2018 CDB Part IB
Plant Development

Lecture 3

Regulation of gene expression by auxin

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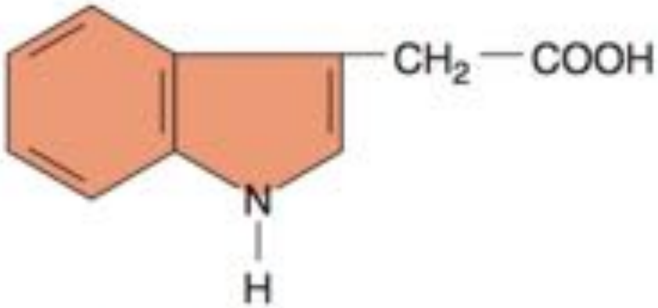
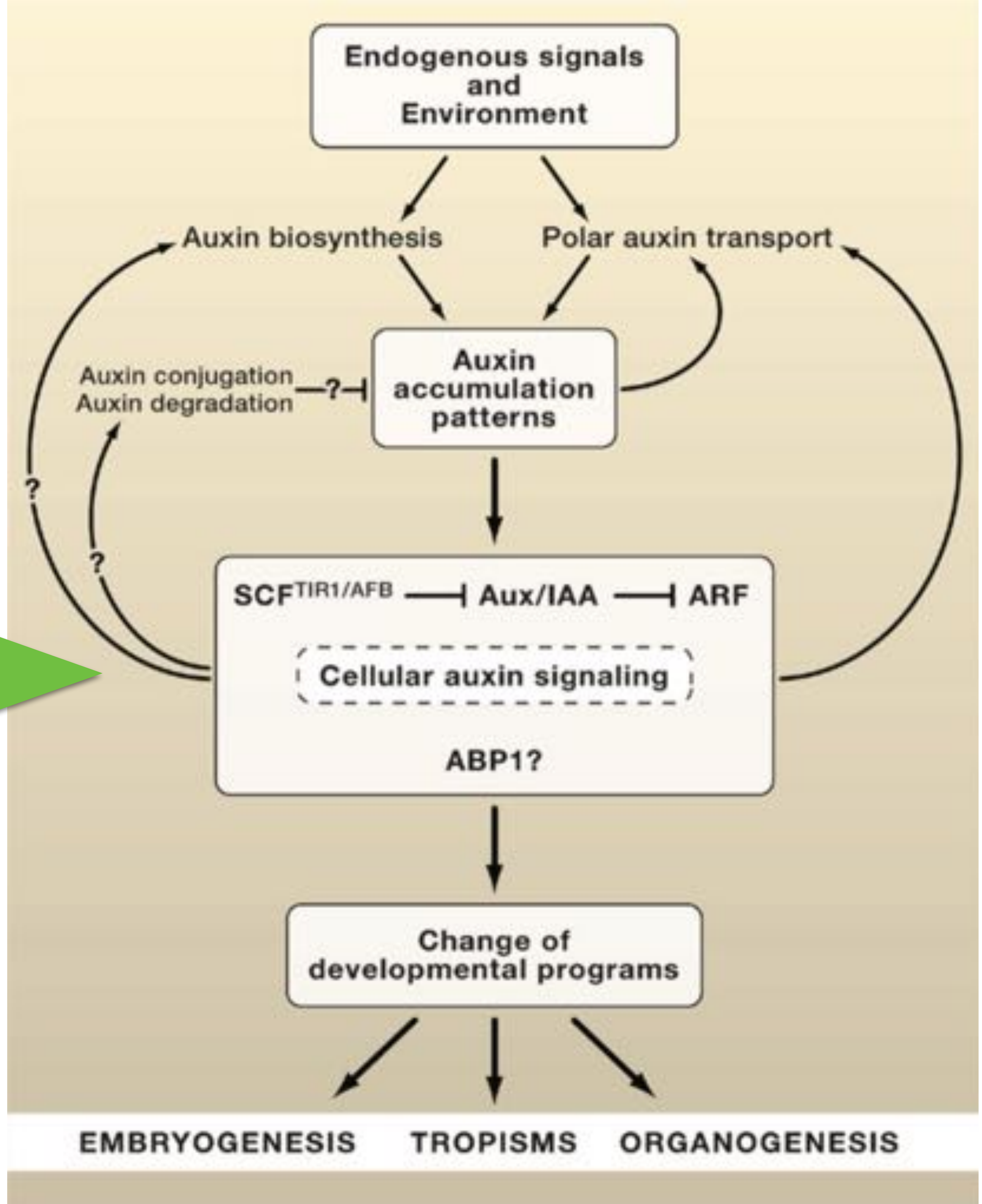
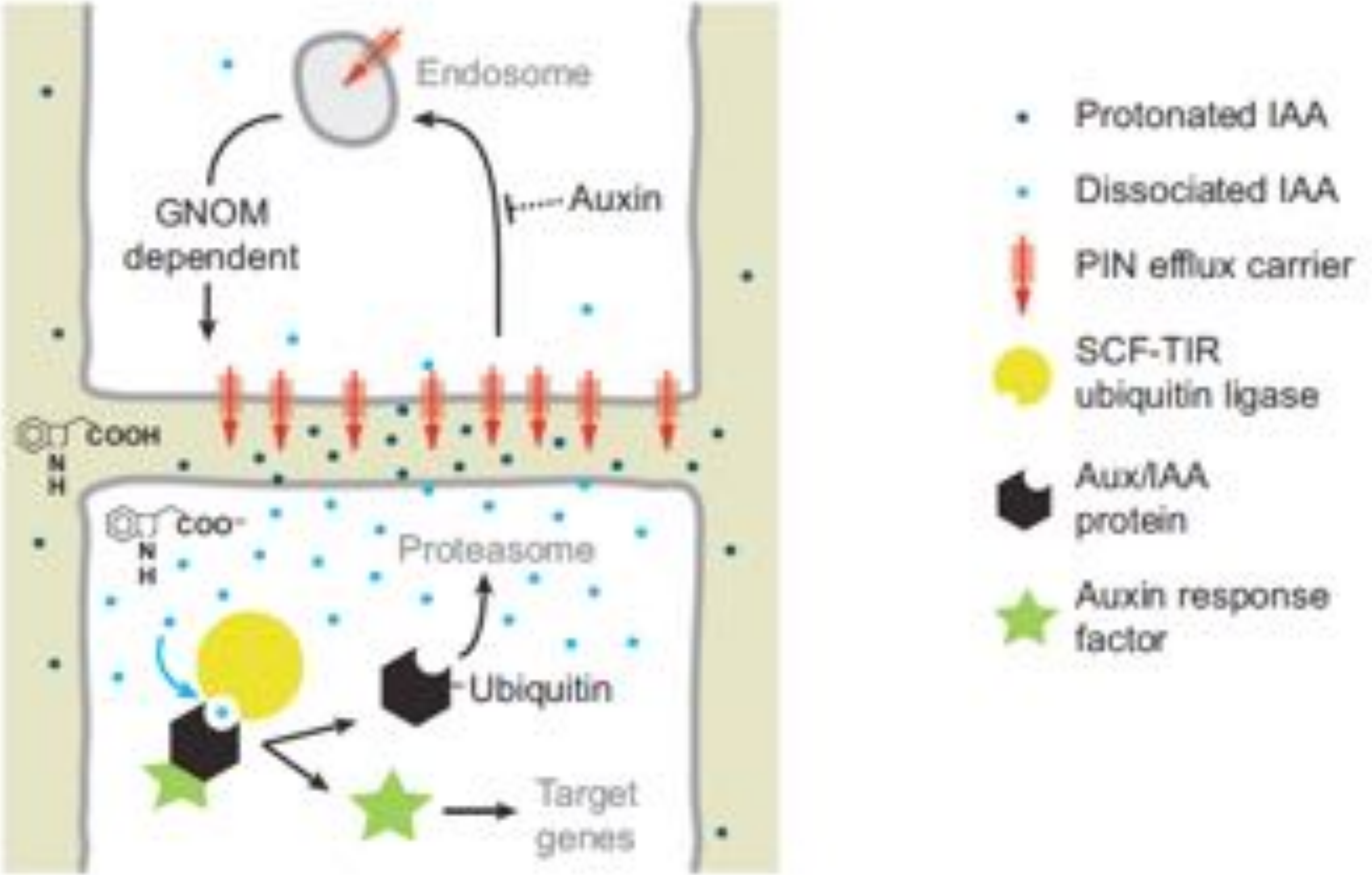


(a) **IAA (Indoleacetic acid)**

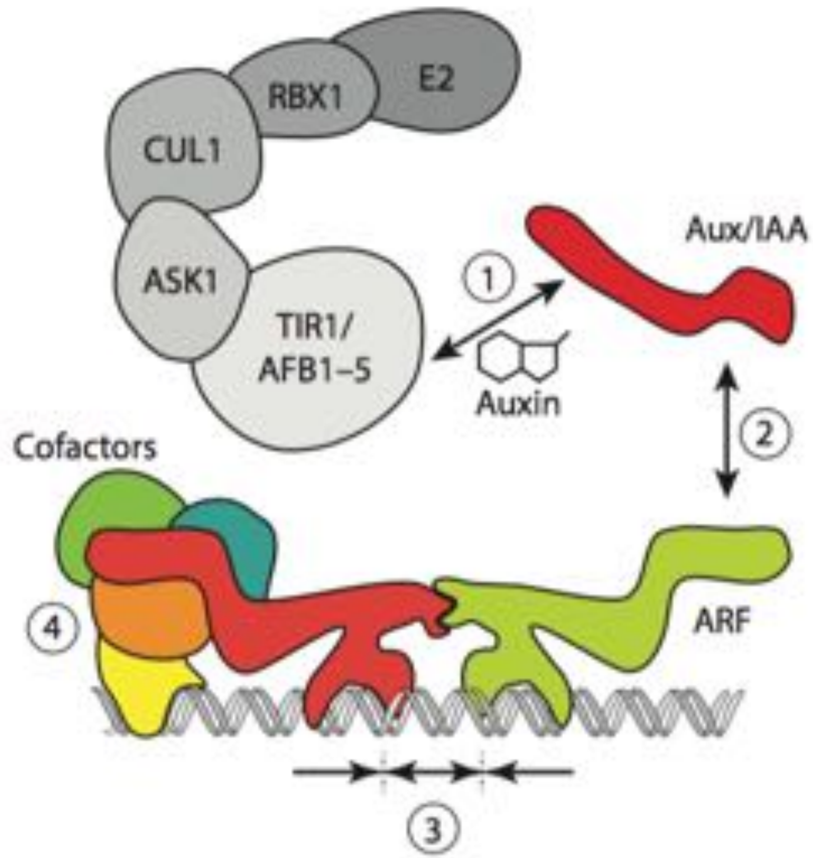



Endosome
GNOM dependent
Auxin
COOH
N H
COO-
Proteasome
Ubiquitin
Target genes
Protonated IAA
Dissociated IAA
PIN efflux carrier
SCF-TIR ubiquitin ligase
Aux/IAA protein
Auxin response factor

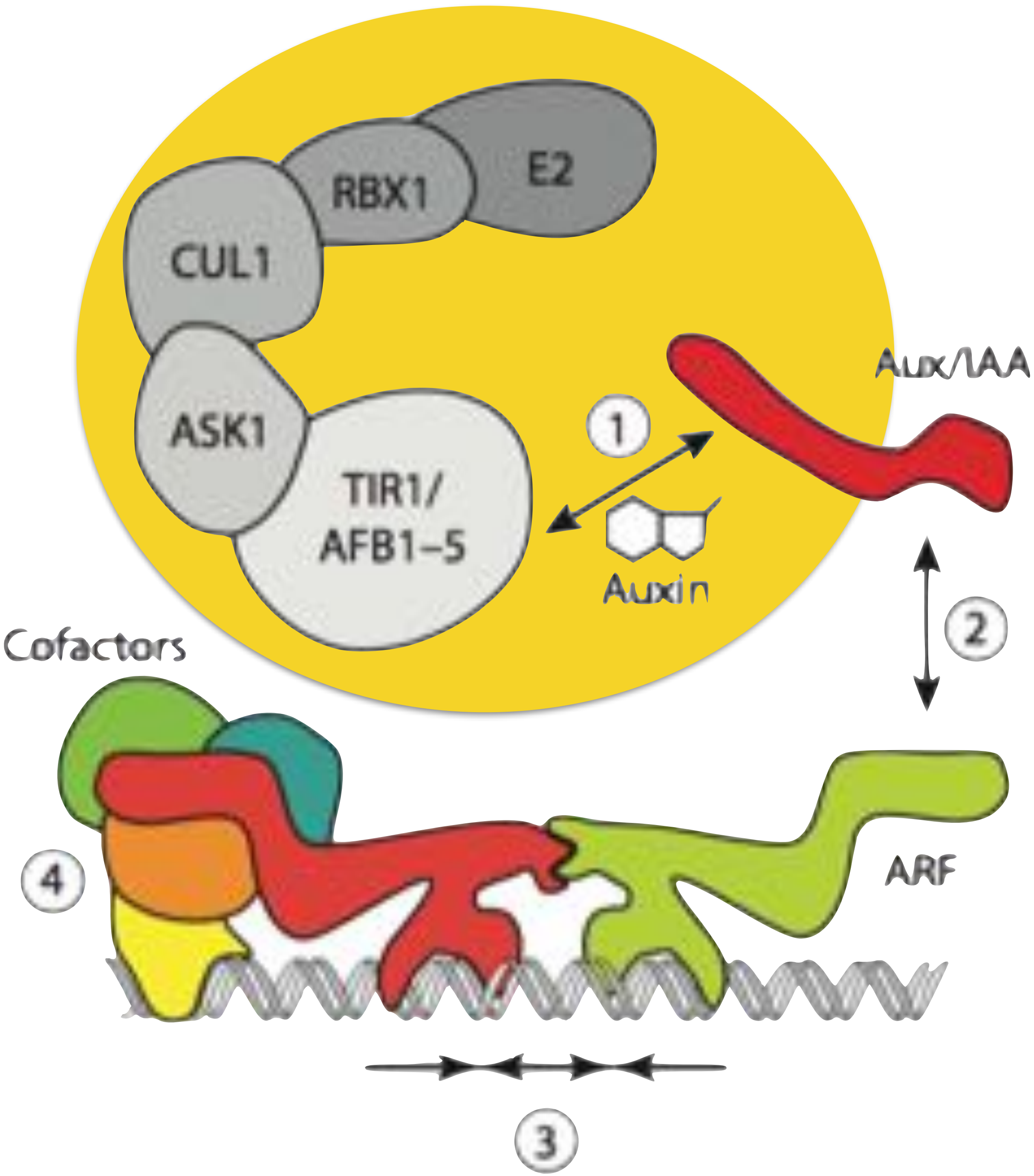
OVERVIEW:

Regulation of gene expression by auxin

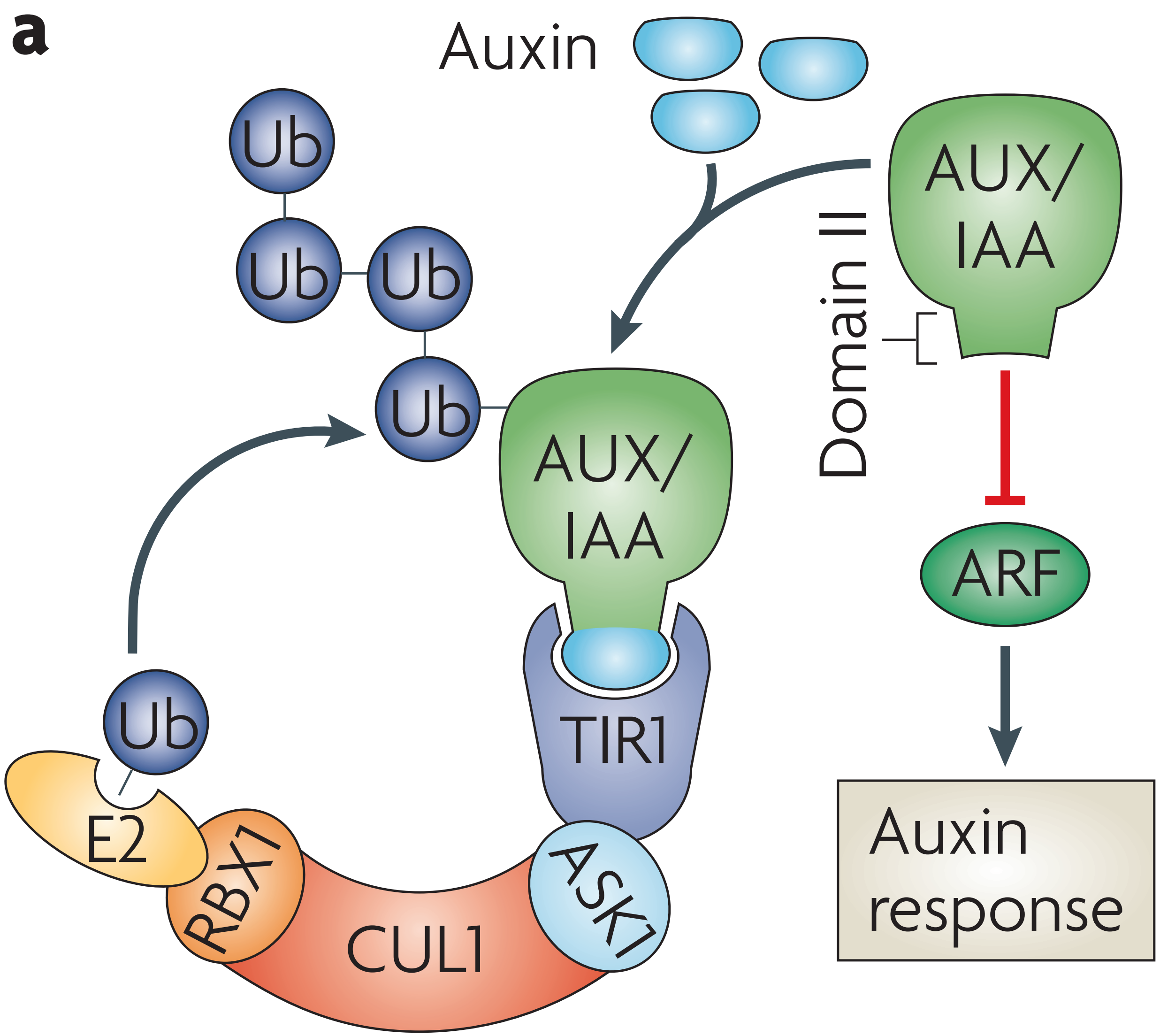
1. Intracellular binding of auxin
2. Targeted degradation of Aux/IAA repressors
3. Selective activation of genes by ARF binding to auxin responsive promoter elements
4. Recruitment of protein co-factors for maintenance of gene expression and chromatin remodelling



1. Intracellular binding of auxin



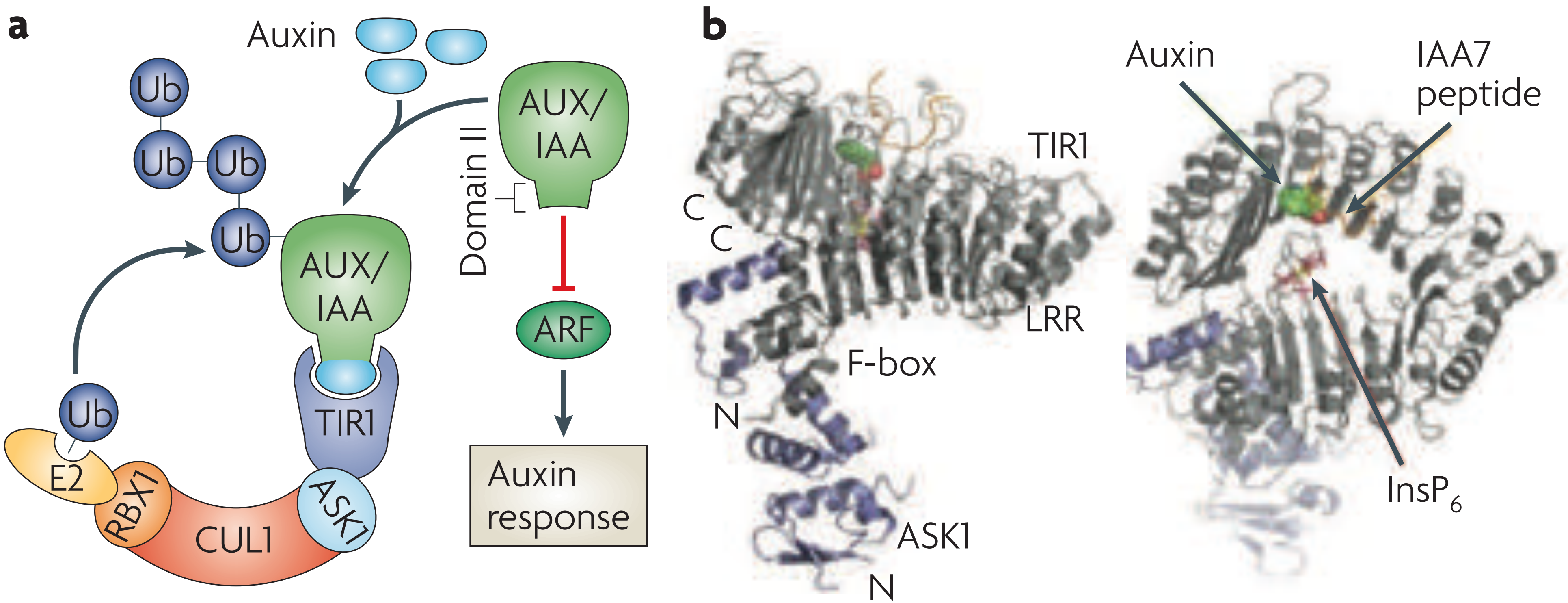
TIR1-mediated mediated binding of auxin



Auxin receptors in Arabidopsis (6)

Gene	Product	Function	Genetic evidence for role in auxin-mediated development	References
TIR1	Transport inhibitor response 1, TIR1 F-box protein	Interacts with ASK1; interacts with Aux/IAAs; auxin increases Aux/IAA affinity; crystal structure shows TIR1-auxin-Aux/IAA complex	Loss-of-function mutations reduce multiple auxin responses	N. Dharmasiri et al. 2003, Dharmasiri et al. 2005b, Gray et al. 1999, Kepinski & Leyser 2005, Ruegger et al. 1998, Tan et al. 2007
AFB1	Auxin F-box protein 1 (AFB1)	Member of TIR1/AFB family; auxin increases Aux/IAA affinity	Loss of function with *tir1*, *afb2*, *afb3* dramatically impairs development	Dharmasiri et al. 2005b
AFB2	Auxin F-box protein 2 (AFB2)	Member of TIR1/AFB family; auxin increases Aux/IAA affinity	Loss of function with *tir1* reduces multiple auxin responses	Dharmasiri et al. 2005b
AFB3	Auxin F-box protein 3 (AFB3)	Member of TIR1/AFB; auxin increases Aux/IAA affinity	Loss of function with *tir1* and *afb2* dramatically impairs development	Dharmasiri et al. 2005b
AFB4	Auxin F-box protein 4 (AFB4)	Member of TIR1/AFB family		Dharmasiri et al. 2005b
AFB5	Auxin F-box protein 5 (AFB5)	Member of TIR1/AFB family	Loss-of-function mutation confers resistance to auxin analogs	Dharmasiri et al. 2005b, Walsh et al. 2006

TIR1-mediated ubiquitination of AUX/IAA proteins



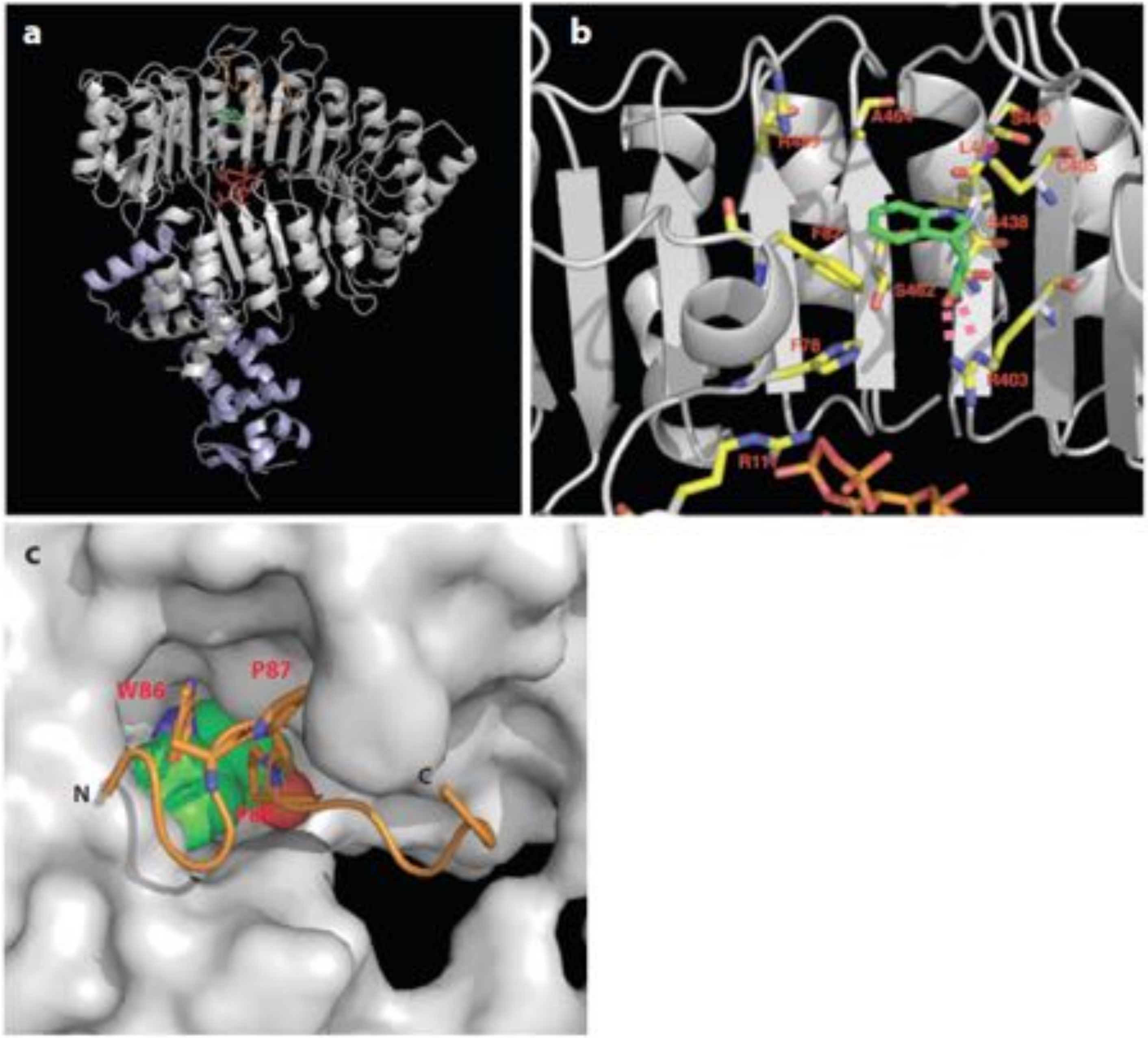
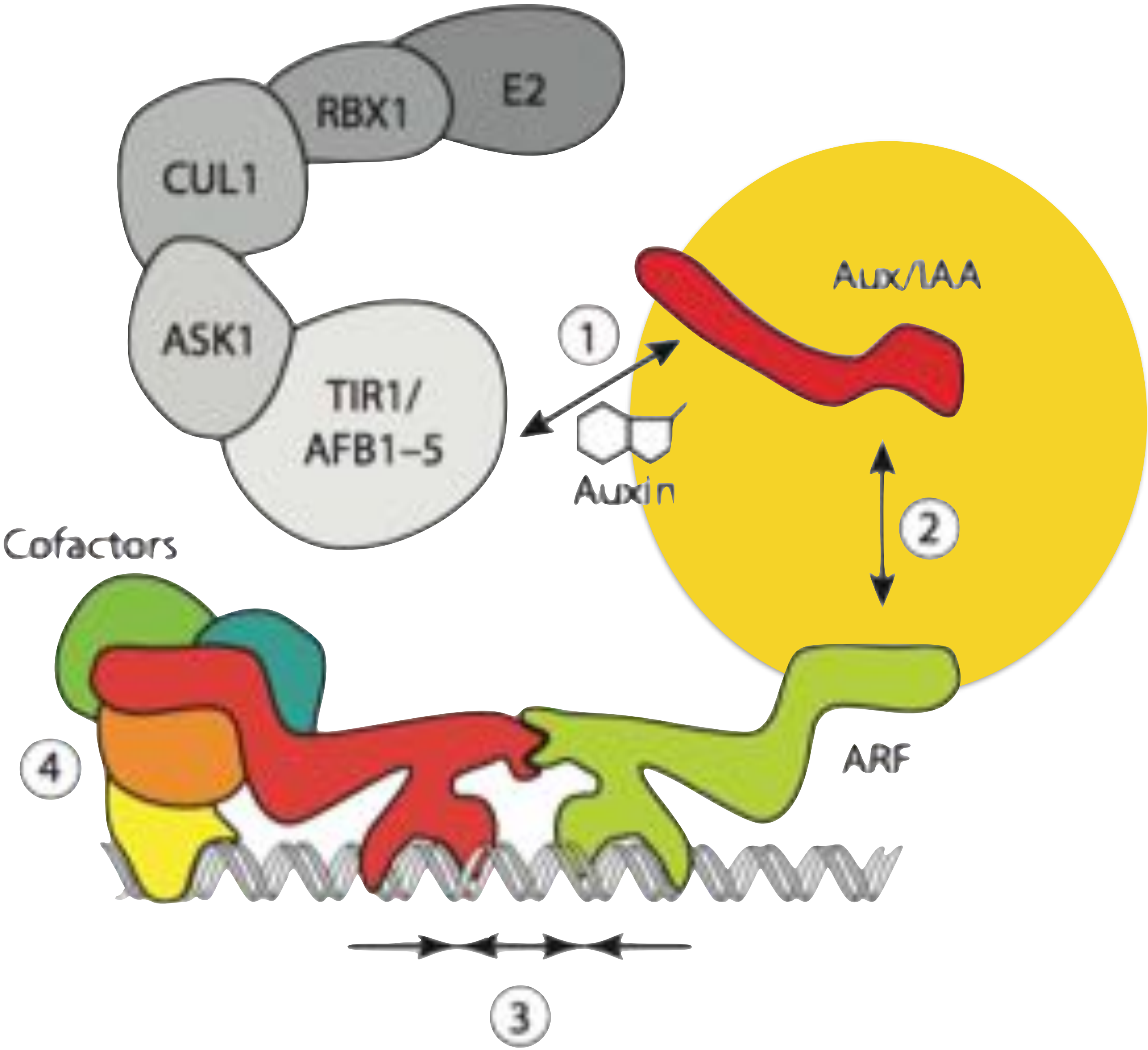
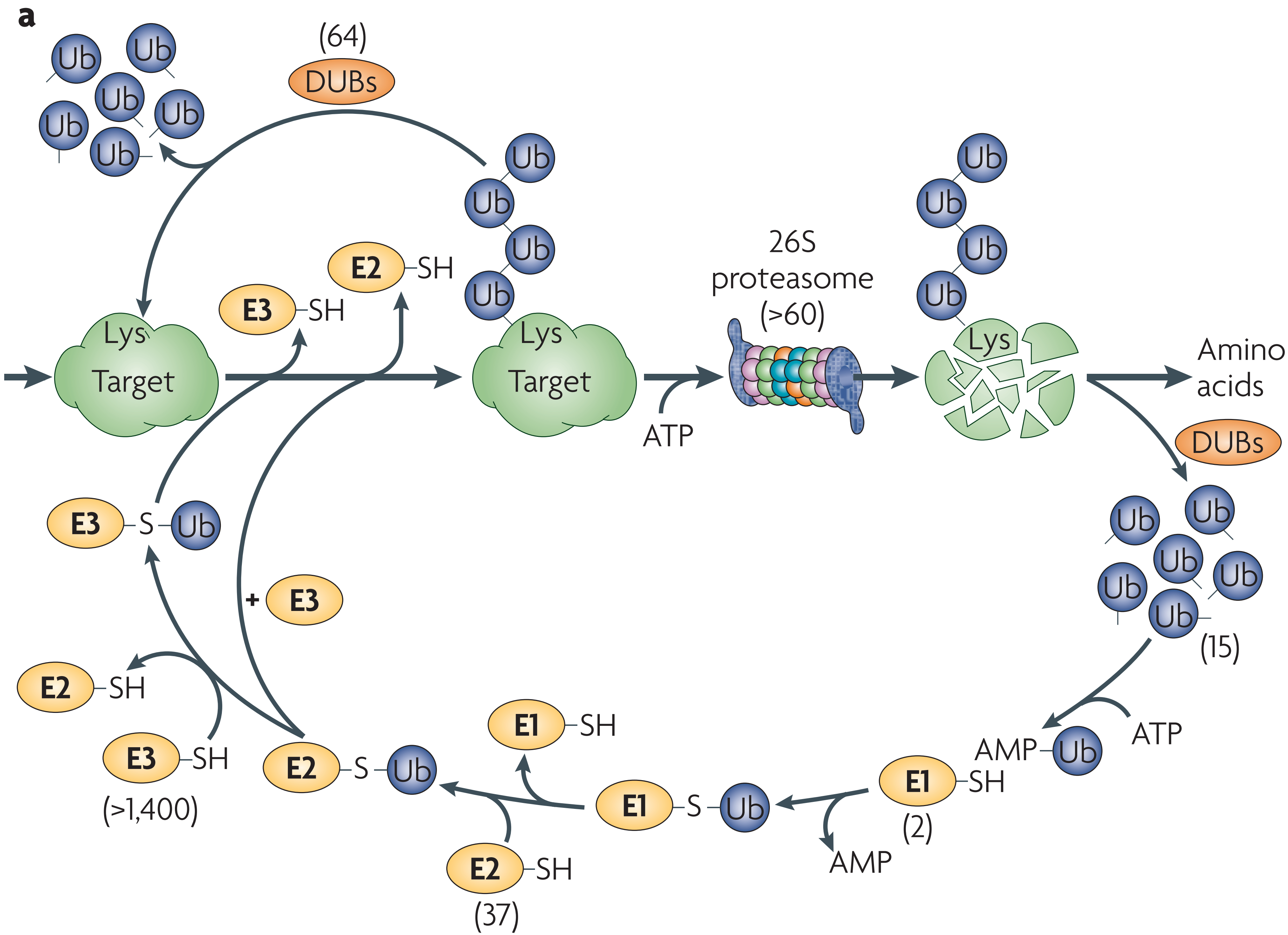


Figure 2

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*).

2. Targeted degradation of AUX/IAA repressors





The ubiquitin–26S proteasome system for protein degradation in Arabidopsis

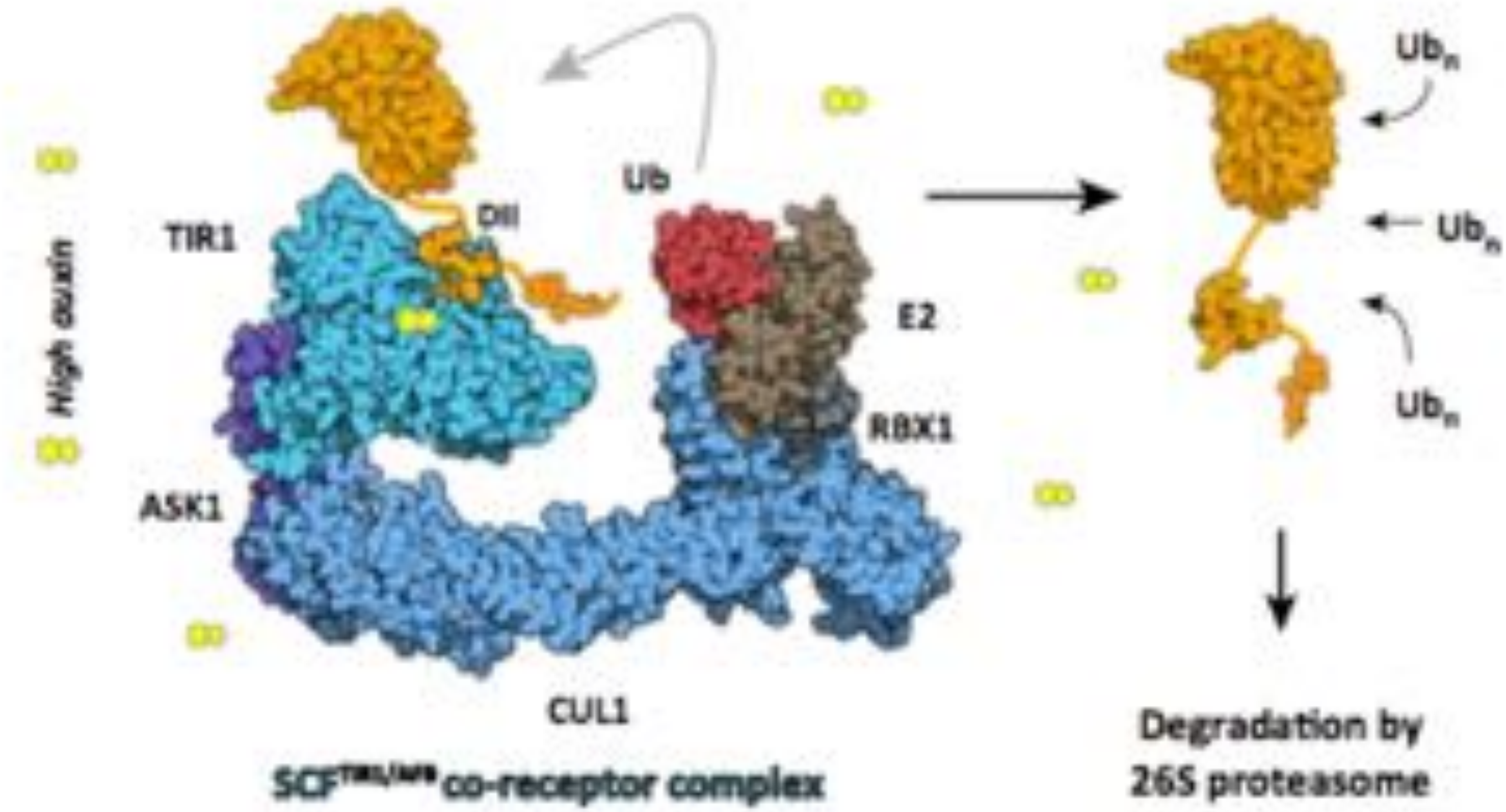
24 Aux/IAA proteins & diverse functions in Arabidopsis

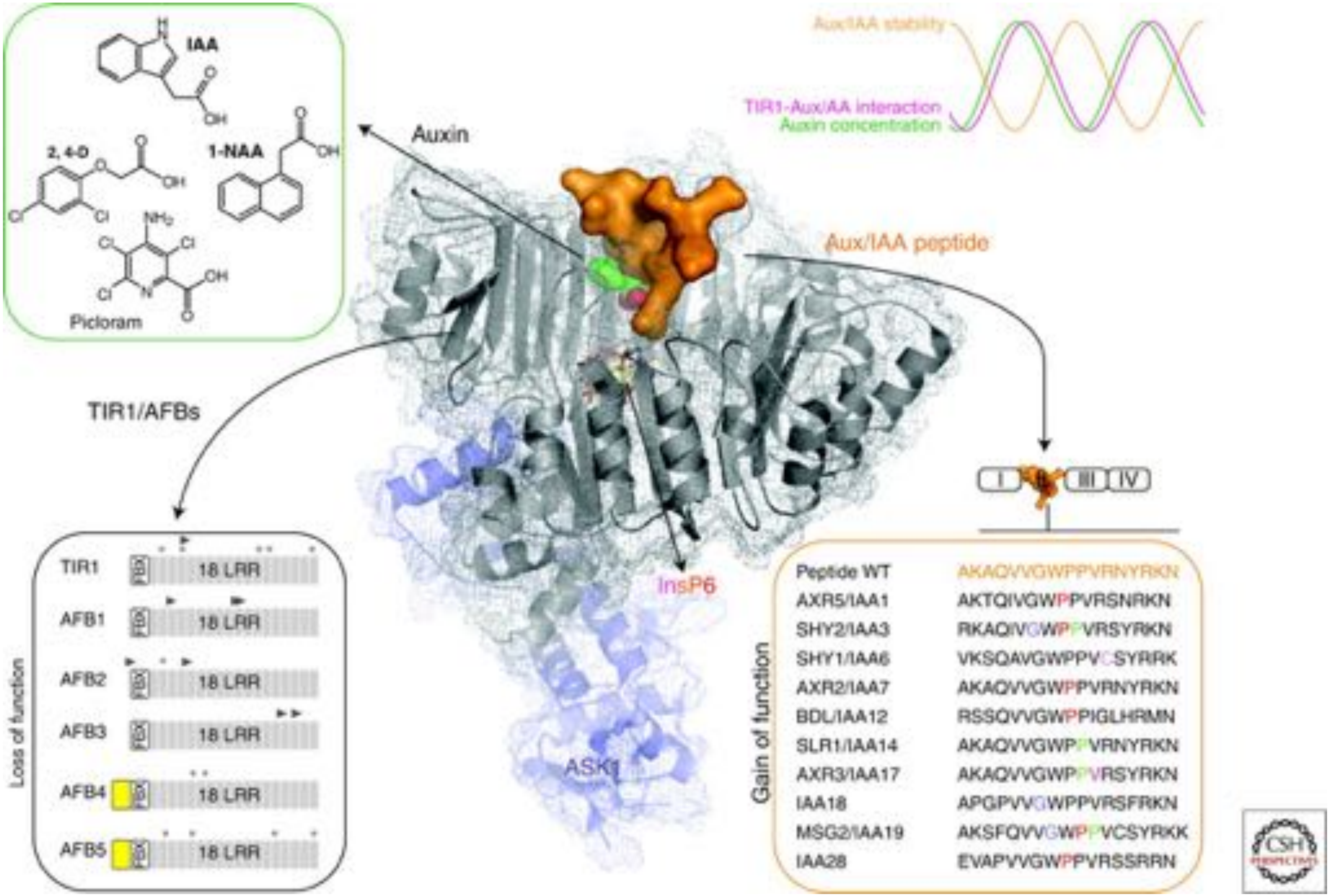
Table 1 Aux/IAA proteins in *Arabidopsis thaliana* and evidence for their roles in auxin-mediated development

Gene	Product	Function	Genetic evidence for role in auxin-mediated development	References
IAA1	IAA1	Auxin decreases protein half-life; *axr5-1* gain-of-function mutation is in degron	*axr5-1* degron mutation reduces multiple auxin responses	Abel et al. 1995; Park et al. 2002; Yang et al. 2004; Zenser et al. 2001, 2003
IAA2	IAA2	Contains domain II degron	Phylogenetic relationship	Abel et al. 1995, Liscum & Reed 2002
IAA3	SUPPRESSOR OF HY2 or SHORT HYPOCOTYL 2 (SHY2/IAA3)	*shy2-1*, *-2*, *-3*, *-6* mutations are in degron	*shy2-1*, *-2*, *-3*, *-6* degron mutations reduce multiple auxin responses	Abel et al. 1995, Kim et al. 1996, Reed 2001, Reed et al. 1998, Soh et al. 1999, Tian & Reed 1999, 2003
IAA4	IAA4	Pea ortholog shows rapid turnover in vivo	Phylogenetic relationship	Abel et al. 1994, 1995; Liscum & Reed 2002
IAA5	IAA5	Contains domain II degron	Phylogenetic relationship	Abel et al. 1995, Liscum & Reed 2002
IAA6	SUPPRESSOR OF HY1 (SHY1/IAA6)	Pea ortholog shows rapid turnover in vivo; *shy1-1* mutation is in degron	*shy1-1*-stabilizing mutation reduces multiple auxin responses	Abel et al. 1994, 1995; Kim et al. 1996; Ramos et al. 2001; Reed 2001
IAA7	AUXIN RESISTANT 2 (AXR2/IAA7)	Auxin decreases protein half-life; protein can interact with TIR1; *axr2-1* mutation is in degron; *axr2-1* mutation abolishes protein interaction with TIR1 and increases protein half-life	*axr2-1*-stabilizing mutations reduce multiple auxin responses	Abel et al. 1995, N. Dharmasiri et al. 2003, Gray et al. 2001, Nagpal et al. 2000, Timpte et al. 1994
IAA8	IAA8	Protein shows rapid turnover in vivo; contains domain II degron	Phylogenetic relationship	Abel et al. 1995, Dreher et al. 2006, Liscum & Reed 2002
IAA9	IAA9	Protein shows rapid turnover in vivo; contains domain II degron	RNAi-reduced levels in tomato increase sensitivity to auxin in multiple developmental processes	Abel et al. 1995, Dreher et al. 2006, Liscum & Reed 2002, Wang et al. 2005
IAA10	IAA10	Contains domain II degron	Phylogenetic relationship	Abel et al. 1995
IAA11	IAA11	Contains domain II degron	Phylogenetic relationship	Abel et al. 1995, Liscum & Reed 2002
IAA12	BODENLOS (BDL/IAA12)	*bdl* mutation is in degron	*bdl* degron mutation reduces multiple auxin responses	Abel et al. 1995; Hamann et al. 1999, 2002; Liscum & Reed 2002
IAA13	IAA13	Contains domain II degron	Degron mutant transgene impairs auxin-related development	Abel et al. 1995, Weijers et al. 2005
IAA14	SOLITARY ROOT (SLR/IAA14)	*slr-1* mutation is in degron	*slr-1* degron mutation reduces multiple auxin responses	Abel et al. 1995; Fukaki et al. 2002, 2005; Vanneste et al. 2005
IAA15	IAA15	Contains domain II degron	Phylogenetic relationship	Liscum & Reed 2002
IAA16	IAA16	Contains domain II degron	Phylogenetic relationship	Liscum & Reed 2002
IAA17	AUXIN RESISTANT 3 (AXR3/IAA17)	Auxin decreases protein half-life; protein can interact with TIR1; *axr3* mutations are in degron and increase protein half-life	*axr3-1* and *-3* degron mutations reduce multiple auxin responses	N. Dharmasiri et al. 2003, Gray et al. 2001, Leyser et al. 1996, Ouellet et al. 2001, Overvoorde et al. 2005, Rouse et al. 1998
IAA18	IAA18	*iaa18-1* mutation is in degron	*iaa18-1* degron mutation reduces multiple auxin responses	Reed 2001
IAA19	MASSUGU 2 (MSG2/IAA19)	*msg2-1* to *-4* mutations are in degron	*msg2-1* to *-4* degron mutations reduce multiple auxin responses	Liscum & Reed 2002, Tatematsu et al. 2004
IAA26	Phytochrome interacting protein 1 (PAP1/IAA26)	Contains domain II degron	Phylogenetic relationship	Liscum & Reed 2002
IAA27	Phytochrome interacting protein 2 (PAP2/IAA27)	Contains domain II degron	Phylogenetic relationship	Liscum & Reed 2002
IAA28	IAA28	Auxin decreases protein half-life; *iaa28-1* mutation is in degron	*iaa28-1* degron mutations reduce multiple auxin responses	Dreher et al. 2006, Rogg et al. 2001
IAA29	IAA29	Contains domain II degron	Phylogenetic relationship	Liscum & Reed 2002
IAA31	IAA31	Auxin decreases protein half-life; imperfect conservation of domain II correlates with a half-life longer than that of other Aux/IAAs in vivo	Phylogenetic relationship	Dreher et al. 2006, Liscum & Reed 2002

(*Continued*)

Table 1 (*Continued*)


High auxin
TIR1
DII
Ub
E2
RBX1
ASK1
CUL1
SCF co-receptor complex
Ubn
Ubn
Ubn
Degradation by
26S proteasome



IAA
2, 4-D
1-NAA
Picloram
Auxin
Aux/IAA stability
TIR1-Aux/IAA interaction
Auxin concentration
Aux/IAA peptide
TIR1/AFBs
InsP6
ASK1
I
III
IV
Loss of function
TIR1 FBX 18 LRR
AFB1 FBX 18 LRR
AFB2 FBX 18 LRR
AFB3 FBX 18 LRR
AFB4 FBX 18 LRR
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

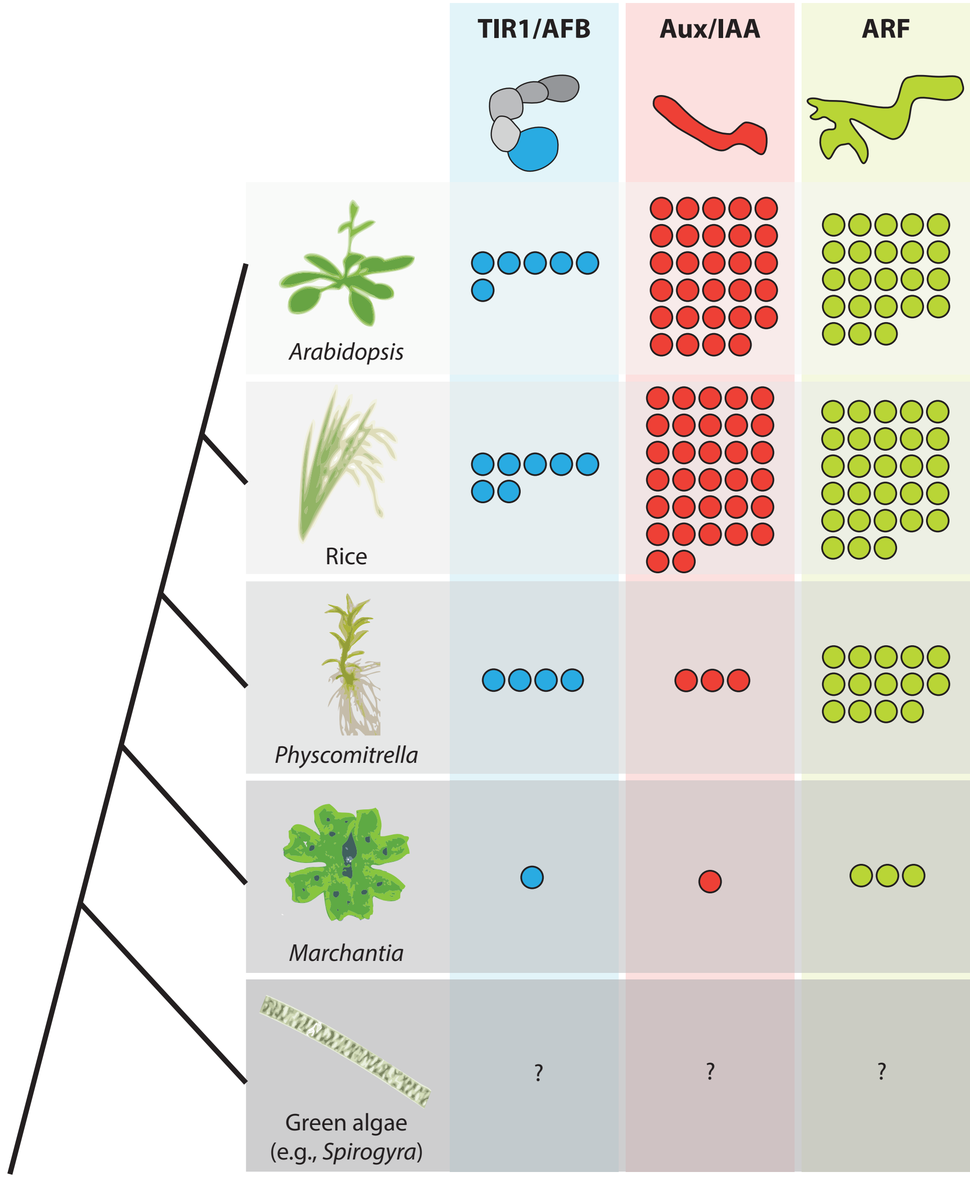
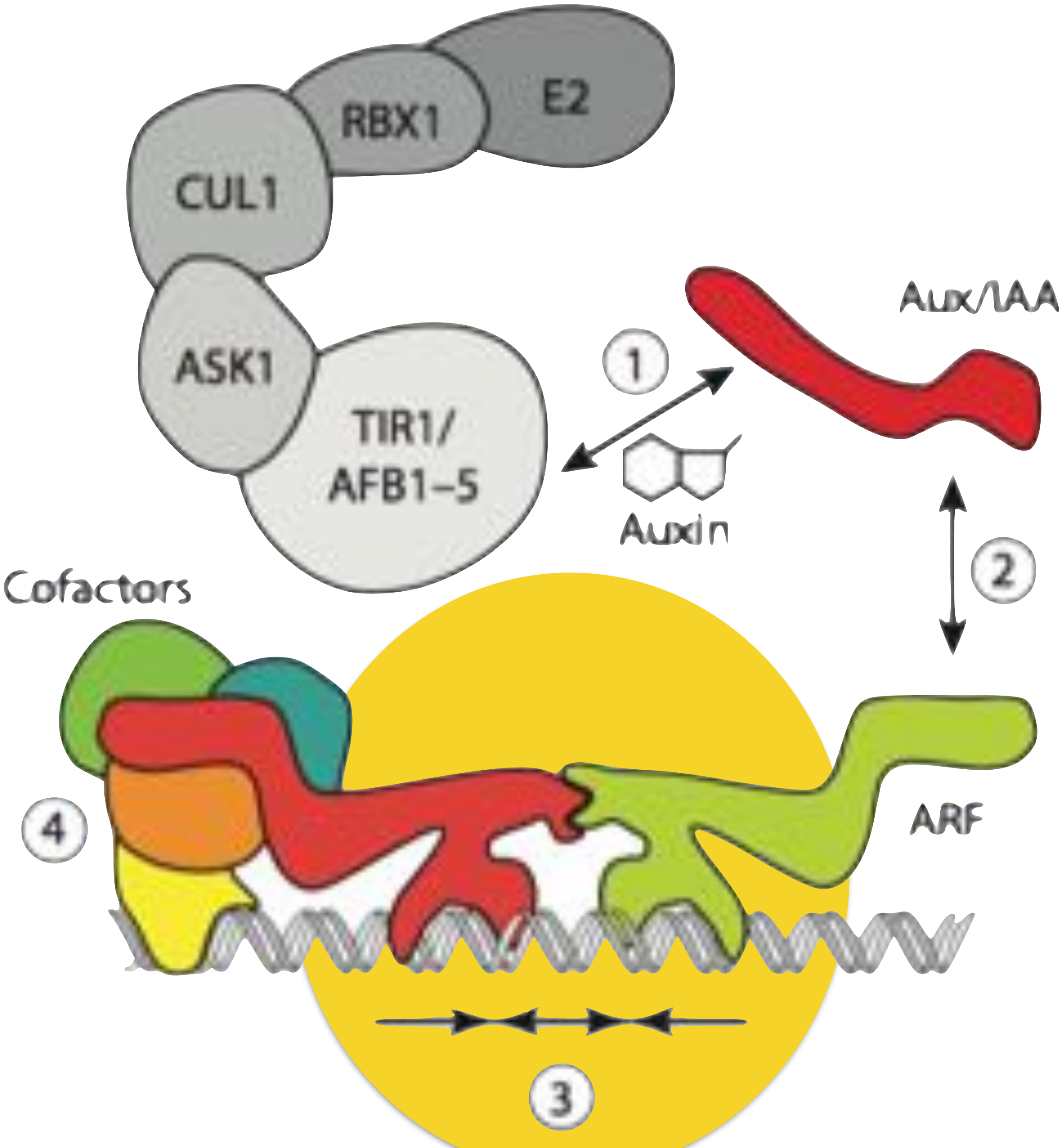
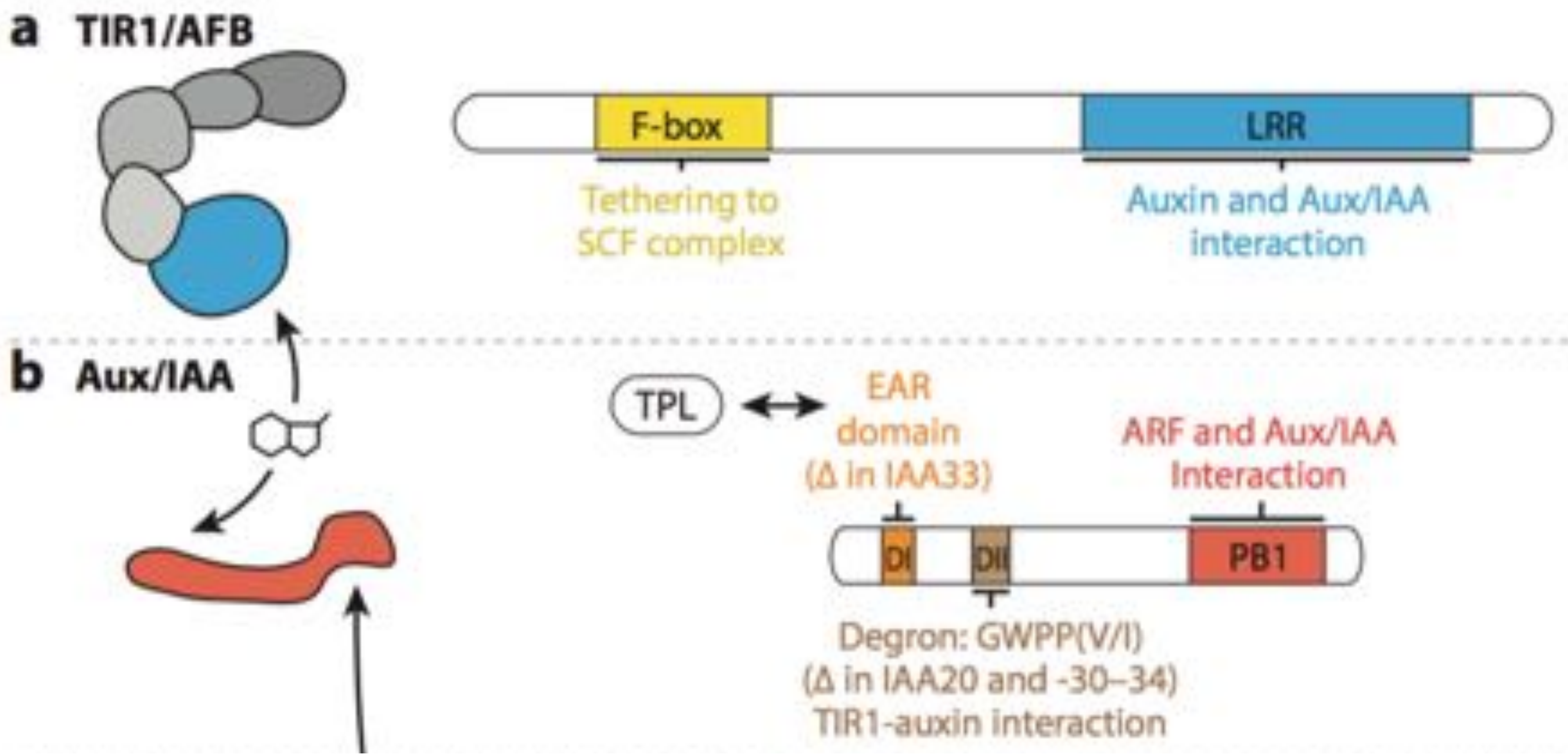


Figure 7

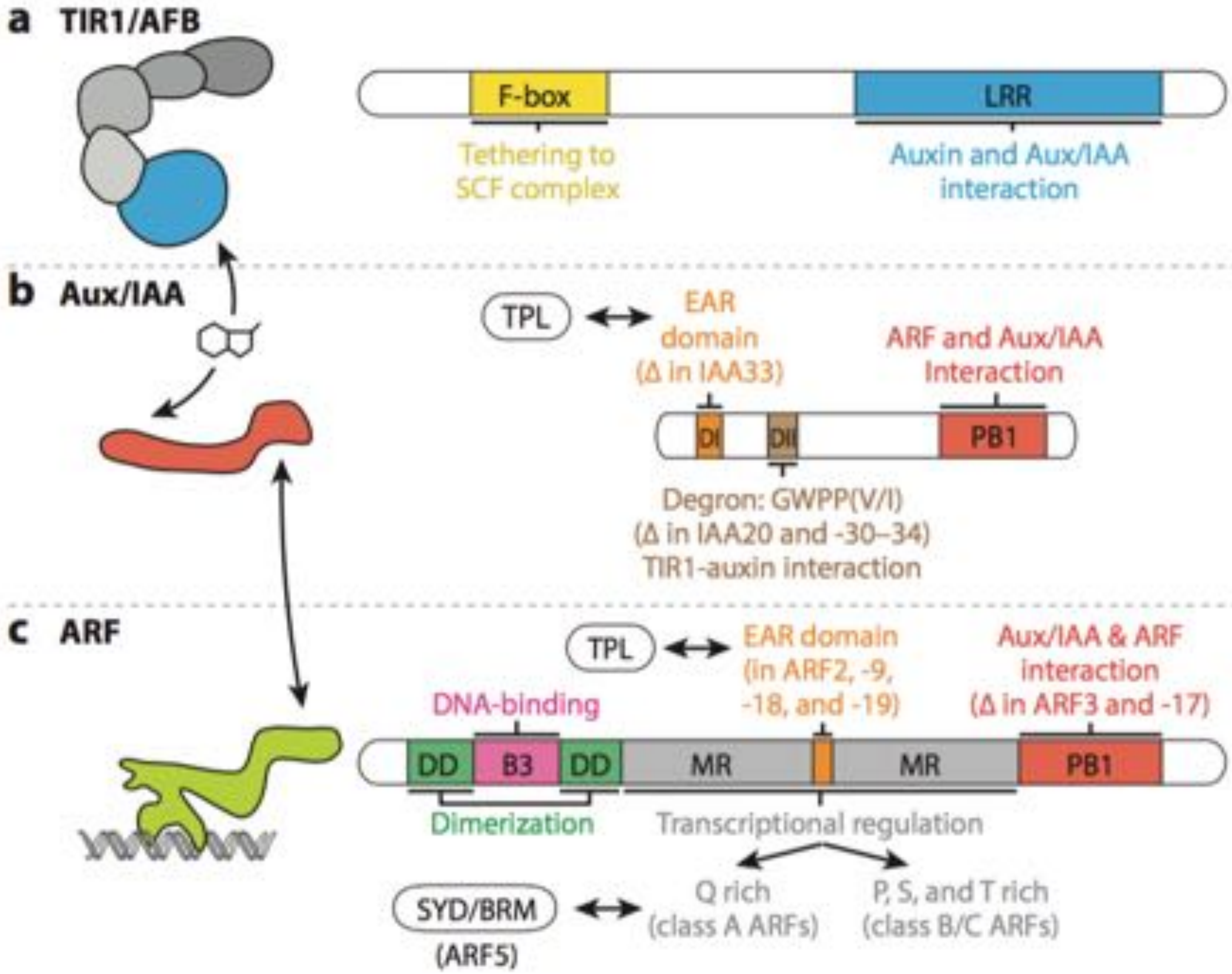
The evolution of the auxin response pathway, showing the distribution of genes encoding TIR1/AFB, Aux/IAA, and ARF proteins in published plant genomes for several plant species. These species represent eudicots (*Arabidopsis*), monocots (rice), mosses (*Physcomitrella*), liverworts (*Marchantia*), and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

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

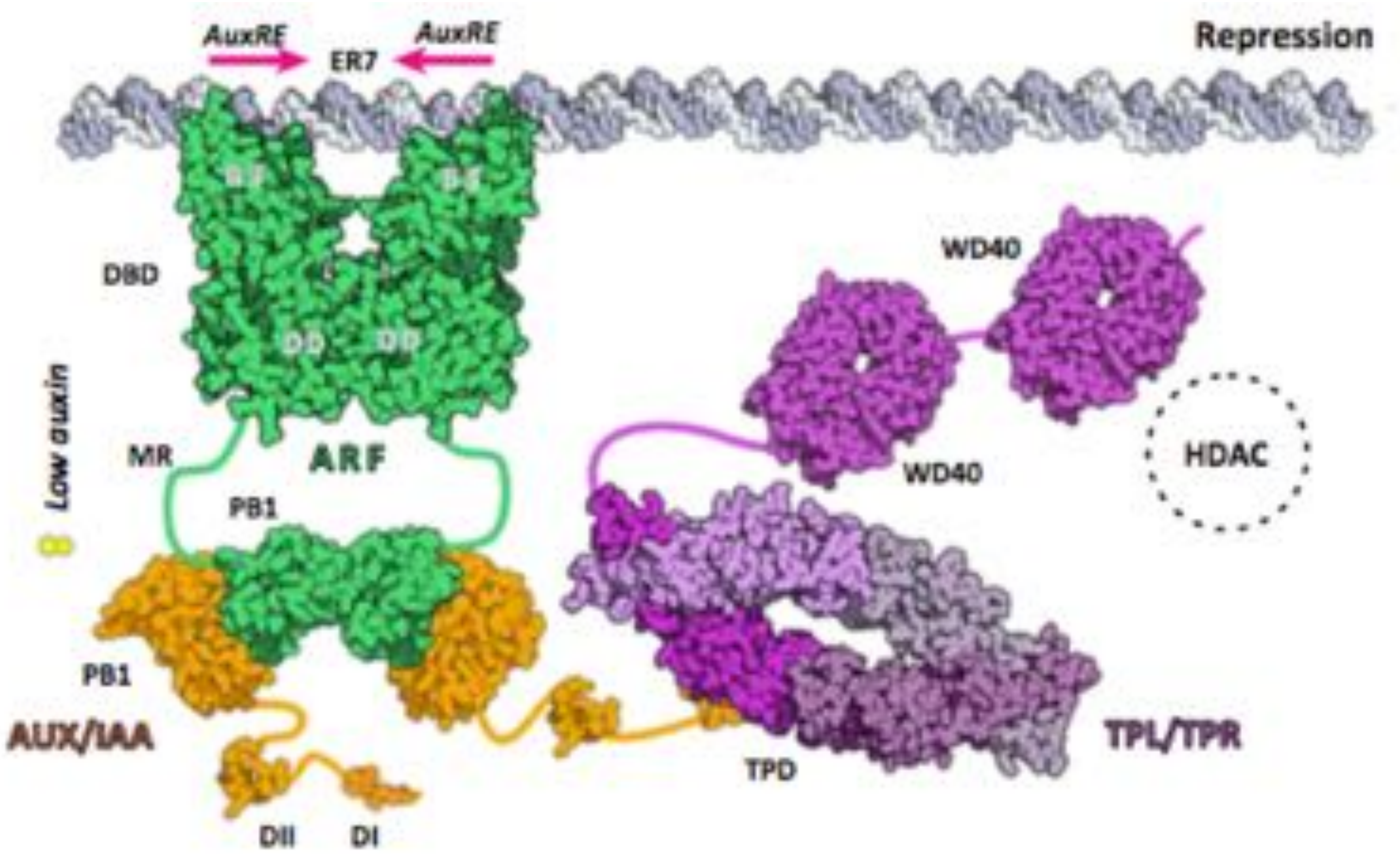




a TIR1/AFB
F-box
LRR
Tethering to SCF complex
Auxin and Aux/IAA interaction
b Aux/IAA
TPL
EAR domain (Δ in IAA33)
ARF and Aux/IAA Interaction
DI
DII
PB1
Degron: GWPP(V/I) (Δ in IAA20 and -30–34) TIR1-auxin interaction



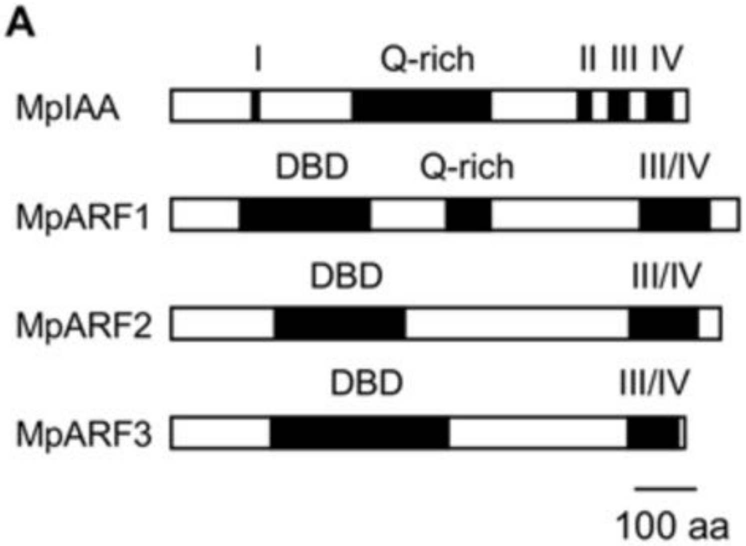
a TIR1/AFB
F-box
LRR
Tethering to SCF complex
Auxin and Aux/IAA interaction
b Aux/IAA
TPL
EAR domain (Δ in IAA33)
ARF and Aux/IAA Interaction
DI
DII
PB1
Degron: GWPP(V/I) (Δ in IAA20 and -30–34) TIR1-auxin interaction
c ARF
TPL
EAR domain (in ARF2, -9, -18, and -19)
Aux/IAA & ARF interaction (Δ in ARF3 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)

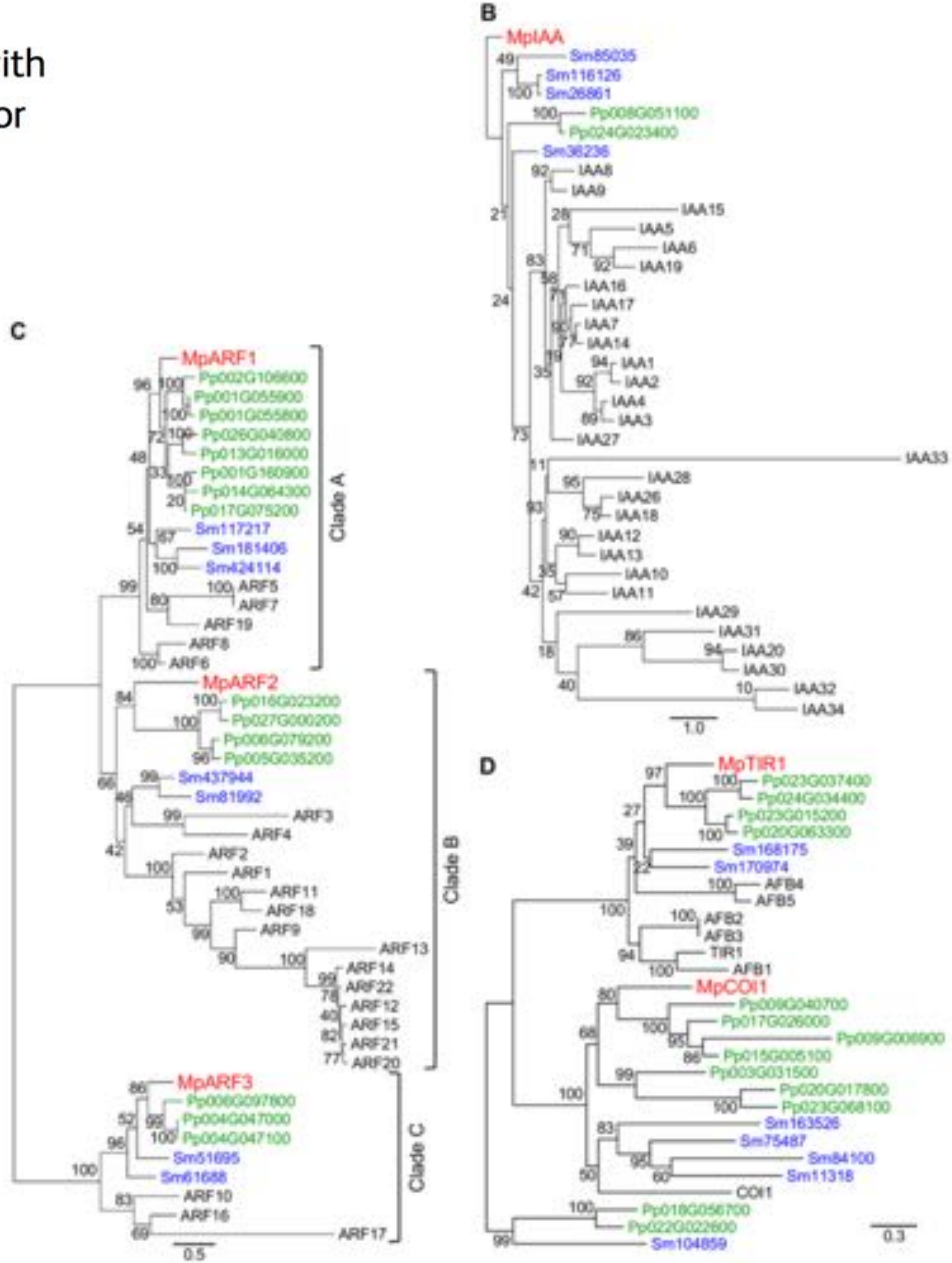

AuxRE
ER7
AuxRE
Repression
DBD
DD
DD
WD40
WD40
HDAC
Low auxin
MR
ARF
PB1
PB1
AUX/IAA
DII
DI
TPD
TPL/TPR

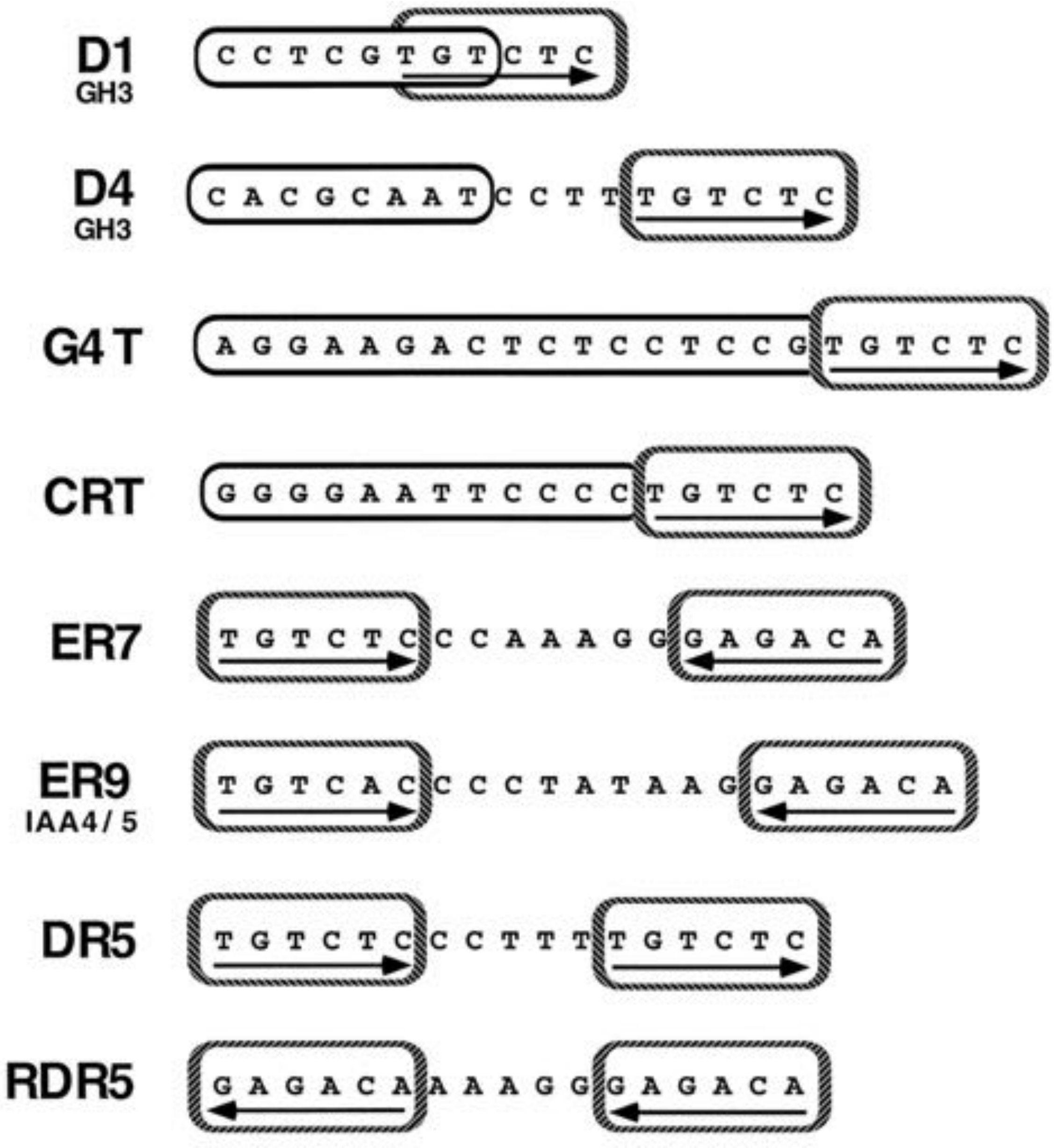
RESEARCH ARTICLE

Auxin-Mediated Transcriptional System with a Minimal Set of Components Is Critical for Morphogenesis through the Life Cycle in *Marchantia polymorpha*

Hirotaka Kato[1], Kimitsune Ishizaki[1,2], Masaru Kouno[1], Makoto Shirakawa[1], John L. Bowman[3,4], Ryuichi Nishihama[1], Takayuki Kohchi[1]*

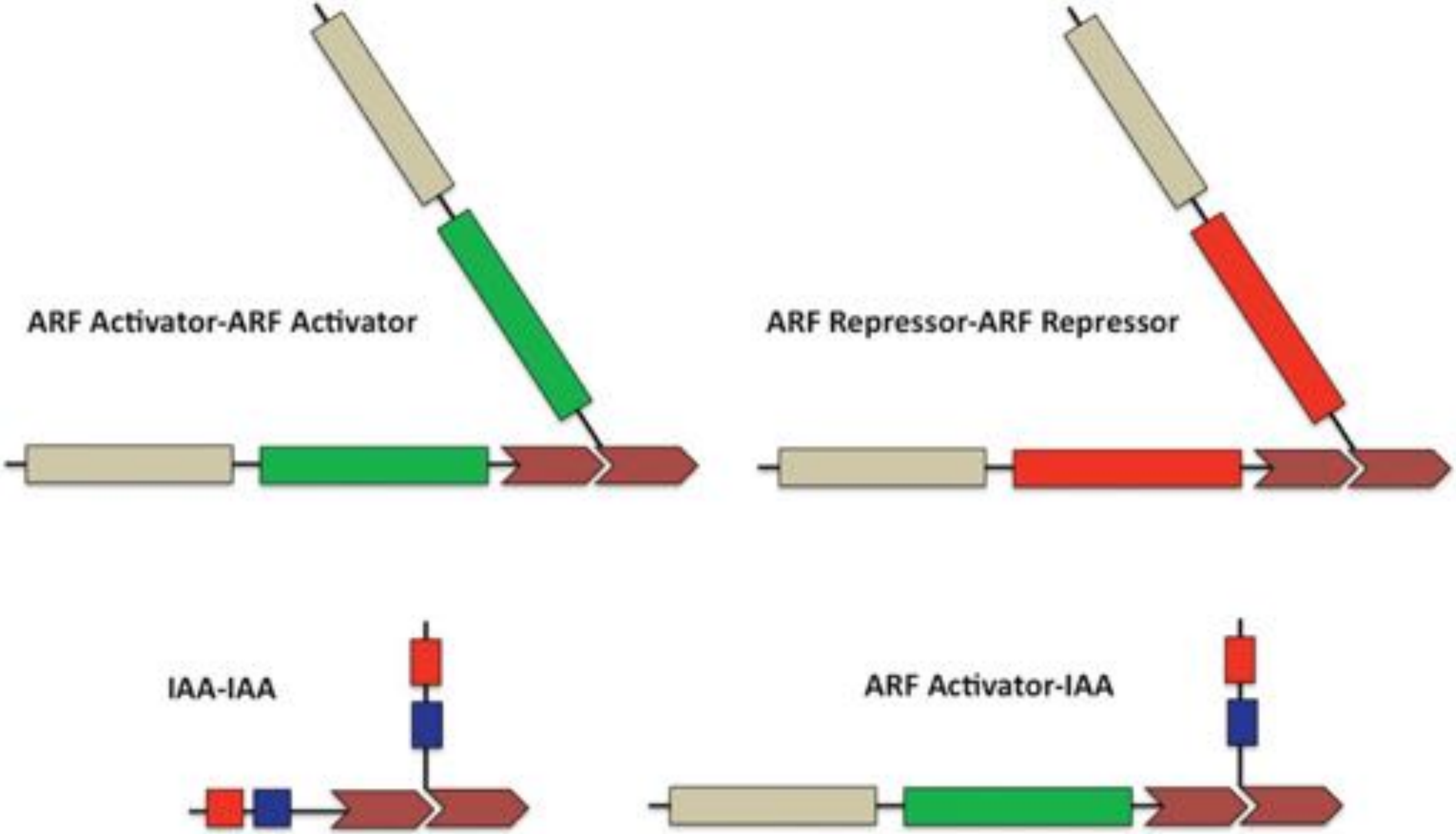




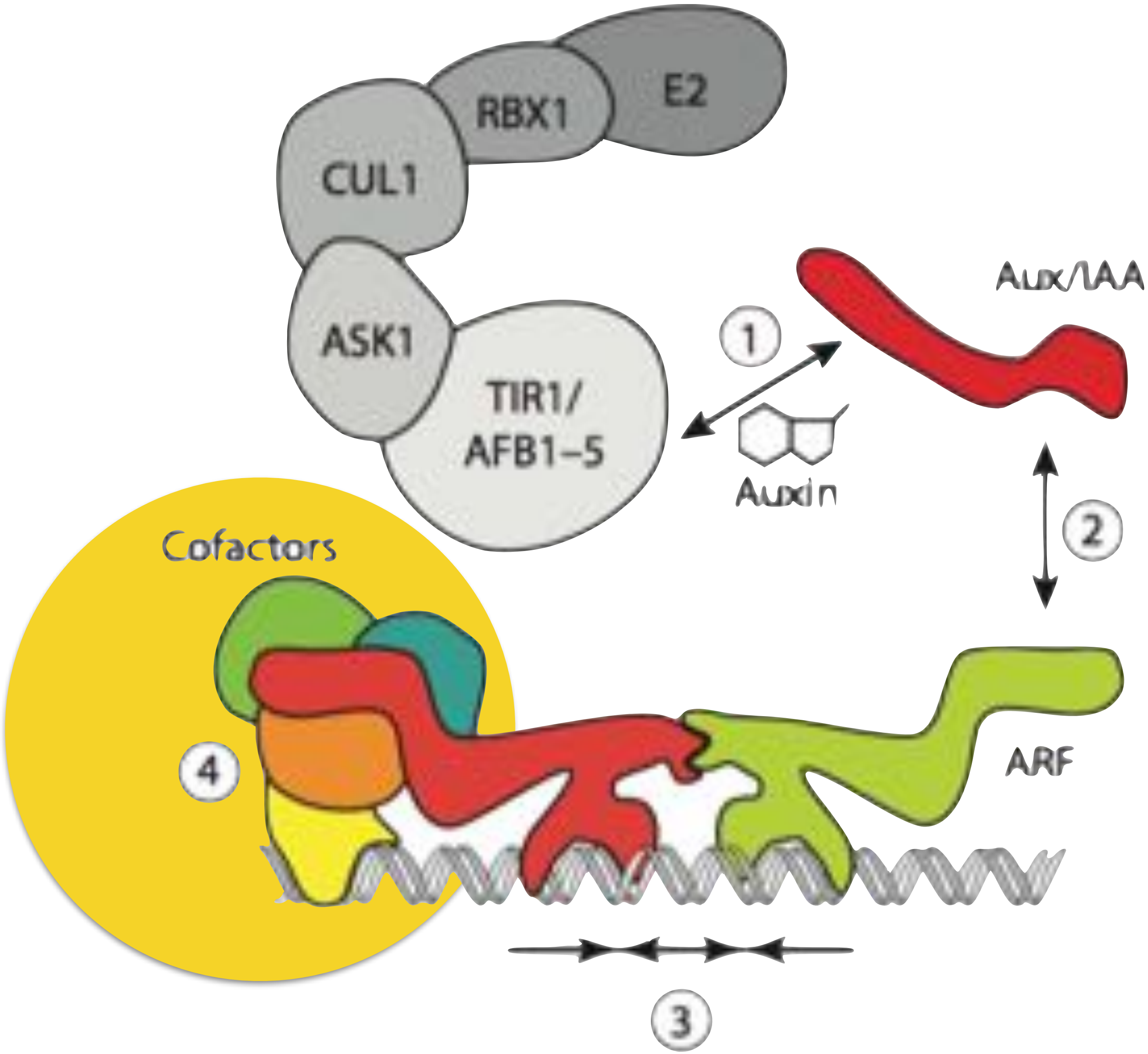


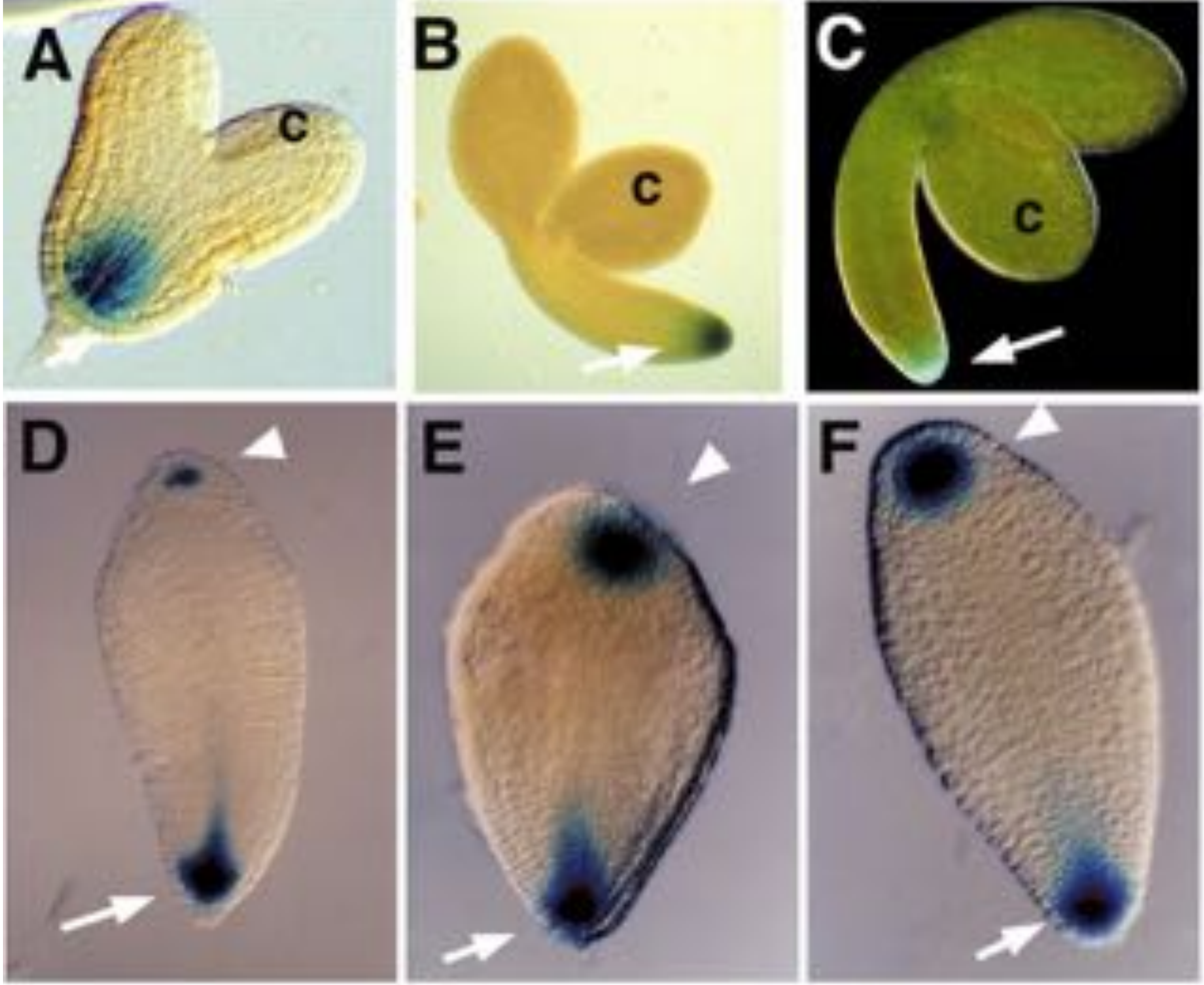
Composite and simple auxin responsive elements (AREs)

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

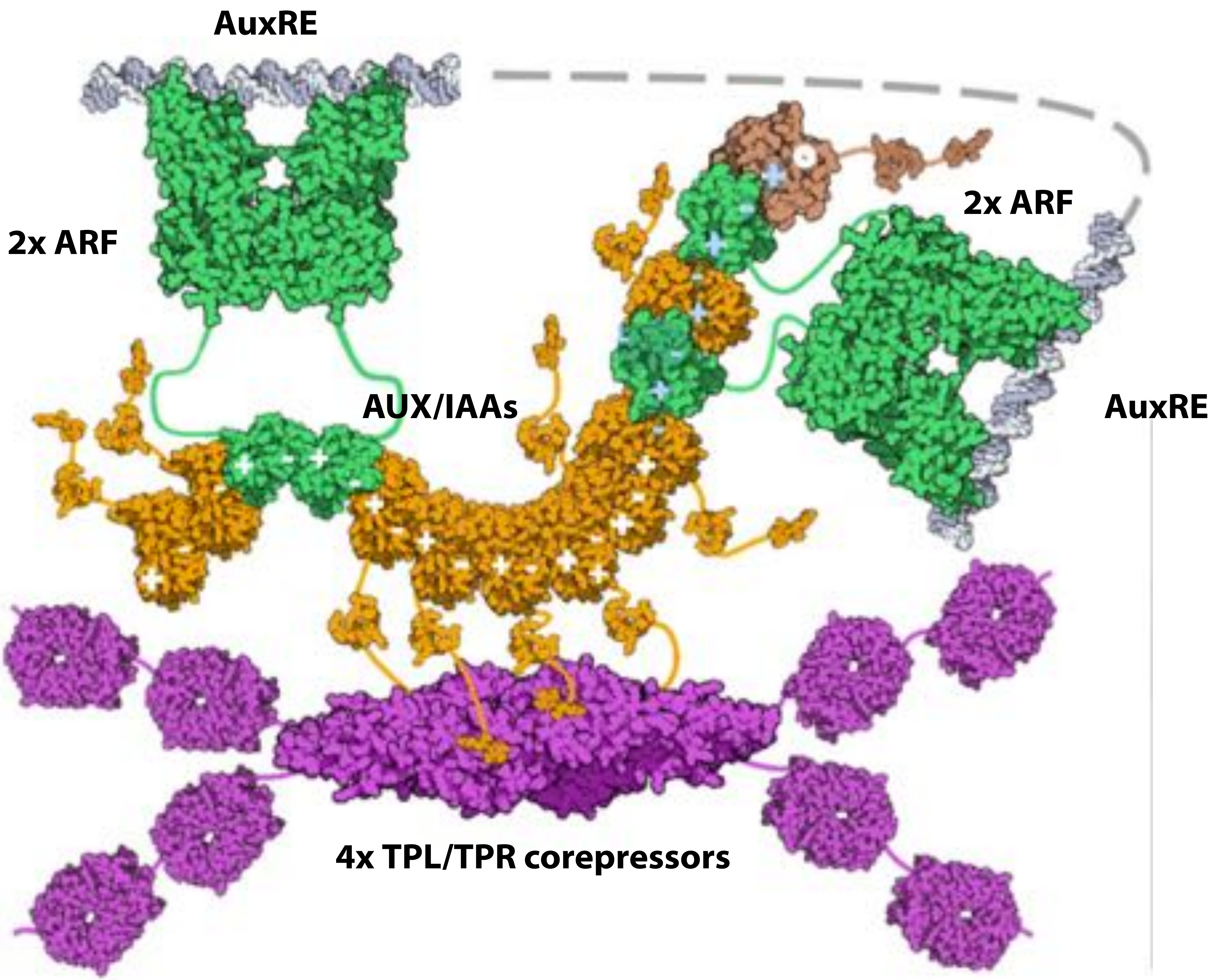

ARF Activator-ARF Activator
ARF Repressor-ARF Repressor
IAA-IAA
ARF Activator-IAA

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

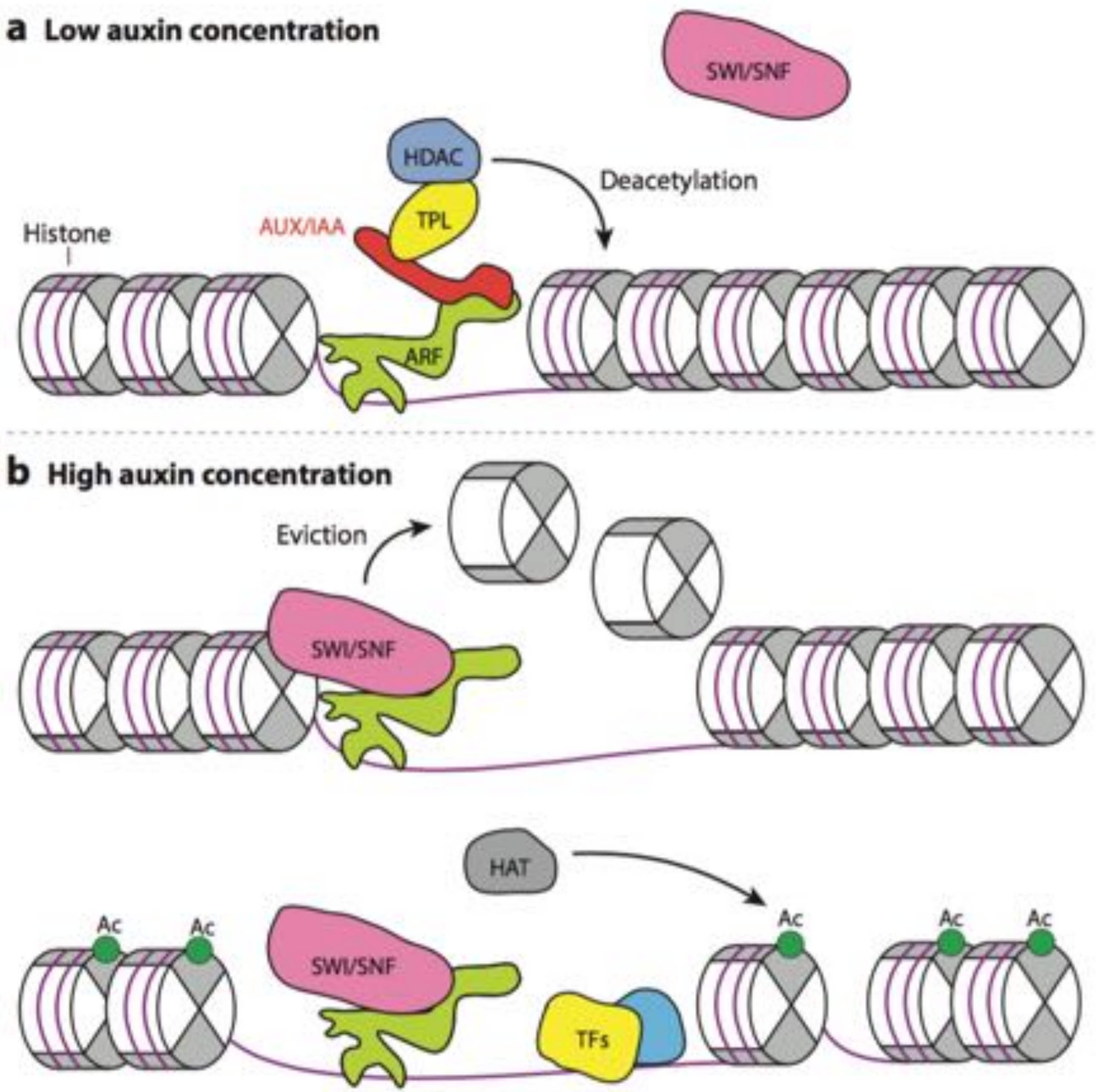




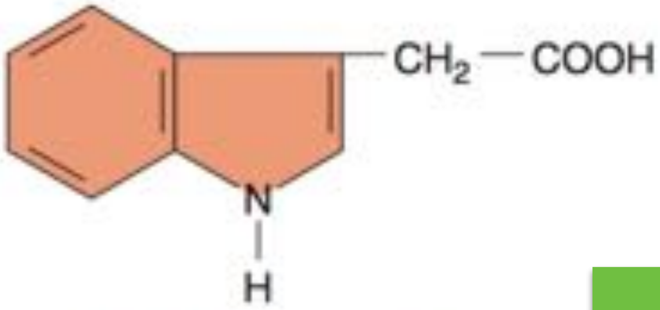
topless **(*tpl*) mutant**



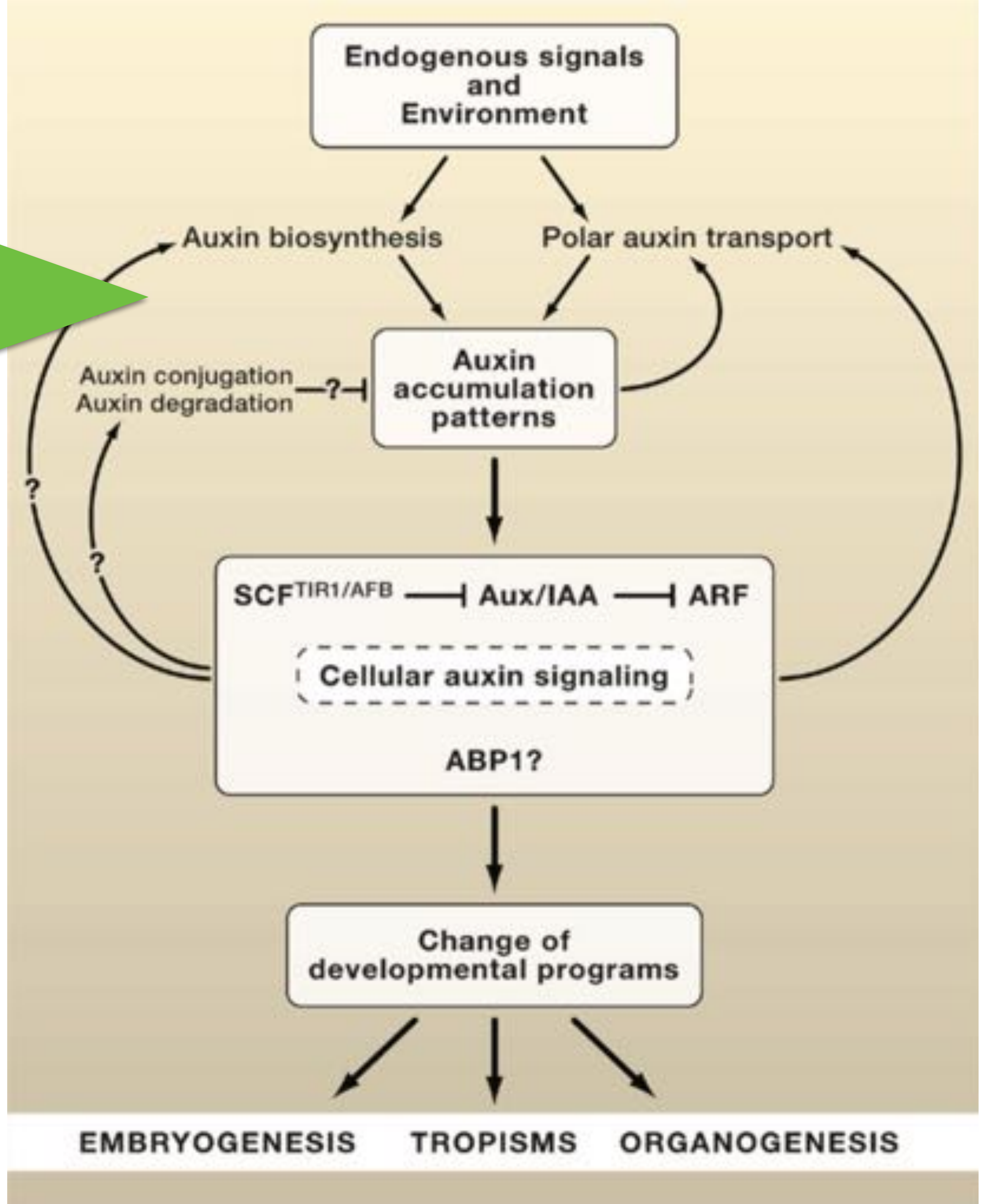
Recognition of composite AuxREs and recruitment of tetrameric TPL/TPR corepressors

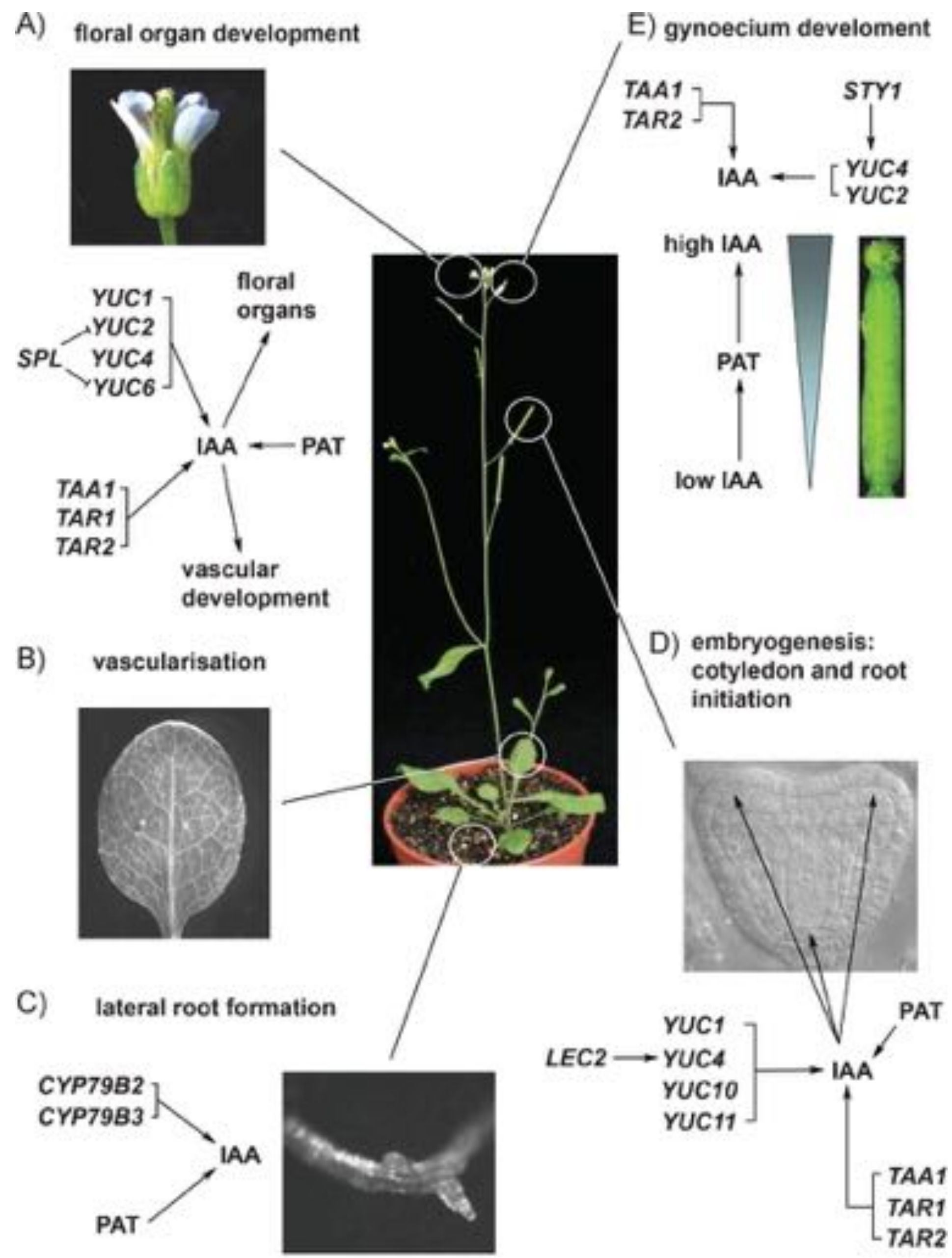


Recruitment of Switch/Sucrose Non-Fermenting (SWI/SNF) and Histone Acetyl Transferase (HAT) complexes for remodelling chromatin



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





Summary of the developmental processes where auxin biosynthesis and polar auxin transport have been shown to be required

Auxin biosynthesis pathways

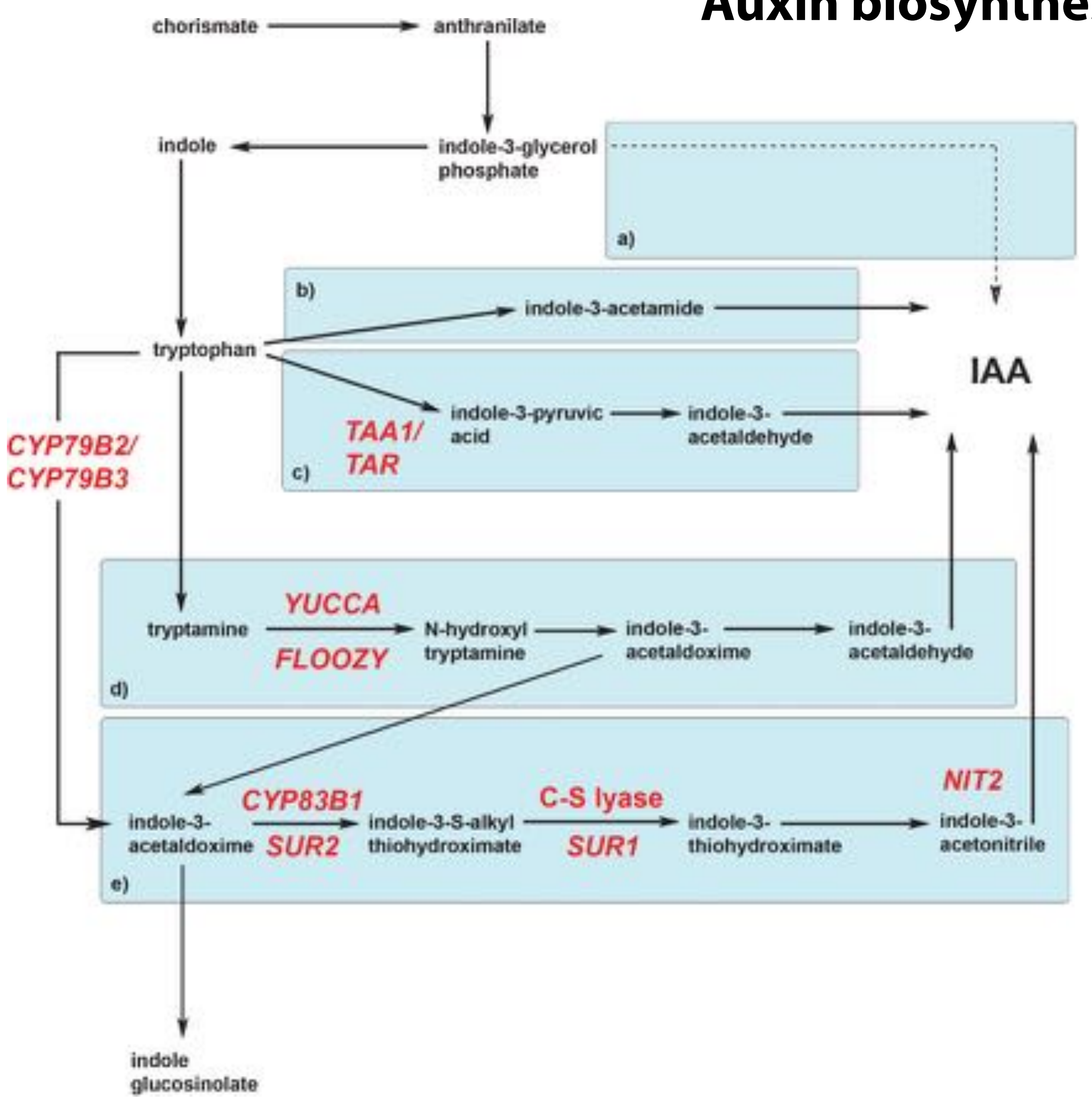


Figure 1. Different auxin biosynthesis pathways, showing a: the postulated tryptophan (Trp)-independent pathway and the four main branches of Trp-dependent synthesis via, b: indole-3-acetamide, c: IPA, d: tryptamine or e: indole-3-acetaldoxime. The positions of enzymes encoded by genes that result in a phenotype when mutated are shown.

Auxin conjugation and degradation

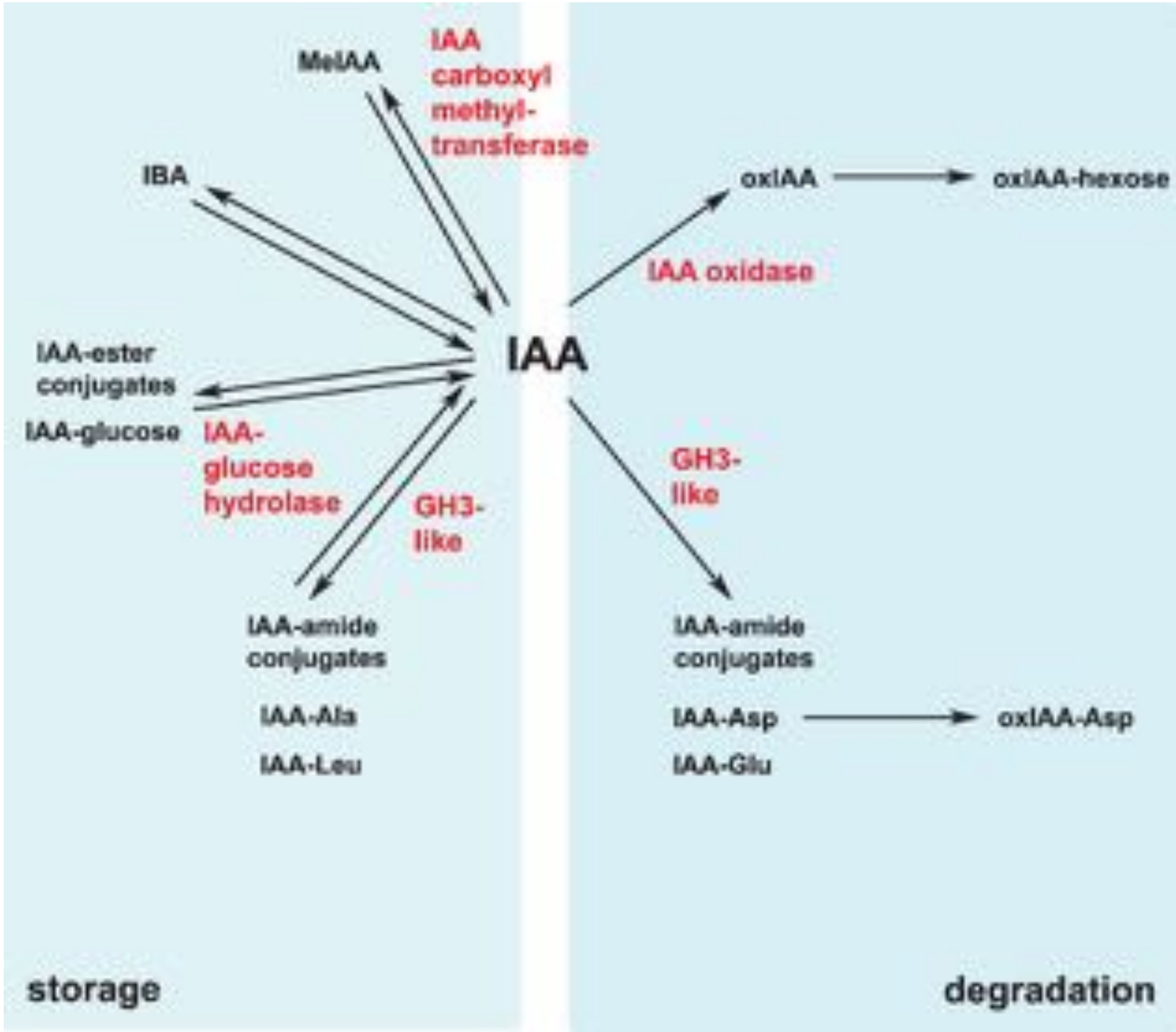
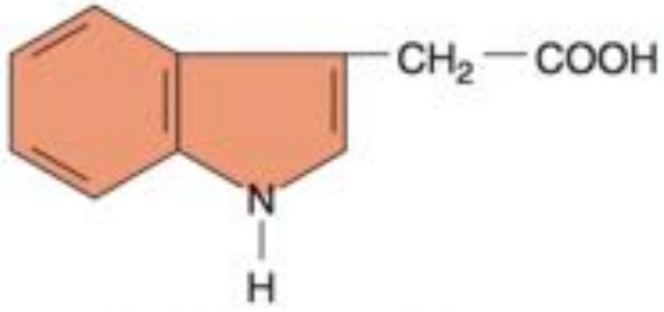
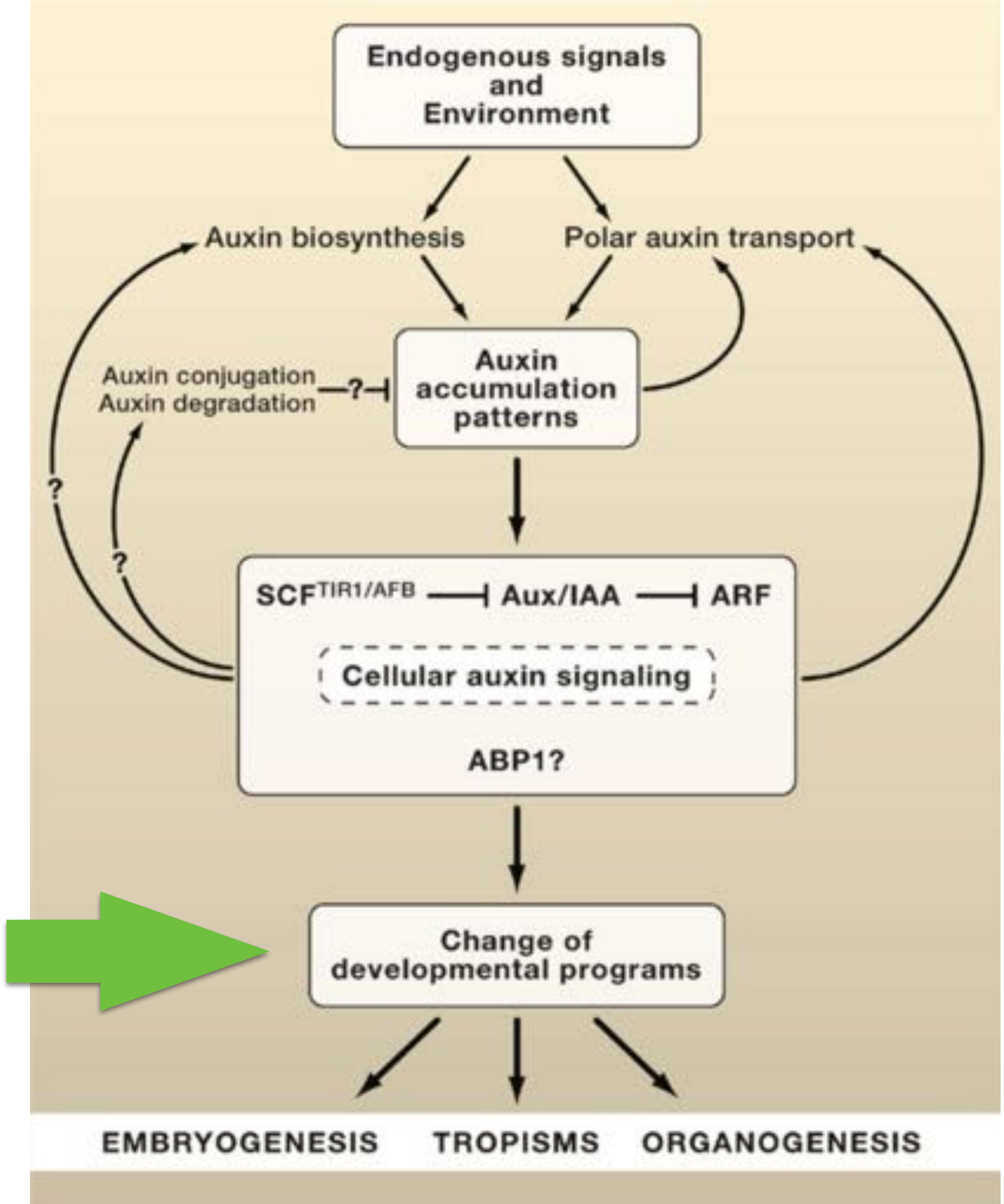


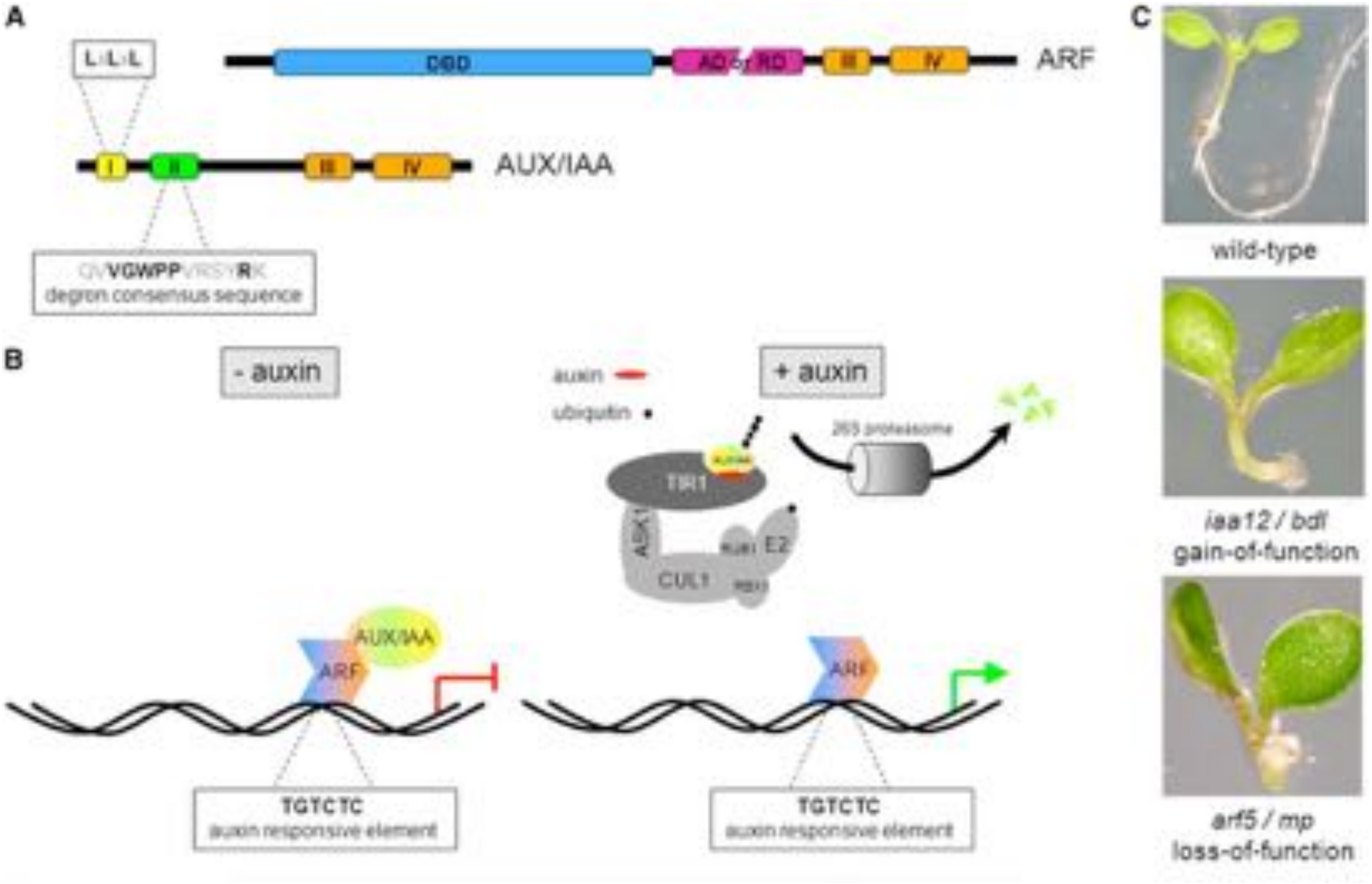
Figure 2. A summary of the main storage and degradation pathways known for IAA.



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



BODENLOS (IAA12) and MONOPTEROUS (ARF5) are required for the establishment of the root apical meristem during embryogenesis



Origin of the root apical meristem during embryogenesis

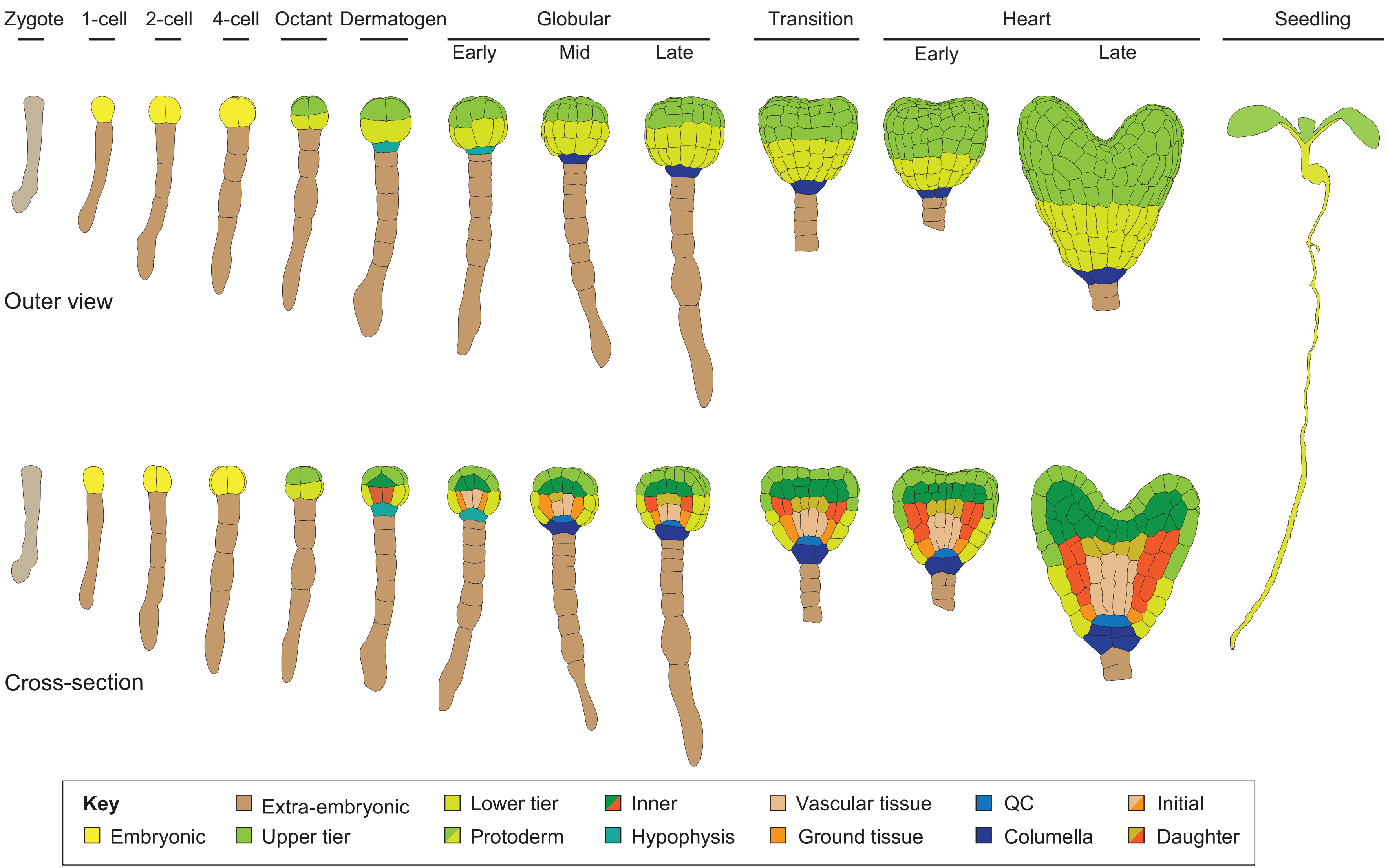
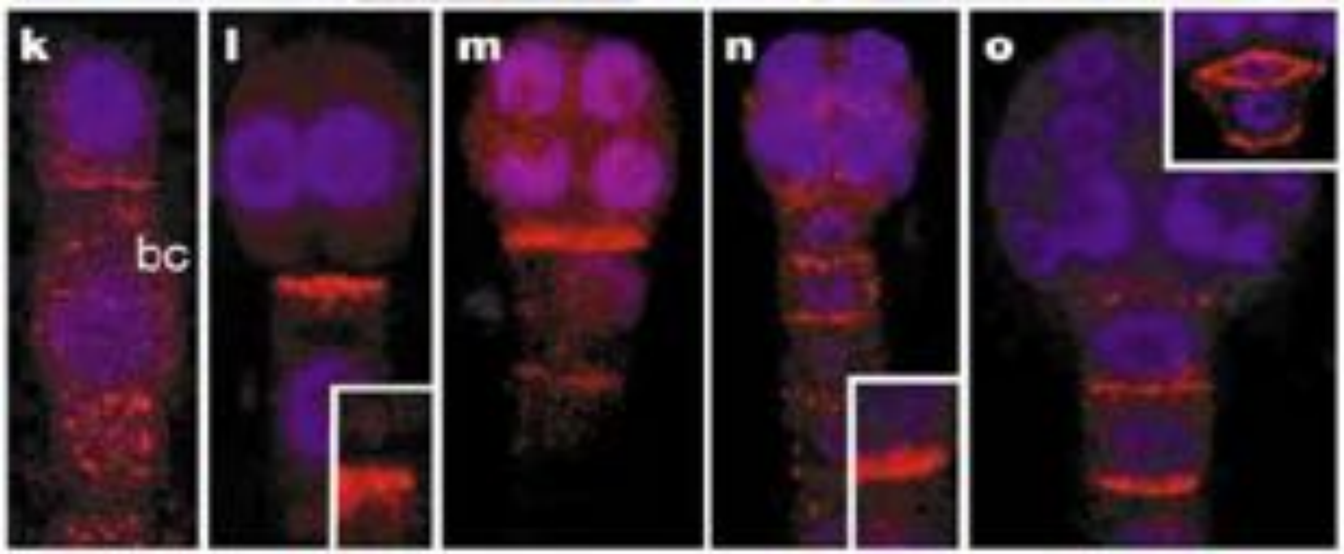
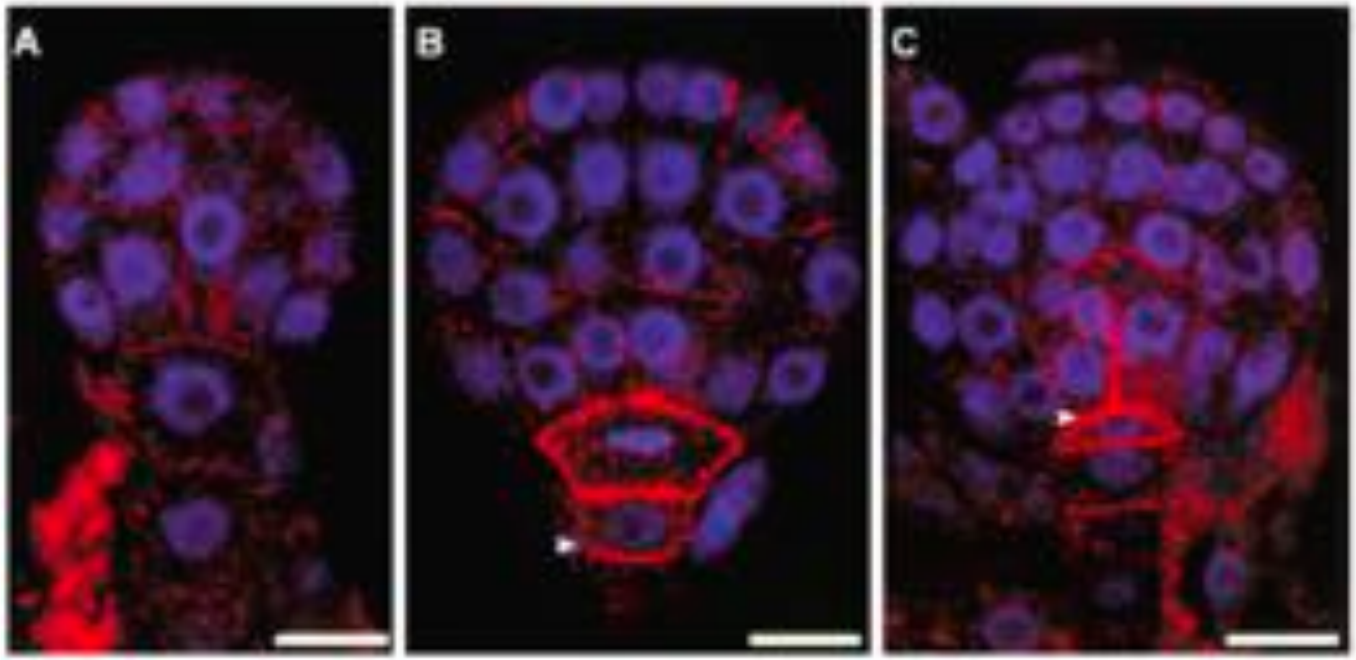


Fig. 1. *Arabidopsis* embryo development. Surface view and longitudinal cross-sections of a developing *Arabidopsis* embryo. Cells are coloured according to their lineage, as indicated in the key. Based on data from Yoshida et al. (2014).

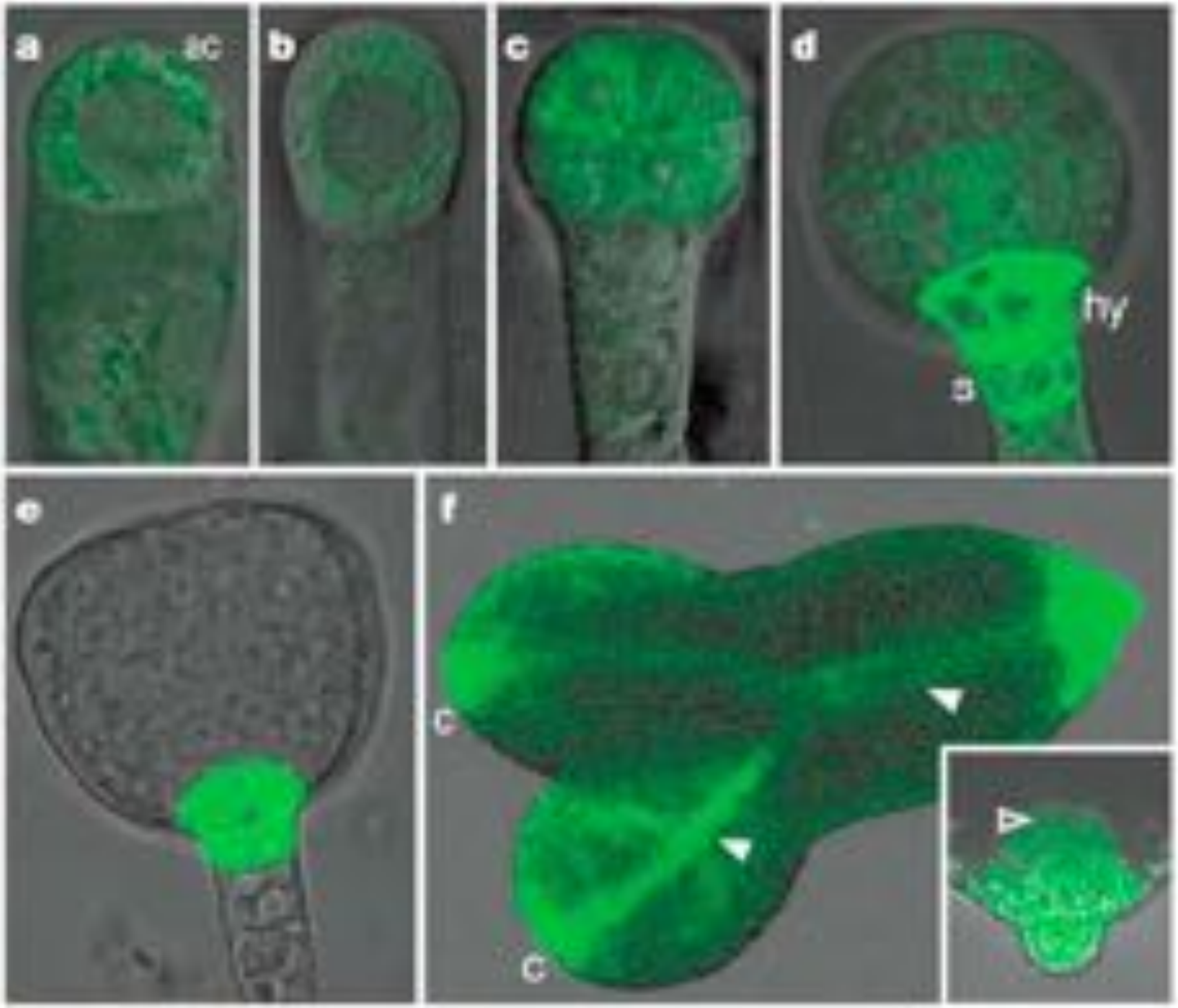
Immunolocalisation of PIN7 in Arabidopsis embryos



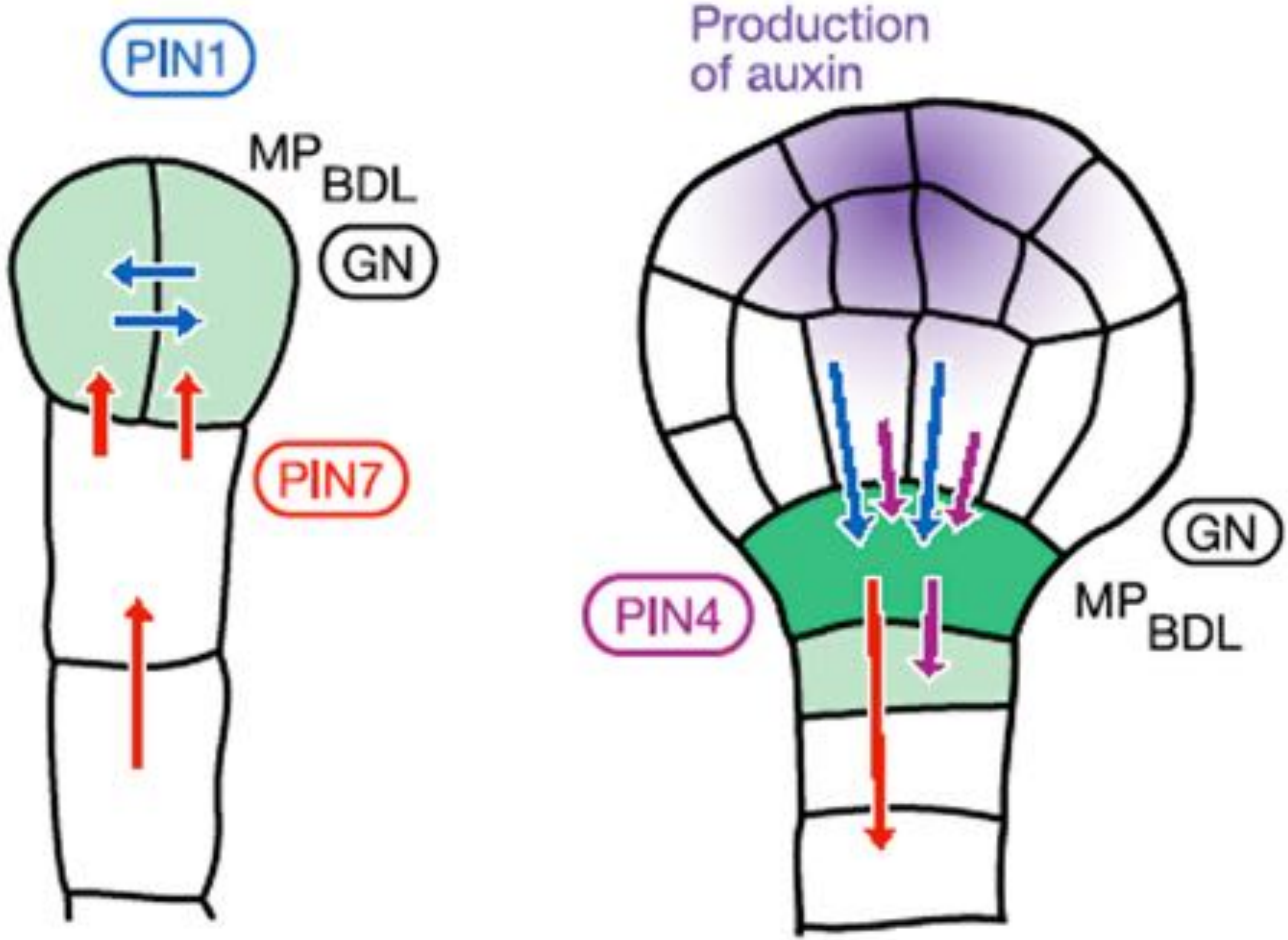
Immunolocalisation of PIN4 in Arabidopsis embryos



Auxin triggered gene expression during embryogenesis



DR5::GFP


PIN1
MP
BDL
GN
PIN7
Production
of auxin
GN
PIN4
MP
BDL

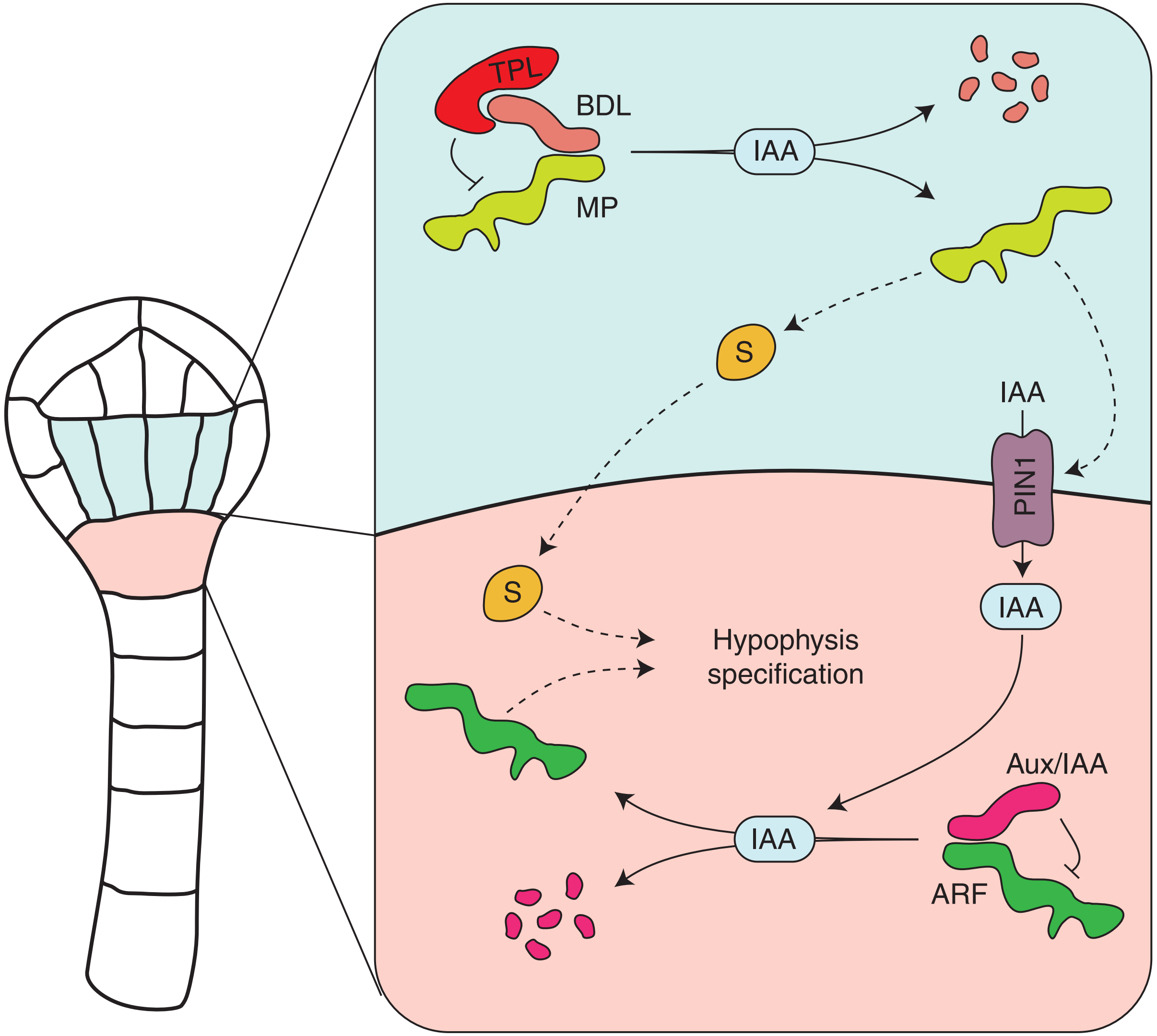
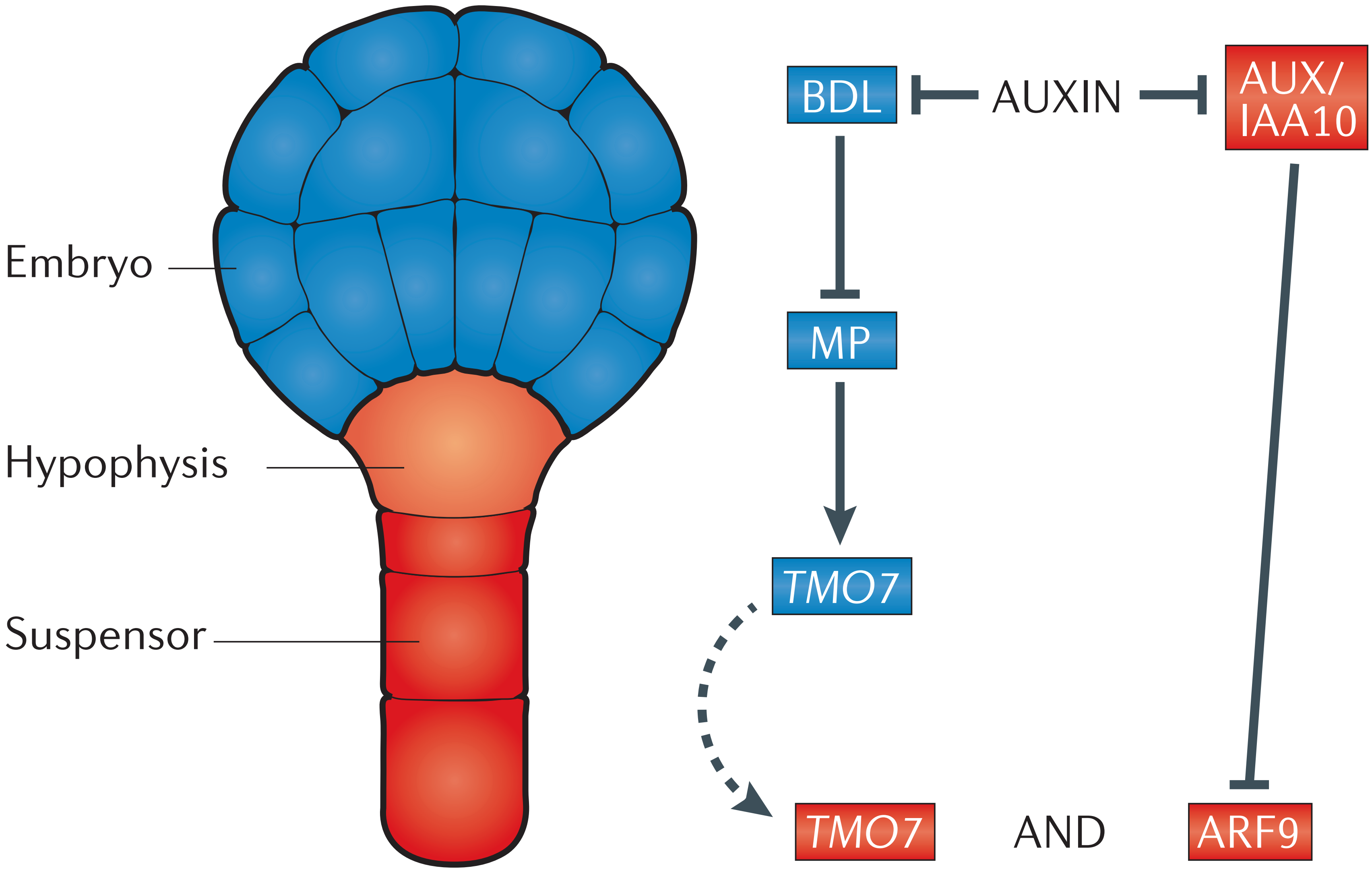
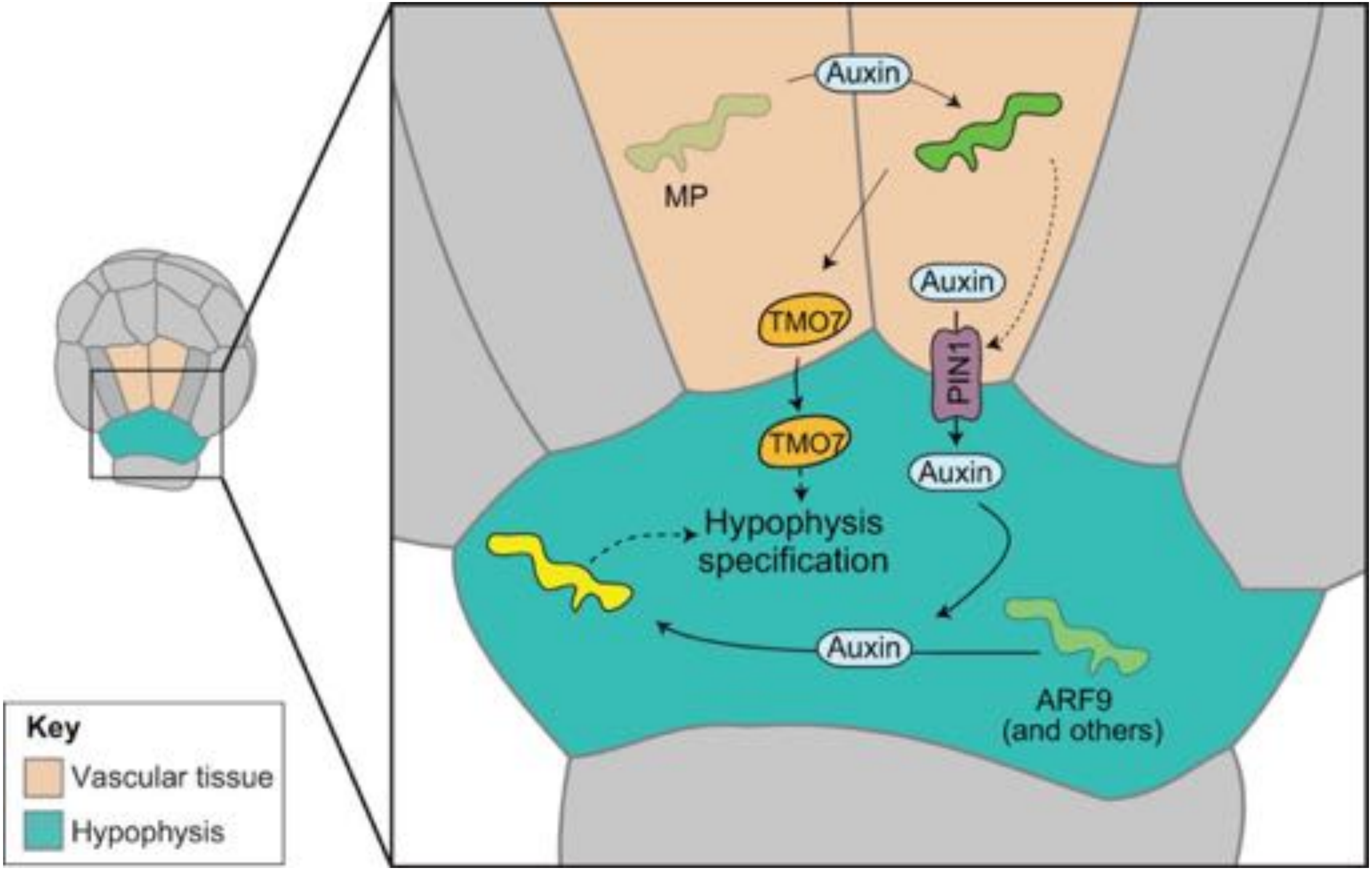


Figure 4. Hypophysis specification in the globular-stage embryo. MP activity is required non-cell-autonomously in the provascular cells (light blue) adjacent to the uppermost suspensor cell (pink) to specify this cell as hypophysis. In the provascular cells, high auxin levels release MP from its inhibitor, the Aux/IAA protein BDL, and the corepressor TPL. Subsequently, MP induces the expression of *PIN1* in the provascular cells, resulting in auxin transport to the uppermost suspensor cell. MP also promotes the transport of a hypothetical signal (S) to the future hypophysis. Here, auxin releases another yet unidentified ARF from a so far unknown Aux/IAA protein to elicit an auxin response that converges with S to specify hypophysis fate.

f Hypophysis determination



Mechanism for auxin-mediated specification of the root apical meristem



Cell-cell communication during specification of the root apical meristem

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