Hormones: communicating with chemicals

•History- discovery of plant hormone. Auxin

•Concepts of hormones

•Auxin levels are regulated by synthesis/degradation, transport, compartmentation, conjugation .

•Polar auxin transport

a. Chemiosmotic model

b. Identifying the molecular players

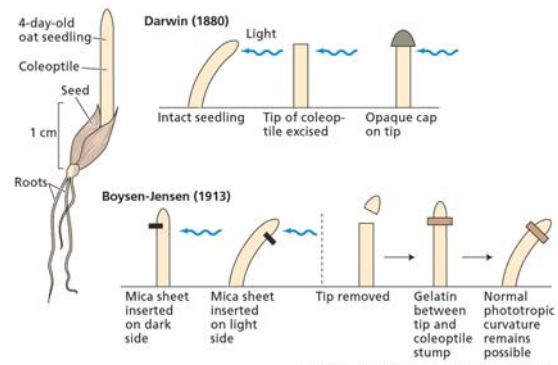
•Uptake- passive
active via AUX1 permease, ABC transporters

•Efflux carriers: PINS, ABC transporters

•Environmental signals can cause changes in hormone levels.

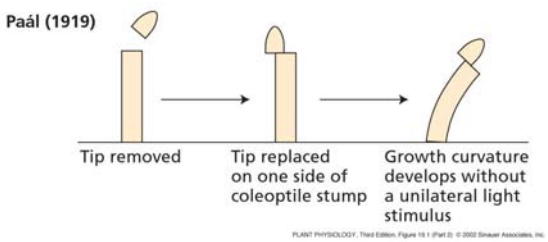
Physiological effects, what changes does each hormone induce?

19-1. History: **Discovery of a growth substance (hormone- auxin)**

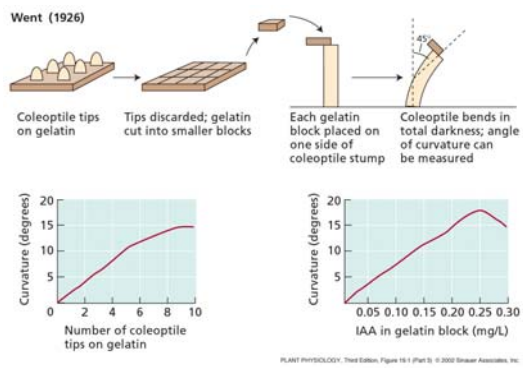


Growth substance is produced at one location and then transported to other parts of the plant.

No light



Curvature was dependent on the **conc** of auxin



Form and function of multicellular organisms depend on efficient communication among cells, and organs. Communication depends on chemical signals.

Concepts

1. Hormones are chemical signals that facilitate intercellular communication.
   Made in certain tissues and *transported* to target tissues.
   Act at very low conc.
2. Level of hormones is regulated.
   By a. Synthesis and degradation: Rate of biosynthesis
   b. Release of conjugated-auxin & conjugation
   c. Transport: influx and efflux
   Life time of chemical messenger is limited.

3. Environmental & developmental signals cause changes in hormonal balance.
Environment cues ---> change [Hormone] --> response

Actions of auxin

Elongation

Differential growth in response to light

Differential growth in response to gravity

Lateral root formation

Maintenance of overall plant polarity

Perturbation of auxin transport will lead to changes in these processes.

Auxin- sites of synthesis

Rapidly dividing and growing tissues

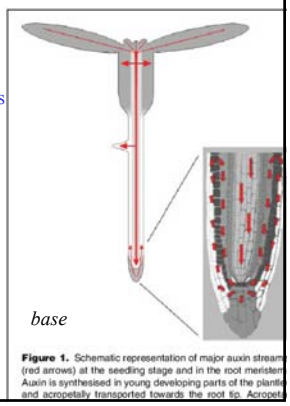
Shoot apical meristem

Young leaves- [from tip to base]

Developing fruits and seeds

acropetally (from the base toward the apex)
basipetally (from the apex toward the base)

Fig. From Benjamins et al 2005 Bioessays

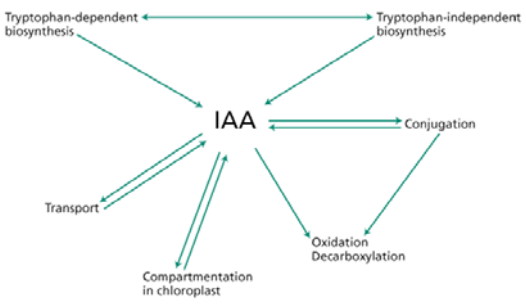


Hydathode-
Has high levels of auxin.
site of auxin synthesis?

DR5::GUS reporter for auxin level

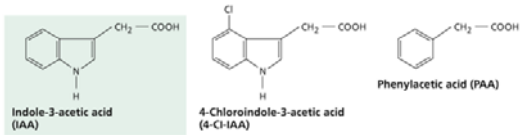
DR5 gene is upregulated by auxin [SAUR]

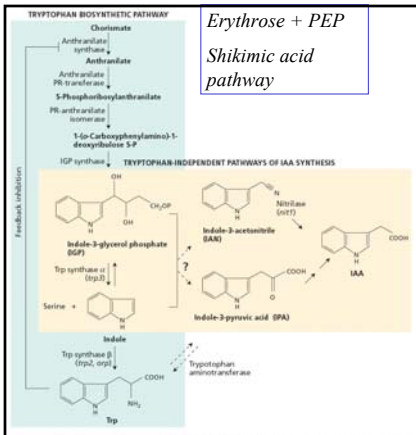
19-12: Taiz 1998. Steady state levels of auxin depends on synthesis, degradation, transport and compartmentation.



19-2. Taiz. Principal auxin in plants is IAA

Synthesized in shoot meristem, young leaves, developing fruits





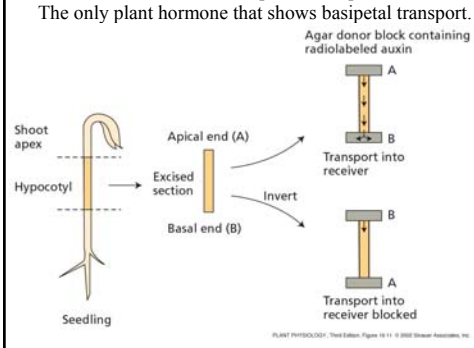
Erythrose + PEP

Shikimic acid pathway

*Auxins are **synthesized** from primary metabolites via multiple pathways*

Auxin is degraded by multiple pathways

19-11. Taiz. Auxin is transported in a polar fashion.
The only plant hormone that shows basipetal transport.



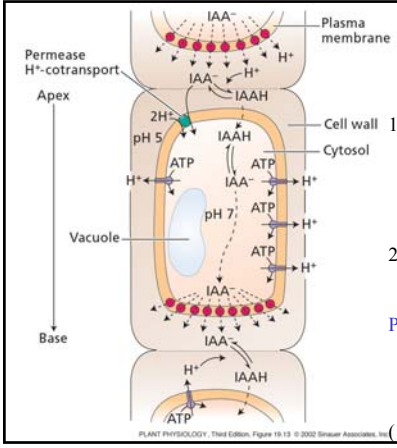
Requires energy: transport is inhibited by no oxygen or metabolic inhibitors.

Taiz, ch 19-12. Roots grow at basal end of inverted bamboo sections

Polar transport is independent of gravity

Stem: vascular parenchyma

Root: more complex



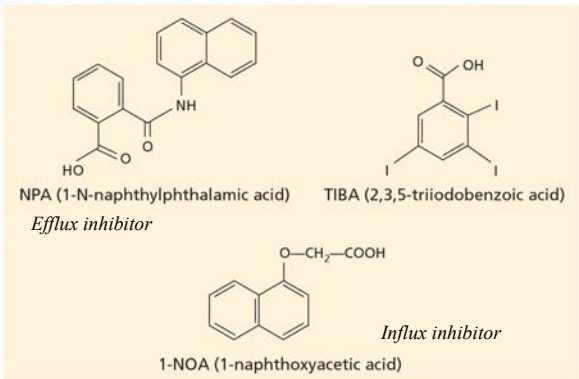
PLANT PHYSIOLOGY, Third Edition, Figure 19.13 © 2002 Sinauer Associates, Inc.

Chemiosmotic Model of polar auxin transport

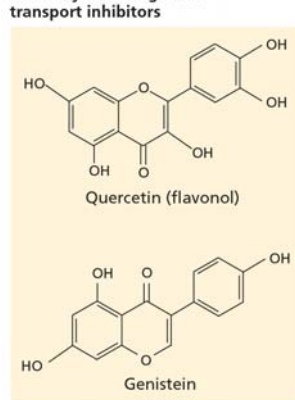
1. Entry/ influx
   a. Passive
   b. Active, H+/IAA symport (AUX1 permease)
2. Efflux.

Polarity set up by efflux carriers if they are located only at the base.

(1977-1990s)



PLANT PHYSIOLOGY, Third Edition, Figure 19.16 (Part 1) © 2002 Sinauer Associates, Inc.



PLANT PHYSIOLOGY, Third Edition, Figure 19.16 (Part 2) © 2002 Sinauer Associates, Inc.

Search for auxin efflux carriers since 1980s

JACOBS M, GILBERT SF (1983)

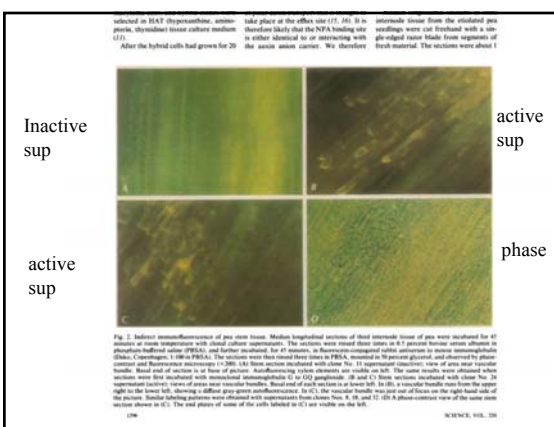
BASAL LOCALIZATION OF THE PRESUMPTIVE AUXIN TRANSPORT CARRIER IN PEA STEM-CELLS

SCIENCE 220 (4603): 1297-1300

Had made mab to pea membrane fraction.

Screened Antibody culture supernatant for ability to inhibit NPA binding to membranes.

One mAb recognized only the basal side of stem cells in immunofluorescence.



Genetic discovery of auxin efflux regulator/facilitator PIN

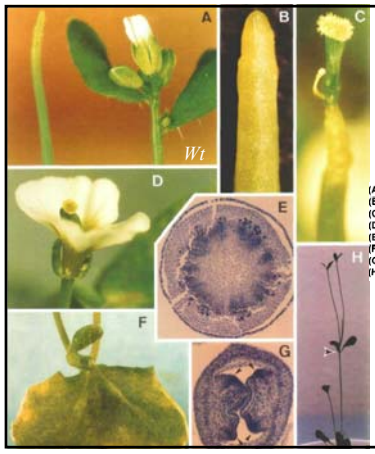
1991 Okada et al. Plant Cell

The Plant Cell, Vol. 3, 677–684, July 1991 © 1991 American Society of Plant Physiologists

Requirement of the Auxin Polar Transport System in Early Stages of *Arabidopsis* Floral Bud Formation

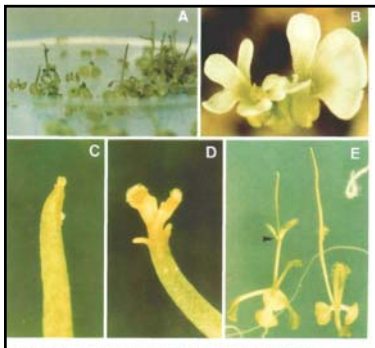
Kiyotaka Okada,[a,1] Junichi Ueda,[b] Masako K. Komaki,[a] Callum J. Bell,[a,2] and Yoshiro Shimura[a,c]
[a] Division I of Gene Expression and Regulation, National Institute for Basic Biology, Okazaki 444, Japan

These mutant phenotypes are exactly the same in wild-type plants cultured in the presence of chemical compounds known as auxin polar transport inhibitors: 9-hydroxyfluorene-9-carboxylic acid or N-(1-naphthyl)phthalamic acid. We tested the polar transport activity of indole-3-acetic acid and the endogenous amount of free indole-3-acetic acid in the tissue of inflorescence axes of the pin1 mutants and wild type. The polar transport activity in the pin 1-1 mutant and in the pin1-2 mutant was decreased to 14% and 7% of wild type, respectively. These observations strongly suggest that the normal level of polar transport activity in the inflorescence axes is required in early developmental stages of floral bud formation in Arabidopsis and that the primary function of the pin1 gene is auxin polar transport in the inflorescence axis.



Pin mutant
Okada et al 91

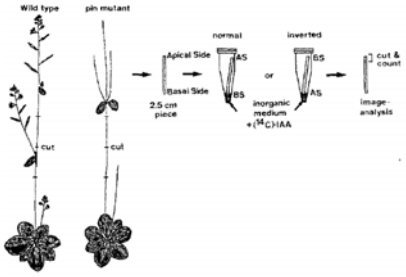
(A) Tops of the inflorescence axis of *Arabidopsis* wild
(B) A mutant inflorescence with no floral buds.
(C) A mutant inflorescence with a pistil-like structure
(D) A mutant inflorescence with aberrant petals and
(E) Transverse section of the top region of the inflores
(F) A deformed leaf.
(G) Transverse section of the pistil-like structure show
(H) A mutant plant with two leaves and two axiliary sh



Wild type plants cultured in auxin transport inhibitors look like pin1 mutants

Okada et al 91

Figure 3. Assay for Transport Activity in the Inflorescence Axis.

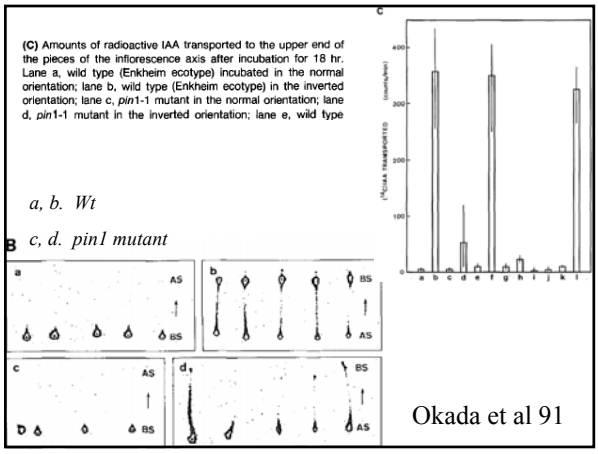


Okada et al 91

(C) Amounts of radioactive IAA transported to the upper end of the pieces of the inflorescence axis after incubation for 18 hr. Lane a, wild type (Enkheim ecotype) incubated in the normal orientation; lane b, wild type (Enkheim ecotype) in the inverted orientation; lane c, *pin*1-1 mutant in the normal orientation; lane d, *pin*1-1 mutant in the inverted orientation; lane e, wild type

a, b. Wt

c, d. pin1 mutant



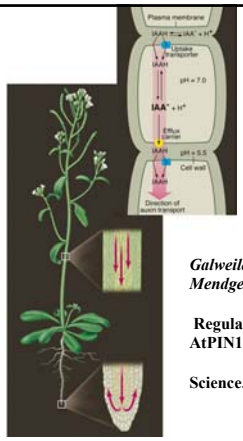
Okada et al 91

Table 2. Endogenous IAA Levels in Wild Type and the *pin*1-1 Mutant

	IAA in ng/g Fresh Wt
Wild type[a]	326.7 ± 2.6
*pin*1-1[b]	25.8 ± 1.7

Plant materials including rosette leaves and inflorescence were used for the measurement.
[a] Plants including *pin*1-1/+ heterozygotes and +/+ homozygotes, which are segregated in the self-fertilized progeny of the heterozygous parent.
[b] *pin*1-1/*pin*1-1 homozygous plants.

Okada et al 91

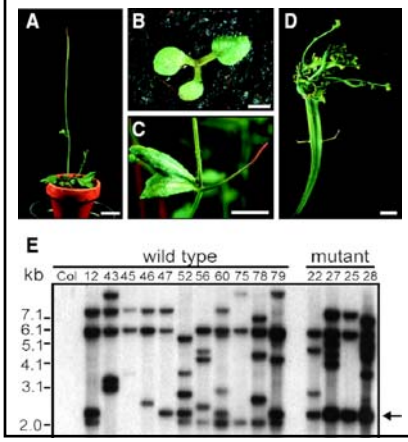


What is the PIN1 gene?

Galweiler L, Guan C, Muller A, Wisman E, Mendgen K, Yephremov A, Palme K.

Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue.

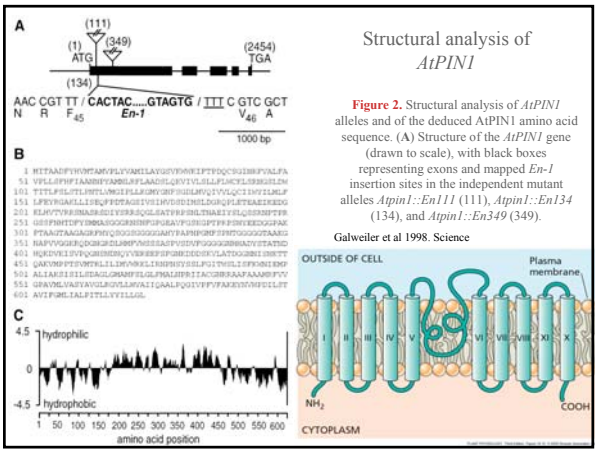
Science. 1998 Dec 18;282(5397):2226-30.



Phenotypic and Southern blot analysis of the transposon insertional mutant *Atpin1::En134*. pin1 mutant

(**A**) The most obvious phenotypic aspect of the homozygous mutant represents the naked, pin-forming inflorescence with no or just a few defective flowers. (**B**) *Atpin1::En134* seedlings showed frequently aberrant cotyledon positioning or triple cotyledons. (**C**) A mutant cauline leaf exhibited abnormal vein branching resulting in the appearance of fused twin or triple leaves. Unusually, the leaf and "pin"-forming axillary shoot have formed in opposite positions. (**D**) Drastically fasciated inflorescence of an aged mutant. (**E**) Southern blot analysis of a segregating *Atpin1::En134* mutant population.

Galweiler et al 1998. Science



Structural analysis of *AtPIN1*

Figure 2. Structural analysis of *AtPIN1* alleles and of the deduced AtPIN1 amino acid sequence. (**A**) Structure of the *AtPIN1* gene (drawn to scale), with black boxes representing exons and mapped *En-1* insertion sites in the independent mutant alleles *Atpin1::En111* (111), *Atpin1::En134* (134), and *Atpin1::En349* (349).

Galweiler et al 1998. Science

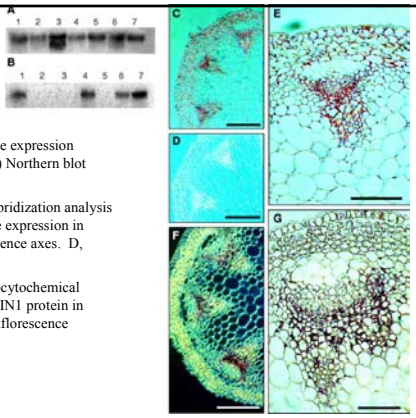


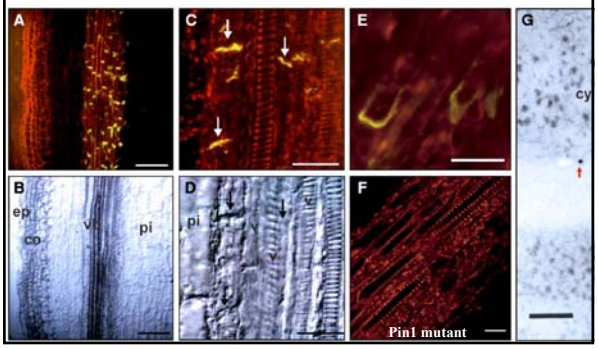
Fig. 3. *AtPIN1* gene expression analysis. (**A** and **B**) Northern blot analysis.

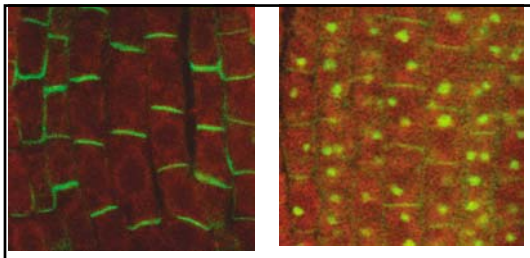
(**C** to **E**) In situ hybridization analysis of the *AtPIN1* gene expression in wild-type inflorescence axes. D, sense

(**F** and **G**) Immunocytochemical localization of AtPIN1 protein in cross sections of inflorescence

Galweiler et al 1998. Science

Figure 4. AtPIN1 immunolocalization in longitudinal *Arabidopsis* tissue sections. (**A** to **F**) Indirect immunofluorescence analysis by laser scanning confocal microscopy. Stem segments of plants were fixed, sectioned. fluorescent (FITC) secondary antibodies AtPIN1 signals are found at the of vessel elements, (Galweiler et al. 1998 Science)

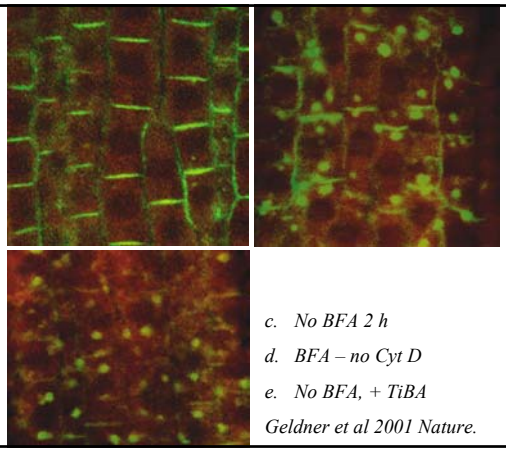




Buffer Control *BFA 2 h*

Role of PIN1

Geldner et al 2001 Nature. Auxin transport inhibitors block PIN cycling and ves traffic. PIN1 localization results from rapid cycling between PM and endosomes..



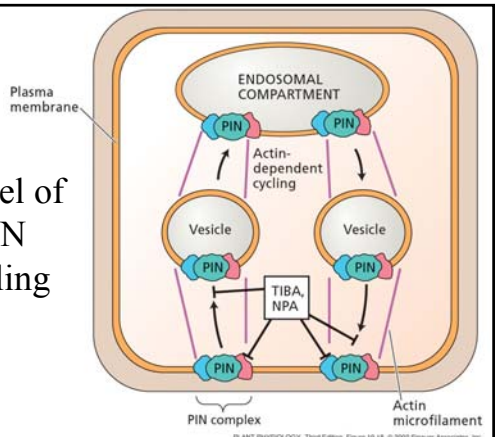
c. No BFA 2 h

d. BFA – no Cyt D

e. No BFA, + TiBA

Geldner et al 2001 Nature.

Model of PIN cycling



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PIN1 is one member of a gene family

PIN1-

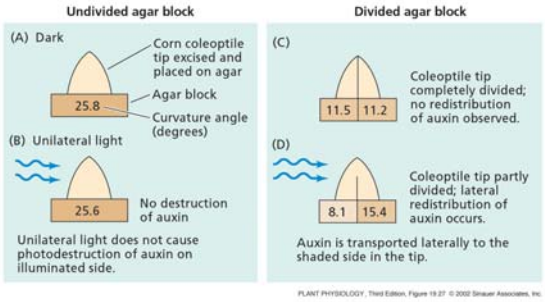
PIN8

Phototropism & Gravitropism

19- 27. Light causes redistribution of auxin to shaded side in phototropism.

Environmental signals regulate hormone levels via transport

E



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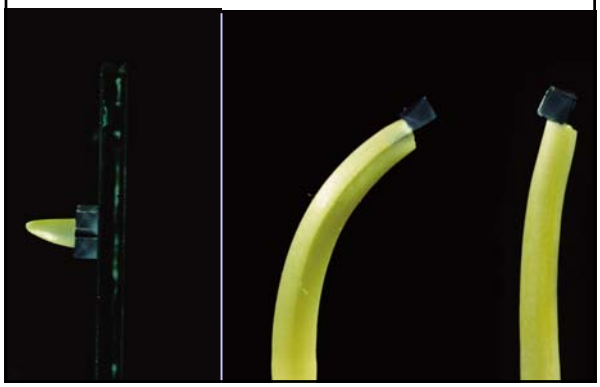
Response to Gravity. Auxin is transported to lower side of horizontal oat tip

Lower half Upper half



Auxin level changes in response to light and to gravity are due to auxin transport.

Light		*Gravity*	
- inhib NPA	*+ Inhib*	*- inhib*	*+ Inhib*

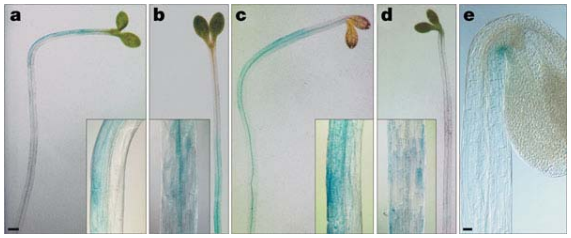
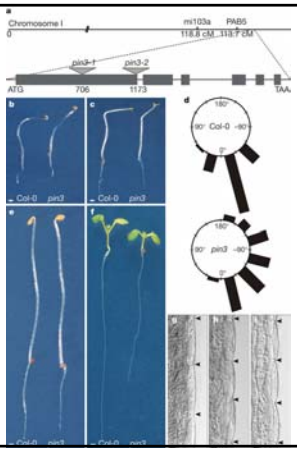


Figure 1 Auxin response during differential hypocotyl growth. a–d, Expression of the DR5::GUS reporter in hypocotyl of untreated (a, c) or auxin efflux inhibitor (NPA)-treated (b, d) wild-type seedlings upon stimulation by light (a, b) or gravity (c, d). Insets show details of DR5::GUS expression. Scale bars, 400 µm. e, Asymmetric expression of DR5::GUS reporter in the apical hook of an etiolated seedling. Scale bar, 50 µm.

Friml J. et al 2002. Nature



PIN3 involved

Figure 2. pin3 mutants.

b, c, **pin3 hypocotyls are defective in gravitropic (b) as well as phototropic** (c) responses.

d, pin3 mutants are defective in root gravitropism

Friml J. et al 2002. Nature

Model: Auxin levels can be changed by location of auxin efflux carriers

Different PINS are expressed at different places, in response to different cues.

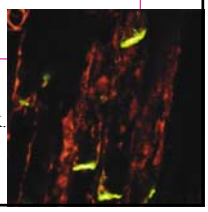
PIN1 Basipetal auxin transport in the stem

PIN2 Basipetal auxin transport, gravitropic response

PIN3 Lateral auxin efflux in gravitropic responses

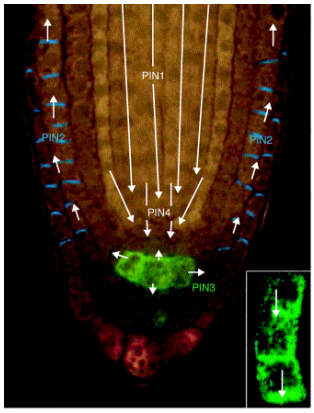
PIN1-GFP is localized to base of cell

PIN3-GFP is localized to lateral side of cell



Friml, J. , Wisniewska, J. , Benkova, E. , Mendgen, K. (2002) Nature 415, 806-809.

Geldner N. 2001 Nature



Current Opinion in Plant Biology

Model

Figure 3. Immunolocalization of the PIN3 protein in the Arabidopsis root apex and probable routes of polar auxin transport (white arrows). PIN3 (in green) is localized symmetrically in columella cells and apparently mediates lateral auxin distribution to all sides of root cap. After the root is turned by 90 degrees away from vertical (i.e. after a gravistimulus is applied), PIN3 rapidly relocates to the bottom side of columella cells (inset), and thus probably regulates auxin flux to the lower side of root. Auxin is further transported through lateral root cap and epidermis cells basipetally by a PIN2-dependent route (polar localization of PIN2 at the upper side of cells is depicted in blue) This basipetal transport also requires AUX1-dependent auxin influx. AUX1 is present in the same cells as PIN3 and PIN2. Auxin is supplied to the root cap by the PIN1- and PIN4-dependent acropetal route (which is depicted in white).

Friml 2003

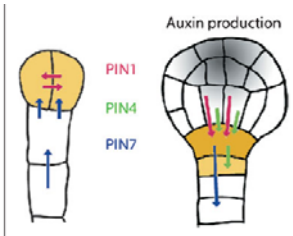


Figure 3. Model describing the role of auxin transport during early embryo development. Various PIN proteins determine the direction of auxin transport to and from the embryo (arrows; PIN1 in purple; PIN4 in green and PIN7 in blue). At the 4-cell stage (left), auxin accumulates in apical cells (orange) due to the action of PIN1 and PIN7. At the globular stage (right), auxin biosynthesis has started in the embryo and the direction of auxin transport is reversed. Auxin accumulates in the hypophyseal region (orange), which is mediated by the action of PIN1 and PIN4. At this stage, PIN7 is no longer apically, but rather basically localised, possibly involved in auxin transport from the embryo to the maternal tissue. Figure adapted from Friml J, Vieten A, Sauer M, Weijers D, Schwarz H et al. 2003 Nature 426:147–153.

Model: Auxin transport during Embryo development

Transport direction changes as embryo develops

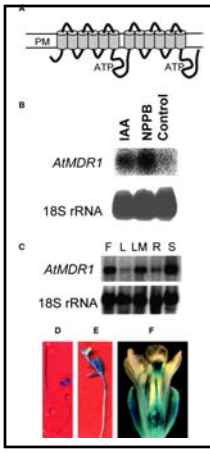
Benjamins et al. 2005 TiPS

Questions?

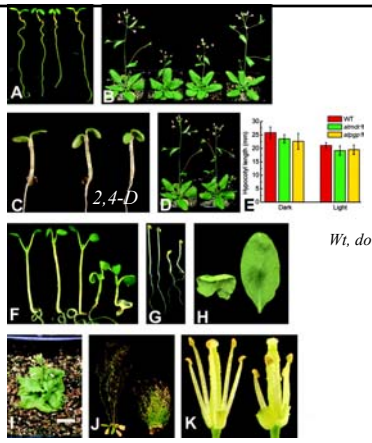
ABC transporters

Evidence for role in auxin transport

PGP1 mediates direct auxin transport



MDR1 expr induced by IAA
Noh et al 2001



Mutant

Wt, double, single, OE MDR1

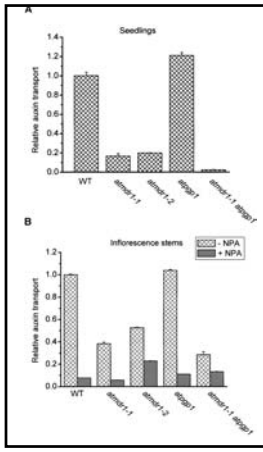
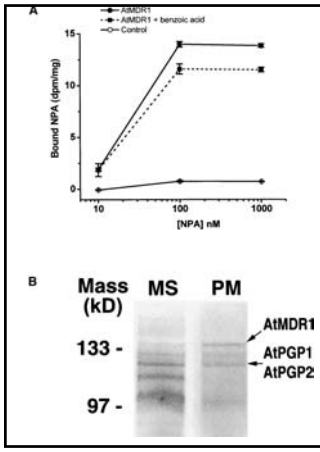
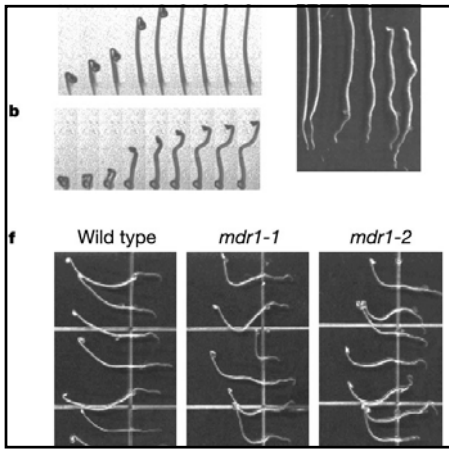


Figure 4. Auxin Transport Activity in the Wild Type and Mutants.

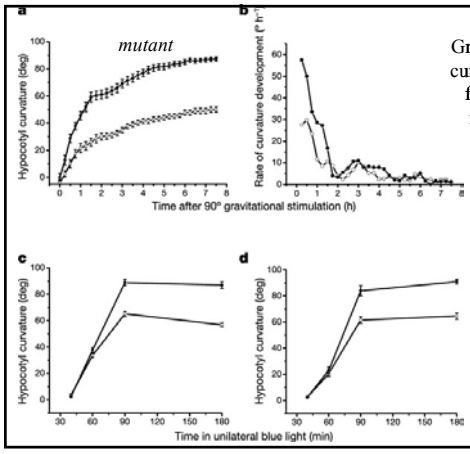


Binding of NPA by MDR expressed in yeast.



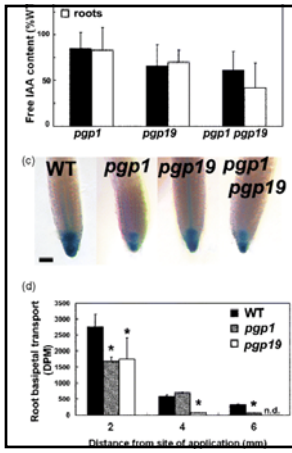
Mdr mutant mislocalize PIN & enhance gravitropic responses

Noh et al 2003 Nature



Gravitropic curvature is faster in mutant

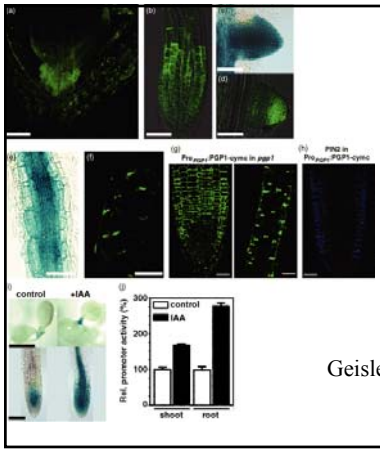
phototropic curvature is faster in mutant



Geisler et al 2005. Plant J. pgp1, pgp19 mutants

Pro_{DR5}:GUS expression in 5-day seedling root

Basipetal ^{3}H-IAA transport from the root tip in 5-day seedlings

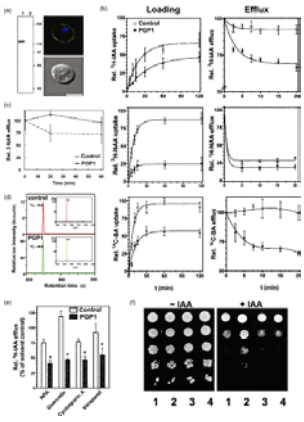


PGP1-cymc localised to root & shoot. Non-polar.

Efflux is reduced in mutant

Geisler et al 2005. Plant J.

PIN1 and PIN2 localization is unaltered in *pgp1* root tips.



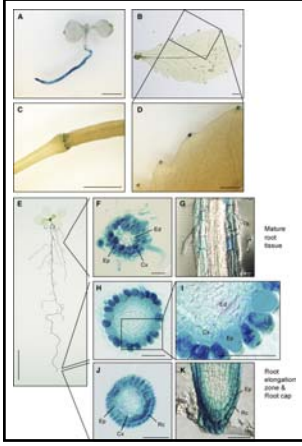
Heterologous expr in yeast : PGP1 mediates auxin efflux

Efflux is inhibited by NPA and Quercetin.

Conclusion:

PGP1 mediates efflux of auxin & auxin metabolites

Geisler et al 2005. Plant J.

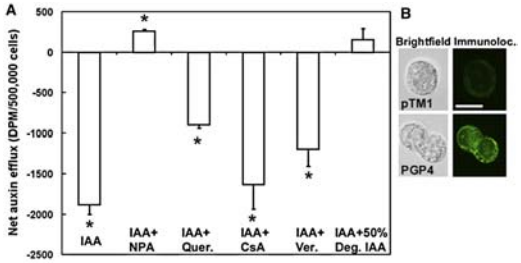


Terasaka et al 2005. Plant Cell

PGP4 catalyze auxin transport in roots

Prom::Gus shows PGP4 is expressed in roots

PGP4-Mediated Auxin Influx in Hela Cells is inhibited by NPA



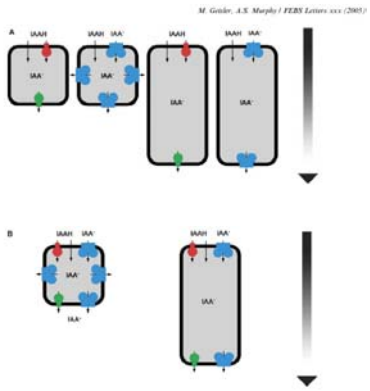
Terasaka et al 2005. Plant Cell

Summary of ABC transporters

PGP1: efflux

PGP4: influx in roots

Questions still unresolved?



models

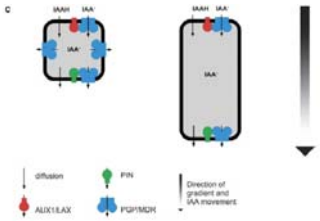


Fig. 3. Model of PGP-, PIN- and AUX1/LAX-mediated auxin transport. In a complementary model of PGP and AUX1/LAX and PIN action (A), auxin would enter the cell via diffusion or AUX1- or AtPGP4-like mediated import. Auxin then exits the cells via an efflux complex that is characterised by PIN proteins. This model works in mature tissues, in which cells are longer and re-diffusion is not a limiting factor. In meristematic tissues, where cells are smaller and auxin concentrations are higher, re-diffusion would inhibit PAT (see text for details). Therefore, in these smaller cells asymmetric distributed transporters, like PGPs, have been postulated to play an essential role in PAT. In an additive model (B), PGPs, and AUX1/LAX and PIN proteins, respectively, would function independently in the same cells. While in a synergistic model (C), individual components would co-function as import and efflux complexes. PIN–PGP pairings have been suggested to provide specificity and directionality to PAT [15] that would favor a synergistic mode of action compared to the additive model, which suggest the function of two independent efflux systems.