A matter of time: auxin signaling dynamics and the regulation of auxin responses during plant development

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Highlights

After a description of the different auxin signaling modes, this review discusses how auxin responses are regulated during plant development, with a particular focus on temporal regulations.

Abstract

As auxin is a major regulator of plant development, studying the signaling mechanisms by which auxin influences cellular activities is of primary importance. In this review, we describe the current knowledge on the different modalities of signaling, from the well-characterized canonical nuclear auxin pathway, to the more recently discovered or re-discovered non-canonical modes of auxin signaling. In particular, we discuss how both the modularity of the nuclear auxin pathway and the dynamic regulation of its core components allow to trigger specific transcriptomic responses. We highlight the fact that the diversity of modes of auxin signaling allows for a wide range of timescales of auxin responses, from second-scale cytoplasmic responses to minute/hour-scale modifications of gene expression. Finally, we question the extent to which the temporality of auxin signaling and responses contributes to development in both the shoot and the root meristems. We conclude by stressing the fact that future investigations should allow to build an integrative view not only of the spatial control, but also of the temporality of auxin-mediated regulation of plant development, from the cell to the whole organism.

Keywords

Auxin signaling, auxin responses, dynamics, nuclear auxin pathway, plant development, regulation, specificity, temporality, timescales

Introduction

Plant development is controlled through a diversity of signals, among which phytohormones play a central role. Auxin is probably the most famous phytohormone, and rightfully so. Since its discovery in 1928 by Fritz Went **(Went, 1928)**, decades of research have established it as a major regulator of plant development. Auxin derives its name from *auxein*, the Greek word for "growth", as it has been first characterized as a positive regulator of plant growth. The spectrum of action of this phytohormone is actually much wider, as auxin controls virtually all aspects of plant development, including embryo polarization, formation of aerial and underground organs, shoot and root growth, stem cell maintenance in the root and shoot apical meristem, vascular patterning, and tropisms, to cite only a few. What confers this pleiotropy to auxin in the regulation of plant development is thought to lie primarily in the diversity of its cellular effects. Indeed, auxin controls both cell elongation and cell proliferation, as well as cell identities, at least in part through regulating the expression of thousands of target genes in a cell-specific manner.

The effects of this small molecule signal on cells can be drastically different depending on the developmental context. Elucidating the molecular mechanisms by which auxin specifically instructs a given cell what to do during development remains a central focus in the auxin field and in plant biology in general. A key to answer the question of how auxin can perform so many functions is indeed to understand how cells read and process the auxin signal, or in other words, to uncover the fundamental rules by which the auxin signaling machinery turns a simple and generalist signal into a precise cell response. Cells perceive auxin through a now well-characterized canonical pathway called the nuclear auxin pathway (NAP; **(Leyser, 2018)**), which leads to the transcriptional reprogramming mentioned earlier. However, it is also well established that auxin can induce very rapid, non-transcriptional responses in cells such as notably ion fluxes **(Rayle, 1973; Zimmermann *et al.*, 1994; Claussen *et al.*, 1997; Philippar**

et al., 1999; Monshausen *et al.*, 2011). In line with this, findings in the recent years have now established not only that auxin signaling activity is molecularly much more diverse than originally thought, but has also identified some of the key molecular mechanisms involved, through the characterization of several non-canonical auxin signaling pathways leading to either NAP-independent transcriptional responses to auxin, or to fast, cytoplasmic, non-transcriptional responses. In this review, we provide a summary of the different modalities of auxin signaling, with a focus on the central question of how the NAP orchestrates space- but also time-specific transcriptional responses to auxin. We also highlight the recently discovered non-canonical modes of auxin signaling and discuss what we currently understand of their function in mediating responses to auxin during development. Finally, we discuss recent findings on the timing of auxin responses, highlighting the fact that different temporalities in auxin signaling are essential for the coordination of developmental events.

The nuclear auxin pathway and the dynamics and specificity of transcriptional responses to auxin

The nuclear auxin pathway core components in canonical auxin sensing

We summarize here briefly our knowledge on the NAP components and refer the reader to recent reviews for more extensive information on the subject **(Leyser, 2018; Morffy and Strader, 2022)**. The general mechanism of the NAP is in its principle remarkably simple, as the whole pathway, that takes place in the nucleus, consists in protein-protein interactions between 3 types of components, each belonging to one protein family: the TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB) co-receptor family, the AUXIN/INDOLE-3-ACETIC ACID INDUCIBLE (Aux/IAA) transcriptional repressor family, and the AUXIN RESPONSE FACTORS (ARF) transcription factor family **(Leyser, 2018)**.

The ARF transcription factors **(Ulmasov *et al.*, 1997; Guilfoyle and Hagen, 2007)** display a conserved structure with 3 regions characteristic of a transcription factor activity: this includes (1) a B3-type DNA-binding domain (DBD) that can also mediate ARF-ARF interactions **(Boer *et al.*, 2014)**, (2) an intermediate region that confers transcription regulation capacity, and (3) a region containing a Phox and Bem1 (PB1) domain, that constitutes a multimerization module **(Nanao *et al.*, 2014; Korasick *et al.*, 2014)** **(Fig. 1A)**. Depending on the composition of the intermediate region, ARFs can act either as transcriptional activators or repressors. ARFs are then categorized in three different clades depending on their effect on transcription: A for the activators, B for the repressors and C whose activity are less well characterized **(Leyser, 2018; Li *et al.*, 2022)**. ARFs bind DNA as dimers on pairs of consensus sequences called auxin response elements (AuxREs; see later), and regulate the activity of auxin-responsive genes, whose promoters are enriched in such elements **(Freire-Rios *et al.*, 2020)**. The total number of ARFs, as well as their number in a given class of ARFs varies significantly across plant species (see later), determining the regulatory potential of the NAP for a given species.

The Aux/IAA transcriptional repressors were first identified as early transcriptional targets of auxin and as proteins inhibiting the expression of auxin-responsive genes **(Ulmasov *et al.*, 1997*b*)**. Most Aux/IAA have a short half-life and display a conserved structure with four domains **(Luo *et al.*, 2018)**. The domain I contain Ethylene-responsive element binding factor-Associated amphiphilic Repression (EAR) motifs allowing to recruit TOPLESS(TPL)/TOPLESS-RELATED(TPR) co-repressors that can themselves recruit histone deacetylases (HDACs) to target loci, thus being responsible for the repression **(Szemenyei *et al.*, 2008)** **(Fig. 1A-B)**. EAR motifs are also found in the intermediate domain of B-class ARFs, allowing them to act as transcriptional repressors **(Causier *et al.*, 2012)**. The Aux/IAA Domain II is a degron domain, which allows for the degradation of the protein through auxin-dependent interaction with TIR1/AFB (see below). Finally, Aux/IAAs harbor a PB1

domain similarly to ARFs **(Han *et al.*, 2014)** and this domain is involved in protein-protein interactions with other Aux/IAAs, and also primarily with class A ARFs **(Vernoux *et al.*, 2011; Guilfoyle, 2015)** **(Fig. 1A-B)**. Similarly to ARFs, plants have a variable number of Aux/IAAs which defines the combinatorial potential with ARFs and also with TIR1/AFBs.

TIR1 or the 6 AFBs auxin co-receptors, first identified in *Arabidopsis thaliana*, are a subunit of the SKF, CULLIN, F-BOX CONTAINING COMPLEX (SCF) ubiquitin ligase complex **(Dharmasiri *et al.*, 2005*a*,*b*; Kepinski and Leyser, 2005)**, that have been shown or suggested to trigger the core mechanism of the canonical nuclear auxin pathway, auxin-dependent Aux/IAA degradation **(Zenser *et al.*, 2001; Gray *et al.*, 2001)**. Auxin binds to a hydrophobic pocket at the TIR1/AFB surface, thus stabilizing $SCF^{TIR1/AFB}$ interaction with Aux/IAA proteins, and enabling SCF-mediated Aux/IAA ubiquitination and subsequent degradation **(Tan *et al.*, 2007)** **(Fig. 1B)**. The degradation of Aux/IAAs relieves their inhibition on class A ARFs (it is less clear whether it affects directly the activity of class B and C ARFs), allowing them to regulate the transcription of auxin target genes **(Leyser, 2018)**.

Amongst the many genes regulated by auxin, distinct sets of genes are regulated depending on the cell type, indicating space-specificity in response to auxin **(Paponov *et al.*, 2008; Bargmann *et al.*, 2013)**. In addition, these responses are also time-specific, as the nature of cell responses directly depend on the duration of exposure to auxin **(Bargmann *et al.*, 2013; McReynolds *et al.*, 2022)**. The molecular mechanisms underlying this space- and time-specificity of transcriptional responses to auxin are not trivial, but an in-depth understanding of the regulation of the NAP can provide some explanations.

A central role for Aux/IAAs in controlling the dynamics of auxin signaling

The transcriptional regulation of auxin target genes depends on the degradation of Aux/IAA, making them pivotal actors of the NAP. There are 29 Aux/IAA members in *Arabidopsis*, most of them displaying the conserved four-domain organization we just described **(Luo *et al.*, 2018)**.

Some Aux/IAAs (IAA20 and IAA29-34) however lack the degron domain required for auxin-induced rapid degradation by SCF **(Dreher *et al.*, 2006; Luo *et al.*, 2018)**, and among them, IAA20 has been shown to be long-lived and insensitive to auxin **(Dreher *et al.*, 2006)**. Interestingly, other factors influence Aux/IAAs degradation rate independently of the degron domain. In particular, rate motifs have been defined as highly conserved degron-flanking sequences that tune Aux/IAAs degradation rate, presumably by modulating Aux/IAA-SCF^{TIR1} interactions **(Moss *et al.*, 2015)**. Furthermore, these Aux/IAA rate motifs belong to flexible, intrinsically disordered regions (IDR) enriched in lysines, that are substrates for ubiquitylation. Variations in the rate motif confers differences in ubiquitylation processivity, as observed between IAA6 and IAA19 **(Winkler *et al.*, 2017)**. This provides another putative mechanism explaining the differences of degradation rates between different Aux/IAAs. Adding to that, the nature of the receptor also impacts Aux/IAA degradation rates, as AFB2 has been reported to promote a faster degradation than TIR1 upon auxin sensing, for several Aux/IAAs, in both *Arabidopsis* **(Havens *et al.*, 2012)** and maize **(Ramos Báez *et al.*, 2020)**.

These properties can be summed up in a combinatorial model of differential auxin sensitivity: TIR1/AFB and Aux/IAA proteins form different co-receptor complexes with different auxin-binding affinities, which confer different degradation dynamics upon auxin sensing **(Calderón Villalobos *et al.*, 2012)** **(Fig. 2, upper part)**. Amongst the many modeling work that studied the properties of the NAP **(Martin-Arevalillo and Vernoux, 2023)**, one identified a potential contribution of ARF-Aux/IAA and Aux/IAA-Aux/IAA dimers respectively in regulating the amplitude and the speed of the auxin transcriptional response, thus identifying specific contributions for Aux/IAAs and their degradation **(Farcot *et al.*, 2015)**, and providing a framework to understand the impact of the regulation of Aux/IAA degradation dynamics. In line with these predictions, a synthetic approach based on NAP reconstruction in yeast has shown that an auxin transcriptional reporter can respond twice faster or slower to auxin

depending on the nature of the Aux/IAA **(Pierre-Jerome *et al.*, 2014)**. Furthermore, this approach showed that the co-expression of two different Aux/IAAs results in response dynamics similar to what happens when only one of the two Aux/IAAs is expressed alone, suggesting that dominance relationships exist between Aux/IAAs **(Pierre-Jerome *et al.*, 2014)**. This hierarchy between different Aux/IAAs could explain, at least in part, how sequential auxin responses can be generated from a single auxin input. Thus, the existence of Aux/IAAs with different degradation rates **(Dreher *et al.*, 2006; Calderón Villalobos *et al.*, 2012; Moss *et al.*, 2015; Winkler *et al.*, 2017)**, could be a key feature that enables the tuning of the temporal dynamics of auxin responses **(Fig. 2, upper part)**.

Regulation of NAP core components: multiple layers of complexity regulating auxin signaling dynamics

Transcriptional regulation of NAP core components

Mechanisms that tune the activity of the different molecular components of the NAP in a given cell are adding extra regulatory layers that can contribute to spatial and temporal specificities in auxin responses. The size of the multigene families encoding NAP components display a remarkable diversity **(Mutte *et al.*, 2018)**. Besides the 29 Aux/IAAs, there are 6 different TIR1/AFBs and 23 ARFs in *Arabidopsis*, while the moss *Physcomitrium patens* contains 4 TIR1/AFBs, 3 Aux/IAAs and 16 ARFs **(Prigge *et al.*, 2010)**. The NAP of the liverwort *Marchantia polymorpha* is even simpler, with only 1 TIR1/AFB, 1 Aux/IAA and 3 ARFs **(Flores-Sandoval *et al.*, 2015; Kato *et al.*, 2015)**. On the other hand, the NAP structure is more complex in maize compared to *Arabidopsis*, with 8 TIR1/AFBs, 34 Aux/IAAs and 33 ARFs **(Ramos Báez *et al.*, 2020)**. Among one organism (we will focus here on the most studied one, *Arabidopsis*), all these components exhibit specific expression patterns in the embryo and the growing plant **(Rademacher *et al.*, 2011; Vernoux *et al.*, 2011)**, suggesting that cell type-

specific expression of a combination of NAP components would enable to generate particular auxin responses depending on the developmental context.

This raises the question of how these expression patterns are generated. *TIR1/AFB* genes expression appears to be mostly regulated post-transcriptionally, as TIR1/AFBs are expressed broadly across the plant **(Dharmasiri *et al.*, 2005*b*)**, and translational fusion of TIR1/AFBs **(Parry *et al.*, 2009)** exhibit much sharper expression patterns than transcriptional fusions **(Dharmasiri *et al.*, 2005*b*)**. In contrast, transcriptional regulation has been shown to be an important feature regulating Aux/IAA and ARFs expression and activity, as demonstrated with promoter-swapping experiments **(Weijers *et al.*, 2005)**. *Aux/IAA* genes expression is regulated by a wide range of transcription factors (TFs): for instance, the HD-ZIP transcription factor HB5 represses the expression of *IAA12* **(De Smet *et al.*, 2013)**, and members of the DREB/CBF transcription factor family promote the expression of both *IAA5* and *IAA19* **(Shani *et al.*, 2017)**. The regulation of *Aux/IAA* genes by DREB/CBF transcription factors occurs in response to abiotic stress **(Shani *et al.*, 2017)**, indicating that environmental conditions may shape auxin responses through transcriptional regulation of NAP components. Furthermore, there are some evidences for an epigenetic regulation of *Aux/IAA* genes: half of the *Aux/IAA* genes are enriched in H3K27 methylation marks **(Lafos *et al.*, 2011)**, and induction of *IAA3* gene expression by light requires GCN5-dependent histone H3 and H4 acetylation **(Benhamed *et al.*, 2006)**. The regulation of class A ARFs expression is particularly interesting, as chromatin at class A *ARF* genes loci is constitutively open, and the expression of these genes is directly controlled by a network of transcriptional repressors, in both the shoot apex and the root tip **(Truskina *et al.*, 2021)**. It has been shown that each gene encoding an activator ARF is regulated by a specific set of transcription factors: as an example, MONOPTEROS/ARF5 is repressed by LBD3, SMZ and KNAT1 **(Truskina *et al.*, 2021)**. Auxin-related phenotypes have been identified in mutants

in these regulators, but their specific effect on spatial and temporal properties of auxin responses during development remains to be studied in details.

Post-transcriptional regulation of NAP core components

As NAP activation is based on auxin-induced degradation of Aux/IAA, one can wonder whether regulation of NAP core components turnover and other post-transcriptional regulations play an additional role in shaping auxin responses. Among regulations at the RNA level, microRNAs (miRNAs) and trans-acting short-interfering RNAs (tasiRNAs) constitute important actors in post-transcriptional regulation of ARF-encoding mRNAs. Class B ARF2, 3 and 4 mRNAs are privileged target of tasiRNAs in inflorescences and siliques **(Williams *et al.*, 2005)**, leaves **(Adenot *et al.*, 2006)**, and roots **(Marin *et al.*, 2010)**. Recent work identified ARF17 as a new example of ARF regulated by RNA interference, which is in this case targeted by a miRNA in the ovule **(Huang *et al.*, 2022)**. These studies show that RNA interference against ARFs contributes to leaf morphogenesis **(Adenot *et al.*, 2006; Fahlgren *et al.*, 2006)**, timing of lateral root initiation **(Marin *et al.*, 2010)**, timing of developmental transitions **(Fahlgren *et al.*, 2006)**, and spatial restriction of auxin signaling capacity **(Huang *et al.*, 2022)**, suggesting that regulation of NAP core components mRNA is essential to orchestrate auxin effects on these developmental processes. Consistent with this idea, tasiRNA-mediated regulation of repressor ARFs seems conserved during evolution, as ARF2, 3 and 4 mRNAs have been shown to be targets of tasiRNAs in the moss *Physcomitrium patens* as well **(Plavskin *et al.*, 2016)**. Apart from ARFs, TIR1/AFBs also appear to be regulated by RNA interference, although the precise mechanism is still unclear **(Parry *et al.*, 2009)**. Another type of regulation was recently identified for ARF5/MONOPTEROS mRNA that undergo alternative splicing in *Arabidopsis* ovule, leading to the production of an ARF5 isoform functioning independently of Aux/IAAs **(Cucinotta *et al.*, 2021)**. Two alternative splicing-derived ARF8 isoforms which display a truncated PB1 domain have also been identified **(Ghelli *et al.*, 2018)**. But whether alternative

splicing of NAP core components is frequent during development and effectively contributes to the regulation of auxin responses remains completely unexplored.

Post-translational protein modifications play an additional role in the regulation of NAP core components activity. The BIN2 kinase has been shown to phosphorylate several *Arabidopsis* ARFs at different sites and to regulate their activity. For instance, BIN2-mediated phosphorylation negatively regulates ARF2 by abolishing its DNA binding and transcription repressor activities **(Vert *et al.*, 2008; Zhao *et al.*, 2016)**. In the case of class A ARF7 and ARF19, BIN2-mediated phosphorylation prevents their interaction with Aux/IAA repressors, enabling these ARFs to constitutively stimulate the expression of their target genes, independently of auxin **(Cho *et al.*, 2014)**. As BIN2 is upregulated by brassinosteroids **(Vert *et al.*, 2008)**, low potassium stress **(Zhao *et al.*, 2016)** and secreted peptides in the root **(Cho *et al.*, 2014)**, this illustrates how a combination of endogenous and exogenous signals can modulate the activity of the NAP, and fine-tune auxin responses. In addition, IAA15 has been identified as a substrate of Mitogen-Activated Protein Kinases, and its stabilization by phosphorylation is required for its action in the root **(Kim *et al.*, 2022)**. This finding suggests that other Aux/IAAs might be regulated by phosphorylation in a similar fashion, but it is still unknown whether phosphorylation acts in the global regulation of the NAP and how it affects the dynamics of signaling.

Another recently identified post-translational protein modification involved in NAP components regulation is SUMOylation. IAA17 is stabilized through SUMOylation by the MMS21 SUMO E3 ligase in the root **(Zhang *et al.*, 2022)**; and ARF7 is inactivated by SUMOylation in particular regions of the root, allowing for a spatially restricted activity of ARF7 that controls root architecture upon differential water availability, a response also called hydropatterning **(Orosa-Puente *et al.*, 2018)**. Interestingly, each family of NAP core components seem to be affected differently by these protein modifications: phosphorylation

and SUMOylation regulate mainly Aux/IAA proteins stability, degradation and turnover, and could thus affect the amplitude and speed of the transcriptional response **(Farcot *et al.*, 2015)** whereas these modifications directly activate or inactivate ARF proteins activity without affecting their turnover and could thus rather affect the specificity of the response. Recent work in *Marchantia polymorpha* identified a mechanism of selective proteasome-mediated degradation of MpARF2, which was shown to regulate auxin transcriptional responses **(Das *et al.*, 2022, Preprint)**. Thus, the turn-over of ARFs can also be regulated.

TIR1 and Aux/IAAs can be S-nitrosylated as well. This protein modification seems to fine-tune TIR1-Aux/IAA interaction in a complex fashion, as both TIR1 **(Terrile *et al.*, 2012)** and Aux/IAA **(Jing *et al.*, 2022*b*)** can be S-nitrosylated, and this either enhances **(Terrile *et al.*, 2012)** or represses **(Jing *et al.*, 2022*b*)** TIR1-Aux/IAA interaction. Adding to that, S-nitrosylation of the ASK1 adaptor protein favours $SCF^{TIR1/AFB}$ complex assembly **(Iglesias *et al.*, 2018)**, providing a new original example of NAP regulation through a modulation of $SCF^{TIR1/AFB}$ complex formation. By targeting Aux/IAA auxin-dependent turn-over, these mechanisms have also the potential to be essential to regulate the temporality of the transcriptional responses.

Finally, nucleo-cytoplasmic partitioning recently emerged in the literature as a novel mechanism of ARF activity regulation. ARF7 and ARF19 have been shown to form cytoplasmic molecular condensates in regions of the root with attenuated auxin responsiveness **(Powers *et al.*, 2019)**. The formation of such molecular condensates involves the intermediary region from the ARFs, which was predicted to be an intrinsically disordered region (IDR) in the case of ARF19, and the PB1 domain **(Powers *et al.*, 2019)**. Several factors have been shown to regulate ARFs condensation, including the newly identified F-box protein AFF1 **(Jing *et al.*, 2022*a*)** as well as the sequence of the IDR itself, which influences condensate morphology **(Emenecker *et al.*, 2021)**. Mutants unable to form ARF cytoplasmic condensates display

disrupted auxin responsiveness, altered transcriptome upon auxin treatment, and developmental defects **(Powers *et al.*, 2019; Jing *et al.*, 2022*a*)**; indicating that ARF nucleo-cytoplasmic partitioning is an important mechanism in the regulation of auxin responses. Only ARF7 and ARF19 molecular condensates have been observed so far, but it is very likely that other types of ARF behave the same, given the conservation of the protein structure that allows the formation of such condensates.

Thus, despite the NAP simple logic, diversity in the number of components and in regulatory mechanisms of the different component likely allows to exquisitely tune specific parameters of the transcriptional response, such as amplitude, speed and sensitivity **(Farcot *et al.*, 2015)**, as well as specificity **(Fig. 2)**, not only in response to auxin but also as a function of other endogenous and exogenous signal.

Target gene specificity in auxin action

The combination of ARFs in a given cell is expected to be a central determinant of response specificity. Recent studies have started to decipher how this specificity is achieved. Auxin Response Elements (AuxREs) are 5'-TGTCnn-3' sequences bound by ARFs. Structural and biochemical studies have demonstrated that ARFs bind AuxRE pairs as dimers, and that different ARFs have different binding preferences for different AuxRE configurations **(Boer *et al.*, 2014)**. AuxRE pairs are characterized by their configuration, which includes both the relative orientation of the two consensus sequences (which can be the same, be inverted or everted), and the nucleotide spacing between them **(Freire-Rios *et al.*, 2020)**. Transcriptomic approaches have established correlations between the enrichment of a particular AuxRE configuration in the promoter, and either up- or down-regulation of the associated gene in response to auxin **(Zemlyanskaya *et al.*, 2016; Stigliani *et al.*, 2019; Freire-Rios *et al.*, 2020)**. Furthermore, genome-wide *in vitro* TF-DNA binding assays have shown that clade and B ARFs bind different regions in the genome and different AuxRE pair configurations, in both

Arabidopsis **(O'Malley *et al.*, 2016; Stigliani *et al.*, 2019)** and maize **(Galli *et al.*, 2018)**. Altogether, these findings suggest that gene specificity of auxin responses may be encoded by AuxRE pairs directly in the promoters of auxin responsive genes, each promoter recruiting a specific set of ARFs depending on its AuxRE composition **(Fig. 2, lower part)**. Still, genome-wide *in vivo* binding data for a large number of ARFs is still lacking to reinforce and validate this model. Given that each cell can express several ARFs from different clades, their interaction is crucial in defining the transcriptional output of the NAP. One of the current models of transcriptional regulation by ARFs is the competition model. Indeed, ARFs can act as transcriptional activators (clade A) or repressors (clade B), and certain promoters have been shown to be bound *in vitro* by both activator and repressor ARFs **(Galli *et al.*, 2018; Stigliani *et al.*, 2019)**, allowing for a potential competition between them. This model is also supported by functional analyses in *Physcomitrium patens* showing that clade B ARFs regulate auxin-induced genes **(Lavy *et al.*, 2016)**, as well as in *Marchantia polymorpha* where the single clade A and clade B ARFs regulate common targets. The expression of an auxin target gene would then depend on the activator:repressor ratio of ARFs bound to the promoter. In this model, auxin perception at the nucleus would push the activity balance towards activator ARFs, resulting in transcriptional activation of the target gene **(Fig. 2, lower part)**.

Investigations of upstream regions of auxin-responsive genes have shown that these regions are not only enriched in AuxREs, but also in cis-regulatory elements from other TF families, including MYB and MYC factors **(Berendzen *et al.*, 2012)**, basic leucine zipper (bZIP) factors **(Berendzen *et al.*, 2012; Cherenkov *et al.*, 2018)**, and basic helix-loop-helix (bHLH) factors **(Cherenkov *et al.*, 2018)**. This is an argument for a potential cooperation between these factors and ARFs in the regulation of auxin-responsive genes **(Fig. 2, lower part)**. Consistent with this idea, the transcription factor bZIP11 has been shown to stimulate the auxin-induced expression of the *GH3.3* gene **(Weiste and Dröge-Laser, 2014)**. Single cis-regulatory elements from other

TF families, termed coupling motifs, are also found in close vicinity to AuxREs (within 50 nucleotides) **(Mironova *et al.*, 2014)**, suggesting that ARFs could associate with these TFs to form heterodimers that directly bind to these coupled binding sites and regulate transcription **(Fig. 2, lower part)**. This is further supported by data showing that some ARFs can heterodimerize *in vitro* with specific MYB factors **(Shin *et al.*, 2007)** and bHLH factors **(Varaud *et al.*, 2011)**. Whether this happens *in vivo* at the loci of auxin-responsive promoters and effectively modulates gene expression remains to be shown.

Altogether, these data shed light on how specificity of auxin transcriptional responses is achieved. Notably, a clear picture of the mechanisms underlying space and time specificity of auxin transcriptional responses emerges: changes during development of tissue- or cell-specific expression of different NAP components and ARF-interacting TFs, combined to the different AuxRE preferences of the different ARFs, would enable the expression of a selected subset of auxin target genes by each cell, in a context-dependent fashion **(Fig. 2)**.

To sum up, the NAP involves three families of core components, assembled in a modular signaling pathway. The molecular properties of each component, coupled to a highly complex transcriptional and post-transcriptional regulation, provide tunable dynamical capacities to nuclear auxin signaling (amplitude, speed, sensitivity), and enable it to trigger specific responses in space and time.

Non-canonical auxin signaling: alternative roles for nuclear auxin pathway core components and fast non-transcriptional auxin responses

An atypical ARF: direct auxin perception by ARF3/ETTIN

Some ARFs lack the PB1 domain which is required for interactions with Aux/IAAs **(Guilfoyle, 2015)**, and are thus not interacting with Aux/IAAs and unlikely to have their activity directly

regulated by auxin. Among them, ARF3/ETTIN (ARF3/ETT) has been shown to regulate a subset of its target genes in an auxin-dependent manner, by interacting with different TFs **(Simonini *et al.*, 2016, 2017)** **(Fig. 3)**. A mechanism for auxin perception through this non-canonical pathway was recently uncovered as ARF3/ETT itself has the ability to bind auxin, thus suggesting that it acts as an auxin receptor **(Kuhn *et al.*, 2020)** **(Fig. 3)**. A possible mechanism is that binding of auxin to ARF3/ETT triggers a conformational change, which modulates the interactions of ARF3/ETT with both its TF partners and DNA, therefore leading to auxin-dependent changes in the expression of ARF3/ETT target genes **(Simonini *et al.*, 2017; Kuhn *et al.*, 2020)**. It is worth to emphasize that ARF3/ETT is the only ARF known to physically bind auxin (and it does so through an ETTIN-specific domain **(Simonini *et al.*, 2016)**), and the first identified DNA-bound hormone receptor in plants. As this mechanism does not depend on Aux/IAAs, it likely provides different dynamical properties that expand the spatial and temporal regulatory potential of auxin-dependent transcriptional regulations. The defects in gynoecium formation, ovule development and lateral root patterning of *arf3/ett* mutants **(Simonini *et al.*, 2016)** highlight the importance of this non-canonical mode of auxin signaling for plant development. Interestingly, the ARF5 and ARF8 splicing isoforms generate protein variants devoided of PB1 as is ARF3/ETT. This constitutes an insight into the evolution of the architecture and the regulation of the NAP: two distinct molecular mechanisms may have been selected and perform the same function, namely providing ARFs with Aux/IAA independency, consequently diversifying the auxin-dependent temporal dynamics.

Thinking outside the F-box: alternative auxin-dependent TIR1/AFB activities

As pointed out in the introduction and despite the rapidity of some auxin-induced transcriptional responses (the earliest mRNA can be detected within 5 minutes after auxin exposure **(McClure *et al.*, 1989)**, auxin is able to trigger even faster developmental responses. For instance, auxin reversibly inhibits root growth within 3 minutes **(Fendrych *et al.*, 2018)**. This phenomenon

requires the formation of the $SCF^{TIR1/AFB}$-Aux/IAA co-receptor complex, suggesting the existence of a TIR1/AFB-mediated non-transcriptional regulation **(Fendrych *et al.*, 2018)**. Other studies have identified the Cyclic Nucleotide-Gated Channel CNGC14 as a key player in the rapid inhibition of root growth by auxin **(Shih *et al.*, 2015)**. An electrophysiological approach showed that a one second auxin pulse is sufficient to trigger a TIR1/AFB-dependent rapid membrane depolarization caused by transient Ca^{2+} ions influx mediated by CNGC14 **(Dindas *et al.*, 2018)**. Further work established AFB1 as a central receptor among TIR1/AFB paralogues for rapid auxin responses in the root, although other paralogues contribute to it **(Prigge *et al.*, 2020; Serre *et al.*, 2021)**. In line with this idea, *afb1* mutants display no membrane depolarization and no root growth inhibition in response to auxin, as well as a delayed early gravitropic response **(Serre *et al.*, 2021)**. This links together AFB1-dependent auxin signaling, membrane depolarization and root growth inhibition; and demonstrates the importance of fast auxin signaling in plant development.

Altogether, these findings indicate that the TIR1/AFBs receptors from the NAP can act non-canonically by triggering rapid, non-transcriptional auxin responses in the root **(Fig. 3)**. However, the precise downstream signaling events leading to membrane depolarization and growth regulation still have to be elucidated (but see next section). Interestingly, a recent study unexpectedly identified an evolutionary conserved adenylate cyclase (AC) amino-acid motif at the C-terminus of TIR1/AFBs **(Qi *et al.*, 2022)**. The AC motif of *Arabidopsis* TIR1, AFB1 and AFB5 was shown to be functional and able to produce cyclic adenosine monophosphate (cAMP) **(Fig. 3)**. Increases in cAMP levels correlating with root growth inhibition were however detected only from 1 hour after auxin treatment, ruling out the involvement of this second messenger in rapid non-transcriptional auxin responses. In contrast, AC activity of TIR1/AFBs was shown to be necessary for the auxin-induced upregulation of genes including *GH3.3*, *GH3.5*, *IAA5*, *IAA19* and *LBD29* **(Qi *et al.*, 2022)**. However, cAMP is known to regulate

Ca^{2+} influx and actin cytoskeleton dynamics **(Gehring, 2010)**. The fact that TIR1/AFBs translate auxin perception in a dual way, Aux/IAA degradation and cAMP production, could then act in the temporal synchronization of the transcriptional and non-transcriptional responses to auxin during developmental responses. The search for downstream effectors of TIR1/AFB-produced cAMP is therefore an important next step to bring light on this question. Interestingly, a recent preprint reported an inhibitory effect of AFB1 on the transcription of auxin target genes from the *Aux/IAA* and *LBD* families **(Dubey *et al.*, 2023, Preprint)** **(Fig. 3)**. This reinforces the idea that TIR1/AFB are key players acting at the interface of transcriptional and non-transcriptional signaling, and suggests that the different TIR1/AFB paralogues might drive differently these two modes of auxin signaling.

Back in business: ABP1-dependent auxin perception

The most famous actor involved in non-canonical auxin signaling is undoubtedly Auxin-Binding Protein 1 (ABP1), first identified in 1972 **(Hertel *et al.*, 1972)**. Since then, numerous studies attempting to link ABP1 auxin-binding capacity to developmental and physiological effects in the plant have been published. These findings were intensively discussed and put into question because of several caveats in the analyses, up to the point that ABP1 ended up being qualified as the "red herring" of auxin biology (reviewed in Napier, 2021 **(Napier, 2021)**). A pivotal discovery in the ABP1 story was the demonstration that the embryo-lethality of a supposedly loss-of-function *abp1* mutation was actually caused by the disruption of another gene next to *ABP1* **(Gao *et al.*, 2015; Dai *et al.*, 2015)**.

However, the tumultuous history of ABP1 recently saw a major turn **(Friml *et al.*, 2022)**. In their work, Friml and co-authors used extensively characterized *abp1* mutant lines as well as a combination of localization studies and proteomics, and reported the following findings: (1) A fraction of cellular ABP1 is secreted to the cell surface, and located at the apoplast; (2) Apoplastic ABP1 interacts in a dose-dependent manner with the transmembrane kinase TMK1

(which had been previously proposed to mediate ABP1-dependent responses upon auxin binding **(Xu *et al.*, 2014)**); (3) Auxin perception by ABP1/TMK1 triggers phosphorylation of ~3000 proteins within two minutes, including plasma membrane H^+-ATPases and myosin XI (see also **(Han *et al.*, 2021)** and **(Roosjen *et al.*, 2022)**) **(Fig. 3)**; and (4) ABP1/TMK1 mediated auxin signaling is crucial for rapid cellular responses to auxin (including H^+-ATPase activation and cytoplasmic streaming), as well as formation of auxin transport channels at the tissue scale, and subsequent vascular regeneration after wounding **(Friml *et al.*, 2022)**.

This study now provides robust evidence that the ABP1-TMK1 module is acting in extracellular auxin perception, and triggers a non-canonical, fast auxin signaling pathway through the massive phosphorylation of proteins **(Fig. 3)**. These findings suggest that ABP1 and TMK1 are likely two of the molecular components that links auxin to the fast non-transcriptional responses we discussed above. The involvement of the ABP1/TMK1 module in vascular regeneration also indicates that extracellular auxin sensing by ABP1 could be essential for canalization **(Hajný *et al.*, 2022)**. Canalization proposes that PIN localization at a membrane is increased by the auxin flux going through it, thus stabilizing the auxin efflux, and explaining notably the formation of veins **(Sachs, 1969)**. Canalization is expected to occur through auxin-dependent relocalization of PIN auxin transporters and a preprint suggests that this could be mediated by the ABP1-dependent phosphorylation of myosin XI **(Han *et al.*, 2021, Preprint)**. Dissecting the molecular chain of events involved in canalization would allow to get a better grasp on vasculature formation, but also in other developmental processes involving dynamic PIN relocalization such as in the shoot apical meristem **(Vernoux *et al.*, 2021)**. Importantly it would also allow to understand how different timescales are bridged during development, here an ultrafast auxin response induced at membrane and a tissue-wide reorganization of polarities that drives tissue differentiation dynamics.

In conclusion, there are several alternative signaling pathways involving either NAP core components (TIR1, AFB1, ARF3) or specific molecular actors (ABP1, TMK), which drive transcriptional or fast non-transcriptional responses to auxin with different dynamics than the NAP itself, therefore adding up to the diversity and complexity of cellular outputs triggered by this phytohormone. Despite being called "non-canonical", the alternative modes of auxin perception and signaling presented above are omnipresent, and their importance in orchestrating plant development is increasingly clear. In the future, understanding the dynamics of auxin-regulated development will without doubts require taking into account both the NAP and the non-canonical auxin signaling mechanisms.

Again, canonical and non-canonical auxin signaling generate responses with different timescales, from second-scale phosphorylations and ion fluxes to hour-scale regulations of gene expression, growth and differentiation. Whether and how these different signaling pathways interact, cooperate or relay information over time during development still remains to be analyzed.

The temporal dimension of auxin signaling: emerging concepts and developmental implications

So far, the question of the contribution of auxin signaling to development has been largely focused on the question of the spatial specificity of auxin responses, in order to understand how different cells can respond differently to auxin. The fact that both non-transcriptional and transcriptional responses to auxin occur on the second to minutes/hour timescales and the need for complex quantifications over time has often led to neglect a possible contribution of the temporality of auxin signaling mechanisms to the control of the dynamics of plant development, except in specific rapid growth responses such as gravitropism as discussed in the previous

section. However, as pointed out above, even in the case of the gravitropic response that occurs just over an hour **(Band *et al.*, 2012)**, how the timescale of the signaling responses and of the growth response are bridged remains elusive. Also, recent work suggest that auxin-induced gene regulation might not always be a rapid process. Here, we pinpoint a few examples that are starting to identify how temporal dynamics of auxin signaling and auxin responses can be regulated, and to help us understand the integration of such regulations in a developmental framework.

Rhythmic auxin-dependent lateral root patterning and development

A first relevant model to question the role of the temporality of auxin signaling in plant development is lateral root (LR) priming. Priming is the establishment of a pre-branching site (PBS), which is a group of pericycle cells that has the competence to form a LR **(Torres-Martínez *et al.*, 2022)**. PBS are robustly established along the growth axis of the primary root, with a constant spacing, every 6 hours **(Moreno-Risueno *et al.*, 2010)**. This is a clear case of reiterative patterning, where future organs are rhythmically determined, with constant spacing and period. PBS are characterized by a cyclic expression of thousands of auxin-responsive genes, and those PBS are established in a region comprising the basal meristem and the elongation zone, termed the oscillation zone (OZ) **(Moreno-Risueno *et al.*, 2010) (Fig. 4, lower right, yellow frame)**. Auxin is involved in controlling this rhythmic process but the underlying mechanism is highly debated. Several mechanistic models have been proposed. The *root clock* model, hypothesizes that cyclic gene expression is cell autonomous **(Moreno-Risueno *et al.*, 2010)**, and emerges from a negative feedback loop involving ARF7 and IAA18, entrained by an initial pulse of auxin in the OZ **(Perianez-Rodriguez *et al.*, 2021) (Fig. 4, lower right, yellow frame)**. Another model, termed *reflux-and-growth*, stipulates non-cell autonomous cyclic gene expression, caused by the combined effect of auxin loading and cell growth, which would lead to oscillations in auxin concentrations, and subsequent expression of auxin target

genes **(Berg *et al.*, 2021)**. The second model was recently refined in a two-step mechanism, where PBS would first be initiated by a transient elevation in auxin concentration in the OZ (*reflux-and-growth*), which would then be followed by a persistent increase in auxin signaling allowing stable PBS establishment **(Santos Teixeira *et al.*, 2022)** **(Fig. 4, lower right, yellow frame)**. In this model, the persistent increase in auxin signaling is hypothesized to rely on temporal integration of auxin information by root cells, similarly to what was recently described in the shoot apical meristem (SAM: see below; **(Galvan-Ampudia *et al.*, 2020)**). Both models constitute examples where temporal properties of the NAP, either generated through its integration within a larger network of oscillating genes or intrinsic to the NAP itself would be crucial for the rhythmicity of organ formation in the root, therefore linking together the temporal properties of auxin signaling and the timing of developmental events.

Considering the development of lateral roots themselves, it has been shown using degradation rate variants of IAA14 that both LR density and the rate of LR development were correlated with the degradation rate of IAA14 variants **(Guseman *et al.*, 2015)**. This constitutes a demonstration that IAA turn-over itself is sufficient to influence the temporal dynamics of developmental events, an important concept that we discussed in the first section of this review. This finding further highlights the interdependency between the timing of lateral root initiation and development and the temporality of the NAP.

Coupling fast and slow auxin responses in the root during gravitropism

In the root, auxin has long been known to be involved in root meristem zonation **(Sabatini *et al.*, 1999; Ding and Friml, 2010)**. Root meristem zonation is defined by a proximo-distal auxin gradient along the root. This gradient enables both the maintenance of a stem cell pool by high auxin signaling in the quiescent center **(Sabatini *et al.*, 1999)** and the differentiation of stem cells triggered by an auxin minimum at the transition zone **(Di Mambro *et al.*, 2017)**. Auxin-dependent proximo-distal patterning of the root meristem is mediated by several effector

proteins, among which PLETHORA (PLT) 1 and 2 play a central role, by translating the auxin gradient in distinct cellular domains **(Galinha *et al.*, 2007; Ding and Friml, 2010)**. In parallel, we have seen that auxin is the central actor of the gravitropic response of the primary root, during which root bending towards gravity requires the accumulation of auxin on the lower side of the root **(Su *et al.*, 2017)**.

How auxin distribution in the root meristem achieves the simultaneous regulation of these different phenomena is not trivial. Indeed, given the mechanisms described above, one would expect a disruption of root meristem patterning due to auxin relocalization during gravitropism. The explanation of why this does not occur lies in the different kinetics of auxin signaling involved in these distinct developmental processes. Indeed, Mähönen *et al.* have shown that the PLT2 gradient is not a direct translation of the auxin gradient, but results instead of slow transcriptional activation of *PLT2* by auxin, followed by a protein gradient formation through growth dilution of PLT2 along the proximo-distal axis, which requires several days to be established **(Mähönen *et al.*, 2014)** **(Fig. 4, lower left, pink frame)**. In contrast, auxin relocalizes within minutes upon gravistimulation of the primary root, and fast non-transcriptional auxin signaling allows for the complete bending of the root towards the new gravity vector to be achieved within 6 hours **(Mähönen *et al.*, 2014)** **(Fig. 4, lower left, pink frame)**. Auxin redistribution in the root during gravitropism is only transient, and occurs within a timescale that is not long enough to perturb the auxin-mediated patterning of the root meristem. This constitutes a remarkable example where distinct kinetics in auxin-regulated networks (the auxin signaling pathway on one side and the auxin-dependent network controlling PLT2 expression) enables the coupling of two auxin-dependent developmental events, without one interfering with the other.

With this finding, we can hypothesize that the conservation of different kinetics of auxin responses through evolution could be explained because it allows a “segregation of duties”,

enabling the same chemically simple molecule to concomitantly regulate distinct developmental processes, in the same tissue but with different timescales.

Temporality of auxin signaling at the shoot apex and the regulation of organogenesis

Organ initiation at the SAM has very recently provided yet another striking example of the importance of the temporality of auxin signaling and responses. In this tissue, aerial organs are sequentially initiated at precise spatial positions, with a particular time period, the plastochron. This spatio-temporal pattern of organ initiation is at the origin of phyllotaxis, the robust geometric arrangement of organs around the stem. Organ initiation is triggered by the accumulation of auxin, which is polarly transported in the SAM by PIN efflux carriers (for review: **(Vernoux *et al.*, 2021)**). Using cutting-edge microscopy and computational approaches, Galvan-Ampudia and colleagues mapped auxin distribution and signaling in the SAM with an unprecedented spatio-temporal resolution **(Galvan-Ampudia *et al.*, 2020)**. They found that at any time several auxin maxima are present at the same time in the organogenetic zone, even at positions where no organ initiation or auxin signaling activity can be detected **(Galvan-Ampudia *et al.*, 2020)**. They also demonstrated an absence of correlation within the organogenetic zone between auxin concentration in a cell detected with the qDII auxin biosensor and the intensity of auxin transcriptional response in the same cell detected with the DR5 auxin-inducible reporter **(Galvan-Ampudia *et al.*, 2020)**. This reinforced the idea that cells experiencing higher concentrations of auxin do not necessarily display a higher transcriptional response. They further demonstrated using quantitative imaging and exogenous applications of auxin that cells require a sustained exposure to auxin over time to integrate this information and to start activating a transcriptional response, leading to a delay of the transcriptional activation close to one plastochron (12h) after auxin concentration builds-up at a given site in the SAM organogenesis domain **(Galvan-Ampudia *et al.*, 2020)** **(Fig. 4, top, blue frame)**. The molecular mechanism by which shoot apical cells temporally integrate the

auxin signal has not known, but genetic and pharmacological data indicates that it could rely on the NAP and on histone acetylation mediated by NAP components **(Galvan-Ampudia *et al.*, 2020)** **(Fig. 4, top, blue frame)**. Interestingly, epigenetic modifications are known to control the timing of gene expression in plants, although on longer timescales than the plastochron **(Sun *et al.*, 2009; Angel *et al.*, 2011)** and at least one example is known in animals, where the timing of activation of a transcriptional response is tuned by progressive histone acetylation during several hours of sustained signaling **(Coda *et al.*, 2017)**. This work thus changes the paradigmatic view that auxin induces genes primarily within minutes up to 1-2 hours, demonstrating that, on the contrary, in the SAM auxin-induced transcription occurs over the timescale of the plastochron. In this developmental context, temporal integration properties intrinsic to the NAP might allow to drive the rhythmic generation of organs by differentiating spatial positions within the SAM, based on the history of their exposure to auxin rather than on an immediate auxin concentration. This also draws an interesting parallel with animal morphogens that can provide temporal information used in development **(Sagner and Briscoe, 2017)** and open the possibility that auxin temporal information could be used in other developmental situations, as suggested for the root **(Santos Teixeira *et al.*, 2022)**.

To conclude this section, these few examples illustrate the key importance of the temporal dynamics of auxin signaling and responses in the control of plant development. Although the underlying molecular mechanisms are not well characterized yet, the temporality of auxin processing by cells provides particular properties suited to each developmental context considered: temporal robustness for reiterative patterning at the shoot and the root apex, and rapidity of growth response for root gravitropism.

Concluding remarks and open questions

Auxin biology has gone a long way since 1928. The progressive elucidation of the intricate complexity of the canonical nuclear auxin pathway, in parallel to the discovery of novel actors mediating a myriad of auxin responses with different properties, has enabled to build an extensive molecular understanding of the effects auxin has on cells. Still, the pleiotropy of this phytohormone remains vertiginous when considering it in a developmental context. Many studies have contributed to shine some light on the mechanisms underlying auxin response specificity, but there are still many unknowns that we have highlighted, in particular on the temporal specificity of auxin responses. On this particular aspect, many questions remain unanswered: How do the different layers of regulation of NAP components impact the kinetics of auxin signaling and response? By which means can a plant cell read its duration of exposure to auxin? How can second- to minute-scale auxin responses control hour- to day-scale developmental events? How do fast and slow auxin signaling interact over time within a given cell? The generalization of the use of quantitative high-resolution imaging and genomics combined with the use of multiscale approaches will surely help to build-up on the available knowledge we have about auxin, and to reach an integrative view of both the spatial control and the temporality of auxin-mediated regulation of plant development, from cells to the whole organism. We believe that tackling this question of temporality in auxin responses will be crucial because development is mostly - if not all - about time.

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Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Fig. 1. The canonical nuclear auxin pathway (NAP).

(A) Functional domains of NAP components protein families, TIR1/AFB, Aux/IAA and ARF; LRR: Leucine-Rich Repeat motif; DI: Domain I; RM: Rate Motifs; DII: Domain II; DBD: DNA-Binding Domain; MR: Middle Region. (B) Canonical mode of auxin sensing in the nucleus. In absence of auxin, the expression of auxin-responsive genes is repressed by HDACs, recruited at the promoter by both Aux/IAAs and class B ARFs via TPL/TPR (TOPLESS/TOPLESS RELATED)-dependent interactions. Perception of nuclear auxin by the $SCF^{TIR1/AFB}$ co-receptor complex triggers the degradation of Aux/IAAs, enabling class A ARFs to activate the expression of their target genes by recruiting SWI/SNF chromatin remodeling complexes and HATs.

Fig. 2. Regulatory mechanisms tuning auxin response dynamics and specificity.

The tuning of the dynamical properties (amplitude, speed and sensitivity) and the spatio-temporal specificity of auxin responses are ensured by both the diversity of NAP core components, the regulation of their activity, and the complexity of their interactions (upper part

of the figure). At the level of the promoter (lower part of the figure), DNA binding properties of ARFs and their interactions with partner transcription factors are thought to provide gene specificity to auxin action. AuxRE: Auxin Response Element; DR: Direct Repeat; IR: Inverted Repeat; ER: Everted Repeat.

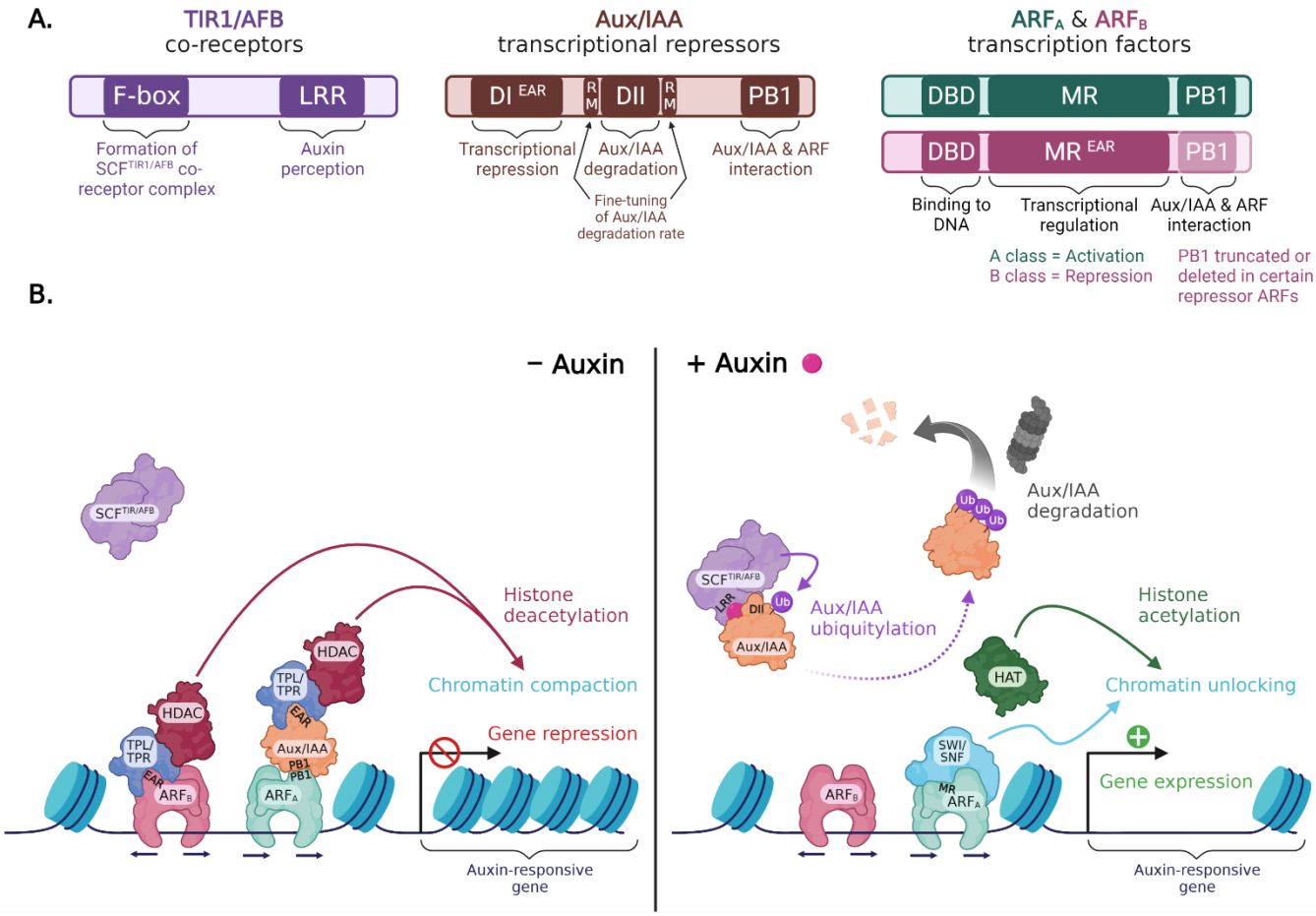
Fig. 3. Canonical vs. Non-canonical auxin signaling.

Left: Canonical auxin signaling, occurring in the nucleus (see Fig. 1 for more details). Right: Non-canonical auxin signaling. This notably includes (1) extracellular auxin perception by the ABP1/TMK module triggering the activation of several cytoplasmic proteins via phosphorylation (light blue); (2) cytosolic auxin perception by TIR/AFB leading to CNGC14 (Cyclic Nucleotide-Gated Channel 14)-dependent calcium influx (green) and the production of cAMP (Cyclic Adenosine Monophosphate), a second messenger (yellow); and (3) direct nuclear auxin perception by the non-canonical ARF3/ETT (ETTIN) (pink). See main text for details.

Fig. 4. Importance of the temporal dynamics of auxin signaling in the control of plant development.

Top, blue frame: Auxin signaling and response dynamics at the shoot apical meristem of *Arabidopsis*. CZ: Central Zone; PZ: Peripheral Zone; Pi: Primordia of age I; Temp. int.: Temporal integration. Left: Cells leaving the CZ require 12 hours of sustained auxin exposure to activate a transcriptional response to auxin. Modified from Galvan-Ampudia et al. 2021. Right: Temporal integration imposes a delay before transcription is activated in response to auxin. Lower left, pink frame: Dual mode of auxin signaling in the root of *Arabidopsis*. PLT: PLETHORA; QC: Quiescent Center; MZ: Meristematic Zone; TZ: Transition Zone; EZ: Elongation Zone. Root patterning requires the upregulation of PLT expression by auxin, which

takes several days to be established, whereas auxin triggers the gravitropic response within hours via rapid non-transcriptional signaling. Lower right, yellow frame: Models of auxin-dependent lateral root patterning in *Arabidopsis*. OZ: Oscillating Zone; LR: Lateral Root; T: Period of the molecular clock. Oscillations of auxin responses along the root could emerge either from a molecular clock intrinsic to the NAP (left), or from the temporal integration of auxin levels, which oscillate due to a *reflux-and-growth* mechanism (right, see text for more details).



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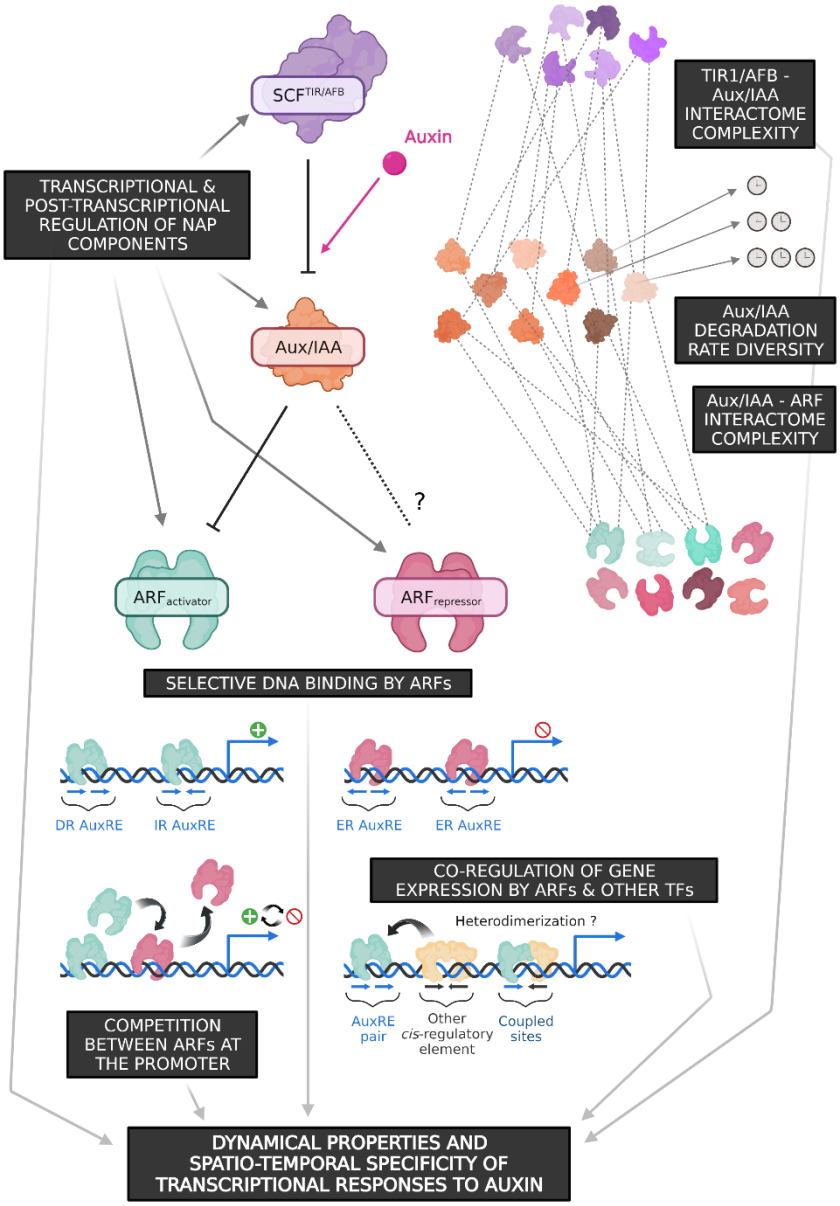

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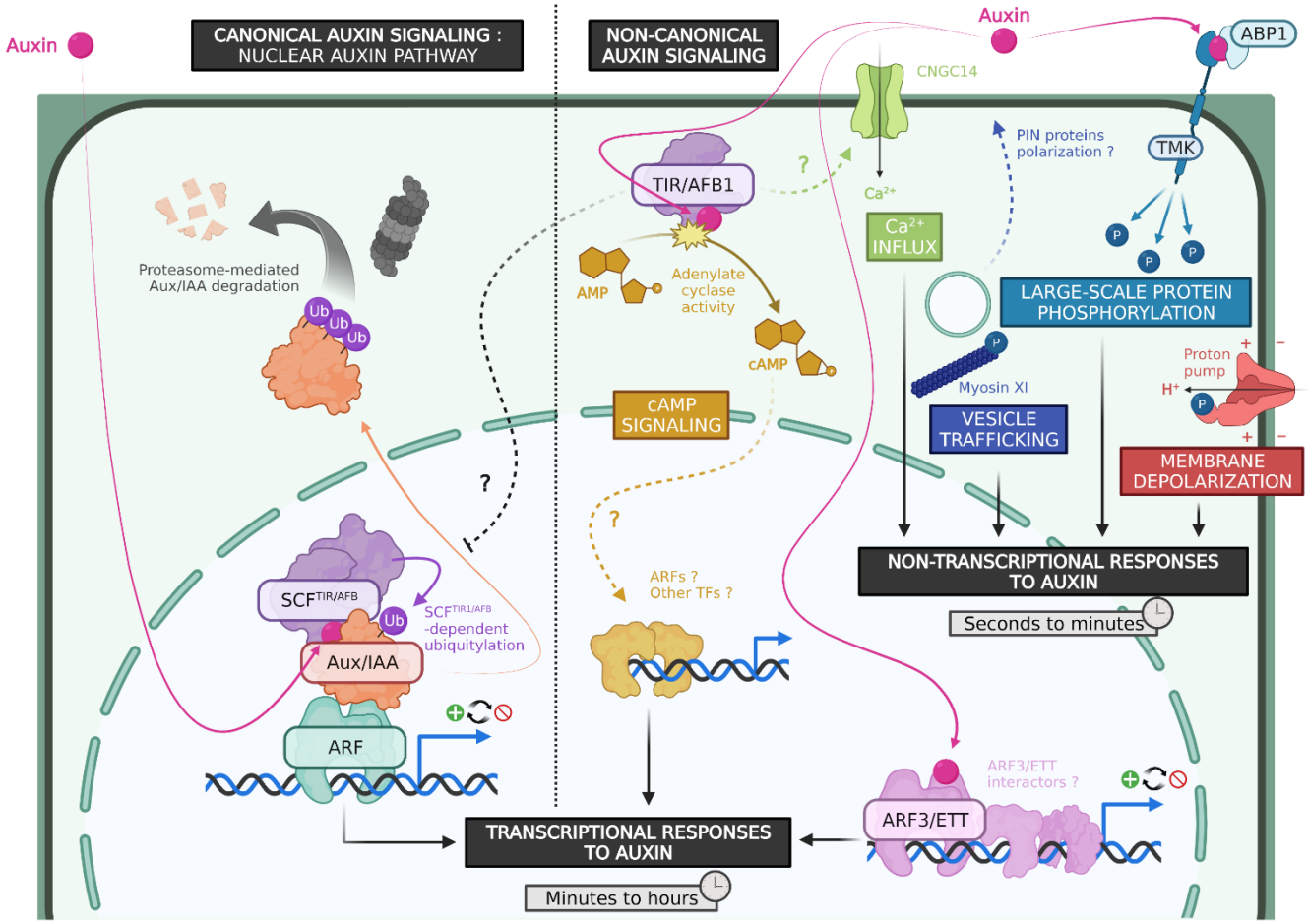

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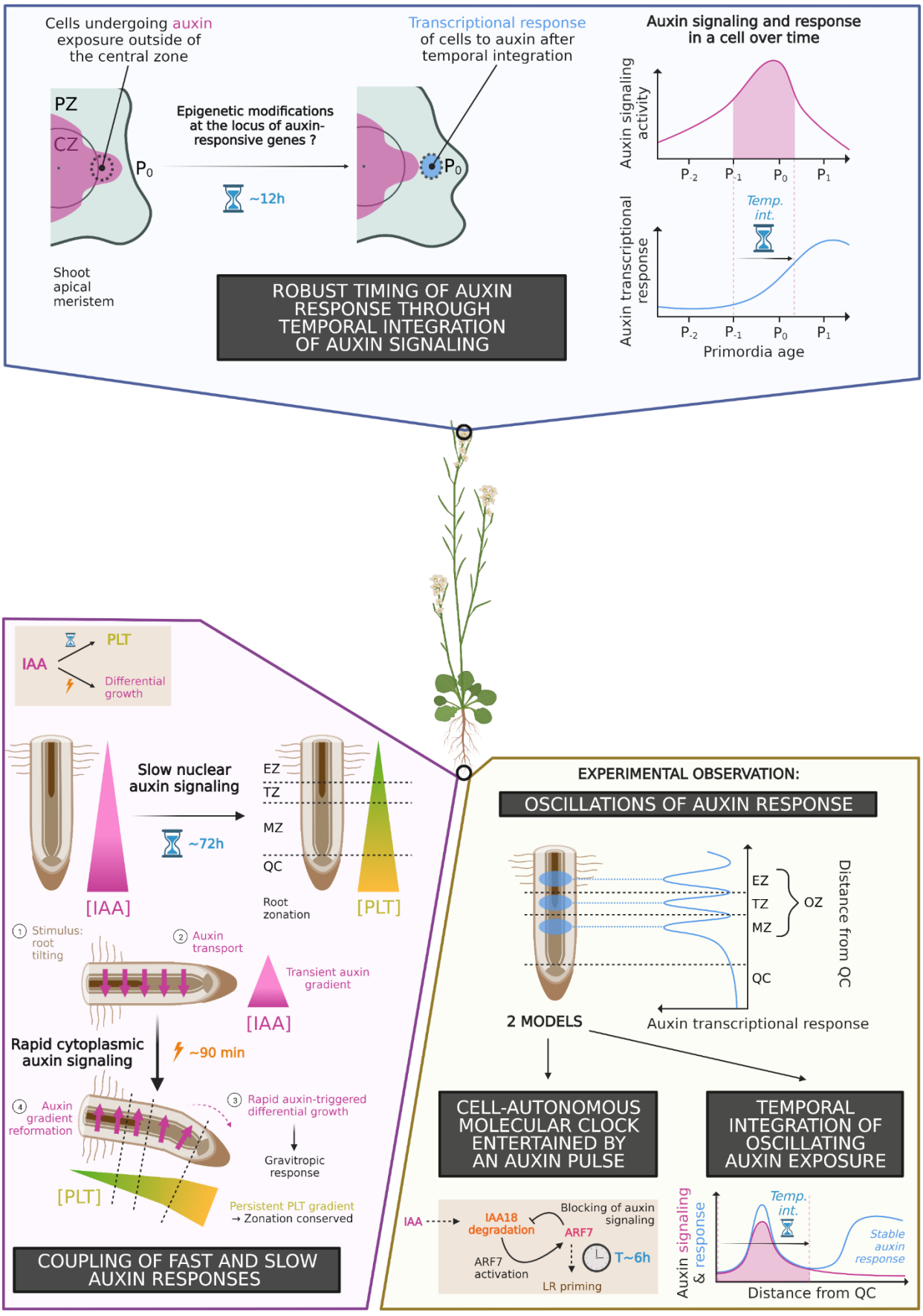
Cells undergoing auxin exposure outside of the central zone
PZ
CZ
P0
Shoot apical meristem
Epigenetic modifications at the locus of auxin-responsive genes ?
~12h
Transcriptional response of cells to auxin after temporal integration
P0
ROBUST TIMING OF AUXIN RESPONSE THROUGH TEMPORAL INTEGRATION OF AUXIN SIGNALING
Auxin signaling and response in a cell over time
Auxin signaling activity
P-2 P-1 P0 P1
Temp. int.
Auxin transcriptional response
P-2 P-1 P0 P1
Primordia age
IAA
PLT
Differential growth
Slow nuclear auxin signaling
~72h
[IAA]
EZ
TZ
MZ
QC
Root zonation
[PLT]
1 Stimulus: root tilting
2 Auxin transport
Transient auxin gradient
[IAA]
Rapid cytoplasmic auxin signaling
~90 min
4 Auxin gradient reformation
3 Rapid auxin-triggered differential growth
Gravitropic response
[PLT]
Persistent PLT gradient → Zonation conserved
COUPLING OF FAST AND SLOW AUXIN RESPONSES
EXPERIMENTAL OBSERVATION:
OSCILLATIONS OF AUXIN RESPONSE
EZ
TZ
MZ
QC
OZ
Distance from QC
Auxin transcriptional response
2 MODELS
CELL-AUTONOMOUS MOLECULAR CLOCK ENTERTAINED BY AN AUXIN PULSE
TEMPORAL INTEGRATION OF OSCILLATING AUXIN EXPOSURE
IAA
IAA18 degradation
Blocking of auxin signaling
ARF7
ARF7 activation
T~6h
LR priming
Auxin signaling & response
Temp. int.
Stable auxin response
Distance from QC

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