

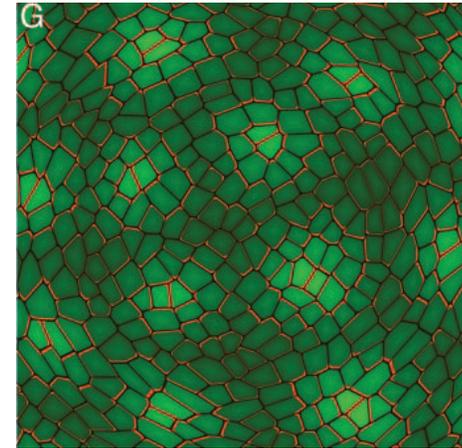
Auxin transport by the numbers: carriers, diffusion, and plasmodesmata

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Bard College at Simon's Rock
March 2014

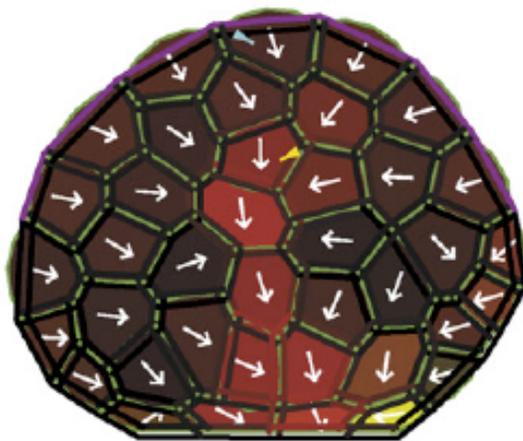
What auxin does...

1. Meristems: auxin accumulation triggers new primordia. Auxin canals initiate vascular strands.
2. Regulate elongation growth.
3. Wound-healing: restores connectivity of vascular tissue.
4. Tropic growth is signaled by rapid changes in auxin distribution.

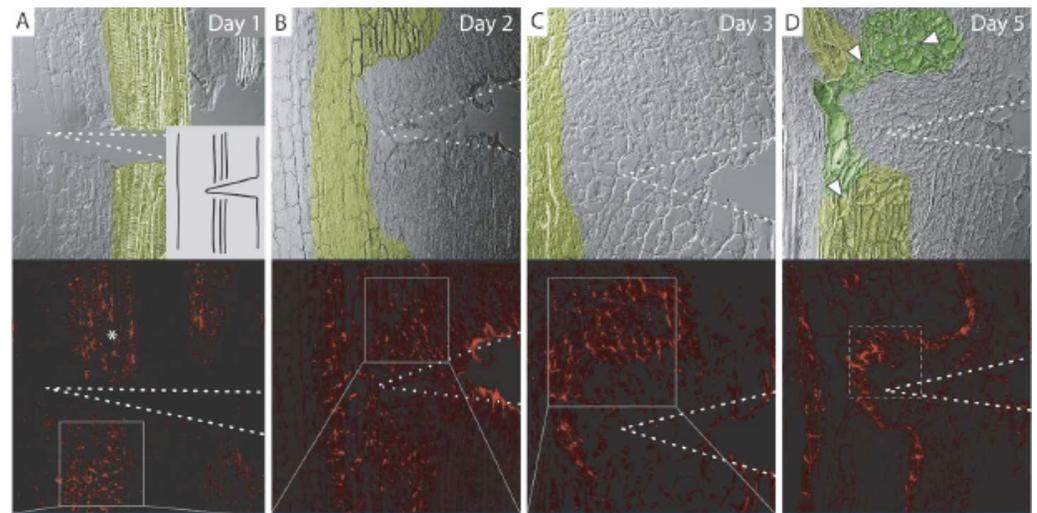
Smith et al. 2006



Merks et al. 2007



Sauer et al. 2006



How auxin gets in/out of a cell

Protonated auxin (IAAH) can diffuse across membrane.

Ionized auxin (IAA⁻) can cross the cell membrane via

PIN efflux carriers (IAA⁻)

AUX/LAX influx carriers (IAA⁻ plus two protons)

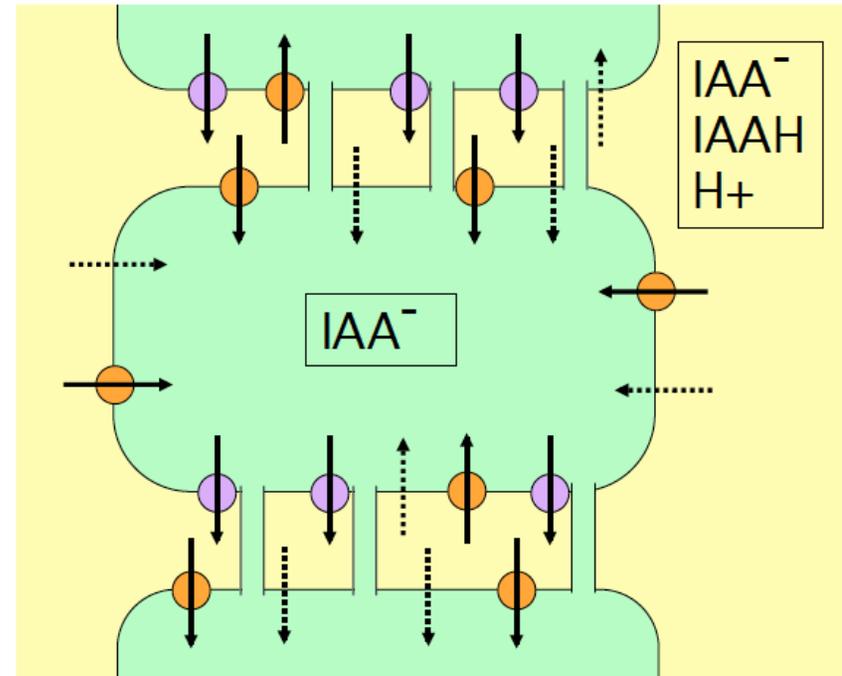
ABCBs (IAA⁻, burns ATP)

Other families of carriers (PILS, WAT1) distribute auxin inside the cell.

IAA⁻ moves through the plasmodesmata

Q: How do we estimate the concentration of auxin inside/outside the cell?

A: need real numbers for these fluxes!



Some vocabulary

Flux J = the rate at which auxin moves in/out of cell
units of moles/s/cell or moles/s/(unit membrane area)

Influx Permeability $P_{\text{influx}} = (\text{influx})/(\text{external concentration})$
units of $\mu\text{m/s}$

Efflux Permeability $P_{\text{efflux}} = (\text{efflux})/\text{internal concentration}$
units of $\mu\text{m/s}$

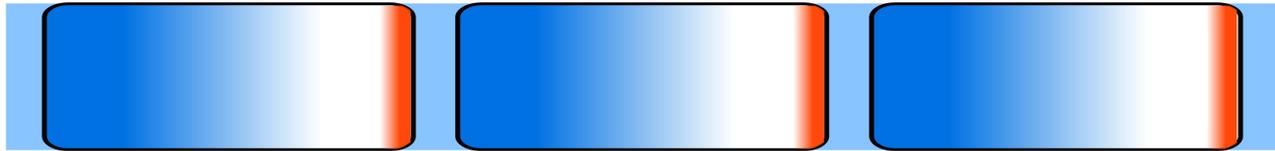
Transport speed v = speed of a labeled pulse of auxin down a stem
units of $\mu\text{m/s}$

None of these is the speed of an auxin “signal”, as determined by the timing of various cellular events.

Part I:

Auxin efflux

Auxin speed vs. Efflux permeability



Single file of plant cells, length L

Auxin concentration

Efflux carriers

Mitchison 1980 showed the speed v

$$v = \frac{P_{efflux}}{1 + \left(\frac{LP_{efflux}}{2D} \right)}$$

D is the diffusion coefficient of auxin inside the cell

If diffusion is fast enough ($D \gg LP$), then $v = P$

Cytoplasmic streaming/mixing will also bring v close to P .

Reviewing auxin transport speed

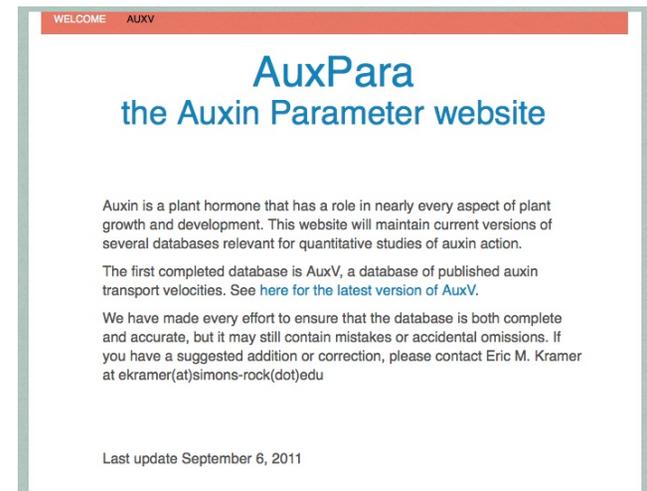
Textbooks generally say 10-20 mm/h, but no comprehensive review.

Kramer et al. 2011 TIPS.

An auxin velocity database **AuxV**

<http://www.simons-rock.edu/AuxPara>

Currently: 95 publications, 44 species



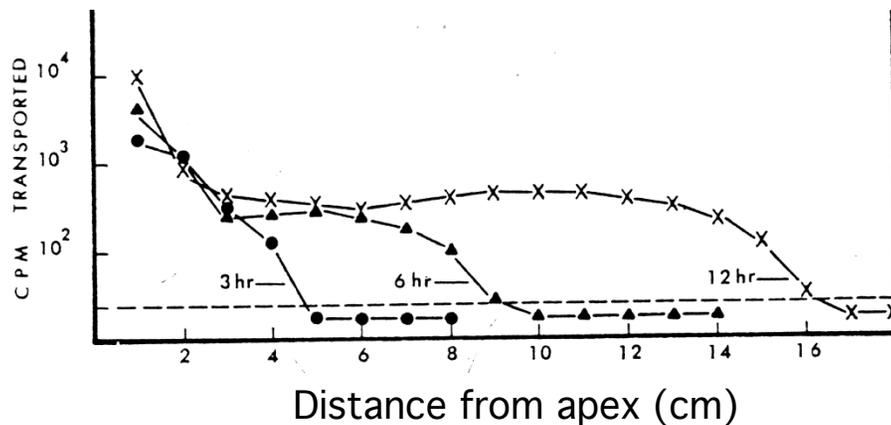
Additions/corrections are welcome!

Evaluation criteria

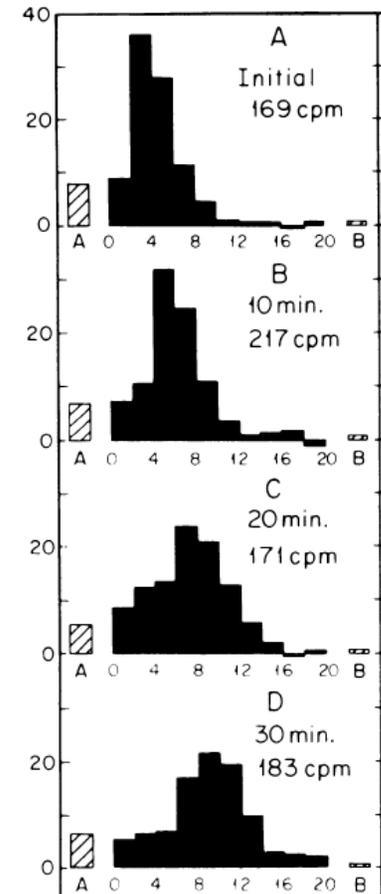
- No bioassays; no *Avena* curvature tests.
- Sample data must be shown.
- Curve fit should be convincing.
- Speed should be calculated correctly, using the solutions to the advection diffusion equation.

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} - v \frac{\partial c}{\partial x}$$

From Hollis & Tepper, 1971
Ash branches, front method

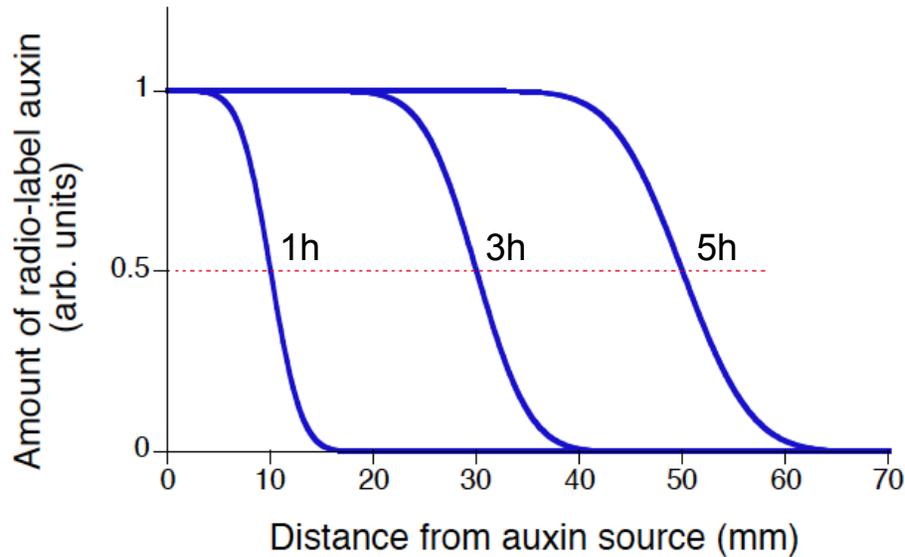


Goldsmith 1967
Corn coleoptiles
Pulse method

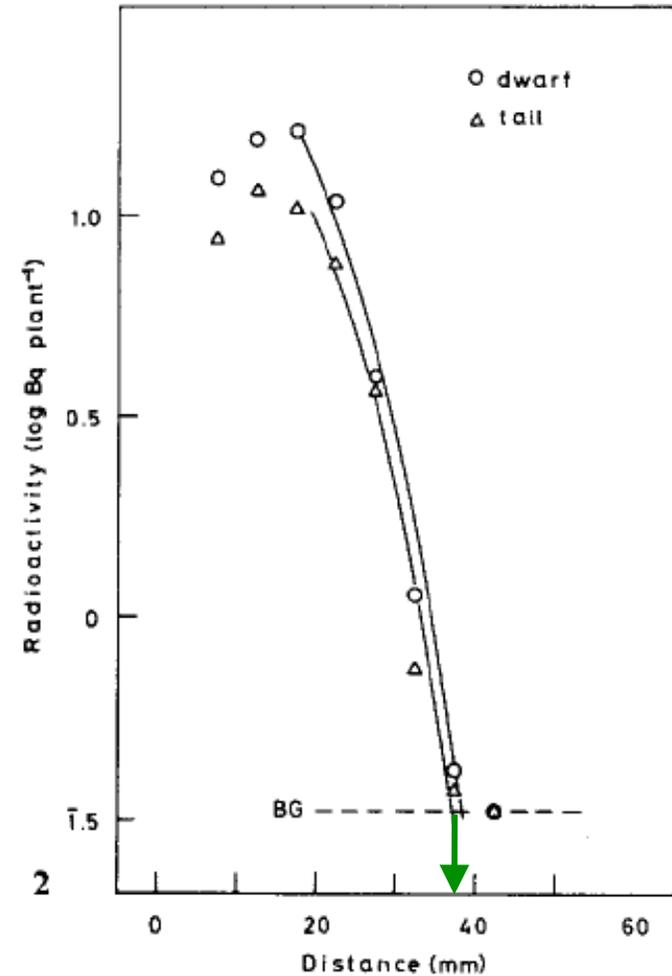


Distance from apex (mm)

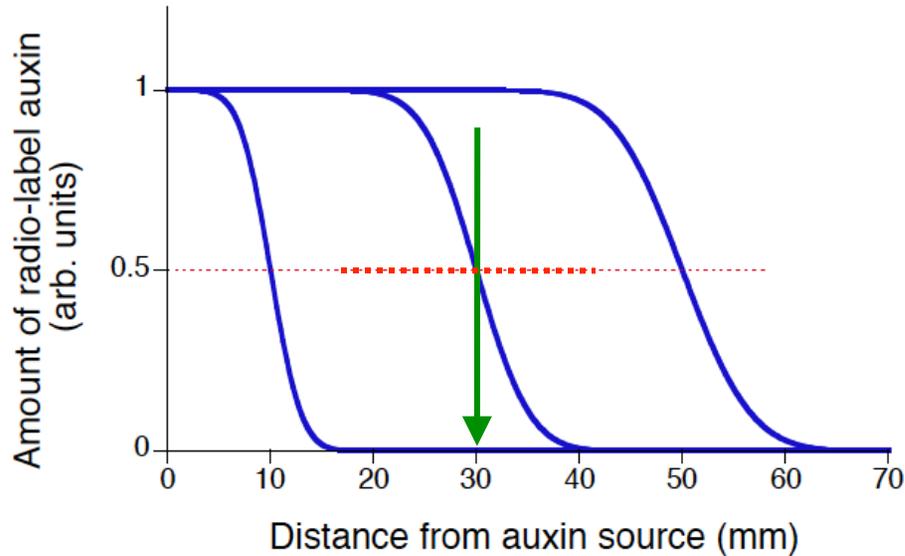
The front method done wrong



Sample data from 1980
(note log scale on y)
Auxin transport in pea stems.
Front method
Transport period = 3 h
Reported speed 12.4 mm/h (tall)
12.7 mm/h (dwarf)



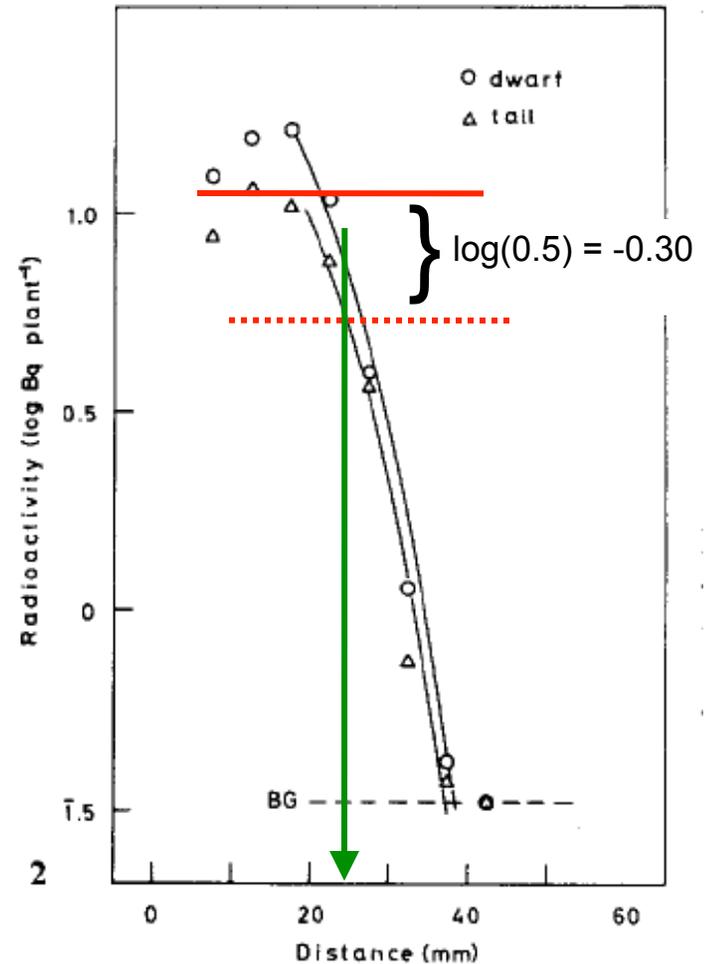
The front method done right



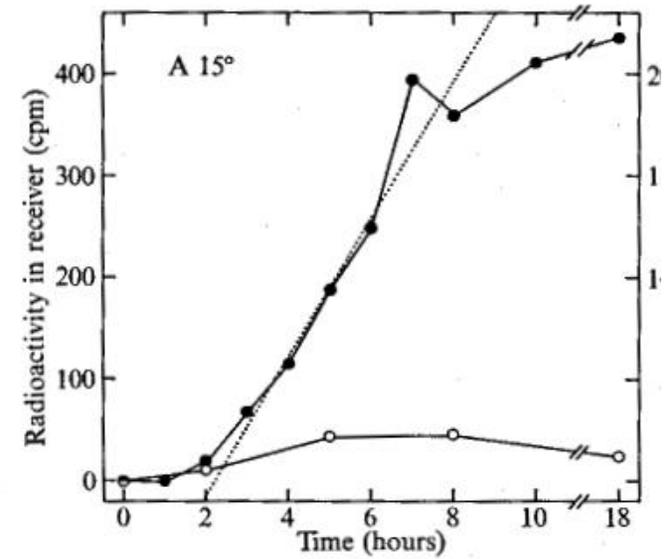
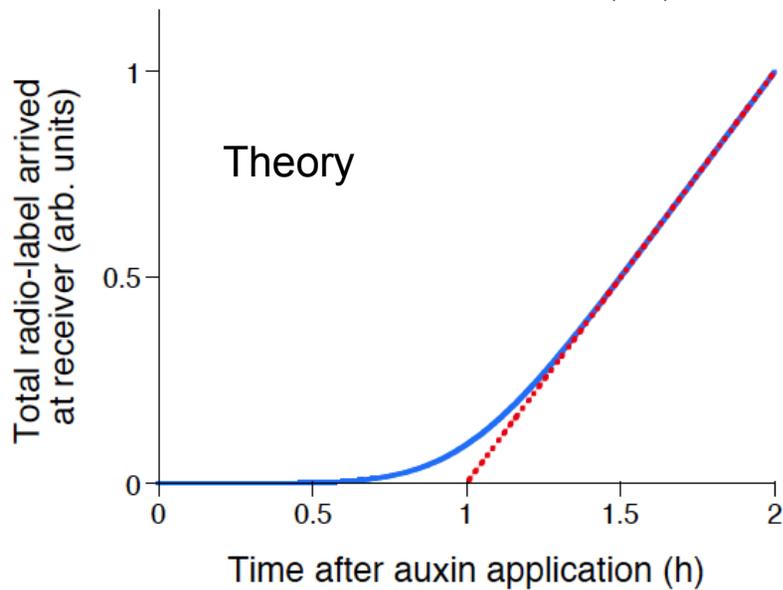
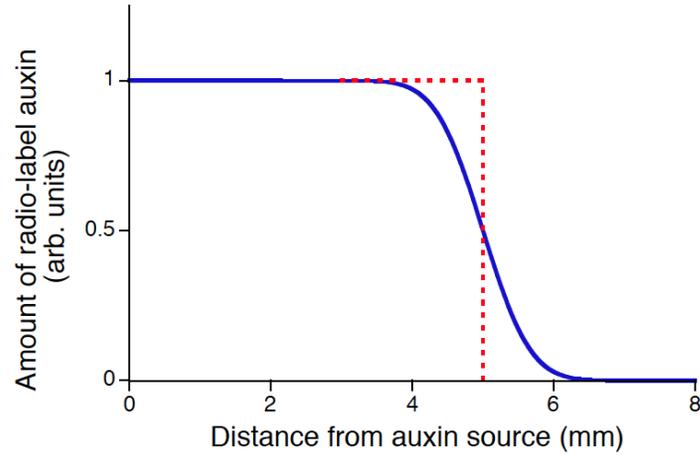
The correct way to measure speed from a front is to track the half-height.

Recalculating 1980 (tall) gives the AuxV value 9.0 ± 0.9 mm/h, a decrease of 27%.

Note implication for bioassays.



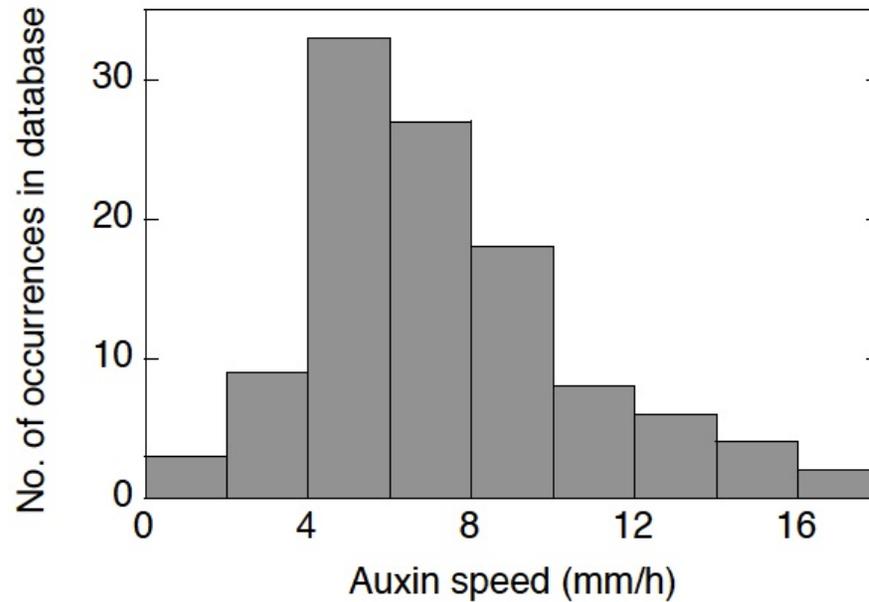
Intercept method



Wilkins & Cane 1970
 Maize radicle, 6 mm segments,
 transport towards root apex.

$$\text{Speed} = (\text{segment length}) / (\text{time intercept})$$

Auxin transport speed



AuxV: 119 speeds in 35 species

(Limit temperature range: 15-30 °C)

Max: 18 mm/h *Zea mays* coleoptiles

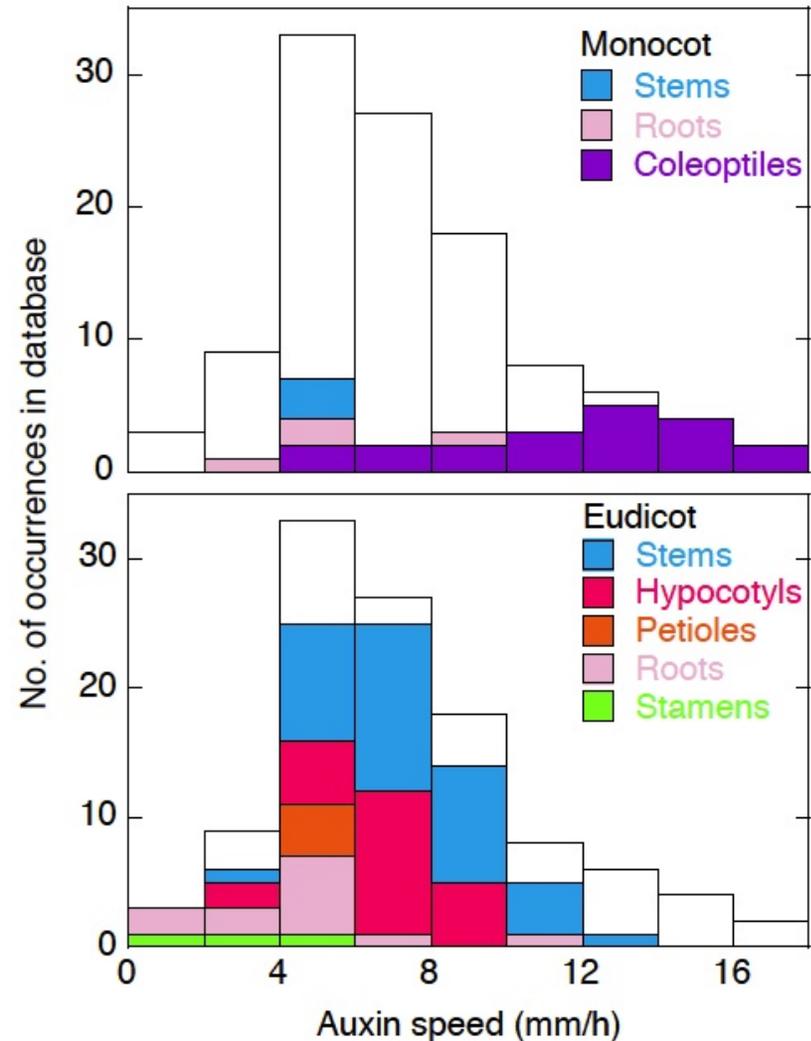
Min: 1.2 mm/h *Cleome hassleriana* stamen filaments

Median: 6.3 mm/h

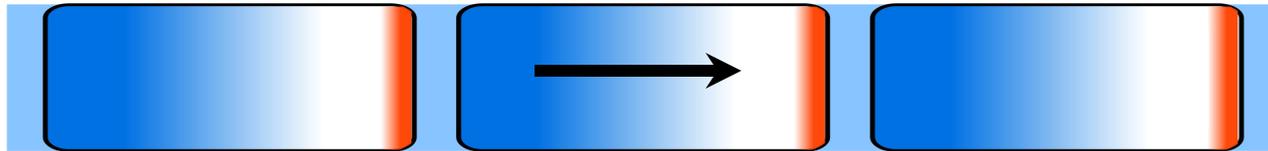
Speed by clade and organ

Coleoptiles are by far the fastest. Since *Avena* coleoptiles were the standard for years, the accepted value for auxin transport became 15 mm/h.

Roots and stamen filaments tend to be the slowest.



Why speed < 20 mm/h?



Mitchison 1980 noted that the speed of auxin transport may be limited by the ability of auxin to diffuse across the cell.

This gives an upper limit to the speed

$$v < 2 * D / L$$

Using $D = (0.4 - 0.1)D_{aq}$ and $L = 50 \text{ mm}$

$$v < 10 - 40 \text{ mm/h}$$

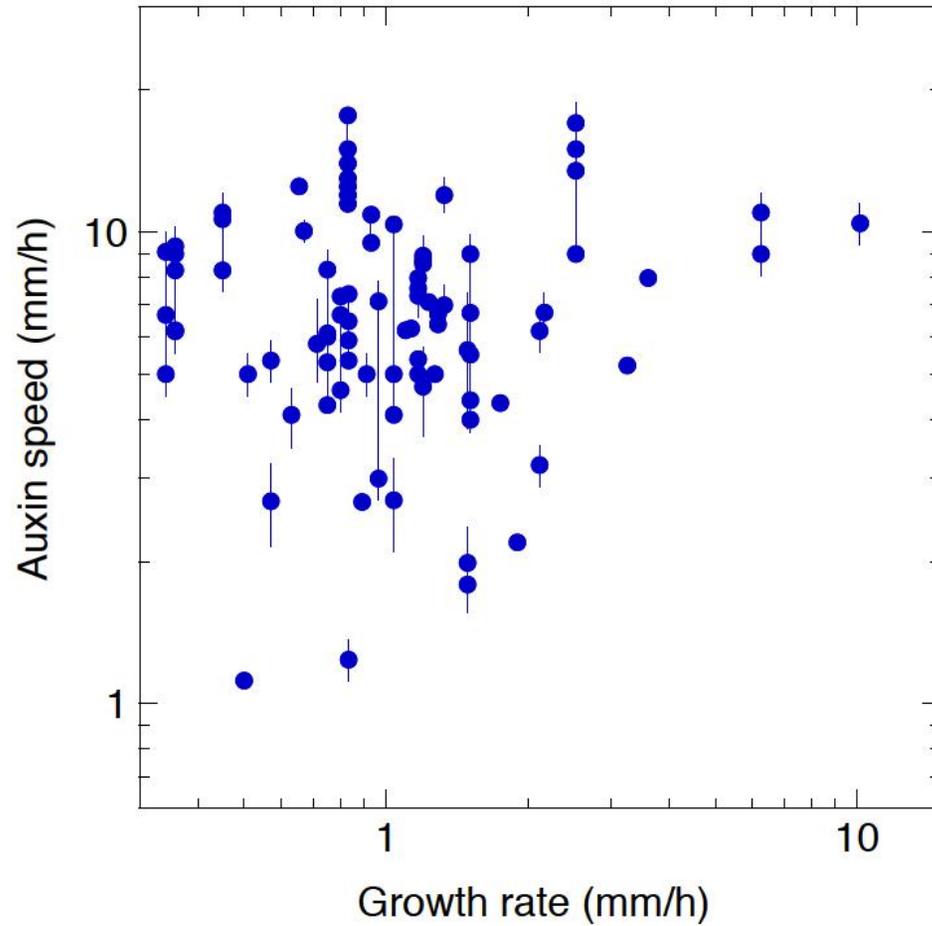
$$v = \frac{P_{efflux}}{1 + \left(\frac{L P_{efflux}}{2D} \right)}$$

Predicts: Longer cells → lower auxin speed
Cytoplasmic streaming might increase speed.

Auxin speed vs growth rate

Find growth rate for each auxin speed in database.

Match species, organ, age, etiolation state, temperature.

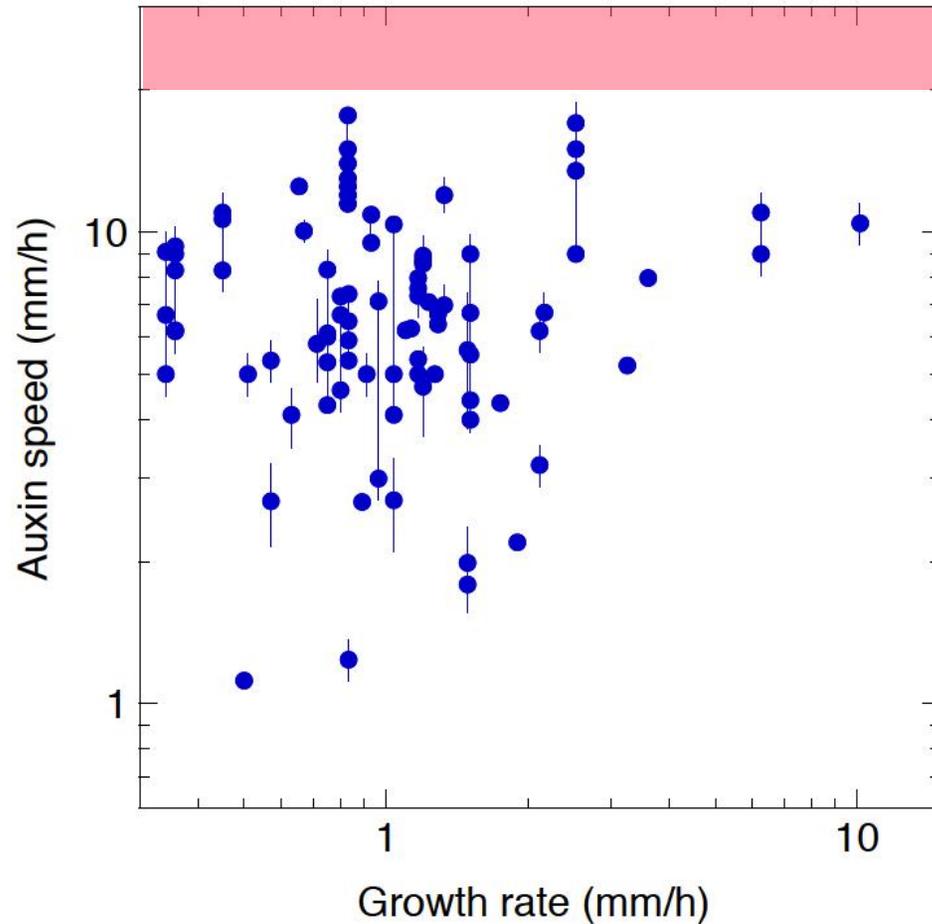


Auxin speed vs growth rate

Find growth rate for each auxin speed in database.

Match species, organ, age, etiolation state, temperature.

Upper bound:
speed < 20 mm/h



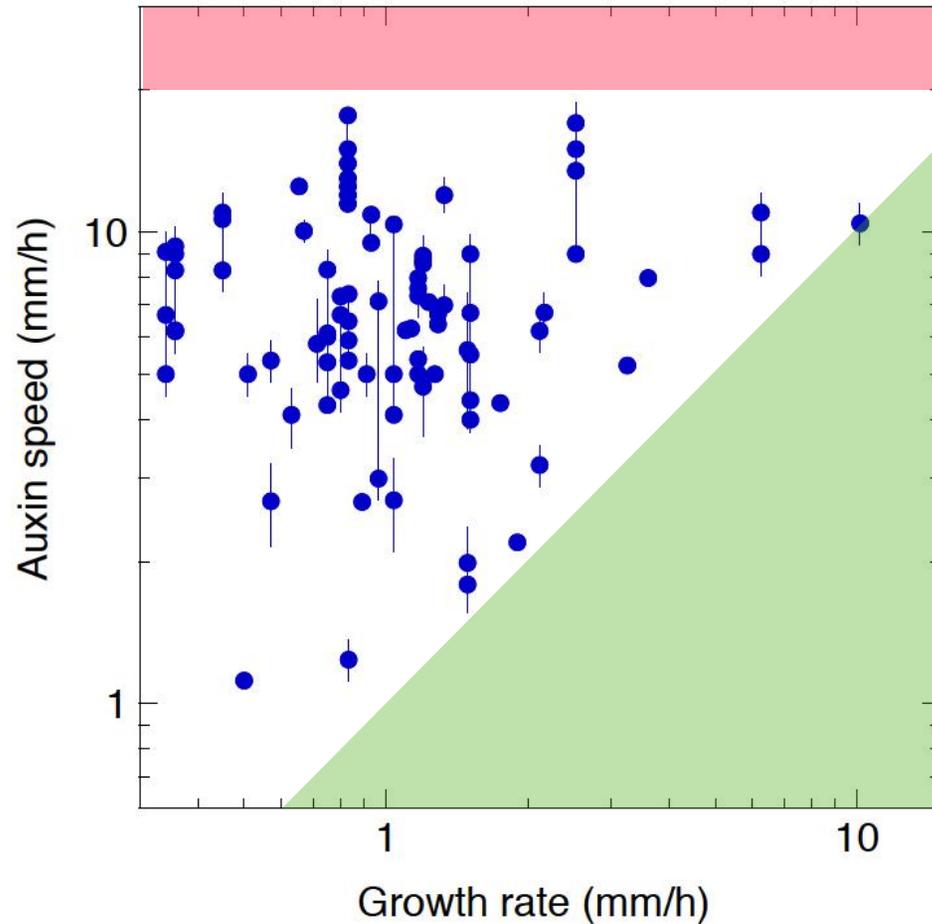
Auxin speed vs growth rate

Find growth rate for each auxin speed in database.

Match species, organ, age, etiolation state, temperature.

Upper bound:
speed < 20 mm/h

Lower bound:
speed > growth rate



Summing up Part I

Auxin speeds vary with species and organ.

Auxin speed has an upper bound imposed by cytoplasmic diffusion.

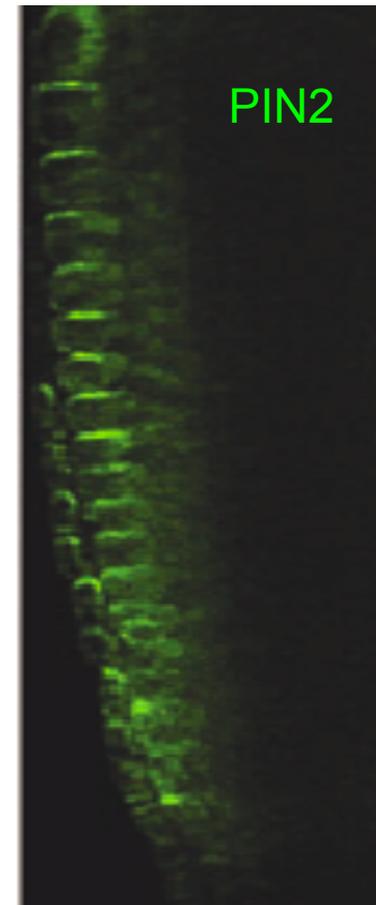
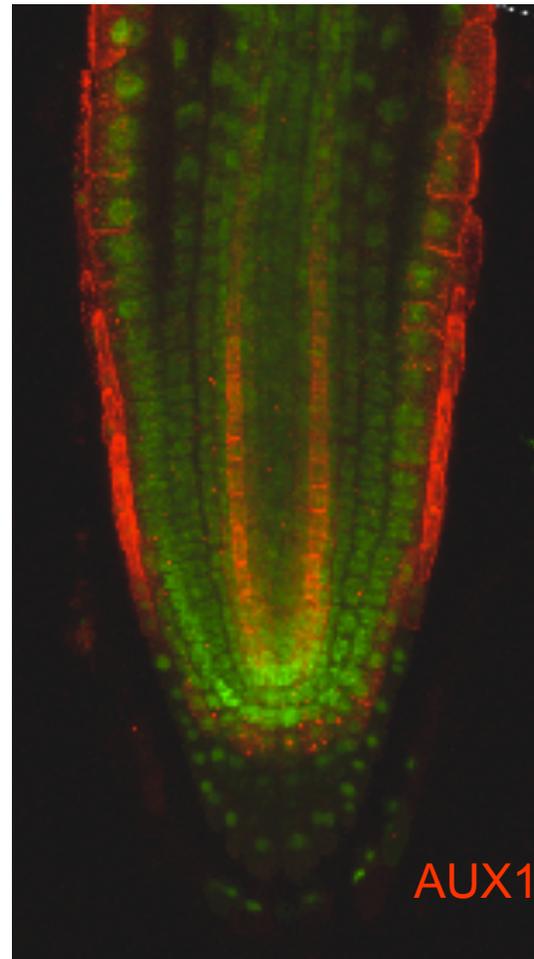
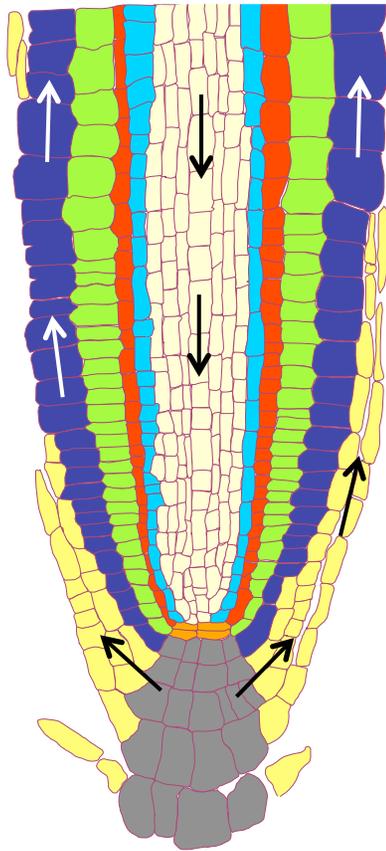
Auxin speed is faster than plant growth rate.

Speed provides estimate for efflux permeability,
median value 6 mm/h

Part II:

Auxin uptake

The root apex

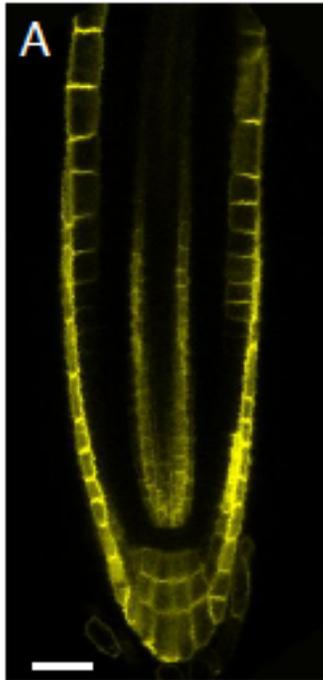


Shin et al. (2005)

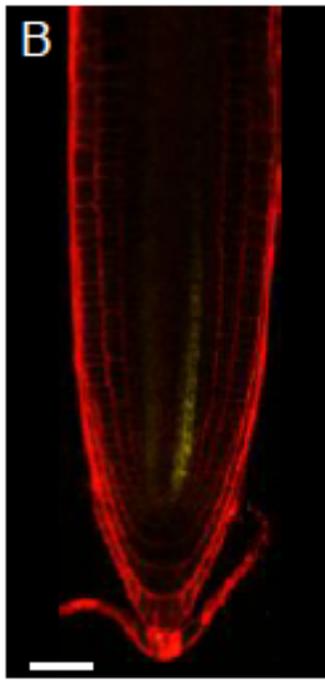
Influx carriers

Peret et al. 2012

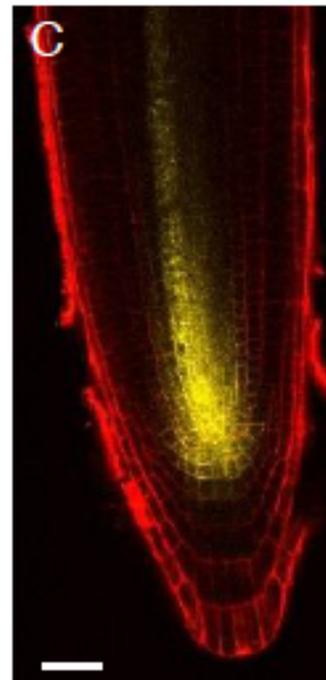
AUX1-YFP



LAX1-VENUS



LAX2-VENUS



LAX3-VENUS



And some ABCB's.

Open questions

Q: What is the permeability of the plasma membrane to IAAH?

A1: Delbarre et al.

Tobacco cell cultures (1994) and leaf protoplasts (1996)

$$P_{\text{diffusion}} = 0.4 - 0.5 \mu\text{m/s}$$

A2: Gutknecht & Walter (1980), artificial lipid bilayers

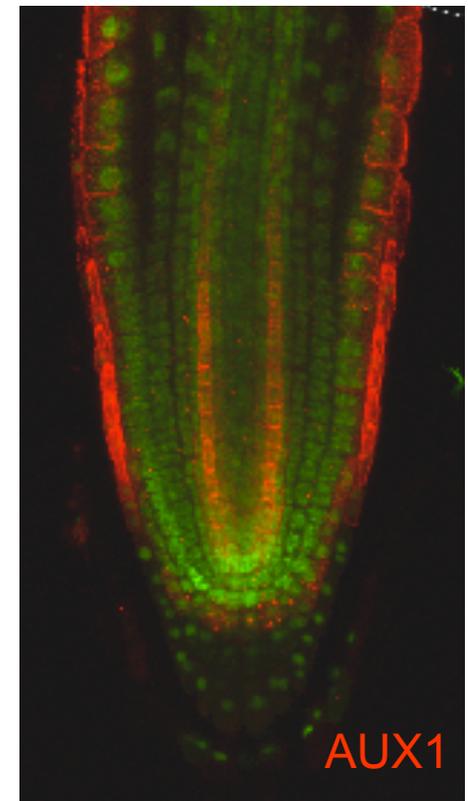
$$P_{\text{diffusion}} = 33 \mu\text{m/s}$$

Q: Can specific expression of an influx carrier partition auxin between adjacent cell layers?

A: Unknown. Need to compare $P_{\text{diffusion}}$ to P_{influx}

Q: Can the auxin influx carrier keep a cell supplied with auxin despite strong efflux?

A: Unknown. Need to compare P_{efflux} to P_{influx}



Earlier work: auxin uptake

The technique of radiolabeled auxin uptake in protoplasts/cell cultures

Rubery 1974 – climbing vine *Parthenocissus tricuspidata*

Raven 1975 – green algae *Hydrodictyon africanum*

Loper & Spanswick 1991 – soybean

Delbarre (1994, 1996) - tobacco

All done before the identification of auxin carriers.

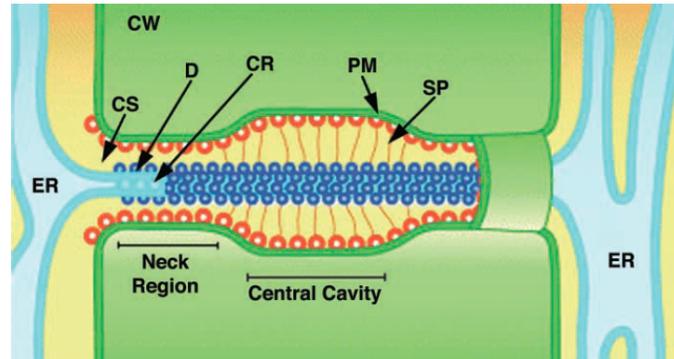
None in *Arabidopsis*.

Part III:

Symplasmic transport in the root meristem

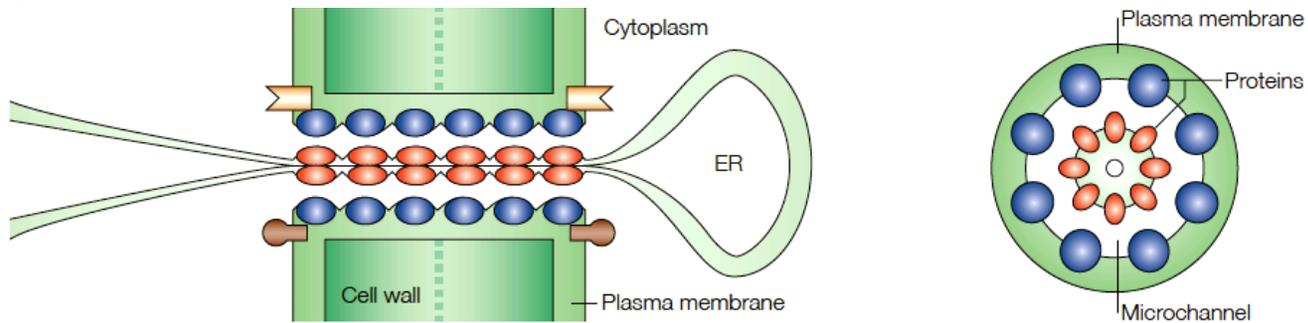
Plasmodesmata

Figure 1. Diagram of longitudinal section of PD spanning the cell wall between adjacent cells. CR, central rod; CW, cell wall; SP, spoke-like connections between the D and PM that may control aperture. Blue and orange circles represent hypothetical PD-specific proteins. Figure from Roberts and Oparka, 2003.



Zambryski 2004

a



Lucas 2004

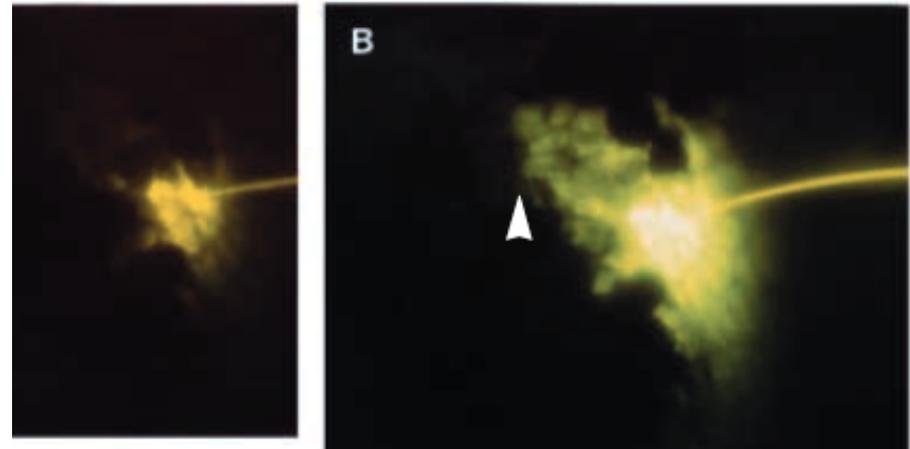
Proteins: movement proteins dock to pd and assist transport of specific proteins.

Small solutes (sugars, amino acids, fluorescent dyes) seem to pass nonspecifically through the lumen of the pd.

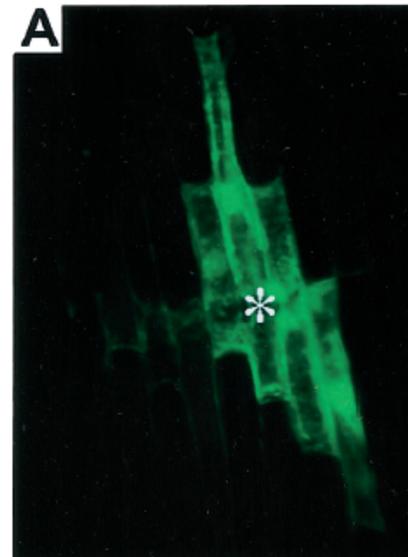
Size exclusion limit (SEL) ~ 700 Daltons.

Hints of fast movement

Rinne et al. 1998: Iontophoresis of lucifer yellow carbohydrazide (LYCH) in the shoot apical meristem of birch
~ 50 micron in 20 s.



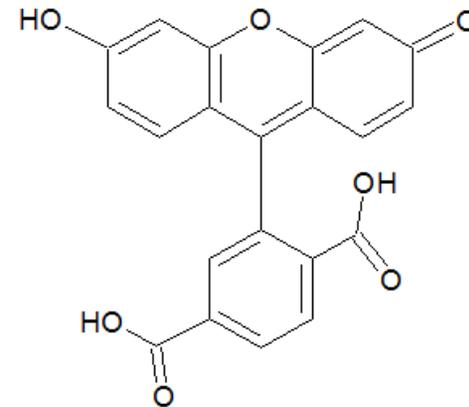
Sivaguru et al. 2000, reported dye coupling “within seconds”, in experiments with LYCH microinjected into peripheral root cells of wheat.



Did injection cause an artifact?

carboxyfluorescein diacetate

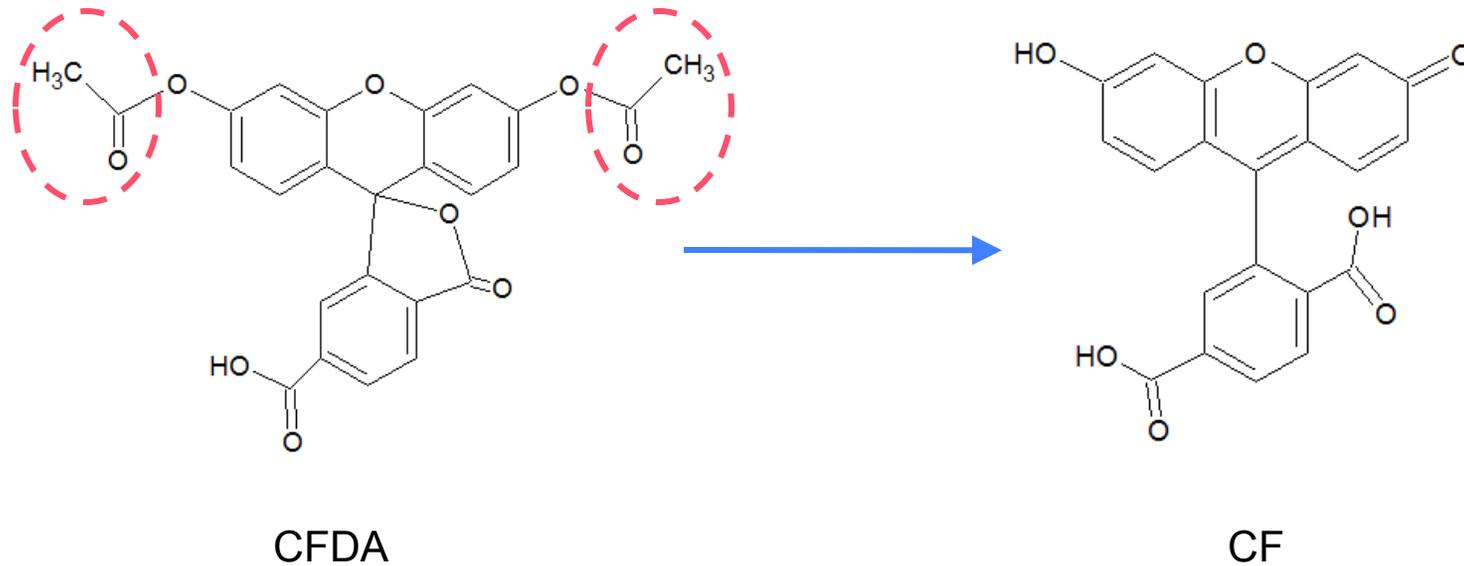
CFDA: the acetate groups are cleaved inside the cell, leaving CF



CF

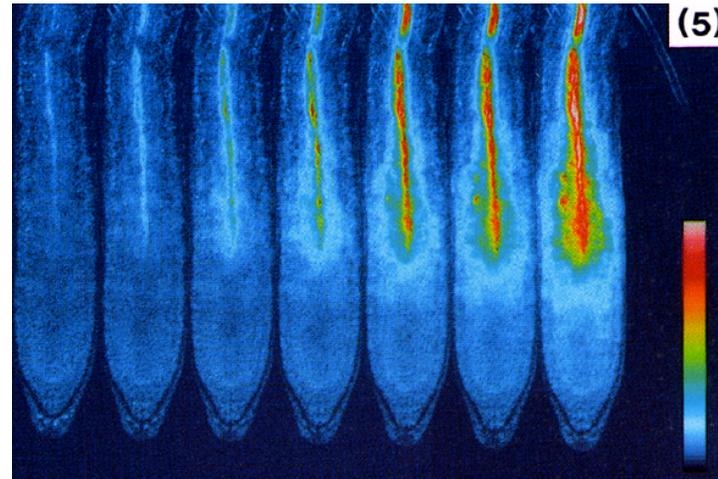
carboxyfluorescein diacetate

CFDA: the acetate groups are cleaved inside the cell, leaving CF

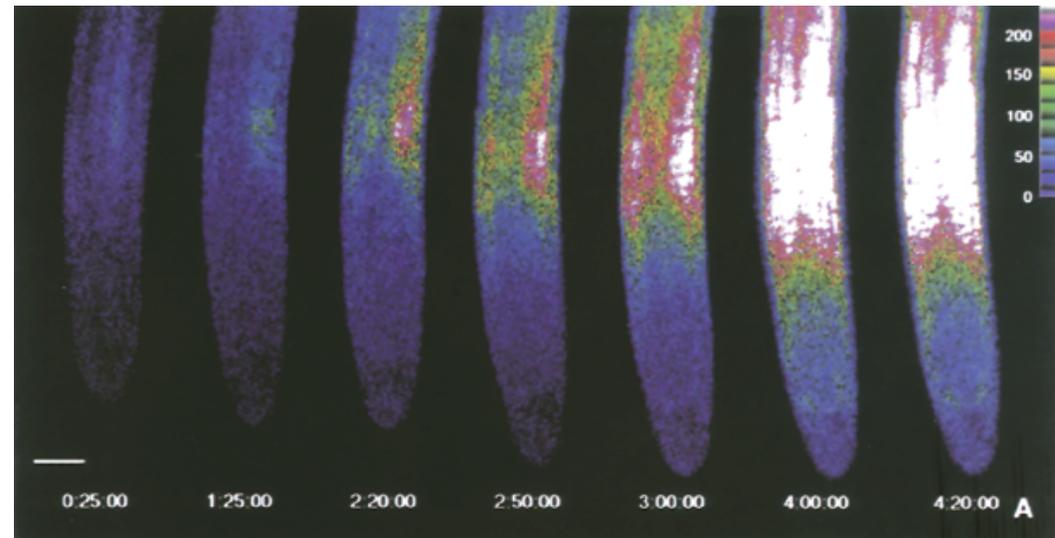


Possible slow movement

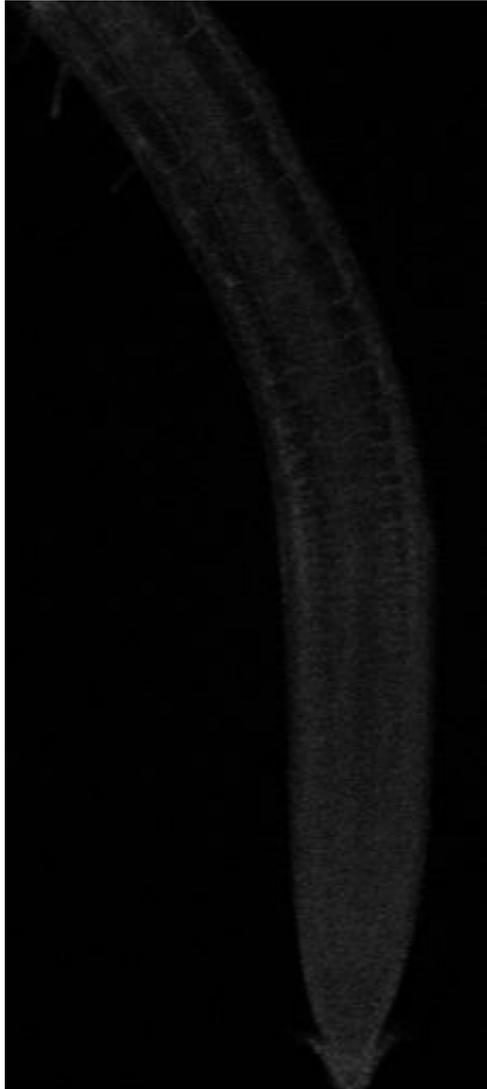
Oparka et al. 1994.
CFDA applied to cotyledon.
Suggest symplasmic
“barrier” to transport at endo-
cort and cort-epi transitions. ~40
min shown.



Zhu et al. 1998. Suggested
“two phase” unloading. 4 h
shown.



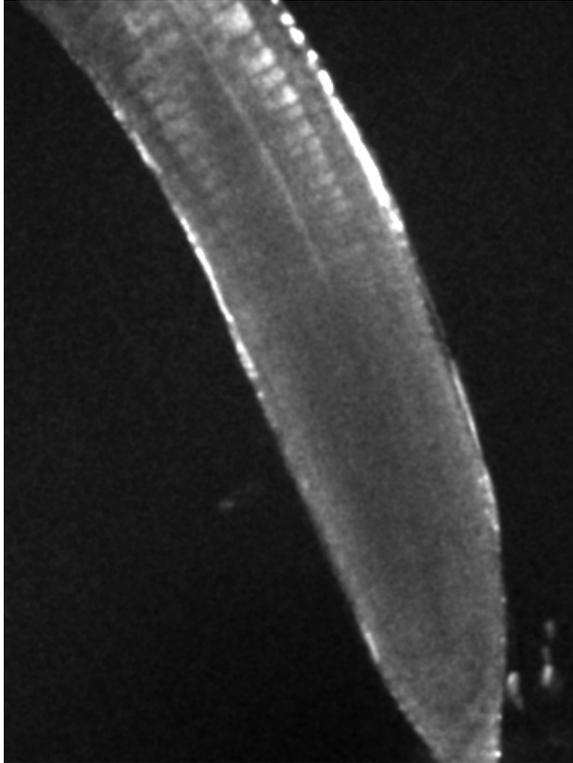
CF phloem unloading



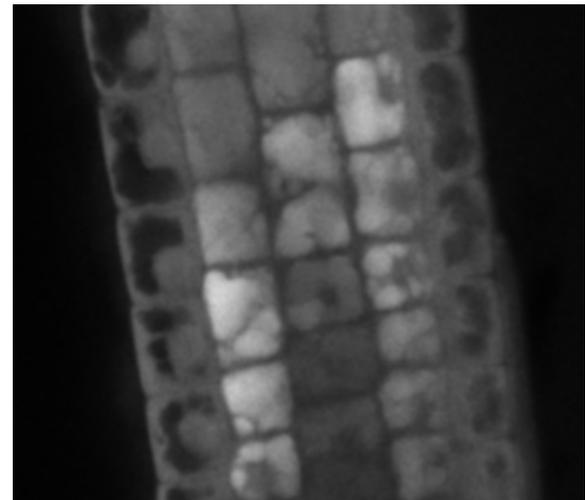
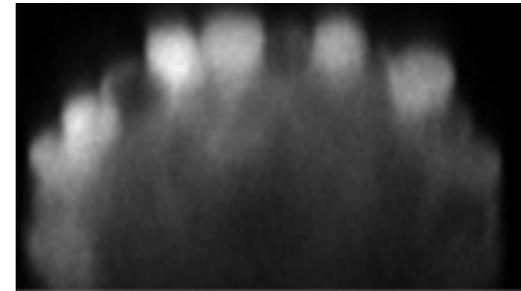
Repeating Oparka1994.
CFDA applied to leaf.
2 hr shown.

CF and vacuoles

Loaded CFDA into a cut leaf.

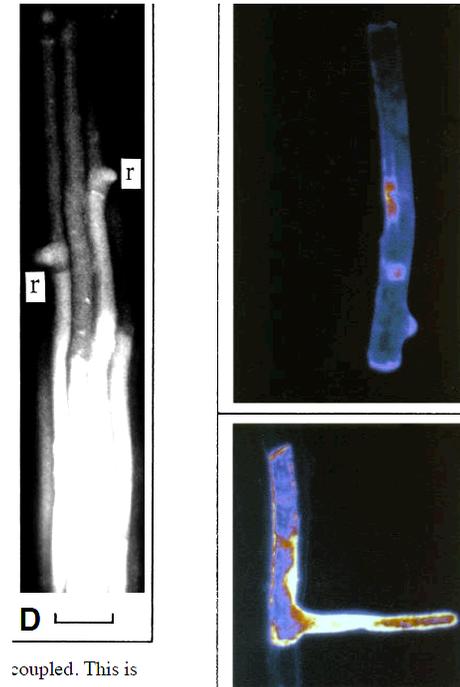


seedling in CFDA 7 min, wash 3 min
after ~1 hour

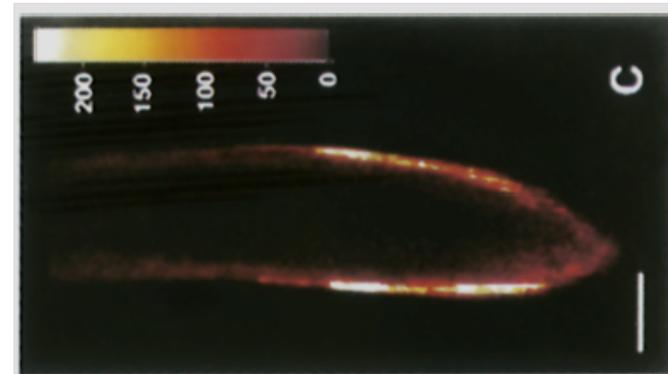


Other signs of symplasmic restriction

Duckett et al. 1994. Local microinjection of CF into root hairs shows limited export.



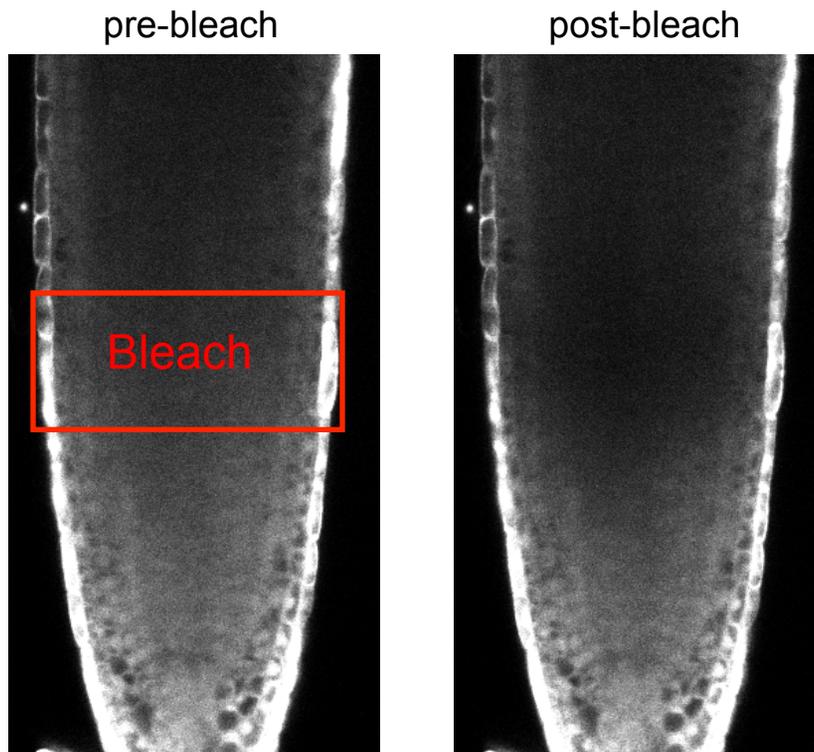
Zhu et al. 1998. CFDA applied to the lateral root cap resulted after 20 minutes in CF visible throughout a cylinder with limited penetration to the inner tissues.



Tissue-scale FRAP

Rutschow, Baskin & Kramer 2011

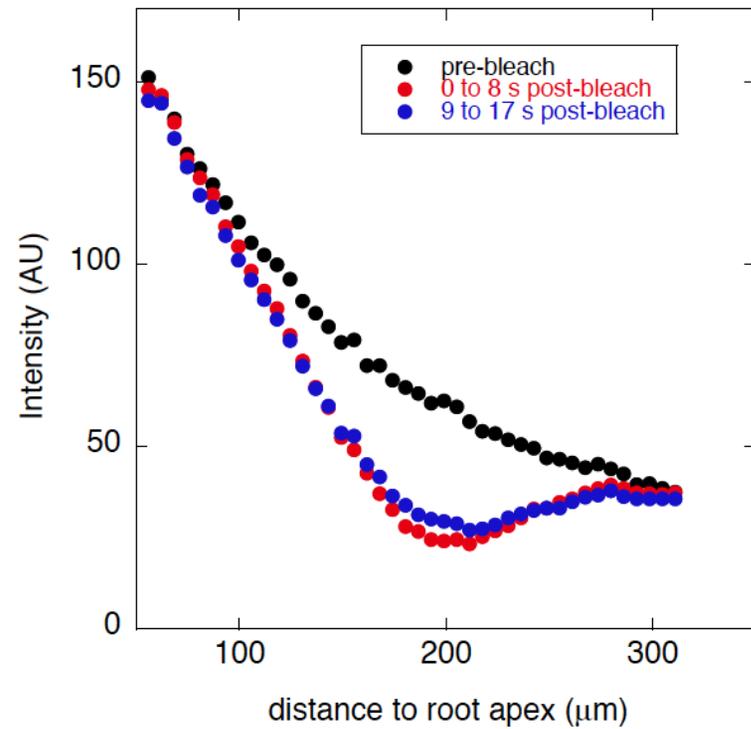
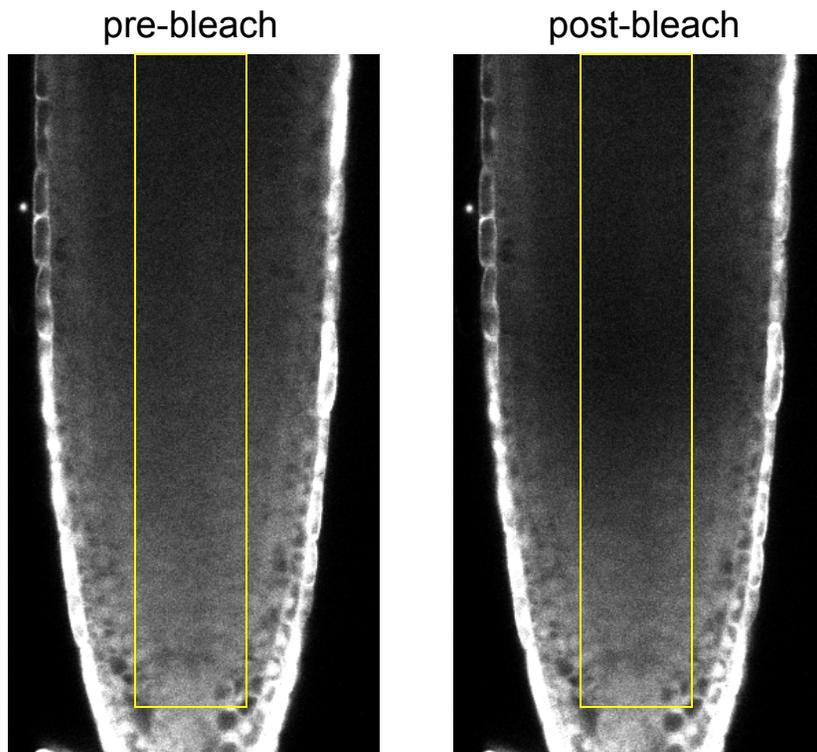
Bleach a zone in meristem for ~40 s, measure recovery.



Tissue-scale FRAP

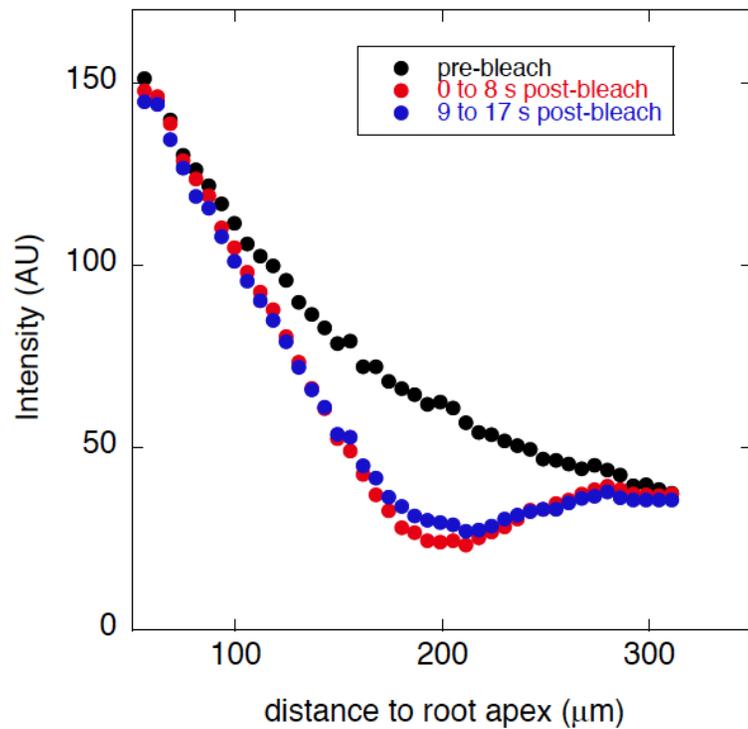
Rutschow, Baskin & Kramer 2011

$$I(x, t) = I_{ss}(x) - \frac{B}{\sqrt{t - t_0}} \exp\left(\frac{-(x - x_0)^2}{4D(t - t_0)}\right)$$

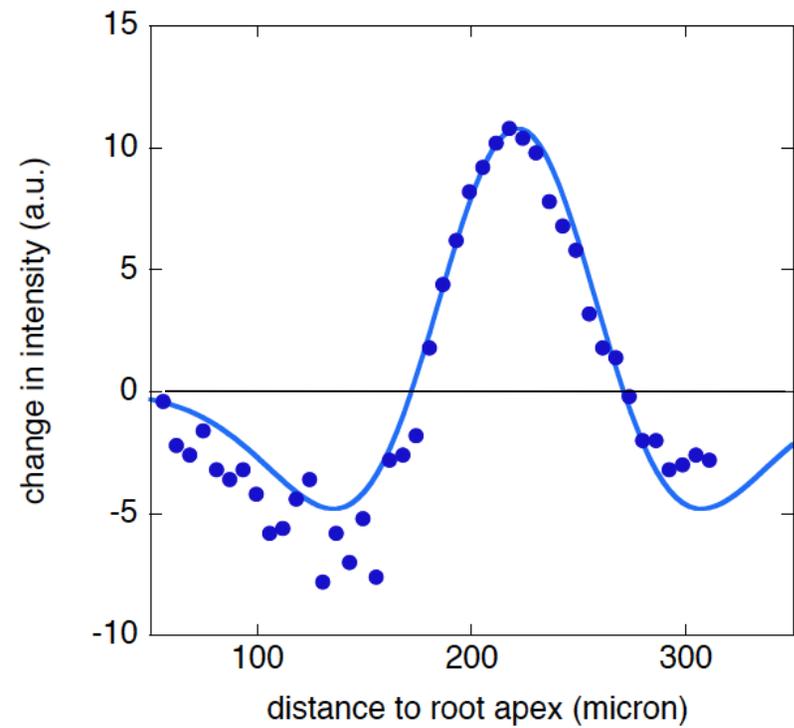


Tissue-scale FRAP

Measuring D

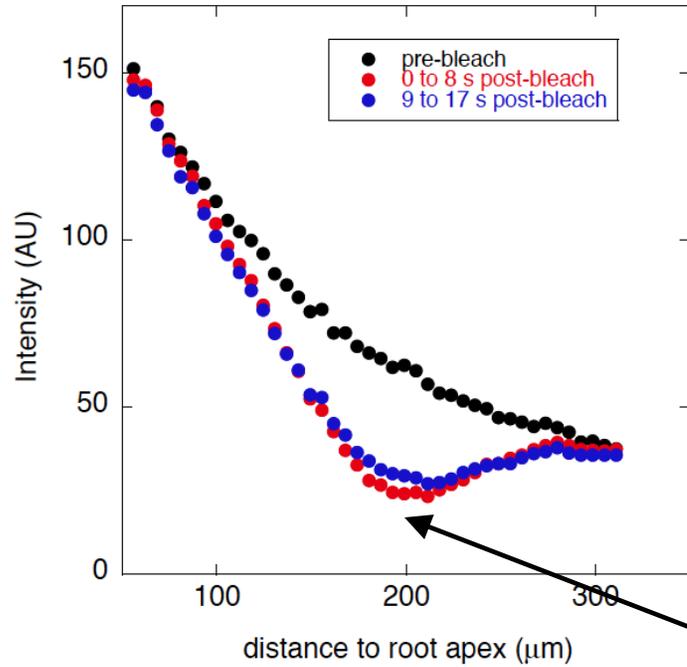


$$F(x, t_1, t_2) = I(x, t_2) - I(x, t_1) \\ = \frac{B}{\sqrt{t_1 - t_0}} \exp\left(\frac{-(x - x_0)^2}{4D(t_1 - t_0)}\right) - \frac{B}{\sqrt{t_2 - t_0}} \exp\left(\frac{-(x - x_0)^2}{4D(t_2 - t_0)}\right)$$



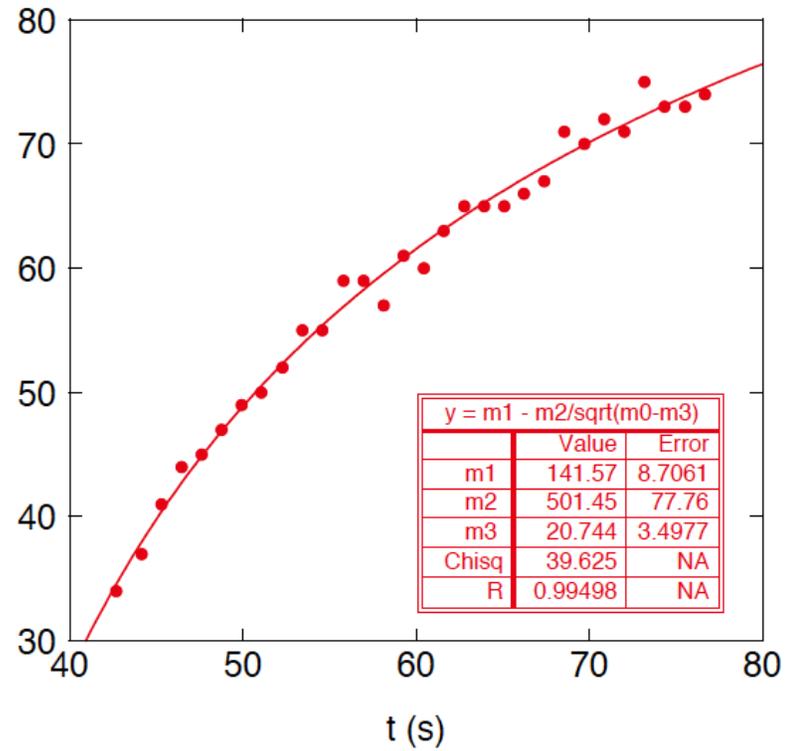
Tissue-scale FRAP

Determining t_0



$$I(x'_0, t) = I_{SS}(x'_0) - \frac{B}{\sqrt{t - t_0}}$$

Trough intensity (a.u.)



Diffusivity of CF in meristem

Our value in WT

$$D = 46 \pm 3 \mu\text{m}^2/\text{s}$$

CF in water, $D_{\text{aq}} = 487 \mu\text{m}^2/\text{s}$

CF in cytoplasm, $D_{\text{cyt}} < 162 \mu\text{m}^2/\text{s}$

Cell-to-cell diffusion is only reduced by a factor of ~ 3 compared to no walls at all!

Bret-Harte & Silk (1994) considered sucrose unloading in the maize root apex, estimated $D = 7 - 14 \mu\text{m}^2/\text{s}$, and found that the carbon flux between phloem and apex was inadequate for growth. Was D too low?

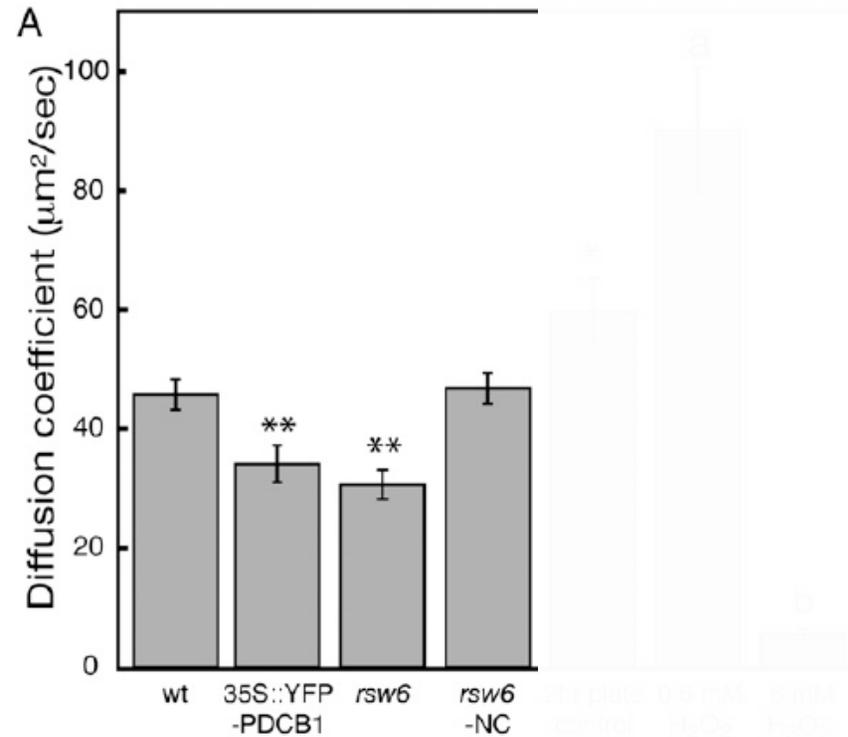
Validation: lines with known pd restriction

35S: YFP-PDCB1

Andy Maule group (Simpson et al. 2009)
Plasmodesmata Callose Binding Protein 1
localizes to pd, overexpression line

rsw6

Baskin group (Bannigan et al. 2006)
radially swollen phenotype at 30° C
NC = normal phenotype at 22° C



Effects of auxin and tryptophan

2 h pre-treatment with auxin or tryptophan
(30 nM IAA or 0.4 mM inhibits root elongation by 50%)

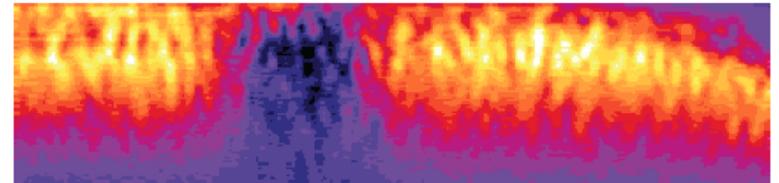
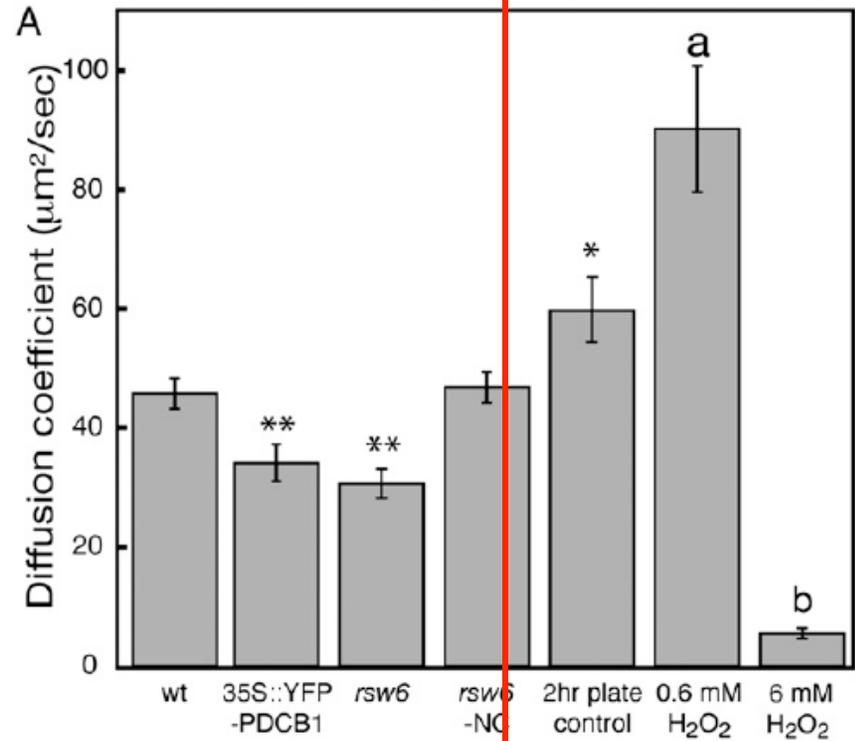
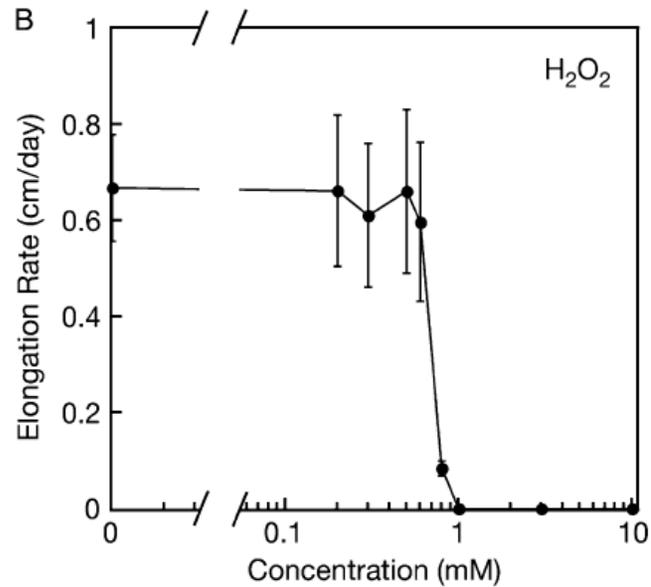
Treatment	D
	$\mu\text{m}^2 \text{s}^{-1}$
Control	59.9 ± 5.4
Trp (0.3 mM)	57.6 ± 5.3
Trp (0.6 mM)	47.4 ± 4.9
IAA (30 nM)	50.7 ± 5.1
IAA (100 nM)	60.5 ± 6.5

24 h pre-treatment with auxin or tryptophan

Treatment	D
	$\mu\text{m}^2 \text{s}^{-1}$
Control	45.9 ± 2.6
Trp (0.3 mM)	45.4 ± 4.9
Trp (0.6 mM)	32.0 ± 4.3^a * $P < 0.05$
IAA (30 nM)	37.5 ± 8.6

Hydrogen Peroxide regulates pd

7 DAG seedlings transferred to H₂O₂ for 2 hours.



From Diffusivity to Permeability

Flux = (permeability) × (concentration difference)

$$J = P (c_2 - c_1)$$

[J] = moles per unit time through unit area

[c] = moles per unit volume

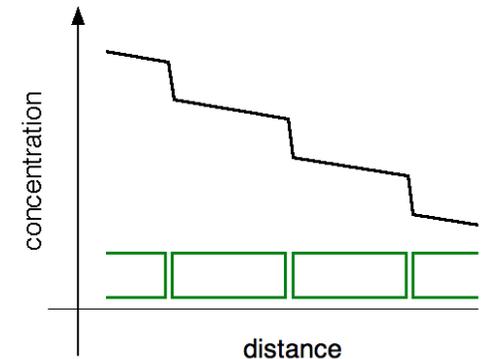
[P] = length over time

The diffusion coefficient isn't a good measure of pd permeability because it includes the effects of both pd and diffusion in the cytoplasm.

Including D_{cyt} for a single file of cells (Crick 1970)

$$P = \frac{D/L}{1 - D/D_{\text{cyt}}}$$

Measure mean cell length in the tissue of interest.

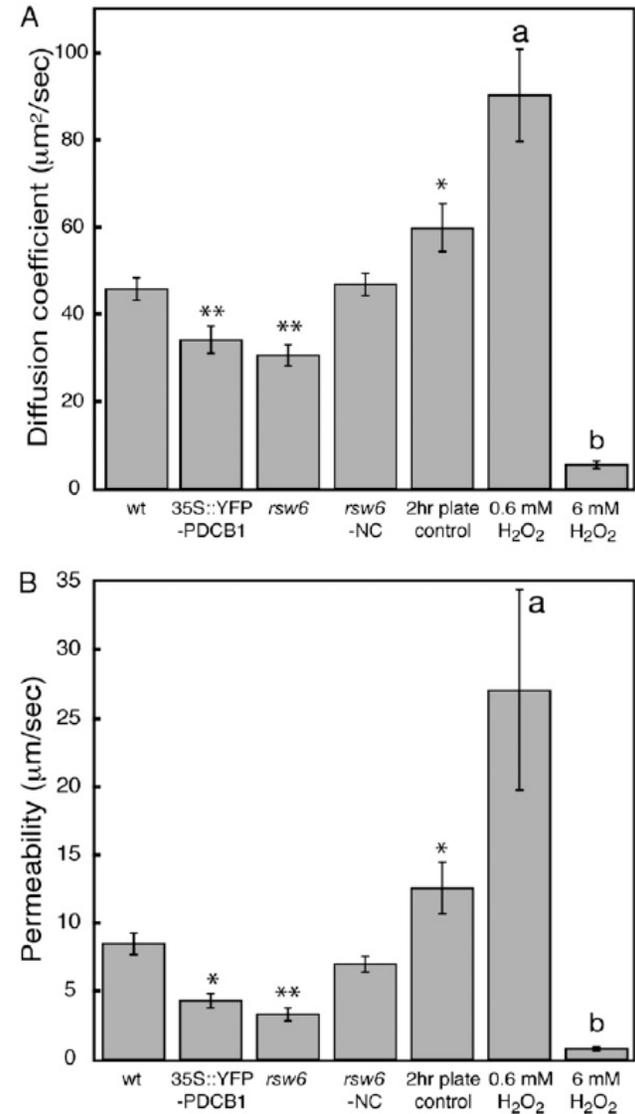


From Diffusivity to Permeability

Statistical significance doesn't change, but differences are magnified.

WT perm: $8.5 \mu\text{m/s} = 29 \text{ mm/h}$

Much faster than auxin carrier values.



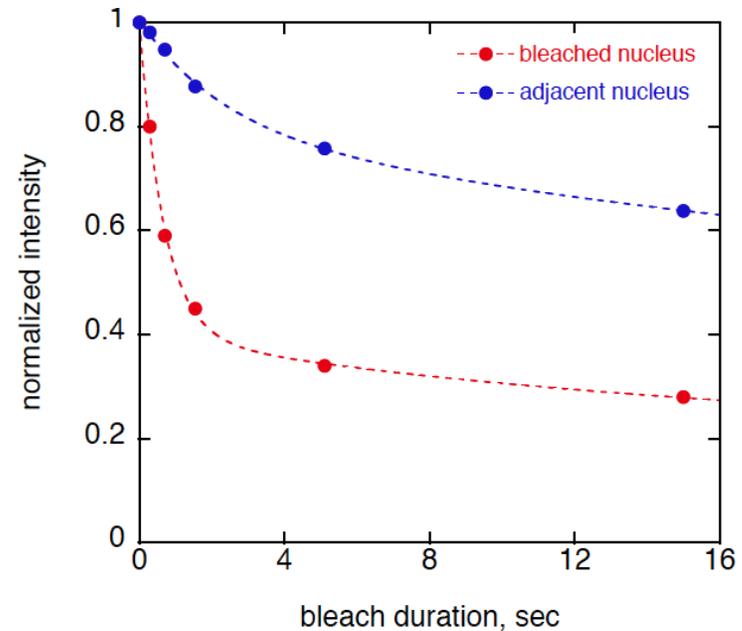
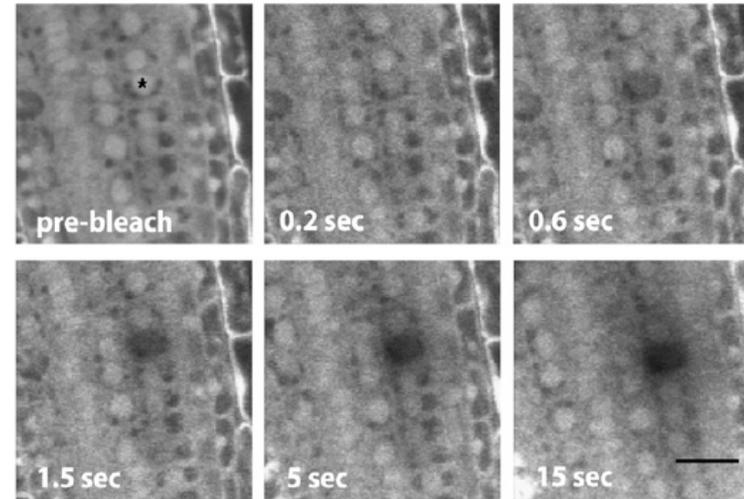
Validation: single-cell bleaches

To validate the bulk bleaches, did a series of single-cell bleaches in the epidermis.

Instead of modeling a complex and poorly-known connectivity, compare two adjacent cells.

$$\frac{dn_1}{dt} = \left(\frac{PA}{V} \right) (n_2 - n_1) - \frac{n_1}{\tau} - b$$

Result: $P = 3.3 \pm 0.8$ micron/s



CF moves, but not auxin??

Conventional wisdom since 1980's was auxin can't move through plasmodesmata.

But: CF larger than IAA

CF charge -2, IAA charge -1

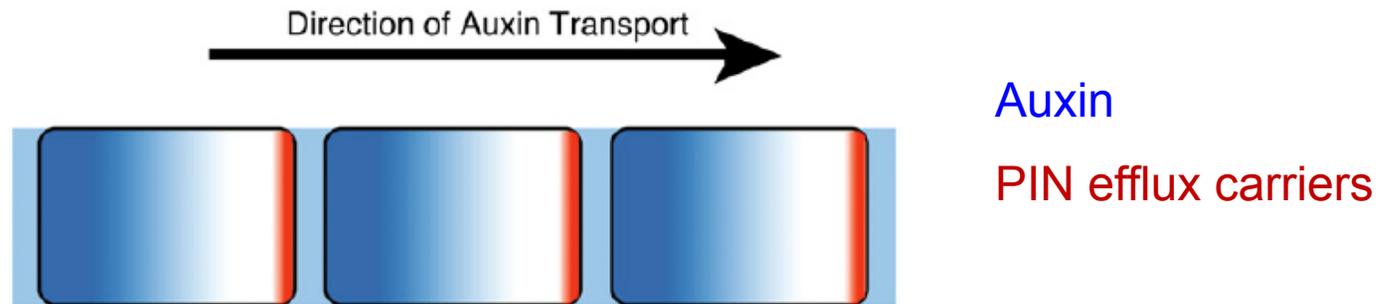
Would require an auxin-specific exclusion mechanism.

New: recent results on GSL8 (Glucan Synthase Like 8) by Han et al. 2014 show that callose synthesis deficit impairs auxin gradients and gravitropism in Arabidopsis seedlings.

Callose is a cell wall constituent that tends to close pd.

Perm value for IAA should be comparable/larger than for CF.

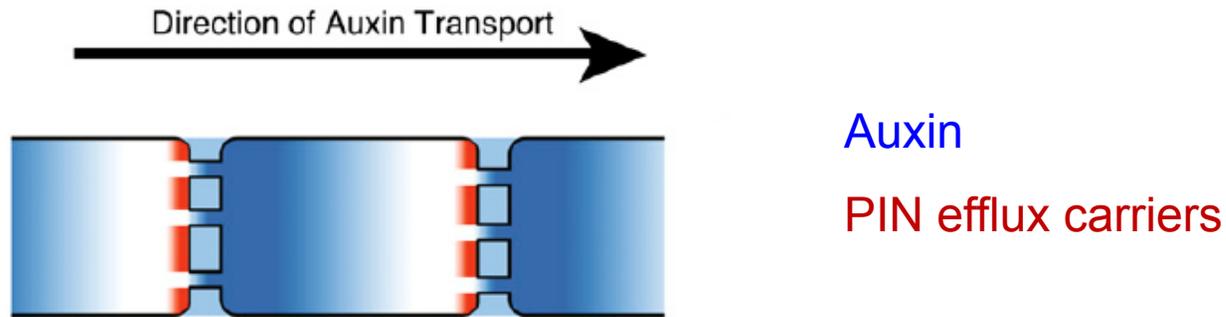
Consequences for Auxin transport



Polar auxin transport is transcellular

What happens if we allow a path for auxin to diffuse backwards, through pd?

Consequences for Auxin transport



If the backward flux is larger than the forward flux, polar transport becomes inefficient, then fails.

$$(\text{backward flux})/(\text{forward flux}) = (\text{p.d. perm})(\text{cell length})/D_{\text{cyt}}$$

Using the WT p.d. perm of $8.5 \mu\text{m/s}$.

For a cell $10 \mu\text{m}$ long, this is 0.45

For a cell $100 \mu\text{m}$ long, this is 4.5

Possible reason why root cells close their pd during maturation?

Summing up Part III

Auxin can move through plasmodesmata.

pd perms are higher than auxin carrier perms in root meristem cells.

Large fluxes through pd do not contradict the usual models of auxin transport, but should be included.

Things that regulate pd conductivity will regulate auxin concentrations. ROS in particular.

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