Trends in Plant Science

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Opinion

A Glimpse beyond Structures in Auxin-Dependent Transcription

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Auxin response factors (ARFs), transcription factors (TFs), and their Aux/IAA (IAA) repressors are central components of the auxin signalling pathway. They interact as homo- and heteromultimers. The structure of their interacting domains revealed a PB1 fold mediating electrostatic interactions through positive and negative faces. Detailed structural analysis revealed additional hydrophobic and polar determinants and started unveiling an ARF/IAA interaction code. Structural progress also shed new light on the DNA binding mode of ARFs showing how they dimerize to bind repeated DNA elements. Here, we discuss the *in vitro* and *in vivo* significance of these structural properties for the ARF family of TFs and identify some critical missing information on how specificity might be achieved in the auxin signalling pathway.

Trends

The conserved domain of the ARF and Aux/IAA formerly known as domain III/IV is a PB1 domain and should be renamed accordingly.

Structures of PB1 domains unveil possible rules for interaction specificity between ARF and Aux/IAA proteins.

Structures of the ARF DNA-binding domain (DBD) reveal domain organisation and a dimerization interface that might drive DNA binding specificity, although *in vivo* tests are still required.

Genome-wide identification of ARF-binding sites is crucially needed to understand how specificity is achieved in auxin-induced transcription.

ARF and IAA Proteins Are Key Players in Auxin-Mediated Transcription

The plant hormone auxin is a morphogenetic regulator key to plant development from embryogenesis onwards. Two types of regulators encoded by multigene families mediate transcriptional responses to auxin: the **auxin response factor** (ARF) (see Glossary) transcription factors, and the Aux/IAA (**IAA**) transcriptional repressors. IAA repressors associate with ARFs through the domain III/IV, a protein–protein interaction domain conserved in both protein families (reviewed in [1]), and recruit the transcriptional co-repressors of the TOPLESS family [2] to prevent the expression of auxin-inducible genes (reviewed in [3]). Auxin, perceived directly by receptor complexes comprising a TIR1/AFB F-box co-receptor and an IAA, signals by triggering IAA ubiquitination and subsequent degradation [4–9]. ARFs can then regulate auxin-responsive genes. In higher plants such as arabidopsis (*Arabidopsis thaliana*), ARFs can either activate (ARF activator) or repress (ARF repressor) transcription (reviewed in [1,3,8]). Thus, protein–protein interactions are at the heart of transcriptional regulation downstream of auxin. Until recently, little was known at the structural level regarding ARF and IAA regulators. A flurry of publications now illuminates both the structural mechanism of ARF interaction with IAAs and ARF dimerization through their DNA-binding domain (DBD) [10–15]. These results were recently reviewed and thoroughly discussed in various publications [3,16–19]. Here, we summarise recent advances in our structural understanding of auxin-regulated transcription and speculate on how these findings change our view and raise new questions on protein–protein and protein–DNA interactions in auxin-mediated transcription. Throughout this opinion article, we use the Ps prefix for *Pisum sativum* proteins and no prefix for arabidopsis proteins.

ARF/IAA PB1 Domains: Comparison and Prospects

Within a year, structures of four III/IV domains from ARF or IAA proteins have been published (Table 1). They all revealed a PB1 (Phox and Bem1) fold as already guessed from primary

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Table 1. Details of Structures Used in this Review[a]

Protein	Domain	Wt or Mutant	Species	Technique	Accession	Refs
ARF7	PB1	Mutant	*Arabidopsis thaliana*	RX	4NJ6, 4NJ7	[10]
ARF5	PB1	Wt	*A. thaliana*	RX	4CHK	[14]
IAA17	PB1	Mutant	*A. thaliana*	NMR	2MUK	[13]
PsIAA4	PB1	Wt	*Pisum sativum*	NMR	2M1M	[12]
ARF1	DBD	Wt	*A. thaliana*	RX	4LDV, 4LDW	[15]
ARF5	DBD	Wt	*A. thaliana*	RX	4LDU	[15]
ARF1	DBD+DNA	Wt	*A. thaliana*	RX	4LDX	[15]

[a]Abbreviations: AuxRE, auxin response element; ARF, auxin response factor; DBD, DNA-binding domain; DD, dimerization domain; ER, everted repeat; FD, flanking domain; IAA, Aux/IAA protein; IR, inverted repeat; RX, X-ray crystallography; NMR, nuclear magnetic resonance; PB1, Phox and Bem1; TF, transcription factor; Wt, wild type.

Glossary

Auxin response factor (ARF): ARFs are transcription factors that bind to auxin response elements in promoters of early auxin response genes.

Direct repeat (DR): *cis*-element recognised by an ARF dimer where two ARF-binding sites are arranged as direct repeat.

Everted repeat (ER): *cis*-element recognised by an ARF dimer where two ARF-binding sites are arranged as everted repeat.

IAA: this term designates Aux/IAA proteins that bind to ARF activators and prevents them from activating auxin-responsive genes.

sequence analysis [20] and we propose this conserved domain should be accordingly renamed the PB1 domain. PB1 domains contain approximately 80 residues, they mediate homo- or hetero-association and are found in animals, fungi, amoebas, and plants (reviewed in [21]). ARF and IAA PB1 domains can be classified as type I/II [21] as they all display a negatively charged face (hereafter named negative face) electrostatically interacting with a positively charged one (named positive face), allowing them to form head-to-tail homo-oligomers. Such a property often leads to recombinant protein oligomerization and aggregation, a problem overcome by weakening their oligomerization: key amino acids of the dimerization interface of ARF7 or IAA17 were mutated and PsIAA4 was manipulated at low pH to reduce the charges of its negative surface (Table 1) [10,12,13]. Only the crystallographic structure of ARF5 was obtained at physiological pH with a wild-type PB1 domain [14], thereby allowing direct observation of an intact PB1 oligomerization interface.

Despite these technical differences, all four structures revealed a very similar interface involving assembly through a positive and a negative face, a feature confirmed using size exclusion chromatography, pull-down experiments, yeast two-hybrid, isothermal titration calorimetry (ITC) and NMR in homotypic (ARF5/ARF5, ARF7/ARF7, PsIAA4/PsIAA4, IAA12/IAA12, and IAA17/IAA17), and heterotypic (ARF5/IAA12, ARF7/IAA17, ARF5/IAA17) interactions [10,12–14] (Figure 1).

Still, previous studies on the ARF and IAA families [22–35] indicated that they do not all interact with each other. These analyses are mostly qualitative and some limited inconsistency exists in available data (discussed in [16]). Nevertheless, the most extensive interaction study in arabidopsis [22] strongly suggested that most of the 29 Aux/IAA (IAA) proteins form complexes with the five ARF activator (ARF5–8,19) PB1 domains. By contrast, despite also bearing a PB1, ARF repressors rarely interact with IAAs. These data were recently complemented by quantitative measurements. An elegant study, using ITC, showed that interaction between ARF5 and IAA17 (equilibrium dissociation constant K_d = 0.07 μM) is stronger than those between two ARF5 (K_d = 0.87 μM) and those between two IAA17 (K_d = 6.6 μM) [12]. Those measurements have an important functional implication: IAA proteins could be stored as a homopolymer in the cell (the 'signalosome' hypothesis as suggested for other oligomeric proteins [36]) and, when ARFs are present, ARF/IAA complexes would preferentially assemble (because this interaction has a lower K_d than ARF/ARF or IAA/IAA interactions) and inhibit ARF activity at the expense of the formation of ARF and IAA homo-oligomers.

Understanding these large affinity differences among PB1 domains and being able to predict interaction specificities from mere examination of protein sequences is a major stake in the auxin