

Branching out

Auxin, auxin transport and
the self-organisation of plant form

Ottoline Leyser

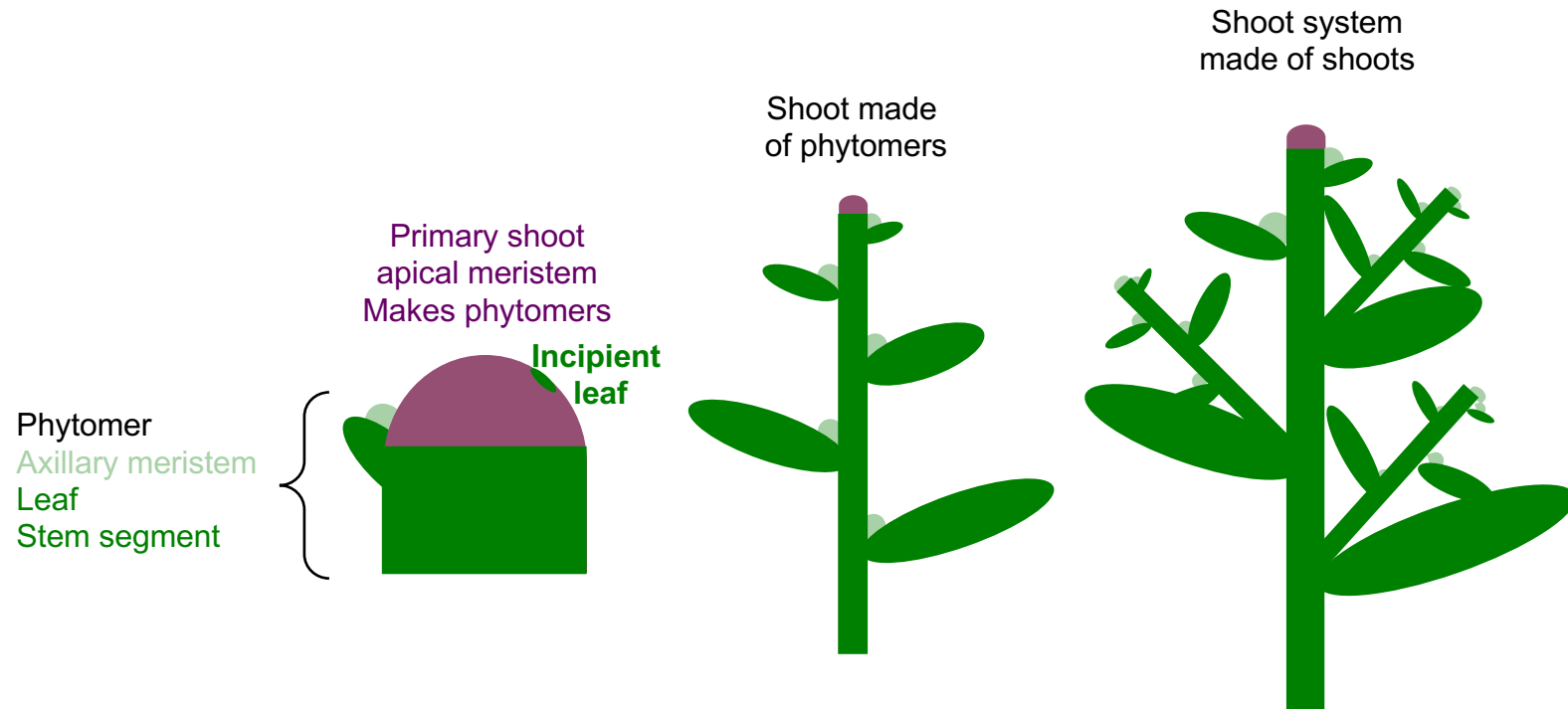


Immobility- a dual problem



A sitting duck in a changing environment

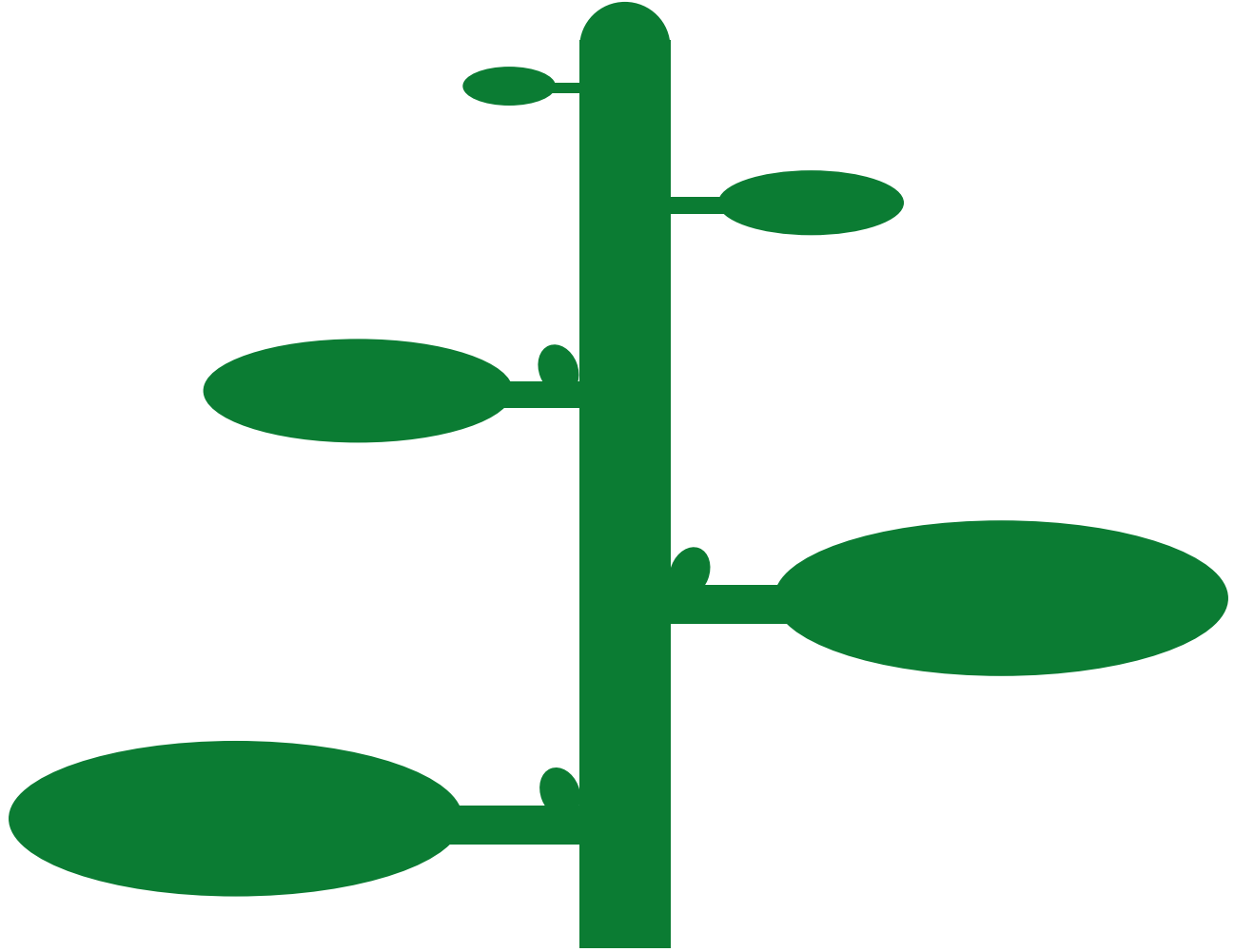
Plant development is modular

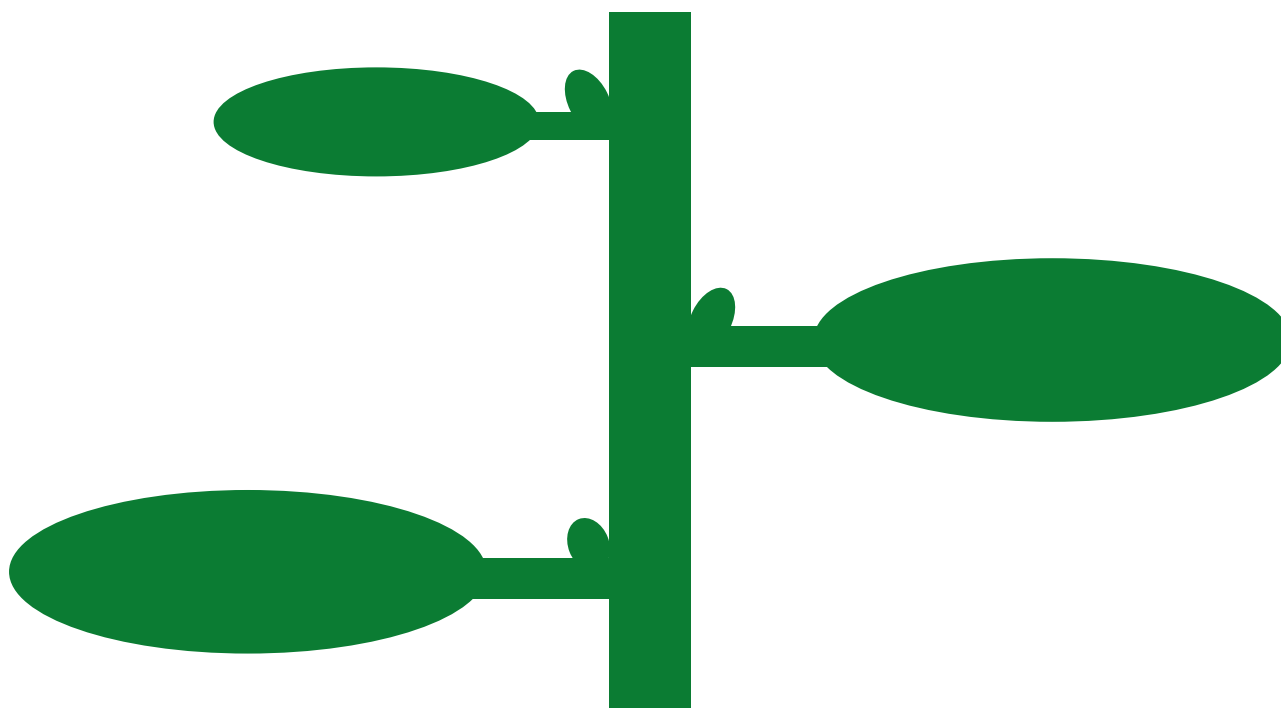


Plant development is plastic

Distributed decision making





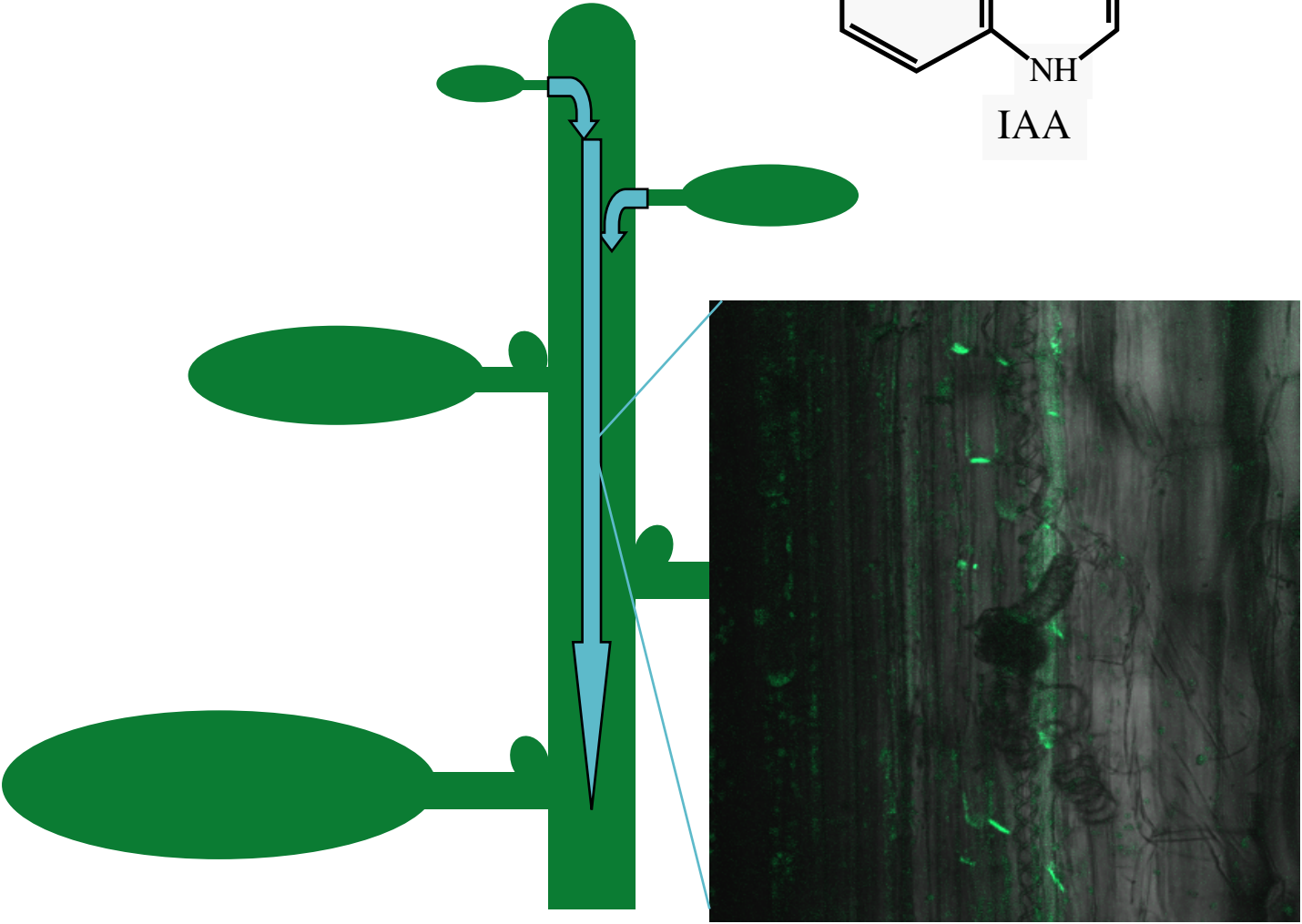
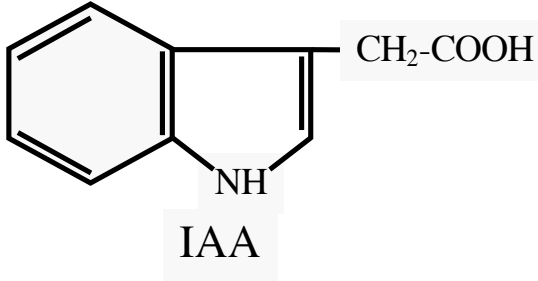


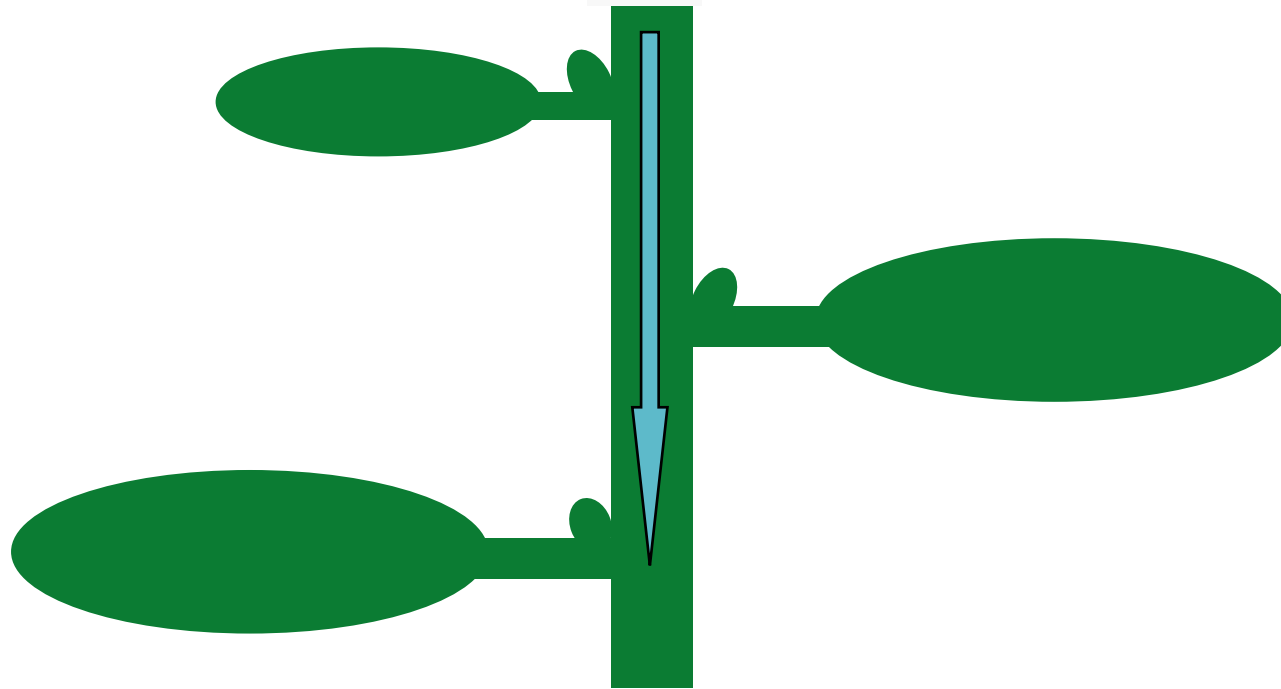
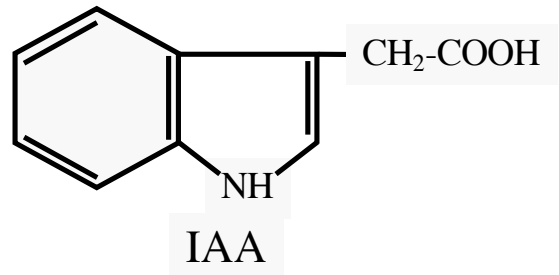
Apical dominance



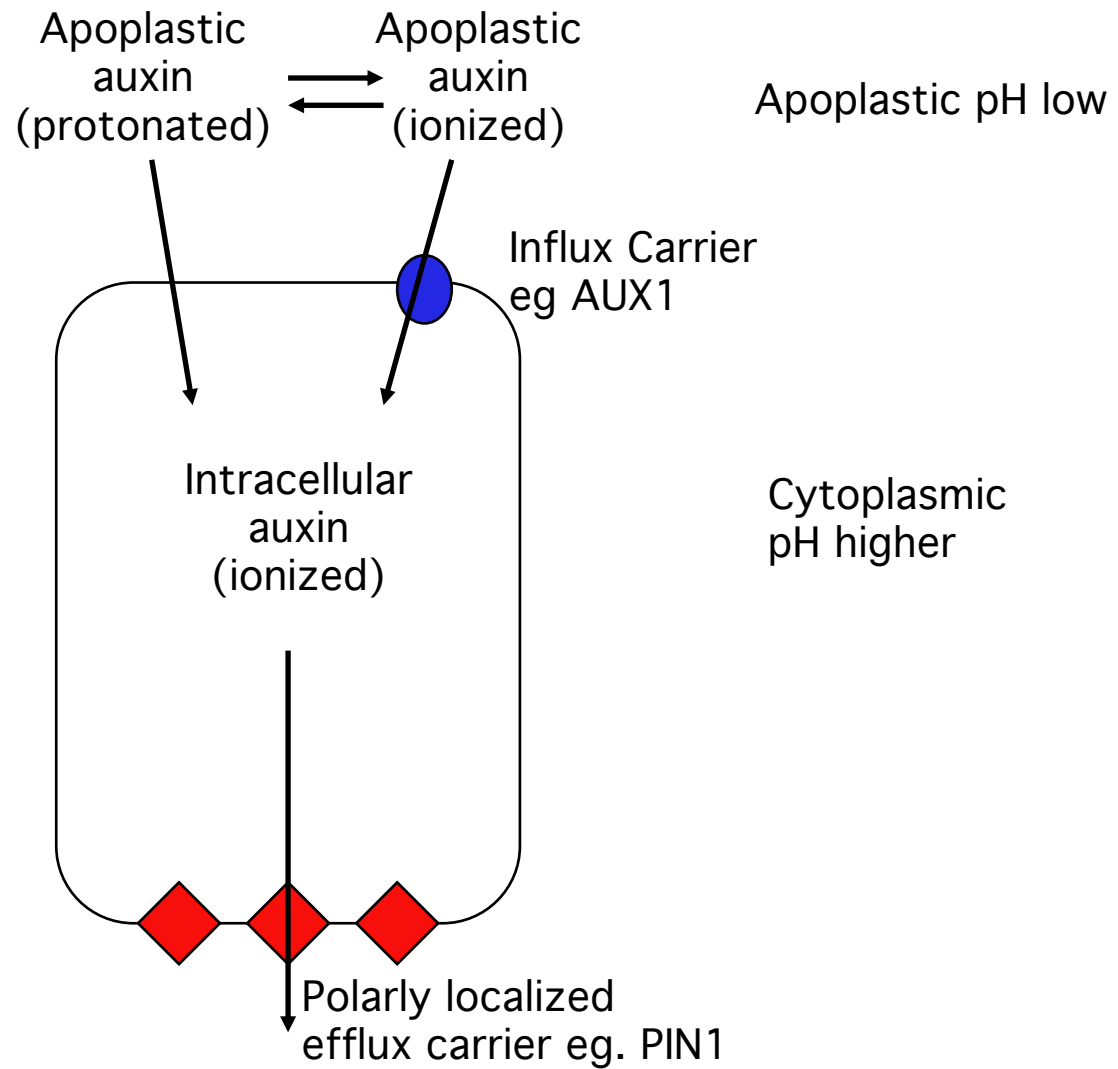
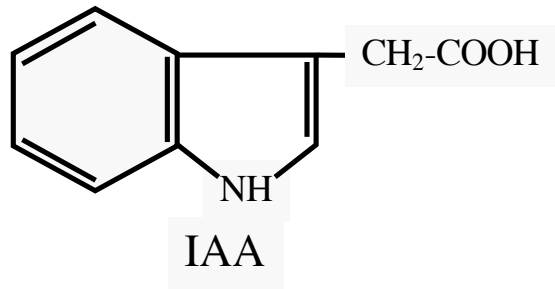


Auxin



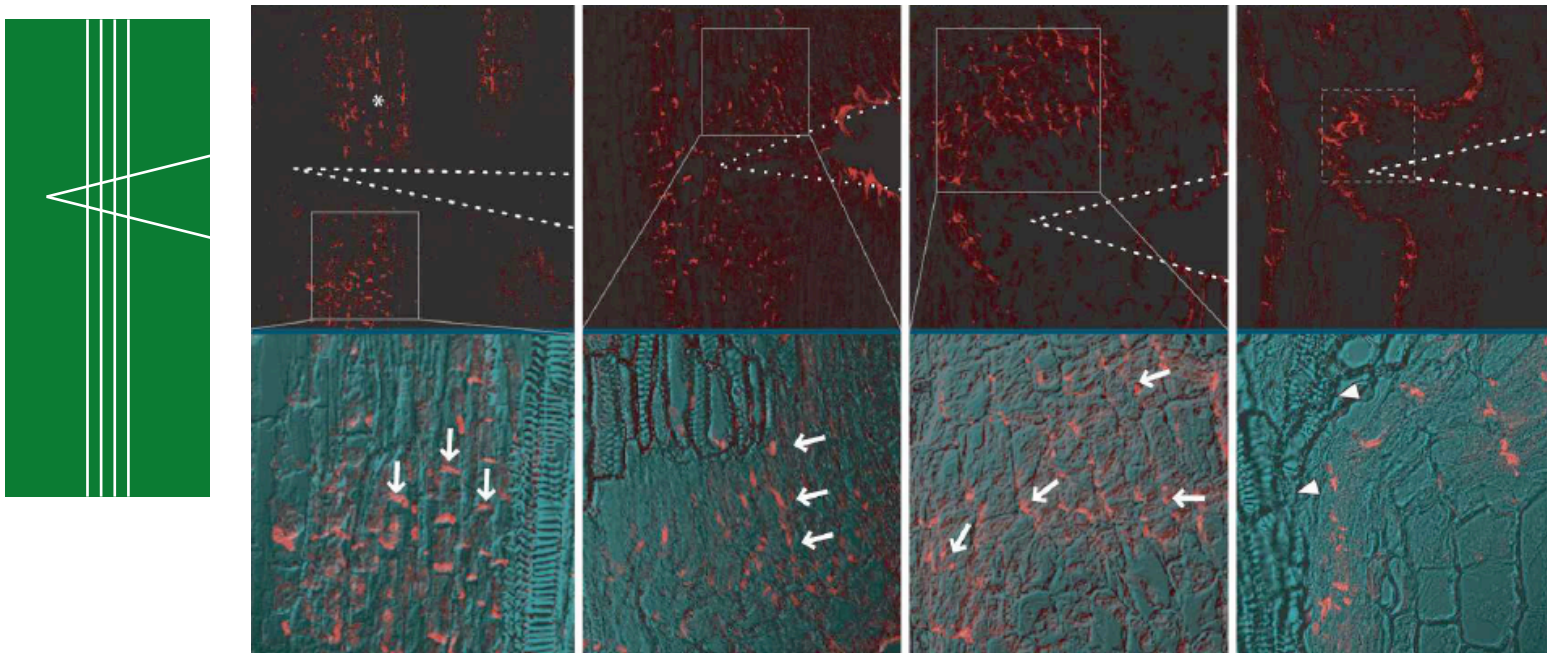


Directional Auxin Transport



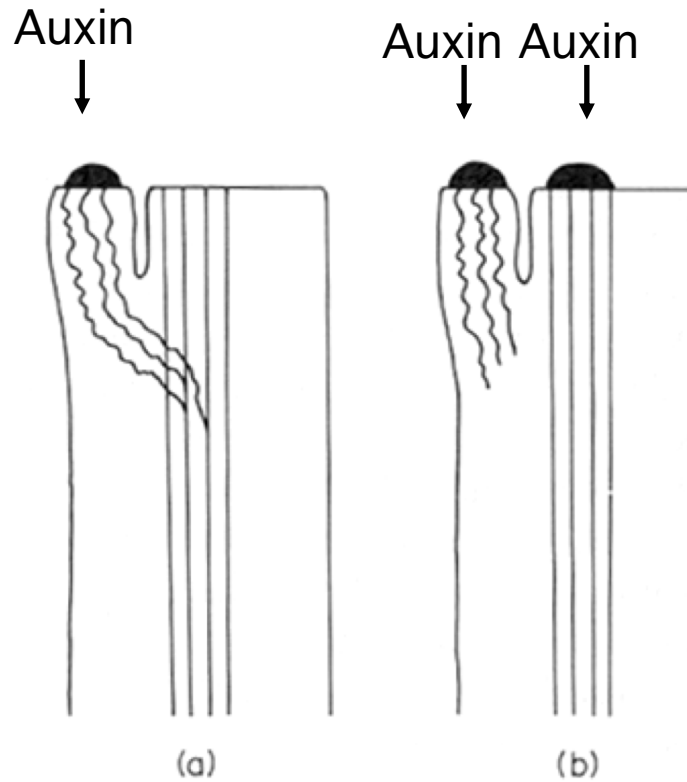
Auxin transport is self-organizing

- Auxin up-regulates and polarizes its own transport
- This is an important factor in wound healing

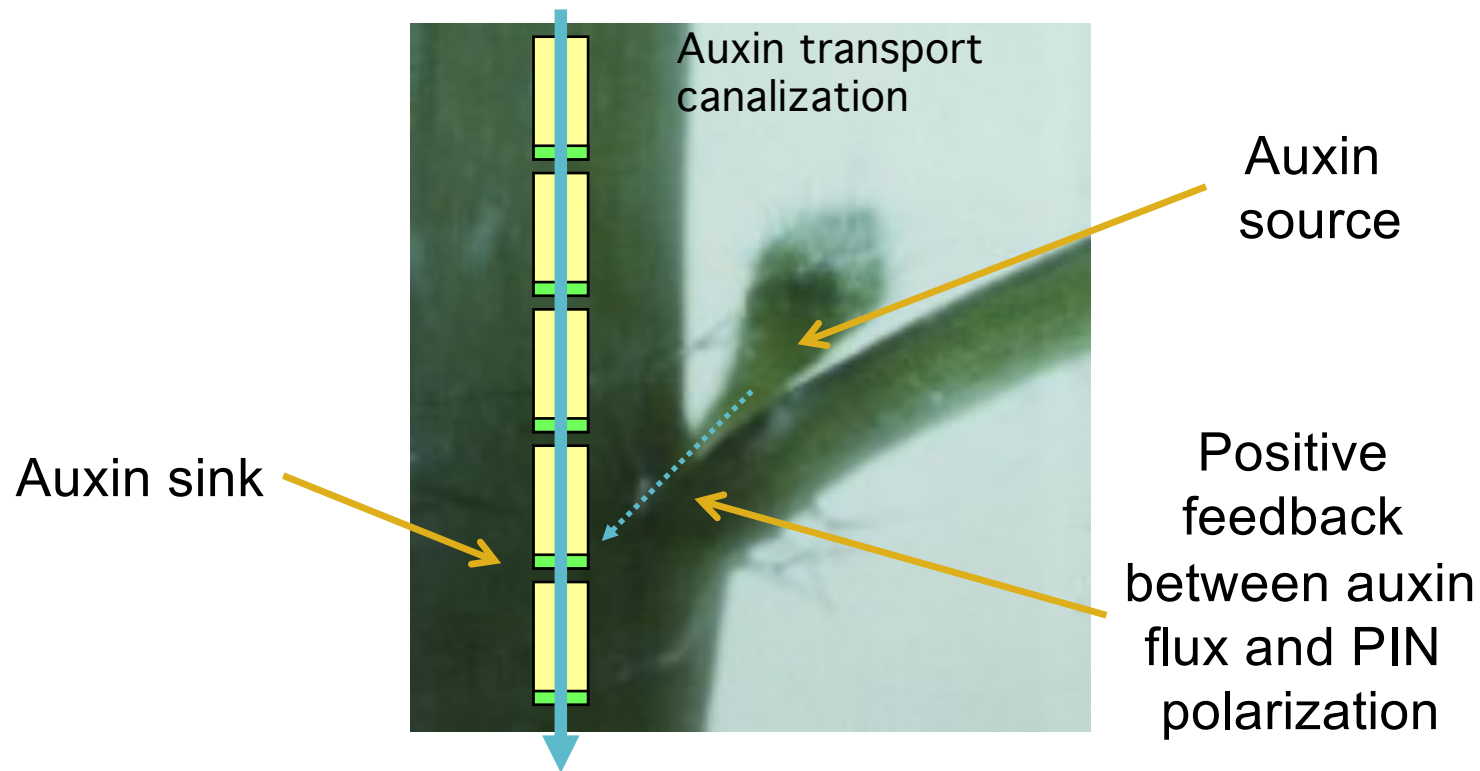


Saur et al G&D 20:2902

Auxin transport canalizes toward auxin sinks

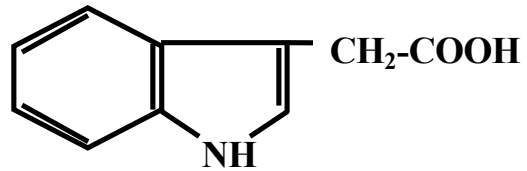


Sachs, 1984
Decapitated pea epicotyls



Hypothesis: For sustained activation, buds must establish canalized auxin transport from the bud apex into the stem

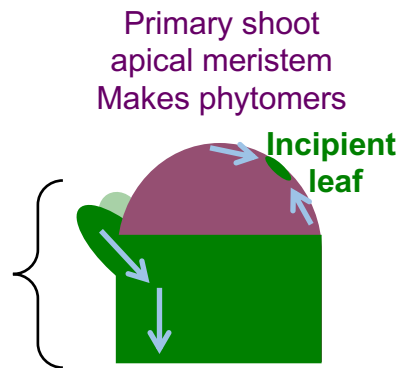
Auxin flow in the shoot system



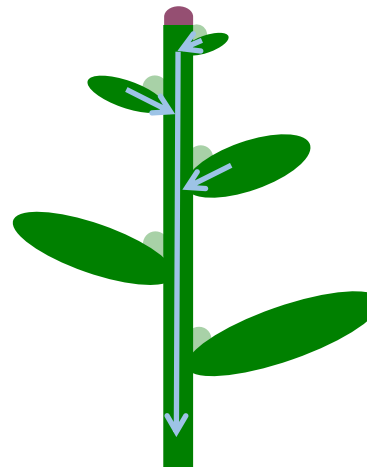
Auxin flow



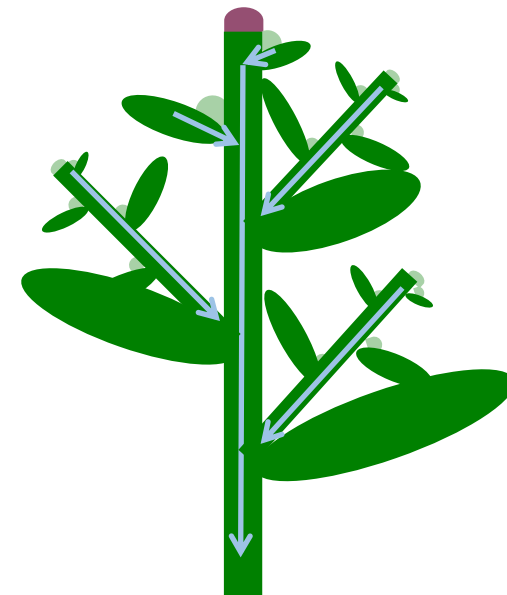
Phytomer
Axillary meristem
Leaf
Stem segment



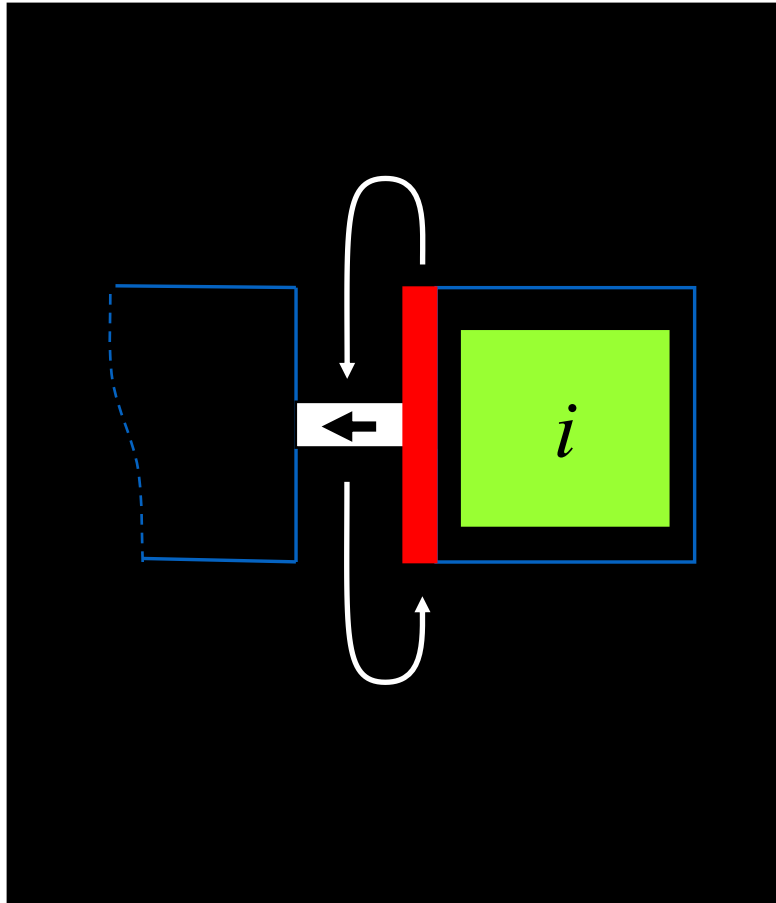
Shoot made of phytomers



Shoot system made of shoots



Canalization theory – feedback loop



$$\frac{d[PIN]}{dt} = \rho \frac{\Phi^n}{K^n + \Phi^n} + \rho_0 - \mu[PIN]$$

Φ - is the flux from *i* to *k*

ρ - PIN insertion constant

μ - PIN removal constant

Przemek Prusinkiewicz

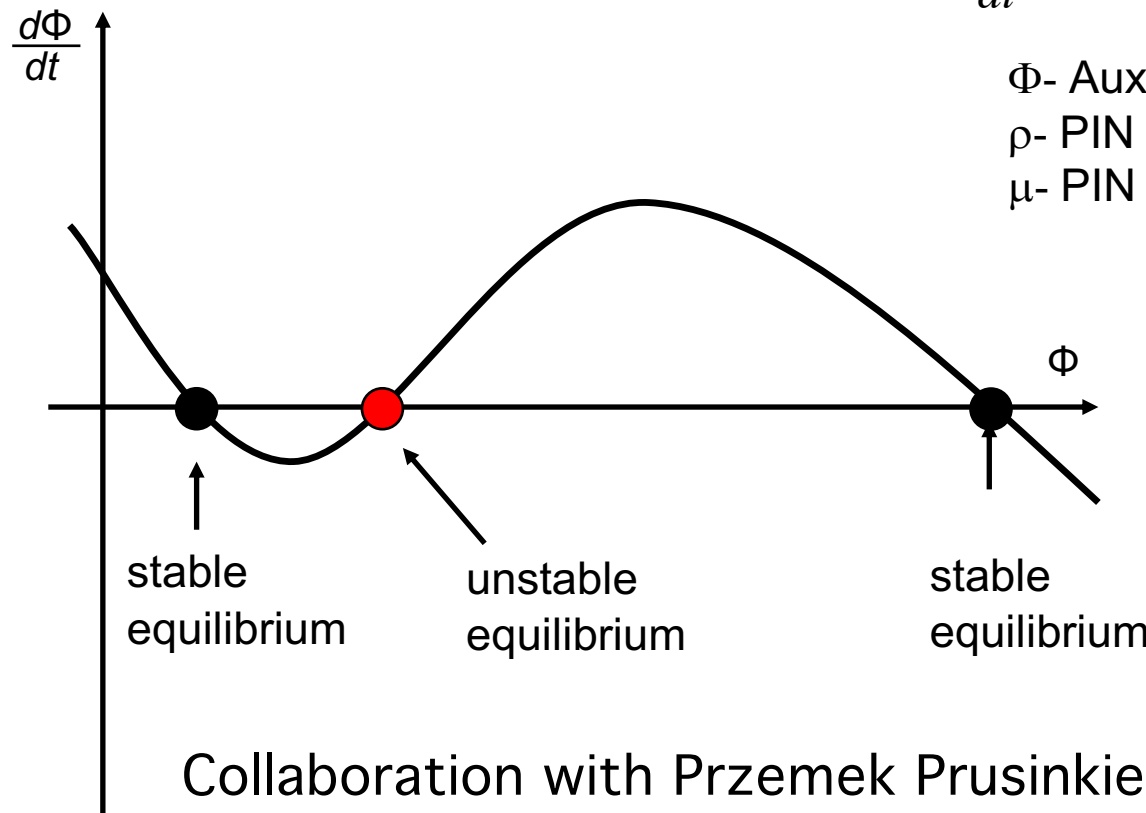
Bistability of a basic auxin transport canalization model

$$\frac{d[PIN]}{dt} = \rho \frac{\Phi^n}{K^n + \Phi^n} + \rho_0 - \mu[PIN]$$

Φ - Auxin flux

ρ - PIN insertion constant

μ - PIN removal constant

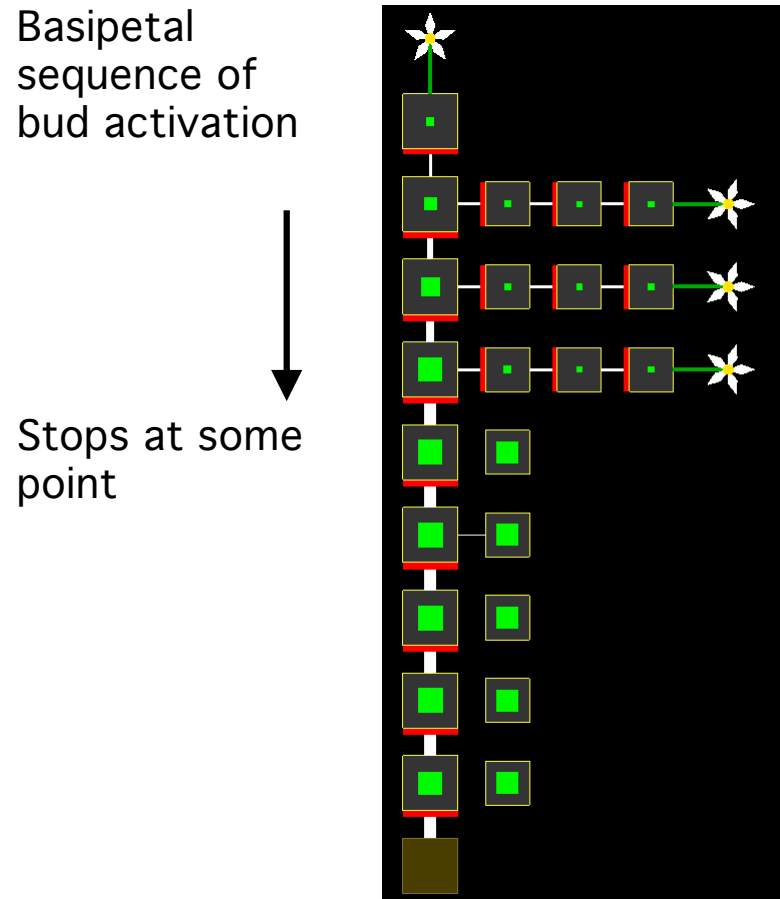


Collaboration with Przemek Prusinkiewicz

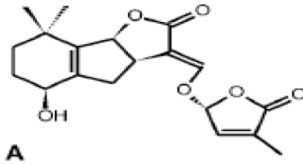
Przemek
Prusinkiewicz



Residual auxin production



Strigolactone deficient mutants are branchy



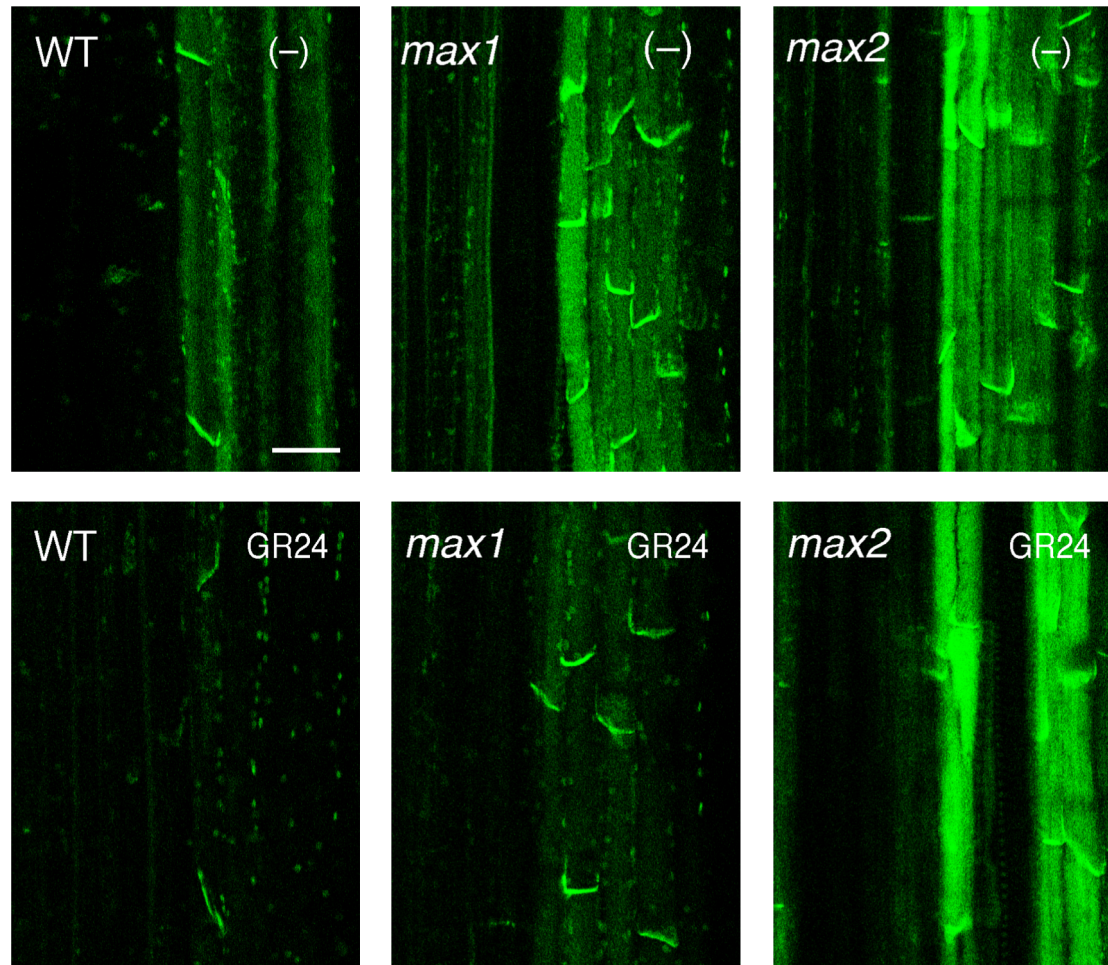
WT

max1



Strigolactone is
upwardly mobile

Strigolactone (GR24) limits auxin transporter accumulation making canalization more difficult



Bistability of a basic auxin transport canalisation model

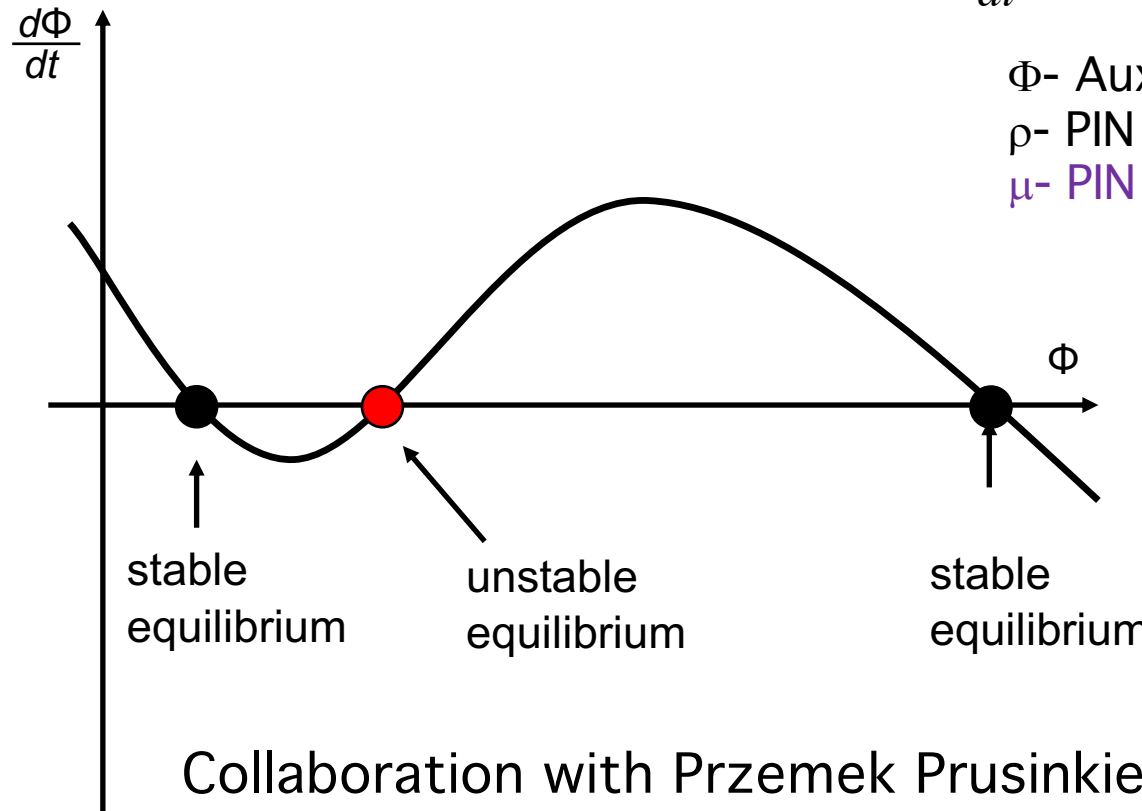
$$\frac{d[PIN]}{dt} = \rho \frac{\Phi^n}{K^n + \Phi^n} + \rho_0 - \mu[PIN]$$

Φ - Auxin flux

ρ - PIN insertion constant

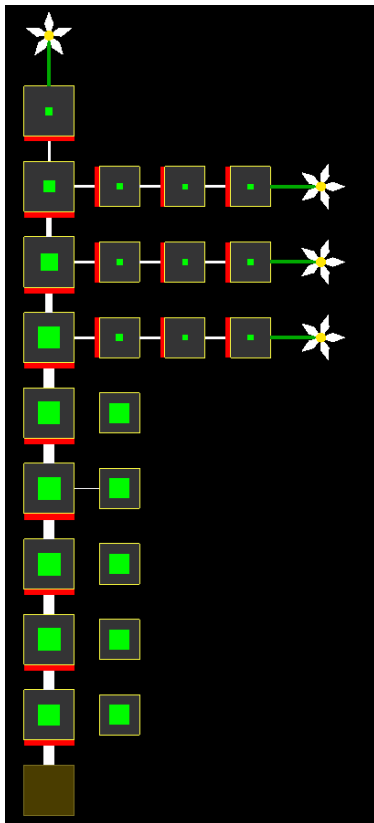
μ - PIN removal constant

Affected by strigolactone

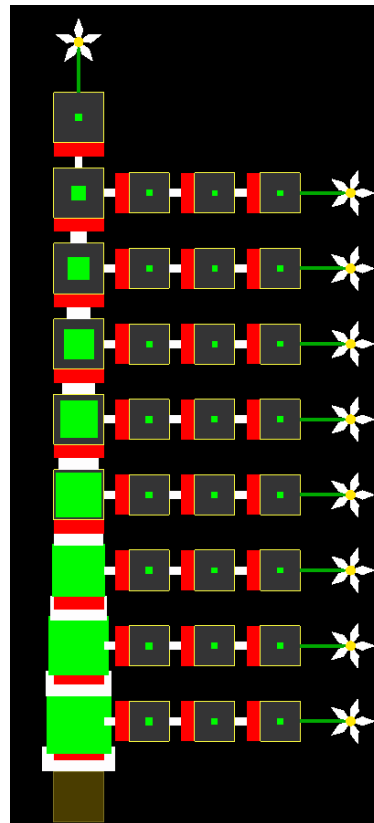


Collaboration with Przemek Prusinkiewicz

Wild type vs. max mutants



WT



max

Residual IAA production

- More branches
- More PIN
- More auxin
 - accumulating basally

Bistability of a basic auxin transport canalisation model

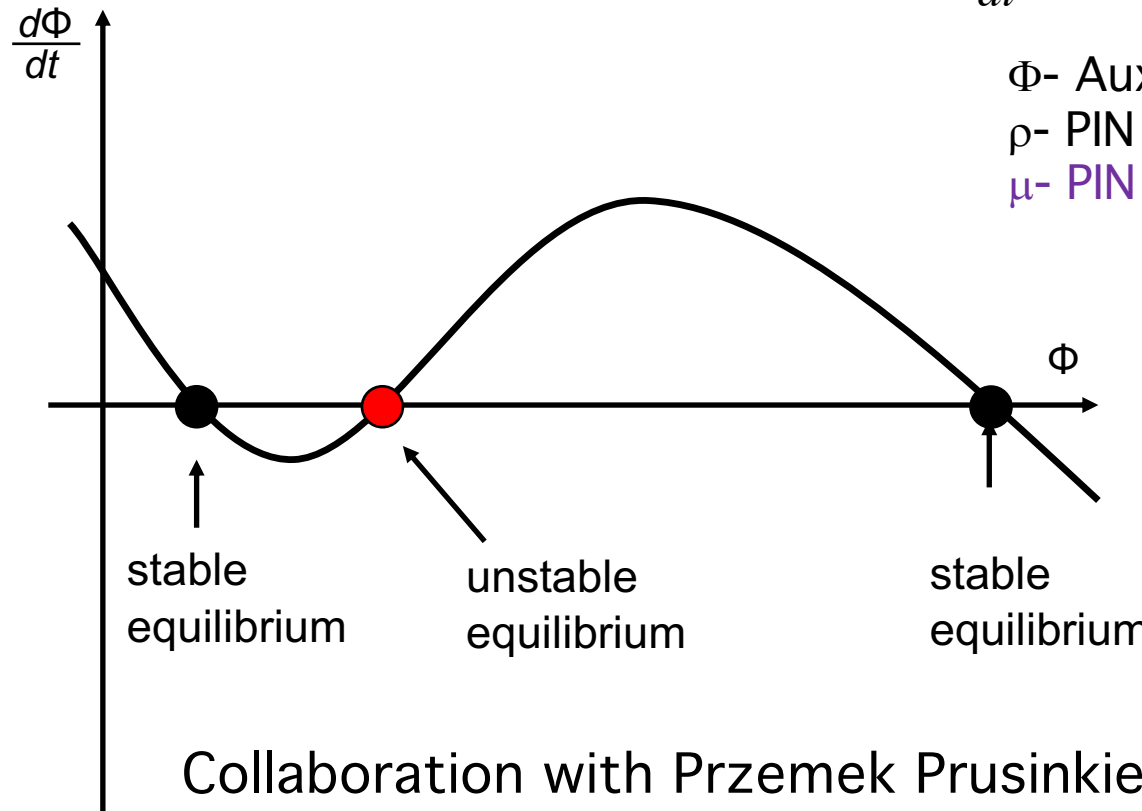
$$\frac{d[PIN]}{dt} = \rho \frac{\Phi^n}{K^n + \Phi^n} + \rho_0 - \mu[PIN]$$

Φ - Auxin flux

ρ - PIN insertion constant

μ - PIN removal constant

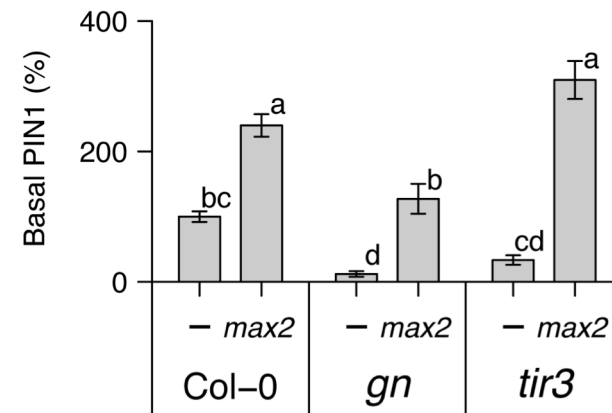
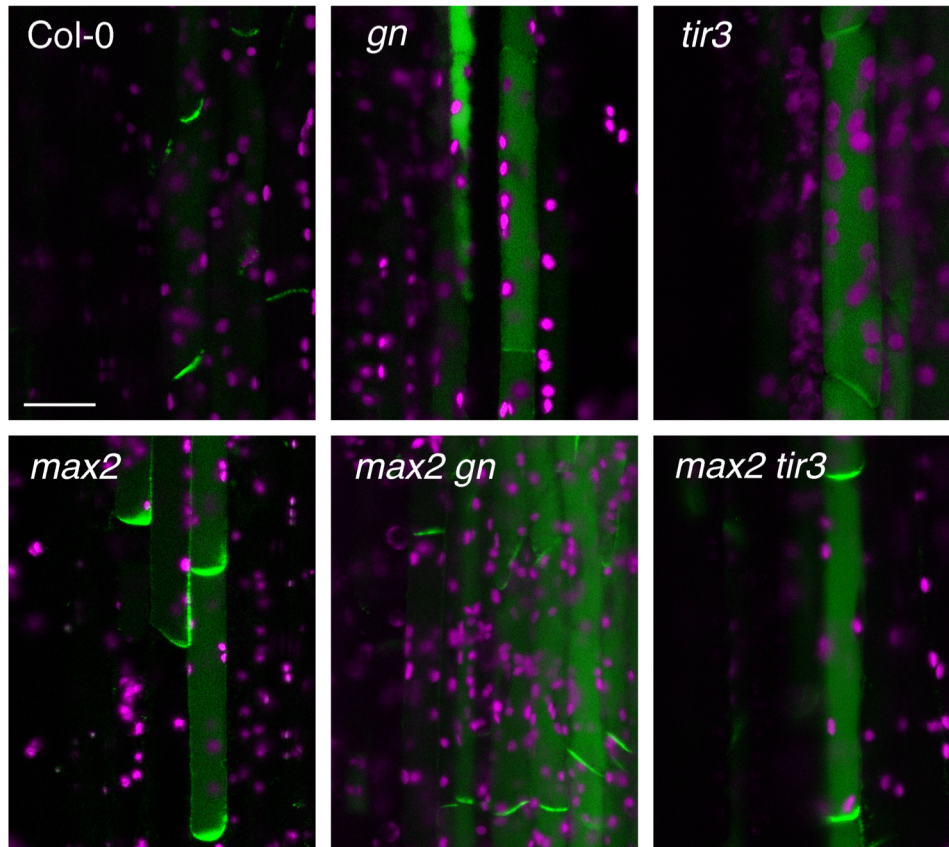
Affected by strigolactone



Collaboration with Przemek Prusinkiewicz

Other genes affecting PIN accumulation

tir3 and *gn* mutants: Reduced basal PIN



Bistability of a basic auxin transport canalization model

$$\frac{d[PIN]}{dt} = \rho \frac{\Phi^n}{K^n + \Phi^n} + \rho_0 - \mu[PIN]$$

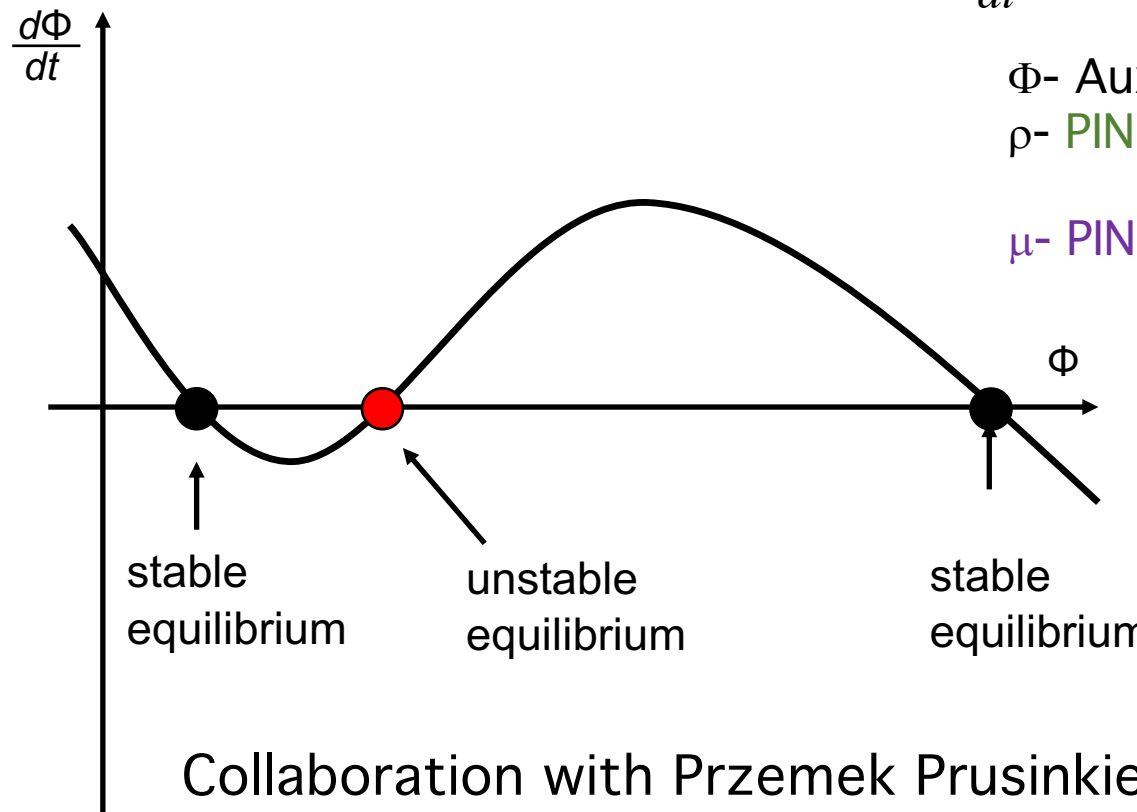
Φ - Auxin flux

ρ - PIN insertion constant

Affected by GN and TIR3

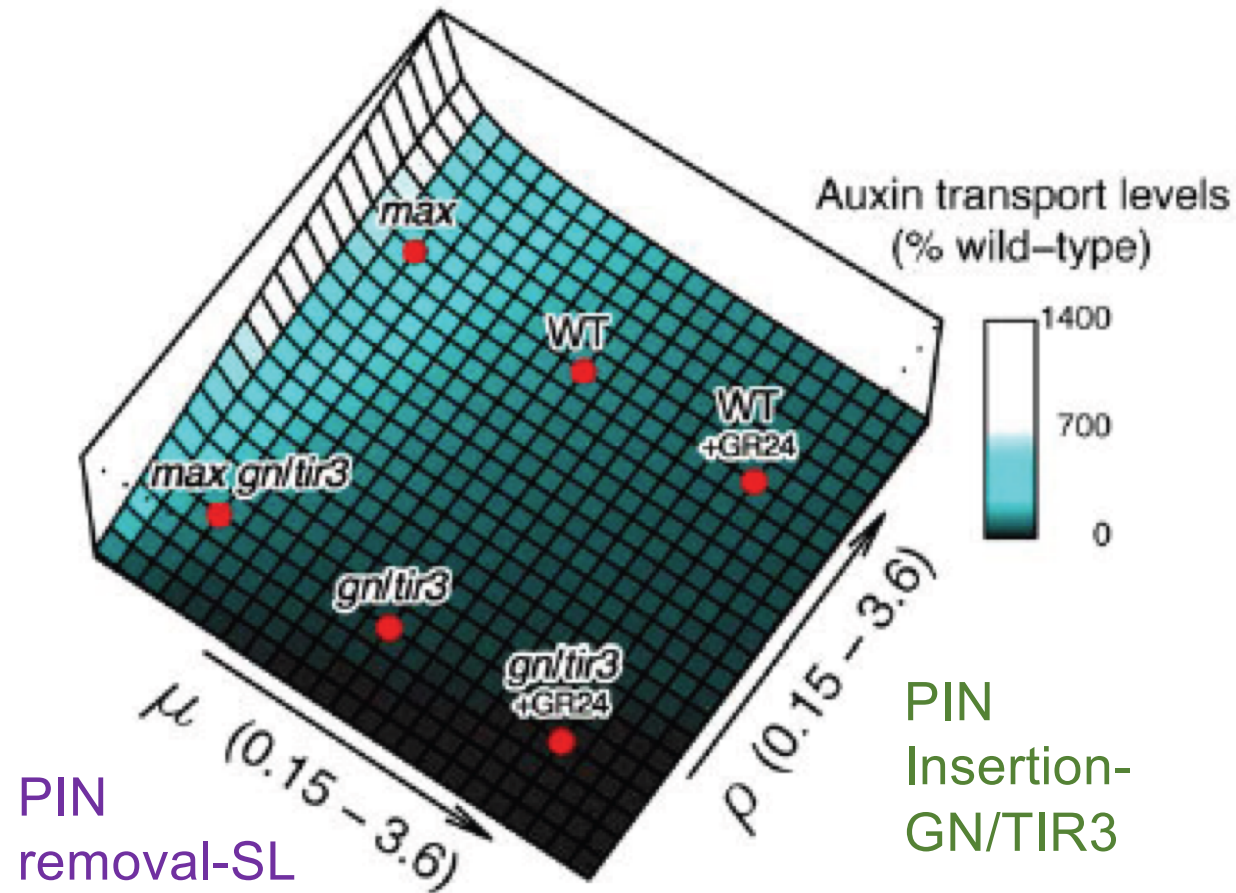
μ - PIN removal constant

Affected by strigolactone

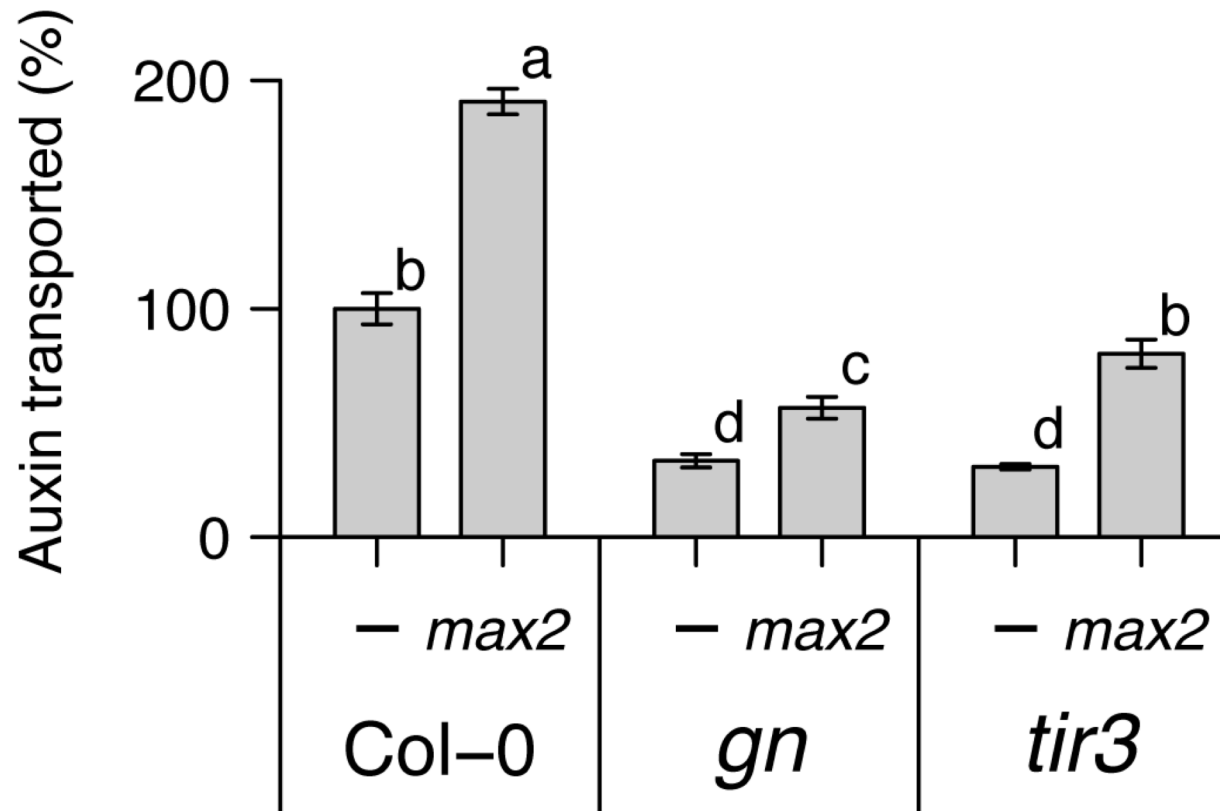


Collaboration with Przemek Prusinkiewicz

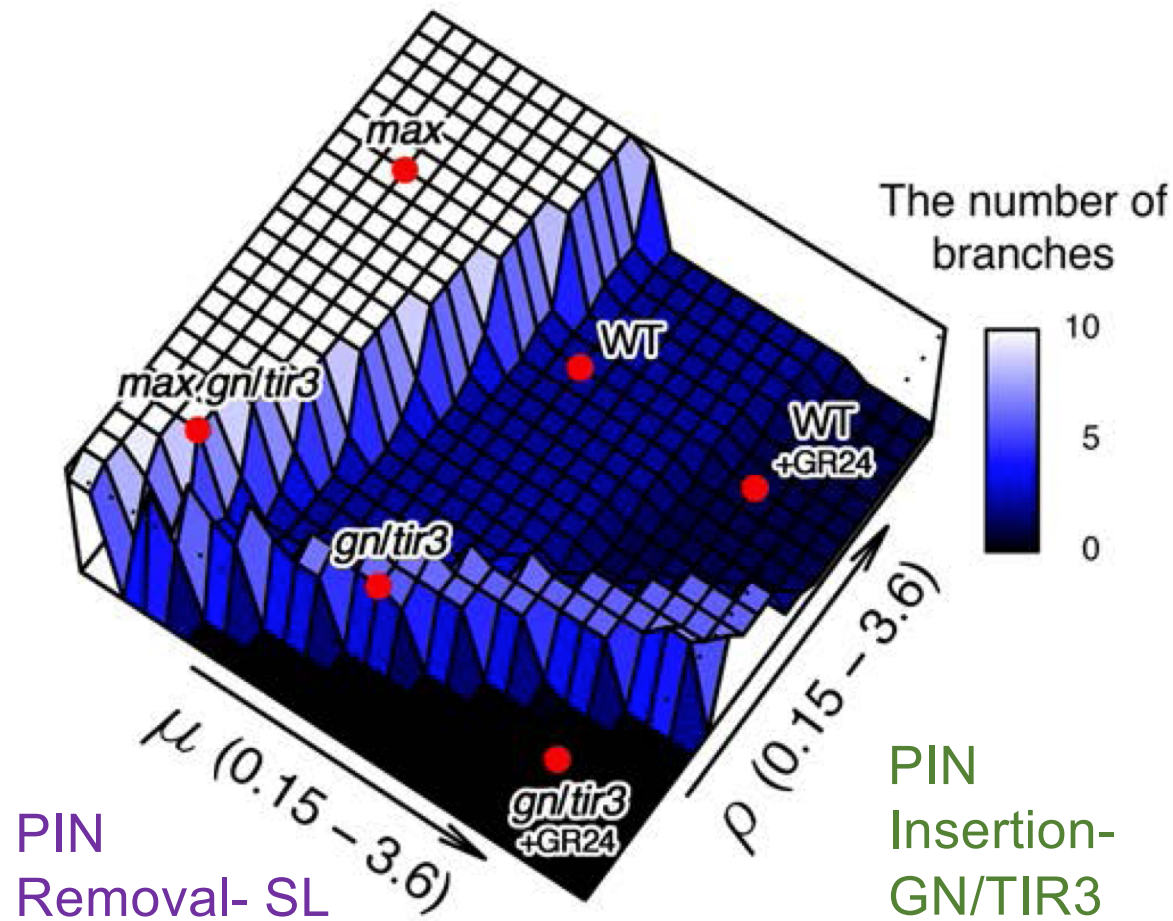
Parameter space exploration- Auxin transport



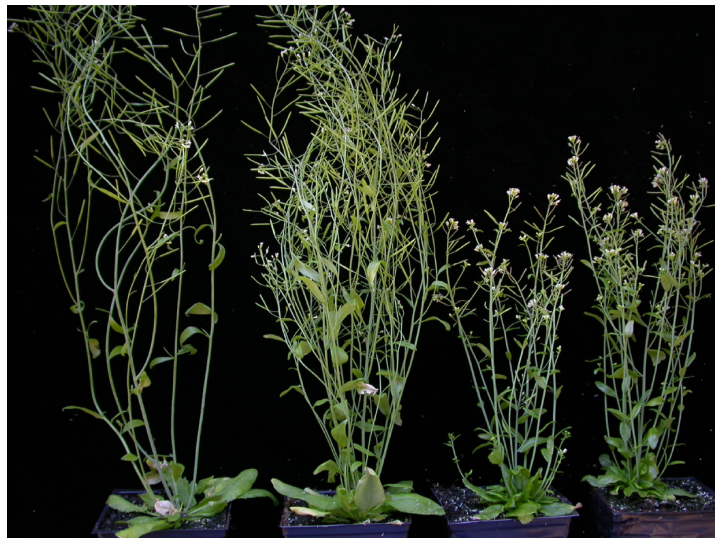
max mutants transport more auxin
tir3 and *gn* transport less auxin



Parameter space exploration- Shoot branching (= buds with canalized auxin transport)



max, *tir3* and *gn* mutants have increased branching

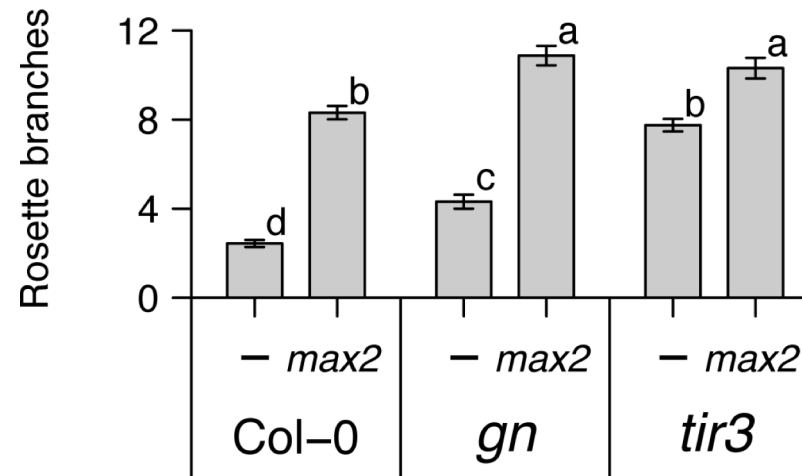


WT

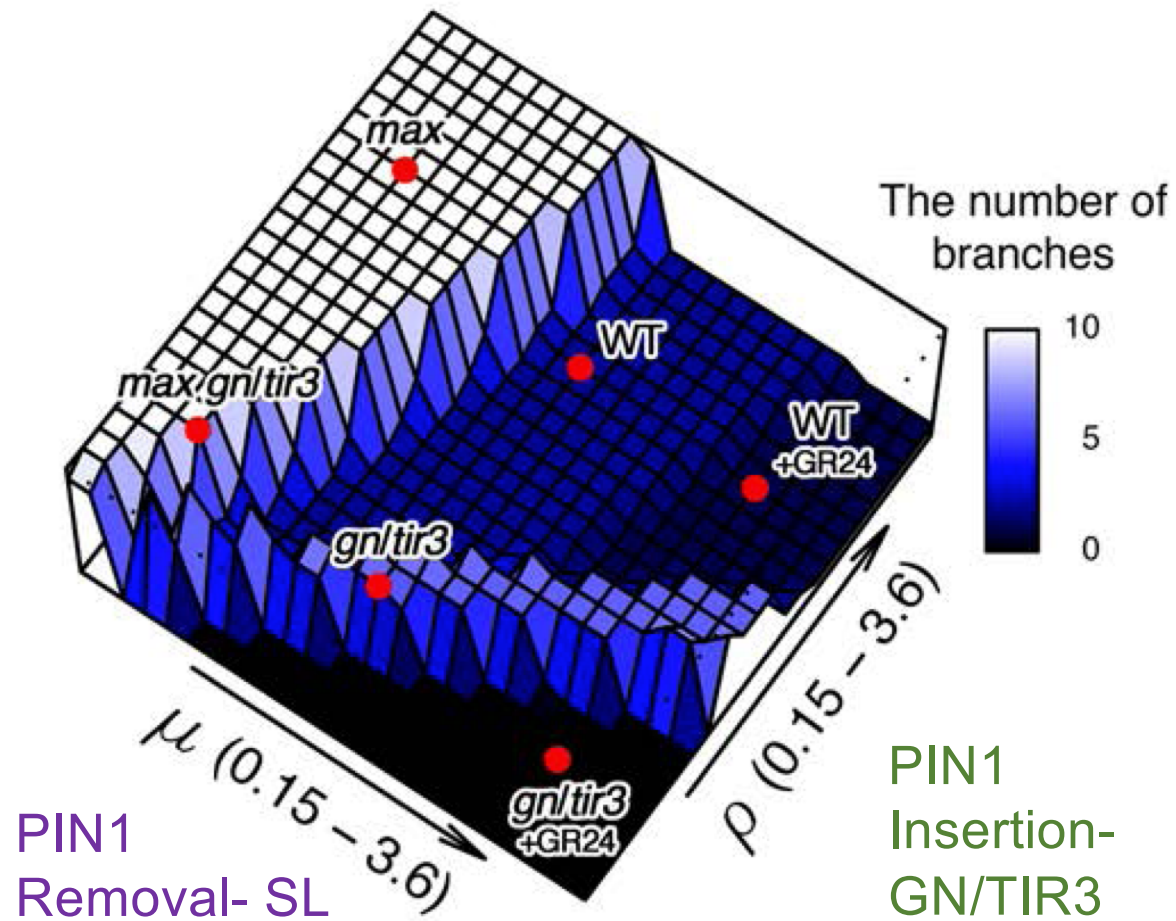
max4

tir3

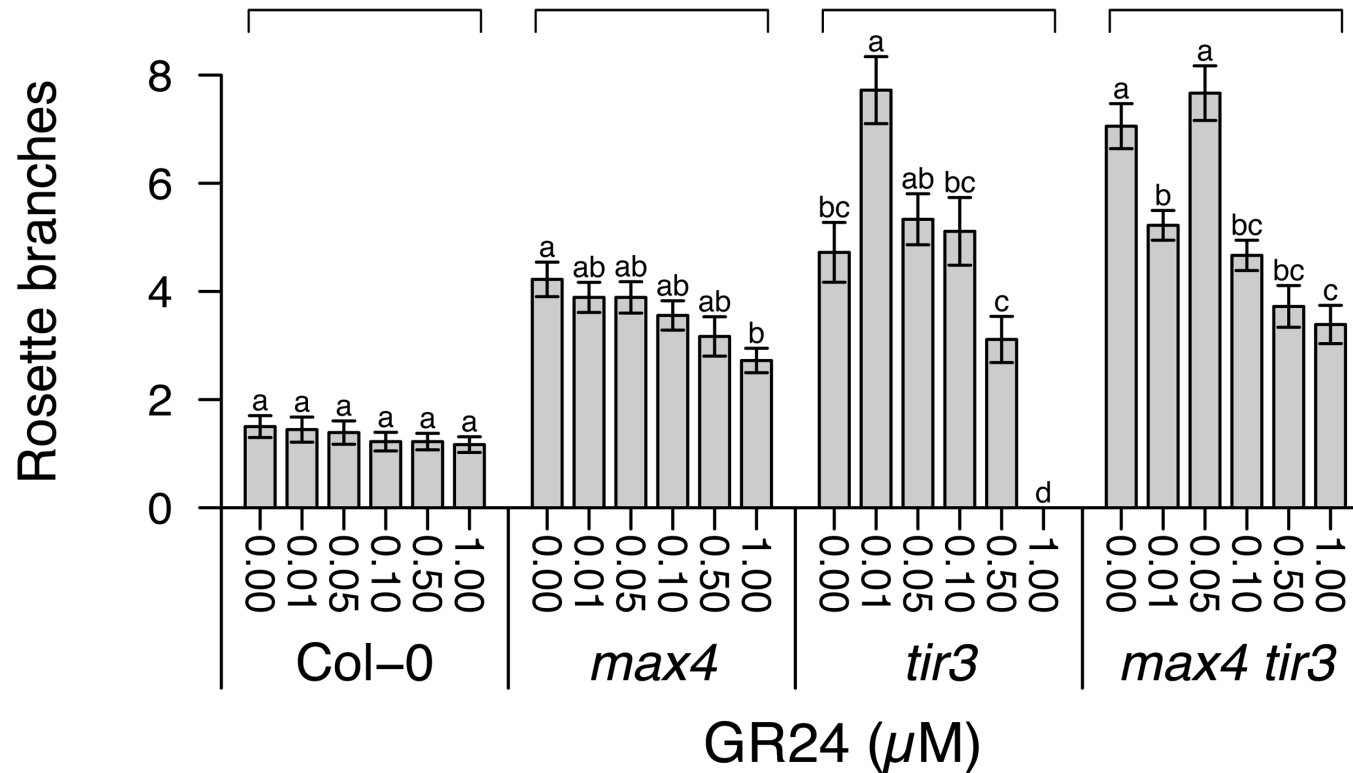
tir3
max4



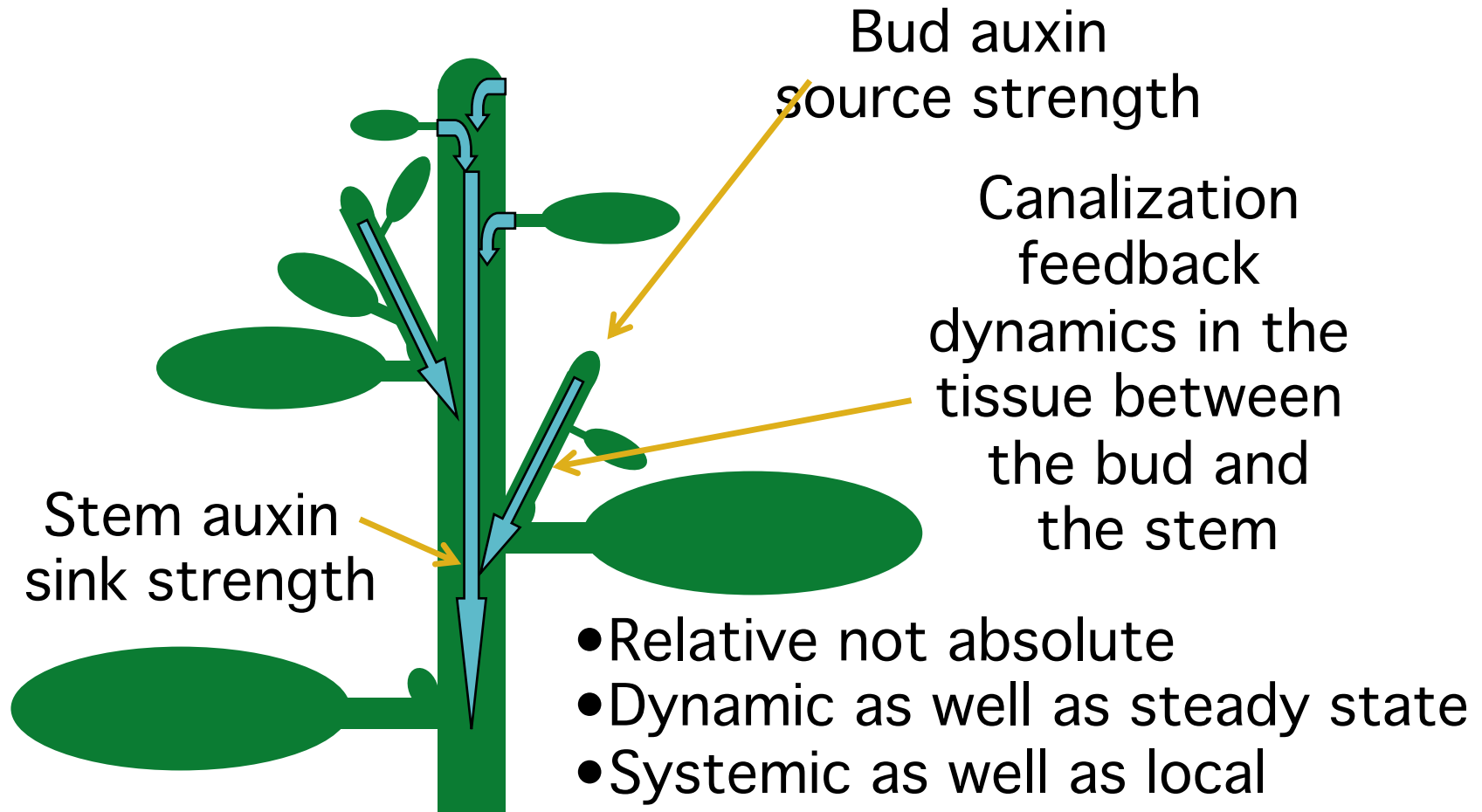
Parameter space exploration- Shoot branching

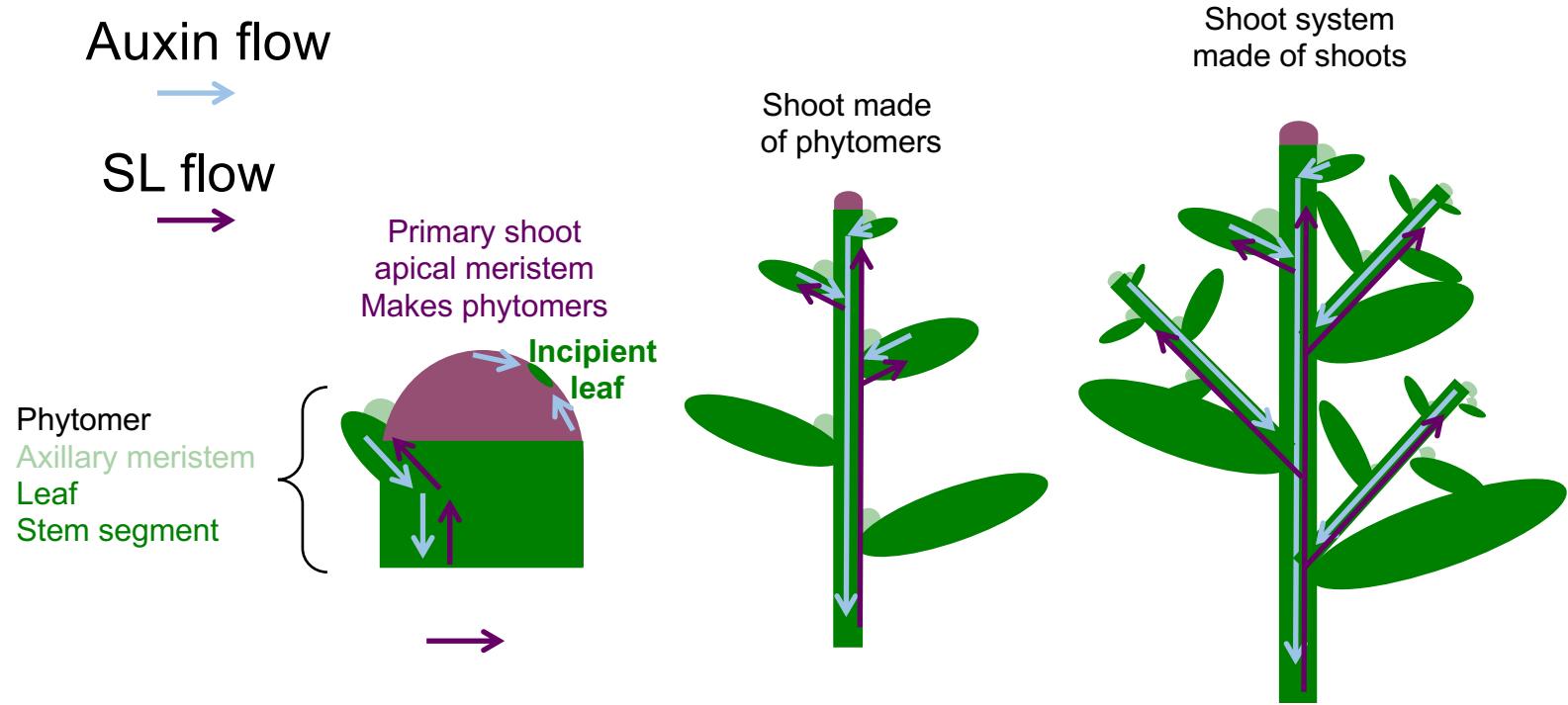


Strigolactone can promote or inhibit branching depending on plant auxin transport status



Competitive canalization as a signal integrator





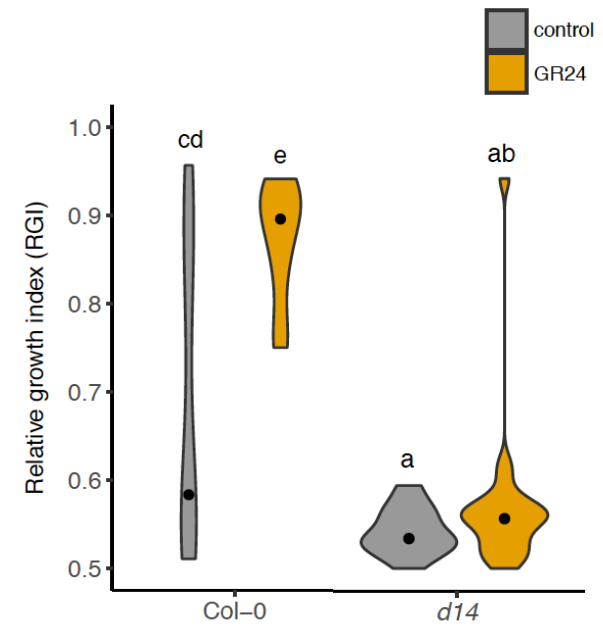
Strigolactone enhances bud-bud competition



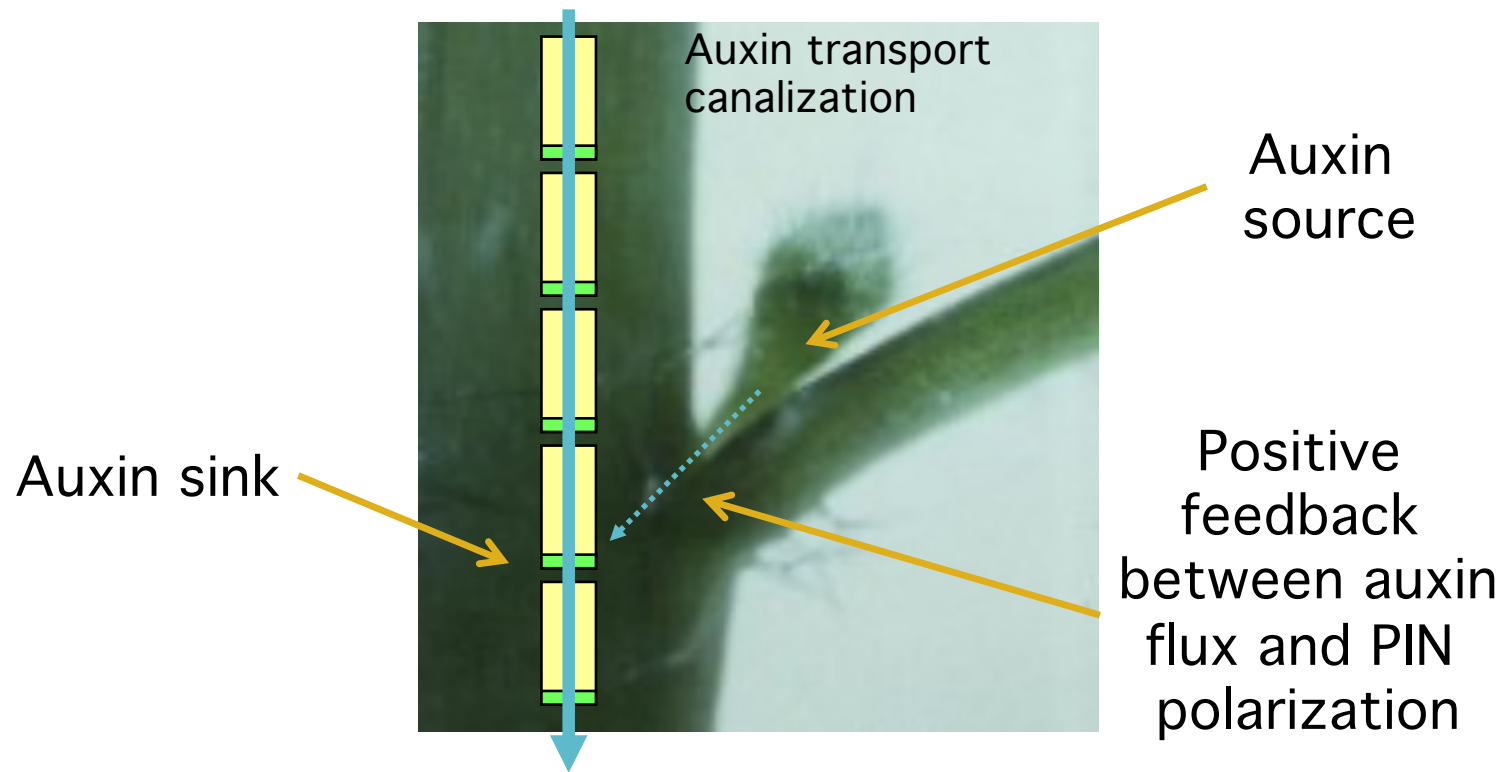
RGI
~0.5

RGI
~1

RGI
~1

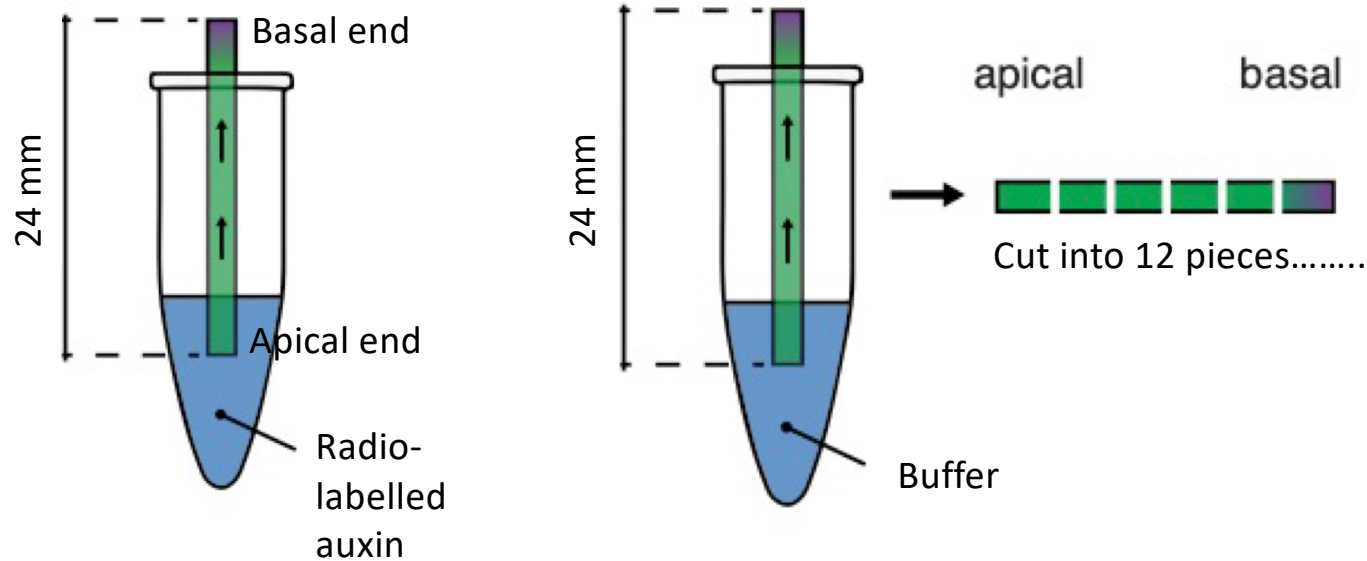


Stem auxin transport is central to branching control

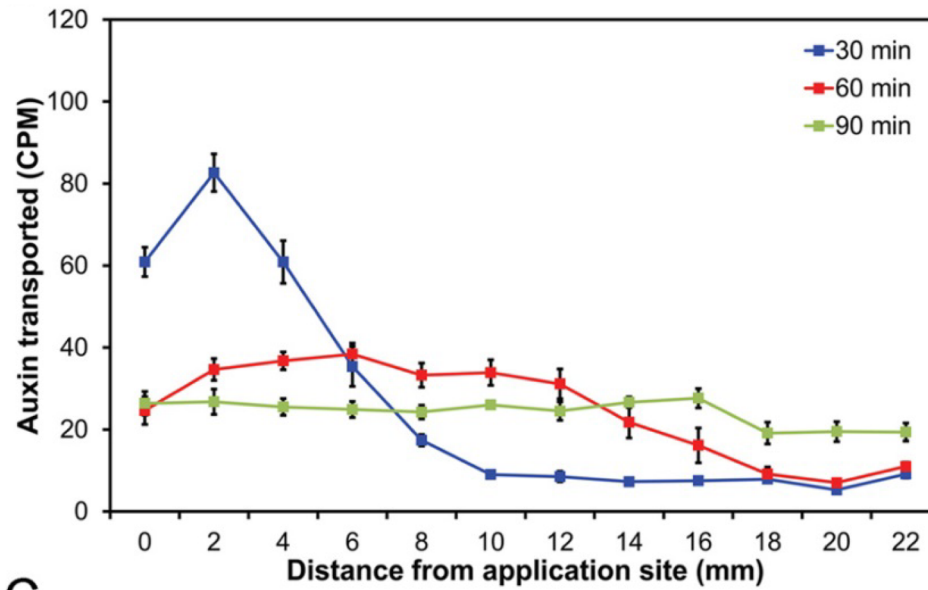


Pulse auxin transport assay

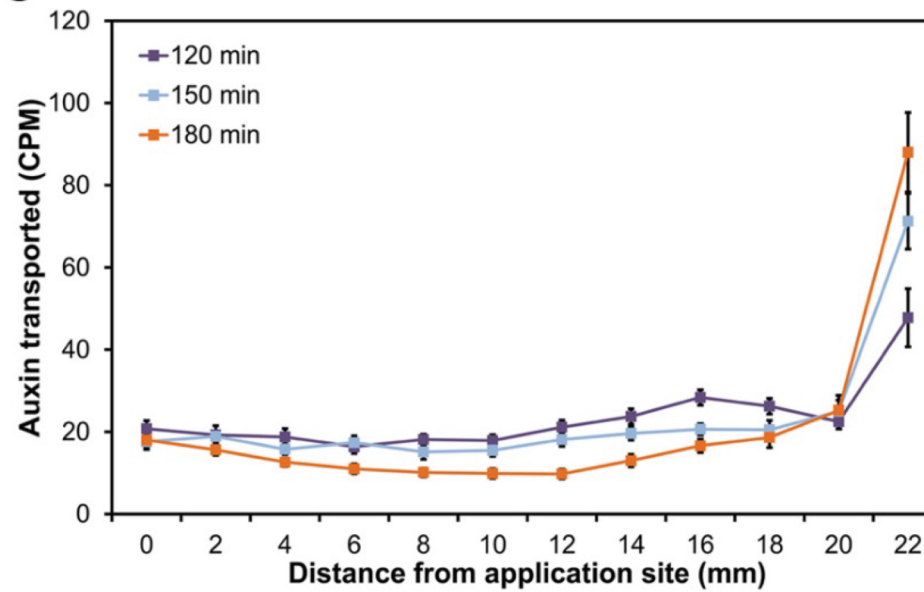
10 minute ^{14}C auxin pulse No treatment for n minutes Cut into 12 2mm pieces



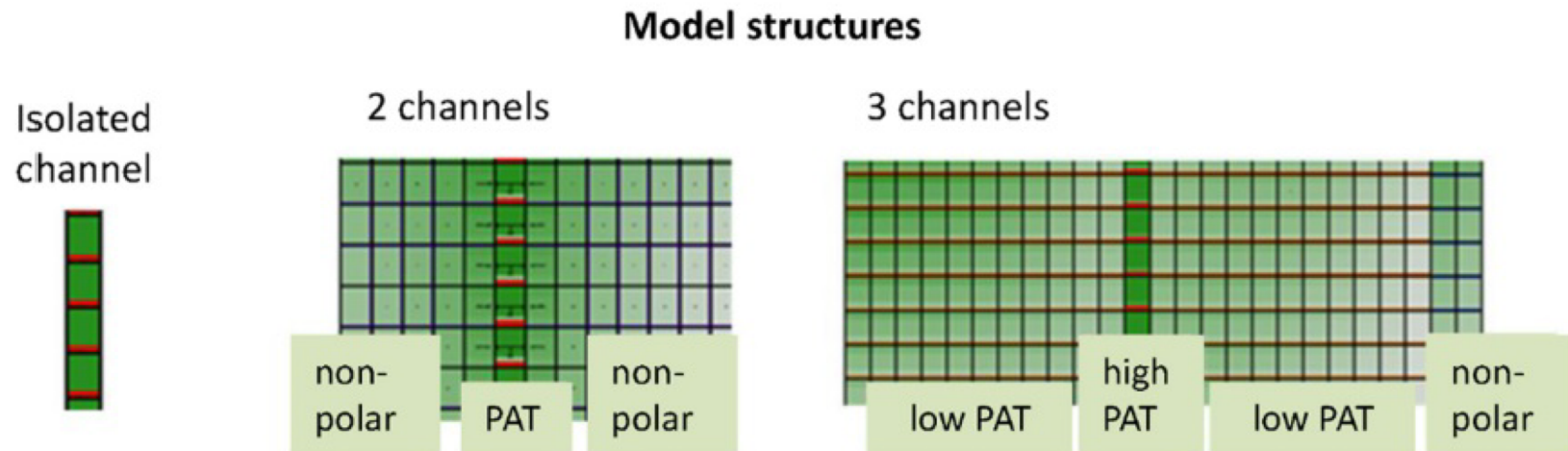
Stem auxin transport dynamics are complex



C



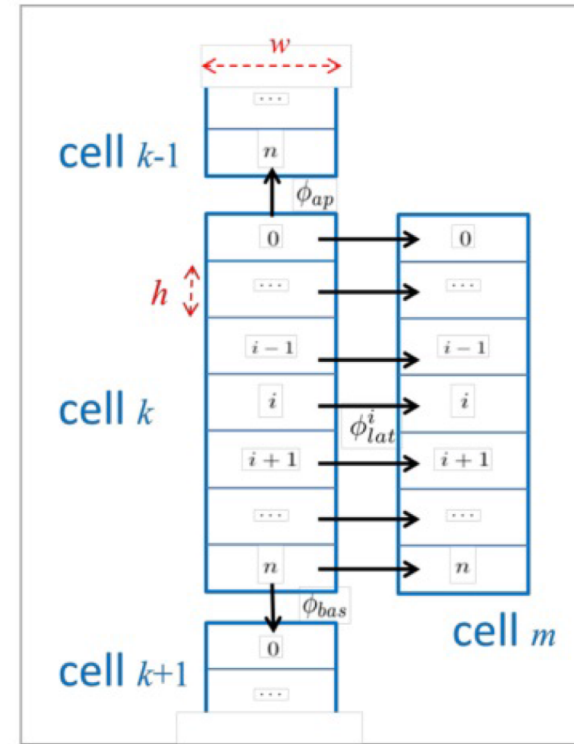
Multi-modal model for stem auxin transport



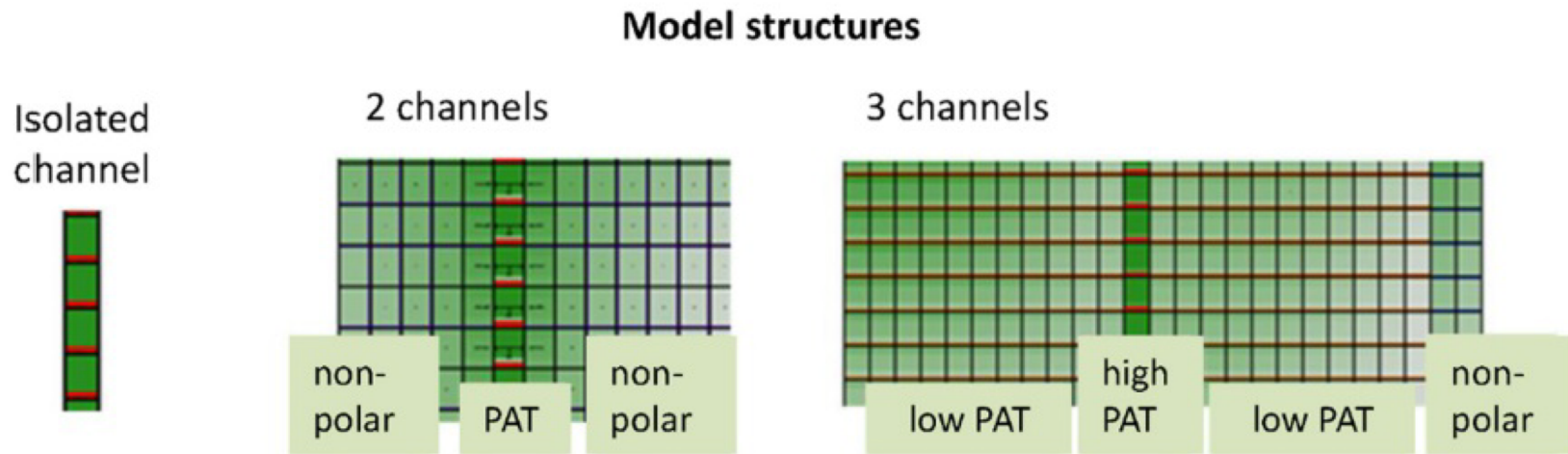
Multi-modal model for stem auxin transport

Auxin movement is modelled by:

- (1) Diffusion inside cells, lengthwise only, using $n+1$ cell sub-divisions
- (2) Direct transport from one cell into the next

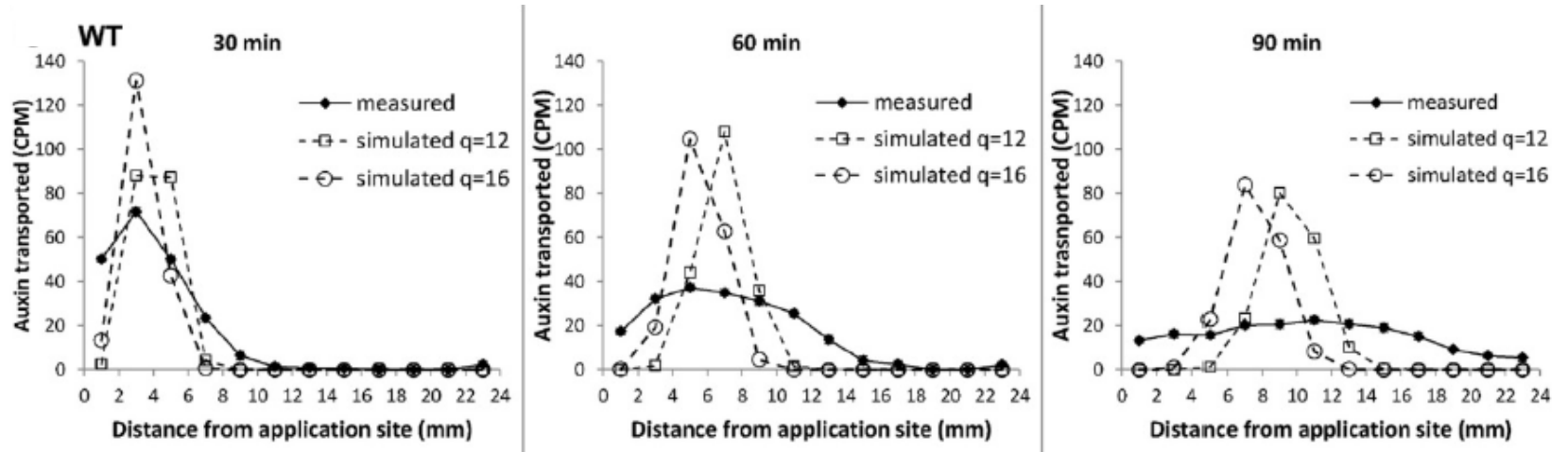


Multi-modal model for stem auxin transport

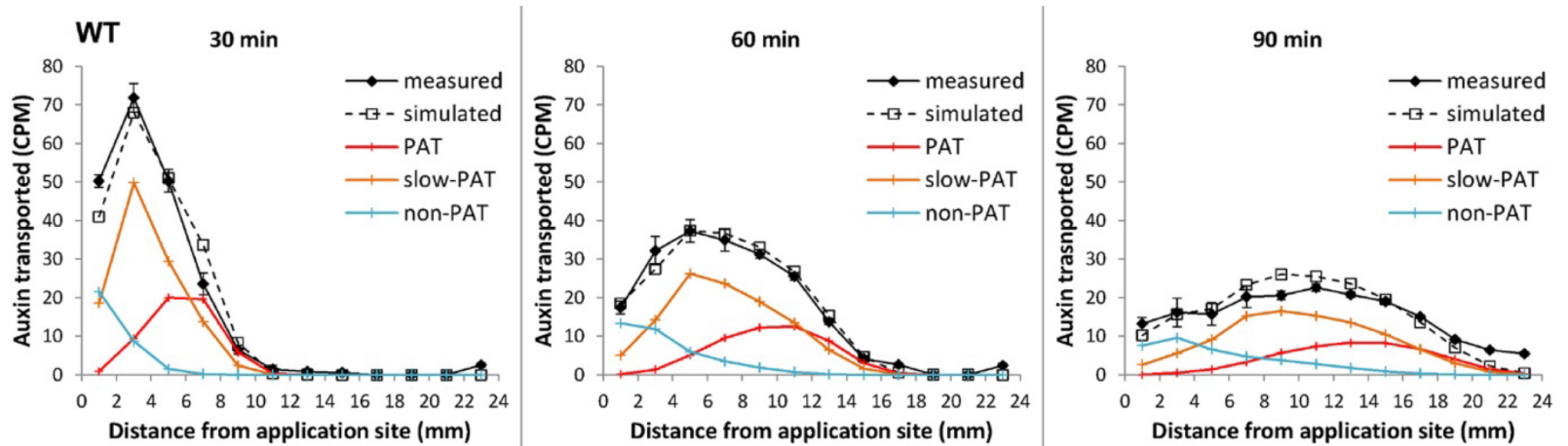


Multi-modal model captures peak spreading

One channel

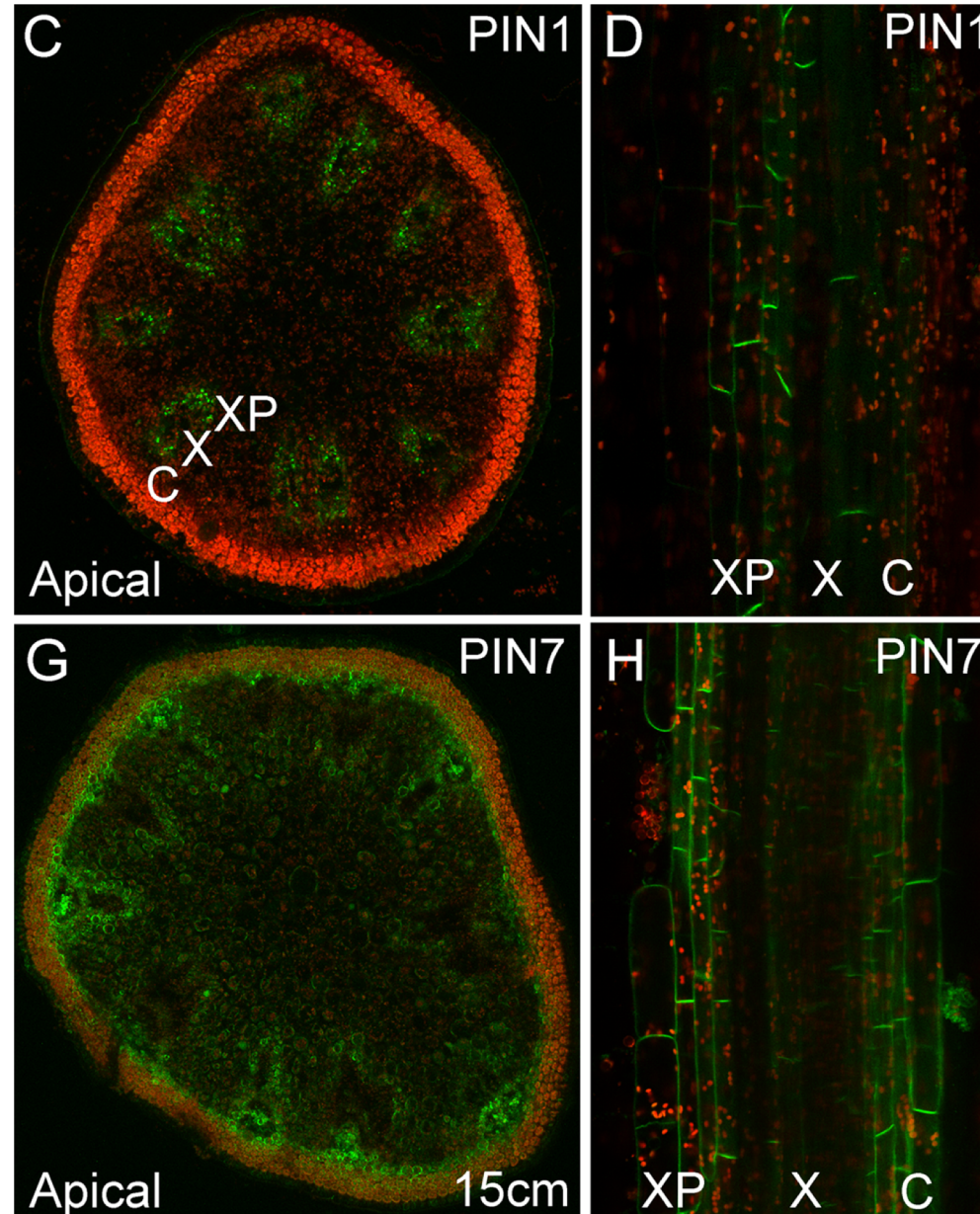


Three channels

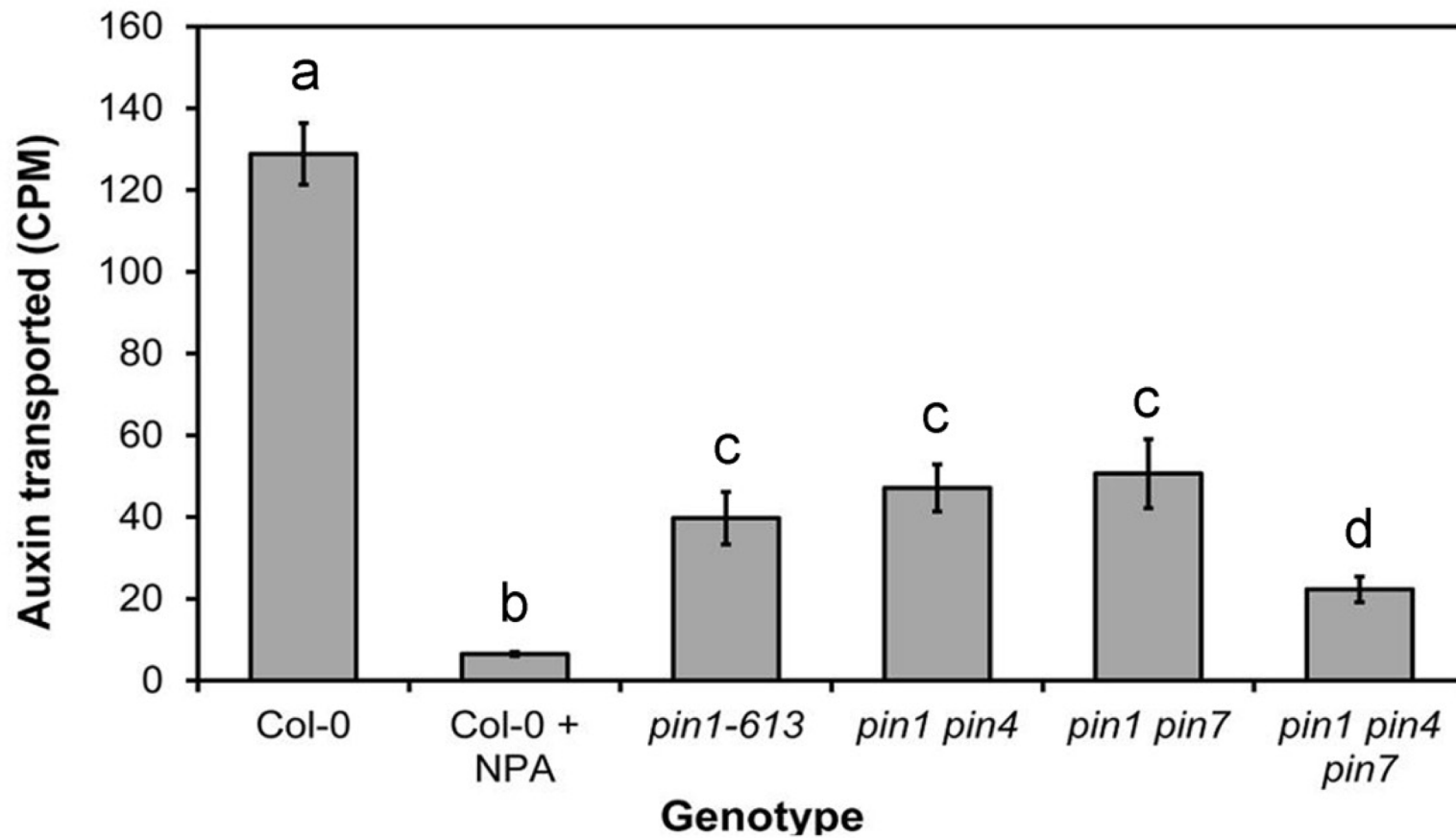


PIN1 accumulates in the polar auxin transport stream

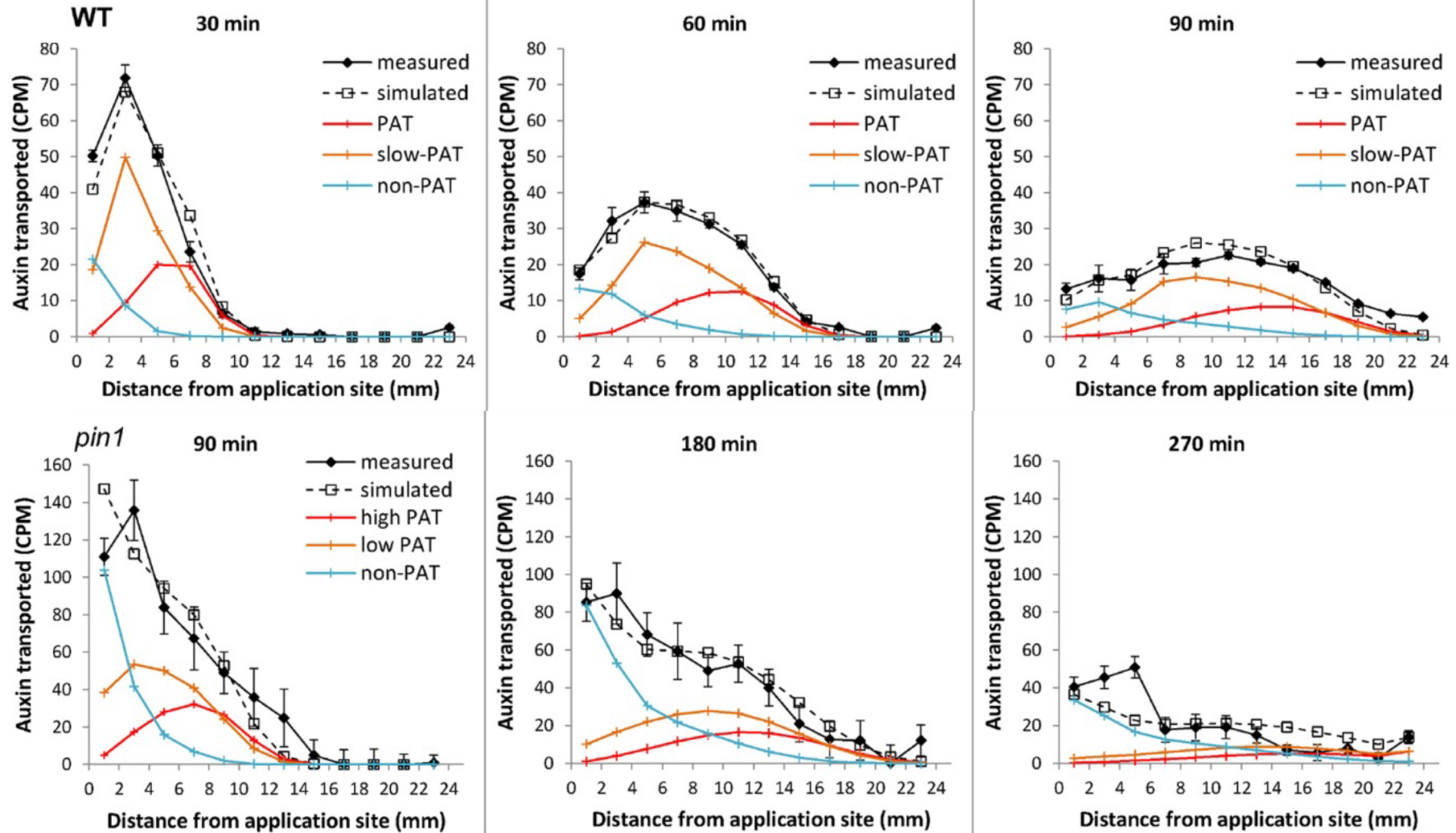
PIN3 4 & 7 accumulate more widely



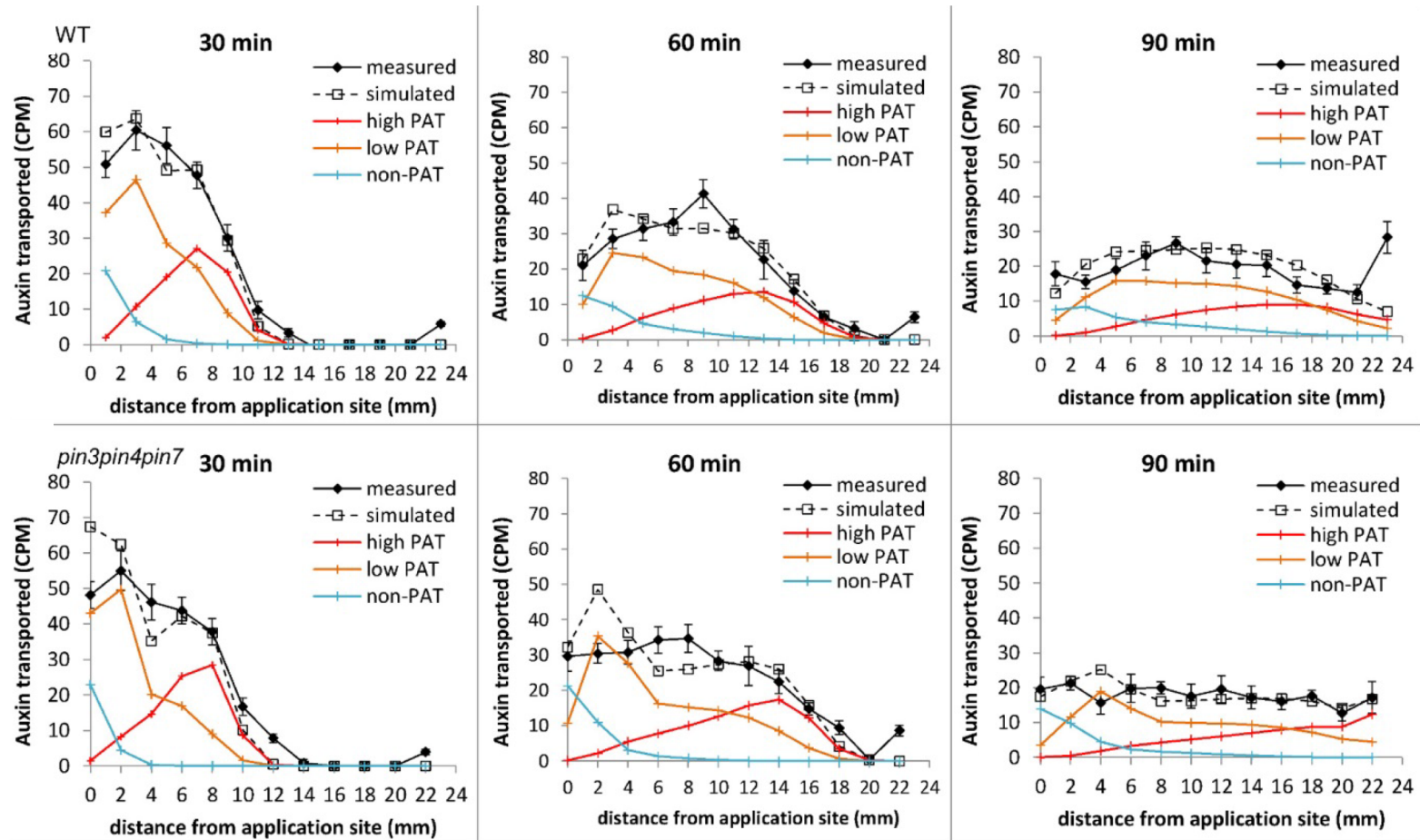
PIN1 and PIN347 contribute additively to bulk stem auxin transport



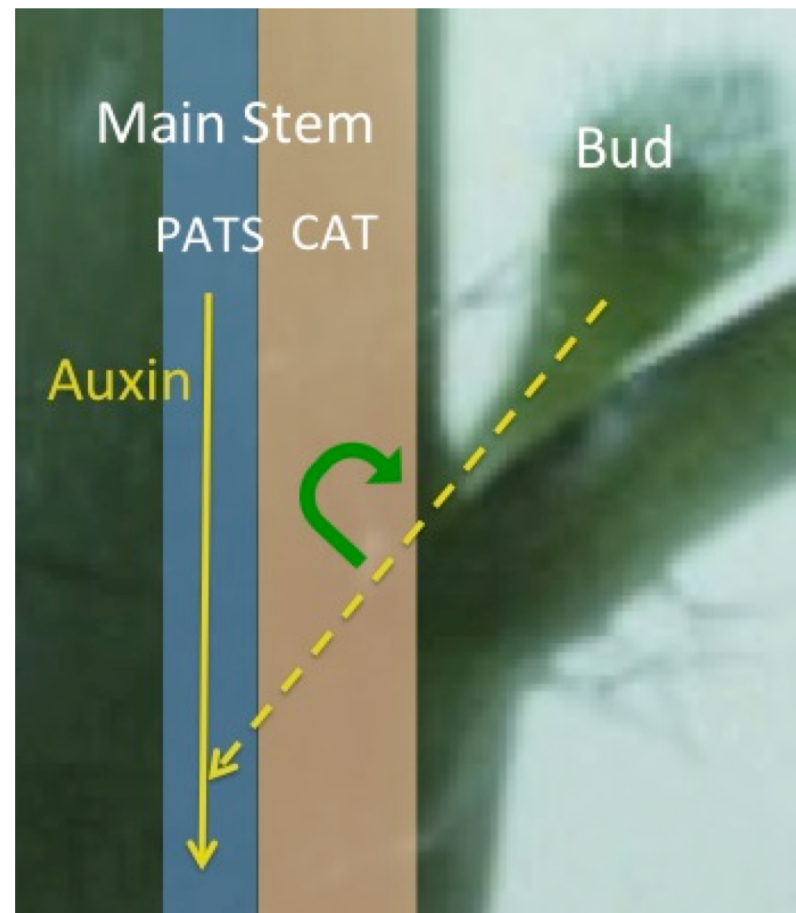
pin1 stem auxin transport dynamics can be modelled by reducing basal auxin transport



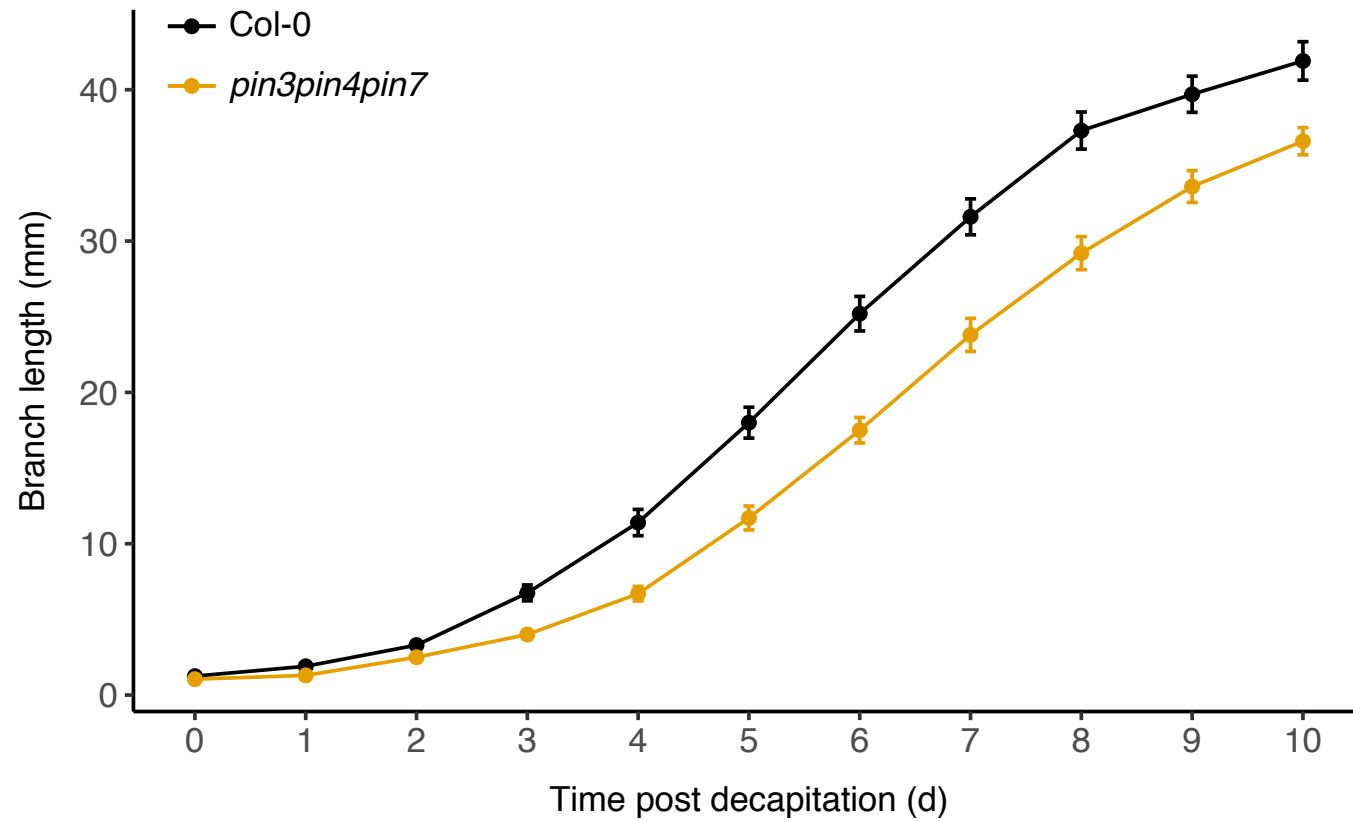
pin347 stem auxin transport dynamics can be modelled by reducing exchange between transport channels



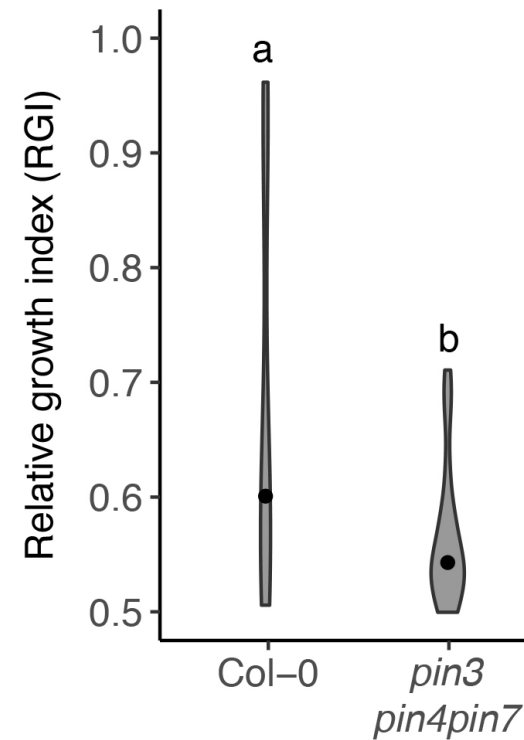
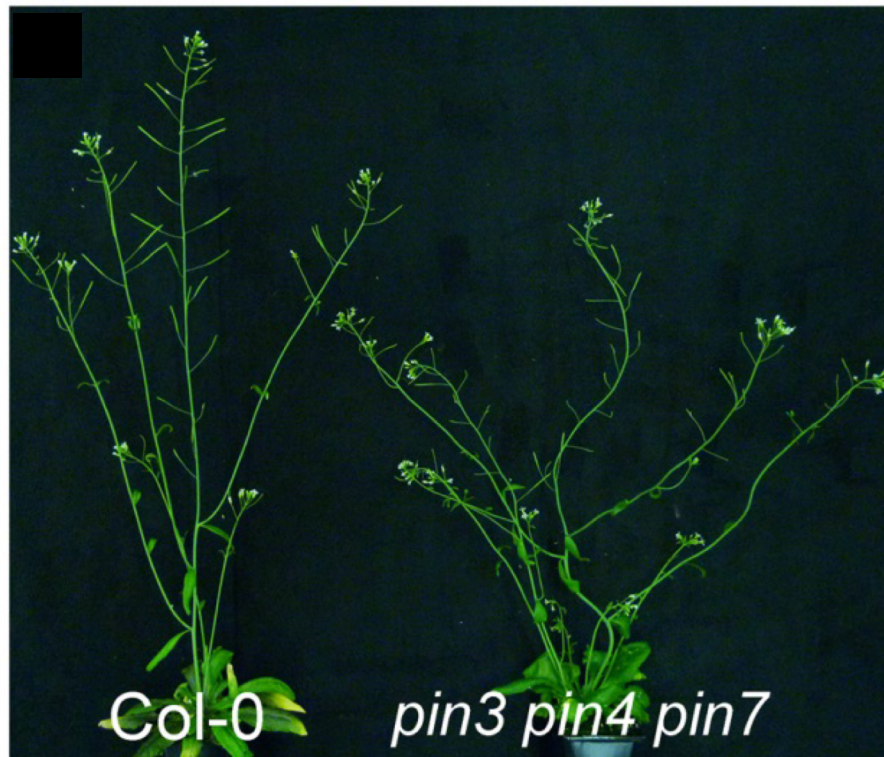
Two-step canalisation



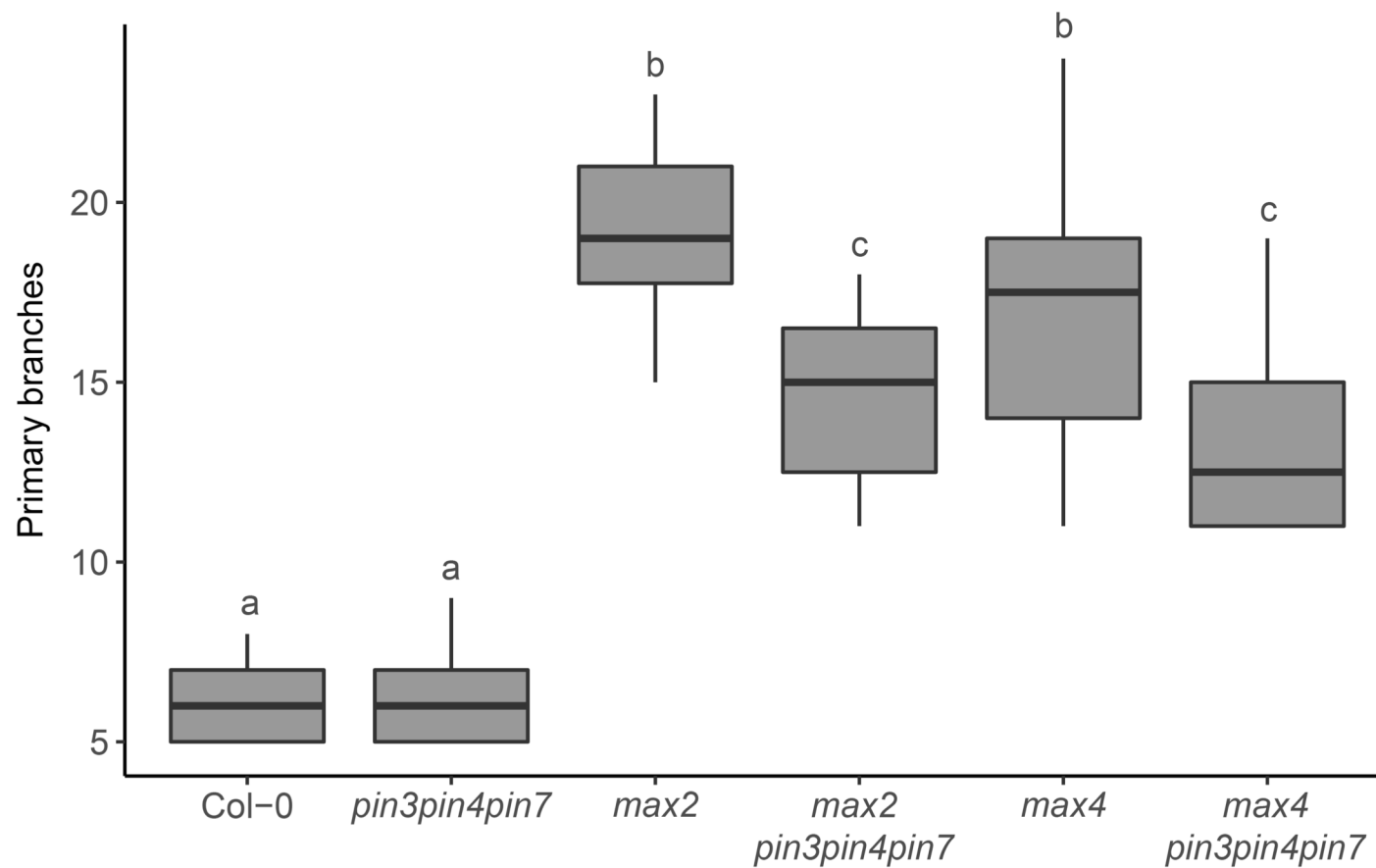
pin347 mutants activate buds slowly



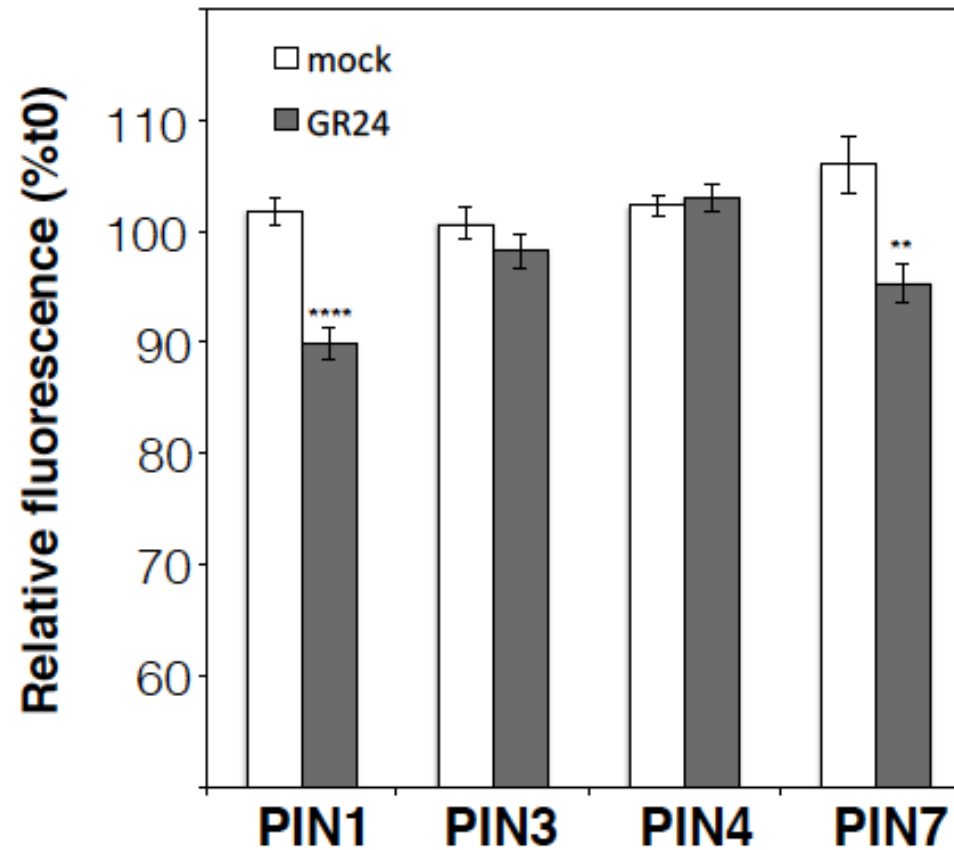
Bud bud competition is reduced in *pin347* mutants



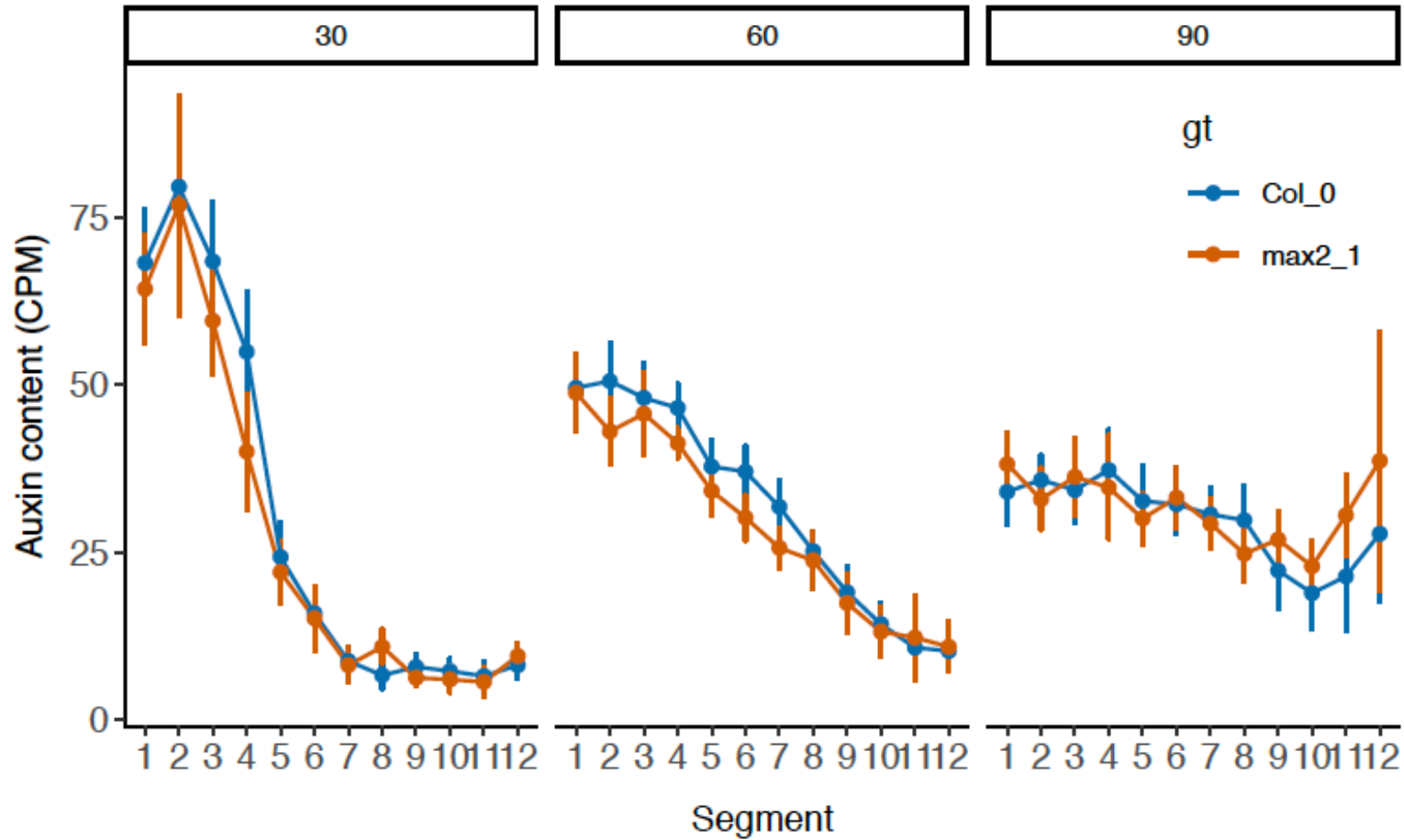
pin347 mutation partially suppresses branching in strigolactone mutants



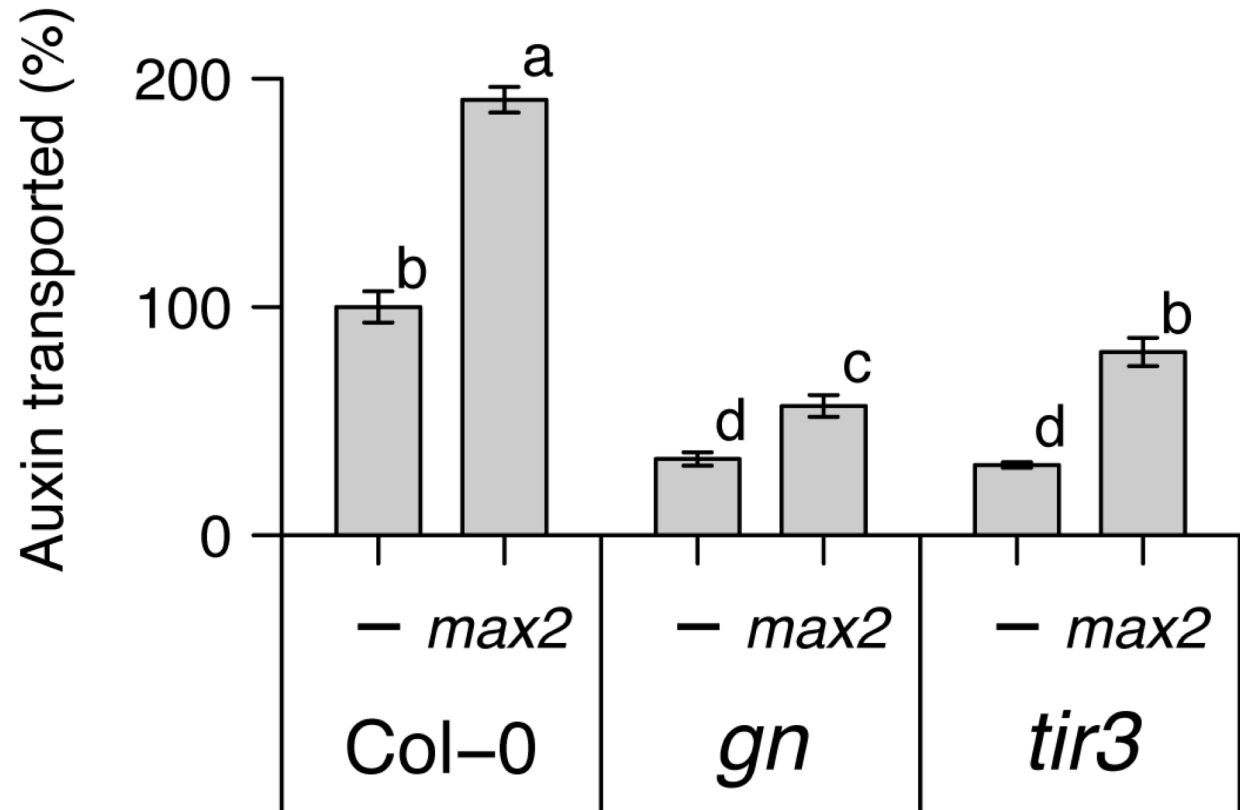
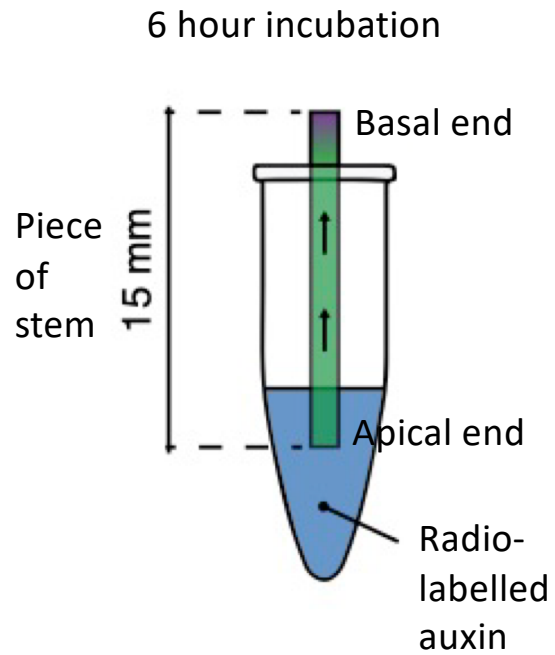
Strigolactone triggers PIN1 and PIN7 endocytosis



Auxin pulse progression is not affected by SL deficiency



Strigolactone affects bulk auxin transport



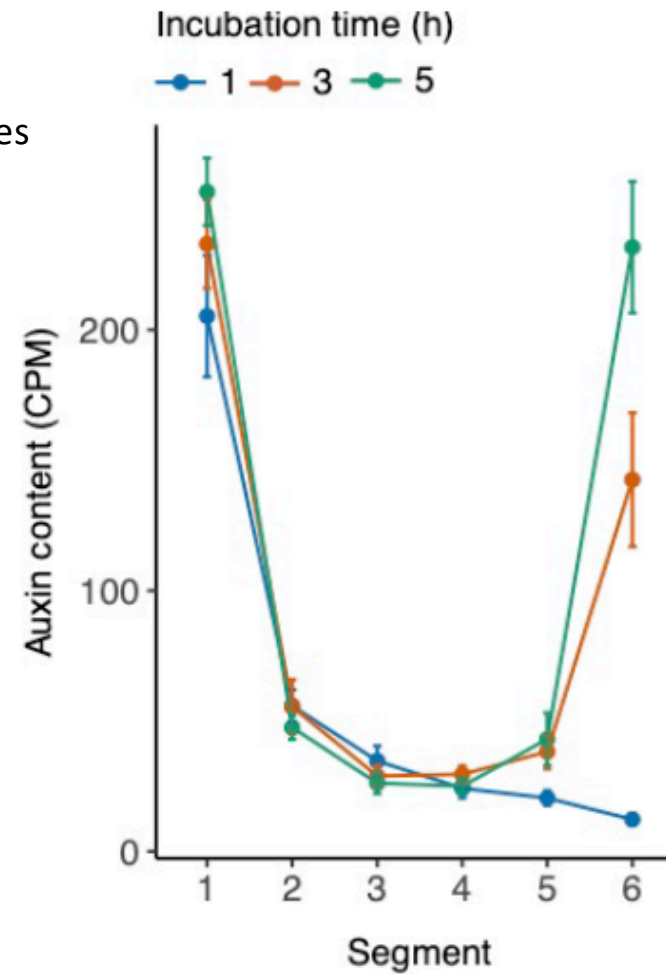
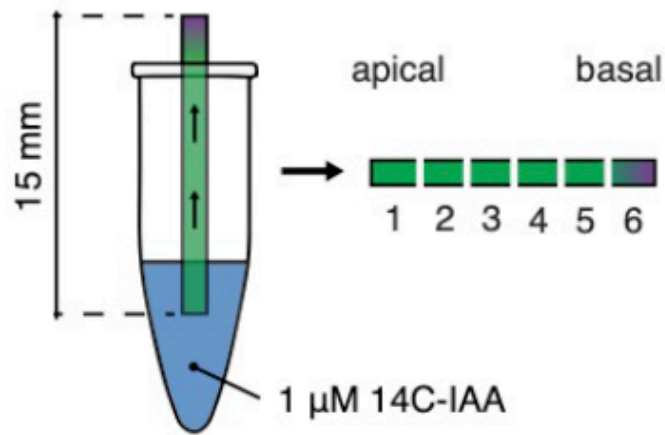
Reconciliation?

- Pulses can be the same shape in SL mutants and WT by increasing transport (basally and otherwise) in both PAT and CAT
- Pulses can be the same, while bulk transport is different if there are different apical loading rules relating, for example, to the length of time of the emersion of the apical end in auxin
- One idea is uptake-limited vs efflux-limited loading

Half-way-house auxin transport assay

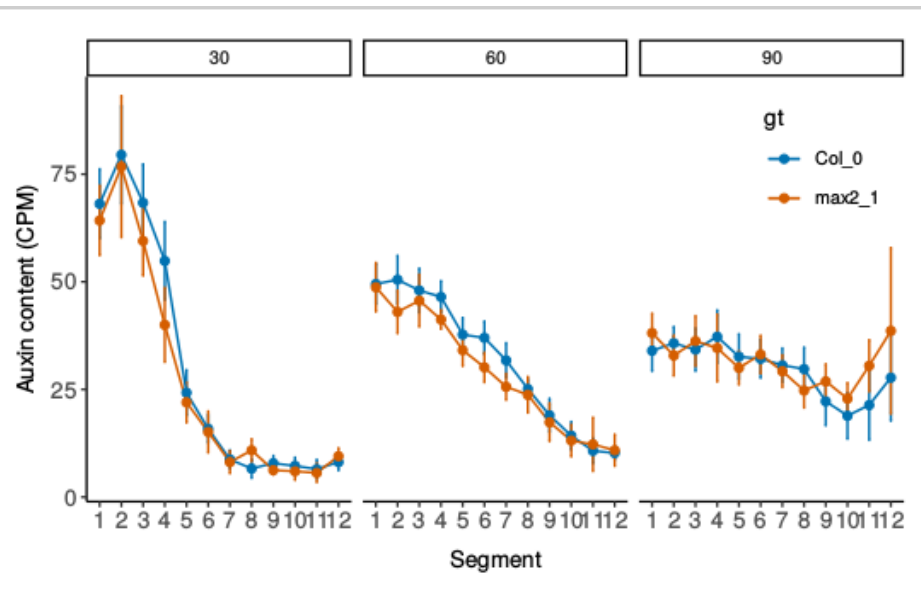
n hour ^{14}C auxin incubation

Cut into 6 2.5mm pieces



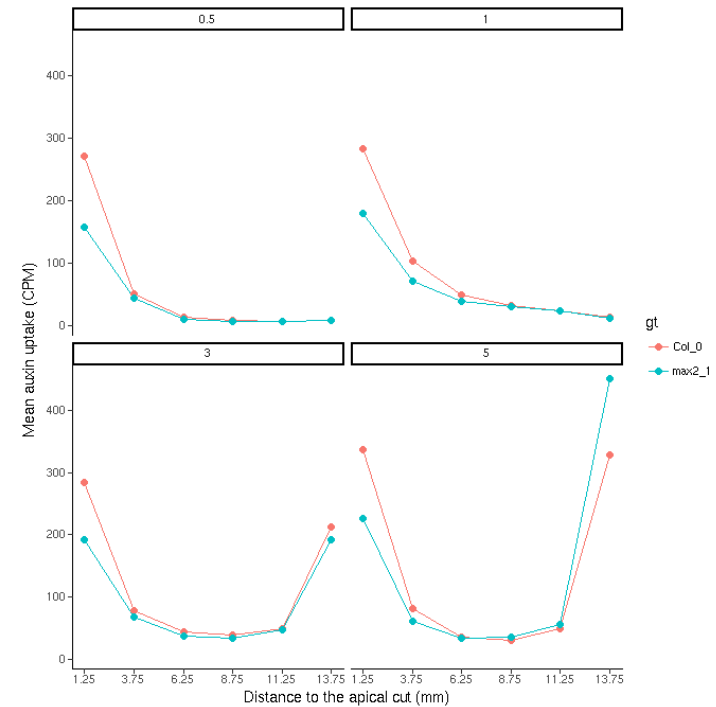
Pulse (10min)

- 24 mm stem segments
- 10 min ^{14}C -IAA ($5\ \mu\text{M}$) application
- 30, 60 or 90 min wait
- CPM measured in 2 mm long sub-segments

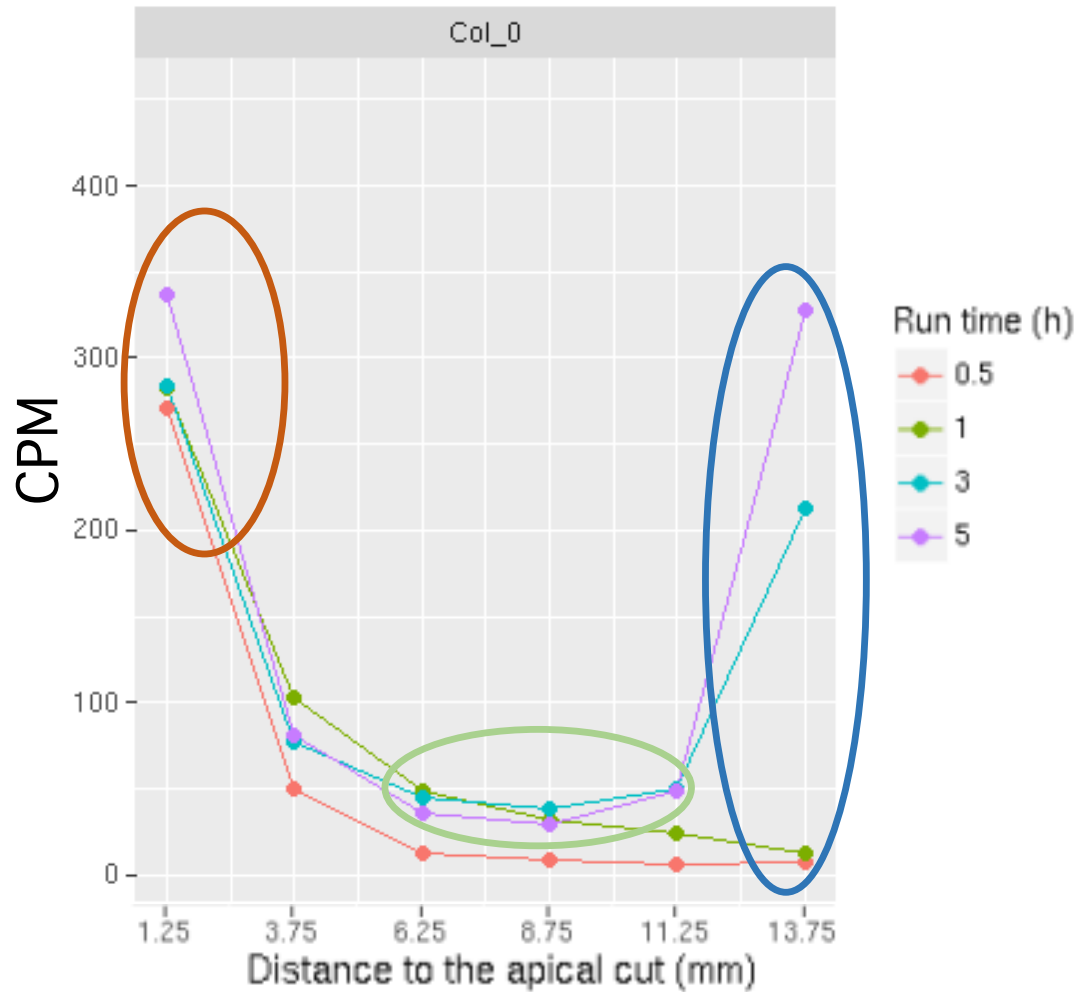


Continuous treatment

- 15 mm stem segments
- 30 min, 1h, 3h or 5h ^{14}C -IAA ($1\ \mu\text{M}$) application
- CPM measured in 2.5 mm long sub-segments
- (Standard bulk transport assay 6hrs, last 5 mm measured)



Interesting features



Bulk auxin transport results reproduced in accumulation of auxin over time at base of stem

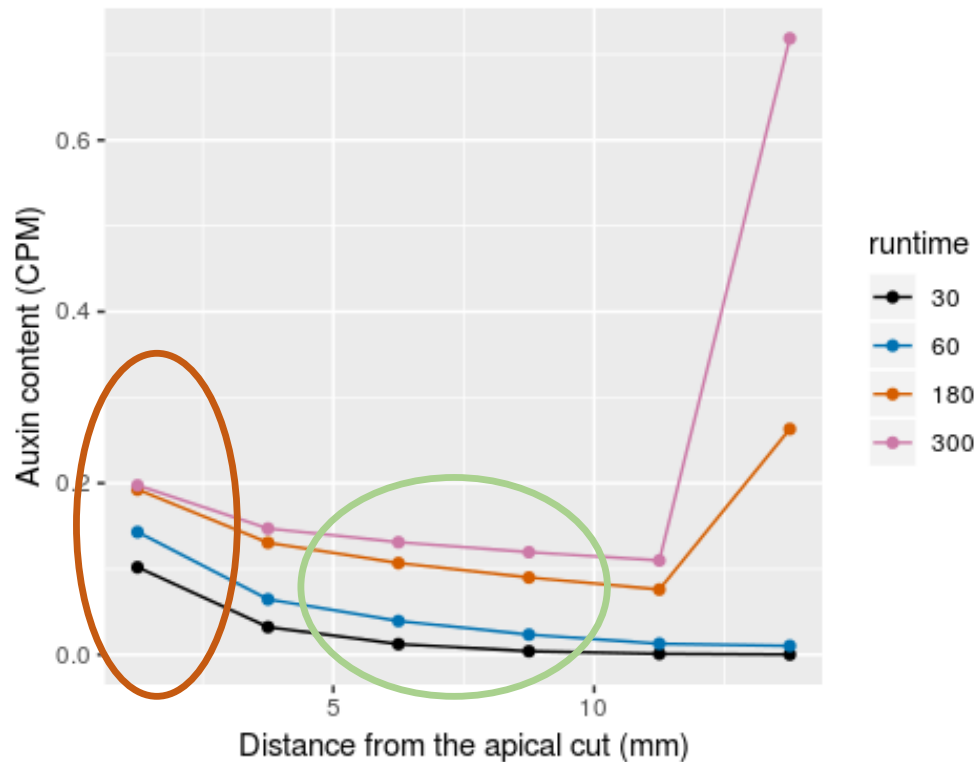
No accumulation of auxin in the middle of the stem over the time course

Auxin level at apex remains higher than middle sections

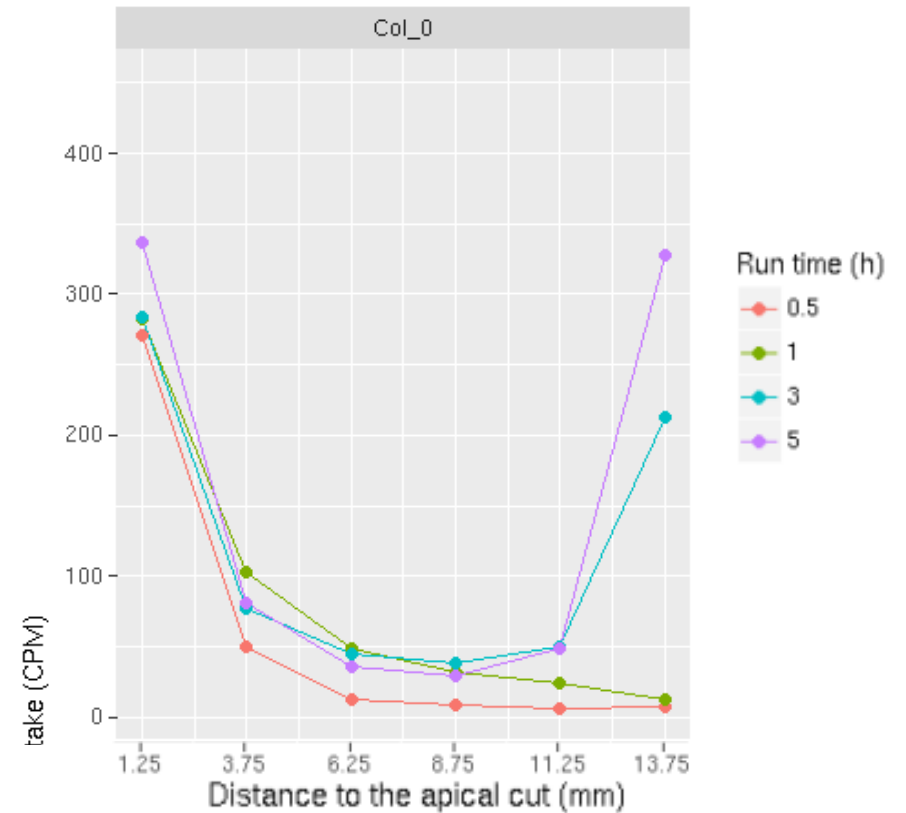
Interesting features poorly captured by model

Middle segment levels continue to rise

Top and middle levels equilibrate

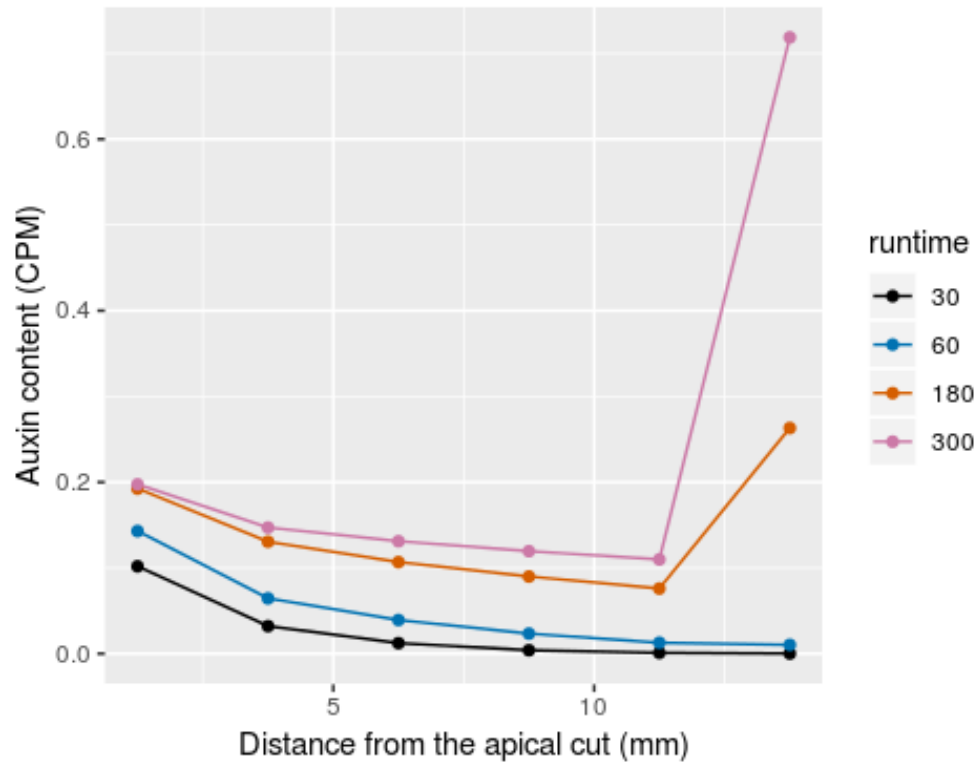


Model

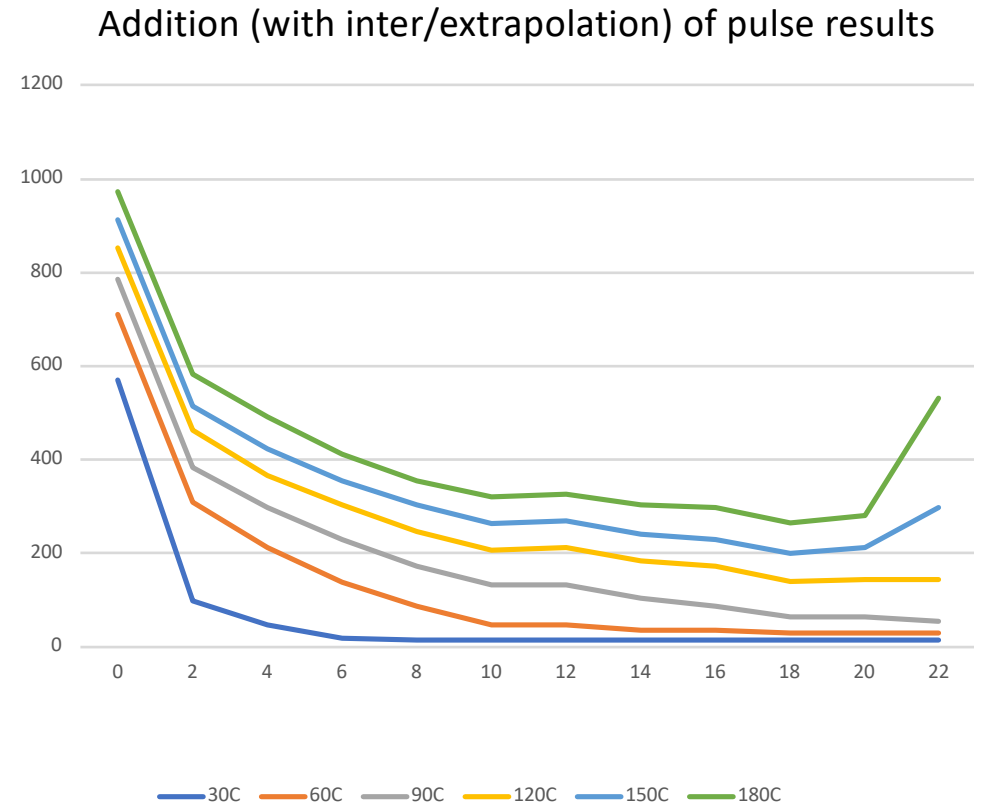


Data

Inter/extrapolation of pulse data matches model in middle but not top segments

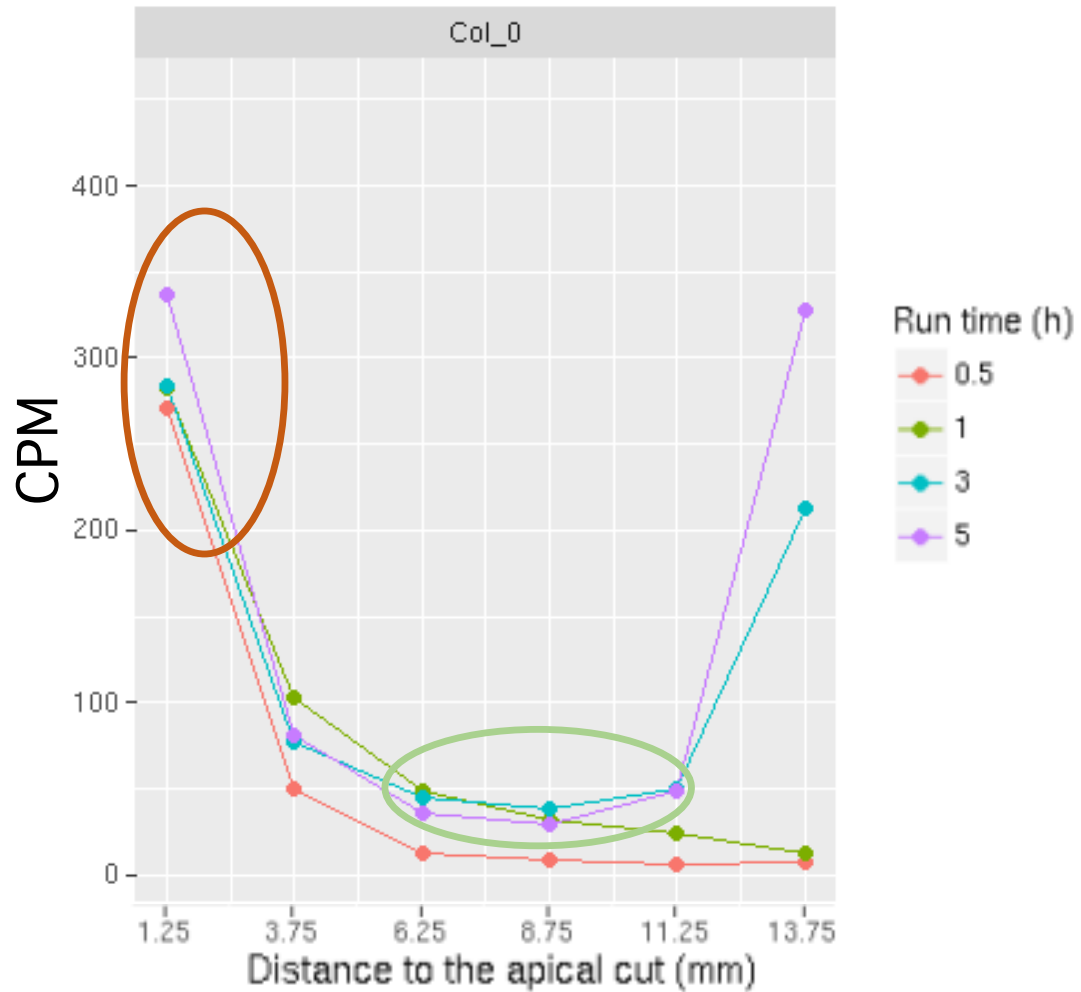


Model



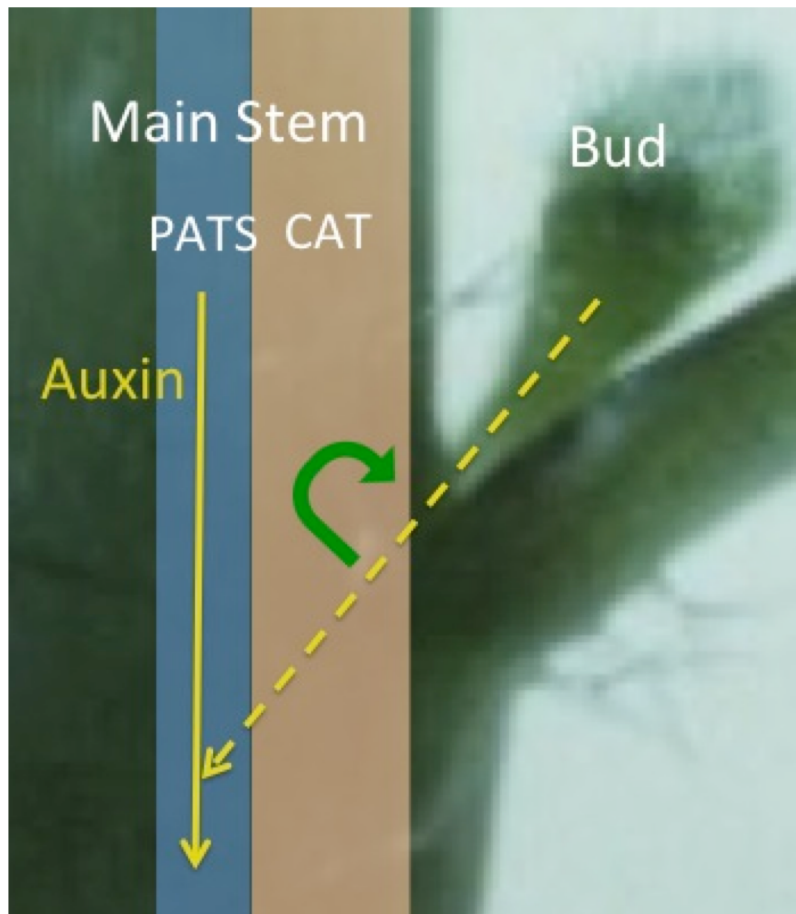
"Data"

Questions



- Accumulation at apical end in all assays- Conjugation and immobilisation of auxin?
- Loading issues?
- Equilibration of middle region not evident in pulse data but clear in continuous application data- Explanations?

Two-step canalisation

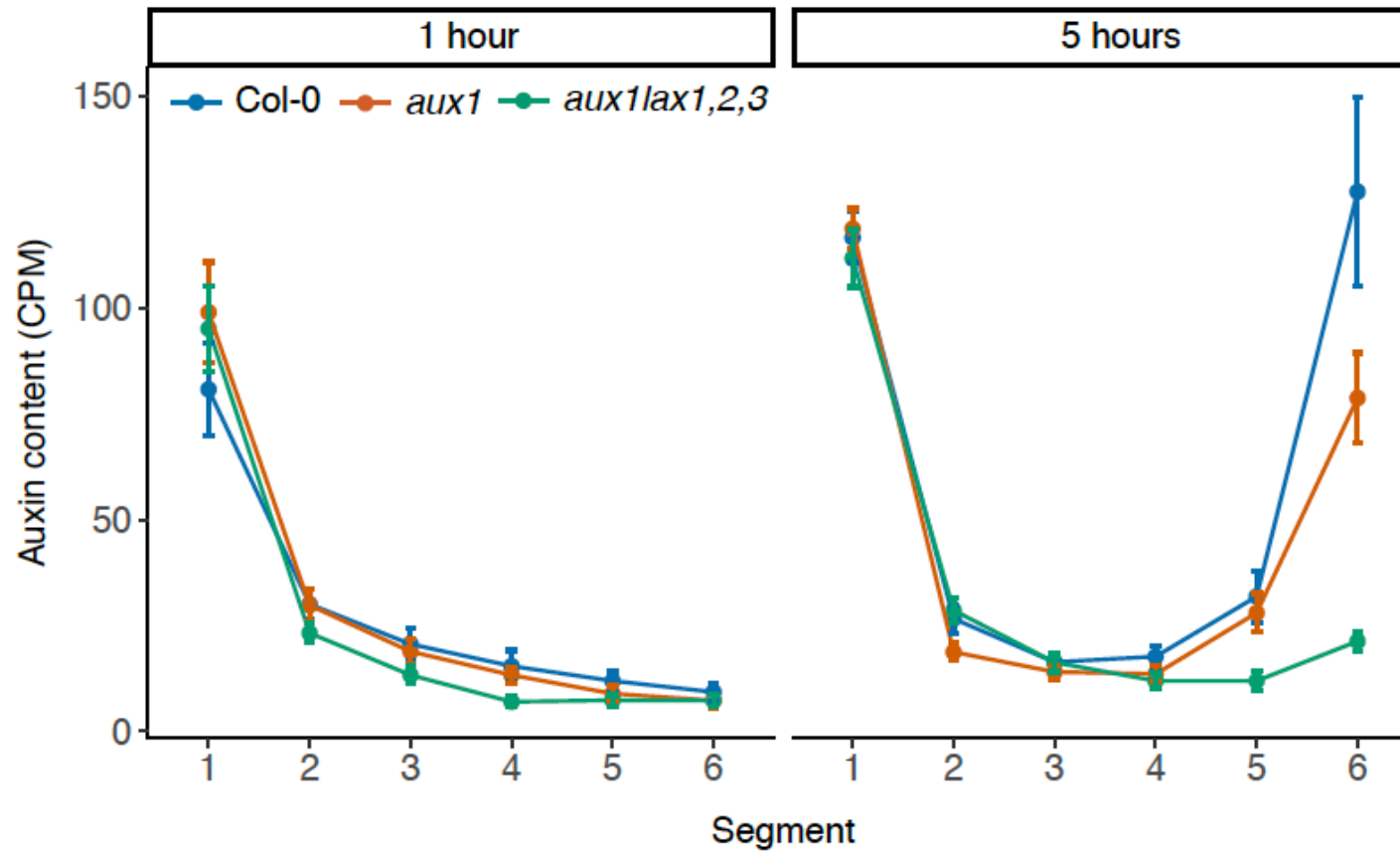


Bigger questions

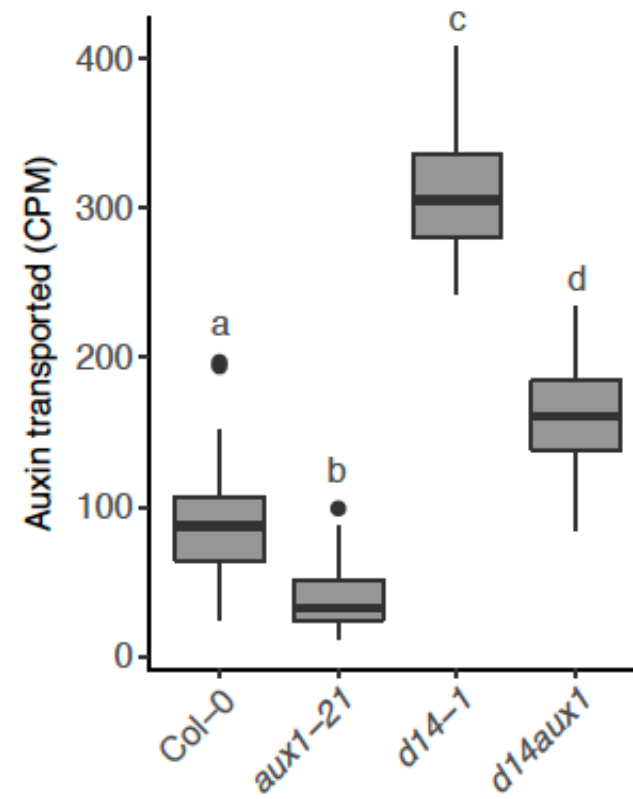
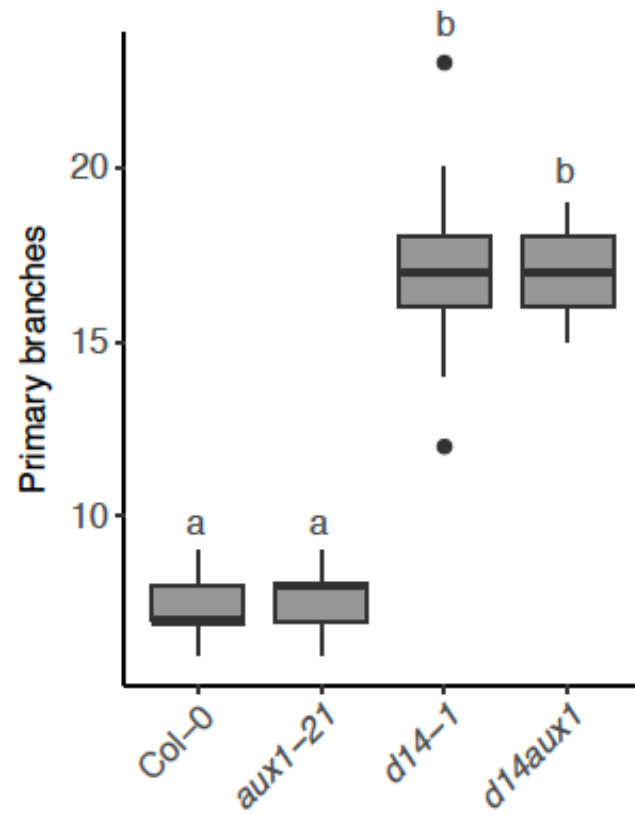
How do the properties of the stem auxin transport network contribute to branching control?

What is actually measured to bring about canalization (what the flux)?

Mutations in the auxin uptake carrier family have a major effect on stem auxin transport

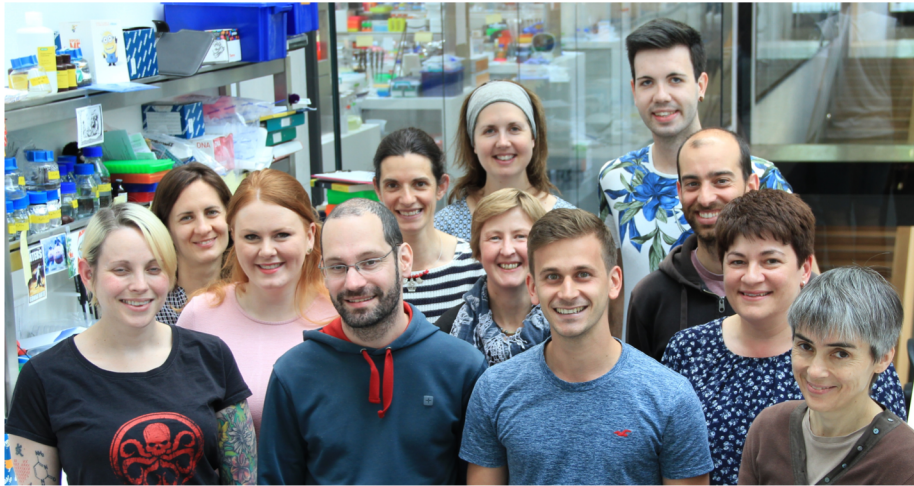


Mutations in the auxin uptake carrier family have no effect on branching, even in SL mutant backgrounds





The Sainsbury Laboratory Cambridge University



Branching and PAT

Genevieve Hines
Sally Ward
Martin van Rongen
Fabrizio Ticchiarelli
Anthony Bridgen
Ruth Stephens
Tanya Waldie
Tom Bennett
Yueyang Liang
Maddy Seale
Devin O'Connor

Collaborators

Karin Ljung (Umea)
Przemek Prusinkiewicz (Calgary)
David Nelson (UC Riverside)
Ning Zheng (U Washington)
Pilar Cubas (Madrid)
Joe Kieber (North Carolina)
Graeme Mitchison
Alison Bentley (NIAB)



Plasticity diversity

Urszula Baster
Hugo Tavares
Stephanie Smith

Bet Hedging

Katie Abley

Phloem

Andrea Paterlini