BiAux, a newly discovered compound triggering auxin signalling

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Abstract

Lateral root (LR) formation, that is vital for plant development, is one of many auxin-modulated processes, but the underlying regulatory mechanism is not yet fully known. Recently, González-García *et al.* discovered the BiAux compound and showed its involvement in lateral root development via regulating specific auxin coreceptors.

The root system, consisting typically of primary root (PR), LRs, and root hairs, anchors the plant in the soil and facilitates the uptake of water and nutrients essential for plant growth and adaptation to diverse environmental conditions. LRs, originating from the pericycle tissue, a single-cell layer surrounding the vascular tissues, play an important role in the root system function. The development of LRs is governed by various endogenous and exogenous factors and is initiated in specific pericycle cells by local accumulation of the plant hormone, auxin. Auxins are low-molecular weight indoles with growth-promoting properties. The most abundant auxin in plants is indole-3-acetic acid (IAA). IAA accumulates in selected pericycle cells due to the activity of PIN auxin transporters, leading these cells to become founder cells (FC) that further developing into LR primordia. During LR development, an IAA gradient forms with the highest concentration at the primordia tip, coordinating cell divisions and differentiation [1]. Consequently, IAA plays a key regulatory role as a morphogenetic trigger during the LR formation. However, the precise coordination of these processes remains to be unravelled. Similar auxin gradient-based mechanisms are versatile and not only govern LR development but also influence embryonic patterning, shoot-derived organogenesis, root meristem organisation, and overall plant architecture, allowing for the plasticity and adaptability of plants to various environmental conditions including climatic changes.

It is currently known that, in plant cells both, transcriptional and non-transcriptional signalling takes place. However, the specific mechanism by which auxin triggers a cascade of cellular events and cellular responses is not fully understood. In transcriptional auxin signalling, nuclear TIR1/AFB receptors recognize and bind a set of natural and synthetic auxins. Some chemicals are structurally similar to auxin precursor L-tryptophan, or are indolic compounds, but they do not bind to auxin receptors, and thus do not elicit an auxin response [2]. This selective action of TIR1/AFB receptors underscores the importance of identifying new auxinic compounds that bind to these receptors, because this could enhance our understanding of auxin perception and downstream signalling pathways. In the presence of IAA, auxin/IAA (Aux/IAA) repressor proteins bind to the $SCF^{TIR1/AFBs}$ component of the ubiquitin ligase complex. This binding leads to the ubiquitination and degradation of the

Aux/IAA repressors by the proteasome. As a result, transcription factors from the AUXIN RESPONSE FACTOR (ARF) family, which are bound to the promoters of auxin-controlled genes, are released from the Aux/IAA repression. This allows for the activation or inhibition of the transcription of many genes, and ultimately regulating specific stages of plant development.

The mechanism of LR formation has been elucidated in detail in arabidopsis (*Arabidopsis thaliana*), thanks to the straightforward root anatomy and the availability of a broad spectrum of mutants and molecular techniques [3]. LR development is regulated by the root clock cycle, which is governed by cyclic auxin pulses, approximately every - 4-6 h [4]. This rhythmic process can be easily tracked using the *DR5::LUC* auxin response reporter and by observing the changes in the expression of those genes, often auxin-inducible, that are responsible for the establishment of the root branching locations, called the pre-branch sites (PBS) (Figure 1A). This root clock cycle takes place in the main root and LR tips in the so-called oscillation zone (OZ). Transcriptomic analyses of the OZ revealed - 2000 genes with a DR5-like oscillatory pattern, called 'in-phase', and - 1400 genes oscillating in an opposite phase, referred to as the 'anti-phase' oscillatory pattern [5] (Figure 1A). Many years of research on LR development have led to the discovery of components of the molecular mechanism controlling auxin oscillations. In addition to auxin biosynthesis and transport, canonical nuclear auxin signalling is involved, including $SCF^{TIR1/AFBs}$ receptors - ARF7, repressed by IAA3, 14, 18, and 28; ARF19 is repressed by IAA14 and 28; ARF5 is inhibited by IAA12 and 28; and ARF6, ARF8 and ARF19 are suppressed by IAA28 - all these proteins play a fundamental role in different stages of LR development (Figure 1B) [6].

Many environmental factors - water, light, nitrogen, phosphorus, and nitric oxide - influence the development of roots. In Arabidopsis roots exposed to light, there is an increased formation of LRs, probably because direct root illumination may act as a stress factor, but the reason for this phenomenon is still unknown. Metabolomic analyses have identified a new chemical compound. BiAux, which significantly accumulates in light-grown roots compared to dark-grown roots. Gonzalez-Garcia *et al*. chemically synthesised this BiAux molecule ($C_{36}H_{38}N_2O_{13}$, 3,3′-bis(2-methoxy-2-oxoethyl)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1H,1′H-2,2′-bisindole), analyzed its role in LR formation, and probed into its underlying molecular mechanism [7]. The authors showed that plants treated with BiAux exhibited more PBSs and thus developed more LRs (Figure 1C). Unlike natural and synthetic auxins, BiAux does not inhibit root growth, which is one of the most typical auxin responses [1]. BiAux-treated plants were characterized by an increase in the length of the main root, the total length of the root system, and smaller but more numerous meristematic cells. At the same time, BiAux-treated plants showed no changes in the appearance of the shoots. Notably, the mechanism of BiAux action is similar in different plant species, because the treatment of tomato plants also improves root growth.

The authors addressed the question of why BiAux causes changes in the architecture of the root system. During BiAux treatment, they observed increased amplitude and altered frequency patterns of the oscillations in OZ, resulting in more numerous pericycle FCs that form PBSs and give rise to primordia and LRs. BiAux also increases LR density in various mutants with LR formation defects (i.e., *axr1-12* or the *tir1 afb1 afb2 afb3* quadruple mutant), confirming the dominant role of BiAux in LR initiation. Docking studies show that BiAux binds to the TIR1 and AFB2 auxin nuclear receptors. However, BiAux binds at a different site from natural auxin. This binding increases the association of IAA18 and 28 proteins with the $SCF^{TIR1/AFB2}$ complex. Consequently, Aux/IAA proteins can undergo ubiquitination and degradation (Figure 1D), and released ARF7 protein can thus activate transcription. Transcriptomic analysis and studies an *arf7* and *arf19* mutants suggested that BiAux acts through ARF7 (Figure 1E) but not ARF19. In plants with a mutation in the *ARF7* gene (but not in *ARF19*), many of the genes activated after BiAux treatment are no longer upregulated. This shows that ARF7 is crucial for LR development triggered by BiAux. In addition, an *arf7* (but not *arf19*) mutant treated with BiAux, exhibited a decreased *DR5::LUC* signal, proving that ARF7 is necessary to increase the number of DR5-positive sites in the BiAux response. Furthermore, analyses of the transcriptome indicated differences in gene expression between roots treated with BiAux, IAA, or both chemicals simultaneously. The differential expression of many genes explains the diverse effects of these two auxinic compounds on root development.

In summary, the elegant study of Gonzalez-Garcia *et al*. describes a novel auxinic chemical, BiAux, which acts, via nuclear auxin signalling as a new compound involved in LR formation. It is interesting to contemplate the implications of this discovery for further mechanistic studies on auxin signalling and auxin-mediated development as well as for biotechnological applications (Figure 1F). For example, will BiAux increase the efficiency of shoot organogenesis *in vitro* from root explants, given that shoot primordia are also formed from pericycle cells at PBSs [8]? BiAux may find application in rooting shoots of species that are difficult to root and hence cannot be efficiently propagated under *in vitro* culture conditions [9]. Treating plants with high concentrations of auxin leads to plant death, which is why auxins are often used as herbicides in agriculture. Because there is a constant search for biologically active compounds that can be used as herbicides [10], investigating whether the simultaneous application of BiAux with natural or synthetic auxins can boost their action as herbicides is also of interest. As hinted, BiAux characterised by Gonzalez-Garcia and co-authors [7] will also be a useful tool for mechanistic studies on auxin signalling and auxin-mediated development. This would aid a better understanding of the relative contributions of individual TIR1/AFB receptors and Aux/IAA repressors to different developmental processes. For example, the fascinating and still largely elusive processes of auxin-induced vascular tissue formation and regeneration in plants can be further dissected by BiAux. This self-organising machinery correlated with canalized auxin flow, individual cell polarity, and intercellular communication seems to depend on both ABP1/TMK-based cell surface [11] and nuclear TIR1 signalling [12], although their specific contributions remian unknown. Thus, BiAux holds great potential for a range of applications in basic and applied research.

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Authors' contributions

B.W. and E.M., conceptualisation; B.W., E.M., and J.F., writing, review and editing; E.M., J.F., funding acquisition; B.W., visualisation; B.W., and E.M., writing – original draft.

Declaration of interests

The authors declare no competing interests.

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Figure 1. Mechanism of BiAux action during the initiation and formation of lateral roots (LRs). (A) The root clock cycle regulating the LR development in control condition. (B) ARF transcription factors (ARF5, 6, 7, 8, 19) and their Aux/IAA repressors (IAA3, 12, 14, 18, 28) coordinate different stages of LR formation. (C) Effect of BiAux on root architecture and root clock. (D) Seedlings grown in light produce more BiAux than dark-grown. BiAux, like IAA, can bind to the nuclear auxin receptors TIR1/AFB2. The presence of BiAux and IAA activates auxin signalling by recruiting IAA18 proteins to the $SCF^{TIR1/AFB2}$ complex, where they undergo ubiquitination and are targeted for degradation. The released transcription factor ARF7 mediates auxin-induced transcription to establish PBSs and drive LR development. (E) At low concentrations of IAA and in the absence of BiAux, the ARF7 transcription factor is repressed by IAA18 and cannot activate the expression of auxin response genes. (F) Possible application of BiAux in research. Abbreviations: ARF, AUXIN RESPONSE FACTOR; ASK1, ARABIDPOSIS SKP1 homolog; Aux/IAA, AUXIN/INDOLE-3-ACETIC ACID; BiAux, 3,3′-bis(2-methoxy-2-oxoethyl)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1H,1′H-2,2′-bisindole); CRs,

chromatin remodelers; CUL1, CULLIN 1; E2, ubiquitin-ligase; IAA, indole-3-acetic acid; LR, lateral root; OZ, oscillation zone; PBS, pre-branch sites; PR, primary root; RBX1, RING-BOX 1; TF, transcription factor; TIR1/AFBs, TRANSPORT INHIBITOR RESISTANT 1/AUXIN SIGNALLING F-BOXES; TPL/TPR, TOPLESS/TOPLESS RELATED; Ub, ubiquitin.