Gene regulatory network models for plant development

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Accumulated genetic data are stimulating the use of mathematical and computational tools for studying the concerted action of genes during cell differentiation and morphogenetic processes. At the same time, network theory has flourished, enabling analyses of complex systems that have multiple elements and interactions. Reverse engineering methods that use genomic data or detailed experiments on gene interactions have been used to propose gene network architectures. Experiments on gene interactions incorporate enough detail for relatively small developmental modules and thus allow dynamical analyses that have direct functional interpretations. Generalities are beginning to emerge. For example, biological genetic networks are robust to environmental and genetic perturbations. Such dynamical studies also enable novel predictions that can lead to further experimental tests, which might then feedback to the theoretical analyses. This interplay is proving productive for understanding plant development. Finally, both experiments on gene interactions and theoretical analyses allow the identification of frequent or fixed evolutionary solutions to developmental problems, and thus are contributing to an understanding of the genetic basis of the evolution of development and body plan.

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Introduction

The way in which the concerted action of multiple genes, along with environmental factors, regulates cell differentiation and development is still an open question in biology. Given the overwhelming number of genes and complexity of interactions that are involved in these processes, schematic and intuitive models are not sufficient to describe them. Hence, quantitative and integrative tools, such as mathematical representations and computational simulations, are becoming paramount. These tools enable structural and dynamic studies of complex assemblages of interconnected genes, proteins and other molecules, which we refer to as gene regulatory networks (GRN).

In Box 1 (see also [1]), we summarize some mathematical and simulation approaches that have been used recently to integrate experimental data on GRN. Such formal theory and methods provide improved understanding of biological systems. During the 20th century, several mathematical models for development were proposed (see timeline in Figure 1). These constitute a solid theoretical framework that might help to pose hypotheses about the conditions that are necessary and sufficient for cell differentiation and pattern formation, but they incorporated unrealistic assumptions about genetic mechanisms or, until very recently, could not be validated because of the scarcity of data. For example, the first GRN models [2], which aimed to represent dynamic and structural aspects of collections of interacting genes, assumed randomly connected networks with the same average number of regulatory interactions per node. By contrast, recent data suggest that actual GRN exhibit skewed distributions and preferred local connectivity patterns [3,4].

The accumulation of data from classical molecular genetic studies of development and functional genomics enables more realistic dynamic GRN models of cell differentiation and morphogenesis. In GRN models, genes, mRNA or proteins correspond to the network nodes and the links among nodes stand for regulatory interactions. These models are being developed with two main approaches. One uses functional genomics to reverse engineer the identity of the network nodes and the regulatory interactions among them (e.g. [5••]). The second approach uses detailed molecular genetic experiments to propose models of GRN architectures for relatively small gene networks (e.g. [6••]). Such networks can be studied thoroughly in relative isolation from the whole, allowing direct functional interpretations. They have enabled analyses of the temporal change of concerted gene activities (i.e. network dynamics) and of the way in which genes are connected to each other (i.e. network structure or architecture).

Box 1 From genes and molecules to gene regulatory networks: mathematical models.

There is always tension between generality and level of detail (and thus tractability) in a model. Depending on the scale involved and the nature of the available information, a suitable mathematical framework can be selected. We present basic terminology and concepts in GRN models and, in this context, introduce different types of models (see review in [48]). As development involves a wide range of scales and mechanisms, a combination of the models presented below, and others, will surely become necessary to understand fundamental aspects of morphogenesis.

Gene regulatory network models

In GRN models, the nodes correspond to genes, messengers or proteins and the edges represent regulatory interactions (activations or inhibitions) among the network components. In these models, gene regulatory interactions are translated into a set of updating rules that determines the nodes' states at every moment (Box Figure 1). These rules make it possible to follow the trajectory from one gene activity configuration to another, starting in each one of the possible configurations of gene activities (initial conditions). Configurations can be tracked until they reach a state that, given the network rules, remains unchanged. This state is called a fix point attractor. Configurations can also follow a trajectory that leads to a so-called periodic attractor, which corresponds to a collection of states among which the configuration cycles indefinitely once it is reached. The set of all initial configurations that lead to a specific attractor, be it fix point or periodic, conform the attractor's basin of attraction. A frequently used analogy is that of a landscape with valleys, in which the bottom of the valleys correspond to attractors and the valley's basin to the basins of attraction. Continuing with the analogy, every point on the landscape matches a gene activity configuration and if a bead were located on one of these points, it would follow a path on the rugged landscape, a trajectory, until it reached a valley. Finally, the topography of the valley, i.e. the number and identity of attractors, is determined by the set of logical rules and network architecture. Cell states that can be characterized by a certain fixed or cyclic gene activity configuration might indeed correspond to the attractors of a complex dynamic system [2,49•].

Discrete *versus* continuous GRN models

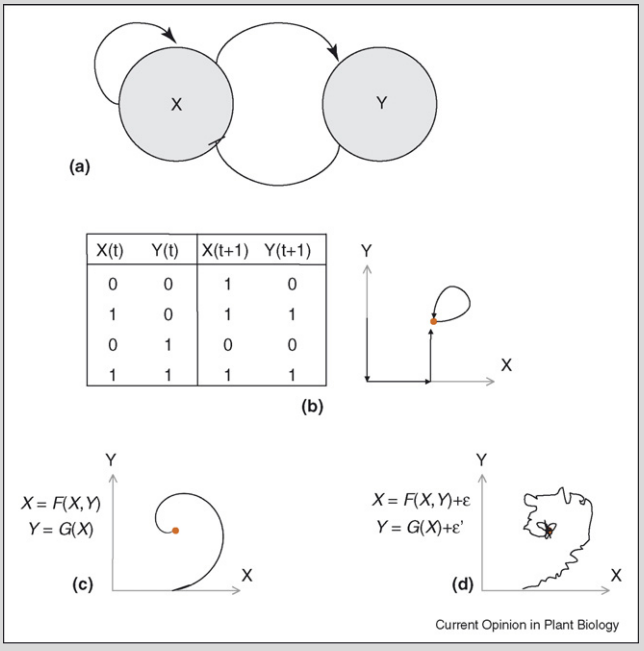
GRN models might incorporate continuous functions (differential equations; e.g. [22]) or discrete functions (difference equations; e.g. [6••]) to describe the rules that govern gene activation kinetics. Continuous implementations can include more detail and yield quantitative predictions, but experimental data that provide parameter estimates for such models are scarce. Despite this, continuous models have proven to be very useful for investigating signal transduction pathways and the circadian clock, both relevant processes in plastic plant development ([34••,35•,50,51•]; see [52] for review).

Different analyses of topologically equivalent continuous and discrete models have shown that both yield equivalent dynamic results ([24,53], although see [54]). In networks that have many non-linearities, the behaviour of the system seems to depend mostly on the GRN topology rather than on specific parameter values. In addition, if gene expression functions and pattern formation time scales are considerably distinct, qualitative discrete systems might be useful. Finally, recent experimental evidence suggests that gene expression is digital and stochastic at the individual cell level, although in cell aggregates gene expression might appear to be continuous [55,56,57•]. Given this, qualitative GRN models that have discrete kinetics of gene activation (e.g. [6••]) (0 ['OFF'] or 1 ['ON'] in the simplest Boolean case [32]) might be the most appropriate representation of complex gene regulatory logics.

Deterministic versus stochastic GRN dynamics

If logical rules or equations that govern the updating of the network states allow us to determine the fates of all states at every moment, the system is deterministic (e.g. [22]). By contrast, stochastic models [58] consider the noise inherent to natural systems that is caused by small numbers of molecules or other sources of uncertainties. In these models, the updating depends partially on a stochastic variable, which introduces a certain amount of uncertainty into the system dynamics.

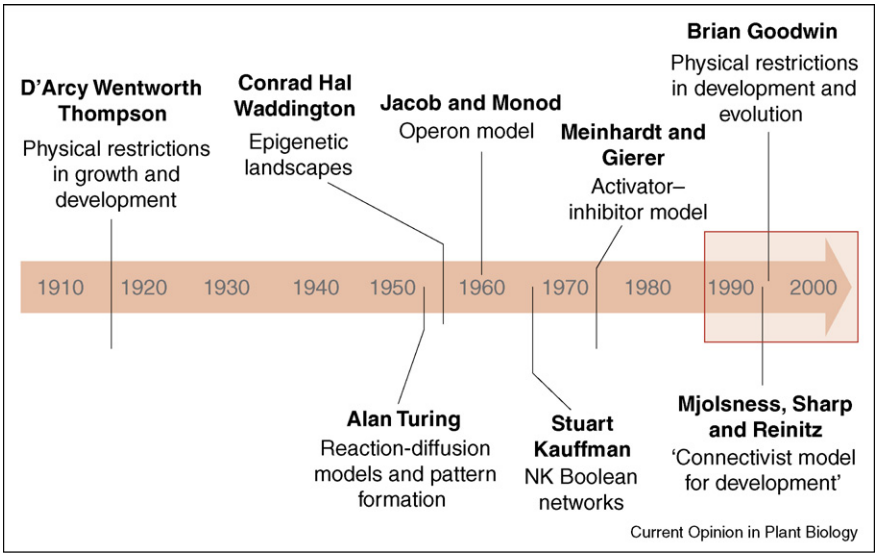
Box Figure 1



Example of a simple two-gene GRN dynamic model. **(a)** Two (X and Y) element GRN with positive (arrowhead edges) and negative (flat end edges) regulatory interactions. **(b)** Logical rules and graphical representation of a two-dimensional discrete trajectory that results from applying the set of rules (explicit only for the 0,1, Boolean case). Note that the 1,1 corresponds to a fix point attractor and the rest of the conditions that lead to this state to its basin of attraction. **(c)** Continuous deterministic case, given by differential equations, and graphical representation of a dynamic scenario qualitatively equivalent to that presented for the Boolean case. **(d)** Stochastic case of a similar dynamical scenario (ε and ε' are sufficiently small stochastic variables).

Here, we review studies of these two approaches for plant systems, also touching upon relevant animal examples. We then highlight the general findings that are emerging from these studies, the efforts to model morphogenesis from coupled GRN in explicit spatiotemporal domains, and the utility of formal dynamical analyses for evolutionary studies. We conclude that the two approaches are complementary for understanding the interplay between the structure and dynamics of GRN, and for uncovering general rules in the logic of the regulation of developmental processes and its links with signalling pathways and other cellular processes.

Figure 1



Some of the twentieth century theoreticians of development and their contributions. The boom of functional genomic technologies and system biology approaches is framed in the red rectangle.

From functional genomic data to gene regulatory networks

Recent powerful experimental technologies and novel statistical methods are being developed to infer GRN architectures from genomic data obtained in microarray experiments (reviewed in [7,8,9•]; for plants see [10]; and see Figure 2). Efficient reverse engineering of GRN architectures depends on collecting data that guarantee a wide exploration of perturbation conditions [11] or phenotypic variations of a cell type [12••], so that correlations among expression levels of different genes can be thoroughly investigated.

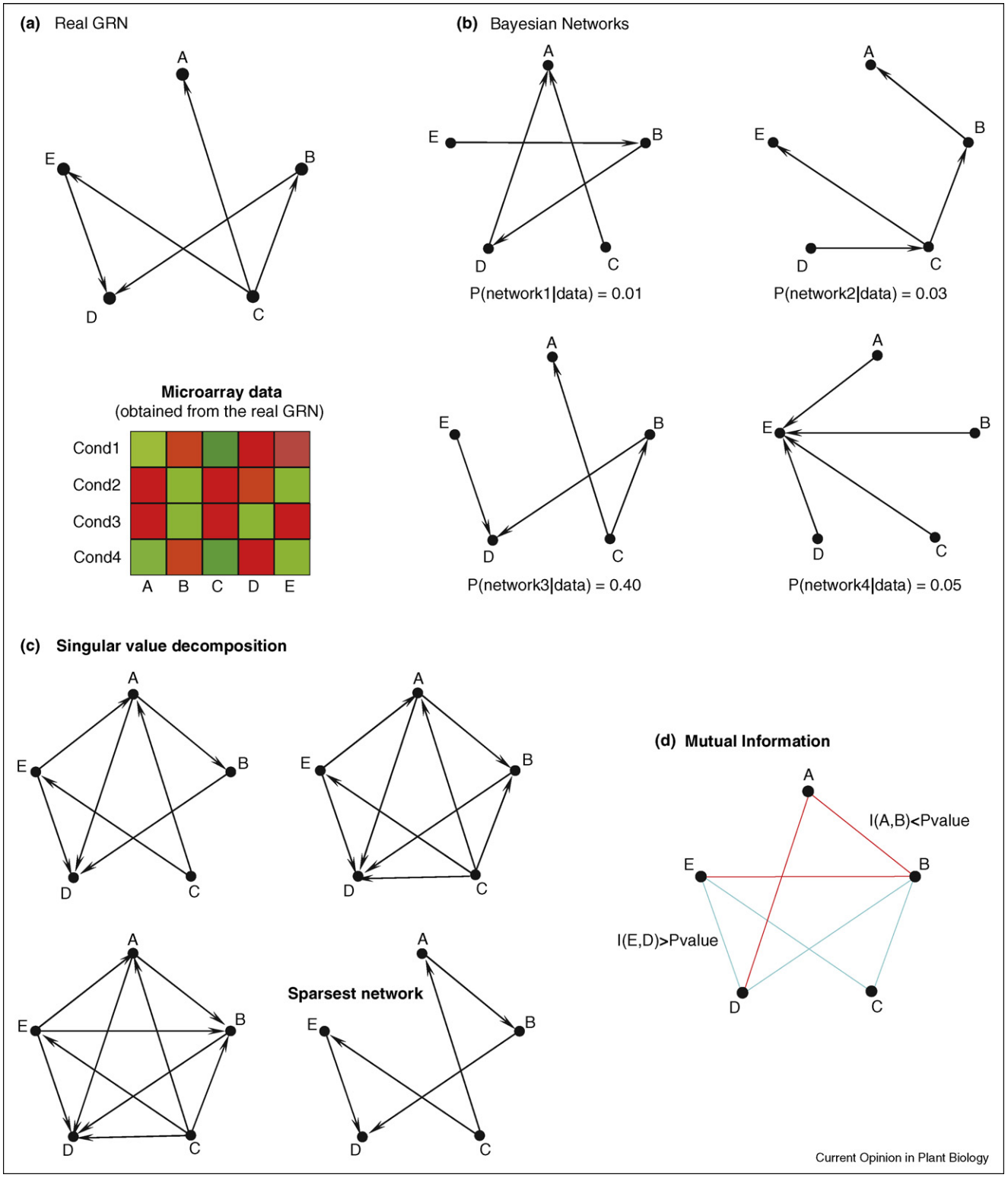
Two GRN architecture inference methods are widely accepted and have a particularly sound theoretical basis (Figure 2). More importantly, networks obtained by these two methods have been extensively validated with experimental data; although to our knowledge, they have not been applied to data from plant systems. The first method is based on Bayesian inference theory ([13]; Figure 2). When applied to gene regulation, the goal of this theory is to find the most probable GRN given the observed expression patterns of the genes to be considered in the network. Thus, the regulatory interactions among genes and their directions are derived from expression data. Different network structures are proposed and then scored on the basis of how well they explain the data. This method has been applied, for example, to infer regulatory modules in *Saccharomyces cerevisiae* [14].

The second method uses 'mutual information' as a measure of correlation between gene expression patterns ([15,16]; Figure 2). A regulatory interaction between two genes is established if the mutual information on their expression patterns is significantly larger than a P-threshold value calculated from the mutual information between random shufflings of the same patterns. In contrast to the Bayesian theory, which tries out whole networks and selects the one that best explains the observed data, the mutual information method constructs a network by selecting or rejecting regulatory interactions between pairs of genes or GRN nodes. This method does not provide the direction of regulatory interactions and has been tested for GRN that underlie the differentiation of human cell types [12••].

Recently, a third method for inferring GRN has been put forward (Figure 2). It assumes that GRN operate near a steady state and approximates its dynamics by a system of linear differential equations. The matrix of the linear system gives the type and strength of regulatory interactions. The system is solved to yield a matrix of gene interactions that matches the gene expression data. This method has been improved to take into account sparcity of connections and to incorporate different sets of microarray data [17]. To our knowledge, this is the first method that has been used to model the structure of an *Arabidopsis thaliana* GRN [5••].

Other recent methods have been tested using detailed data for relatively small model networks (for plant examples see [18,19], and for *Drosophila* [20•]) and might be useful as complementary techniques. Reverse engineering methods, in general, should be part of a recursive

Figure 2



The organism whose GRN is to be inferred is exposed to different conditions and microarray data is obtained accordingly. **(a)** A known or 'real' GRN consisting of A, B, C, D and E genes. Note that the expression patterns of the genes vary under different environmental conditions (cond1, . . ., cond4); the 'richness' of the patterns, which depends in part on the conditions tested, is important to discover gene interactions. **(b)** In Bayesian Network inference, different architectures are discriminated by calculating the probability of a network architecture given the observed data, P(network/data). The network that has the highest probability is selected. In this case, we see that the network that best explains

process in which previous or additional functional data are also considered to propose and validate former and novel regulatory interactions.

Dynamic models for small GRN of developmental modules

Development consists of processes that can be logically isolated, probably because of an underlying modularity in the global GRN [21]. This encourages the analysis of gene sub-networks for modules that are structurally and functionally isolated from the rest and that have been thoroughly studied in terms of molecular genetics, therefore allowing the introduction of dynamic models.

Pioneering work on this approach, developed in Odell's laboratory [22,23], shows that the gene network that determines *Drosophila* segment polarity is robust for different initial conditions or parameter values that affect, among other things, the strength of interactions and the exact kinetic functions of the genes (or proteins) [22]. Robustness to initial conditions supports evidence suggesting that the studied GRN performs its function semi-autonomously, irrespective of its interactions with other genes outside the network [21]. The robustness of the GRN studied by von Dassow and collaborators is further supported by the fact that a Boolean model of the segment polarity GRN recovers the same patterns for the *Drosophila* segment polarity genes as those recovered by the continuous model [22,24]. Similarly, the neurogenic and proneural GRN in *Drosophila*, also studied in Odell's laboratory [25,26], is a robust module. This latter study further suggested that structural alterations, and not only parameter changes, are tolerated by the GRN.

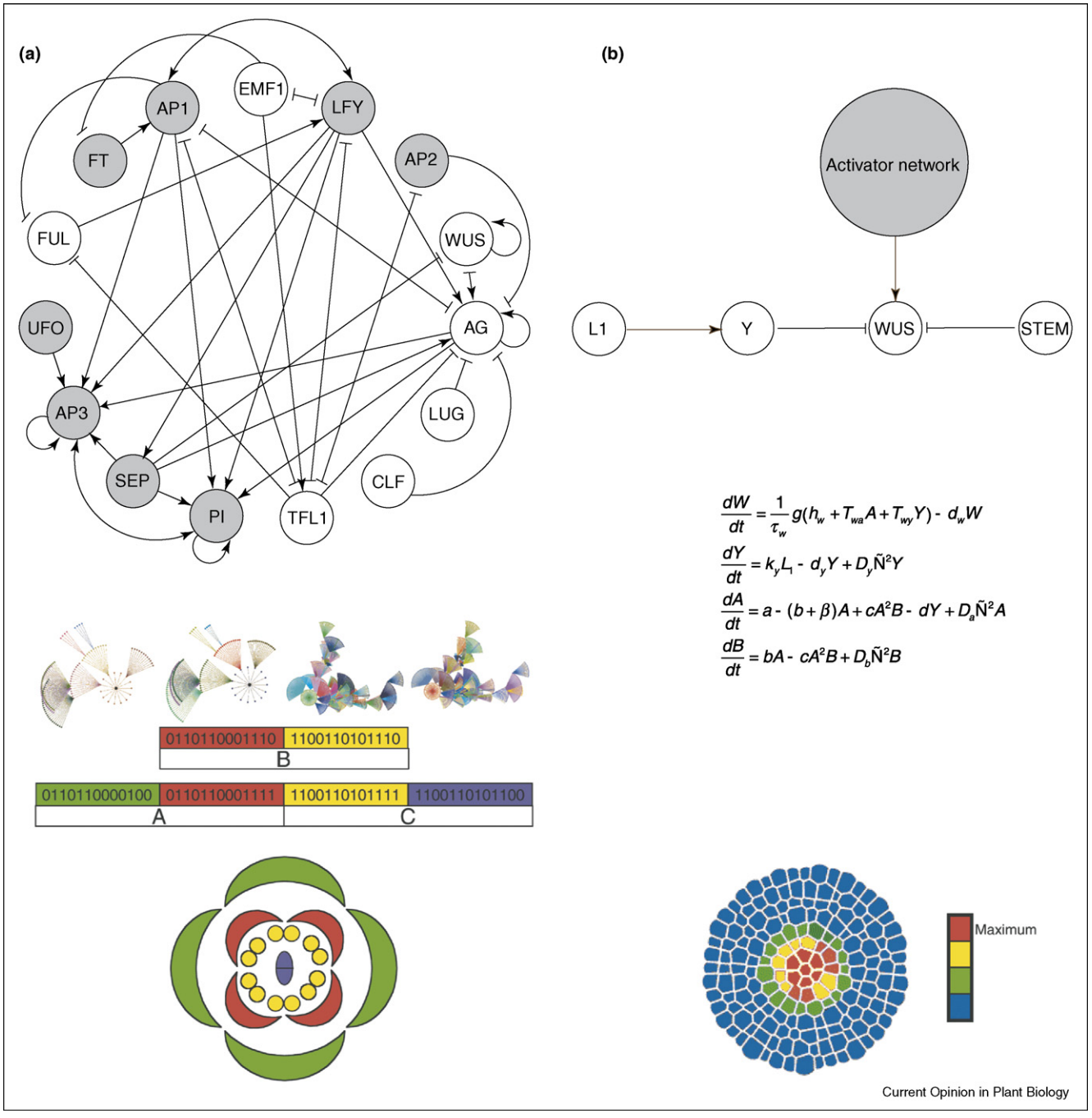
Few similar studies have been put forward for plant systems. The first dynamic models of plant GRN were proposed for the specification of floral organ and root epidermis cell types in *A. thaliana* [27,28]. These studies used Boolean models, grounded on available experimental data, to describe the gene activity profile that characterizes the state of single cells. Thomas [29] proposed a logical approach to analyze the dynamics of Boolean GRN (Box 1) that was based on identifying functional positive and negative feedback loops. Such analyses might also be used to identify functional sub-modules within GRN and were applied to *A. thaliana* floral identity GRN [30]. The results mostly coincided with predictions made by the now classical ABC model of floral organ specification [31] or with the steady states predicted by a different approach [28].

The ABC model postulates that the combination of three classes of genes (A, B and C) underlies the specification of primordial floral organ cells in plants; with A genes alone specifying sepal primordia, A+B petals, B+C stamens and C alone carpel. Furthermore, ABC functions seem to be conserved over a wide array of flowering plant species. The ABC combinatorial model does not, however, explain the conservation of this model, or the logic and dynamics of gene regulation involving ABC and non-ABC genes that underlie the gene activation profiles observed in floral organ specification. An updated GRN floral model [6••] showed that, given the interaction rules extracted from experimental information, all possible initial gene activity configurations converge to few fixed gene activity states, also called attractors (Box 1). Such attractors match the gene expression profiles of the cells of inflorescence meristems and of sepal, petal, stamen and carpel primordia. In addition, genetic perturbations of this GRN reproduce patterns observed in reported mutants ([6••]; Figure 3a). By recovering all of the gene activation profiles that correspond to either inflorescence meristem cells or cells of each one of the four floral organs, and no more, this GRN model [6••] provides a dynamic explanation for the ABC model. It seems to incorporate the key elements of a developmental module that underlies the ABC model of floral organ specification. This study also showed that the steady states are robust to changes in the interaction rules [6••,32], consistent with the fact that the overall floral plan is widely conserved among flowering plants. Furthermore, this study suggests that, even though qualitative models do not consider the detailed kinetic functions of gene activation (Box 1), such models might provide an adequate representation of the logic of regulation, and are useful integrative tools for detecting holes in experimental data and for generating novel predictions. For example, the floral organ identity GRN ([6••]; see [28,30] for other examples) predicted that the gene *AGAMOUS* should self-activate. This was confirmed by independent parallel experiments [33].

Another recent example of a relatively small plant network that has been grounded on detailed experimental data has identified the essential components of the abscisic acid signal transduction pathway, which controls stomatal opening and closure depending on the water balance of the plant [34••]. Although this GRN controls a physiological rather than a developmental process, it constitutes another example of the power of qualitative representations to integrate data into a dynamic analysis. The model outputs are consistent with experimental

(Figure 2 Legend continued) the data is the third one (P[network/data] = 0.40). **(c)** Using Singular Value Decomposition, the family of feasible solution architectures, constricted by the data, are obtained. The final network is selected on the assumption that the most (biologically) relevant network is the sparsest one. The example shows the family of solution networks that are consistent with the data. At the end, the sparsest one is selected. **(d)** The Mutual Information (I) of different pairs of genes is measured. If I is lower than a P-threshold value, then the interaction is rejected (red links in the example network). If I is larger than the P-threshold value, the interaction is accepted (blue links). In this way, the GRN is constructed interaction by interaction.

Figure 3



Gene regulatory network models for plants. **(a)** Single cell GRN dynamic model for cell specification in *Arabidopsis* floral organs [6••]. The topology of the 15 gene GRN is shown according to [6••], with activations as arrowheads and repressions as flat heads. Below the GRN, the basins of attraction that lead to each of the four floral primordial cell types (sepals, green; petals, red; stamens, yellow; and carpels, purple) are shown. Each attractor is defined by the steady-state activations of the fifteen genes (OFF - '0' and ON - '1'), and they match those observed experimentally and predicted by the ABC model. Shaded genes in the GRN correspond to those active ('1') in the petal attractor. **(b)** Continuous spatiotemporal model for the mRNA expression pattern of the *WUSCHEL* (*WUS*) gene in the *Arabidopsis* SAM [22]. The simple GRN proposed is shown with nodes and edges as in (a). The equations of the model proposed are shown, with concentration of *WUS* (W) and A, Y and B as variables of a reaction–diffusion Brussellator system. Below the equations, WUS protein concentration is shown in a cellular lattice simulation of the SAM (modified from [45••]).

data on stomata dynamics in wildtype and in mutant or pharmacologically treated plants. The model also allowed a number of clear and novel predictions, some of which have been tested experimentally. This work also suggests that network modules of signal transduction pathways are robust in the face of diverse perturbations (see also [35•]).

From GRN to morphogenetic patterns and evolution

Experimentally supported GRN models have made it possible to propose some generic aspects of development. For example, dynamic GRN models of the functional gene modules studied to date suggest that cell-type determination depends mainly on global aspects of GRN architecture and dynamics, rather than on the precise values of parameters for kinetic functions such as gene activation or protein degradation. Additionally, GRN characterization in diverse systems tends to support the claim of some theoreticians (Figure 1) that a limited number of mechanisms are capable of generating and maintaining heterogeneities during morphogenesis. For example, the so-called activator–inhibitor system has been frequently found among documented GRN [36]. This system is a type of reaction–diffusion system, which has been widely used to address how spatial heterogeneities or patterns might arise in living organisms. In activator–inhibitor systems, two elements interact (the activator element positively regulates itself and the inhibitor element, whereas the latter negatively regulates the activator) and diffuse, giving rise to different spatial patterns.

As described above, GRN that are grounded on experimental data generally also seem to be functionally robust under perturbations. Apparently, particular structural traits (e.g. feedback loops) could underlie such generic robustness [29,37]. Further documentations of GRN should provide a more complete analysis of the interplay between structure and function. This might be more feasible in small and well-characterized sub-networks or modules in which functional and evolutionary interpretations are more direct than in global networks that have been inferred from genomic data. Nonetheless, the modular and reverse engineering genomic approaches should feedback from each other, and from more theoretical approaches that aim at uncovering general principles for network assemblage and dynamics [38,39•].

Comparative approaches are promising in searching for generalities, and, in their broadest sense, these approaches should consider both plants and animals [40]. Correlations of structural and dynamic aspects of GRN with variation in the morphological traits that are regulated by such networks [41••,42] are already being explored. For example, von Dassow and collaborators [21,22] suggested that the robustness and alterations of the segment polarity gene network in insects could underlie the overall conservation of body plan and the origin of long and short germ-band insects, respectively. Also, the GRN for floral organ specification ([6••], Figure 3a) seems to be robust even in the face of gene duplications, and thus could underlie the conserved basic floral plan of Eudicot flowering plants. At the same time, it could also account for the observed divergent phenotypes of mutants of flowering species that have duplicated floral genes [6••].

Conclusions

Mathematical models have proven to be very useful in developmental biology, but we still lack a formulation based on experimental facts that can account for biological phenomena at different scales and, most importantly, for the emergence of robust yet evolvable spatiotemporal patterns. There has been a recent burst of mathematical models invoking non-linear dynamic mechanisms to address spatiotemporal patterns of morphogens in plants (see review in this issue, e.g. [43•]) and animals (e.g. [44•]). However, these do not incorporate explicit complex GRN. The first efforts in this direction for plant systems are being made by Jonsson and collaborators [45••]. These authors used quantitative gene expression data from *in vivo* live confocal microscopy to create dynamic and spatially explicit computational templates. They have explicitly incorporated a small GRN that regulates shoot apical meristem (SAM) size and maintenance and have modeled the expression pattern of the gene *WUSCHEL* in a simulated SAM domain (Figure 3b). For this end, they applied the so-called 'connectivist model' [46,47], which unfortunately has the limitation of allowing only for paired gene–gene interactions.

Although simplified versions of GRN are relatively tractable, they might not be helpful in recovering the robust patterns observed in organisms [25,26,37]. This encourages the consideration of experimentally grounded complex GRN coupled in realistic cellular contexts. To this end, multidisciplinary work aimed at building hybrid models (see Box 1) and at incorporating available empirical information will definitely help to address the major task of building models that accurately capture the essential aspects of multi-scale processes during development.

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