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Opinion

Necessity for modeling hormonal crosstalk in arabidopsis root development?

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Hormones play vital roles in plant root development. Mathematical models have been employed to study hormone functions. However, models developed by different research groups focus on different aspects of hormones and therefore cannot be used to study root growth as an integrative system that involves the functions of all hormones. To use modeling to study root development, the crosstalk nature of hormones requires the further development of mathematical models to understand their interplay in the context of diverse experimental data. This opinion article discusses what new insights can be developed by modeling hormonal crosstalk beyond experimental data. We propose that one integrative model should be developed to integrate all experimental data for elucidating root growth.

Highlights

Phytohormones play vital roles in root development. The activity of each hormone cannot change independently of others. The development of mathematical models is required to understand their interplay.

The necessity for modeling hormonal crosstalk in root development in *Arabidopsis thaliana* is explored, and we discuss new insights that can be developed by modeling and artificial intelligence (AI). Important questions addressed include: is regulation of auxin biosynthesis by cytokinin and ethylene required for the simultaneous patterning of auxin, cytokinin, and ethylene? How does patterning of a given PIN-FORMED (PIN) change in other *pin* mutants, and what role does it play in patterning auxin, cytokinin, and ethylene? How is patterning of ethylene-related gene expression related to hormonal crosstalk?

We propose that one integrative model should be developed to integrate all experimental data for elucidating root growth.

The need for integrative modeling in arabidopsis root growth

In *Arabidopsis thaliana* primary root growth is determined by the balance between three fundamental processes: (i) cell division in the root apical meristem (RAM), (ii) elongation of the cells that leave the RAM, and (iii) cell differentiation. All of these processes are known to be regulated by hormones, including auxin, cytokinin, ethylene, gibberellic acid (GA), abscisic acid (ABA), brassinosteroids (BRs), salicylic acid (SA), jasmonic acid (JA), and strigolactone [1–9].

Experimental measurements reveal that multiple hormones exhibit distinctive patterning of their distribution in the arabidopsis root. For example, there is a distinct auxin maximum in the organizing quiescent center (QC) of the root apex [10], and the cytokinin concentration is at maximum levels in the lateral root cap, columella, columella initials, and QC cells in the primary root tip [11]. ABACUS biosensors reveal that ABA forms distinct patterns in the arabidopsis root [12]. Moreover, GA gradients are observed in the growing root [13] and GAs accumulate in the elongating endodermal cells of the arabidopsis root [14].

Importantly, the activities of these hormones depend on cellular context and exhibit either synergistic or antagonistic interactions [1–4,8,9,15,16]. For example, ethylene positively regulates auxin biosynthesis and transport [3,17–20], cytokinin promotes auxin biosynthesis [21], and auxin upregulates cytokinin biosynthesis [22]. In addition, ethylene biosynthesis is positively regulated by auxin and cytokinin [23]. It is also known that cytokinin regulates spatially specific ethylene production to control root growth [24]. Furthermore, auxin requirements for a meristematic state in roots depend on a dual BR function [25], and ABCB19, an auxin transporter [26], is also a transporter of BR [27]. Therefore, the activity of each hormone cannot change independently of other hormones in space and time.

Due to the complexity in the functions of multiple hormones, mathematical models have been employed as a useful tool for studying their interactions. Recent reviews have comprehensively

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covered many aspects of hormone-related mathematical models ([28,29] and references therein). Despite the progress in modeling the roles of hormones in arabidopsis root development, models developed by different research groups focus on different aspects of hormones; however, arabidopsis primary root growth involves the functions of all hormones. The crosstalk nature of hormones requires the development of mathematical models to understand their interplay [30]. Here, we discuss the relationship between hormonal crosstalk and the emergence of the simultaneous patterning of multiple hormones, and what new insights can be developed by modeling rather than interpretation of experimental data alone. We propose that one integrative model should be developed to integrate all experimental data for elucidating root growth.

What insights can be developed by modeling hormonal crosstalk beyond experimental data?

As discussed, experimental data show that distinct patterns of multiple hormones exist simultaneously. These patterns do not emerge independently. Thus, how patterns of multiple hormones are related to each other is an important question, especially since 'relative' hormone concentrations have developmental outcomes [31]. However, although experimental data can show the coexistence of patterning for multiple hormones, it is difficult to elucidate experimentally how this simultaneous patterning emerges. To this end, modeling hormone crosstalk can develop new insights, as demonstrated by modeling simultaneous auxin and cytokinin patterning arising from crosstalk with ethylene [30].

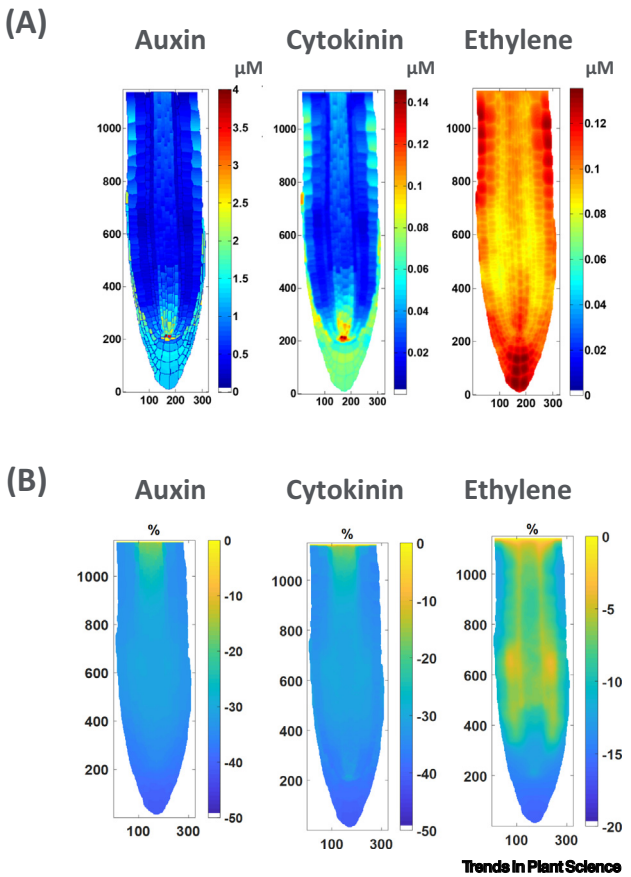

Figure 1. Modeling regulation of auxin biosynthesis by cytokinin and ethylene and the emergence of auxin, cytokinin, and ethylene patterning. (A) Auxin, cytokinin, and ethylene patterning in the absence of regulation of auxin biosynthesis by cytokinin or ethylene. (B) Percentage change of auxin, cytokinin, and ethylene concentration in the absence of regulation of auxin biosynthesis by cytokinin or ethylene relative to the respective counterpart in the presence of regulation of auxin biosynthesis by cytokinin or ethylene. The details for the hormonal crosstalk model and parameters used are described in [30].

Is regulation of auxin biosynthesis by cytokinin and ethylene required for the emergence of simultaneous patterning of auxin, cytokinin, and ethylene?

Experimental observations reveal that auxin biosynthesis is positively regulated by both cytokinin [21,32] and ethylene [3,17–20,33,34]. Modeling hormonal crosstalk can integrate metabolism, transport and signaling of auxin, cytokinin, and ethylene into an integrative system [2,30]. Since regulation of auxin biosynthesis by cytokinin and ethylene is a part of the crosstalk between the three hormones, modeling crosstalk can develop further insights into whether the regulation of auxin biosynthesis by cytokinin and ethylene patterning is necessary for the simultaneous patterning of auxin, cytokinin, and ethylene.

Figure 1A shows that an auxin pattern still emerges, albeit with reduced auxin concentrations, in the absence of regulation of auxin biosynthesis by cytokinin or ethylene, and when all auxin transporters are fixed as in the wild type [30]. This indicates auxin influx and efflux transporters are key players in generating auxin patterning. Moreover, both cytokinin and ethylene patterning still emerges. Thus, regulation of auxin biosynthesis by cytokinin and ethylene is not required for forming simultaneous qualitative patterning of auxin, cytokinin, and ethylene. However, Figure 1B shows that the regulation of auxin biosynthesis by cytokinin and ethylene quantitatively alters the percentage change in the concentration of auxin, cytokinin, and ethylene relative to the wild type in different cells. This implies that, although regulation of auxin biosynthesis by cytokinin and ethylene is not required for the emergence of patterning for all three hormones, it does quantitatively and nonlinearly change the cellular concentrations of all three hormones in the root tip, in particular (and important developmentally) it modifies their relative concentrations. By considering that plant responses to auxin concentrations in different cells are threshold-dependent and that auxin concentrations must be finely regulated during plant growth [35], regulation of auxin biosynthesis by cytokinin and ethylene can play important roles in plant growth due to its role in changing both absolute and relative concentrations of all three hormones. Thus, modeling hormonal crosstalk has revealed two aspects about the regulation of auxin biosynthesis by cytokinin and ethylene. On the one hand, this regulation is important for regulating the concentrations of auxin, cytokinin, and ethylene, which is compatible with experimental observations. On the other hand, this regulation is not required for the emergence of the simultaneous qualitative patterning of auxin, cytokinin, and ethylene, demonstrating that modeling hormonal crosstalk develops novel insights into how regulation of auxin biosynthesis is related to the emergence of the patterning of auxin, cytokinin, and ethylene.

Since modeling can analyze the patterning relationship between auxin, cytokinin, and ethylene in the presence or absence of the regulation of auxin biosynthesis by cytokinin and ethylene, as summarized in Figure 1, it is also able to analyze the role of any components relating to the three hormones. For example, transporters for cytokinin and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), though not for the diffusible gas ethylene, have been experimentally identified [36,37]. Thus, since auxin, cytokinin, and ethylene form a crosstalk network, a further question about the patterning relationship between auxin, cytokinin, and ethylene is how those transporters (although they do not transport auxin directly) regulate the simultaneous patterning of these three hormones. The methodology developed for modeling hormonal crosstalk [2,30,38,39] should be able to address the question when the cellular locations of cytokinin and ACC transporters are experimentally determined.

In addition, other hormones that also have transporters interact with auxin, cytokinin, and ethylene [36,37]. By developing crosstalk networks and by integrating their metabolism with transport, further research should be able to establish the patterning relationship among auxin, cytokinin, ethylene, and other hormones.

How does the patterning of specific PINs change in other *pin* mutants, and what role does this play in patterning auxin, cytokinin, and ethylene?

It has been shown experimentally that the patterning of PIN1 changes in the *pin3*, *pin4*, or *pin7* single mutants [40]. Experimental observations also show changes in PIN2 patterning in the *pin3 pin4 pin7* triple mutant, where a clear increase in PIN2 level in the vasculature emerges [41]. Modeling the crosstalk between auxin, cytokinin, and ethylene reveals that changes in PIN1 or PIN2 patterning in various *pin* mutants may be explained by regulation of PIN1 or PIN2 metabolism and translocation by auxin, cytokinin, and ethylene [30]. Since PIN1 or PIN2 patterning changes in various *pin* mutants, the question remains as to what role this change plays in patterning auxin, cytokinin, and ethylene. Modeling hormonal crosstalk could develop insights into this question [30].

Figure 2A shows that the modeled change in PIN2 patterning for the *pin3 pin4 pin7* triple mutant predicts a significant increase in PIN2 concentrations in the vasculature, which is consistent with experimental observation [41]. In addition, in the *pin3*, *pin4*, and *pin7* single mutants, the region of the modeled PIN1 expression extends shootward up the vasculature [30]. This is also similar to experimental observations [40]. Although both experimental data and modeling results show that the patterning of PIN1 and PIN2 changes for the *pin3*, *pin4*, and *pin7* single mutants and for the *pin3 pin4 pin7* triple mutant, only modeling can further develop the role of such changes in the patterning of auxin, cytokinin, and ethylene. For example, Figure 2B–D shows that the change in patterning of PIN1 and PIN2 in the *pin3 pin4 pin7* triple mutant results in more auxin, cytokinin, and ethylene accumulation in the cells in the vasculature and the columella initial cells [30], even though PIN1 and PIN2 do not themselves transport cytokinin or ethylene. This implies that effects of these gene mutants are not restricted to a simple gene–protein relationship, but they are epistatic and influence the whole hormonal crosstalk network. Since auxin, cytokinin, and ethylene all regulate gene expression, the effects of a gene mutant (e.g., *pin3 pin4 pin7* triple mutant) can potentially have multifaceted roles in root development via the actions of multiple hormones.

It has been shown experimentally that patterning of auxin, cytokinin, and ethylene and patterning of auxin efflux transporters (PINs) are mutually regulated [41–45]. Modeling hormonal crosstalk

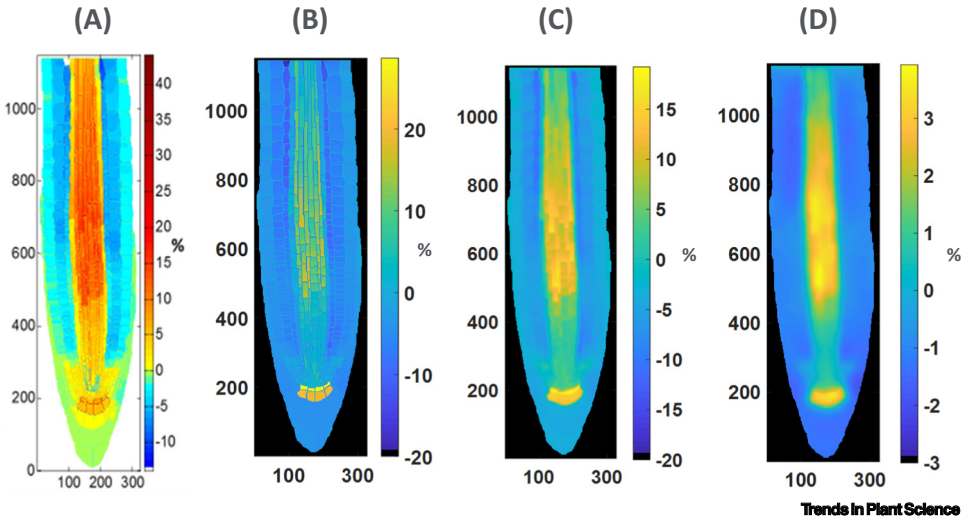

Figure 2. Modeling PIN1 or PIN2 patterning changes in the *pin3,4,7* triple mutant linked to patterning of auxin, cytokinin, and ethylene. (A) PIN1 or PIN2 patterning change in *pin3,4,7* mutants. (B–D) Percentage change of auxin (B), cytokinin (C), and ethylene (D) patterning relative to their respective counterpart for PIN1 or PIN2 patterning to remain at wild-type levels. The details for the hormonal crosstalk model and parameters used are described in [30].

can develop more insights into this mutually regulated relationship beyond experimental data alone, as demonstrated in Figure 2.

How is gene expression patterning related to hormonal crosstalk?

As mobile signals, all hormones in arabidopsis regulate gene expression spatiotemporally [46–48]. Since multiple hormones form complex crosstalk networks and regulate gene expression either synergistically or antagonistically, modeling hormonal crosstalk is required to elucidate the relationship between gene expression and the actions of multiple hormones.

Ethylene is synthesized from its precursor ACC by the enzyme ACC-oxidase (ACO) [49]. Figure 3 shows patterning of the ethylene biosynthesis rate predicted by modeling the crosstalk between auxin, cytokinin, and ethylene [30]. When the simultaneous patterning of auxin and cytokinin is studied in the context of the crosstalk between auxin, cytokinin, and ethylene [30], neither ethylene concentration patterning nor the ethylene biosynthesis rate patterning could be compared with experimental observations because of a lack of experimental data. However, the tissue-specific expression pattern of the five ACO members of arabidopsis has recently been determined experimentally [50]. The predicted patterning of ethylene biosynthesis rate in Figure 3 compares favorably with experimental observations.

Modeled ethylene biosynthesis rate is high in the root tip, including the columella, root cap, and QC (Figure 3). In the central region of the transition and elongation zones, the ethylene biosynthesis rate is also relatively high. These predictions are qualitatively similar to the experimental observations for the tissue-specific expression pattern of the five ACO members [50]. Since ethylene

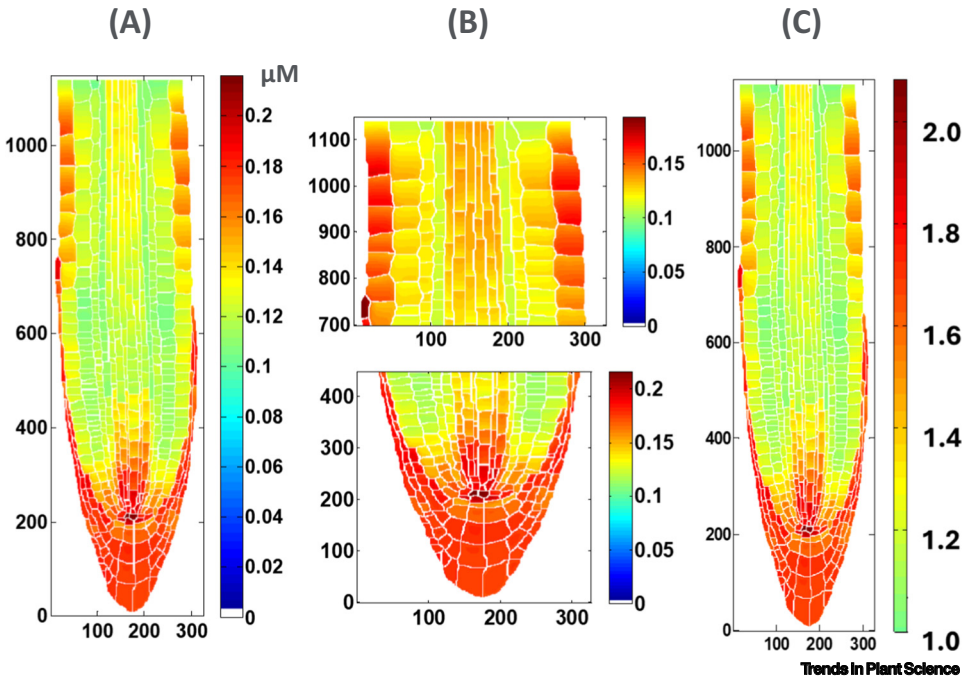

Figure 3. Predicted ethylene biosynthesis rate patterning. Modeled data are qualitatively similar to the experimental observations for the tissue-specific expression pattern of the five 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (ACO) members, as observed by Houben *et al.* [50]. (A) Predicted ethylene biosynthesis rate patterning. (B) Zoom-in of A shows the details of predicted ethylene biosynthesis rate patterning. (C) Relative ethylene biosynthesis rate to quantitatively show the range of ethylene biosynthesis rate. The details for the hormonal crosstalk model and parameters used are described in [30].

biosynthesis is catalyzed by the five ACOs, it is reasonable to consider that the ethylene biosynthesis rate correlates with the tissue-specific expression pattern of the ACOs. Thus, modeling the crosstalk between auxin, cytokinin, and ethylene naturally and logically explains the experimental observations of the expression pattern of the ACOs. Furthermore, modeling reveals that emergence of the tissue-specific expression pattern of the five ACO members [50] is due to the regulation of ethylene biosynthesis by both auxin and cytokinin [30]. However, there is a noticeable discrepancy between modeling prediction and experimental observations in the epidermal cells of the transition and elongation zones. The modeled high ethylene biosynthesis rate in the epidermal cells in these zones is not similar to experimental observations for the tissue-specific expression pattern of the five ACO members [50]. This discrepancy may indicate that the equation for modeling the ethylene biosynthesis rate [30] may need refining and expanding further. In particular, the kinetic parameters of the five ACOs are significantly different [50], and cytokinin differentially regulates the activity of the five ACOs [24]. The hormonal crosstalk model [30] simplified the ethylene biosynthesis rate equation into a single kinetic equation. In addition, Figure 3C shows quantitative fold-change of ethylene biosynthesis rate in the root. It shows that the ethylene biosynthesis rate in the QC, root cap, and columella cells is approximately twice that in cortex and pericycle cells at the transition zone. However, to elucidate how this quantitative fold-change can be compared with the fold-change of the protein levels of the five ACO members described in [50] requires further model development to explicitly include the five proteins [30]. Importantly, in addition to its ability to predict gene expression patterns, modeling hormonal crosstalk is able to identify knowledge gaps that need to be explored in the future. Figure 3 demonstrates that modeling hormonal crosstalk can be a powerful tool for predicting gene expression patterning because modeling the crosstalk between auxin, cytokinin, and ethylene naturally explains the expression pattern of the ACOs.

In addition, POLARIS (PLS) represents a crosspoint at which ethylene and auxin interact [51]. The expression of the *PLS* gene is upregulated by auxin and downregulated by ethylene [52]. Since auxin, cytokinin, and ethylene form a crosstalk network [2,30] in which PLS regulates the crosstalk of the three hormones, while *PLS* gene expression is also regulated by auxin and ethylene, modeling of hormonal crosstalk can be used to analyze how changes in the biosynthesis or degradation of auxin, cytokinin, or ethylene can lead to the change in *PLS* expression, as demonstrated in Figure 4.

Figure 4A shows a distinct but limited change in *PLS* expression when auxin biosynthesis is not regulated by cytokinin or ethylene. This limited change reflects the fact that auxin and ethylene antagonistically regulate *PLS* expression. If auxin biosynthesis is modeled to be independent of cytokinin or ethylene (Figure 4A), auxin concentration is reduced, which in turn plays a role in reducing ethylene concentration since ethylene biosynthesis is positively regulated by auxin [23,53,54]. The reductions in auxin and ethylene concentration play antagonistic roles in *PLS* expression: reducing auxin concentration decreases *PLS* expression, while reducing ethylene concentration enhances *PLS* expression. Thus, the simultaneous reduction in auxin and ethylene concentrations leads to limited change in *PLS* expression. The distinct change in *PLS* expression in Figure 4A reflects the fact that the regulation of *PLS* expression by auxin and ethylene is nonlinear. This demonstrates that modeling the crosstalk of auxin, cytokinin, and ethylene is able to analyze the antagonistic effects of auxin and ethylene on *PLS* expression.

Similarly, Figure 4B,C also shows distinct but limited change in *PLS* expression if ethylene biosynthesis rate is not regulated by auxin or cytokinin, or if cytokinin degradation is not regulated by the ethylene signaling pathway. Thus, modeling hormonal crosstalk between auxin, cytokinin, and ethylene can be used to elucidate the dependence of a gene's expression (in this case *PLS*) on

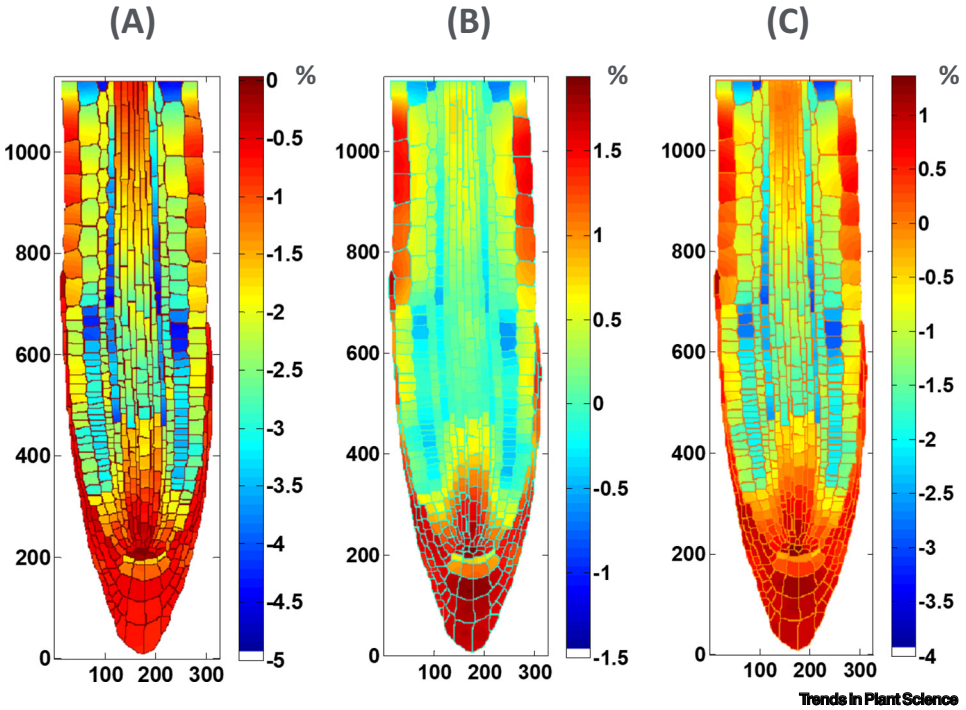

Figure 4. Modeling analysis of the effects of auxin, cytokinin, and ethylene crosstalk on POLARIS (*PLS*) gene expression. Percentage change of *PLS* gene expression relative to its expression in wild type for the following three cases. (A) Auxin biosynthesis is regulated by neither cytokinin nor ethylene. (B) Ethylene biosynthesis is regulated by neither auxin nor cytokinin. (C) Cytokinin degradation is not regulated by the ethylene signaling pathway. The details for the hormonal crosstalk model and parameters used are described in [30].

the biosynthesis or degradation of auxin, cytokinin, and ethylene. Since the percentage change in Figure 4 is generally small, whether or not this modeled output can be experimentally tested depends on the resolution of experimental measurements. However, the small percentage change in Figure 4 reflects the fact that auxin and ethylene antagonistically regulate *PLS* expression. This may imply that, when two hormones (e.g., auxin and ethylene) antagonistically regulate gene expression (e.g., *PLS*), gene expression level may be insensitive to changes in the biosynthesis or degradation rate of the two hormones. Whether or not this kind of trade-off also exists in the expression of other genes remains to be investigated.

The two examples shown in Figures 3 and 4 demonstrate that, when gene expression (such as for *ACO* or *PLS*) is regulated by multiple hormones, hormone crosstalk modeling is required to elucidate how each hormone, directly or indirectly, regulates such expression, since the concentrations of multiple crosstalk hormones such as auxin, cytokinin, and ethylene cannot change independently.

The examples summarized in Figures 1–4 show that modeling hormonal crosstalk can develop further novel insights into crosstalk between auxin, cytokinin, and ethylene beyond experimental observations alone. In principle, the network for crosstalk of all hormones can be established following the accumulation of experimental data.

Can one hormonal crosstalk model integrate all experimental data for elucidating root growth?

A wide range of experimental data on hormones is available in the literature. It is evident that the pace of accumulation of such data is accelerating. However, due to the complex interplay

between multiple hormones in root growth, many outstanding questions remain to be addressed (see Outstanding questions). For example, a common assumption is that natural free auxin (IAA) is synthesized in the meristematic region of the arabidopsis root [17]. However, based on the enzyme parameters and tryptophan concentration, it is also hypothesized that auxin biosynthesis may be located mainly in dying cells such as in the root cap [55]. Therefore, how is auxin patterning regulated if auxin biosynthesis varies between different cells? In addition, it has been demonstrated experimentally that auxin produced in shoots is insufficient to support root development if *YUC3*, *YUC5*, *YUC7*, *YUC8*, and *YUC9* auxin biosynthesis genes are inactivated in the root [56]. What, therefore, is the relationship between auxin patterning in the root and auxin production in the shoot? To address these questions as an integrative system of plant root development, modeling hormonal crosstalk provides a valuable methodology.

Previous mathematical modeling of the roles of auxin transport and biosynthesis in auxin patterning in arabidopsis root has overlooked the role of auxin biosynthesis sites in auxin patterning, and so the quantitative relationship between auxin patterning in the root and auxin production in the shoot remains elusive. Grieneisen *et al.* [57] showed how auxin transport alone could generate robust auxin gradients in roots with non-local sources of auxin. They concluded that 'auxin transport is sufficient to generate a maximum and gradient guiding root growth'. Band *et al.* [43] developed models by manually assigning auxin biosynthesis rates, assuming that minor biosynthesis and degradation of auxin take place in all root cells while higher auxin biosynthesis rates occur in the QC and columella initials. If locally synthesized auxin is removed from these models, auxin patterning is not qualitatively affected [43], which would indicate that local auxin biosynthesis is not an important auxin source in the root. Moreover, Mellor *et al.* [58] developed a model to examine the role of plasmodesmata in auxin patterning in the arabidopsis root without exploring the role of auxin biosynthesis. In a recent spatiotemporal hormonal crosstalk model developed by us [30], the regulation of auxin biosynthesis by ethylene and cytokinin is thoroughly investigated, and the emergence of simultaneous auxin and cytokinin patterns in the arabidopsis root is examined in the context of the crosstalk between auxin, cytokinin, and ethylene; however, the model did not consider the role of tryptophan concentration [55] in auxin biosynthesis and patterning. Since tryptophan is a precursor of the TAA–YUC pathway [59], auxin biosynthesis should be examined in the context of tryptophan distribution in the arabidopsis root [55] in the future.

Importantly, auxin biosynthesis is regulated by other hormones such as cytokinin and ethylene; therefore, auxin biosynthesis forms an important crosstalk intersection between auxin and other hormones. Since existing models have not properly integrated the roles of auxin biosynthesis into auxin patterning in the root, further modeling development is required to explore how the regulation of auxin biosynthesis by other hormones plays its role in auxin patterning in the root. Thus, hormonal crosstalk needs to be further expanded and explored. Since all experiments relating to arabidopsis root development use wild types as reference points, integrating all experimental data to develop an *in silico* wild-type root is possible. Such an *in silico* root would be a valuable platform for developing deeper understanding of the roles of multiple hormones in root development.

Here we propose that – in order to develop deeper insights into the functions of multiple hormones in root development than those that can be gained from experimental data alone – one hormonal crosstalk model integrating all experimental data relating to all hormones, as summarized in Figure 5, should be developed by closely combining experimental data and modeling.

Root images and other experimental data can be used to construct a hormonal crosstalk network. It has been demonstrated that the crosstalk network for auxin, cytokinin, and ethylene

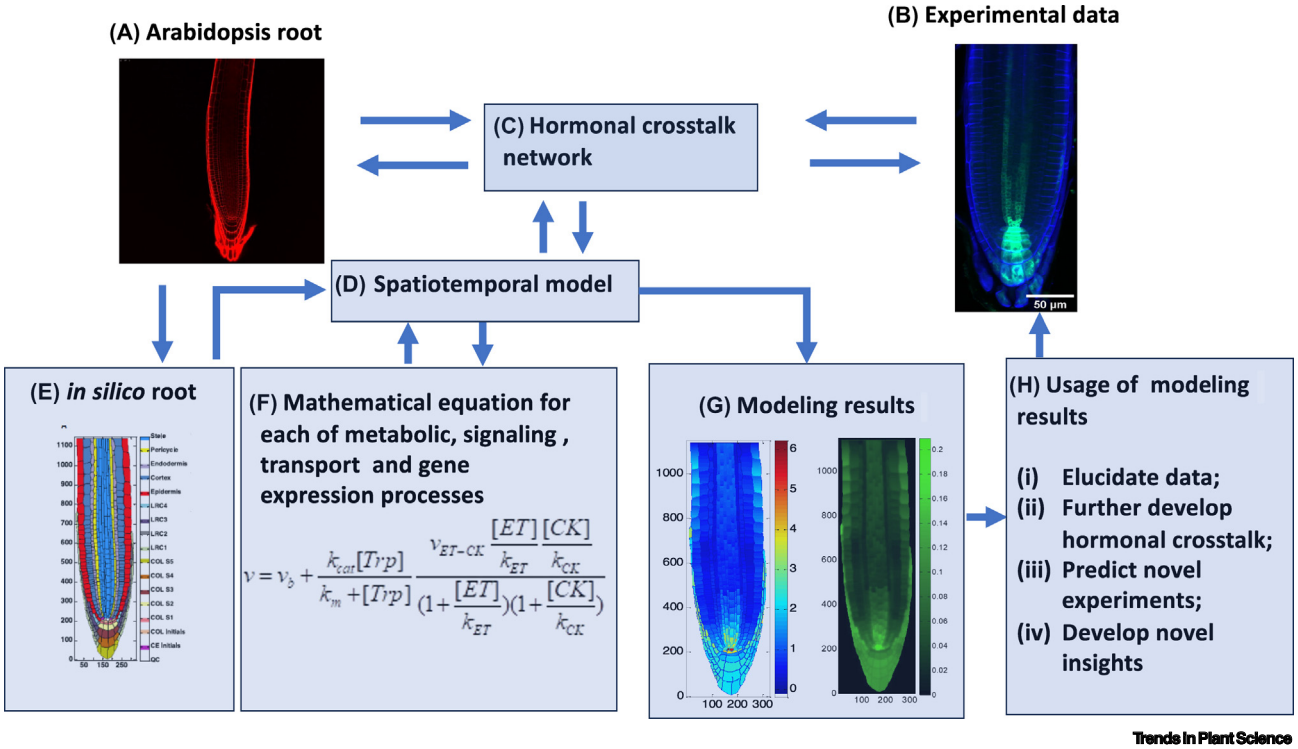

Figure 5. A schematic description of the development of one hormonal crosstalk model for integrating all experimental data. Experimental data (A,B) are used to construct and validate spatiotemporal model of hormonal crosstalk and to predict experimental outcomes (C–H). Modeling results are used to design novel experiments for further improving model development and for developing insights into the functions of multiple hormones (H). Panel (E) is reproduced from [2] with permission.

can be constructed by integrating and interrogating a variety of experimental data [2,30]. The regulatory relationships as described by hormonal crosstalk can be used to derive kinetic equations for each of the metabolic, signaling, transport, and gene expression processes. The combination of kinetic equations with an *in silico* root structure allows the study of the spatiotemporal dynamics of all components in the hormonal crosstalk system. For example, this approach can reveal how auxin and cytokinin patterns can emerge simultaneously [30]. The modeled spatiotemporal dynamics of multiple hormones, multiple transporters, and gene expression can be used to (i) elucidate experimental data, (ii) further develop the hormonal crosstalk network by comparing the similarities of experimental and modeling results, (iii) predict new experiments for further experimental validation, and (iv) develop mechanistic insights into the relationships between multiple hormones such as the role of PIN1 or PIN2 patterning change in the *pin3,4,7* triple mutant for patterning auxin, cytokinin, and ethylene (Figure 2).

Each hormone has its own transport, biosynthesis, and inactivation pathways. Thus, crosstalk among multiple hormones occurs at all levels of gene expression, signal transduction, and metabolism. Modeling crosstalk between multiple hormones needs to integrate these different regulatory components. Therefore, a crosstalk model is a multilevel model that integrates signaling, transport, gene expression, and metabolism, and can be analyzed as an integrative system. Moreover, depending on the research objectives, different modules of the model can also be extracted at different levels. For example, metabolism of cytokinin and ethylene has been investigated using kinetic modeling [60,61]. Metabolism of auxin and cytokinin has also been studied using constraint-based modeling [62]. If a researcher is interested in how the concentration of

auxin, cytokinin, and ethylene is regulated without exploring signaling, transport, or gene expression processes, Figure 6A shows an example of how to model the crosstalk of these three hormones at a metabolic level in a homogeneous cell. Figure 6A shows that (i) the biosynthesis or degradation of each auxin, cytokinin, or ethylene is regulated by the other two hormones based on a variety of experimental observations [3,17–24]; (ii) the concentration of auxin,

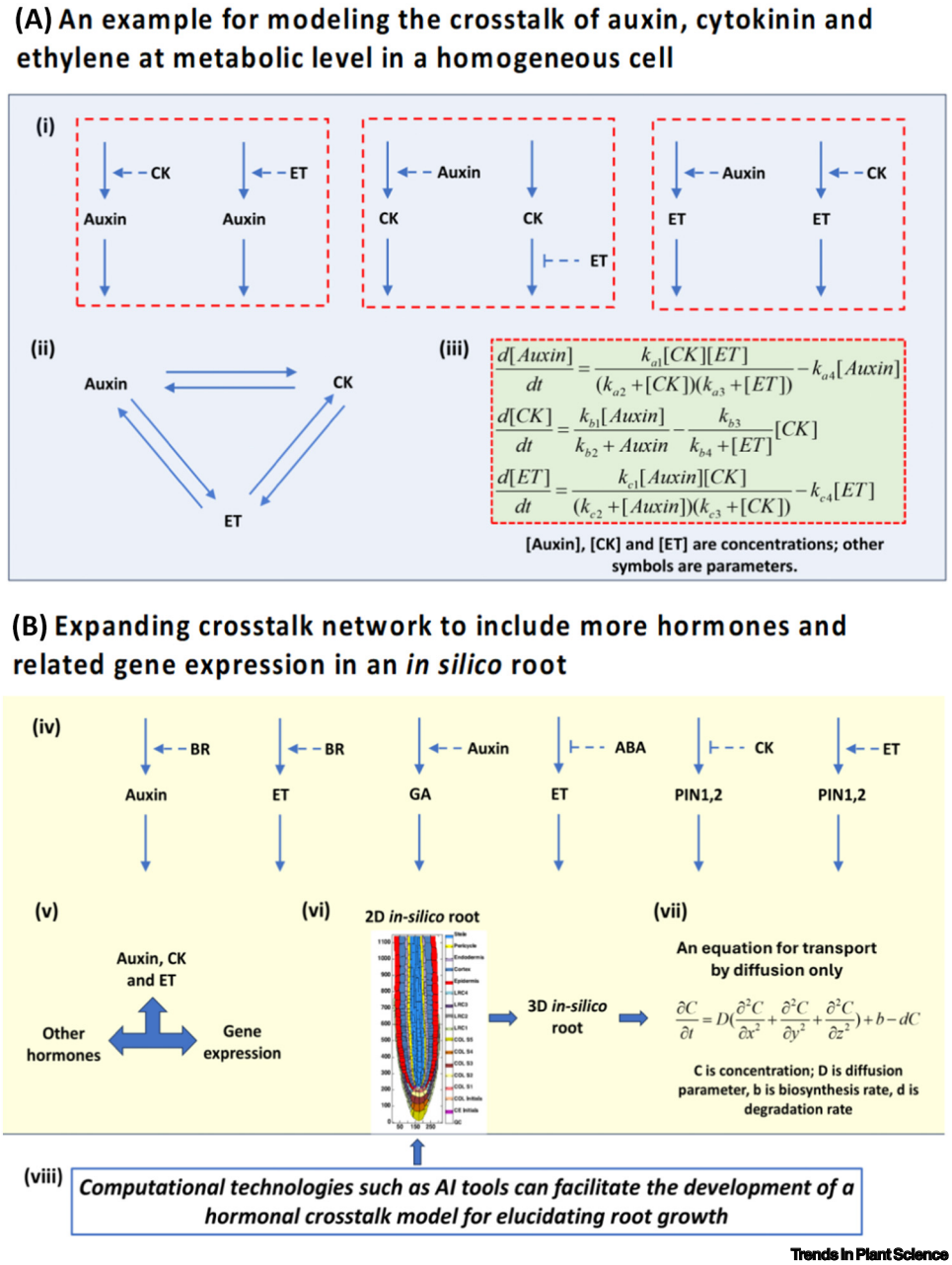

Figure 6. (A) An example for modeling the crosstalk of auxin, cytokinin, and ethylene at the metabolic level in a homogeneous cell. (B) Expanding crosstalk network to include more hormones and related gene expression in an *in silico* root. The details of Figure 6 are described in the text. Abbreviations: ABA, abscisic acid; AI, artificial intelligence; BR, brassinosteroid; CK, cytokinin; ET, ethylene; GA, gibberellic acid. Panel (vi) is reproduced from [2] with permission.

cytokinin, and ethylene is mutually regulated; and (iii) mathematical equations can be used to study the dynamics of the three hormones. Since Figure 6A describes only the regulation at a metabolic level, it is a minimal integrative model for describing the crosstalk of the concentration of the three hormones. Figure 6B then shows how to expand a minimal crosstalk network to include more hormones and related gene expression in an *in silico* root. Figure 6B describes how (i) gene expression of auxin transporters and other hormones can be integrated into the metabolic crosstalk network between auxin, cytokinin, and ethylene [25,33,53,63–65]; (ii) the concentrations of all hormones and the components of all related gene expression can be integrated into an integrative system; (iii) an *in silico* root can be constructed; (iv) mathematical equations can be used to study the spatiotemporal dynamics of all hormones and all components for related gene expression; and (v) computational technologies such as AI tools can be used to facilitate the development of a single hormonal crosstalk for all hormones to elucidate plant growth, as discussed later.

Plant hormonal signaling has been extensively studied using several different models ([28,29] and references therein). Responses of various genes to hormonal signals have been modeled [22,25,33,53,60–83]. These models have been extensively used to study many aspects of hormonal crosstalk. Integrating existing segmented models together would be important for further developing a single integrative model for hormonal crosstalk.

Moreover, multiple hormones work together to regulate a complex network with a large number of interacting proteins [84]. In principle, this kind of protein network can be integrated into a hormonal crosstalk network since the protein network is itself regulated by multiple hormones. In addition, the structural aspects of the core nuclear auxin signaling components (TIR1/AFBs, Aux/IAAs, and ARFs) have been extensively studied [84–92]. Furthermore, the structures of three PIN-formed (PIN) proteins PIN1, PIN3, and PIN8 reveal their mechanism of auxin efflux [89–91]. This important progress combined with other advances in auxin biology provides a platform for a more comprehensive understanding of the systems biology of auxin [92]. Future development of an integrative hormonal crosstalk model can incorporate this knowledge to develop further insights into the roles of those protein structures in hormonal crosstalk in the arabidopsis root, likely unachievable by experiment alone (see Outstanding questions).

Advances in experimental and computational technologies can facilitate the development of a single hormonal crosstalk model for elucidating root growth

Development of one hormonal crosstalk model for elucidating root growth requires the integration of a wide range of interacting processes, including signaling, transport, gene expression, and metabolic processes, all in the context of an accurate *in silico* root construct. Advances in experimental and computational technologies can facilitate the development of such a model.

In recent years, a wide range of live imaging techniques for plant hormones – including the imaging of the transcriptional output of hormones and the measurement of hormonal input – have been developed [93]. Moreover, multiscale microscopy can reveal plant cell structure [94], and mass spectrometry technologies have progressed to quantify multiple hormones, although the cellular concentrations of hormones are usually very low [95]. Thus, hormone metabolism and signaling can be experimentally investigated in the context of genome-scale metabolism [96]. Therefore, advances in experimental technologies open opportunities to acquire a multitude of diverse experimental data.

However, bridging the data to the development of one integrative hormonal crosstalk model for elucidating root growth requires meeting the challenges of managing large and complex data

sets. AI possesses powerful computational technologies, offering the possibility to build a virtual cell [97]. Thus, there are multiple avenues by which AI can facilitate the development of one hormonal crosstalk model by iteratively combining experimental data with all aspects of modeling development, as schematically summarized in Figure 5.

First, due to the complexity of cell morphology and connectivity in plant tissues in the arabidopsis root, construction of an *in silico* root requires AI to explore morphological features and 3D cell connectivity (see Outstanding questions). It has been shown that construction of a cell atlas of plant tissues can be automated [98]. AI can assist image processing to enable semi-automated quantification of cell division and elongation dynamics at the tip of the arabidopsis root [99].

Second, current development of a hormonal crosstalk network relies on (i) manually interrogating and integrating biological knowledge in the literature, and (ii) combining the knowledge with novel experimental measurements in the laboratory [2,30]. Furthermore, AI-related computational approaches can integrate 'omics' data to develop large-scale models of signaling networks [100], and so they can be used to facilitate the construction of a crosstalk network for all hormones.

Third, development of a spatiotemporal model for hormonal crosstalk requires the formulation of kinetic equations for all processes involved in the crosstalk. Moreover, the equations need to be parameterized. Previously, we have shown that Bayesian uncertainty analysis, one of the important technologies in AI, is able to comprehensively explore a 32-dimensional parameter space for a hormonal crosstalk network without spatial setting [101,102]. It is anticipated that one integrative hormonal crosstalk model that integrates all hormones will have far more than 32 parameters. However, with the computational power of AI following established methods [100–102], exploring a parameter space with a large number of parameters should be possible. In addition, AI can automate many, if not all, model development processes. Previously, we demonstrated a recovery principle so that auxin transporter patterning can be searched based on a targeted auxin patterning [39]. If the process for pattern searching is automated using AI, it is anticipated that the relationship between patterns of hormones (e.g., auxin and cytokinin) and their metabolism, as well as transport, can be more thoroughly and logically investigated within one integrative system.

Concluding remarks

The outputs of a single integrative hormonal crosstalk model can be diverse since the model includes signaling, transport, gene expression, and metabolic processes of all hormones. Examples of these outputs can include hormone concentration and patterning (e.g., Figure 1), transporter concentration and patterning (Figure 2), and gene expression patterning (Figure 3). Experimental technologies – such as live imaging techniques [93], multiscale microscopy [94], mass spectrometry [95] and 'omics' data [100] – can be used to measure diverse components. The measurements will be compared with model outputs, and AI technologies used to facilitate the interaction between experiments and modeling.

In addition, due to the complexity of hormonal crosstalk in the arabidopsis root, it is anticipated there will be major challenges in each step of developing one integrative hormonal crosstalk model (Figure 5). For example, each of the multi-omics datasets is inevitably noisy. How AI could help to build one hormonal crosstalk model in the context of noisy data will be a major challenge. Furthermore, since hormonal crosstalk is involved in all levels of metabolism, signaling, and transport, it is inevitable that experimental measurements cannot reveal all components involved. Thus, how to deal with missing links will be another challenge. There is a wide diversity of experimental data currently available in the literature, and additional novel data are being generated; therefore, remaining questions to be tackled include how to use computational technologies

Outstanding questions

How is auxin patterning regulated if auxin biosynthesis varies between different cells? Both tryptophan and gene expression play important roles in regulating auxin biosynthesis rate. The effects of both tryptophan concentration and gene expression level on auxin biosynthesis rate can be integrated into existing equations for modeling auxin biosynthesis. An in-depth understanding of auxin patterning requires further exploration of the quantitative role of auxin biosynthesis sites in auxin patterning.

What is the relationship between auxin patterning in the root and auxin production in the shoot? It is generally assumed that auxin transport plays a key role in auxin patterning in the arabidopsis root. However, experimental observations show that auxin shoot-to-root transport is insufficient to compensate for a reduction in the auxin biosynthesis rate in the root. Thus, how auxin shoot-to-root transport and auxin biosynthesis locally in the root work together to regulate auxin patterning need to be further explored. A hormonal crosstalk model can be used to investigate this by examining auxin patterning while varying shoot-to-root transport and local auxin biosynthesis, to determine whether they have distinct roles in auxin patterning.

What kinetic equations should be used to describe the interplay between all hormones at multiple levels? Development of one hormonal crosstalk model for elucidating root growth requires the integration of a wide range of interacting processes including signaling, transport, gene expression, and metabolic processes. Thus, regulation of a hormone by other hormones can occur at multiple levels in the hormonal crosstalk network. Therefore, kinetic equations need to be used to scrutinize the regulatory mechanisms identified by various experimental observations, and the mechanisms should be used to derive the equations.

How can we accurately construct a 3D digital root for modeling hormonal crosstalk? Experimental measurements of the morphological features and cell connectivity in the arabidopsis root provide a foundation for addressing this

such as AI to assess the reliability of those data, and then how to use reliable data to construct a hormonal crosstalk model. In addition to those AI-related challenges, developing one integrative hormonal crosstalk model may face further computational challenges. For example, a pattern of oscillating gene expression regulated by auxin can emerge in the arabidopsis root [75,103]. How such an emergent property is related to hormonal crosstalk during root development needs to be addressed, but the existing AI-related computational tools possess a limited power to deal with the biological mechanisms for generating complex dynamics. Thus, in order to unravel the biological mechanism underpinning the emergence of complex dynamics such as oscillating gene expression in the context of hormonal crosstalk, other computational tools need to be further explored.

Nevertheless, by iterating experimental measurements with the development of one integrative hormonal crosstalk model, the role of multiple hormones in arabidopsis root growth can be more clearly elucidated. For example, a classical hormonal crosstalk is that the auxin-to-cytokinin ratio controls plant development [104,105]. Once a single integrative hormonal crosstalk model is developed, relationships between all interconnected hormones can be established, within which the auxin-to-cytokinin ratio is only a subset. Thus, root growth can be further elucidated in the context of the relationships between multiple interconnected hormones combined into a single integrated system.

question. Experimental images need to be converted into a suitable *in silico* 3D root structure for modeling analysis, and AI-related computational tools can facilitate image analysis. This approach has already proved successful for a 2D root tip. While a 3D version will be more complex, requiring significantly more data points, it should still be possible to create an *in silico* 3D root based on experimental images.

How can we incorporate the protein network regulated by multiple hormones into a hormonal crosstalk network? Experimental and bioinformatic analysis about protein network regulation by multiple hormones provides a foundation for addressing this question. Since some proteins have enzymatic activities that regulate the metabolism of multiple hormones, the concentrations of proteins and hormones can be mutually regulated. A first step for integrating two networks could be to add known protein–hormone relationships to a hormonal crosstalk network.

How can we incorporate data such as PIN1, PIN3, and PIN8 structures into a hormonal crosstalk model? No modeling framework currently exists to integrate structural data with hormonal crosstalk. However, structural data of PIN1, PIN3, and PIN8 can shed light on how auxin transport through membranes is voltage-gated. Since auxin transport is a part of one integrative hormonal crosstalk network, structural data can be integrated into the hormonal crosstalk network. In addition, the structural data of hormonal receptor complexes would likely be important determinants of signal perception; they can also be integrated into hormonal crosstalk network by integrating their roles in signaling perception.

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Declaration of interests

The authors declare no conflicts of interest.

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