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Das, Shubhajit; Weijers, Dolf; Borst, Jan Willem

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Opinion

Auxin Response by the Numbers

Shubhajit Das,[1,@] Dolf Weijers,[1,*,@] and Jan Willem Borst[1,*,@]

Auxin is fundamental to the growth and development of land plants, and acts in large part through the control of gene activity. Genetic and biochemical analysis of the nuclear auxin signaling pathway (NAP) has led to the establishment of a generic model for auxin-dependent gene regulation. To understand how this dynamic system operates in living cells, quantitative data are needed. For this, the liverwort *Marchantia polymorpha* provides a useful model system. Its limited number of NAP components, combined with experimental approaches to determine concentrations, binding affinities, and turnover rates, will enable a new, quantitative view on the mechanisms that allow auxin to control plant growth and development.

Highlights

Quantitative data are essential for the establishment of a predictive auxin response network.

Developmental stage-specific auxin responses are dependent on protein concentrations, binding affinities, and protein turnover.

Marchantia polymorpha is a unique model system for studying protein networks *in vivo*.

The Nuclear Auxin Signaling Pathway

Auxin-induced physiological and transcriptional responses are at the heart of land plant growth and development [1–3]. Auxin is perceived by TRANSPORT INHIBITOR RESPONSE1/AUXIN-BINDING F-BOX (TIR1/AFB), an F-box protein in the **$SCF^{TIR1/AFB}$ complex** (see Glossary) [4,5], and its Auxin/INDOLE-3-ACETIC ACID (Aux/IAA) substrate [6]. At low cellular auxin concentrations, Aux/IAA repressors interact with DNA-binding AUXIN-RESPONSE FACTORS (ARFs) via their shared **PB1 domain** and prevent ARF-driven transcription. At high auxin levels, the $SCF^{TIR1/AFB}$ complex initiates ubiquitination and degradation of Aux/IAA repressors, allowing ARFs to regulate gene transcription [7,8]. Based on phylogeny, ARFs are divided into three classes: A, B, and C. Transient assays showed that A-ARFs tend to activate transcription, whereas B/C-ARFs are considered repressors [9].

Over the past two decades, fluorescent transcriptional and translational reporters have been used to map the activity of NAP genes and proteins in various plant tissues [10–12]. In addition, the network of protein–protein interactions (PPIs) and protein–DNA interactions among the response components has been established [13–16]. These investigations provided a qualitative view of the functions of each signaling protein family and a generic mechanistic model for auxin-driven gene activation. However, it is well known that responses to auxin in plant tissues are extremely diverse; for example, high auxin inhibits root growth, yet promotes hypocotyl growth [17–19]. This demonstrates that qualitative information alone is not sufficient to explain differential auxin sensitivities and cell/tissue-specific responses. The dynamics and diversity of auxin responses could be encrypted in cellular protein abundance, PPI/protein–DNA interaction affinities, complex stoichiometry, turnover rates and so on. Here, we argue that systematic quantitative analysis of auxin response is the next frontier in auxin research.

The Arabidopsis Auxin Response Network: A Tangled Web of Paralogs

Whole-genome duplications have had a central role in the evolution of land plants. From **Charophytes** through **Angiosperms**, genome duplications have inflated gene families with multiple **paralogs** and allowed their **sub/neofunctionalization**, thereby expanding genome complexity, spurring physiological innovations [20]. Higher paralog numbers in Angiosperms gave rise to multiple **auxin-response proteins** (ARP) with overlapping or, in some cases, nonredundant functions [21]. In arabidopsis (*Arabidopsis thaliana*), six AtTIR1s, 23 AtARFs, and 29 AtAux/IAAs constitute the NAP [22] (Figure 1). These 58 proteins create thousands of possible interaction

[1]Laboratory of Biochemistry, Wageningen University, Stippeneng 4, 6708WE Wageningen, The Netherlands

*Correspondence:
dolf.weijers@wur.nl (D. Weijers) and janwillem.borst@wur.nl (J.W. Borst).
[@]Twitter: @marquantia (S. Das), @dolfweijers (D. Weijers), and @jwborst (J.W. Borst).

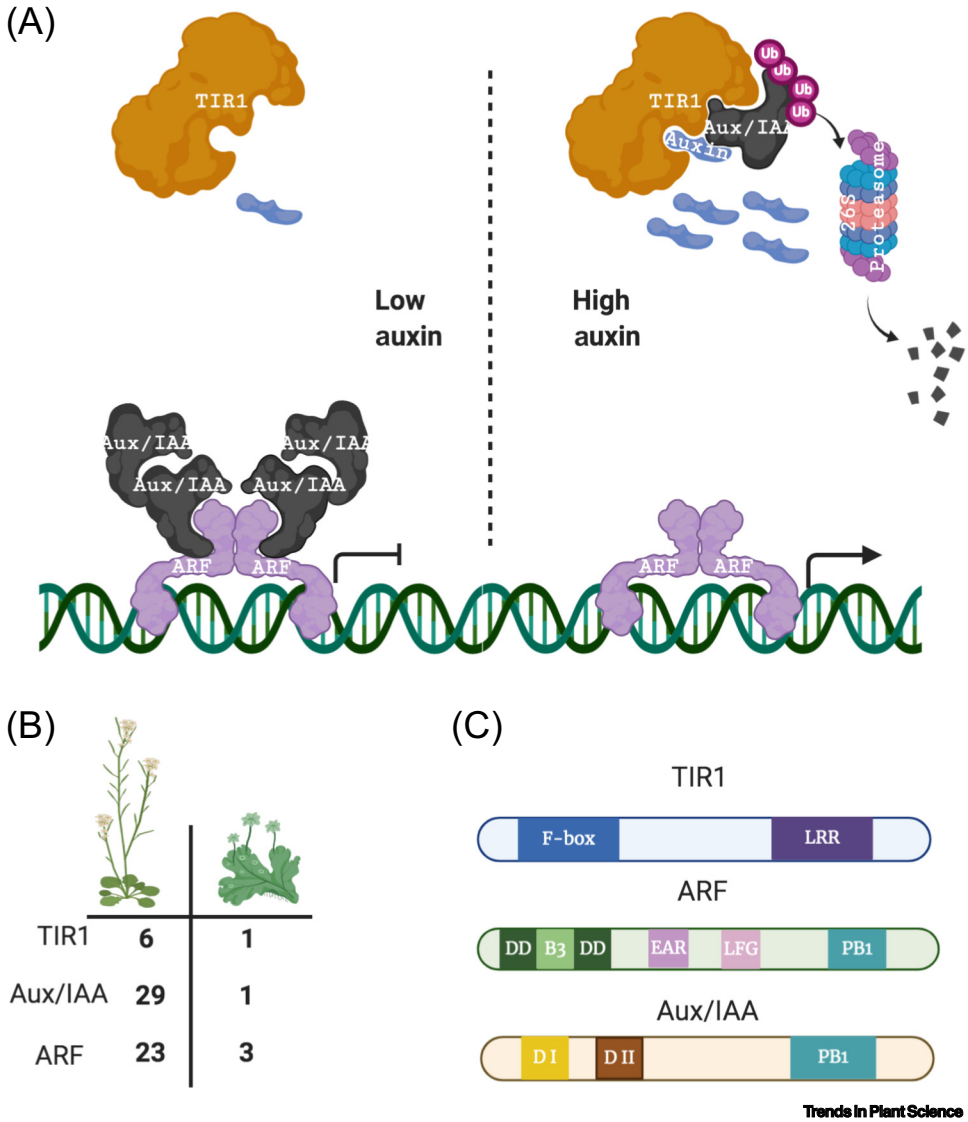

Figure 1. Schematic Overview of Auxin Signaling and Components Involved. (A) Schematic representation of the nuclear auxin-signaling pathway (NAP). At low cellular auxin levels, the hormone is not perceived by the TRANSPORT INHIBITOR RESPONSE1/AUXIN-BINDING F-BOX (TIR1/AFB) receptor, allowing the Auxin/INDOLE-3-ACETIC ACID (Aux/IAA) repressors to persist and bind to AUXIN-RESPONSE FACTOR (ARF) transcription factors (TFs). This interaction prevents ARFs from driving gene transcription. At high cellular auxin concentrations, the hormone is perceived by the TIR1-Aux/IAA co-receptor complex, which is followed by ubiquitination and proteasomal degradation of Aux/IAA proteins. In the absence of Aux/IAA repressors, ARFs activate auxin-induced gene transcription. (B) *Arabidopsis thaliana* harbors multiple auxin response proteins, whereas *Marchantia polymorpha* has only one response protein of each family. (C) Domain architecture of auxin response proteins: the TIR1 receptor has an N-terminal F-box domain for interaction with other SCF complex subunits. A C-terminal leucine-rich-repeat (LRR) domain mediates binding to auxin and Aux/IAA repressors. ARF TFs have a B3 DNA-binding domain inserted between two halves of a dimerization domain (DD) at the N terminus. The unstructured middle region (MR) of ARFs has a class-dependent amino acid composition. Class-A ARFs have glutamine-rich MRs, whereas Class-B and C ARFs have proline/serine-rich MRs. Some ARFs have EAR and LFG motifs for the interaction with TOPLESS (TPL). The C-terminal ends of ARFs and Aux/IAA have a Phox and Bem1 (PB1) domain for ARF-ARF and ARF-Aux/IAA oligomerization. At the N terminus of Aux/IAA repressors, a domain 1 (DI) mediates TPL recruitment, whereas domain 2 (DII) harbors a degron motif for the recognition and degradation of Aux/IAA by the TIR1-SCF complex. A C-terminal PB1 domain allows oligomerization. Figure created using BioRender (https://biorender.com/).

Glossary

Angiosperms: seed-bearing flowering plants with a complete vascular system.
Auxin-response proteins (ARPs): all protein families (mainly ARFs, Aux/IAA, TIR, and TOPLESS) involved in auxin signaling.
Auxin-responsive elements (AuxREs): sequence-specific DNA motifs in the promoter of auxin-regulated genes; serve as ARF-binding sites.
Charophytes: extant group of green algae that are sister to land plants.
DII-Venus: auxin reporter based on the Aux/IAA DII degron motif fused to a Venus fluorophore. In the presence of auxin, this reporter protein is degraded and, thus, serves as a 'negative' sensor for the presence of auxin .
Diffusion coefficient: proportionality constant between molar flux and concentration gradient.
Direct Repeat 5 (DR5): a synthetic reporter designed with a tandem direct repeat of an 11-base pair region containing the AuxRE TGTCTC, used to visualize cells with a high auxin response.
Dissociation constant (Kd): equilibrium constant to signify reversible dissociation rate of a multimeric complex.
DNA-binding domain (DBD): specific protein domain in a transcription factor that allows DNA binding.
DR5v2: version of the DR5 promoter with the high-affinity ARF binding site TGTCGG as the AuxRE sequence.
Fluorescence lifetime: fluorophore-specific time elapsed between fluorophore excitation and return to ground state by emission of photons.
LFG motif: a TOPLESS binding motif conserved in class-B ARFs.
Liquid-liquid phase separation (LLPS): spontaneous demixing of a homogeneous protein solution into membrane-less condensed liquid-like droplets, which may function as storage, partitioning, or active transcriptional sites.
Neofunctionalization: acquisition of a completely new function by a paralog after gene duplication.
Paralogs: copies of the same gene generated by genome duplications.
PB1 domain: a conserved oligomerization domain present in both animal and plant kingdoms; a C-terminal PB1 domain mediates homo- and heterotypic ARF and Aux/IAA interactions.

combinations, which makes it almost impossible to quantitatively describe the system. However, a qualitative AtARF-Aux/IAA interaction network has been obtained with yeast-2-hybrid (Y2H) assays (Box 1) showing that AtAux/IAAs can both homodimerize and heterodimerize with class-A ARFs, whereas interactions are more limited with class-B/C ARFs [23,24]. These studies provided a static, binary interactome for AtARF-Aux/IAA interactions, but lack binding affinities and interaction dynamics.

Auxin strongly influences plant growth and, therefore, any mutation in key signaling genes is expected to manifest into a phenotype. However, due to high genetic redundancy, single mutations in AtTIR1/AFB genes do not cause strong defects [25]. Likewise, only a few loss-of-function mutants of AtAux/IAA (IAA3, 7, and 28) with growth defects have been reported [26–29]. Out of the 23 AtARFs, only five show strong phenotypes as single mutants [30], which impedes the dissection of the individual function of each NAP protein. In all these families, higher-order mutants reveal biological function [11], supporting the notion of functional redundancy.

Beyond redundancy, partial functional diversification among paralogous proteins in arabidopsis generates a complex set of cell-specific auxin-response systems. It is well understood that

Ratiometric version of 2 D2s (R2D2): semiquantitative reporter of auxin activity comprising a mutant DII-ntdTomato and DII-n3xVenus.
$SCF^{TIR1/AFB}$ complex: E3 ubiquitin ligase complex comprising Skp, Cullin, and F-box proteins. It catalyzes the ubiquitination of proteins and targets them for degradation by the 26S proteasome.
Subfunctionalization: changes in paralogous genes leading to each paralog retaining a subset of the ancestral gene function
Turnover rate: continuous balance between protein synthesis and degradation rates.

Box 1. Methods for Quantifying Auxin Response Network

Yeast-2-hybrid (Y2H) assays are genetic tools based on the transcriptional activation of a reporter gene upon reconstitution of two transcription factor domains, each fused to a bait or prey protein. Upon interaction between the bait and prey proteins, the split transcription factor is reconstituted, activating a reporter gene.

Fluorescence correlation spectroscopy (FCS) is a single-molecule technique that tracks fluorescent molecules in a small diffraction limited confocal volume ($<10^{-15}$ l). Due to diffusion, fluorescent molecules cause fluorescence fluctuation signals, which are autocorrelated in time. The generated autocorrelation curve can reveal the diffusion coefficient.

Isothermal titration calorimetry (ITC) is a label-free technique that utilizes the thermodynamic principle of heat exchange during a molecular binding reaction to determine the dissociation constant (Kd), stoichiometry, and cooperativity of PPIs or protein–DNA interactions in a titration-based protocol.

Microscale thermophoresis (MST) is an infrared laser-based optical method that allows Kd measurements in buffer solutions or cell lysates. This immobilization-free technique requires fluorescent protein labeling, and tracks fluorescence changes due to temperature and thermophoresis.

Surface plasmon resonance (SPR) uses the principle of resonance angle change of refracted polarized light due to the refractive index change of a metal (gold or silver) surface by the binding/unbinding of an analyte (e.g., protein) to immobilized ligand (e.g., DNA/protein).

Single-molecule Förster resonance energy transfer (smFRET) is a technique that enables the study of protein interactions, conformational dynamics, intramolecular distance, and protein complex stoichiometry at a single-molecule resolution. It relies on non-radiative resonance energy transfer between donor and acceptor fluorophores that have overlapping emission and absorption spectra.

Fluorescence lifetime microscopy (FLIM) is a fluorescence lifetime-based quantification method of FRET efficiency between fluorophores.

Bimolecular fluorescence complementation (BiFC) is a protein complementation assay based on reconstituting a fluorescent protein from two split fragments, upon interaction of the proteins fused to each half.

Fluorescence recovery after photobleaching (FRAP) can be used to deduce protein diffusion rates based on the fluorescence recovery rate within a photobleached region in a cell.

FCCS is a dual fluorophore version of FCS that allows researchers to probe dynamic protein interactions.

Pair correlation function (pCF) is used to track the path of diffusion of a labeled molecule by correlating intensity fluctuations at multiple points in an image.

intracellular auxin perception results in liberation of ARFs from Aux/IAAs, allowing activation of auxin-responsive genes. However, this activation model does not clarify the roles of the different ARF classes. Recently, analysis of the minimal auxin-response system of *Marchantia polymorpha* (hereafter Marchantia) provided insight into these roles [31].

The Marchantia Auxin-Response System: A Rudimentary Design

The liverwort *M. polymorpha* [32] has an auxin-response network operating with the least possible number of signaling components: one copy of MpTIR1 and MpAux/IAA, and three MpARFs representing each phylogenetic class [33,34]. Out of the three MpARFs, MpARF1 acts as an activator, whereas MpARF2 and MpARF3 are repressors [31,33,34] This simple network dramatically reduces the number of protein interaction combinations and opens an opportunity for *in vivo* quantitative research.

While Marchantia has been a model bryophyte for almost a century, modern genetic tools for this species were unavailable until recently. Progress in T-DNA transformation methods, CRISPR/Cas gene editing, and homologous recombination have paved the way for modern genetics and

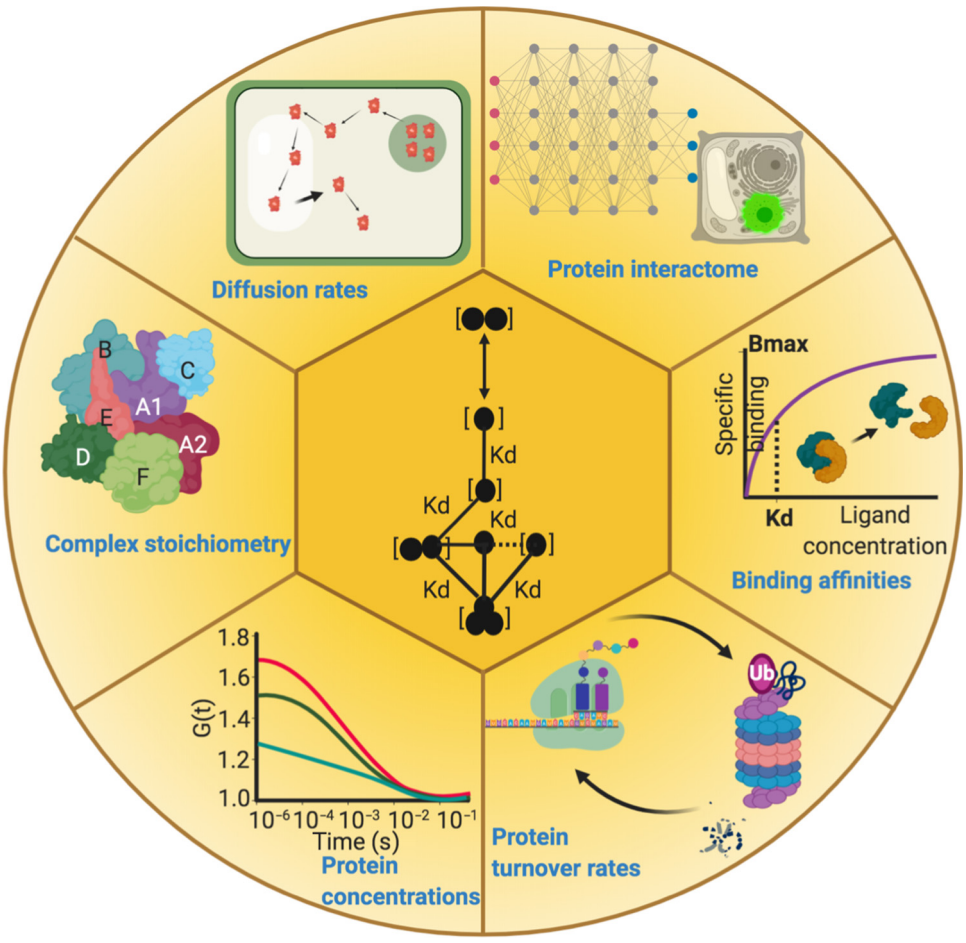

Figure 2. Key Quantitative Parameters that Can Regulate Nuclear Auxin Signaling. Determination and integration of these parameters will allow development of a quantitative mathematical model for output prediction of auxin signaling network. Figure created using BioRender (https://biorender.com/).

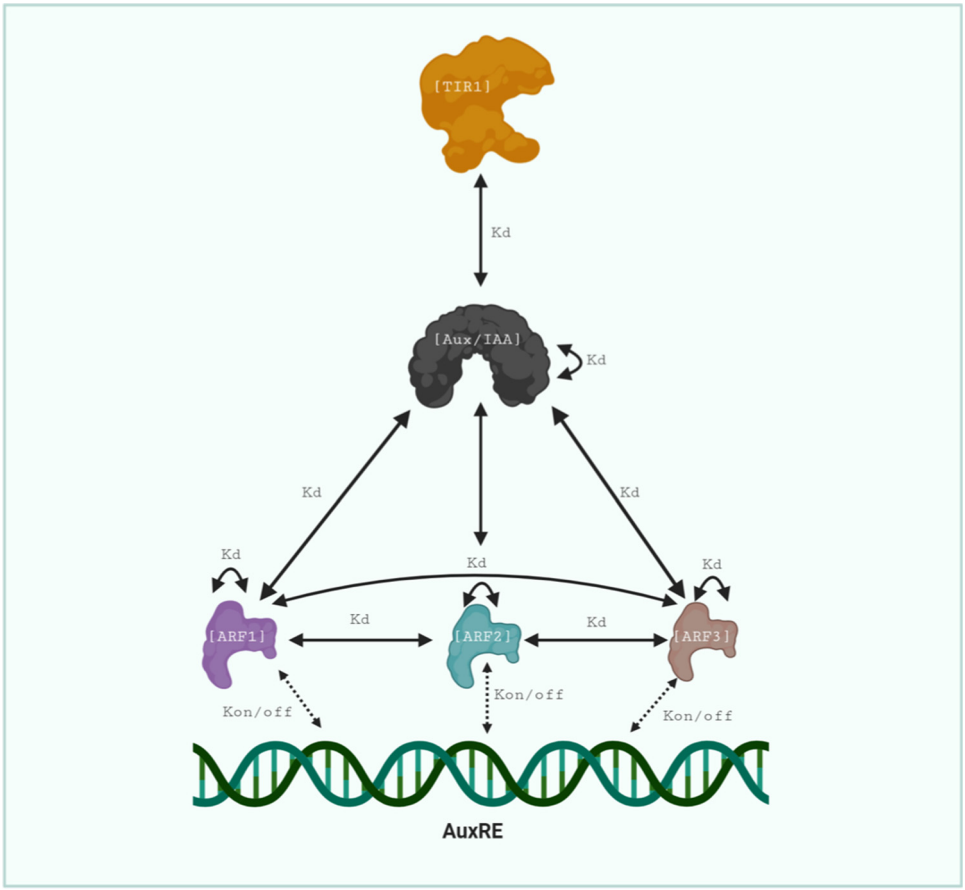

Figure 3. A Hypothetical Quantitative Model of the Auxin Response Protein Network in *Marchantia polymorpha*. Figure created using BioRender (https://biorender.com/). Abbreviations: ARF, AUXIN-RESPONSE FACTOR; Aux/IAA, Auxin/INDOLE-3-ACETIC ACID; AuxRE, auxin-responsive element; Kd, dissociation constant; TIR1/AFB, TRANSPORT INHIBITOR RESPONSE1/AUXIN-BINDING F-BOX.

genomics on this haploid model [35–41]. Therefore, Marchantia represents the epitome of a model system for both evolutionary and quantitative auxin research.

Protein Concentrations Define Response Output

A long-standing hypothesis on auxin signaling is the competition between class-A and B ARFs for binding similar **auxin-responsive elements** (AuxREs) [23,31,42]. Distinct intracellular ARF concentrations could rationalize this hypothesis because contrasting levels of class-A and B ARFs could dictate which ARF binds an AuxRE. Differing expression profiles of MpARFs in genomic knock-in translational fusion lines further endorse this notion [31]. Additionally, auxin sensitivity could be highly dependent on the concentration of TIR1, because low concentrations of this receptor could dampen the auxin response. At high auxin levels, the Aux/IAA concentration depletes and favors ARF dimerization. Therefore, the concentration of ARPs within the cell is a determinant of homo–heteromer or monomer–oligomer equilibria. However, determining native protein concentrations from a single plant cell is unattainable due to technical limitations, resulting in our sparse understanding of how response-protein abundance modulates auxin signaling. Utilizing the simplicity of Marchantia NAP, and if all ARPs are fluorescently labeled at endogenous locus, quantification of absolute protein concentrations would be possible with fluorescence

correlation spectroscopy (FCS) and raster image correlation spectroscopy (RICS) (Figure 2) [43], but these approaches remain challenging in plants. Furthermore, a key modulator of the native protein concentration is the **turnover rate** of a protein, which in turn can depend on post-translational modifications (PTMs) and its cooperative stability in a complex. Most NAP components are reported to undergo PTM [44,45] and oligomerization [46–49,59,60]. Hence, it is imperative to learn how native protein concentrations are affected by these modifications.

Binding Affinities Define the Response Network

The auxin-response network is defined by PPIs and protein–DNA interactions. For example, ARFs and Aux/IAAs have a shared C-terminal PB1 domain, which mediates their homo- and heterotypic interactions [15,16]. Crystallography studies on wild-type and mutated PB1 domains of AtARF5, AtARF7, and AtIAA17 revealed the presence of opposite electrostatic interfaces in this domain, which may facilitate oligomerization [46,47]. To understand the logic behind preferential binding and stable/transient complex formation by ARPs, it is essential to determine **dissociation constants** (Kds) of all biomolecular interactions. Quantification of Kds of purified proteins or isolated protein domains can be performed using biophysical methods, such as isothermal titration calorimetry (ITC), microscale thermophoresis (MST), surface plasmon resonance (SPR), single-molecule Förster resonance energy transfer (smFRET), and FCS (Figure 3).

In arabidopsis, binding affinities of different TIR1/AFB-Aux/IAA co-receptor members were determined using SPR [50]. The results showed that all AtAux/IAA degron peptides have rapid dissociation rates from AtAFB5 (K_{off} ranging from 1.1 to 1.9 × 10^{-2} s^{-1}) compared with AtTIR1 (K_{off} ranging from 3.3 to 5.8 × 10^{-3} s^{-1}) [50]. ITC experiments with PB1 domains of ARF and Aux/IAA showed that PB1 heterodimerization of ARF5-Aux/IAA17 has a higher affinity (Kd ~0.07 μM) than either ARF5 (Kd 0.2–0.9 μM) or Aux/IAA17 (Kd 6.5 μM) homodimerization, and values of ARF7 homodimerization support these differences [51–53]. However, it should be noted that these distinct affinities for homo- versus heterotypic PB1 interactions, derived from a few Aux/IAA and ARF proteins in arabidopsis, may not reflect the typical behavior of these protein families. Apart from the PB1 domain, the N-terminal **DNA-binding domain** (DBD) can also facilitate AtARF1 and AtARF5 dimerization [14]. Hence, the binding constant of full-length ARFs and Aux/IAAs is required to understand the significance of divergent interaction affinities.

In addition to variable PPI affinities, ARF–DNA affinity is another important regulatory module. ARFs can bind AuxRE motif as dimers [14,54]. Differential DNA-binding affinities among ARFs influence which ARF dimer forms and binds to the promoter to regulate transcription. Two well-known ARF-binding DNA motifs are TGTCTC [16,55] and TGTCGG [14], both known to be bound by class-A and class-B ARFs. However, there are experimental evidences that different ARF classes may have different preferences for AuxREs. For example, in genome-wide DAPseq experiments, class-A ARFs showed higher enrichment for the TGTCGG motif, whereas class-B ARFs were enriched for TGTCTC [42]. Additionally, ARFs may have nonoverlapping DNA-binding sites between different classes, further suggesting variable ARF–DNA binding preferences. Recent bioinformatics analysis of *cis* elements showed that auxin-induced genes may have other TGTCNN motifs in their promoter besides the two hexameric consensus motifs [56–58]. Identification of novel AuxRE elements and determination of ARF-AuxRE binding affinities could explain the basis for specificity of ARF-induced gene regulation. To determine ARF–AuxRE affinities, smFRET would be the method of choice because it can track binding and unbinding dynamics. Recently, smFRET analysis revealed that MpARF1-DBD (Kd ~12 nM) has higher AuxRE binding affinity than MpARF2-DBD (Kd ~61 nM), suggesting a competitive advantage for ARF1 [31]. A similar mode of binding affinities was found in smFRET with AtARF1 (class-B) and AtARF5 (class-A), where class-A AtARF5 binds to DR5 and IR8 motifs with higher affinity than class-B AtARF1 does [59].

These independent observations encourage a systematic survey of PPI/protein–DNA interaction analysis to understand auxin response. Due to the lack of adaptable *in vivo* Kd determination tools, most studies have used *in vitro* methods, as discussed earlier. These *in vitro* Kd values are good starting points, but it is important to note that *in vivo* Kd values can be affected by PTMs, the presence of binding competitor, and intracellular crowding among others and, thus, could be different to known *in vitro* Kd values.

Regulation of NAP by Dynamic Stoichiometry of ARP Complexes

In auxin signaling, most protein interactions are transient, context specific and restricted to specific tissues, cell types, or even organelles [60]. Therefore, imaging-based PPI assays offering dynamic analysis can illuminate these interactions. Recently, the interaction between overexpressed MpTOPLESS (MpTPL) and MpARF1 andMpARF2 was tested in arabidopsis protoplasts using FRET quantified by **fluorescence lifetime** microscopy (FLIM). MpTPL and MpARF1 did not interact, whereas a clear interaction between MpTPL and MpARF2 was observed. Interestingly, the interaction was abrogated in the MpARF2 **LFG motif** mutant, indicating a role for this motif in the MpTPL–MpARF2 association [31]. Future FRET-FLIM studies with natively expressed ARPs would allow spatial resolution of protein interactions and enrichment of quantitative models for tissue-specific output prediction.

Although many protein complexes within the core auxin-response network are known, the stoichiometry of those complexes remains unclear. Crystal structure analysis of the N terminus of AtTPL suggested that it forms tetramers. This observation was corroborated by a protoplast-based repression assay where impaired TPL tetramerization by mutation showed higher **Direct Repeat 5 (DR5)** reporter activity [48]. However, the functional significance of TPL oligomerization is still unknown. Likewise, crystallography of Aux/IAA proteins [46,47] and bimolecular fluorescence complementation (BiFC) and Y2H assays of TIR1 [49] suggest possible oligomerization of these ARPs. Having multiple ARPs that can oligomerize raises the questions of what the actual stoichiometry of ARPs is in a transcriptional regulatory complex and how important these oligomerizations are in auxin output. Therefore, dissection of protein complexes with all constituting proteins expressed at endogenous level is essential. An emerging mechanism of ARF complex formation is their condensate formation by **liquid-liquid phase separation (LLPS)**. Arabidopsis ARF7 and ARF19 have been shown to phase separate in the cytoplasm of differentiated root cells as a partitioning mechanism to diminish auxin response [61]. Whether condensate formation is a conserved phenomenon among MpARFs would be the next question to address. Nuclear condensate formation of ARPs could regulate transcriptional complex stoichiometry and act as a transcriptional hub of auxin-response genes.

Protein Diffusion as a Parameter

Subcellular protein abundance, PPI/protein–DNA interactions are limited by protein diffusion rates. Transcription factors (TFs), such as ARFs, may diffuse freely within the nucleus in a 3D motion or be facilitated by the electrostatic attraction between DNA and TFs. The facilitated form of diffusion includes 1D sliding motion along the DNA length, and the intersegmental transfer and hopping of TFs [62,63]. Due to slow 3D diffusion, TFs obtain access to the entire genome, whereas facilitated diffusion allows TFs to rapidly switch between genes during a quick transcriptional response. Another important purpose served by random 3D diffusion is the continuous collision between various proteins, thereby allowing them to find interaction partners and form protein complexes. Calculating diffusion dynamics of ARPs can provide better insights into their oligomerization status, ARF–DNA binding dynamics, and probability of interaction. Relatively fast or slow diffusion rates of ARPs could indicate whether it is part of a large protein complex or a free monomer. Based on variable diffusion rates of ARFs, one could differentiate DNA-bound and

freely diffusing ARFs. However, the intracellular environment is nonhomogeneous and, therefore, the final diffusion rates of a protein can differ based on its subcellular location, the temperature, molecular weight, concentration, and so on. **Diffusion coefficients** can be measured *in vivo* using fluorescence recovery after photobleaching (FRAP) and FCS/fluorescence cross-correlation spectroscopy (FCCS), while RICS and pair correlation function (pCF) enable the path of movement/diffusion of a labeled molecule to be tracked. In contrast to measuring protein concentrations by FCS (see earlier), the use of RICS to determine diffusion coefficientssuffer less from autofluorescence and, therefore, is a promising approach.

Concluding Remarks

For almost a century, plant biologists have been intrigued by the incredible functional diversity of the small auxin molecule. Even though extensive qualitative research has been conducted, the entire operational complexity of the NAP is still a conundrum. Recent genome analysis and phylogenetic studies have indicated the presence of a simple auxin signaling network in marchantia, which has opened opportunities to explore auxin signaling from a quantitative perspective.

The use of genetic and biochemical approaches has established a substantial qualitative background to advance towards quantitative investigations of auxin signaling. Semiquantitatively visualizing the auxin response has become possible with the development of fluorescent reporters for auxin activity, such as DR5, **DII-Venus**, **DR5v2**, and **ratiometric version of 2 D2s** (R2D2) [64,65]. These reporters revealed differences in auxin sensitivity and transcriptional responses across plant tissues. Simultaneous advancements in hardware technologies and implementation of functional imaging modalities have promoted quantitative analysis in auxin biology.

Determination of PPI/protein–DNA interaction affinities will explain how structural features of these proteins contribute to a dynamic and specific auxin response. Estimation of *in vivo* ARF concentrations would clarify how competition for the same DNA motif binding between different ARFs is regulated. Protein turnover and diffusion rates will reflect on how local concentrations of response proteins are maintained within a cell. ARF protein complex stoichiometry analysis will help determine the actual constituents of auxin-induced transcriptional activator or repressor complexes. Beyond these properties, it will also be critical to determine how PTMs of NAP components influence activity and response output. The various microscopy tools mentioned earlier will help achieving these goals. To deal with the multitude of quantitative data generated, interdisciplinary collaborations will be required that will lead to an integrative mathematical auxin-response model. Mathematical models will help us answer biological questions that are difficult to address experimentally. Over the past decade, several mathematical models have been developed to understand aspects of auxin and other plant hormone signaling [66,67]. Such models can describe various scales, from the molecular to the cellular and tissue levels. Each allows different questions to be addressed and requires a different level of detail and granulation. The simplicity of Marchantia anatomy could bring us closer to integrating these scales in multilevel models. Since auxin signaling is wired tightly with other plant hormone signaling pathways, a complete mathematical model of the auxin system grounded in measurement of most (or all) possible parameters may also be a starting point to address how the auxin system connects to other hormone signaling pathways. Finally, in the foreseeable future, the challenge will be to develop quantitative models for other plant hormone signaling pathways and their amalgamation into one unified model of plant development (see Outstanding Questions).

Outstanding Questions

How different are MpARF turnover rates, and do variable turnover rates add specificity to the NAP?

Do developmental and environmental inputs modulate A/B-ARF stoichiometry to regulate gene expression?

How many ARF and Aux/IAA proteins constitute a transcriptional regulatory complex?

What is the biological significance of A/B-ARF heteromerization?

If and how do PTMs, oligomerization, and LLPS of ARPs control response output?

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Declaration of Interests

The authors have no interests to declare.

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