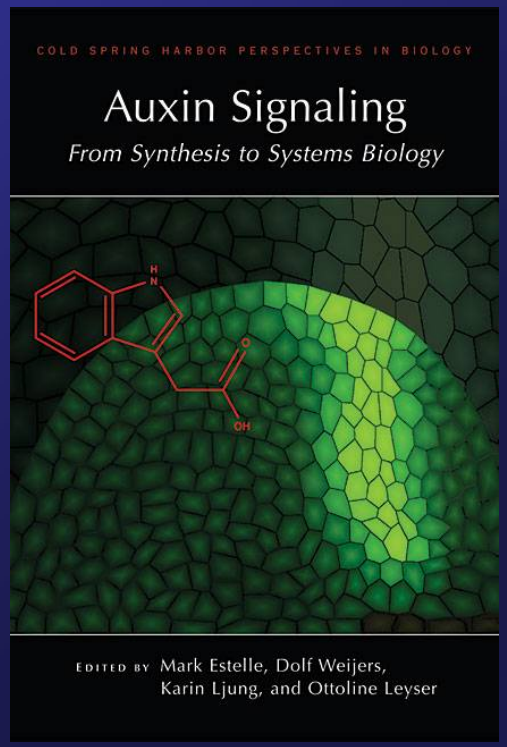
SFRZE

2022

5) Physiology of plant hormones auxins: receptors and signaling



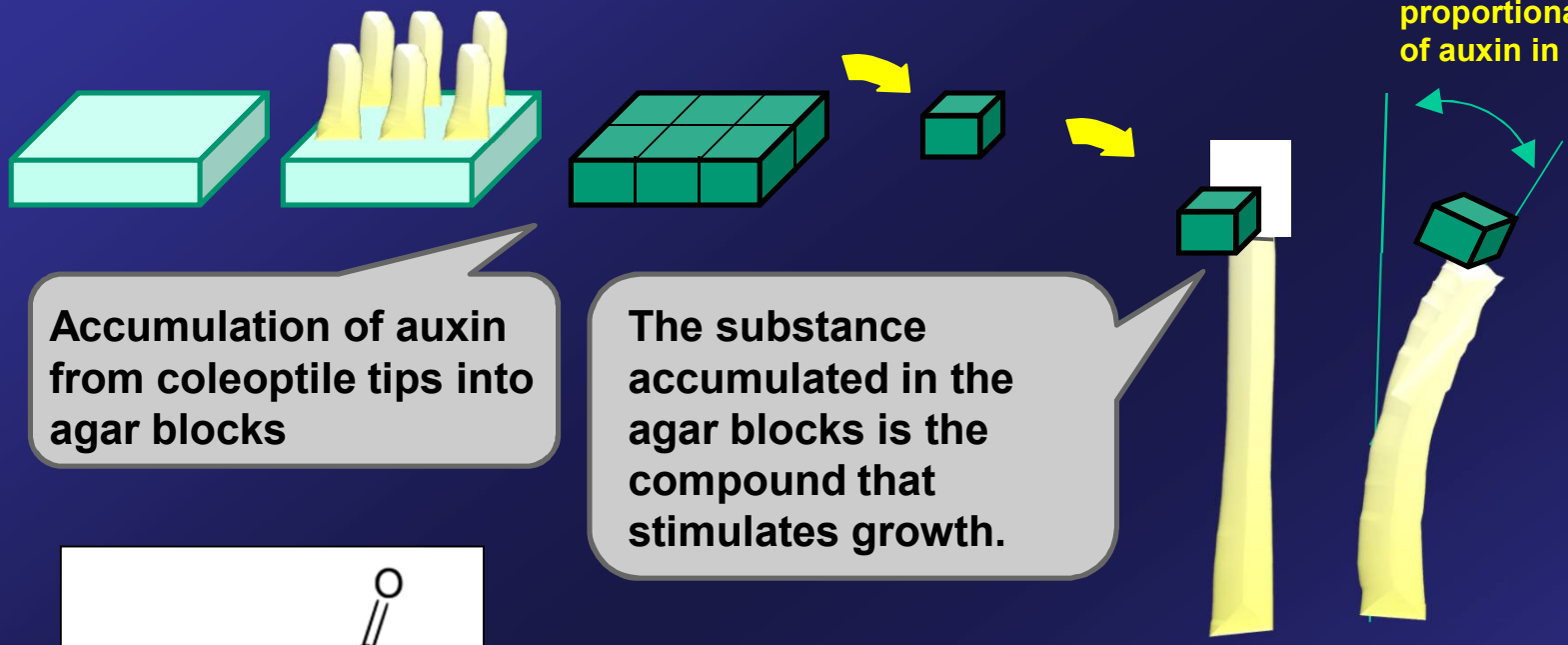
a) Auxin receptor TIR1

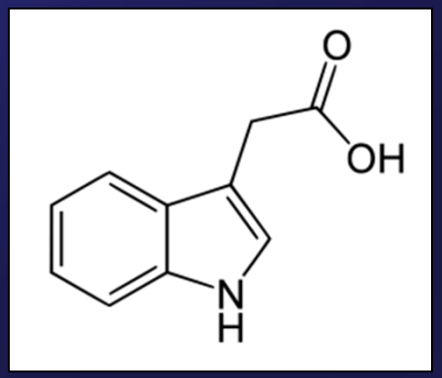
b) Auxin receptor ABP1

Estelle M et al. (2011) Auxin Signaling: From Synthesis to Systems Biology; Cold Spring Harbor Laboratory

Martin Fellner
Laboratoř růstových regulátorů
PřF UP v Olomouci a ÚEB AVČR

In 30th auxin has been isolated and it was shown that it stimulates growth.





IAA - Indole-3-acetic acid

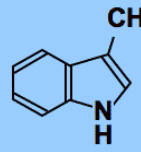
This experiment showing auxin-induced growth stimulation was used as a basis for auxin purification.

Redrawn from Went, F.W. (1935) Auxin, the plant growth-hormone. Bot. Rev. 1: 162-182.

Auxins – important plant hormones involved in spread spectrum of growth and developmental processes.

Naturally Occurring Auxins

indole-3-acetic acid

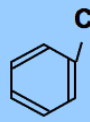


IAA

4-chloroindole-3-acetic acid

4-Cl-IAA

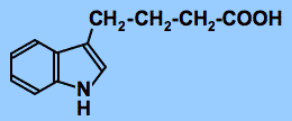
phenylacetic acid



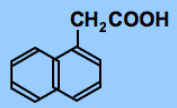
PAA

Commonly Used Synthetic Auxins

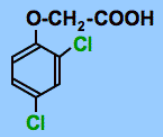
indole-3-butyric acid



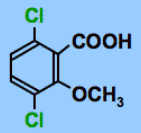
naphthalene acetic acid



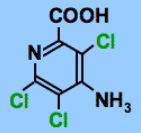
2,4-dichlorophenoxy-acetic acid



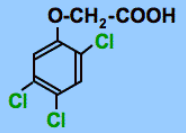
2-methoxy-3,6-dichlorobenzoic acid



4-amino-3,5,6-trichloropicolinic acid



2,4,5-trichlorophenoxy-acetic acid



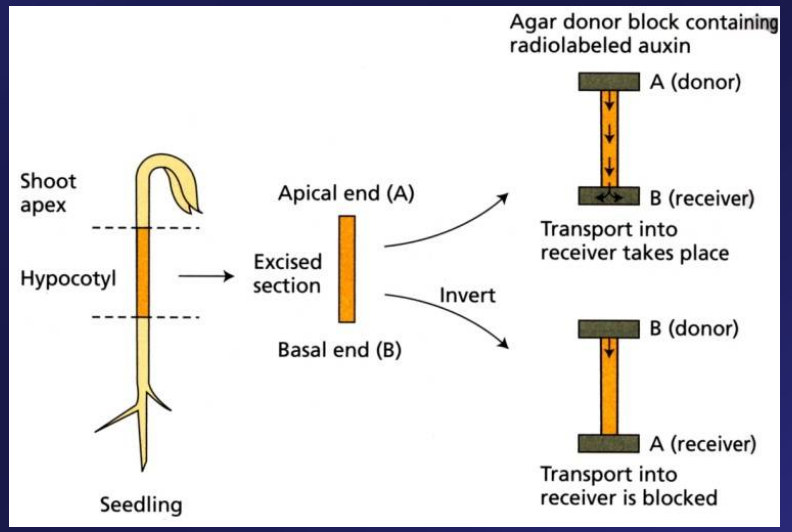
- embryogenesis
- stem elongation
- apical dominance
- photo- and gravitropism
- lateral root formation

Cellular level:

- cell division
- cell expansion
- cell differentiation

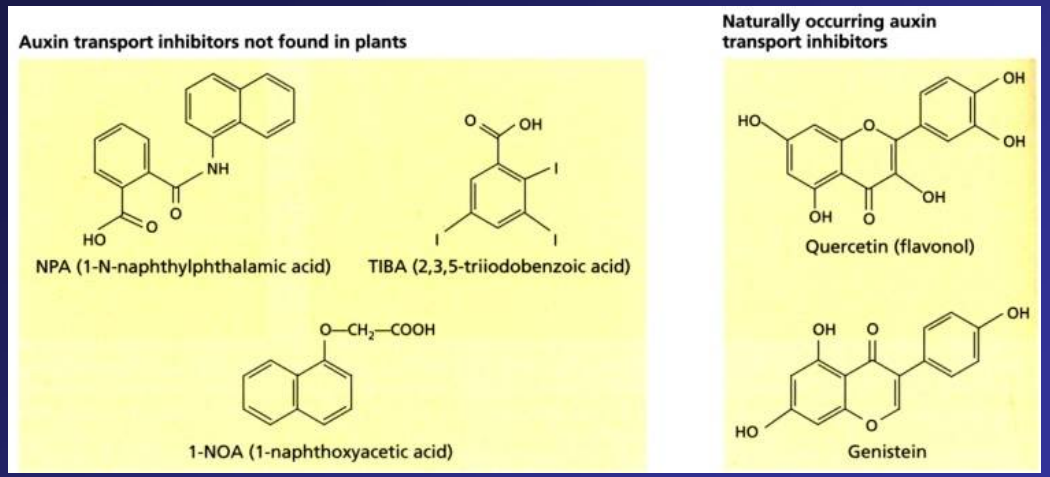
Auxin transport

- Polar (basipetal) transport from apex, coleoptile; in roots acropetal transport predominates (is not affected by orientation)
- Apoplastic transport, xylem and phloem transport



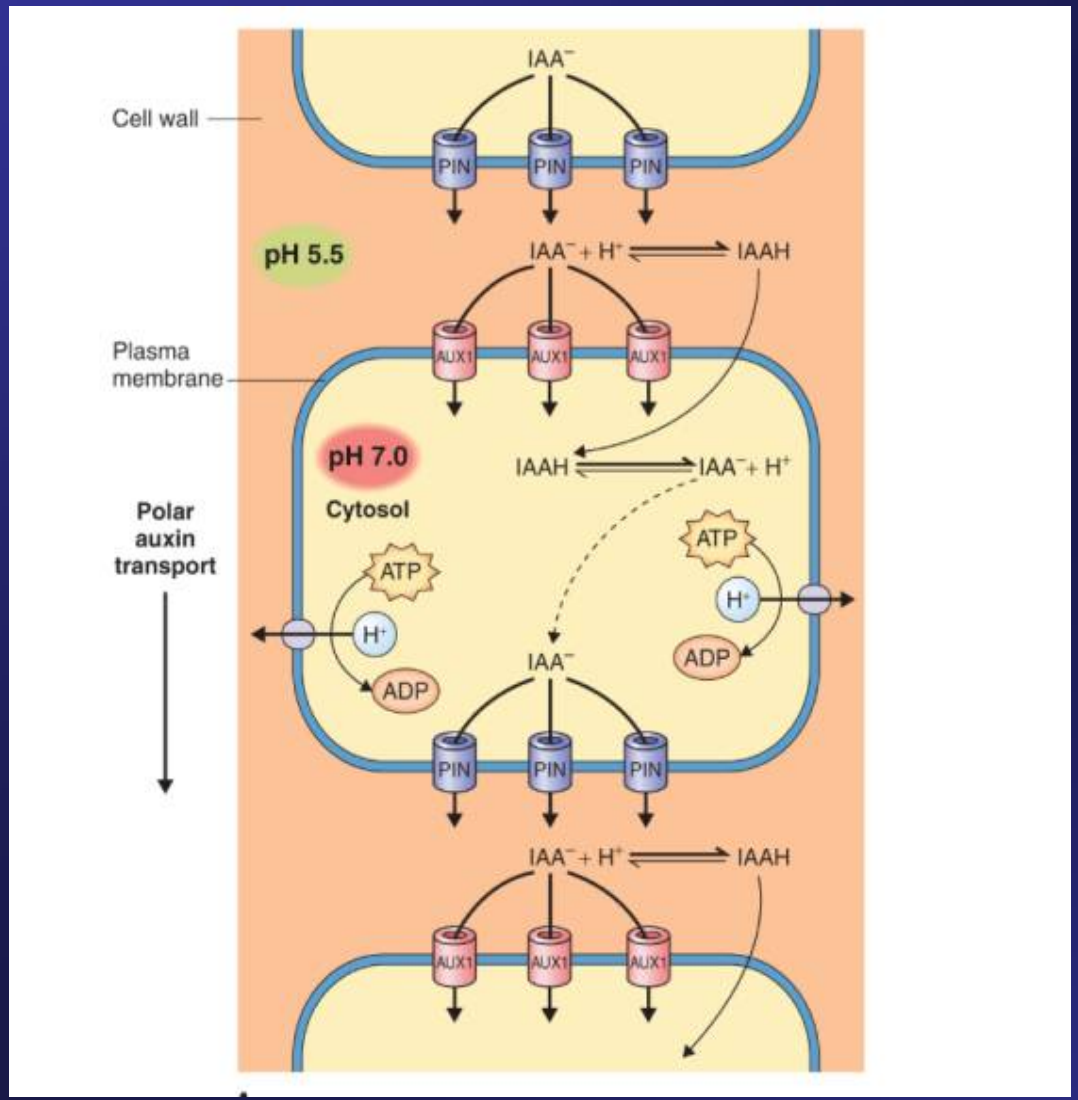
Transport inhibitors

NPA: 1-N-Naphthylphthalamic acid
TIBA: 2,3,5-triiodobenzoic acid
1-NOA: 1-naphthoxyacetic acid
quercetin, genistein



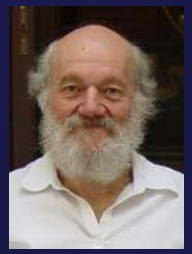
Influx

- Passive diffusion of IAAH (lipophilic)
- Active permease - AUX1 carrier (symport $2H^+/IAA^-$)



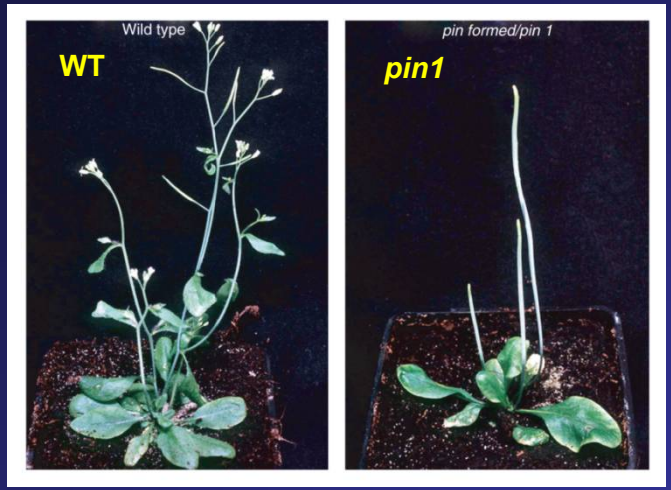
Efflux

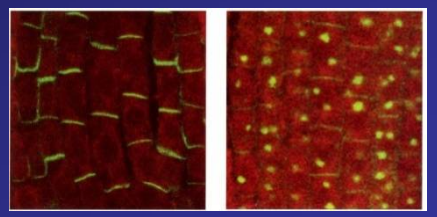
- Active - carrier: PIN proteins, P-glycoproteins (ATP-dependent carrier)



John Raven

Mary Helen Goldsmith



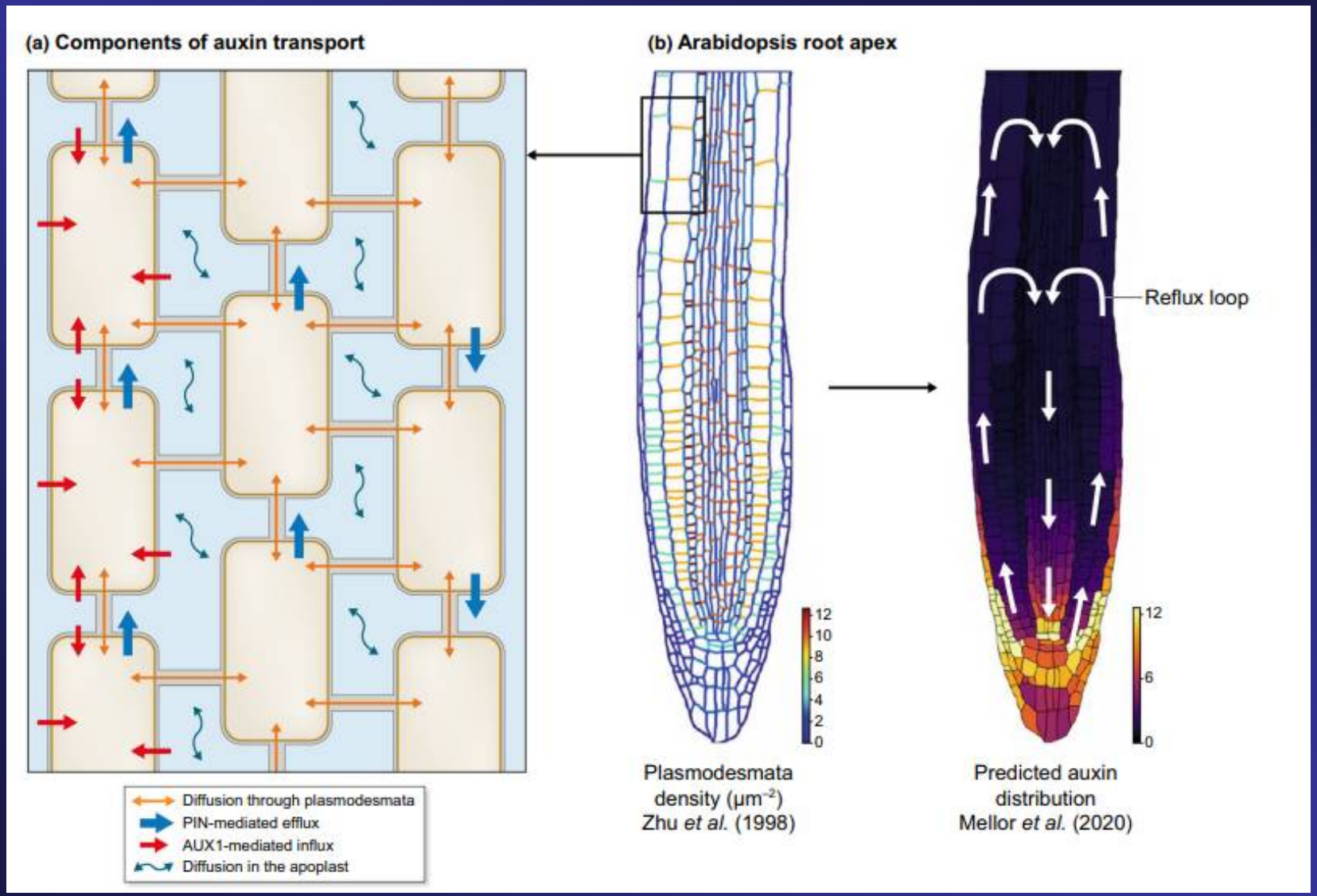


Accumulation of PIN1 on plasma membrane

Accumulation of PIN1 in nucleus

Update 2021

Band LR (2021) New Phytologist 231: 1686–1692



Auxin signaling

Auxin receptor TIR1

Ottoline Leyser
University of Cambridge

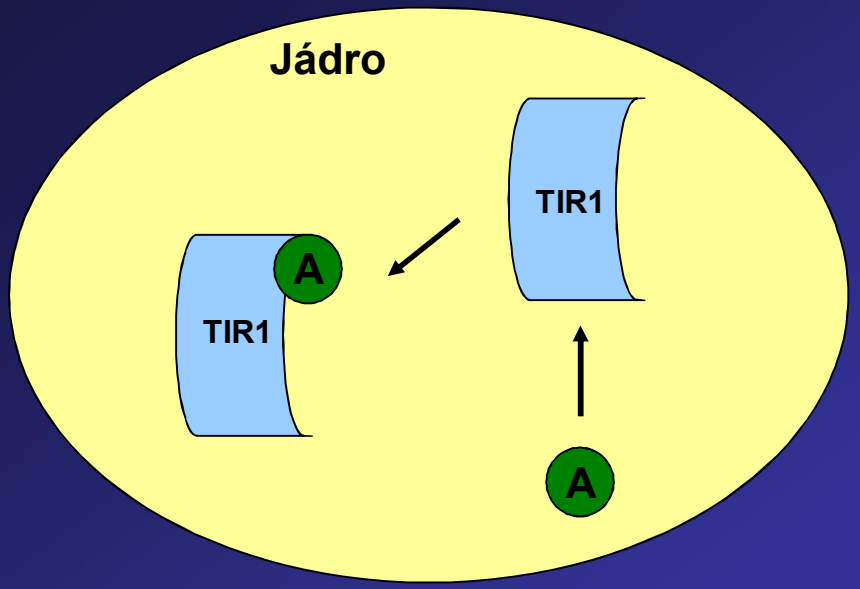
Mark Estelle
University of California
San Diego

Intracellular receptor TIR1 (Transport Inhibitor Response 1) in *Arabidopsis*

Kepinski and Leyser 2005

Dharmasiri *et al*. 2005

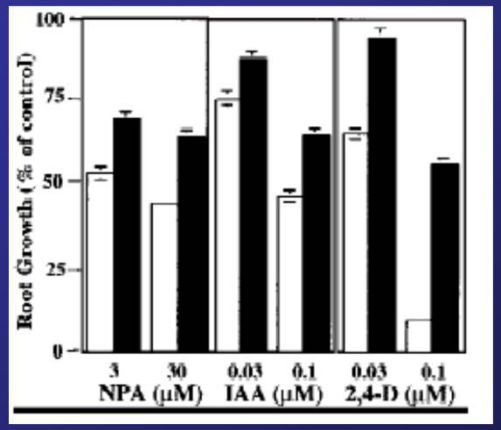
Auxin binds directly to TIR1 in nucleus



1) First intracellular auxin receptor

2) Receptor mediates transcriptional responses to auxin

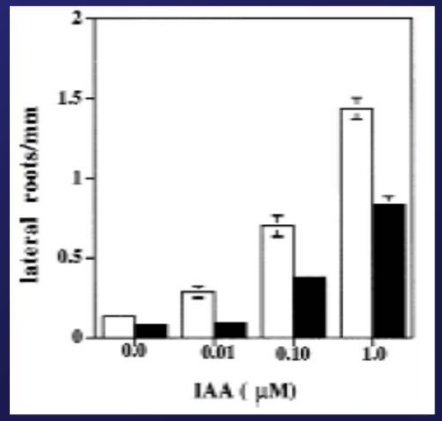
tir1-1 mutant in *Arabidopsis:*



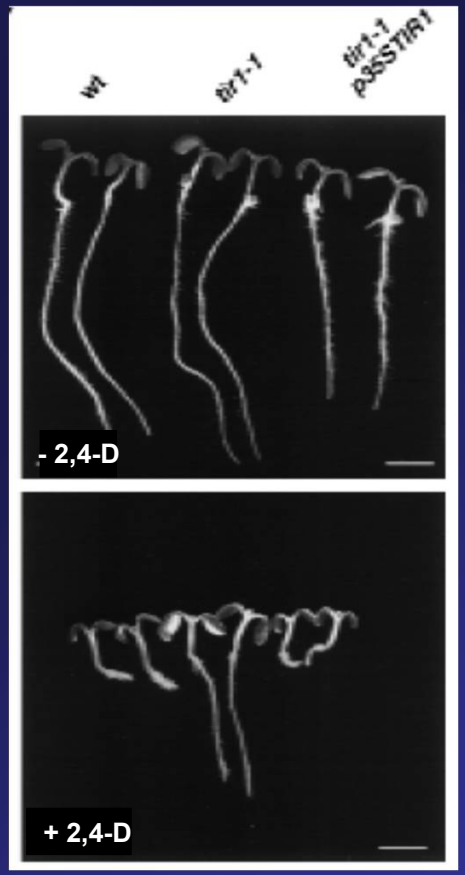
wt

tir1

***tir1-1* – resistant to the inhibitor of polár auxin transpor (NPA) and to k auxins**



***tir1-1* – reduced formation of lateral roots**

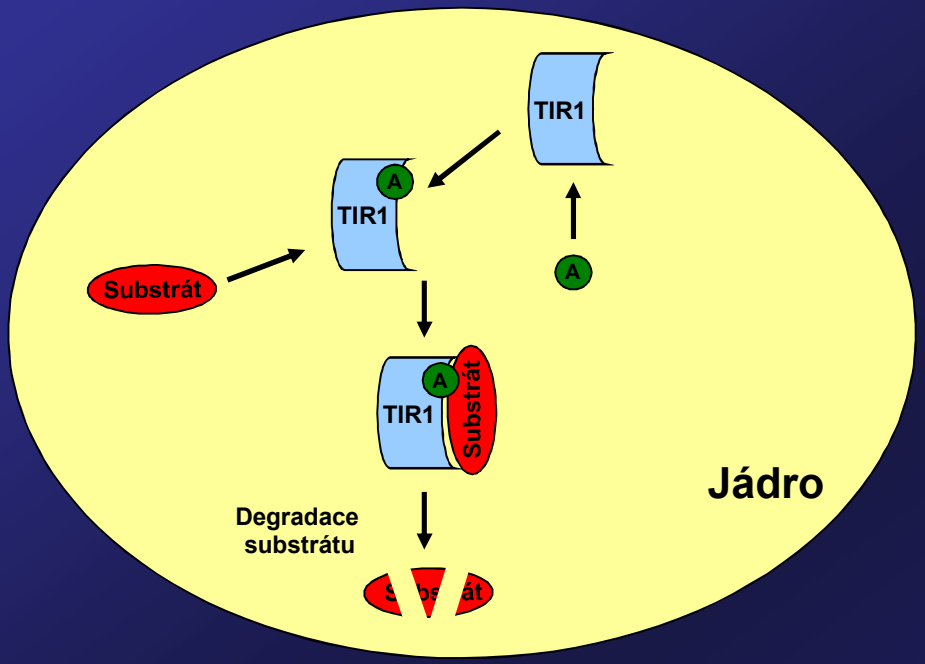


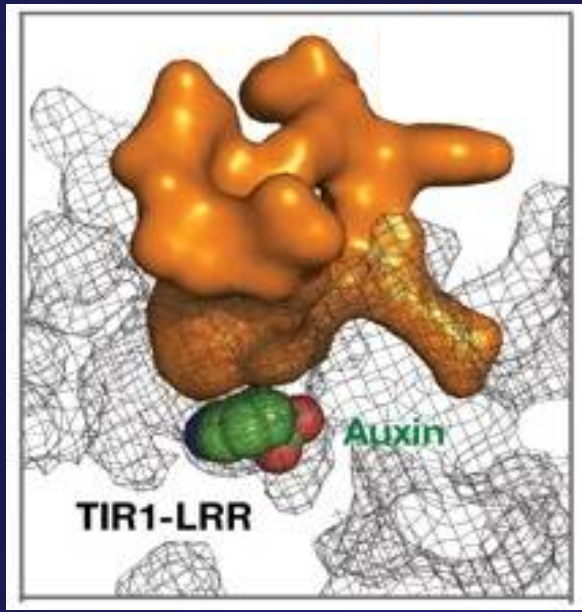
- TIR1 contains a serie of leucine repeats and series F-box motives
- F-box proteins are involved in processes mediated by ubiquitination

Responses to auxin depend on the modifications of proteins caused by ubiquitination

TIR1 functions in ubiquitination conjugate pathway, which directs proteins to proteolytic degradation.

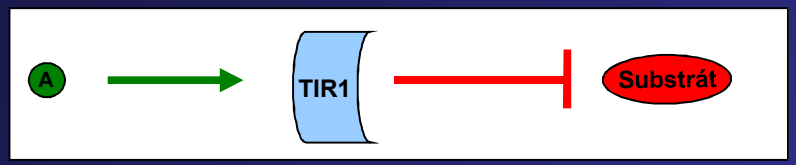
F-box – mediates transcriptional responses to auxin





Binding of auxin to TIR1 enables the binding of a substrate protein (= repressor of transcription of auxin-induced genes). This leads to the degradation of the substrate protein and thus to the release of the expression of auxin-induced genes.

Auxin acts as "a glue" allowing TIR1 to bind to the substrate.



Ubiquitination - necessary regulatory mechanism in plant and animal cells

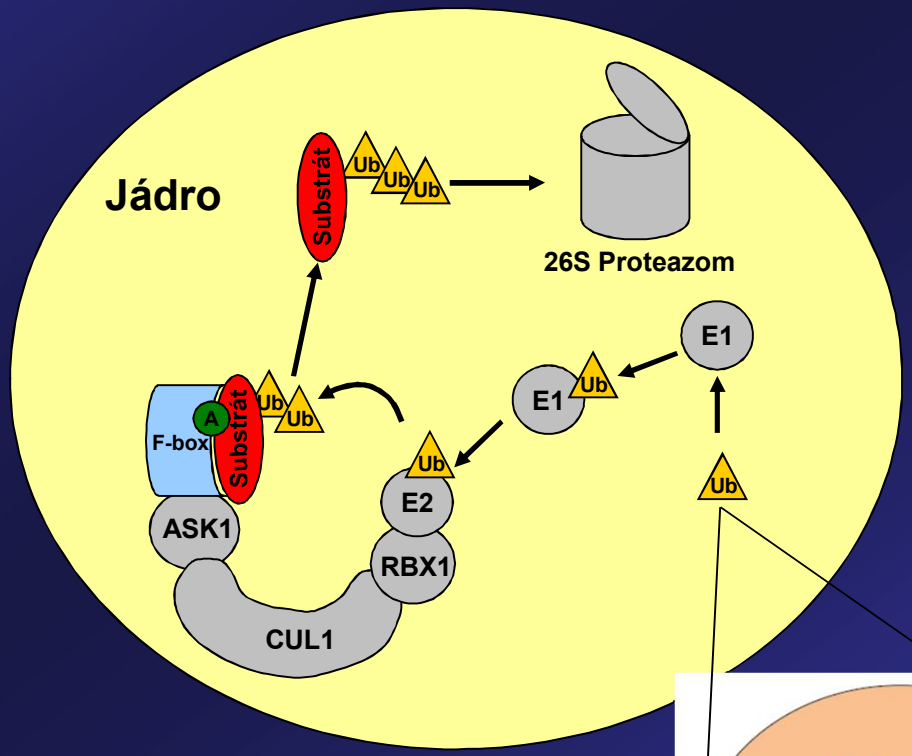
Ub — Ubiquitin

E1 — Ubiquitin-activating enzyme

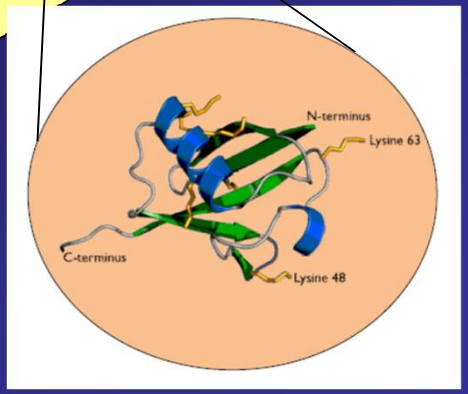
E2 — Ubiquitin-conjugation enzyme

F-box — F-box of E3 ubiquitin-ligase

Substrát — Target protein



Ubiquitin



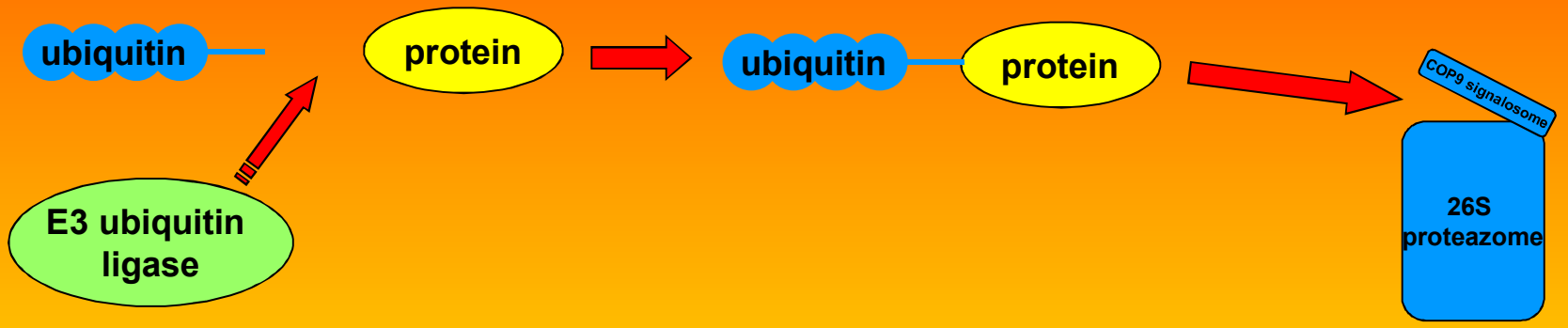
The target protein is poly-ubiquitinated and targeted to the 26S proteasome where it is degraded.

Similarity in light signaling.

COP1 functions as an E3 ubiquitin ligase – a securing enzyme in the cell protein degradation (proteolysis)

Proteolysis mediated by proteazome needs protein ubiquitin.

Ubiquitination – common mechanism of protein degradation in organims



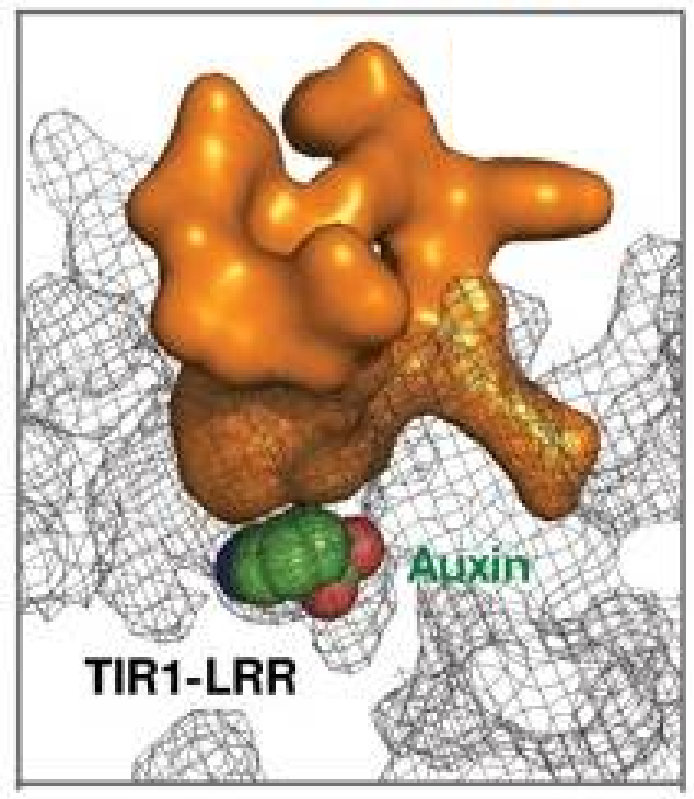
UPDATE 2012

Lau OS, Deng XW (2012) TIPS 17: 584-593

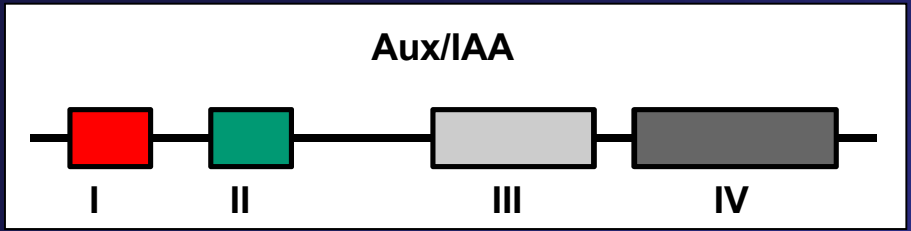
Recent information about the function of COP1 in plants.

What is a substrate protein?

Substrate ?



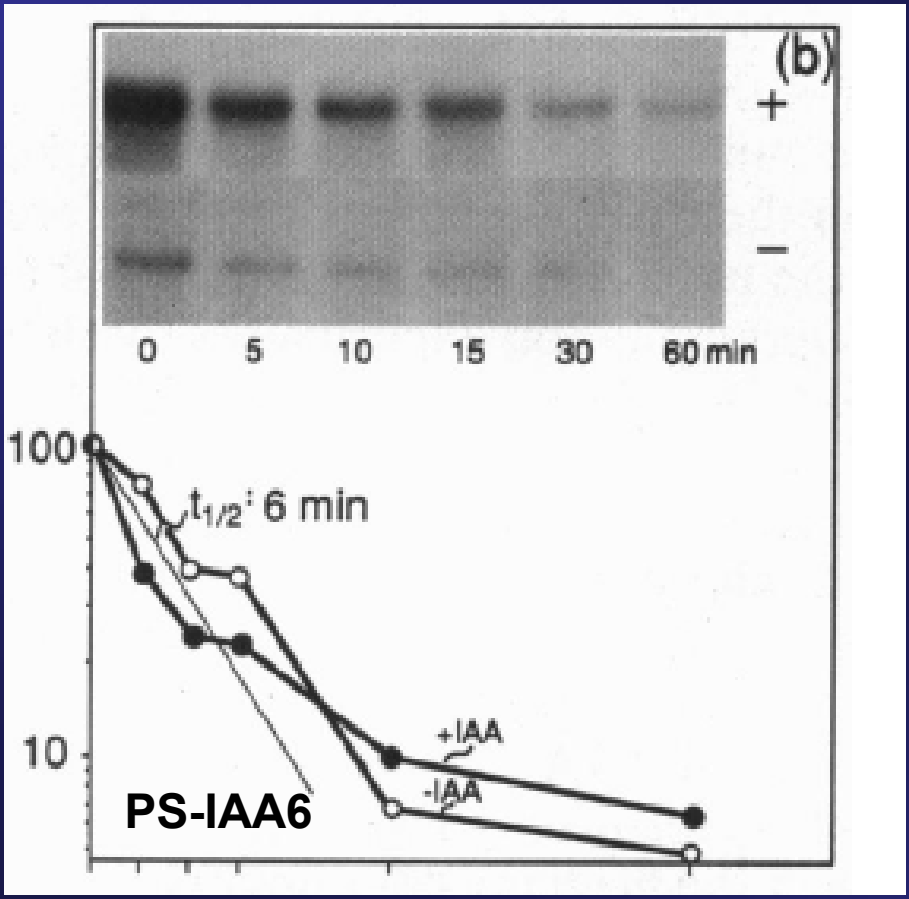
Aux/IAA proteins – nuclear proteins, which inhibit auxin responses. *Arabidopsis* – 29 types of proteins identified.



The Aux/IAA-GUS fusion protein shows nuclear accumulation.

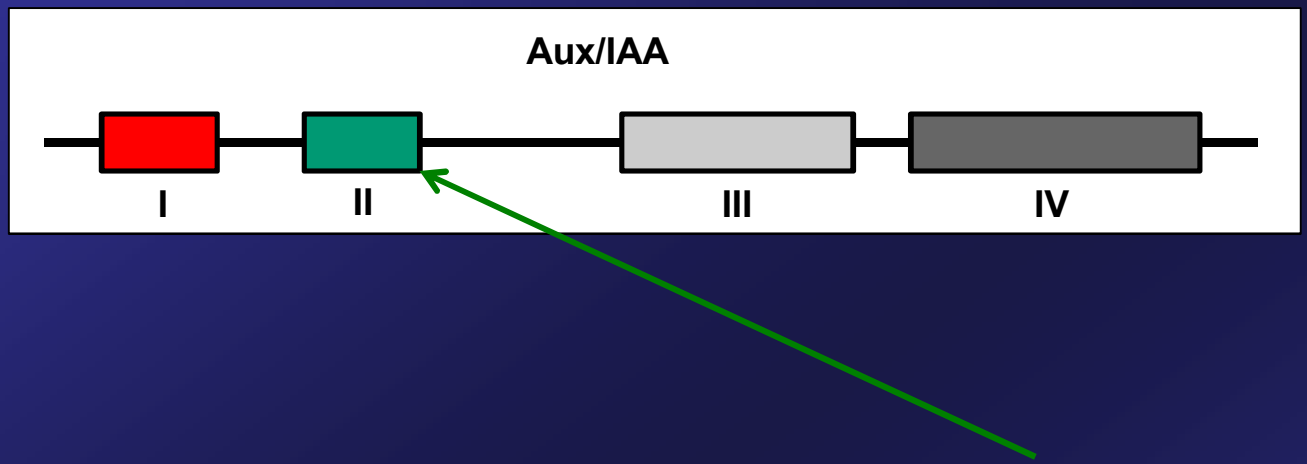
Aux/IAA are short-live proteins

Aux/IAA have a regulation function



Aux/IAA are destabilized in the presence of auxin.

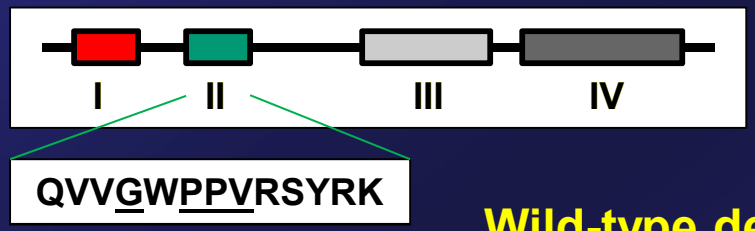
Aux/IAA proteins have protein-destabilizing domain.



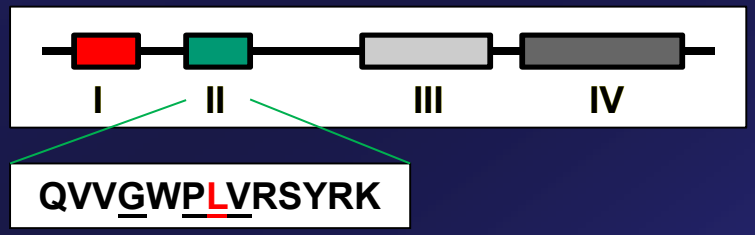
A short amino acid sequence called a degron - required for auxin-induced instability of the Aux/IAA protein

A change in one amino acid in the degron domain results in greater stability of the Aux/IAA protein => auxin resistance

Through the degron domain, Aux/IAA binds to TIR1



Wild-type degron



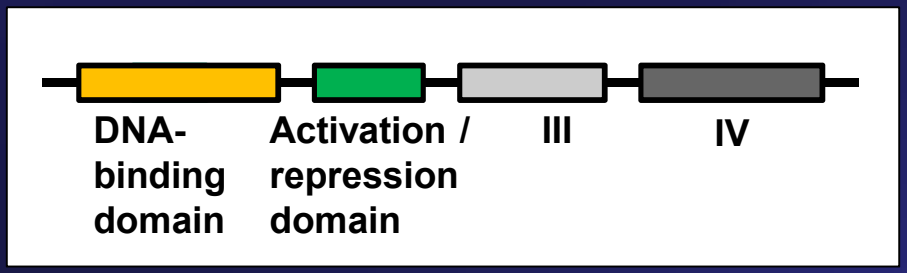
Mutant degron

A mutation in the degron domain prevents the Aux/IAA protein from binding to TIR1 and thus prevents the degradation of Aux/IAA => the mutant Aux/IAA protein is more stable.

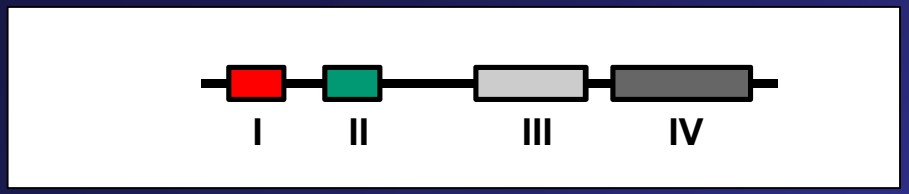
Transcription factors ARF (Auxin Response Factors) – short-lived proteins; binds to a DNA sequence called *AuxRE* (Auxin Response Element). ARFs stimulate the expression of auxin-inducible genes.

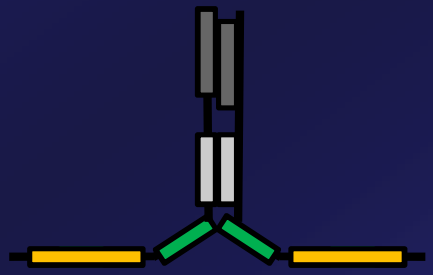
***Arabidopsis* – 23 ARFs**

ARF

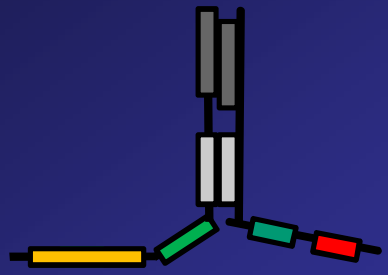


Aux/IAA



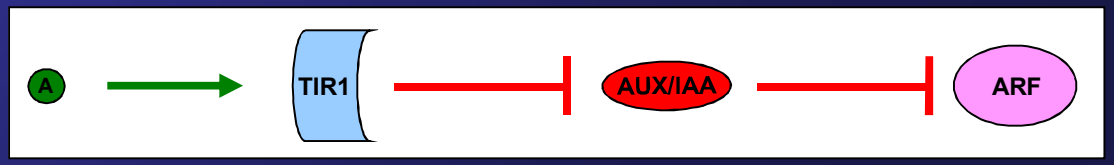


ARF homodimer



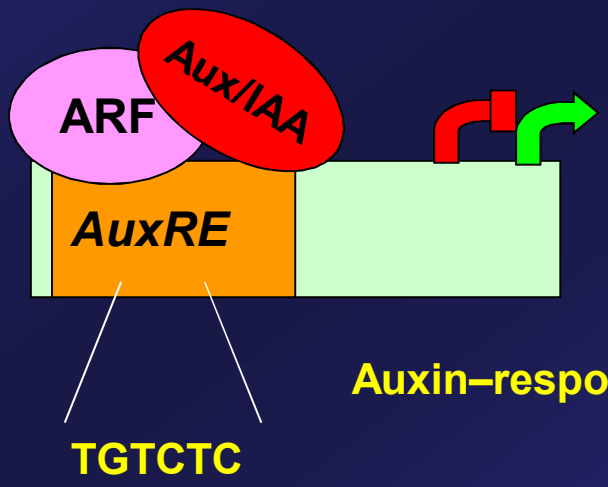
ARF Aux/IAA heterodimer

Aux/IAA proteins block transcriptional factors ARF



The transcriptional result (activation or repression) depends on the concentration of auxin, and thus on the final concentration of the transcription factors ARF and Aux/IAA in the cell and on their mutual affinity and affinity to the DNA binding site.

[Auxin] → [Aux/IAA] → Transcriptional result



Auxin–responsive gene

Binding ARF to *AuxREs*

Activation of gene expression

Repression of gene expression

TIR1- mediated auxin signaling

A – auxin

TIR1 – F-box subunit of E3-ubiquitin ligase; auxin receptor

AUX/IAA – repressor of transcription of auxin-induced genes

ARF – transcription factor (expression activator)

AuxRE – auxin-responsive element

Ub Ubiquitin

E1 Ubiquitin-activating enzyme

E2 Ubiquitin-conjugation enzyme

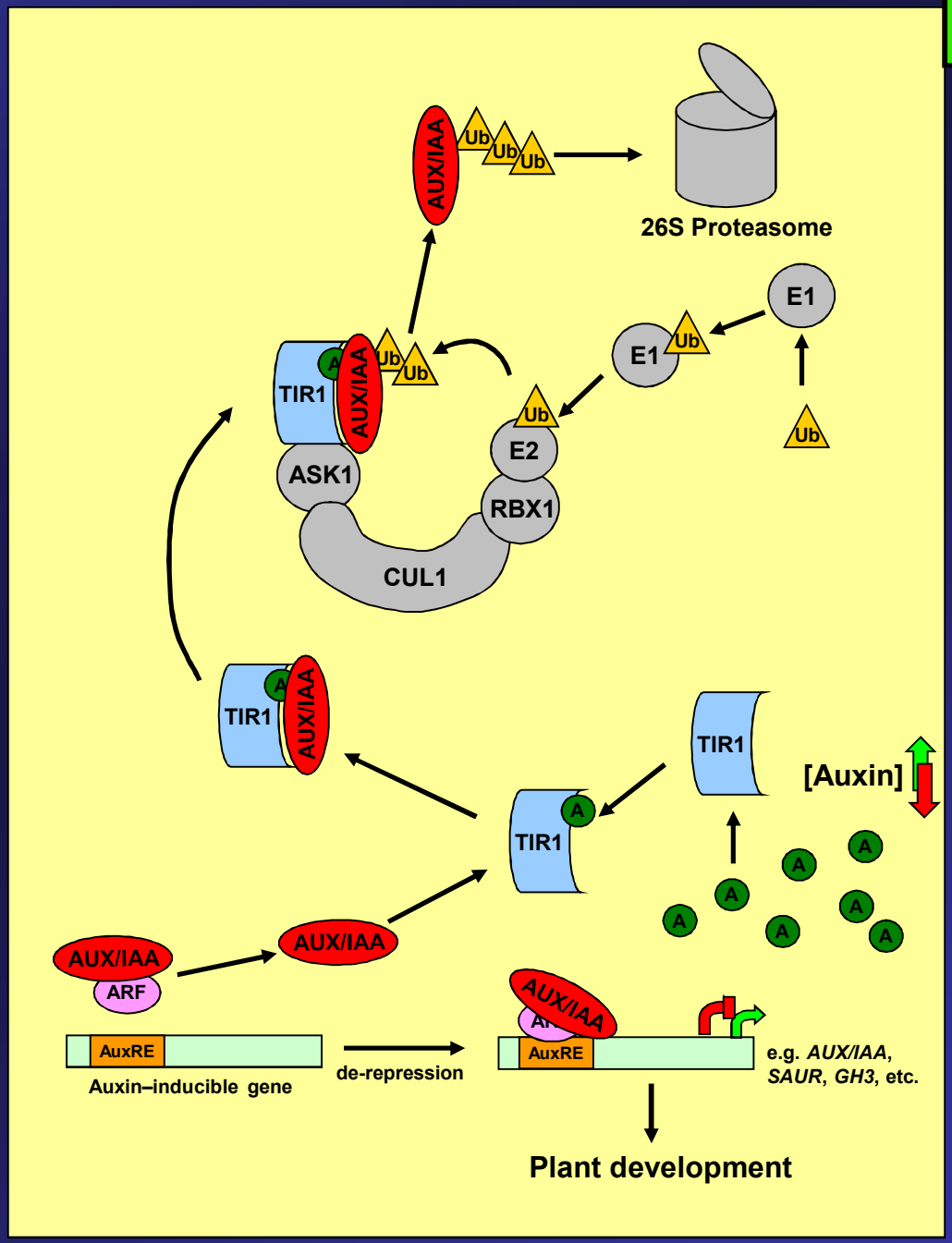
Auxin functions as „a glue“ enabling connection of TIR1 with substrates.

Similar mechanisms for F-box proteins:

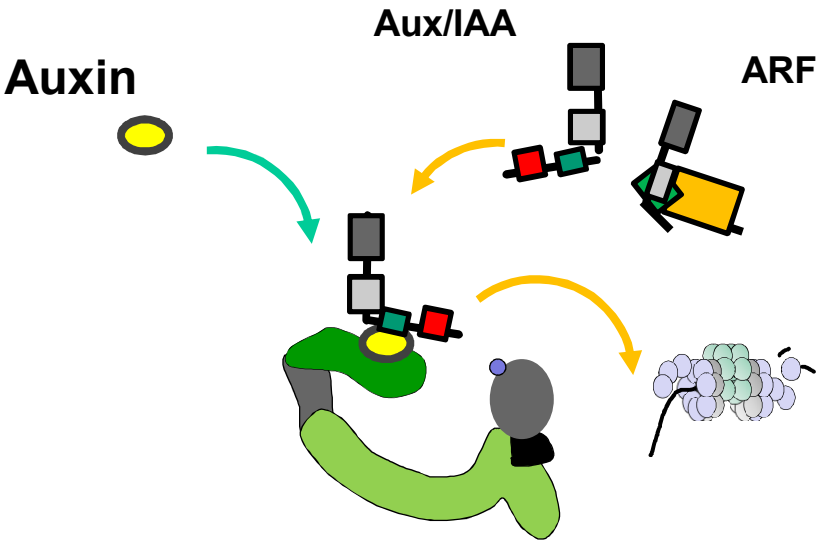
COP1 – CONSTITUTIVE PHOTOMORPHOG. 1

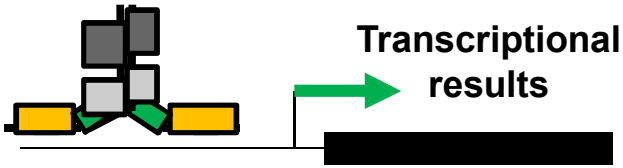
COI – CORONATINE INSENSITVE 1

ZTL – ZEITLUPE, etc.



The diversity of auxin responses correlates with the diversity of signaling proteins.





The number of potential interactions between ARFs and Aux/IAA proteins is large. This creates a high potential (ability) to precisely control the expression of auxin-induced genes in many combinations.

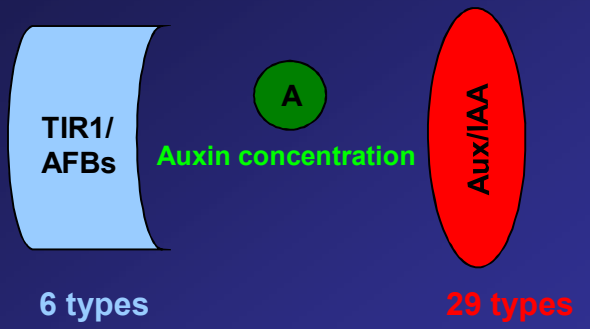
The diversity of auxin responses is further enhanced by the different auxin affinity of TIR1 receptors and Aux/IAA substrate proteins.

6 types of TIR1 receptors: TIR1, AFB1, AFB2, AFB3, AFB4 a AFB5

29 types of protein substrates Aux/IAA

TIR1/AFBs receptors have different affinities for Aux/IAA substrates depending on auxin concentration. At different auxin concentrations, different Aux/IAA substrates bind to different TIR1/AFBs receptors. Some Aux/IAA substrates require a high auxin concentration to bind to the TIR1/AFBs receptor.

The TIR1/AFBs-Aux/IAA complex is called co-receptor system. Aux/IAA affinity to auxin is determining the overall affinity systém.



Auxin - induced genes

Primary (early) genes – directly activated by TIR1/AFBs-Aux/IAA signalizací

Secondary genes – code for proteins, which are not directly involved in the induction of primary auxin response.

Primary genes - induction: minutes to hours; 3 functions

Transcription – early genes encode proteins regulating the expression of late genes

Signaling – early genes are involved in intercellular communication or signaling between cells

Conjugation/catabolism of auxins – early genes encode proteins involved in the elimination of IAA either by conjugation or degradation

Primary genes for growth and development

AUX/IAA – auxin-stimulated expression within 6–60 min; the resulting protein lives for about 7 minutes.

SAUR – auxin-stimulated expression within 2–5 min; expression does not require the synthesis of new proteins; genes do not contain introns, they encode very similar peptides of unknown function.

- Induce cell expansion (SAUR19)
- Induce cell division (SAUR76)
- Coordinate cell divisions and expansion (SAUR41)

} UPDATE 2013-2014

GH3 (*Gretchen Hagen 3*) – auxin-stimulated expression within 5 min; GH3 encodes acyl-acid-amido synthases - function in IAA conjugation - inactivation of IAA and jasmonic acid; GH3 expression reflects the amount of endogenous auxin => DR5 synthetic promoter.

Prof. Gretchen Hagen
University of Missouri

Secondary (late) genes

Auxin-stimulated expression within 2–4 hrs; often induced by stress

Signaling pathway through the receptor TIR1 is not the only auxin signaling pathway.

Homologs TIR1: AFB1, AFB2, AFB3 – the same functions as TIR1

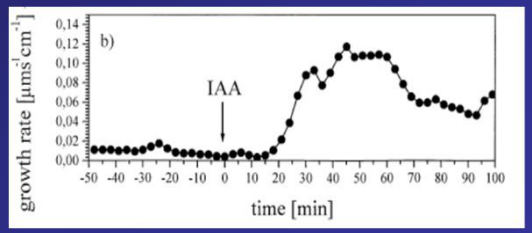
However: 1) Quadruple mutant – functional plant → ReceptorsTIR1 and AFB are not essential

Dharmasiri *et al*. 2005b

2)

Auxin induces cell elongation with lag phase 8-15 min => rapid response excludes involvement of TIR1, i.e. gene expression and protein synthesis

Auxin functions via signaling pathway involving another receptor → Receptor ABP1



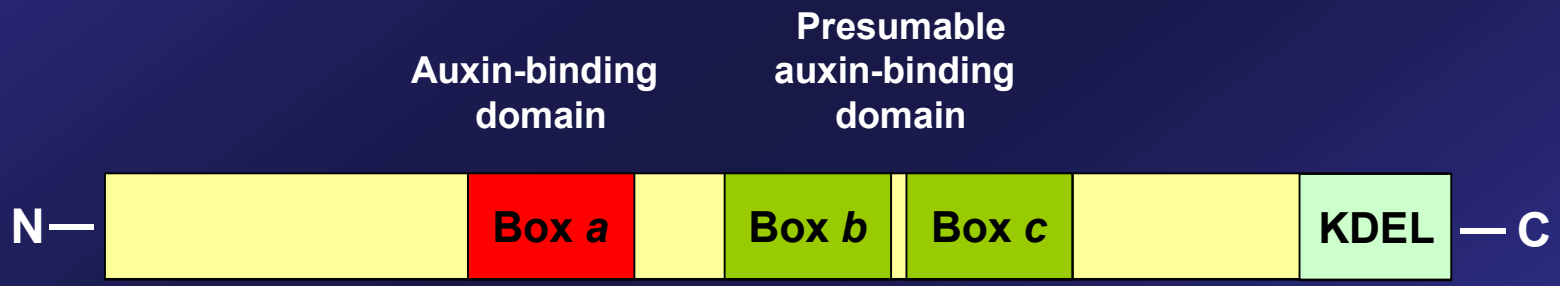
Steffens and Lüthen 2000

Auxin receptor ABP1

1972 – identification of ABP1 (Auxin-Binding Protein 1) in isolated membranes of maize coleoptile cells; binding of radioactive auxin in the membrane

1985 – isolation of ABP1 protein in maize; 22 kD

End of 80th – cloning and structure of ABP1



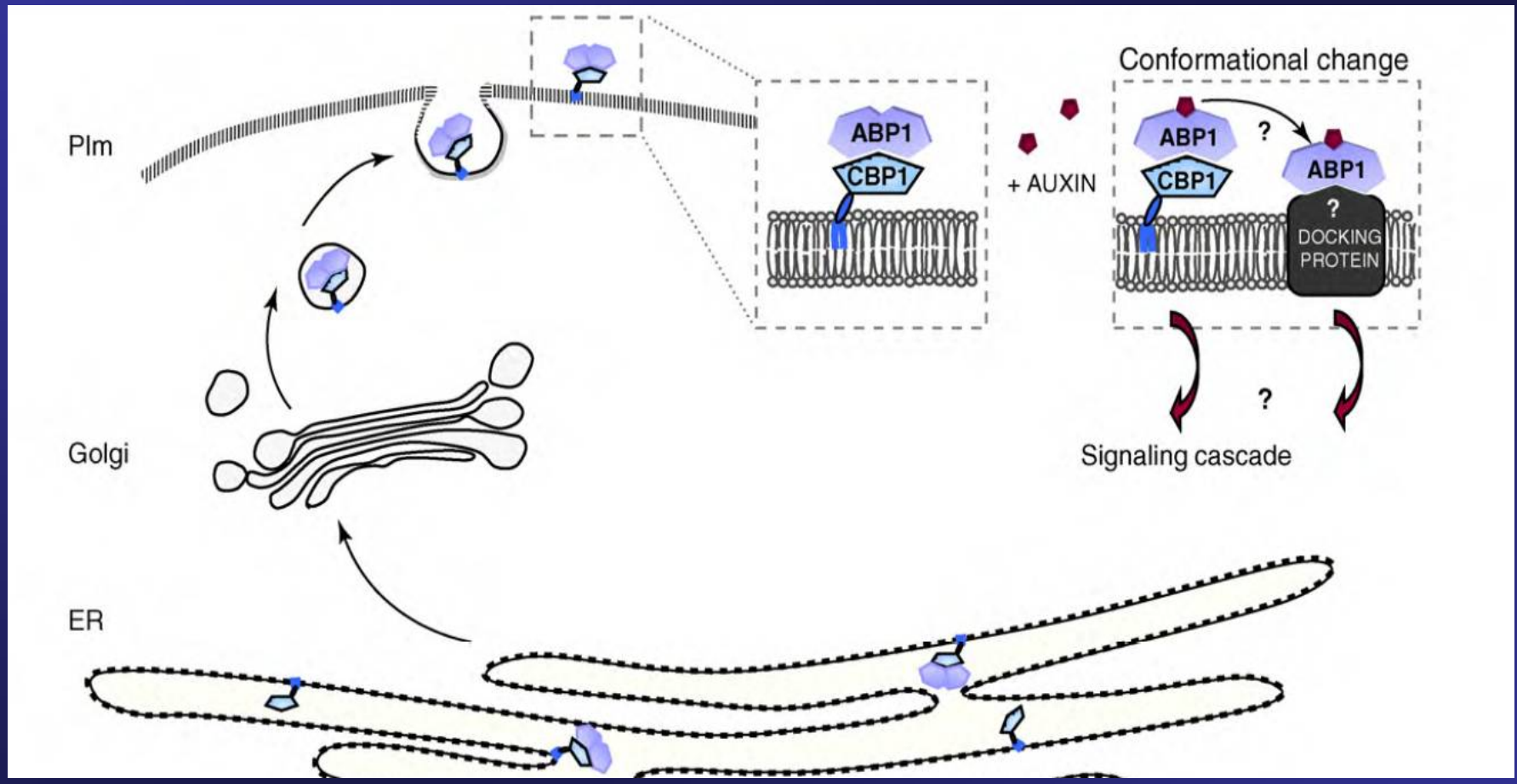
Auxin binds to plasma membrane fraction.

BUT ?

Protein ABP1 has not transmembrane domain, but has s KDEL domain.

Transport of ABP1 from ER and „Docking“ model

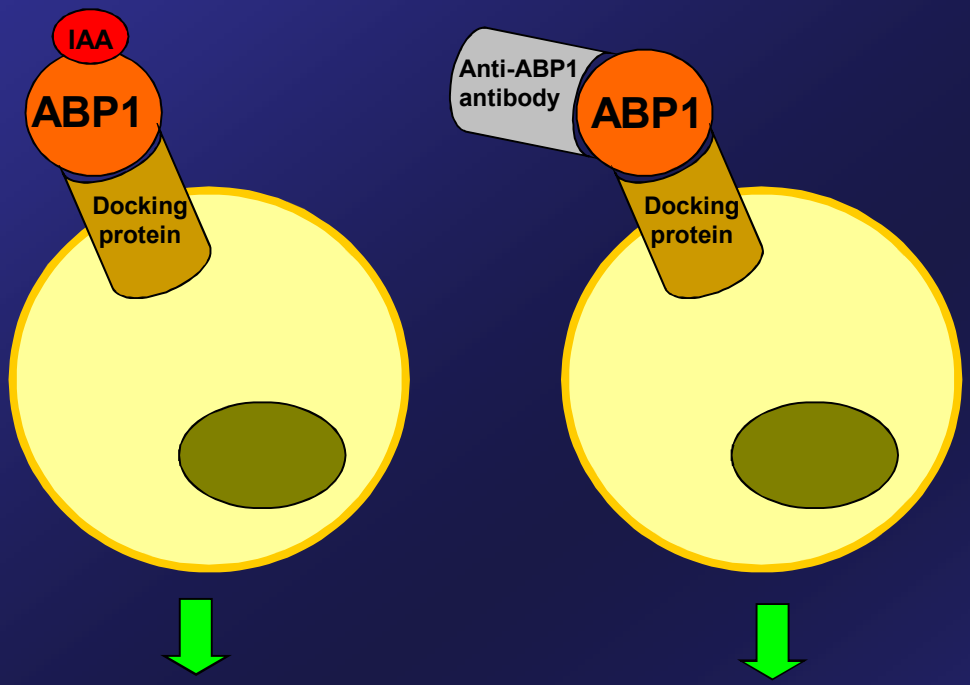
Transport of ABP1 from endoplasmic reticulum to apoplast and binding to a docking protein.



The majority of ABP1 is localized in the endoplasmic reticulum (ER). A small part of ABP1 is secreted by vesicles into the apoplast by an as yet unknown mechanism and attached to the plasma membrane (Plm). Secretion of ABP1 has been demonstrated in various types of cell cultures. ABP1 is also regularly found in the culture medium. Brefeldin - an inhibitor of vesicular transport - inhibits the secretion of ABP1 into the culture medium.

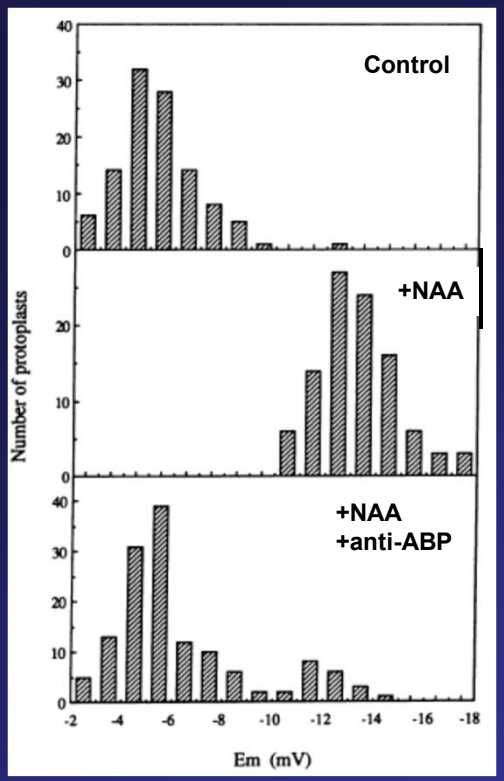
What is (was) the evidence that ABP1 is an extracellular auxin receptor?

Electrophysiological experiments on protoplasts



Membrane hyperpolarisation

Membrane hyperpolarisation is blocked



Barbier-Brygoo H et al. (1989)

Patch-clamp technique: Auxin-induced increase in ATP-dependent current can be blocked by antibodies against ABP1

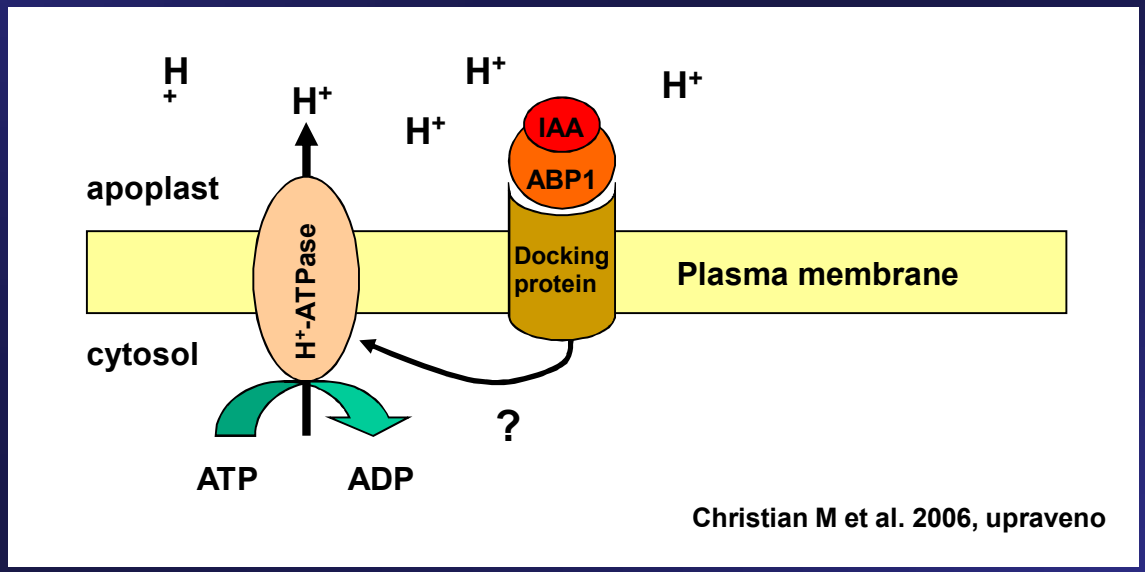
Acid growth theory, proton pump and K^+ channels

Hager A et al. (1971)
Rayle D and Cleland R (1970)

Rayle D and Cleland R (1970)

Auxin → Excretion of H^+ to apoplast → Lowering of pH in apoplast

Activation of enzymes (expansins) loosening the cell wall



Christian M et al. 2006, upraveno

Binding of auxin to ABP1

↓

Stimulation of H^+-ATPase

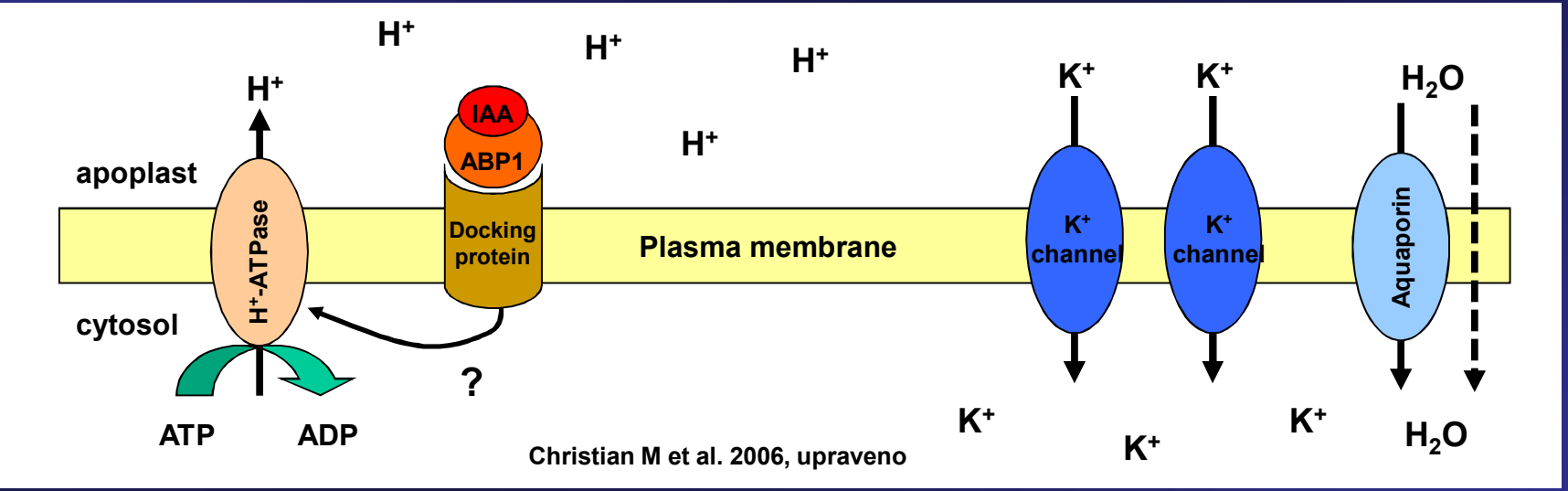
↓

Plasma membrane hyperpolarization
Apoplast acidification

The conditions for growth is a turgor. But auxin itself does not increase turgor.

Accumulation of H^+ in apoplast → Compensation of charge in cytosol

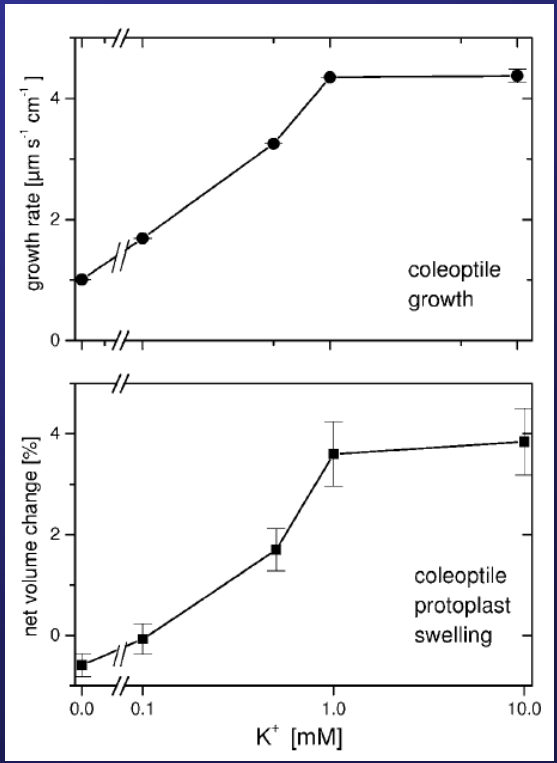
Inward-rectifying K^+ channels



K^+ accumulation in cytosol → Transport of H_2O into cell → Turgor → GROWTH

Presence of K^+ : conditions of sustained acidification and growth

Evidence for the involvement of K^+ channels in auxin-induced growth



Christian M et al. (2006)

- auxin-induced elongation of maize coleoptile is dependent on K+ concentration

- auxin-induced enlargement of protoplasts is dependent on the K+ concentration

- TEA (K+ channel inhibitor) inhibits auxin-induced elongation

- ZMK1 (*Zea mays* K^+ channel 1) is expressed in dependence on auxin concentration

- auxin-induced ZMK1 expression correlates with auxin-induced growth

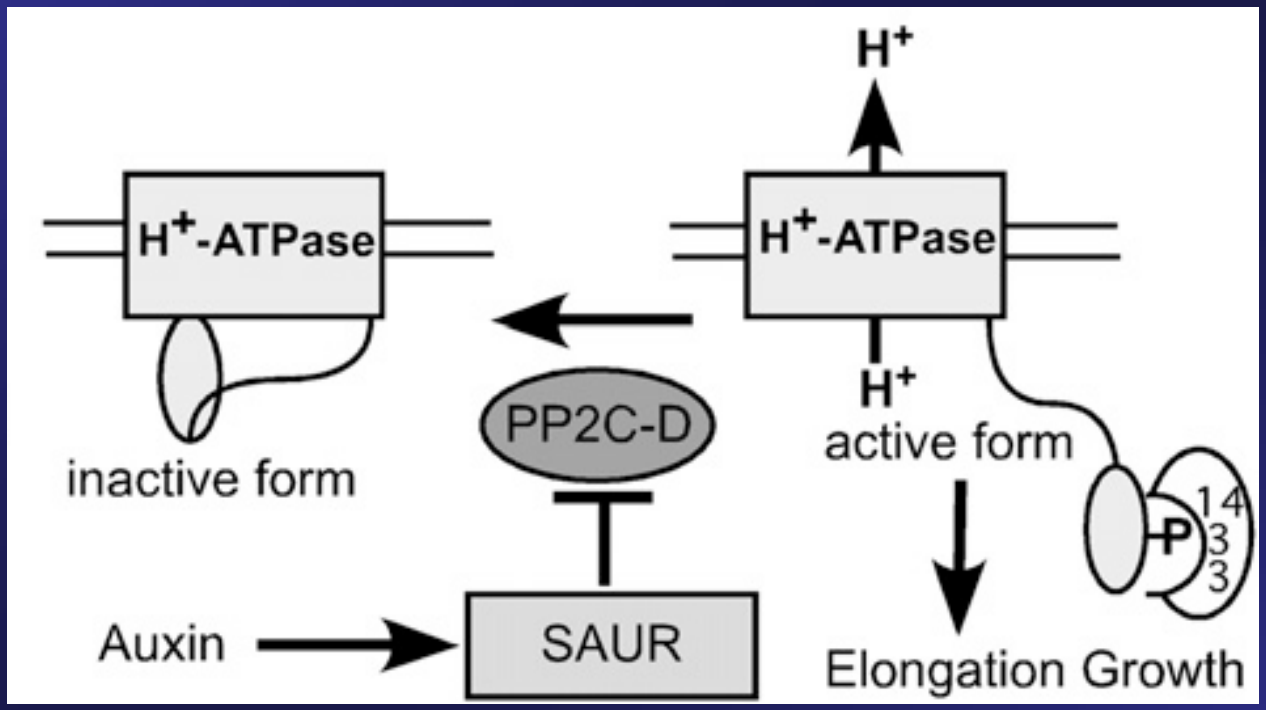
- ZMK1 knockout plants are embryo lethal

- plants with overexpressed ZMK1 are supersensitive to auxin

Update 2014

Spartz AK et al. (2014) Plant Cell 26: 2129-2142

H^+-ATPase (proton pump) activity is stimulated by SAUR19 proteins.



Mechanism:

H^+-ATPase is active only when its C-terminal domain is phosphorylated and bound to the 14-3-3 protein. This unblocks the catalytic site of the pump - the pump is active.

If the proton pump is dephosphorylated, it is not active. This dephosphorylation occurs by the protein phosphatase PP2C-D. When SAUR19 is expressed, it interacts with PP2C-D and thereby inactivates it. The proton pump is then not dephosphorylated and is therefore active.

Update 2021

Gelová Z et al. (2021) Developmental roles of Auxin Binding Protein 1 in *Arabidopsis thaliana*. Plant Science 303, art.no: 110750

By studying the *abp1* loss-of-function mutant, the role of ABP1 in auxin signaling involved in gene transcription was challenged.

BUT!

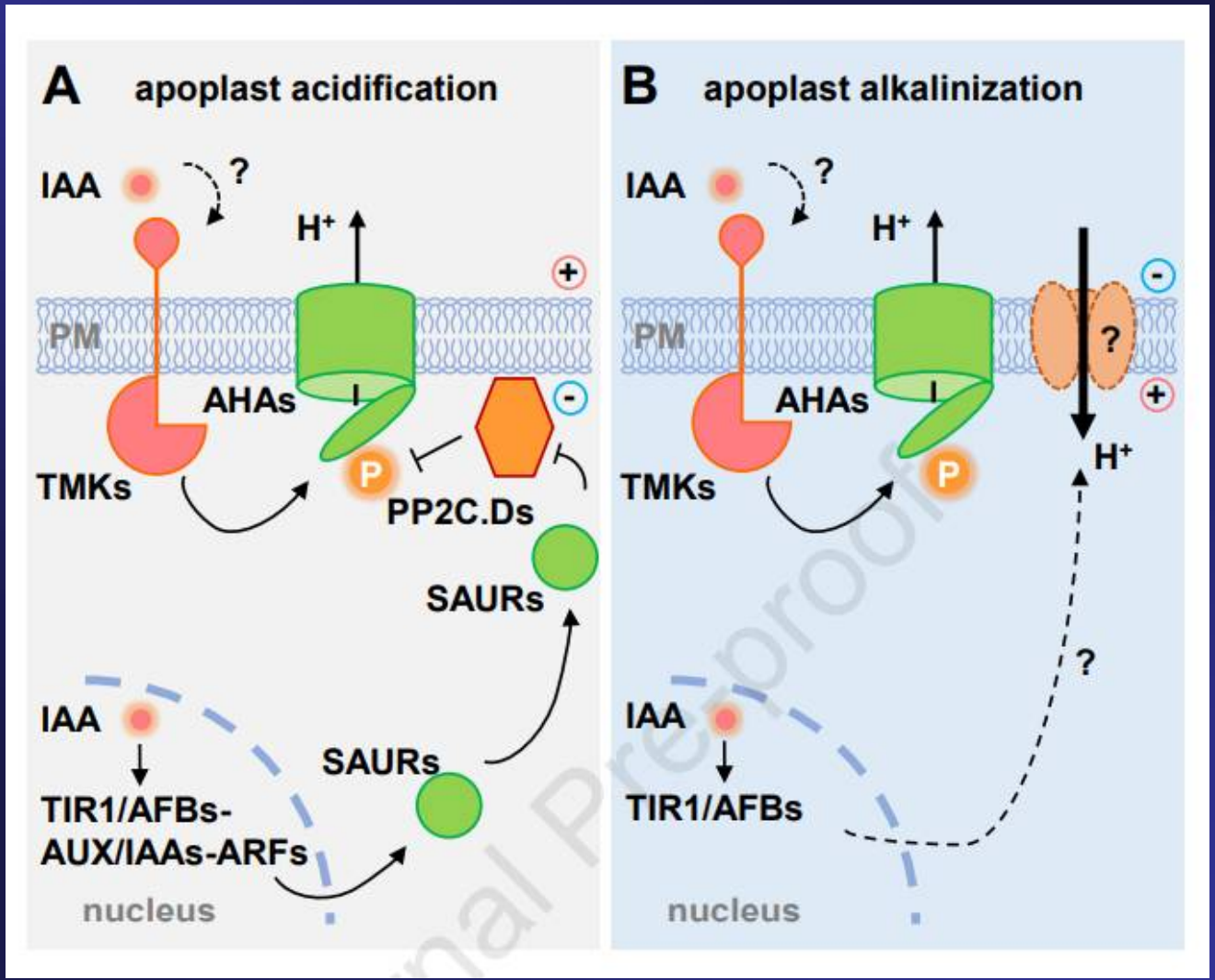
ABP1 gain-of-function mutants show:

- weakened effect of auxins on the polar distribution of PIN proteins
- attenuated brefeldin-dependent intracellular aggregation of PIN protein

ABP1 is significantly involved in plant development. However, the mechanism of action of ABP1 is unknown. The role of ABP1 may be masked by functional redundancy.

Update 2021

Peng Y, Tan S (2021) TMK: A Crucial Piece of the Acid Growth Puzzle. Molecular Plant, November 19



Update 2021

Review o ABP1

Napier R (2021) The story of Auxin-Binding Protein 1 (ABP1)
Cold Spring Harb Perspect Biol doi: 10.1101/cshperspect.a039909

Review on the role of phosphorylation and dephosphorylation (post-translational modification) in auxin biosynthesis, transport and signaling

Tan S et al. (2021) Pho-view of auxin: Reversible protein phosphorylation in auxin biosynthesis, transport ans signaling. Molecular Plant 14: 151-165