From Genome to Phenotype: Modeling the interaction of physical and chemical signals in plant meristems

Meyerowitz Lab and many collaborators
Needs to understand tissues, morphogenesis and development:

- Image Analysis
- Computational Models of Chemical Signals
- Physical Models
- Connection between Physical and Chemical Models and Substrate
- Connection between Chemical and Physical Models
Where the action is: The *Arabidopsis* shoot apical meristem
Shoot apical meristem
35S::YFP29-1, every 2 hours and 30 minutes 65 hours
Lineage analysis

Developing lineages were traced by tracking individual cell divisions over 5 days.
Animation of tracked and smoothed nuclear trajectories
Principal directions of growth
Flower Development, Too
Previous work has shown:-

• Application of auxin paste to plant meristems causes lateral organ outgrowth at the site of application (Snow and Snow, 1937).

• When auxin transport is blocked, either in pin1 mutants or in plants treated with chemical phytotropins lateral organs do not initiate (Okada et al., 1991).

• Auxin application can rescue organ initiation when transport is blocked (Reinhardt et al., 2000).

Taken together, these data suggest that endogenous auxin is required in some way for lateral outgrowth on the meristem flanks.
Micro-array analysis of auxin-induced genes in *pin1-1* mutants

Two days after treatment

Three days after treatment
Examples of expression profiles

PIN1
AUX1
IAA4
AP2-like
Zinc finger
SAUR-like

Zinc finger
Giberellin beta-hydroxylase
Polyubiquitin
PIN1 expression is auxin induced

Auxin induced genes on pin1-1 apex

PIN1:GFP and DR5 expression

PIN1:GFP and DR5 expression

30 mins
3 hrs
5 hrs
Time lapse imaging of PIN1GFP in the meristem confirms expression dynamics!
Green: PIN1-GFP
Red: Plasma Membrane Dye
Clues to a model.....
Model

1) Auxin efflux carrier moves auxin, and its gene is auxin-induced - so rate of transport from a cell depends on the auxin level in the cell.

2) Local high auxin concentration causes new primordia, and it gets high locally by transport and diffusion.

3) Auxin efflux carrier is polarized in cells, and points toward neighboring cells with the highest auxin concentration.
Equations

\[
\frac{dP_i}{dt} = F_{\text{creation}}(a_i) - K_{Pd} P_i
\]

\[
\frac{da_i}{dt} = K_p - K_d a_i - T \sum_{j}^{N_j} (a_i P_{ij} - a_j P_{ji}) + D \left( \sum_{j}^{N_i} a_j - N_i a_i \right)
\]

\[
P_{ij} = P_i \frac{a_j}{\sum_{k}^{N_i} a_k} \propto P_i a_j
\]

\[P_i, a_i - \text{PIN1, auxin concentration in cell i}\]

\[T - \text{transport strength, D - diffusion strength}\]
Peak formation including radial growth
Polarity reversal is abrupt and has a sharp boundary
The auxin concentration model may account for PIN1 reversal.
Conclusions

• The distribution of auxin is involved in patterning PIN1 expression and can influence PIN1 polarity.

• PIN1 polarity also responds to signals generated by neighboring cells.

• These observations support a proposed model for phyllotaxis based on feedback between PIN1 polarity and auxin levels within neighboring cells.

• THIS IS A NEW CLASS OF DEVELOPMENTAL MODEL - NOT REACTION-DIFFUSION, NOT MUTUAL INHIBITION, BUT REGULATED TRANSPORT OF A MORPHOGEN
pinoid apex, pPIN1::PIN1-GFP

IAA spot added at arrow
Question

How does a cell *directionally* detect the auxin concentration of its neighbors?

- Auxin itself as a signal?
- Auxin induces a different diffusible substance?
- Auxin induces an ephrin-type cell-to-cell protein?
- Auxin causes cell expansion, physical force affects neighboring cells?: Could this explain expansin and PME experiments?
Can stress really be directionally sensed by meristem cells?
Microtubule patterns in the SAM

Movie S1

Fig. 2
Stress Pattern in SAM
Model surface extracted from real template - Calculated stress match real microtubules.
MT orientations 6 hrs following compression
Simulation of cell ablation
Double cell ablations also match and argue against chemical morphogen model.
Question

How does a cell directionally detect the auxin concentration of its neighbors?

Why do cell wall relaxers have the same effect as auxin in inducing new primordia?

• Auxin causes cell expansion, PIN1 moves to the membrane adjacent to the most stressed wall?
Responses to single cell ablations indicate local signaling can modulate PIN1 polarity. Response occurs within two hrs.
PIN1 and microtubule interphase are generally aligned.
Reorientation (at a distance) is NPA insensitive

Plants treated with 100μM NPA for 24 hrs

MTs

PIN1

Plants treated with 100μM NPA for 24 hrs
Most stressed wall/PIN1?
Conclusion

• Cell polarity in response to ablation is not easily perturbed by inhibition of auxin transport but fits patterns predicted by mechanical models

• Phyllotaxis is likely to involve a mechanical-chemical coupling
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