



**HAL**  
open science

## Auxin response factors are keys to the many auxin doors

Coralie Cancé, Raquel Martin-Arevalillo, Kenza Boubekour, Renaud Dumas

### ► To cite this version:

Coralie Cancé, Raquel Martin-Arevalillo, Kenza Boubekour, Renaud Dumas. Auxin response factors are keys to the many auxin doors. *New Phytologist*, 2022, 235, pp.402-419. 10.1111/nph.18159. hal-03692354

**HAL Id: hal-03692354**

**<https://hal.science/hal-03692354v1>**

Submitted on 25 Jul 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

# ARFs are keys to the many auxin doors

Coralie Cancé<sup>1,a</sup>, Raquel Martin-Arevalillo<sup>2,a,\*</sup>, Kenza Boubekeur<sup>1</sup> and Renaud Dumas<sup>1,\*</sup>

Address authors:

<sup>1</sup>Univ. Grenoble Alpes, CNRS, CEA, INRAE, IRIG-DBSCI-LPCV, 38000 Grenoble, France.

<sup>2</sup>Laboratoire de Reproduction et Développement des Plantes, Univ. Lyon, ENS de Lyon, UCB Lyon1, CNRS, INRA, Lyon, France.

<sup>a</sup>Co-first author

\*Authors for correspondence:

Raquel Martin-Arevalillo, [raquel.martin@ens-lyon.fr](mailto:raquel.martin@ens-lyon.fr) and Renaud Dumas, [renaud.dumas@cea.fr](mailto:renaud.dumas@cea.fr).

Total word count (excluding summary, references, tables, figures and boxes)	7931	Number of Tables	0
Summary word count	164	Number of Boxes	0
Number of Figures	7	Number of Supporting Information files	0

## Contents

- Summary
- I. Introduction
- II. Structure and function of ARF domains
  - II.A- The DNA binding domain
  - II.B- The Middle Region
  - II.C- The C-terminal domain PB1
- III. Role of ARF domains mediated interactions in auxin signaling
  - III.A- ARFs and auxin-dependent signaling
  - III.B- ARFs and auxin-independent signaling
  - III.C- Modulation of auxin signaling
  - III.D- Crosstalk with other hormonal signaling pathways
- IV. Conclusions
- Acknowledgments
- Funding
- References

## Summary

In plants, most developmental programs depend on auxin action. The best described model of auxin signaling pathway that explains most, but not all, auxin transcriptional responses relies on a derepression mechanism. Repressors called Aux/IAAs (Auxin/Indole-3-Acetic Acid) interact with ARFs (Auxin Response Factors), the transcription factors of the auxin signaling pathway, leading to a repression of ARF-controlled genes. Auxin induces Aux/IAA degradation, releases ARFs and activates transcription. However, this elegant model is not suitable for all ARFs. Indeed, in *Arabidopsis thaliana* which has 22 ARFs, only 5 of them fit into the model since they are the ones able to interact with Aux/IAAs. The 17 left have a limited capacity to interact with the repressors and their mechanism is still unclear. ARF-Aux/IAA differential interaction is one of the many examples of ARFs biochemical and structural diversification that affects ARFs action and therefore, auxin transcriptional responses. A deeper understanding of the structural properties of ARFs is fundamental to better explain the action of auxin in plants.

## Key words

ARF, domain, interactions, Nuclear Auxin Pathway.

## I- Introduction

Auxin is a key regulator of a myriad of processes involved in plant development. Many of its functions are evident at the organ and plant level such as flower initiation, tropisms, or lateral roots formation. However, the real action takes place at a much smaller scale, inside plant cell nuclei, where auxin triggers transcriptional changes leading to the activation or repression of the sets of genes implicated in the different auxin-controlled developmental programs. These transcriptional changes are the result of the transduction of auxin signal through a series of proteins that constitute the Nuclear Auxin Pathway (NAP) and that ultimately ends in the main characters of this review, the Auxin Response Factors (ARFs). Monopteros (MP) was the first protein to join the NAP family thanks to the observation of the *Arabidopsis thaliana mp* mutant phenotype, shared by *pin formed-1*, a mutant impaired in auxin transport (Przemeck et al., 1996). The later discovery of several ARF genes in *A.*

*thaliana* revealed that one of them, ARF5, was related to *mp* phenotype establishing a clear link between this family of proteins and auxin signaling (Hardtke, 1998; Ulmasov, 1997). Further work identified the other protein families involved in auxin signaling. Nowadays we know that the NAP is constituted by three core components: the Auxin/Indole-3-Acetic Acid (Aux/IAA) repressors, the TRANSPORT INHIBITOR RESPONSE 1 (TIR1)/AUXIN-SIGNALING F-BOX (AFB) receptors and the ARF Transcription Factors (TFs). TOPLESS/TOPLESS-related (TPL/TPR) corepressors, although not specific to auxin signaling, are also involved in the NAP. In the last decades we have gained huge insight into how these proteins modulate specific auxin inputs into particular transcriptional outputs. Most of this knowledge comes from studies done in the plant *A. thaliana* (note that further genes nomenclature will be mostly referred to this plant as *At*) and has given rise to different models, the best known being the one based on a de-repression mechanism: Aux/IAs interact with ARFs, and recruit co-repressor complexes that block ARF TFs. ARFs are released from this repression after auxin-triggered Aux/IAA degradation.

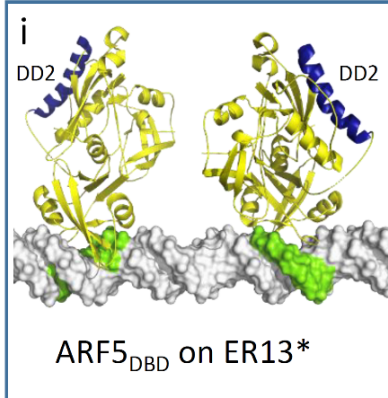
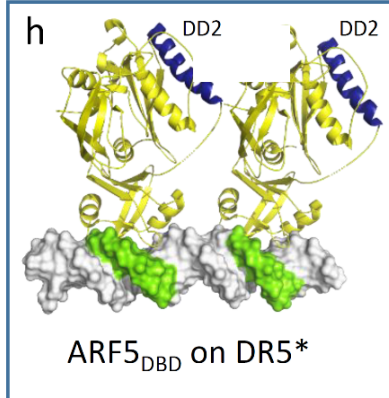
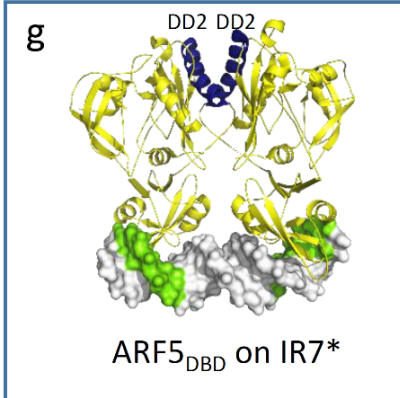
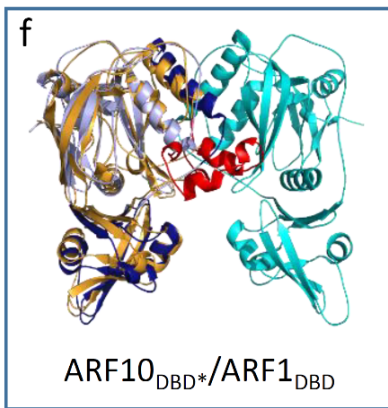
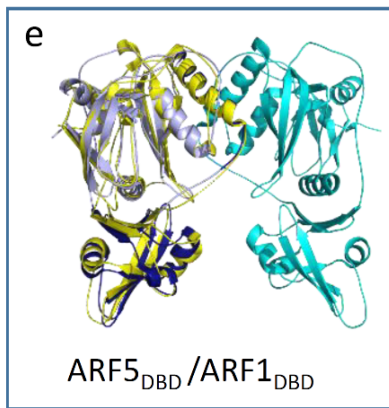
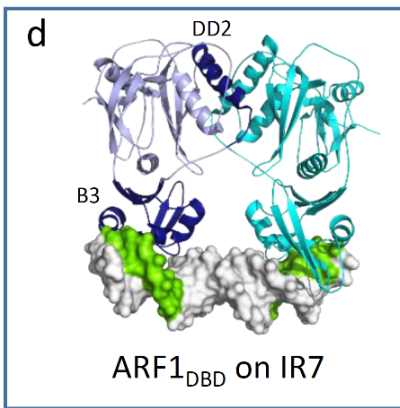
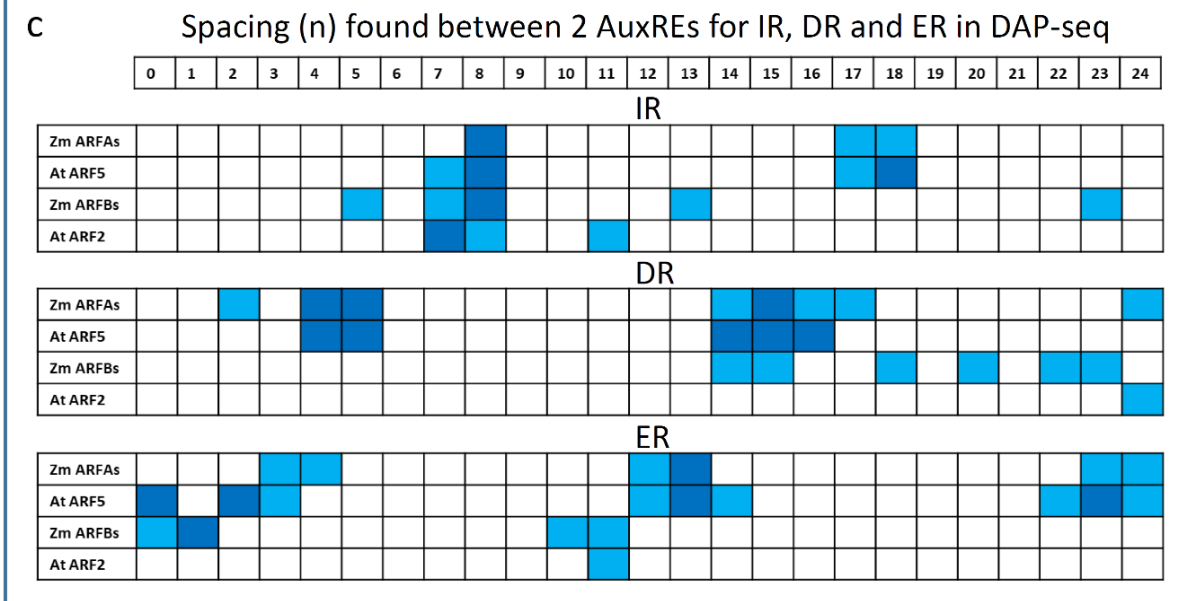
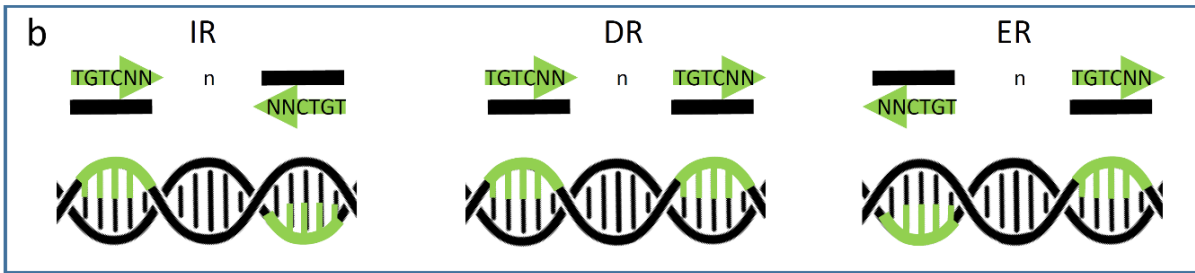
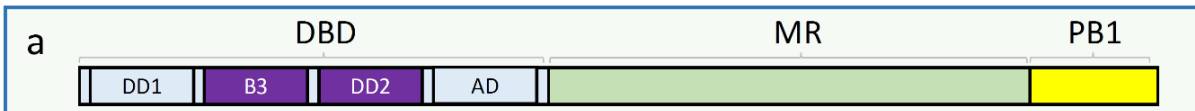
Recent studies have proposed an evolutionary history of the NAP (Bowman et al., 2017; Flores-Sandoval et al., 2018; Martin-Arevalillo et al., 2019; Mutte et al., 2018) rooting ARFs origins to early-charophyte algae where a single proto-ARF gene existed. A first duplication event led to proto-ARF diversification into two classes (A/B and C) in the late-divergent charophytes. A second division of class A/B into classes A and B took place in the first land plants giving the three evolutive classes described today: A, B, and C. Further duplications happened within the 3 classes that enlarged and diversified ARFs family in higher land plants (Mutte et al., 2018). No matter their class, ARFs are composed of three domains, a DNA Binding Domain (DBD) followed by a Middle Region (MR) and a C-terminal Phox and Bem1 (PB1) domain (Korasick et al., 2014; Nanao et al., 2014) that assemble in a modular structure, enabling them to work independently from one another (Fig. 1a). Despite their high degree of conservation, ARF domains diversified along evolution, gaining different capacities to bind DNA, to recruit partners or even different functions. We are only starting to understand how these differences affect ARF behavior and mechanisms of action in the control of auxin responses. In this review we will first describe the structural properties of the different domains of the 3 ARF classes. Then we will link key properties to different ARFs' involvement in the NAP in an auxin-dependent or independent context and in various

developmental processes. Many questions have been answered but many more remain to be answered.

## **II- Structure and function of ARF domains**

### **II.A- The DNA binding domain**

As with every TF, ARFs possess a domain that mediates interaction with DNA, the DNA Binding Domain, DBD. The specificity of a TF for DNA determines which genes are bound and thus, controlled by it. Therefore, ARF-DBDs and their DNA binding properties will be fundamental in deciding which genes are regulated by auxin or not. ARF-DBD is located at the N-terminus of the protein with a sequence of about 350 amino acids (Fig. 1a). Interaction with DNA happens through a B3 subdomain unique to plants (Swaminathan et al., 2008; Tim Ulmasov et al., 1999). B3 subdomains are also present in other plant TF families as LAV (LEAFY COTYLEDON2 (LEC2)-ABSCISIC ACID INSENSITIVE-3 (ABI3)-VAL), protein Related to ABI/VP1 (RAV) and REPRODUCTIVE MERISTEM (REM) (Swaminathan et al., 2008) each with different DNA binding specificities (Yamasaki et al., 2013). ARF-B3s bind sequences of 6 bp known as Auxin Response Elements (AuxRE) initially identified in promoters of auxin responsive genes as a TGTCTC sequence (Liu et al., 1994). However, studies of ARFs binding to DNA revealed that variations in the two last nucleotides of an AuxRE were permitted and could play a role in ARFs affinity for DNA. Notably, AtARF1,3,5 have a higher affinity for TGTCGG than the classic TGTCTC (Boer et al., 2014; Franco-Zorrilla et al., 2014). The increased affinity of ARFs for TGTCGG found its explanation in crystallographic studies of AtARF1 showing stronger interaction of a histidine residue (His136) with the last two G bases of the TGTCGG (Boer et al., 2014; Freire-Rios et al., 2020). This opens the possibility that small changes in B3-DNA interface modify the strength of the interactions modulating gene expression. Indeed, versions of the auxin transcriptional reporter DR5 chaining together consecutive TGTGNN sequences with variations in the two last nucleotides of the TGTCNN element present different patterns of expression *in planta* (Liao et al., 2015; Lieberman-Lazarovich et al., 2019). Exhaustive studies of different ARF affinities for different AuxREs could help understanding if and how the strength of these interactions translates into different auxin transcriptomic responses.



**Figure 1. ARFs specificities for DNA.** **a.** ARFs contain 3 domains: the DBD, the MR and the PB1 domain. The DBD contains two dimerisation domains (DD1 and DD2) flanking a B3 subdomain, and an ancillary domain (AD). **b.** Configuration of repeated AuxRE: IR, DR and ER motifs. *n* indicates the number of bp between two AuxREs (TGTCNN) for each motif. **c.** Summary of DAP-seq experiments done on *A. thaliana* (AtARF5 and AtARF2) and maize ARFs (different class A (ZmARF4,16,18,27,29,34,35) and class B (ZmARF7,10,13-14,25,36,39))(from Galli et al., 2018; O'Malley et al., 2016; Stigliani et al., 2019. For the clarity of the figure, the spacing *n* has been limited here to 24. Light and dark colors represent preferred and highly preferred motifs, respectively. **d.** Structure of AtARF1-DBD (class B) dimer in complex with IR7 (PDB code 4LDX) (two monomers in blue; B3 subdomain and DD2 are colored in dark blue in one monomer) **e.** Superimposition of one monomer of AtARF5-DBD (class A) (yellow, PDB code 4LDU) on AtARF1-DBD (blue). **f.** Superimposition of a model of one monomer of AtARF10-DBD (class C) (orange with its DD2 in red) on AtARF1-DBD (blue). **g-i.** Structure models of AtARF5-DBD dimer docked on IR7 (g), DR5 (h) and ER13 (i). Structures were made using PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC. ER and IR nomenclature corresponds to the new nomenclature used since 2020 (Freire-Rios et al., 2020) (the old ER7 is now IR7; the old IR13 is now ER13; DR remains unchanged). AtARF10-DBD model was generated by RoseTTaFold (Baek et al., 2021). Abbreviations used: DBD (DNA Binding Domain), MR (Middle Region), PB1 (Phox and Bem1), DD (Dimerization Domain), AD (Ancillary Domain), IR<sub>n</sub> (Inverted Repeat spaced by *n* nucleotides), ER<sub>n</sub> (Everted Repeat spaced by *n* nucleotides), DR<sub>n</sub> (Direct Repeat spaced by *n* nucleotides).

Several studies suggested that ARFs bind repeated AuxREs (Freire-Rios et al., 2020; Guilfoyle et al., 1998; Martin-Arevalillo et al., 2019; Tim Ulmasov et al., 1999; T. Ulmasov et al., 1999; Ulmasov, 1997). However, knowledge on ARF interactions with double AuxREs was limited to studies done using small, isolated DNA sequences tested *in vitro* for their binding to ARFs. The recently developed DNA Affinity Purification and Sequencing (DAP-seq) technology provided a genome-wide perspective to the understanding of ARF DNA binding (O'Malley et al., 2016). DAP-seq uses recombinant TFs that are combined *in vitro* with a library of fragmented genomic DNA permitting the identification of the genomic regions potentially bound by the TF of interest. Contrary to TF-DNA interaction tests such as Electrophoretic Mobility Shift Assays (EMSAs) or Yeast One-Hybrid assays (Y1H), where single DNA sequences are used, DAP-seq allows identification of preferential binding sites in a genomic context and of surrounding elements that might influence binding. DAP-seq was applied to *A. thaliana* and maize genomes to determine the specificity of class A and B ARFs. Although there are some differences between AtARFs and maize ARFs, binding rules have begun to emerge (Galli et al., 2018; O'Malley et al., 2016; Stigliani et al., 2019). In the genomic regions bound by ARFs three configurations of double AuxREs are enriched: Inverted Repeats (IR,

double sites in which two AuxREs are oriented towards each other in different strands of DNA), Direct Repeats (DR, two AuxREs following each other in the same DNA strand) and Everted repeats (ER, two AuxREs oriented backwards each over in different strands of DNA) (Fig. 1b) (Please note that different nomenclatures have been used to refer to the orientation of double AuxREs; In this review we use the most recent one from Freire-Rios et al., 2020). Class A ARFs bind all three configurations whereas class B binds mainly to IR sites, indicating that the orientation of the two AuxREs is fundamental for the specificity of ARFs binding to DNA (Fig. 1c). Another element determinant for the specificity of the binding is the spacing between both sites. Very specific spacings were detected for each binding with a wider permission again for class A ARFs (IR7-8 and IR18; DR 4-5, DR14-16 and DR25; and ER3, ER13 and ER23) compared to the class B ARFs (IR7-8) (Fig. 1c).

ARFs available structural studies partially explain how orientation and spacing of two AuxREs are main determinants of ARFs binding. Indeed, AtARF1-DBD structure revealed that regions flanking the B3 subdomain fold into a Dimerization Domain (DD1 and DD2) enabling AtARF1 to form a dimer when binding to IR7 (Fig. 1d). In this structure, the DD functions as a caliper placing two DBDs at a distance that allows ARF dimers to bind IR motifs spaced specifically by 7 or 8 bases (IR7/8) whereas other spacings are less favored (Boer et al., 2014). The same group also determined the structure of AtARF5 (class A) (Boer et al., 2014). Superimposition of both structures (Fig. 1e) shows a high conservation between ARF-DBDs from A and B classes, coherent with their similar preferences for IR motifs with a 7/8 nucleotides spacing. DAP-seq has not been applied to class C ARFs yet but EMSA interaction tests suggest that ARFs from this class present a different binding behavior. In contrast to class A and B ARFs, AtARF10 (class C) binds IR motifs with relaxed spacing specificity (IR4-9) (Martin-Arevalillo et al., 2019). Although the structure of the DBD of class C ARFs has not been determined, structure models disclosed that class C DBDs possess an inserted disordered region within the DD2 that could modify dimerization and explain their different behavior (Martin-Arevalillo et al., 2019) (Fig. 1f).

Whereas enrichment in IR7/8 fits with precedent results and could be explained by the caliper model, it is difficult to interpret the binding to DR and ER motifs with the same logic. Indeed, structure modeling of AtARF5-DBD on DR5 or ER13 suggests that the known DD2 may not be involved in ARFs dimerization on DR and ER motifs (Fig. 1h,i). Given the



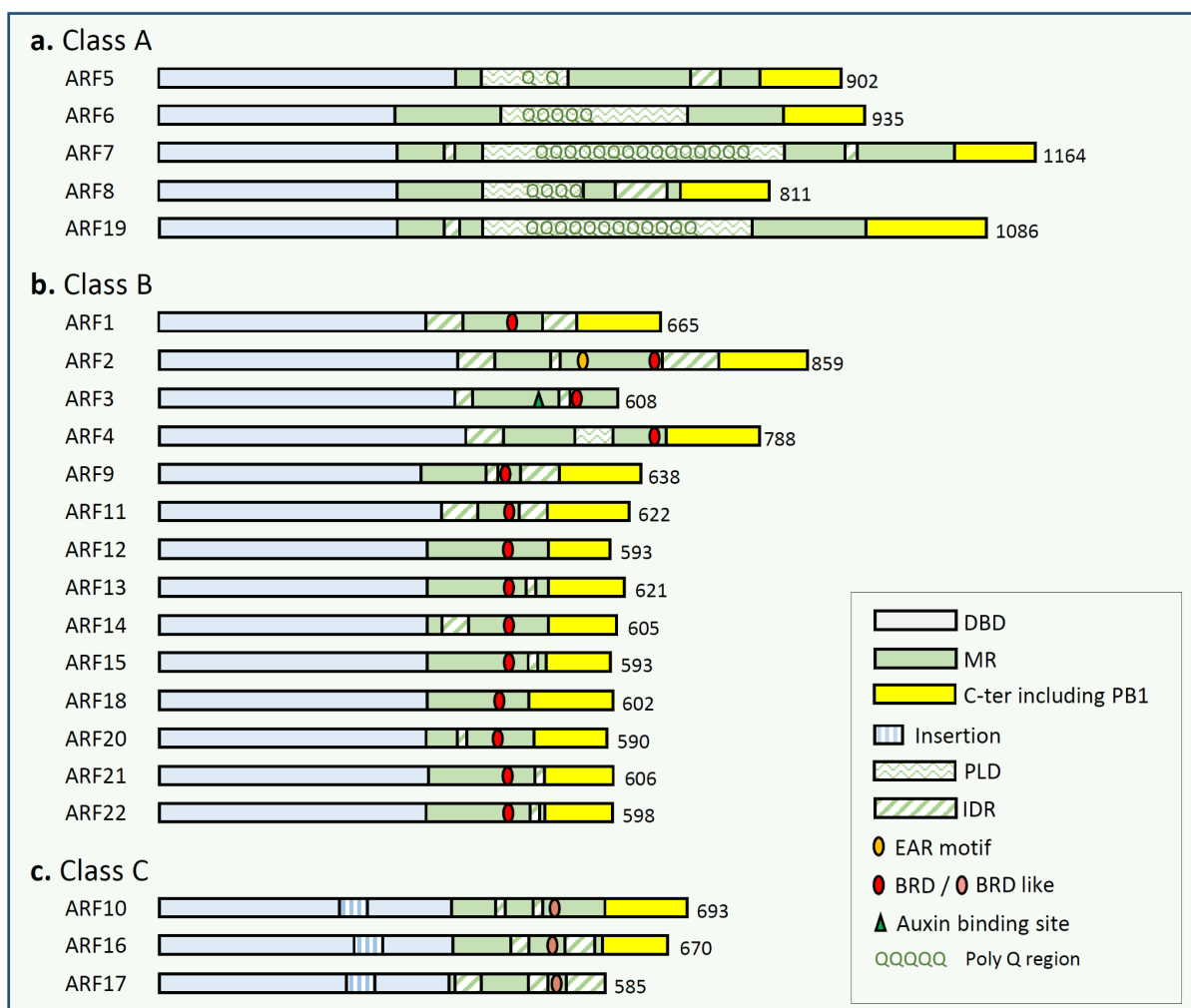
similarity between class A and B ARF-DBD structures, it is unlikely that their differential binding to ER and DR motifs comes from this domain and could rather rely on the MR or PB1 domains.

Interestingly, ARFs binding to DNA could be affected by other TFs. The DBD of the three ARF classes (AtARF5,6 (class A), AtARF1,2 (class B) and AtARF16,17 (class C)) have been shown to interact with G-box-binding factor-1/2 basic Leucine Zipper (GBF1/2 bZIP) TFs (Smit et al., 2020). More specifically, enrichment of G-box motifs was observed in the vicinity of AtARF5 AuxRE motifs, which may modulate the interaction of GBF2 with AtARF5 to subsequently control the activity of AtARF5 (Smit et al., 2020). Interactions involving the MR or PB1 domains can also lead to a modulation of ARF binding to DNA allowing a cross-talk between different signaling pathways. This will be discussed into more details in section III.

Altogether, differences in B3 subdomains and dimerization interfaces are fundamental structural features that determine ARFs specificities for DNA and therefore, that decide upon which genes are regulated in response to auxin. For a better understanding of ARF-DNA binding rules, DNA-binding tests at a genome scale, such as DAP-seq or ChIP-seq will be fundamental in the future, especially for members of class C ARFs, for which we lack any kind of information. Further structural work could be useful to identify ARFs binding modes as dimers to various types of AuxRE configuration and could permit to better understand the different DNA binding preferences from the three ARF classes. ARFs class A and B DBD structure similarity and shared DD open the possibility of ARFs heterodimerization through their DBD, an option that has not been explored yet. According to their structure we could imagine that only certain heterodimerization modes are possible and again, this would be motif dependent. Heterodimerization between DBD of class A and B ARFs via their DD seems possible on IR 7/8 motifs due to their similar binding mode. However, heteromerization on DR or ER motifs as well as heterodimerization between the DBD of a class A or B ARF with a class C ARF having an insertion in its DD seems unlikely. It would be attractive in the future to determine whether motif-dependent heterodimerization between class A and B ARFs could contribute to the modulation between activation and repression of different promoters.

## **II.B- The Middle Region**

In between the N-terminal DBD and the C-terminal domain, ARFs present a region of variable length named the Middle Region (MR). Contrary to the high conservation observed for the DBD, ARF-MR are very variable domains. Still, some general properties characterize MR from different ARF classes. Class A ARF-MR are rich in glutamine residues and highly disordered. Their Intrinsically Disordered Regions (IDR) are highly hydrophobic and predicted to form Prion-Like Domains (PLD (Powers et al., 2019)). On the other hand, class B and C MRs are marked by a high content of serine, proline and threonine (Guilfoyle et al., 1998; Tim Ulmasov et al., 1999) and predictions suggest a lower degree of disorder compared to class A (Roosjen et al., 2018) (Fig. 2).



**Figure 2.** Schematic organization of ARF-MR domains. **a.** Class A. **b.** Class B. **c.** Class C. Intrinsic Disordered Region (IDR), Prion Like Domain (PLD), Amphiphilic Repression Motif associated with the Ethylene-Responsive element binding factor (EAR), B3 Repression Domain (BRD), insertion sequences, auxin binding sites and glutamine-rich regions (Poly Q region) are indicated.

While the DBD decides which genes are regulated, the MR is the one determining the way they are regulated. Transient expression of different ARF-MR fused to Gal4-DBD in protoplasts revealed that ARFs can either activate or repress transcription and that their capacity to do either one or the other relies exclusively on their MR (Tiwari, 2003). ARFs are therefore classified according to their function into activators and repressors. This classification must be considered carefully. Activator ARFs belong all to the phylogenetic class A and their capacity to activate has been extensively shown. This is not the case for the rest of the ARFs. Classes B and C comprise the repressors. However, only certain members within class B have actually been proven to act as such (Tiwari, 2003). As for class C ARFs, certain publications pointed their role as repressors but more recent work puts under question their implication in the auxin pathway (Flores-Sandoval et al., 2018; Kato et al., 2020). If ARFs function is not clear, how they carry it out is even more of a mystery. The main hypothesis is that the MR acts as an interaction domain for recruiting different types of cofactors. Evidence for this are interactions described with chromatin remodelers.

The MR of the activator AtARF5 interacts with the chromatin remodelers SPLAYED (SYD) and BRAHMA (BRM) that help chromatin opening and further activation of genes in response to auxin (Wu et al., 2015). How this interaction happens and if other activator ARFs possess this same property is yet to be studied. MR of the other class A ARFs (AtARF6-8,19) share a longer stretch of glutamine residues in association with IDRs and PLDs compared to AtARF5 (Fig. 2a). Could this affect their binding to BRM or SYD? Interestingly, some class B ARFs (AtARF3,18) seem also able to bind chromatin remodelers (Efroni et al., 2013) despite their very different MR composition. The role of these interactions at the level of chromatin remodeling has not been studied yet.

On the other hand, most of class B ARF-MRs contain repression domains (R/KLFG sequences) within ordered sequences associated with the recruitment of the C-terminal part of TPL/TPR co-repressors. R/KLFG sequences are also present on other B3 subdomain-containing proteins (RAV proteins) and for this reason have been referred to as the B3 Repressor Domain (BRD) (Choi et al., 2018) (Fig. 2b). Class C ARFs possess a BRD-like domain with a slightly different sequence (VLFG) (Fig. 2c). In addition, the MR of some class B ARFs (AtARF2 or certain *Physcomitrella patens* ARFs for instance) also contains an Amphiphilic Repression Motif associated with the Ethylene-Responsive element binding

factor (EAR motif, sequence LxLxLx) (Causier et al., 2012; Causier et al., 2012; Choi et al., 2018) identical to that found on Aux/IAAs and which allows interaction with the N-terminal part of TPL/TPRs (Ke et al., 2015; Martin-Arevalillo et al., 2017) (Figs. 2,3). However, interaction with TPL has only been detected for certain class B ARFs. Whether this property is translatable to the whole class or shared by class C ARFs remains unexplored.

Apart from chromatin remodelers and corepressors, a large number of transcriptional regulators have been identified as interactors of class A ARF-MR (BRASSINOSTEROID INSENSITIVE 2 kinase (BIN2), PHYTOHORMONE-INTERACTING FACTOR 4 TF (PIF4), BRASSINAZOLE-RESISTANT 1 TF (BZR1), BR-ENHANCED EXPRESSION 2 TF (BEE2), HOMOLOG OF BEE2 INTERACTING WITH IBH1 TF (HBI1), DELLA repressor (Hu et al., 2018; Oh et al., 2014) and class B ARF-MR (BIN2, *Carica papaya* ETHYLENE-INSENSITIVE3-like 1 (CpEIL1)) (Vert et al., 2008; Zhang et al., 2020) using Yeast-Two-Hybrid (Y2H) and pull-down experiments suggesting a role of the MR as scaffold for signaling cross-talk. Additional interactors have not been reported for class C MR.

Besides acting as a protein-protein interaction platform, recent studies suggest that PLDs present in certain ARF-MRs could play a role in protein availability. Powers *et al* demonstrated that AtARF7,19 ARF-MR (together with the PB1) is involved in the formation of micron-sized protein assemblies that accumulate in the cytoplasm. Because of their size, these assemblies may prevent the diffusion of proteins from the cytoplasm limiting TF availability in the nucleus (Powers et al., 2019). Condensation of ARFs into liquid droplets could also affect interactions between protein partners or DNA and thus have a role in activating gene expression, as shown for other IDR-containing TFs (Boija et al., 2018).

A last and surprising role for ARF-MR has been recently described for ETTIN (AtARF3). AtARF3 lacks the PB1 domain and its MR contains a C-terminal subdomain specific to this protein, ES (ETT-specific). Recent experiments showed that AtARF3-ES binds auxin. Two close regions mediate this binding: a serine patch in positions 523-529 and a Tryptophan residue in position 505, both conserved in ARF3 homologs in other plant species (Kuhn et al., 2020; Simonini et al., 2018a) (Fig. 2b). Auxin binding to AtARF3-MR modulates its interactions with corepressors and over ten TFs as described below (Kuhn et al., 2020; Simonini et al., 2018a, 2017).

Potential roles as hub for interactors, auxin perception, control of protein availability or modulation of DNA binding have added up in the last years to the classic roles of the MR as determinant of ARFs functions. However, while the identity of interactors and the role of these interactions is well known for some ARFs, it remains completely unexplored for most ARFs. The study of these domains is particularly difficult owing to their inherently disordered nature and poor conservation. More exhaustive studies of protein-protein interactions and identification of the specific regions involved in such interactions might help to clarify in the future if mechanisms such as TPL/TPR or BRM/SYD recruitment are applicable or not to all ARFs within a class or if interactions are ARF-specific. Moreover, interactions of some class B ARF with the remodelers BRM and SYD bring up the possibility of class B ARFs having a dual role as activators and repressors depending on the cofactor recruited. Understanding the physiological importance of such interactions would be a first step to test this possibility.

### **II.C- The C-terminal domain PB1**

Moving forward to the C-terminus of ARF proteins we find a small domain of 80-100 amino acids, formerly referred as Domain III/IV (DIII/IV). DIII/IV are also present in Aux/IAA proteins (Guilfoyle, 2015; Kim et al., 1997; Ouellet et al., 2001; Ulmasov et al., 1997). Resolution of the 3D structures of ARFs and Aux/IAA DIII/IV (AtARF5 (Nanao et al., 2014); AtARF7 (Kim et al., 2020; Korasick et al., 2014), AtAux/IAA17 (Han et al., 2014; Kim et al., 2020); *Pisum sativum* Aux/IAA4 (Dinesh et al., 2015)) revealed that these two domains actually fold into one single Phox and Bem1 (PB1) domain (Guilfoyle and Hagen, 2012; Nanao et al., 2014). PB1 domains are also present in fungi, animals and amoeba and in several protein families in plants.

ARFs and Aux/IAA-PB1 show a similar 3D structure with one negative and one positive face that allow PB1-PB1 interactions in a head-to-tail manner through electrostatic interactions and hydrogen bonds (Fig. 3a). ARF and Aux/IAA-PB1s interact according to a well-established network (Piya et al., 2014; Vernoux et al., 2011). On one hand, most PB1s of class A ARFs interact with most PB1s of class A ARFs and with Aux/IAAs. However, heterotypic interactions are stronger than ARF or Aux/IAAs homotypic interactions. This is explained by a higher number of electrostatic bonds between ARF and Aux/IAA-PB1s (Han et al., 2014; Kim et al., 2020; Korasick et al., 2015; Parcy et al., 2016).

On the other hand, Aux/IAAs and class B and C ARF-PB1 interactions are very limited. Only some class B (AtARF1, 2, 4, 9, 12-15, 18, 20, 22) and C (AtARF10, 16) ARFs interact with a small number of AtAux/IAAs (Li et al., 2011; Piya et al., 2014; Trigg et al., 2017; Vernoux et al., 2011; Wang et al., 2013). No PB1 structure has been obtained for class B and C ARFs yet but structural models show small differences in amino acids involved in hydrogen bonds or hydrophobic contacts that could explain their lower capacity to interact with Aux/IAAs (Parcy et al., 2016).

As the de-repression model proposes, ARFs-Aux/IAA heterotypic interaction is fundamental for auxin signaling since it establishes the connection between ARFs and auxin. However, ARF PB1-PB1 interactions are also important. They have been shown to be fundamental for ARFs function in *Marchantia polymorpha* where class A and class B ARFs lacking their PB1 failed to complement *arf* mutants. Interestingly, chimeric ARFs where their PB1 was substituted by the Sterile Alpha Motif (SAM) oligomerization domain of LEAFY TF (LFY) partially rescued the mutants (Kato et al., 2020). These experiments suggest an important role for PB1 oligomerization other than interactions with Aux/IAAs. Also, ARF PB1 mutants affected in their capacity to homodimerize had a reduced capacity to bind DNA and to activate gene expression in yeast (Pierre-Jerome et al., 2016). ARF PB1-PB1 interaction could help the formation of alternative dimeric configurations that would allow interaction with DR and ER motifs not explained by the DBD structure, as previously presented (Stigliani et al., 2019); or promote ARFs oligomerization allowing their binding to multiple sites on DNA as suggested for LFY SAM oligomerization Domain (Sayou et al., 2016).

PB1 described interactions with proteins outside the NAP are limited to a small number of proteins (AtARF8 with the TF BIG PETALp (BPEp) (Varaud et al., 2011); AtARF1,7 and Aux/IAA3,19 with the TF MYB77 (Shin et al., 2007)). However, it is easily imaginable that these are not the only cases. In plants, many other PB1-containing proteins have been identified, including NIN-LIKE PROTEIN TF (NLP) involved in nitrate signaling (Hsin et al., 2021), protein kinases, OCTICOSAPEPTIDE /PHOX/BEMP1 (PB1) DOMAIN-CONTAINING PROTEIN/TETRATRICOPEPTIDE REPEAT (TPR)-CONTAINING PROTEIN (PHOX), CBS DOMAIN-CONTAINING PROTEIN (CBSCBSPB1), or NEXT TO BRCA1 GENE 1 (NBR1) (Mutte and Weijers, 2020). It will be important to determine whether these proteins are also capable of interacting with ARFs and/or Aux/IAAs PB1, thereby establishing points of crosstalk between

pathways. Moreover, some class B PB1s (AtARF12-15, 20-22) contain a conserved EAR motif. Although the functionality of this repression domain remains totally unexplored it opens the possibility of TPL-recruitment both through the MR and PB1 domains.

ARF-PB1 consensus function is to establish interactions. Whether interactions happen with Aux/IAAs, with other proteins outside the NAP or with itself seems to be specific to each ARF class. The biochemical determinants of such specificity are yet to be discovered. Structural information for ARF-PB1 from classes B and C could help to better understand such specificities. PB1 role for classes B and C remains unexplained as only some members interact with Aux/IAAs. Although the importance of such interactions remains to be investigated, it suggests that some class B and C ARFs function similarly to class A ARFs. If PB1 is not used to interact with Aux/IAA in most class B and C ARFs, one may wonder about its role. Several class B ARFs lack this domain in flowering plants (Paponov et al., 2009), raising the question of its importance for this class. This is the case for AtARF3, 17 (Fig. 2). The effect of the lack of PB1 is not known for AtARF17. However, its absence in AtARF3 is thought to disconnect it from the regulation of Aux/IAA degradation, retaining only regulation related to direct auxin binding to its MR. Also, isoforms of ARFs usually containing a PB1 can be produced without it by alternative splicing like AtARF13 (Okushima, 2005) and AtARF5 (Cucinotta et al., 2021) yielding a form insensitive to Aux/IAA repression. Lastly, additional roles for this domain as binding to DNA are yet to be explored. DNA binding studies with ARF mutants unable to homo-dimerize through their PB1 could provide insight into this matter.

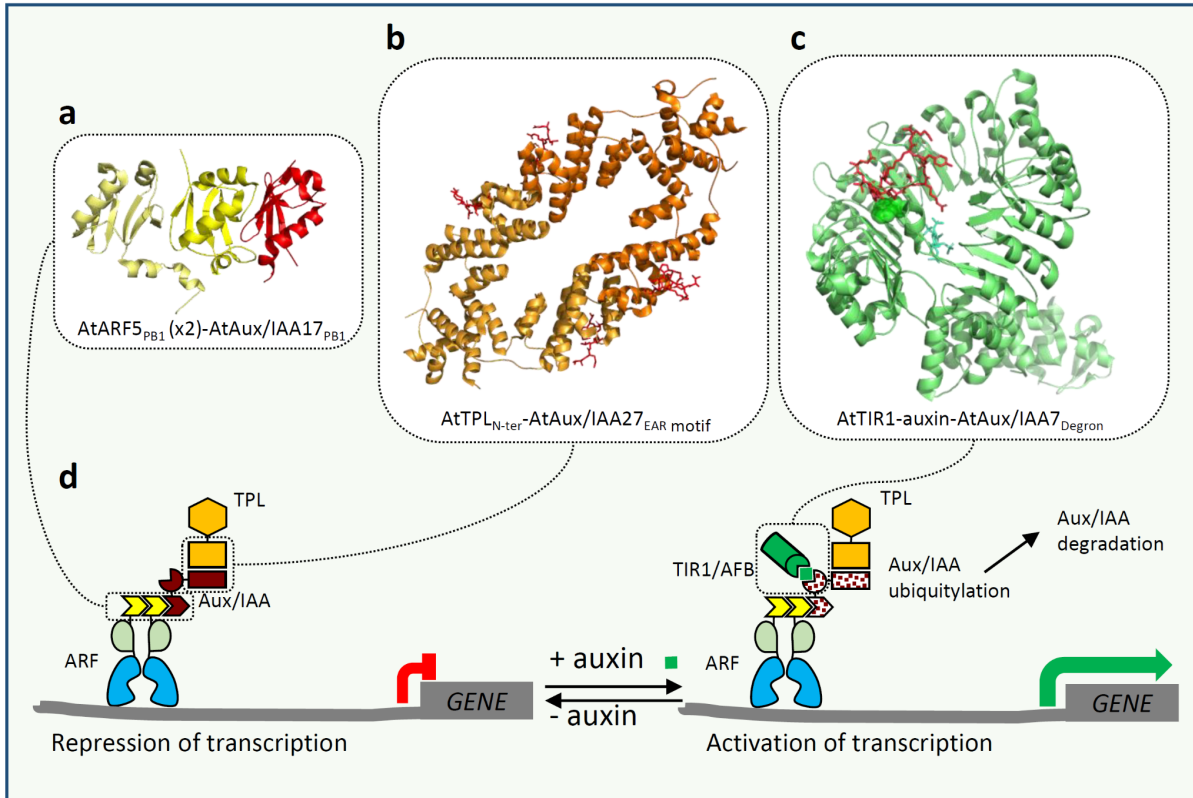
### **III- Role of the ARF domains mediated interactions in auxin signaling**

In this section we will focus on how the previously explained diversity of ARF structural and biochemical features drives ARFs different mechanisms in the context of the NAP. ARFs work together with 3 other proteins: TIR1/AFB auxin receptors and Aux/IAA repressors, specific to the auxin signaling pathway and TPL/TPR, a family of plant co-repressors implicated in many other signaling pathways (Fig. 3). These families of proteins interact with each other in 4 modules of interactions (Aux/IAA-ARFs (Fig. 3a), Aux/IAA-TPL (Fig. 3b), TIR1-Aux/IAA (Fig. 3c) and ARFs-DNA (Fig. 1b)) that perceive, transmit and translate auxin signal into a transcriptional output (Fig. 3d). Several crystallographic works have allowed us to

understand the structural details of these modules. Aux/IAAs are divided in three domains DI (EAR motif), DII (degron) and PB1 that interact with TPL/TPR, TIR1/AFB and ARF, respectively. Through its EAR motif and PB1, Aux/IAA bring TPL/TPR together with larger co-repressor complexes to the vicinity of ARFs, repressing expression of ARF-regulated genes (Hagen, 2015; Szemenyei et al., 2008). Increase of auxin levels inside a cell drives the formation of a co-receptor complex between the TIR1/AFB receptor, auxin and Aux/IAA DII. TIR1/AFB proteins belong to the SKP1-CUL1-F-box protein (SCF) E3 ubiquitin ligase complex (Gray et al., 1999; Ruegger et al., 1997) that triggers ubiquitination and further degradation of Aux/IAAs by the 26S proteasome. Aux/IAAs degradation leads to removal of TPL/TPR co-repressor complexes allowing ARFs to activate the genes under their control (Gray et al., 1999; Ruegger et al., 1997; Ulmasov et al., 1997).

Each of these protein families is composed of several members in high order plants (6 TIR1/AFB receptors, 5 TPL/TPR, 33 Aux/IAAs and 22 ARFs in *A. thaliana*). Numerous *in vitro* experiments have shown that different combinations of these members could alter the mechanism of the NAP as highlighted for the TIR1/AFB-Aux/IAA complexes. *In vitro* and Y2H studies have shown that the amino acids composition of the DII and regions flanking it influence their interaction with different TIR/AFB members, affecting Aux/IAAs degradation rates and ultimately the dynamics of auxin response (Calderón Villalobos et al., 2012; Dreher et al., 2006; Havens et al., 2012; Niemeyer et al., 2020; Pierre-Jerome et al., 2014; Ramos et al., 2001; Winkler et al., 2017). Although other interactions are not that well characterized, the ability of the various NAP members to interact will most certainly affect the final response. Therefore, understanding them and integrating them into the context of the full pathway is fundamental for a better comprehension of the auxin-triggered transcriptional response. In the previous section we focused on the diverse biochemical and structural features of the three ARF domains from different evolutive classes and within each class. Along the next section we will link these properties to ARFs' different mechanisms and roles in auxin signaling pathway and in auxin controlled developmental processes.





**Figure 3.** Auxin signaling and modules of interaction. **a.** Structure of AtAux/IAA17 PB1 (red) AtARF5 PB1 (yellow) complex (PDB codes 6L5KN and 4CHK). **b.** AtTPL N-terminus (orange) in complex with AtAux/IAA27 EAR motif (red sticks)(PDB code 5N9V). **c.** AtTIR1 (green)-auxin (green spheres)-AtAux/IAA7 degron (red sticks) complex (PDB code 2P1Q). **d.** De-repression model of the Nuclear Auxin Pathway.

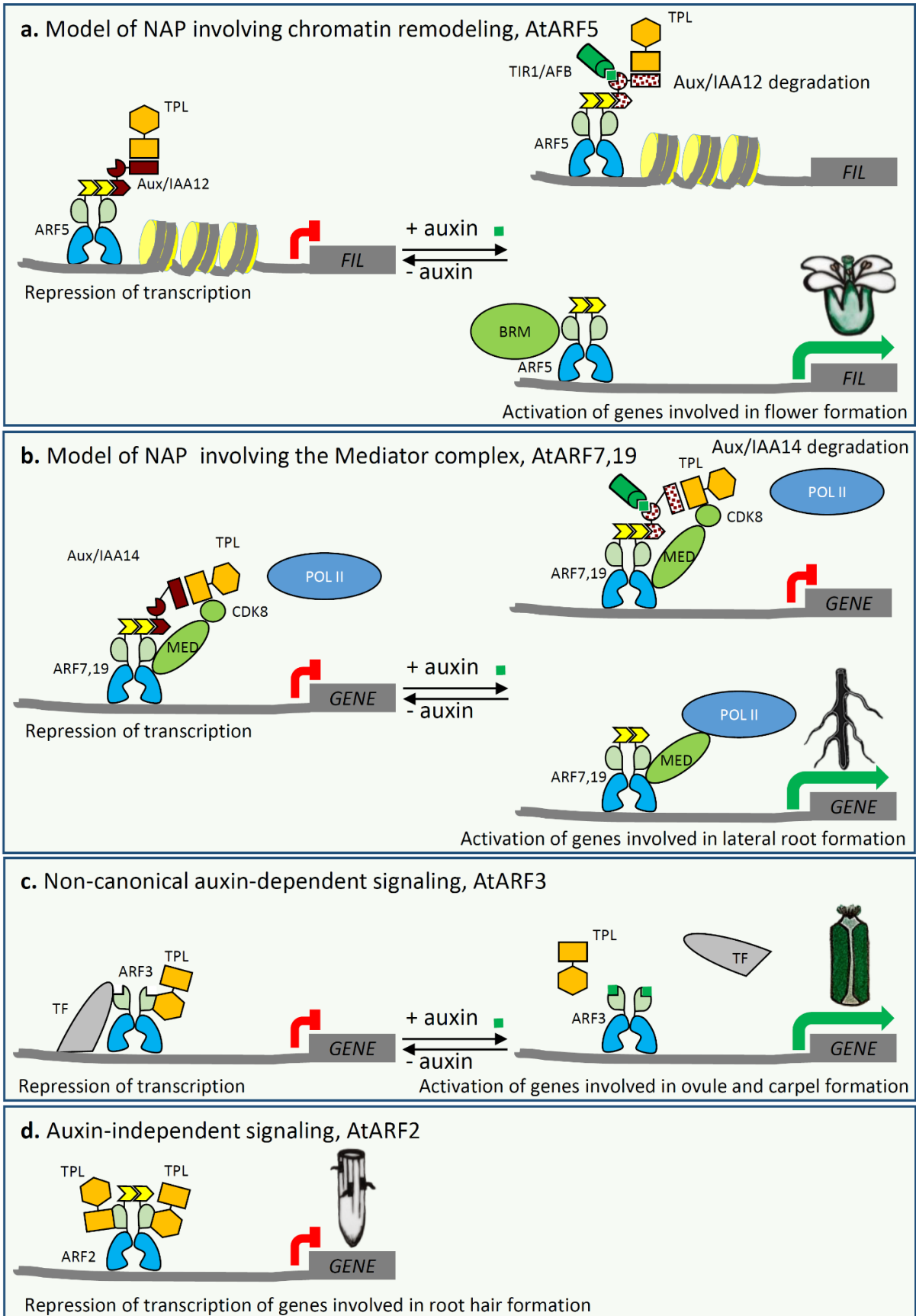
### III.A- ARFs and auxin-dependent signaling

Probably the clearest example of how different ARF properties might determine their role in the NAP is the differential capacity of the three ARF classes to interact with Aux/IAs through their PB1. As explained in section II.C, differences in ARF-PB1 determine that this interaction involves mainly class A ARF, leaving out most ARFs from other classes. For this reason, class A ARFs are considered to work in an auxin-dependent manner. Recent publications have shown the relevance of class A ARFs-Aux/IAA interactions in different developmental contexts and have revealed two possible mechanisms of transcriptional induction dependent on such interactions.

The first one concerns changes in chromatin states in response to auxin, a mechanism studied in the context of flower formation. MP has a fundamental role in flower

development: in response to auxin, it regulates genes implicated in flower formation. Chromatin immunoprecipitation experiments have revealed that MP, TPL and histone deacetylase HDA19 co-localize on gene loci involved in flower formation. Exogenous auxin treatment on inflorescences reduced TPL and HDA19 signals, events coinciding with chromatin opening, deposition of acetylation marks and localization of BRM and SYD chromatin remodelers on such loci. These findings led to a model according to which in the absence of auxin, Aux/IAA bound to MP-PB1, recruits TPL/TPR and HDA19 but also prevents BRM/SYD binding to MP acting as a physical impediment (Fig. 4a). Both events contribute to chromatin closure at the promoters of MP-regulated genes as shown on the *FILAMENTOUS FLOWER (FIL)* promoter. Auxin treatment triggers Aux/IAA degradation resulting in the eviction of TPL/TPR and HDA19 and later recruitment of BRM/SYD, allowing chromatin decompression and larger accessibility of transcription factors on the promoters of AtARF5-regulated genes (Wu et al., 2015) (Fig. 4a). This change in chromatin state mediated by both auxin and physical interactions of MP with Aux/IAA and BRM/SYD enables iterative initiation of floral primordia and may orchestrate other auxin-dependent cell fate reprogramming events (Weijers and Wagner, 2016; Wu et al., 2015).

The second mechanism involves RNA polymerase II (Pol II) recruitment through the mediator multiprotein complex, discovered in the context of auxin-controlled lateral roots formation (LRs) (Fig.4b). At low auxin concentration, AtARF7/19 interacts with the mediator complex and with AtAux/IAA14 that recruits TPL which in turn interacts with CDK8, a dissociable part of the mediator complex blocking Pol II enrollment (Ito et al., 2016; Leydon et al., 2021). Increase in auxin concentrations degrades AtAux/IAA14 and removes TPL and CDK8 permitting ARF7/19-mediator complex to recruit Pol II and leads to transcriptional activation of ARF7/19 regulated genes (Ito et al., 2016; Leydon et al., 2021) (Fig. 4b). Recently, it has been proposed that this system could constitute a sort of promoter pausing, a mechanism that has not been described in plants before (Morffy and Strader, 2021).



**Figure 4.** ARFs mechanism of transcriptional regulation **a.** Auxin signaling involving chromatin remodeling (AtARF5). **b.** Auxin signaling involving the Mediator complex

(AtARF7,19). **c.** Non-canonical auxin signaling (AtARF3). **d.** Auxin-independent signaling (AtARF2). For clarity of the figure, only one developmental mechanism is shown for each ARF. However, as indicated in the text, each ARF can be involved in several developmental processes.

These two mechanisms are not necessarily exclusive. We could imagine that they control transcriptional programs at different speeds. Indeed, the promoter pausing system is only dependent on Aux/IAA degradation and thus, compatible with the fast auxin-transcriptional responses observed only a few min after auxin induction. On the contrary, ARF-chromatin remodelers recruitment and chromatin changes could be associated to slower auxin transcriptional responses. This scenario seems promising, but it is still missing a huge amount of information: interactions with SYD/BRM have only been described for MP. They involve ARF5-MR but the specific region mediating them has not been determined yet. Similarly, interactions with the Mediator complex have only been shown for AtARF7/19 and we do not know how they happen or the domain of the ARFs implicated in the interactions. Are their MR also responsible for them? Could all class A ARF recruit both SYD/BRM and Mediator complex and trigger both transcriptional induction programs? Or rather, would different ARFs have the capacity to recruit either one or the other and decide upon which mechanism of transcriptional activation will be launched and control in this way the kinetics of the response? It will be of interest in the future to determine first, the specific regions implicated in such interactions and second, to extend interaction studies to all class A ARFs to determine if they share the same mechanism of transcriptional activation.

Both mechanisms lie on Aux/IAs acting as a physical impediment that is removed after auxin presence. Although the general rule establishes that only class A activators PB1 interact with Aux/IAs, certain interactions have been detected for classes B and C ARFs PB1 with a limited number of Aux/IAs. The importance of some of these interactions between class B and C ARFs and Aux/IAs has been demonstrated *in vivo*. AtARF9 is a class B ARF that in Y2H was found to interact with many Aux/IAA proteins (Vernoux et al., 2011). AtARF9-AtAux/IAA10 interaction was also observed *in vivo* and seems central to a suspensor-specific auxin response mechanism mediating hypophysis specification and preventing transformation to embryo identity (Rademacher et al., 2012). ARF10/16 class C ARFs interact with AtAux/IAA5. Although this interaction is part of a non-canonical

mechanism of auxin signaling (it will be further explained in Section III.C), it could establish the missing link between ARF C and auxin signaling.

The relevance of the rest of the described class B and C interactions with Aux/IAA and what PB1 features make them possible needs to be investigated since they open the door to an auxin-dependent action of certain class B and C ARFs in developmental contexts or tissues where their Aux/IAA partners are present.

Until a few years ago, auxin-dependent ARFs action was exclusively associated to their PB1 capacity to interact or not with Aux/IAAs. AtARF3 and its MR-ES domain changed this scenario. AtARF3 has a non-canonical auxin mechanism based on its ES domain ability to interact reversibly with auxin (Kuhn et al., 2020; Simonini et al., 2018a, 2018b, 2017, 2016) (Fig. 4c). Auxin binding to AtARF3 modulates its interaction with proteins (PROBABLE POLYOL TRANSPORTER 6 (PLT6)), TFs (KANADI 1 and 2 (KAN1,2), BABYBOOM (BBM), FIL, INDEHISCENT (IND), KNOTTED1-LIKE TALE 1 and 3 (KNAT 1,3), REPLUMLESS (RPL), TARGET UNDER ETTIN CONTROL (TEC1), TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR 4 TF (TCP4)) and corepressors (TPL/TPR, transcriptional corepressor SEUSS) involved in a wide range of developmental processes such as gynoecium morphogenesis, lateral root emergence, ovule development, and primary branch formation (Chung et al., 2019; Franks et al., 2002; Kelley et al., 2012; Pfluger and Zambryski, 2004; Simonini et al., 2018a, 2018b, 2017, 2016). Direct AtARF3-auxin interactions dissociates AtARF3-TPL complex, enabling the switch from a repressive chromatin state to a depressive state in a reversible way, an important mechanism for gynoecium development and floral initiation (Chung et al., 2019; Kuhn et al., 2020) (Fig. 4c). AtARF3 was also found to interact with BRM and SYD in Y2H (Efroni et al., 2013). This suggests that AtARF3-driven chromatin changes could also happen thanks to chromatin remodelers recruitment like MP activation mechanism. AtARF3 remains the only ARF described to be able to bind auxin and it constitutes the first example of DNA-binding hormone receptors in plants, characteristic of animal hormone signaling mechanisms.

### **III.B- ARFs and auxin-independent signaling**

Despite the few exceptions of interactions with Aux/IAA or the special case of AtARF3, class B and C ARFs must have alternative and auxin-independent mechanisms of action. These

ARFs are classed as repressors and several hypotheses attempt to explain how they do so. These include TPL recruitment, competition with class A ARFs for DNA or sequestration of class A ARFs.

TPL/TPR potential recruitment relies on ARF-MR. Repression through AtARF2-MR-TPL interactions is fundamental for root hairs formation. AtARF2-MR contains both an EAR motif and a BRD domain that allows binding to the N-terminal and C-terminal parts of TPL/TPR (Choi et al., 2018) (Fig. 4d). Individual mutations in these two repressive domains weakly affect AtARF2 inhibition of root hairs, whereas the double mutation strongly compromises it. These two mutations also affect flowering time and seed size, two phenotypes characteristic of *arf2* mutants. These results suggest that the EAR motif and the BRD domain cooperate to facilitate AtARF2 TPL/TPR recruitment to fulfill its repressive functions in different biological contexts (Choi et al., 2018). Although other ARF-TPL interactions have been shown *in vitro* the implications of such interactions and the domains involved in them remains to be studied. BRD domains conservation on other class B and C ARFs suggests that they could happen in the same way.

Secondly, ARFs could compete for binding to the same target gene, a mechanism dependent on their DBD properties. Although this idea is not well supported by *in vivo* evidence yet, class B and class A ARF-DBDs do share binding motifs and target genes (Boer et al., 2014; Lavy and Estelle, 2016; O'Malley et al., 2016; Stigliani et al., 2019). For competition to happen, both proteins should bind the shared target/motif within the same range of affinities. Single-molecule FRET affinity assays showed that DBDs from class A and B ARFs possess affinities for IR7/8 compatible with the theory of competition, with higher affinities calculated for class A ARFs ( $K_d$  MpARF1 =  $12 \pm 3$  nM,  $K_d$  AtARF5-DBD =  $9 \pm 4$  nM) compared to class B ARFs ( $k_d$  MpARF2 =  $62 \pm 3$  nM,  $K_d$  AtARF1 =  $140 \pm 80$  nM) (Freire-Rios et al., 2020; Kato et al., 2020). This suggests that at the same protein concentration, class A ARFs have a binding advantage on IR motifs. The class B/class A ARFs stoichiometry would then determine which of the two proteins binds to a shared target modulating its expression. Both proteins show different patterns of accumulation in *M. polymorpha* thallus, delimiting zones with different stoichiometries that could possess different sensitivities and transcriptional responses to auxin (Kato et al., 2020). Such a competition mechanism is likely

promoter dependent. Auxin-responsive genes with DR and ER promoter sites, preferably bound by class A ARFs, could be less affected by class B ARF competition.

Some PB1-PB1 interactions have been found between ARFs from different classes (Kato et al., 2015; Trigg et al., 2017; Vernoux et al., 2011). These interactions could sequester class A activator ARFs repressing them or bring TPL co-repressor complexes close to class A similarly to Aux/IAAs. Due to the similarity of their DBD structures, class A ARFs sequestering could also happen through DBD heterodimerization. However, such a repressive mechanism remains a hypothesis and we lack any biological evidence of it for now.

### **III.C- Modulation of auxin signaling**

Post-transcriptional and post-translational modifications also alter ARFs' intrinsic biochemical properties. Although very few examples exist for such processes, the resulting ARF variants have important effects in the modulation of the NAP.

Alternative spliced forms exist for several ARF genes involved in different developmental contexts. MP transcript generates two isoforms: MP and MP11ir, that translates into a protein lacking the PB1 domain. Interestingly, MP11ir partially complements *mp* mutant suggesting that certain functions of MP bypass its interaction with Aux/IAAs and therefore, could work in an auxin-independent manner (Cucinotta et al., 2021; Krogan et al., 2012) (Fig. 5a). MP11ir has an essential role in ovule development, activating genes (TARGET OF MONOPTEROS 3 (TMO3), TMO5, CUP-SHAPED COTYLEDON 1 (CUC1) and 2 (CUC2)) in different regions of the ovule characterized by low auxin concentrations (Cucinotta et al., 2021). AtARF8 alternative splicing also generates isoforms that lack a part of their PB1, ARF8.2 and ARF8.4. The latter contains an additional Leucine-rich sequence in its MR. These two ARF8 isoforms are involved in anther development (Ghelli et al., 2018). A third example is AtARF4 isoform  $\Delta$ ARF4 that loses a Ser-rich region conserved between ARF4 orthologs. The two AtARF4 transcripts possess different functions during carpel development (Finet et al., 2013). Whether there are more splicing variants or the mechanisms underlying the existent ones are still to be studied. PB1 loss could modulate transcriptional responses to different auxin levels, as it seems to be the case for MP11ir, whereas changes in ARFs MR could affect protein-protein interactions.

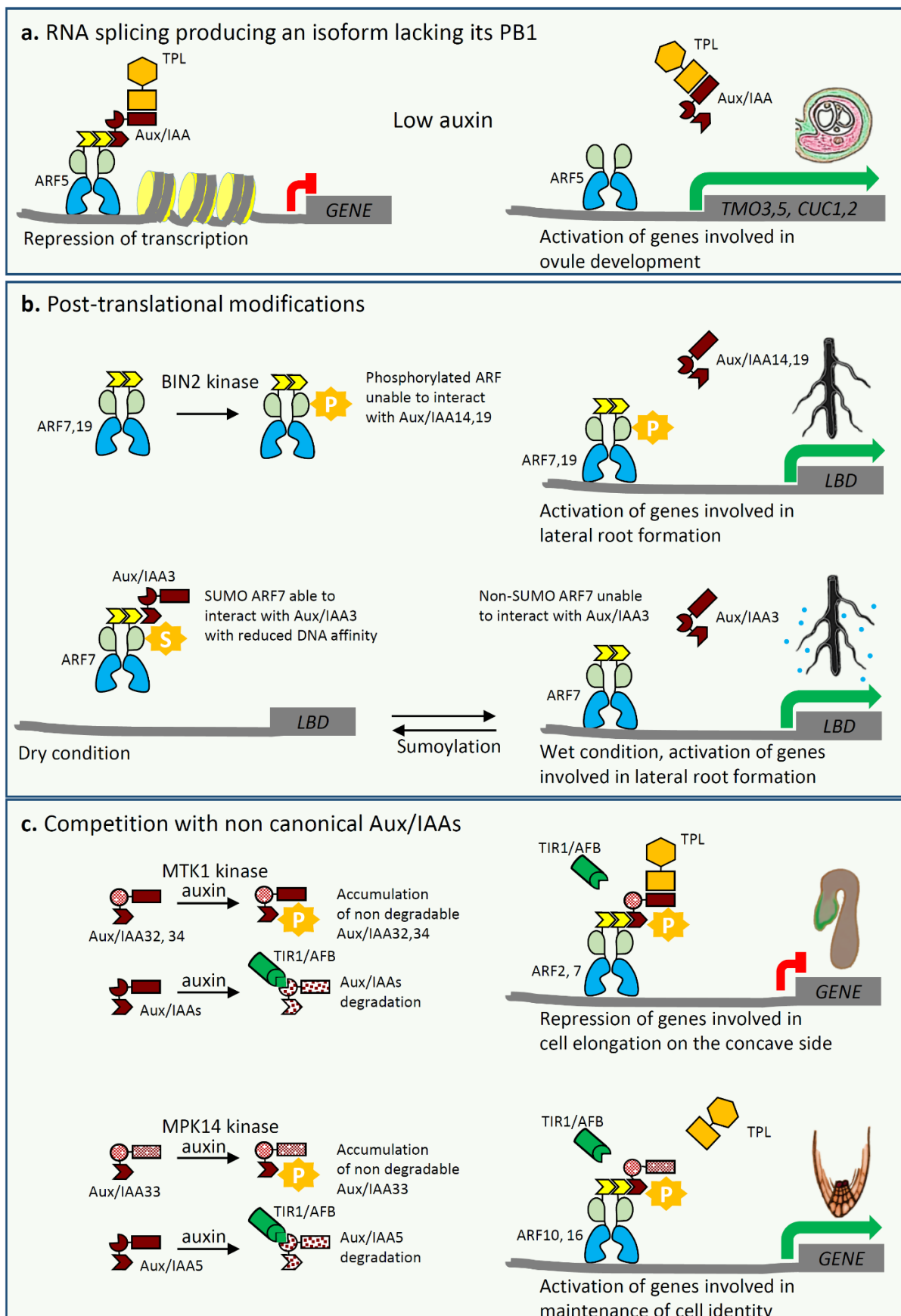
Three different kinds of post-translational modifications have been reported for ARFs: phosphorylation, sumoylation and ubiquitination. While phosphorylation and sumoylation affect Aux/IAA interactions and DNA binding (Fig 5b), ubiquitination induces protein degradation. AtARF2 was found to be phosphorylated by BIN2 kinase or under low K concentrations. In both cases, this modification leads to a decrease in DNA binding and reduced repressor activity (Vert et al., 2008; Zhao, 2018). AtARF7 and AtARF19 MR interaction with BIN2 leads also to their phosphorylation reducing their ability to interact with AtAux/IAA14,19 whereas it enhances their binding to *LATERAL ORGAN BOUNDARIES-DOMAIN (LBD) LBD16* and *LBD19* genes. Altogether this results in an auxin-independent increase in the activation of LBD genes involved in lateral root formation (Cho et al., 2014). In the same developmental context of LR formation, AtARF7 sumoylation controls root branching in response to different water conditions, a process known as hydropatterning (Orosa-Puente et al., 2018). Under dry conditions AtARF7 undergoes sumoylation. AtARF7 sumoylated protein interacts with AtAux/IAA3 and has a reduced DNA binding capacity. When the roots come in contact with water, AtARF7 is no longer sumoylated, allowing its binding to DNA and preventing its interaction with AtAux/IAA3. Through this modification AtARF7 induces an asymmetric expression of its target gene *LBD16* in lateral root founder cells in response to water conditions. This mechanism does not replace the canonical auxin mechanism (AtAux/IAA3 is still able to interact with TPL/TPR and TIR1/AFB), but rather modulates it. Finally, the clearest example of ARF ubiquitination has been described for AtARF6. The mechanism is induced by abscisic acid and leads to the degradation of the protein (Li et al., 2020).

Diversity within the other NAP protein families also modulates auxin signaling. A notable example are non-canonical Aux/IAAs (AtAux/IAA32-34) that lack Domain II and therefore are insensitive to auxin-dependent TIR1/AFB mediated degradation (Fig 5c). Certain ARFs heterodimerize with non-canonical Aux/IAA; how can this interaction control their activity? AtAux/IAAs 32 and 34 have a fundamental role in apical hook development (Cao et al., 2019). This process requires auxin concentration gradients with maximum hormone levels at the concave side of the hook that inhibit cell elongation generating differential growth and curvature of the hook. At high auxin concentrations, cleaved TMK1 kinase phosphorylates AtAux/IAA32,34 leading to their stabilization and accumulation. These non-degradable



Aux/IAAs will maintain their interactor ARFs repressed inhibiting gene expression and growth at the concave side. Lower auxin concentrations will trigger enough canonical auxin responses to induce gene expression and growth on the convex side of the hook. The combination of two auxin signaling mechanisms permits perception of different auxin concentrations and corresponding modulation of the transcriptional response. Several ARFs from different classes interact with AtAux/IAA32 and 34 and these interactions suppress their activity either as repressor or activator (Cao et al., 2019).

In addition to the absence of Domain II, AtAux/IAA33 does not contain an EAR motif, preventing its interaction with TPL/TPR. Modulation of auxin responses by this non-degradable and non-repressor protein happens through competition with canonical Aux/IAAs. This mechanism has been described for the control of stem cell identity in the root. When auxin levels increase, MPK14 phosphorylates AtAux/IAA33. Like in the previous example, this phosphorylation stabilizes the protein that accumulates. High AtAux/IAA33 levels will compete with AtAux/IAA5 for the interaction with ARF10/16, that control root distal stem cell identity. At the same AtAux/IAA5 levels decrease because of its auxin-dependent degradation (Lv et al., 2019).



**Figure 5.** Modulation of auxin signaling **a.** RNA splicing. **b.** post-translational modifications. **c.** competition with non-canonical Aux/IAAs.

### **III.D- Crosstalk with other hormonal signaling pathways**

Plant hormone signaling pathways are deeply interconnected to co-regulate plant development. ARFs constitute one of the levels of cross-talk of auxin responses with other phytohormones. Indeed, ARFs can establish direct cross-talk through interaction with proteins from other signaling pathways. These interactions happen through the three ARF domains and can alter their properties. The previously described interactions with BIN2 are a good example of this (Fig. 5b). BIN2 is a component of brassinosteroids (BRs) signaling pathway. Its interaction with different ARFs modulates their properties and constitutes a point of cross talk between auxin and BRs hormones. Indirect or genetic cross-talk can happen through ARFs regulating expression of genes involved in other hormonal pathways or the other way round. Below we discuss some examples of protein/protein interactions through ARFs that connect the auxin signaling pathway to different hormonal signaling pathways for different development processes.

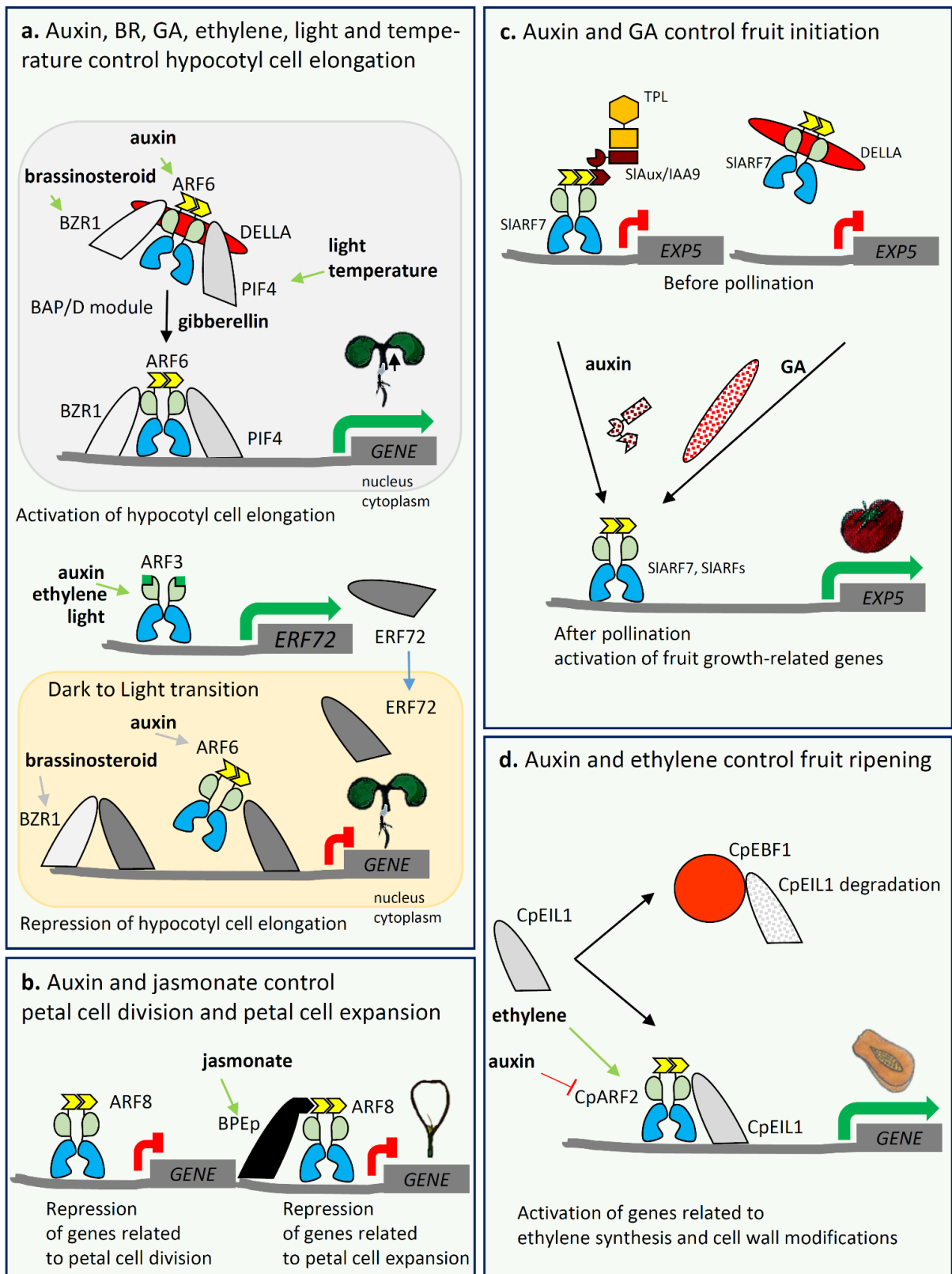
**Auxin, BRs, gibberellins (GA) and ethylene control hypocotyl cell elongation.** The elongation of hypocotyl cells involves a crosstalk between auxin, BR, GA and external stimuli such as light/temperature. Through direct interactions between AtARF6, BZR1 (BRs response), PIF4 (light response) and RGA (GA response) the 4 signals converge on promoter of genes that regulate the elongation of hypocotyl cells. Like auxin, GAs work through a de-repression mechanism driving degradation of DELLA repressors. The RGA DELLA protein interaction with ARF6 blocks its binding to DNA. In the presence of GA, RGA is degraded allowing the AtARF6/BZR1/PIF4 complex to form, bind DNA, and cooperatively regulate transcription (Oh et al., 2014)(Fig. 6a). Both ARF6 interactions involve its MR suggesting a competition between RGA and BZR1/PIF4. Y2H experiments showed that AtARF8 can also interact with BZR1 and PIF4 while AtARF1 and AtARF7 cannot. Moreover, class A AtARFs 7 and 8 can also interact with RGA while AtARF1 cannot. Such differences in interactions suggest different roles of ARFs within the class A and between class A and B in the crosstalk between auxin and these three signals.

ARF6/BZR complex is also regulated by ethylene in response to light. Light induces translocation of ERF72 (ethylene response) to the nucleus where it will repress the

ARF6/BZR complex and transcription of genes involved in hypocotyl elongation (Liu et al., 2018).

**Auxin and Jasmonate control petal growth.** Jasmonate and auxin control the number and size of petal cells through a variety of genetic and physical interactions (Brioudes et al., 2009; Szécsi et al., 2006). Jasmonate induces the expression of the transcription factor BIGPETAL (BPEp), that interacts with AtARF8-PB1 domain (Fig. 6b). At early stages of flower development, AtARF8/BPEp complex co-represses genes involved in cell proliferation. At later stages, physical interactions between AtARF8 and BPEp contribute to repress the transcription of genes involved in cell expansion (Varaud et al., 2011). The competition between Aux/IAA and BPEp for the binding to ARF8 was not discussed in this context.

**Auxin and GA control fruit initiation in tomato.** *Solanum lycopersicum* class A SIARF7 interacts through its MR with a DELLA protein. In the absence of auxin and GA, SIARF7/SIAux/IAA9 and SIARF7/SIDELLA complexes additively repress gene transcription. After pollination, auxin and GA induce SIAux/IAA and SIDELLA degradation, enabling SIARF7 and other class A ARFs to activate fruit growth-related gene (Hu et al., 2018) (Fig. 6c).



**Figure 6.** Crosstalk between Auxin signaling and other hormonal signaling pathways. **a.** Auxin, BR, GA, ethylene and light/temperature control hypocotyl cell elongation. **b.** Auxin and jasmonate control petal cell division and petal cell expansion. **c.** Auxin and GA control fruit initiation. **d.** Auxin and ethylene control fruit ripening.

**Auxin and ethylene control fruit ripening in papaya.** Auxin-ethylene cross-talk in papaya (*Carica papaya*) is mediated by interactions between CpARF2 and CpEIL1 (interaction domains not determined), a transcription factor that controls ethylene biosynthesis and cell wall modification-related genes. The interaction of CpARF2 with CpEIL1 weakens the interaction of CpEIL1 with the F-box protein CpEBF1, preventing its ubiquitinylation and 26S-proteasome dependent degradation. Stabilization of CpEIL1 by CpARF2 on promoters of genes related to ethylene biosynthesis and cell wall modification will result in their activation and papaya fruit ripening (Zhang et al., 2020) (Fig. 6d).

These few examples show the importance of class A and class B ARFs in the cross-talk between different hormonal signaling. They also highlight the complexity of these mechanisms with the various roles that ARFs of the same class can play.

#### **IV – Conclusion**

The more we learn about ARF proteins the more we realize that their mechanisms of action cannot be restricted to what we know about their most emblematic representant, MP, or to an activator or repressor role. A wide variety of transcriptional regulatory mechanisms appear to exist for the ARFs. Through this review, we have gone through the known diverse properties of ARF-DBD, MR and PB1 domains and put forward the unknown aspects of ARFs biochemical diversity and function. Important general questions remain to be answered: Are all class A ARFs able to condensate in large protein assemblies and to interact with chromatin remodelers or the mediator complex to induce transcription? Are all class B ARFs repressors? Can some of them act in an auxin-dependent manner? What is the DNA specificity and role of class C ARFs? What does seem clear is that ARF biochemical properties are intimately linked to their function and that figuring out the firsts is fundamental for unraveling the latter and ultimately understanding auxin signaling. Answers to those questions will be fundamental pieces to complete the simplified de-repression model of auxin signaling puzzle.

		Class A	Class B	Class C
DBD	Structure	AtARF5	AtARF1 MpARF2	no
	Binding to AuxREs			?
	Interaction with TFs			
MR	Structure	no	no	no
	Composition	Q-rich/PLD	S/T/P-rich	S/T/P-rich
	Transcriptional regulation			?
	Aggregation		?	?
	Interaction with coregulators			?
	Post-translational modifications		?	?
				?
		?	?	
	Interaction with TFs			?
PB1	Structure	AtARF5/7	no	no
	Aux/IAA heterodimerization			
	ARF homodimerization		?	?
	Heterodimerization with other TFs		?	?

**Figure 7.** A schematic representation of known ARF properties for each class (A, B and C) and characterized protein domain (Phox/Bem1p (PB1), middle region (MR) and DNA binding domain (DBD)). Outstanding questions to be determined are indicated with a question mark.

**Acknowledgments.** We thank the teams Flo\_Re (Laboratory of Cellular and Plant Physiology, CEA Grenoble, France) and Hormonal Signaling and Development (Plant Reproduction and Development laboratory, ENS Lyon, France)

**Funding.** This work was supported by the Agence Nationale de la Recherche [ANR-18-CE12-0014, ChromAuxi], a PhD fellowship from the ENS Lyon and University Grenoble Alpes [to C.C.] and the Grenoble Alliance for Cell and Structural Biology [ANR-10-LABX-49-01].



## References

- Baek, M., DiMaio, F., Anishchenko, I., Dauparas, J., Ovchinnikov, S., Lee, G.R., Wang, J., Cong, Q., Kinch, L.N., Schaeffer, R.D., Millán, C., Park, H., Adams, C., Glassman, C.R., DeGiovanni, A., Pereira, J.H., Rodrigues, A.V., van Dijk, A.A., Ebrecht, A.C., Opperman, D.J., Sagmeister, T., Buhlheller, C., Pavkov-Keller, T., Rathinaswamy, M.K., Dalwadi, U., Yip, C.K., Burke, J.E., Garcia, K.C., Grishin, N.V., Adams, P.D., Read, R.J., Baker, D., 2021. Accurate prediction of protein structures and interactions using a three-track neural network. *Science* 373, 871–876. <https://doi.org/10.1126/science.abj8754>
- Boer, D.R., Freire-Rios, A., van den Berg, W.A.M., Saaki, T., Manfield, I.W., Kepinski, S., López-Vidrieo, I., Franco-Zorrilla, J.M., de Vries, S.C., Solano, R., Weijers, D., Coll, M., 2014. Structural Basis for DNA Binding Specificity by the Auxin-Dependent ARF Transcription Factors. *Cell* 156, 577–589. <https://doi.org/10.1016/j.cell.2013.12.027>
- Boija, A., Klein, I.A., Sabari, B.R., Dall'Agnesse, A., Coffey, E.L., Zamudio, A.V., Li, C.H., Shrinivas, K., Manteiga, J.C., Hannett, N.M., Abraham, B.J., Afeyan, L.K., Guo, Y.E., Rimel, J.K., Fant, C.B., Schuijers, J., Lee, T.I., Taatjes, D.J., Young, R.A., 2018. Transcription Factors Activate Genes through the Phase-Separation Capacity of Their Activation Domains. *Cell* 175, 1842–1855.e16. <https://doi.org/10.1016/j.cell.2018.10.042>
- Bowman, J.L., Kohchi, T., Yamato, K.T., Jenkins, J., Shu, S., Ishizaki, K., Yamaoka, S., Nishihama, R., Nakamura, Y., Berger, F., Adam, C., Aki, S.S., Althoff, F., Araki, T., Arteaga-Vazquez, M.A., Balasubramanian, S., Barry, K., Bauer, D., Boehm, C.R., Briginshaw, L., Caballero-Perez, J., Catarino, B., Chen, F., Chiyoda, S., Chovatia, M., Davies, K.M., Delmans, M., Demura, T., Dierschke, T., Dolan, L., Dorantes-Acosta, A.E., Eklund, D.M., Florent, S.N., Flores-Sandoval, E., Fujiyama, A., Fukuzawa, H., Galik, B., Grimanelli, D., Grimwood, J., Grossniklaus, U., Hamada, T., Haseloff, J., Hetherington, A.J., Higo, A., Hirakawa, Y., Hundley, H.N., Ikeda, Y., Inoue, K., Inoue, S., Ishida, S., Jia, Q., Kakita, M., Kanazawa, T., Kawai, Y., Kawashima, T., Kennedy, M., Kinose, K., Kinoshita, T., Kohara, Y., Koide, E., Komatsu, K., Kopschke, S., Kubo, M., Kyojuka, J., Lagercrantz, U., Lin, S.-S., Lindquist, E., Lipzen, A.M., Lu, C.-W., De Luna, E., Martienssen, R.A., Minamino, N., Mizutani, Masaharu, Mizutani, Miya, Mochizuki, N., Monte, I., Mosher, R., Nagasaki, H., Nakagami, H., Naramoto, S., Nishitani, K., Ohtani, M., Okamoto, T., Okumura, M., Phillips, J., Pollak, B., Reinders, A., Rövekamp, M., Sano, R., Sawa, S., Schmid, M.W., Shirakawa, M., Solano, R., Spunde, A., Suetsugu, N., Sugano, S., Sugiyama, A., Sun, R., Suzuki, Y., Takenaka, M., Takezawa, D., Tomogane, H., Tsuzuki, M., Ueda, T., Umeda, M., Ward, J.M., Watanabe, Y., Yazaki, K., Yokoyama, R., Yoshitake, Y., Yotsui, I., Zachgo, S., Schmutz, J., 2017. Insights into Land Plant Evolution Garnered from the *Marchantia polymorpha* Genome. *Cell* 171, 287–304.e15. <https://doi.org/10.1016/j.cell.2017.09.030>
- Brioudes, F., Joly, C., Szűcs, J., Varaud, E., Leroux, J., Bellvert, F., Bertrand, C., Bendahmane, M., 2009. Jasmonate controls late development stages of petal growth in *Arabidopsis thaliana*: Role of jasmonate in petal growth control. *Plant J.* 60, 1070–1080. <https://doi.org/10.1111/j.1365-313X.2009.04023.x>
- Calderón Villalobos, L.I.A., Lee, S., De Oliveira, C., Ivetac, A., Brandt, W., Armitage, L., Sheard, L.B., Tan, X., Parry, G., Mao, H., Zheng, N., Napier, R., Kepinski, S., Estelle, M., 2012. A combinatorial TIR1/AFB–Aux/IAA co-receptor system for differential sensing of auxin. *Nat. Chem. Biol.* 8, 477–485. <https://doi.org/10.1038/nchembio.926>
- Cao, M., Chen, R., Li, P., Yu, Y., Zheng, R., Ge, D., Zheng, W., Wang, X., Gu, Y., Gelová, Z., Friml, J., Zhang, H., Liu, R., He, J., Xu, T., 2019. TMK1-mediated auxin signalling regulates differential growth of the apical hook. *Nature* 568, 240–243. <https://doi.org/10.1038/s41586-019-1069-7>

- Causier, B., Ashworth, M., Guo, W., Davies, B., 2012. The TOPLESS Interactome: A Framework for Gene Repression in Arabidopsis. *PLANT Physiol.* 158, 423–438. <https://doi.org/10.1104/pp.111.186999>
- Causier, Barry, Lloyd, J., Stevens, L., Davies, B., 2012. TOPLESS co-repressor interactions and their evolutionary conservation in plants. *Plant Signal. Behav.* 7, 325–328. <https://doi.org/10.4161/psb.19283>
- Cho, H., Ryu, H., Rho, S., Hill, K., Smith, S., Audenaert, D., Park, J., Han, S., Beeckman, T., Bennett, M.J., Hwang, D., De Smet, I., Hwang, I., 2014. A secreted peptide acts on BIN2-mediated phosphorylation of ARFs to potentiate auxin response during lateral root development. *Nat. Cell Biol.* 16, 66–76. <https://doi.org/10.1038/ncb2893>
- Choi, H.-S., Seo, M., Cho, H.-T., 2018. Two TPL-Binding Motifs of ARF2 Are Involved in Repression of Auxin Responses. *Front. Plant Sci.* 9. <https://doi.org/10.3389/fpls.2018.00372>
- Chung, Y., Zhu, Y., Wu, M.-F., Simonini, S., Kuhn, A., Armenta-Medina, A., Jin, R., Østergaard, L., Gillmor, C.S., Wagner, D., 2019. Auxin Response Factors promote organogenesis by chromatin-mediated repression of the pluripotency gene SHOOTMERISTEMLESS. *Nat. Commun.* 10, 886. <https://doi.org/10.1038/s41467-019-08861-3>
- Cucinotta, M., Cavalleri, A., Guazzotti, A., Astori, C., Manrique, S., Bombarely, A., Oliveto, S., Biffo, S., Weijers, D., Kater, M.M., Colombo, L., 2021. Alternative Splicing Generates a MONOPTEROS Isoform Required for Ovule Development. *Curr. Biol.* 31, 892-899.e3. <https://doi.org/10.1016/j.cub.2020.11.026>
- Dinesh, D.C., Kovermann, M., Gopalswamy, M., Hellmuth, A., Calderón Villalobos, L.I.A., Lillie, H., Balbach, J., Abel, S., 2015. Solution structure of the PsIAA4 oligomerization domain reveals interaction modes for transcription factors in early auxin response. *Proc. Natl. Acad. Sci.* 112, 6230–6235. <https://doi.org/10.1073/pnas.1424077112>
- Dreher, K.A., Brown, J., Saw, R.E., Callis, J., 2006. The *Arabidopsis* Aux/IAA Protein Family Has Diversified in Degradation and Auxin Responsiveness. *Plant Cell* 18, 699–714. <https://doi.org/10.1105/tpc.105.039172>
- Efroni, I., Han, S.-K., Kim, H.J., Wu, M.-F., Sang, Y., Steiner, E., Birnbaum, K.D., Hong, J.C., Eshed, Y., Wagner, D., 2014. Regulation of leaf maturation by chromatin-mediated modulation of cytokinin responses 17.
- Finet, C., Berne-Dedieu, A., Scutt, C.P., Marlétaz, F., 2013. Evolution of the ARF Gene Family in Land Plants: Old Domains, New Tricks. *Mol. Biol. Evol.* 30, 45–56. <https://doi.org/10.1093/molbev/mss220>
- Flores-Sandoval, E., Eklund, D.M., Hong, S.-F., Alvarez, J.P., Fisher, T.J., Lampugnani, E.R., Golz, J.F., Vázquez-Lobo, A., Dierschke, T., Lin, S.-S., Bowman, J.L., 2018. Class C ARFs evolved before the origin of land plants and antagonize differentiation and developmental transitions in *Marchantia polymorpha*. *New Phytol.* 218, 1612–1630. <https://doi.org/10.1111/nph.15090>
- Franco-Zorrilla, J.M., López-Vidriero, I., Carrasco, J.L., Godoy, M., Vera, P., Solano, R., 2014. DNA-binding specificities of plant transcription factors and their potential to define target genes. *Proc. Natl. Acad. Sci.* 111, 2367–2372. <https://doi.org/10.1073/pnas.1316278111>
- Franks, R.G., Wang, C., Levin, J.Z., Liu, Z., 2002. *SEUSS*, a member of a novel family of plant regulatory proteins, represses floral homeotic gene expression with *LEUNIG*. *Development* 129, 253–263. <https://doi.org/10.1242/dev.129.1.253>
- Freire-Rios, A., Tanaka, K., Crespo, I., van der Wijk, E., Sizentsova, Y., Levitsky, V., Lindhoud, S., Fontana, M., Hohlbein, J., Boer, D.R., Mironova, V., Weijers, D., 2020. Architecture of DNA elements mediating ARF transcription factor binding and auxin-responsive gene expression in *Arabidopsis*. *Proc. Natl. Acad. Sci.* 117, 24557–24566. <https://doi.org/10.1073/pnas.2009554117>
- Galli, M., Khakhar, A., Lu, Z., Chen, Z., Sen, S., Joshi, T., Nemhauser, J.L., Schmitz, R.J., Gallavotti, A., 2018. The DNA binding landscape of the maize AUXIN RESPONSE FACTOR family. *Nat. Commun.* 9, 4526. <https://doi.org/10.1038/s41467-018-06977-6>

- Ghelli, R., Brunetti, P., Napoli, N., De Paolis, A., Cecchetti, V., Tsuge, T., Serino, G., Matsui, M., Mele, G., Rinaldi, G., Palumbo, G.A., Barozzi, F., Costantino, P., Cardarelli, M., 2018. A Newly Identified Flower-Specific Splice Variant of *AUXIN RESPONSE FACTOR8* Regulates Stamen Elongation and Endothecium Lignification in Arabidopsis. *Plant Cell* 30, 620–637. <https://doi.org/10.1105/tpc.17.00840>
- Gray, W.M., del Pozo, J.C., Walker, L., Hobbie, L., Risseuw, E., Banks, T., Crosby, W.L., Yang, M., Ma, H., Estelle, M., 1999. Identification of an SCF ubiquitin-ligase complex required for auxin response in Arabidopsis thaliana. *Genes Dev.* 13, 1678–1691. <https://doi.org/10.1101/gad.13.13.1678>
- Guilfoyle, T.J., 2015. The PB1 Domain in Auxin Response Factor and Aux/IAA Proteins: A Versatile Protein Interaction Module in the Auxin Response. *Plant Cell Online* 27, 33–43. <https://doi.org/10.1105/tpc.114.132753>
- Guilfoyle, T.J., Hagen, G., 2012. Getting a grasp on domain III/IV responsible for Auxin Response Factor–IAA protein interactions. *Plant Sci.* 190, 82–88. <https://doi.org/10.1016/j.plantsci.2012.04.003>
- Guilfoyle, T.J., Ulmasov, T., Hagen, G., 1998. The ARF family of transcription factors and their role in plant hormone-responsive transcription. *Cell. Mol. Life Sci. CMLS* 54, 619–627. <https://doi.org/10.1007/s000180050190>
- Hagen, G., 2015. Auxin signal transduction. *Essays Biochem.* 58, 1–12. <https://doi.org/10.1042/bse0580001>
- Han, M., Park, Y., Kim, I., Kim, E.-H., Yu, T.-K., Rhee, S., Suh, J.-Y., 2014. Structural basis for the auxin-induced transcriptional regulation by Aux/IAA17. *Proc. Natl. Acad. Sci.* 111, 18613–18618. <https://doi.org/10.1073/pnas.1419525112>
- Hardtke, C.S., 1998. The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO J.* 17, 1405–1411. <https://doi.org/10.1093/emboj/17.5.1405>
- Havens, K.A., Guseman, J.M., Jang, S.S., Pierre-Jerome, E., Bolten, N., Klavins, E., Nemhauser, J.L., 2012. A Synthetic Approach Reveals Extensive Tunability of Auxin Signaling. *Plant Physiol.* 160, 135–142. <https://doi.org/10.1104/pp.112.202184>
- Hsin, K.-T., Yang, T.-J., Lee, Y.-H., Cheng, Y.-S., 2021. Phylogenetic and Structural Analysis of NIN-Like Proteins With a Type I/II PB1 Domain That Regulates Oligomerization for Nitrate Response. *Front. Plant Sci.* 12, 672035. <https://doi.org/10.3389/fpls.2021.672035>
- Hu, J., Israeli, A., Ori, N., Sun, T., 2018. The Interaction between DELLA and ARF/IAA Mediates Crosstalk between Gibberellin and Auxin Signaling to Control Fruit Initiation in Tomato. *Plant Cell* 30, 1710–1728. <https://doi.org/10.1105/tpc.18.00363>
- Ito, J., Fukaki, H., Onoda, M., Li, L., Li, C., Tasaka, M., Furutani, M., 2016. Auxin-dependent compositional change in Mediator in ARF7- and ARF19-mediated transcription. *Proc. Natl. Acad. Sci.* 113, 6562–6567. <https://doi.org/10.1073/pnas.1600739113>
- Kato, H., Ishizaki, K., Kouno, M., Shirakawa, M., Bowman, J.L., Nishihama, R., Kohchi, T., 2015. Auxin-Mediated Transcriptional System with a Minimal Set of Components Is Critical for Morphogenesis through the Life Cycle in *Marchantia polymorpha*. *PLOS Genet.* 11, e1005084. <https://doi.org/10.1371/journal.pgen.1005084>
- Kato, H., Mutte, S.K., Suzuki, H., Crespo, I., Das, S., Radoeva, T., Fontana, M., Yoshitake, Y., Hainiwa, E., van den Berg, W., Lindhoud, S., Ishizaki, K., Hohlbein, J., Borst, J.W., Boer, D.R., Nishihama, R., Kohchi, T., Weijers, D., 2020. Design principles of a minimal auxin response system. *Nat. Plants* 6, 473–482. <https://doi.org/10.1038/s41477-020-0662-y>
- Ke, J., Ma, H., Gu, X., Thelen, A., Brunzelle, J.S., Li, J., Xu, H.E., Melcher, K., 2015. Structural basis for recognition of diverse transcriptional repressors by the TOPLESS family of corepressors. *Sci. Adv.* 1, e1500107–e1500107. <https://doi.org/10.1126/sciadv.1500107>
- Kelley, D.R., Arreola, A., Gallagher, T.L., Gasser, C.S., 2012. ETTIN (ARF3) physically interacts with KANADI proteins to form a functional complex essential for integument development and polarity determination in Arabidopsis. *Development* 139,

- 1105–1109. <https://doi.org/10.1242/dev.067918>
- Kim, J., Harter, K., Theologis, A., 1997. Protein-protein interactions among the Aux/IAA proteins. *Proc. Natl. Acad. Sci.* 94, 11786–11791. <https://doi.org/10.1073/pnas.94.22.11786>
- Kim, Y., Park, C., Cha, S., Han, M., Ryu, K.-S., Suh, J.-Y., 2020. Determinants of PB1 Domain Interactions in Auxin Response Factor ARF5 and Repressor IAA17. *J. Mol. Biol.* 432, 4010–4022. <https://doi.org/10.1016/j.jmb.2020.04.007>
- Korasick, D.A., Chatterjee, S., Tonelli, M., Dashti, H., Lee, S.G., Westfall, C.S., Fulton, D.B., Andreotti, A.H., Amarasinghe, G.K., Strader, L.C., Jez, J.M., 2015. Defining a Two-pronged Structural Model for PB1 (Phox/Bem1p) Domain Interaction in Plant Auxin Responses. *J. Biol. Chem.* 290, 12868–12878. <https://doi.org/10.1074/jbc.M115.648253>
- Korasick, D.A., Westfall, C.S., Lee, S.G., Nanao, M.H., Dumas, R., Hagen, G., Guilfoyle, T.J., Jez, J.M., Strader, L.C., 2014. Molecular basis for AUXIN RESPONSE FACTOR protein interaction and the control of auxin response repression. *Proc. Natl. Acad. Sci.* 111, 5427–5432. <https://doi.org/10.1073/pnas.1400074111>
- Krogan, N.T., Ckurshumova, W., Marcos, D., Caragea, A.E., Berleth, T., 2012. Deletion of *MP/ARF5* domains III and IV reveals a requirement for *Aux/IAA* regulation in *Arabidopsis* leaf vascular patterning. *New Phytol.* 194, 391–401. <https://doi.org/10.1111/j.1469-8137.2012.04064.x>
- Kuhn, A., Ramans Harborough, S., McLaughlin, H.M., Natarajan, B., Verstraeten, I., Friml, J., Kepinski, S., Østergaard, L., 2020. Direct ETTIN-auxin interaction controls chromatin states in gynoecium development. *eLife* 9, e51787. <https://doi.org/10.7554/eLife.51787>
- Lavy, M., Estelle, M., 2016. Mechanisms of auxin signaling. *Development* 143, 3226–3229. <https://doi.org/10.1242/dev.131870>
- Leydon, A.R., Wang, W., Gala, H.P., Gilmour, S., Juarez-Solis, S., Zahler, M.L., Zemke, J.E., Zheng, N., Nemhauser, J.L., 2021. Repression by the *Arabidopsis* TOPLESS corepressor requires association with the core mediator complex. *eLife* 10, e66739. <https://doi.org/10.7554/eLife.66739>
- Li, J.-F., Bush, J., Xiong, Y., Li, L., McCormack, M., 2011. Large-Scale Protein-Protein Interaction Analysis in *Arabidopsis* Mesophyll Protoplasts by Split Firefly Luciferase Complementation. *PLoS ONE* 6, e27364. <https://doi.org/10.1371/journal.pone.0027364>
- Li, K., Wang, S., Wu, H., Wang, H., 2020. Protein Levels of Several *Arabidopsis* Auxin Response Factors Are Regulated by Multiple Factors and ABA Promotes ARF6 Protein Ubiquitination. *Int. J. Mol. Sci.* 21, 9437. <https://doi.org/10.3390/ijms21249437>
- Liao, C.-Y., Smet, W., Brunoud, G., Yoshida, S., Vernoux, T., Weijers, D., 2015. Reporters for sensitive and quantitative measurement of auxin response. *Nat. Methods* 12, 207–210. <https://doi.org/10.1038/nmeth.3279>
- Lieberman-Lazarovich, M., Yahav, C., Israeli, A., Efroni, I., 2019. Deep conservation of cis-element variants regulating plant hormonal responses. *Plant Cell* tpc.00129.2019. <https://doi.org/10.1105/tpc.19.00129>
- Liu, Kun, Li, Y., Chen, X., Li, L., Liu, Kai, Zhao, H., Wang, Y., Han, S., 2018. ERF72 interacts with ARF6 and BZR1 to regulate hypocotyl elongation in *Arabidopsis*. *J. Exp. Bot.* 69, 3933–3947. <https://doi.org/10.1093/jxb/ery220>
- Liu, Z.B., Ulmasov, T., Shi, X., Hagen, G., Guilfoyle, T.J., 1994. Soybean GH3 promoter contains multiple auxin-inducible elements. *Plant Cell* 6, 645–657. <https://doi.org/10.1105/tpc.6.5.645>
- Lv, B., Yu, Q., Liu, J., Wen, X., Yan, Z., Hu, K., Li, H., Kong, X., Li, C., Tian, H., Ding, Z., 2019. Non-canonical AUX/IAA protein IAA33 competes with canonical AUX/IAA repressor IAA5 to negatively regulate auxin signaling. *EMBO J.* 14.
- Martin-Arevalillo, R., Nanao, M.H., Larrieu, A., Vinos-Poyo, T., Mast, D., Galvan-Ampudia, C., Brunoud, G., Vernoux, T., Dumas, R., Parcy, F., 2017. Structure of the

- Arabidopsis TOPLESS corepressor provides insight into the evolution of transcriptional repression. *Proc. Natl. Acad. Sci.* 114, 8107–8112. <https://doi.org/10.1073/pnas.1703054114>
- Martin-Arevalillo, R., Thévenon, E., Jégu, F., Vinos-Poyo, T., Vernoux, T., Parcy, F., Dumas, R., 2019. Evolution of the Auxin Response Factors from charophyte ancestors. *PLOS Genet.* 15, e1008400. <https://doi.org/10.1371/journal.pgen.1008400>
- Morffy, N., Strader, L.C., 2021. Plant promoter-proximal pausing? *Nat. Plants* 7, 862–863. <https://doi.org/10.1038/s41477-021-00970-6>
- Mutte, S.K., Kato, H., Rothfels, C., Melkonian, M., Wong, G.K.-S., Weijers, D., 2018. Origin and evolution of the nuclear auxin response system. *eLife* 7, e33399. <https://doi.org/10.7554/eLife.33399>
- Mutte, S.K., Weijers, D., 2020. Deep Evolutionary History of the Phox and Bem1 (PB1) Domain Across Eukaryotes. *Sci. Rep.* 10, 3797. <https://doi.org/10.1038/s41598-020-60733-9>
- Nanao, M.H., Vinos-Poyo, T., Brunoud, G., Thévenon, E., Mazzoleni, M., Mast, D., Lainé, S., Wang, S., Hagen, G., Li, H., Guilfoyle, T.J., Parcy, F., Vernoux, T., Dumas, R., 2014. Structural basis for oligomerization of auxin transcriptional regulators. *Nat. Commun.* 5. <https://doi.org/10.1038/ncomms4617>
- Niemeyer, M., Moreno Castillo, E., Ihling, C.H., Iacobucci, C., Wilde, V., Hellmuth, A., Hoehenwarter, W., Samodelov, S.L., Zurbriggen, M.D., Kastiris, P.L., Sinz, A., Calderón Villalobos, L.I.A., 2020. Flexibility of intrinsically disordered degrons in AUX/IAA proteins reinforces auxin co-receptor assemblies. *Nat. Commun.* 11, 2277. <https://doi.org/10.1038/s41467-020-16147-2>
- Oh, E., Zhu, J.-Y., Bai, M.-Y., Arenhart, R.A., Sun, Y., Wang, Z.-Y., 2014. Cell elongation is regulated through a central circuit of interacting transcription factors in the Arabidopsis hypocotyl. *eLife* 3, e03031. <https://doi.org/10.7554/eLife.03031>
- Okushima, Y., 2005. Functional Genomic Analysis of the AUXIN RESPONSE FACTOR Gene Family Members in Arabidopsis thaliana: Unique and Overlapping Functions of ARF7 and ARF19. *PLANT CELL ONLINE* 17, 444–463. <https://doi.org/10.1105/tpc.104.028316>
- O'Malley, R.C., Huang, S.C., Song, L., Lewsey, M.G., Bartlett, A., Nery, J.R., Galli, M., Gallavotti, A., Ecker, J.R., 2016. Cistrome and Epicistrome Features Shape the Regulatory DNA Landscape. *Cell* 165, 1280–1292. <https://doi.org/10.1016/j.cell.2016.04.038>
- Orosa-Puente, B., Leftley, N., Srivastava, A.K., Hill, K., Truskina, J., Bhosale, R., Morris, E., Srivastava, M., Kümpers, B., Goh, T., Fukaki, H., Vermeer, J.E.M., Vernoux, T., Dinneny, J.R., French, A.P., Bishopp, A., Sadanandom, A., Bennett, M.J., 2018. Root branching toward water involves posttranslational modification of transcription factor ARF7 5.
- Ouellet, F., Overvoorde, P.J., Theologis, A., 2001. IAA17/AXR3: Biochemical Insight into an Auxin Mutant Phenotype 14.
- Paponov, I.A., Teale, W., Lang, D., Paponov, M., Reski, R., Rensing, S.A., Palme, K., 2009. The evolution of nuclear auxin signalling. *BMC Evol. Biol.* 9, 126. <https://doi.org/10.1186/1471-2148-9-126>
- Parcy, F., Vernoux, T., Dumas, R., 2016. A Glimpse beyond Structures in Auxin-Dependent Transcription. *Trends Plant Sci.* 21, 574–583. <https://doi.org/10.1016/j.tplants.2016.02.002>
- Pfluger, J., Zambryski, P., 2004. The role of *SEUSS* in auxin response and floral organ patterning. *Development* 131, 4697–4707. <https://doi.org/10.1242/dev.01306>
- Pierre-Jerome, E., Jang, S.S., Havens, K.A., Nemhauser, J.L., Klavins, E., 2014. Recapitulation of the forward nuclear auxin response pathway in yeast. *Proc. Natl. Acad. Sci.* 111, 9407–9412. <https://doi.org/10.1073/pnas.1324147111>
- Pierre-Jerome, E., Moss, B.L., Lanctot, A., Hageman, A., Nemhauser, J.L., 2016. Functional analysis of molecular interactions in synthetic auxin response circuits. *Proc. Natl. Acad. Sci.* 113, 11354–11359. <https://doi.org/10.1073/pnas.1604379113>

- Piya, S., Shrestha, S.K., Binder, B., Stewart, C.N., Hewezi, T., 2014. Protein-protein interaction and gene co-expression maps of ARFs and Aux/IAAs in Arabidopsis. *Front. Plant Sci.* 5. <https://doi.org/10.3389/fpls.2014.00744>
- Powers, S.K., Holehouse, A.S., Korasick, D.A., Schreiber, K.H., Clark, N.M., Jing, H., Emenecker, R., Han, S., Tycksen, E., Hwang, I., Sozzani, R., Jez, J.M., Pappu, R.V., Strader, L.C., 2019. Nucleo-cytoplasmic Partitioning of ARF Proteins Controls Auxin Responses in Arabidopsis thaliana. *Mol. Cell* 76, 177-190.e5. <https://doi.org/10.1016/j.molcel.2019.06.044>
- Przemeck, Gerhard K.H., Mattsson, J., Hardtke, Christian S., Sung, Z.R., Berleth, T., 1996. Studies on the role of the Arabidopsis gene MONOPTEROS in vascular development and plant cell axialization. *Planta* 200. <https://doi.org/10.1007/BF00208313>
- Rademacher, E.H., Lokerse, A.S., Schlereth, A., Llavata-Peris, C.I., Bayer, M., Kientz, M., Freire Rios, A., Borst, J.W., Lukowitz, W., Jürgens, G., Weijers, D., 2012. Different Auxin Response Machineries Control Distinct Cell Fates in the Early Plant Embryo. *Dev. Cell* 22, 211–222. <https://doi.org/10.1016/j.devcel.2011.10.026>
- Ramos, J.A., Zenser, N., Leyser, O., Callis, J., 2001. Rapid Degradation of Auxin/Indoleacetic Acid Proteins Requires Conserved Amino Acids of Domain II and Is Proteasome Dependent. *Plant Cell* 13, 2349–2360. <https://doi.org/10.1105/tpc.010244>
- Roosjen, M., Paque, S., Weijers, D., 2018. Auxin Response Factors: output control in auxin biology. *J. Exp. Bot.* 69, 179–188. <https://doi.org/10.1093/jxb/erx237>
- Ruegger, M., Dewey, E., Hobbie, L., Brown, D., Bernasconi, P., Turner, J., Muday, G., Estelle, M., 1997. Reduced naphthylphthalamic acid binding in the tir3 mutant of Arabidopsis is associated with a reduction in polar auxin transport and diverse morphological defects. *Plant Cell* 9, 745–757. <https://doi.org/10.1105/tpc.9.5.745>
- Sayou, C., Nanao, M.H., Jamin, M., Posé, D., Thévenon, E., Grégoire, L., Tichtinsky, G., Denay, G., Ott, F., Peirats Llobet, M., Schmid, M., Dumas, R., Parcy, F., 2016. A SAM oligomerization domain shapes the genomic binding landscape of the LEAFY transcription factor. *Nat. Commun.* 7, 11222. <https://doi.org/10.1038/ncomms11222>
- Shin, R., Burch, A.Y., Huppert, K.A., Tiwari, S.B., Murphy, A.S., Guilfoyle, T.J., Schachtman, D.P., 2007. The Arabidopsis Transcription Factor MYB77 Modulates Auxin Signal Transduction. *PLANT CELL ONLINE* 19, 2440–2453. <https://doi.org/10.1105/tpc.107.050963>
- Simonini, S., Bencivenga, S., Trick, M., Østergaard, L., 2017. Auxin-Induced Modulation of ETTIN Activity Orchestrates Gene Expression in Arabidopsis. *Plant Cell* 29, 1864–1882. <https://doi.org/10.1105/tpc.17.00389>
- Simonini, S., Deb, J., Moubayidin, L., Stephenson, P., Valluru, M., Freire-Rios, A., Sorefan, K., Weijers, D., Friml, J., Østergaard, L., 2016. A noncanonical auxin-sensing mechanism is required for organ morphogenesis in *Arabidopsis*. *Genes Dev.* 30, 2286–2296. <https://doi.org/10.1101/gad.285361.116>
- Simonini, S., Mas, P.J., Mas, C.M.V.S., Østergaard, L., Hart, D.J., 2018a. Auxin sensing is a property of an unstructured domain in the Auxin Response Factor ETTIN of Arabidopsis thaliana. *Sci. Rep.* 8, 13563. <https://doi.org/10.1038/s41598-018-31634-9>
- Simonini, S., Stephenson, P., Østergaard, L., 2018b. A molecular framework controlling style morphology in *Brassicaceae*. *Development* 145, dev158105. <https://doi.org/10.1242/dev.158105>
- Smit, M.E., Llavata-Peris, C.I., Roosjen, M., van Beijnum, H., Novikova, D., Levitsky, V., Sevillem, I., Roszak, P., Slane, D., Jürgens, G., Mironova, V., Brady, S.M., Weijers, D., 2020. Specification and regulation of vascular tissue identity in the *Arabidopsis* embryo. *Development* dev.186130. <https://doi.org/10.1242/dev.186130>
- Stigliani, A., Martin-Arevalillo, R., Lucas, J., Bessy, A., Vinos-Poyo, T., Mironova, V., Vernoux, T., Dumas, R., Parcy, F., 2019. Capturing Auxin Response Factors Syntax Using DNA Binding Models. *Mol. Plant* 12, 822–832. <https://doi.org/10.1016/j.molp.2018.09.010>

- Swaminathan, K., Peterson, K., Jack, T., 2008. The plant B3 superfamily. *Trends Plant Sci.* 13, 647–655. <https://doi.org/10.1016/j.tplants.2008.09.006>
- Szécsi, J., Joly, C., Bordji, K., Varaud, E., Cock, J.M., Dumas, C., Bendahmane, M., 2006. BIGPETALp, a bHLH transcription factor is involved in the control of Arabidopsis petal size. *EMBO J.* 25, 3912–3920. <https://doi.org/10.1038/sj.emboj.7601270>
- Szemenyei, H., Hannon, M., Long, J.A., 2008. TOPLESS Mediates Auxin-Dependent Transcriptional Repression During Arabidopsis Embryogenesis. *Science* 319, 1384–1386. <https://doi.org/10.1126/science.1151461>
- Tiwari, S.B., 2003. The Roles of Auxin Response Factor Domains in Auxin-Responsive Transcription. *PLANT CELL ONLINE* 15, 533–543. <https://doi.org/10.1105/tpc.008417>
- Trigg, S.A., Garza, R.M., MacWilliams, A., Nery, J.R., Bartlett, A., Castanon, R., Goubil, A., Feeney, J., O'Malley, R., Huang, S.C., Zhang, Z.Z., Galli, M., Ecker, J.R., 2017. CrY2H-seq: a massively multiplexed assay for deep-coverage interactome mapping. *Nat. Methods* 14, 819–825. <https://doi.org/10.1038/nmeth.4343>
- Ulmasov, T., 1997. ARF1, a Transcription Factor That Binds to Auxin Response Elements. *Science* 276, 1865–1868. <https://doi.org/10.1126/science.276.5320.1865>
- Ulmasov, T., Hagen, G., Guilfoyle, T.J., 1999. Dimerization and DNA binding of auxin response factors. *Plant J.* 19, 309–319. <https://doi.org/10.1046/j.1365-313X.1999.00538.x>
- Ulmasov, T., Hagen, G., Guilfoyle, T.J., 1999. Activation and repression of transcription by auxin-response factors. *Proc. Natl. Acad. Sci.* 96, 5844–5849. <https://doi.org/10.1073/pnas.96.10.5844>
- Ulmasov, T., Murfett, J., Hagen, G., Guilfoyle, T.J., 1997. Aux/IAA Proteins Repress Expression of Reporter Genes Containing Natural and Highly Active Synthetic Auxin Response Elements. *Plant Cell, American Society of Plant Physiologists* 9, 1963–1971.
- Varaud, E., Brioudes, F., Szecsi, J., Leroux, J., Brown, S., Perrot-Rechenmann, C., Bendahmane, M., 2011. AUXIN RESPONSE FACTOR8 Regulates Arabidopsis Petal Growth by Interacting with the bHLH Transcription Factor BIGPETALp. *Plant Cell* 23, 973–983. <https://doi.org/10.1105/tpc.110.081653>
- Vernoux, T., Brunoud, G., Farcot, E., Morin, V., Van den Daele, H., Legrand, J., Oliva, M., Das, P., Larrieu, A., Wells, D., Guédon, Y., Armitage, L., Picard, F., Guyomarc'h, S., Cellier, C., Parry, G., Koumproglou, R., Doonan, J.H., Estelle, M., Godin, C., Kepinski, S., Bennett, M., De Veylder, L., Traas, J., 2011. The auxin signalling network translates dynamic input into robust patterning at the shoot apex. *Mol. Syst. Biol.* 7, 508. <https://doi.org/10.1038/msb.2011.39>
- Vert, G., Walcher, C.L., Chory, J., Nemhauser, J.L., 2008. Integration of auxin and brassinosteroid pathways by Auxin Response Factor 2. *Proc. Natl. Acad. Sci.* 105, 9829–9834. <https://doi.org/10.1073/pnas.0803996105>
- Wang, S., Hagen, G., Guilfoyle, T.J., 2013. ARF-Aux/IAA interactions through domain III/IV are not strictly required for auxin-responsive gene expression. *Plant Signal. Behav.* 8, e24526. <https://doi.org/10.4161/psb.24526>
- Weijers, D., Wagner, D., 2016. Transcriptional Responses to the Auxin Hormone. *Annu. Rev. Plant Biol.* 67, 539–574. <https://doi.org/10.1146/annurev-arplant-043015-112122>
- Winkler, M., Niemeyer, M., Hellmuth, A., Janitza, P., Christ, G., Samodelov, S.L., Wilde, V., Majovsky, P., Trujillo, M., Zurbriggen, M.D., Hoehenwarter, W., Quint, M., Calderón Villalobos, L.I.A., 2017. Variation in auxin sensing guides AUX/IAA transcriptional repressor ubiquitylation and destruction. *Nat. Commun.* 8, 15706. <https://doi.org/10.1038/ncomms15706>
- Wu, M.-F., Yamaguchi, N., Xiao, J., Bargmann, B., Estelle, M., Sang, Y., Wagner, D., 2015. Auxin-regulated chromatin switch directs acquisition of flower primordium founder fate. *eLife* 4. <https://doi.org/10.7554/eLife.09269>
- Yamasaki, K., Kigawa, T., Seki, M., Shinozaki, K., Yokoyama, S., 2013. DNA-binding domains of plant-specific transcription factors: structure, function, and evolution.

- Trends Plant Sci. 18, 267–276. <https://doi.org/10.1016/j.tplants.2012.09.001>
- Zhang, X., Adamowski, M., Marhava, P., Tan, S., Zhang, Y., Rodriguez, L., Zwiewka, M., Pukyřová, V., Sánchez, A.S., Raxwal, V.K., Hardtke, C.S., Nodzyński, T., Friml, J., 2020. Arabidopsis Flippases Cooperate with ARF GTPase Exchange Factors to Regulate the Trafficking and Polarity of PIN Auxin Transporters. *Plant Cell* 32, 1644–1664. <https://doi.org/10.1105/tpc.19.00869>
- Zhao, Y., 2018. Essential Roles of Local Auxin Biosynthesis in Plant Development and in Adaptation to Environmental Changes. *Annu. Rev. Plant Biol.* 69, 417–435. <https://doi.org/10.1146/annurev-arplant-042817-040226>