

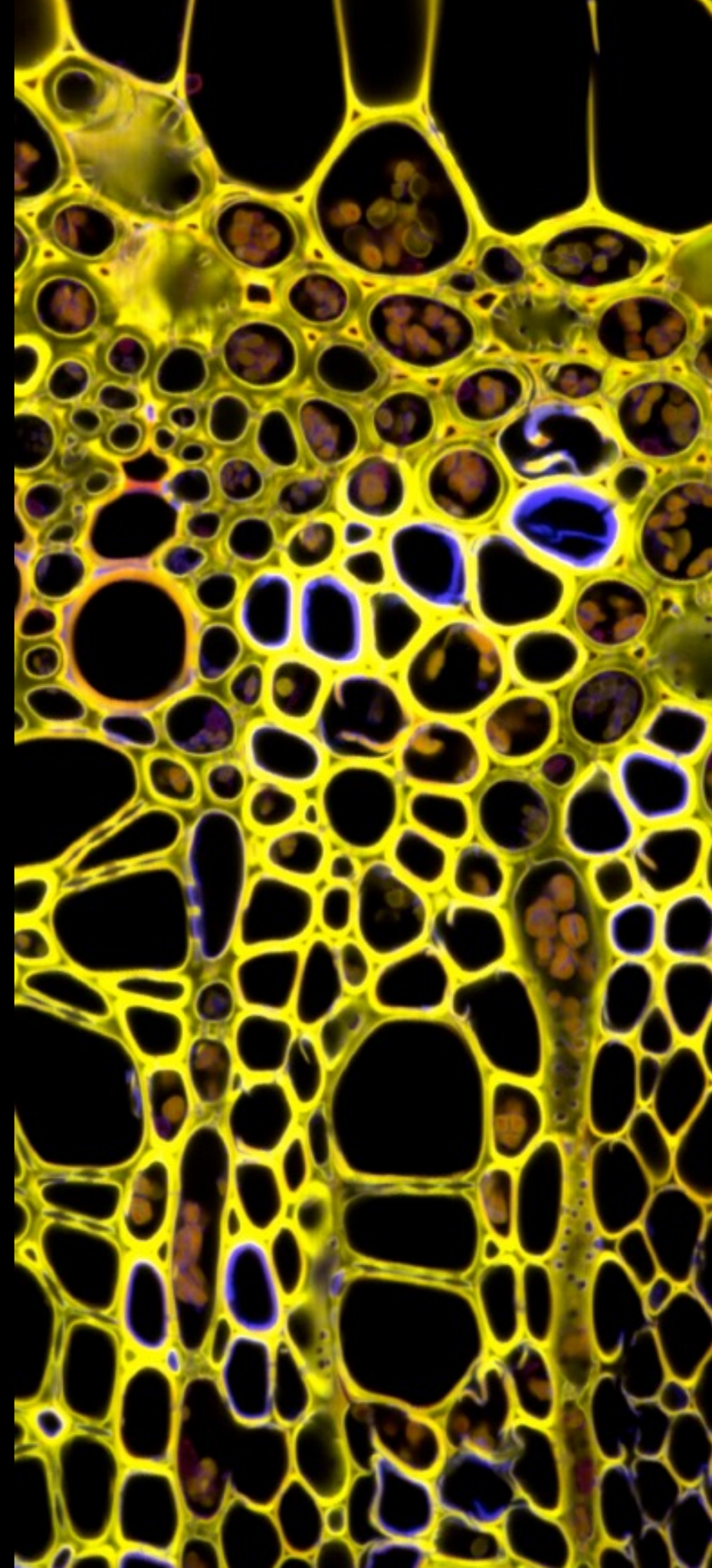
CDB Part IB

Plant Development

Lecture 2:

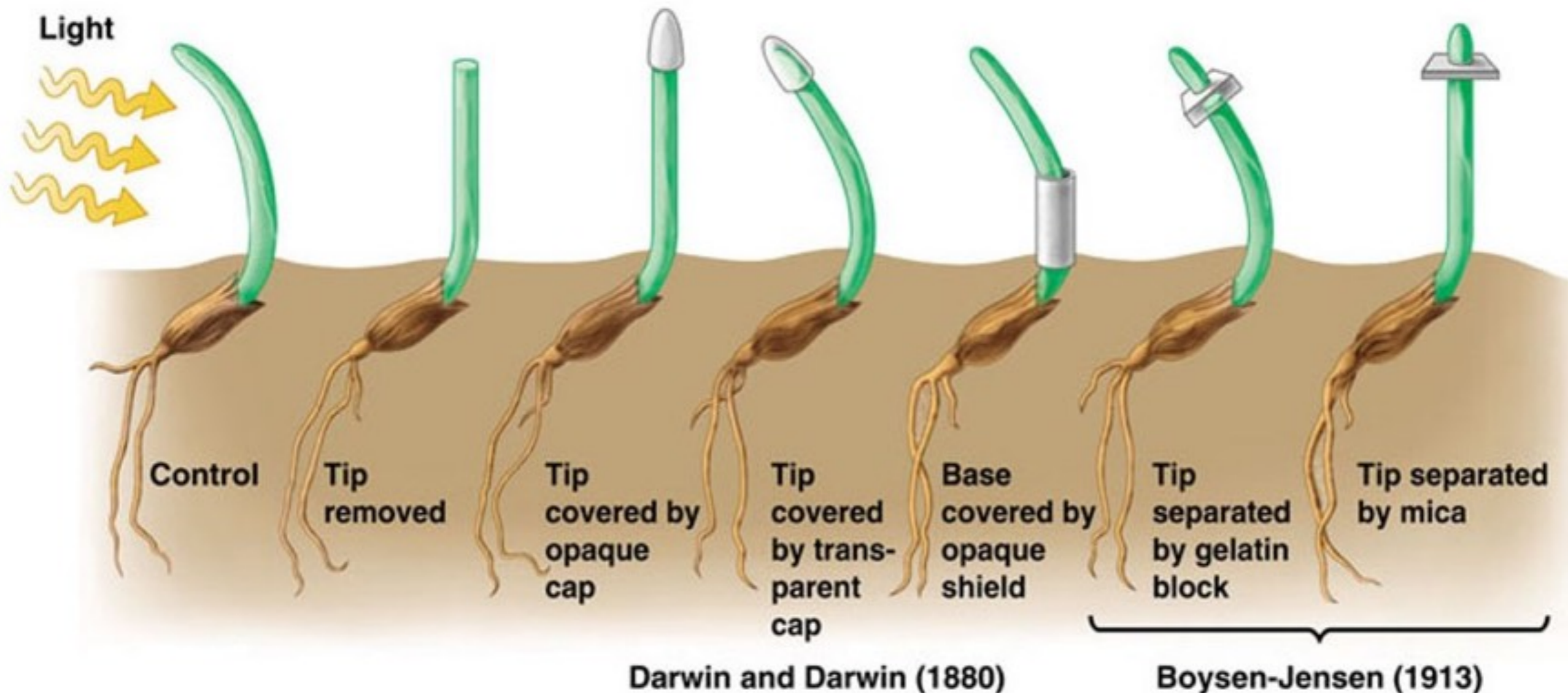
Polarity, auxin traffic and auxin response

Jim Haseloff
Department of Plant Sciences



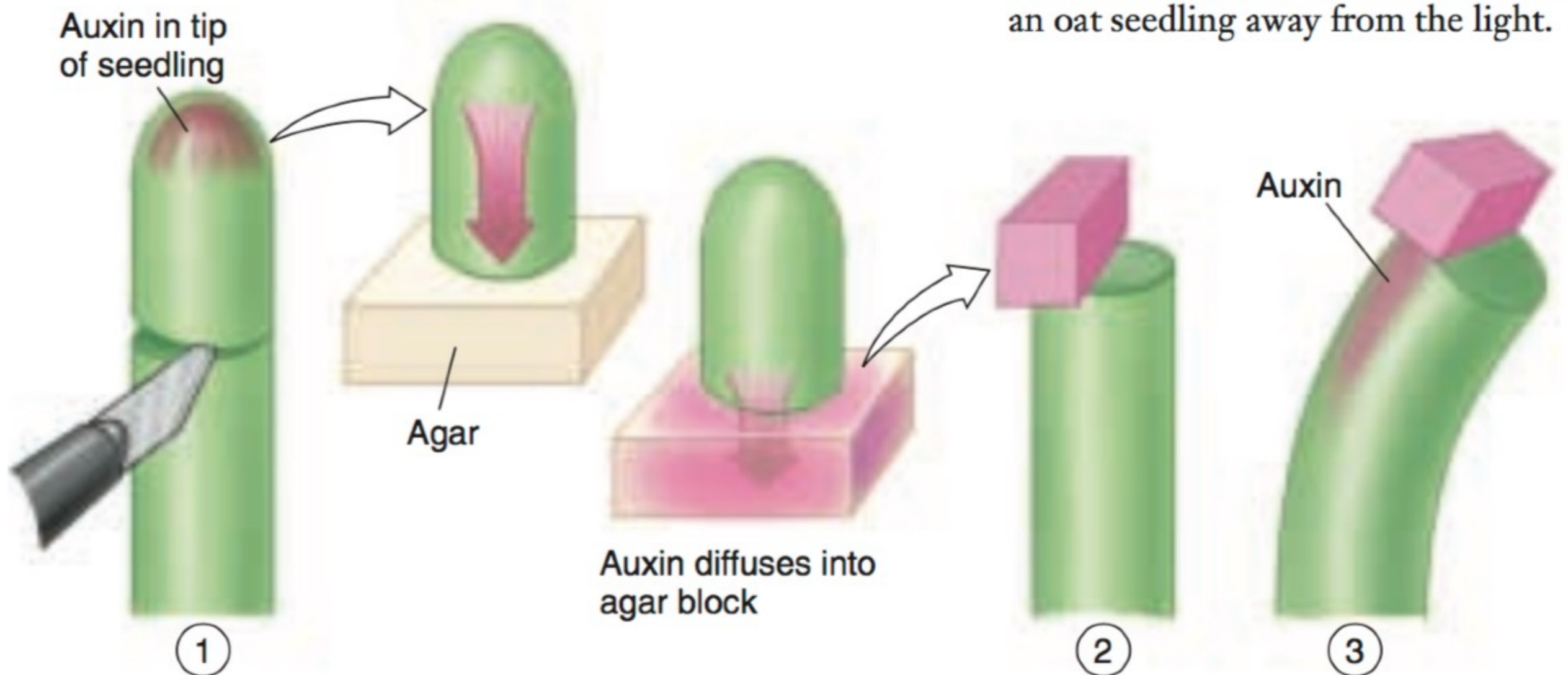
Signalling in phototropism

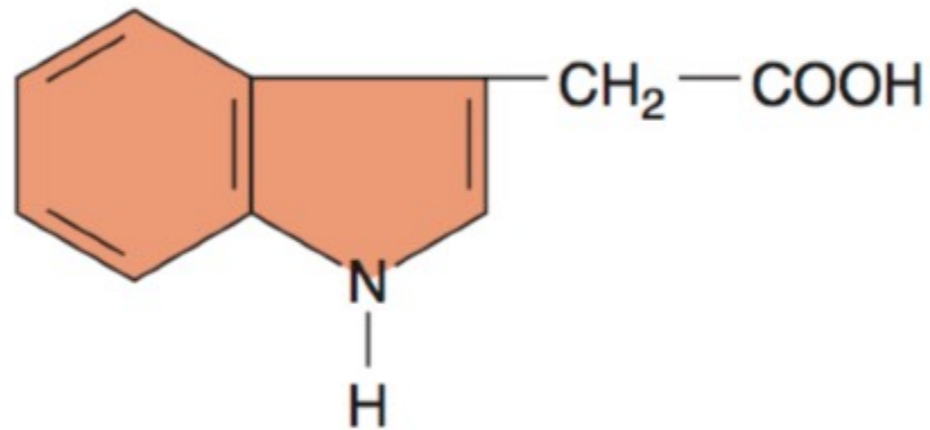
Oat seedlings were placed in a dark box with a window for light entry. Seedlings bend towards the source of light. Simple experiments demonstrated that site for reception of the environmental stimulus was distant from the site of action. This implied the existence of a mobile signal.



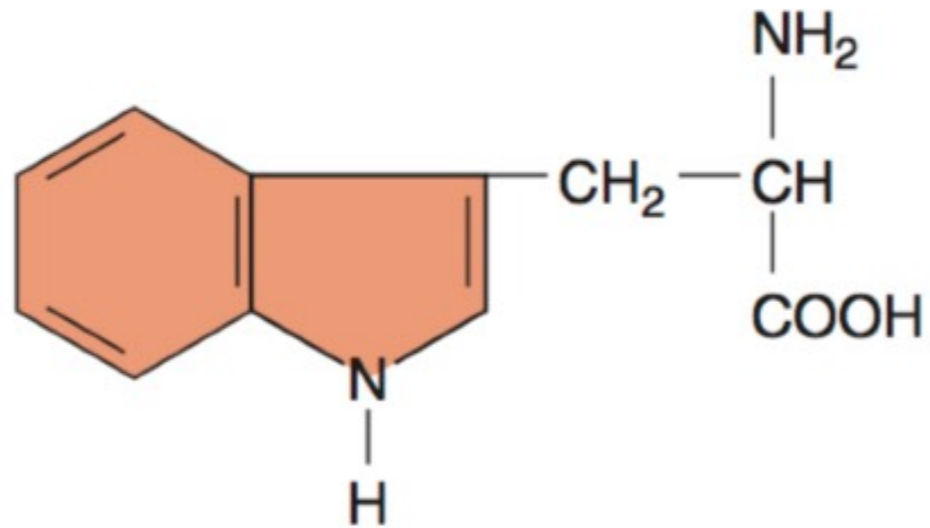
Characterisation of the chemical signal in plant tissues, named auxin

Frits Went's experiment. (1) Went removed the tips of oat seedlings and put them in agar, an inert, gelatinous substance. (2) Blocks of agar were then placed off-center on the ends of other oat seedlings from which the tips had been removed. (3) The seedlings bent away from the side on which the agar block was placed. Went concluded that the substance that he named *auxin* promoted the elongation of the cells and that it accumulated on the side of an oat seedling away from the light.

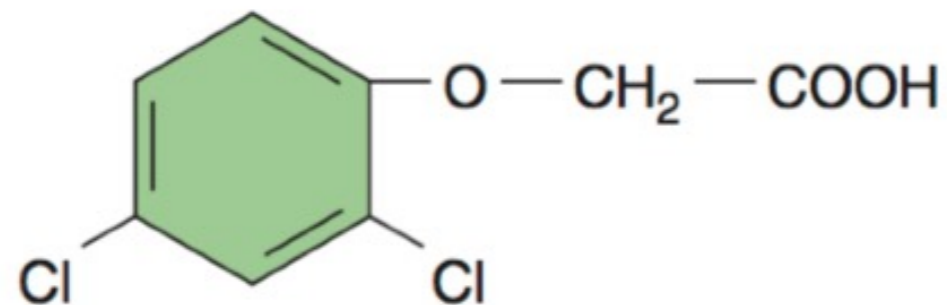




(a) **IAA (Indoleacetic acid)**



(b) **Tryptophan**



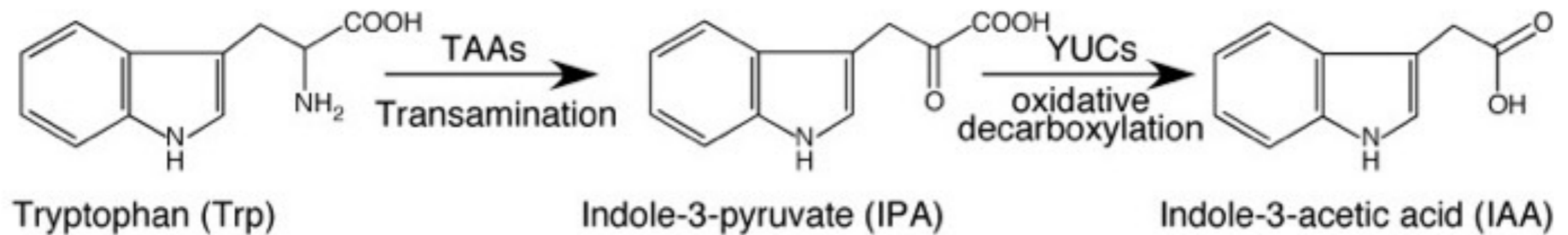
(c) **Dichlorophenoxyacetic acid
(2,4-D)**

Auxin and apical-basal polarity:

Apical-basal polarity and the coordination of indeterminate growth and branching in plants is maintained by traffic of growth regulators.

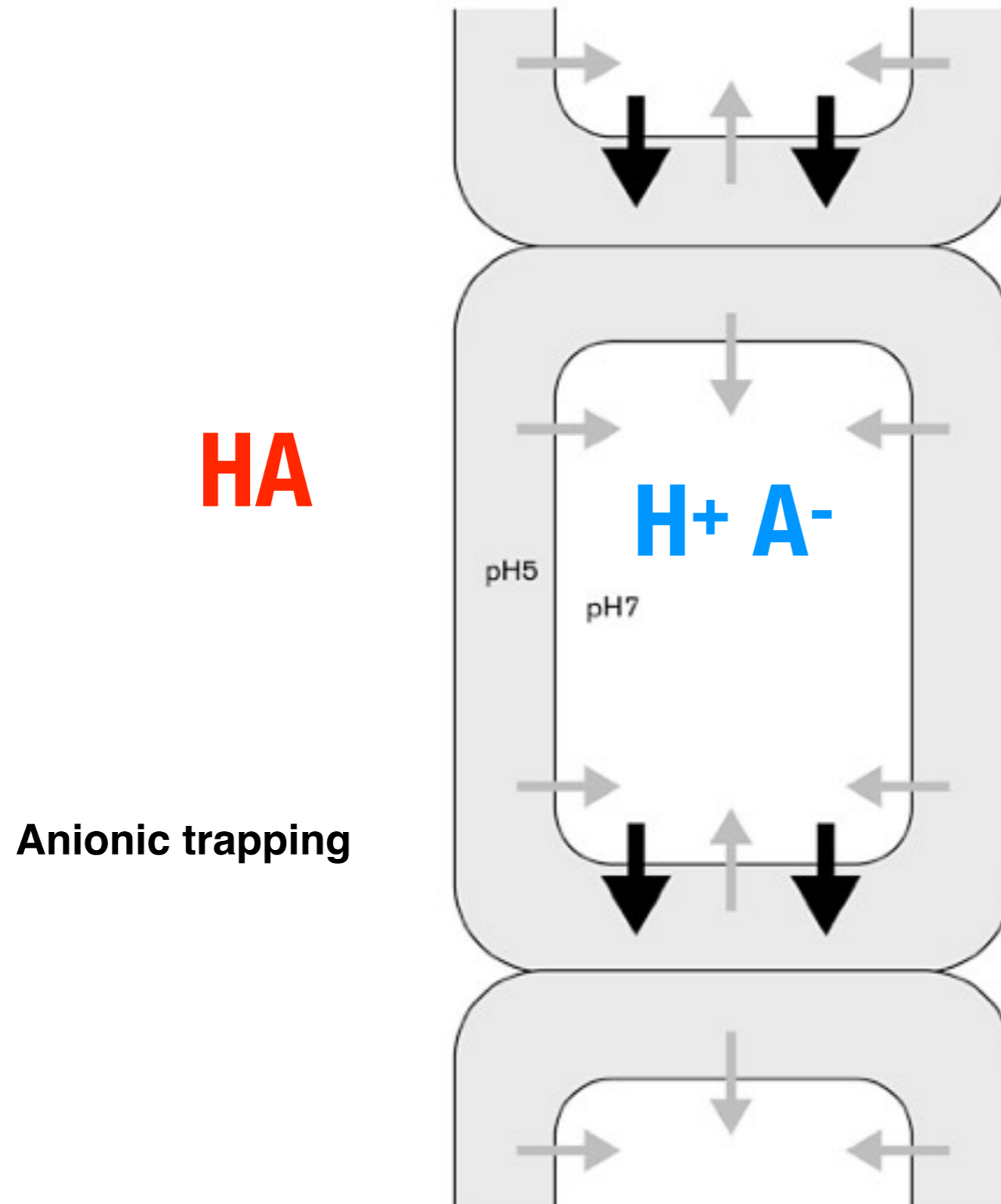
These are not passive gradients, but are the product of active cellular transport.

Auxin Biosynthesis



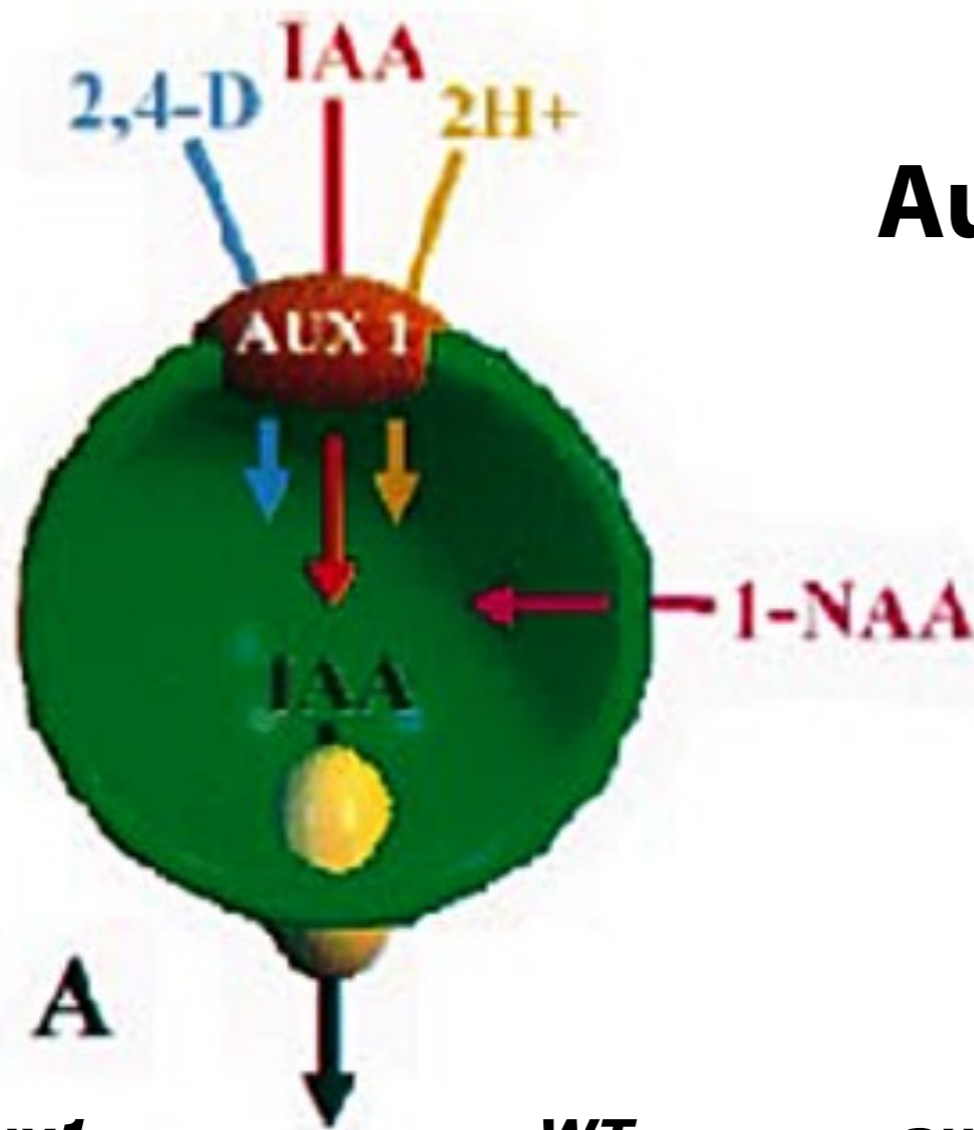
Auxin is synthesised from the amino acid tryptophan in two steps

The pathway of auxin traffic through the plant is determined by the activities of influx and efflux carriers.

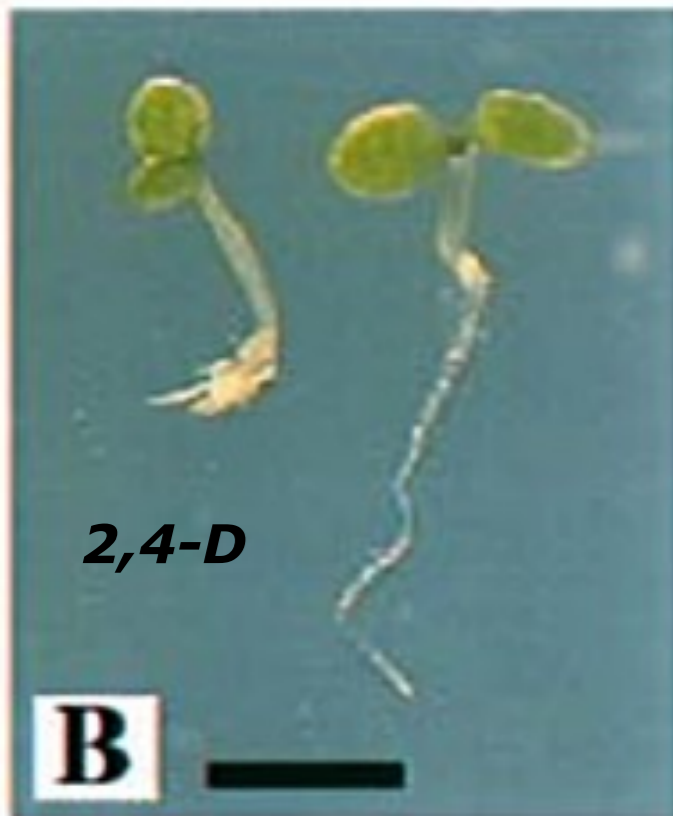


Auxin influx carrier: AUX1

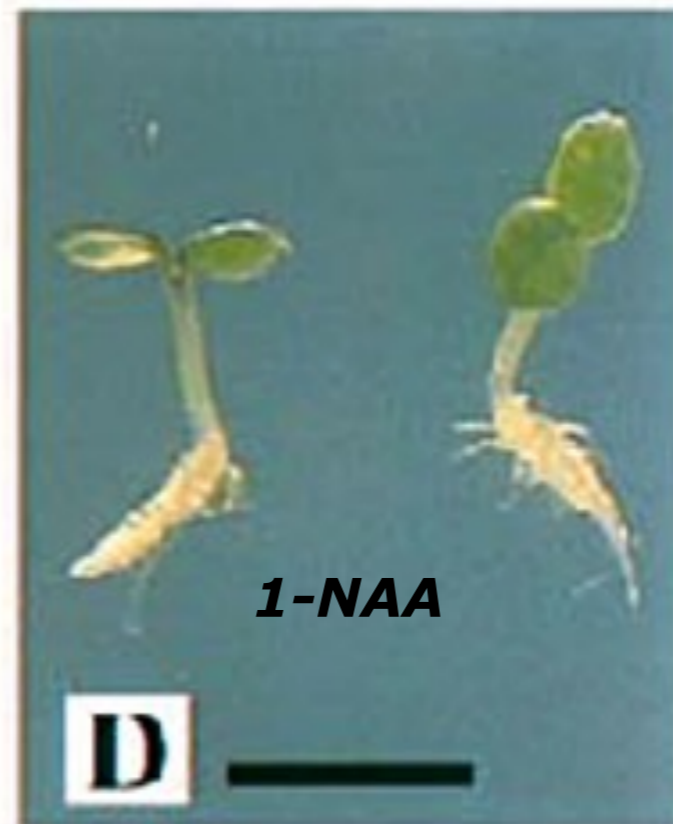
The *aux1* mutant confers resistance to the herbicide 2,4-D, an auxin mimic



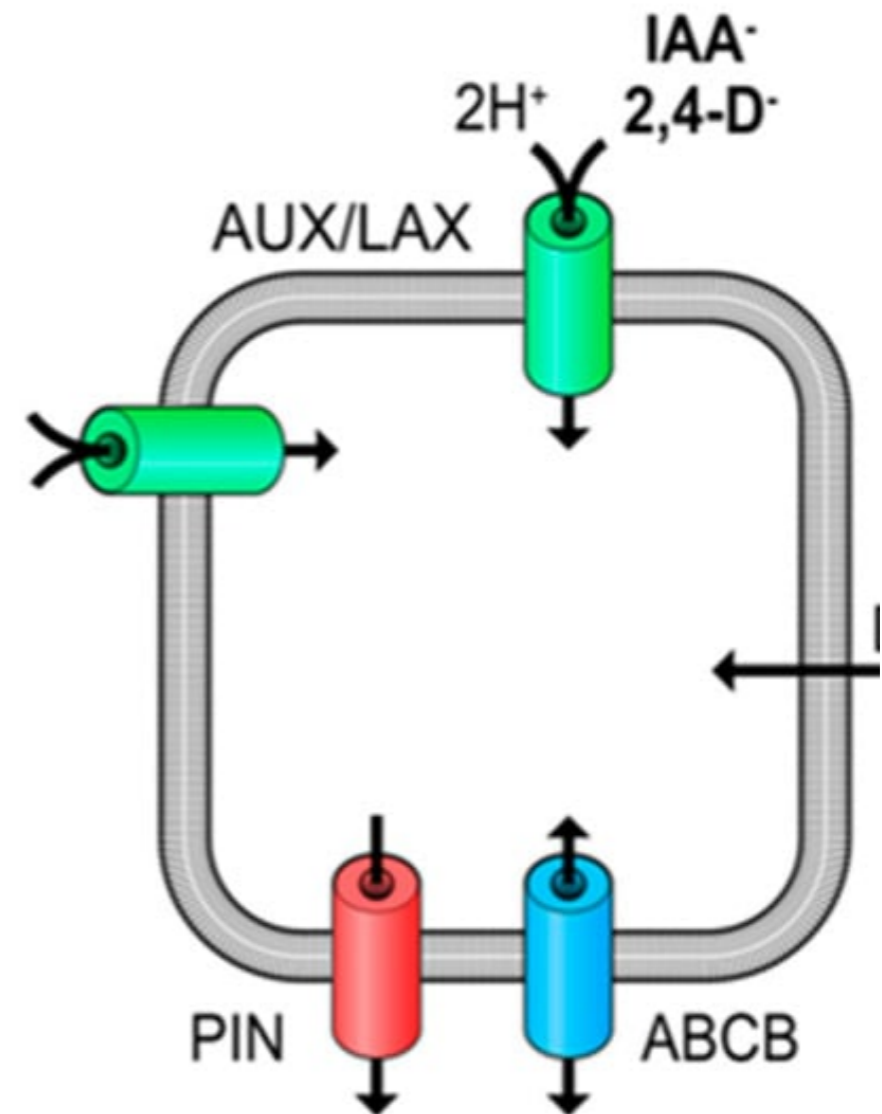
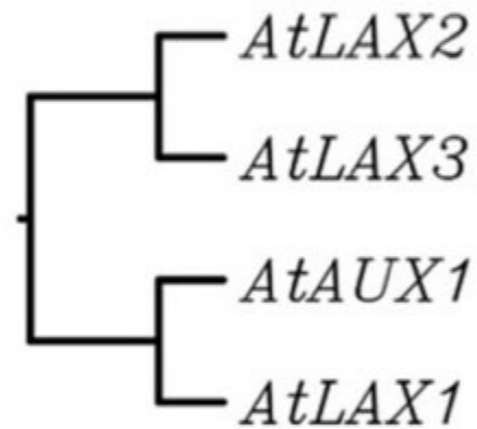
WT *aux1*



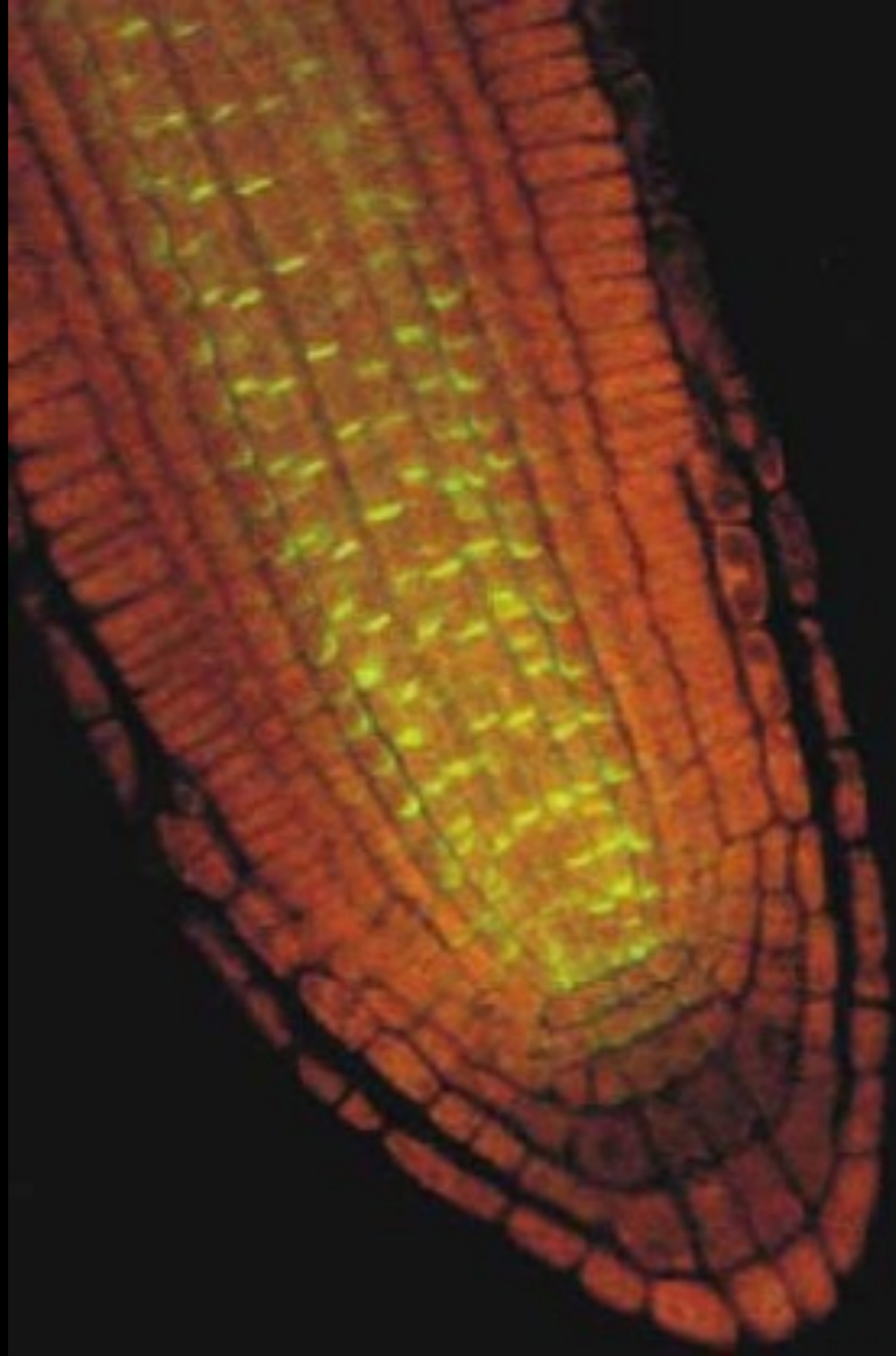
WT *aux1*



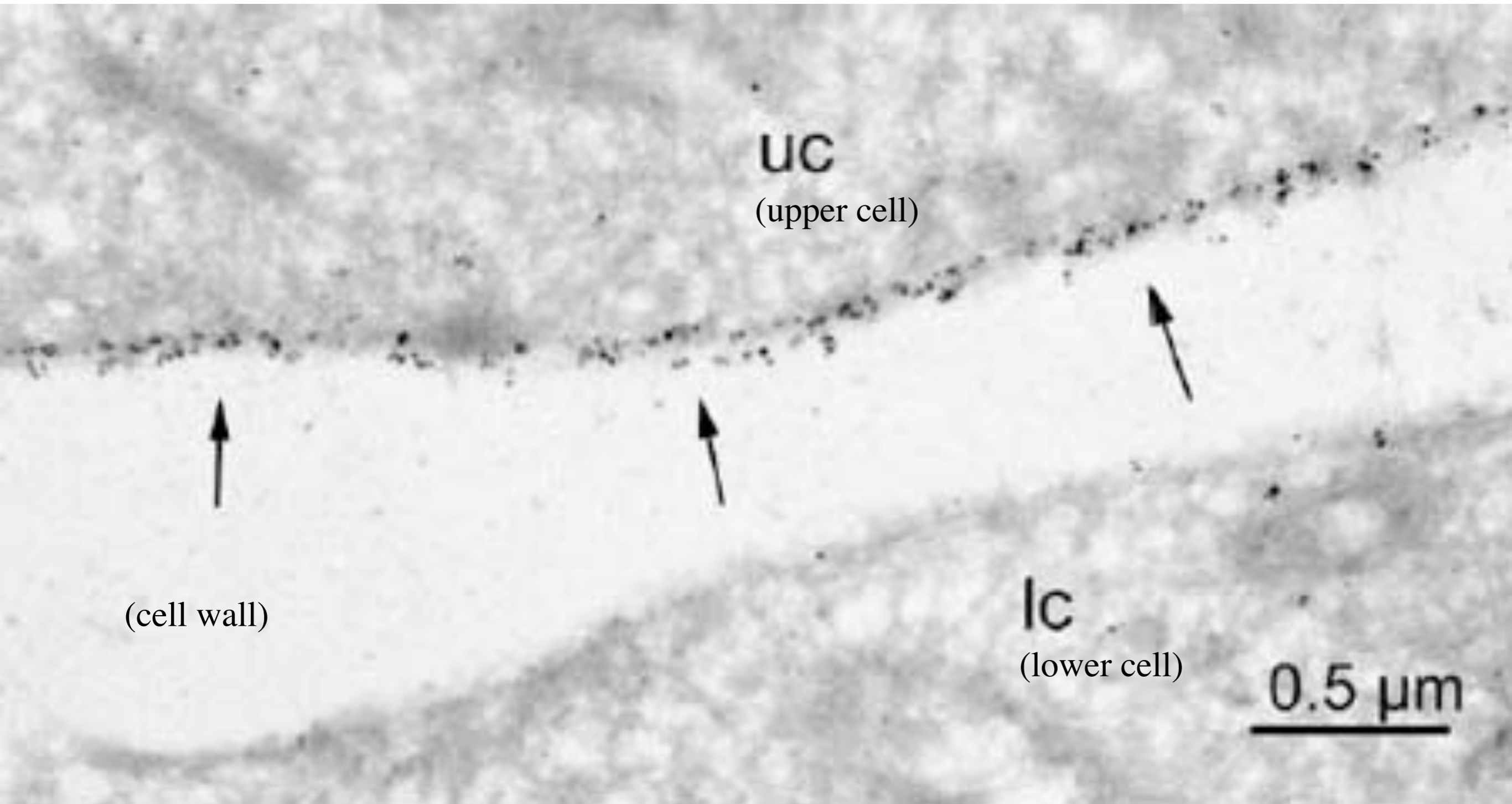
Active auxin importers: the AUXIN1/LIKE-AUX1 (AUX/LAX) family



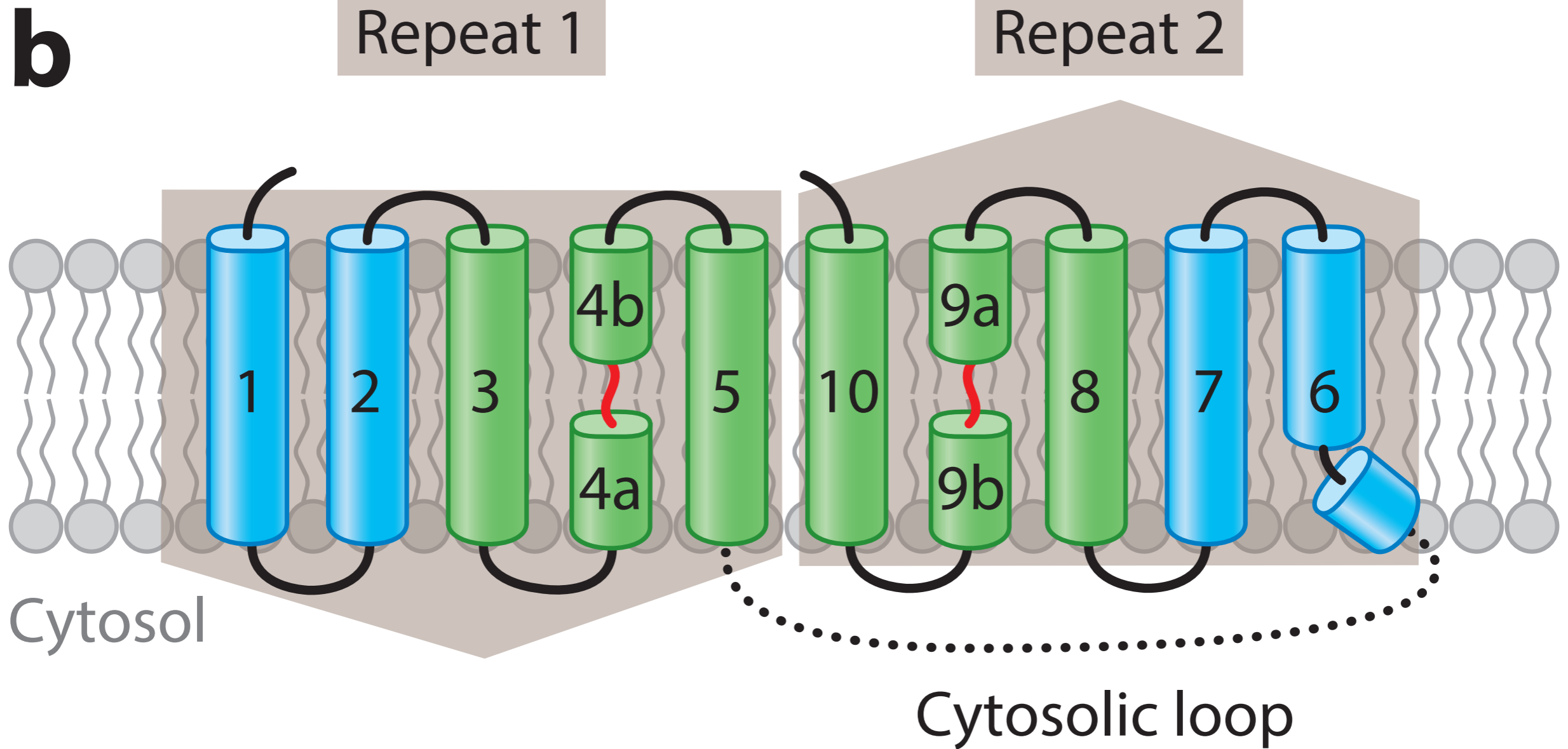




PIN1 is plasma membrane localised with a polar distribution within the cell



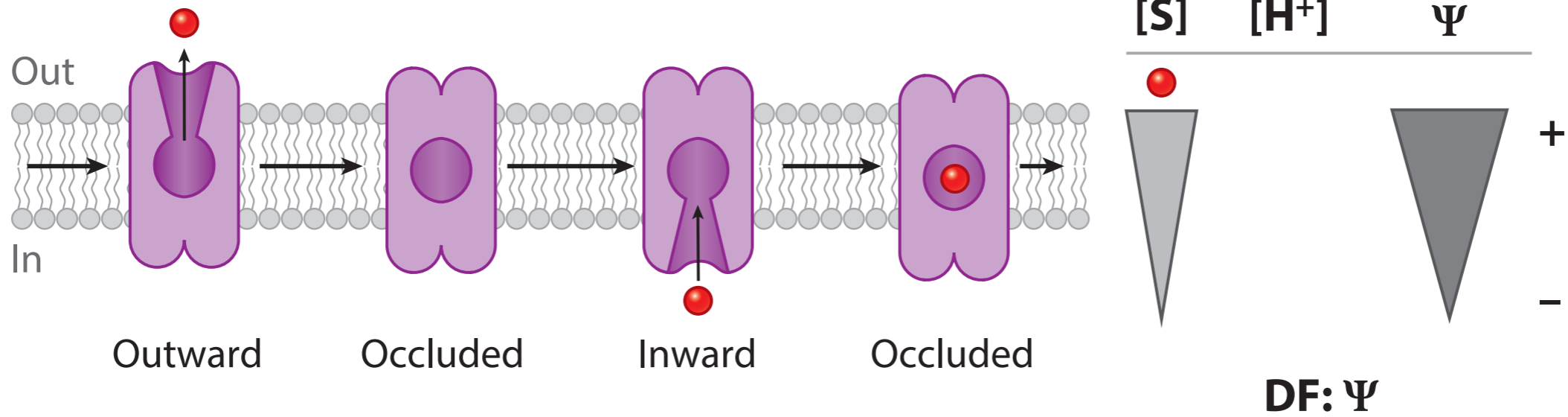
PINs proteins are a family of integral membrane proteins found in the plasma or ER membranes



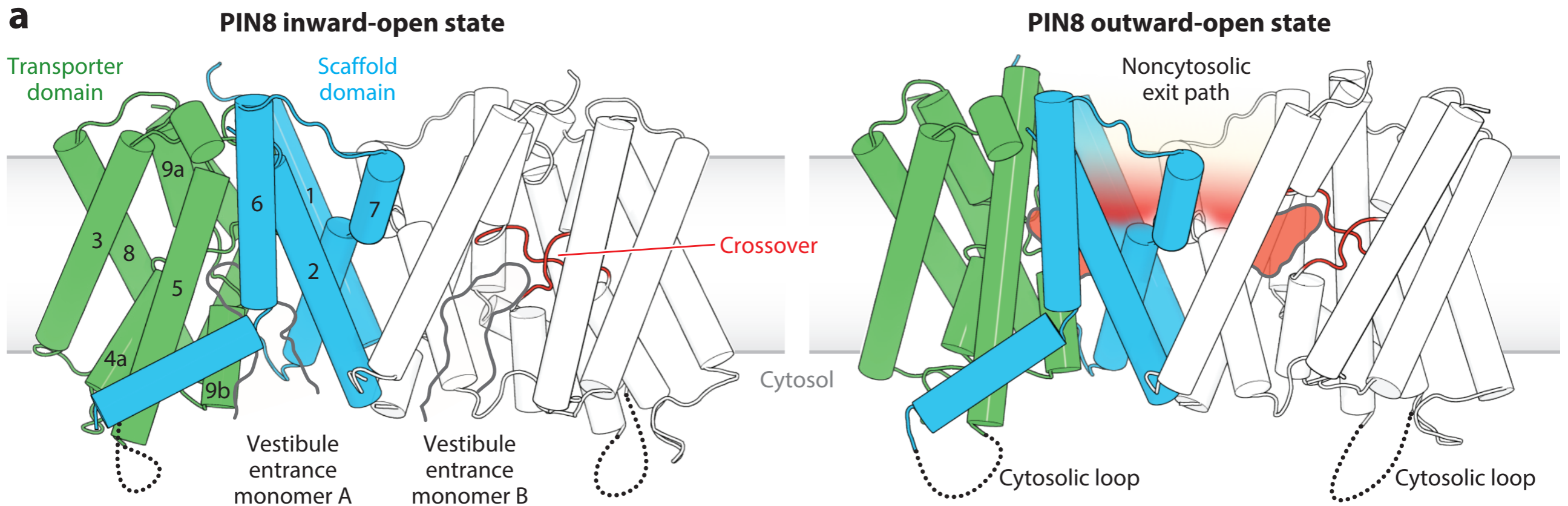
Scaffold domain: M1–M2 + M6–M7

Transporter domain : M3–M5 + M8–M10

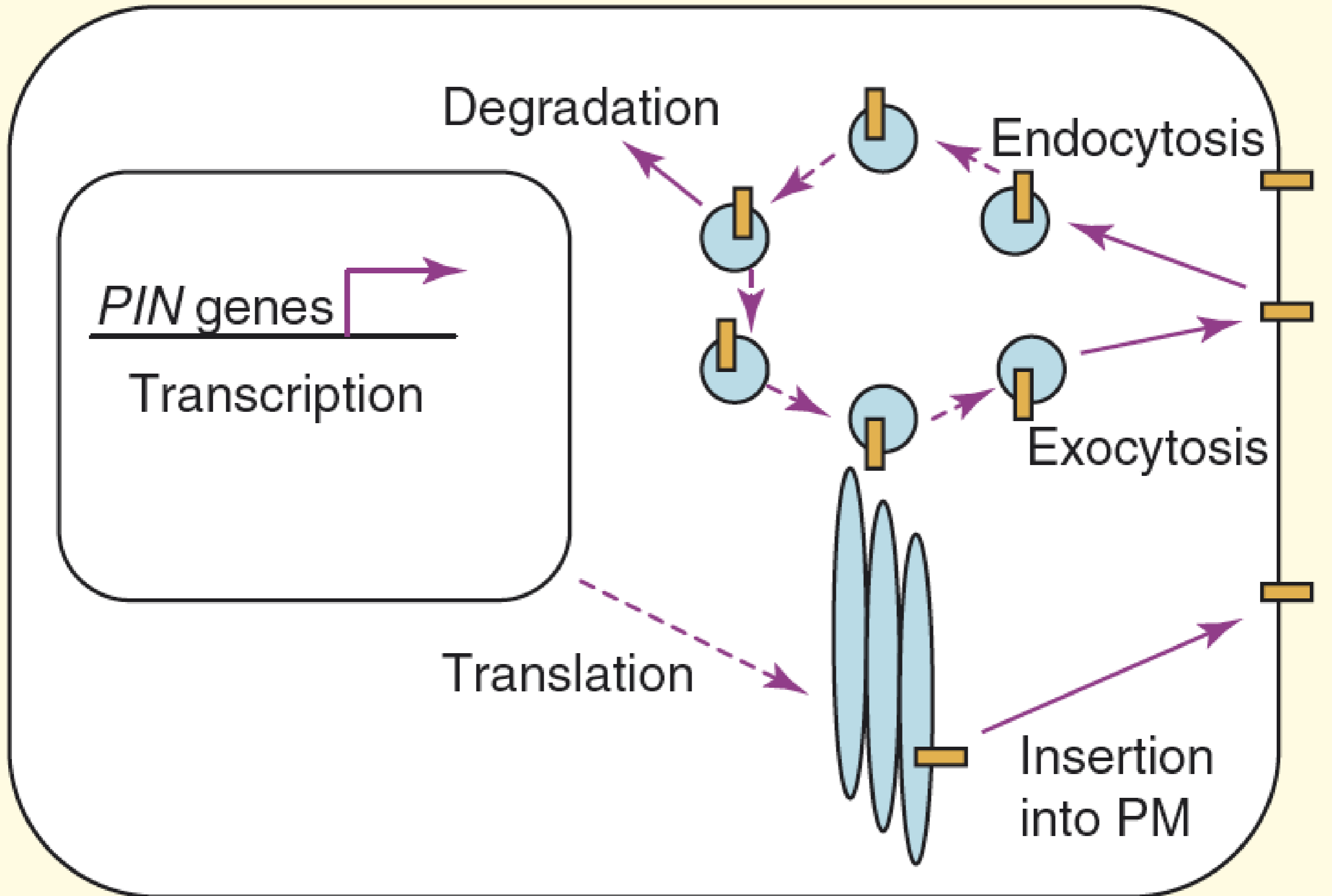
C Uniport



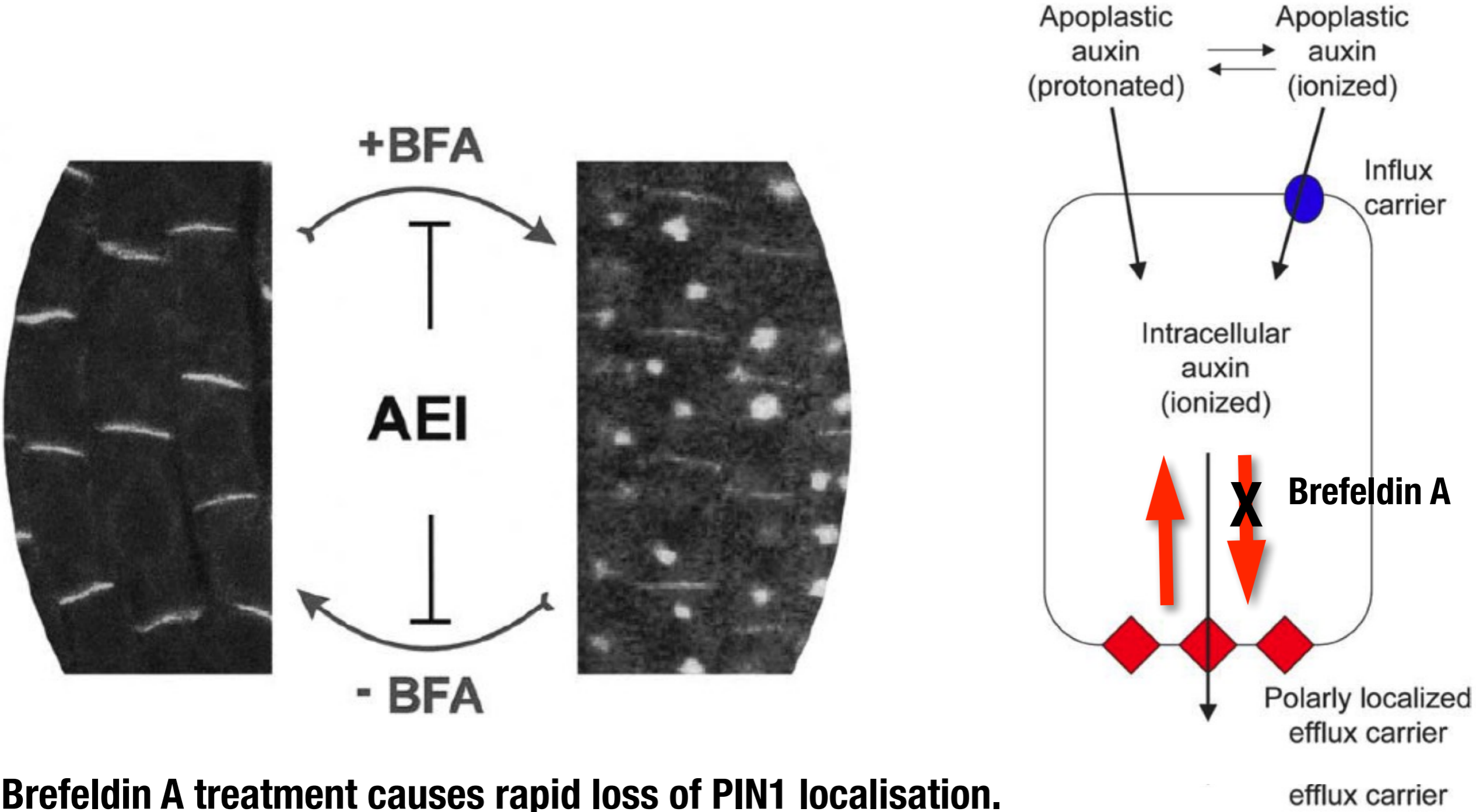
PINs form dimers at the membrane to export auxin



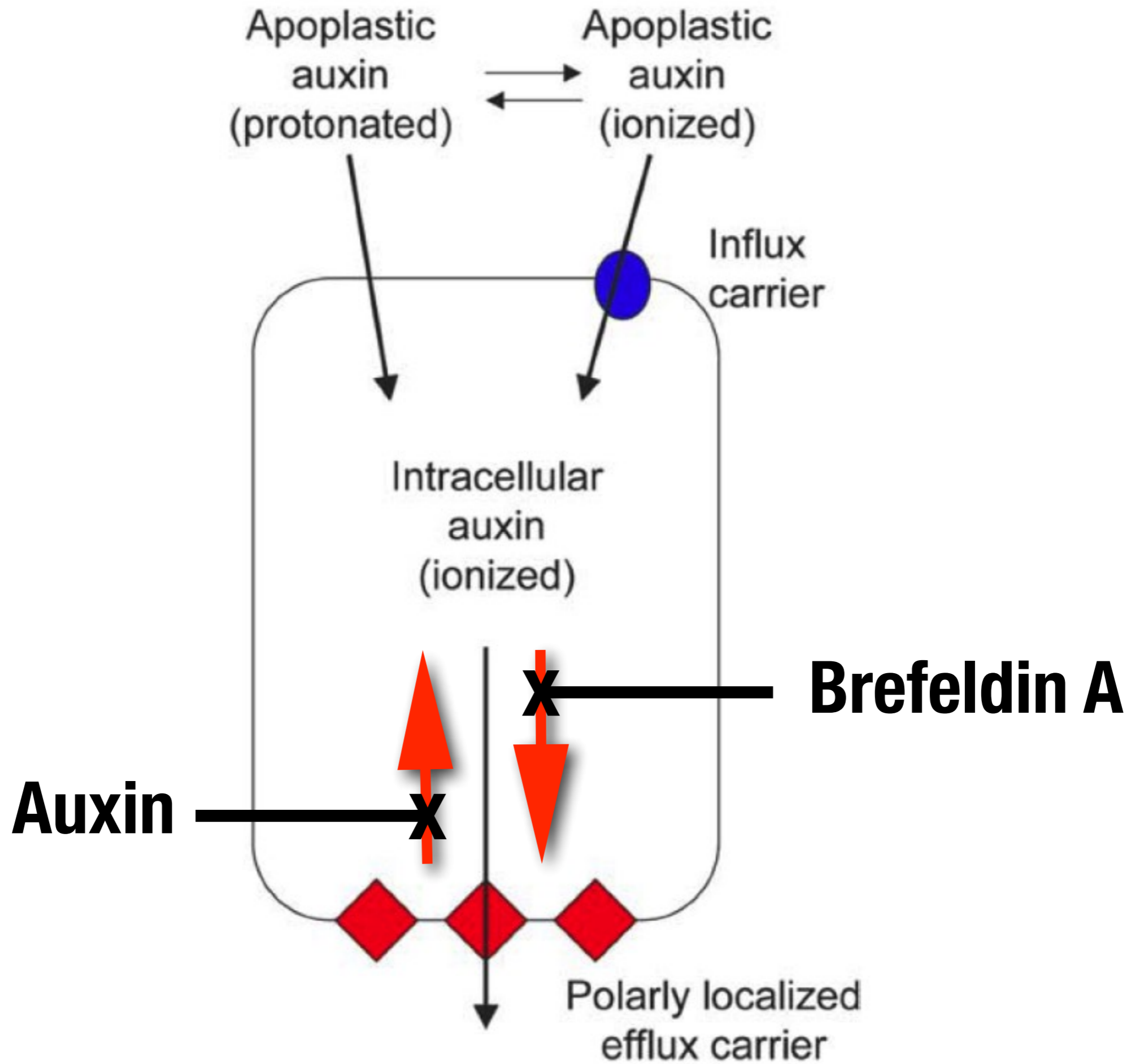
Feedback through regulated expression and localisation of PIN genes

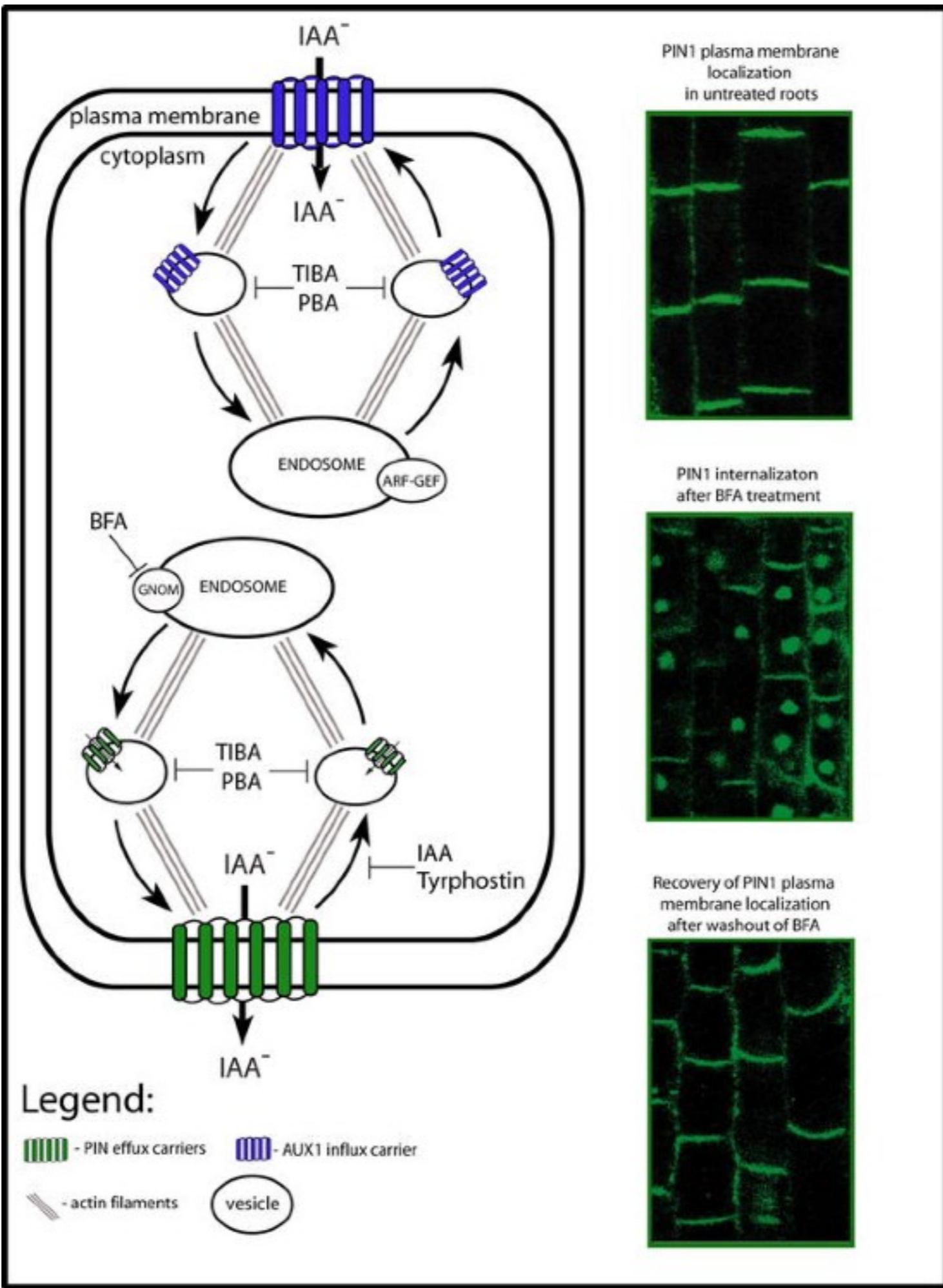


Asymmetric localisation of the PIN1 auxin efflux transporter is a dynamic process and requires the maintenance of polar secretion.

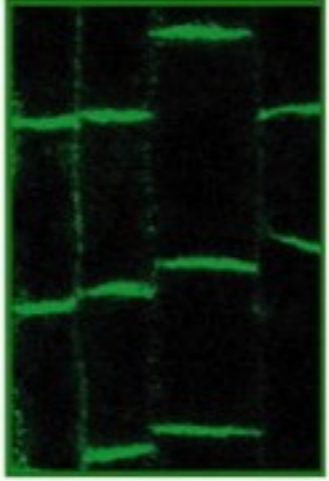


Brefeldin A treatment causes rapid loss of PIN1 localisation.

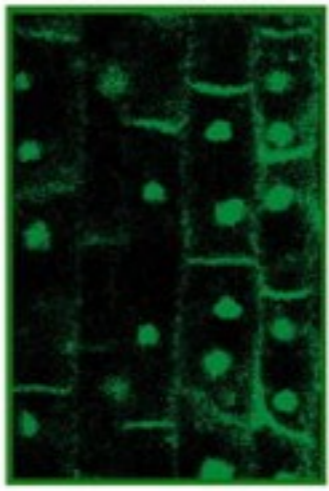




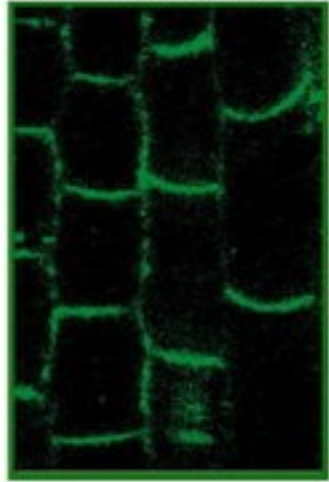
PIN1 plasma membrane localization in untreated roots



PIN1 internalization after BFA treatment



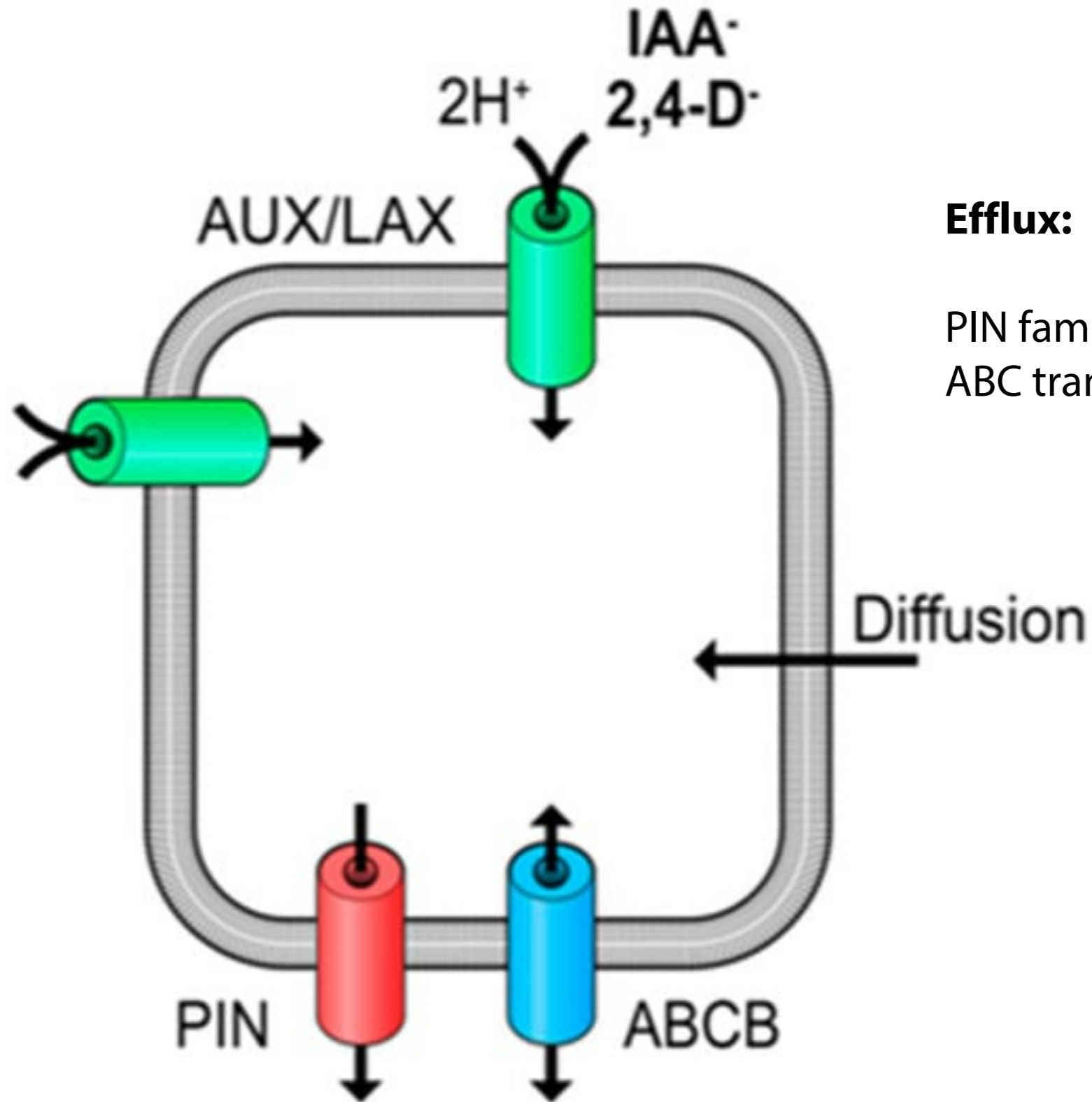
Recovery of PIN1 plasma membrane localization after washout of BFA



Auxin traffic is determined by the the balance of activities of influx and efflux carriers.

Influx:

AUX/LAX family
Diffusion

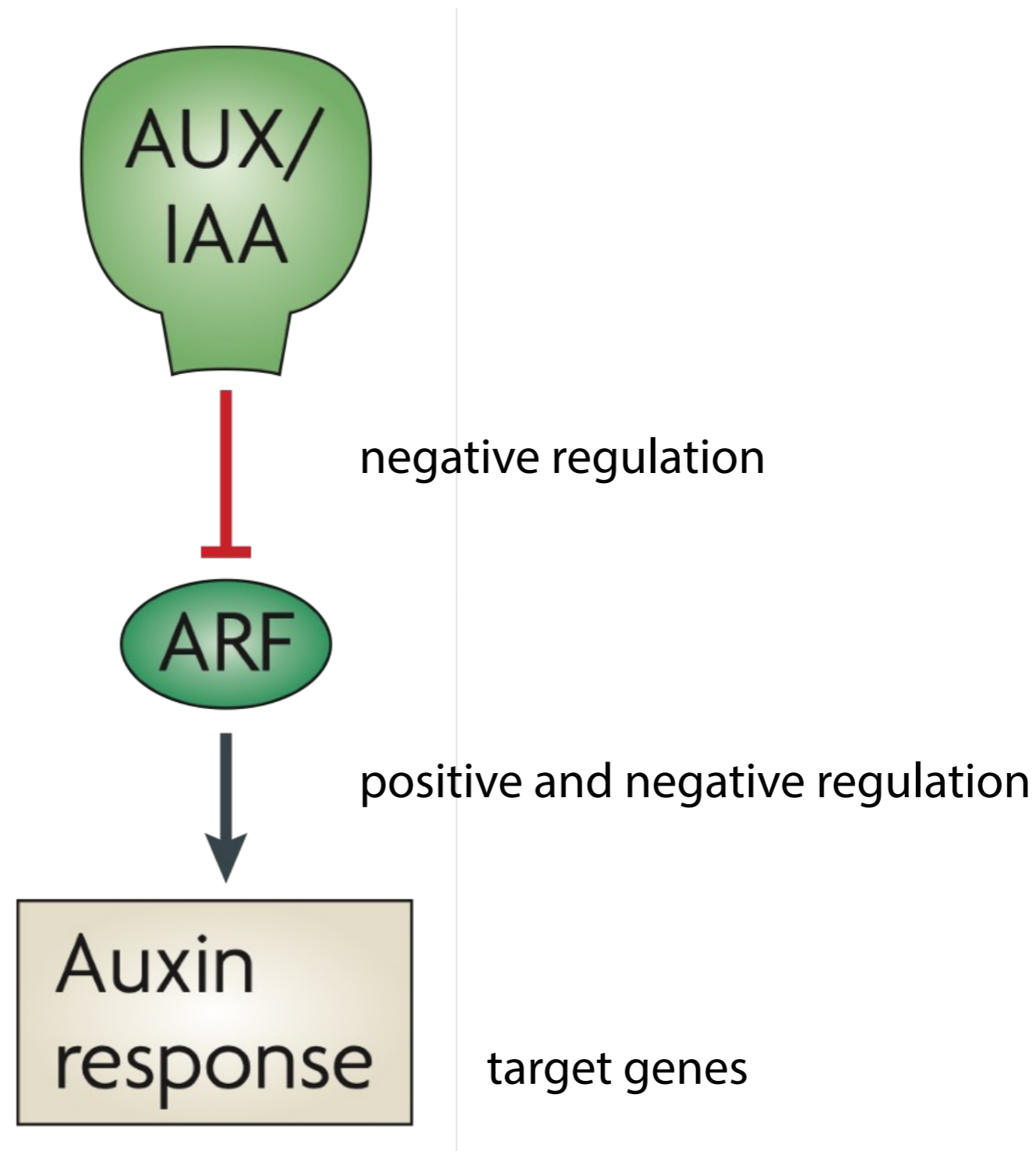


Efflux:

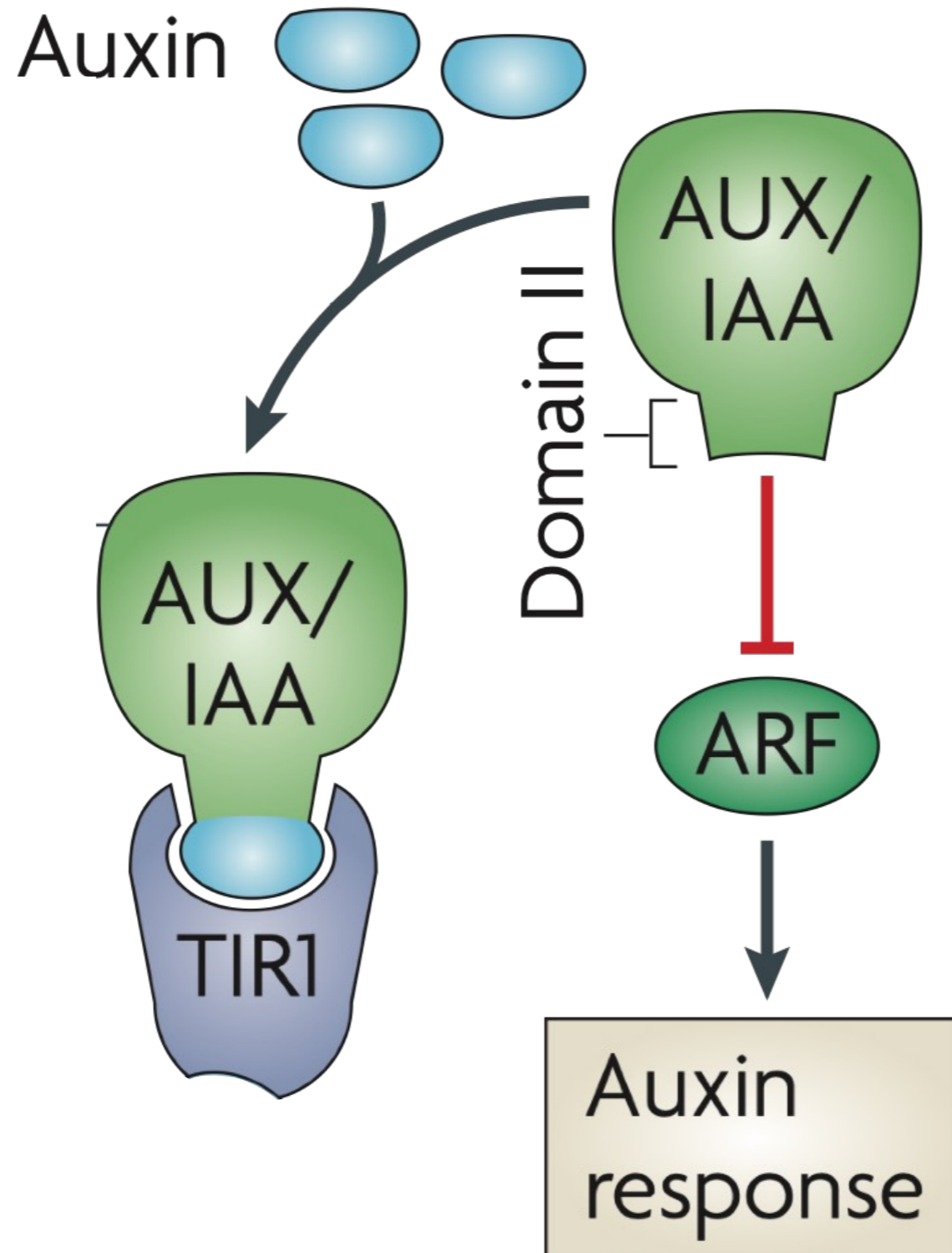
PIN family
ABC transporter family

How is auxin flux or accumulation converted to states of gene expression?

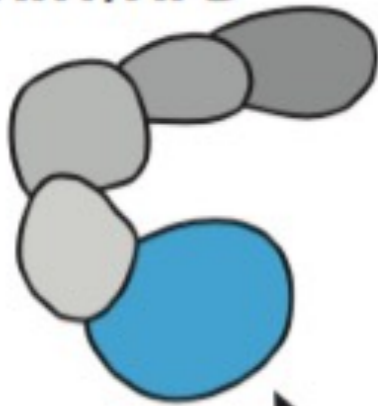
Auxin responses are mediated by a set of positive and negative gene regulators, the AUX/IAA and auxin response factors (ARFs)



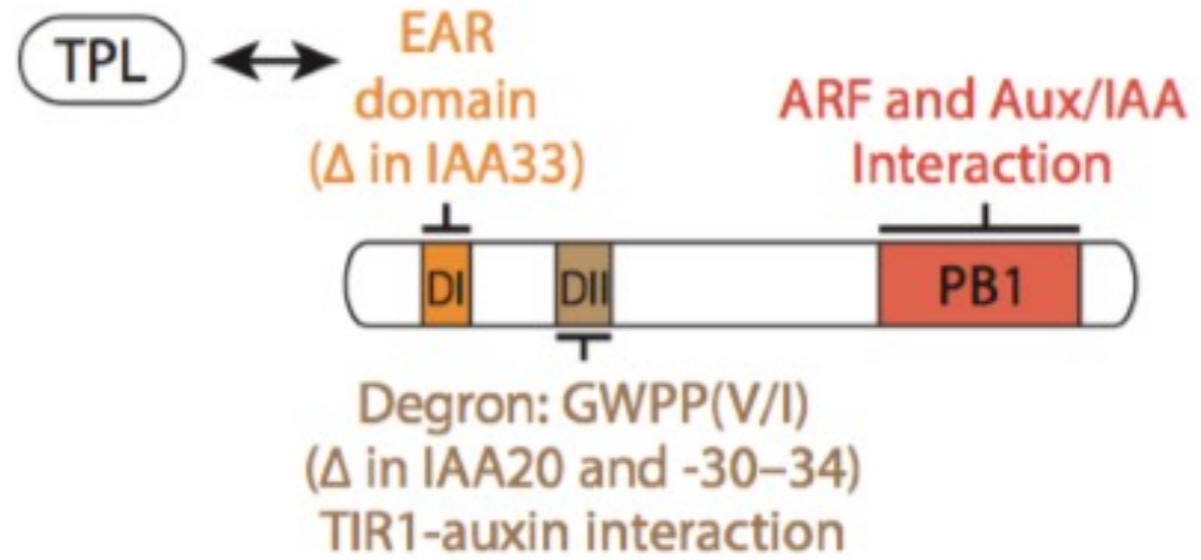
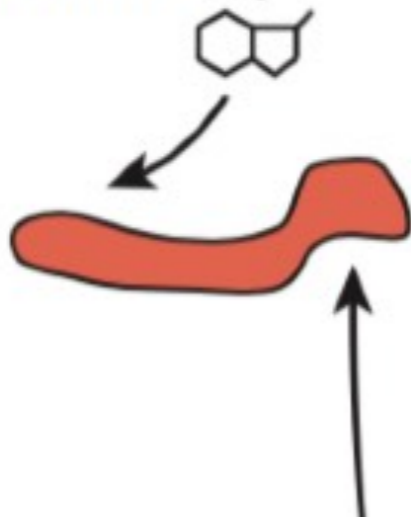
Auxin molecules trigger intracellular binding of AUX/IAA proteins to an F-box protein, TIR1



a TIR1/AFB



b Aux/IAA



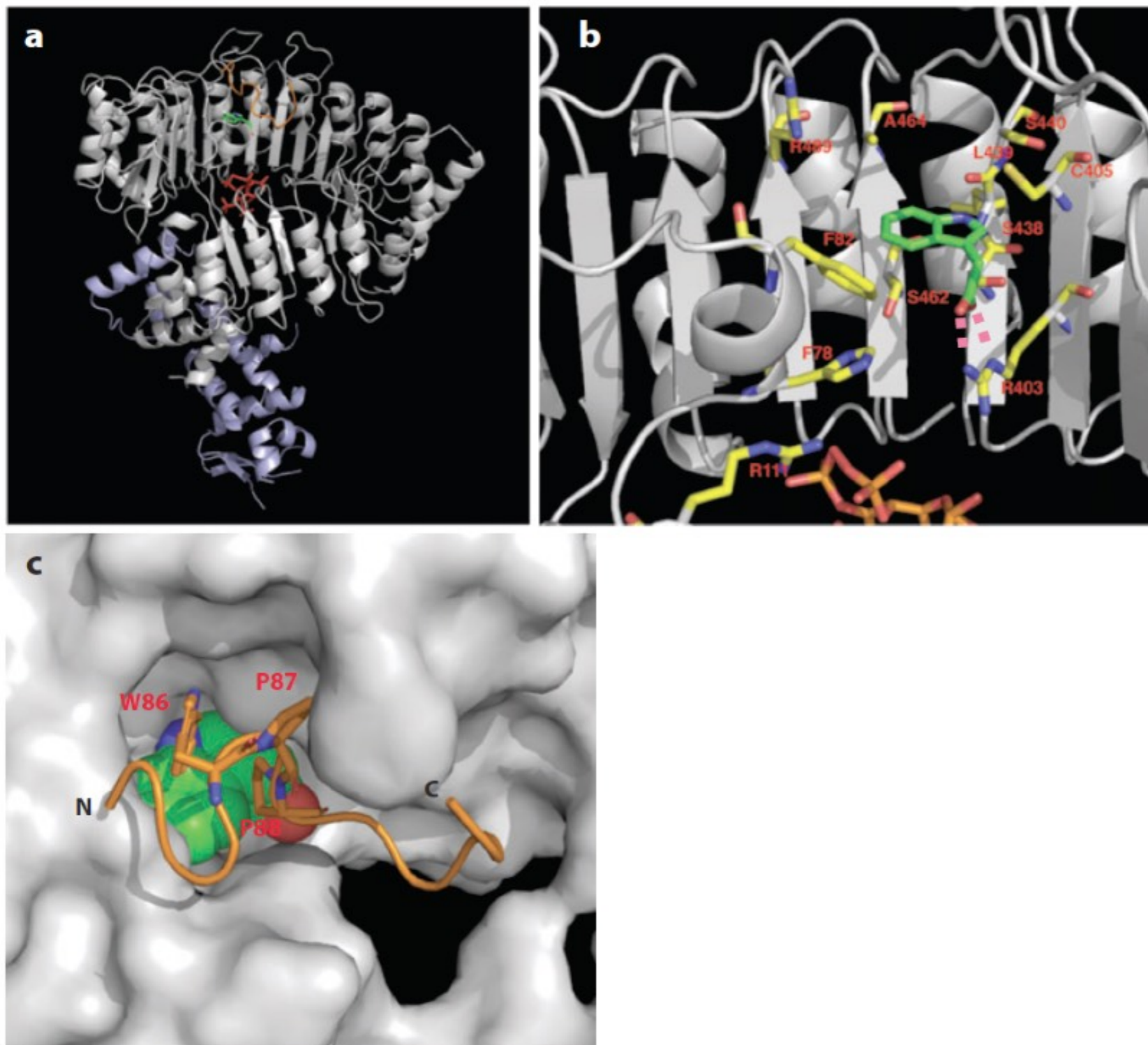
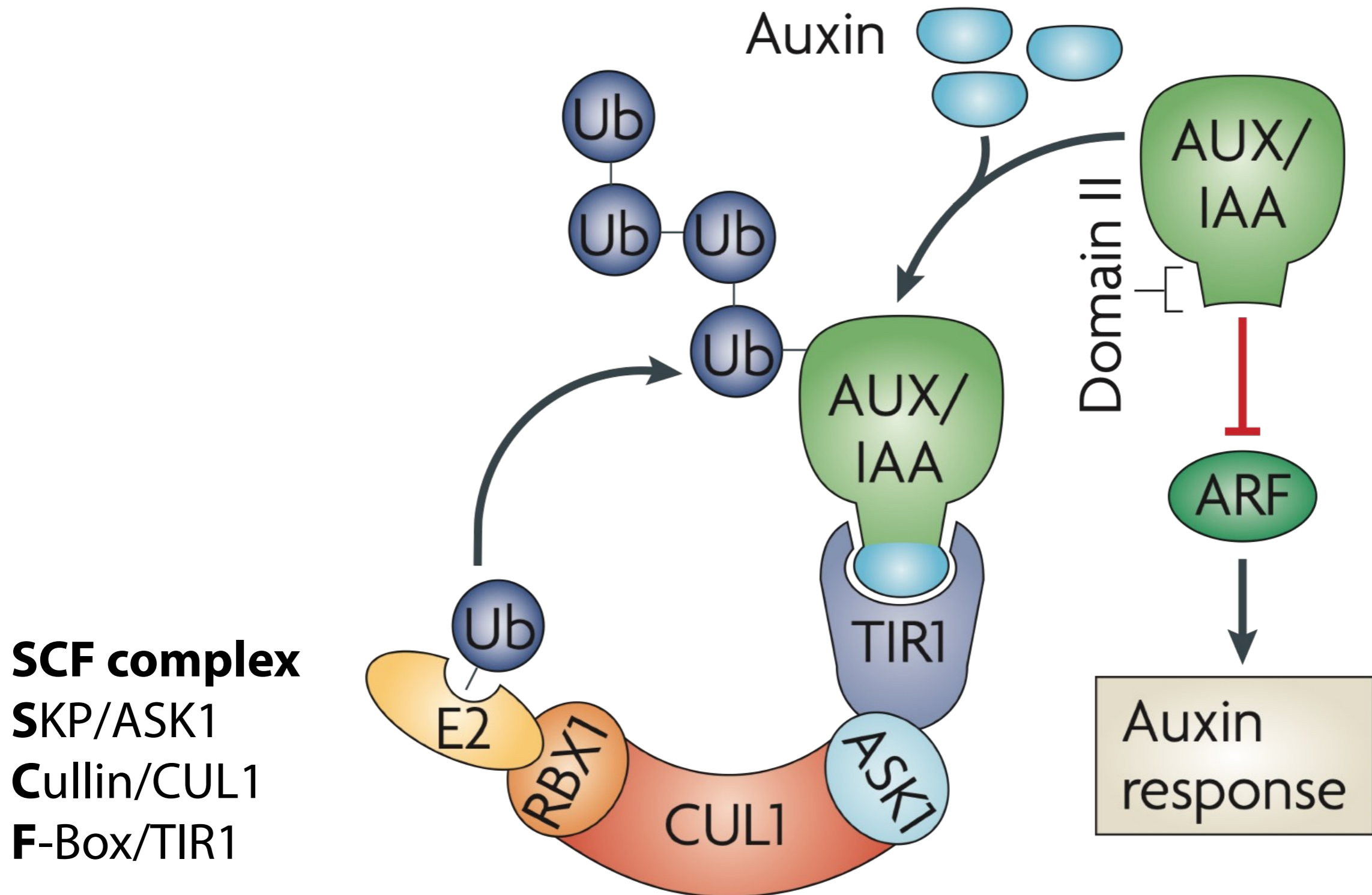


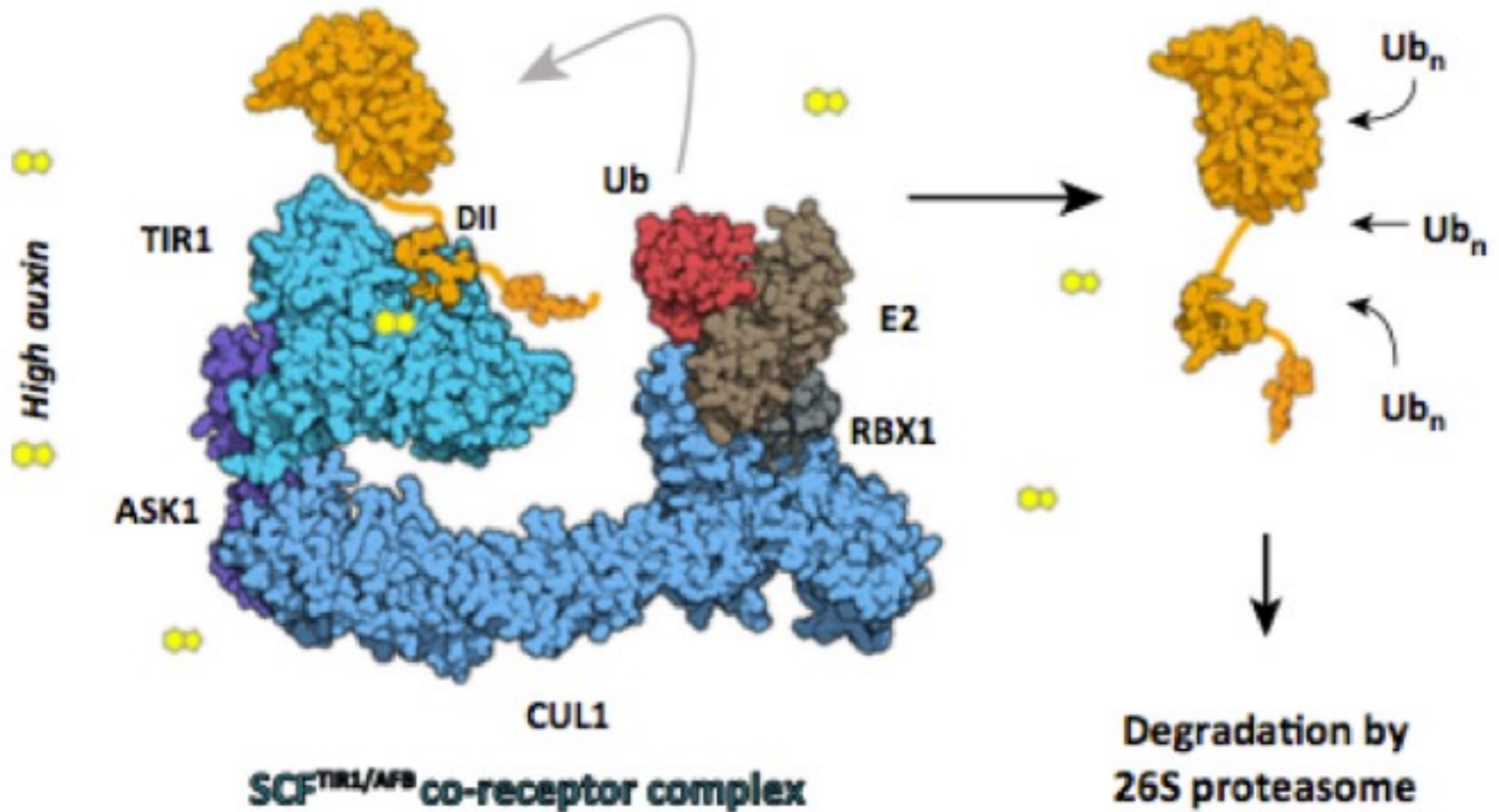
Figure 2

Auxin perception by the F-box protein TIR1. (a) Structure of TIR1 (gray) in complex with ASK1 (dark blue), indole-3-acetic acid (IAA) (green), Aux/IAA domain II peptide (orange), and inositol hexakisphosphate (red). (b) Close-up of the auxin-binding pocket occupied by IAA (green). Surrounding TIR1 residues are shown in yellow. Dashed pink lines indicate hydrogen bonds between the carboxyl group of IAA and conserved R403. (c) Surface view of TIR1 in complex with IAA (green) and domain II peptide (orange).

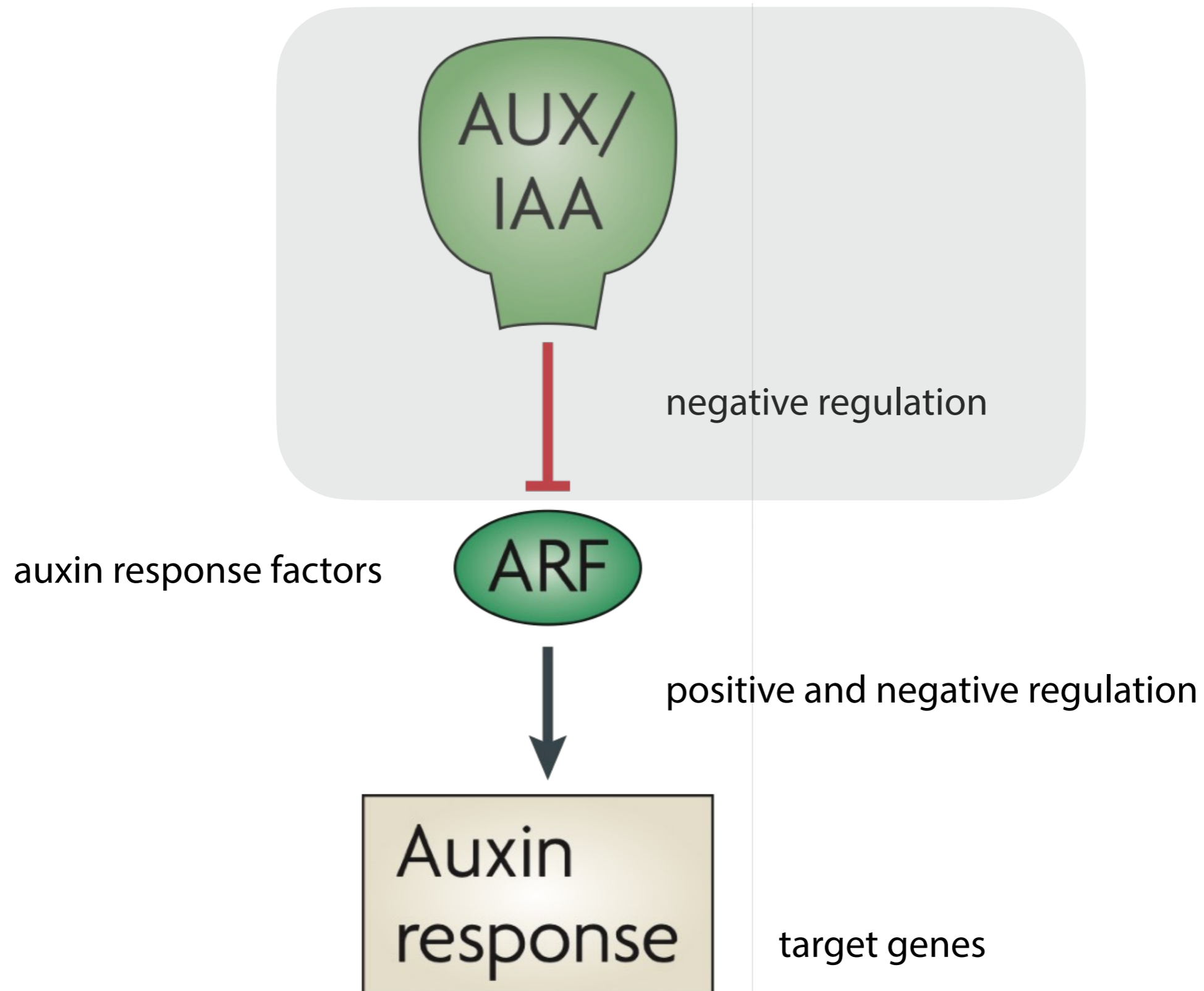
The transient TIR1-AUX/IAA protein complex targets the AUX/IAA protein for degradation in the 26S proteasome via ubiquitination



The transient TIR1-AUX/IAA protein complex targets the AUX/IAA protein for degradation in the 26S proteasome via ubiquitination



The auxin-mediated loss of AUX/IAA proteins derepresses ARF functions



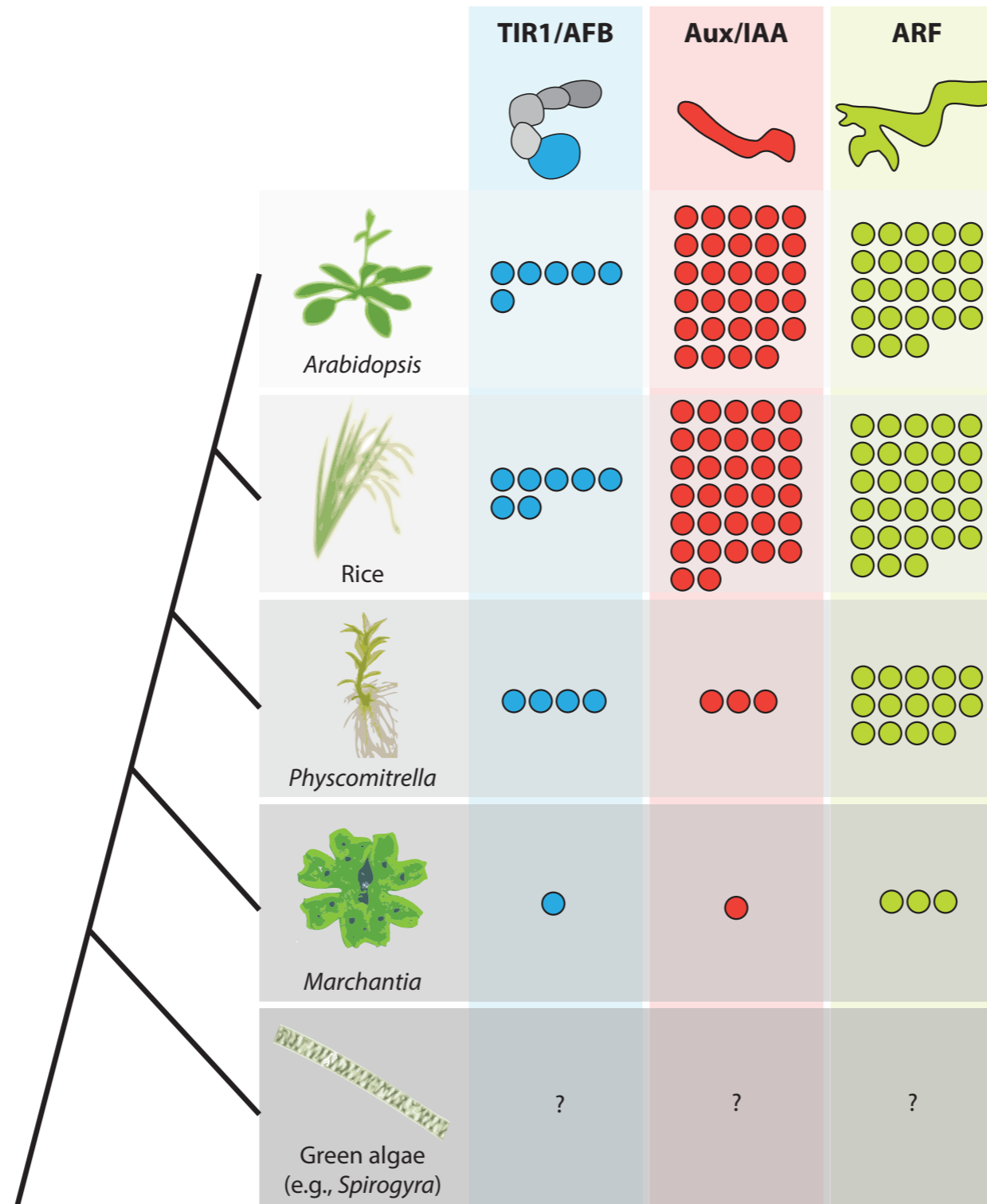
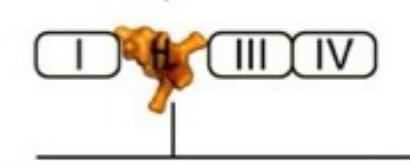
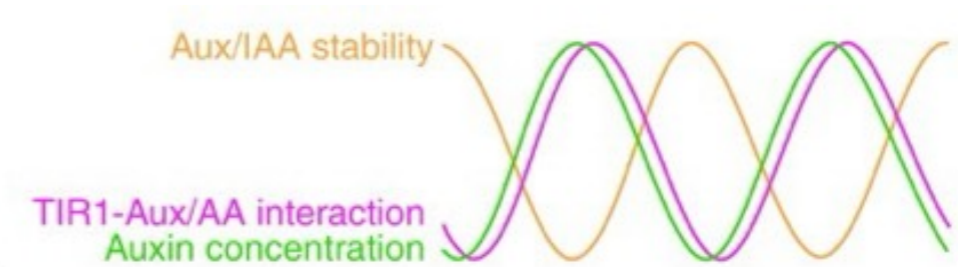
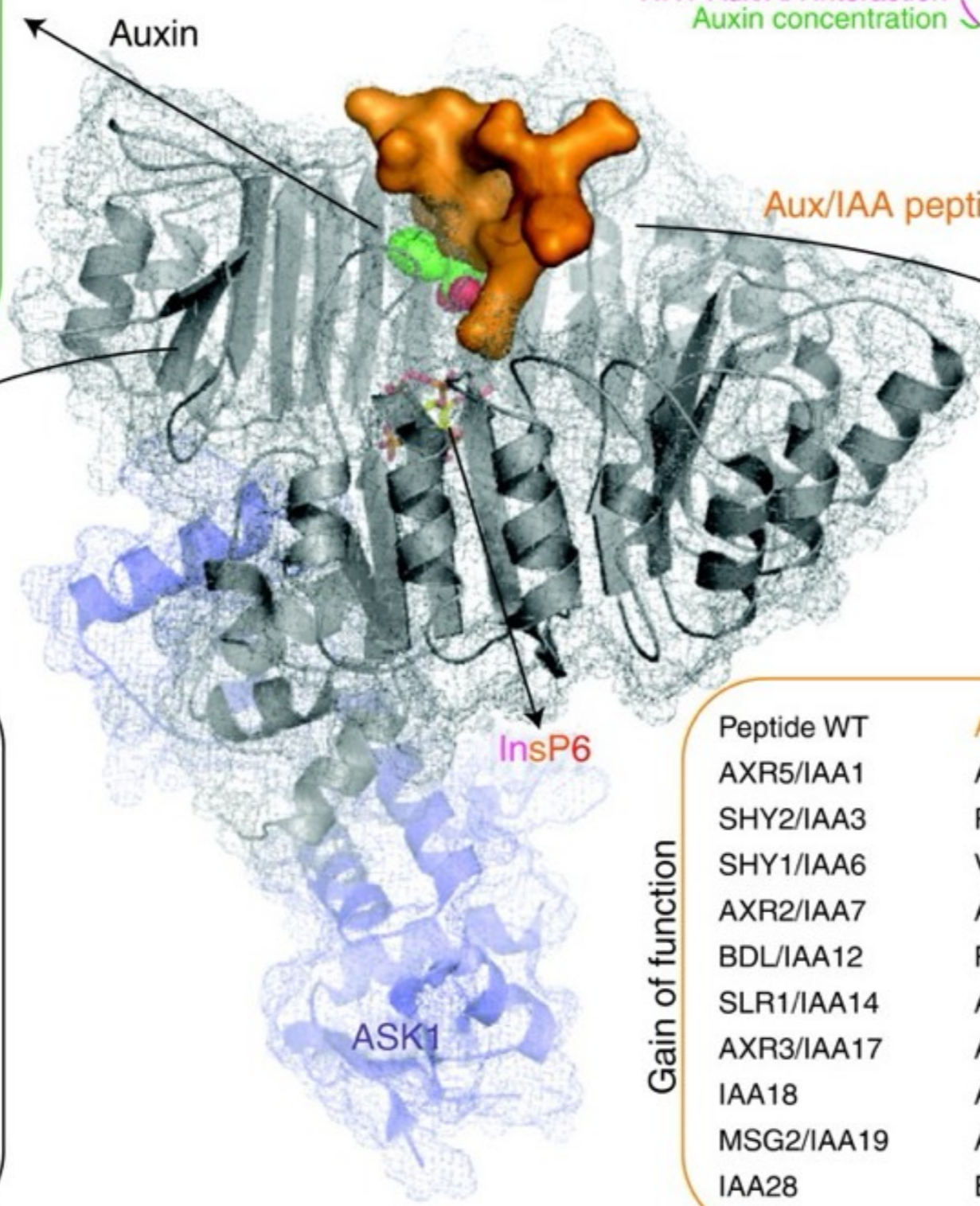
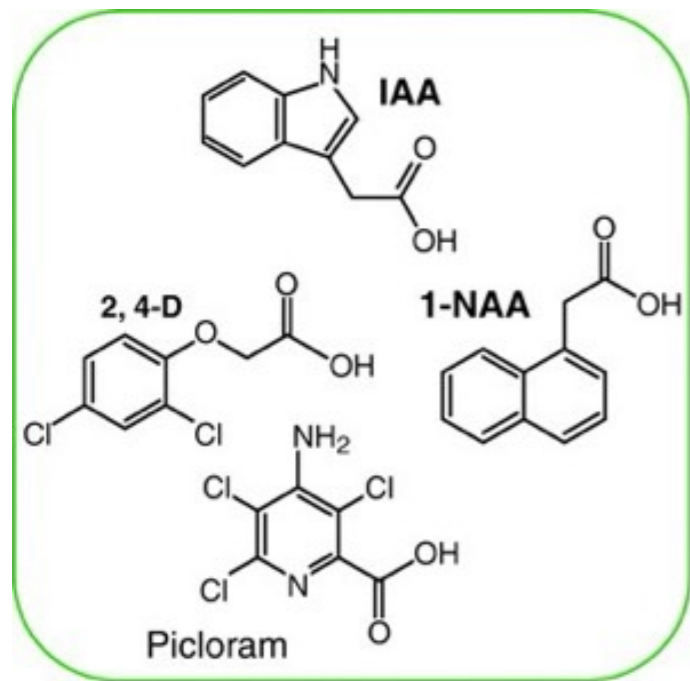
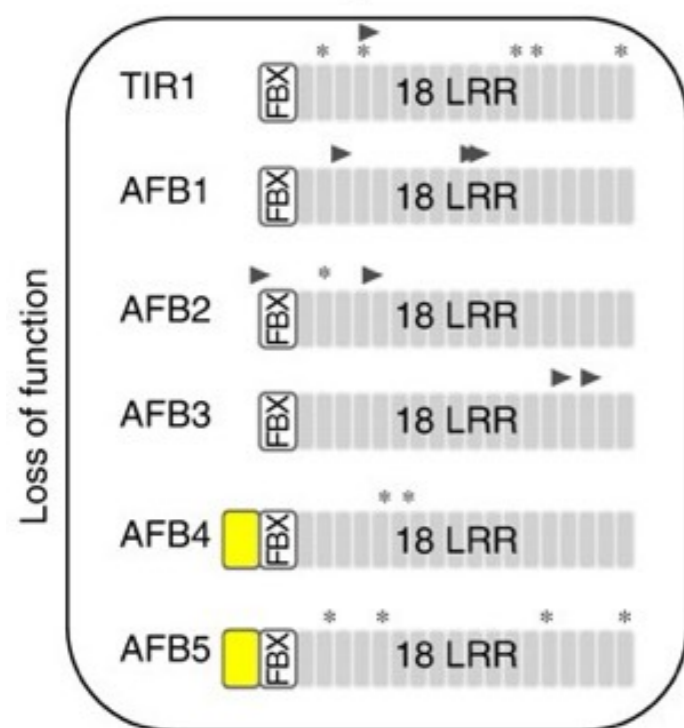


Figure 7

The evolution of the auxin response pathway, showing the distribution of genes encoding TIR1/AFB, Aux/IAA, and ARF proteins in published plant genomes for several plant species. These species represent eudicots (*Arabidopsis*), monocots (rice), mosses (*Physcomitrella*), liverworts (*Marchantia*), and green algae (*Spirogyra*, as an example of charophytes). The tree on the left-hand side indicates the divergence order but is not drawn to scale. Protein abbreviations: ARF, AUXIN RESPONSE FACTOR; Aux/IAA, AUXIN/INDOLE-3-ACETIC ACID; TIR1/AFB, TRANSPORT INHIBITOR RESISTANT 1/AUXIN SIGNALING F-BOX.



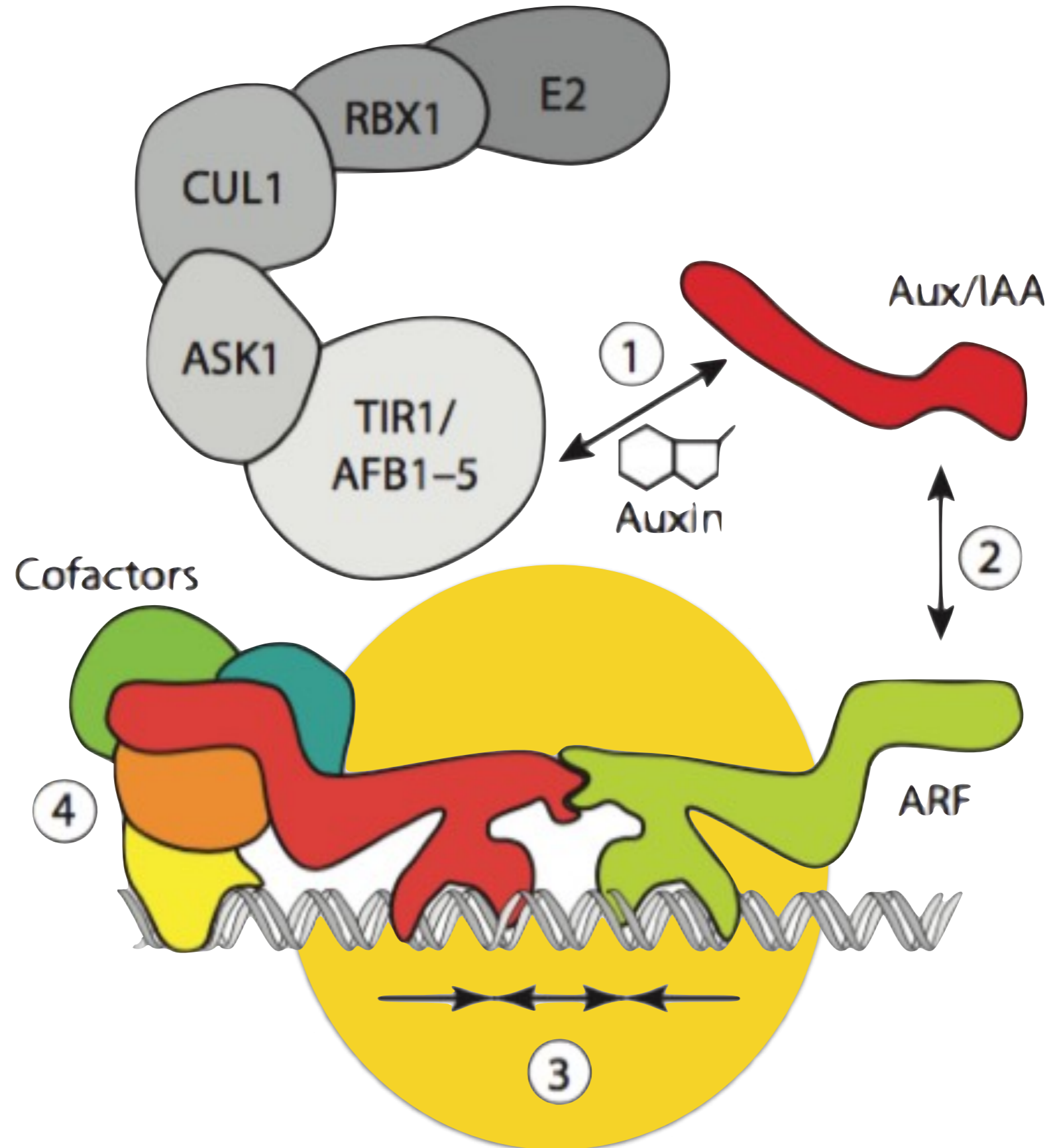
TIR1/AFBs



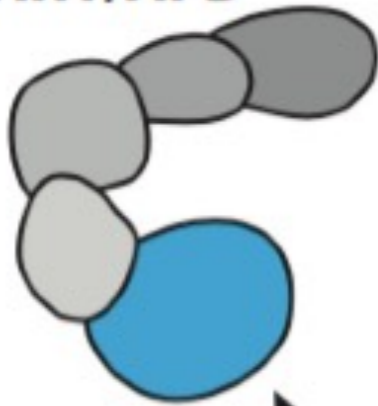
Gain of function

Peptide WT	AKAQVVGWPPVRNYRKN
AXR5/IAA1	AKTQIVGWPPVRSNRKN
SHY2/IAA3	RKAQIVGWPPVRSYRKN
SHY1/IAA6	VKSQAVGWPPVCSYRRK
AXR2/IAA7	AKAQVVGWPPVRNYRKN
BDL/IAA12	RSSQVVGWPPIGLHRMN
SLR1/IAA14	AKAQVVGWPPVRNYRKN
AXR3/IAA17	AKAQVVGWPPVRSYRKN
IAA18	APGPVVGWPPVRSFRKN
MSG2/IAA19	AKSFQVVGWPPVCSYRKK
IAA28	EVAPVVGWPPVRSSRRN

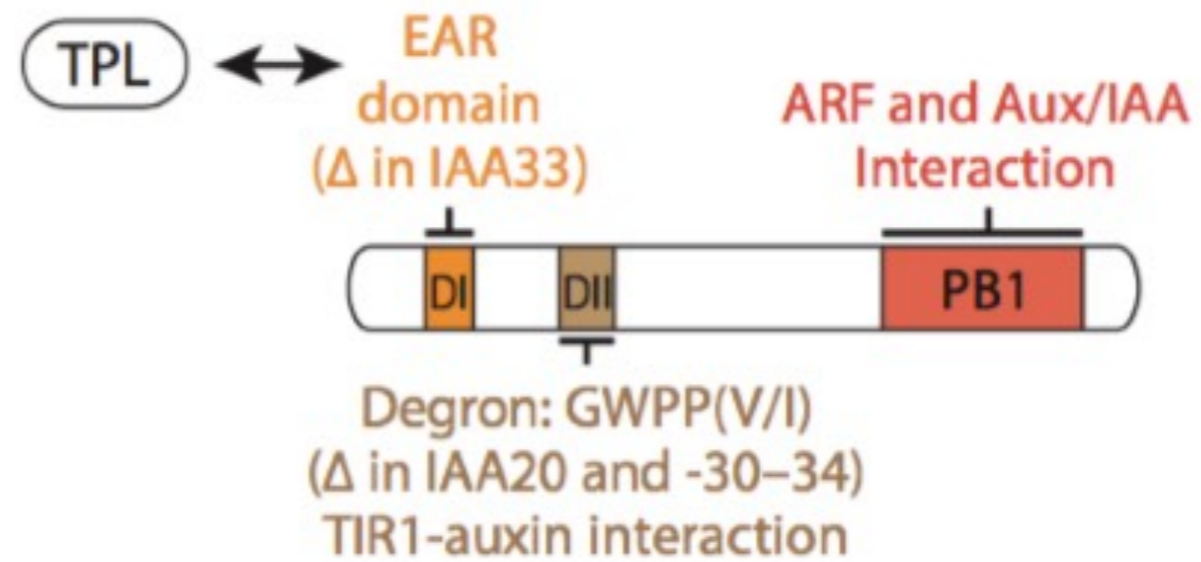
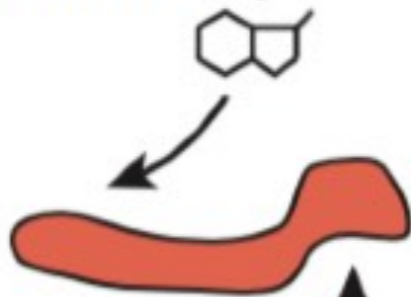
3. Selective activation of genes by ARF binding to auxin responsive promoters



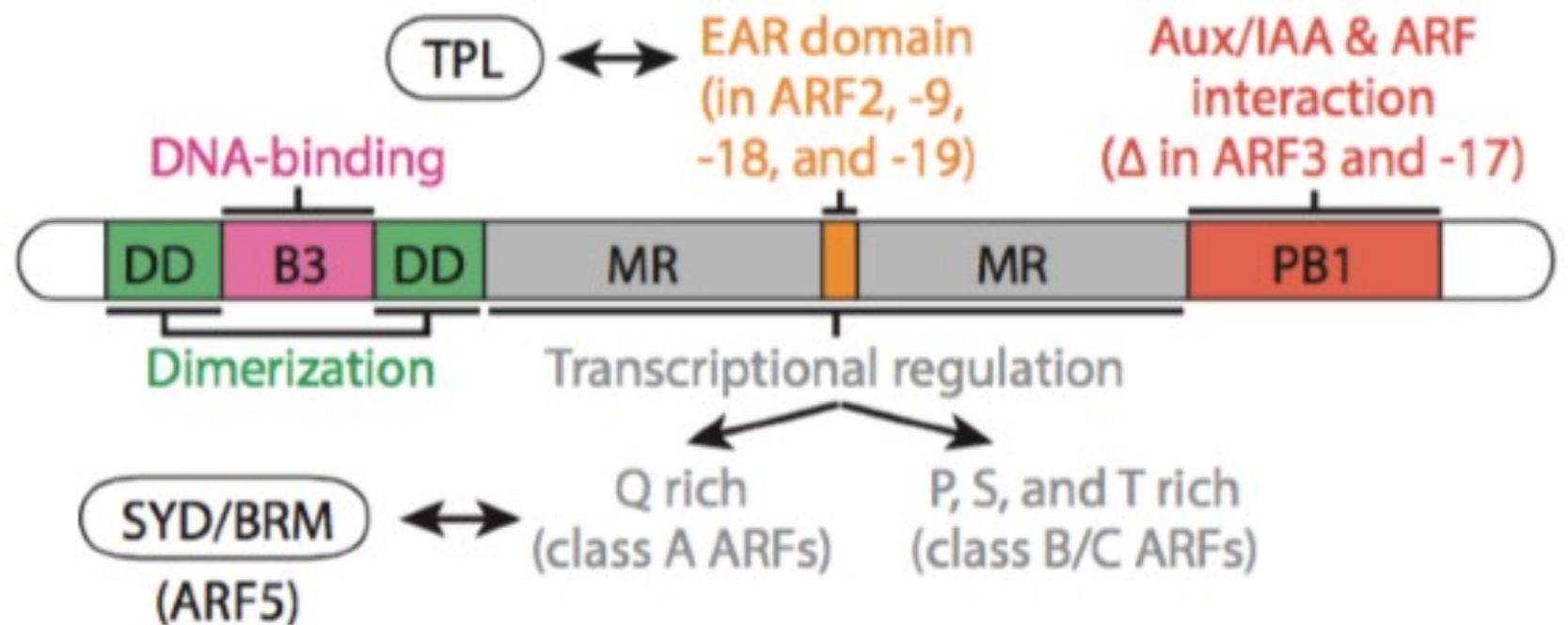
a TIR1/AFB

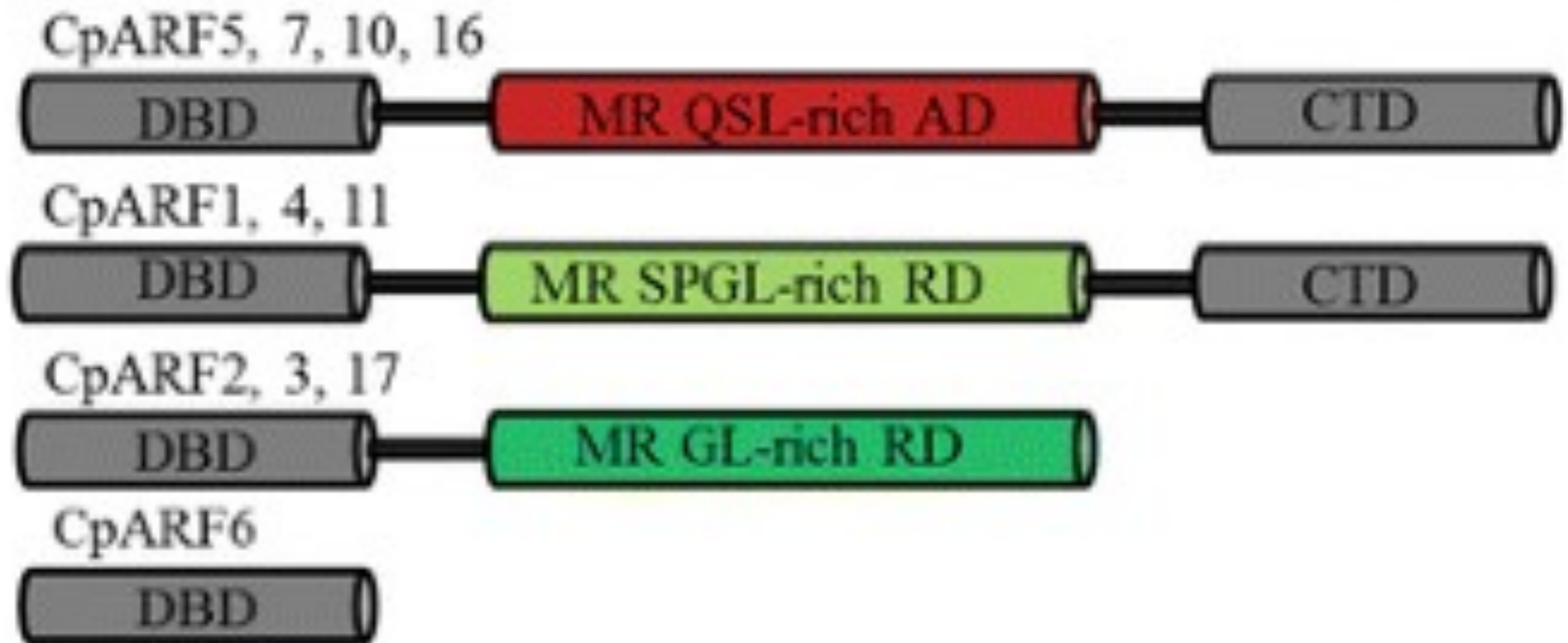


b Aux/IAA



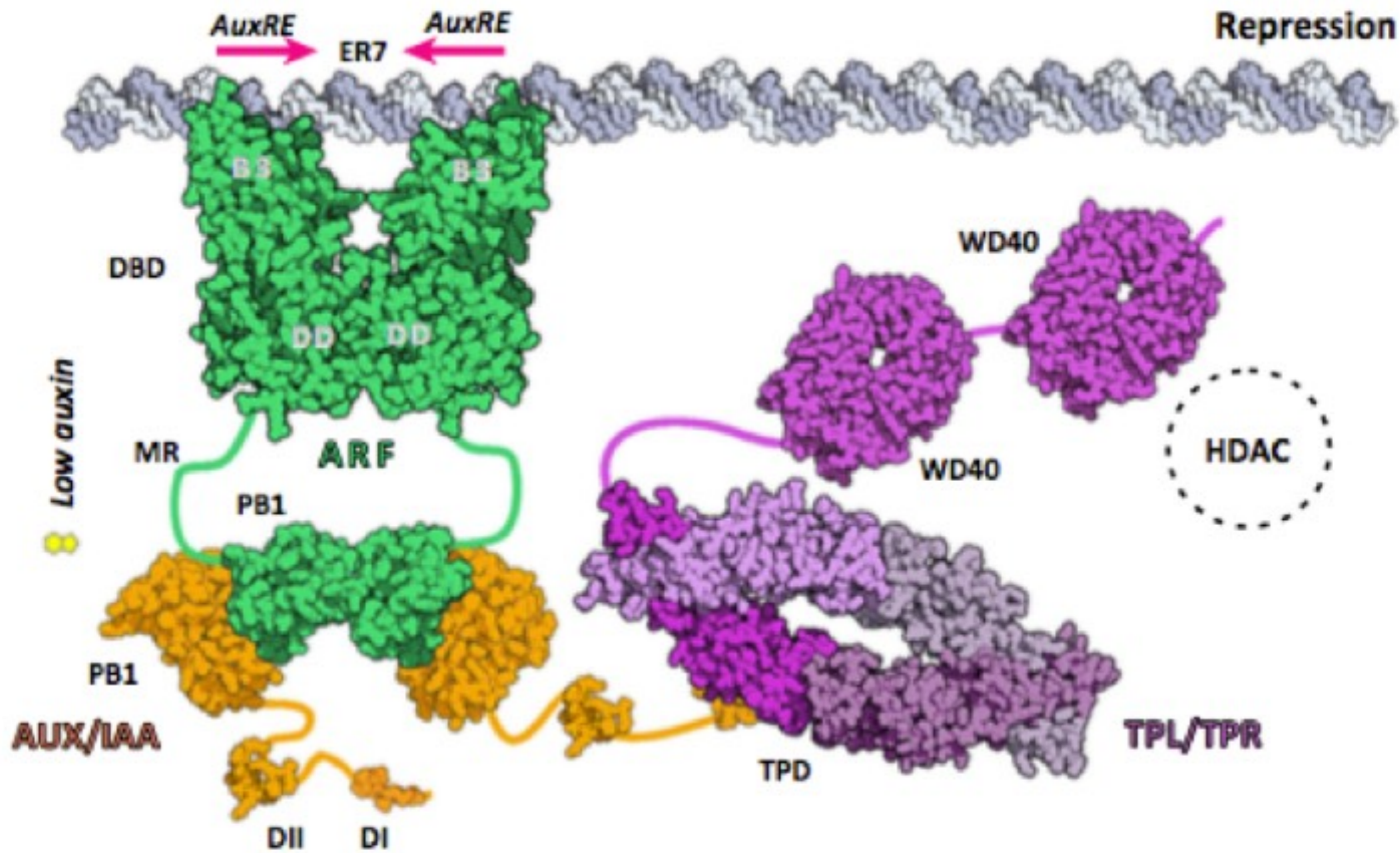
c ARF

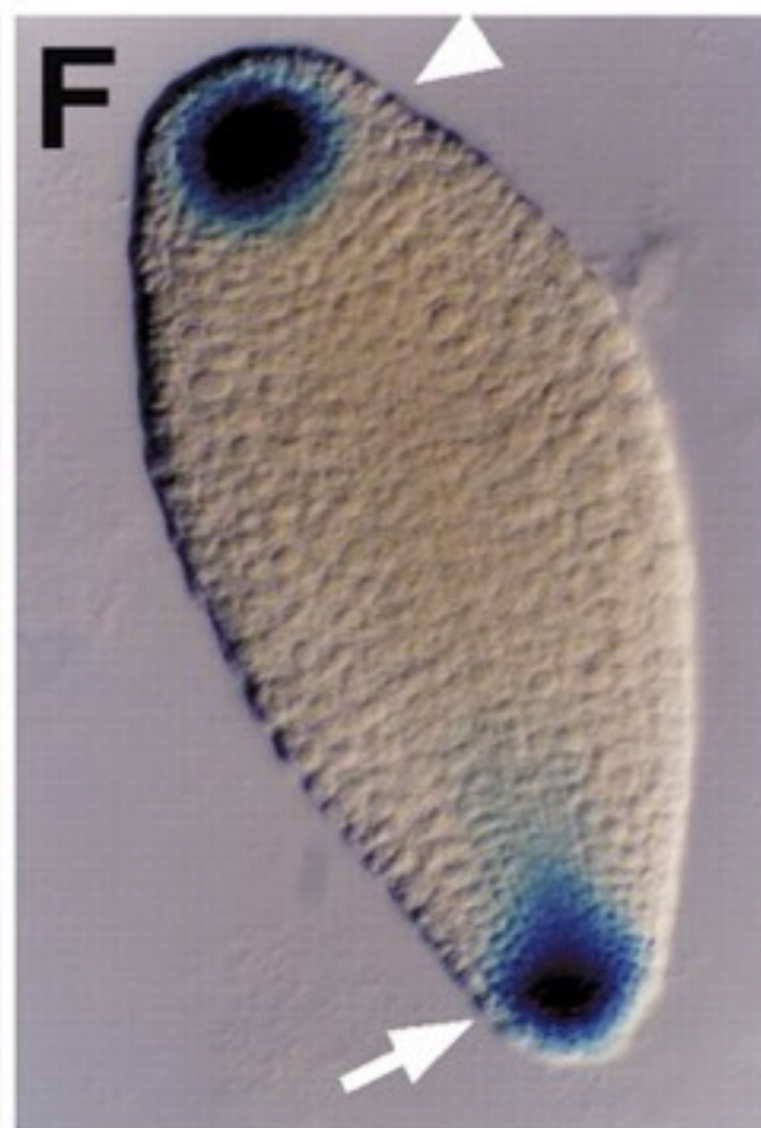
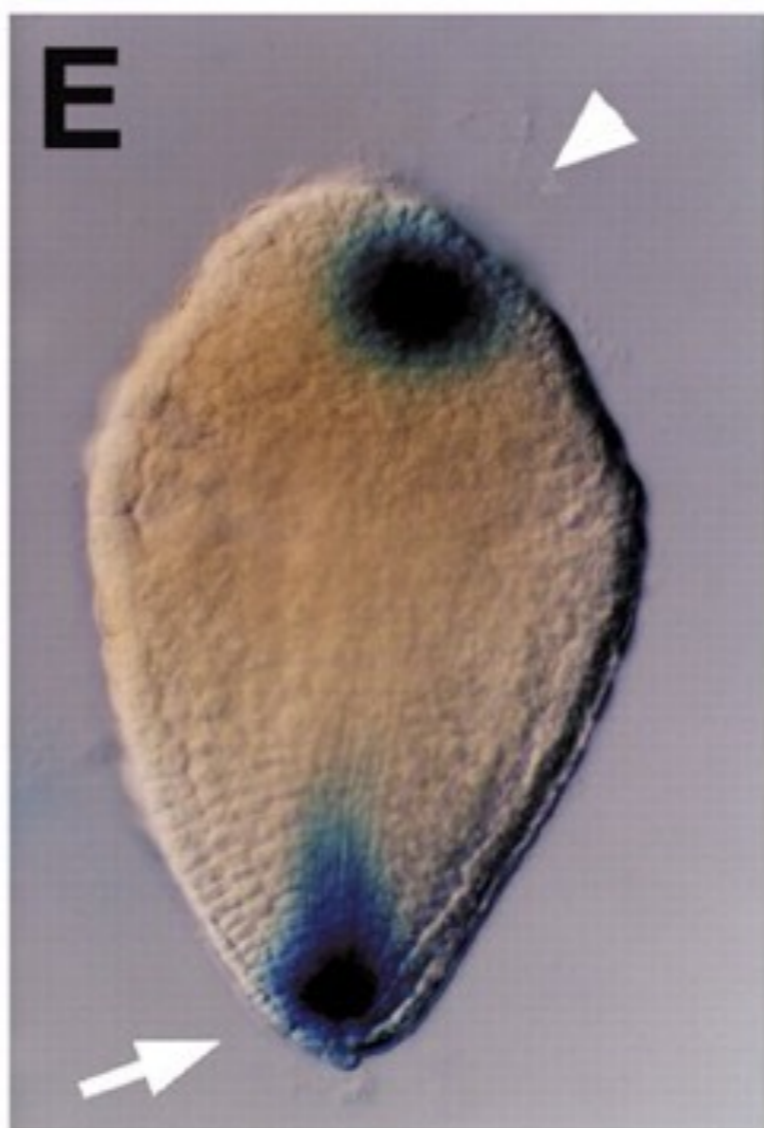
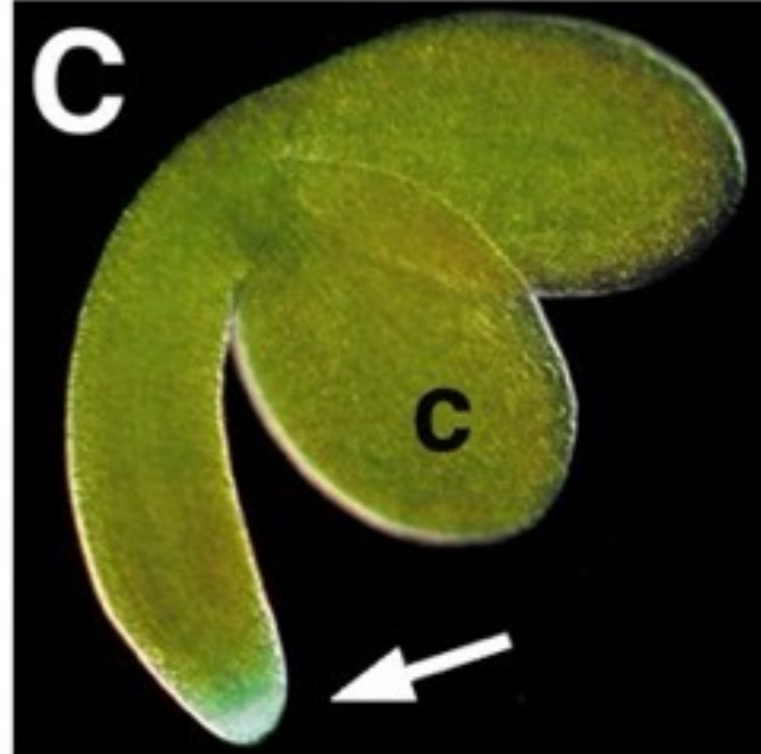
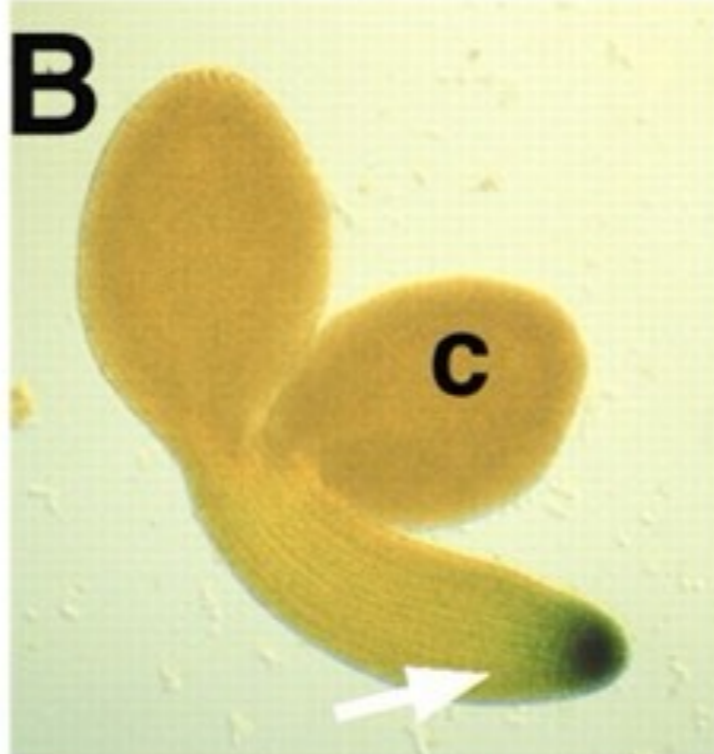
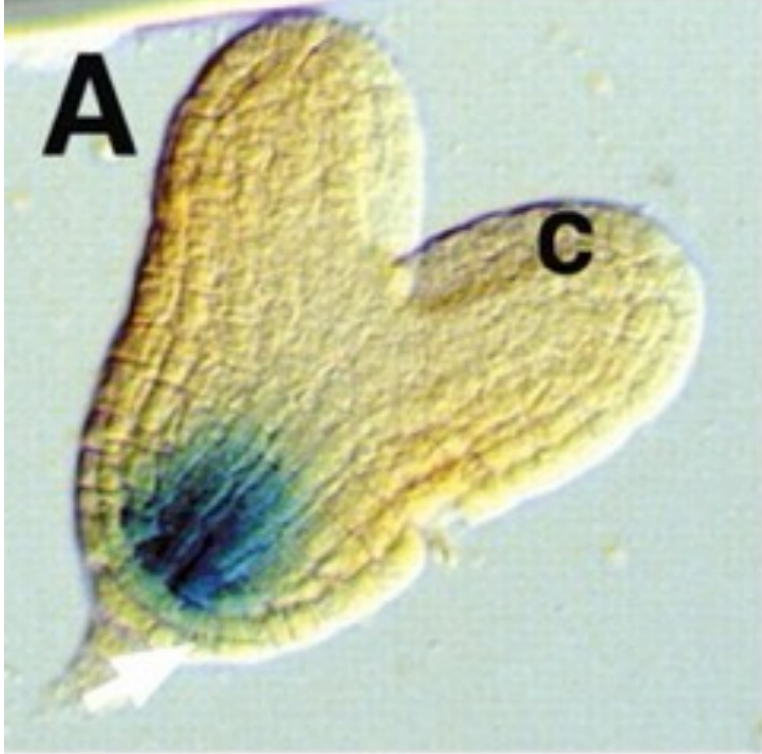




The protein structure of ARFs.

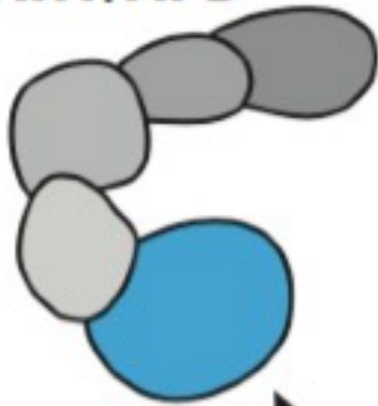
DBD, DNA-binding domain; CTD, C-terminal dimerization domain; MR, middle region; RD, repression domain; AD, activation domain;



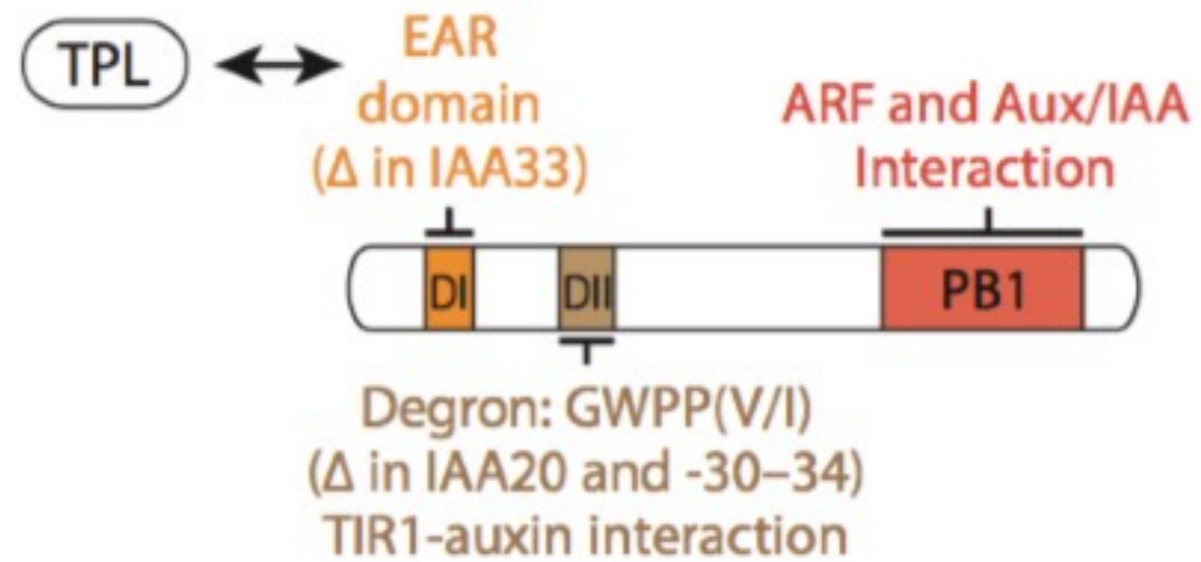
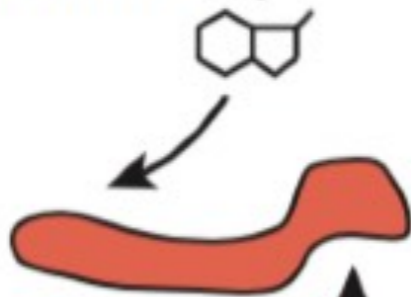


***topless (tpl)* mutant**

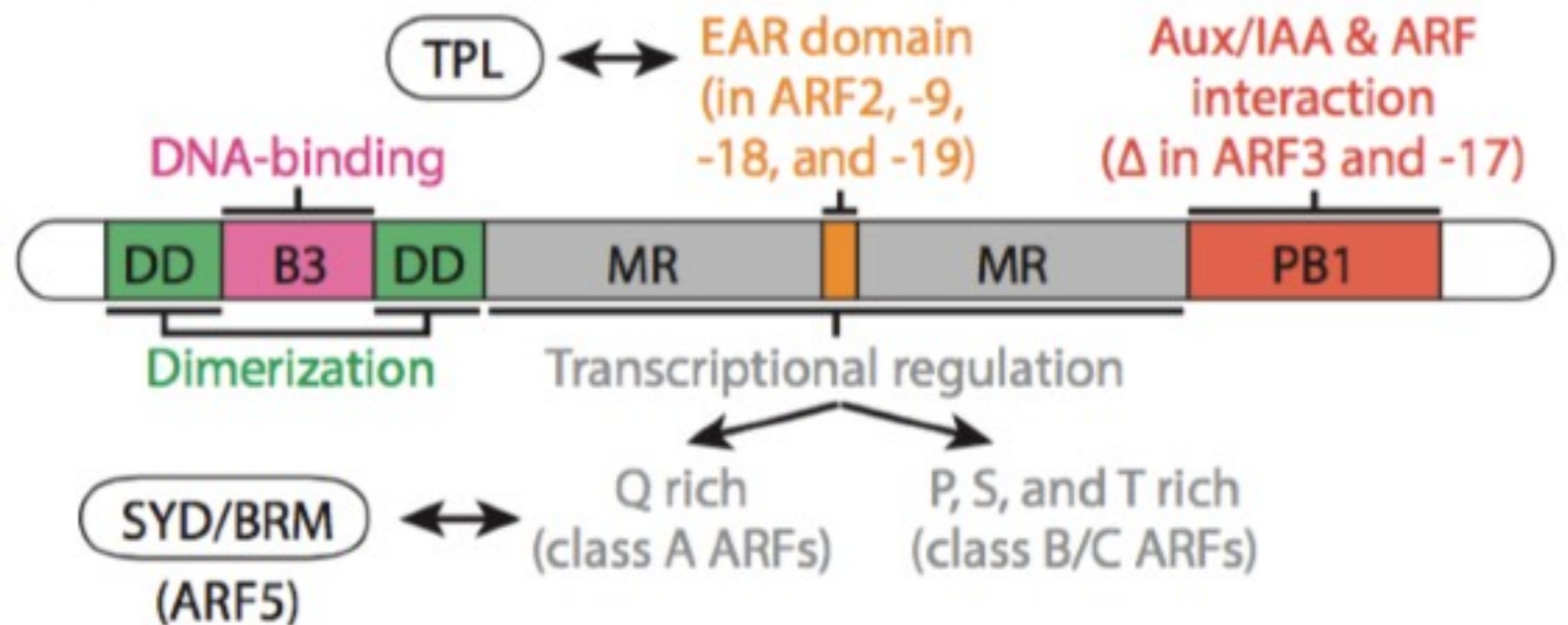
a TIR1/AFB



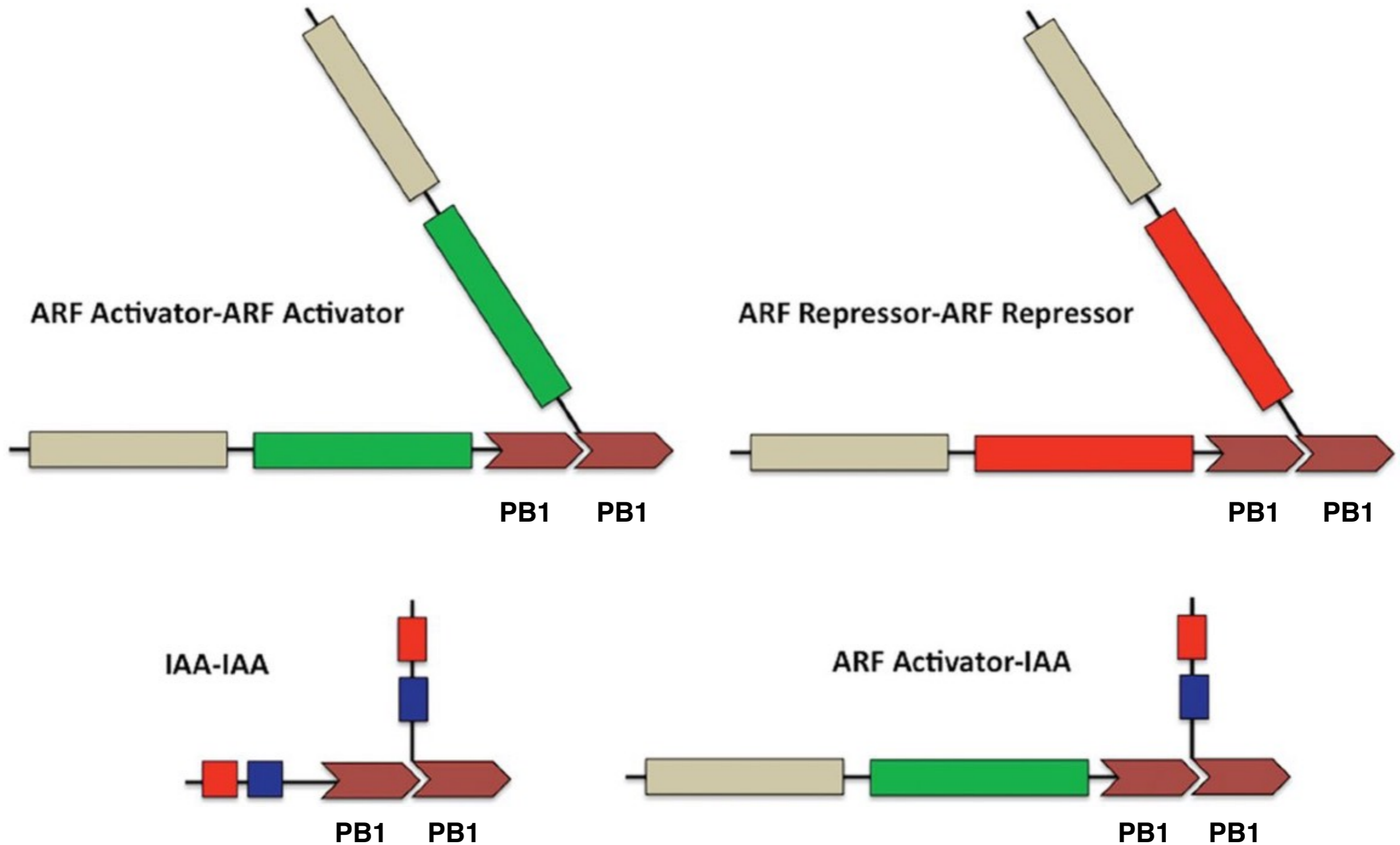
b Aux/IAA

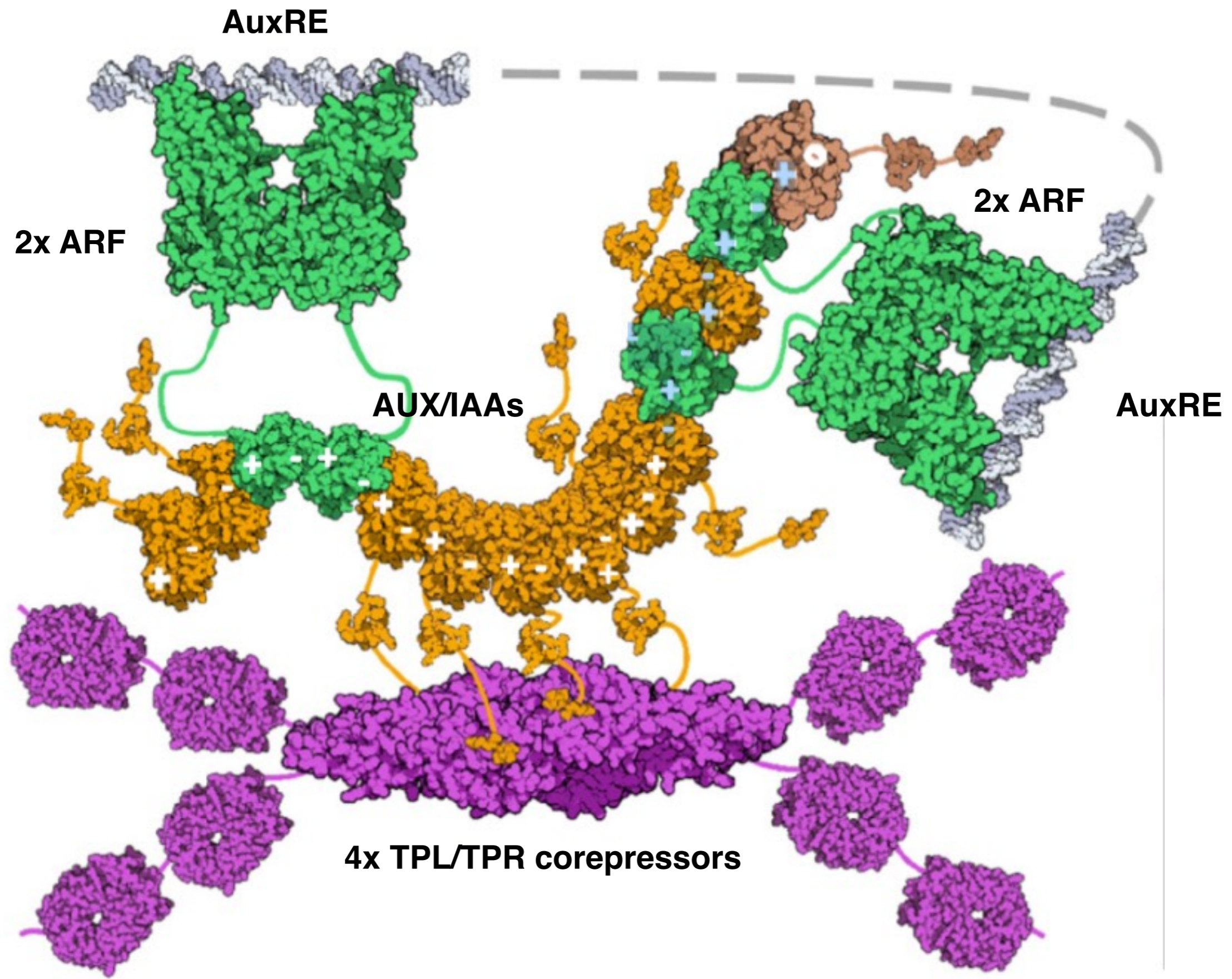


c ARF



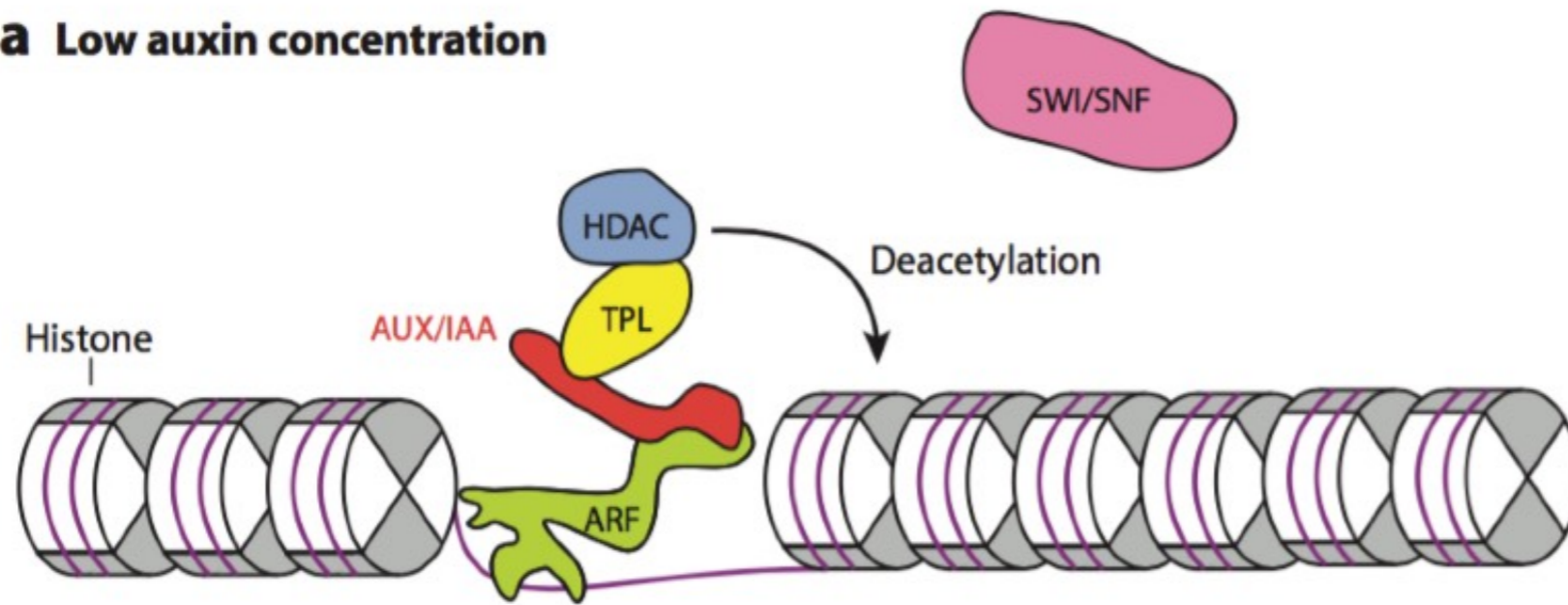
Protein Binding domains (PB1) are found on AUX/IAA and ARF proteins and allow formation of protein complexes



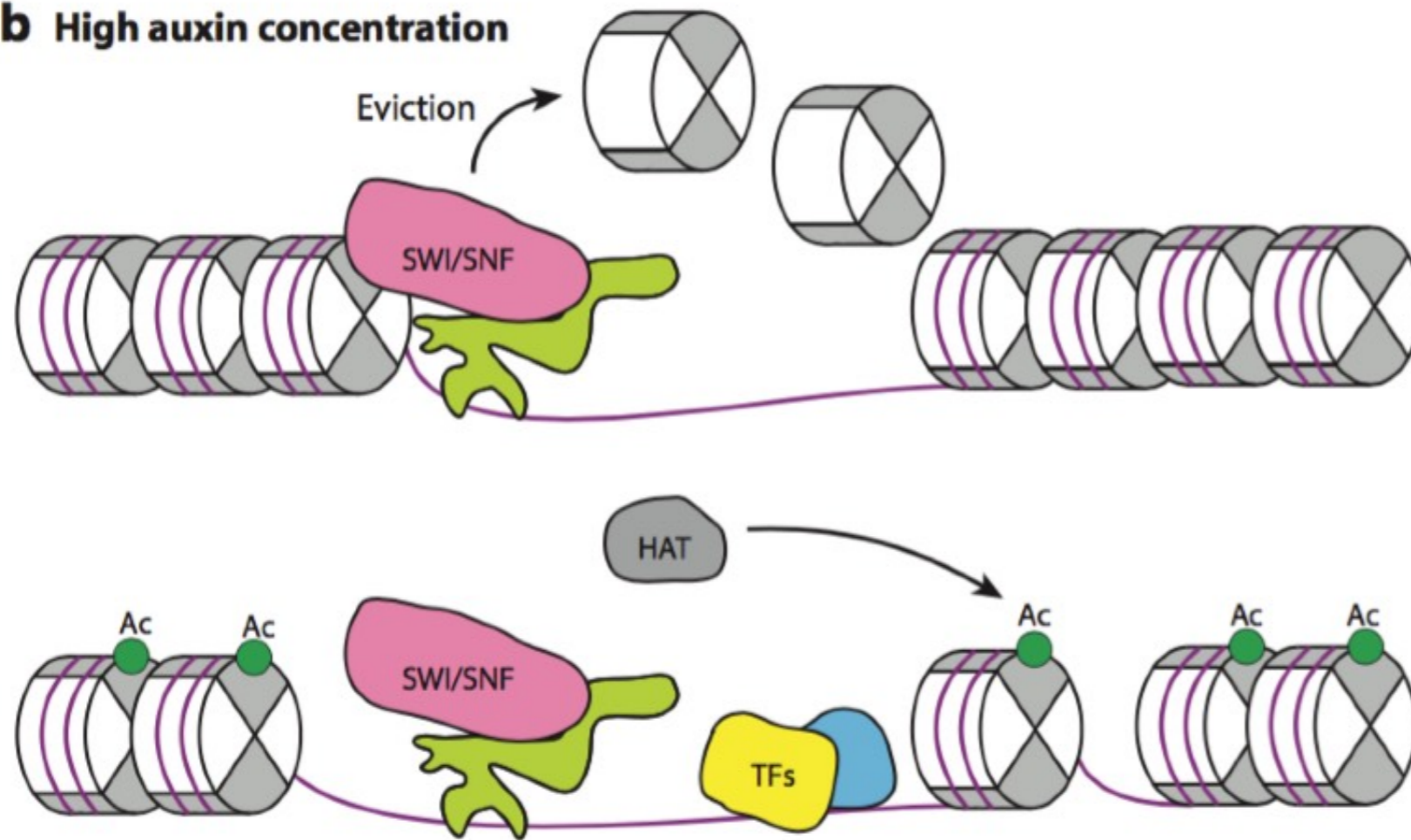


Recognition of composite AuxREs and recruitment of tetrameric TPL/TPR corepressors

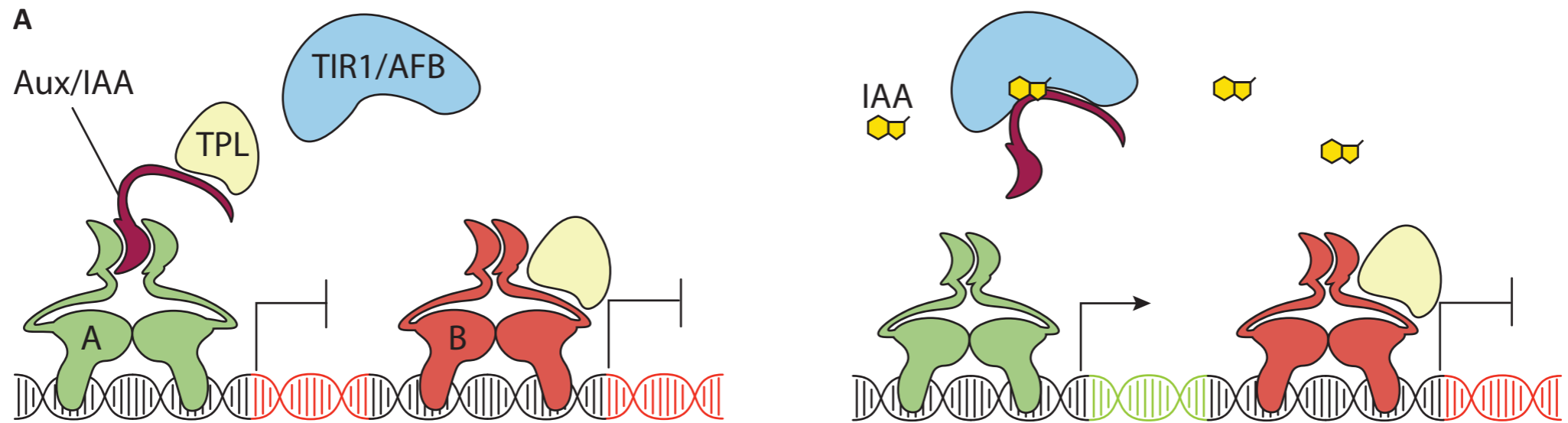
a Low auxin concentration



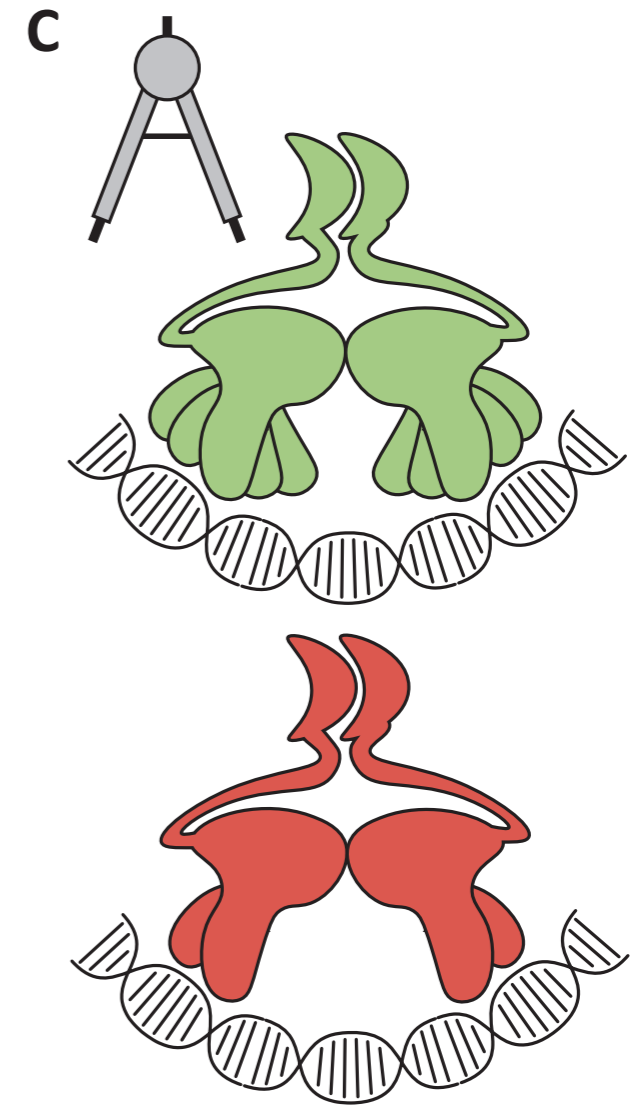
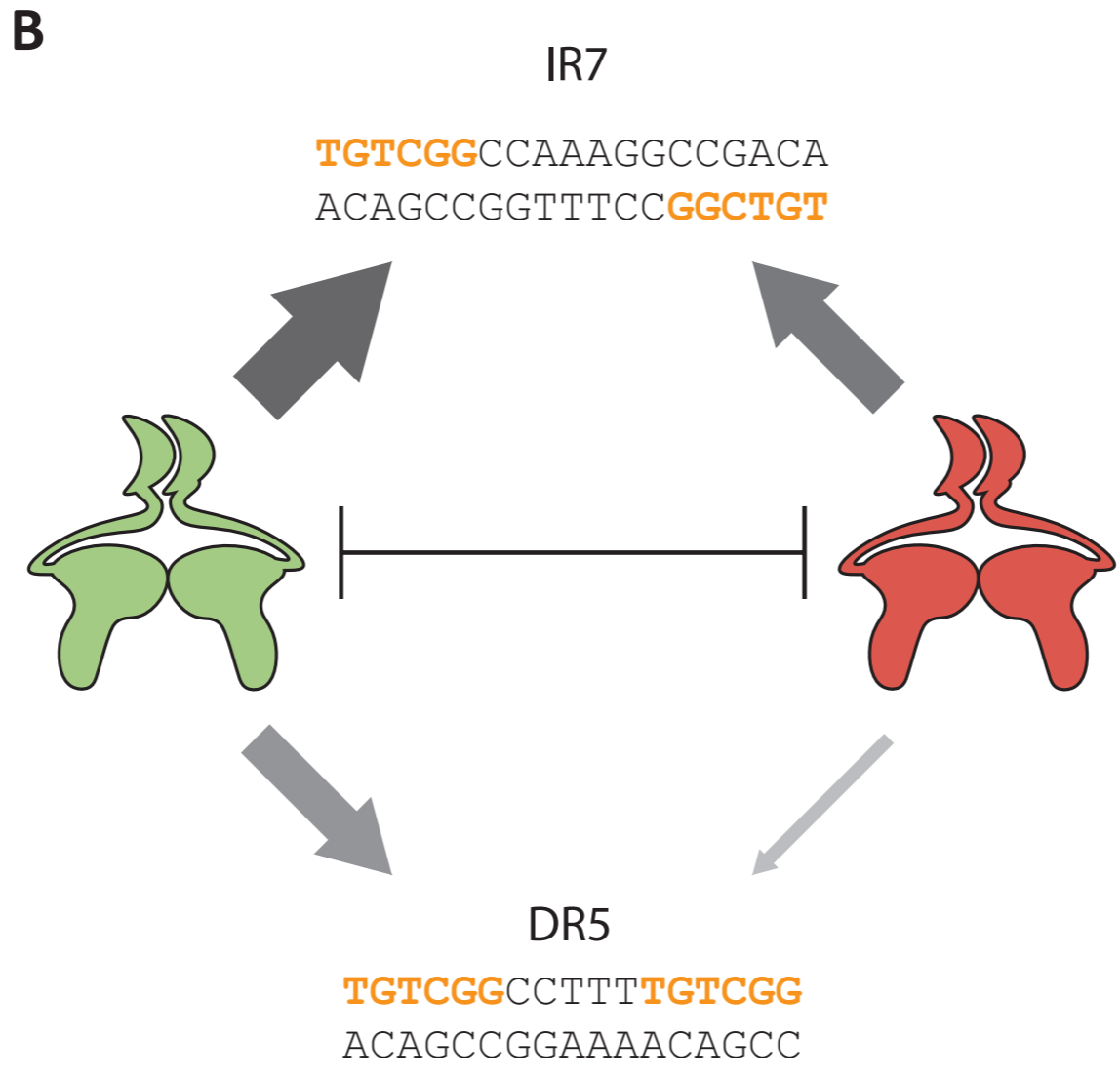
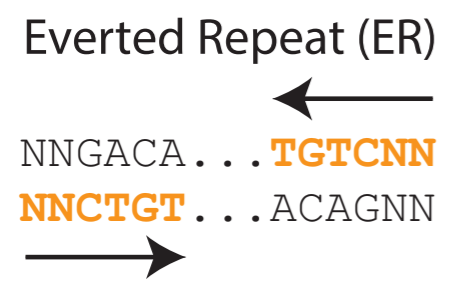
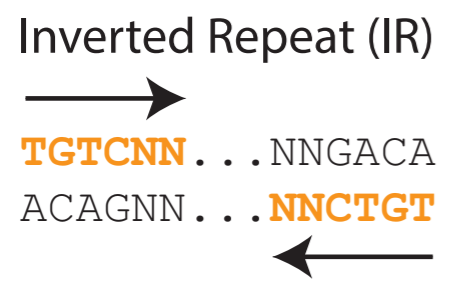
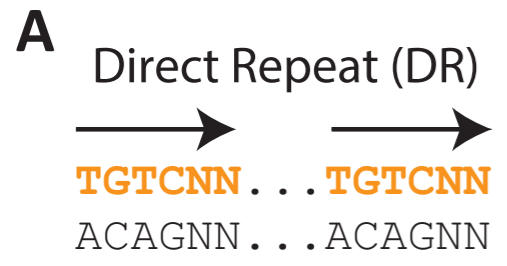
b High auxin concentration



Recruitment of Switch/Sucrose Non-Fermenting (SWI/SNF) and Histone Acetyltransferase (HAT) complexes for remodelling chromatin

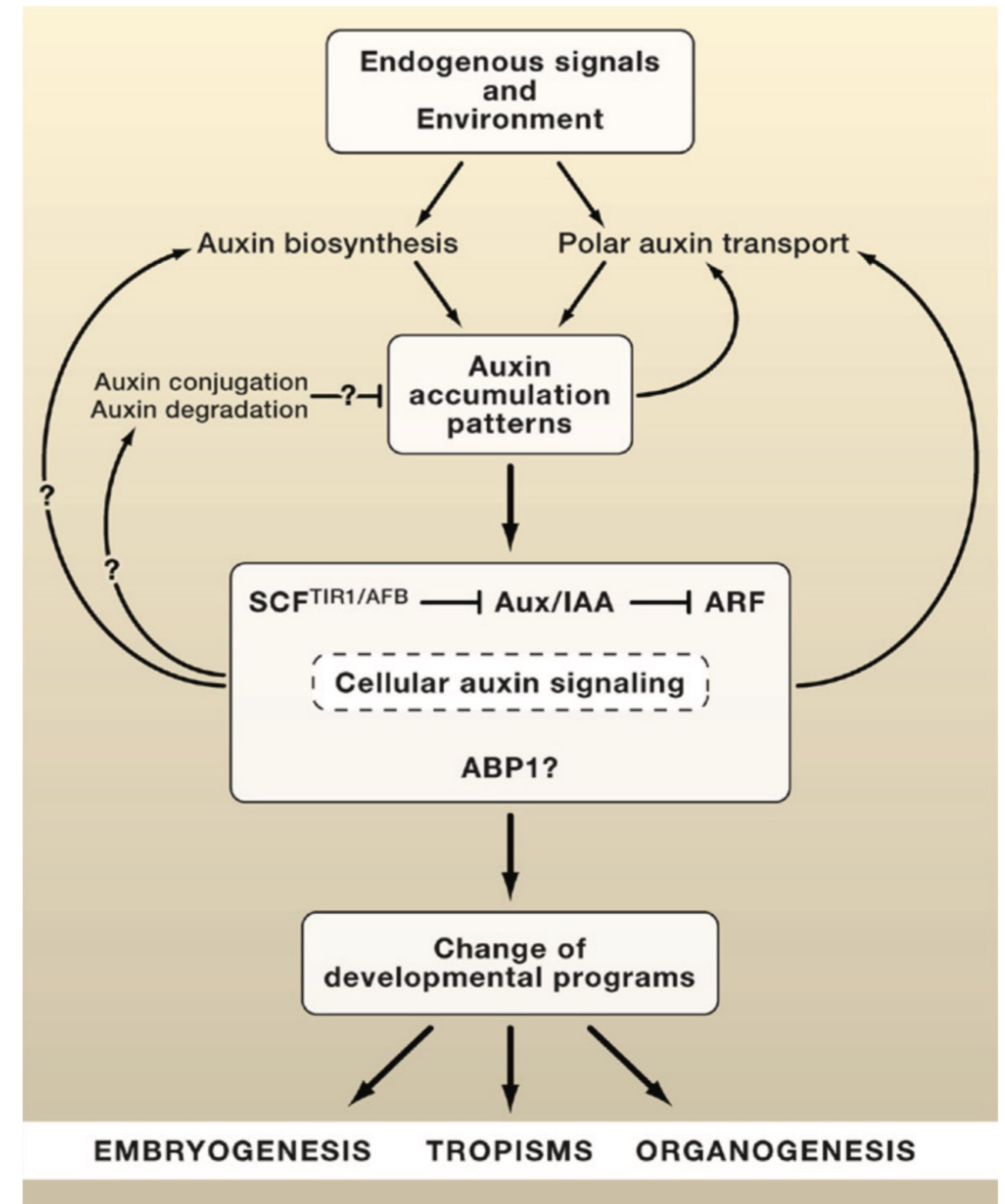
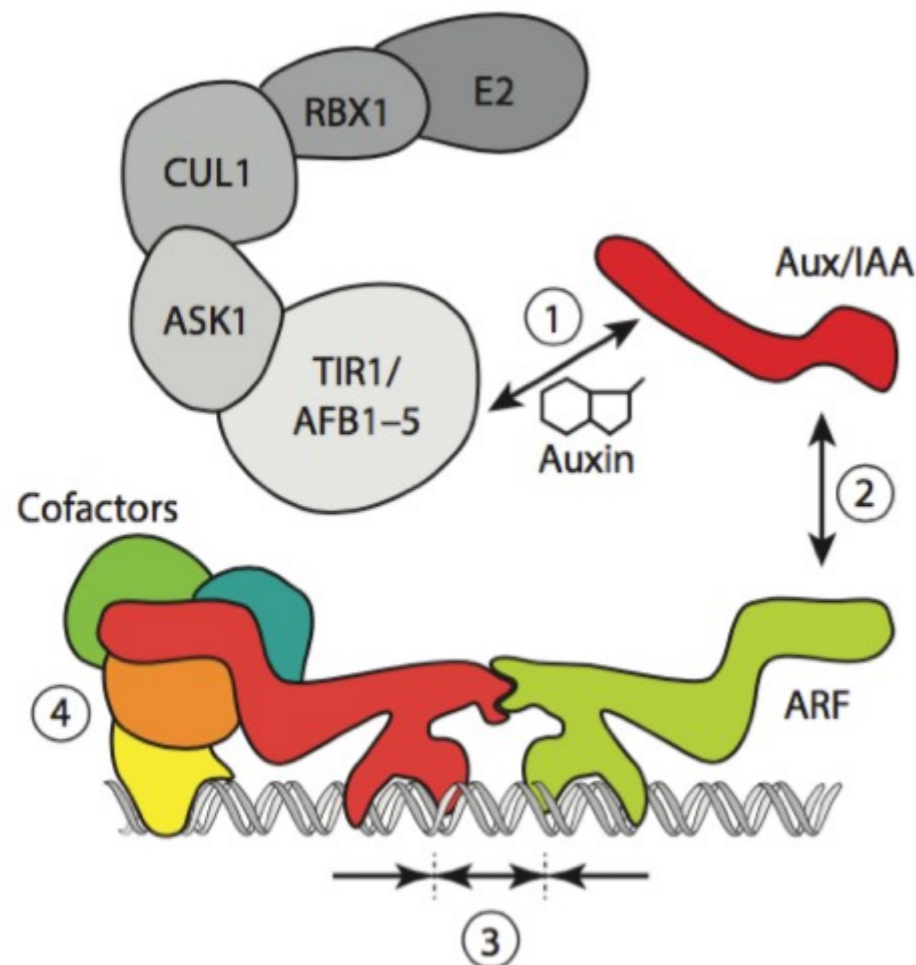


Generating diverse patterns of gene expression from a simple mechanism



Regulation of gene expression by auxin

1. Intracellular binding of auxin
2. Targeted degradation of Aux/IAA repressors
3. Selective activation of genes by ARF binding to auxin responsive promoter elements
4. Recruitment of protein co-factors for maintenance of gene expression
5. Cell-cell communication



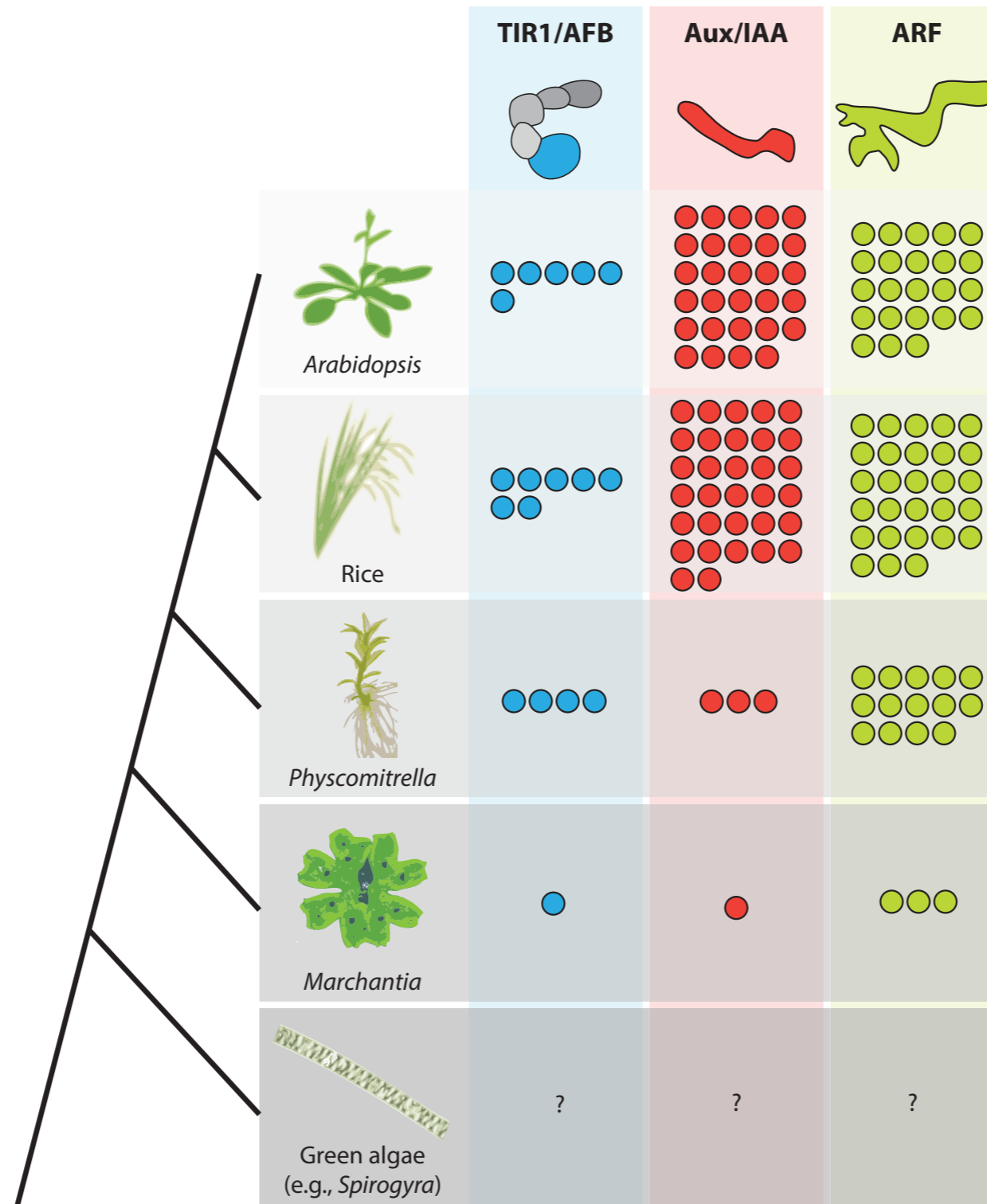


Figure 7

The evolution of the auxin response pathway, showing the distribution of genes encoding TIR1/AFB, Aux/IAA, and ARF proteins in published plant genomes for several plant species. These species represent eudicots (*Arabidopsis*), monocots (rice), mosses (*Physcomitrella*), liverworts (*Marchantia*), and green algae (*Spirogyra*, as an example of charophytes). The tree on the left-hand side indicates the divergence order but is not drawn to scale. Protein abbreviations: ARF, AUXIN RESPONSE FACTOR; Aux/IAA, AUXIN/INDOLE-3-ACETIC ACID; TIR1/AFB, TRANSPORT INHIBITOR RESISTANT 1/AUXIN SIGNALING F-BOX.