Towards mechanistic models of plant organ growth

Dirk De Vos1,2,*, Abdiravuf Dzhurakhalov1,2,*, Delphine Draelants2,*, Irissa Bogaerts1,*, Shweta Kalve1,*, Els Prinsen1, Kris Vissenberg1, Wim Vanroose2, Jan Broeckhove2 and Gerrit T. S. Beemster1,

1 Department of Biology, University of Antwerp, Belgium
2 Department of Mathematics and Computer Science, University of Antwerp, Belgium

* These authors contributed equally and should be considered joint first authors.
† To whom correspondence should be addressed. E-mail: gerrit.beemster@ua.ac.be

Received 17 October 2011; Revised 23 January 2012; Accepted 23 January 2012

Abstract

Modelling and simulation are increasingly used as tools in the study of plant growth and developmental processes. By formulating experimentally obtained knowledge as a system of interacting mathematical equations, it becomes feasible for biologists to gain a mechanistic understanding of the complex behaviour of biological systems. In this review, the modelling tools that are currently available and the progress that has been made to model plant development, based on experimental knowledge, are described. In terms of implementation, it is argued that, for the modelling of plant organ growth, the cellular level should form the cornerstone. It integrates the output of molecular regulatory networks to two processes, cell division and cell expansion, that drive growth and development of the organ. In turn, these cellular processes are controlled at the molecular level by hormone signalling. Therefore, combining a cellular modelling framework with regulatory modules for the regulation of cell division, expansion, and hormone signalling could form the basis of a functional organ growth simulation model. The current state of progress towards this aim is that the regulation of the cell cycle and hormone transport have been modelled extensively and these modules could be integrated. However, much less progress has been made on the modelling of cell expansion, which urgently needs to be addressed. A limitation of the current generation models is that they are largely qualitative. The possibilities to characterize existing and future models more quantitatively will be discussed. Together with experimental methods to measure crucial model parameters, these modelling techniques provide a basis to develop a Systems Biology approach to gain a fundamental insight into the relationship between gene function and whole organ behaviour.

Key words: Cell division, cell expansion, hormones, modelling, Systems Biology.

Why do we need modelling?

With the advent of the ‘genomics era’, following the sequencing of the Arabidopsis genome (TAGI, 2000), plant biologists are witnessing an explosion of genomes that are being sequenced. One way to view these genome sequences is as a ‘parts list’ for building functional plants. Therefore, the challenge for molecular plant biologists is to understand how the tens of thousands of genes encoded in each of these genomes work together. The complex regulatory networks formed by these genes result in highly reproducible growth and developmental patterns in the function of the environmental conditions encountered. Genomics approaches are now extensively used to map the organization of genome-wide genetic networks into sets of interacting functional modules. However, understanding and predicting how these networks ultimately control growth and development is still extremely challenging, if not impossible.

Inversely, classical developmental (molecular) genetics approaches have focused for years on key regulatory modules by meticulous functional characterization of the genes involved, through monitoring their behaviour, by altering their expression or function genetically, and/or by administering chemical perturbations. These approaches have yielded a treasure of information on dozens of regulatory modules involved in different aspects of plant development.
With the exception of the interaction between hormonal pathways, the functional interaction between various regulatory pathways is hardly ever studied. Nevertheless, most of these pathways ultimately affect growth and development and therefore somehow are all part of the complete regulatory circuitry of the plant.

The conclusion from the above is that there is a clear need for a new approach that integrates the knowledge obtained from genome-wide and molecular genetics studies in order to come to a mechanistic understanding of plant growth regulation. This approach will have to be able to cope with, on the one hand, the sheer number of genes involved and, on the other hand, the frequently non-linear interactions resulting from complex interactions such as feedback and feed-forward loops, competition for substrates and substrate saturation. These complications make it impossible for the human brain to understand the global network fully. Moreover, the genetic networks are intertwined at the molecular level with the entire complexity of plant metabolites, but also at the higher level with cell-cell and organ-organ interactions (Beemster et al., 2003), so the approaches to integrate our experimentally obtained knowledge need to be multi-scale.

One approach to start tackling this challenge is to build computer simulation models ‘from the bottom-up’, essentially reverse-engineering the plant by focusing on those modules that most directly link to growth and extend from there. This task involves converting the current knowledge of regulatory processes into sets of mathematical equations and testing these to see if, together, they lead to realistic behaviour. When they don’t lead to realistic behaviour, this indicates gaps in our knowledge. This then needs to be addressed experimentally, creating a Systems Biology feedback loop between experiments and adaptation of the models (Kitano, 2002). In this contribution, the approaches and tools that are currently available to experimental biologists to achieve this ambitious goal will be looked at. Furthermore, the progress that has already been made in modelling several key regulatory processes, what the limitations of the current models are, and the possible solutions to overcome these will be discussed.

The cell as the central unit of plant growth

Essential for building a model is determining its scope. As indicated, plant growth regulation is a multi-scale process, encompassing molecular, cellular, tissue/organ, and whole-plant levels. Currently, it may be too ambitious to cover this entire spectrum, but it seems feasible to address the molecule to organ scale as a first step. Given that many developmental studies focus on single organs, this correlates closely with the available knowledge. It is clear that, in this context, the unit of the plant cell plays a crucial role. Not only does it form an organizational level between molecule and organ, but cells can also be seen as basic units of the plant that semi-autonomously interact with their direct environment and compartmentalize the molecular interaction networks that govern their development. This perspective is in line with the evolution of higher plants from unicellular organisms. Moreover, in terms of developmental processes, the complexity at the cellular level is relatively limited. Cells can grow, divide, die, or retain their current size. In contrast to animal systems, migration does not play a significant role in plants due to the presence of the cell wall. To start building mechanistic models of organ growth it follows that at least three regulatory modules need to be implemented: a cell cycle module that regulates cell division, a cell expansion module, and a signalling module that spatially co-ordinates these processes at the organ scale. It is not coincidental that models for each of these modules have separately been developed over the past few years. These can serve as a basis for modules to include in whole organ models.

Modelling frameworks

A modelling framework makes it possible to formulate the ‘biological rules’ that describe the interactions involved in the various regulatory modules, usually in terms of mathematical equations. In addition, it provides a simulator capable of executing these rules and producing visual and/or numerical output. To this end, a number frameworks have been developed that are suitable for simulating multi-cellular plant systems. An overview of them is listed in Table 1. Each of these frameworks has its strengths and weaknesses and it is therefore useful for the aspiring Systems Biologist to review them here.

L-studio and Virtual Laboratory (VLAB) are based on an underlying fractal generator with continuous parameters (CPFG). L-studio is available on Windows and its counterpart VLAB on UNIX/Linux. Development of VLAB has apparently stopped about a decade ago. L-studio supports descriptive and mechanistic models for plant development. Distributed since 1999 (Karwowski and Prusinkiewicz, 2004) the simulator is originally based on L-systems (Lindenmayer, 1975) and has since been extended to vertex-vertex (vv) systems (Smith, 2006). They can be used to model plant morphology and plant growth, in which plants are described as an arrangement of functional modules such as buds or leaves. L-studio can also include the interaction between plants and their environment, for example, for simulating the diffusive transport of water in the soil, for calculating the distribution of light in a plant canopy, etc.

L-studio is not an open source project. It is distributed in binary format, including extensive documentation and sample models. An evaluation version, as well as the price and licensing information for the full version are available upon request.

OpenAlea (Pradal et al., 2008) is a modelling and simulation framework in plant ecophysiology. It consists of a set of components to analyse, visualize, and model the functioning and growth of plant architecture, implemented in the Python programming language (Chun, 2001). A key goal of this project is the capability to exchange, integrate, and
re-use existing tools for the rapid development of models. The use of Python, an interpreted language, for its implementation facilitates this and also ensures that it can be deployed on all platforms. It also provides a user-friendly environment with an interface to the inner structure of its plant models. Elementary programming skills (Python) are needed to formulate new models in OpenAlea (as with CellModeller and VirtualLeaf) but a visual programming environment is included (Visualea) to support high-level model design.

GreenLab (Cournède et al., 2006) is a functional–structural plant model for the fast construction of plants and for the calculation of yields in terms of organs in environmental applications concerning the rationalization of agriculture and forest exploitation. Note that the structural model is more aimed at organogenesis or morphogenesis studies, while the functional model is more geared towards ecophysiology studies. GreenLab, with the GreenSciLab, tool can simulate different botanical plant architectures, plant growth processes, the effects of climate conditions on plant growth, and calibrate the model from experimental data on real plants. This tool has a user-friendly interface and visual output. It is well documented and freely available.

CellModeller is an environment for simulating two-dimensional multicellular models of plant tissues where the mechanical properties of the cell wall have been taken into account (Dupuy et al., 2008). The plant is defined by various structural entities at different scales represented in different levels of the model: vertices, walls, cells, tissues, and organs. The relationships between entities in different levels are defined by vertical functions, while interactions between units of the same level (i.e. neighbouring relationships) are referred to as horizontal functions. The behaviour and properties of these entities can be controlled through user-specified models, using the Python scripting language. The biomechanical description of cell wall dynamics as cell wall response to turgor pressure through cell wall viscosity is well developed in this simulator. It is an open source project, available on all platforms, and it is distributed under the GNU General Public License. The distribution includes documentation and a tutorial.

VirtualLeaf is a recently developed modelling and simulation tool of plant tissue morphogenesis (Merks et al., 2011). It is a cell-based framework that uses an energy minimization method for describing the expansion, division, and shape change of cells, including cell wall mechanics. The cell walls are flexible as they consist of multiple viscoelastic elements linked by nodes. A proxy for turgor pressure has been implemented as a target area that each cell would reach in the absence of counteracting cell wall forces. The intracellular diffusion and reaction of chemicals operate within each cell and cell wall. Ordinary differential equations are used to describe biochemical and genetic regulatory networks and diffusive and active transport. VirtualLeaf defines the biological entities (such as cells, cell walls, transporter proteins, signalling molecules) as C++ classes. Models are defined through plugins, written in the C++ programming language (Stroustrup, 2005), using those classes. VirtualLeaf is an open source project, distributed under the GNU General Public License and is available on all platforms. It includes several demonstration models and a tutorial is available (see Supplementary data in Merks et al., 2011). Detailed instruction in its usage in the form of an experimental protocol is also available (Merks and Guravage, 2012).

Biological regulatory networks allow cells or organs to adjust their biochemical behaviour to internal and environmental changes. GINsim (Gene Interaction Network simulator) is a simulator for the qualitative modelling and analysis of genetic regulatory networks (Naldi et al., 2009). In GINsim the user specifies a model of a genetic regulatory network in terms of asynchronous, multivalued logical functions, and analyses its qualitative dynamical behaviour. A regulatory network is modelled as a regulatory graph, with nodes representing genes or regulatory products and with arcs representing the interactions between them. Graphical settings of genes and interactions can be interactively modified using either default or user-defined parameters. This logical regulatory graph is the input and a dynamical graph is generated as the output. Modelling attributes determine the dynamical behaviour of the network. GINsim is well documented, freely available for academic users and available on all platforms.

COPASI (Complex Pathway Simulator) is a software for creating and solving mathematical models of biochemical networks and analysing their dynamics (Hoops et al., 2006). It can be used both through a graphical and a command line interface. No knowledge of mathematics is required: the
mathematical model (differential equations) is automatically generated from the molecular model (chemical reactions). A molecular model is implemented in a straightforward way by defining the chemical reactions in a simple format. COPASI is capable of performing time-dependent simulations using a deterministic or stochastic framework, depending on the user preference. It is equipped with a number of diverse optimization algorithms that can be used to minimize or maximize any variable of the model, as well as for estimating parameter values that best fit a set of data provided by the user. A number of practical examples such as steady state and time-course simulations, stoichiometric analyses, sensitivity analysis (including metabolic control analysis), are provided (Mendes et al., 2009). COPASI is a very well documented open source, platform-independent and user-friendly software.

This overview illustrates the activity in the development of simulation frameworks suitable for modelling various aspects of plant development. Most of these simulators aim to make the modelling accessible to experimental biologists with a basic understanding of computer programming and mathematics. They do so by providing a framework that takes care of all basic simulator functions (input/output, visualization, solving mathematical equations) and provides building blocks, at the highest level of abstraction possible, in the framework, to formulate models. Nevertheless, at present, most studies using these tools have involved mathematicians and computer scientists that directly or indirectly interact with experimental biologists.

**Cell cycle**

The cell cycle is a central module in the context of multi-scale modelling of growth and development of higher plants as it drives the increase of cell numbers required to sustain organ growth. Essentially, the eukaryotic cell cycle is responsible for balancing the cell’s ‘growth cycle’ in which it increases its size, with the duplication of DNA and redistribution of this genetic information to the newly formed daughter cells. Rather than an endless repetition of cycling through G1, S, G2, and M phases, cell cycle activity in plants is flexible. Duration of the cycle can vary widely depending on genetic background and environmental conditions. This phenomenon shows that cell growth and division are not strictly coupled and that the cell cycle is composed of different, and to some extent independent, sub-modules. A final aspect of cell cycle regulation is that, at some point, cells exit the cycling activity to start differentiation. The occurrence of distinct cell cycle states in the context of growth and development implies that the transitions between successive cell cycle phases (checkpoints) should be considered as regulatory (sub-)modules. In eukaryotic cells these cell cycle regulatory modules are strongly conserved. They are all, to some extent, concerned with regulating cyclin dependent Ser/Thr kinases (CDK) activity. Indeed, the central cell cycle ‘engine’ consists of a catalytic CDK-subunit that phosphorylates different substrate proteins, with exchangeable regulatory cyclins that determine the substrate specificity (Dudits et al., 2011; Inzé and De Veylder, 2006). The target proteins then facilitate the different transitions (G1/S or ‘Start’, G2/M, intra-M or ‘Finish’).

In addition to a generally conserved basis of cell-cycle control, the plant cell cycle has a unique feature in which inhibitory phosphorylation of CDK (Nurse, 1990) plays a less prominent role. CDC25 does not seem to play a role in phosphoregulation at G2/M (Dissmeyer et al., 2010). Inversely the role of cyclin-dependent kinase inhibitors (CKIs), that regulate CDKs by binding and thereby competing with other interacting factors, appear to play a more central role, possibly replacing the CDC25 function (Verkest et al., 2005; Churchman et al., 2006; Peres et al., 2007; Dissmeyer et al., 2009). Their levels also appear to be controlled by ubiquitin-mediated proteolysis (Marrocco et al., 2010). Moreover, a relatively unique feature of the plant cell cycle is that the G2 and M phases can simply be bypassed. This results in endoreduplication, i.e. consecutive DNA replications without any cell division, leading to an increased ploidy (Breuer et al., 2010).

The dynamical properties of the general eukaryotic cell cycle have been successfully modelled around a number of recurring structural motifs (Fig. 1). Double positive or negative feedback loops can act like switches (Tyson et al., 2003). This regulatory motif allows a system to change its state in a discontinuous way in response to an input signal. Depending on the direction of the change in signal, the

![Fig. 1. Basic eukaryotic cell cycle model with regulatory motifs. The principal regulatory interactions as found in generic cell cycle models (Csikasz-Nagy et al., 2006) with a central CDK:Cyclin complex. Solid arrows represent 1 or more reactions (e.g. protein expression is indicated by arrows pointing to Cyclin and CKI), dashed arrows represent regulatory interactions. The CDK:Cyclin activity is regulated by temporal cyclin degradation (via APC and CDC20), reversible binding to an inhibitor (CKI), and (de-)phosphorylation by an inhibitory kinase (WEE) or an activatory phosphatase (CDC25). These three mechanism all include feedback from the active CDK:Cyclin complex as illustrated in the boxes alongside the corresponding regulatory network motifs discussed in the text (pointed and T shaped arrows indicating positive and negative influences, respectively).](https://academic.oup.com/jxb/article-abstract/63/9/3325/582466)
This mechanism has been shown to govern start (G1/S) and finish (intra M) transitions in budding yeast cells (Cross et al., 2002). In models for cell cycle control in fission yeast and generic models the intra-M transition module is proposed to be an oscillator, which alternates in a continuous way between two states and is typically based on a negative-feedback loop: CDK-CYCB activating the APC, which activates CDC20, which subsequently degrades CYCB (Tyson et al., 2003; Csikasz-Nagy et al., 2006). Importantly, a mathematical model can be built up from these motifs, possibly with finer molecular detail added, while conserving the qualitative behaviour of the respective modules. On top of this regulatory layer, so-called surveillance mechanisms are active that can stop the cell at the various checkpoints. Depending on the type of cell and the conditions, this can be size (so the growth and cell cycle can be balanced), DNA damage and repair, completion of DNA replication, and convergence of replicated chromosomes to the metaphase plane (Kastan and Bartek, 2004). In model simulations, this is often represented by changes in the parameters that govern the system’s dynamics. As an example, Zwolak et al. (2009) proposed that in frog egg cell extracts, unreplicated DNA would halt G2/M progression by activating the inhibitory Weel kinase and inhibiting the activatory CDC25 phosphatase. In the yeast model by Tyson and Novak (2001) the cell mass governs the reaction kinetics in various ways to act as a ‘sizer’ mechanism i.e. cell cycle progression is dependent on reaching a critical size.

In contrast to general cell cycle behaviour, relatively limited progress has been made in the computational modelling of the plant cell cycle. This may be attributed to the fact that there has been somewhat of a lag in the characterization of the molecular reaction network. Beemster et al. (2006) have proposed a computational model for cell cycle regulation in Arabidopsis in the context of leaf development. SIMplex (Vercruysse and Kuiper, 2005), a dedicated software tool (http://www.psb.ugent.be/cbd/papers/sim-plex/), was used to produce the model that consists of piece-wise linear differential equations governed by a set of logical rules. A growth factor drives synthesis of specific cyclins and subsequent formation of S-phase and M-phase promoting factors (SPF and MPF, respectively). Despite its simplicity, it qualitatively captures the behaviour of a proliferating cell, even reproducing the entry into the endocycle. This approach combines elements of Boolean networks (composed only of rules, with discrete state variables) and differential equation (DE) models (composed of rate equations, with continuous state variables). The strategy of using hybrid models (consisting in part of an automaton) to overcome the lack of specific kinetic information has been applied before in cell cycle modelling (Novak and Tyson, 1995; Singhania et al., 2011). Tyson and Novak (2001) demonstrated that reasonable models of the control system in yeast cells, frog eggs, and cultured mammalian cells can be obtained by adding specific pieces to a simple generic model based on the antagonistic interactions between CDKs and the APC. These models are all built upon two major irreversible transitions (Start and Finish) between two stable states (G1 and S-G2-M) of the dynamical system. Dissmeyer et al. (2009, 2010) have modified this DE-based generic cell cycle model by replacing the classical G2/M module based on inhibitory phosphorylation by two alternatives. The first mechanism relies on activation by MPF of an alternative M-phase cyclin and the second mechanism on a G2-phase-specific CDK inhibitor (itself feedback inhibited by CDK). Simulations with these model versions have demonstrated that these mechanisms can produce a distinct G2 phase as required for the plant cell cycle. Importantly, the above modelling studies have shown that, especially for intricately wired networks such as the plant cell cycle, they are indispensable to understand and predict a system’s behaviour.

Given their sessile life style, growth and development of plants is, in comparison with animals, more influenced by the environment. Not surprisingly, the list of factors involved in cell cycle regulation is long. Auxin and cytokinins are considered indispensable for plant cell cycle progression (Hartig and Beck, 2006), whereas other hormones play a leading part in more specific conditions (Del Pozo et al., 2005). Besides plant hormones, many factors play a role in cell cycle regulation, for example, sucrose, light, wounding, and ROS (Dudits et al., 2011). It is evident that, to obtain a better understanding of how cell cycle regulation affects the patterns of growth and development and vice versa, these factors should be integrated into future cell cycle models, eventually leading to powerful, multi-scale, spatio-temporal models.

**Cell expansion**

Final plant size and shape are determined by the production of cells and the subsequent, often anisotropic, expansion of these cells during, and especially after, they have ceased to divide. In contrast to cell cycle regulation, the presence of the cell wall on the one hand and vacuoles that determine the major volume of the mature cell on the other hand, make the regulation of the cell-expansion process unique to plants.

Early scientists like Julius Sachs (1882) already described that when parenchyma cells expand in a growing root, their morphological changes were correlated with the volume increase of the central vacuole. Cell turgor and water uptake were discussed as being instrumental in causing expansion, concomitant with stretching of the walls.

Up to now, however, an adequate and generally accepted molecular model of the cell wall structure and how this structure allows both an increase in surface and an incorporation of new wall material still remains elusive. Most models only differ in the types of interaction and spatial arrangement of the different components (Cosgrove, 2000). One widely used model states that plant primary cell walls...
are a composite material consisting of cellulose microfibrils embedded in a highly hydrated pectin matrix and tethered by hemicelluloses (Carpita and Gibeaut, 1993). The specific spatial arrangements of the load-bearing components make cell walls that can be mechanically highly anisotropic and different classes of cell wall-modifying enzymes can regulate wall behaviour at different developmental stages (Cosgrove, 2005). Furthermore, it is generally accepted that microtubules serve as guides for the cellulose synthase complexes and that, by doing so, they also influence the mechanical anisotropy of the cell walls (Paredez et al., 2006).

In contrast to the situation in the regulation of the cell cycle, models of regulation of cell expansion focus largely on physical properties, rather than molecular interactions controlling them. An important first step in the modelling of cell expansion was the summary of experimental data on wall extensibility by Lockhart (1965) in a rather straightforward formula that has continued life as the ‘Lockhart equation’:

\[ r = \varphi(P - Y) \]

where \( r \) is growth rate, \( \varphi \) is extensibility of the cell wall, \( P \) is turgor pressure (the source of cell wall stress), and \( Y \) represents the yield threshold (the minimum pressure required for growth). This equation clearly states that the rate of cell expansion correlates with a difference between turgor pressure and wall stress threshold, and with the extensibility of the wall, which is dependent on arrangements of components and even on the location in the organ (Crowell et al., 2011). This emphasizes that key players of the cell elongation process, potentially capable of changing any of these parameters, are to be found in the symplast as well as in the apoplast. Undoubtedly different sets of genes and proteins must be expressed at different stages of the expansion process and/or rendered active or inactive. Several of these crucial genes and proteins are starting to emerge, but the complete picture is far from clear. Links with hormone metabolism and transport complicate the picture even further.

Wall loosening proteins such as expansins and xyloglucan endotransglucosylase/hydrolases (XTHs) and other lytic enzymes are active in expanding cells (McQueen-Mason et al., 1992; Vissenberg et al., 2000). They exert specific effects on wall polymers and their interactions, and hence on the biomechanical properties of the wall in general (Cosgrove, 2005; Van Sandt et al., 2007). To study the molecular basis of cell wall extension several in vitro techniques can provide answers to different questions. Measurements of breaking strength, and elastic and plastic compliance can indicate changes in cell wall structure. On the other hand, wall stress relaxation and creep measurements give clues both to changes in wall structure and to wall-loosening processes (Cosgrove, 2011) and can be used to monitor the effect of proteins on cell wall behaviour (Cosgrove, 2005; Van Sandt et al., 2007). Findings resulting from these approaches can be used to model changes in cell wall behaviour (yielding and extensibility) when for example XTHs exert their typical transglucosylase action on xyloglucan (XET) or when only hydrolase action (XEH; Nishitani and Vissenberg, 2007) is present.

Next to the loosening of walls and their turgor-driven stretching, new cell wall material needs to be laid down to keep the growth rate high for some time. This can be seen by the reduction in maximal cell size when for example cellulose biosynthesis is reduced in a mutant background or by chemical interference, in different organs as well as in cell cultures (Arioli et al., 1998; Fagard et al., 2000; Vissenberg et al., 2000; Lane et al., 2001).

In recent years the first models that simulate the cell expansion process have appeared. Corson et al. (2009) modelled changes in cell shape that were observed in the shoot apical meristem of Arabidopsis upon disruption of cell wall microfibril orientation by treatment with oryzalin. Their model, based on a set of ordinary differential equations, describes the elastic behaviour and plastic yielding of cell walls under turgor pressure generated by solute concentrations in the cells. With this approach, they were able to simulate the dynamic behaviour of cell growth, resulting in realistic geometrical properties of cells in normal and perturbed tissues.

Qian et al. (2010) used a finite element method of a fibre-reinforced hyperelastic composite material to model the mechanical properties of onion epidermis in relation to the orientation and distribution of the microfibrils in the walls. They related this model to extensiometry data that show the relationship between microfibril orientations, stress applied to the wall and the resulting strain rates (Suslov and Verbelen, 2006). They developed an inverse modelling approach that allowed the mechanical parameter set to be found that leads to a good correlation of the simulated data with the experimental observations.

After models that capture the essentials of the physical basis of cell expansion have been developed, a next challenging step will be to incorporate the molecular level regulation. This will allow linking of these models to upstream regulatory signals. This is necessary to go from the more or less uniform tissues that they have been tested on to the spatially and temporally complex situation in growing organs.

Hormone signalling

The classical plant hormones all affect growth and hence must directly or indirectly impinge on the regulation of cell division and cell expansion. Broadly speaking, two functions can be discriminated. The first is the generation of morphogen gradients, which determine the locations where cells actively divide and expand. The second, superimposed function is the signalling of environmental conditions (often stress) that among many other effects result in an adjustment of the growth rate. Here the focus will only be on the first function as, for modelling organ growth per se, one needs some form of morphogen that determines cell cycle and cell growth activity. Auxins are the best studied plant hormones in this context. More recently, gibberellins (GA) have also been shown to be instrumental in controlling the transition from proliferation to cell expansion (Achard
et al., 2009; Ubeda-Tomás et al., 2009), but more research is needed to understand how concentrations of active GA are linked to this function.

At the level of isolated plant cells, cell division and cell expansion are mainly controlled by auxins, in collaboration with cytokinins. The auxin:cytokinin ratio represents an important signal in the formation of cell phenotype and also in the onset and maintenance of the cell division process. They appear to influence different phases of the cell cycle. Auxins exert an effect on DNA replication, while cytokinins exert control over the events leading to mitosis (Machakova et al., 2008). Differences in the concentration of these hormones have indeed been linked to the spatial occurrence of cell cycle activity in a growing organ. To establish concentration gradients, active transport plays a crucial role. For auxin, the molecular components that determine this transport have been identified as PIN and AUX1 auxin efflux and influx transporter families, respectively (Paciorek and Friml, 2006). These components form an auxin transport network that mediates local auxin distribution and triggers different cellular responses in various developmental contexts. Auxin function in the cells is mediated by a cascade where auxin binds to TIR1, the F-box subunit of the ubiquitin ligase complex SCF(TIR1), thereby stabilizing the interaction between TIR1 and Aux/IAA substrates. This interaction results in Aux/IAA ubiquitination and subsequent degradation. As a result, the inhibitory action of Aux/IAA proteins on transcription factor activity of the Auxin Response Factor (ARF) family is released. These ARFs then activate auxin responsive elements (AuxREs) that mediate the auxin-response regulation of genes involved in many processes among which are cell division and expansion (Mockaitis and Estelle, 2008). Auxin is known to induce the expression of some members of the XTH family that modify cell wall components (Vissenberg et al., 2015). In addition, it is capable of inducing the acidification of the apoplast (Kotake et al., 2000), thereby inducing cell growth (Kotake et al., 2000).

The knowledge concerning the cell and molecular biology of auxins has formed the basis for several mathematical models that simulate auxin-mediated plant development (Fig. 2). Mathematical formulation of polar auxin transport rules, mainly in the form of sets of coupled differential equations, has led to an impressive array of models that explain auxin distribution patterns in the context of many developmental processes in the model plant, Arabidopsis. Swarup et al. (2005) used such a model to explain the importance of AUX1 expression in the epidermal tissue during a gravitropical response. Three independent papers, published back to back, modelled the accumulation of auxin at the shoot apical meristem in order to understand phyllotactic patterning (de Reuille et al., 2006; Jönsson et al., 2006; Smith et al., 2006). In contrast to these studies, Stoma et al. (2008) proposed a flux-based PIN polarization mechanism to explain auxin distribution at the shoot meristem. Flux-based and concentration-based mechanisms were combined into a dual polarization model by Bayer et al. (2009). Grieiinessen et al. (2007) modelled auxin transport in roots based on microscopic observations of PIN localization to explain the occurrence of an auxin maximum in the Arabidopsis root tip and, by extension, the spatial occurrence of cell division and expansion, resulting in a growing root tip. Further development of this work resulted in a mechanistic model for lateral root initiation (Laskowski et al., 2008), Jones et al. (2009) modelled auxin transport in the epidermis of the root tip to explain the differentiation of trichoblast and atrichoblast cell files. No fewer than three different mechanisms were proposed to describe the role of auxin transport in the formation of leaf vascular patterns. The first is a reaction-diffusion model which assumes a single diffusible substance (in contrast to the classical model that combines the slow diffusion of an activator molecule with faster diffusion of an inhibitor, that is transcriptionally stimulated by the activator (Fig. 2)), while assuming uniform local auxin production (Dimitrov and Zucker, 2006). The second is a canalization model (where auxin flow orients the PIN transporters to amplify transport in that direction further, i.e. down the gradient transport (Rolland-Lagan and Prusinkiewicz, 2005). The third is a PIN-mediated transport model where PIN expression is dependent on auxin concentration and orientation is towards neighbours with higher concentration (Merks et al., 2007; Wabnik et al., 2010). Bilborough et al. (2011) modelled the interaction between PIN1, auxin, and CUC2, explaining the formation of serrations at the leaf margin. Lucas et al. (2008) used stochastic modelling to build an auxin transport-based model of root branching in Arabidopsis thaliana.

All these models have in common that they confirm the central role of active auxin transport and auxin signalling in plant morphogenesis. These studies have substantially expanded our mechanistic understanding of diverse processes like embryonic development, stem cell maintenance, root and shoot architecture, and tropic growth responses. However, they also highlight the need to unify the underlying mechanisms in accordance with the central role of auxin as a regulator of growth and development. Further steps in auxin modelling will therefore need to assess the level of detail, i.e. which processes and how many levels of organization to incorporate in order to obtain suitable models for the developmental process under study (ten Tusscher and Scheres, 2011). However, the usefulness of the auxin models also implies the need for similar models for other hormones, particularly cytokinin and gibberellin, in the context of organ growth and development. However, currently, there is insufficient knowledge on the localization of synthesis and on transport mechanisms on these hormones to construct similar models.

Parameter space exploration: transitions in the steady-state patterns of hormone transport

As outlined above, most models describe the biological system with the help of sets of coupled differential evolution
Implicitly, these equations contain a range of parameters that determine the behaviour of the system. For the majority of these parameters, no experimental variables are available. Currently, most modelling papers only present the (realistic) behaviour of the model for a standard set of parameters. However, once established most models allow for a more refined evaluation of the model parameters. Answers to questions such as, ‘Across what range of values do the models yield stable solutions?’ and ‘Which of the parameters are crucial for the overall behaviour and which ones are less important?’, provide crucial information about the model and pose testable hypothesis for the experimental biologist.

Mathematical analysis techniques have been developed for this purpose and their usefulness will be illustrated here in the context of the transport of auxin hormones in a simple linear file of cells with a static cell geometry, using the model of Smith et al. (2006). This model consists of two equations for every cell. One equation models the evolution of the PIN1 protein and the other equation describes the evolution of the hormone auxin (IAA). Both equations are dependent on the concentration of PIN1 and the concentration of IAA in that cell, but they are also dependent on those concentrations in the adjacent cells with, at most, distance 2. This leads to a non-linear coupled system of evolution equations that can yield a stable pattern.

**Fig. 2.** Auxin transport models. (A) Classical reaction-diffusion mechanism with slow diffusion of an activator molecule (here auxin) and faster diffusion of an inhibitor, that is (transcriptionally) stimulated by the activator. The resulting local activation and lateral inhibition can lead to characteristic leaf venation patterns (cf. Meinhardt et al., 1998). Pointed and T shaped arrows represent activation and inhibition, respectively. (B) In cellular models of auxin transport, auxin auto-activation corresponds to the activation of PIN exporters that transport auxin in the direction of higher fluxes (canalization) or higher concentrations. (C) Schematic representation of plant cells with PIN transporters located in the plasma membrane as well as the endosome. Polar re-distribution of PIN transporters is proposed to depend on a mechanism that senses the auxin concentration in neighbouring cells (or, for example, in the apoplast as proposed by Wabnik et al., 2010) or the auxin flux. This signal could feed back on PIN translocation preferentially retaining PINs at one side of the cell.
of equally spaced auxin maxima. This pattern can be visualized, for example, using reporter genes. The non-linearity is a consequence of the active transport between the cells.

The question one could address is how this pattern varies when model parameters are changing. For most parameters in the model, only a range of possible values is known and no particular values have yet been experimentally determined. It is still not clear which role each of the parameters plays in the pattern formation.

However, if one of the parameter values is changed, the stability properties of the pattern might change, resulting in a new pattern or periodic fluctuations of the concentrations over time. It is therefore important to understand how the stability properties of the pattern solution depend on the parameters of the model.

The study of the relationship between the stability of a solution and the parameters of the corresponding dynamical system is known as bifurcation analysis (Seydel, 1994). Such an analysis identifies the stable and unstable solutions and the bifurcation points that mark the transitions between them. At bifurcation points the number of solutions change. This analysis usually leads to a bifurcation diagram that highlights the connections between stable and unstable branches as the parameters change. This is illustrated here with the bifurcation results for the model of Smith et al. (2006) applied on a row of 20 cells with the IAA transport coefficient \( T \) as the varying parameter (Fig. 3). For the other parameters of the model, similar scenarios can be calculated. It was observed for some of the parameters that there is a constant solution with an equal concentration in all cells. This stable solution does not change in time and peaks are absent. However, when the parameters are changed beyond a critical threshold, this equilibrium is suddenly replaced by a stable pattern of peaks or a periodic motion of the concentration. When the stable pattern of peaks appears, the transition point is known as a branch bifurcation (Fig. 3). When a periodic motion appears, the transition is known as a Hopf bifurcation.

The above illustration of a bifurcation analysis of the auxin patterns shows its usefulness to explore the relationship between all possible patterns and the parameters used to model the transport of hormones. With the help of bifurcation analysis, for each given mathematical model the ‘parameter space’ can be explored and the stable patterns that can emerge can be identified. It is also possible to compare multiple models that aim to describe the same biological system. Furthermore the genesis of a given pattern can be linked to a particular parameter.

For a low-dimensional system of coupled ODEs, such as a model for a row of a few cells studied in the paper, there are excellent numerical continuation tools that automatically identify bifurcation points and switch between branches and produce the bifurcation diagram. Examples are AUTO (Doedel et al., 1997) and PyDsTool (Clewley et al., 2004).

**Perspectives**

The above review has illustrated the growing integration of modelling in biological research and the developments that will increase the power of these approaches to dissect the behaviour of the models and hence the biological system under study. No doubt these developments will deliver powerful new tools for a thorough understanding of plant growth and development.

It is also clear that for this approach to reach its full potential, computational and mathematical frameworks need to be developed further, but, equally important, biologists also need to evaluate the types of experiments and measurements required to support the modelling. Biologists will need to develop strategies to validate models at underlying organizational levels such as the cell and molecular levels. At the cellular level, methods to quantify cell division and expansion accurately, both at high spatial and temporal resolution, have been developed (Florani and Beemster, 2006). Unfortunately, these methods are still rather labour-intensive, but several steps are currently being automated, so that, in future, the capacity for such analyses will increase.

At the molecular level, many tools are available to plant biologists to study various aspects of the models qualitatively. Particularly in the model species, Arabidopsis, a wide range of reporter lines are available that report the transcriptional activity of well-studied genes and are therefore likely to become part of the modelling activity. In particular, for the study of auxin transport and accumulation, many extremely useful reporter lines and tools have been developed. For other hormones such tools are still largely missing, but may become available in the next few years. Measurements of metabolites, of interactions of metabolites, as well as of rates of synthesis, decay, and transport are extremely difficult. Nevertheless, efforts should be made to develop strategies to measure such quantities. Further integration of modelling in biological research will increase the need for such measurements.

The need for measurements of key model parameters is a first example of how modelling can direct the biological experiments that need to be developed in order to increase our understanding of the biological system under study. It clearly shows how models can direct research to unanswered pertinent questions (Systems Biology). By integrating new detailed knowledge such models can gradually provide increasingly accurate descriptions of specific developmental processes. Besides this increasing detail, combining different sub-models of parts of the plant in a joint framework will allow the plant and, ultimately, a crop as a whole, to be accurately represented. Such knowledge-based models will ultimately replace phenomenological models build on a statistical basis that are currently most commonly used in modelling field crops. The long-term perspective of having models built on the function of detailed gene networks will enable crops to be engineered with improved growth characteristics specifically tailored for high yields of particular organs under a defined set of environmental conditions.
Fig. 3. Bifurcation analysis of hormone transport in a linear file of 20 cells. (a) The bifurcation diagram that depicts the concentration of the growth hormone auxin in cell number 5 versus the IAA transport coefficient T. The solid lines indicate the stable solutions and the dashed lines the unstable solutions. The other plots illustrate the steady-state auxin patterns in all cells for the specific places indicated in the bifurcation diagram. For small values of T, there is only the constant solution with equal concentrations in all cells shown as the horizontal line in the diagram. When the parameter T becomes larger, this solution loses its stability at a branch point (BP1). In this branch point there is an exchange of stability to the other branch, also shown in the diagram. There are two stable parts on this other solution branch with patterns. The solution patterns in area 2 show one big peak as displayed in (c). This pattern can appear when the IAA transport coefficient T is large. The other stable pattern shows two small peaks as displayed in (g). This pattern appears in area 4 for a very limited range of the IAA transport coefficient T.
Acknowledgements

This work was funded by a GOA research grant, ‘A Systems Biology Approach of Leaf Morphogenesis’ granted by the research council of the University of Antwerp and a return grant from the Belgian Federal Science Policy Office (BELSPO) to DDV.

References


