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Abstract:

Cellular patterning in the Arabidopsis root is coordinated via a localized auxin concentration maximum in the root tip, requiring the regulated expression of specific genes. The activities of plant hormones such as auxin, ethylene and cytokinin depend on cellular context and exhibit either synergistic or antagonistic interactions. Due to the complexity and nonlinearity of spatiotemporal interactions both of hormones and gene expression in root development, modelling plant hormone gradients requires a systems approach in which experimental data and modelling analysis are closely combined. Modelling therefore allows a predictive interrogation of highly complex and non-intuitive interactions between components in the system. Important factors to be considered when modelling hormone gradients include the construction of a hormonal crosstalk network, the formulation of kinetic equations, and the construction of an *in silico* root map. A modelling approach enables the analysis of relationships between multiple hormone gradients, predictions on how hormone gradients emerge under the action of hormonal crosstalk, and the prediction and elucidation of experimental results from mutant roots.

Key concepts:

- Patterning in Arabidopsis root development is coordinated via a localized auxin concentration maximum in the root tip, requiring the regulated expression of specific genes.
- The activities of plant hormones such as auxin, ethylene, cytokinin, abscisic acid, gibberellin and brassinosteroids depend on cellular context and exhibit either synergistic or antagonistic interactions.
- Auxin concentration and the associated regulatory and target genes are regulated by diverse interacting hormones and gene expression and therefore cannot change independently of the various crosstalk components in space and time.
- Other hormone concentrations, such as ethylene and cytokinin concentrations, and expression of the associated regulatory and target genes are also interlinked.
- Modelling plant hormone gradients requires a systems approach where experimental data and modelling analysis are closely combined.
- A hormonal crosstalk network describes the regulatory relationships between hormones and their associated genes.

- A kinetic equation can be formulated for any regulatory relationship following thermodynamic and kinetic principles.
- Construction of an *in silico* root map enables the study of multicellular cell-cell communications in Arabidopsis root development.
- Modelling hormone gradients enables the analysis of relationships between multiple hormone gradients, predictions on how hormone gradients emerge under the action of hormonal crosstalk, and the prediction and elucidation of experimental results from mutant roots.
- Modelling auxin gradients can also incorporate different mechanisms of polar auxin transport and the interaction of hormone gradients and root growth.

Key words: Arabidopsis; root development; hormone gradients; hormonal crosstalk; mathematical modelling; kinetics; *in silico* root map; gene expression; metabolic regulation; systems biology.

Introduction

Plants are sessile organisms and therefore they must adapt their growth and architecture to a changing environment. A major challenge in plant developmental biology is to understand how plant growth in different ecosystems is coordinated by interacting hormones and genes. Arabidopsis root development is an excellent model system for studying plant development (see also doi: 10.1002/9780470015902.a0020121.pub2). Experimental data on Arabidopsis root development, accumulated over many years, have shown that the complexity of the interactions between hormones and gene expression in the root is multi-faceted, with the following features. 1) the activities of hormones such as auxin, ethylene, cytokinin, abscisic acid, gibberellin and brassinosteroids depend on cellular context and exhibit either synergistic or antagonistic interactions (Garay-Arroyo *et al.*, 2012); 2) cellular patterning in the Arabidopsis root is coordinated via a localized auxin concentration maximum in the root tip, requiring the regulated expression of specific genes (Sabatini *et al.*, 1999); 3) auxin is directionally transported through plant tissues, providing positional and vectorial information during development (Vanneste and Friml, 2009; Adamowski and Friml, 2015); 4) auxin concentration and the associated regulatory and target genes are regulated by diverse interacting hormones and gene expression and therefore cannot change independently of the various crosstalk components in space and time (Garay-Arroyo *et al.*, 2012); 5) other hormone concentrations, such as ethylene and cytokinin concentrations, and expression of the associated regulatory and target genes are also interlinked (e.g. To *et al.*, 2004; Shi *et al.*, 2012); and 6) transport of other hormones, such as cytokinin, from the shoot to the root in the phloem (Bishopp *et al.*, 2011; Schaller *et al.*, 2015) in combination with local biosynthesis, degradation and diffusion, could also be an important factor affecting the interaction of hormones and gene expression in Arabidopsis root development.

Based on these and other experimental observations, it is clear that Arabidopsis root development and response to varying environmental conditions involves a complex network of overlapping interactions between plant signalling hormones and gene expression, collectively described as ‘hormonal crosstalk’ (Liu *et al.*, 2014; Moore *et al.*, 2015a). One of the important properties of hormonal crosstalk in root development is that a change in one signalling component leads to changes in other signalling components. Therefore, in order to understand the establishment and roles of plant hormone gradients in root development, important questions to address include how hormone concentrations and expression of their

associated regulatory and target genes are mutually related, and how patterning of both hormones and gene expression emerge under the action of hormonal crosstalk. Patterning of signalling gradients is functionally linked to patterning of gene expression and cellular organization.

Due to the complexity and nonlinearity of spatiotemporal interactions of both hormones and gene expression in root development, it is increasingly evident that mathematical modelling is a valuable tool for studying hormone gradients (Voß *et al.*, 2014; Liu *et al.*, 2014). This article reviews key aspects of modelling plant hormone gradients in Arabidopsis root development, with emphasis on combining modelling analysis with experimental data. Specifically, this article covers the construction of a hormonal crosstalk network based on experimental data; formulation of kinetic equations for all processes in the network; construction of an *in silico* root map to study multicellular cell-cell communications; and developing models of hormone gradients in the Arabidopsis root.

Construction of a hormonal crosstalk network based on experimental data

An important aspect of modelling hormone gradients is the construction of a hormonal crosstalk network based on experimental data. Such a network describes the regulatory relationships of hormones and expression of the associated regulatory and target genes. We use the interaction of three hormones (auxin, ethylene, and cytokinin) and expression of associated genes (e.g. *PIN1*, *PIN2*, *AUX1* and *ETR1*) as an example to discuss network construction.

In the root tip, the biosynthesis of auxin is activated by ethylene (Stepanova *et al*, 2007; Swarup *et al*, 2007; Ljung 2013), and can be inhibited by cytokinin (Nordstrom *et al.*, 2004, Jones *et al.*, 2011; Ljung, 2013). It is known that both auxin and cytokinin can synergistically activate the biosynthesis of ethylene (Vogel *et al.*, 1998; Stepanova *et al.*, 2007). However, ethylene can also be synthesized without exogenous auxin and cytokinin application, such as in its role in root hair production (Tanimoto *et al.*, 1995). In addition, it has also been shown that auxin can induce a rapid downregulation of cytokinin biosynthesis (Nordstrom *et al.*, 2004). This and other experimental evidence reveals that the metabolism of auxin, ethylene and cytokinin involves mutual regulation.

It is also known that auxin enters the cell both due to diffusion and by the action of influx carriers (AUX/LAX family; Bennett *et al.*, 1996; Band *et al.*, 2014; Péret *et al.*, 2012). Auxin leaves the cell via efflux carriers (PINs and PGPs; Petrasek *et al.*, 2006; Adamowski and Friml, 2015). In addition, experimental observation shows that ethylene may positively regulate AUX1 activity (Ruzicka *et al.*, 2007). The Arabidopsis genome contains eight PIN genes, and different PINs have different locations and may play different roles in auxin biology (Grunewald and Friml, 2010; Peer *et al.*, 2011). In addition, hormones may regulate PIN levels differentially. Auxin positively regulates levels of several PIN proteins (e.g. PIN1 and PIN2) in different developmental contexts (Chapman and Estelle, 2009). Ethylene also upregulates PINs (eg PIN2) to remove auxin from the more distal region of the root tip (Ruzicka *et al.*, 2007). In addition, cytokinin can negatively regulate levels of PIN1, PIN2 and PIN3, but positively regulates PIN7 levels (Ruzicka *et al.*, 2007). This and other experimental evidence reveals that the activities both of auxin influx and efflux carriers are regulated by hormones. Since the activities of auxin influx and efflux carriers in turn affect auxin distribution and concentration, the expression of auxin influx and efflux carriers and the concentrations of hormones are regulated by each other.

Experimental data accumulated over many years can be used to describe the regulatory relationships between hormones and expression of the associated regulatory and target genes. A regulatory network showing these relationships can be constructed and is termed the "hormonal crosstalk network" (Liu *et al.*, 2010; 2013; 2014; Moore *et al.*, 2015a). An example of a hormonal crosstalk network is shown in Figure 1, which describes the regulatory relationships between auxin, ethylene, cytokinin, PIN1, PIN2, AUX1 and the activity of other associated genes (Moore *et al.*, 2015a). This hormonal crosstalk network incorporates a variety of experimental data.

---Figure 1 here---

Formulation of kinetic equations for all processes in the hormonal crosstalk network

Another important aspect of modelling hormone gradients is the formulation of kinetic equations for all processes in the hormonal crosstalk network. Figure 2 schematically describes a methodology for formulating kinetic equations based on experimental data. In

principle, a kinetic equation can be formulated for any regulatory relationship (e.g. inhibition, activation or both) and for the interaction between any signalling or metabolic components (e.g. mRNA, protein, metabolite and effectors such as calcium ions). We use here the regulation of auxin metabolism by cytokinin to illustrate the application of this methodology.

---Figure 2 ---

Experimental data show that exogenous application of cytokinin may reduce the endogenous auxin concentration (Nordstrom *et al.*, 2004). Based on these data, we ask the following question: is the regulation of auxin concentration by cytokinin at the gene expression level, metabolic level, or both? Interrogation of other experimental data indicates that genes involved in auxin metabolism are differentially expressed in response to altered cytokinin levels and/or responsiveness to cytokinin in Arabidopsis (Jones, *et al.*, 2011). Thus, we may consider that regulation of auxin concentration by cytokinin is at the gene expression level. Then, we ask the following question: is it known by which process (or processes) this regulation at gene expression level occurs? Interrogation of experimental data may not clearly determine which process (biosynthesis, degradation or both) of gene expression is regulated. For example, it is unknown whether the transcriptional synthesis or post-transcriptional degradation of mRNA is regulated in this case. We can assume three possibilities for the regulation of gene expression: regulation of biosynthesis, degradation or both. To account for the negative regulation of auxin biosynthesis by cytokinin at the transcriptional level, we may derive the following kinetic equation for the rate of auxin biosynthesis, following thermodynamic and kinetic principles (Leskovac, 2003, Klipp *et al.*, 2009; Sauro, 2011).

$$v_b^{auxin} = \frac{k_{1b}}{1+\frac{[ck]}{k_{1b1}}}[S_0] \quad \text{(equation 1)}$$

where $[ck]$ is the cytokinin concentration and $[S_0]$ represents the concentration of the auxin precursor. k_{1b} and k_{1b1} kinetically describe the maximum auxin biosynthesis rate and the degree to which cytokinin negatively regulates auxin biosynthesis. Similarly, we can derive kinetic equations for the regulation of auxin degradation, or for the simultaneous regulation of auxin biosynthesis and degradation (Moore *et al.*, 2015b).

At this stage, we ask another question: does the regulation of auxin concentration by cytokinin also occur at the level of metabolic conversion, in addition to the regulation at the gene expression level? Experimental data may not provide that information. However, this does not necessarily mean that the regulation of auxin concentration by cytokinin only occurs at the gene expression level. For example, it is plausible that cytokinin-mediated transcriptional regulation may change the concentration of some other metabolites that can be the effectors of auxin metabolism. For this case, we need to derive a kinetic equation that includes the regulation at both gene expression and metabolic levels. If we assume that the regulation of auxin biosynthesis by cytokinin at the metabolic level follows an uncompetitive inhibition mechanism (Leskovac, 2003; Klipp *et al.*, 2009; Sauro, 2011), the kinetic equation for the regulation of auxin concentration by cytokinin at both the gene expression and metabolic levels can be described by equation 2.

$$v_b^{auxin} = \frac{k_{2b}}{1+\frac{[ck]}{k_{2b1}}} \frac{\frac{1}{1+\frac{[ck]}{k_{2b3}}}[S_0]}{\frac{k_{2b2}}{1+\frac{[ck]}{k_{2b3}}}+[S_0]} \qquad \text{(equation 2)}$$

where k_{2b} describes the maximum auxin biosynthesis rate. The term $\frac{k_{2b}}{1+\frac{[ck]}{k_{2b1}}}$ describes how the regulation of auxin biosynthesis by cytokinin at the gene expression level changes the maximum auxin biosynthesis rate by changing enzyme concentration. The term $\frac{1}{1+\frac{[ck]}{k_{2b3}}}$ describes how the regulation of auxin biosynthesis by cytokinin at the metabolic level changes the maximum auxin biosynthesis rate by inhibiting the conversion of the auxin precursor, S_0, into auxin. The term $\frac{k_{2b2}}{1+\frac{[ck]}{k_{2b3}}}$ describes how the regulation of auxin biosynthesis by cytokinin at the metabolic level changes the binding affinity between the auxin precursor, S_0, and the enzyme catalysing the conversion from auxin precursor to auxin.

Based on the above analysis, equation 1 should be used if the regulation of auxin concentration by cytokinin is at the transcriptional level only. However, equation 2 should be used if the regulation of auxin concentration by cytokinin occurs at both the transcriptional and metabolic levels. Thus, the formulation of kinetic equations must be based on experimental data.

When a process is regulated by multiple hormones, a kinetic equation can be derived based on experimental data, following thermodynamic and kinetic principles. For example, auxin biosynthesis can be stimulated by ethylene and inhibited by cytokinins (Nordstrom *et al.*, 2004; Ruzicka *et al.*, 2007; Stepanova *et al.*, 2007). Following thermodynamic and kinetic principles (Leskovac, 2003; Klipp *et al.*, 2009; Sauro, 2011), a kinetic equation for auxin biosynthesis that incorporates various regulatory relationships can be derived (Liu *et al.*, 2010; Moore *et al.*, 2015a; 2015b). Using the methodology described in Figure 2, kinetic equations for all processes in a hormonal crosstalk network (Figure 1) can be derived based on experimental data.

Construction of an *in silico* root map to study multicellular cell-cell communications in Arabidopsis root development

The combination of a hormonal crosstalk network (Figure 1) with the derived kinetic equations for all processes in the network (Figure 2) enables computational analysis of how the components in the hormonal crosstalk network depend on each other in a cell. In order to model hormone gradients in the root, we also need to describe how cell to cell communications occur in the root and at the shoot-root boundary. Thus, we need to construct an *in silico* root map. Figure 3 describes the root structure and the *in silico* root map, represented by a digital matrix.

---Figure 3 here---

The root structure describes a root which is 10 cells wide with 4 epidermal/cortical cell files, 2 pericycle cell files and 4 vascular cell files, with 3 distal tiers of columella cells. The root is 35 cells in length, including 3 tiers of columella cells, 12 tiers in the meristematic zone (MZ) and 20 tiers in the elongation zone (EZ). Cell wall thickness is 2 μm, and the width of all cells is 20 μm. Cell length varies along the longitudinal root axis. Cells in the columella and MZ regions are 28 μm long, including the cell walls. Cell length increases at the same rate from 28 μm at the proximal end of the MZ, by 15% per cell tier, to a length of 64 μm over 7

cell tiers into the EZ. The length of these 7 cell tiers was 28 μm, 32 μm, 36 μm, 42 μm, 48 μm, 56 μm and 64 μm, to give an overall root tip length of 1594 μm (Grieneisen *et al.*, 2007; Moore *et al.*, 2015a).

The root map (Moore *et al.*, 2015a) is a digital matrix. Each point in the root map has a code which describes the property of that point within the root and at the shoot-root boundary. For example, "0" in the root map indicates that this point is within the cytosol where hormones can be synthesized and degraded following the kinetic equations (Figure 2). "1", "2" or "3" in the root map indicate that this point is within the cell wall. However, the kinetic parameters for plasma membrane localisation of PIN1 or PIN2 at "1", "2" or "3" in the root map are different, leading to polarised PIN1 or PIN2 protein positions. Thus, the *in silico* root map describes how cell-cell communications occur (Moore *et al.*, 2015a).

Modelling hormone gradients to study the spatiotemporal dynamics of hormonal crosstalk in Arabidopsis root development

Combination of a hormonal crosstalk network (Figure 1), the derived kinetic equations for all processes in the network (Figure 2), and the *in silico* root map (Figure 3) enables modelling of hormone gradients in root development.

The first step in modelling hormone gradients in root development is the parameterisation of the hormonal crosstalk network (Figure 1) and the transport processes in the root map (Figure 3). Parameterisation is a challenging task, since there are many unknown parameters. We have proposed the following process for parameterisation (Liu *et al.*, 2010; Moore *et al.*, 2015a). First, the parameter values available in the literature should be used. For example, following experimental data, the diffusion coefficient for auxin is set to 220 $\mu m^2\ s^{-1}$ (Rutschow *et al.*, 2011), and the PIN efflux permeability is in the range of 0.5–5 $\mu m\ s^{-1}$ (Kramer *et al.*, 2011). Second, other unknown parameters should be adjusted to meet certain criteria derived from experimental observations. Once these criteria are met, these parameters for a wild type root should be used for model predictions. No further modifications of any parameter are allowed for the wild type root. Third, a number of different parameter sets (e.g. three) for defining the same wild type root should be compared, and they should lead to similar predictions. Fourth, the robustness of modelling results should be evaluated by changing unknown parameters. Following the above procedure, the hormonal crosstalk network (Figure 1) and the transport processes in the root map (Figure 3) can be parameterised (Moore *et al.*, 2015a).

After parameterising the hormonal crosstalk network (Figure 1) and the transport processes in the root map (Figure 3), the spatiotemporal dynamics of hormonal crosstalk in Arabidopsis root development can be studied and predicted. In principle, since the concentration of any component in the hormonal crosstalk network (Figure 1) at any location of the root map (Figure 3) can be calculated, the gradient of any component in the root (Figure 3) can also be calculated. Figure 4 shows the modelled gradients of auxin, ethylene, cytokinin, auxin efflux carriers PIN1 and PIN2, and auxin influx carrier AUX1 in a wild type Arabidopsis root. Since the gradient of each component can be calculated, the modelled gradient can be compared with its experimental counterpart (Moore *et al.*, 2015a). Analysis of the similarities or differences between the modelled and experimental gradients develops insights into how hormone gradients emerge as a result of the interactions of multiple hormones and their associated genes. Moreover, since the gradients of auxin, ethylene and cytokinin are calculated simultaneously, the relationships of the three hormones can be compared and understood as an integrated system. For example, Figure 4 shows that an auxin concentration maximum is established at or close to the QC while the ethylene concentration is relatively low in the same region. Thus, the signalling of both auxin and ethylene at or close to the QC can be simultaneously analysed based on the patterning of auxin and ethylene in this region.

---Figure 4 here---

Furthermore, since the concentration of any component in the hormonal crosstalk network (Figure 1) at any location in the root map (Figure 3) can be calculated, the concentration average of any component can be predicted for different cell types or tissues. Figure 5 shows predictions for auxin concentration profiles along the longitudinal root axis for three cell types and relative auxin concentration in different tissues in the wild type Arabidopsis root. The predictions of tissue-specific hormone concentrations can therefore be compared with their experimental counterparts. The mutual effects of auxin, ethylene and cytokinin (Figure 1) on their tissue-specific concentrations can also be analysed in detail.

---Figure 5 here ---

Arabidopsis mutant roots can also be analysed, since a mutant root simply corresponds to changes in the values of certain parameters of the wild type root. For example, the *pls* mutant

root can be studied by setting the transcription rate of the *PLS* gene to be zero. Mutant roots can be compared to a wild type root, and can also be compared to each other.

In summary, modelling hormone gradients under the action of hormonal crosstalk in a root enables the study of relationships of multiple hormones and their associated genes, the hormone gradients in different cell types or tissues, and the relationships of mutant and wild type roots. Novel insights can therefore be developed into how multiple hormones coordinate the regulation of root development, and so novel experiments can be designed. For example, modelling hormone gradients reveals how the gradients of PIN1 and PIN2 proteins in wild type and mutant roots are regulated by the *PLS* gene, providing new insights into its function (Moore *et al.*, 2015a).

Other important aspects of modelling hormone gradients

a. Mechanisms of polar auxin transport

Most PIN proteins have a polar cellular distribution, leading to directed auxin transport across only those plasma membranes where PIN proteins are localised (Blilou *et al.*, 2005). Our discussion so far has focused on the localisation of PIN1 and PIN2 proteins based on experimental observations. Many other modelling efforts have focused on the mechanisms of polar auxin transport. A recent excellent review has discussed those models and mechanisms (van Berkel *et al.*, 2013). These models can be divided into two main classes: flux-based and concentration-based models, and they can be used to study the mechanisms of polar auxin transport (van Berkel *et al.*, 2013). In general, these models are based on the assumptions that cells can sense the auxin of their neighbouring cells. However, it is difficult to establish the biological mechanisms whereby cells sense auxin levels in neighbouring cells. An alternative mechanism for tissue cell polarity is that intracellular partitioning can form the basis for generating polar auxin transport using bidirectional exchange of information between cells (Abley *et al.*, 2013). This mechanism does not require cells to sense the auxin in their neighbouring cells.

Although the study of mechanisms of polar auxin transport has predominantly focused on the relationship between auxin patterning and the localisation of PIN proteins (van Berkel *et al.*, 2013; Bennett *et al.*, 2014), it is evident that auxin influx carriers are also important for auxin patterning (Band *et al.*, 2014; Moore *et al.*, 2015a). The mechanistic roles of auxin influx

carriers in polar auxin transport should therefore be further examined, as experimental evidence shows that the distribution of AUX1 protein in the root is not uniform (Band *et al.*, 2014) and influx carriers contribute to the shoot vasculature (Fàbregas *et al.*, 2015).

b. **Interaction of growth and hormone gradients**

Recent studies have shown that growth and hormonal patterning can influence each other (De Rybel *et al.*, 2014; Mahonen *et al.*, 2014). It has also been shown that growth-induced hormone dilution can explain the dynamics of plant root cell elongation (Band *et al.*, 2012). In the root map shown in Figure 3, although root cell elongation (therefore root growth) has been incorporated, only a steady-state root structure has been considered (Moore *et al.*, 2015a). Furthermore, it has been shown that genetic control of plant development can override a geometric division rule in a 3-dimensional model of a growing embryo in early Arabidopsis embryogenesis (Yoshida *et al.*, 2014). This shows how genetic control plays a vital role in plant development. Since hormonal crosstalk describes the regulatory relationships of multiple hormones and associated genes, hormonal crosstalk therefore includes the key genetic information for root development. Therefore the combination of hormonal crosstalk with a 3-dimensional growing root should be able to further elucidate the relationships between multiple hormone gradients and growth.

Summary

Modelling plant hormone gradients requires a systems approach in which experimental data and modelling analysis are closely combined. Experimental data are used to construct a hormonal crosstalk network, to formulate kinetic equations, and to construct an *in silico* root map. Moreover, experimental data are also used to calibrate the spatiotemporal hormonal crosstalk network model, to analyse the relationships between multiple hormone gradients, to predict how hormone gradients emerge under the action of hormonal crosstalk, and to elucidate and predict experimental results for mutant roots. The combination of experimental and modelling analysis enables the study of plant hormone gradients as an integrated system, in which the causal relationships of all components can be established. To date, this approach has led to an improved understanding of, for example, how PIN proteins play a key role in auxin patterning and root growth (Grieneisen *et al.*, 2007); how asymmetric auxin gradients

link to gravitropsim (Band *et al*. 2012; see also doi: 10.1002/9780470015902.a0025267); how hormonal crosstalk influences the size of the root meristem (de Vos *et al*., 2014); and how this crosstalk can lead to the emergence of gene expression patterns (Liu *et al.* 2010; Moore *et al*. 2015a).

In principle, a hormonal crosstalk network can be extended to include any hormonal signalling components (Hill *et al.*, 2013; Naseem *et al.*, 2012); kinetic equations can include any degree of complexity in the regulatory relationships (Leskovac, 2003; Klipp *et al.*, 2009; Sauro, 2011); and construction of an *in silico* root map can include any level of detail within a 3-dimensional root structure (Barbier de Reuille *et al.*, 2015, Yoshida *et al.*, 2014). In addition, modelling auxin gradients can also incorporate different mechanisms of polar auxin transport (van Berkel *et al.*, 2013) and the interaction of hormone gradients and root growth (De Rybel *et al.*, 2014; Mahonen *et al.*, 2014).

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Further Reading

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Figure Legends

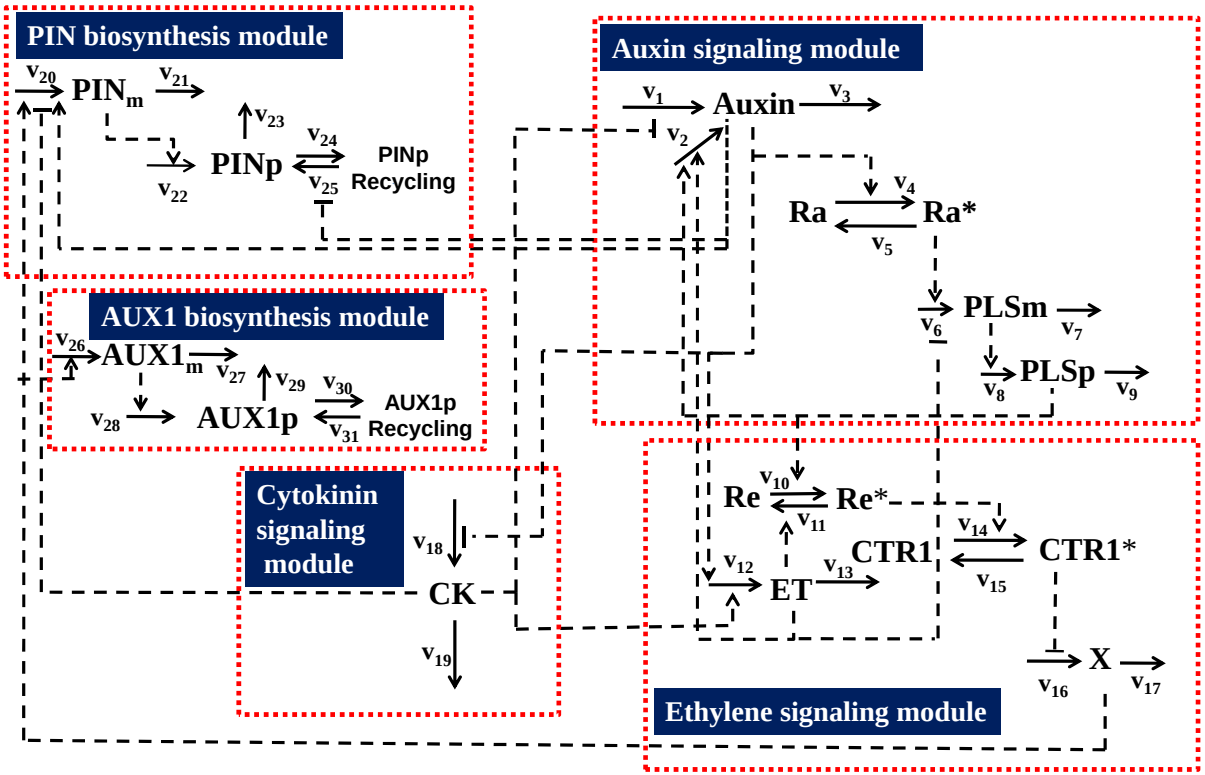
Figure 1. The hormonal crosstalk network constructed based on experimental data (from Moore *et al.*, 2015a, with permission). **Symbols**: Auxin: Auxin hormone, ET: ethylene, CK: Cytokinin, PINm: PIN mRNA, PINp: PIN protein, PLSm: POLARIS mRNA, PLSp: POLARIS protein, X: Downstream ethylene signalling, Ra*: Active form of auxin receptor, Ra: Inactive form of auxin receptor, Re*: Active form of ethylene receptor, ETR1. Re: Inactive form of ethylene receptor, ETR1, CTR1*: Active form of CTR1, CTR1: Inactive form of CTR1, AUX1m: AUX1 mRNA, AUX1p: AUX1 protein.

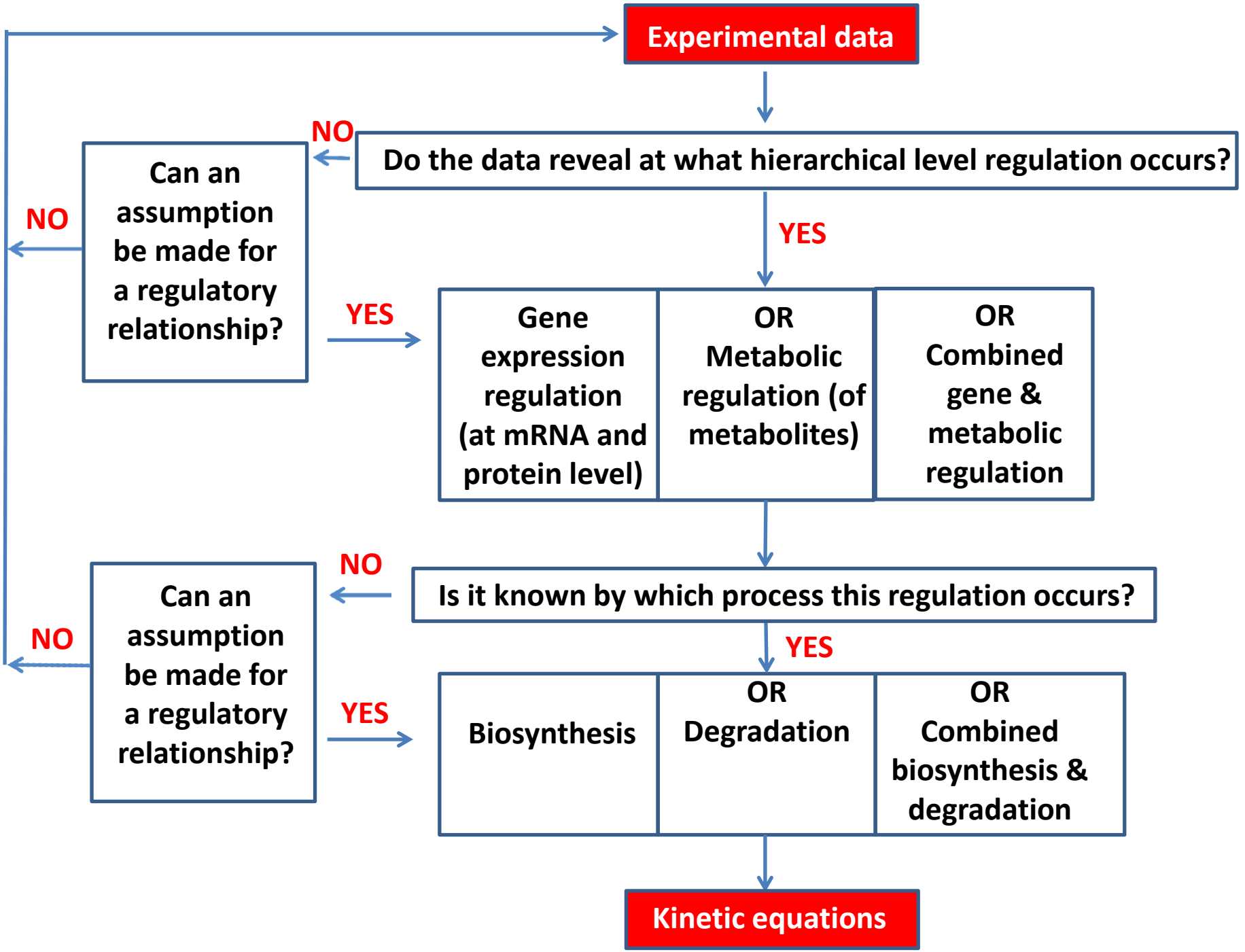
Figure 2. Methodology for formulating kinetic equations based on experimental data. A kinetic equation can be formulated for any regulatory relationship following thermodynamic and kinetic principles.

Figure 3. Root structure and construction of an *in silico* root map. A. A simple rectangular multicellular root showing the developmental regions and the different tissue layers. EZ: elongation zone; MZ: meristematic zone; COL: columella; QC: quiescent centre. B. An example matrix of grid points which code for a MZ cell tier in the root map. Cell files: E: epidermal/cortex; P: pericycle; V: vascular. Grid point codes: 0, cytosol; 1, 2 and 3, cell wall grid points. PIN protein is cycled from the nearest neighbour cytosolic grid point to the cell wall grid points using different rate constants: 1: low, 2: medium, 3: high.

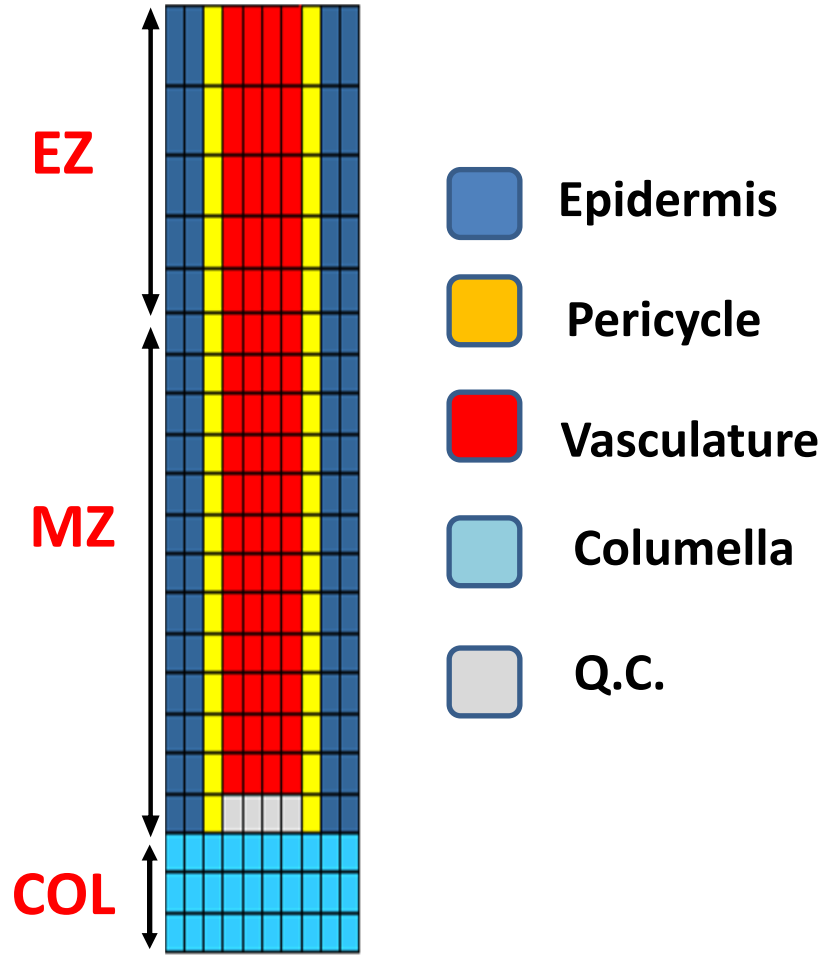
Figure 4. Modelled colour maps demonstrating concentration gradients for auxin, ethylene and cytokinin hormones, and the PIN and AUX1 auxin carrier proteins in the root. Selected expanded views show species gradients in detail. EZ: elongation zone; MZ: meristematic zone; COL: columella; QC: quiescent centre. Red arrows indicate the location of the expanded views in each colour map.

Figure 5. Modelling predictions for the average auxin concentration in different cell types or tissues. A. Individual auxin profiles showing the average auxin concentration down the longitudinal axis of the root for the three different cell types (epidermal, pericycle and vascular cells), indicating that the auxin maximum is predominantly established in the central vascular tissues at or close to the quiescent centre (QC). B. Average auxin concentrations relative to the QC in different regions of the root, with the maximum occurring in the QC region. QC: quiescent centre; COL: columella; STE: stele; END: endodermis; CO: cortex; EP: epidermis; MZ: meristematic zone; EZ: elongation zone.

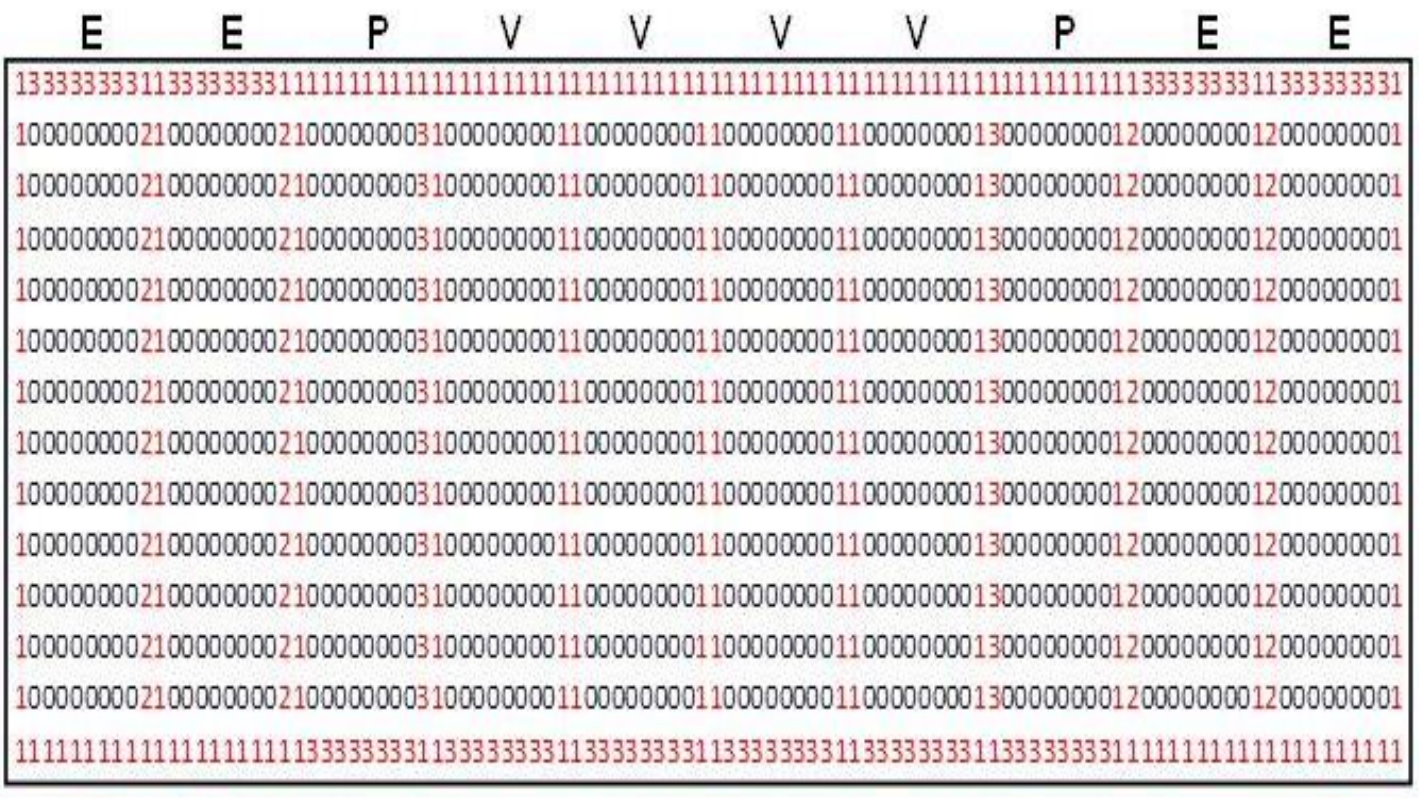

PIN biosynthesis module
Auxin signaling module
AUX1 biosynthesis module
Cytokinin signaling module
Ethylene signaling module
PINp Recycling
AUX1p Recycling
Auxin
Ra
Ra*
PLSm
PLSp
AUX1m
AUX1p
PINm
PINp
Re
Re*
CTR1
CTR1*
CK
ET
X

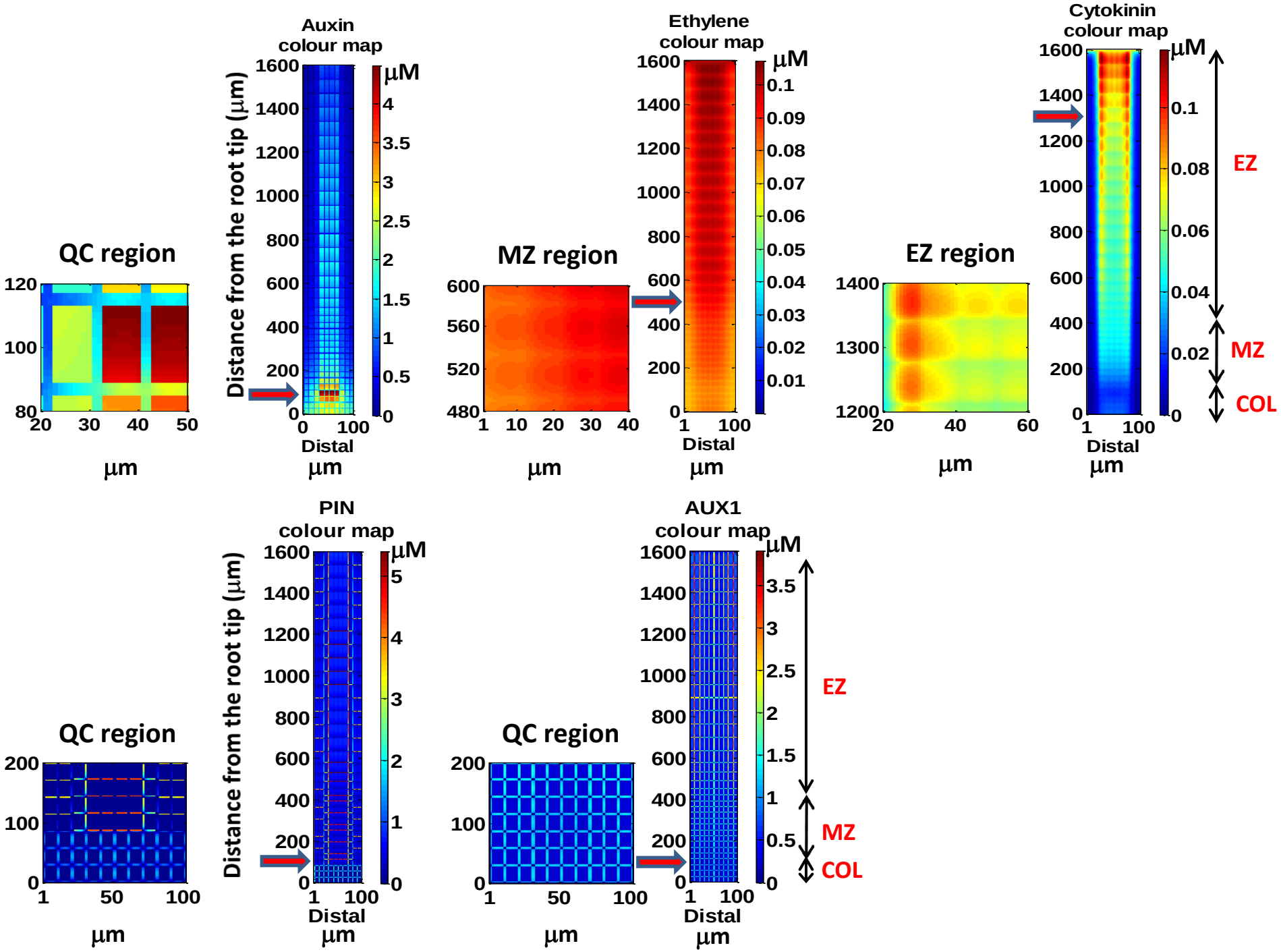

Experimental data
Do the data reveal at what hierarchical level regulation occurs?
NO
Can an assumption be made for a regulatory relationship?
NO
YES
Gene expression regulation (at mRNA and protein level)
OR
Metabolic regulation (of metabolites)
OR
Combined gene & metabolic regulation
YES
Is it known by which process this regulation occurs?
NO
Can an assumption be made for a regulatory relationship?
NO
YES
Biosynthesis
OR
Degradation
OR
Combined biosynthesis & degradation
YES
Kinetic equations

A

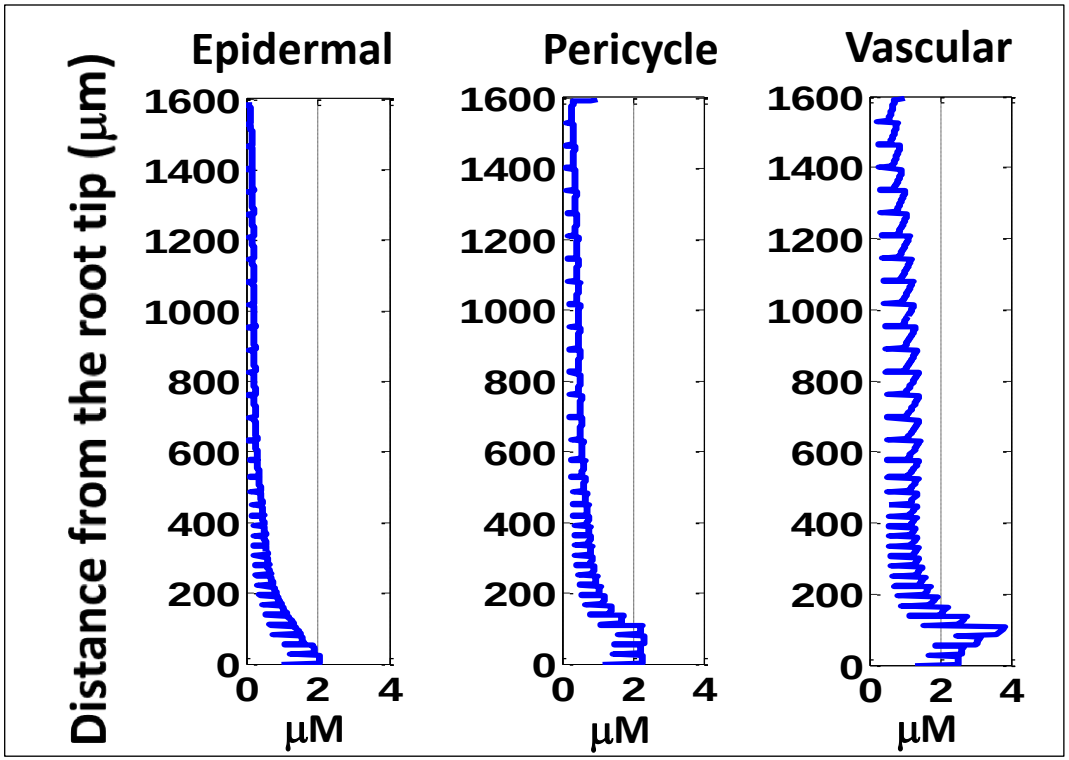

EZ
MZ
COL
Epidermis
Pericycle
Vasculature
Columella
Q.C.

B

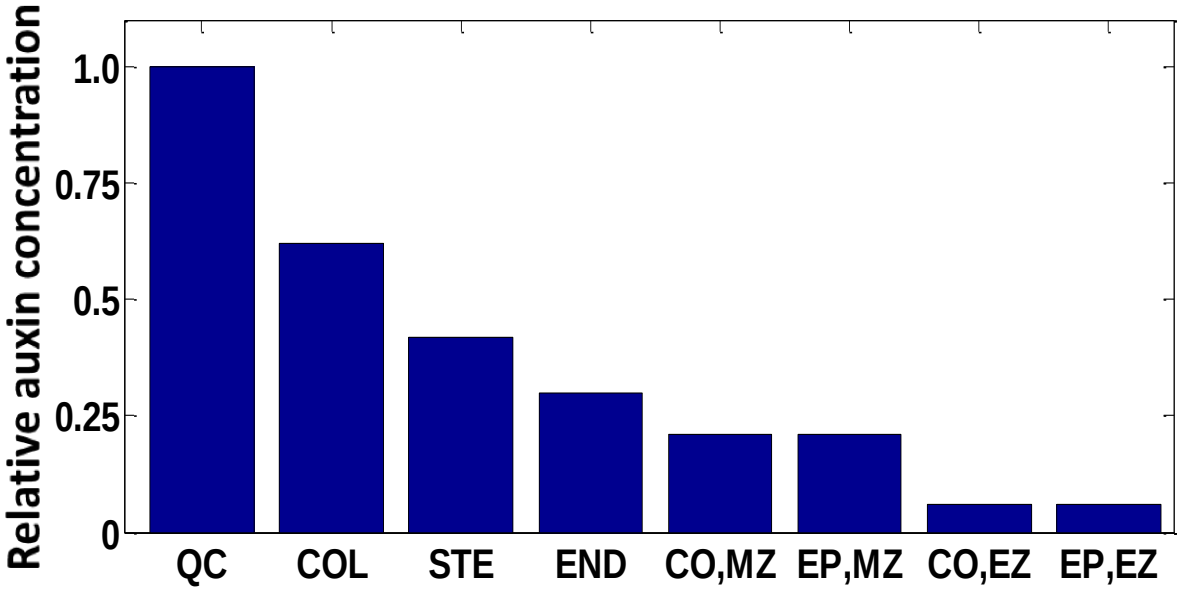

E E P V V V V P E E


QC region
120
100
80
20
30
40
50
µm
Auxin
colour map
Distance from the root tip (µm)
1600
1400
1200
1000
800
600
400
200
0
0
100
Distal
µm
µM
4
3.5
3
2.5
2
1.5
1
0.5
0
MZ region
600
560
520
480
1
10
20
30
40
µm
Ethylene
colour map
1600
1400
1200
1000
800
600
400
200
0
1
100
Distal
µm
µM
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
EZ region
1400
1300
1200
20
40
60
µm
Cytokinin
colour map
1600
1400
1200
1000
800
600
400
200
0
1
100
Distal
µm
µM
0.1
0.08
0.06
0.04
0.02
0
EZ
MZ
COL
QC region
200
100
0
1
50
100
µm
PIN
colour map
Distance from the root tip (µm)
1600
1400
1200
1000
800
600
400
200
0
1
100
Distal
µm
µM
5
4
3
2
1
0
QC region
200
100
0
1
50
100
µm
AUX1
colour map
1600
1400
1200
1000
800
600
400
200
0
1
100
Distal
µm
µM
3.5
3
2.5
2
1.5
1
0.5
0
EZ
MZ
COL

A


Epidermal
Pericycle
Vascular
Distance from the root tip (μm)
1600
1400
1200
1000
800
600
400
200
0
0
2
4
μM

B


Relative auxin concentration
1.0
0.75
0.5
0.25
0
QC
COL
STE
END
CO,MZ
EP,MZ
CO,EZ
EP,EZ