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Modelling the molecular interactions in the flower developmental network of *Arabidopsis thaliana*

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Abstract

We present a dynamical model of the gene network controlling flower development in *Arabidopsis thaliana*. The network is centered at the regulation of the floral organ identity genes (*AP1*, *AP2*, *AP3*, *PI* and *AG*) and ends with the transcription factor complexes responsible for differentiation of floral organs. We built and simulated the regulatory interactions that determine organ specificity using an extension of hybrid Petri nets as implemented in *Cell Illustrator*. The network topology is characterized by two main features: (1) the presence of multiple autoregulatory feedback loops requiring the formation of protein complexes, and (2) the role of spatial regulators determining floral patterning. The resulting network shows biologically coherent expression patterns for the involved genes, and simulated mutants produce experimentally validated changes in organ expression patterns. The requirement of heteromeric higher-order protein complex formation for positive autoregulatory feedback loops attenuates stochastic fluctuations in gene expression, enabling robust organ-specific gene expression patterns. If autoregulation is mediated by monomers or homodimers of proteins, small variations in initial protein levels can lead to biased production of homeotic proteins, ultimately resulting in homeosis. We also suggest regulatory feedback loops involving miRNA loci by which homeotic genes control the activity of their spatial regulators.

Keywords: dynamical model, flower development, gene network

Introduction

Complex regulatory interactions between transcription factors and the prevalence of autoregulation are common themes in gene regulatory networks. Recent evidence suggests that direct molecular interactions form the basis of most regulatory processes: transcription factors generally bind a high number of sites in the genome, and can thus potentially directly influence the expression of hundreds of genes [Farnham, 2009]. This

paradigm may hold for all eukaryotes, since it is supported by data from animals (see, e.g., Li *et al.*, 2008), and plants [e.g. Kaufmann *et al.*, 2009; Oh *et al.*, 2009].

Floral development is initiated in response to a variety of internal and environmental stimuli, such as temperature and light (reviewed in Putterill *et al.*, 2004). Different floral induction pathways are controlled by a small set of flowering time genes, which in turn activate the floral meristem identity genes *APETALA1* (*AP1*) and *LEAFY* (*LFY*). These meristem identity genes stimulate the expression of floral organ identity genes. Organ identity genes act in a combinatorial fashion to specify the different types of floral organs: sepals, petals, stamens and carpels. According to the 'floral quartet model', the proteins encoded by floral homeotic genes assemble into distinct multimeric complexes in an organ-type specific manner [Theissen and Saedler, 2001]. All of the floral homeotic proteins present in those complexes belong to the MADS-box family of transcription factors. Evidence from yeast n-hybrid studies suggests that higher-order complex formation is mediated mostly by members of the SEPALLATA (SEP) subfamily of MADS-domain proteins [Honma and Goto, 2001; Immink *et al.*, 2009], which are required for specification of the identities of all 4 types of floral organs. The *SEPALLATA* subfamily consists of 4 largely redundant genes (*SEP1*-*SEP4*). Combined loss-of-function mutations in each of the 4 genes lead to homeotic conversion of all types of floral organ to leaf-like organs [Ditta *et al.*, 2004].

The formation of multimeric protein complexes seems to be not only required for the regulation of downstream targets, but may also play a role in positive autoregulation of floral homeotic genes. In addition to initial upregulation by upstream factors, autoregulation has been observed for key floral homeotic genes, like *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) [Jack *et al.*, 1994; Krizek and Meyerowitz, 1996; Hill *et al.*, 1998; Honma and Goto, 2000], *AGAMOUS* (*AG*) [Gomez-Mena *et al.*, 2005] and *SEPALLATA3* (*SEP3*) [Kaufmann *et al.*, 2009].

The requirement for autoregulation involving heterodimer formation has so far been characterized primarily for AP3 and PI, which are the two floral homeotic B function proteins specifying petal and stamen identity. However, genome-wide binding data for SEP3 indicate that it binds to the promoters of almost all of the floral homeotic genes, and induction experiments also show that it can upregulate the expression of these genes [Kaufmann *et al.*, 2009]. Since SEP3 is a key mediator of heteromeric higher-order complex formation between floral homeotic proteins, and autoregulation is observed for nearly all floral homeotic proteins, this likely indicates that autoregulation is mediated by higher-order complexes, although the initial expression of floral homeotic genes is unaffected in *sepallata* triple mutants [Pelaz *et al.*, 2000]. Thus, the combination of results from protein-protein and protein-DNA interaction studies as well as genetic evidence suggest a complex scenario for the establishment of the different floral organ identities by multiple direct protein-protein and regulatory interactions. Despite the fact that not all interactions have been confirmed *in planta* yet, current evidence allows us to generate a model for interactions during early flower development. Furthermore, other recent evidence suggests post-transcriptional control mechanisms in the network, such as the role of the microRNA miR172 in the translational repression of the spatial regulator APETALA2 (AP2) [Chen, 2004].

The complexity of direct molecular interactions necessitates the use of novel computational tools to understand the flowering process, optimally those which would allow for the explicit modelling of transcription, translation and protein binding reactions. A limitation at this point is a general lack of quantitative data for these different processes, restricting the modelling to generic estimates.

Ordinary differential equations are widely accepted as a modelling method for biological pathways [Sun and Zhao, 2004]. However, this method carries the disadvantage of being difficult to represent schematic information such as pathway models illustrated using biological elements such as mRNAs and proteins. The estimation of the required parameters for a simulation, especially in the case of a gene regulatory network, is also an open issue. In this regard, Petri nets offer an attractive alternative for simple construction, visualization and simulation of gene regulatory networks.

A Petri net is a mathematical model used for the representation and analysis of concurrent processes. Petri nets are described in part by the visual elements "place", "transition", "arc", and "token" (Fig. 1). Arcs are directed connections from places to transitions (input) and from transitions to places (output). Places can contain tokens, and a transition which has all places connected by input arcs with tokens, will transfer these

tokens (in discrete units) to the places connected by output arcs.

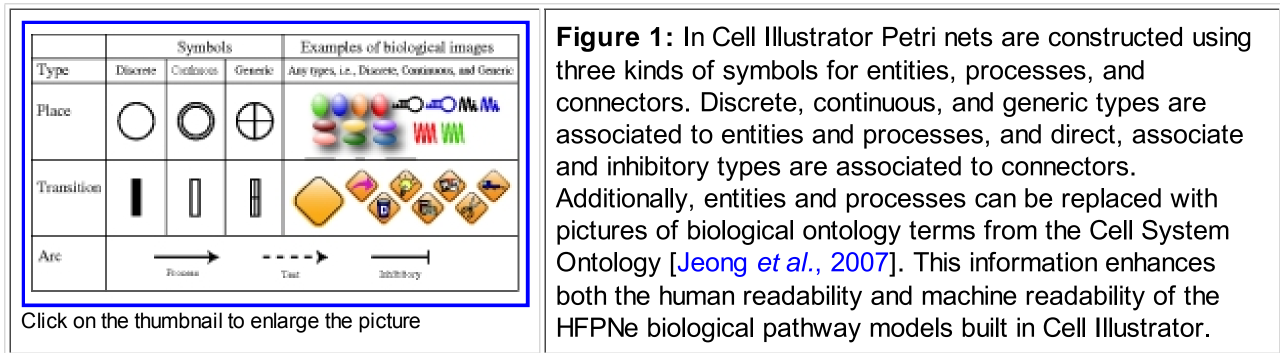

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Figure 1: In Cell Illustrator Petri nets are constructed using three kinds of symbols for entities, processes, and connectors. Discrete, continuous, and generic types are associated to entities and processes, and direct, associate and inhibitory types are associated to connectors. Additionally, entities and processes can be replaced with pictures of biological ontology terms from the Cell System Ontology [Jeong *et al.*, 2007]. This information enhances both the human readability and machine readability of the HFPNe biological pathway models built in Cell Illustrator.

Since the proposal of the original Petri net [Petri, 1962], various types of Petri nets have been developed, e.g. timed Petri net, continuous Petri net and Hybrid Petri net (HPN) [Nagasaki *et al.*, 2005]. Hybrid Petri nets have new types of place and transition that can receive continuous token values, along with the classic discrete ones. Like other proposed Petri nets, Hybrid Petri nets also expand the original Petri net by introducing the concepts of "inhibitory arc" and "test arc"; the inhibitory arc will stop a transition from working if the value on the place it is connected to (its input) is higher than a certain threshold (weight in Petri net terms), which in biology might easily represent a transcriptional repressor or an enzyme inhibitor. The test arc will not consume any tokens from the place it is connected to, making an easy analogy to an enzyme, which is not consumed by the reaction it catalyses.

Pioneering works by the groups of Reddy and Hofestädt were among the first to apply Petri nets to the modelling of biological pathways [Reddy *et al.*, 1993; Hofestädt and Thelen, 1998]. An advanced HPN has been also applied to the modelling of lambda phage pathway [Matsuno *et al.*, 2000]. Furthermore, Hybrid Functional Petri nets (HFPN) and its extension (HFPNe) were developed by expanding the HPN to be more powerful and suitable for biological pathway modelling and simulation [Matsuno *et al.*, 2003; Nagasaki *et al.*, 2004].

HFPN cannot only handle both discrete and continuous events at once, but also allows any kind of functions to be assigned to the delay, weight and speed parameters. HFPNe was introduced to facilitate the handling of any kind of objects within the concept of a Petri net. To handle these objects, new generic elements for place and transition were introduced (see Fig. 1). Using HFPNe, complicated biological pathway processes can be modelled, for example, networks involving gene regulation, signal transduction and metabolical reactions, as well as other biological processes that are not normally treated in biological pathways, such as alternative splicing and frame-shifting [Nagasaki *et al.*, 2004].

Differential equations can be easily modelled using a subset of HFPNe elements, by assigning continuous values for place and transition and suspending the weight parameters evaluation. Nagasaki *et al.*, have described a detailed formal definition and the properties of HFPNe [Nagasaki *et al.*, 2004; Nagasaki *et al.*, 2005].

In HFPNe, to bridge the gap between computer science and biology, the Petri net terms of place, transition, arc, and token are renamed to the more intuitive terms entity, process, connector, and content, respectively.

Cell Illustrator is a software implementation that includes HFPNe as well as an extended graphical user interface for building and simulating biological networks. By using Cell Illustrator a researcher can directly draw a network map using icons to represent Petri net elements (entities, processes and connectors), assign speed rules to the processes and directly simulate the dynamics of the network. A plot with the concentration change of the different entities is displayed during simulation time.

Models of the flowering network have been described and simulated in the past, originally as a Boolean gene network [Mendoza and Alvarez-Buylla, 1998] which included only 10 genes, but was later refined to a logical network including 15 genes [Espinosa-Soto *et al.*, 2004]. These network analyses could correctly identify the

steady state gene activation patterns for each of the floral organs, and demonstrated the importance of the network architecture above of that of the initial parameters assumed.

In the model presented here, we include both direct regulation between genes (mediated by a protein) and the formation and regulatory effect of heterodimeric transcription factor complexes, thus creating a larger and more complex network. Explicit translation reactions in the model also allow for the inclusion of post-transcriptional regulation, such as translational inhibition by miRNAs. Individual transcription reactions dependent upon different regulatory elements can be associated with each gene, enabling us to distinguish distinct transcription factor binding events, allowing promoter elements bound by different transcription factors to be separated and providing the ability to model binding-site competition. Different floral homeotic protein complexes may compete for binding sites, as is suggested by overlapping DNA-binding preferences of different floral homeotic factors (reviewed in Melzer *et al.*, 2006). Our model assumes that organ-specific developmental programmes are stabilized by autoregulatory loops involving all members of a floral homeotic protein complex, which is further supported by our perturbation analysis results.

Methods

Modelling and simulation software: Cell Illustrator

The Cell Illustrator software implements the HFPNe architecture with highly tuned modelling and simulation graphical user interfaces [Nagasaki *et al.*, 2003; 2009b]. Publicly available models created on Cell Illustrator are maintained in two websites (http://www.csml.org/ and http://genome.ib.sci.yamaguchi-u.ac.jp/~gon). The genetic network controlling flower development was implemented using the latest Cell Illustrator Online 4.0 version (http://www.cellillustrator.com/ [Nagasaki *et al.*, 2009a]).

Construction of the network

The blueprint of the regulatory network was built by compiling information from current literature, and ensuring that each proposed entity and connector is qualitatively supported by genetic data and, whenever possible, by molecular data that confirms a direct physical interaction. The basic module used in this network was composed of a transcription reaction producing an mRNA, connected to a translation reaction producing a protein and degradation reactions for both of the mRNA and protein products (Fig. 2). The network was then made using the underlying HFPNe architecture in Cell Illustrator by first drawing the entities involved on a canvas, and then connecting them by intermediary processes such as transcription, translation, activation, inhibition and degradation. Finally speed rules were set to reproduce biologically meaningful simulations. All entities can receive any floating-point value, and all reactions can receive any given speed rule [Doi *et al.*, 2004; Nagasaki *et al.*, 2004]. As additional elements, the network included binding reactions between proteins and the degradation of the resulting protein complexes. Regulatory connections (activation, inhibition) were made between transcription factors and the transcription of mRNAs (treated as reactions), or, in the case of the miRNA miR172, between the miRNA and the regulated translation reaction. When evidence of different regulatory elements was available, independent transcription reactions were added corresponding to each of the regulatory elements in play; as an example, *AG* is known to be regulated by LFY and MADS transcription factor complexes, so in the final model *AG* had independent transcription reactions for each regulating factor or complex. Competition between different MADS complexes for the same sites in positive regulatory interactions was implemented by assuming that the sites can become saturated, and the production of mRNA would reach a certain maximal transcription speed, beyond which different levels of competing transcription factors would make no further contribution to the process. In order to reflect this, a threshold value for transcription speed was set on transcription reactions regulated by common factors in order to make the different contributions additive only before the saturation threshold was reached.

The network includes static entities, whose concentration does not change during the simulation, and dynamic entities, which are actually playing an active role in the simulation. The flowering time complexes FD/FT and SOC1/AGL24 are static entities which are set as the starting point of the network (Fig. 3). Their concentration

is set to a constant value of 1, and their activity is set to act as a pulse, activating the downstream entities only during a short time interval (see methods for details). All other entities in the network with the exception of the spatial regulators SUP, UFO and miR172 are dynamic: the meristem identity genes *AP1*/*CAL*, *LFY* and *AP2* are the first entities activated in the simulation, followed downstream by the organ identity genes *SEP*, *AP3*, *PI* and *AG*. The production of these organ identity transcription factors leads to the formation of protein dimers (SEP/AP1, AP3/PI, SEP/AG) and higher-order complexes (SEP/AP1/AP3/PI and SEP/AP3/PI/AG).

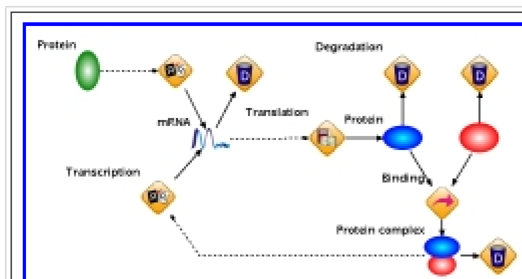

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Figure 2: Basic network structure implemented in Cell Illustrator. The basic regulatory module used to build this model consists of a transcription reaction producing an mRNA, followed by a translation reaction producing a protein. A protein can form complexes through a binding reaction, and either single proteins or protein complexes can act as activators of transcription. All entities involved in the simulation have a degradation reaction associated.

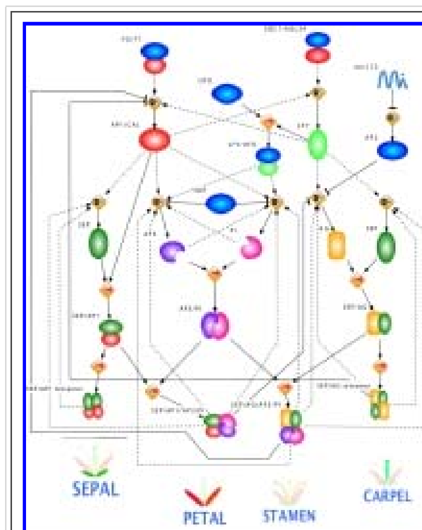

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Figure 3: Simplified representation of the network considered in this work, in which only proteins, protein complexes and miRNAs are depicted. The SEP entity has been duplicated for the sake of visual clarity. Reaction processes are depicted by 2 kinds of yellow diamonds, transcription (black and white icon) and protein binding (red arrow). Dashed arrows correspond to associative processes, solid black arrows correspond to direct processes and solid lines with blunt ends correspond to inhibitory reactions. The organ specified by each end transcription factor complex is indicated at the bottom of the figure. The individual protein and miRNA names depicted in the network are, from top left to bottom right: FD/FT: FLOWERING LOCUS D/FLOWERING LOCUS T, UFO: UNUSUAL FLORAL ORGANS, SOC1/AGL4: SUPPRESSOR OF CONSTANS OVEREXPRESSION/AGAMOUS-LIKE 24, miR172: microRNA 172, AP1/CAL: APETALA1/CAULIFLOWER, LFY: LEAFY, AP2: APETALA2, SUP: SUPERMAN, SEP: SEPALLATA, AP3: APETALA3, PI: PISTILLATA, AG: AGAMOUS.

Even though some of the factors that control spatial expression domains of organ identity genes in flower development are already known (e. g. *SUP*, *UFO*, *miR172*), the upstream regulation of these spatial control genes remains to be elucidated. In this network model, the simulation started from a set of 4 different initial conditions that replicate the known activities of these spatial regulators. These initial conditions depend on the value of the 3 spatial regulators UFO, SUP and miR172.

The concentration values for the entities of the spatial regulators SUP and miR172 were set to 0 or 1 as on/off states. The translation speed of UFO was chosen to maintain the biological congruence of the network, at 0.5 units per simulation cycle in active state, and 0 when inactive. According to the model network, high concentrations of UFO would sequester the protein LFY and alter the expression patterns of *AP1*/*CAL* and *SEP*, which are regulated by LFY alone; thus the chosen speed allows the presence of free LFY protein.

The initial conditions sets were then chosen as follows:

- *SUP* on, *miR172* off and *UFO* off leading to sepal formation: The presence of AP2 inhibits expression of *AG*. Since *UFO* is off and *SUP* is on, there is no AP3/PI production, leading to the expression of SEP/AP1 as steady-state TF complex.
- *SUP* off, *miR172* off and *UFO* on leading to petal formation: The expression of UFO leads to the formation of the dimer UFO/SEP which activates the expression of *AP3*/*PI*. The presence of AP2 inhibits expression of *AG* and allows expression of *AP1*, leading to the formation of the steady-state TF

complex SEP/AP1/AP3/PI.

- *SUP* off, *miR172* on and *UFO* on leading to stamen formation: inhibition of *AP2* by miR172 allows *AG* to be expressed, and the presence of UFO activates the expression of *AP3*/*PI*. AP1 is transiently expressed before the TF complexes that inhibit it are formed, but once AG complexes are formed, *AP1* is inhibited, leading to the formation of the steady-state TF SEP/AP3/PI/AG.
- *SUP* on, *miR172* on and *UFO* off leading to carpel formation: SUP inhibits *AP3*/*PI*, and the inhibition of *AP2* by miR172 allows expression of *AG*, leading to SEP/AG formation. This factor also inhibits *AP1* when formed but allows a transient expression in early simulation time.

The final network is provided as a file in Cell Illustrator's CSML format, and can be inspected and simulated by using the free Cell Illustrator Player program launched from a web browser (https://cionline.hgc.jp/cifileserver/apps/usersman/main).

Experimental support for interactions described in the network

Floral meristem identity genes. The closely related MADS-box genes *APETALA1* and *CAULIFLOWER* (*AP1*/*CAL*) as well as the NonMADS transcription factor *LEAFY* (*LFY*) control the initial specification of flowers in response to different floral induction pathways [Huala and Sussex, 1992; Mandel *et al.*, 1992; Weigel *et al.*, 1992]. All three genes are expressed at the earliest stages of floral meristem development and LFY and AP1 are known to positively upregulate each others' expression [Liljegren *et al.*, 1999]. *AP1* and *CAL* are two closely related paralogous MADS-box genes with highly redundant function [Kempin *et al.*, 1995], and are therefore treated as one functional molecule in our network. AP1 has a second role at later stages of flower development in the specification of sepal and petal identity [Mandel *et al.*, 1992]. AP1 and CAL form dimers with SEPALLATA (SEP) MADS-domain proteins [Pelaz *et al.*, 2001; Castillejo *et al.*, 2005] and interact in a higher-order complex with APETALA3 (AP3) and PISTILLATA (PI) [Honma and Goto, 2001]. According to the 'quartet model' of flower development, the AP1/SEP protein complex establishes sepal identity, while the AP1/SEP/AP3/PI complex specifies petal identity [Theissen and Saedler, 2001]. At later stages of flower development, AP1/CAL is inhibited by AGAMOUS (AG) in stamens and carpels [Gustafson-Brown *et al.*, 1994]. Since, according to the 'floral quartet' model, the principal functional AG complexes in these floral organs are AG/SEP/AP3/PI and AG/SEP, respectively, we assume in our model that both of these complexes can repress the expression of *AP1*/*CAL*.

Genetic data and the results of gene expression microarray experiments suggest that LFY and AP1 positively regulate the expression of floral homeotic genes *AP3*, *PI* and *AG* [Huala and Sussex, 1992; Weigel and Meyerowitz, 1993; Busch *et al.*, 1999; Honma and Goto, 2000; Lohmann *et al.*, 2001; Wellmer *et al.*, 2006; Chae *et al.*, 2008].

For the upregulation of *AP3* and *PI*, LFY requires UNUSAL FLORAL ORGANS (UFO) as a cofactor [Wilkinson and Haughn, 1995; Lee *et al.*, 1997]. LFY and UFO form functional protein complexes in plants, which are required for binding of LFY to the promoter of *AP3* [Chae *et al.*, 2008]. The direct binding of a LFY/UFO complex to the promoter of *PI* has not yet been demonstrated. However, since LFY and UFO also activate *PI* expression via a common promoter region [Honma and Goto, 2000], we assume in our model that the LFY/UFO complex acts in a similar manner on the *PI* promoter.

Floral homeotic genes. Aside from the initial activation by AP1 and LFY, there are two major aspects of regulation of floral homeotic genes: (1) autoregulation via multiprotein complexes, and (2) the presence of spatial factors that permit or prohibit the expression of floral homeotic genes in certain tissues within the floral meristem. We implemented 3 known factors that spatially modulate homeotic gene expression: (a) UFO, which is required together with LEAFY for the activation of *APETALA3* and *PISTILLATA*. (b) APETALA2 (AP2), which negatively regulates *AG* in sepal and petal primordia [Drews *et al.*, 1991; Bomblies *et al.*, 1999]. (c) SUPERMAN (SUP), which negatively regulates *AP3* and *PI* in carpel primordia [Bowman *et al.*, 1992; Yun *et al.*, 2002].

The *SEPALLATA* genes (*SEP1*-*SEP4*) are closely related, highly redundant MADS-box genes which are

required for the specification of the identities of all types of floral organ due to upregulation of other floral organ identity genes [Pelaz *et al.*, 2000; Ditta *et al.*, 2004]. Protein-protein interaction data suggest that they form larger complexes with all other floral organ identity proteins belonging to the MADS-box transcription factor family [Honma and Goto, 2001]. According to the current model of flower development, each of the complexes is specific for a certain type of floral organ: the SEP/AP1 complex for sepals, the SEP/AP1/AP3/PI complex for petals, SEP/AG/AP3/PI for stamens and SEP/AG for carpels (Fig. 1) [Honma and Goto, 2001; Theissen and Saedler, 2001]. Only little is known about the regulation of SEP gene expression, however expression microarray data suggest that *SEP* genes are activated by AP1/CAL, LFY and AG [Schmid *et al.*, 2003; Gomez-Mena *et al.*, 2005; Wellmer *et al.*, 2006]. There are several indications from genetic data that floral homeotic genes can positively upregulate their own expression, and that SEP genes are required for this upregulation. AP3 and PI, which act together in the specification of petal and stamen identity, depend on each other in the autoregulatory process [Jack *et al.*, 1994]. Heterodimerization of the two gene products is required for the positive autoregulation. Since also *SEP*, *AP1* (petals) and *AG* (stamen) gene products are able to upregulate of *AP3* and *PI* [Gustafson-Brown *et al.*, 1994; Pelaz *et al.*, 2000; Gomez-Mena *et al.*, 2005], we assume in our model that the AP1/SEP/AP3/PI (petal) and AG/SEP/AP3/PI (stamen) protein complexes are the functional complexes for upregulation of *AP3* and *PI in planta*.

Gomez-Mena *et al.*, 2005, demonstrated that AG can upregulate its own expression. Since both, the *AG* and *SEP* gene products can upregulate *AG* expression, and AG and SEP proteins can interact with each other as well as with AP3 and PI in a higher-order protein complex [Honma and Goto, 2001], and SEP3 and AG bind to regulatory elements in the *AG* locus [Kaufmann *et al.*, 2009], we assume in the model that the AG/SEP (carpel) and the AG/SEP/PI/AP3 (stamen) protein complexes are functional in this process.

Simulation parameters

The initial activation of the network, through the entities FD/FT, SOC1/AGL24 and a generic transcription reaction acting on *AP2* was given the speed 1 during the first 10 simulation cycles (a simulation cycle corresponds to one Petri net time unit), and 0.01 thereafter as basal activity.

Transcription speeds were set so that the sum of transcription activation speeds from reactions simultaneously acting on an entity was equal or lower than 1, and each activation speed depended linearly on the concentration of the activation factor under this limit. For every factor activating a given entity, an independent transcription reaction was set, and the maximal speed of each reaction was chosen so that the maximal combined speed of all the reactions that could be active simultaneously did not exceed 1. As an example, the AG mRNA is transcriptionally induced by LFY and, independently, also by the complexes SEP/AG and SEP/AP3/PI/AG, so the maximal activation speed for each transcription reaction was set to 0.33.

Translation speeds were set as the mRNA concentration divided by 5. Binding speeds were set as the product of the concentration of the monomers divided by a constant, which was chosen to better represent the biological implications of the network. In this case, the binding reactions were faster for the petal and stamen complexes (the product of the monomer's concentration divided by 2) than for the sepal and carpel complexes (the monomer's concentration divided by 5) following the supposition that the heterodimers may have higher binding affinity than the homodimers [de Folter *et al.*, 2005]. The degradation speeds were set as the concentration of an mRNA divided by 5, and the concentration of a protein divided by 10, under the assumption that proteins are more stable than mRNAs [Matsuno *et al.*, 2003]. The degradation speed of a protein complex was set as the concentration of the complex divided by 15.

Exception from these rules were implemented to address the dependence of AP3 and PI on each other. AP3 and PI stabilize each others expression and function as obligate heterodimers [Jack *et al.*, 1994; Hill *et al.*, 1998; Tilly *et al.*, 1998]. To represent this dependence in the network, we assume that the single monomer is extremely unstable and degrades fast, so we set the degradation speed of the protein to be 10 times the protein translation speed if the partner was absent. Reaction thresholds were set to 0.1, which implies the requirement of a low protein concentration before the reaction becomes active.

The only exceptions to this rule were applied in the cases of the inhibition of AG by the petal complex and the

activation of *AG* by LFY, where the inhibition and activation thresholds were set to 1. In the first case, the fact that low level presence of the petal complex in early stamen conditions would block the stamen complex formation by inhibiting the transcription of *AG*. In the second case, LFY tends to be expressed at a very low level, but still over the 0.1 threshold set in general. Under conditions where AG is also expressed, the activity of low level LFY on AG would lead to an overproduction of this protein, while the network structure suggests that AG depends on feedback from the protein complexes in which it is present to keep its expression level. These threshold changes would have the biological implication that the petal complex and LFY have a lower affinity to bind to the *AG* promoters than AP2 or the stamen and carpel complexes. Further experimental data is necessary to elucidate the binding affinity differences of these transcription factor complexes. Additionally, *AG* expression is also induced by other factors, e.g. WUSCHEL [Lenhard *et al.*, 2001; Lohmann *et al.*, 2001], which were not considered in our simple model.

Results

Network simulation

The network was simulated under the 4 initial conditions described above, until the expression levels of the proteins reached a steady state (Fig. 4). The relative protein expression levels of each simulation are in accordance with those currently known for each floral organ, and provide a qualitative description of the dynamics behind the specification of floral organ identities [Krizek and Fletcher, 2005]. Fig. 4 presents results of simulations for each type of floral organ. In all cases, the higher-order protein complex specific for a certain type of floral organ can be recovered as the predominant component in the system after reaching the equilibrium. According to the simulation, the petal and sepal identity factor AP1(/CAL) is transiently expressed during carpel- and stamen initiation, leading also to transient formation of high-order complexes involving these proteins. This is consistent with experimentally determined mRNA and protein expression patterns indicating that AP1 is found in all floral whorls at the earliest stages of flower development [Mandel *et al.*, 1992; Urbanus *et al.*, 2009], and reflects the dual role of *AP1* and *CAL* as flower meristem identity genes. Thus, the model predicts that different higher-order complexes may transiently coexist at early time-points of flower meristem development.

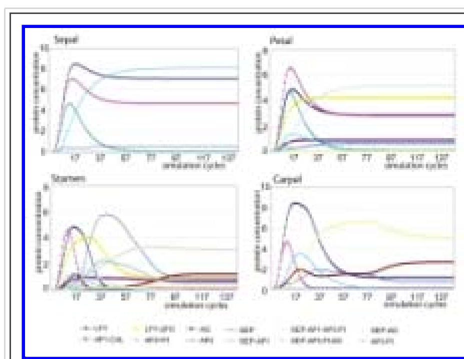

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Figure 4: Simulation results for each of the 4 types of floral organs, dependent on the initial conditions described in the methods. The expression (Y axis) corresponds to a protein concentration value (arbitrary units), and time corresponds to the simulation cycle number. The color coded lines correspond to single and complex factors as described in the bottom table. In every case, the highest expression value in the steady state corresponds to the complex known to be responsible for the differentiation of each organ.

In order to further test our model, simulations of various known mutants were performed. The networks of mutant scenarios were simulated by setting the translation speed of the targeted entity to 0. As can be seen in Fig. 5, mutation of the *LFY* gene under the stamen conditions results in a transient low-level activation of AP1/CAL and SEP proteins, which is not sufficient to trigger persistent upregulation of floral homeotic genes. In agreement with this, the *lfy* mutant produces leaf-like organs instead of stamens. Similar results were obtained for the other floral organs. In contrast, *ag* and *ap3* mutants form petal (AP1/SEP/AP3/PI) and carpel (AG/SEP) complexes in the third whorl, respectively, which is consistent with the homeotic mutant phenotypes that are described in the literature [Bowman *et al.*, 1989; Yanofsky *et al.*, 1990; Jack *et al.*, 1992].

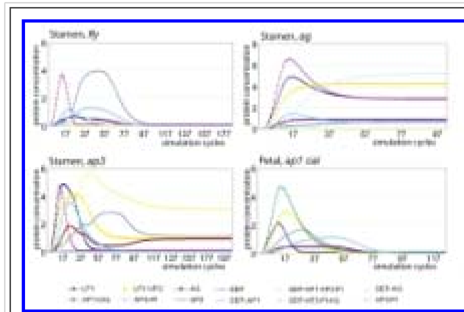

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Figure 5: Simulation of the network under different mutant contexts. The mutant described in each plot was simulated by setting the translation speed of the mutated gene to 0. The steady state expression profiles coincide with the phenotypes of experimentally characterized mutants.

Network robustness and response to stochastic oscillations

The response of the network to stochastic oscillations was measured by setting the value of independent entities to oscillate at random within a given interval during the simulation time. It was observed that the requirement of protein complexes as end regulators of the network leads to dampening of noise and to a more stable concentration of the complexes themselves. As a result, the regulatory effect of these oscillations on target entities is also reduced. This effect arises if one of the partners of a dimer complex has an aberrant expression, but the other remains under normal control, the concentration of the dimer will show an oscillation whose magnitude is reduced with respect to the monomer. This dampening effect is even more pronounced for higher-order complexes. The corresponding variation of the concentration of the final transcription factor complexes is found to be about one fifth of the variation of the chosen single monomer.

Changes in organ-specific steady states of gene expression were observed when random noise on the AP2 protein concentration was introduced. Under the initial conditions for sepal development, *AG* was transiently activated because the concentration of AP2 reached a value under the activity threshold that is required for inhibition of *AG*. AG, in turn, inhibited the production of AP1 which resulted in the disappearance of the sepal complex (Fig. 6). In the case of petal and stamen conditions, the petal complex is formed at relatively low concentration. Here the petal complex further inhibits the expression of *AG*, resulting in an indirect activation effect on AP1 which, while it does not recover the expression pattern of the original model, allows for sustained AP1 production and a low level formation of the petal complex SEP/AP1/AP3/PI. It is important to note that if a basal level of AP2 protein expression is kept, the inhibitory effect of stochastic fluctuations of AP2 on sepal development is absent. Stochastic fluctuation of AG leads to defects in organ-specific protein complexes: the stamen complex is formed instead of the petal complex and the carpel complex instead of the sepal complex, leading to floral homeotic conversions. The simulation thus mimics a situation in which AG is ectopically expressed. Notably, the sensitivity of petal and sepal steady-states of gene expression are in line with data suggesting that *AG* is repressed by multiple mechanisms in developing sepals and petals, which have for sake of focus and simplicity not been included in our model [see, e.g., Krizek *et al.*, 2000; Bao *et al.*, 2004].

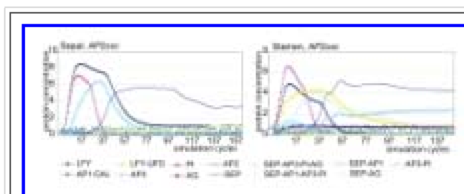

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Figure 6: Protein complexes mediate dampening of stochastic oscillations. By setting the value of a given entity to a random number, the overall response of the network to stochastic oscillations is observed. In this case the value of a given entity is set to oscillate at random between 0 and 1 under the given initial conditions. The only cases found to be sensitive to these oscillations are the random activation of AP2, which does not form a protein complex under sepal and stamen initial conditions, a situation which disrupts expression patterns.

The role of transcription factor complexes in stabilizing network dynamics

According to the 'floral quartet model' [Theissen and Saedler, 2001], floral homeotic proteins belonging to the

MADS-box family assemble in a combinatorial fashion into organ-specific, higher-order protein complexes. In order to test whether heteromeric protein complexes could play a role in the stabilization and robustness of the regulatory network, an attempt was made to rebuild the whole network so that the autoregulatory links we redirected to start at their single monomers instead of protein complexes. In this case the complexes were still produced as end products but they played no further role in regulation within the network. Simulations were made following the same set of starting conditions as in the previous model. While the correct steady state expression profiles for sepal, petal and carpel were observed (data not shown), stable expression of the stamen complex was unattainable (Fig. 7). This was traced to the presence of two separate positive autoregulatory feedback loops of the AP3/PI complex and AG acting on the expression of their respective genes. Since AP3/PI and SEP/AG heterodimers assemble into the higher-order SEP/AP3/PI/AG complex, the concentration of any free heterodimer depends on the concentration of the other heterodimer. For instance, if the production of AG is higher than that of AP3 and PI, the SEP/AG dimer will bind to all the AP3/PI dimers available to form the higher-order complex, and since AP3 and PI are positively autoregulated, the titration of the AP3/PI complex by SEP/AG will lead to a decrease and eventual halt of the transcriptional activation of *AP3* and *PI*. This decrease in *AP3* and *PI* transcription causes formation of the carpel complex SEP/AG. On the other hand, if the production speed of AP3 and PI is higher than that of AG, free AG, which under this assumption activates its own expression, will be sequestered by SEP/AP3/PI and will be unable to activate its own expression, leading to the loss of the stamen complex (Fig. 7). In this case feedback by the higher-order complex, not by the single monomers, is a prerequisite in order to maintain a stable expression of the steady-state transcription factor complex.

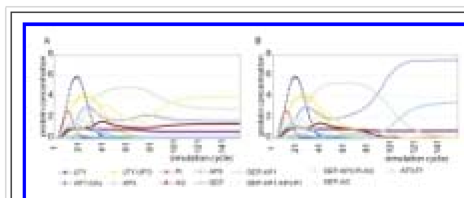
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Figure 7: Instability of the stamen network assuming simple feedback. The presence of feedback loops of AP3/PI and AG to their respective own genes and the fact that these assemble into a higher-order complex makes the network extremely sensitive to differences in the production of the monomers. A) assuming standard parameters, the production of AG is slightly higher than that of AP3/PI, the sequestering of AP3/PI by the SEP/AG complex leads to the collapse of its expression, causing a final expression pattern corresponding to the carpel, with the accumulation of the SEP/AG complex. B) If the speed of AP3/PI production is adjusted to be higher than that of AG, the expression of AG collapses leading to the accumulation of AP3/PI.

Extension of the network: Feedback loops controlling spatial regulators

Recently published ChIP-seq data [Kaufmann *et al.*, 2009] supports the idea that some of the spatial patterning genes might be under feedback control from MADS-box transcription factor complexes. In particular, binding of SEP3 to the promoters of *AP2* and three *miR172* orthologs suggest that SEP3 complexes (e.g. with AP1) could stabilize the expression of AP2 in the outer whorls and that other SEP3 complexes might stabilize miR172 expression in the inner whorls. In order to test the feasibility of such potential regulatory interactions, we extended the network to include regulatory reactions from the sepal and petal MADS complexes to activate the transcription of *AP2* and from the stamen and carpel complexes to activate the transcription of *miR172*. This change implies that the miR172 entity, which was not playing an active role in the previous simulations, now is included in the modelling dynamics. However, the previous requirements on the initial conditions set to direct the formation of each flower organ, namely, its initial value is set to 1 in stamen and carpel conditions and 0 in sepal and petal, is maintained. Simulations of the extended network suggest that the inclusion of these interactions maintains the correct expression patterns of each floral organ, and may act as redundant control mechanisms to further stabilize the organ-specific gene expression patterns. For example, in the case of the protein AG, the sustained presence of AP2 would insure that the inhibition of AG remains constant until the sepal or petal complexes can be formed (Fig. 8). On the other hand, feedback activation of *miR172* allows the stabilization of the spatial boundary between the outer and inner whorls through the translational inhibition of AP2.

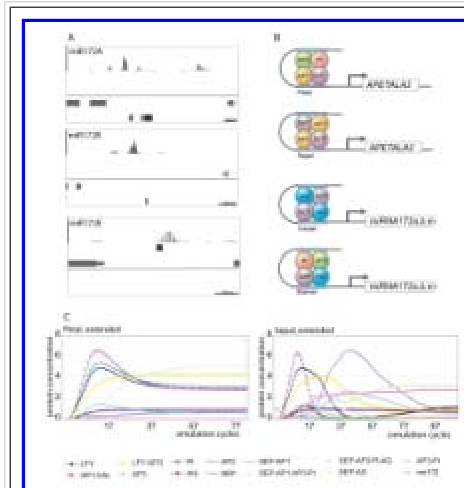
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Figure 8: Inclusion of feedback regulatory reactions on AP2 and miR172. A) SEP3 binding patterns at 3 *miR172* loci as revealed by ChIP-seq. The ChIP-seq peaks are shown in the upper panel in each figure, with genomic loci indicated beneath. In all cases, the main peak is downstream of and close to the locus. B) Potential regulatory interactions of different complexes on *AP2* and *miR172* loci which were added to our model. For simplification, the 3 different miRNA loci are treated as one entity in our model. C) (Bottom left) A transcriptional activation process was added between the petal complex (AP1/SEP/AP3/PI) and *AP2*, leading to the sustained expression of *AP2* in the petal. The presence of AP2 in sepals and petals would add a redundant repression control on *AG*. (Bottom right) Activation of *miR172* by the stamen complex (AG/SEP3/AP3/PI). The explicit maintenance of *miR172* expression by the stamen complex allows the translational inhibition of AP2, and thus stabilizes the expression of *AG*.

Discussion

A dynamic molecular regulatory network of early flower development

The model presented here predicts relative concentrations of mRNA, proteins and protein complexes at the earliest time points of the flower developmental programme, when the identities of the different types of floral organs are specified. We model transitory and final states of expression of the different components in each floral whorl. A limitation of the current model is the lack of experimentally determined quantitative data for estimating the production and degradation speeds of the different components, allowing us to use only generic estimates. However, we find that our model, which is based on (qualitative) genetic as well as molecular evidence, is capable of reproducing current knowledge on the timing of expression and organ-specific complex formation of transcription factors. The model is also able to correctly reproduce the outcome of different loss-of function mutants, suggesting that model simulations can be used to formulate biological hypotheses which subsequently can be experimentally tested.

Robustness through interaction: the role of heteromeric protein complexes in network stability

MADS-domain proteins form an "intrafamily" protein-protein interaction network ([de Folter *et al.*, 2005]; reviewed in [Kaufmann *et al.*, 2005]). This network evolved by a series of duplication events, associated with rounds of whole-genome duplication [Veron *et al.*, 2007]. The family presumably originated from a homodimerizing ancestral protein which was present in green algae [Tanabe *et al.*, 2005]. Autoregulation and positive regulation by interaction partners are common features among MADS-box transcription factors, with the most well-known examples characterized for floral homeotic proteins, which were analyzed in our model.

In order to analyze the role of higher-order MADS protein complexes in autoregulation, we designed the model in a manner that allows for autoregulation by heteromeric complexes as opposed to single proteins. We find that allowing autoregulation by single proteins can destabilize the network under certain conditions. This is especially the case when the formation of a higher-order complex relies mostly on two independently regulated heterodimers. If additional factors stabilize the expression of both heterodimers, the correct organ-type specific higher-order complex may still be formed. This suggests that SEP proteins as mediators of higher-order complex formation are required to integrate and balance the expression of different homeotic proteins, in order to produce stable floral structures. Experimental quantification of differences in protein interaction preferences and DNA-binding affinity, as well as specific levels of activation by certain proteins/complexes, would help to clarify the role of these complexes at the regulatory level. Evidence for the idea that regulatory circuits of some floral organs may be more sensitive to loss of higher-order complex

formation than others comes from the *pistillata-5* (*pi-5*) mutant, in which the formation of the AP3/PI/SEP3 complex is reduced due to a mutation in the protein interaction domain of the PISTILLATA protein. The *pi-5* mutant shows floral homeotic conversion of petals into sepals, but has no defect in stamen identity [Yang *et al.*, 2003].

In addition, our model supports the idea that different higher-order complexes coexist, at least transiently, and it also proposes that higher-order complexes and heterodimers may coexist. Different DNA binding affinities of MADS-domain proteins would allow for the co-occurrence of different complexes without disrupting the expression patterns of the target genes. This becomes relevant since under petal and stamen conditions, which depend upon the formation of the heterotetramer complexes SEP/AP1/AP3/PI and SEP/AP3/PI/AG, the formation of sepal and carpel complexes, SEP/AP1 and SEP/AG respectively, cannot be ruled out. A goal of future research should be to obtain more molecular *in planta* support as well as quantitative data for the physical interactions that are present in our model, in order improve the modelling of the flowering process.

Feedback on spatial regulators

Recent evidence from whole-genome approaches to identify DNA-binding sites of transcription factors in vivo (ChIP-seq and ChIP-CHIP) suggests a multitude of direct cross-talk between different transcriptional regulators and feedback/feedforward loops. One example is the binding of SEP3 complexes to the promoter of *AP2* and the miRNA loci which negatively regulate *AP2*. These findings suggest the presence of feedback loops acting on the spatial regulator AP2, in addition to yet unknown processes that achieve the early activation of *AP2* and its miRNA repressors. The result of our stochastic response simulations on AP2, the only entity that disrupted the network's expression pattern, points to the fact that in the current model the regulation of AP2 is rather simple, in contrast to almost every other entity. Given the central role of AP2 in spatial patterning, it is likely that more complex elements and interactions are involved in the transcriptional control of the *AP2* gene. Complex feedback mechanisms involving miRNA loci were recently also described for the control of floral transition [Wu, G., *et al.*, 2009], and may play a general role in developmental transitions and pattern formation. Which other processes might control the expression of spatial regulators remains an open question. In animals, concentration gradients of morphogens are important to set developmental pre-patterns. In plants, hormones like auxin have been suggested to act in similar fashion to morphogens in animals, however a role of this hormone in orchestrating floral homeotic gene expression has not yet been demonstrated. Thus a major challenge in the future will be to unravel the upstream processes driving the expression of spatial regulators of floral homeotic genes.

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References

- Bao, X., Franks, R. G., Levin, J. Z. and Liu, Z. (2004). Repression of *AGAMOUS* by *BELLRINGER* in floral and inflorescence meristems. Plant Cell **16**, 1478-1489.
- Bomblies, K., Dagenais, N. and Weigel, D. (1999). Redundant enhancers mediate transcriptional repression of *AGAMOUS* by *APETALA2*. Dev. Biol. **216**, 260-264.
- Bowman, J. L., Smyth, D. R. and Meyerowitz, E. M. (1989). Genes directing flower development in *Arabidopsis*. Plant Cell **1**, 37-52.
- Bowman, J. L., Sakai, H., Jack, T., Weigel, D., Mayer, U. and Meyerowitz, E. M. (1992). *SUPERMAN*, a regulator of floral homeotic genes in *Arabidopsis*. Development **114**, 599-615.

- Busch, M. A., Bomblies, K. and Weigel, D. (1999). Activation of a floral homeotic gene in *Arabidopsis*. Science **285**, 585-587.
- Castillejo, C., Romera-Branchat, M. and Pelaz, S. (2005). A new role of the Arabidopsis *SEPALLATA3* gene revealed by its constitutive expression. Plant J. **43**, 586-596.
- Chae, E., Tan, Q. K.-G., Hill, T. A. and Irish, V. F. (2008). An *Arabidopsis* F-box protein acts as a transcriptional co-factor to regulate floral development. Development **135**, 1235-1245.
- Chen, X. (2004). A microRNA as a translational repressor of *APETALA2* in *Arabidopsis* flower development. Science **303**, 2022-2025.
- de Folter, S., Immink, R. G. H., Kieffer, M., Pařenicová, L., Henz, S. R., Weigel, D., Busscher, M., Kooiker, M., Colombo, L., Kater, M. M., Davies, B. and Angenent, G. C. (2005). Comprehensive interaction map of the *Arabidopsis* MADS box transcription factors. Plant Cell **17**, 1424-1433.
- Ditta, G., Pinyopich, A., Robles, P., Pelaz, S. and Yanofsky, M. F. (2004). The *SEP4* gene of *Arabidopsis thaliana* functions in floral organ and meristem identity. Curr. Biol. **14**, 1935-1940.
- Doi, A., Fujita, S., Matsuno, H., Nagasaki, M. and Miyano, S. (2004). Constructing biological pathway models with hybrid functional Petri nets. In Silico Biol **4**, 0023.
- Drews, G. N., Bowman, J. L. and Meyerowitz, E. M. (1991). Negative regulation of the *Arabidopsis* homeotic gene *AGAMOUS* by the *APETALA2* product. Cell **65**, 991-1002.
- Espinosa-Soto, C., Padilla-Longoria, P. and Alvarez-Buylla, E. R. (2004). A gene regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles. Plant Cell **16**, 2923-2939.
- Farnham, P. J. (2009). Insights from genomic profiling of transcription factors. Nat. Rev. Genet. **10**, 605-616.
- Gómez-Mena, C., de Folter, S., Costa, M. M. R., Angenent, G. C. and Sablowski, R. (2005). Transcriptional program controlled by the floral homeotic gene *AGAMOUS* during early organogenesis. Development **132**, 429-438.
- Gustafson-Brown, C., Savidge, B. and Yanofsky, M. F. (1994). Regulation of the *Arabidopsis* floral homeotic gene *APETALA1*. Cell **76**, 131-143.
- Hardy, S. and Robillard, P. N. (2008). Petri net-based method for the analysis of the dynamics of signal propagation in signaling pathways. Bioinformatics **24**, 209-217.
- Hill, T. A., Day, C. D., Zondlo, S. C., Thackeray, A. G. and Irish, V. F. (1998). Discrete spatial and temporal *cis*-acting elements regulate transcription of the *Arabidopsis* floral homeotic gene *APETALA3*. Development **125**, 1711-1721.
- Hofestädt, R. and Thelen, S. (1998). Quantitative modelling of biochemical networks. In Silico Biol. **1**, 0006.
- Honma, T. and Goto, K. (2000). The *Arabidopsis* floral homeotic gene *PISTILLATA* is regulated by discrete *cis*-elements responsive to induction and maintenance signals. Development **127**, 2021-2030.
- Honma, T. and Goto, K. (2001). Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature **409**, 525-529.
- Huala, E. and Sussex, I. M. (1992). *LEAFY* interacts with floral homeotic genes to regulate Arabidopsis floral development. Plant Cell **4**, 901-913.
- Immink, R. G. H., Tonaco, I. A. N., de Folter, S., Shchennikova, A., van Dijk, A. D. J., Busscher-Lange, J., Borst, J. W. and Angenent, G. C. (2009). SEPALLATA3: the 'glue' for MADS box transcription factor complex formation. Genome Biol. **10**, R24.
- Jack, T., Brockman, L. L. and Meyerowitz, E. M. (1992). The homeotic gene *APETALA3* of Arabidopsis

thaliana encodes a MADS box and is expressed in petals and stamens. Cell **68**, 683-697.

- Jack, T., Fox, G. L. and Meyerowitz, E. M. (1994). Arabidopsis homeotic gene *APETALA3* ectopic expression: transcriptional and posttranscriptional regulation determine floral organ identity. Cell **76**, 703-716.
- Jeong, E., Nagasaki, M., Saito, A. and Miyano, S., (2007). Cell system ontology: representation for modelling, visualizing, and simulating biological pathways. In Silico Biol. **7**, 623-638.
- Kaufmann, K., Melzer, R. and Theissen, G. (2005). MIKC-type MADS-domain proteins: structural modularity, protein interactions and network evolution in land plants. Gene **347**, 183-198.
- Kaufmann, K., Muiño, J. M., Jauregui, R., Airoldi, C. A., Smaczniak, C., Krajewski, P. and Angenent, G. C. (2009). Target genes of the MADS transcription factor SEPALLATA3: integration of developmental and hormonal pathways in the *Arabidopsis* flower. PLoS Biol. **7**, e1000090.
- Kempin, S. A., Savidge, B. and Yanofsky, M. F. (1995). Molecular basis of the cauliflower phenotype in *Arabidopsis*. Science **267**, 522-525.
- Koh, G., Teong, H. F. C., Clément, M.-V., Hsu, D. and Thiagarajan, P. S. (2006). A decompositional approach to parameter estimation in pathway modelling; a case study of the Akt and MAPK pathways and their crosstalk. Bioinformatics **22**, e271-e280.
- Krizek, B. A. and Meyerowitz, E. M. (1996). Mapping the protein regions responsible for the functional specificities of the *Arabidopsis* MADS domain organ-identity proteins. Proc. Natl. Acad. Sci. USA **93**, 4063-4070.
- Krizek, B. A. and Fletcher, J. C. (2005). Molecular mechanisms of flower development: An armchair guide. Nat. Rev. Genet. **6**, 688-698.
- Krizek, B. A., Prost, V. and Macias, A. (2000). *AINTEGUMENTA* promotes petal identity and acts as a negative regulator of *AGAMOUS*. Plant Cell **12**, 1357-1366.
- Lee, I., Wolfe, D. S., Nilsson, O. and Weigel, D. (1997). A *LEAFY* co-regulator encoded by *UNUSUAL FLORAL ORGANS*. Curr. Biol. **7**, 95-104.
- Lenhard, M., Bohnert, A., Jürgens, G. and Laux, T. (2001). Termination of stem cell maintenance in *Arabidopsis* floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. Cell **105**, 805-814.
- Li, X.-Y., MacArthur, S., Bourgon, R., Nix, D., Pollard, D. A., Iyer, V. N., Hechmer, A., Simirenko, L., Stapleton, M., Luengo Hendriks, C. L., Chu H. C., Ogawa, N., Inwood, W., Sementchenko, V., Beaton, A., Weiszmann, R., Celniker, S. E., Knowles, D. W., Gingeras, T., Speed, T. P., Eisen, M. B. and Biggin, M. D. (2008). Transcription factors bind thousands of active and inactive regions in the *Drosophila* blastoderm. PLoS Biol. **6**, e27.
- Liljegren, S. J., Gustafson-Brown, C., Pinyopich, A., Ditta, G. S. and Yanofsky, M. F. (1999). Interactions among *APETALA1*, *LEAFY*, and *TERMINAL FLOWER1* specify meristem fate. Plant Cell **11**, 1007-1018.
- Lohmann, J. U., Hong, R. L., Hobe, M., Busch, M. A., Parcy, F., Simon, R. and Weigel, D. (2001). A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. Cell **105**, 793-803.
- Mandel, M. A., Gustafsonbrown, C., Savidge, B. and Yanofsky, M. F. (1992). Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. Nature **360**, 273-277.
- Matsuno, H., Doi, A., Nagasaki, M. and Miyano, S. (2000) Hybrid Petri net representation of gene regulatory network. Pac. Symp. Biocomput. **5**, 338-349.
- Matsuno, H., Tanaka, Y., Aoshima, H., Doi, A., Matsui, M. and Miyano, S. (2003). Biopathways representation and simulation on hybrid functional Petri net. In Silico Biol. **3**, 0032.
- Melzer, R., Kaufmann, K. and Theißen, G. (2006). Missing links: DNA-binding and target gene specificity of floral homeotic proteins. *In*: Advances in Botanical Research, Vo. 44, Developmental

genetics of the flower, Soltis, D., Soltis P. and Leebens-Mack, J. H. (eds.), Academic Press. pp. 209-236.

- Mendoza, L. and Alvarez-Buylla, E. R. (1998). Dynamics of the genetic regulatory network for *Arabidopsis thaliana* flower morphogenesis. J. Theor. Biol. **193**, 307-319.
- Nagasaki, M., Doi, A., Matsuno, H. and Miyano, S. (2003). Genomic Object Net: I. A platform for modelling and simulating biopathways. Appl. Bioinformatics **2**, 181-184.
- Nagasaki, M., Doi, A., Matsuno, H. and Miyano, S. (2004). A versatile petri net based architecture for modelling and simulation of complex biological processes. Genome Inform. **15**, 180-197.
- Nagasaki, M., Doi, A., Matsuno, H. and Miyano, S. (2005) Computational modeling of biological processes with Petri net-based architecture, Bioinformatics Technologies, Springer, Berlin Heidelberg, pp. 179-242.
- Nagasaki, M., Doi, A., Matsuno, H. and Miyano, S. (2009a). Foundations of Systems Biology - Using Cell Illustrator and Pathway Databases. Springer, Berlin Heidelberg.
- Nagasaki, M., Saito A. and Miyano, S. (2009b). Cell Illustrator 4.0: A Computational Platform for Systems Biology. In Silico Biology **10**, 0002.
- Oh, E., Kang, H., Yamaguchi, S., Park, J., Lee, D., Kamiya, Y. and Choi, G. (2009). Genome-wide analysis of genes targeted by PHYTOCHROME INTERACTING FACTOR 3-LIKE5 during seed germination in *Arabidopsis*. Plant Cell **21**, 403-419.
- Pelaz, S., Ditta, G. S., Baumann, E., Wisman, E. and Yanofsky, M. F. (2000). B and C floral organ identity functions require *SEPALLATA* MADS-box genes. Nature **405**, 200-203.
- Pelaz, S., Gustafson-Brown, C., Kohalmi, S. E., Crosby, W. L. and Yanofsky, M. F. (2001). *APETALA1* and *SEPALLATA3* interact to promote flower development. Plant J. **26**, 385-394.
- Peterson, J. L. (1981) Petri Net Theory and the Modelling of Systems. Prentice Hall.
- Petri, C. A. (1962). Fundamentals of a theory of asynchronous information flow. IFIP Congress 1962, 386-390.
- Putterill, J., Laurie, R. and Macknight, R. (2004). It's time to flower: the genetic control of flowering time. Bioessays **26**, 363-373.
- Reddy, V. N., Mavrovouniotis, M. L. and Liebman, M. N. (1993). Petri net representations in metabolic pathways. Proc. Int. Conf. Intell. Syst. Mol. Biol. **1**, 328-336.
- Sato, Y., Hashiguchi, Y. and Nishida, M. (2009). Evolution of multiple phosphodiesterase isoforms in stickleback involved in cAMP signal transduction pathway. BMC Syst. Biol. **3**, 23.
- Schmid, M., Uhlenhaut, N. H., Godard, F., Demar, M., Bressan, R., Weigel, D. and Lohmann, J. U. (2003). Dissection of floral induction pathways using global expression analysis. Development **130**, 6001-6012.
- Sun, N. and Zhao, H. (2004). Genomic approaches in dissecting complex biological pathways. Pharmacogenomics **5**, 163-179.
- Tanabe, Y., Hasebe, M., Sekimoto, H., Nishiyama, T., Kitani, M., Henschel, K., Münster, T., Theissen, G., Nozaki, H. and Ito, M. (2005). Characterization of MADS-box genes in charophycean green algae and its implication for the evolution of MADS-box genes. Proc. Natl. Acad. Sci. USA **102**, 2436-2441.
- Theißen, G. and Saedler, H. (2001). Plant biology. Floral quartets. Nature **409**, 469-471.
- Tilly, J. J., Allen, D. W. and Jack, T. (1998). The CArG boxes in the promoter of the *Arabidopsis* floral organ identity gene *APETALA3* mediate diverse regulatory effects. Development **125**, 1647-1657.
- Troncale, S., Tahi, F., Campard, D., Vannier, J.-P. and Guespin, J. (2006). Modelling and simulation with Hybrid Functional Petri Nets of the role of interleukin-6 in human early haematopoiesis. Pac. Symp.

Biocomput. **11**, 427-438.

- Urbanus, S. L., de Folter, S., Shchennikova, A. V., Kaufmann, K., Immink, R. G. H. and Angenent, G. C. (2009). In planta localisation patterns of MADS domain proteins during floral development in *Arabidopsis thaliana*. BMC Plant Biol. **9**, 5.
- Veron, A. S., Kaufmann, K. and Bornberg-Bauer, E. (2007). Evidence of interaction network evolution by whole-genome duplications: A case study in MADS-box proteins. Mol. Biol. Evol. **24**, 670-678.
- Walhout, A. J. M. (2006). Unraveling transcription regulatory networks by protein-DNA and protein-protein interaction mapping. Genome Res. **16**, 1445-1454.
- Weigel, D. and Meyerowitz, E. M. (1993). Activation of Floral Homeotic Genes in *Arabidopsis*. Science **261**, 1723-1726.
- Weigel, D., Alvarez, J., Smyth, D. R., Yanofsky, M. F. and Meyerowitz, E. M. (1992). *LEAFY* controls floral meristem identity in Arabidopsis. Cell **69**, 843-859.
- Wellmer, F., Alves-Ferreira, M., Dubois, A., Riechmann, J. L. and Meyerowitz, E. M. (2006). Genome-wide analysis of gene expression during early *Arabidopsis* flower development. PLoS Genet. **2**, e117.
- Wilkinson, M. D. and Haughn, G. W. (1995). *UNUSUAL FLORAL ORGANS* Controls Meristem Identity and Organ Primordia Fate in *Arabidopsis*. Plant Cell **7**, 1485-1499.
- Wu, G., Park, M. Y., Conway, S. R., Wang, J. W., Weigel, D. and Poethig, R. S. (2009)_The sequential action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. Cell **138**, 750-9.
- Yang, Y., Xiang, H. and Jack, T. (2003). *pistillata-5*, an *Arabidopsis* B class mutant with strong defects in petal but not in stamen development. Plant J. **33**, 177-188.
- Yanofsky, M. F., Ma, H., Bowman, J. L., Drews, G. N., Feldmann, K. A. and Meyerowitz, E. M. (1990). The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. Nature **346**, 35-9.
- Yun, J.-Y., Weigel, D. and Lee, I. (2002). Ectopic expression of *SUPERMAN* suppresses development of petals and stamens. Plant Cell Physiol. **43**, 52-57.