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Feature Review

Structural Biology of Nuclear Auxin Action

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Auxin coordinates plant development largely via hierarchical control of gene expression. During the past decades, the study of early auxin genes paired with the power of *Arabidopsis* genetics have unraveled key nuclear components and molecular interactions that perceive the hormone and activate primary response genes. Recent research in the realm of structural biology allowed unprecedented insight into: (i) the recognition of auxin-responsive DNA elements by auxin transcription factors; (ii) the inactivation of those auxin response factors by early auxin-inducible repressors; and (iii) the activation of target genes by auxin-triggered repressor degradation. The biophysical studies reviewed here provide an impetus for elucidating the molecular determinants of the intricate interactions between core components of the nuclear auxin response module.

Auxin Sensing in the Nucleus

Indole-3-acetic acid (IAA), the principal auxin in the embryophytes, plays a critical role in orchestrating plant development and adaptive growth in response to environmental cues [1,2]. The small, tryptophan-related molecule coordinates the myriad of underlying processes largely via hierarchical control of nuclear gene expression [3,4]. The auxin signaling pathway is surprisingly short and direct as suggested by the rapid kinetics of primary gene activation [5]. After cellular uptake and diffusion into the nucleus [6], the hormone binds to and thereby rearranges core components of the auxin sensing apparatus, which immediately triggers the activation of early response genes by derepression [4]. The combination of plant molecular biology and *Arabidopsis* genetics identified the major components of the core auxin response circuit and biochemical studies uncovered the mode of nuclear auxin action [3,7]. During the past few years, biophysical approaches have begun to unravel on the atomic scale the structural basis of auxin perception and the manifold interactions determining specificity in auxin signaling [8]. In this review we focus on recent studies employing structural biology to understand the molecular logistics of auxin signal transduction [9–17].

The Core Auxin Response Module

When auxin levels are low, members of the AUXIN/IAA-INDUCIBLE (AUX/IAA) family of transcriptional repressors interact with DNA-binding proteins of the AUXIN RESPONSE FACTOR (ARF) family [18,19], which specifically occupy auxin-responsive promoter elements (*AuxRE*s) in numerous auxin-regulated genes [20] (Figure 1, Key Figure). AUX/IAA proteins repress ARF function either passively by sequestering ARF proteins away from their target promoters [21] or actively by recruiting TOPLESS (TPL)/TPL-RELATED (TPR) corepressors, which leads to chromatin inactivation and silencing of ARF target genes [22–25]. A rise in nuclear auxin concentration is registered by auxin-promoted assembly of coreceptor complexes that comprise an F-box protein from the TRANSPORT INHIBITOR RESPONSE 1 (TIR1)/AUXIN SIGNALING F-BOX PROTEIN (AFB) family and an AUX/IAA member [9,26,27]. TIR1/AFBs are specificity-lending subunits of nuclear S-PHASE KINASE ASSOCIATED PROTEIN 1-CULLIN-F-BOX PROTEIN

Trends

Auxin governs plant development via gene expression controlled by a few nuclear components. Auxin-promoted assembly of $SCF^{TIR1/AFB}$ complexes recruits AUX/IAA repressors for proteolysis, causing derepression of ARF activators and gene induction via auxin-responsive DNA elements.

Structural biology has gained enormous momentum and the first high-resolution models allow unparalleled insights into DNA recognition by ARFs, into interaction modes of AUX/IAAs with ARFs and corepressors, and into auxin-triggered AUX/IAA degradation.

It has increasingly becoming clear that the key players are embedded in complex molecular networks of post-translational modifications and interactions with various ligands that provide multiple nodes for signal integration and response specification.

The biophysical approach will firmly expand and refine our understanding of the auxin response pathway beyond the nuclear core module, which will be tested by synthetic biological studies *in planta* and in heterologous *in vivo* systems.

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(SCF)-type E3 ubiquitin-protein ligases ($SCF^{TIR1/AFB}$) and mediate substrate recognition. Formation of ternary TIR1/AFB:auxin:AUX/IAA coreceptor complexes sequesters AUX/IAAs for polyubiquitylation and subsequent 26S proteasome-dependent degradation [9,28]. Thus, rapid auxin-stimulated proteolysis of AUX/IAA repressors links auxin perception to the control of nuclear gene expression and represents the pivot of auxin signaling. In the simplest scenario, auxin-initiated AUX/IAA removal relieves ARF repression and activates the transcription of primary genes. Remarkably, such a minimal auxin response circuit, comprising a member of each of the four protein families, is sufficient to reconstitute *AuxRE*-dependent activation of reporter genes in yeast [29].

Variations on the Main Theme Specify the Numerous Auxin Responses

Diversification of the auxin sensing machinery is believed to specify the multitude of responses to the hormone. The core parts of the auxin response apparatus are encoded by six *TIR1/AFB*, 29 *AUX/IAA*, 23 *ARF*, and five *TPL/TPR* genes in *Arabidopsis thaliana* [27]. For each family, the developmental regulation of cell type-specific mRNA expression at multiple levels, the cellular control of protein abundance and activity, and the functional diversification of protein domains provide a vast repertoire for combinatorial interactions between the core components [30–32]. The imaginable complexity is likely to be necessary for appropriate interpretation of the context-specific information of auxin distribution profiles in a field of cells, which may range from steep maxima to distinct minima [33,34]. Such complex auxin gradients are often modified by internal and external cues and have been implicated in the nonlinear regulation of numerous auxin-mediated processes relevant to the adaptation of plant form and function. Differential expression of *AUX/IAA* multigene family members seems to be significant for tuning auxin responses because AUX/IAAs notably determine the affinities of the coreceptor pairs for auxin and its structural analogs [26,35]. A broad range of auxin concentration can be differentially sensed by the numerous TIR1/AFB:AUX/IAA coreceptor combinations, which results in different AUX/IAA degradation rates [26,35–37]. The AUX/IAA repressors engage in sophisticated AUX/IAA:ARF interaction networks and are often products of early auxin genes, which establish robust negative feedback loops [38]. Finally, ARF-dependent selection of downstream target genes is thought to confer specificity on the countless auxin responses [20].

Recognition of Auxin-Responsive DNA Elements

Structure and Composition of AuxREs

Auxin rapidly induces (2–30 min) primary response genes of three families known as *AUX/IAA*s, *GH3*s, and *SAUR*s [5]. Select members of each family were established as experimental models to study their function and transcriptional regulation by auxin [5,39]. Refined *GH3* promoter deletion and linker scanning analyses identified the canonical TGTCTC-type *AuxRE* found in many early auxin genes. However, the core hexamer TGTCTC motif confers auxin responsiveness only when at least duplicated (direct, inverted, or everted repeats) or coupled to a second, different promoter element in an overlapping or disjointed arrangement (composite *AuxRE*) [40–42]. A comparison of several transcript profiling studies revealed that the early response to auxin (<30 min) comprises mostly upregulated mRNAs [43,44]. Computational analyses of the genome-wide distribution of TGTCTC-type *AuxRE*s showed a strong association with the transcriptional start sites or proximal promoter regions of auxin-induced genes and recognized the presence of several coupling elements to form composite *AuxRE*s, including additional TGTCTC-type elements or the binding sites of bZIP and MYB transcription factors [45–47].

Interaction of ARF Proteins and AuxREs

Using multiple tandem copies of inverted TGTCTC repeats as a bait, the founding member of the *Arabidopsis* ARF family, ARF1, was selected in a yeast one-hybrid screen and shown to bind *in vitro* to distinctly spaced palindromic TGTCTC motifs (e.g., the ER7 element) [48]. Most ARF proteins contain three separable regions of specific functions: the conserved N-terminal

Key Figure

Model of the Nuclear Auxin Response Core Module Including Currently Available Macromolecular Structures

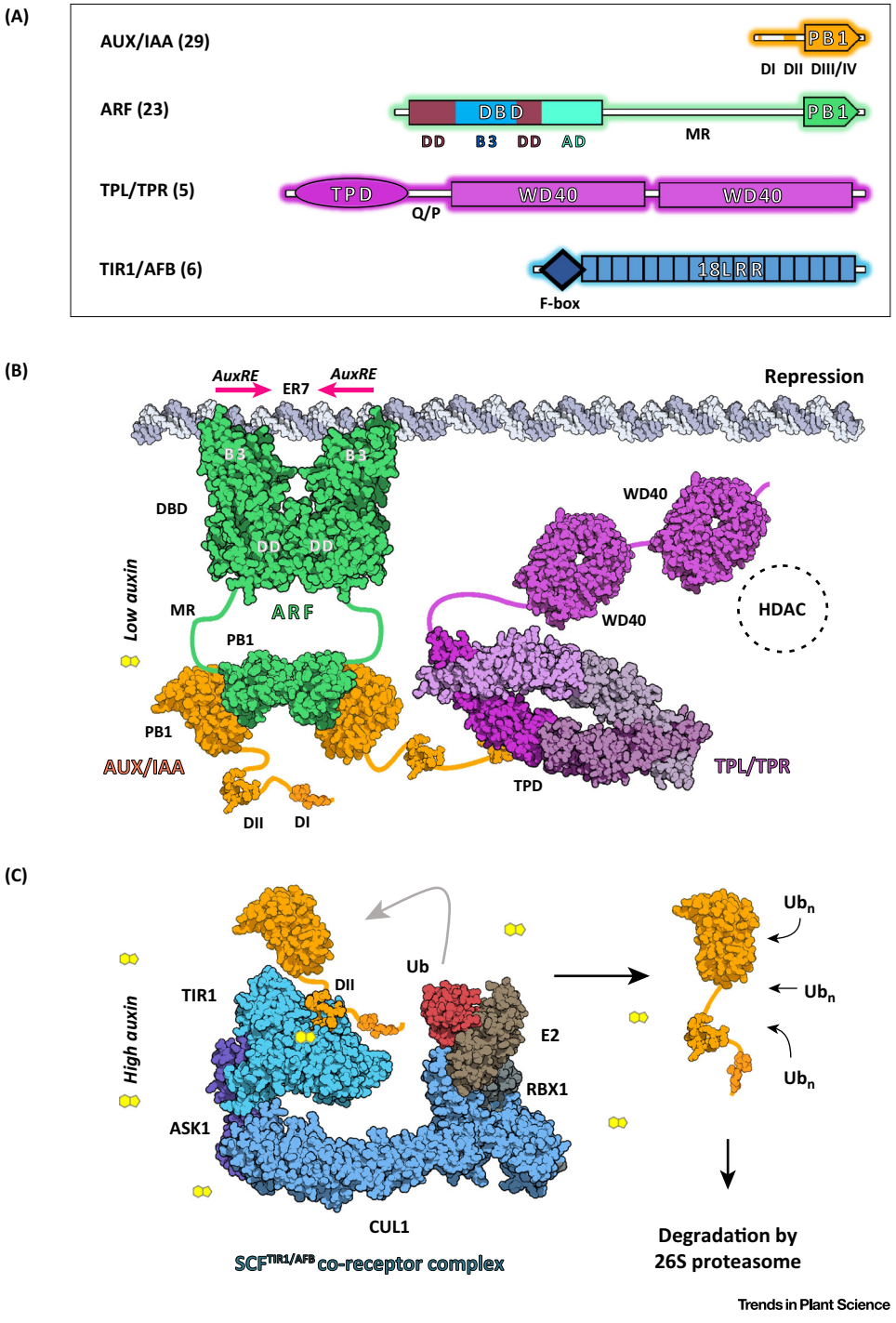


Figure 1. (A) Consensus domain structure of AUX/IAA repressors, ARF activators/repressors, TPL/TPR corepressors, and TIR1/AFB F-box proteins. Depicted are the four conserved domains I–IV (DI–DIV) of AUX/IAAs, which share the C-terminal

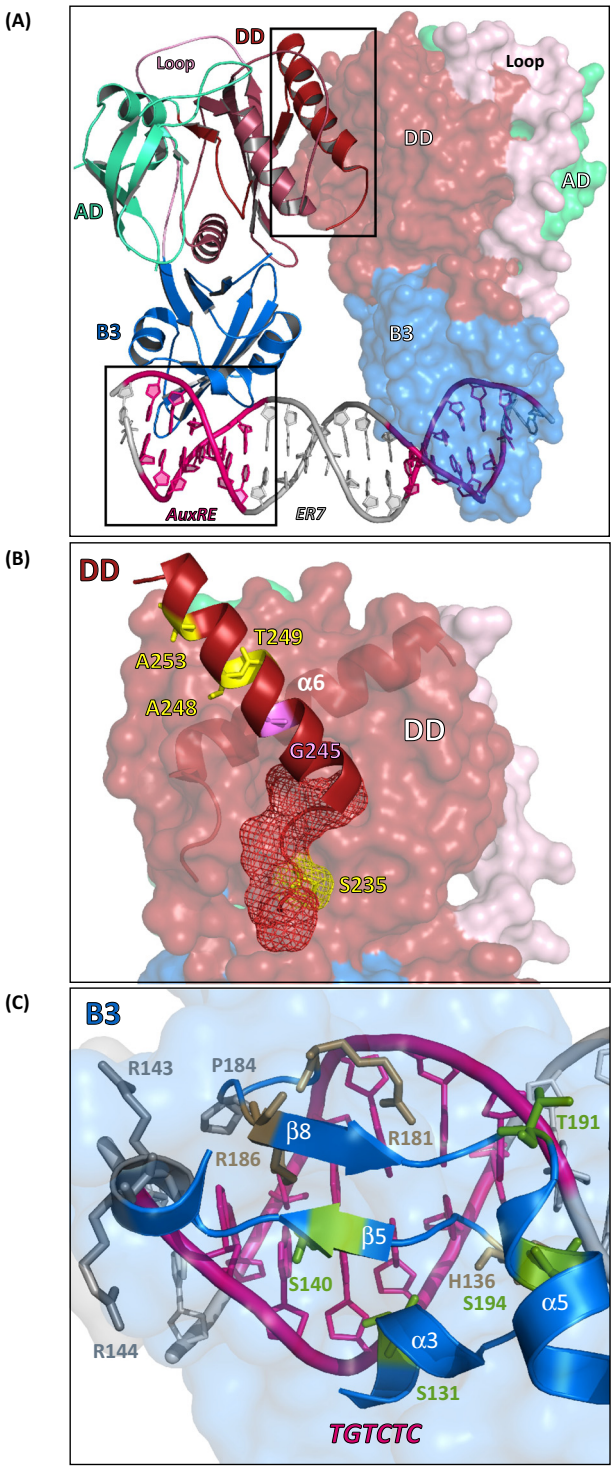
DNA-binding domain (DBD), the variable middle region (MR) of biased amino acid composition determining either ARF activator function (Q-rich as in ARF5-8 and ARF19) or ARF repressor activity (S, P, L/G-rich), and the C-terminal protein–protein interaction domain shared between the ARF and AUX/IAA families [20,49] (Figure 1A). Early work showed that all of the ARFs tested bound with specificity on palindromic *AuxRE*s; however, robust DNA recognition requires ARF dimerization and the first four nucleotides of the TGTCTC motif [50,51]. The core DBD region of the 22 full-length ARFs is related to the plant-specific B3 domain [52], but additional flanking residues are necessary for efficient *AuxRE* binding [50].

The high-resolution DBD crystal structures of the ARF5 activator and the phylogenetically distant ARF1 repressor were recently reported, which allowed unprecedented insight into the mechanism of ARF:DNA interaction [10] (Figure 2). Both ARF DBDs fold into three distinct subdomains. The regions at both flanks of the B3-type subdomain, which adopts a seven-stranded open β-barrel structure, fold into a second, structurally novel subdomain (DD) that forms a highly curved, 'taco'-like five-stranded β sheet. A distinct surface of the DD subdomain facilitates robust ARF dimerization via a network of hydrogen bonds and hydrophobic interactions (Figure 2B). The C-terminal residues of each ARF DBD form a third, ancillary subdomain (AD) of unknown function that is related to the Tudor domain (a five-stranded β-barrel-like structure) and interacts tightly with the DD subdomain [10]. It was noted that the plant B3 domain is similar to the DNA-contacting domain of bacterial endonucleases, classified as a DNA-binding pseudobarrel [53], which is likely to have been inserted into a bromodomain/WD40 repeat protein during ARF evolution [10,49]. A series of biophysical studies and genetic complementation tests provided solid evidence that the ARF DBD also dimerizes in solution and is sufficient for *in vivo* dimerization of full-length ARF5 independent of its C-terminal protein interaction domain and that DBD dimerization is essential for ARF5 function *in planta*. Interestingly, the residues at the dimerization interface of the DBD are highly conserved between ARFs, which suggests that homodimerization and perhaps heterodimerization is a general property of ARF DBDs [10] (see Outstanding Questions).

ARF 'Molecular Calipers' Gauge Composite AuxREs

The co-crystal structure of the ARF1 DBD bound to the palindromic ER7 *AuxRE* revealed a U-shaped DBD dimer [10] (Figure 2A). The two B3-type subdomains are positioned at the tips of

PB1 domain with ARF proteins (DIII/IV). A variable middle region (MR) separates the ARF PB1 domain and the N-terminal DNA-binding domain (DBD), which comprises a plant-specific B3-type (B3), a dimerization (DD), and an ancillary (AD) subdomain. TPL/TPR proteins share an N-terminal tetramerization domain, named the TOPLESS domain (TPD), which is separated by a glutamine- and proline-rich spacer from C-terminal WD40 repeat β-propeller domains. TIR1/AFB proteins possess the F-box domain followed by 18 leucine-rich repeat (LRR) motifs. (B) Under low-auxin conditions, transcription of primary auxin genes is actively repressed by inactivation of promoter-bound ARF activators. In the composite model, the ARF5 dimer (green) binds two everted TGTCTC motifs (*AuxRE*) separated by 7 bp (ER7 oligonucleotide) via its B3-type subdomains [10]. Dimerization of the N-terminal ARF5 DBD is mediated by the DD subdomain and the C-terminal ARF5 PB1 domain may further stabilize ARF5 dimerization [11]. The ARF PB1 domain is also thought to mediate ARF oligomerization (not shown) by directional interactions and ARF heteromerization with AUX/IAA repressors (orange) via the AUX/IAA PB1 domain (here shown for IAA17 [13]). The structure of the ARF5 MR remains to be determined (green line). The AUX/IAA model also shows the fold of the IAA7 degron peptide (DII) [9] and the extended conformation of the IAA1 EAR motif (DI) [17]. The structures of the connecting loops are unknown (orange lines). The EAR motif interacts with TPL/TPR corepressors via their N-terminal TPD (here shown for OsTPR2 [17]), which tetramerizes (shades of purple). Thus, four AUX/IAA EAR motifs may cooperate in the recruitment of TPL/TPR corepressor tetramers (note that only one OsTPR2 monomer is depicted; see also Figure 3B). The C-terminal WD40 repeat domains of TPL/TPR corepressors sequester chromatin-modifying enzymes such as the histone deacetylase complex (HDAC, broken circle), leading to transcriptional repression. (C) Under high-auxin conditions (yellow small molecule), ARF derepression and transcriptional activation is triggered by sequestering AUX/IAAs (orange) via their degron peptide (DII) to the SCF^{TIR1} E3 ubiquitin ligase complex, which comprises the TIR1 F-box protein (marine blue), the ASK1 adapter (dark blue),the CUL1 scaffold (light blue), the RING finger protein RBX1 (grey). The SCF^{TIR1} complex interacts with an E2 ubiquitin-conjugating enzyme (brown), which presents an activated ubiquitin (Ub, red). Polyubiquitylation (Ub_n) of AUX/IAAs leads to subsequent degradation via the 26S proteasome. The structural models were generated using the following PDB ID entries: ARF5 DBD dimer (4LDU), ARF5 PB1 (4CHK), IAA17 PB1 (2MUK), OsTPR2 TPD:IAA1 DI (5C7F), yeast WD40 (1ERJ), ASK1-TIR1:auxin:IAA7 DII degron (2P1Q), human CUL1–RBX1 (1LDJ), and human E2 ubiquitin-conjugating enzyme (4MDK).



Trends in Plant Science

Figure 2. The 'Molecular Caliper' Concept for Cooperative Recognition of Composite *AuxRE*s by ARF DBD Dimers. (A) The co-crystal structure of the ARF1 DBD:ER7 complex (PDB ID: 4LDX). The two everted TGTCTC motifs of the ER7 oligonucleotide are highlighted in pink and its 7-bp spacer in grey. The two ARF1 DBD monomers are shown as cartoon (left) and surface (right) presentations. The three subdomains of the DNA-binding domain (DBD) are colored as follows: B3-type (B3), blue; dimerization (DD), dark red; ancillary (AD), green; and variable loop connecting B3 and DD, pale pink. The two boxes frame the protein (DD):protein (DD) and protein (B3): DNA (ER7) interaction interfaces, which are enlarged in (B) and (C), respectively. The flexible loop varies among ARF members and is thought to generate various 'molecular calipers' that recognize *AuxRE*s of differently spaced TGTCTC elements. (B) Molecular interactions at the ARF1 DBD dimerization interface. The DD helix 6 (α6) of each monomer is juxtaposed and centered at a conserved glycine residue (G245). Flanking residues at the same face of the helix (A248, T249, A253) make hydrophobic contacts whereas the preceding short loop with a serine residue (S235) at its center fits into a groove of the opposite monomer [10]. (C) Molecular interactions at the ARF1 DBD:DNA interface. The ARF1 B3 subdomain recognizes a single TGTCTC element of the ER7 *AuxRE* mainly at the major groove via nucleobase contacts (olive: R181, R186, H136), backbone interactions (green: S131, S140, T191, S194), and other conserved DNA-interacting residues (grey: P184, R143, R144), which are supported by two adjacent β strands (β5 and β8), two helices (α3 and α5), and their connecting loops [10].

the U stems and contact the inverted TGTCTC elements at both ends of the double-stranded ER7 oligonucleotide. Flexible loops connect the B3-type and DD subdomains of the DBD dimer. This architecture explains the preferential binding of ARF1 to palindromic *AuxRE*s with distinct spacing and the proposed cooperativity of ARF1:DNA interaction [48,50,51], which was confirmed by binding assays using mutated *AuxRE*s and dimerization-defective ARF DBD variants [10]. Ten residues of the B3-type subdomain, positioned on two adjacent β strands, two helices, and their connecting loops, bind to the major-groove TGTCTC motif and were validated by biophysical and genetic experiments (Figure 2C). Interestingly, the DNA-binding residues are also almost completely conserved within the ARF family. In agreement, an unbiased interrogation of the hexamer sequence space by protein binding microarrays indicated that both ARF1 and ARF5 prefer the same *AuxRE* motif (the TGTCGG high-affinity site in addition to the prototype TGTCTC element) although the two proteins have different biochemical and biological functions [10,20,54]. While the critical DNA-contacting and dimerization-mediating residues are highly conserved in both ARF DBD subdomains and within the entire ARF family, the largest amino acid sequence variation is found in the B3–DD connecting loops. This observation prompted the authors to test whether ARF DBD dimerization provides a means to recognize palindromic *AuxRE*s of uniquely spaced TGTCTC-type elements. The ARF1 and ARF5 DBD homodimers discriminate between palindromic *AuxRE*s of different spacer lengths because of higher ARF5 intradomain DBD flexibility. Thus, the various possible ARF dimers were hypothesized to provide an assortment of "molecular calipers" to measure and recognize uniquely spaced, palindromic TGTCTC-type *AuxRE* elements [10] (see Outstanding Questions). Multiple composite *AuxRE*s are often found in ARF target genes, including many members of the *AUX/IAA* family [3,38,39]. A computational study of datasets derived from mutational promoter analyses of various early auxin genes predicts a highly multiplicative mode of *AuxRE* cooperation during gene repression in the absence of auxin [55]. This prediction is consistent with ARF occupation of TGTCTC-type *AuxRE*s regardless of cellular auxin status and with active repression of ARF activators by AUX/IAA proteins, as suggested previously [51,56].

Control of ARF Activators by AUX/IAA Interactions

Domain Organization of AUX/IAA Repressors

Members of the ARF and AUX/IAA families interact directly via their homologous C-terminal regions [18,19]. The importance of ARF:AUX/IAA interaction for ARF repression was demonstrated in transfection assays with ARF proteins lacking the C-terminal interaction domain, which result in constitutive high auxin responses [49,57]. The primary structures of most AUX/IAAs share four regions of sequence conservation known as domains I–IV (DI–IV) [30] (Figure 1A). The N-terminal DI recruits TPL/TPR corepressors to target promoters [23,58,59], the degron motif of central DII interacts with TIR1/AFBs and is required for auxin-dependent coreceptor assembly [4,9], and the C-terminal DIII/IV mediate homotypic as well as heterotypic interactions within and between the AUX/IAA and ARF families [18,19]. While the N-terminal region of most AUX/IAAs (comprising DI and DII) is predicted to be in large part intrinsically disordered, a bioinformatics analysis of the C-terminal DIII/IV of both ARF and AUX/IAA family members indicated a single protein–protein interaction domain [60]. This domain is related in secondary structure to the more ancient Phox/Bem1p (PB1) domain and in tertiary structure to the globular ubiquitin-like β-grasp fold [60,61]. Canonical PB1 domains comprise two helices and a mixed five-stranded β sheet and are classified into three types depending on the conservation and presence of oppositely charged and oriented surface patches [62]. PB1 domains may expose a conserved acidic cluster (D-X-D/E-X-D-X_n-D) known as the octicosapeptide repeat, p40phox, Cdc24p, atypical PKC interaction domain (OPCA) motif (type I), a basic surface patch with an invariant lysine residue as its hallmark (type II) or both characteristic structural features (type I/II). Electrostatic interactions between the two different faces drive specific PB1 dimer formation between type I and type II PB1 domains or multimerization by directional front-to-back association of type I/II PB1 monomers [62,63].

The PB1 Domain of ARF and AUX/IAA Proteins

The high-resolution structures of the C-terminal regions of two ARF (ARF5 and ARF7) and two AUX/IAA (IAA17 and *Pisum sativum* IAA4) proteins were recently solved by X-ray crystallography [11,12] and NMR spectroscopy [13,14], respectively. These studies confirmed that the protein–protein interaction domains of both families adopt the characteristic ubiquitin-like β-grasp fold, which only minimally deviates from the canonical PB1 domain (Figure 3A). The four reported PB1 structures contain an additional α helix (α3) at the C-terminus and the loop connecting DIII (β1–α1) and DIV (β3–α3) of the IAA17 PB1 domain contains an insertion that folds into an additional α helix (α1′) [13]. While C-terminal helix α3 is likely to be a general feature of most ARF and AUX/IAA PB1 domains, long insertions (~15 residues) forming helix α1′ are present in only a subset of AUX/IAAs. The functions of neither helix (α1′ and α3) is known; however, they do not obstruct the canonical PB1 fold nor its propensity for protein–protein interaction [13].

The presence of crucial basic (invariant lysine) and acidic (OPCA motif) residues suggests that most ARF and AUX/IAA proteins harbor type I/II PB1 domains mediating electrostatic front-to-back oligomerization [60]. Indeed, *in vitro* protein aggregation impeded the structural studies, which was overcome by the introduction of charge-neutralizing mutations on either surface patch of the PB1 domains [12,13] or by protonation of the acidic OPCA motif at low pH [14]. As in all PB1 domains, the type I/II structural features are found on opposite faces of the DIII/IV structures of ARF5, ARF7, IAA17, and PsIAA4 [11–14]. Biophysical studies confirmed front-to-back interaction of the arrested PB1 dimers (comprising two oppositely mutated monomer units retaining charge complementarity at their interface) and revealed that additional charged and uncharged residues contribute to directional PB1 interactions [11–14]. The type I/II PB1 interaction modus was further validated *in vivo* by yeast two-hybrid assays [11,12,14].

A refined thermodynamic and structural analysis of the ARF7 PB1 interface showed that ARF7 PB1 dimerization is not only driven by electrostatic forces between the invariant lysine and the main cluster of acidic residues in the OPCA motif but also by electrostatic interactions of a second set of charged residues, which includes a conserved arginine on the basic face and a minor cluster of conserved acidic residues on the OPCA face [15] (Figure 3A). Residues of these two PB1 dimer-stabilizing 'electrostatic prongs' [15], which are also evident in the ARF5 and PsIAA4 PB1 dimerization interface [11,14], are conserved in ARF and AUX/IAA PB1 domains and thought to facilitate intra- and interfamily protein–protein interactions of ARF and AUX/IAA members [15]. The specificity of these versatile combinations is likely to be determined by additional contacts surrounding the core set of electrostatic interactions to fine-tune auxin response [14,15]. This is suggested by the broad range of PB1 domain affinities determined for IAA17 and PsIAA4 self-interaction (each $K_D \sim 6.5$ μM), ARF5 and ARF7 homodimerization ($K_D \sim$ 0.2–0.9 μM), and ARF5:IAA17 heterodimerization ($K_D \sim 0.07$ μM), which covers almost two orders of magnitude [13,15]. Interestingly, the reported binding constants for IAA17 and PsIAA4 PB1 self-interaction are remarkably similar, yet enthalpy and entropy changes are quite different for the two associations, which is likely to be a consequence of clear topologic differences between the AUX/IAA PB1 interfaces [13,14]. Similarly, each IAA17 and ARF5 PB1 homodimer interface is strikingly unique in charge density and distribution. Because the IAA17 PB1 interface is not significantly altered on interaction with ARF5 PB1, optimal combinations of complementary bonded and non-bonded contacts are presumably major specificity determinants of AUX/IAA and ARF PB1 domain interactions [13]. A systematic yeast two-hybrid analysis of the AUX/IAA PB1 and ARF PB1 interactome indicated very limited ARF PB1 dimerization, frequent AUX/IAA PB1 homo- and heterodimerization, and preferential interaction of AUX/IAAs with ARF activators [19]. The latter observation was confirmed by interrogating full-length ARF and AUX/IAA proteins in yeast [64].

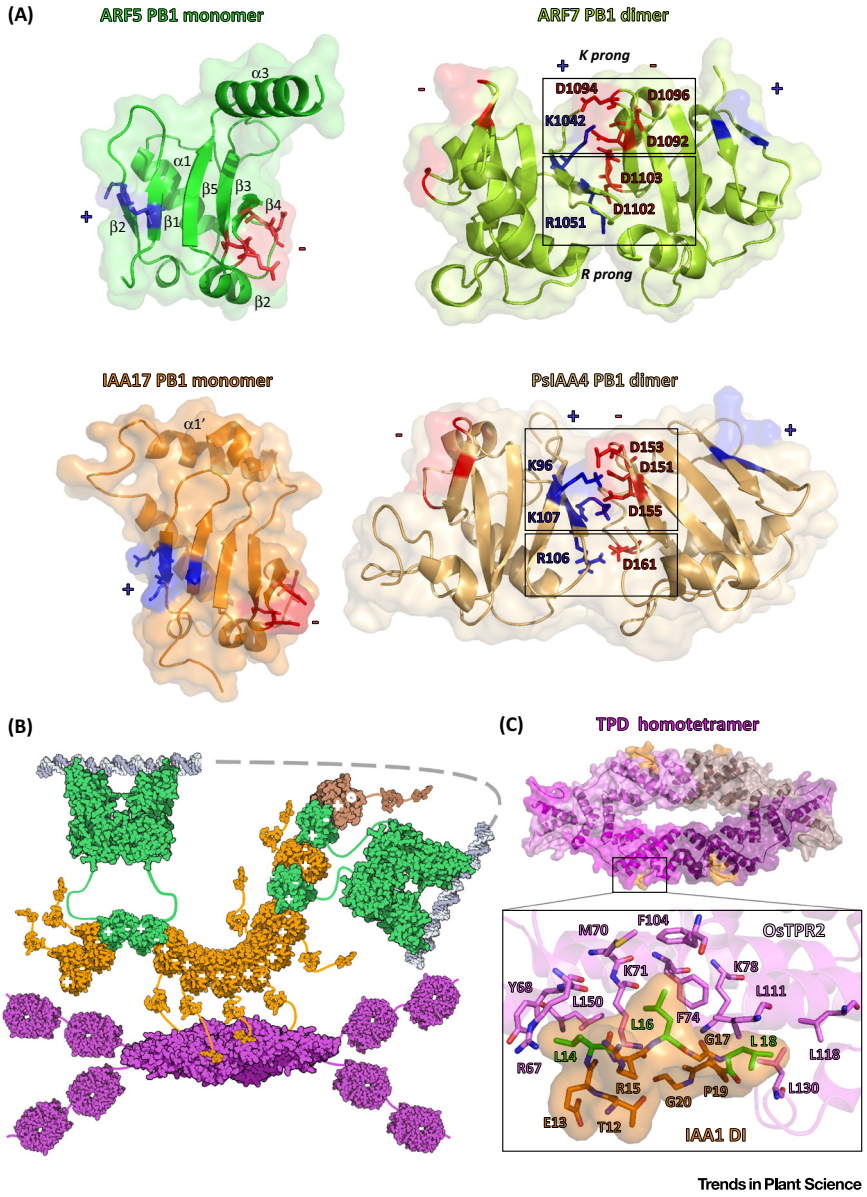


Figure 3. The 'Molecular Magnet' Concept for PB1 Domain Interactions in Auxin Response. (A) High-resolution structures of the type I/II PB1 domains of ARF5 (4CHK) [11], ARF7 (4NJ6) [12], IAA17 (2MUK) [13], and PsIAA4 (2M1M) [14]. Structural models of PB1 domain monomers of ARF5 and IAA17 are shown on the left. The oppositely charged and positioned faces are labeled in blue (invariant lysine residue-centered basic patch, [+]) and in red (acidic OPCA motif, [−]). Structural models of PB1 domain homodimers of ARF7 and PsIAA4, which interact in a front-to-back fashion, are shown on the right. The two boxes in each dimer model highlight the two prongs of electrostatic interactions that stabilize the PB1 dimer [15]. The invariant lysine residue on β1 interacts with the acidic DXDXD stretch of the OPCA motif (K prong), whereas a conserved arginine residue on β2 interacts with additional conserved acidic residues on the OPCA face (R prong). (B) Hypothetical model of higher-order complex formation by type I/II PB1 domain (+/−) polymerization of ARF (green) and AUX/IAA (orange) proteins. The model is based on indirect evidence for ARF:AUX/IAA multimerization *in planta* [11,12] and on direct evidence for polymerization of mammalian PB1 domain-containing proteins by cryoelectron microscopy [65]. Filamentous helical PB1 domain scaffolds may allow the recognition of complex composite *AuxRE*s and efficient recruitment of tetrameric TPL/TPR corepressors (purple) [17]. AUX/IAAs or other proteins containing type II (or type I) PB1 domains (light brown, +/o) may terminate scaffold extension. (C) Structural model of the TPD homotetramer of OsTPR2 (shades of purple) complexed with four EAR peptides of IAA1 DI (orange). The box shows the interacting IAA1 DI and TPD residues in stick presentation (PDB ID: 5C7F) [17]. The conserved leucine residues of the EAR motif are shown in green.

PB1 Domain 'Molecular Magnets' Multimerize ARF and AUX/IAA Proteins

A first series of experiments *in planta*, using protoplast transfection assays [11] and transgenic lines [12], confirmed the predicted role of PB1-mediated multimerization in the control of ARF function in auxin signaling. For example, only overexpression of an ARF7 variant with both of its PB1 faces mutated caused constitutive activation of an *AuxRE*-dependent reporter gene in *arf7* protoplasts, which suggests that endogenous AUX/IAA repressors can bind to either face of the ARF7 PB1 domain [11]. In agreement, the severe auxin-related plant phenotypes, which are caused by the overexpression of stabilized IAA16 (a dominant DII mutation) and by the resulting constitutive repression of endogenous ARFs, cannot be recapitulated when stabilized IAA16 with mutations on either PB1 face is overexpressed. This is consistent with heterotrimer formation (IAA16:ARF7:IAA16) or with the possibility of IAA16 multimerization [12,49]. The modular type I/II PB1 domain of AUX/IAA and ARF proteins is well suited for the assembly of assorted protein complexes via directional (hetero)oligomerization. It is of note that the PB1 domains of some AUX/IAA and ARF proteins may be classified as type I or type II and could thus terminate multimerization if incorporated as a capping subunit into a growing chain [30]. These scaffold-like complexes may acquire additional subunits via noncanonical PB1 interactions to enable specificity and fidelity in signal transduction [62]. An interesting feature of type I/II PB1 domains is their intrinsic potential to polymerize into helical filaments with significant curvature and pitch variation. This ability was recently reported in mammals for the PB1-containing signaling scaffold protein p62/SQSTM1 [65] and for the PB1-like domain protein Par-3 [66,67]. Thus, it is tempting to speculate that long helical polymers comprising various ARF and AUX/IAA proteins provide rotationally shifted 'molecular caliper' arrangements for recognizing multiple and more distantly positioned composite *AuxRE*s in auxin-regulated promoters (Figure 3B) (see Outstanding Questions).

Regulation of PB1 Domain Assembly in Auxin Response

The assembly state of p62/SQSTM1 is affected by multiple post-translational modifications, including phosphorylation by cAMP-dependent protein kinase at a serine residue on the basic face of its PB1 domain, which disrupts homopolymerization or interaction with OPCA motif-containing PB1 domains [68]. A recent study showed that the brassinosteroid-regulated GSK3-like kinase BRASSINOSTEROID-INSENSITIVE2 (BIN2) phosphorylates ARF7 and ARF19 activators, which suppresses their interaction with AUX/IAAs, facilitates AUX/IAA degradation, and enhances ARF DNA binding and target gene activation [69]. BIN2 phosphorylates two serine residues in the Q-rich MR of ARF7; however, it is unclear how this modification disrupts ARF7 interaction with AUX/IAAs. BIN2 also phosphorylates ARF2, which inhibits the repressor activity of ARF2 by preventing its binding to *AuxRE*s presumably in competition with ARF activators [70]. Thus, BIN2-dependent phosphorylation of ARF activators and repressors potentiates auxin response and provides a node for signal integration [71]. Evidence for AUX/IAA phosphorylation and its relevance is limited [72]. However, it is of note that clades of ARF and AUX/IAA proteins feature conserved threonine or serine residues near the invariant lysine of the basic PB1 face. The high affinity and complexity of PB1 domain multimerization highlights the need for post-translational control of ARF:AUX/IAA interactions to fine-tune auxin responses.

In addition to the canonical PB1 domain-mediated interactions of ARF and AUX/IAA proteins, members of both families may recruit other transcription factors. For example, at least some ARFs have been reported or implicated to specifically interact with members of the MYB family (MYB77) [73], the bHLH family (BIGPETAL, PIF4) [74,75], and the bZIP family (bZIP11) [76] and with plant-specific transcriptional regulators such as BZR1/BZR2 [75,77] and BRX [78]. The PB1 domain or the MR of ARFs is required for these interactions, which often support cooperative recognition of promoter elements and are likely to integrate auxin response with other signaling pathways, as best understood for auxin–brassinosteroid crosstalk [71].

ARF Repression by AUX/IAA-Dependent Recruitment of TPL/TPR Corepressors

The N-terminal DI of most AUX/IAAs binds TPL/TPR proteins via its ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR-ASSOCIATED AMPHIPHILIC REPRESSION (EAR)-like motif (D/E-L-X-L-X-L), which is the prototypic repressor motif found in many plant transcriptional regulators [24,25]. The TPL/TPR family is related to the Tup1/Groucho-like corepressors and their C-terminal WD40 domains are believed to recruit histone deacetylase complexes and associated chromatin-modifying enzymes (Figure 1). The recently solved crystal structures of the N-terminal TOPLESS domain (TPD) of *Oryza sativa*TPR2 in complex with the EAR motifs from *Arabidopsis* IAA1 and IAA10 revealed a novel fold of nine helices followed by a zinc finger, which oligomerizes into a donut-like tetramer [17]. A shallow groove on each TPD monomer binds, via hydrophobic and positively charged residues, a single EAR motif peptide of extended conformation (Figure 3C). Biophysical studies have indicated that a single EAR peptide only weakly interacts with OsTPR2 whereas a tetrameric bacterial protein displaying four EAR peptides binds with much higher (>200-fold) affinity. Thus, AUX/IAA repressor multimerization is likely to facilitate synergistic recruitment of TPL/TPR tetramers, which may provide expanded scaffolds of eight seven-bladed WD40 β-propeller domains to mediate the assembly of chromatin remodeling complexes and the interaction with the transcription preinitiation complex [17] (Figure 3B). While TPL/TPR corepressor complexes are tethered to auxin-responsive promoters via the interaction between AUX/IAA repressors and ARF activators, members of the ARF repressor class, which do not bind AUX/IAAs [19,64], may interact directly with TPL/TPR corepressors [25]. However, the mechanisms of transcriptional regulation by ARF repressors, and ARF activators, are not well understood (see Outstanding Questions).

Auxin-Facilitated Removal of AUX/IAA Repressors

Auxin Sequesters AUX/IAAs to SCF Complexes Like 'Molecular Glue'

Auxin perception triggers the destruction of AUX/IAA repressors and its degradation rates largely determine the rates of ARF-dependent gene activation [29]. Genetic and biochemical studies in pursuit of auxin receptors led to the identification of TIR1 and its closely related F-box proteins AFB1–AFB5 [31,79–81]. While modification of protein substrates is often required for recognition by CULLIN scaffold-type E3 ligases, auxin binding to TIR1 alone is sufficient to promote recruitment of AUX/IAAs to $SCF^{TIR1/AFB}$ complexes *in vitro*. Interestingly, auxin binding does not induce an allosteric switch or profound conformational change; instead, auxins fill a polar gap in the bottom of the AUX/IAA-recruiting pocket positioned on the leucine-rich repeat (LRR) domain of TIR1 to create a continuous hydrophobic protein interaction surface, much like an adhesive or 'molecular glue' [9] (Figure 4). The coiled AUX/IAA degron peptide of 13 amino acid residues (conserved DII) seals the hydrophobic pocket by packing its core GWPPV motif against the indolyl moiety of the hormone, which is believed to remain trapped until AUX/IAA ubiquitylation and destruction ensues [82]. Thus, auxin perception promotes the assembly of TIR1/AFB:auxin:AUX/IAA coreceptor complexes, which display a wide range of auxin binding affinities *in vitro* ($K_D \sim$ 10 nM to 1 μM) [26]. The auxin sensitivities of the coreceptor pairs are influenced by either partner and broadly correlate with AUX/IAA degradation kinetics [26,36,37,83], which in turn appear to set the pace for developmental auxin responses [84]. Thus, the repertoire of various coreceptor combinations provides the molecular basis for the wide range of dynamic auxin responses.

Regulation of Auxin Coreceptor Formation

AUX/IAA half-lives, which vary widely from 6 to 80 min [85–88], are determined by TIR1/AFB identity and abundance as well as by intrinsic properties of AUX/IAA proteins [19,26,27,36,83,89]. Genetic evidence suggests that TIR1 and AFB2 are the major nuclear auxin receptors in *Arabidopsis* promoting auxin response [31,90]. Synthetic approaches in yeast provided direct evidence that the two F-box proteins confer rapid auxin-induced degradation of degron-harboring AUX/IAAs, whereas the remaining four AFBs do not affect

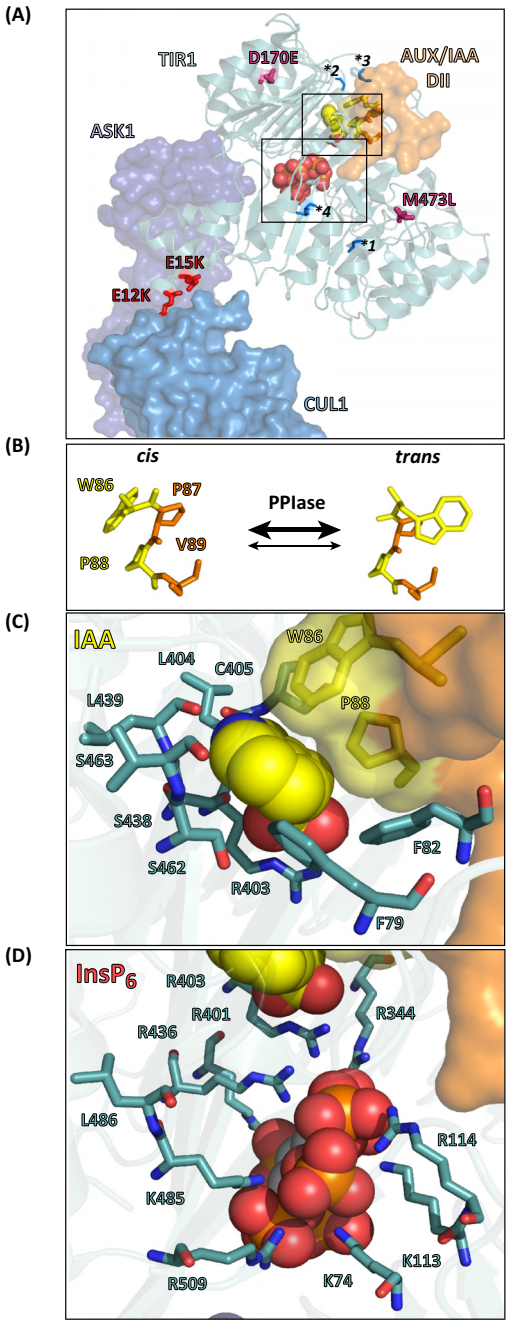


Figure 4. The 'Molecular Glue' Concept for Auxin Perception by SCF^{TIR1}:AUX/IAA Coreceptor Complexes. (A) Structural model of the substrate recognition wing of the SCF^{TIR1} E3 ubiquitin ligase. Shown are the ASK1–TIR1:auxin:IAA7 DII degron complex (PDB ID: 2P1Q) together with the N-terminal part of human CUL1 (PDB ID: 1LDJ). Auxin (IAA, yellow spheres) occupies a pocket on the top surface of the TIR1 LRR domain. An $InsP_6$ cofactor (red spheres) is positioned in the center of the solenoid fold. The coiled degron peptide (orange surface presentation) covers the pocket and places its conserved GWPPV fold (stick presentation) on top of the auxin indole ring. Auxin binding extends the hydrophobic interaction surface of TIR1 and thus facilitates AUX/IAA docking without substantial conformational changes. Two mutations on the TIR1 LRR surface, D170E and M473L (pink sticks), increase the affinity for AUX/IAAs [83], whereas other mutations (blue sticks) abrogate TIR1 function; for example, *1 (*tir1-1*, G147D), *2 (*tir1-2*, G441D), *3 (*tir1-6*, P409S), and *4 (*tir1-7*, L112Q). Mutations (E12K, E15K) that disrupt TIR1 and CUL1 interaction (red sticks) lead to auxin insensitivity [93]. The two boxes frame the auxin binding pocket and the $InsP_6$ binding site, which are enlarged in (C) and (D), respectively. (B) The *cis* conformation of the first proline residue in the WPPV motif is necessary for the maintenance of the coiled binding conformation of the degron peptide, which is facilitated by a cyclophilin-type peptidyl-prolyl *cis/trans* isomerase (PPIase) [16]. (C) A close-up view of the spatial arrangement of the TIR1 auxin binding pocket, illustrating the hydrophobic stacking (yellow) between the indole ring of IAA (center space-filled molecule, carboxylate group in red) and the WPPV motif of the coiled degron, with its critical residues (W86 and P88) shown in stick presentation. (D) A close-up view of the spatial arrangement of the $InsP_6$ binding site (center space-filled molecule in red). The $InsP_6$ cofactor anchors the auxin compound (upper space-filled molecule) via salt bridges (R344, R401) between one of its phosphate groups and the carboxylate group of IAA.

degradation rates although they interact with AUX/IAAs in the presence of auxin [19,26,36,37]. The DII is essential for auxin-dependent AUX/IAA degradation and dominant mutations, foremost in its nearly invariant GWPPV core motif, abolish AUX/IAA binding to TIR1/AFBs and cause auxin-resistant phenotypes [4]. Additional residues flanking DII impact coreceptor formation and AUX/IAA degradation [26,36,87,89], which indicates the importance of a broader DII context in the regulation of AUX/IAA proteolysis by post-translational modification or ligand interaction.

The first evidence for control of AUX/IAA protein level by post-translational modification was recently reported in rice (*Oryza sativa*). A cyclophilin-type peptidyl-prolyl *cis/trans* isomerase (PPIase), encoded by *LATERAL ROOTLESS2* (*LRT2*), directly regulates the binding of OsIAA11 to OsTIR1 and thus OsIAA11 stability. NMR spectroscopy demonstrated that LRT2 efficiently catalyzes the *cis/trans* conformational exchange in the core GWPPV motif of the OsIAA11 degron peptide [16]. Notably, the crystal structure of the TIR1:auxin:IAA7 coreceptor complex revealed that the *cis*-conformation of the first proline residue is necessary for the maintenance of the coiled binding conformation of the IAA7 degron peptide [9] (Figure 4). Because LRT2-like proteins are conserved in land plants and their functional loss leads to similar auxin-insensitive phenotypes [91,92], it was proposed that the cyclophilin-catalyzed *cis/trans* isomerization of AUX/IAA transcriptional repressors may represent a general mechanism to accelerate their proteolysis [16].

Screening for novel TIR1 variants in yeast identified mutations disrupting TIR1 binding to the CULLIN 1 scaffold, which causes growth defects related to sequestration of AUX/IAAs by stabilized TIR1 [93]. This observation suggests accelerated degradation of TIR1 on assembly into $SCF^{TIR1/AFB}$ complexes. Interestingly, the opposite dynamics was noted for the TIR1-related F-box protein CORONATINE INSENSITIVE 1 (COI1), which is stabilized once bound to SCF^{COI1} [94]. The SCF^{COI1} E3 ubiquitin–protein ligase recruits jasmonate ZIM-domain (JAZ) transcriptional repressors on binding to the bioactive jasmonic acid (JA) conjugate JA–Ile, which, much like auxin, acts as a molecular glue in the assembly of COI1:JA–Ile:JAZ coreceptor complexes [95]. The auxin and jasmonate coreceptors share a very similar architecture in which an inositol polyphosphate cofactor ($InsP_6$ and $InsP_5$, respectively) positioned in the interior of each LRR domain plays a critical role in arresting the carboxylate anchor group of the hormone ligand [9,95] (Figure 4). Remarkably, a recent study showed that an inositol pyrophosphate ($InsP_7$) binds more efficiently to the JA–Ile coreceptor than the less anionic $InsP_6$ and $InsP_5$ polyphosphates and that the binding pocket may even accommodate a single $InsP_8$ molecule [96]. Direct binding assays combined with structural data suggest that only simultaneous detection of both JA–Ile and $InsP_x$ ligands promotes coreceptor formation and subsequent proteasomal degradation of JAZ repressors to activate JA-responsive gene expression [95,96]. The authors propose that 'coincidence detection' of two unrelated small molecules adds yet another layer to hormone response regulation that is determined by the $InsP_x$ signature of a given tissue [95]. Inositol pyrophosphates are increasingly recognized as signaling molecules in yeast, animals, and plants [97] and it remains to be seen how this proposed mechanism applies to the regulation of auxin coreceptor assembly (see Outstanding Questions).

Proteasomal Destruction of AUX/IAA Repressors

Experimental data indicate that at least some AUX/IAA repressors are polyubiquitylated by SCF^{TIR1} [88,98] and degraded by the 26S proteasome [28,86], which is likely to apply to all DII-containing AUX/IAAs. Specific ubiquitylation is often the rate-limiting step in proteolysis and E3 ubiquitin ligases typically form an isopeptide bond between the terminal carboxylate group of ubiquitin and the ε-amino group of lysine residues within the substrate [99]. Surprisingly, an attempt to identify preferred lysine ubiquitylation sites on rapidly degraded IAA1 concluded that exhaustive lysine-to-arginine substitutions did not considerably stabilize the mutant protein nor impair its ability to heterodimerize with IAA7 or to interact with TIR1 [88]. The same study obtained evidence for noncanonical oxyester-linked ubiquitylation of serine and threonine side chains on the lysine-free IAA1 variant, but also for a mixture of isopeptide and oxyester linkages on wild-type IAA1. The latter observation suggests that ubiquitylation at multiple AUX/IAA sites is a robust process that supports complex AUX/IAA degradation dynamics depending on intrinsic substrate properties [88]. Dissection of the broader, variable DII context of AUX/IAAs with different half-lives identified several degron-flanking 'rate motifs' that differentially affect auxin-dependent coreceptor assembly and

AUX/IAA turnover [89]. The uncoupling of AUX/IAA binding to SCF^{TIR1} from AUX/IAA degradation dynamics suggests regulatory processes that modify such rate motifs and may include canonical or noncanonical ubiquitylation [88,100] or other post-translational modifications, such as phosphorylation [72] (see Outstanding Questions). Lastly, given that the PB1 domain of AUX/IAA and ARF proteins adopts a ubiquitin-like β-grasp fold [60,62] and that certain proteins appear to be degraded by the proteasome in a ubiquitin-independent manner [100], it is tempting to speculate that PB1-mediated multimerization may mimic polyubiquitylation and thus affect AUX/IAA degradation rates.

Concluding Remarks

Since the first structural model of a plant hormone receptor, the ASK1–TIR1:auxin:IAA7 DII degron complex, was reported [9], biophysical, structural, and synthetic biological studies on central auxin signaling components and their dynamic interactions have gained enormous momentum, particularly during the past 3 years [10–17,26,29,33–37,64]. Nuclear auxin action is executed by only a few key players encoded by diverse multigene families. It has become increasingly clear that these core components are embedded in complex molecular networks of post-translational modifications and protein interactions with varied ligands, which are likely to provide multiple nodes for signal integration and response specification. Structural biology has delivered the first high-resolution models of auxin perception, of transcription factor interaction modes, and of ARF:DNA complexes. The discovered principles have also made possible the development of sensitive auxin reporters [33,101] and of versatile, inducible degradation systems [102–104]. The biophysical approach is poised to firmly expand our unparalleled insights beyond the core module to include additional subunits, adaptor proteins, or chaperones [105] of $SCF^{TIR1/AFB}$ E3 ligases, ARF:AUX/IAA PB1 domain scaffolds, TPL/TRP corepressor assemblies, and transcriptional ARF activator/repressor complexes (see Outstanding Questions). Structure–function and kinetic studies of dynamic complex formation and remodeling will lead to enhanced understanding of the auxin response pathway controlling primary gene expression, which will be further tested *in planta* and by synthetic biological studies in heterologous *in vivo* systems.

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Outstanding Questions

The discovery that ARF1 and ARF5 homodimerize via their DBD raises the question of whether DD-dependent homo- and heterodimerization is a general property of ARF proteins and whether combinatorial ARF dimer formation generates a repertoire of 'molecular calipers' for recognizing different *AuxRE*s.

The precise mechanisms of how ARF proteins function as activators or repressors of gene transcription remain to be established.

What are the precise structural determinants that promote selective interactions between ARF activators and AUX/IAA repressors via their PB1 domains?

What is the extent (composition, stoichiometry) and *in vivo* significance of PB1-mediated multimerization of ARFs and AUX/IAAs? Are helical PB1 scaffolds formed for distant *AuxRE* recognition that further integrate other PB1 domain-containing proteins?

Inherent to this question is how the formation of ARF:AUX/IAA complexes is regulated by post-translational modifications such as phosphorylation or ubiquitylation.

Does heterotetramerization of TPL/TPR corepressors add another layer of regulation and signal integration in auxin response? What determines the specificity of AUX/IAA:TPL/TPR interactions? What is the composition and structure of TPL/TPR-recruited protein complexes?

Another set of open questions centers on the regulation of auxin-dependent $SCF^{TIR1/AFB}$ coreceptor assembly. How are full-length AUX/IAAs structured when bound to TIR1/AFBs? How do degron-flanking regions and their post-translational modification affect AUX/IAA recruitment? What is the role of inositol poly- and pyrophosphates as cofactors? What other proteins sustain a fully functional $SCF^{TIR1/AFB}$-dependent network in the nucleus?

What are the intrinsic properties of AUX/IAA proteins and the regulatory processes that determine the sites and extent of polyubiquitylation as well as the rate of degradation? Are associated TPL/TPR corepressor complexes corecruited to the $SCF^{TIR1/AFB}$ complex and copolyubiquitylated?

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