CDB Part IB
Plant Development

Lecture 2.

Polarity, auxin traffic and auxin response

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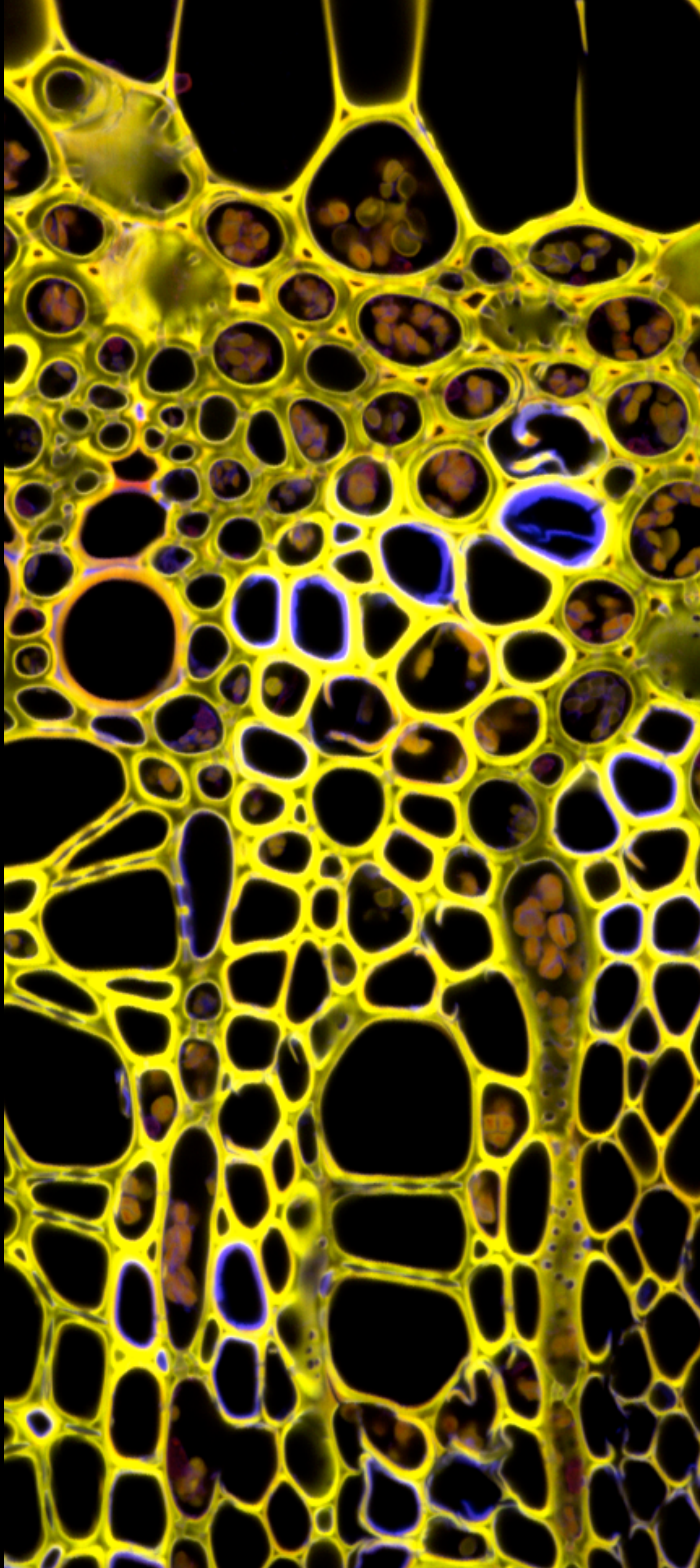
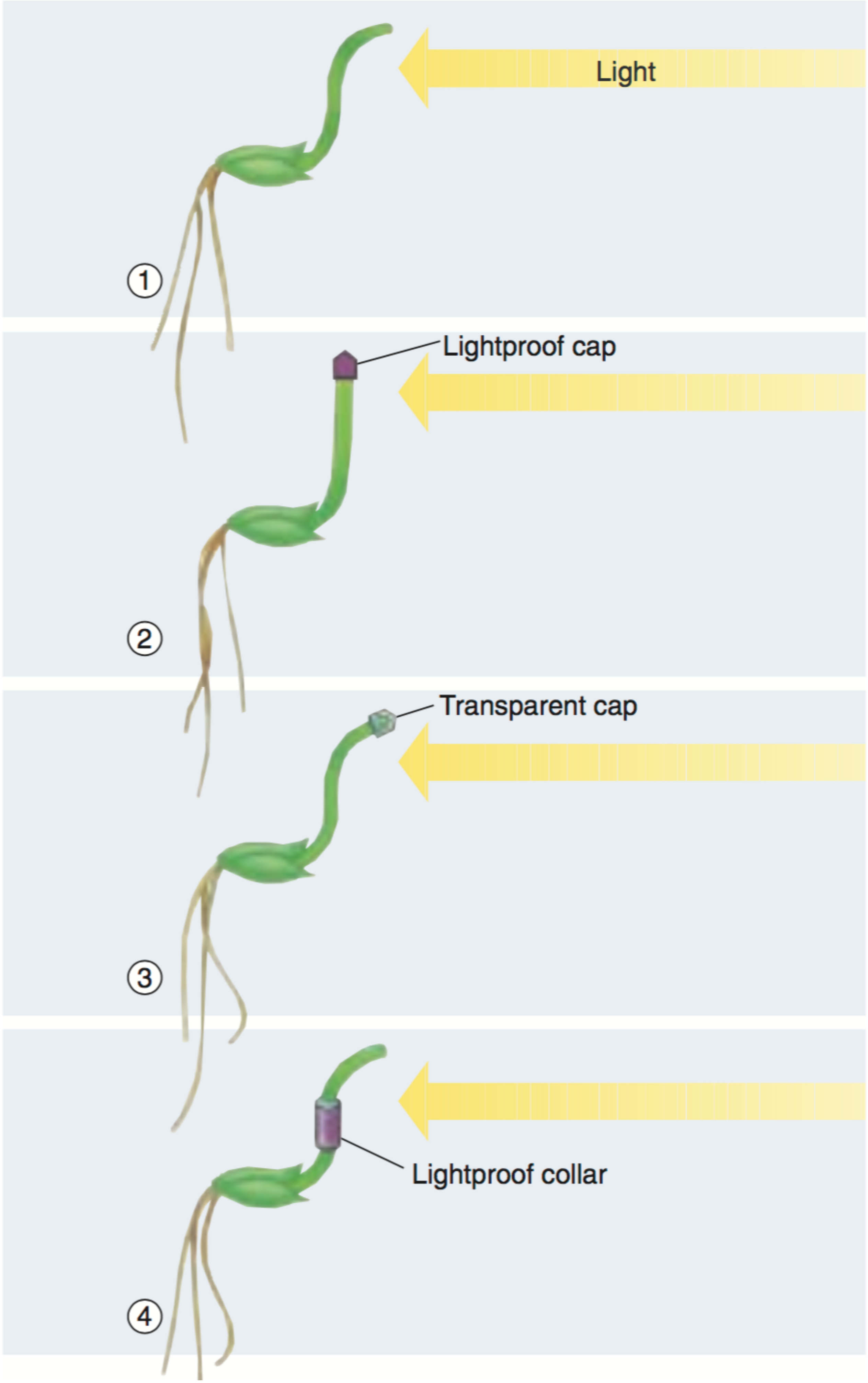


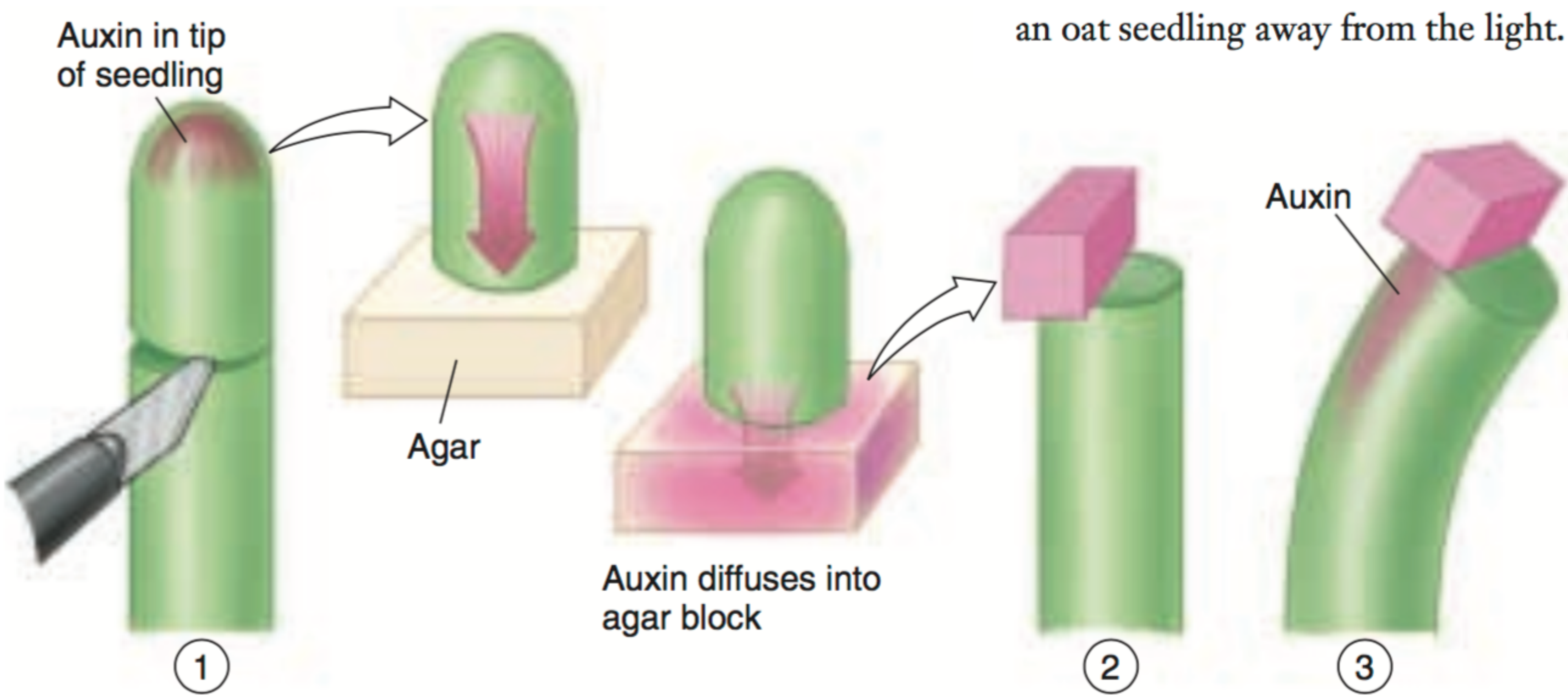
Charles and Francis Darwin's experiments on signalling during plant phototropism

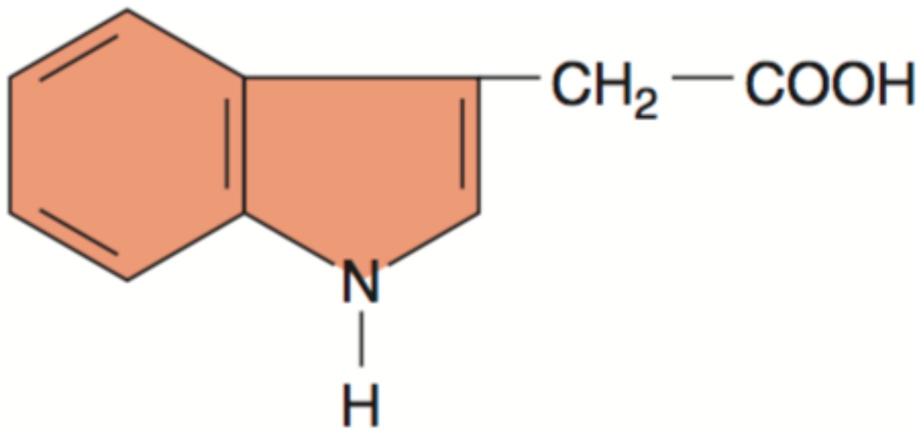


The Darwins' experiment. (*a*) Young grass seedlings normally bend toward the light. (*b*) The bending (*1*) did not occur when the tip of a seedling was covered with a lightproof cap (*2*), but did occur when it was covered with a transparent one (*3*). When a collar was placed below the tip (*4*), the characteristic light response took place. From these experiments, the Darwins concluded that, in response to light, an "influence" that caused bending was transmitted from the tip of the seedling to the area below, where bending normally occurs.

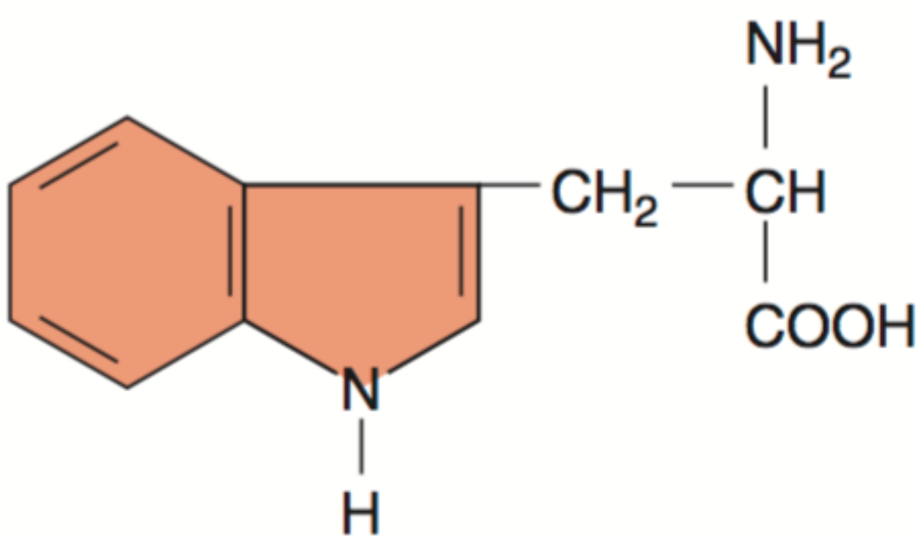
Demonstration of auxin signaling in plant tissues

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.

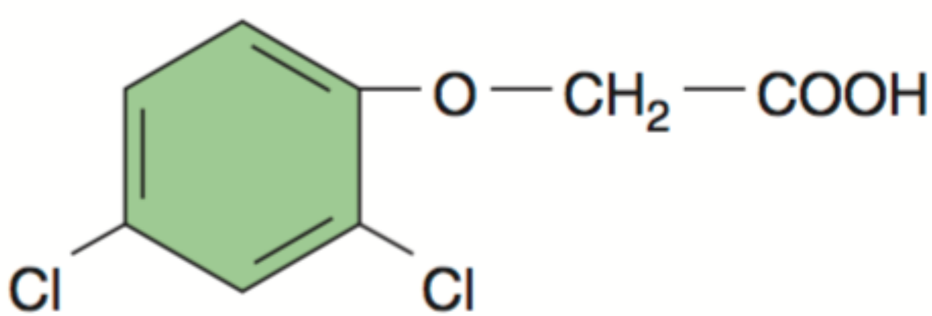




(a) **IAA (Indoleacetic acid)**



(b) **Tryptophan**



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

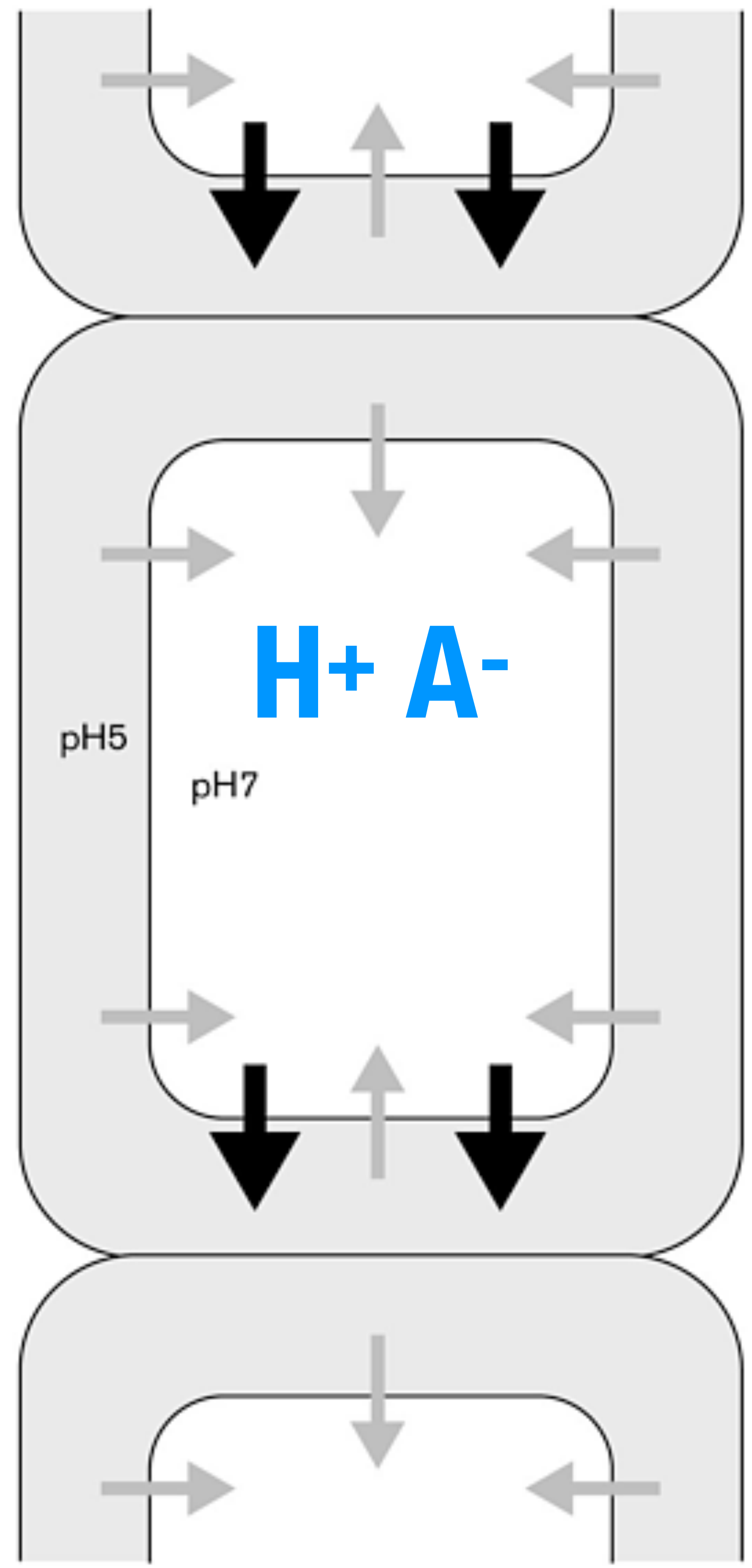
Auxin and apical-basal polarity:

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.

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

HA

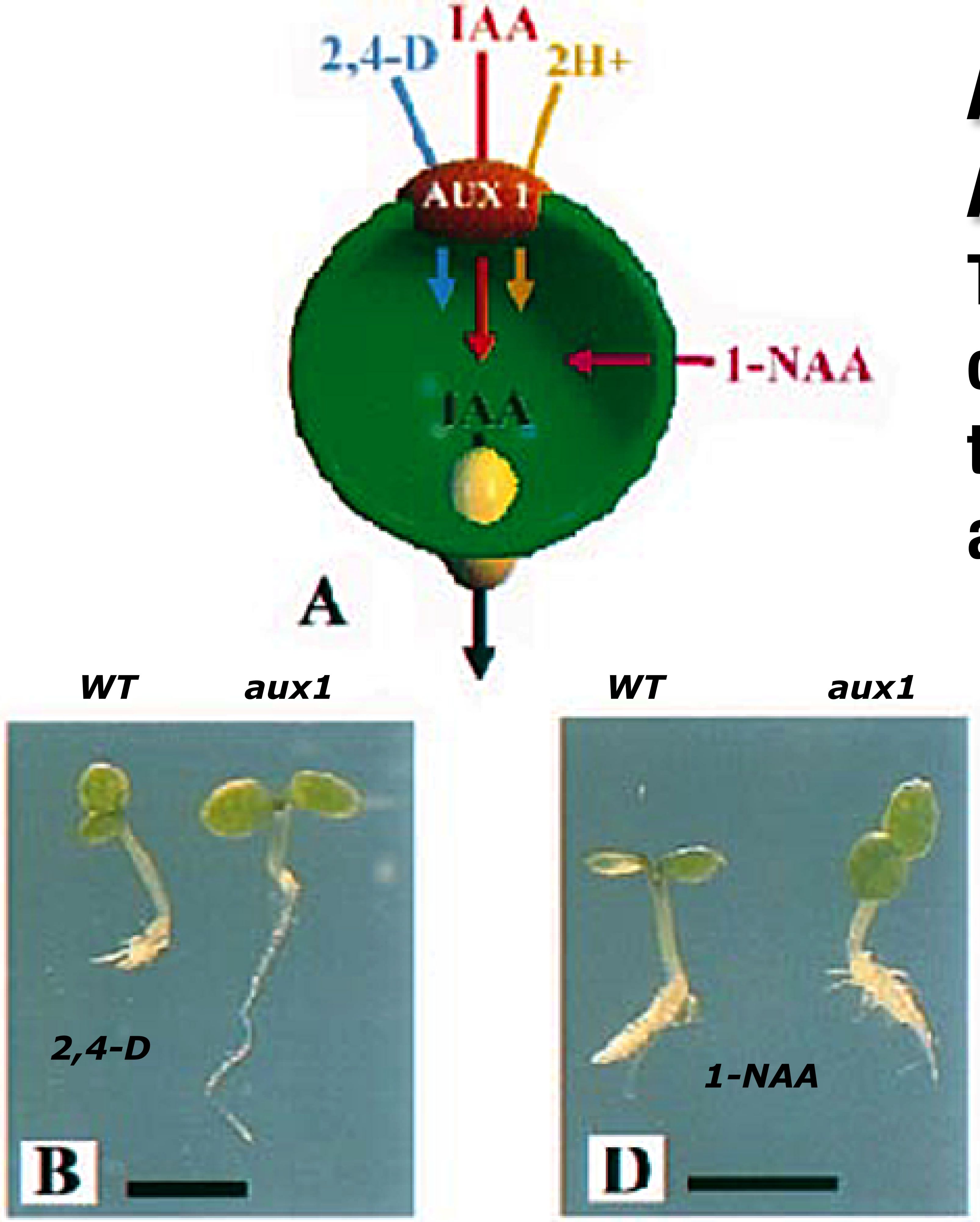


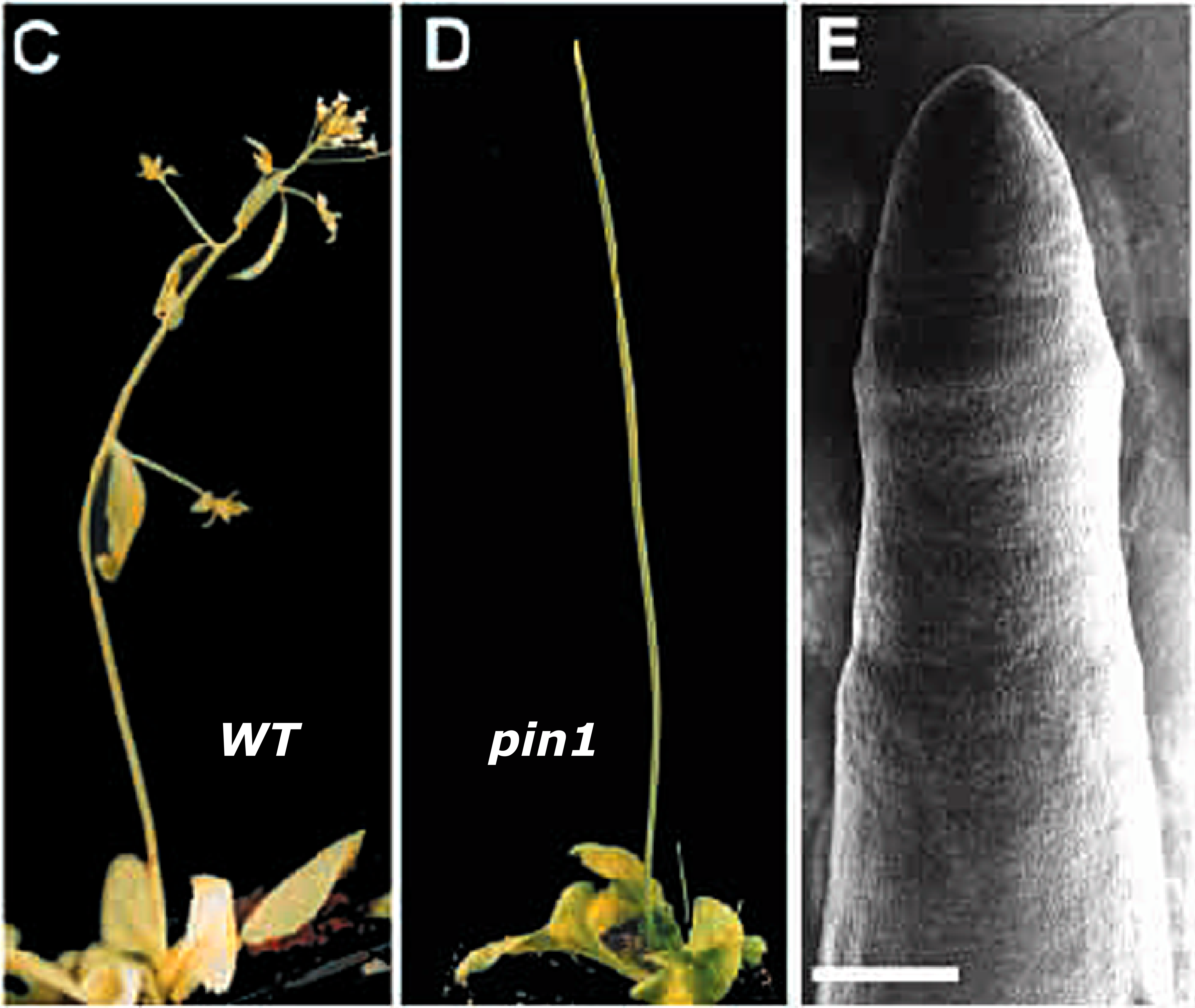
Anionic trapping

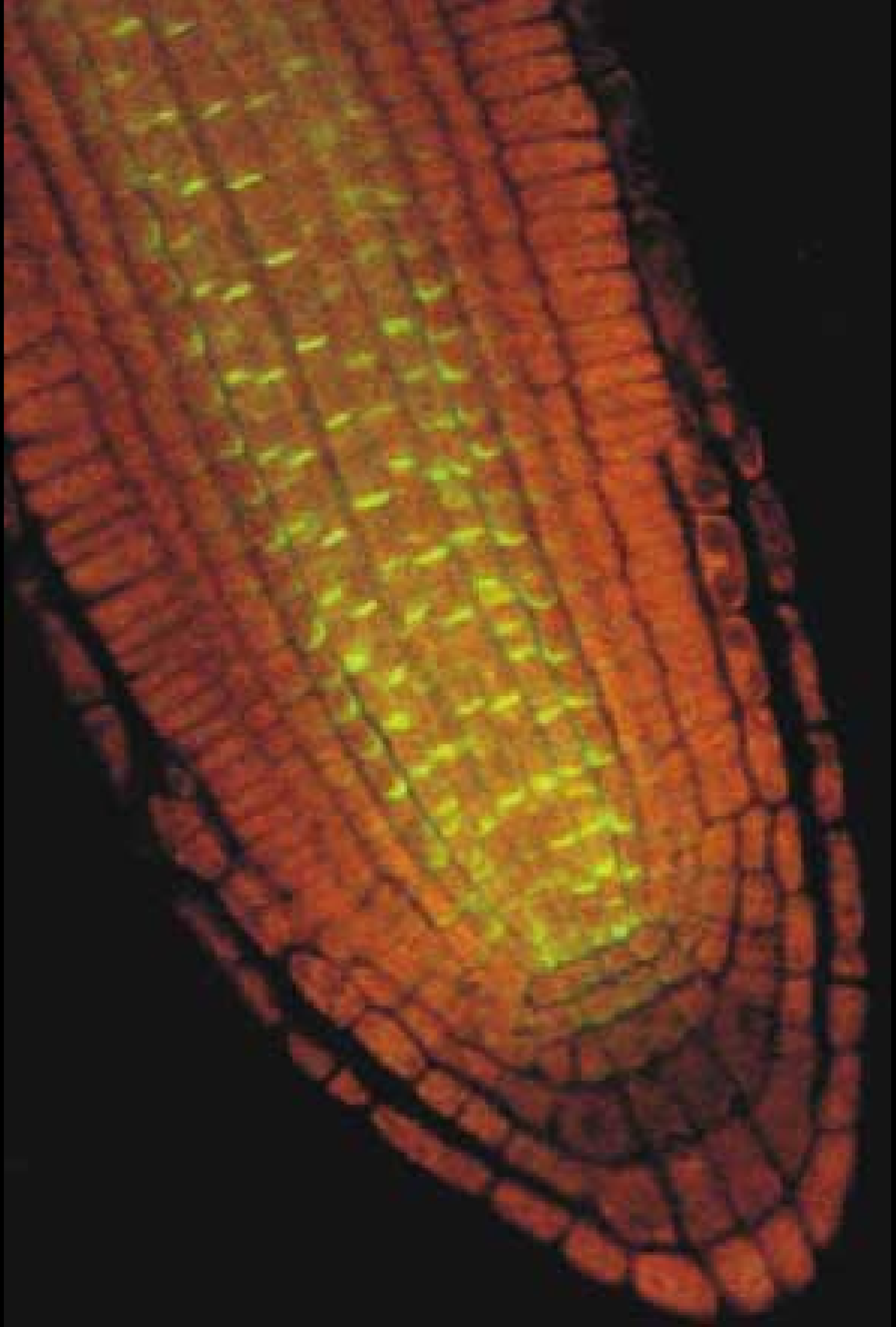
Current Opinion in Plant Biology

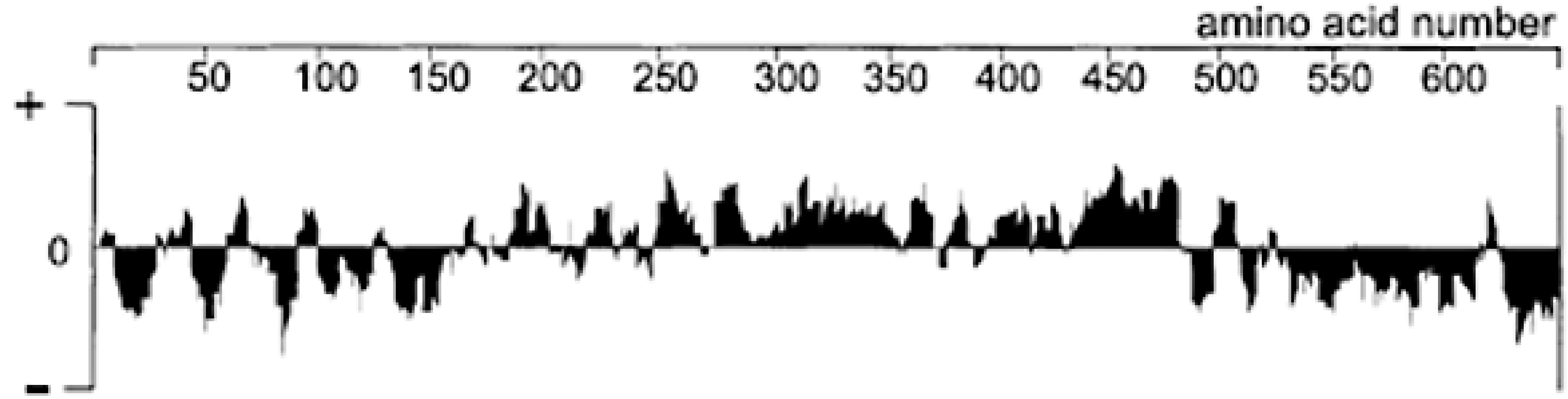
Auxin influx carrier: AUX1

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

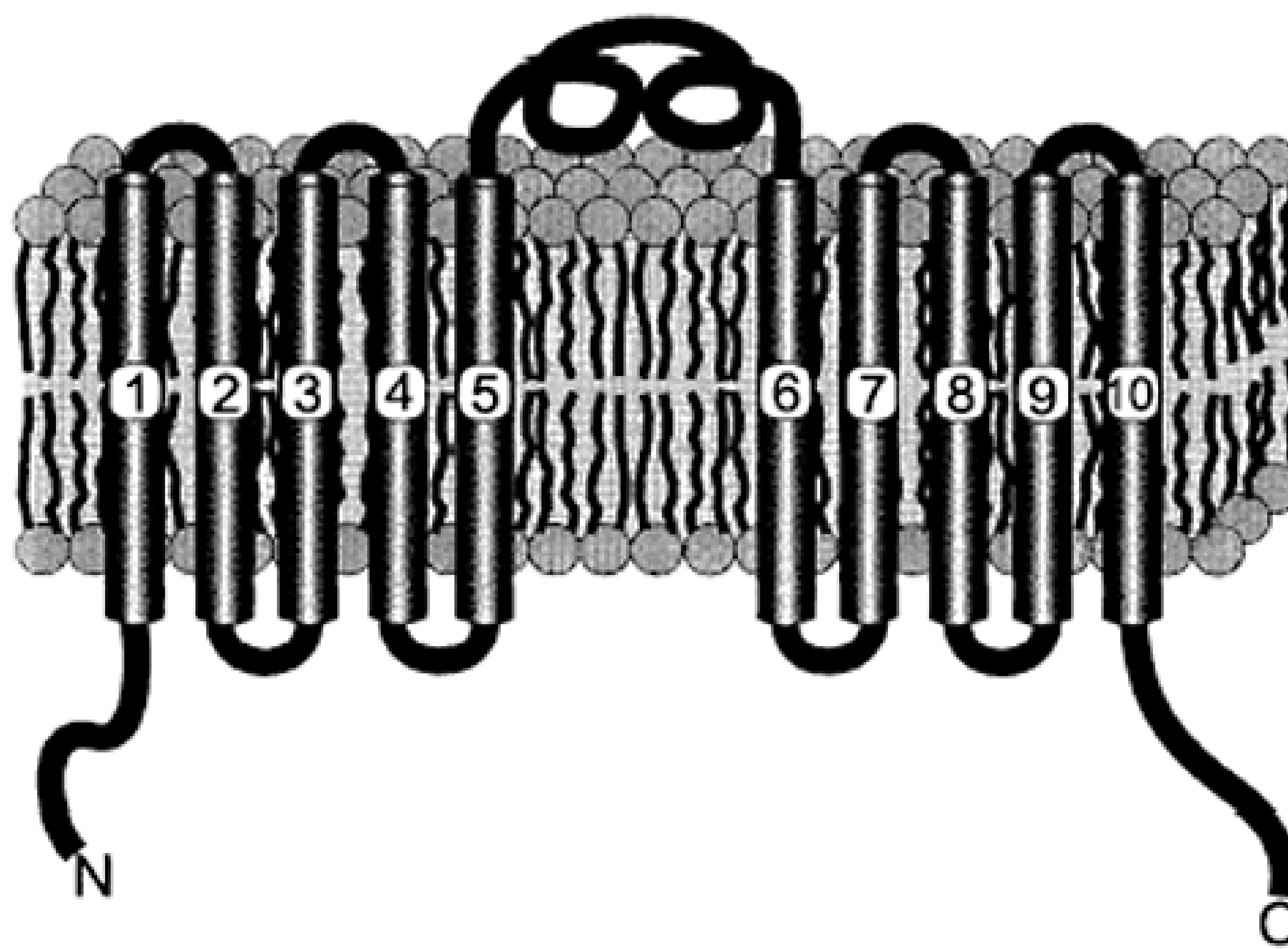



C
WT
D
pin1
E

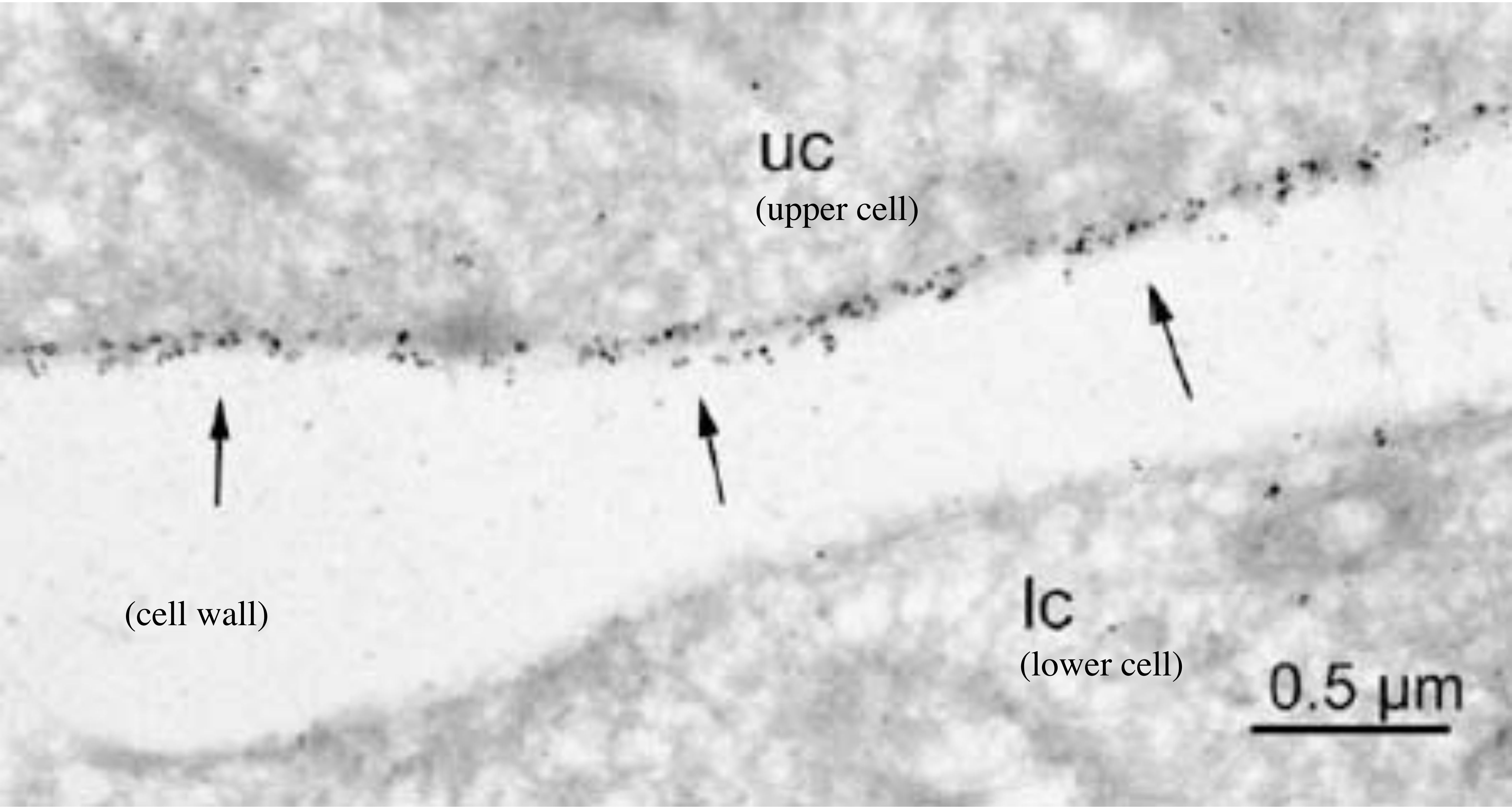




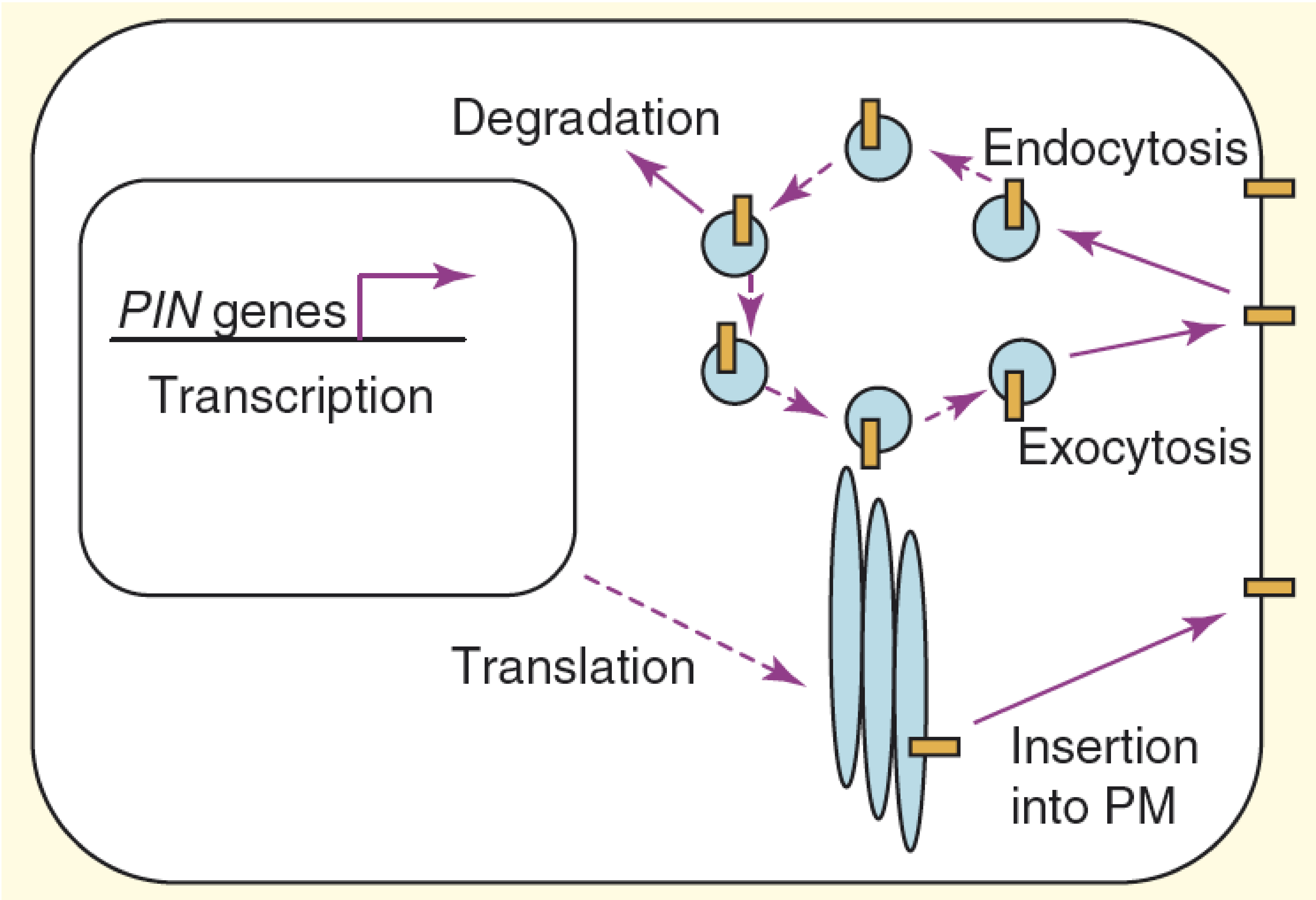
PIN1 auxin efflux carrier



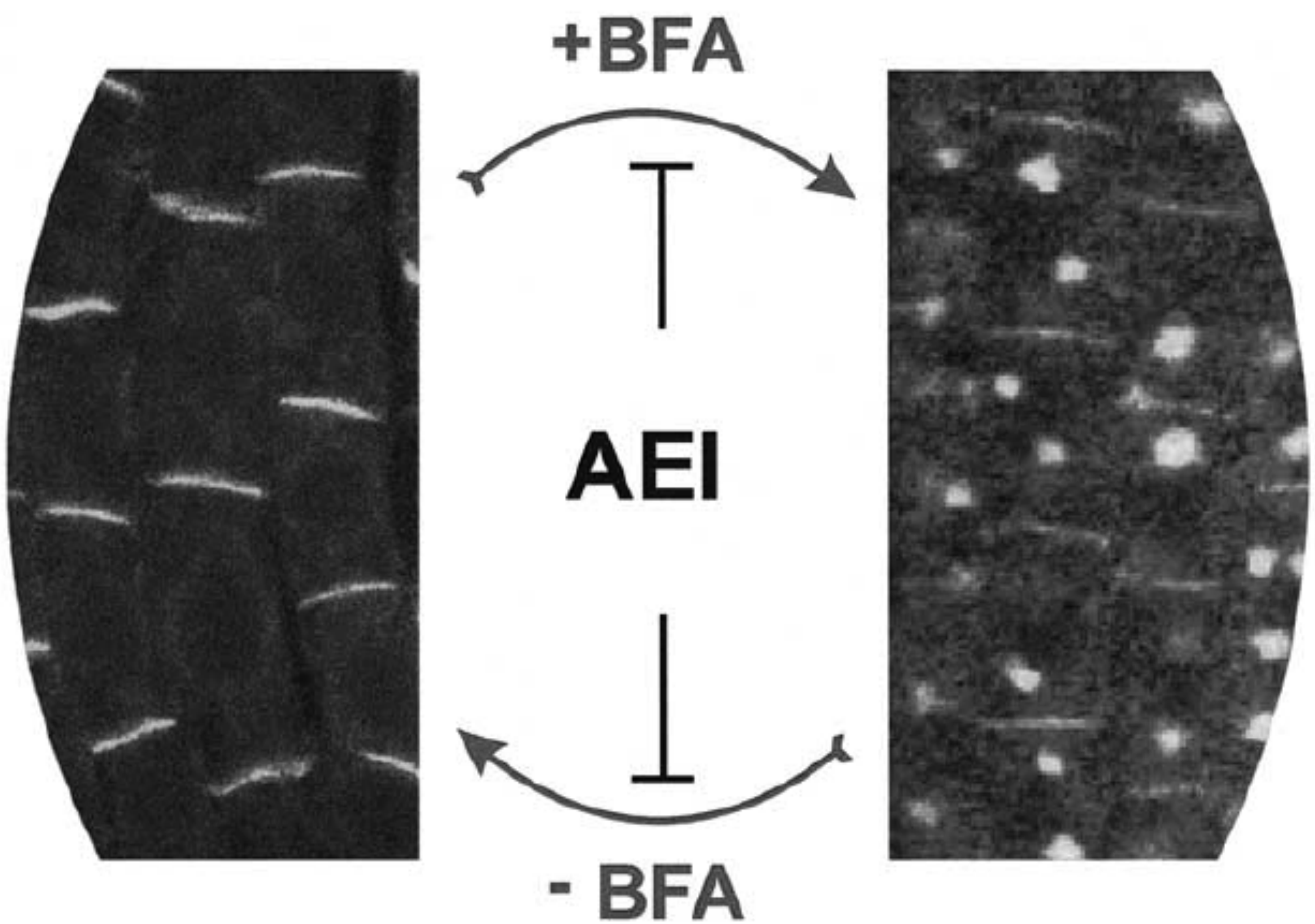
PIN1 is plasma membrane localised with a polar distribution within the cell

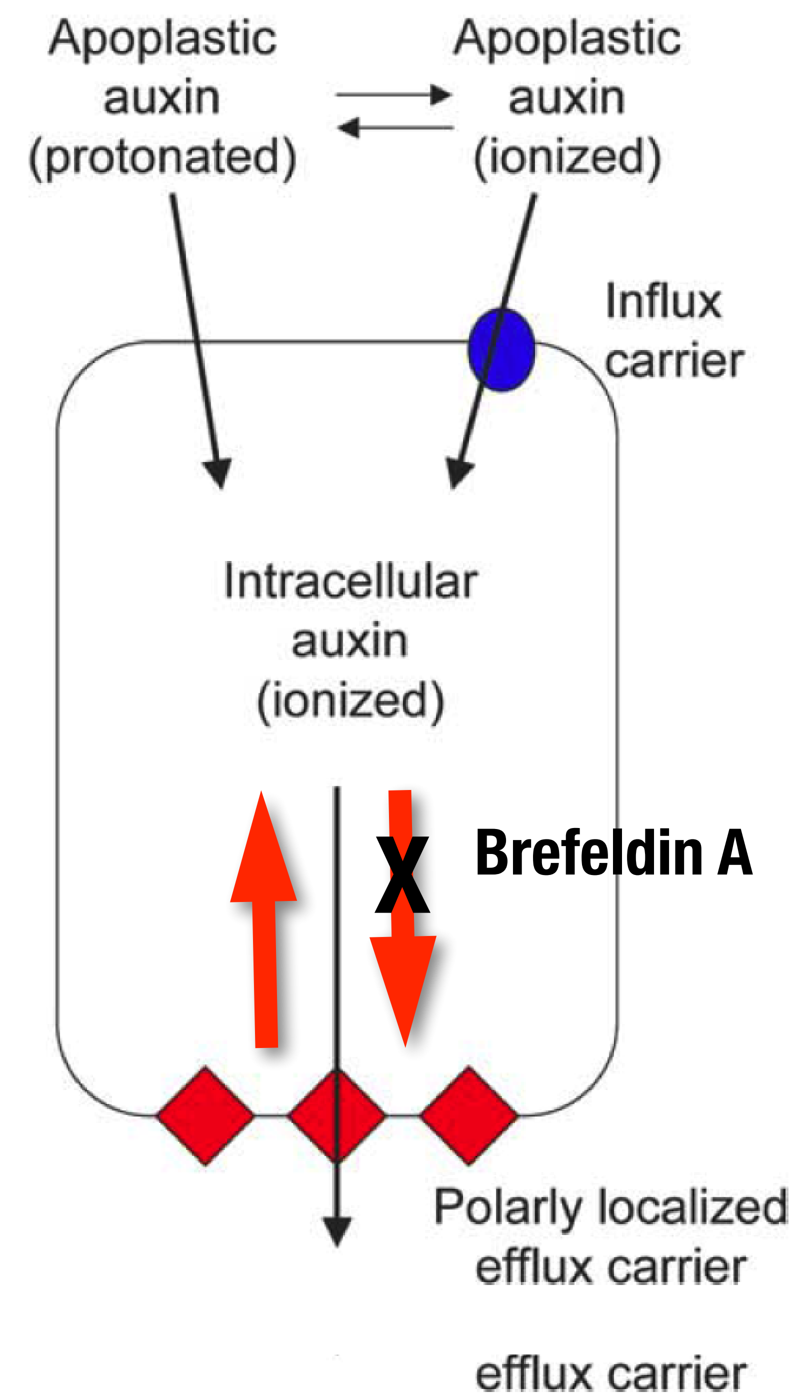


Feedback through regulated expression and localisation of PIN genes

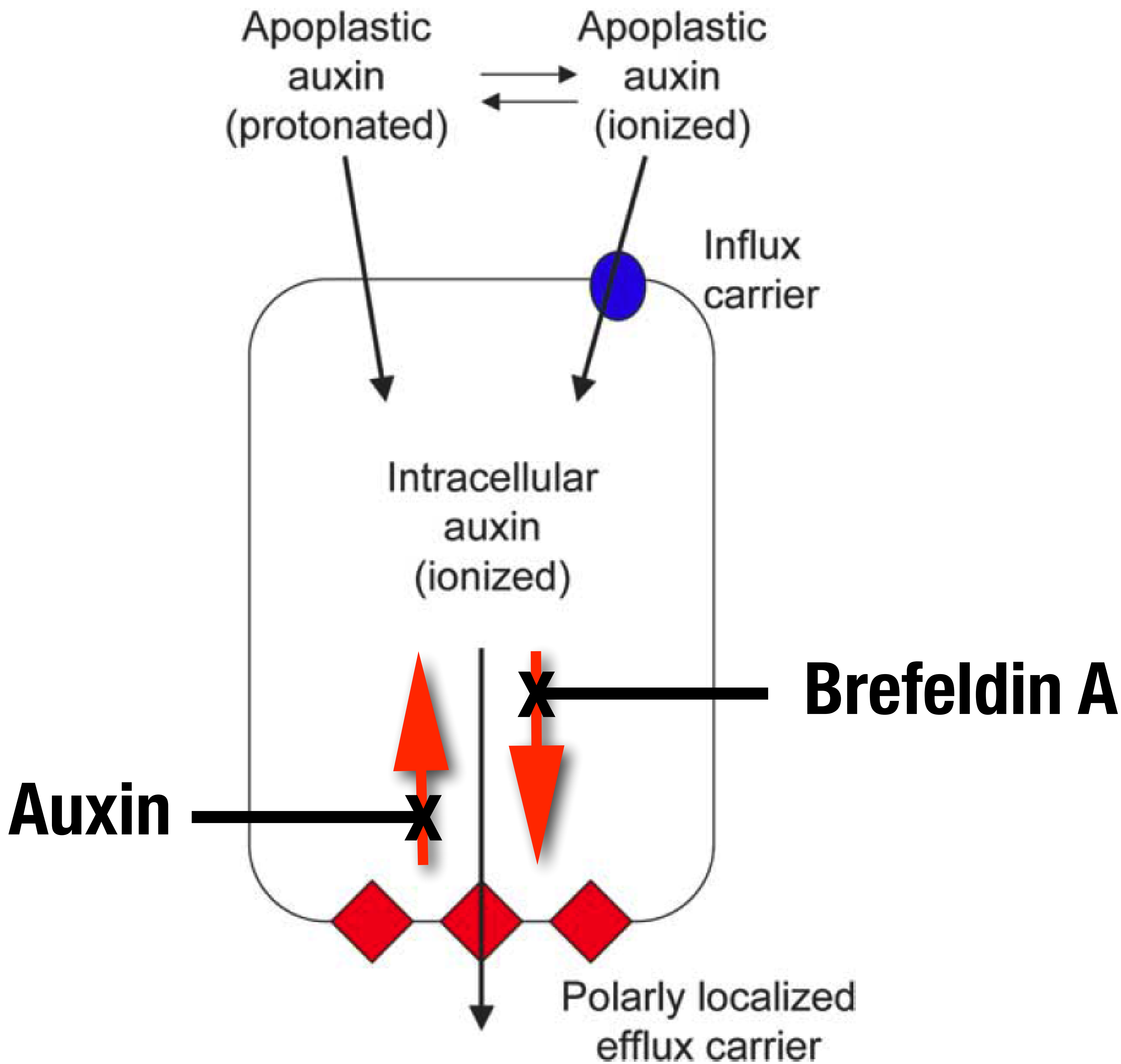


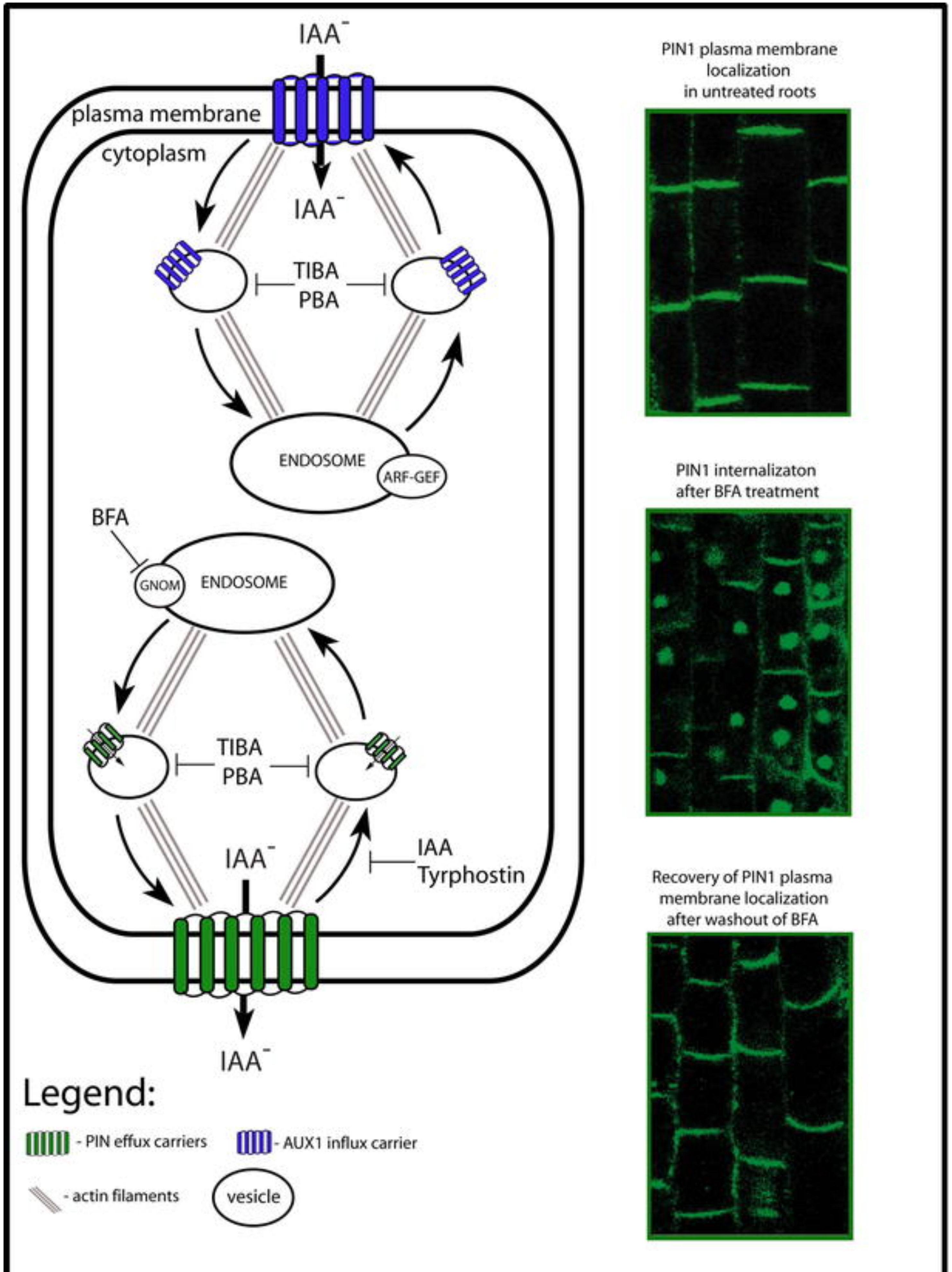
Asymmetric localisation of the PIN1 auxin efflux transporter is a dynamic process and requires the maintenance of polar secretion.

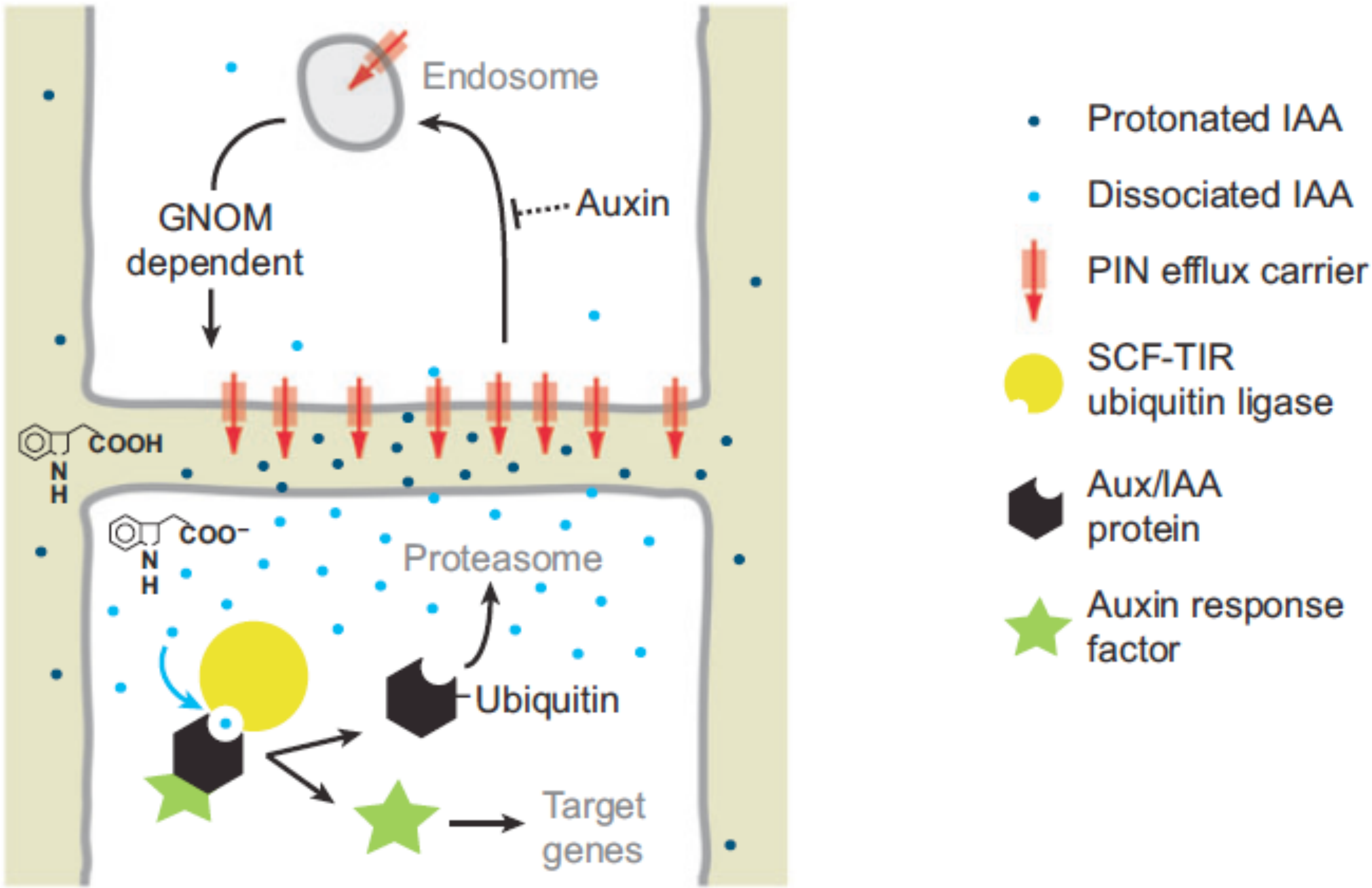




Brefeldin A treatment causes rapid loss of PIN1 localisation.


Apoplastic auxin (protonated)
Apoplastic auxin (ionized)
Influx carrier
Intracellular auxin (ionized)
Brefeldin A
Auxin
Polarly localized efflux carrier


IAA⁻
plasma membrane
cytoplasm
IAA⁻
TIBA
PBA
ENDOSOME
ARF-GEF
BFA
GNOM
ENDOSOME
TIBA
PBA
IAA⁻
IAA
Tyrphostin
IAA⁻
Legend:
- PIN effux carriers
- AUX1 influx carrier
- actin filaments
vesicle
PIN1 plasma membrane localization in untreated roots
PIN1 internalizaton after BFA treatment
Recovery of PIN1 plasma membrane localization after washout of BFA

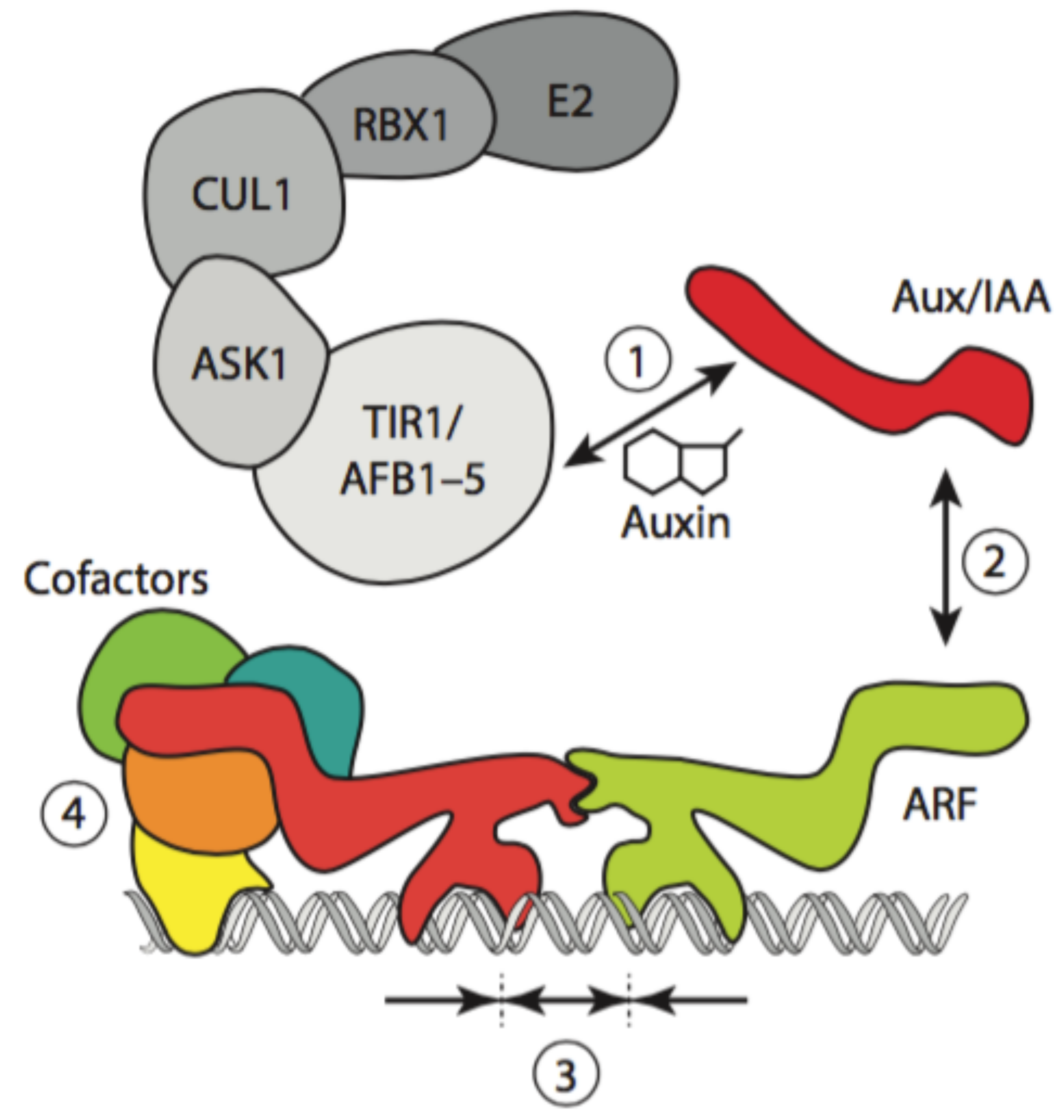

Endosome
GNOM
dependent
Auxin
COOH
N
H
COO⁻
N
H
Proteasome
Ubiquitin
Target
genes
Protonated IAA
Dissociated IAA
PIN efflux carrier
SCF-TIR
ubiquitin ligase
Aux/IAA
protein
Auxin response
factor

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

OVERVIEW:

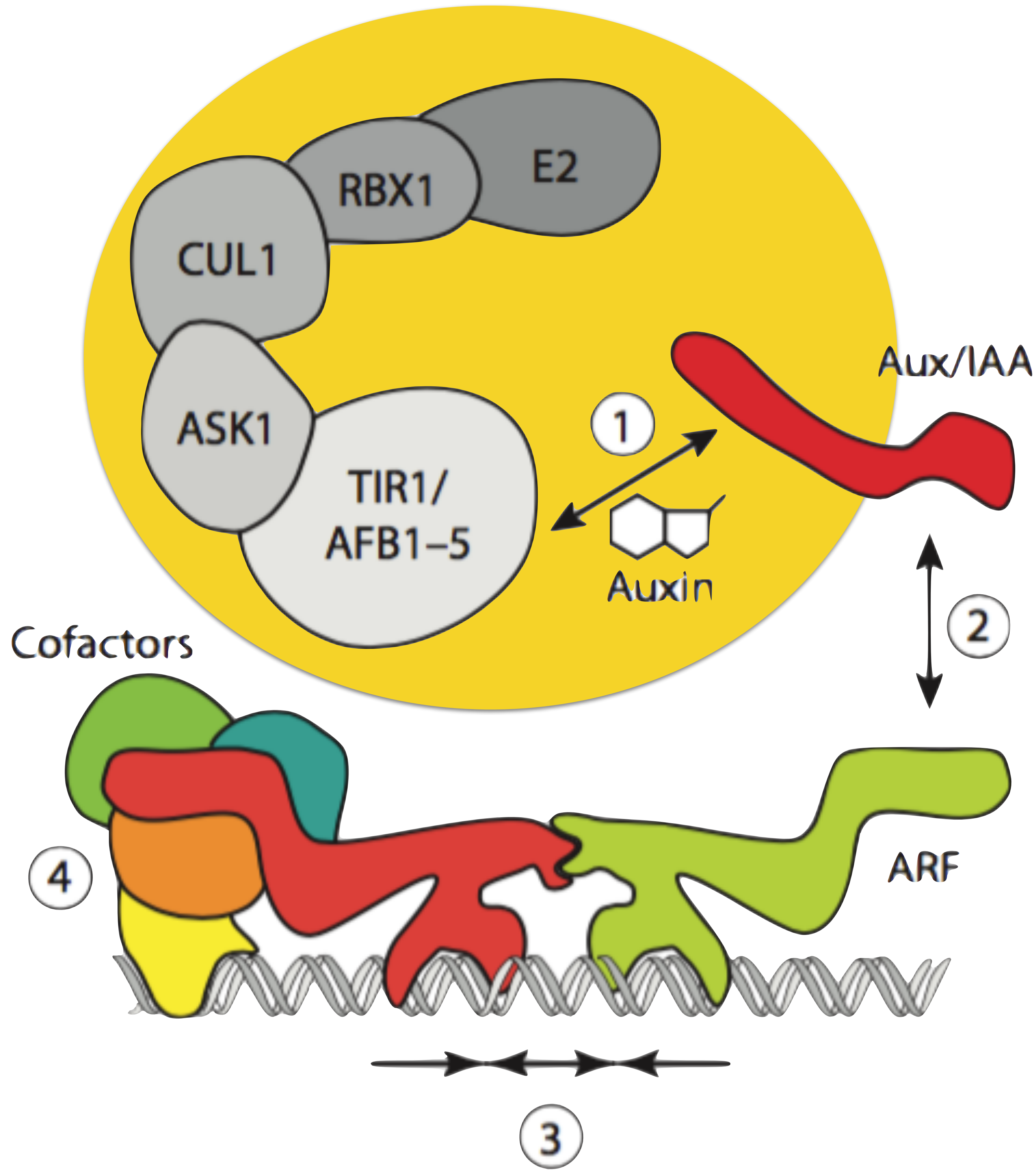
Regulation of gene expression by auxin

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 and chromatin remodelling

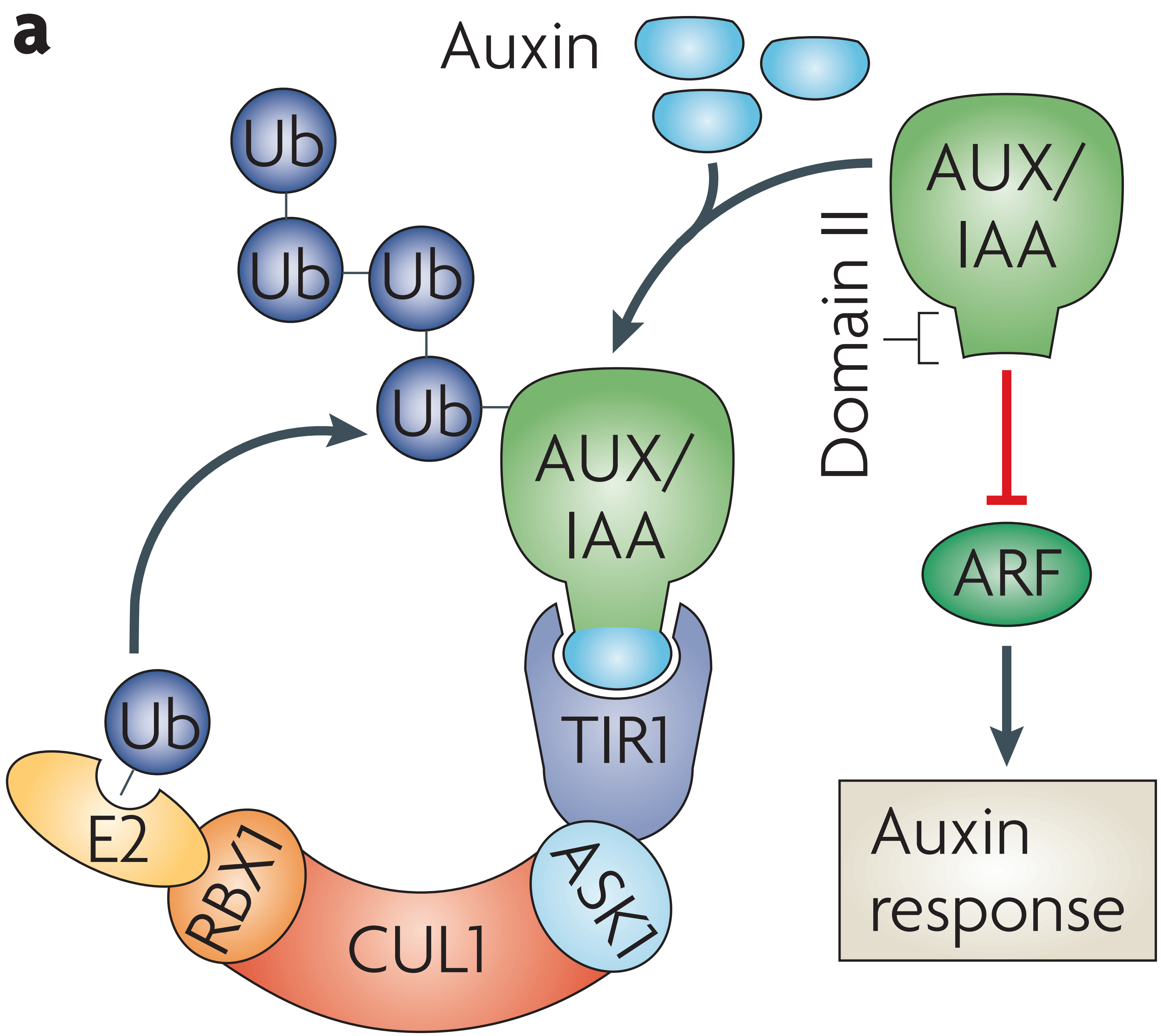


1. Intracellular binding of auxin

SCF complex
SKP/ASK1
Cullin/CUL1
F-Box/TIR1



TIR1-mediated mediated binding of auxin



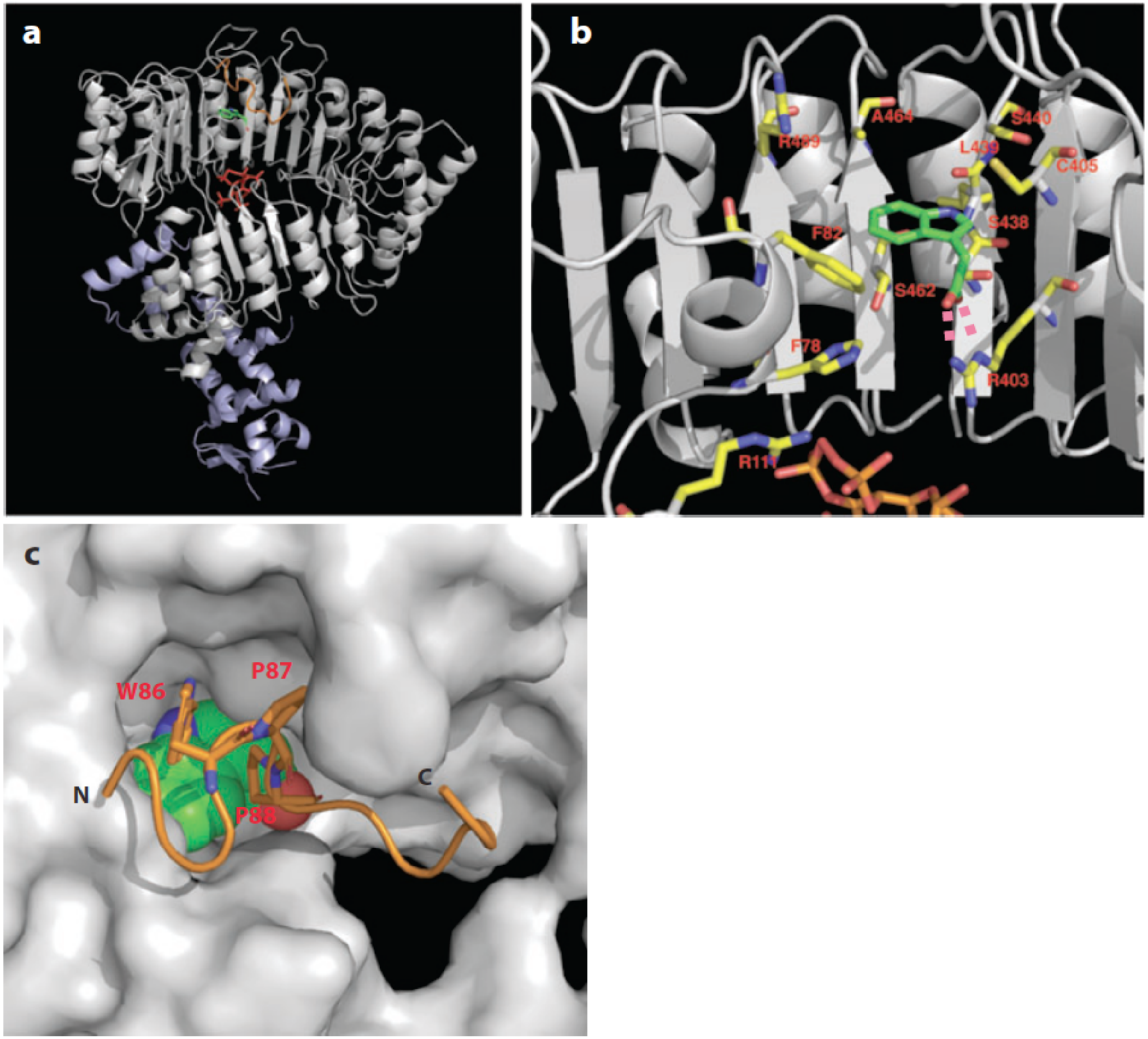
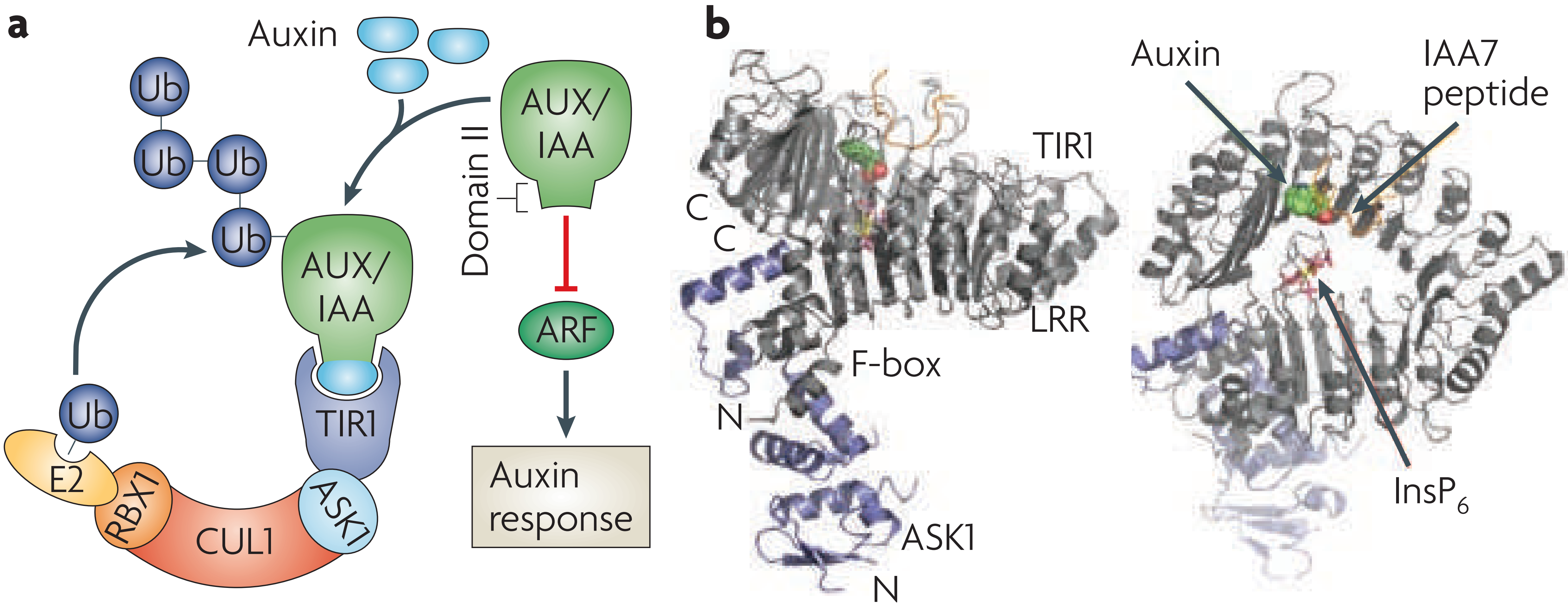


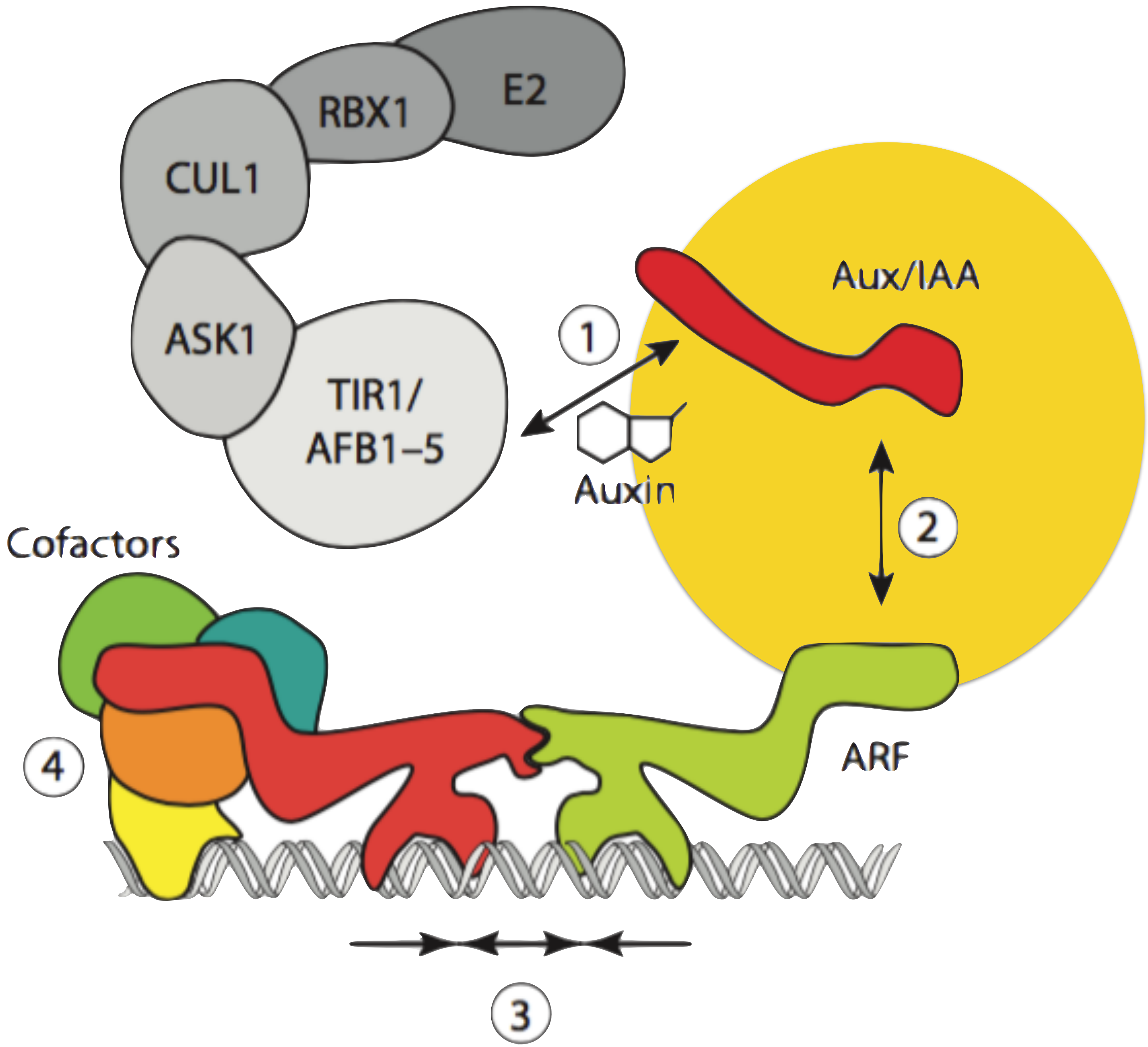
Figure 2

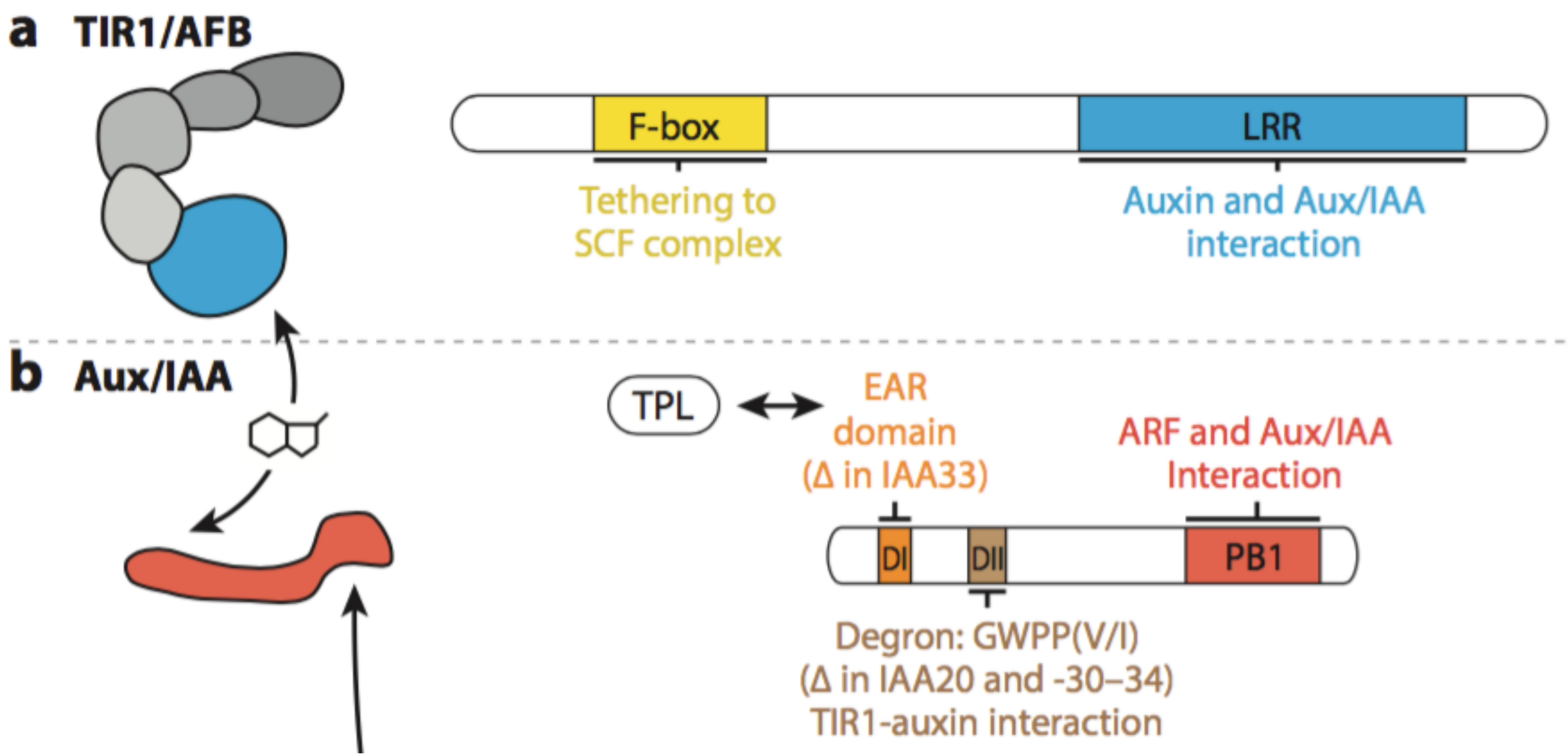
Auxin perception by the F-box protein TIR1. (*a*) Structure of TIR1 (*gray*) in complex with ASK1 (*dark blue*), indole-3-acetic acid (IAA) (*green*), Aux/IAA domain II peptide (*orange*), and inositol hexakisphosphate (*red*). (*b*) Close-up of the auxin-binding pocket occupied by IAA (*green*). Surrounding 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*).

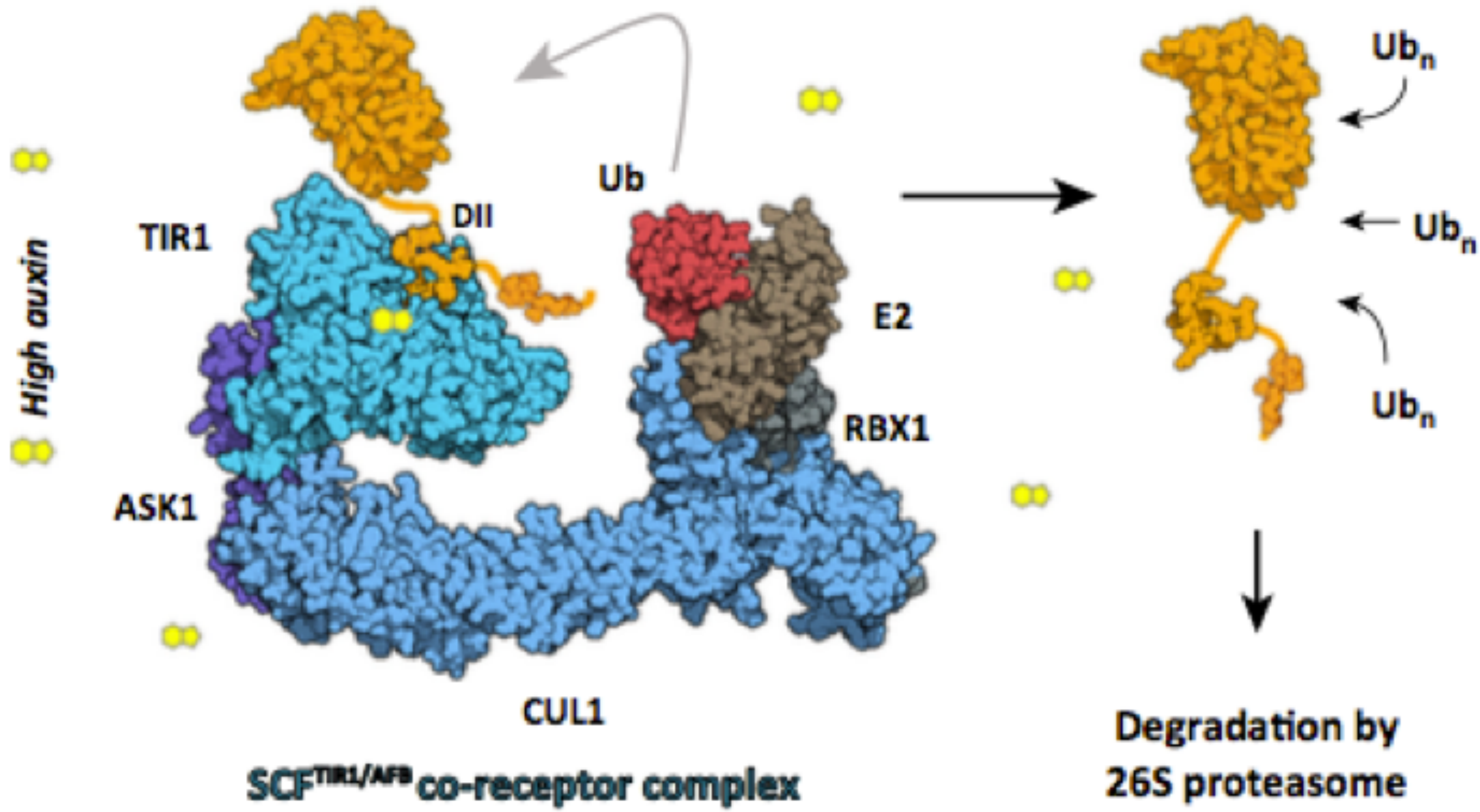
TIR1-mediated ubiquitination of AUX/IAA proteins



2. Targeted degradation of AUX/IAA repressors




a TIR1/AFB
F-box
LRR
Tethering to
SCF complex
Auxin and Aux/IAA
interaction
b Aux/IAA
TPL
EAR
domain
(Δ in IAA33)
ARF and Aux/IAA
Interaction
DI
DII
PB1
Degron: GWPP(V/I)
(Δ in IAA20 and -30–34)
TIR1-auxin interaction


High auxin
TIR1
DII
Ub
E2
RBX1
ASK1
CUL1
SCF$^{TIR1/AFB}$ co-receptor complex
Ub$_n$
Ub$_n$
Ub$_n$
Degradation by 26S proteasome

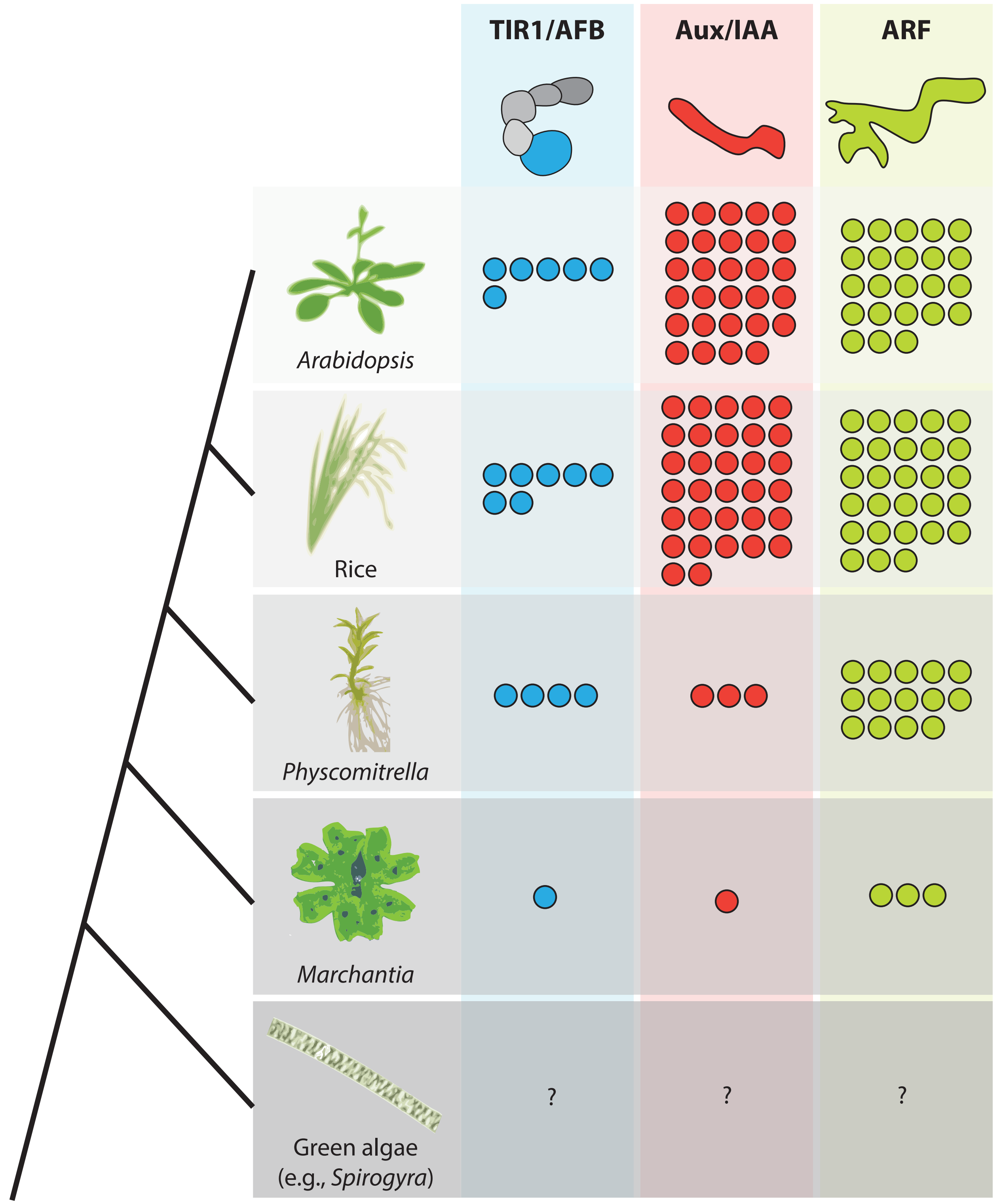
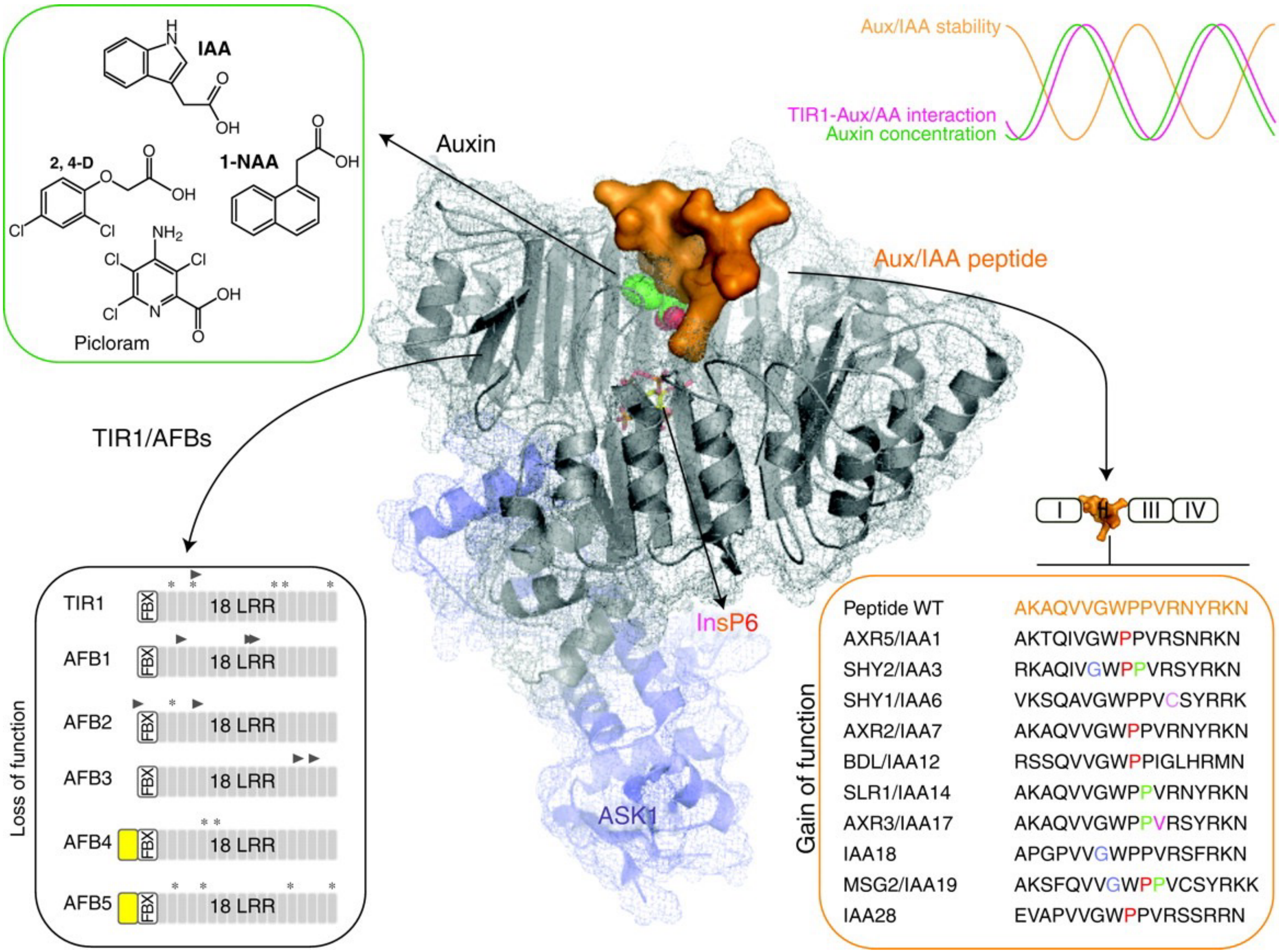
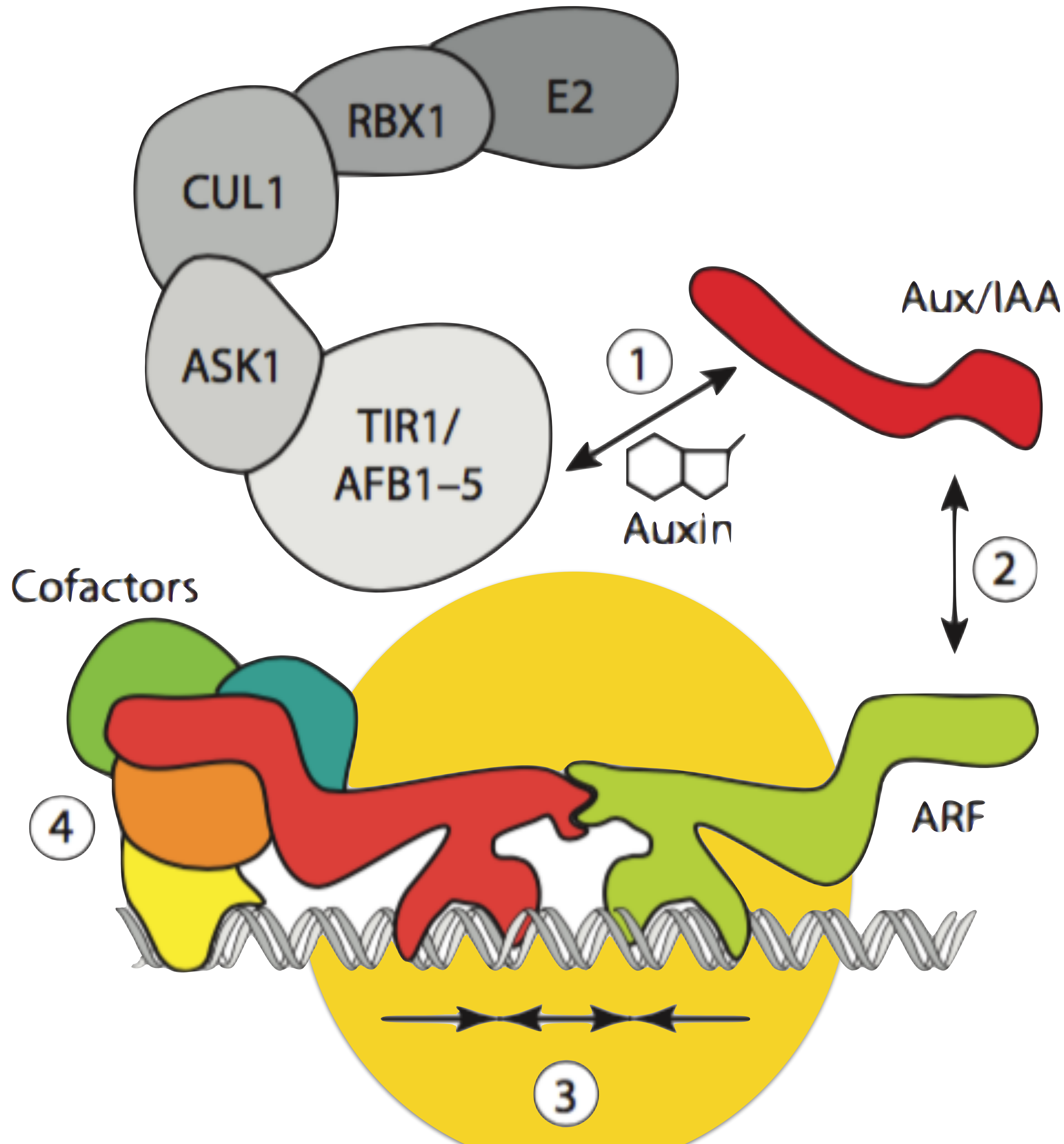


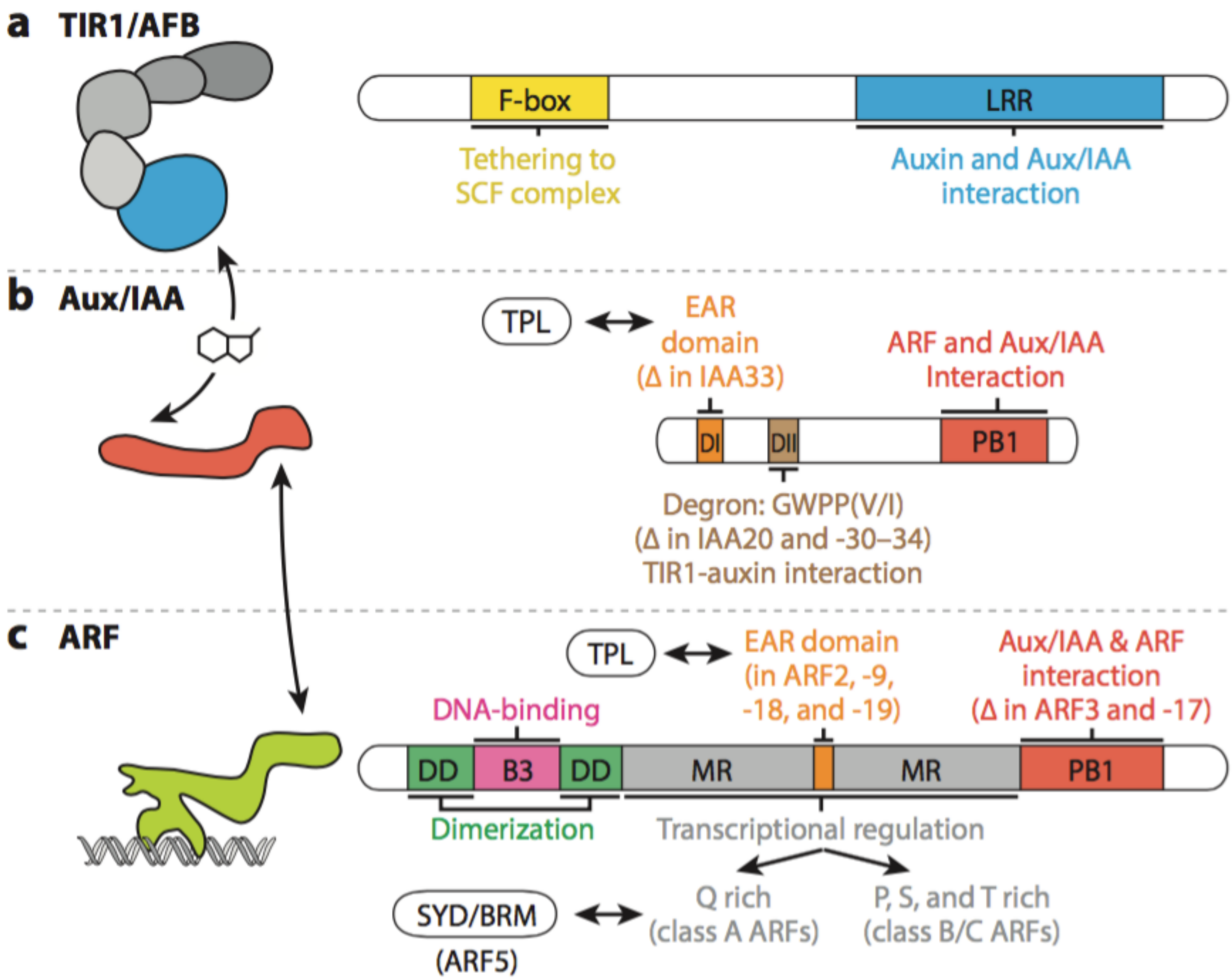
Figure 7

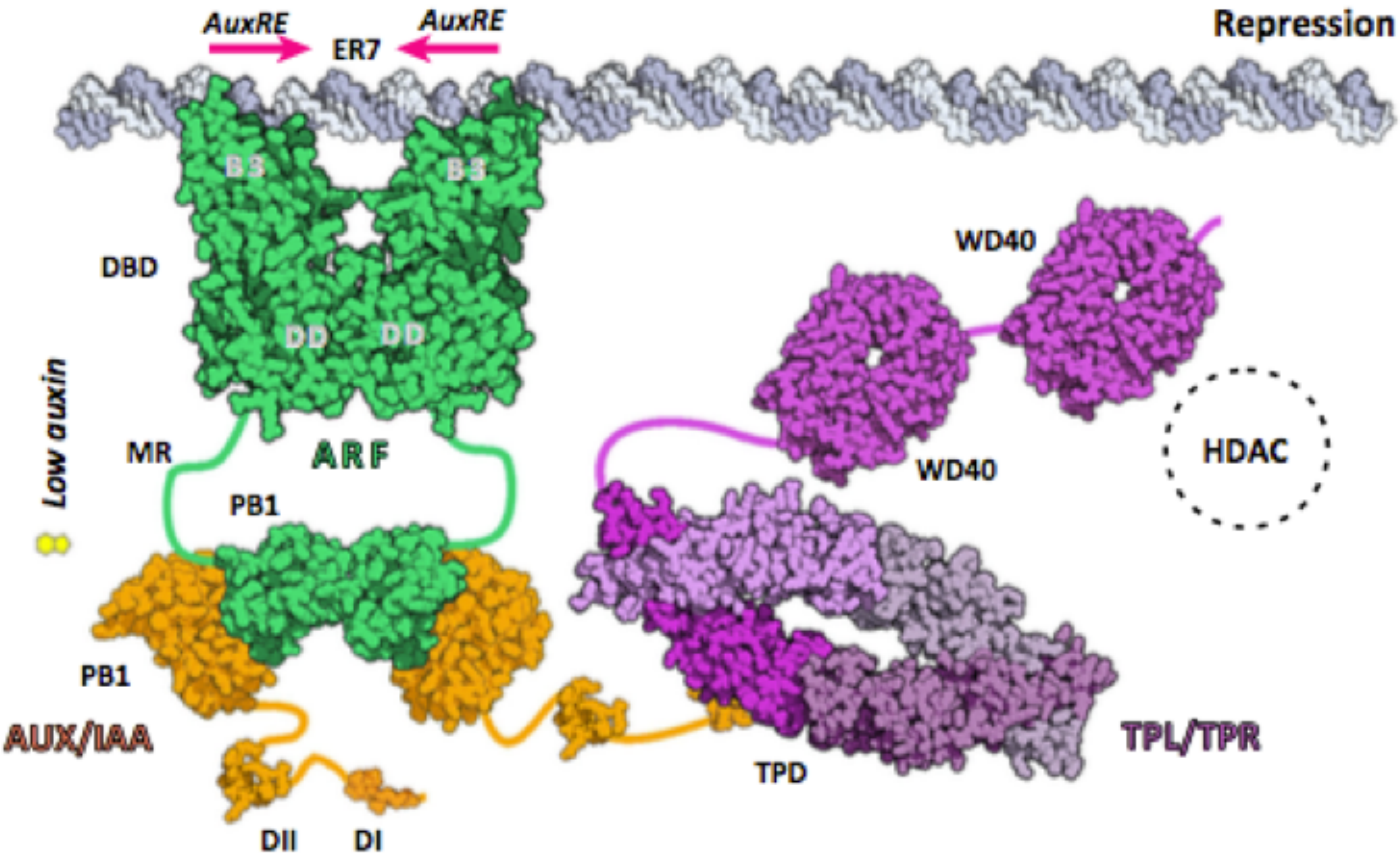
The evolution of the auxin response pathway, showing the distribution of genes encoding TIR1/AFB, Aux/IAA, and ARF proteins in published plant genomes for several plant species. These species represent eudicots (*Arabidopsis*), monocots (rice), mosses (*Physcomitrella*), liverworts (*Marchantia*), and 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.

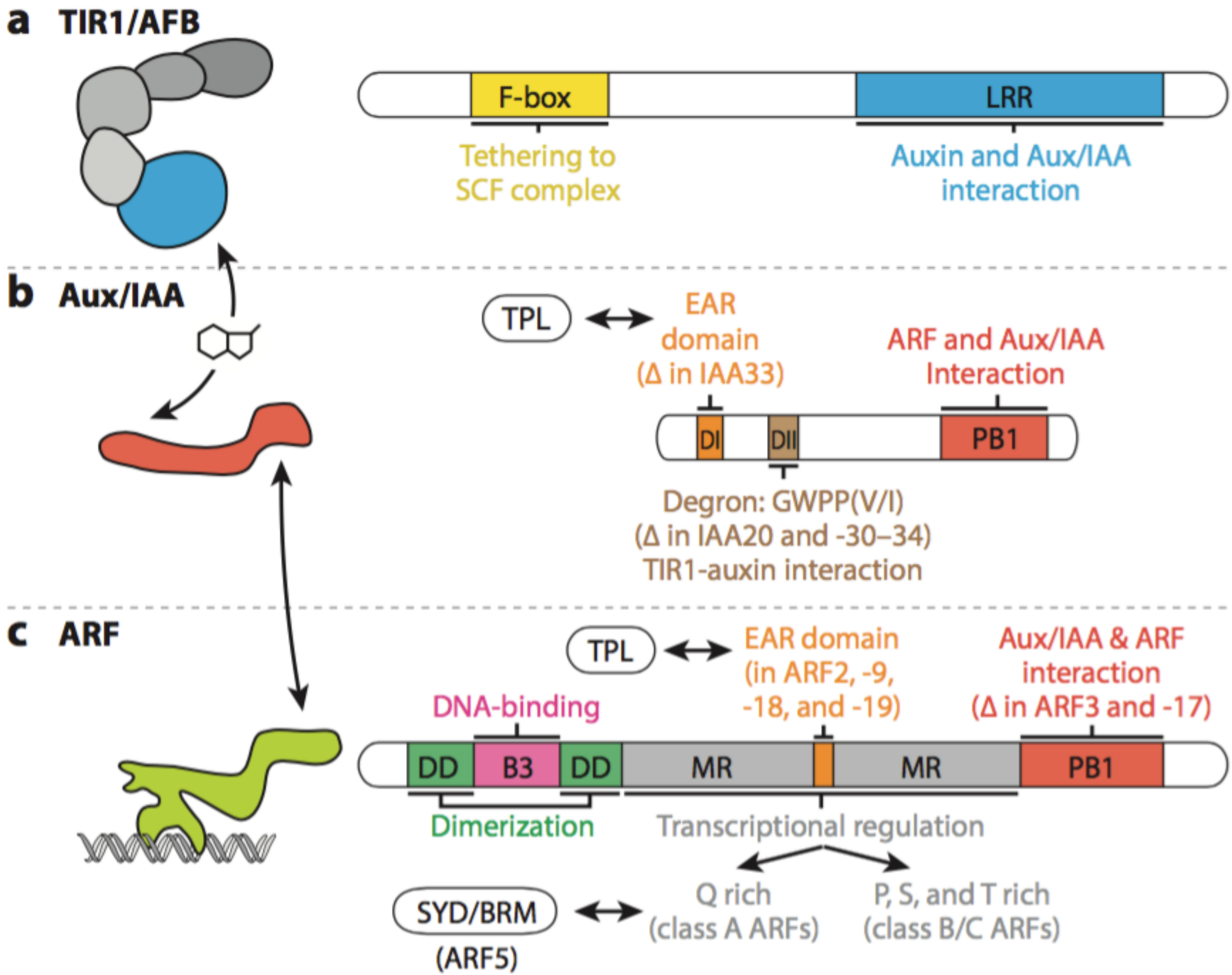

IAA
2, 4-D
1-NAA
Picloram
Auxin
Aux/IAA stability
TIR1-Aux/AA interaction
Auxin concentration
Aux/IAA peptide
TIR1/AFBs
InsP6
ASK1
I
II
III
IV
Loss of function
TIR1
AFB1
AFB2
AFB3
AFB4
AFB5
FBX
18 LRR
Gain of function
Peptide WT AKAQVVGWPPVRNYRKN
AXR5/IAA1 AKTQIVGWPPVRSNRKN
SHY2/IAA3 RKAQIVGWPPVRSYRKN
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
F-box
LRR
Tethering to SCF complex
Auxin and Aux/IAA interaction
b Aux/IAA
TPL
EAR domain (Δ in IAA33)
ARF and Aux/IAA Interaction
DI
DII
PB1
Degron: GWPP(V/I) (Δ in IAA20 and -30–34) TIR1-auxin interaction
c ARF
TPL
EAR domain (in ARF2, -9, -18, and -19)
Aux/IAA & ARF interaction (Δ in ARF3 and -17)
DNA-binding
DD
B3
DD
MR
MR
PB1
Dimerization
Transcriptional regulation
Q rich (class A ARFs)
P, S, and T rich (class B/C ARFs)
SYD/BRM
(ARF5)


AuxRE
ER7
AuxRE
Repression
B3
B3
DBD
DD
DD
WD40
Low auxin
MR
ARF
WD40
HDAC
PB1
PB1
AUX/IAA
TPL/TPR
TPD
DII
DI


a TIR1/AFB
F-box
LRR
Tethering to SCF complex
Auxin and Aux/IAA interaction
b Aux/IAA
TPL
EAR domain (Δ in IAA33)
ARF and Aux/IAA Interaction
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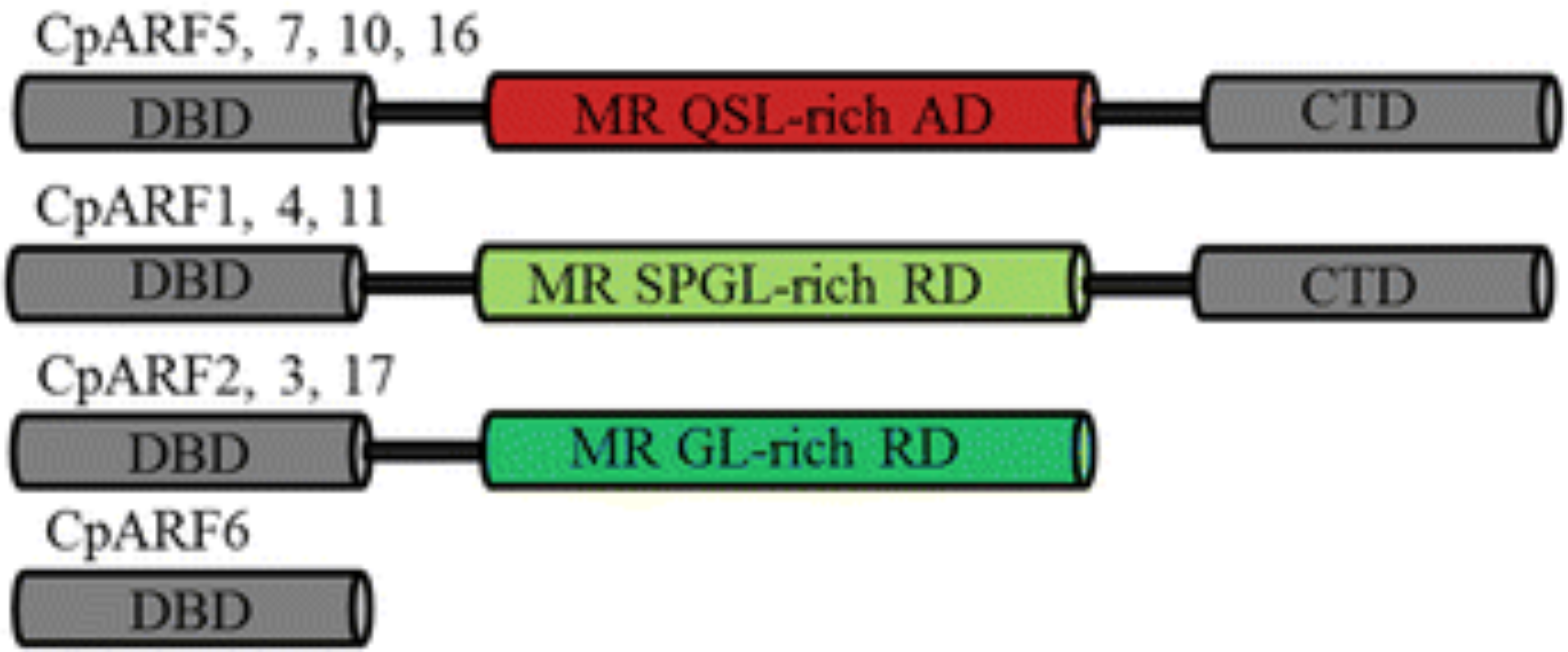
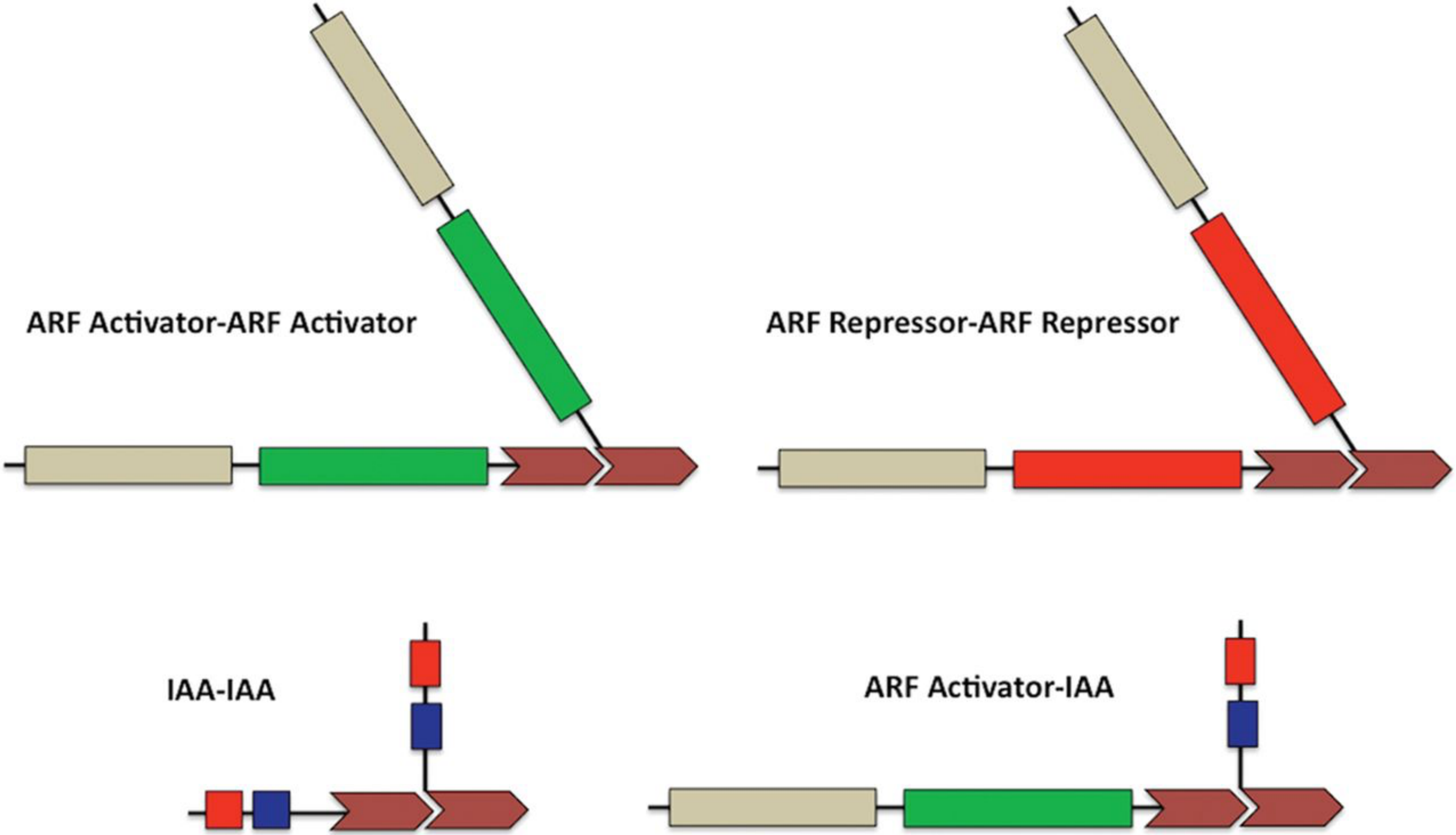
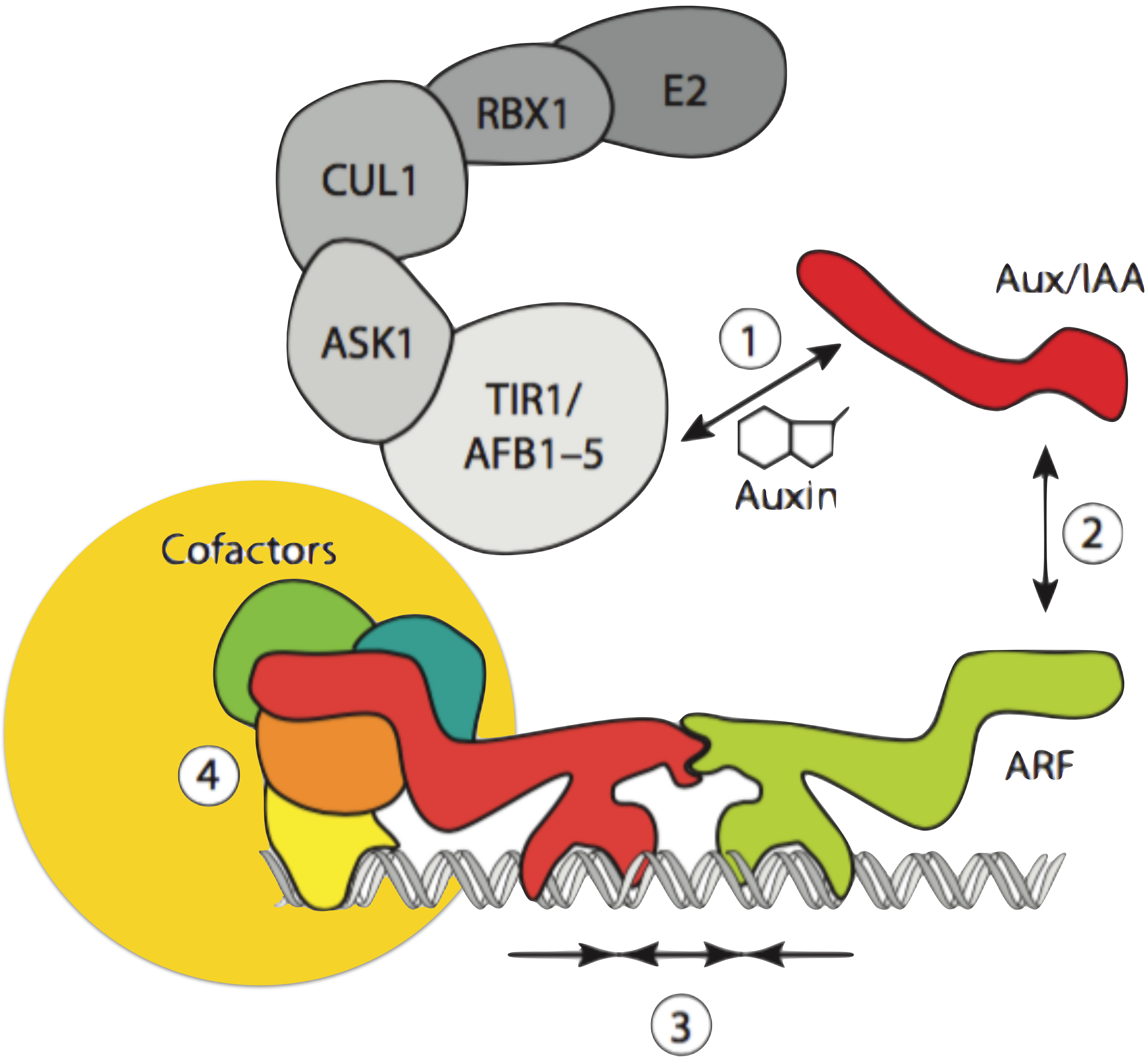
DII
PB1
Degron: GWPP(V/I)
(Δ in IAA20 and -30–34)
TIR1-auxin interaction
c ARF
TPL
EAR domain (in ARF2, -9, -18, and -19)
Aux/IAA & ARF interaction (Δ in ARF3 and -17)
DNA-binding
DD
B3
DD
MR
MR
PB1
Dimerization
Transcriptional regulation
Q rich (class A ARFs)
P, S, and T rich (class B/C ARFs)
SYD/BRM
(ARF5)

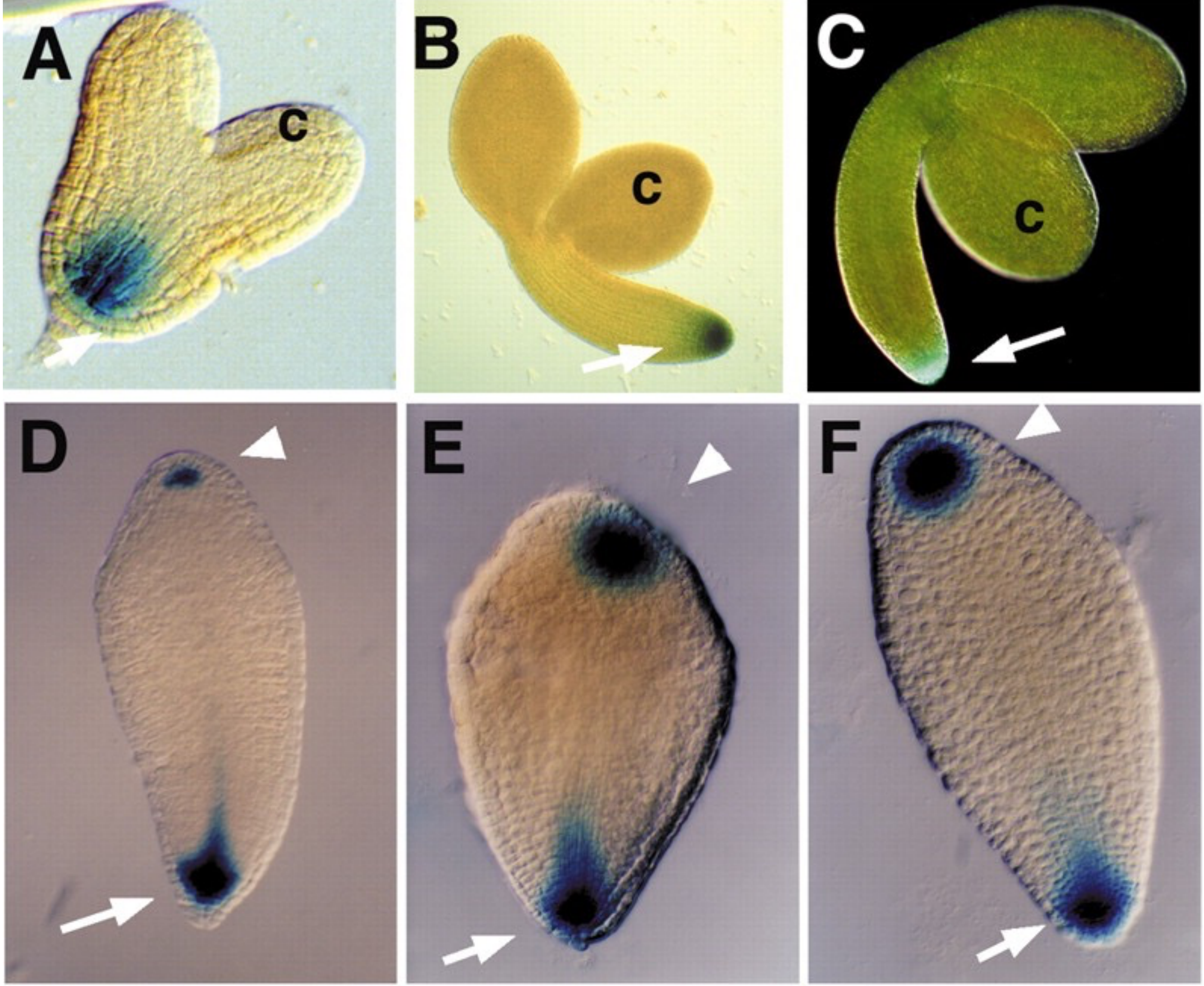


The protein structure of ARFs.
DBD, DNA-binding domain; CTD, C-terminal dimerization domain; MR, middle region; RD, repression domain; AD, activation domain;

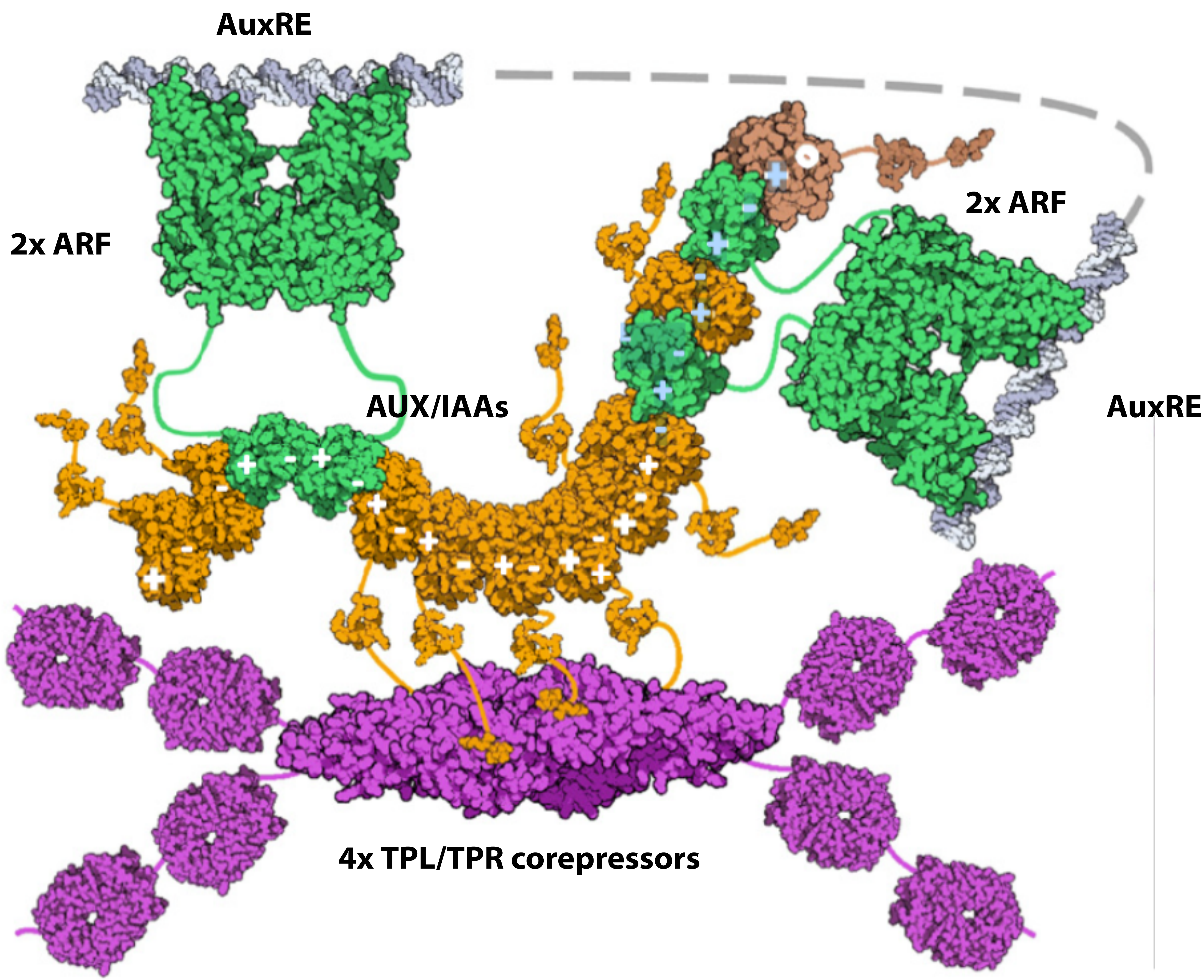

ARF Activator-ARF Activator
ARF Repressor-ARF Repressor
IAA-IAA
ARF Activator-IAA

4. Recruitment of protein co-factors for maintenance of gene expression

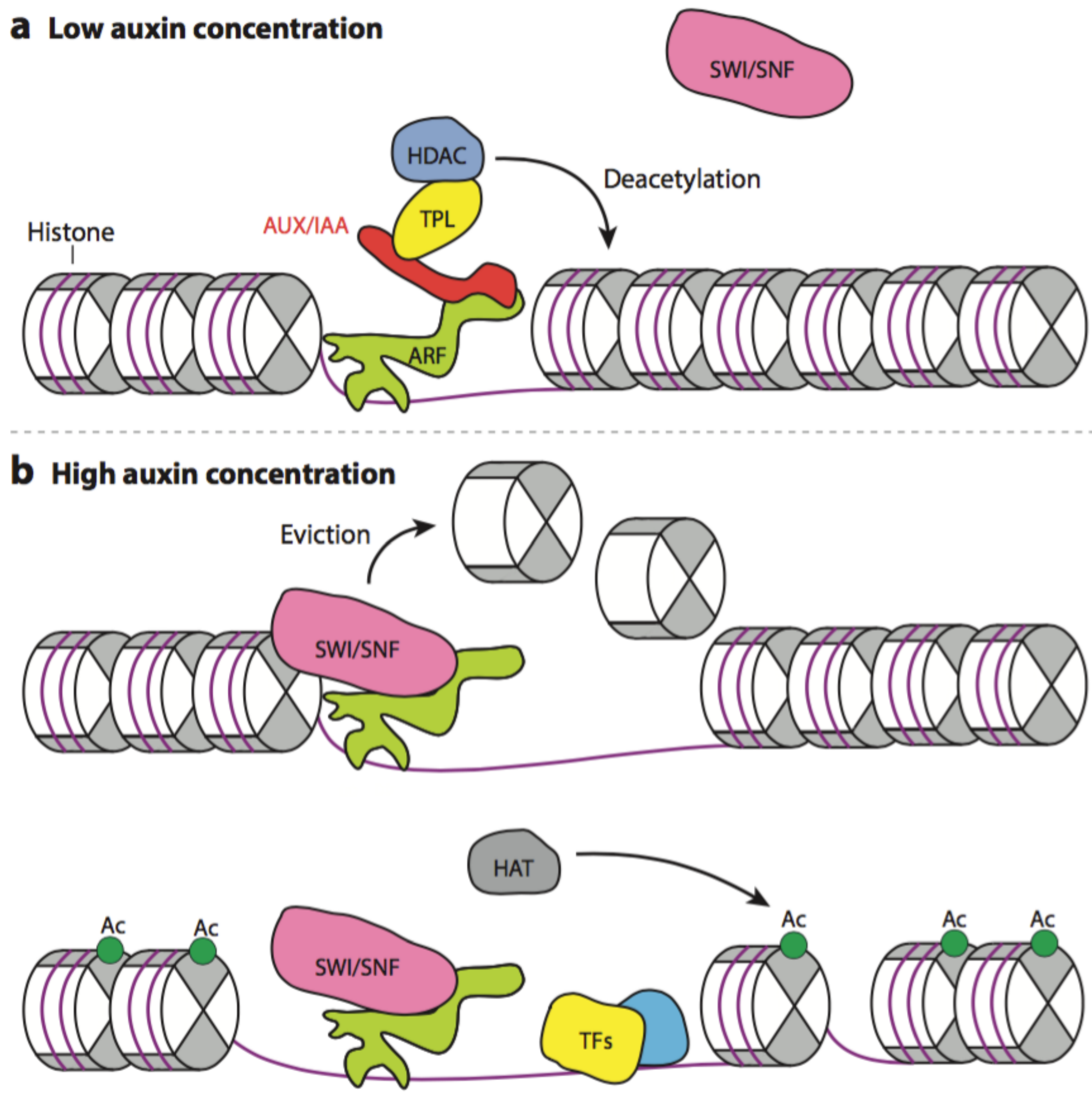




topless **(*tpl*) mutant**



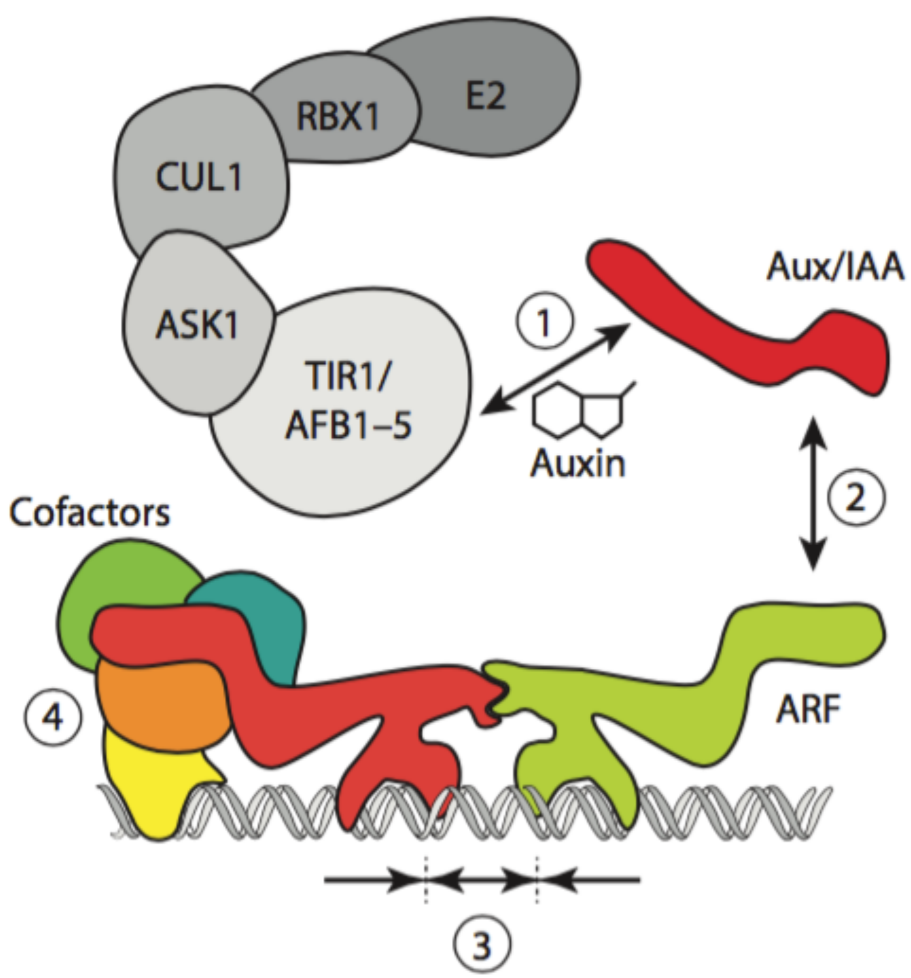
Recognition of composite AuxREs and recruitment of tetrameric TPL/TPR corepressors

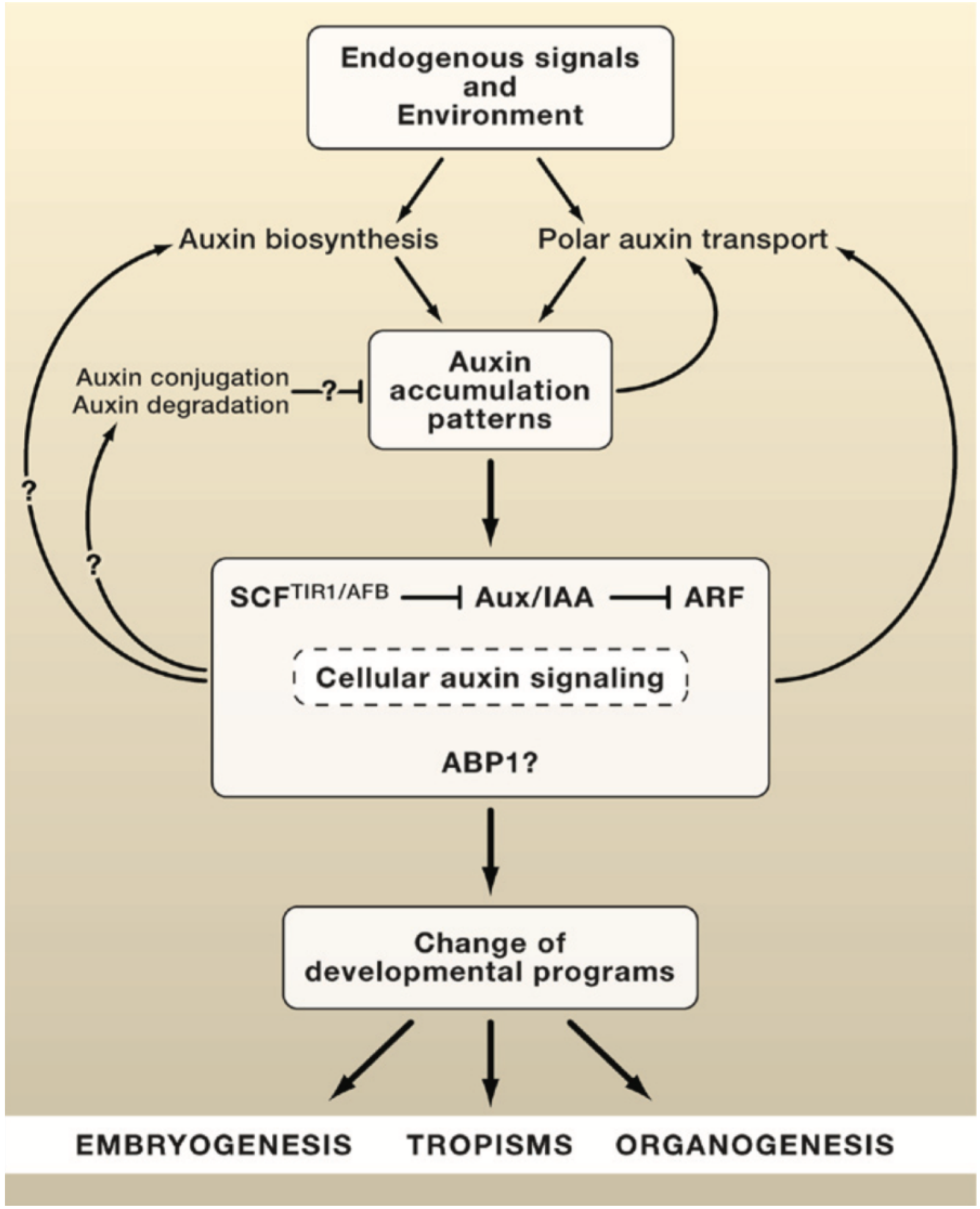


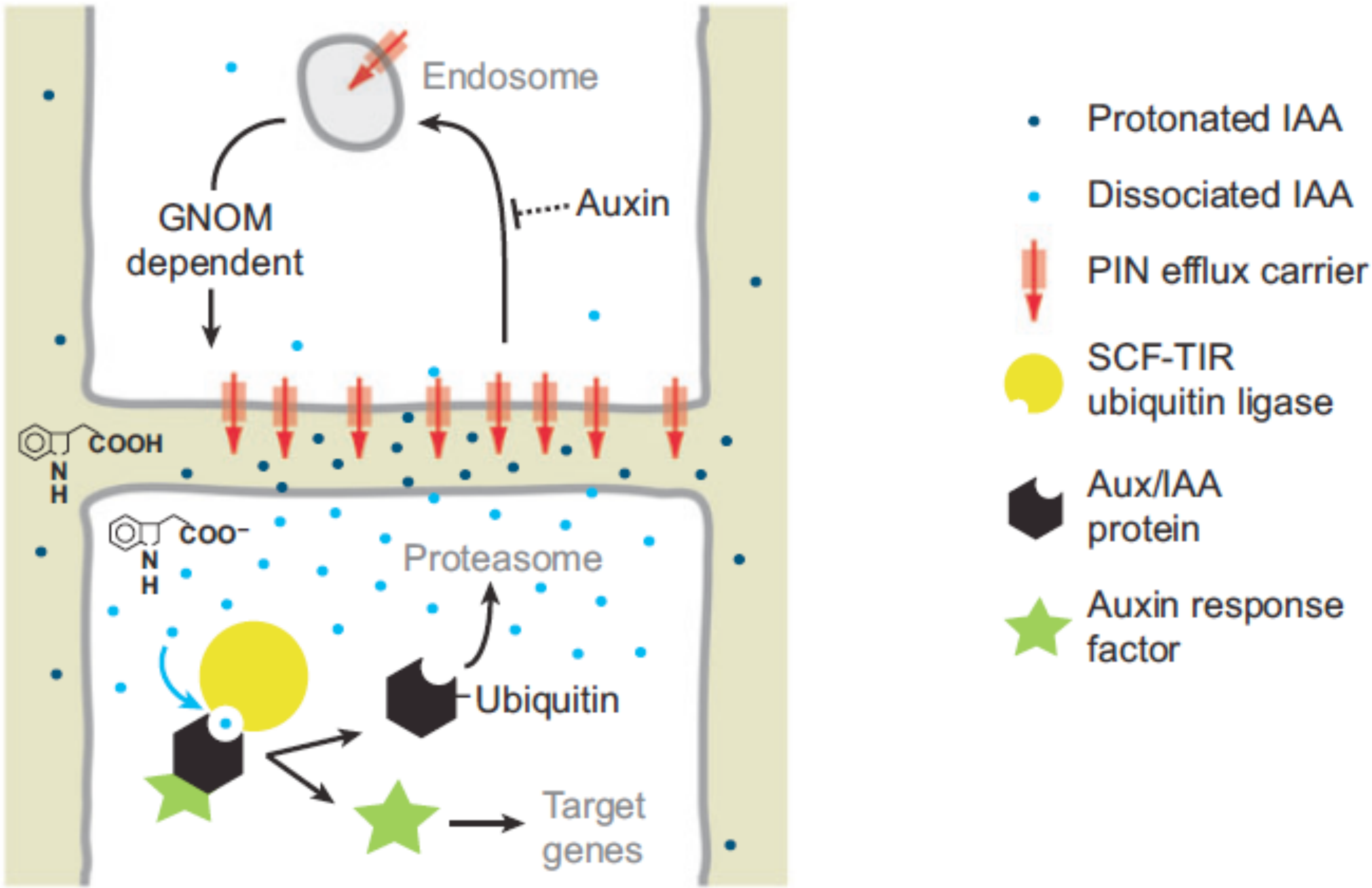
Recruitment of Switch/Sucrose Non-Fermenting (SWI/SNF) and Histone Acetyl Transferase (HAT) complexes for remodelling chromatin

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







Endosome
GNOM
dependent
Auxin
COOH
N
H
COO⁻
N
H
Proteasome
Ubiquitin
Target
genes
Protonated IAA
Dissociated IAA
PIN efflux carrier
SCF-TIR
ubiquitin ligase
Aux/IAA
protein
Auxin response
factor