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Abstract (120 words)

Recent years have seen an impressive increase in our knowledge of the topology of plant hormone signaling networks. The complexity of these topologies has motivated the development of models for several hormones to aid understanding of how signaling networks process hormonal inputs. Such work has generated essential insights into the mechanisms of hormone perception and of regulation of cellular responses such as transcription in response to hormones. In addition, modeling approaches have contributed significantly to exploring how spatio-temporal regulation of hormone signaling contributes to plant growth and patterning. New tools have also been developed to obtain quantitative information on hormone distribution during development and to test model predictions, opening the way for quantitative understanding of the developmental roles

of hormones.

Main text: 2262 words

Introduction

Plant development is driven by multiple extracellular signaling molecules, the most important of which are plant hormones. Hormones, primarily auxin, cytokinin (CK), gibberellin (GA), abscissic acid (ABA), brassinosteroid, jasmonic acid and ethylene, are small molecules with chemical structures analogous to primary or secondary metabolites . In each cell, perception of hormones stimulates signaling networks that regulate gene expression and will eventually lead to cellular decisions, for example a change in growth dynamics or the acquisition of a new cell identity. The identification of hormone signaling network components and understanding of how the activity of these components is affected upon hormone perception, including possible feedback regulation, has recently allowed the generation of essential information regarding the topology of the networks controlling gene transcription in response to hormones (Figure 1). A key remaining challenge is to infer the dynamic properties of these signaling networks from knowledge of their topology, in order to understand how hormonal inputs are transformed into appropriate output responses. This is not intuitive but can be addressed by generating mathematical models of the signaling networks that can subsequently be used to predict the properties of the networks. In this review, we will specifically highlight advances in the last two years relying on modeling approaches to understand hormone signaling and function during development.

Cell-autonomous processing of hormonal signals

The most studied hormone in plant biology is unquestionably auxin. Auxin regulates gene expression primarily by promoting the degradation of Aux/IAA transcriptional regulators. In the presence of auxin, Aux/IAA proteins act as auxin co-receptors together with a TIR1/AFB F-box protein of an SCF complex . This interaction leads to the ubiquitination and degradation of Aux/IAAs . The degradation of Aux/IAAs releases their repression of the ARF transcription factors, allowing ARFs to regulate the expression of their targets, which include most of the *Aux/IAA* genes (Figure 1A). Several modeling approaches have led to important insights into how cells process the auxin signal. Considering the topology described above and an ARF that can activate the transcription of an *Aux/IAA* gene(Figure 2A), a first model of the signaling pathway * predicted two different behaviour for *Aux/IAA* induction in response to exogenous auxin: 1- an increase in transcription before reaching a steady state level; 2- transcription peaking then decreasing to reach a steady state. Both these kinetics can be observed *in planta* and likely result from combinations of Aux/IAAs and ARFs with different biochemical properties. This is notably consistent with the distinct half-lives and binding affinities observed for different Aux/IAAs . Oscillations in *Aux/IAA* transcription levels, despite constant auxin levels, were also obtained using the model. This is reminiscent of oscillations of the *DR5* auxin-inducible reporter (reporting

auxin signaling transcriptional output [11-13]) that have been proposed to play a role in positioning new lateral roots along the root primary axis [14]. Although *DR5* oscillations appears to be under the control of an auxin-independent clock mechanism [15], these simulations suggest that they might also result, in part, from the properties of the auxin signaling pathway itself.

The demonstration, using quantitative real-time PCR, that the *MONOPTEROS(MP)/ARF5* gene can be induced upon exogenous application of auxin motivated the development of another model to analyze the dynamics of induction of the MP-target *BODENLOS/IAA12* [16]* (Figure 2B). The model suggests that an additional positive feedback loop involving MP allows for a switch-like activation of both *MP* and *BDL* in response to auxin, consistent with experimental analysis of the transcription dynamics of the two genes. Both MP and BDL are essential for the regulation of cell identities during both embryonic and post-embryonic development and such a feedback mechanism could be essential for the role of auxin in cell fate decision during development.

Further exploration of the auxin signaling pathway was achieved using a third model based on supplementary information on the topology of the auxin signaling network obtained from a full Aux/IAA-ARF interactome [19]**. Only 5 of the 23 *Arabidopsis* ARFs are predicted to act as transcriptional activators as seen for MP, whilst 18 of them seem likely to act as repressors [20]. Analysis of the Aux/IAA-ARF interactome showed that, while Aux/IAAs and activating ARFs interact, repressing ARFs have very limited interactions with other proteins **. This suggests a scenario

where repressing ARFs regulate transcription mostly by competing with activating ARFs for binding sites in the promoters of auxin-inducible genes (Figure 2C). The model predicts that co-expression of the activating ARFs repressing ARFs provides buffering properties to the auxin signaling network, allowing the stabilization of auxin-induced transcription in conditions where the auxin input shows variations. Supporting this prediction, it was found that auxin levels monitored with the DII-VENUS auxin biosensor [21]** fluctuate in the shoot apical meristem (SAM), while gene expression monitored using *DR5* is stable [19]**. It is important to stress that this model considers different topologies for the auxin signaling network to the others discussed above (Figure 2). Further work is needed to evaluate the diversity of these topologies *in planta* and to determine whether the conclusions drawn from these studies can be generalized. This will notably require the generation of expression datasets for the effectors of auxin signaling from several other tissues [19,22].

A model has also been developed for the GA signaling pathway **. GA signaling shares important similarities with that of auxin, in the sense that GA triggers degradation of transcriptional repressors of the DELLA family [24,25] (Figure 1B). On the other hand, GA biosynthesis genes (GA20ox, GA3ox) [26] and the GA receptor GID1 [27] are positively regulated by DELLA proteins, thus creating a complex circuit of negative feedback loops. The model was parameterized using both existing and new datasets, including time-course expression data after exogenous GA application for both GA signaling and biosynthesis genes [23]**. The predictive value of the model was demonstrated by showing that it

predicted accurately gene expression dynamics after GA application in a mutant accumulating bioactive GA. The model further suggests that the GA20ox feedback loop dominates the GA3ox loop, providing insights into the relative importance of different feedback loops in the modulation of the sensitivity of cells to GA.

Proteins of the PYR/PYL/RCAR family act as intracellular receptors of ABA . These receptors bind and inactivate several PP2Cs, triggering ABA signaling. A combination of structural and biochemical approaches demonstrated that the PYR/PYL/RCAR proteins can act as either monomeric or dimeric receptors [30]*. Dimeric receptors need to dissociate before binding PP2Cs, and have a lower affinity for ABA. Simulations using a model of ABA receptor activation indicate that the balance between the two types of receptors can significantly modulate the sensitivity of the cell to ABA, and thus provide testable hypotheses for further exploration of ABA perception [30]*.

Differential hormone signaling capacities contribute to growth and patterning

Systems biology analysis of, and modeling approaches to hormone signaling have also shed new light on how differential hormone signaling capacities across tissues contribute to growth and patterning. Auxin can activate expression of the *DR5* reporter at the periphery of the SAM, but not in the meristem center [19,31]. An extensive analysis of the spatial expression pattern of *ARF* and *Aux/IAA* genes in the SAM explains this spatial pattern of auxin response [19]**. Most *Aux/IAA*s and both

activating and repressing *ARFs* are expressed in the meristem, more weakly in the center than at the periphery. These differences in expression levels were considered in the last of the auxin signaling models discussed above (Figure 2B) resulting in the prediction that a low level of both types of ARFs led to low sensitivity to auxin, whereas higher levels could trigger a higher sensitivity to auxin [19]**. This difference in auxin sensitivity is supported by the demonstration that auxin indeed accumulates at the center of the SAM without triggering an activation of DR5 in these cells [19]**.

In the root apex, cells divide in the meristem at the root tip and then are displaced into the elongation zone where they rapidly increase in length to finally stop growing when entering the maturation zone. Gibberellins regulate both cell elongation and division by promoting the degradation of DELLA proteins, which function as growth repressors during root development [32,33]. A multicellular model that incorporates the GA signaling network in each cell of the root has tested the effect of cell elongation on GA concentrations and how resulting changes in GA levels feed back onto cell elongation [34]**. Simulations predict that dilution of GA concentrations resulting from cell expansion in the elongation zone creates a GA gradient along the root axis, a prediction supported by transcriptomic data. In the model, GA dilution contributes to a progressive stabilization of the DELLA proteins during growth and provides a plausible mechanism explaining the growth patterns at the root tip.

Hormone signaling in non-cell autonomous developmental

regulation

Cell-cell communication is essential throughout plant development and notably in the maintenance of stem cell niches. In the SAM, the size of the stem cell niche is regulated by a negative feedback loop between the transcription factor WUSCHEL (WUS) and the small secreted peptide CLAVATA3 (CLV3). WUS is expressed in a small subapical region and positively regulates CLV3 transcription in the cells above, which in turn repress WUS expression, thus creating a negative feedback loop [35]. CKs are also essential for stem cell maintenance and models have been developed to test how CKs participate in positioning the WUS and CLV3 expression domains. Computational modeling indicates that CK acts both in a CLV-dependent and independent manner to activate WUS production and regulate its distribution [36,37]*. In addition, an accumulation of bioactive CK in the L1 layer is predicted to lead to differences in CK concentrations in the meristem that could, together with CLV3, provide positional cues for specifying the WUS domain *. This is consistent with the specific expression of a member of the LONELY GUY (LOG) family of CK biosynthetic enzymes in the L1.

Auxin also represents a key non-cell autonomous signal since it is transported between cells in a polar fashion. The polarity of the auxin flux is mainly controlled by polar localization of efflux carriers of the PIN-FORMED (PIN) family [39]. Auxin is a key regulator of its own transport since it regulates both PIN transcription and localization at the plasma membrane. Modeling approaches suggest either sensing of auxin concentration in neighboring cells or sensing of auxin flux across the

plasma membrane as plausible mechanisms for the polarization of PINs in tissues [40-43]. A recent mechanistic model tested how modulation of PIN trafficking by an extracellular auxin receptor together with regulation of *PIN* transcription through intracellular auxin signaling could lead to tissue polarization [44]**. This model, like previous flux-based models, can reproduce spatial patterns of PIN polarization observed during vascular formation. The combination of extracellular auxin sensing, possibly by the ABP1 auxin receptor [45,46], and intracellular auxin sensing by the TIR/AFB-Aux/IAA-ARF pathway could thus provide the cell with a flux-sensing mechanism controlling PIN polarization during tissue development. The obtention of further information about the molecular mechanisms underlying PIN polarization is still necessary to elucidate how PIN networks establish spatio-temporal patterns of auxin during development. However, the modeling described above establishes an exciting framework around which to perform this exploration.

Towards a quantitative understanding of plant hormone signaling

Testing the predictions of models relies on the production of quantitative data. In the case of hormone signaling, acquiring quantitative information about the spatio-temporal distribution of hormones during developmental processes is absolutely crucial. Because they are small molecules, plant hormones are difficult to detect with cellular definition using classical histochemical approaches or direct biochemical measurements. A major step forward in the quantification of auxin distribution was provided by the development of the DII-VENUS sensor

mentioned above [21]**. DII-VENUS is a synthetic protein comprising an Aux/IAA auxin-binding domain fused to a nuclear-localized YFP variant (VENUS) and expressed under the constitutive 35S promoter. Aux/IAAs exhibit rapid turnover and the use of VENUS, a fast-maturating YFP, means that the detection of DII-VENUS fluorescence in tissues is inversely correlated to endogenous auxin levels [21]**. Endogenous auxin levels can be predicted directly from DII-Venus fluorescence levels using a simplified auxin signaling model parameterized by quantifying DII-VENUS degradation after auxin treatment [47]**. When applied to the analysis of auxin redistribution during root gravitropism, this approach shows that an asymmetric distribution of auxin across the root is established within minutes of a gravitational stimulus. In the light of this study, we anticipate that the development of new hormone biosensors, together with modeling, should allow the generation of high-resolution spatiotemporal maps of hormone distribution during development.

Synthetic reconstruction of hormone signaling pathways in unicellular organisms is also emerging as an interesting means of obtaining quantitative data from these pathways. The TIR1/AFB F-box proteins and Aux/IAAs form pairs of co-receptors with a wide range of binding affinity for auxin [4]. This system can be reconstructed in yeast [48,49] and has been used to quantify Aux/IAA degradation dynamics in the presence of auxin, demonstrating that each AFB-Aux/IAA couple has a characteristic degradation behavior in response to auxin in yeast [49]**. Such a synthetic biology approach can be viewed as a means of performing biological simulations of a given network module. It thus

represents a powerful complementary strategy to computational modeling for exploring the properties of signaling pathways, and in particular for the extraction of quantitative data.

Conclusions

The recent development of computational models for plant hormone signaling pathways has provided key insights into how signaling pathways process hormone signals and integrate these signals during plant development. This is only a first step in the exploration of the properties of these pathways. Indeed, several cycles between a model and biological experiments are required to fully assess its predictive value and to identify missing elements. In this interplay between modeling and biological experiments, the development of new tools and quantitative approaches to explore the spatio-temporal distribution of hormones, and possible links with changes in identity and growth patterns, will be instrumental. In addition, models for additional hormone signaling pathways still need to be developed. A key future challenge will be to be develop computational approaches to analyze the coupling between different signaling pathways in order to explore how hormones interact during plant developmental processes.

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References and recommended reading:
*** of special interest**
**** of outstanding interest**

1. Jaillais Y, Chory J: **Unraveling the paradoxes of plant hormone signaling integration**. *Nat Struct Mol Biol* 2010, **17**:642-645.
2. Santner A, Estelle M: **Recent advances and emerging trends in plant hormone signalling**. *Nature* 2009, **459**:1071-1078.
3. Shan X, Yan J, Xie D: **Comparison of phytohormone signaling mechanisms**. *Curr Opin Plant Biol* 2012, **15**:84-91.
4. Calderon Villalobos LI, Lee S, De Oliveira C, Ivetac A, Brandt W, Armitage L, Sheard LB, Tan X, Parry G, Mao H, et al.: **A combinatorial TIR1/AFB-Aux/IAA co-receptor system for differential sensing of auxin**. *Nat Chem Biol* 2012, **8**:477-485.
5. Dreher KA, Brown J, Saw RE, Callis J: **The Arabidopsis Aux/IAA protein family has diversified in degradation and auxin responsiveness**. *Plant Cell* 2006, **18**:699-714.
6. Ramos JA, Zenser N, Leyser O, Callis J: **Rapid degradation of auxin/indoleacetic acid proteins requires conserved amino acids of domain II and is proteasome dependent**. *Plant Cell* 2001, **13**:2349-2360.
7. Zenser N, Ellsmore A, Leasure C, Callis J: **Auxin modulates the degradation rate of Aux/IAA proteins**. *Proc Natl Acad Sci U S A* 2001, **98**:11795-11800.
8. Tiwari SB, Wang XJ, Hagen G, Guilfoyle TJ: **AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin**. *Plant Cell* 2001, **13**:2809-2822.
* 9. Middleton AM, King JR, Bennett MJ, Owen MR: **Mathematical Modelling of the Aux/IAA Negative Feedback Loop**. *Bulletin of Mathematical Biology* 2010, **72**:1383-1407.
The authors develop and analyze an ordinary differential equation (ODE) model of the auxin signaling pathway, including a negative feedback on Aux/IAA. They show that this system reproduces the known temporal profiles of response to auxin, and is furthermore able to present spontaneous oscillations under constant auxin input.
10. Abel S, Nguyen MD, Theologis A: **The PS-IAA4/5-like family of early auxin-inducible mRNAs in Arabidopsis thaliana**. *J Mol Biol* 1995, **251**:533-549.
11. Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ: **Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements**. *Plant Cell* 1997, **9**:1963-1971.
12. Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, et al.: **An auxin-dependent distal organizer of pattern and polarity in the**

Arabidopsis root. *Cell* 1999, **99**:463-472.
13. Benkova E, Michniewicz M, Sauer M, Teichmann T, Seifertova D, Jurgens G, Friml J: **Local, efflux-dependent auxin gradients as a common module for plant organ formation**. *Cell* 2003, **115**:591-602.
14. De Smet I, Tetsumura T, De Rybel B, Frey NF, Laplaze L, Casimiro I, Swarup R, Naudts M, Vanneste S, Audenaert D, et al.: **Auxin-dependent regulation of lateral root positioning in the basal meristem of Arabidopsis**. *Development* 2007, **134**:681-690.
15. Moreno-Risueno MA, Van Norman JM, Moreno A, Zhang JY, Ahnert SE, Benfey PN: **Oscillating Gene Expression Determines Competence for Periodic Arabidopsis Root Branching**. *Science* 2010, **329**:1306-1311.
* 16. Lau S, De Smet I, Kolb M, Meinhardt H, Jurgens G: **Auxin triggers a genetic switch**. *Nat Cell Biol* 2011, **13**:611-615.
The authors show that ARF5 regulates both its own promoter and IAA12, which in turn represses ARF5. This constitutes a system of two intricated feedback loops, which is shown by the authors to trigger switch-like responses to auxin inputs.
17. Hamann T, Benkova E, Baurle I, Kientz M, Jurgens G: **The Arabidopsis BODENLOS gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning**. *Genes Dev* 2002, **16**:1610-1615.
18. Hardtke CS, Berleth T: **The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development**. *Embo J* 1998, **17**:1405-1411.
** 19. Vernoux T, Brunoud G, Farcot E, Morin V, Van den Daele H, Legrand J, Oliva M, Das P, Larrieu A, Wells D, et al.: **The auxin signalling network translates dynamic input into robust patterning at the shoot apex**. *Mol Syst Biol* 2011, **7**:508.
In this paper both the spatial expression maps in the SAM and the interactome of all ARF and Aux/IAA family members are experimentally determined. This information is used to build an ODE model of this pathway and to predict a differential behaviour in the center and the periphery of the SAM (see main text for details). These predictions are confirmed by observations relying on the DII-Venus sensor [22•].
20. Guilfoyle TJ, Hagen G: **Auxin response factors**. *Curr Opin Plant Biol* 2007, **10**:453-460.
** 21. Brunoud G, Wells DM, Oliva M, Larrieu A, Mirabet V, Burrow AH, Beeckman T, Kepinski S, Traas J, Bennett MJ, et al.: **A novel sensor to map auxin response and distribution at high spatio-temporal resolution**. *Nature* 2012, **482**:103-106.
This paper introduces and characterizes a novel auxin sensor, DII-VENUS. The authors demonstrate that the fluorescence levels of DII-VENUS are inversely related to auxin levels in most tissues. They further show that the sensor provide obtain high spatio-temporal information on auxin distribution during development.
22. Rademacher EH, Moller B, Lokerse AS, Llavata-Peris CI, van den Berg

W, Weijers D: **A cellular expression map of the Arabidopsis AUXIN RESPONSE FACTOR gene family**. *Plant J* 2011, **68**:597-606.

** 23. Middleton AM, Ubeda-Tomas S, Griffiths J, Holman T, Hedden P, Thomas SG, Phillips AL, Holdsworth MJ, Bennett MJ, King JR, et al.: **Mathematical modeling elucidates the role of transcriptional feedback in gibberellin signaling**. *Proc Natl Acad Sci U S A* 2012, **109**:7571-7576.
The authors present an ODE model of the GA signaling pathway. The validity of this model is quantitatively assessed through parameter fitting, using new and previously published datasets. It provides an example where modeling leads to quantitative predictions about a complex signaling system.

24. Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP: **The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses**. *Genes Dev* 1997, **11**:3194-3205.

25. Fu X, Richards DE, Fleck B, Xie D, Burton N, Harberd NP: **The Arabidopsis mutant sleepy1gar2-1 protein promotes plant growth by increasing the affinity of the SCFSLY1 E3 ubiquitin ligase for DELLA protein substrates**. *Plant Cell* 2004, **16**:1406-1418.

26. Yamaguchi S: **Gibberellin metabolism and its regulation**. *Annu Rev Plant Biol* 2008, **59**:225-251.

27. Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YI, Kitano H, Yamaguchi I, et al.: **GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin**. *Nature* 2005, **437**:693-698.

28. Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E: **Regulators of PP2C phosphatase activity function as abscisic acid sensors**. *Science* 2009, **324**:1064-1068.

29. Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TF, et al.: **Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins**. *Science* 2009, **324**:1068-1071.

* 30. Dupeux F, Santiago J, Betz K, Twycross J, Park SY, Rodriguez L, Gonzalez-Guzman M, Jensen MR, Krasnogor N, Blackledge M, et al.: **A thermodynamic switch modulates abscisic acid receptor sensitivity**. *EMBO J* 2011, **30**:4171-4184.
The authors use biochemical and structural approaches to demonstrate that the members of PYR/PYL/RCAR family of ABA receptors can be either monomeric and dimeric receptors , with different affinity to ABA. They further use modeling to explore how ABA signaling can be modulated by the relative abundance of momomeric and dimeric receptors.

31. Barbier de Reuille P, Bohn-Courseau I, Ljung K, Morin H, Carraro N, Godin C, Traas J: **Computer simulations reveal properties of the cell-cell signaling network at the shoot apex in Arabidopsis**. *Proc Natl Acad Sci U S A* 2006, **103**:1627-1632.

32. Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van

Der Straeten D, Peng J, Harberd NP: **Integration of plant responses to environmentally activated phytohormonal signals**. *Science* 2006, **311**:91-94.

33. Fu X, Harberd NP: **Auxin promotes Arabidopsis root growth by modulating gibberellin response**. *Nature* 2003, **421**:740-743.

** 34. Band LR, Ubeda-Tomas S, Dyson RJ, Middleton AM, Hodgman TC, Owen MR, Jensen OE, Bennett MJ, King JR: **Growth-induced hormone dilution can explain the dynamics of plant root cell elongation**. *Proc Natl Acad Sci U S A* 2012, **109**:7577-7582.
This paper shows that embedding GA signaling in a growing tissue allows to explain the root growth pattern, in particular via growth induced GA dilution. The authors use an adapted version of the model in [24••], which they project on a growing tissue, taking dilution into account, and compare this multi-scale model to experimental data, showing that dilution of GA during growth provides a plausible explanation to growth patterns in the root tip.

35. Sablowski R: **Plant stem cell niches: from signalling to execution**. *Curr Opin Plant Biol* 2011, **14**:4-9.

* 36. Gordon SP, Chickarmane VS, Ohno C, Meyerowitz EM: **Multiple feedback loops through cytokinin signaling control stem cell number within the Arabidopsis shoot meristem**. *Proc Natl Acad Sci U S A* 2009, **106**:16529-16534.
The authors demonstrate that CK signaling induces the WUS gene via two paths, implying CLAVATA proteins or not. WUS being itself an activator of CK signaling, this leads to multiple positive feedback loops whose role is analyzed using a mathematical model. Predictions on relative expression domains of different actors are formulated, and confirmed experimentally.

* 37. Yadav RK, Perales M, Gruel J, Girke T, Jonsson H, Reddy GV: **WUSCHEL protein movement mediates stem cell homeostasis in the Arabidopsis shoot apex**. *Genes Dev* 2011, **25**:2025-2030.
The authors observe a migration of the WUS protein beyond its synthesis domain. They show that in a computational model, this displacement is crucial in regulating the stem cell population in the shoot apex.

* 38. Chickarmane VS, Gordon SP, Tarr PT, Heisler MG, Meyerowitz EM: **Cytokinin signaling as a positional cue for patterning the apical-basal axis of the growing Arabidopsis shoot meristem**. *Proc Natl Acad Sci U S A* 2012, **109**:4002-4007.
This paper shows by a combination of experimental and computational approaches that a spatial, gradient-like, patterning of CK signaling and CLAVATA in a population of growing cells can explain WUS patterning in the shoot apex. This implies the prediction of a plausible feedback between WUS and CK biosynthesis.

39. Grunewald W, Friml J: **The march of the PINs: developmental plasticity by dynamic polar targeting in plant cells**. *EMBO J* 2010, **29**:2700-2714.

40. Smith RS, Guyomarc'h S, Mandel T, Reinhardt D, Kuhlemeier C, Prusinkiewicz P: **A plausible model of phyllotaxis**. *Proc Natl Acad*

Sci U S A 2006, **103**:1301-1306.
41. Jonsson H, Heisler MG, Shapiro BE, Meyerowitz EM, Mjolsness E: **An auxin-driven polarized transport model for phyllotaxis**. *Proc Natl Acad Sci U S A* 2006, **103**:1633-1638.
42. Stoma S, Lucas M, Chopard J, Schaedel M, Traas J, Godin C: **Flux-based transport enhancement as a plausible unifying mechanism for auxin transport in meristem development**. *PLoS Comput Biol* 2008, **4**:e1000207.
43. Bayer EM, Smith RS, Mandel T, Nakayama N, Sauer M, Prusinkiewicz P, Kuhlemeier C: **Integration of transport-based models for phyllotaxis and midvein formation**. *Genes Dev* 2009, **23**:373-384.
** 44. Wabnik K, Kleine-Vehn J, Balla J, Sauer M, Naramoto S, Reinohl V, Merks RM, Govaerts W, Friml J: **Emergence of tissue polarization from synergy of intracellular and extracellular auxin signaling**. *Mol Syst Biol* 2010, **6**:447.
The authors propose a novel model of auxin transport, where local accumulation of auxin transporters is controlled by a mechanistically detailed system involving both intra- and extra-cellular auxin signaling. This model reproduces realistic auxin patterns, including venation patterns, yet relying on plausible mechanistic processes, and no abstract hypothesis of flux or gradient sensing.
45. Robert S, Kleine-Vehn J, Barbez E, Sauer M, Paciorek T, Baster P, Vanneste S, Zhang J, Simon S, Covanova M, et al.: **ABP1 mediates auxin inhibition of clathrin-dependent endocytosis in Arabidopsis**. *Cell* 2010, **143**:111-121.
46. Xu T, Wen M, Nagawa S, Fu Y, Chen JG, Wu MJ, Perrot-Rechenmann C, Friml J, Jones AM, Yang Z: **Cell surface- and rho GTPase-based auxin signaling controls cellular interdigitation in Arabidopsis**. *Cell* 2010, **143**:99-110.
** 47. Band LR, Wells DM, Larrieu A, Sun J, Middleton AM, French AP, Brunoud G, Sato EM, Wilson MH, Peret B, et al.: **Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism**. *Proc Natl Acad Sci U S A* 2012, **109**:4668-4673.
This paper investigates gravitropism in roots, using the DII-Venus reporter [22•]. A model, based on the work in [9•], allows the authors to quantitatively follow auxin redistribution during gravitropic response. This leads them to show that the auxin differential between the upper and lower part of the root is not only established very rapidly when a root is positioned at a 90° angle but also lost very rapidly when reaching an angle close to 45°.
48. Nishimura K, Fukagawa T, Takisawa H, Kakimoto T, Kanemaki M: **An auxin-based degron system for the rapid depletion of proteins in nonplant cells**. *Nat Methods* 2009, **6**:917-922.
** 49. Havens KA, Guseman JM, Jang SS, Pierre-Jerome E, Bolten N, Klavins E, Nemhauser JL: **A synthetic approach reveals extensive tunability of auxin signaling**. *Plant Physiol* 2012, **160**:135-142.

The authors have implemented auxin induced Aux/IAA degradation

in yeast, for different pairs of auxin co-receptors. This synthetic biology approach, coupled with ODE modeling, allows for quantitative predictions, in particular about distinct signaling kinetics for different pairs of co-receptors. This quantitatively confirms the view that different elements of the auxin pathway lead to a variety of signaling capacities, due to distinct kinetic parameters.

Figure legends :

Figure 1. Examples of hormone signaling pathways.

A. In the presence of auxin, Aux/IAA transcriptional repressors act together with TIR1/AFB F-box proteins as auxin co-receptors. This interaction leads to the ubiquitination and subsequent degradation of Aux/IAAs, allowing ARF transcription factors to regulate the expression of auxin response genes. B. GA signaling is analogous to auxin signaling in the sense that GA promotes the degradation of DELLA repressors thus allowing the expression of GA response genes. However, unlike auxin, the GA receptor is not an F-box protein. Instead, GA binding to the receptor GID1 promotes the interaction between GID1 and the GID2/SLY1 F-box protein, which catalyzes the ubiquitination of DELLA proteins. C. CK perception by AHKs causes phosphorylation of the phosphotransfer proteins AHP1-5, which tranfers the phosphoryl group to type A and B ARR transcription factors thus inducing transcription of CK-regulated targets. CK binding also results in the rapid AHP-dependent nuclear translocation of the transcription factor CRF.

Figure 2. Different network topologies used in auxin signaling models.

Network topologies for three published models of the auxin signaling pathway are displayed. The three models are discussed in the text. Only the elements that were explicitly included as variables or control parameters in the models are shown. A. Model from reference [9•]. B. Model from reference [16•]. C. Model from reference [19••].

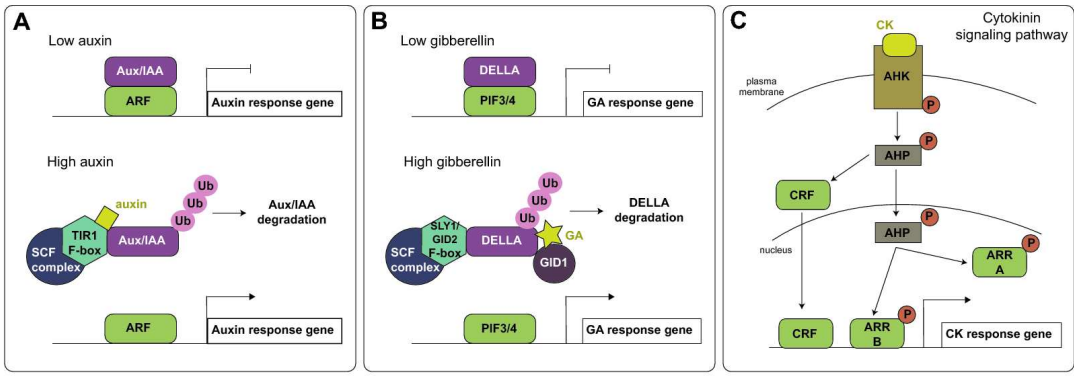

Figure 1

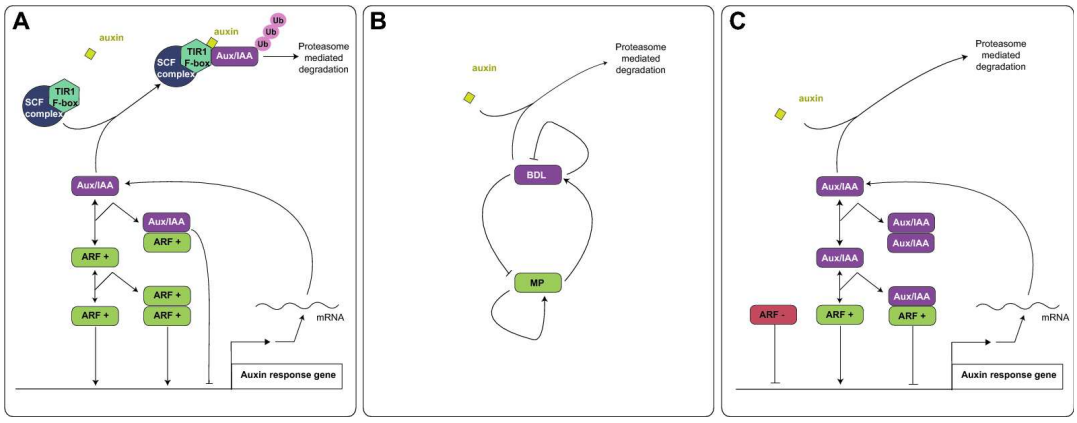

Figure 2