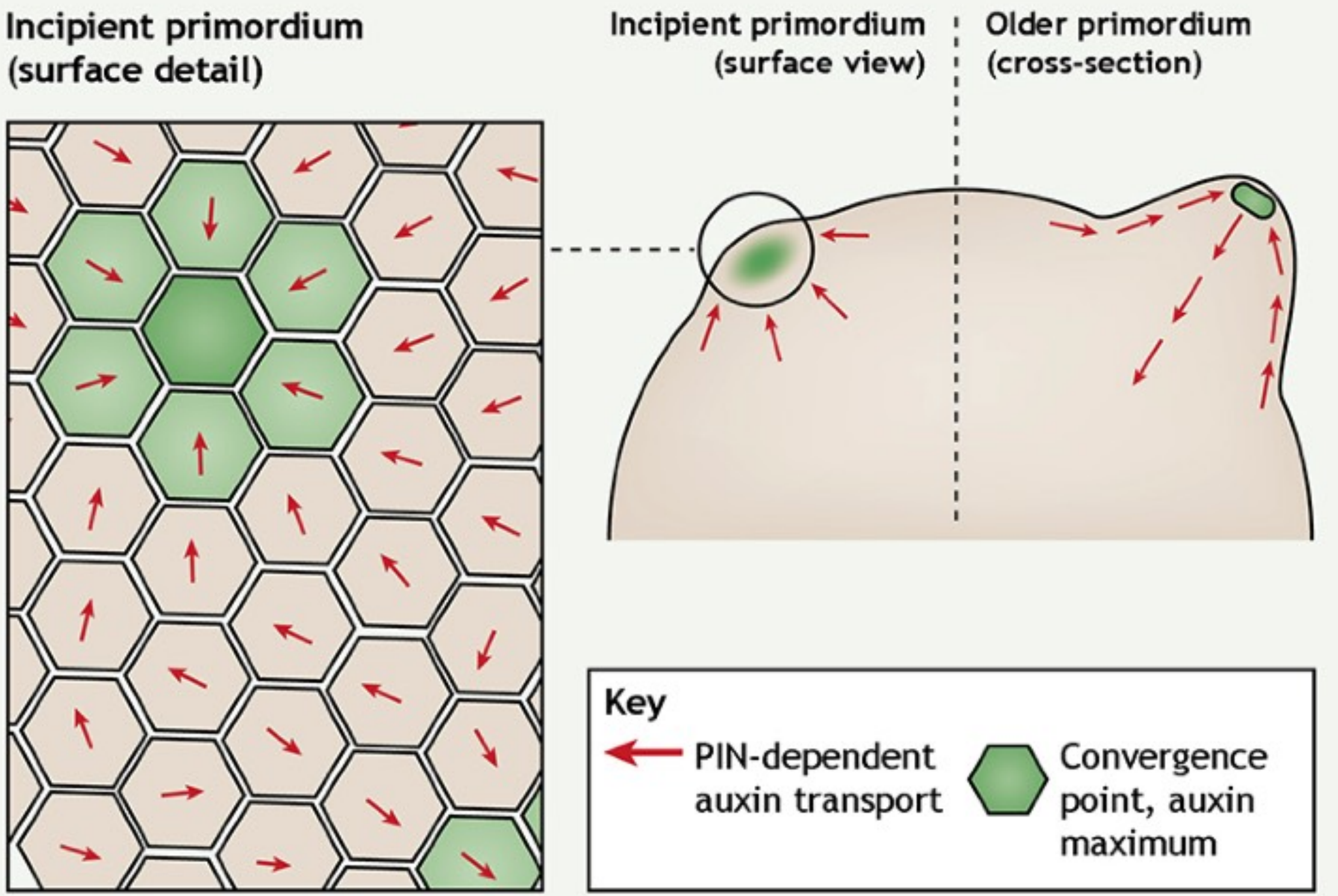


The *Clavata* and *Wuschel* genes form part of a feedback regulated circuit, controlling cell proliferation within each meristem

Wus activates a local cytokinin response in the meristem

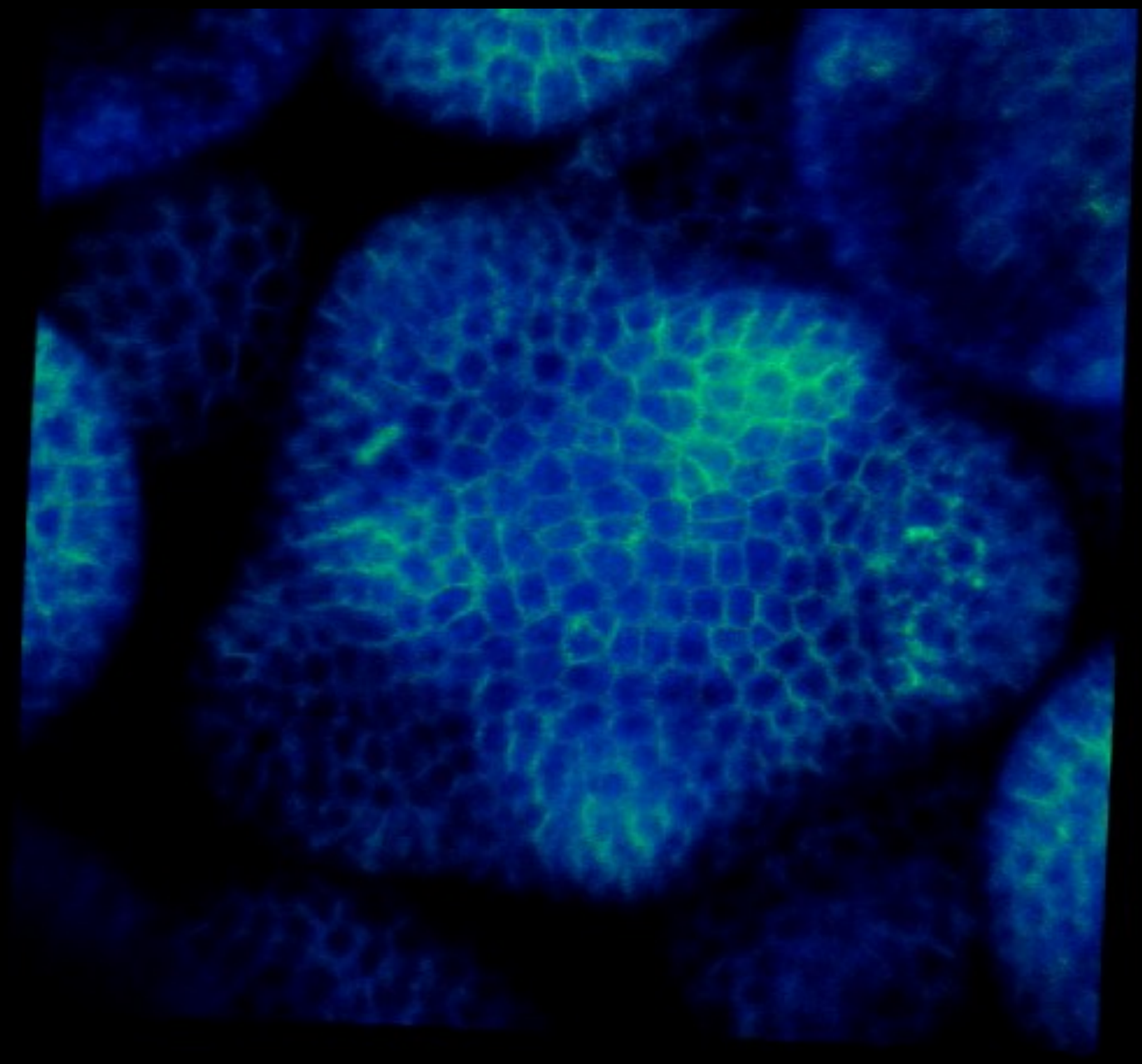


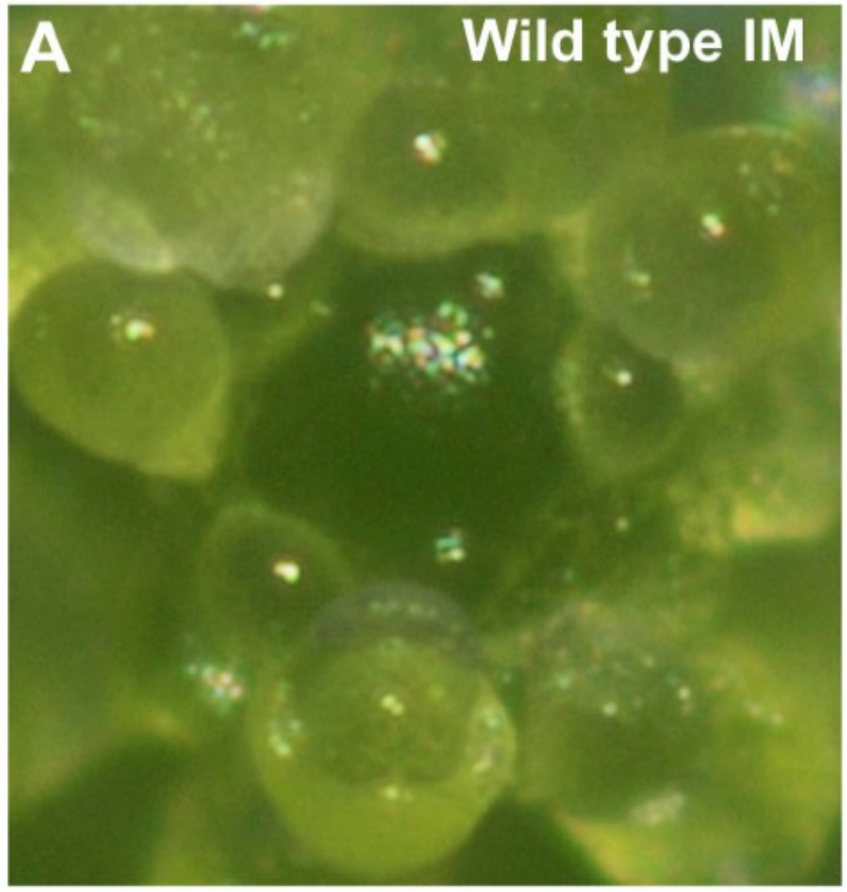
Auxin regulated feedback initiates meristem outgrowth

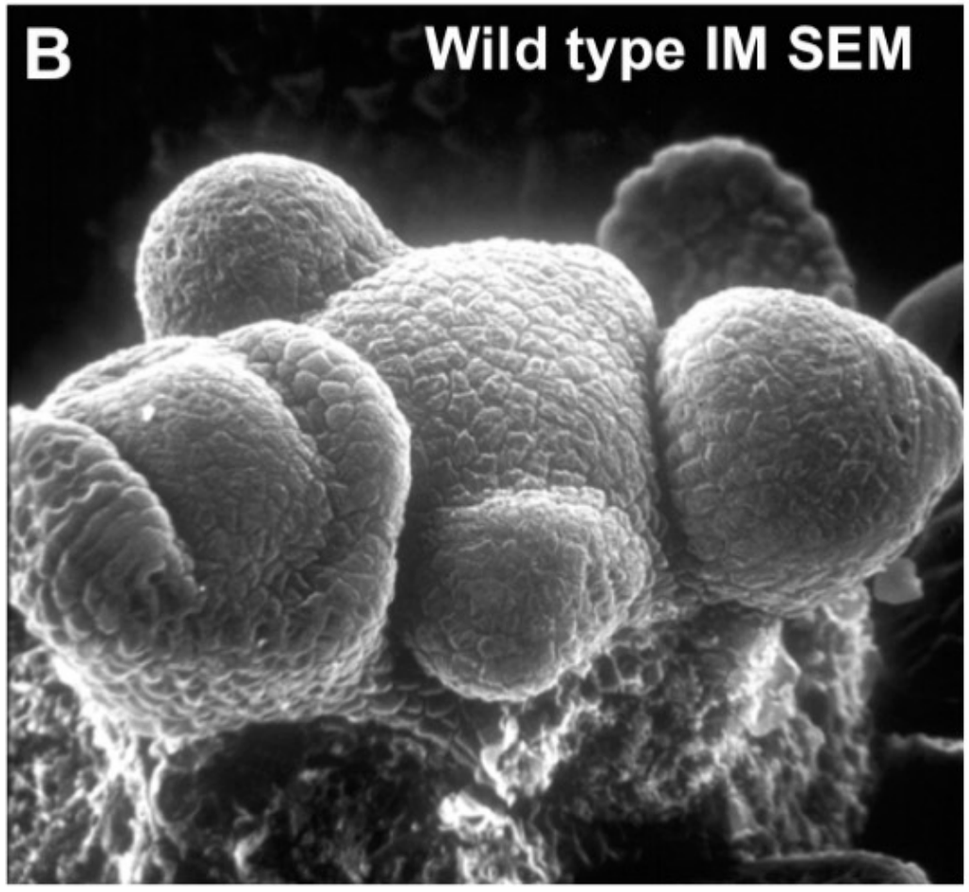


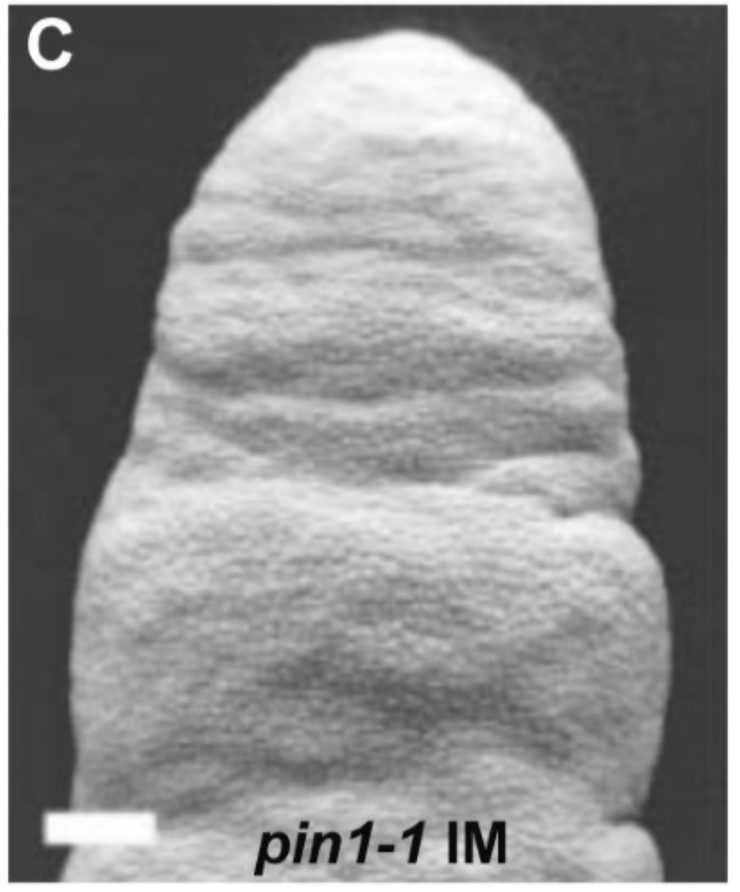
Competitive interactions between self-reinforcing flows of auxin are responsible for initiation and spacing of shoot meristem primordia

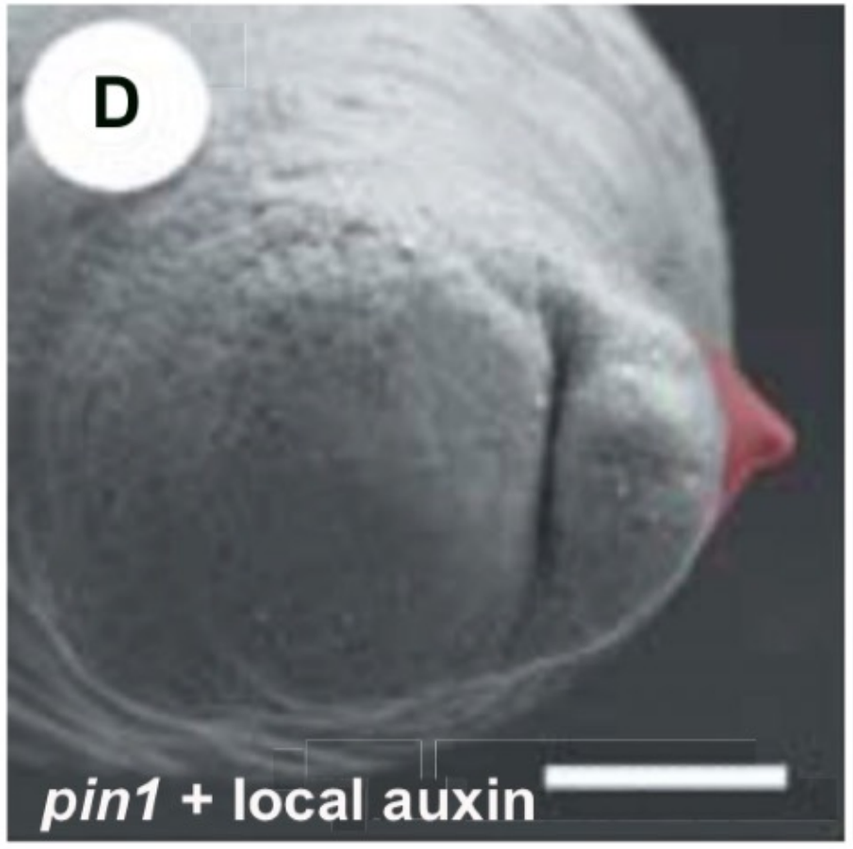
PIN1:GFP distribution in the Arabidopsis shoot apical meristem

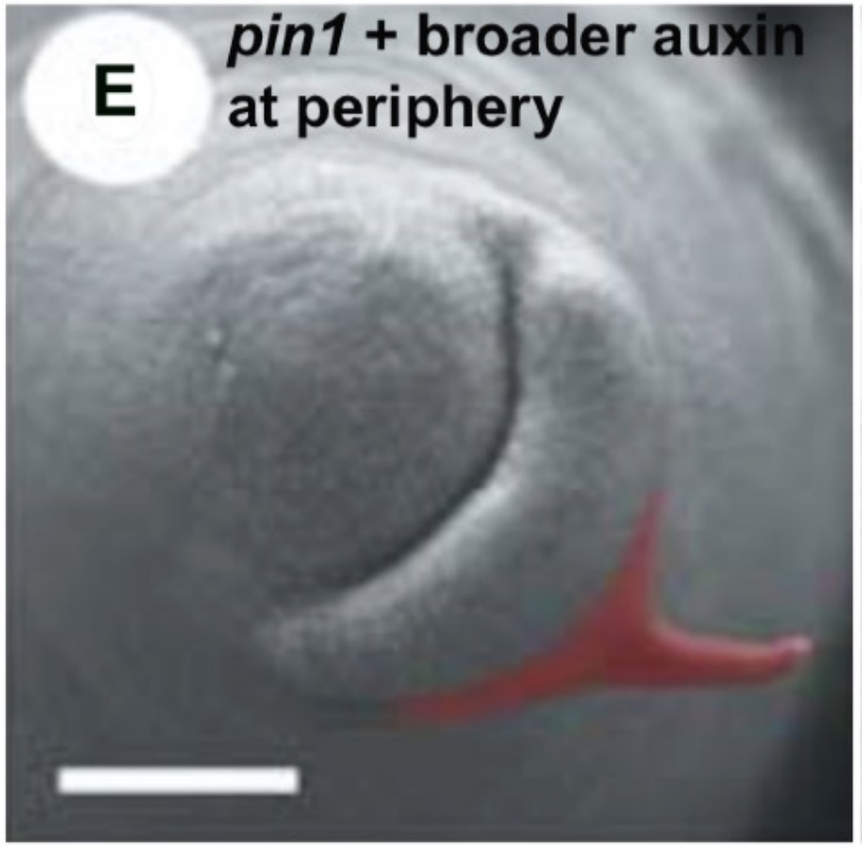


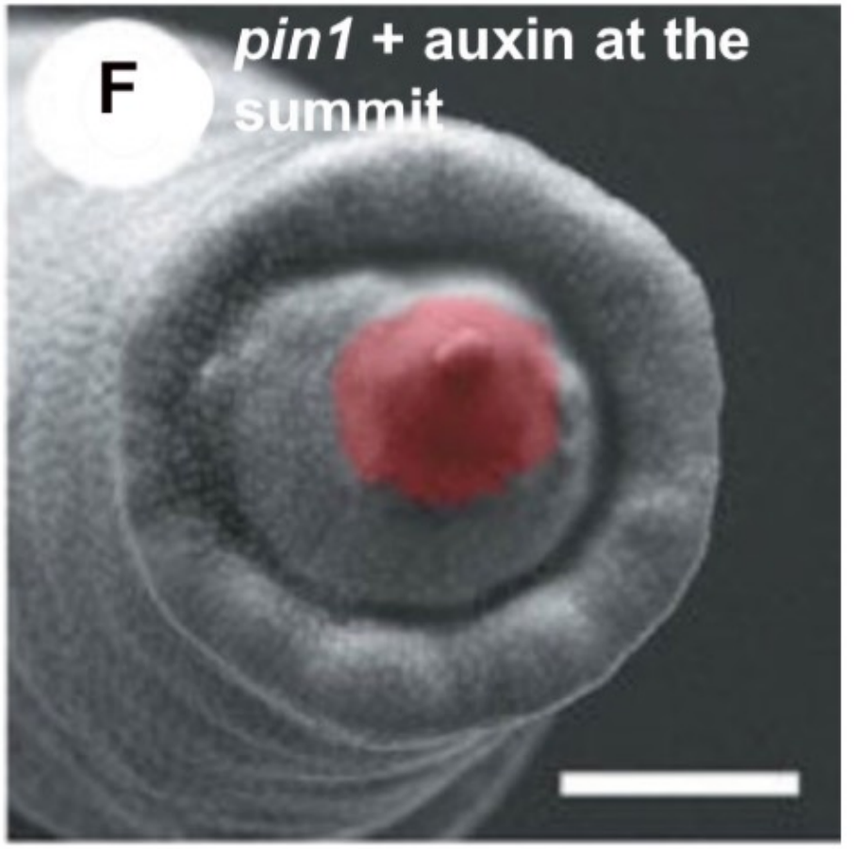












Local application of auxin induces outgrowth of primordia in *pin1* mutants

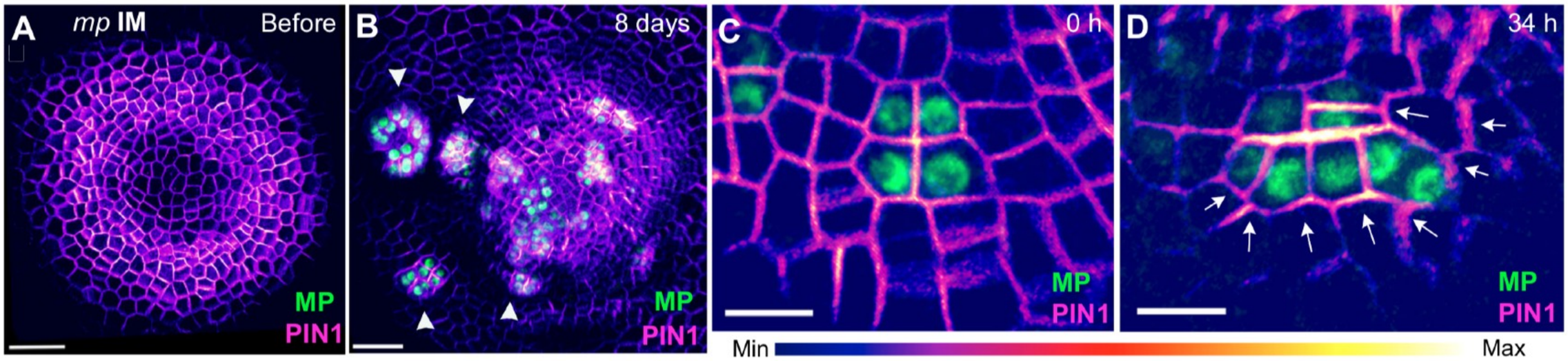


Fig. 6. MONOPTEROS (MP) orients PIN1 polarity non-cell-autonomously. (A,B) Confocal projection of *mpB4149* mutant IM before (A) and 8 days after (B) the induction of MP-YPet clones (green). PIN1 expression is in magenta. Arrowheads indicate organs initiating from cells expressing MP clones. (C) Magnified view of an *mpB4149* mutant apex containing a 4-cell MP-YPet clone (green), showing PIN1-GFP polarity and expression (magenta) 2 days after induction (i.e. when the clone was first visible). (D) Magnified view of the MP-YPet clone shown in C 36 h later, showing an increase in PIN1 expression within the clone and polarization of PIN1-GFP (magenta) in neighboring cells directed towards the clone (arrows). Scale bars: 30 µm A,B; 10 µm in C,D. Adapted from Bhatia et al. (2016).

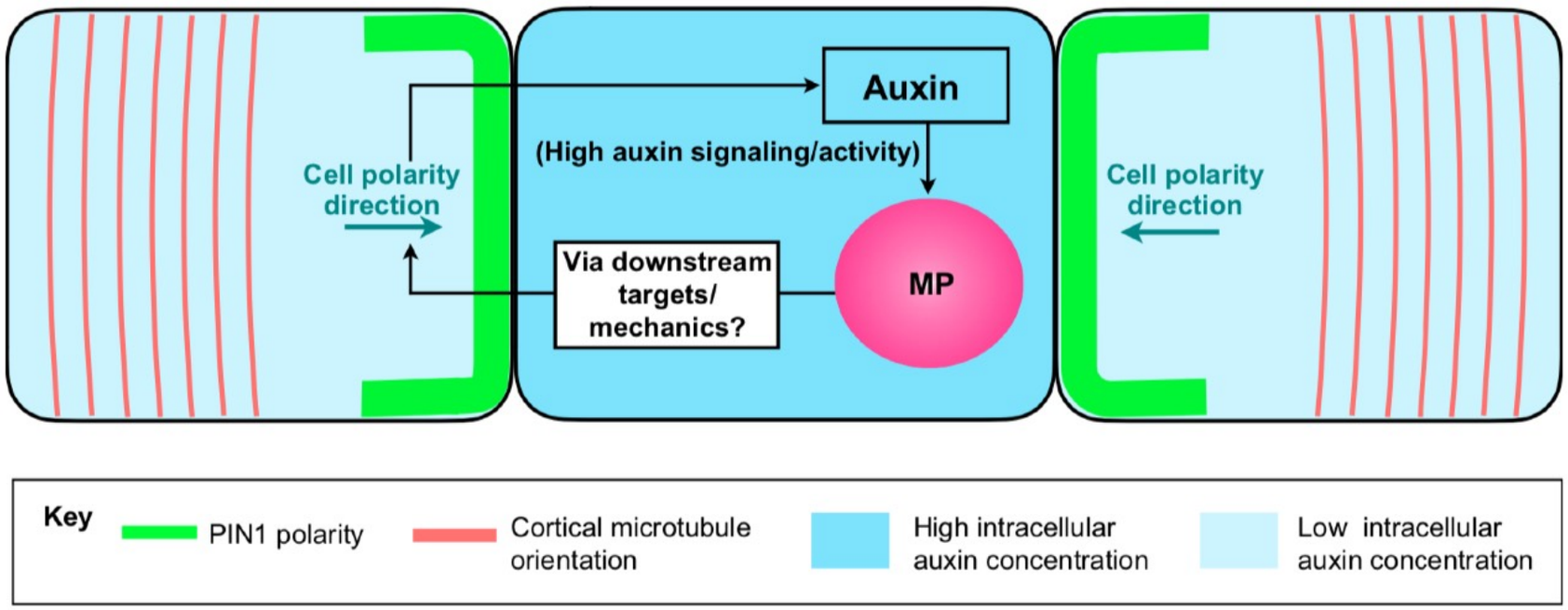
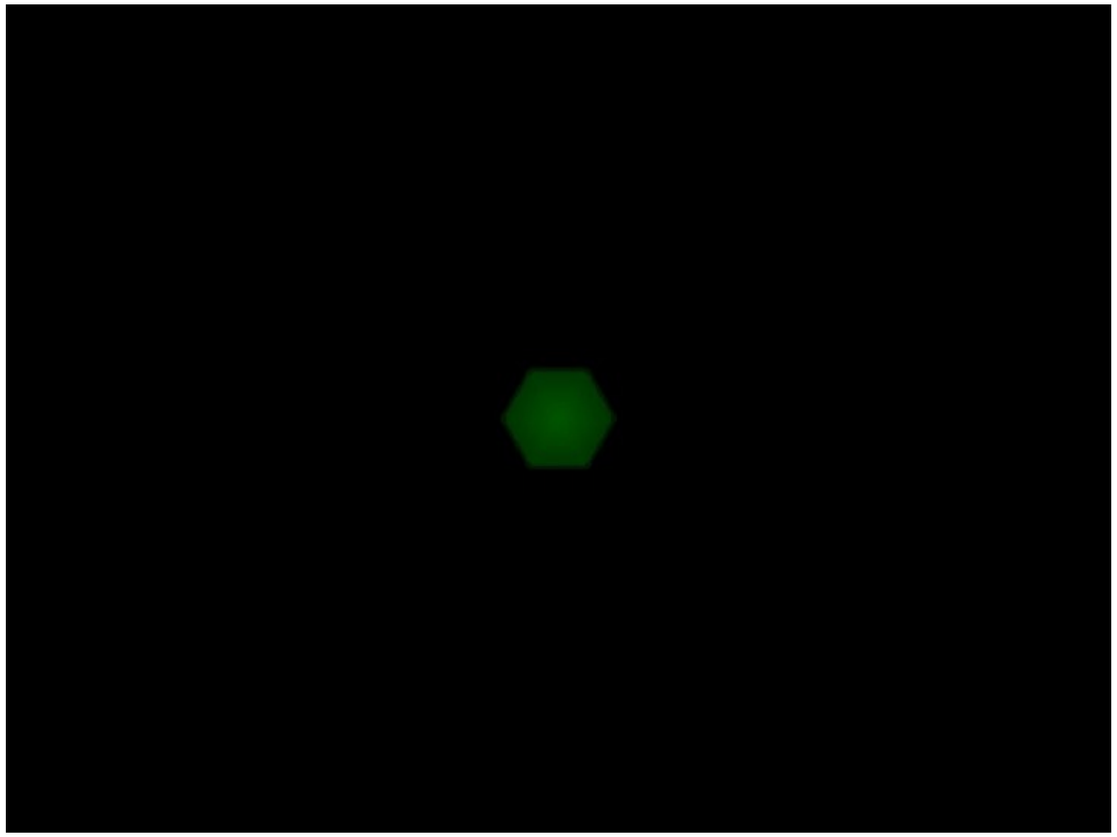
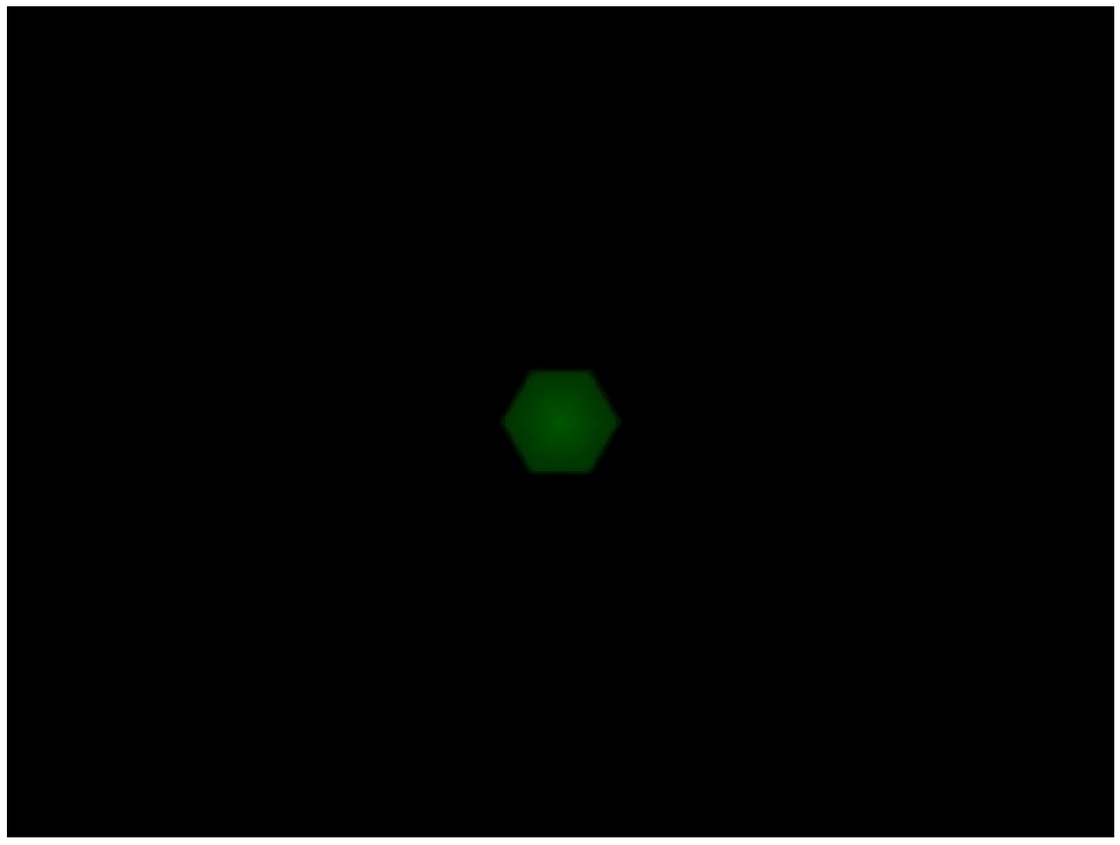
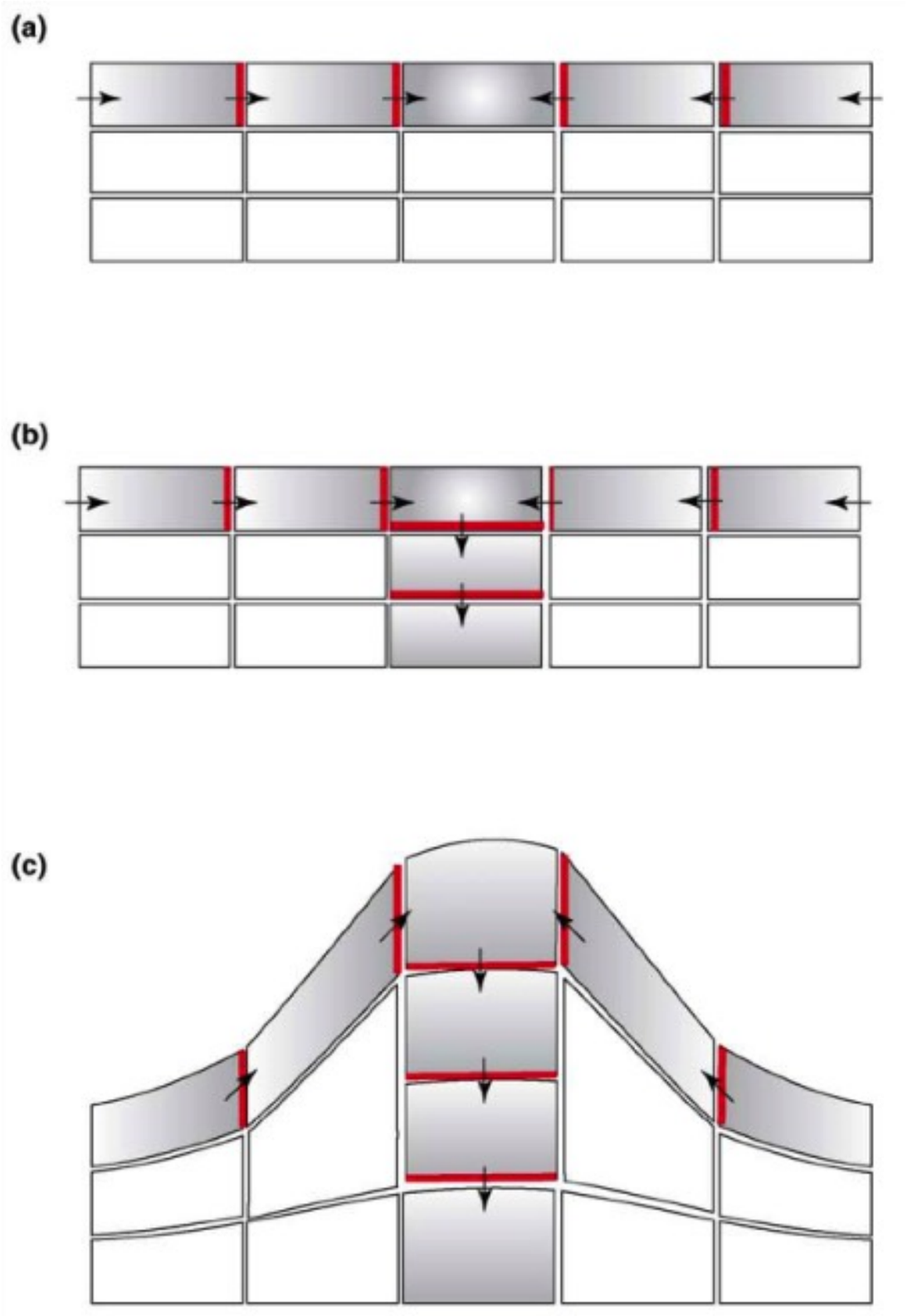


Fig. 8. A feedback loop at the cell-cell communication level generates cell polarity patterns at the tissue level. A positive-feedback loop between auxin abundance and its transport orients PIN1 polarity and microtubule orientations in the neighboring cells non-cell-autonomously, towards the cell with high auxin. This loop acts via a localized auxin transcriptional response mediated by MP activity. This substantiates the proposed up-the-gradient model in organizing complex cell polarity patterns underlying visible phyllotactic patterns. How MP acts to mediate such a response is yet to be discovered; it might act through its downstream targets or via altering cellular mechanics but these hypotheses need to be tested *in vivo*. See Bhatia et al. (2016).

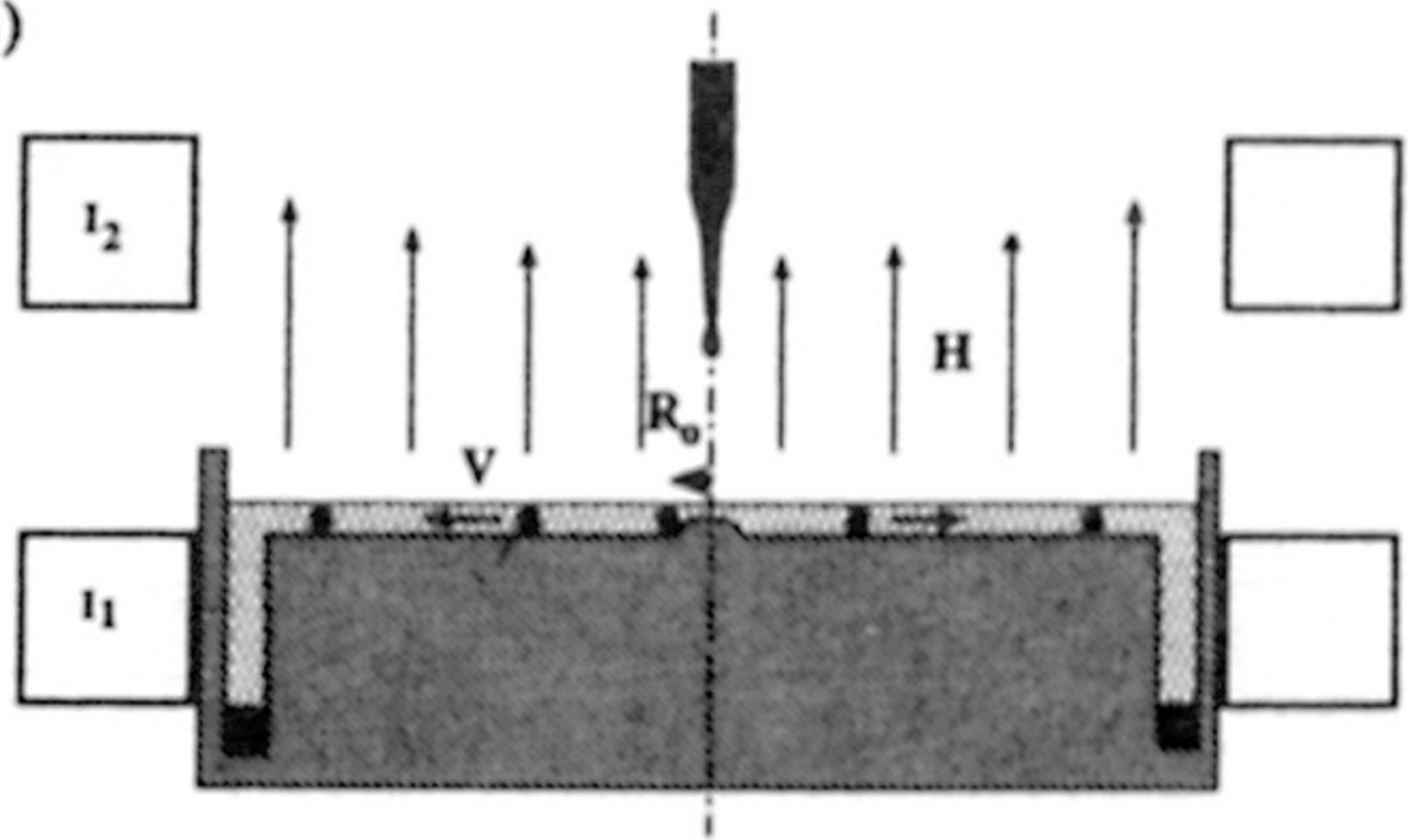
Primordial outgrowths in the meristem are triggered by local influxes of auxin

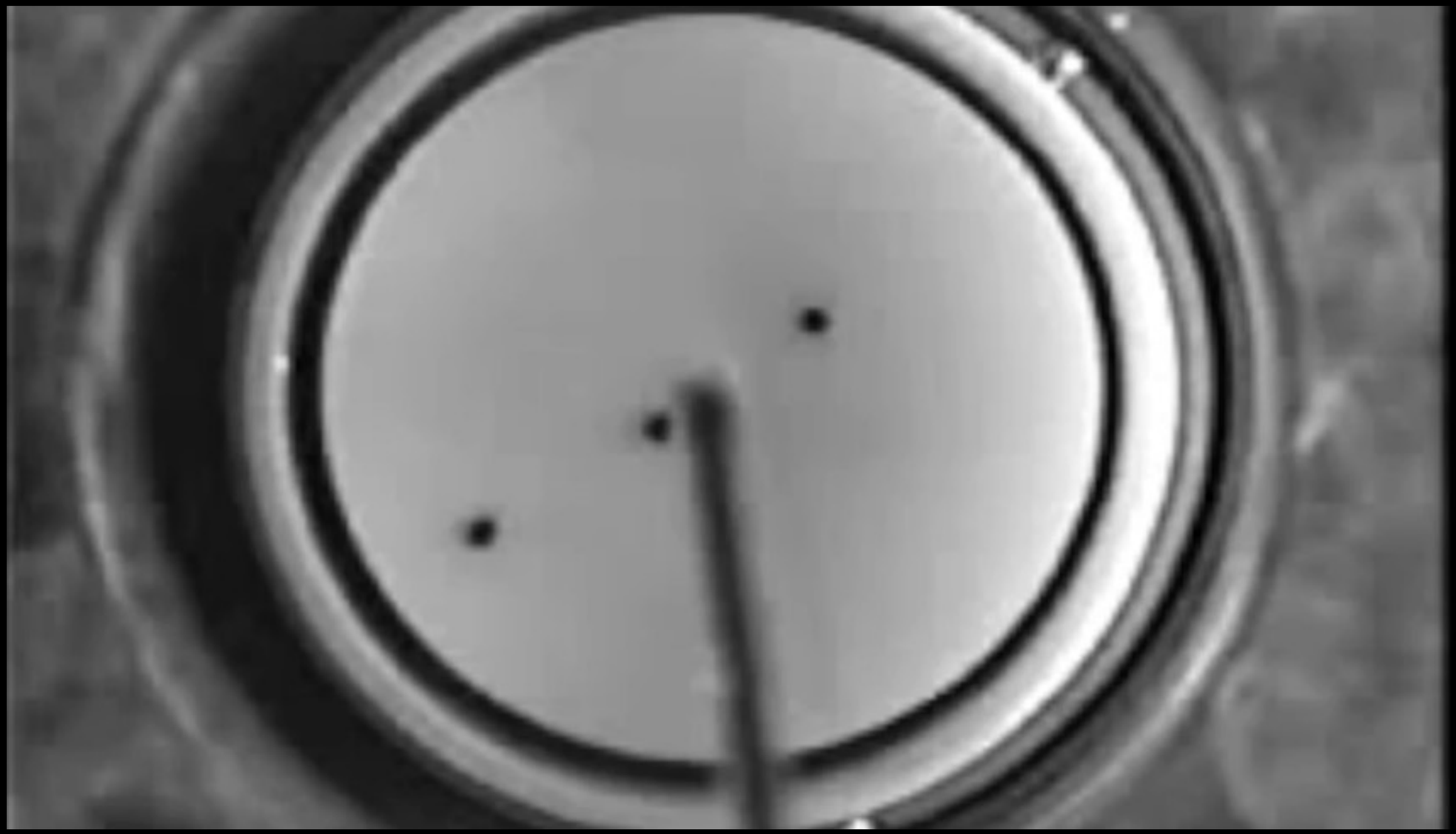


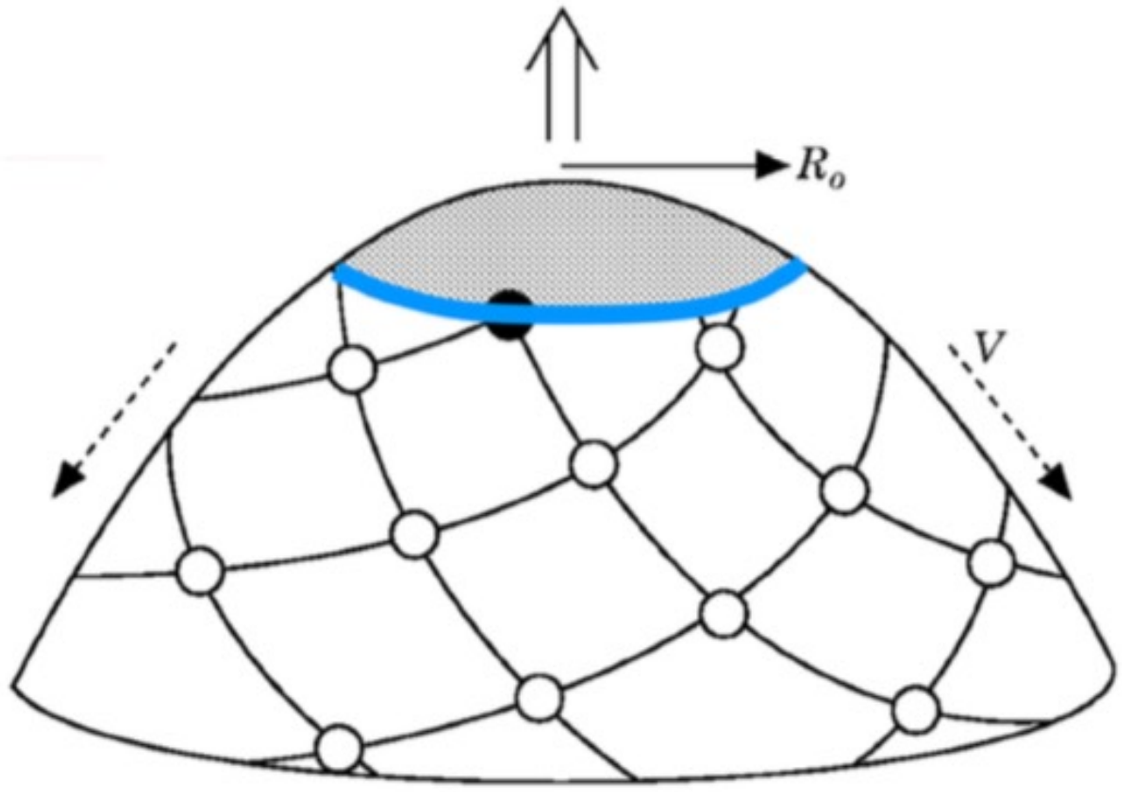




(b)







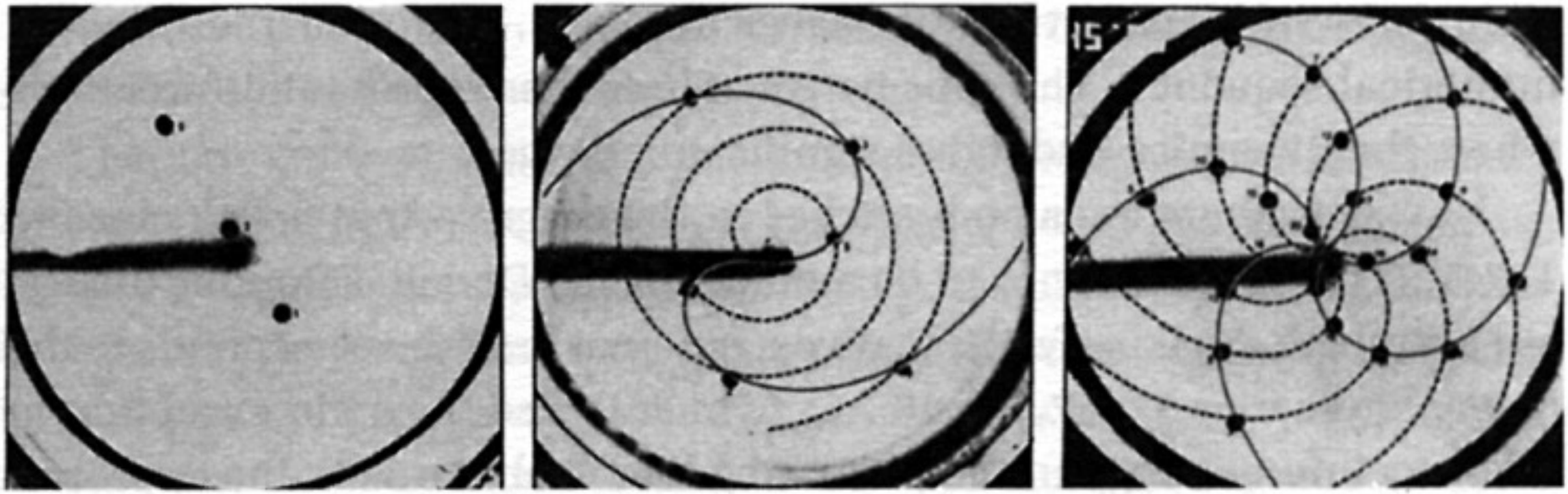


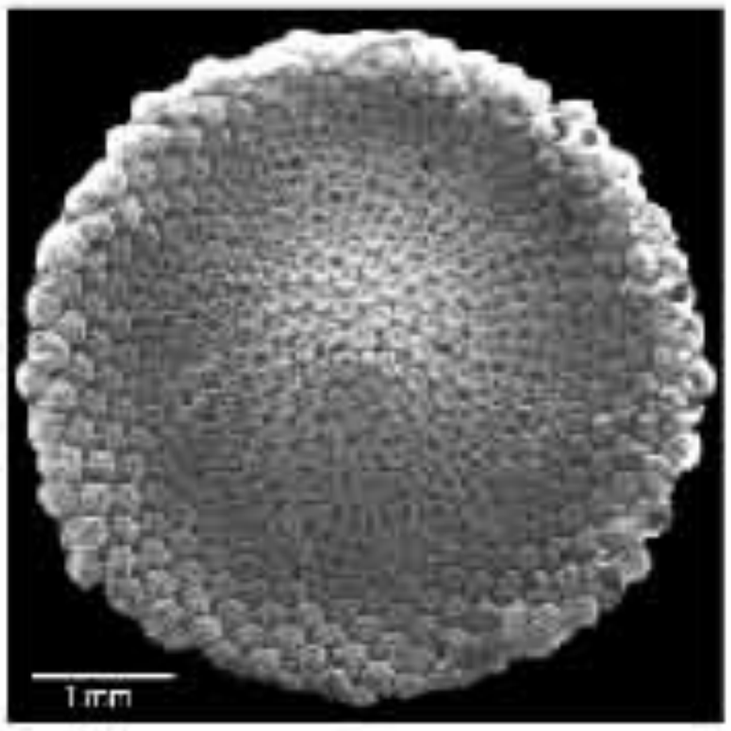
Figure 50
Fibonacci spirals observed in an experiment with electrically charged oil drops.

Emergence of patterns at microscopic scales

Artichoke

Sunflower

Magnolia

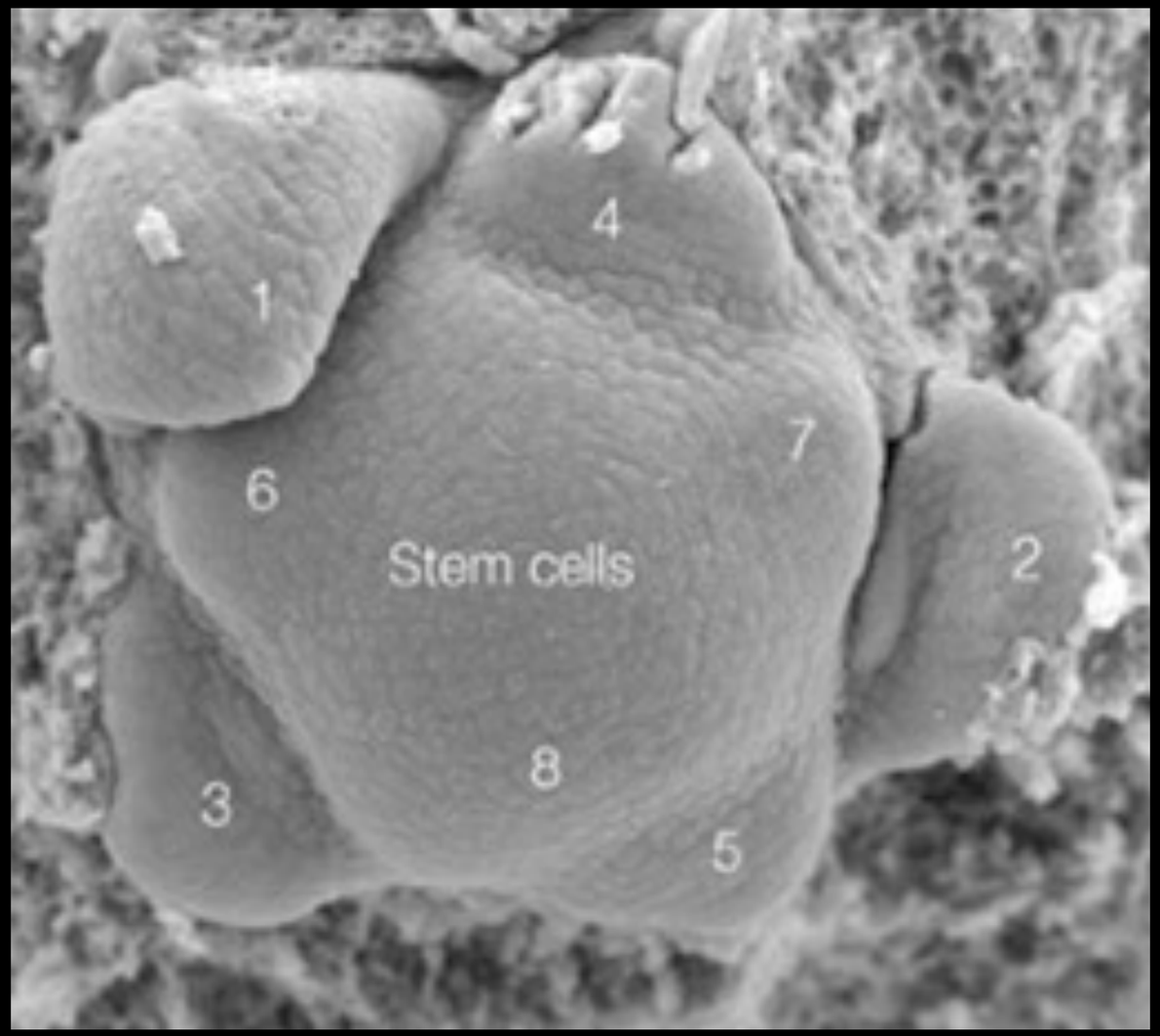


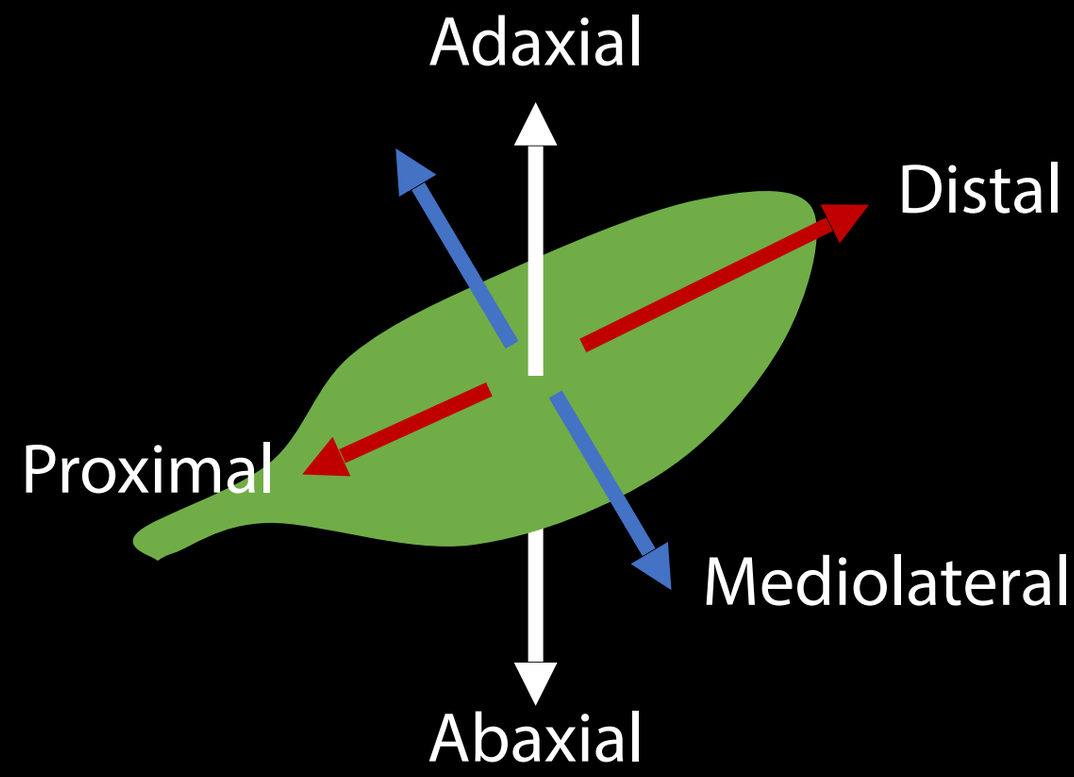
Plant organs and the Fibonacci series:

1 petal: Lily
2 petals: Euphorbia
3 petals: Iris
4 petals: Arabidopsis, Fuchsia (decussate arrangement, not spiral)
5 petals: Buttercup, wild rose, Larkspur, columbine (Aquilegia), pinks
8 petals: Delphinium
13 petals: Ragwort, corn marigold, cineraria, some daisies
21 petals: Aster, black-eyed susan, chicory
34 petals: Plantain, Pyrethrum
55, 89 petals: Michaelmas daisies, the Asteraceae family

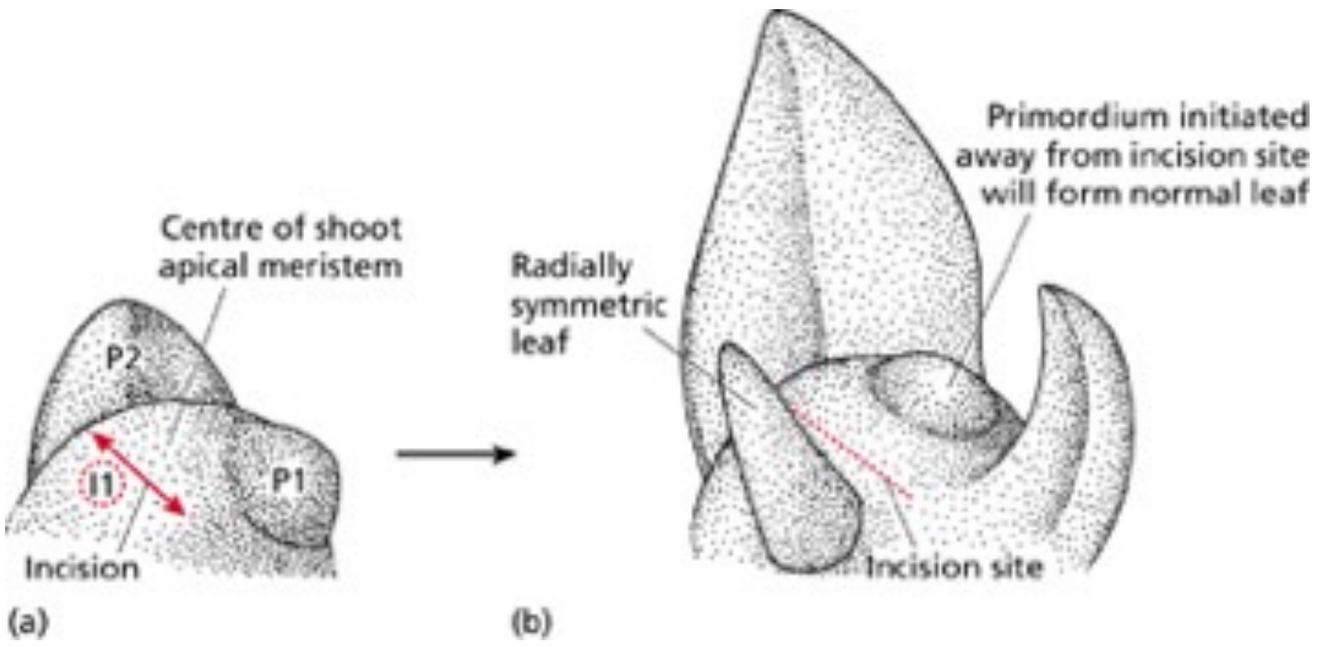
(With some variation in numbers due to noise)

Specification of leaf axes





Emerging leaf primordia:
signals from the apex determine adaxial-abaxial polarity



WT *phantastica*

Waites and Hudson (1994) Development 121:2143-2154

Feedback-regulated auxin traffic and responses play a key role in coordination of whole plant growth

1. Plant body plans are flexible, and are built step-wise through a series of local interactions.
2. Auxin is a mobile informational molecule and its directed traffic plays an important role in establishing key landmarks during cellular development in plants.
3. Auxin triggers specific genetic responses in cells via Aux/IAA and ARF pathways.
4. Genetic responses can trigger coordinated behaviour in adjacent cells, creating feedback systems e.g. PIN1-MP, WUS-CLV3 across meristems
5. Competition for auxin can cause lateral inhibition and result in spatial patterning in responsive tissues, e.g. phyllotactic patterning.
6. PIN-mediated transport of auxin results in coupling of cells, and formation of long-distance interactions that can regulate the balance of growth across tissues or the entire plant.