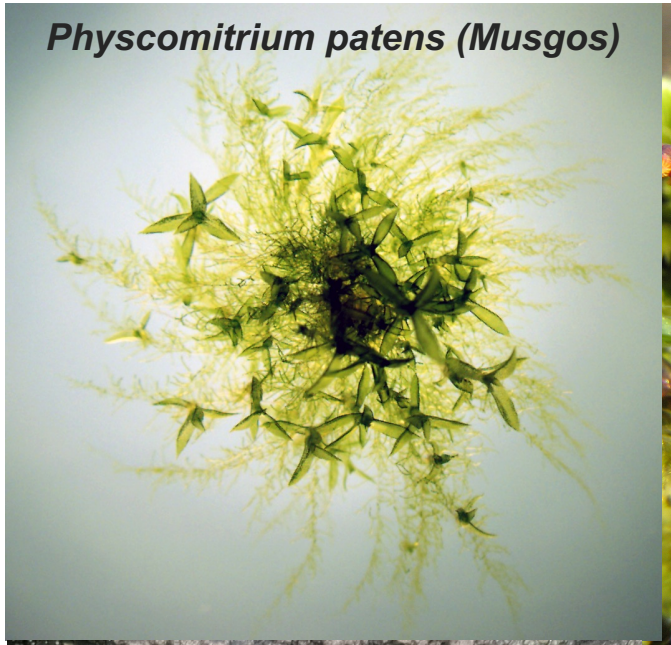
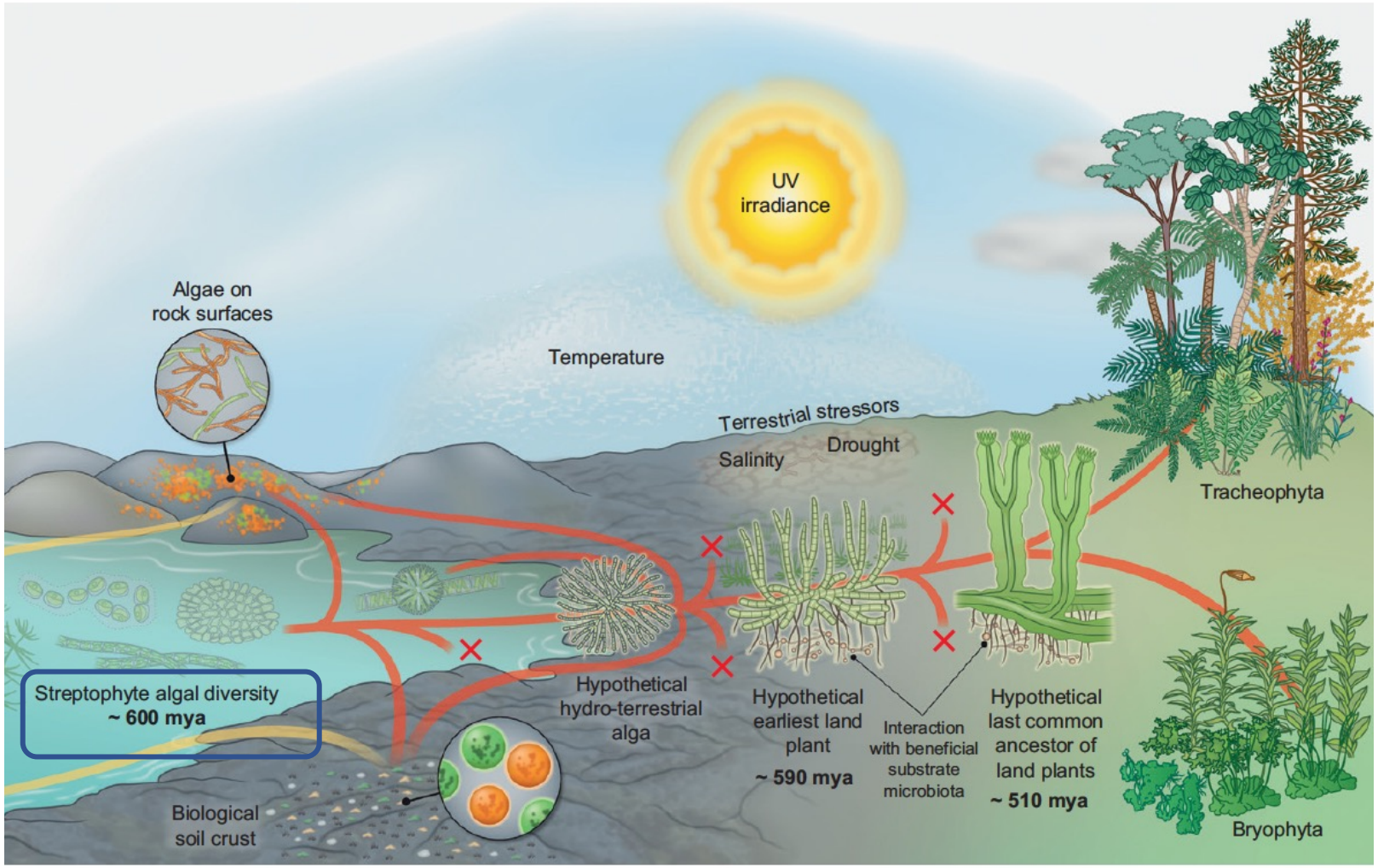


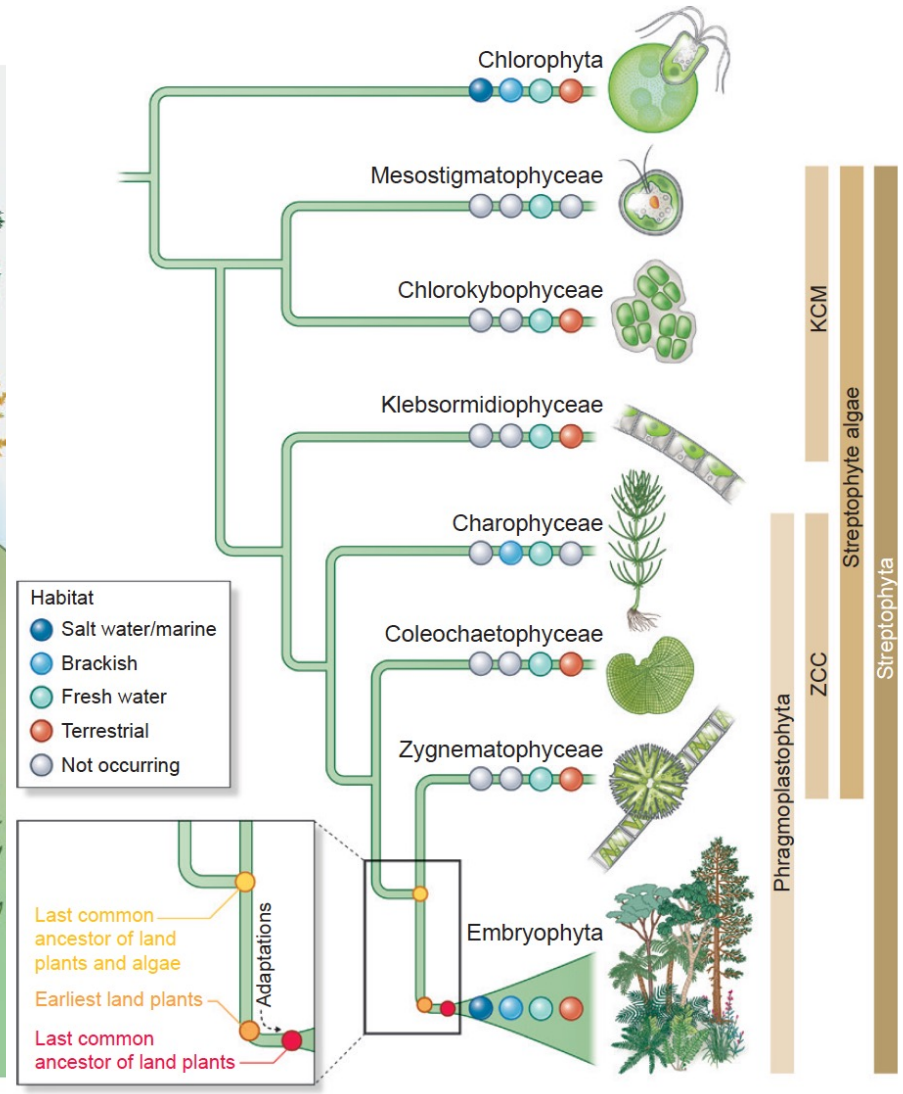
LAS BRIÓFITAS como MODELOS de crecimiento y desarrollo en la evolución vegetal

Laura Saavedra
laura.saavedra@conicet.gov.ar
BMV 2024

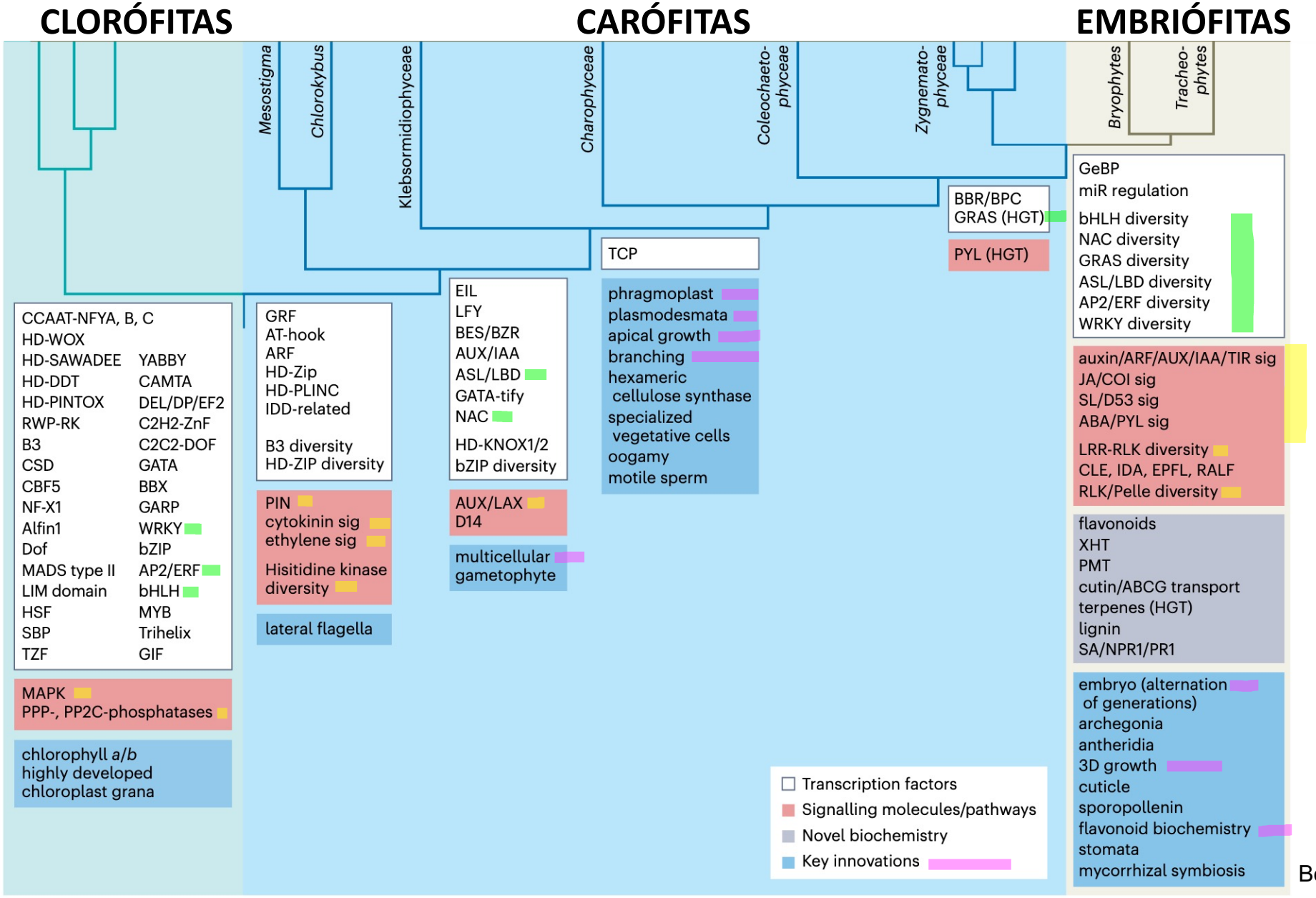
CONICET INTA UNC
U D E A

Escenario evolutivo de la conquista terrestre de las embriófitas





Ensamblado progresivo del conjunto genético de Embriófitas



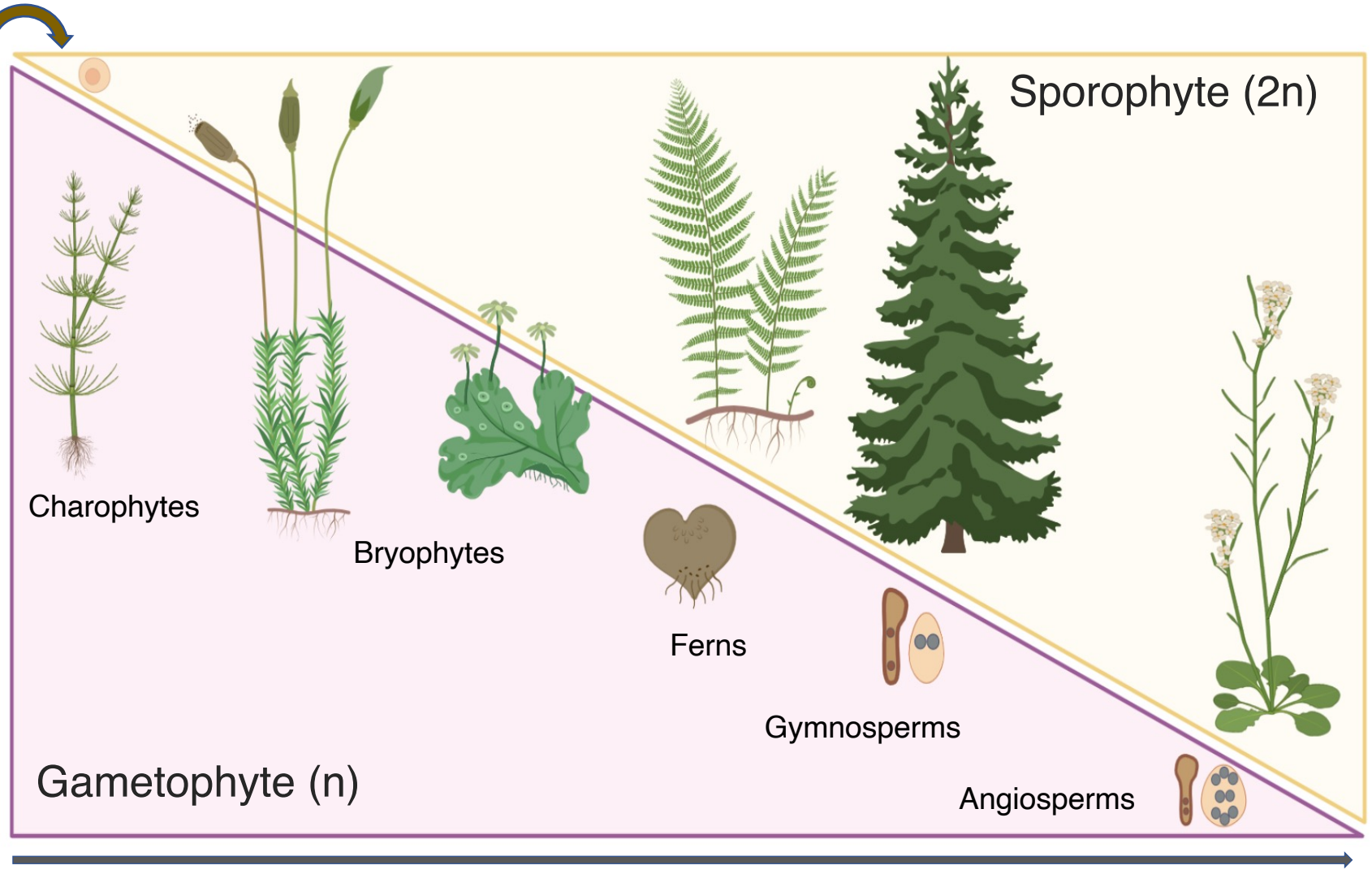
Bowman.J, ARPB (2022)

Evolución de la alternancia de generaciones

Cigoto: célula única

Algas verdes y carofitas:
HAPLONTES

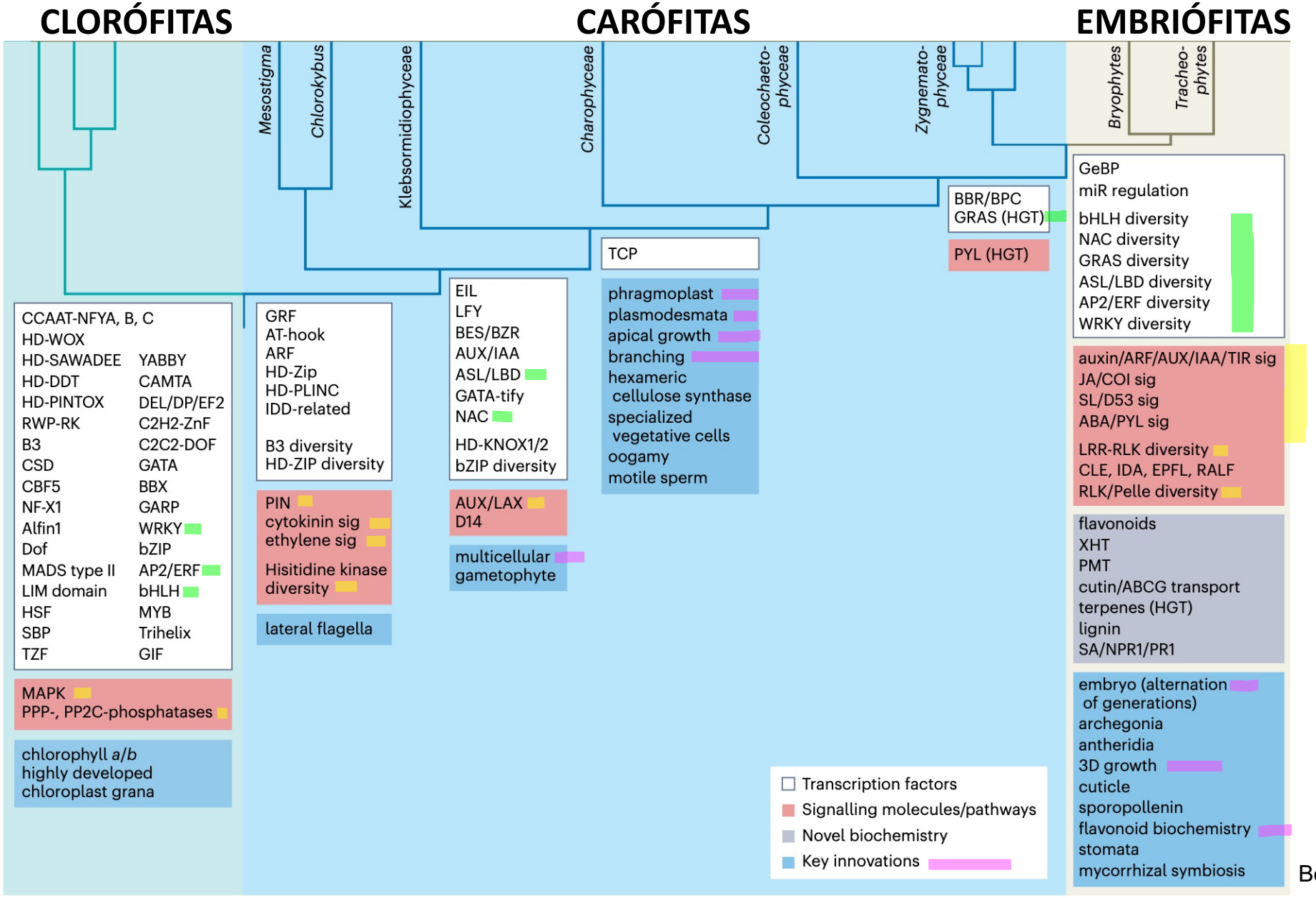
Embriófitas**:**
HAPLO-DIPLONTES



Reducción tamaño Gametofito (n)
Aumento tamaño Esporofito (2n)

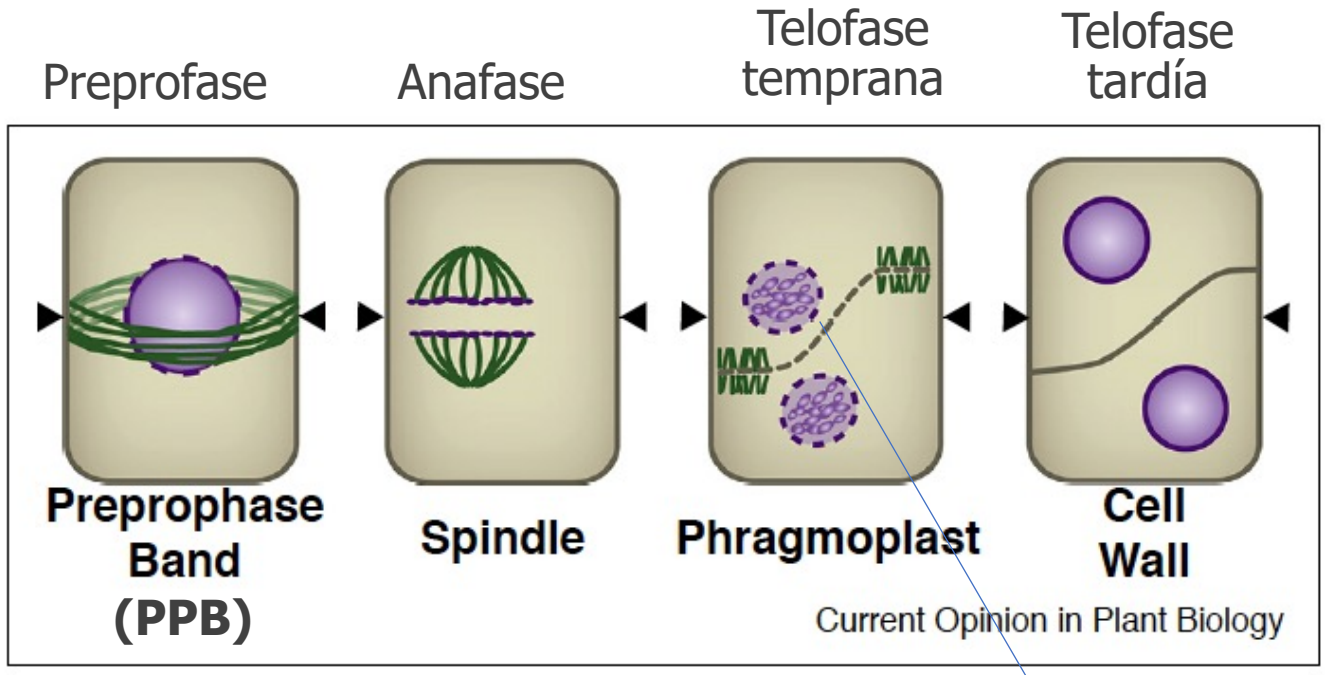
Complejidad multicelular

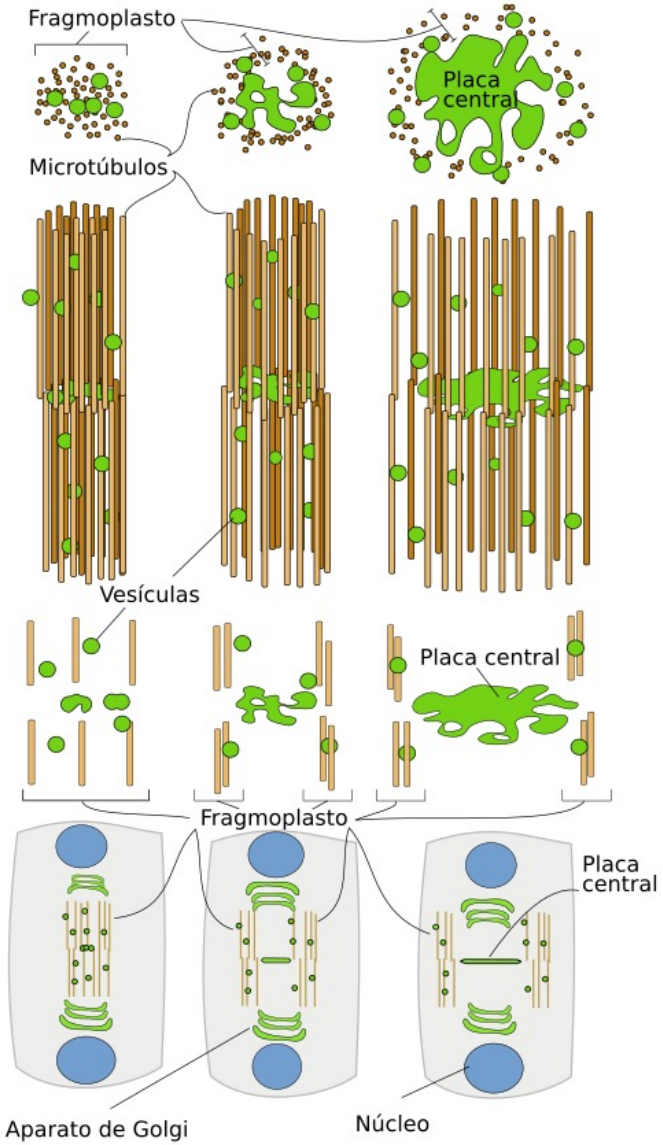
Ensamblado progresivo del conjunto genético de Embriófitas



Bowman.J, ARPB (2022)

División celular: PPB y Fragmoplasto surgen en algas CCZ

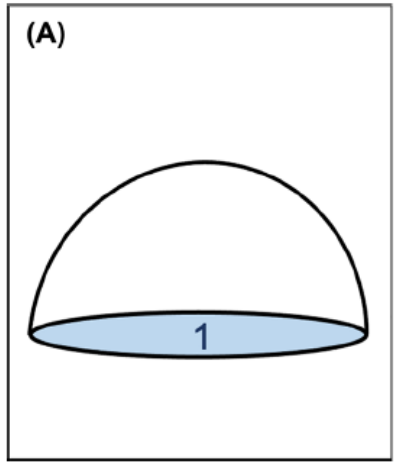


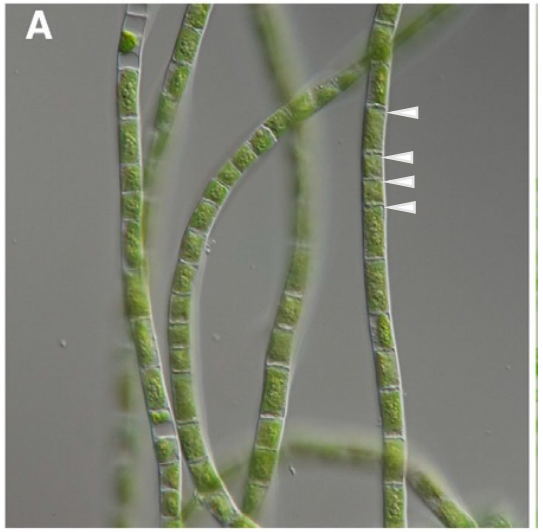


- **PPB:** Establece Plano/orientación de división celular
- **Fragmoplasto:** Andamio para placa celular en sitio definido por PPB

Colonización de la superficie terrestre: transición 2D-3D

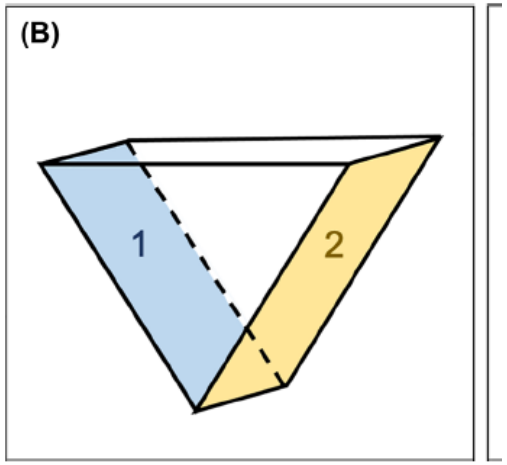
1 plano





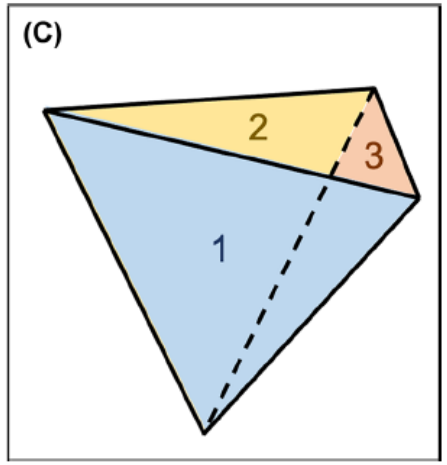
ALGAS

2 planos

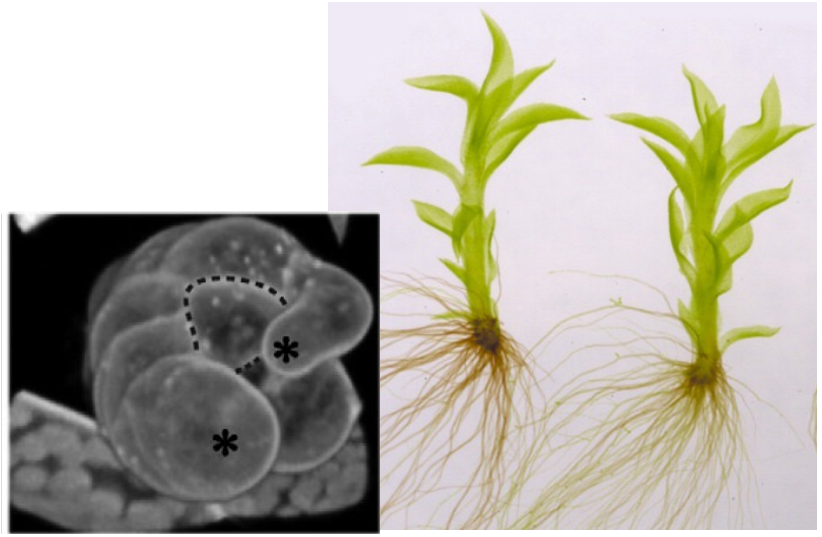


ALGAS

3 planos

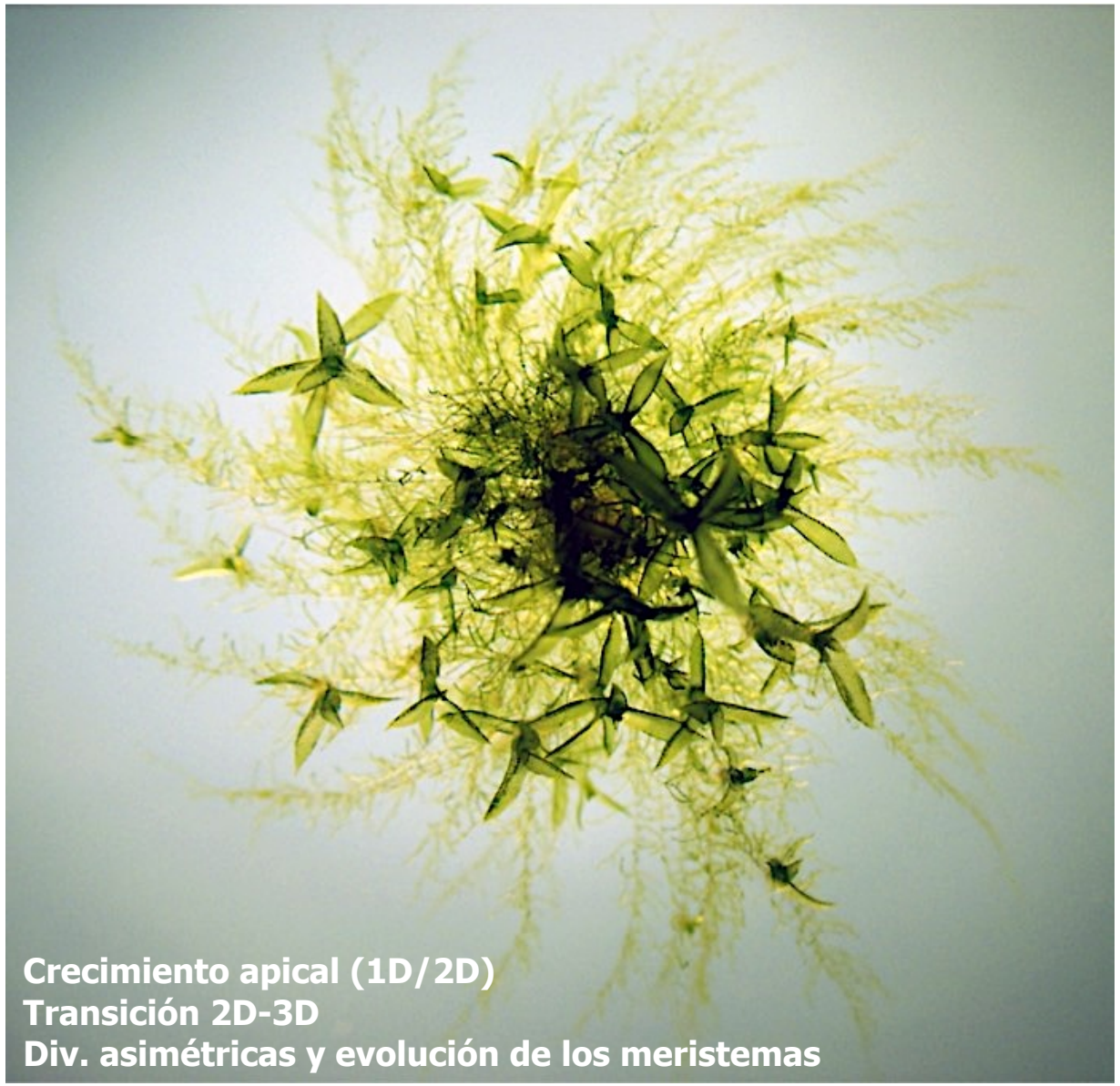


Capacidad de rotar en 3/más planos de orientación las divisiones celulares de la célular madre

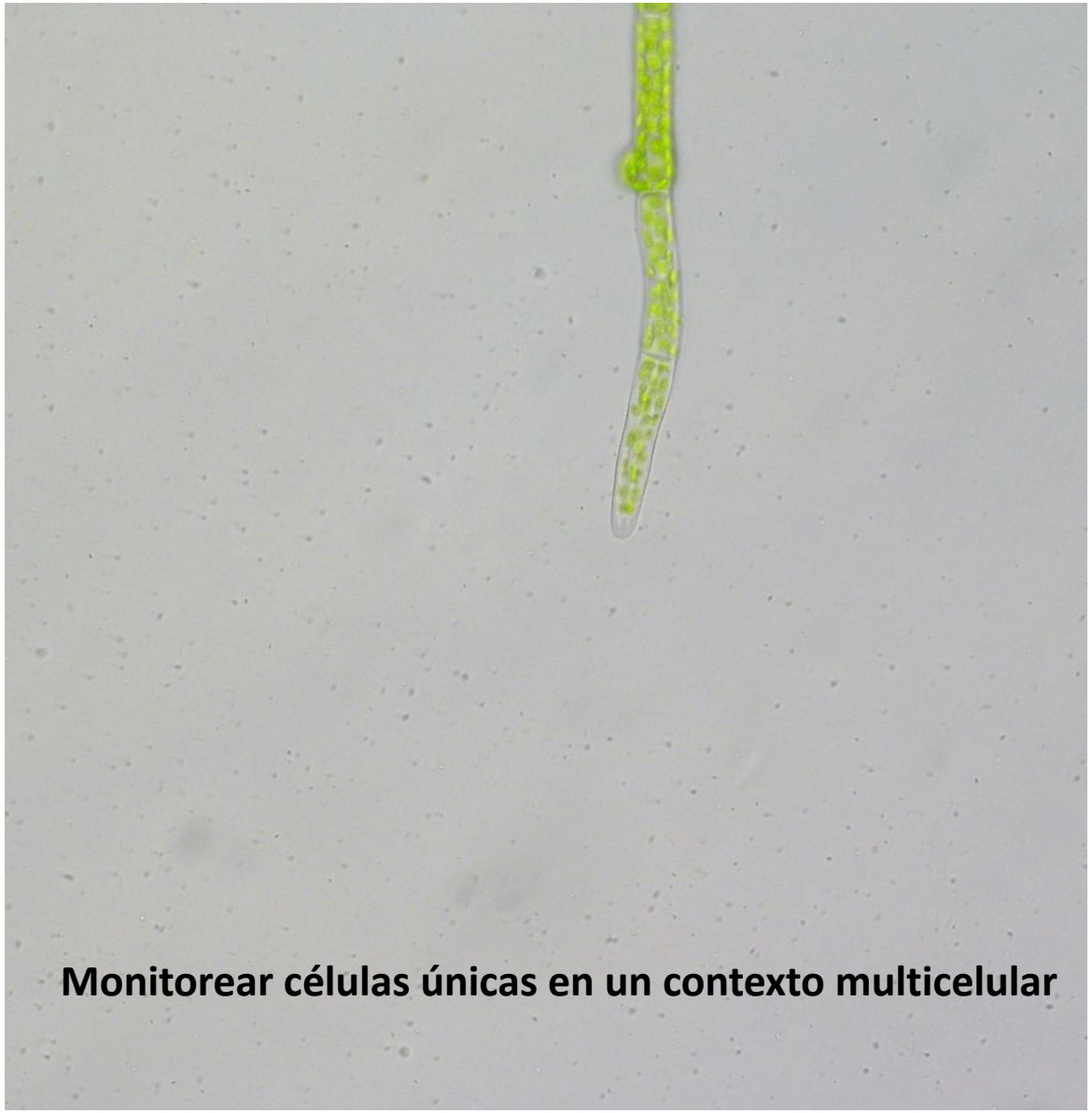


EMBRIÓFITAS

PHYSCOMITRIUM PATENS: monitorear en tiempo y espacio

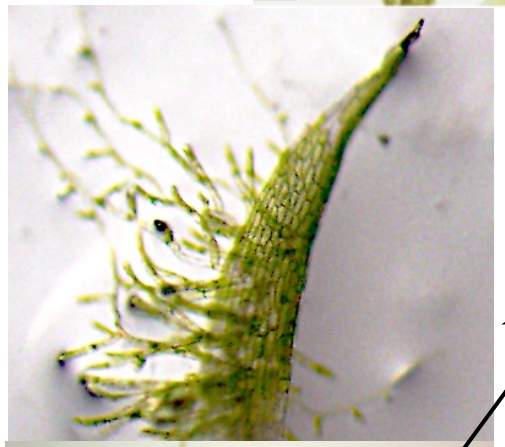


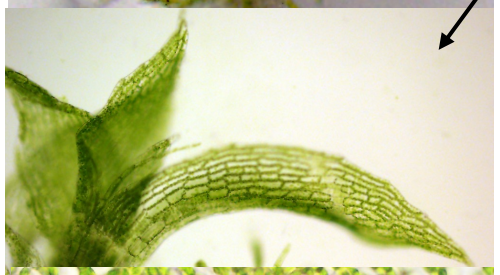
Crecimiento apical (1D/2D)
Transición 2D-3D
Div. asimétricas y evolución de los meristemas

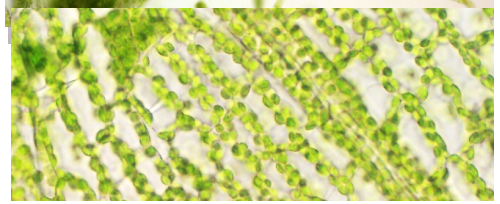


Monitorear células únicas en un contexto multicelular

Ciclo de vida de *Physcomitrium patens*







Esporofito (2n)

Germinación espora

Cloronema*

1D

Protonema

CKK

AUX

Caulonema*

1D

2D

Ramificaciones

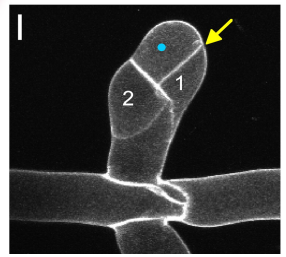
Estructuras reproductivas

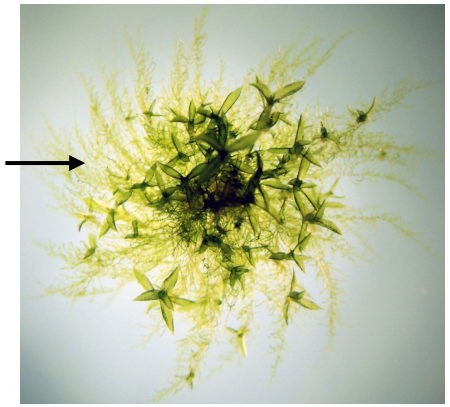
Gametoforo con rizoides*

3D

CKK

Yemas



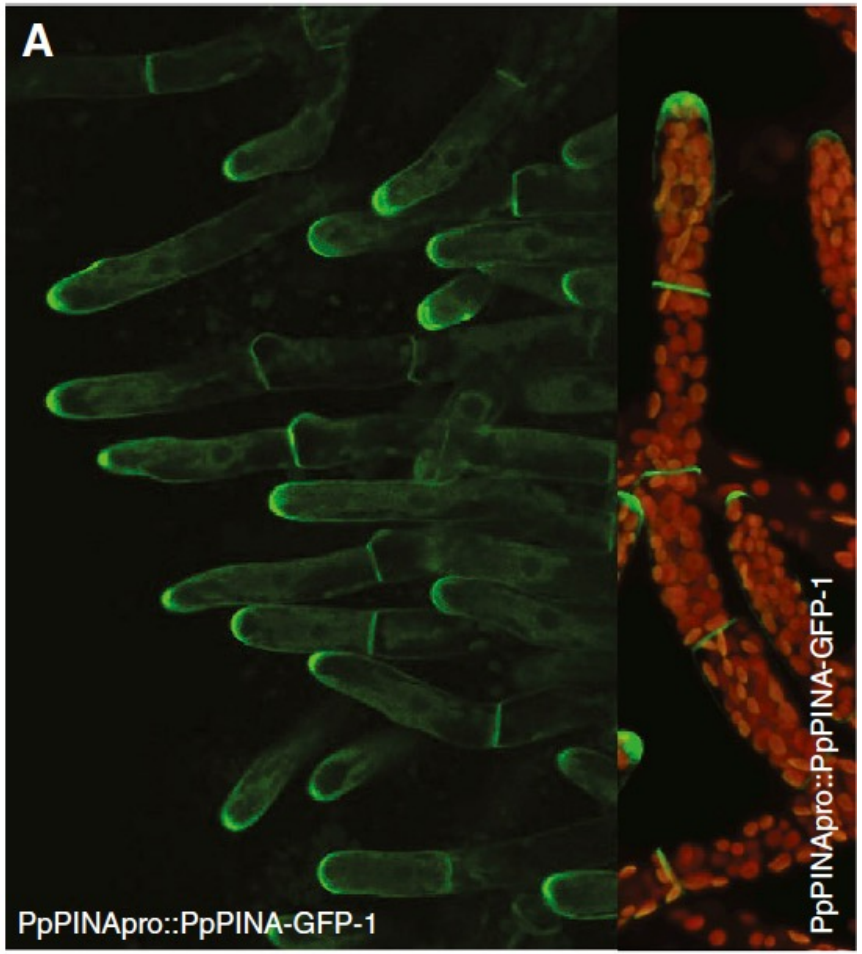


*** Crecimiento apical**

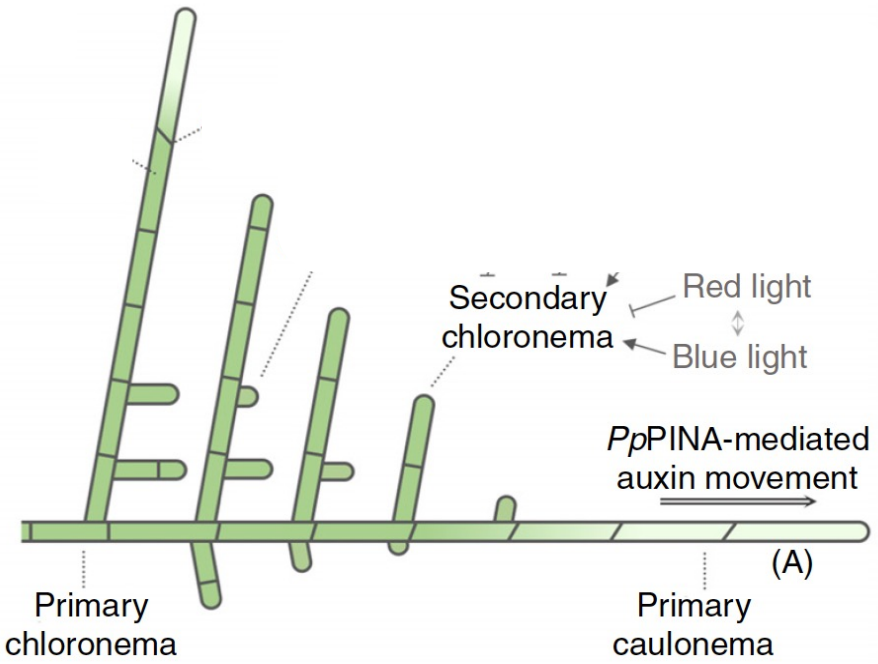
Auxinas promueven la diferenciación cloronema-caulonema

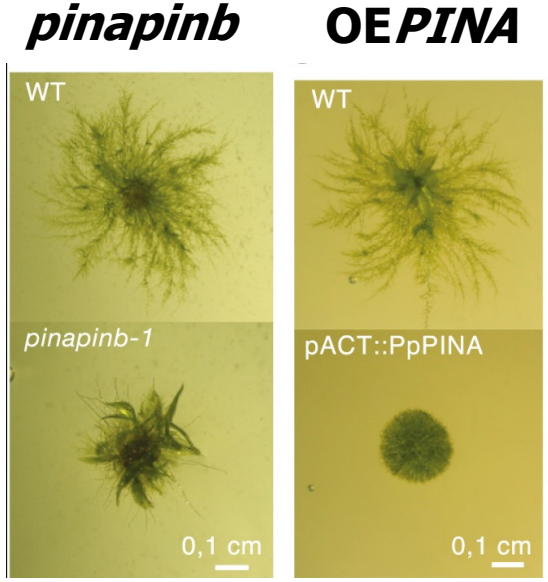
PIN-dependent auxin transport from the base of protonemal filaments to and out of the tip regulates the cellular auxin levels in cells along the filament, which regulates the chloronema-to-caulonema transition.

PpPINApro::PpPINA-GFP

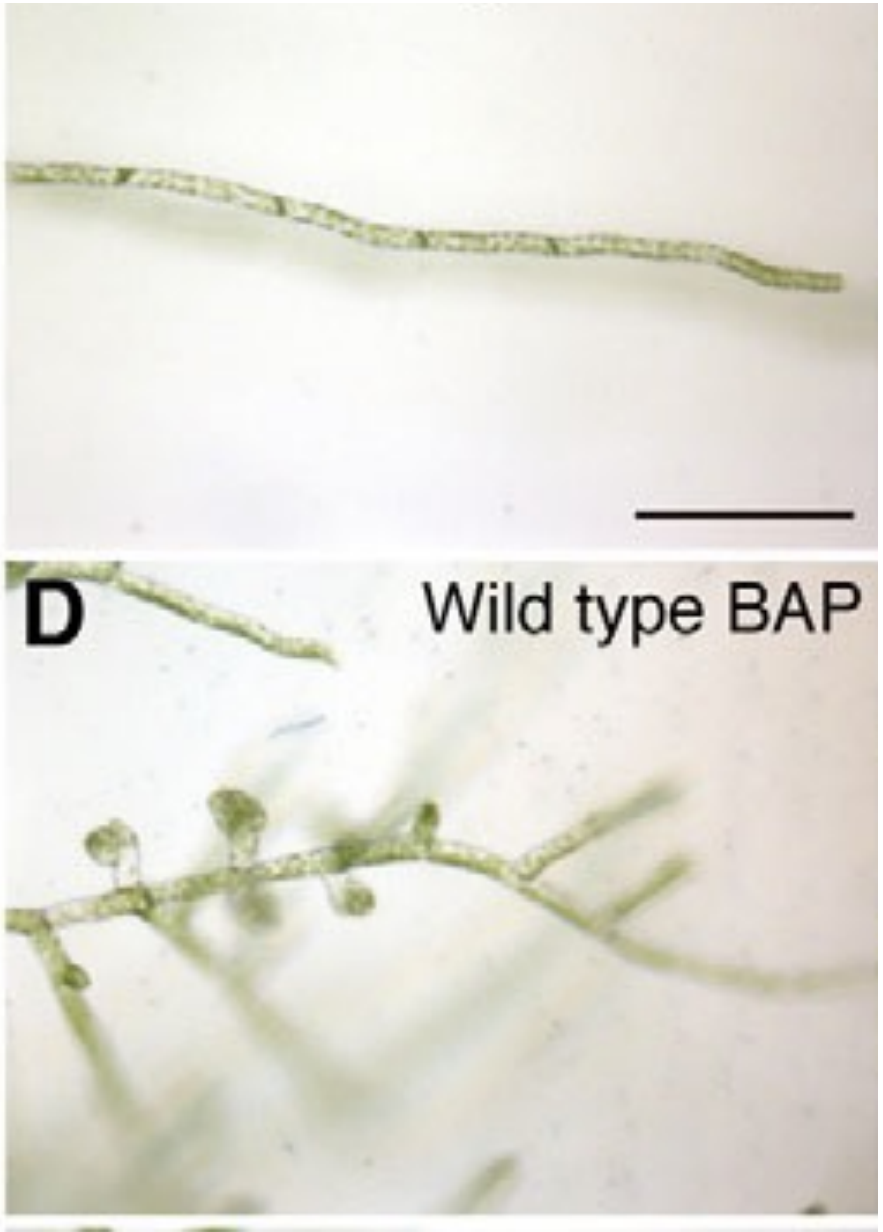


"Apical Dominance"



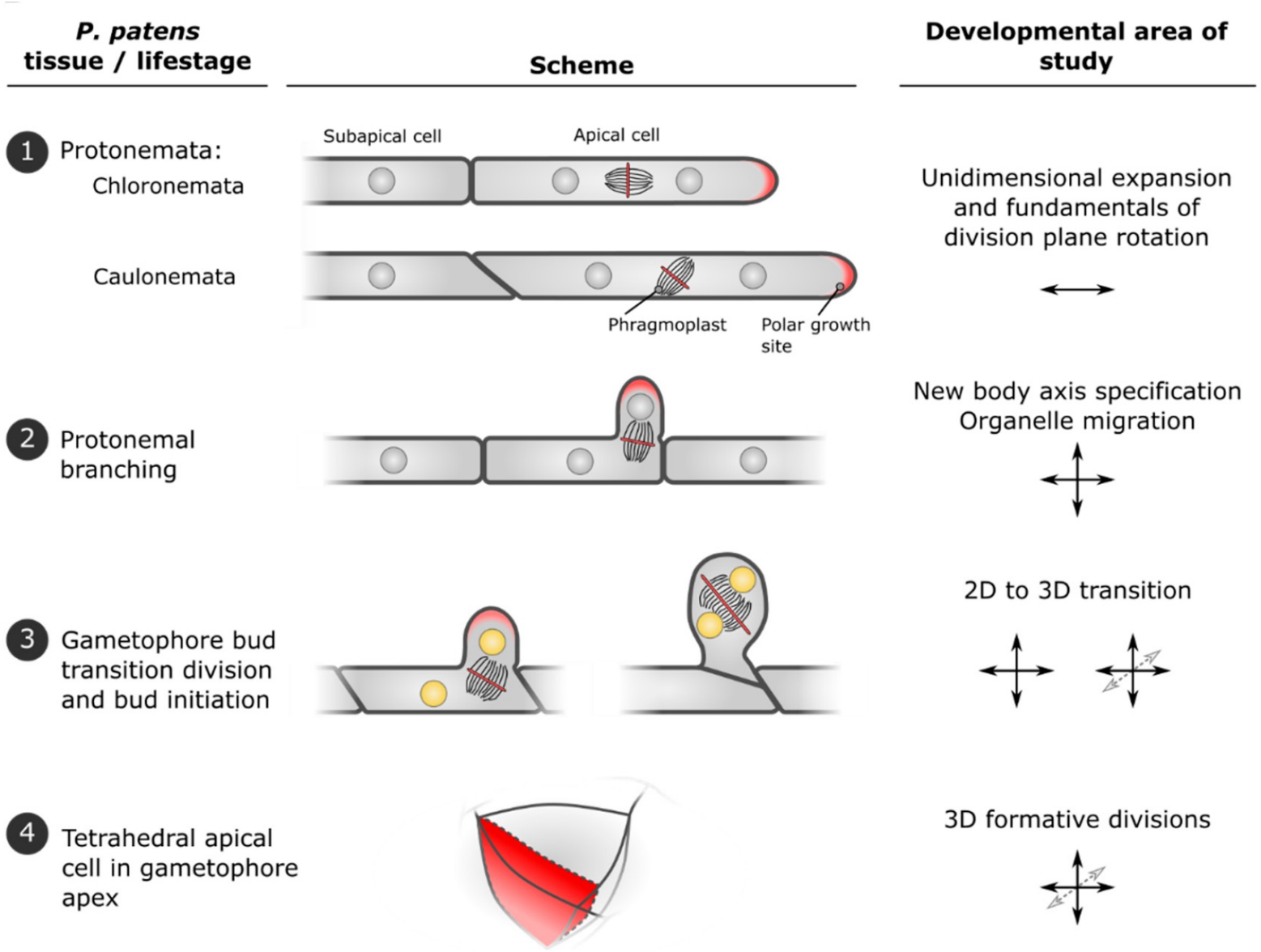


Citocininas promueven la formacion de yemas



La aplicación exógena de **CKs** promueve la formación de células apicales de gametóforo a partir de protonema, pero tambien inhibie el pasaje de cloronema a caulonema.

Modelo para estudios 1D, 2D, 3D y reprogramación celular



ROPs

Las etapas del desarrollo de *P. patens* están marcadas por **divisiones celulares características** y **el establecimiento de nuevos ejes de crecimiento**.

ROPs

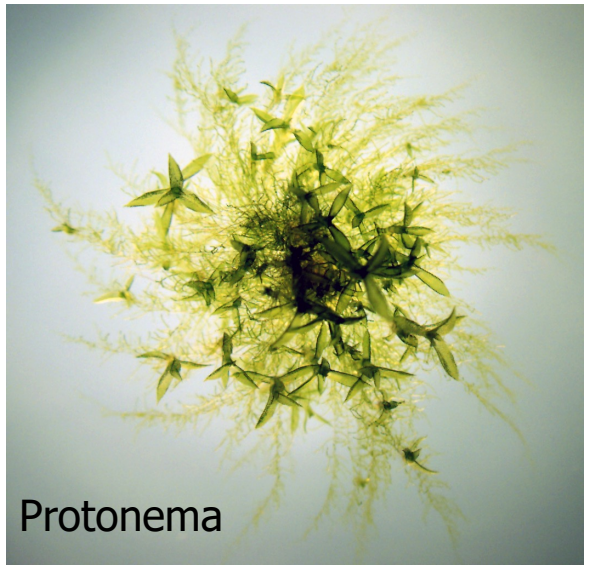
2D vs 3D, ABPs, CLV, KNOX

Evolución meristemas

Modelo para estudios 1D: crecimiento polar

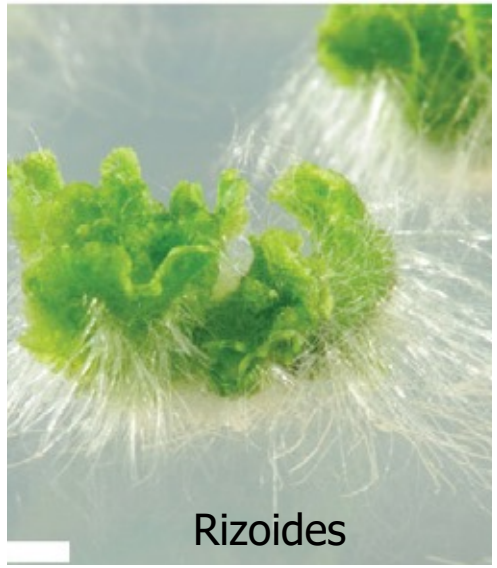
BRIÓFITAS

Physcomitrium patens



Formación del cuerpo de la planta

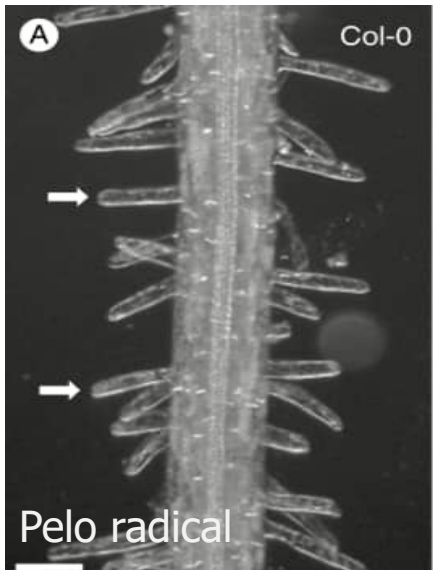
Marchantia polymorpha



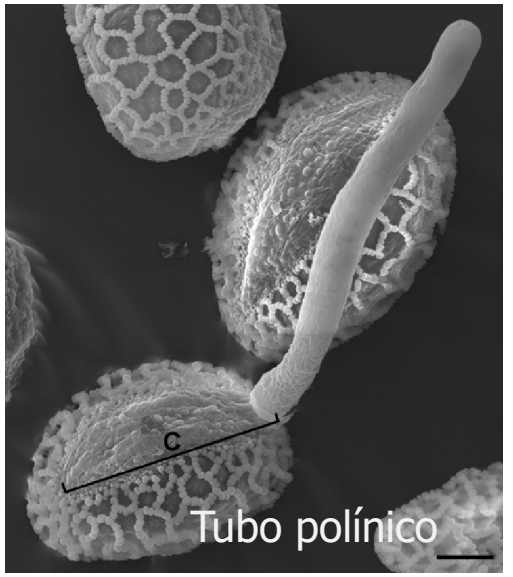
Anclaje al sustrato

TRAQUEÓFITAS

Arabidopsis thaliana

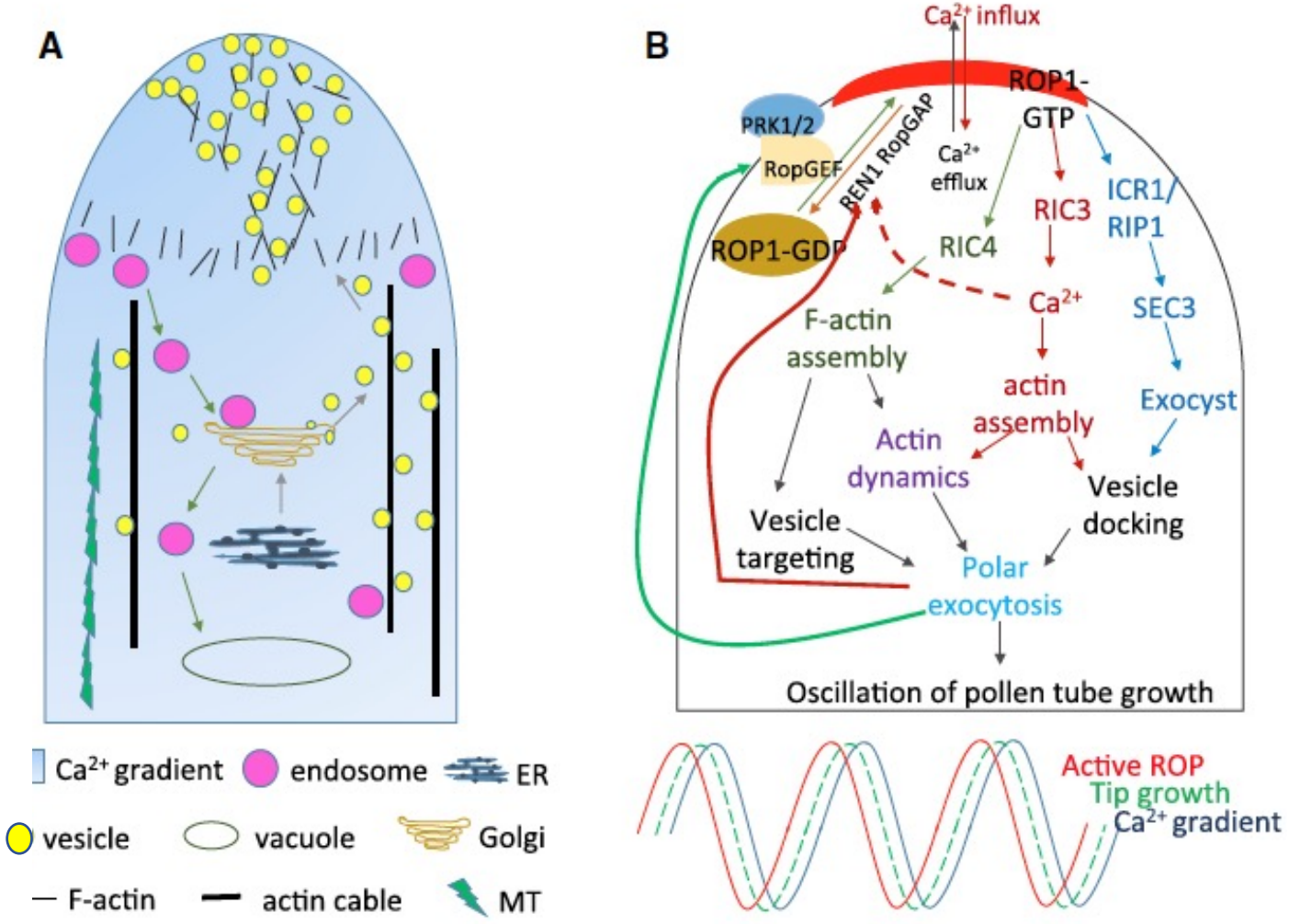


Absorción de nutrientes
Interacción con microorganismos benéficos



Fertilización

Modelo para estudios 1D: crecimiento polar



ROPs polarize tip growth by promoting:

- calcium signaling
- Cytoskeleton remodeling
- vesicle fusion
- exocytosis

Crecimiento 1D-2D (protonema) en *P. patens*

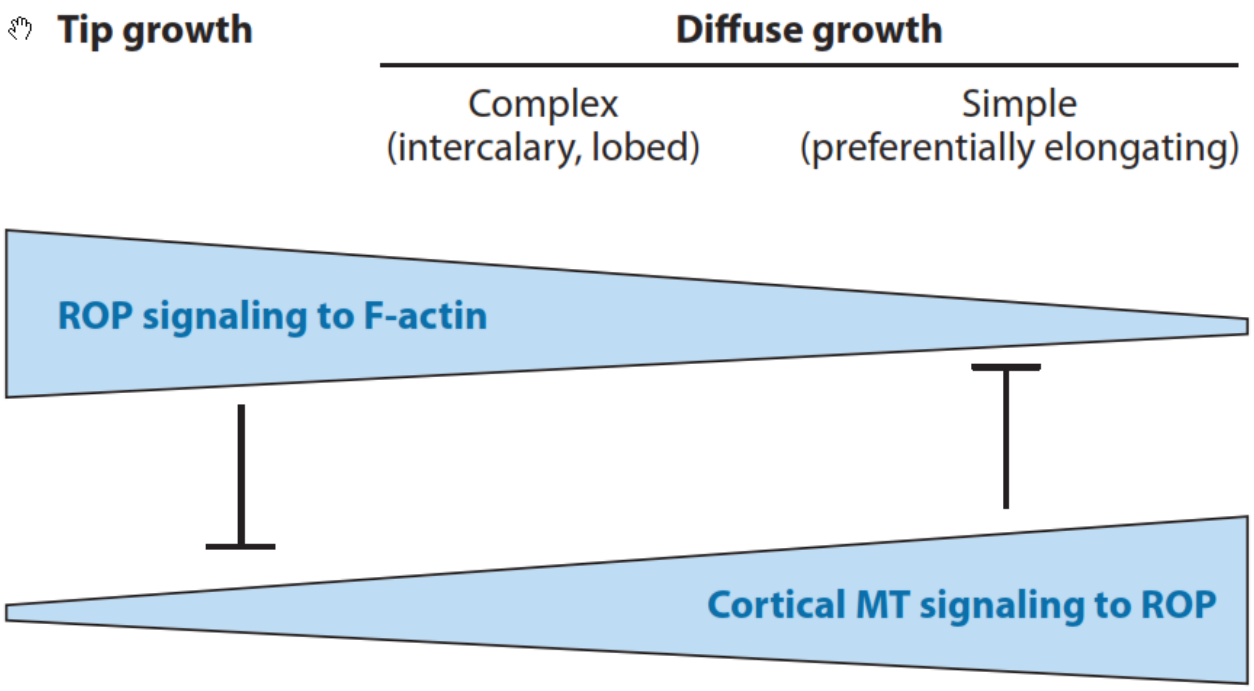


Figure 5

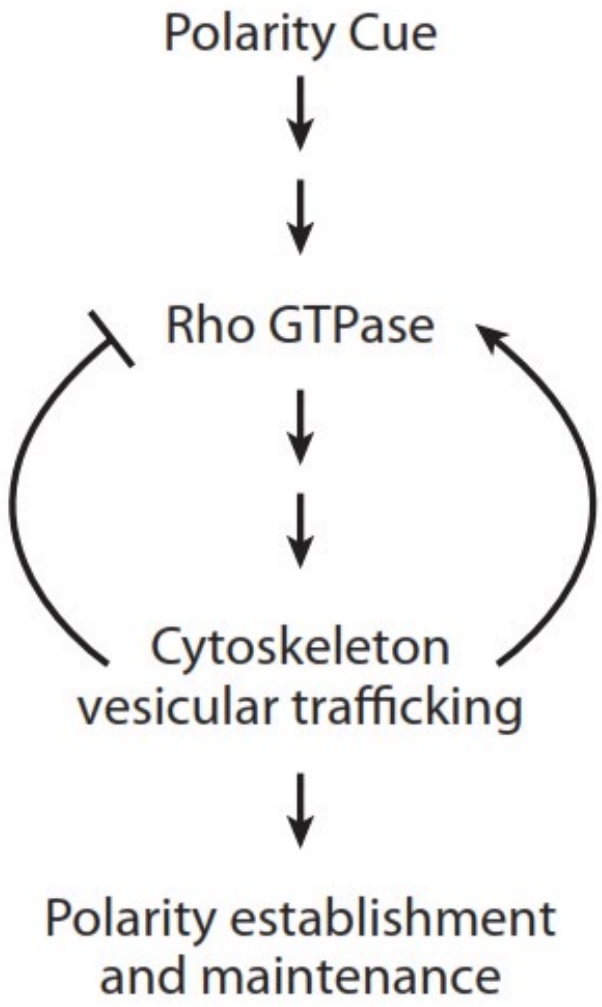
A schematic model for a unifying concept of the mechanisms underlying the control of cell morphogenesis through tip growth or polarized diffuse growth.

Time lapse spinning disc LSCM of Lifeact-mEGFP in a moss chloronema cell. Vidali, et al. 2009

Apical F-actin network
Focal point of F-actin

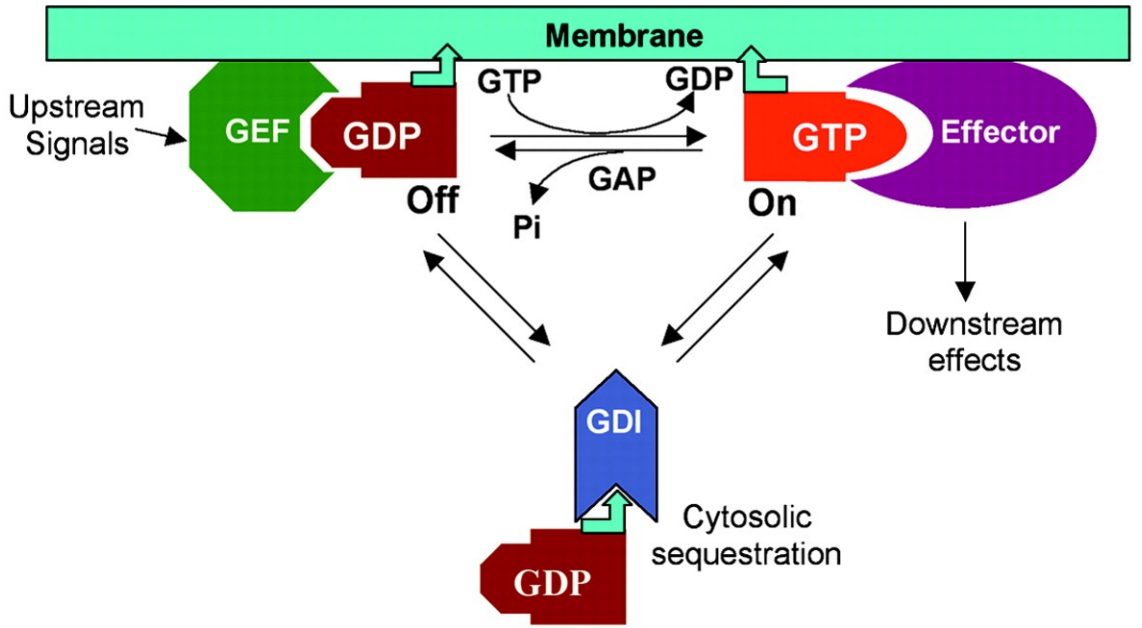
Lifeact is a short actin-binding peptide that is used to visualize filamentous actin (F-actin)

ROPs: reguladores maestros de la polaridad celular



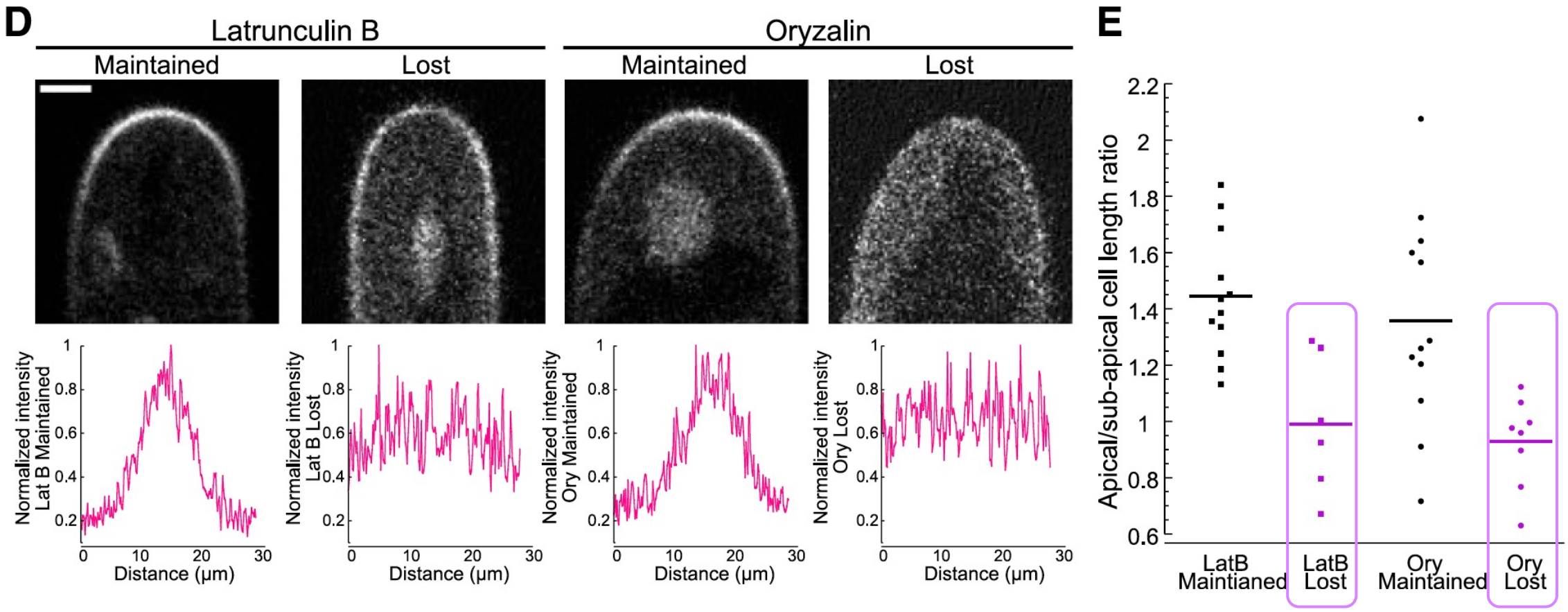
Rho of Plants (ROPs): Positional Cue

GTPasas pequeñas



•Most Rho GTPases cycle between an active GTP-bound and an inactive GDP-bound form, a process that is regulated by guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs). In their GTP-bound form, they interact with a diverse range of different targets to induce cellular responses.

El citoesqueleto afecta la polaridad de ROP, ciclo celular-dependiente



Cheng et al. (2020) The Plant Cell

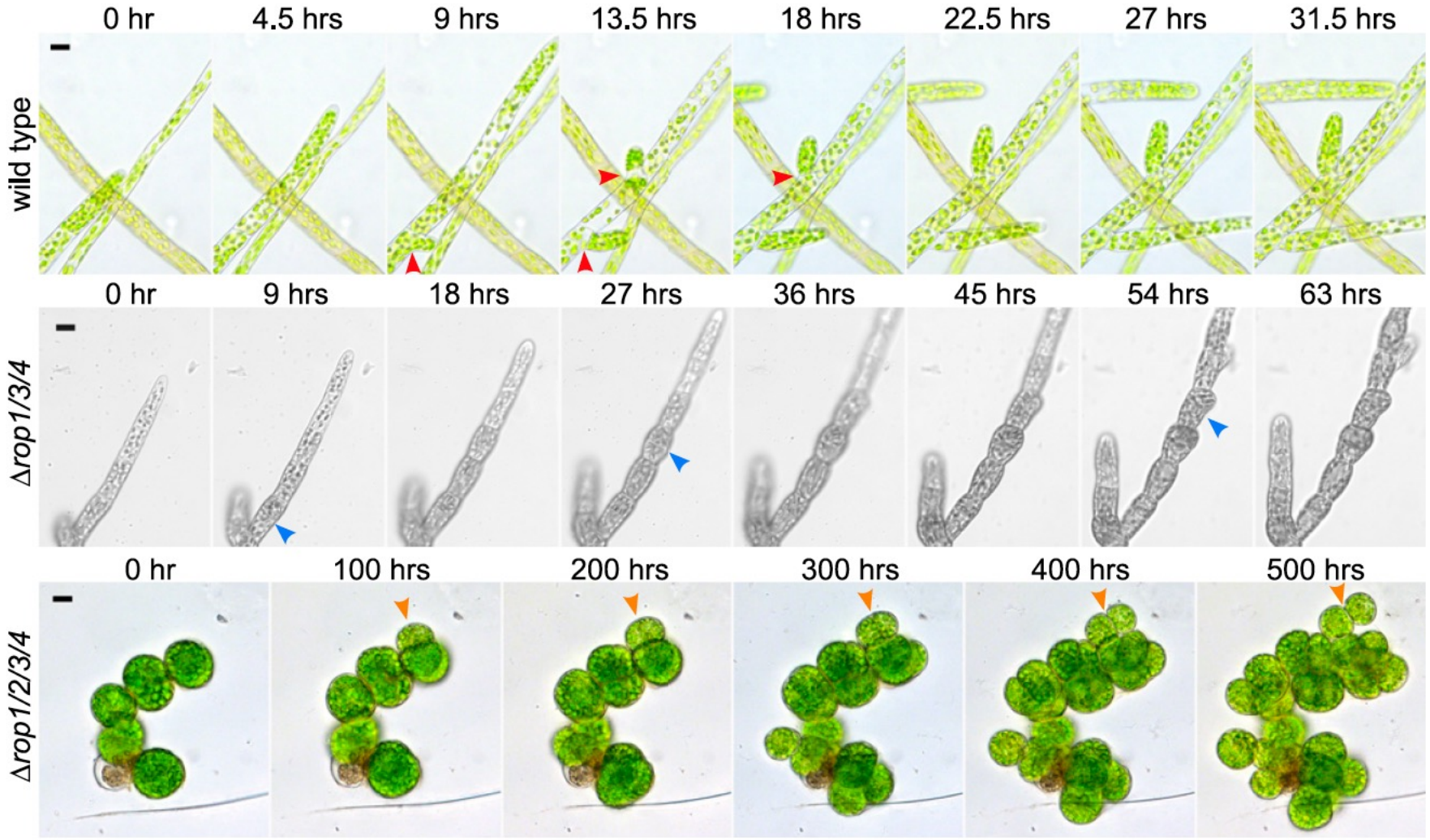
La actina y los microtúbulos poseen un rol en el mantenimiento de la localización de ROP al inicio del ciclo celular.

ROPs son esenciales para el crecimiento polar en *P. patens*

11 genes en *A. thaliana*
4 genes en *P. patens*

Fenotipo intermedio

Células esféricas
Expansión isotrópica
(No hay diferenciación caulo, gametoforos)

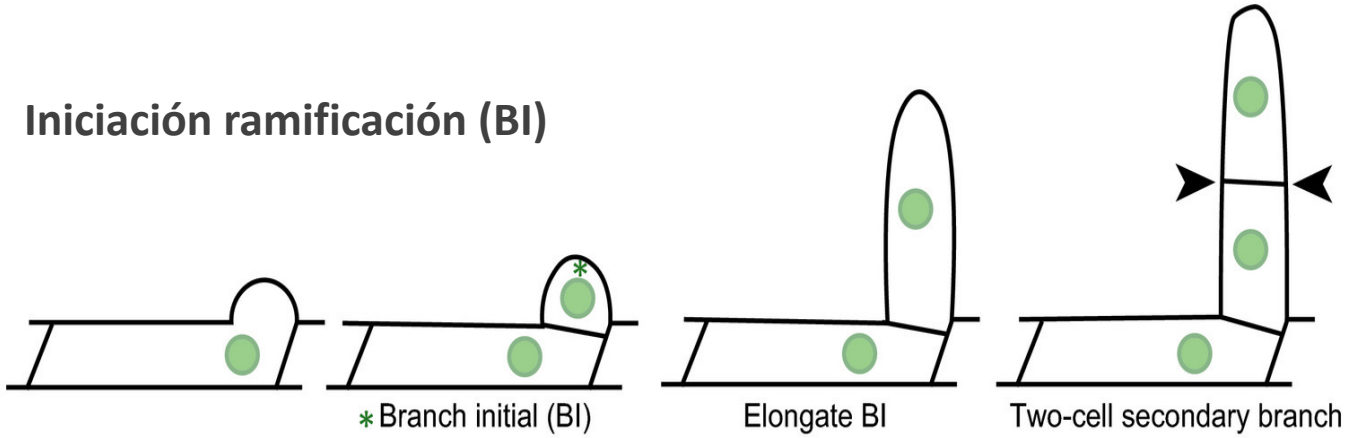


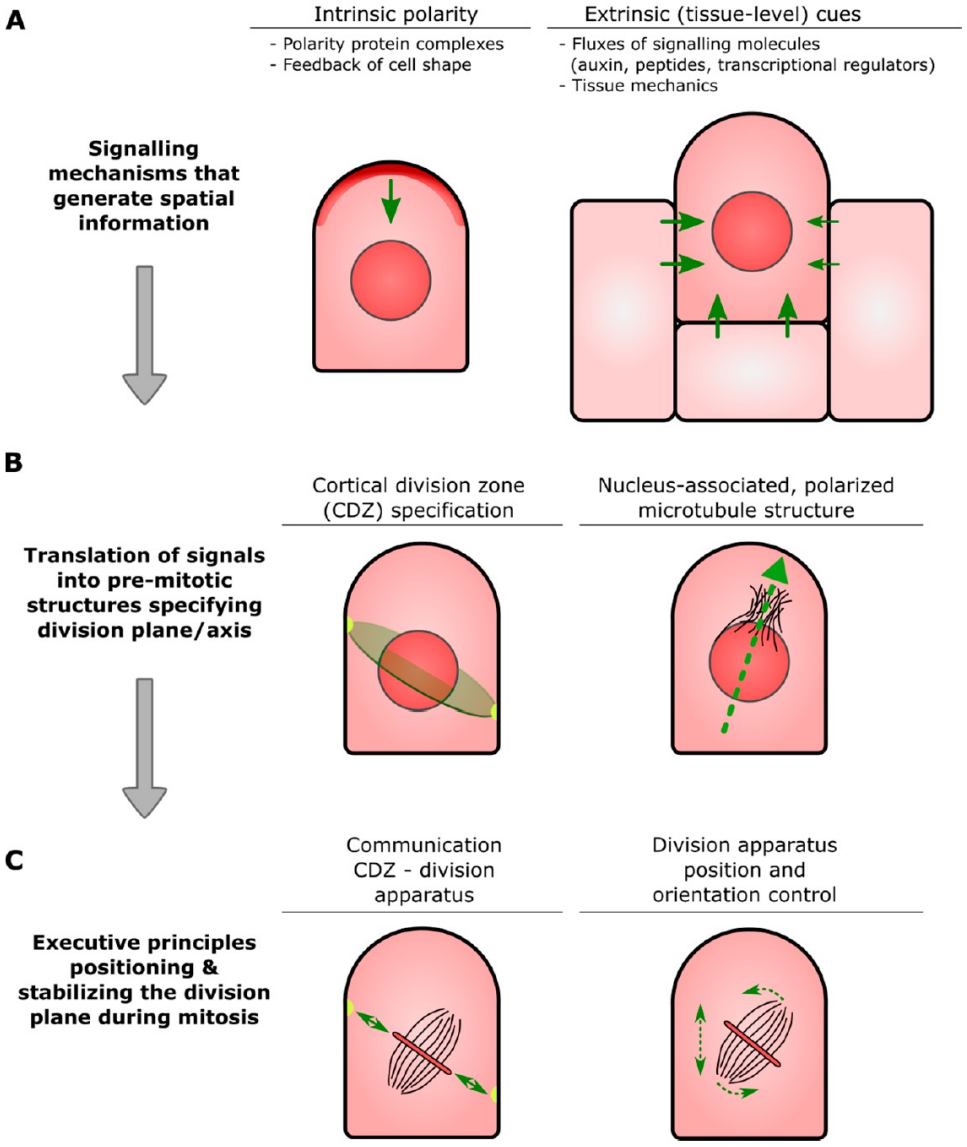
Cheng et al. (2020) The Plant Cell

Factores que controlan las **divisiones asimétricas**

¿Participa ROP como señal posicional para la formación de una ramificación lateral inicial?

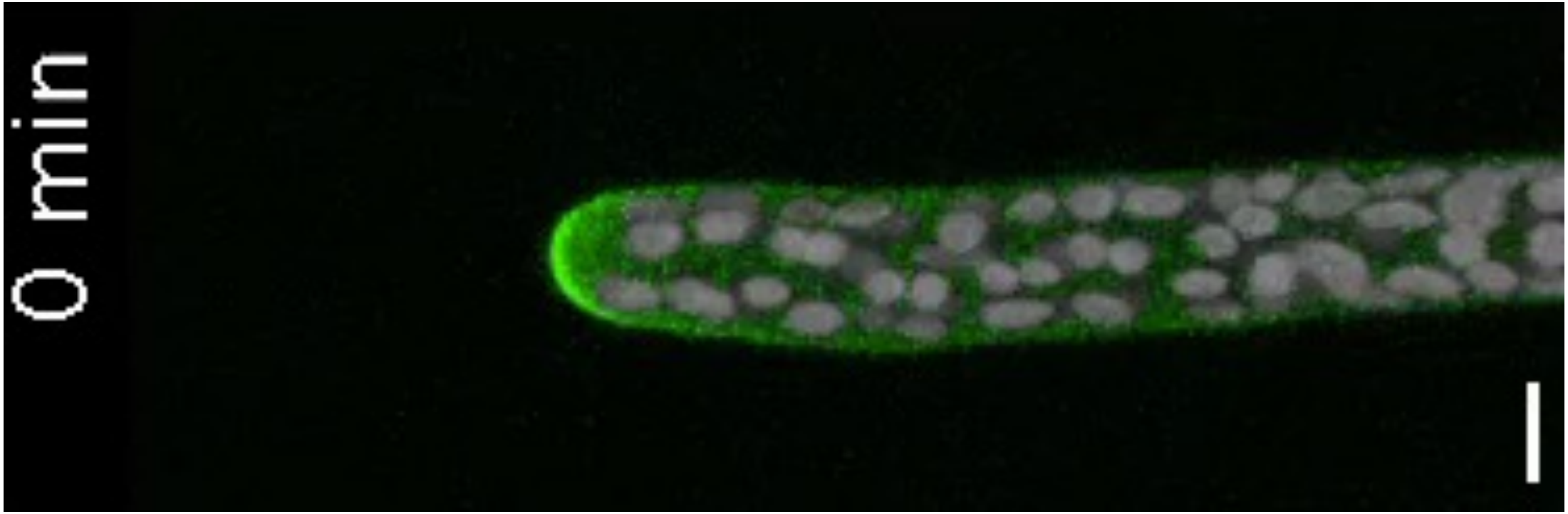
Iniciación ramificación (BI)





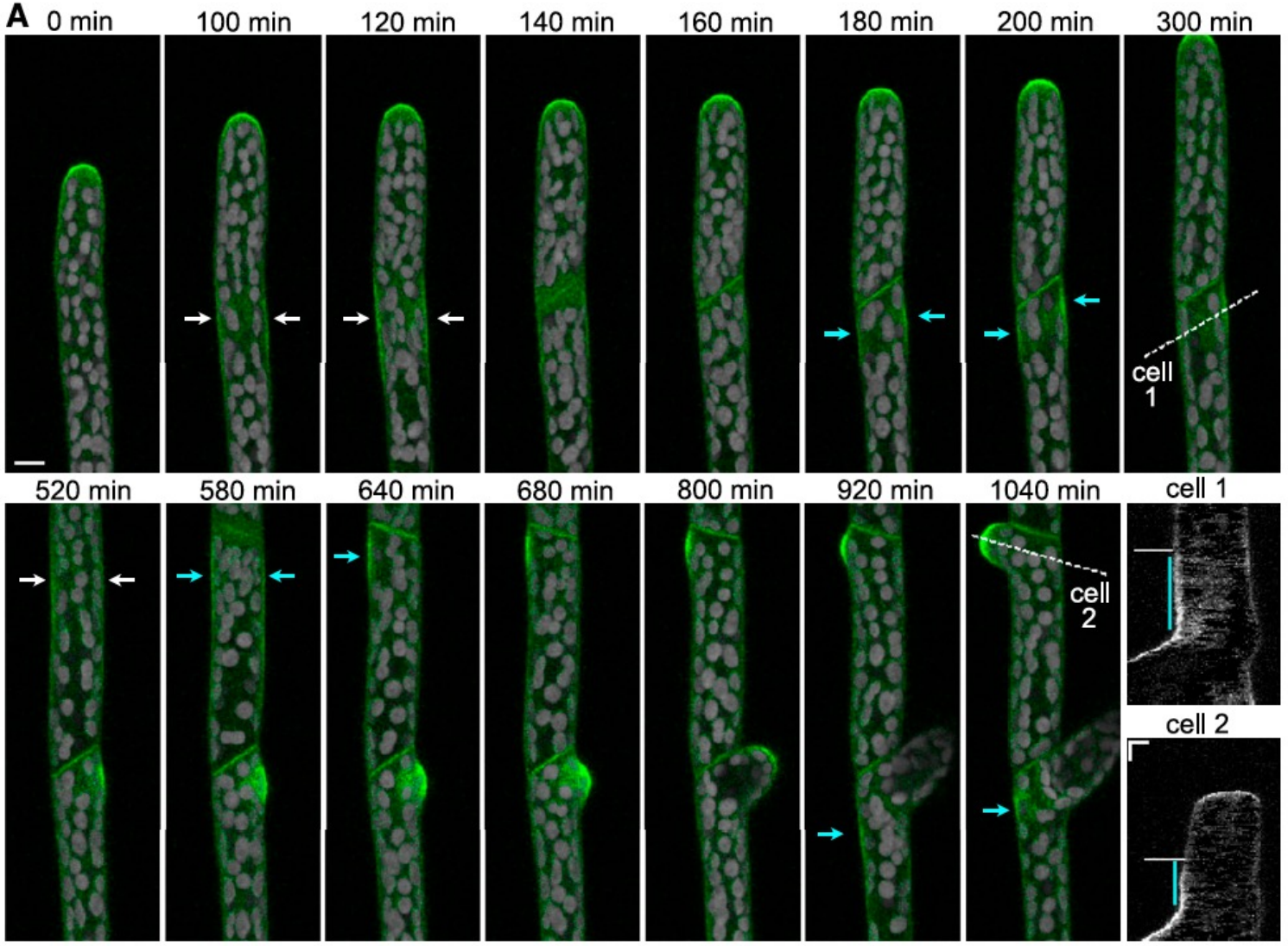
ROP en el establecimiento de la polaridad celular en ramificación

ROP4-NG (ROP-NeonGreen) localizes to the future branch site hours before a branch emerges.



Cheng et al. (2020) The Plant Cell

ROP en el establecimiento de la polaridad y división celular



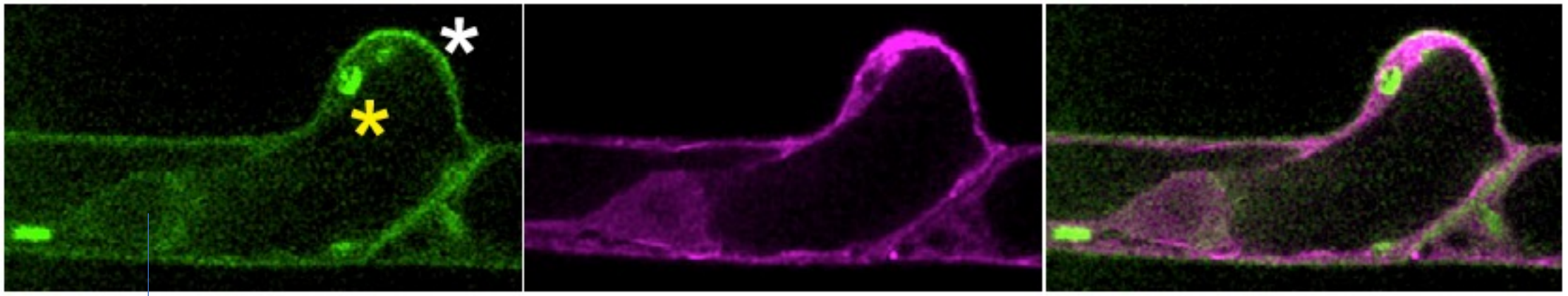
Cheng et al. (2020) The Plant Cell

1- ROP localizes to **the apical plasma membrane** of tip-growing protonemata**.**

2- ROP accumulates at the **cell cortex in the middle of the apical cell** before it is observed in the developing cell plate.

3- ROP localizes to the **future branch site hours before a branch emerges**.

Las **ROP GTPasas** y la **actina** inician la formación de "bulges" polarizados en las células previo a su ramificación



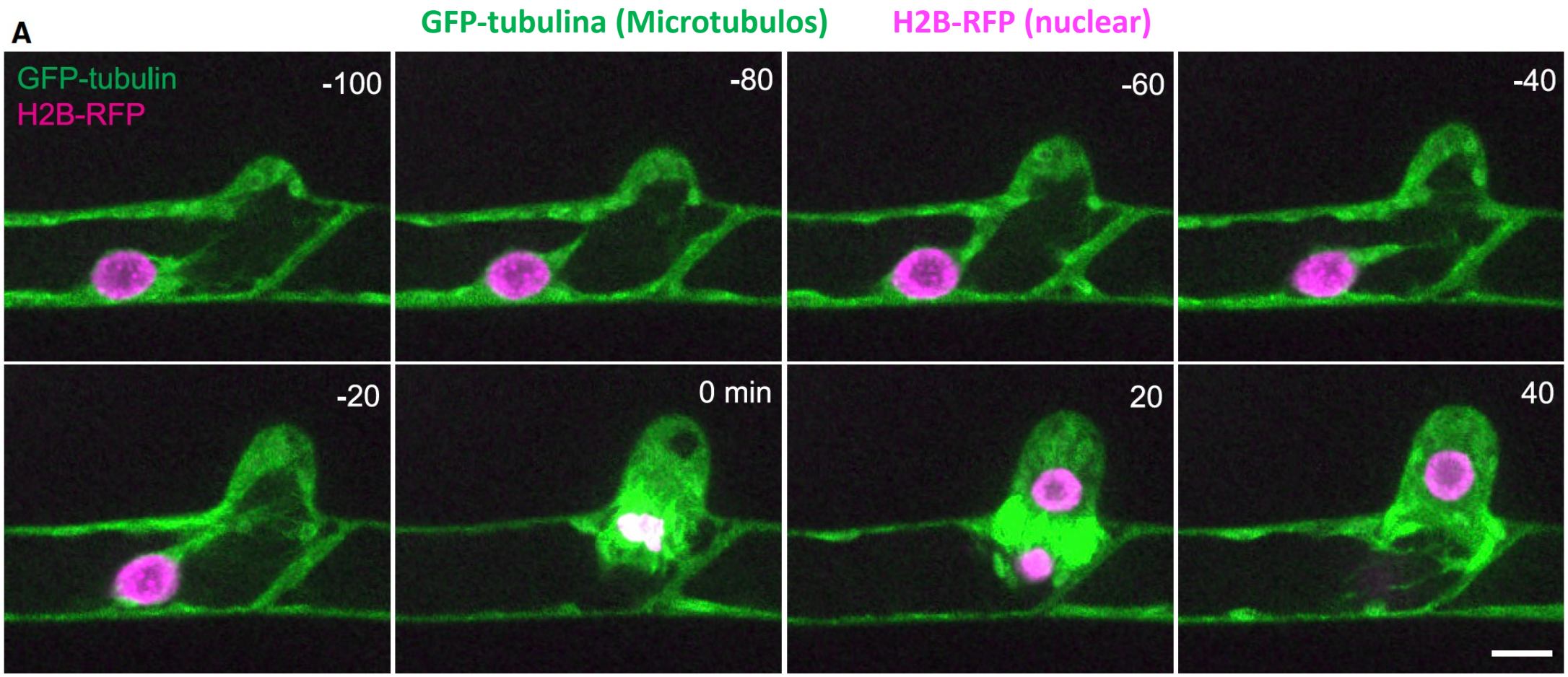
Núcelo

* mNG-ROP4

* Chloroplast

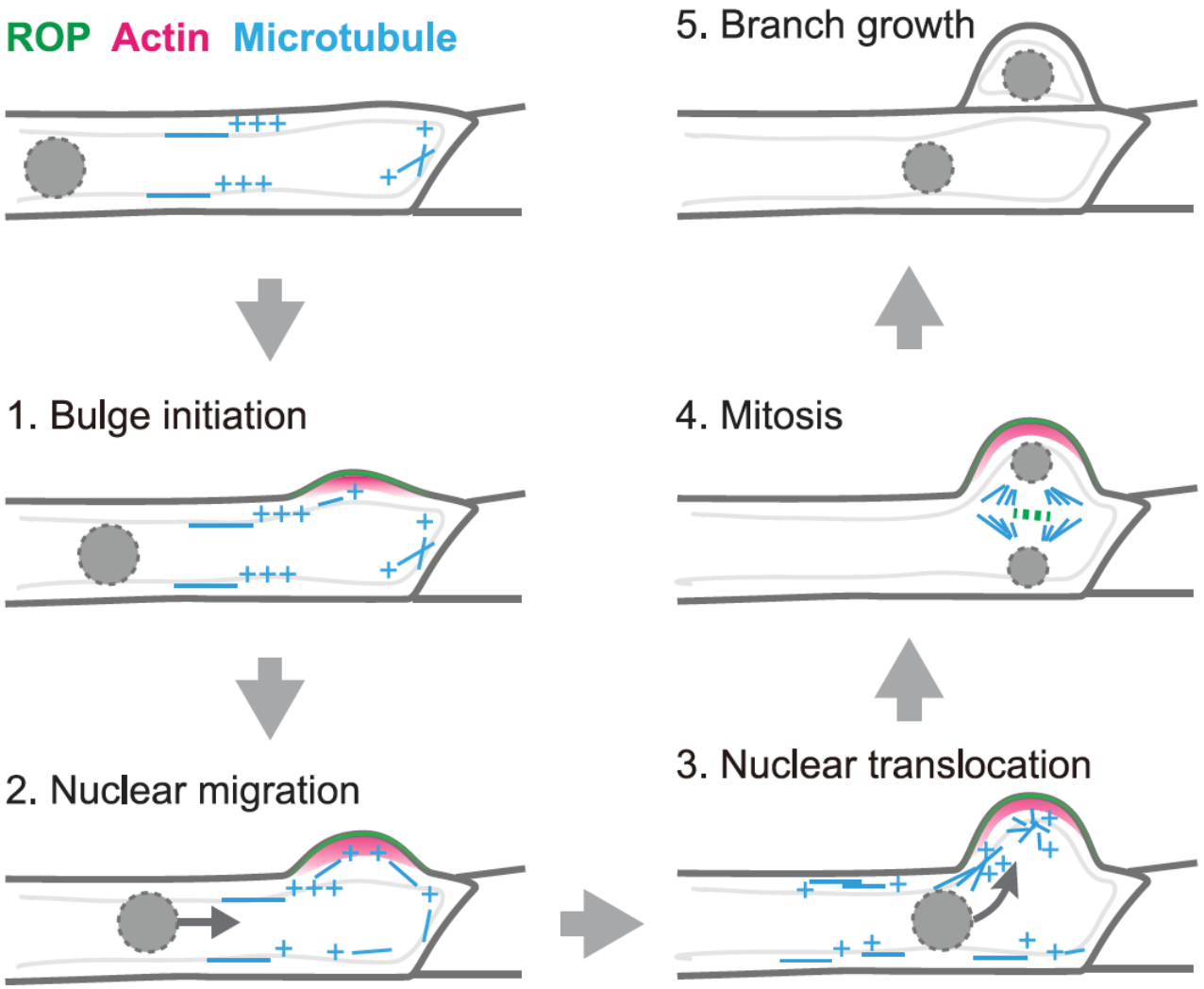
Yi & Goshima (2020) Current Biology

Ramifiación (Branch): migración nuclear, MT y establecimiento nuevo eje division celular



Yi & Goshima (2020) Current Biology

Branching: migración nuclear, MT y establecimiento nuev eje división



Yi & Goshima (2020) Current Biology

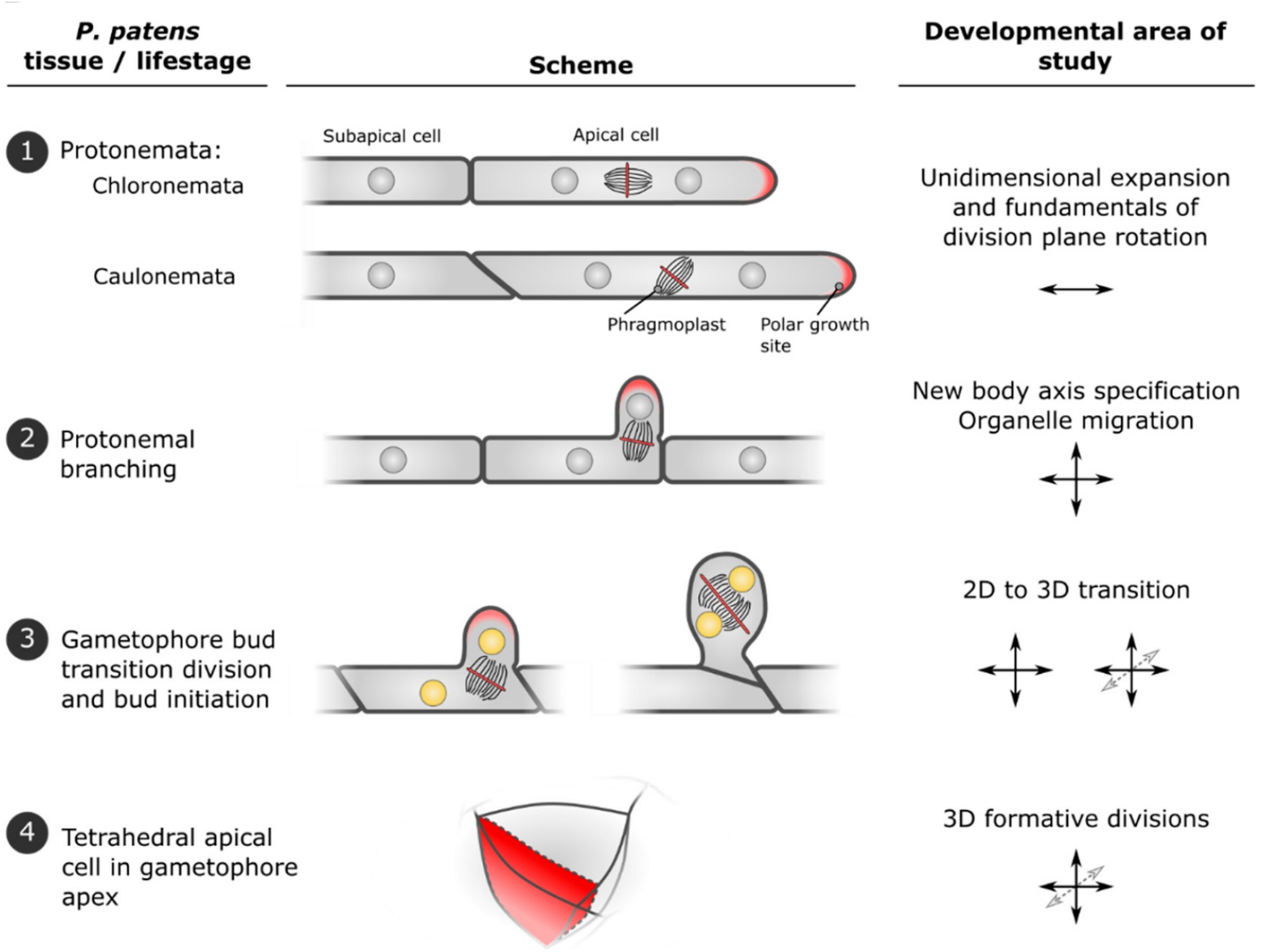
1- Las **ROP GTPasas** y la **actina** inician la formación de "bulges" (abultamientos) polarizados en las células previo a su ramificación

2- lo que a su vez dirige la migración nuclear dependiente de los microtúbulos y

3- la posterior división celular asimétrica

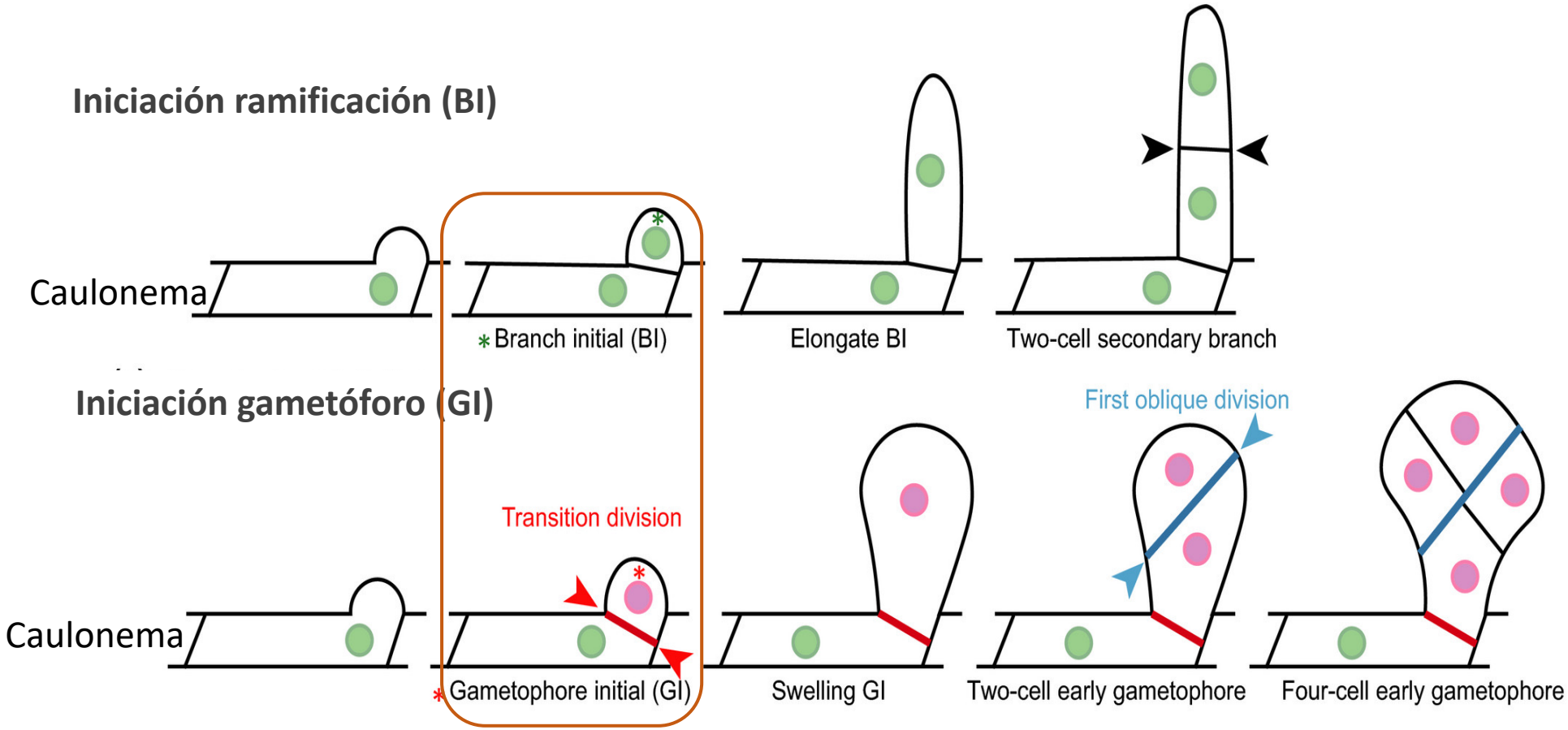
4- Formacion de una ramificación

Modelo para estudios 1D, 2D, 3D y reprogramación celular

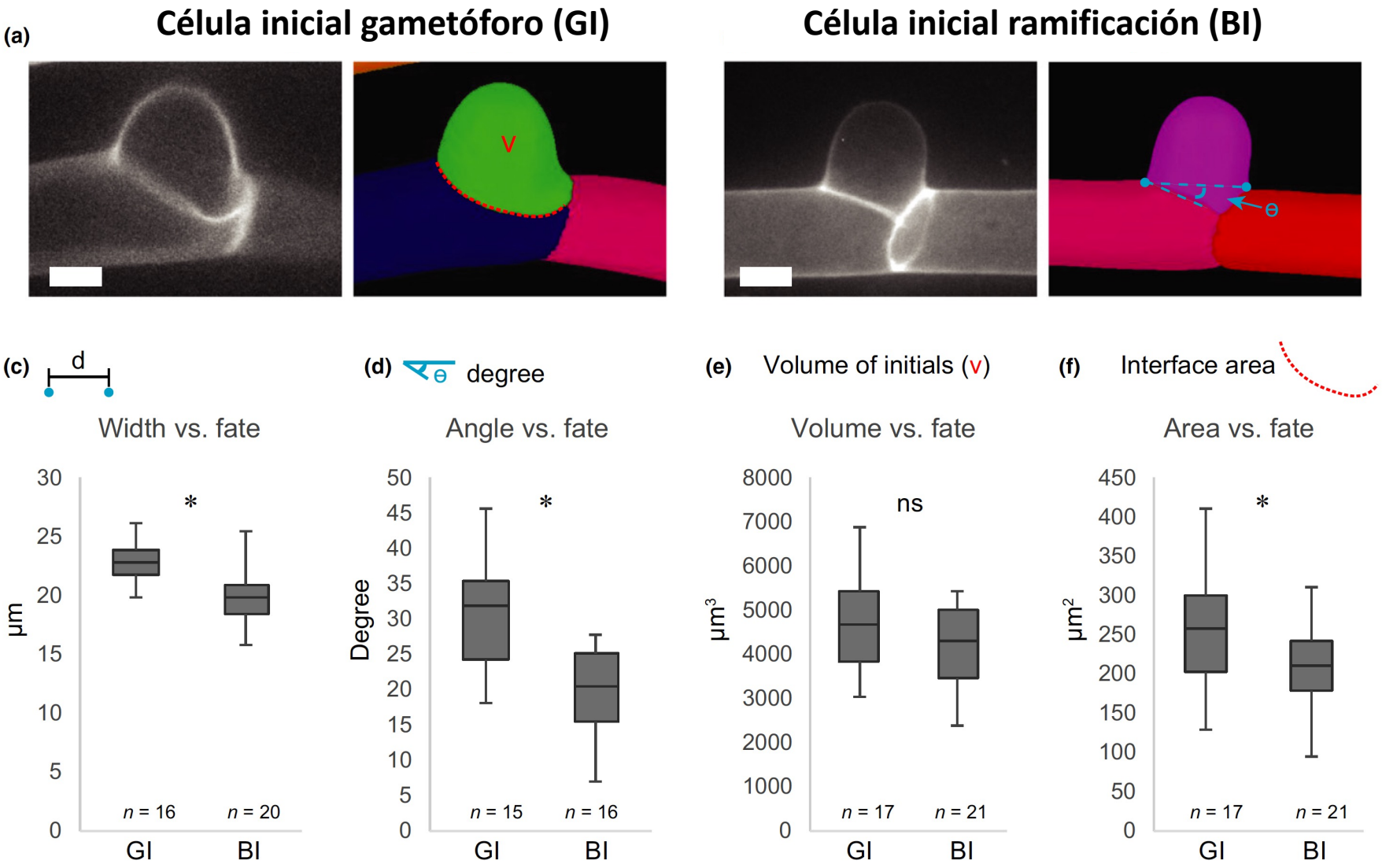


Crecimiento 2D (protonema) o 3D (yemas) en *P. patens*

¿Cómo se determina el destino celular (CELL FATE) de gametóforo inicial (GI) en lugar de la ramificación lateral inicial (BI)?



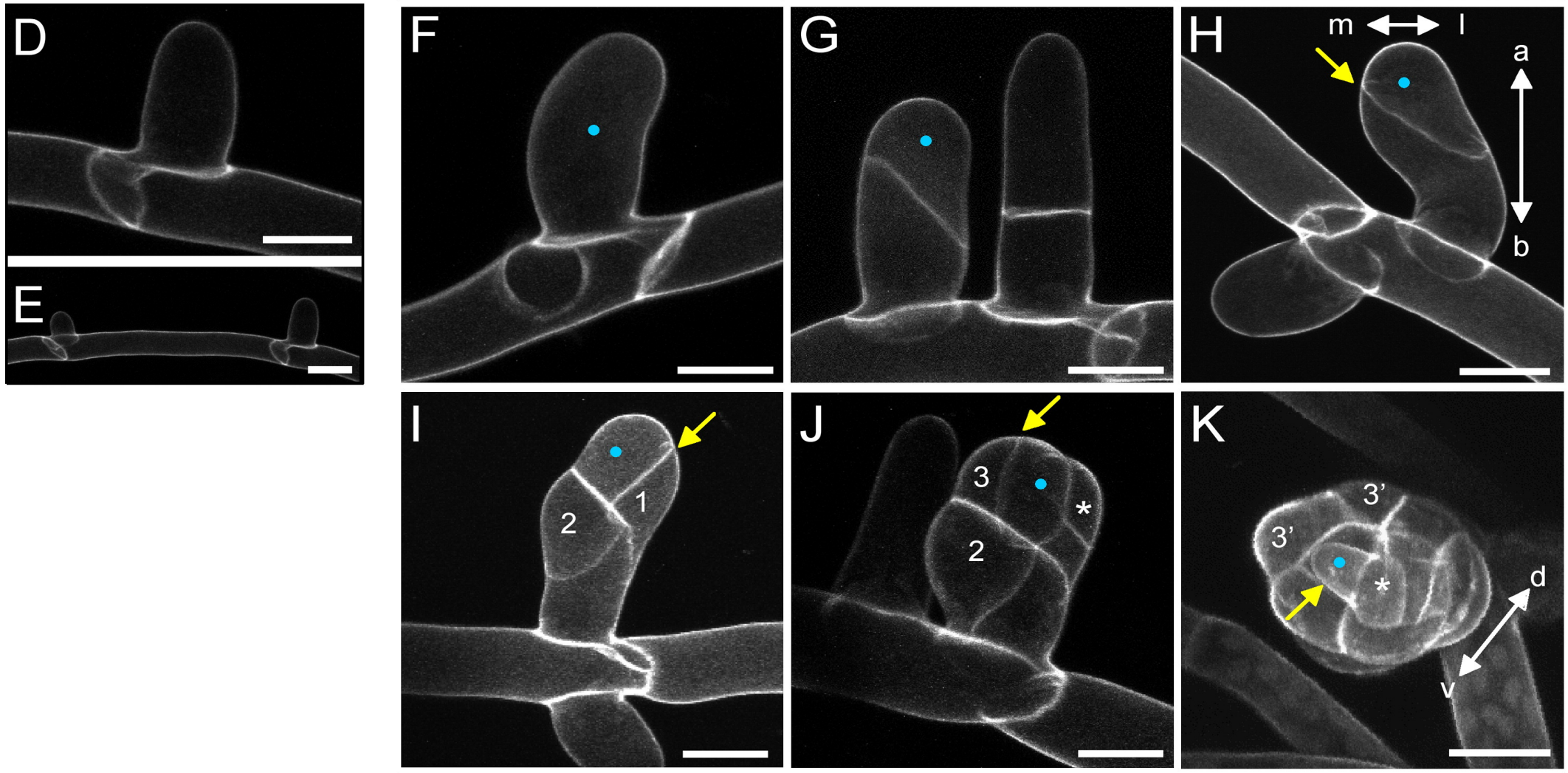
1- Geometrical cues: PREDICEN DESTINO CELULAR GI vs. BI



Transición 2D (protonema) a 3D (yemas) en *P. patens*

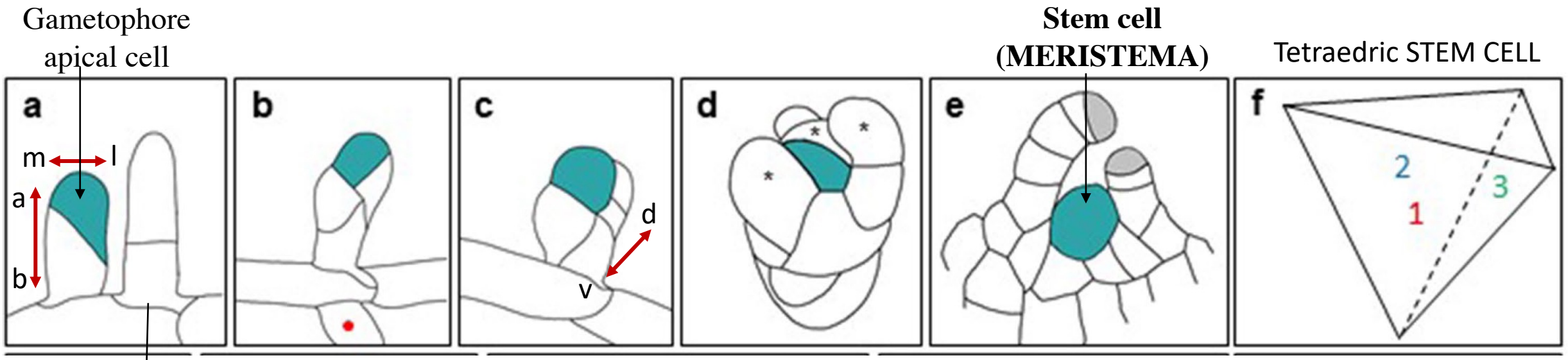
Ramificación
(Branch initial)

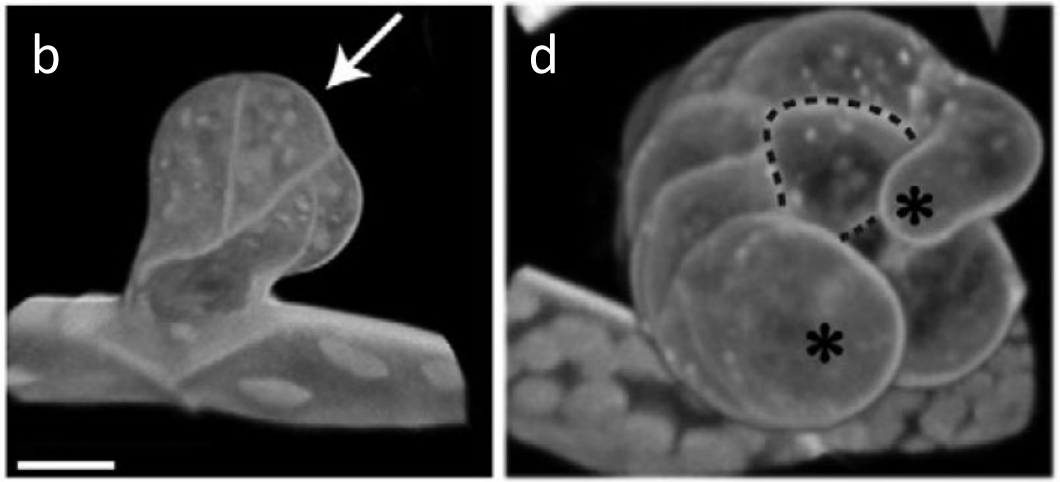
Inicio Yema
(Bud Initial)



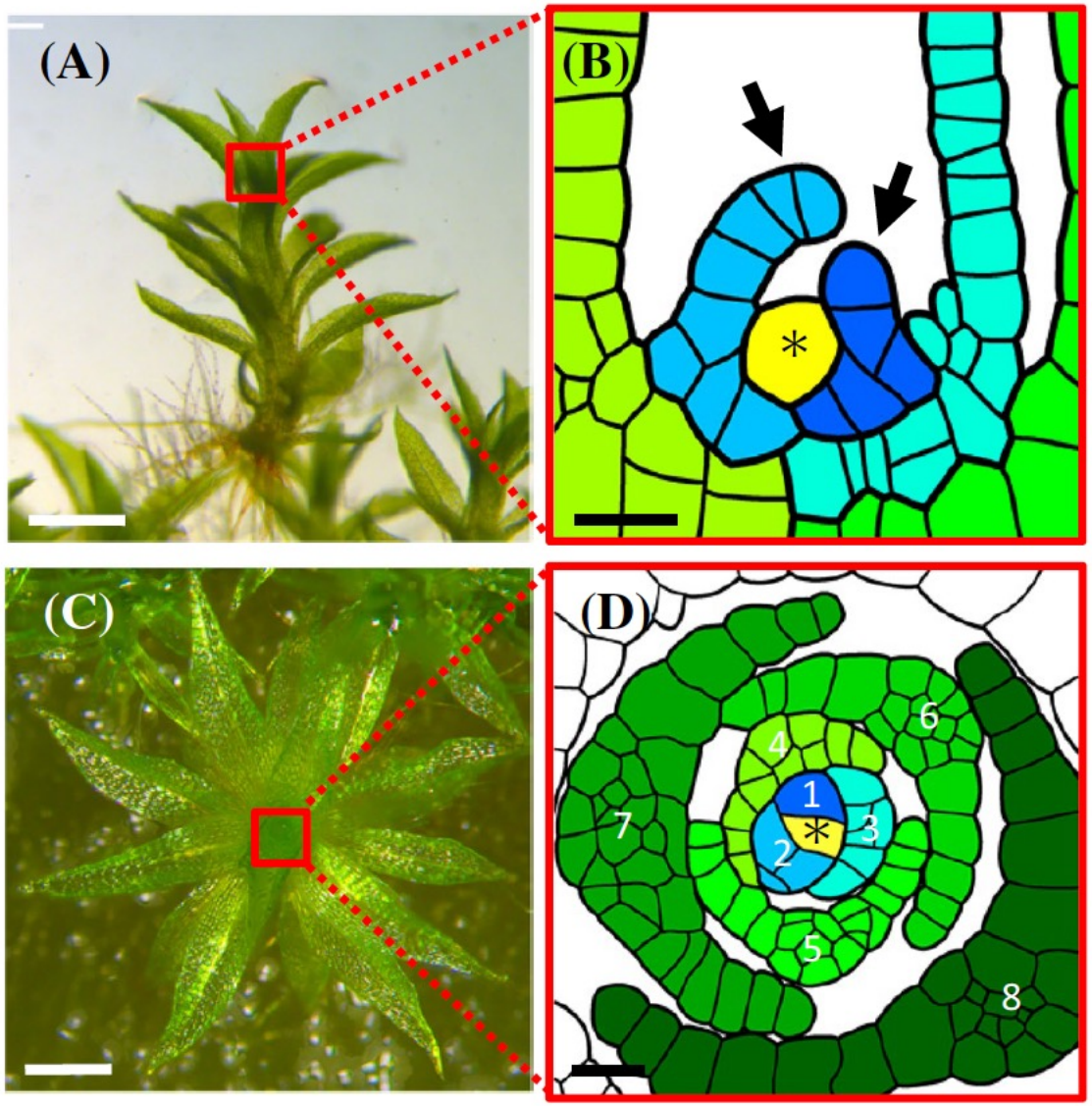
Harrison et al. (2009) Current Biology

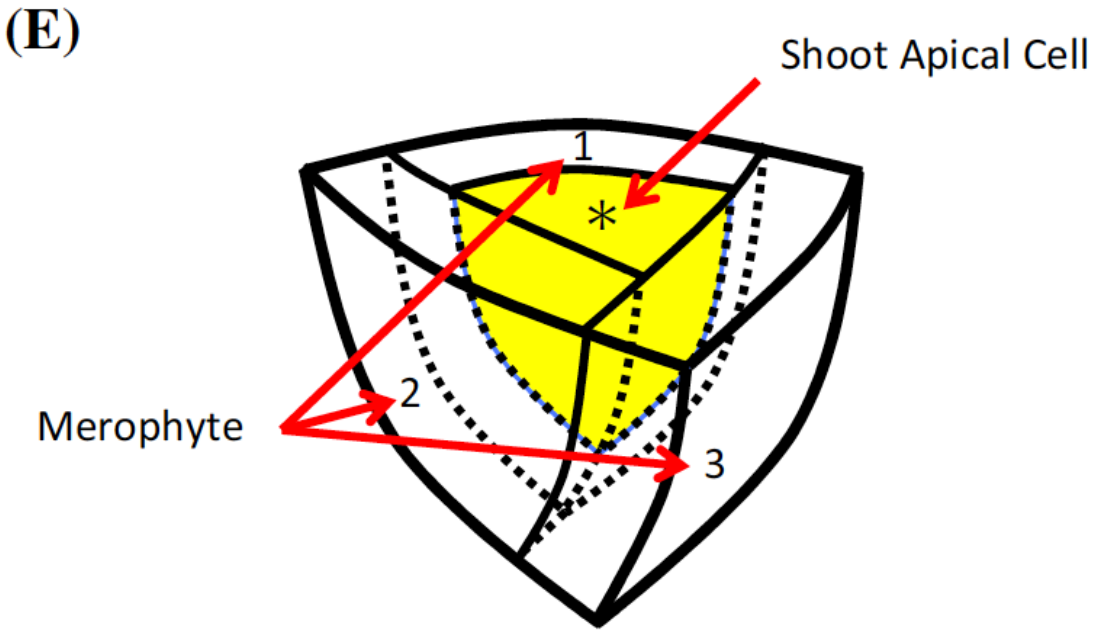
Transición 2D (protonema) a 3D (yemas) en *P. patens*





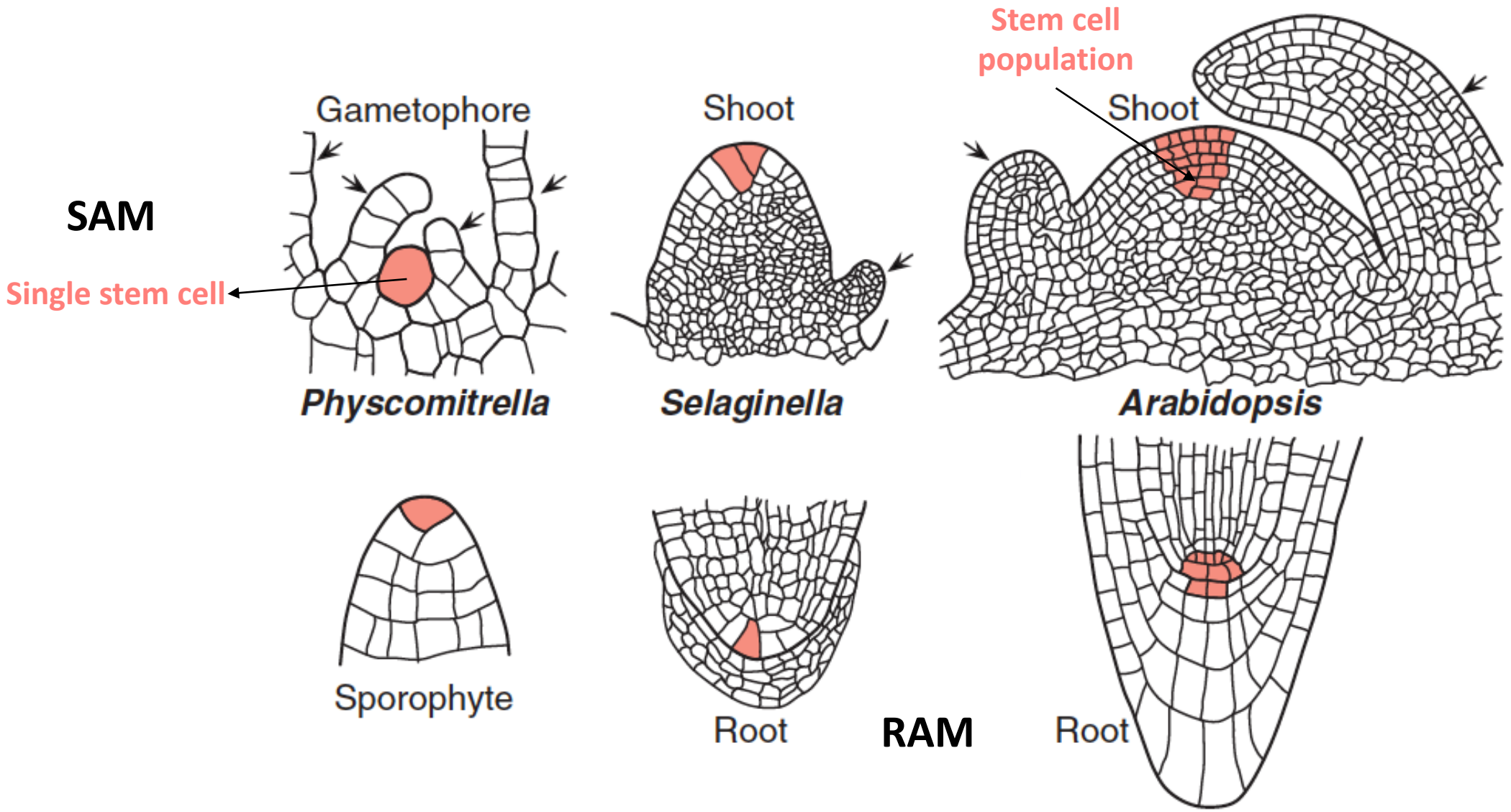
Meristema compuesto por una única celula (single stem cell)





An **asterisk** indicates the **shoot apical cell**. Use of the **same color** indicates **clonal tissue originating from a single merophyte**. **Arrows** in panel b indicate **leaf primordia**. **Numbers** in panel d indicate the **developmental order of leaf primordia**.

Evolución de los meristemas

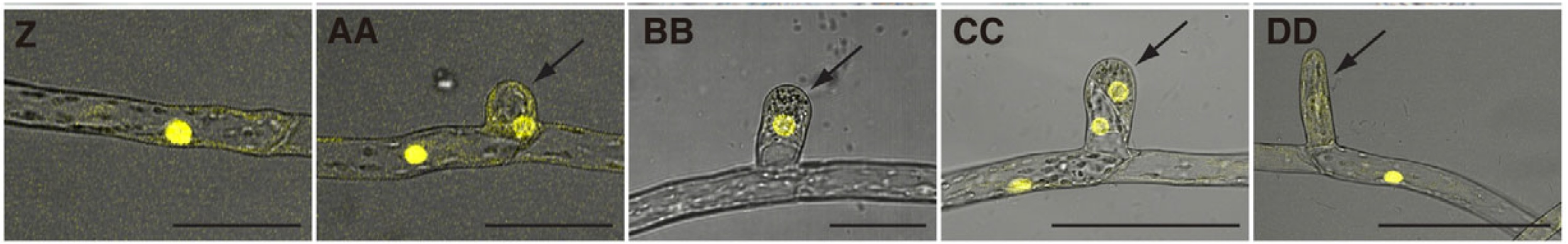


Prigee and Bezanilla, Development (2010)

2- Parental APBs (**A**integumenta, **P**lethora, **B**abyboom) TFs

PpAPBs: Regulators maestros de la transición 2D-3D

Knock In *PpAPB4*$_{pro}$*::PpAPB4-citrina*



Caulonema

Side Branch Initial
and Parental Caulonema

Swollen Gametophore
Apical cell

Gametophore
Apical cell
And its daugther

Seconday protonema
Apical cell

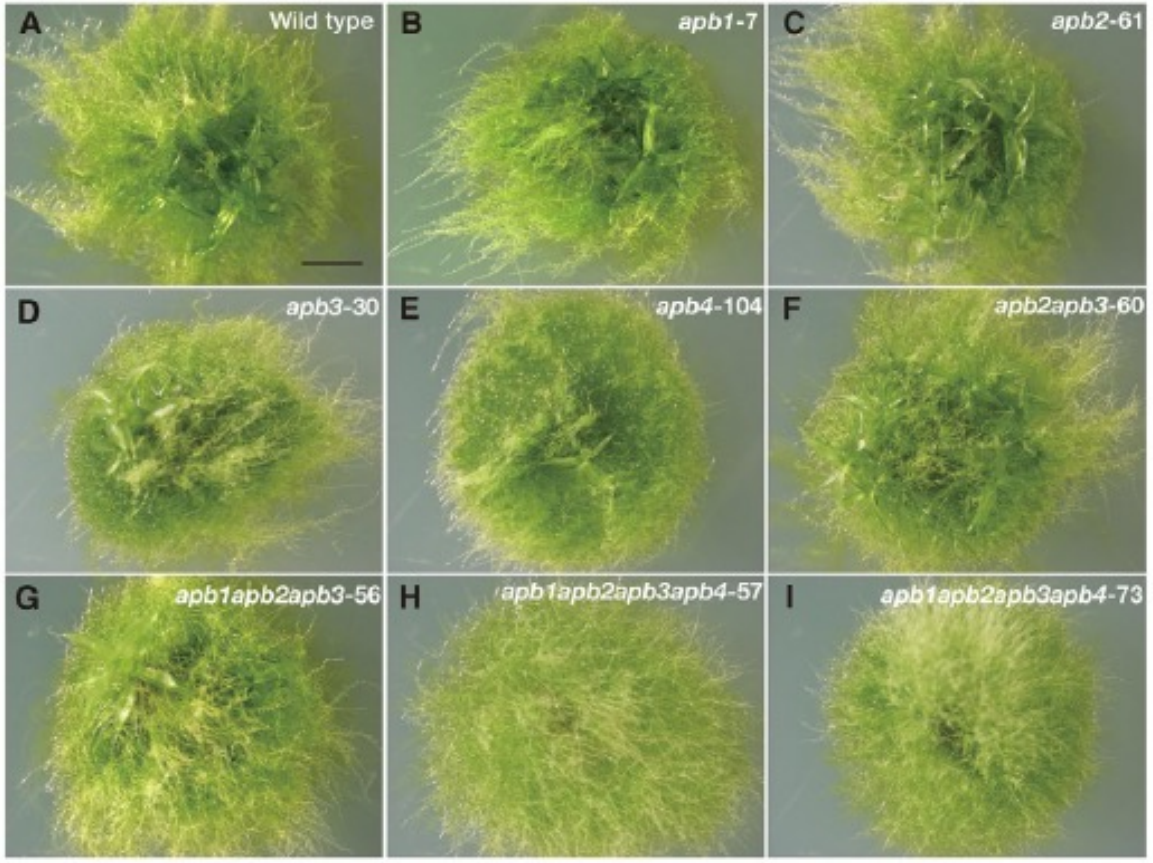
Aoyama et al., Development (2012)

Son regulados positivamente por auxinas

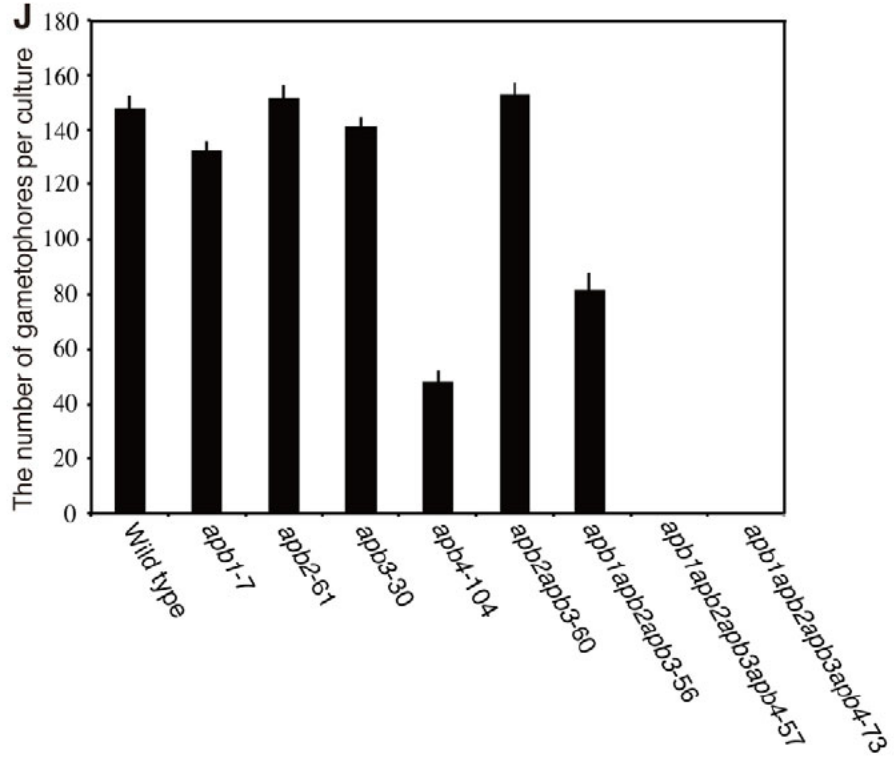
2- Parental APBs (**A**integumenta, **P**lethora, **B**abyboom) TFs

AP2-type transcription factors determine stem cell identity in the moss *Physcomitrella patens*

Tsuyoshi Aoyama[1,2], Yuji Hiwatashi[1,2], Mikao Shigyo[3], Rumiko Kofuji[4], Minoru Kubo[1,5], Motomi Ito[3] and Mitsuyasu Hasebe[1,2,5,*]



PpAPB promuevan la formación de células initiales del gametofito (GIs)

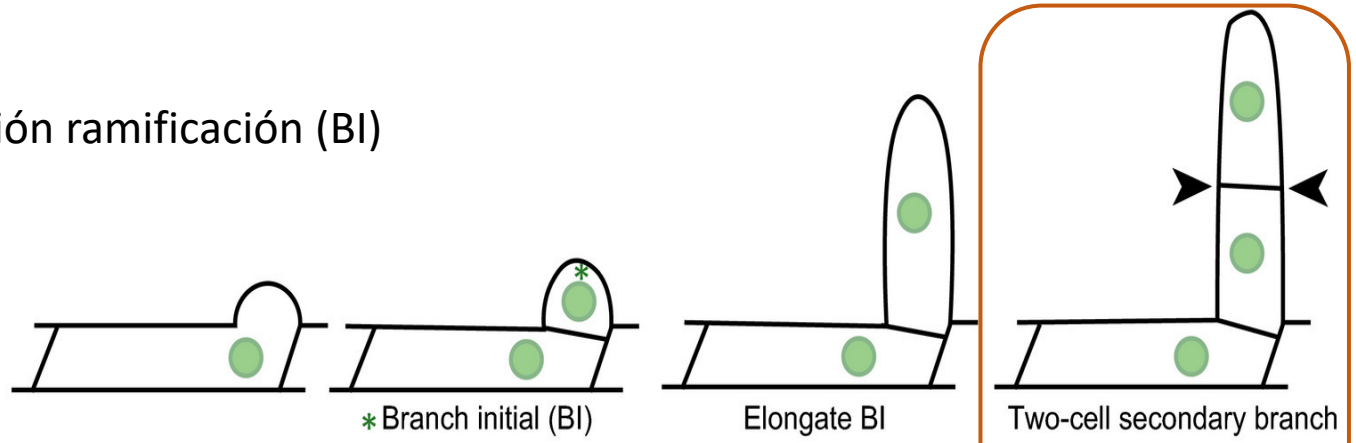


Crecimiento 2D (protonema) o 3D (yemas) en *P. patens*

¿Qué mecanismo regula la orientación del plano de división celular para la transición 3D?

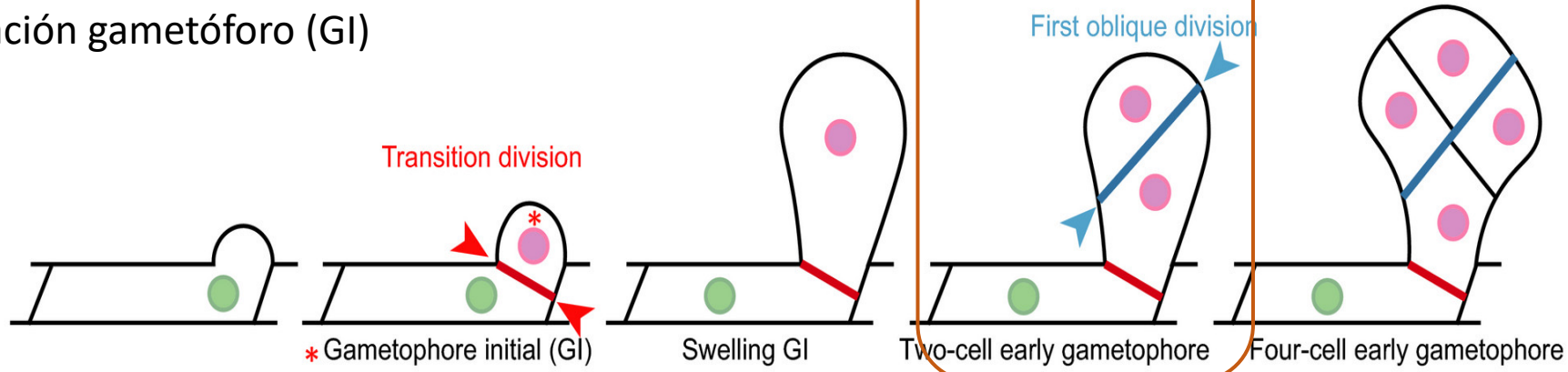
Iniciación ramificación (BI)

Caulonema

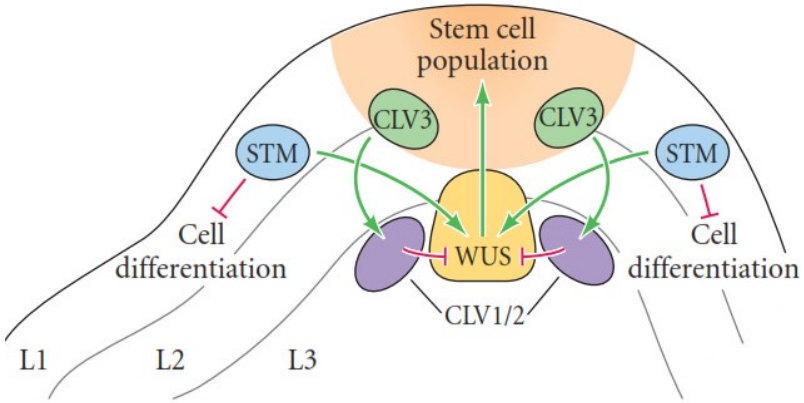


Iniciación gametóforo (GI)

Caulonema

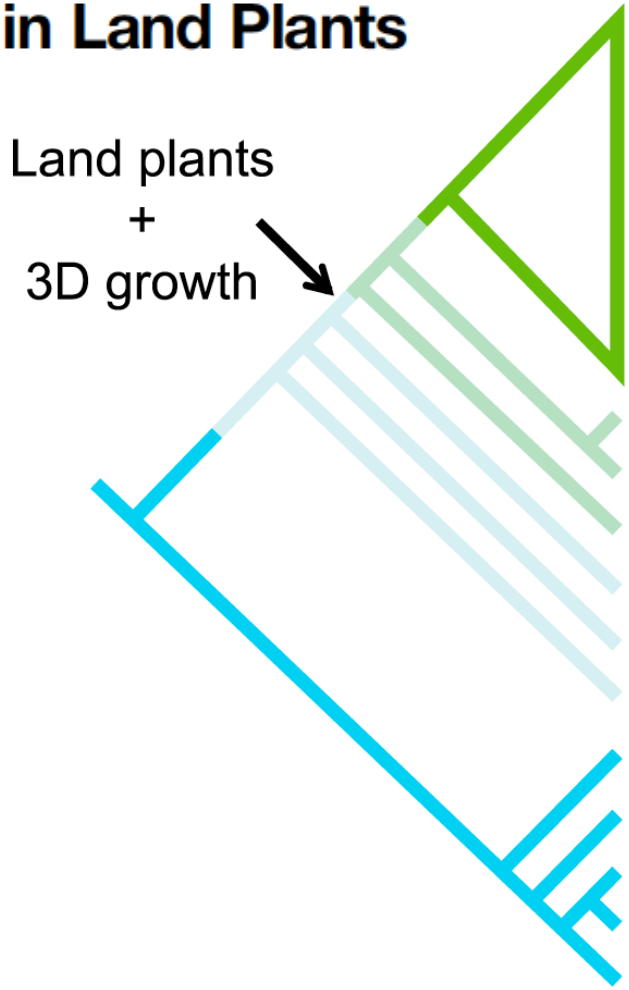


Arabidopsis



3- Clavata

CLAVATA Was a Genetic Novelty for the Morphological Innovation of 3D Growth in Land Plants

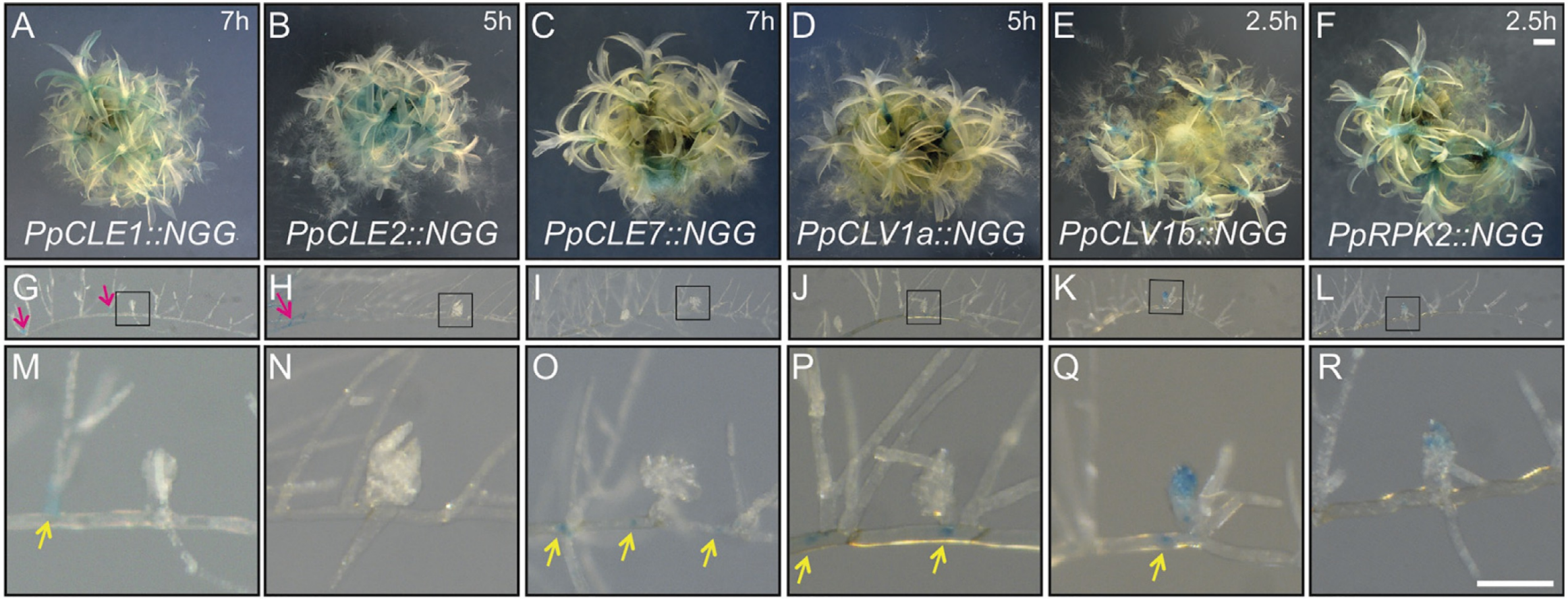


Clade	Species	*CLE*	*TDIF*	*CLV1/BAM*	*RPK2*	*CLV2*	*CRN*
VP	*Arabidopsis thaliana* (G)	32	5	4	1	1	1
	Glycine max (G)	38	6	10	5	2	2
	Oryza sativa (G)	36	3	4	2	1	1
	Amborella trichopoda (G)	8	1	5	2	1	1
	Picea abies (G)	52	17	4	3	1	1
	Diplazium wichurae (T)	2	0	2	2	0	0
	Selaginella moellendorfii (G)	15	4	3	1	0	0
L	*Marchantia polymorpha* (G)	2	1	1	1	0	0
M	*Physcomitrella patens* (G)	7	0	2	1	0	0
H	*Anthoceros agrestis* (G)	1	1	1	0	0	0
Cha	*Coleochaete nitellarum* (T)	0	0	0	0	0	0
	Spirogyra sp. (G)	0	0	0	0	0	0
	Chara braunii (G)	0	0	0	0	0	0
Chl	*Ostreococcus tauri* (G)	0	0	0	0	0	0
	Chlorella vulgaris (G)	0	0	0	0	0	0
	Volvox carteri (G)	0	0	0	0	0	0
	Chlamydomonas reinhardtii (G)	0	0	0	0	0	0
	Ulva spp. (T)	0	0	0	0	0	0

Whitewoods et al., Current Biology (2018)

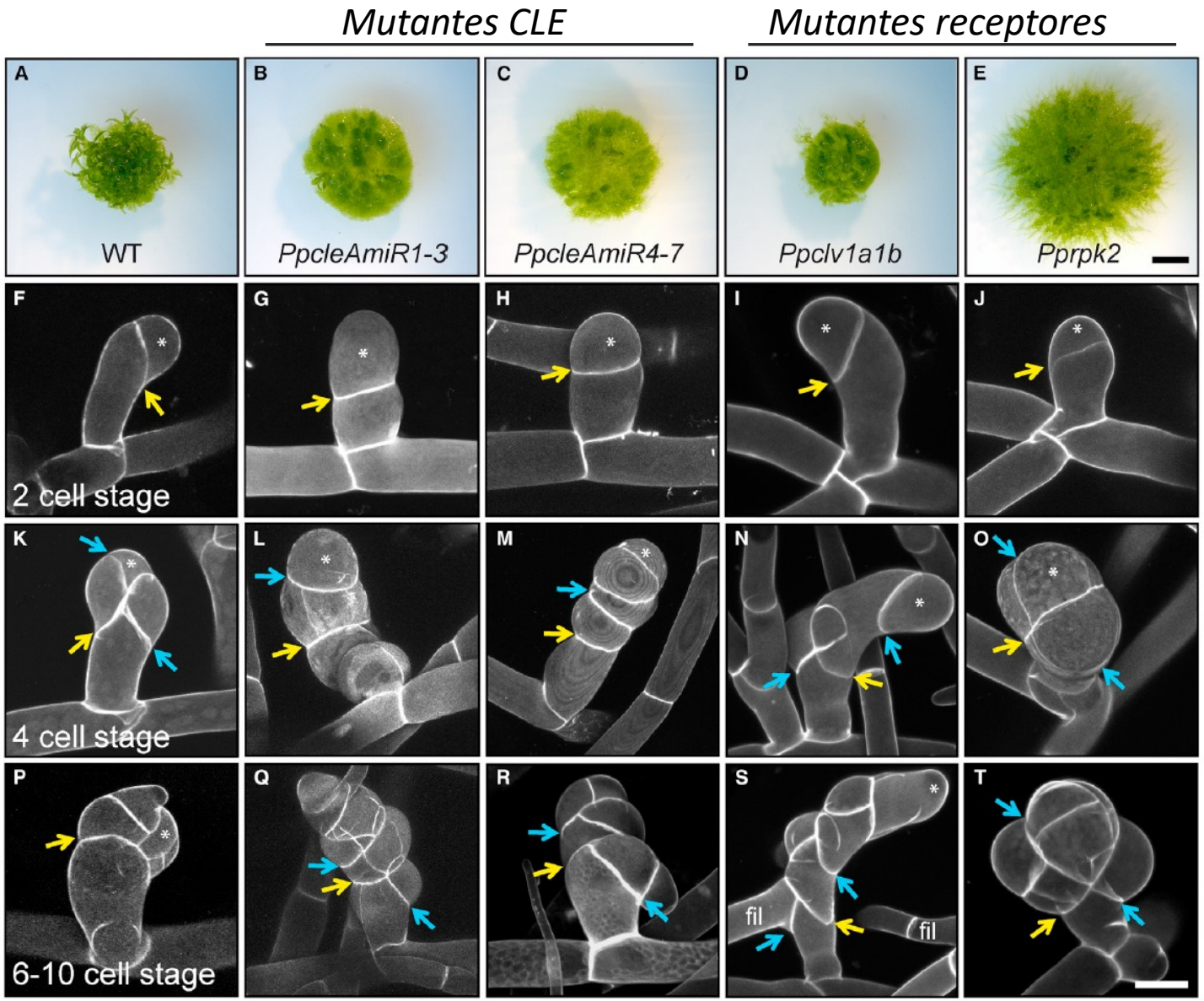
Expresión CLE-Clavata principalmente en crecimiento 3D

Promoter::NLSGFPGUS (promoter::NGG) fusion lines for PpCLE1, PpCLE2, PpCLE7, PpCLV1a, PpCLV1b, and PpRPK2



Whitewoods et al., Current Biology (2018)

Mutantes *clv* tienen defectos en la transición 2D-3D



Whitewoods et al., Current Biology (2018)

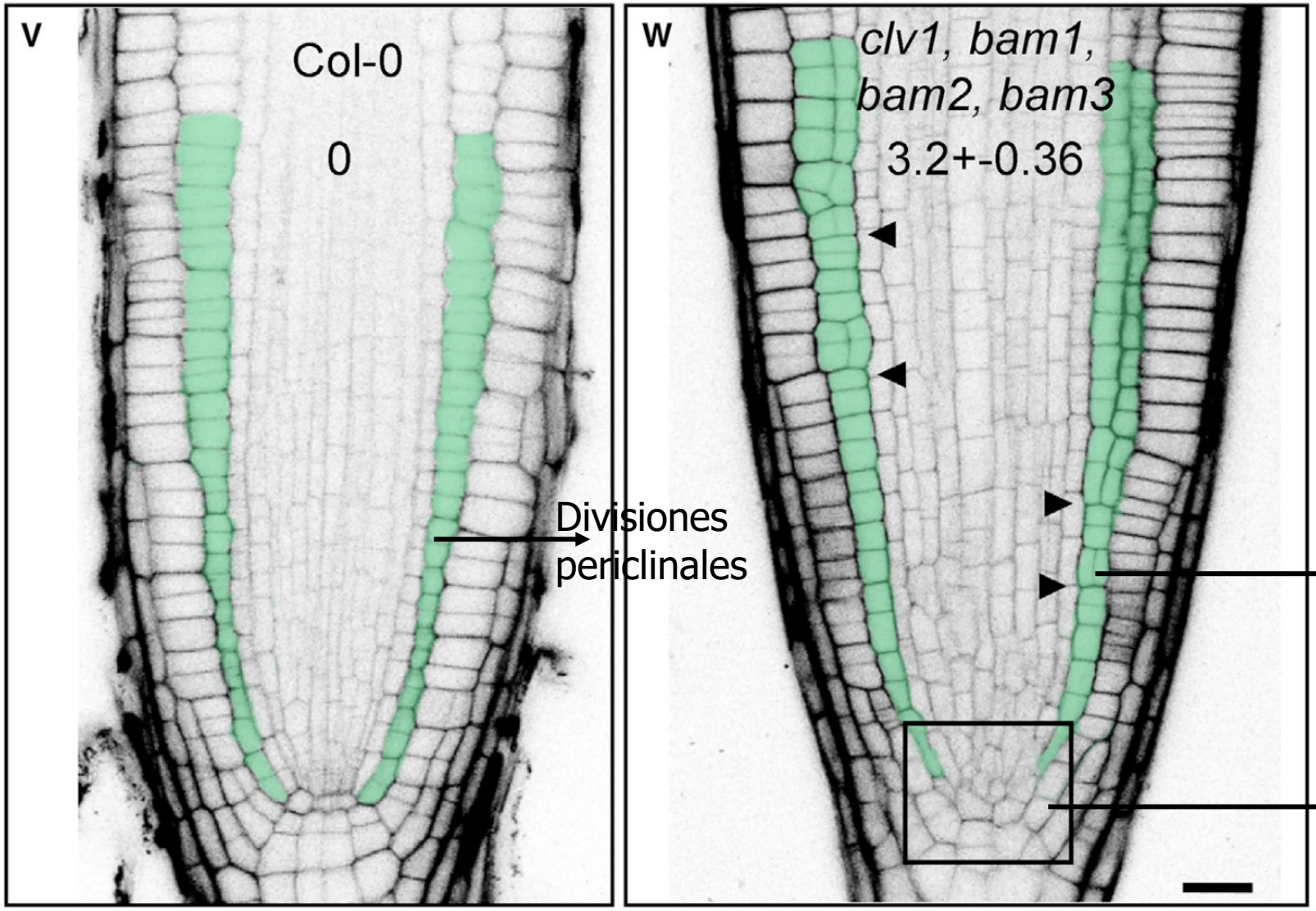
Relatively normal 2D growth phase

3D Plane orientation defects in the first division

↓

WT and mutant gametophore development diverge at the single-cell-stage after cell fate is specified

CLV orienta el plano de division celular en Physcomitrella y Arabidopsis

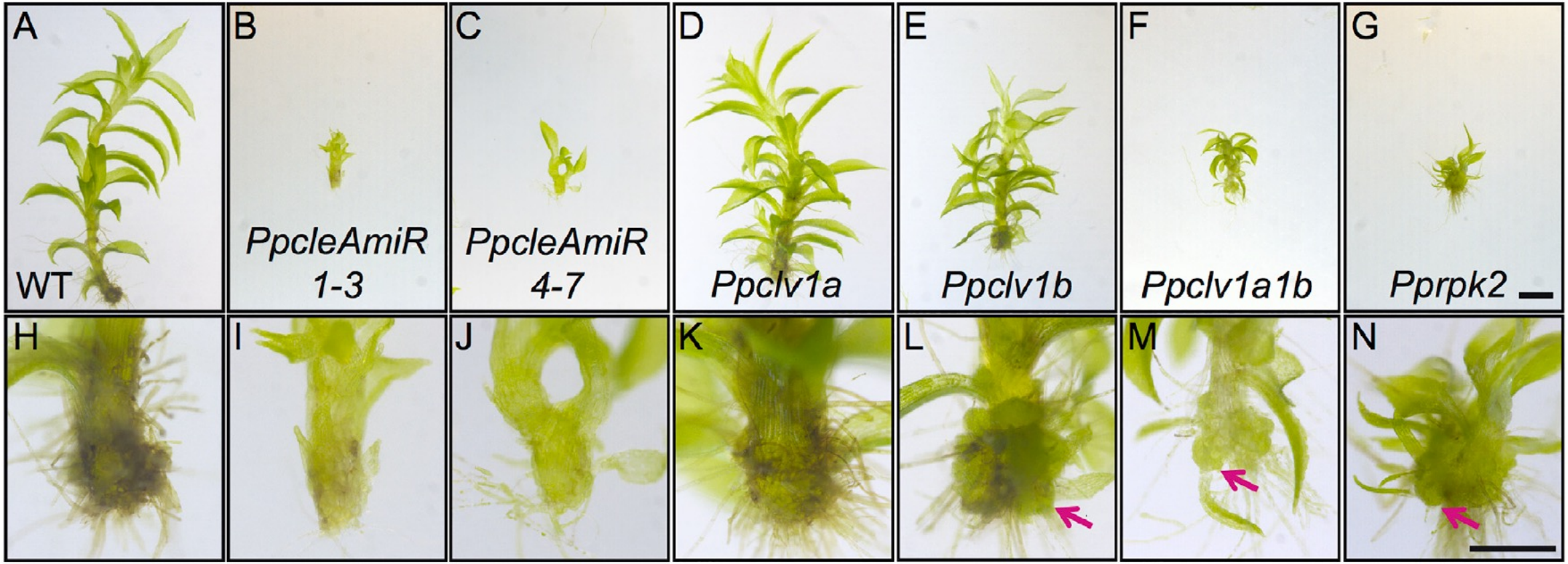


CLV role in cell division plane orientation is conserved between Physcomitrella and Arabidopsis.

Whitewoods et al., Current Biology (2018)

Mutantes *clv* tienen defectos en el desarrollo de gametóforos

All mutants have a strong cell Fate and/or proliferation defects, **developing a callus-like mass at the gametophore base, inability to differentiate**.

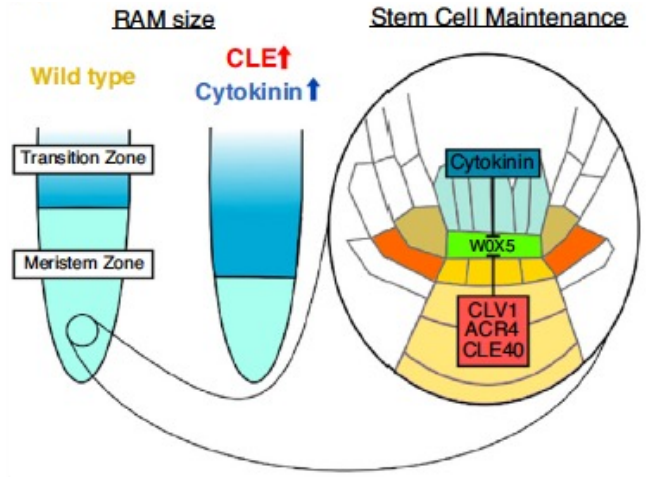


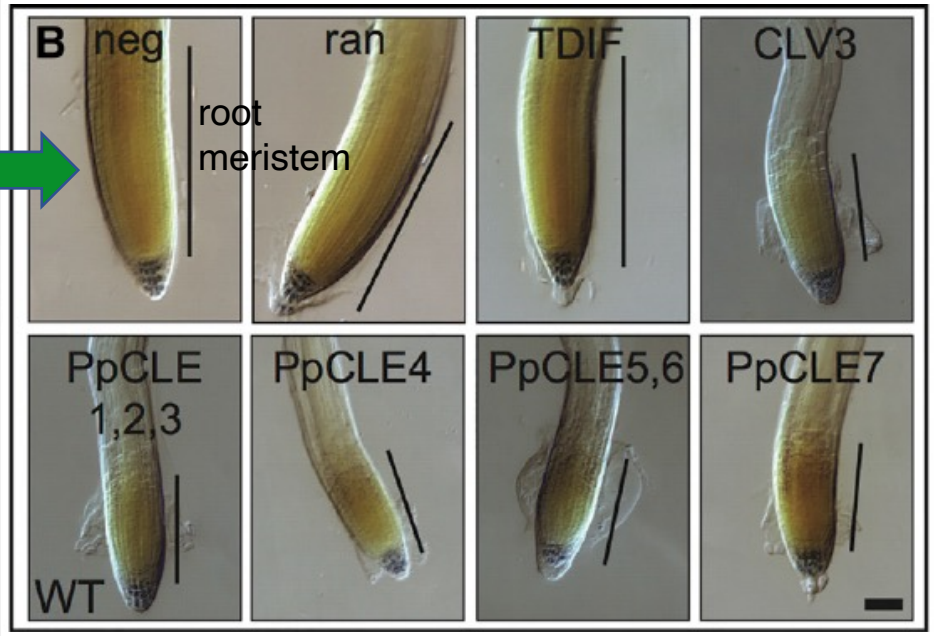
Whitewoods et al., Current Biology (2018)

Los peptidos CLE actuan sobre receptores conservados *Pp* y *At*.

La adición de péptidos CLV3/PpCLEs arresta la función meristemática radical en Arabidospsis

CLE → Cytokinin → Difer.





Whitewoods et al., Current Biology (2018)

Relevancia de estudios de Clavata en *Physcomitrium patens*

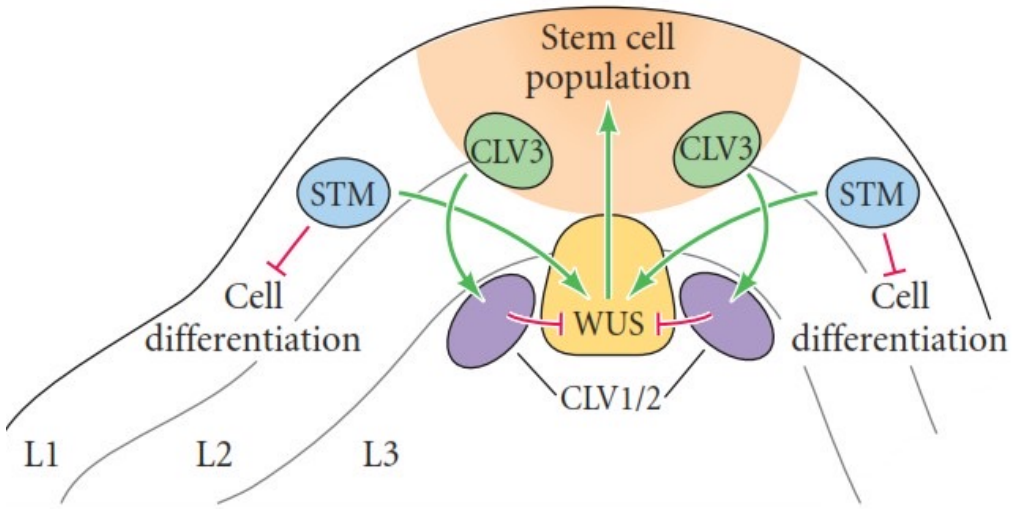
- La vía CLV se originó con las plantas terrestres.

- La capacidad adquirida por las plantas para **orientar las divisiones de células madre en múltiples planos permitió la diversificación** al posibilitar que las plantas desarrollaran ejes verticales con órganos dispuestos en múltiples orientaciones, un paso crucial en la evolución del brote (shoot).

- La proliferación y función de las células madre regulada por CLV (SAM) es probable que sea una característica ancestral de las plantas terrestres. En briófitas CLAVATA actúa independiente de WUSCHEL-like (WOX).

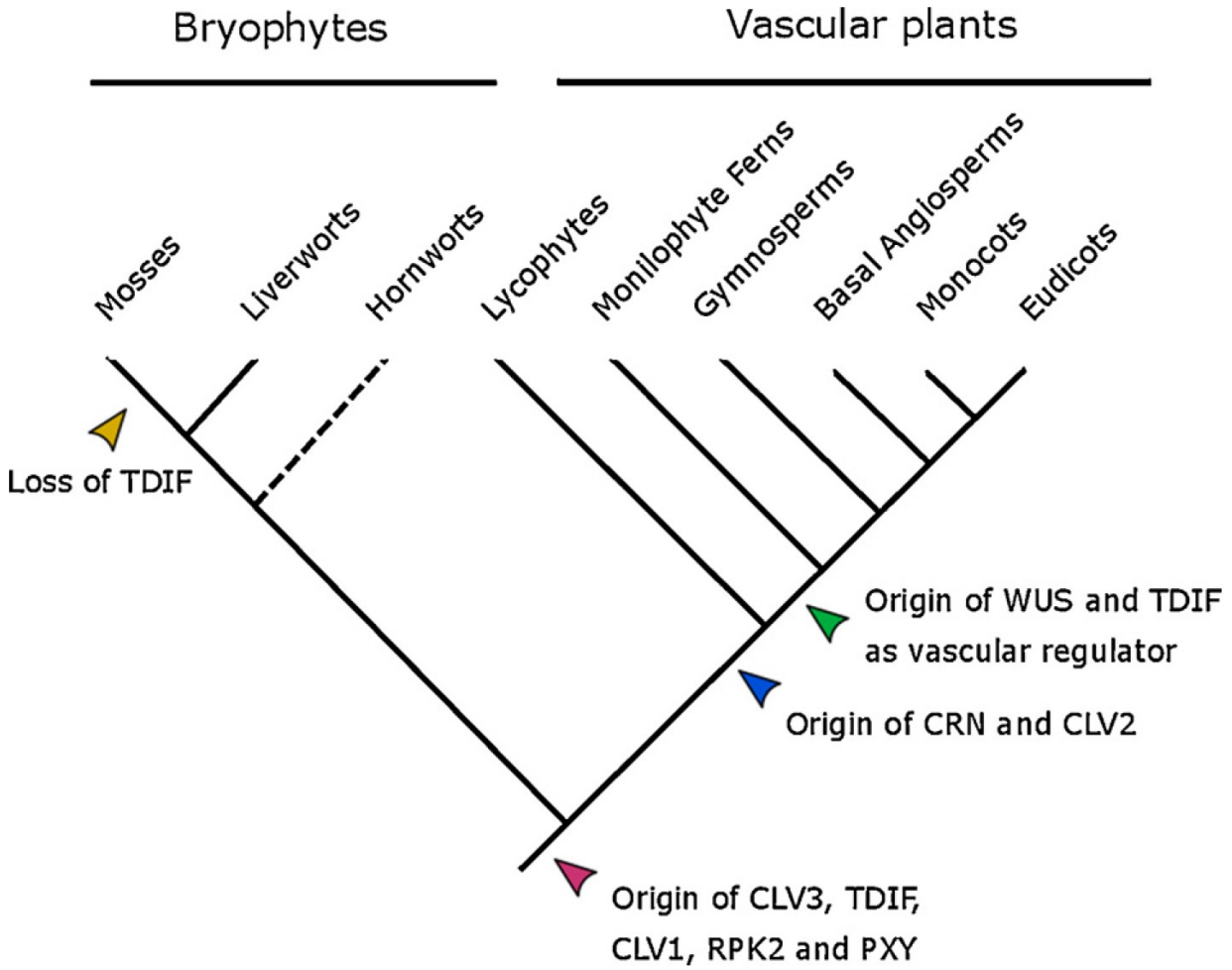
Evolución de redes génicas regulatorias: *CLV-WUS*

Arabidopsis



WUS (KNOX type I):

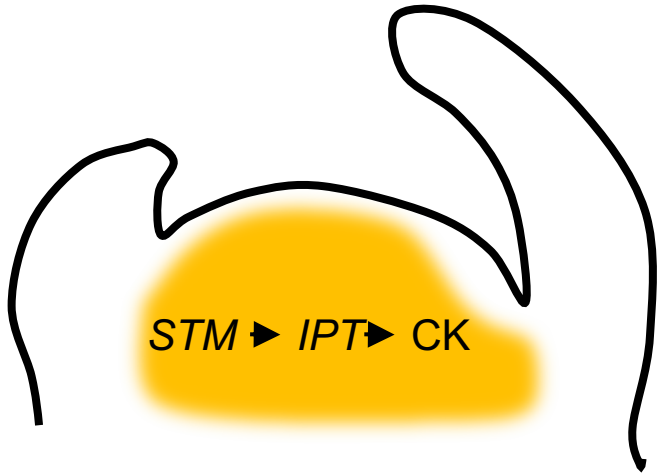
- Promotes Stem Cell identity
- Direct target of CKK signaling
- Ausente en briófitas, evoluciona en plantas vasculares



Genes *KNOX I* en *P. patens: MKN2, MKN4, MKN5* en esporofito

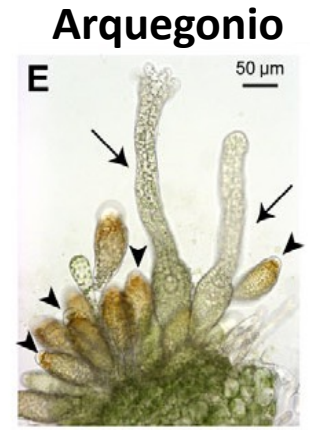
Arabidopsis

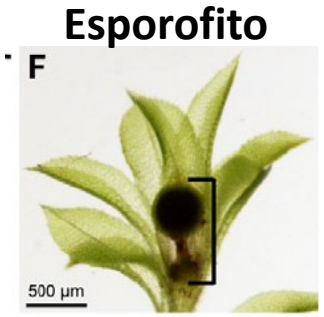
Los genes *KNOX1* actúan en parte estimulando la síntesis de CKs

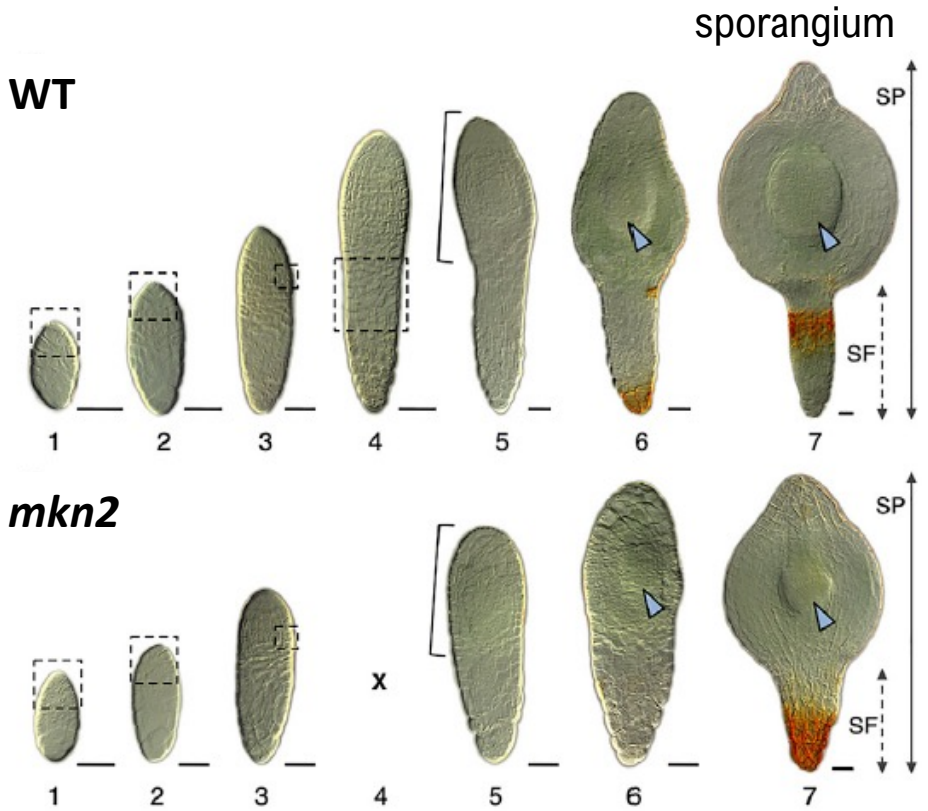


Regulan la proliferación del meristema
Indeterminación del meristema

PpMNK2* se expresa durante la fase esporofítica en *P. patens







Coudert et al., Current Biology (2019)

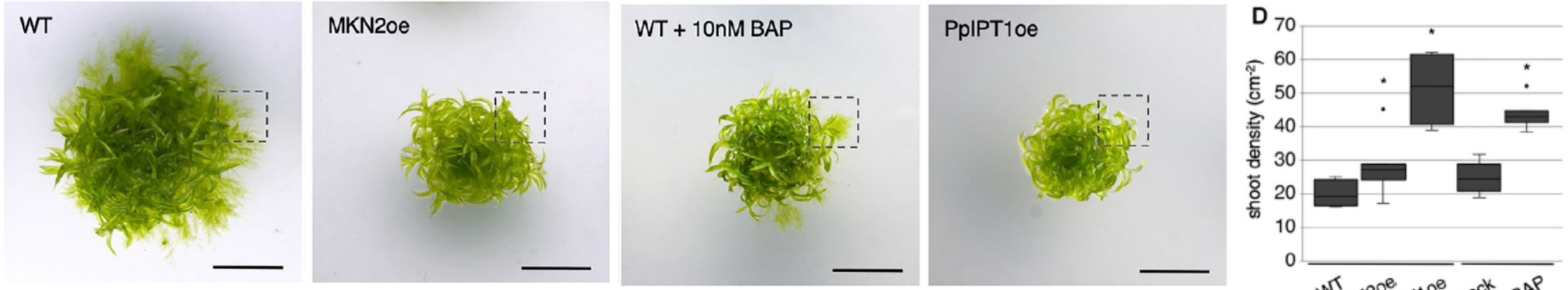
4: proliferative activity in an intercalary region

***PpMKN2* promueve la división de la región intercalar del esporofito**

Genes *KNOX I* en *P. patens*: *MKN2* y citoquininas

Sobreexpresión *MKN2*

Sobreexpresión *IPT:* CK synthesis

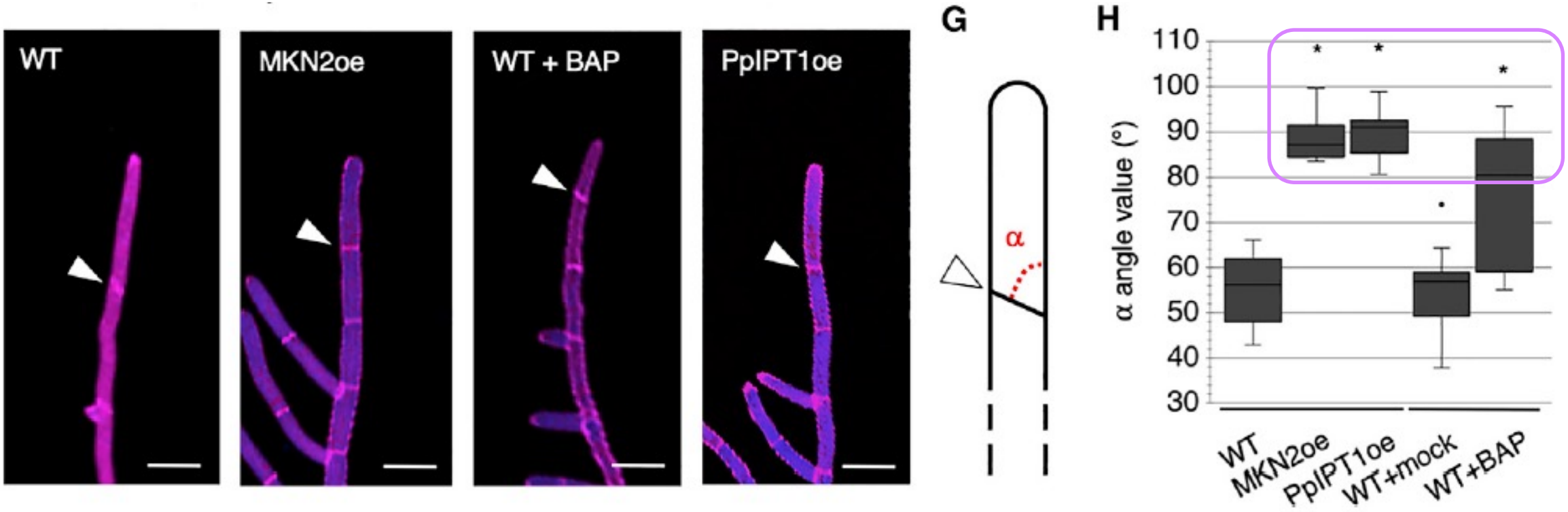


Coudert et al., Current Biology (2019)

Plant spread was reduced to about 40% of the WT area

…. Defective chloronema-to-caulonema transition??

Genes *KNOX I* en *P. patens*: *MKN2* y citoquininas

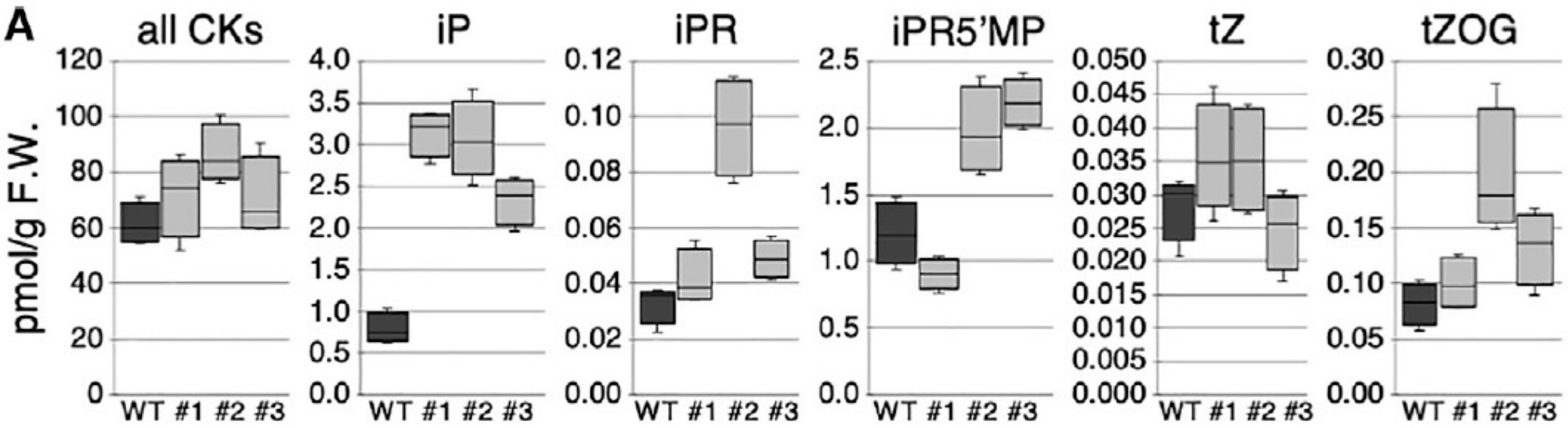


Coudert et al., Current Biology (2019)

Ectopic MKN2 expression in gametophytes suppresses the normal switch from chloronema to caulonema identity! ……

Perturbations in cytokinin levels??

Expresión ectópica de *MNK2* acumula citoquininas

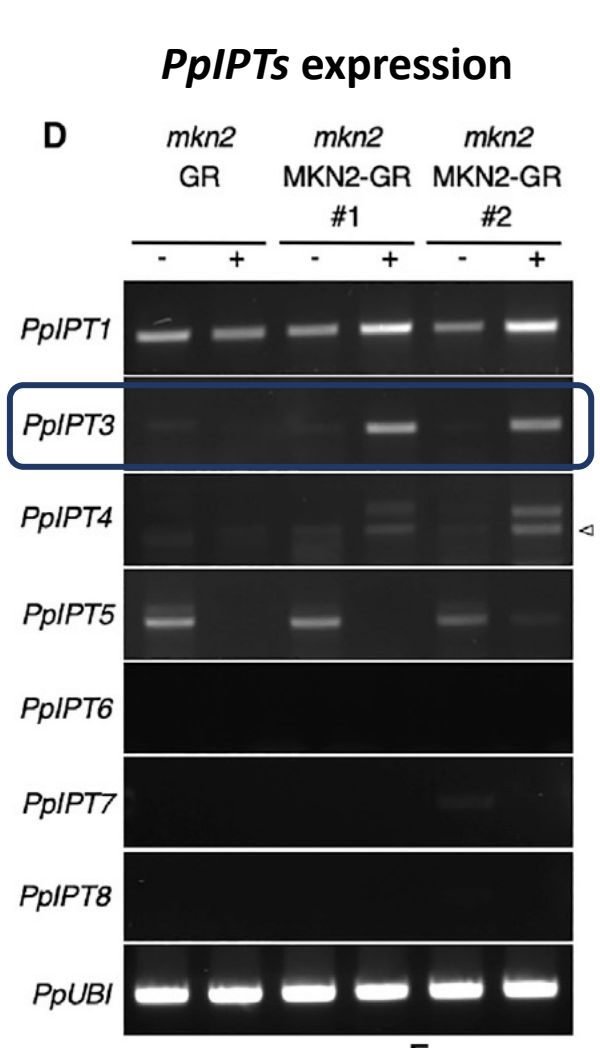


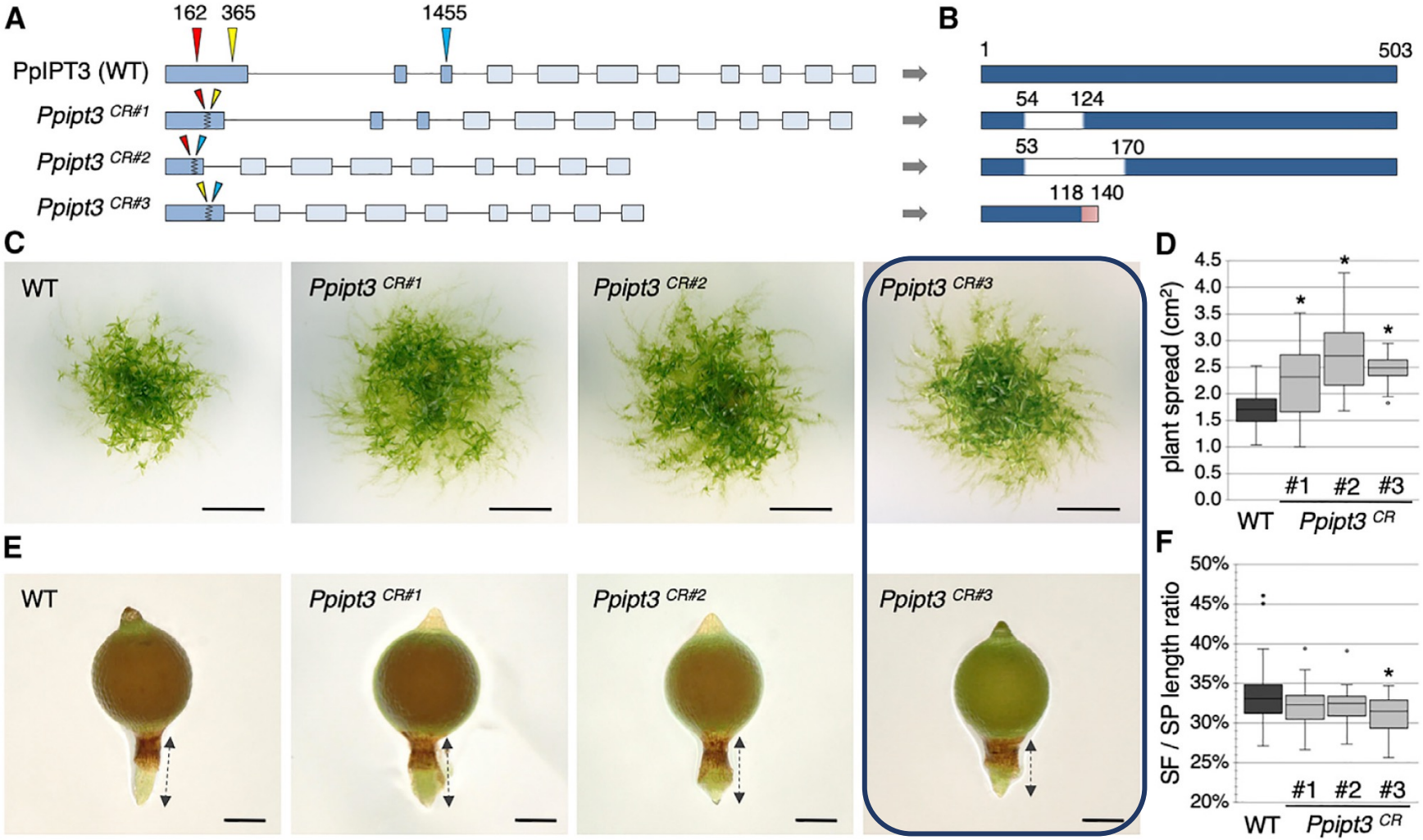
Coudert et al., Current Biology (2019)

Cytokinin (CK) profiling showed a global **increase in CK levels in three independent *MKN2* oe transgenic lines relative to WT** controls…..

***PpMKN2* acts via *PpIPTs*???**

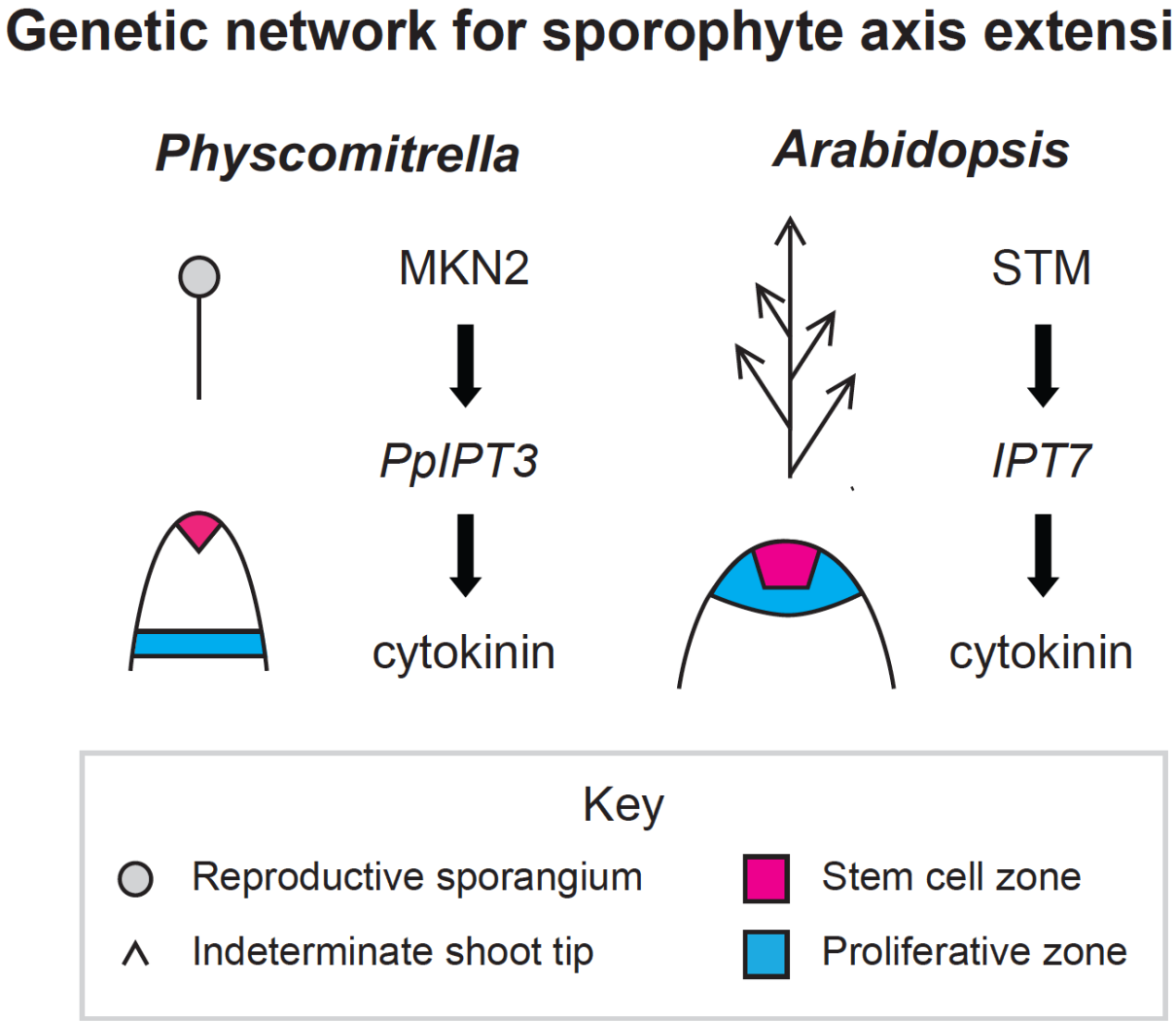
PpIPT3 Promueve la Extensión Axial del Esporofito de *P. patens*





Coudert et al., Current Biology (2019)

Evolución de los Meristemas: crecimiento indeterminado

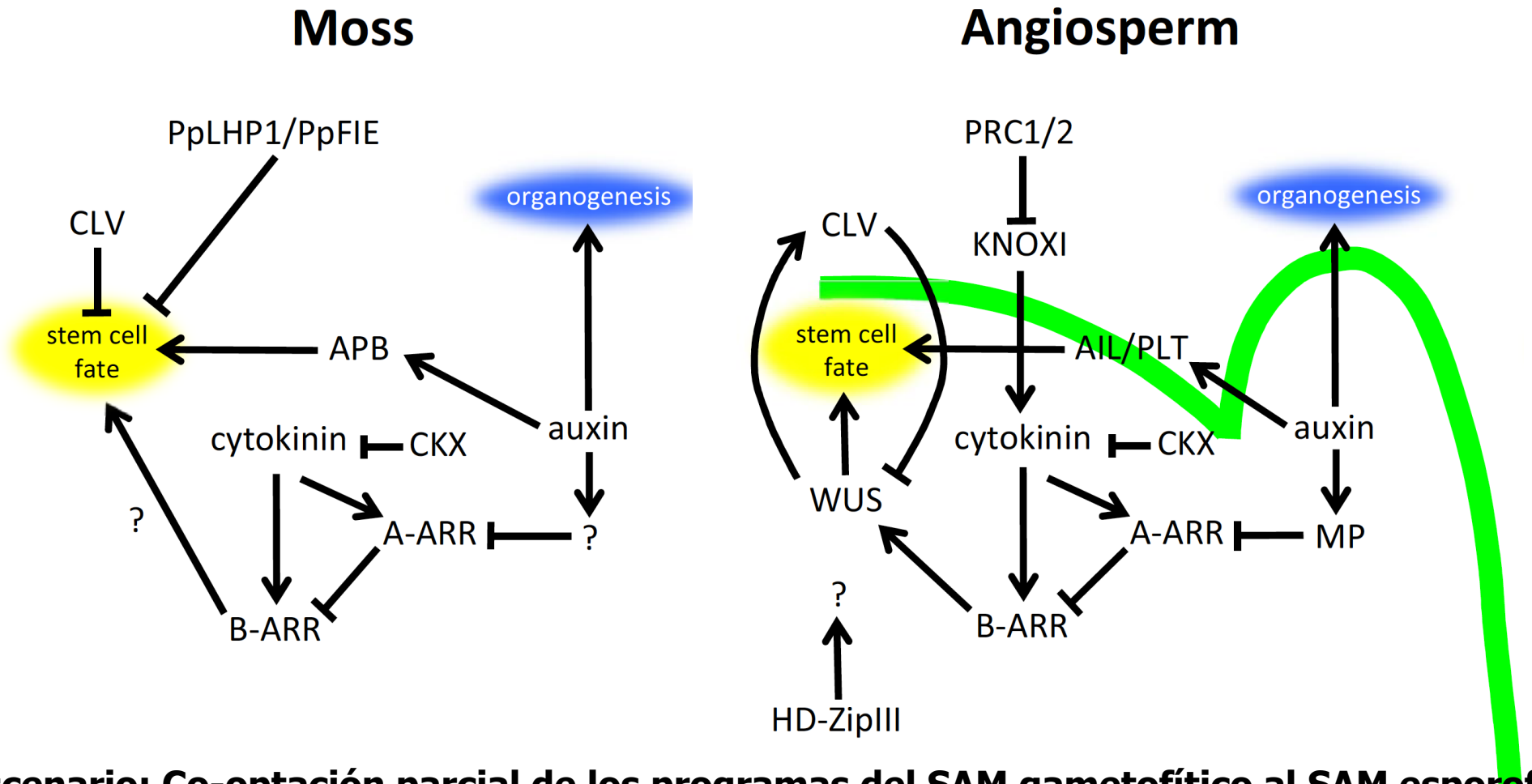


The activation of CK biosynthesis by a class I KNOX protein in Physcomitrella suggests that a KNOX-cytokinin regulatory module is conserved between vascular plants and mosses.

Vascular plant KNOX-cytokinin-regulated meristem functions may have derived directly from a functional unit operating in their ancestors.

There is genetic homology between the proliferative zones of vascular plant meristems and the intercalary region of moss setae.

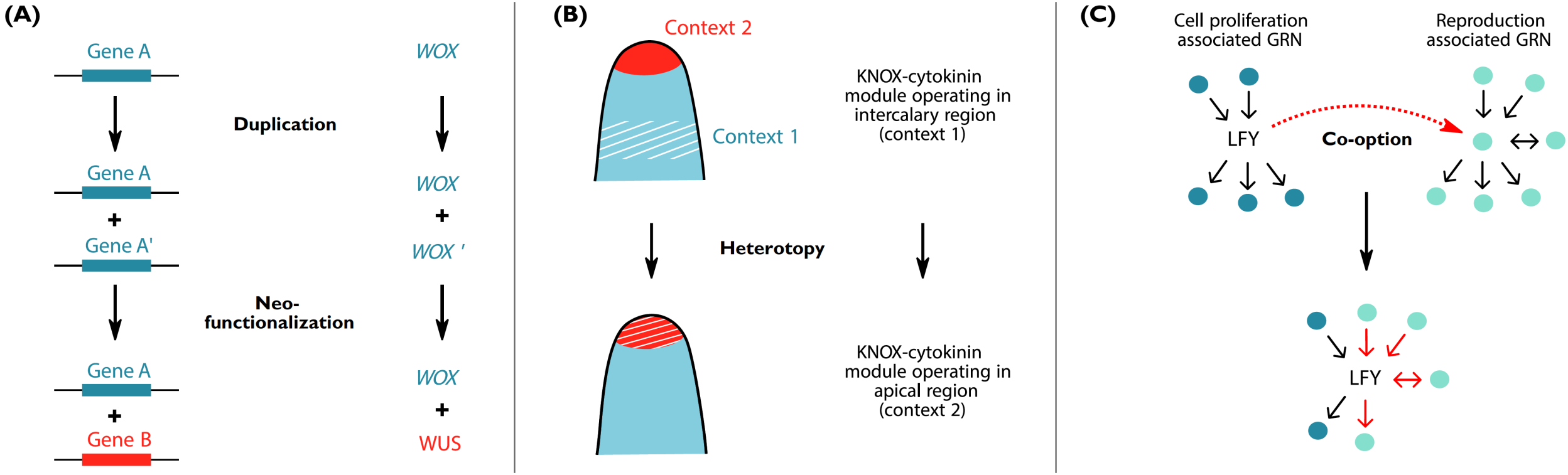
Rutas de señalización para el establecimiento y mantenimiento de células madre pluripotentes en SAM



Posible escenario: Co-optación parcial de los programas del SAM gametofítico al SAM esporofítico y el reclutamiento de reguladores adicionales en las líneas de plantas vasculares.

Evolutionary mechanisms underpinning meristem diversification in land plants

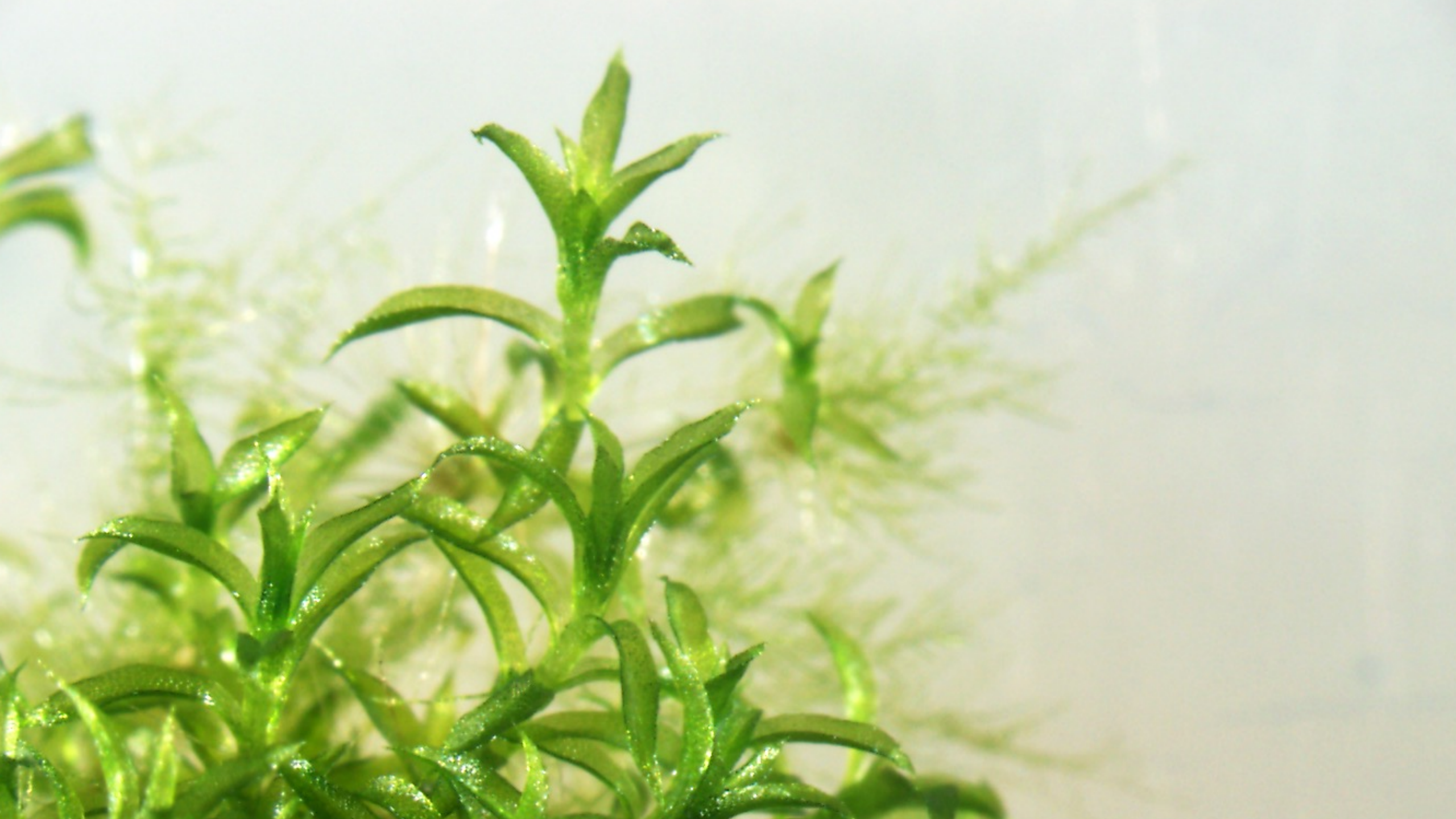
Elaboration of new modules



CLE-CLV en gametofito Pp
CLE-CLV-WUS regulan tamaño SAM esporofito Aa

KNOXI (MKN2, IPT3) meristema intercalar esporofito Pp
KNOX I (STM, IPT7) en SAM Aa

LFY bloquea 1 división celular cigoto Pp
LFY acriva genes homeóticos para los órganos florales en Aa



KNOX1/KNOX2 gene duplication and neofunctionalization – instrumental in the establishment of alternation of generations in land plants

Gene duplication: key drivers in generating ***evolutionary novelty***. Following gene duplication, paralogs can undergo a process of **neofunctionalization**, supplying a genetic basis for **morphological novelty**.