CellPress
OPEN ACCESS

Trends in
Plant Science

Forum

Role of cAMP in TIR1/AFB auxin signaling: open issues

Jiří Friml [1,*]

The canonical mechanism by which the phytohormone auxin regulates transcription has been one of the cornerstones of plant signaling. The recent unexpected discovery of cyclic AMP (cAMP) as a second messenger in this pathway has revised its foundations while leaving many open questions and gaps in our understanding; these will be discussed in this forum article.

Auxin is a major endogenous regulator of plant growth and development. It is among the longest-studied plant hormones, and mechanistic insights into its action have often inspired studies of other signaling pathways. After the initial discovery that auxin induces transcription of hundreds of genes, follow-up studies identified essential auxin response sequences in their promoters [1]. This provided an entry point for the identification of transcription factors that bind to these elements (auxin response factors, ARFs) and their repressors, the auxin/indole-3-acetic acid (Aux/IAA) proteins [2]. In parallel, genetic screens for auxin-insensitive mutants identified components of the ubiquitin ligase machinery that marks proteins for degradation. The most prominent of these, TIR1 (transport inhibitor response 1), acts as an F-box component of the SCF (Skp1–Cullin–F-box) ubiquitin ligase complex, which recognizes specific ubiquitination substrates. The discovery that Aux/IAAs are the elusive substrates of TIR1 brought these two experimental threads together [3], culminating in the early 2000s with the realization that TIR1 and its homologs (auxin-related F-box proteins, AFBs) serve as auxin receptors with Aux/IAAs as co-receptors [4]. The ensuing model is remarkably simple: auxin acts as a molecular glue, bringing together TIR1/AFBs and Aux/IAAs. As a consequence, Aux/IAAs are ubiquitinated and degraded, thereby releasing ARFs from repression and allowing them to mediate transcription. This model elegantly explained existing experimental observations and inspired the identification of similar repressor-degradation mechanisms in other pathways. It appeared complete and required no major updates for almost 20 years.

The first cracks in this foundation appeared when live imaging revealed that root gravitropism and the underlying auxin-triggered growth inhibition occur too rapidly (within 30 s) to involve a transcriptional mechanism, yet still require TIR1/AFB receptors [5], which at that time were linked solely to transcriptional regulation. This motivated a search for additional biochemical functions of TIR1/AFBs beyond ubiquitination, and led to the discovery of a sequence motif resembling those found in animal adenylyl cyclases (ACs), enzymes that produce cAMP, a prominent second messenger in animal cells. The possibility that TIR1 might generate cAMP initially appeared preposterous, as cAMP was generally not considered a major signaling component in plants [6]. Nonetheless, *in vitro* enzymatic AC assays with TIR1/AFBs across species suggested that all of them likely possess AC activity. Notably, these *in vitro* AC activities increase in the simultaneous presence of auxin and Aux/IAA co-receptors, consistent with elevated cAMP levels in roots after auxin treatment. Thus, TIR1/AFB–Aux/IAA association following auxin perception not only leads to Aux/IAA ubiquitination but also to increased cAMP production [7].

The initial hypothesis following these unexpected findings was straightforward: TIR1/AFB ubiquitin ligase activity mediates transcriptional regulation, whereas cAMP is responsible for non-transcriptional root growth inhibition. However, plants expressing engineered TIR1/AFB variants lacking AC activity still exhibited normal rapid root growth inhibition responses. By contrast, auxin-induced transcription was severely compromised, along with downstream developmental processes such as sustained root growth inhibition, shoot growth promotion, root hair elongation, and lateral root organogenesis. Most surprising was that all these defects occurred while auxin-induced Aux/IAA degradation proceeded normally (Figure 1A–C). It was even more striking that when other unrelated AC enzymes were fused to stabilized Aux/IAA proteins, they were able to activate ARF-mediated transcription and downstream development (Figure 1D). Importantly, AC-inactive variants of these fusion proteins were ineffective. Thus, cAMP produced within the context of canonical auxin signaling is sufficient to activate transcription even in the presence of stabilized Aux/IAA repressors [8]. This not only adds an unexpected component to the TIR1/AFB–Aux/IAA mechanism but also revises the original notion that auxin-induced Aux/IAA degradation is a necessary and sufficient step in mediating transcriptional regulation. Without cAMP production, this simply does not apply; on the contrary, cAMP production appears at least partially able to alleviate Aux/IAA-mediated ARF repression (Figure 1).

Despite the fact that all the key new observations have been verified by independent approaches, and the whole set is internally consistent, the emerging picture [9] is less satisfying than the original model and leaves several apparent gaps in our understanding, discussed in the following sections.

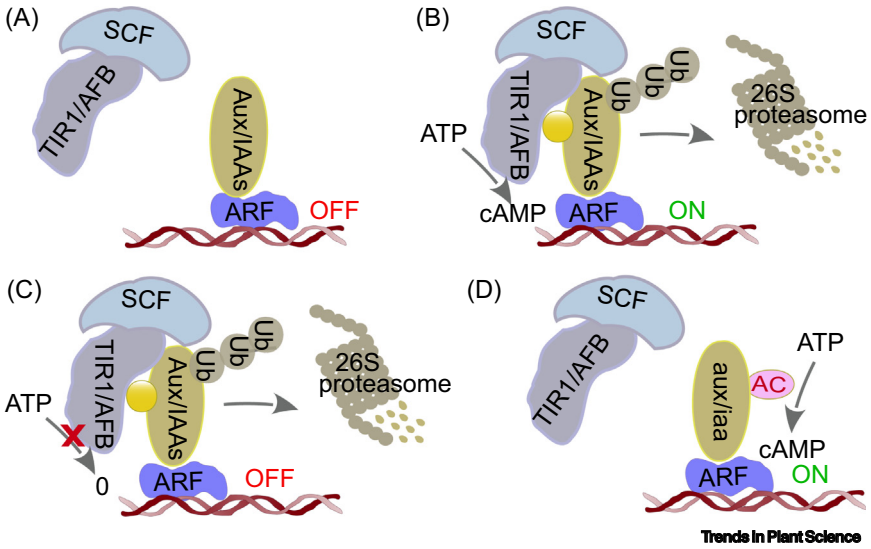

Figure 1. A simplified scheme of the cyclic AMP (cAMP)-dependent auxin signaling pathway. (A) Low auxin: stable Aux/IAA (auxin)/indoleacetic acid) repressors interact with auxin response factor (ARF) transcription factors, inhibiting them and thereby keeping transcription off. (B) High auxin: auxin (yellow circle) acts as a molecular glue between Aux/IAAs and transport inhibitor response 1 (TIR1)/auxin-related F-box proteins (AFBs). Consequently, cAMP production is induced and the SCF (Skp1–Cullin–F-box) ubiquitin (Ub) ligase complex is recruited to Aux/IAAs, leading to their ubiquitination and proteasome-dependent degradation. ARFs are activated; transcription is on. (C) High auxin, mutated TIR1 adenylyl cyclase (AC) activity: in the presence of auxin, the SCF complex is tethered to Aux/IAAs, which are ubiquitinated and degraded. However, without cAMP production, ARFs remain inactive and transcription is off. (D) Low auxin, ectopic cAMP production by an unrelated AC fused with stabilized Aux/IAA: ARFs are activated and transcription is on even without high auxin and in the presence of stabilized Aux/IAAs.

Function and targets of TIR1/AFB-produced cAMP

The most obvious question concerns the function and downstream targets of TIR1/AFB-produced cAMP. Given that cAMP production near ARFs and Aux/IAAs is sufficient to activate ARF-mediated transcription, the most likely scenario is that cAMP acts directly on these components. Possible mechanisms to be tested include whether cAMP can modify the interaction between Aux/IAAs and ARFs or enhance ARF binding affinity to promoters. An alternative explanation – namely, that cAMP acts on an unknown target which in turn regulates ARFs and/or Aux/IAAs – seems less likely, since decades of extensive genetic and biochemical studies would likely have identified such critical components.

Low *in vitro* AC activities and modest activation by auxin

An important issue is that *in vitro* TIR1/AFB AC activities are approximately two orders of magnitude lower than those detected for canonical mammalian ACs, and the auxin perception-induced AC activity increase is small. Whether this reflects similarly low activity and a modest auxin effect *in vivo* remains unclear. Regardless, even these apparently low activities are physiologically meaningful: they rescue AC-deficient bacterial strains and lead to significant cAMP increases *in planta* [7] and, notably, cAMP can be routinely detected in plant tissues [6,10]. It is also possible that the low AC activities are needed to keep the signaling spatially confined and cAMP action specific to close-by targets.

Strong developmental defects in *aux/iaa* mutants

One prominent set of pre-existing data is not easily reconciled with the new observations. Dominant mutations in Aux/IAAs that prevent their interaction with TIR1/AFBs stabilize these repressors even in the presence of auxin, causing strong developmental phenotypes. Notable examples include *bdl/iaa12* (embryonic root formation), *shy2/iaa3* (hypocotyl growth), *slr/iaa14* (lateral root organogenesis), or *axr3/iaa17* (auxin sensitivity and root growth) [2,3]. Why do these defects occur if TIR1/AFB-produced cAMP can activate transcription even in the presence of stabilized Aux/IAA repressors, and, even more strikingly, if ectopic AC activity attached to these stabilized aux/iaas can at least partly rescue those phenotypes [8]? Notably, both *in vitro* and *in planta* studies show that mutated aux/iaa proteins prevent auxin-induced cAMP production. This may result from the depletion of endogenous Aux/IAAs in the presence of stabilized aux/iaas [8], though the exact mechanism remains unclear. Nevertheless, the inability of these mutants to produce cAMP after auxin perception explains their pronounced phenotypic defects.

The role of Aux/IAA degradation

What, then, is the role of auxin-induced Aux/IAA degradation if it is neither sufficient nor strictly required for transcriptional activation? The ubiquitin ligase activity of SCF^{TIR1} and, by association, Aux/IAA degradation appear relevant, as mutants defective in SCF components or *tir1* alleles expressing TIR1 unable to interact with the SCF complex display auxin-related phenotypic defects [11]. Furthermore, the complete *aux/iaa* knockout mutant in moss shows partly auxin-insensitive phenotypes [12]. Because auxin-induced TIR1–Aux/IAA complex formation is required for cAMP production, Aux/IAA degradation likely functions as a negative feedback mechanism. However, this

alone cannot explain the extensive radiation of the Aux/IAA family observed in higher plants (29 members in *Arabidopsis* spp.), suggesting more diversified regulatory roles. Given the interaction specificity of Aux/IAA–ARF pairs, different Aux/IAAs may act as scaffolds, positioning cAMP-producing TIR1/AFBs near specific ARFs to drive developmental programs. In this scenario, Aux/IAA degradation would both release the ARF–Aux/IAA–TIR1/AFB complex and terminate cAMP production, a hypothesis that remains to be tested. Notably, only a subset of developmental stages and processes were tested and shown to depend dominantly on cAMP production [8], thus it is possible that in other processes the Aux/IAA degradation will manifest more.

Broader roles of cAMP beyond auxin signaling

Finally, is the role of cAMP restricted to auxin signaling? Observations of cAMP and cGMP involvement in other processes – for example, correlating with phases of cell cycle [13] – suggest a versatile, yet underappreciated, role for cyclic nucleotides in plant signaling [10]. Given how complete the canonical auxin mechanism appeared and how unexpected cAMP involvement was, similar surprises may await in other pathways. The most obvious candidate is jasmonic acid (JA), whose receptor COI1 (CORONATINE INSENSITIVE1) is the closest homolog of TIR1/AFBs and also acts as an F-box protein. The mechanism mirrors auxin: JA induces COI1 interaction with JAZ repressors, which are ubiquitinated and degraded, releasing transcription factors from inhibition [14]. It is tempting to speculate that cAMP may also serve as an essential component in this pathway. Regardless of whether cAMP is confirmed as a versatile second messenger in plants, auxin-related studies have already revealed that plants have evolved their own unique way of utilizing cAMP in signaling [15].

Concluding remarks

So, where does this all leave us? The new observations and the resulting tentative model do not invalidate any previous experimental results. Aux/IAAs still act as TIR1/AFB co-receptors and are degraded in response to auxin. They still act as ARF repressors, and the interactions of specific Aux/IAA–ARF pairs remain functionally relevant. To this mix, auxin-induced cAMP production by TIR1/AFBs has been added, replacing Aux/IAA degradation as the necessary step of signal transduction. Follow-up studies – particularly those focused on structural aspects and identification of cAMP target(s) – will clarify how these components work together. Exciting times lie ahead for understanding the mechanism of auxin signaling and beyond.

Acknowledgments

I apologize to colleagues whose relevant work I was unable to cite due to space limitations. This work was funded by the European Union (ERC, CYNIPS, 101142681) and Austrian Science Fund (FWF; 37051-B). I thank Drs Huihuang Chen, Yuanrong Pei, Jason Reed, Linlin Qi, and Dolf Weijers for inspiration and critical input.

Declaration of interests

The author declares no competing interests.

[1]Institute of Science and Technology Austria (ISTA), 3400 Klosterneuburg, Austria

*Correspondence:
jiri.friml@ist.ac.at (J. Friml).

https://doi.org/10.1016/j.tplants.2025.10.018

References

1. Abel, S. *et al.* (1996) DNA elements responsive to auxin. *BioEssays* 18, 647–654
2. Guilfoyle, T.J. (2015) The PB1 domain in auxin response factor and aux/IAA proteins: a versatile protein interaction module in the auxin response. *Plant Cell* 27, 33–43
3. Dharmasiri, S. and Estelle, M. (2002) The role of regulated protein degradation in auxin response. *Plant Mol. Biol.* 49, 401–408
4. Mockaitis, K. and Estelle, M. (2008) Auxin receptors and plant development: a new signaling paradigm. *Annu. Rev. Cell Dev. Biol.* 24, 55–80
5. Fendrych, M. *et al.* (2018) Rapid and reversible root growth inhibition by TIR1 auxin signalling. *Nat Plants* 4, 453–459
6. Świeżawska-Boniecka, B. and Szmidt-Jaworska, A. (2025) Cyclic nucleotides in plants: from obscure messengers to central regulators. *Front. Plant Sci.* 16, 1618243
7. Qi, L. *et al.* (2022) Adenylate cyclase activity of TIR1/AFB auxin receptors in plants. *Nature* 611, 133–138
8. Chen, H. *et al.* (2025) TIR1-produced cAMP as a second messenger in transcriptional auxin signalling. *Nature* 640, 1011–1016
9. Vanneste, S. *et al.* (2025) Mechanisms of auxin action in plant growth and development. *Nat. Rev. Mol. Cell Biol.* 26, 648–666
10. Gehring, C. and Turek, I.S. (2017) Cyclic nucleotide monophosphates and their cyclases in plant signaling. *Front. Plant Sci.* 8, 1704
11. Yu, H. *et al.* (2015) Untethering the TIR1 auxin receptor from the SCF complex increases its stability and inhibits auxin response. *Nat Plants* 1, 14030
12. Lavy, M. *et al.* (2016) Constitutive auxin response in *Physcomitrella* reveals complex interactions between Aux/IAA and ARF proteins. *eLife* 5, 13325
13. Ehsan, H. *et al.* (1998) Effect of indomethacin on cell cycle dependent cyclic AMP fluxes in tobacco BY-2 cells. *FEBS Lett.* 422, 165–169
14. Katsir, L. *et al.* (2008) Jasmonate signaling: a conserved mechanism of hormone sensing. *Curr. Opin. Plant Biol.* 11, 428–435
15. Qi, L. and Friml, J. (2023) Tale of cAMP as a second messenger in auxin signaling and beyond. *New Phytol.* 240, 489–495