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

Phytomer
Axillary meristem
Leaf
Stem segment

Primary shoot
apical meristem
Makes phytomers

Incipient leaf

Shoot made of phytomers

Shoot system made of shoots

Plant development is plastic
Distributed decision making
Apical dominance
Auxin

IAA

\[
\text{NH} \quad \text{CH}_2-\text{COOH}
\]
Directional Auxin Transport

Apoplastic auxin (protonated) ↔ Apoplastic auxin (ionized)

Influx Carrier eg AUX1

Apoplastic pH low

Intracellular auxin (ionized)

Cytoplasmic pH higher

Polarly localized efflux carrier eg. PIN1
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

- Phytomer
- Axillary meristem
- Leaf
- Stem segment

Primary shoot apical meristem
Makes phytomers

Incipient leaf

Auxin flow

Shoot made of phytomers

Shoot system made of shoots

Chemical structure: \( \text{C}_6\text{H}_4\text{NH} - \text{CH}_2\text{COOH} \)
Canalization theory – feedback loop

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

\(\Phi\) - is the flux from \(i\) to \(k\)

\(\rho\) - PIN insertion constant

\(\mu\) - PIN removal constant

Przemek Prusinkiewicz
Bistability of a basic auxin transport canalization model

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

- \( \Phi \): Auxin flux
- \( \rho \): PIN insertion constant
- \( \mu \): PIN removal constant

Collaboration with Przemek Prusinkiewicz
Przemek Prusinkiewicz
Residual auxin production

Basipetal sequence of bud activation

Stops at some point
Strigolactone deficient mutants are branchy

Strigolactone is upwardly mobile

WT  max1
Strigolactone (GR24) limits auxin transporter accumulation making canalization more difficult.
Bistability of a basic auxin transport canalisation model

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

- \(\Phi\) - Auxin flux
- \(\rho\) - PIN insertion constant
- \(\mu\) - PIN removal constant

Affected by strigolactone

Collaboration with Przemek Prusinkiewicz
Wild type vs. max mutants

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

Residual IAA production
Bistability of a basic auxin transport canalisation model

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

- \( \Phi \): Auxin flux
- \( \rho \): PIN insertion constant
- \( \mu \): 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

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

- \(\Phi\) - Auxin flux
- \(\rho\) - PIN insertion constant
- \(\mu\) - PIN removal constant

Affected by GN and TIR3
Affected by strigolactone

Collaboration with Przemek Prusinkiewicz
Parameter space exploration - Auxin transport

- PIN Insertion-GN/TIR3
- PIN removal-SL
- Auxin transport levels (% wild-type)
*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
Parameter space exploration - Shoot branching

PIN1
Removal - SL

PIN1
Insertion - GN/TIR3
Strigolactone can promote or inhibit branching depending on plant auxin transport status.
Competitive canalization as a signal integrator

• Relative not absolute
• Dynamic as well as steady state
• Systemic as well as local

Bud auxin
source strength

Canalization
feedback
dynamics in the
tissue between
the bud and
the stem

Stem auxin
sink strength
Auxin flow

SL flow

Phytomer
Axillary meristem
Leaf
Stem segment

Primary shoot
Apical meristem
Makes phytomers

Incipient leaf

Shoot made of phytomers

Shoot system made of shoots
Strigolactone enhances bud-bud competition

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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
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 \textit{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

6 hour incubation

Piece of stem

Radio-labelled auxin

Auxin transported (%)

- max2
- max2
- max2

Col-0

gn

tir3
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

14C auxin incubation

Cut into 6 2.5mm pieces
**Pulse (10min)**

- 24 mm stem segments
- 10 min $^{14}$C-IAA (5 μM) 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 μM) 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
Inter/extrapolation of pulse data matches model in middle but not top segments.
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.
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