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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