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A Gene Regulatory Network Model for Cell-Fate Determination during *Arabidopsis thaliana* Flower Development That Is Robust and Recovers Experimental Gene Expression Profiles [W]

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Flowers are icons in developmental studies of complex structures. The vast majority of 250,000 angiosperm plant species have flowers with a conserved organ plan bearing sepals, petals, stamens, and carpels in the center. The combinatorial model for the activity of the so-called ABC homeotic floral genes has guided extensive experimental studies in *Arabidopsis thaliana* and many other plant species. However, a mechanistic and dynamical explanation for the ABC model and prevalence among flowering plants is lacking. Here, we put forward a simple discrete model that postulates logical rules that formally summarize published ABC and non-ABC gene interaction data for Arabidopsis floral organ cell fate determination and integrates this data into a dynamic network model. This model shows that all possible initial conditions converge to few steady gene activity states that match gene expression profiles observed experimentally in primordial floral organ cells of wild-type and mutant plants. Therefore, the network proposed here provides a dynamical explanation for the ABC model and shows that precise signaling pathways are not required to restrain cell types to those found in Arabidopsis, but these are rather determined by the overall gene network dynamics. Furthermore, we performed robustness analyses that clearly show that the cell types recovered depend on the network architecture rather than on specific values of the model's gene interaction parameters. These results support the hypothesis that such a network constitutes a developmental module, and hence provide a possible explanation for the overall conservation of the ABC model and overall floral plan among angiosperms. In addition, we have been able to predict the effects of differences in network architecture between Arabidopsis and *Petunia hybrida*.

INTRODUCTION

Monod and Jacob (1961) first proposed that complex networks of gene interactions regulate cell differentiation. The first formal models of genetic regulation of cell differentiation anticipated that real biological genetic networks would be too complex to be analyzed without the use of formal mathematical and/or computational tools (Kauffman, 1969). However, because the early models made some assumptions that were biologically unrealistic (Aldana and Cluzel, 2003), experimentalists largely ignored them. Relatively complete genetic descriptions of developmental programs are now available in several model organisms, providing the necessary inputs for developing biologically realistic dynamic models of gene regulatory networks in cell differentiation (Hasty et al., 2002). Such models should aid at building a formal framework for studies of developmental mechanisms and their evolution.

Dynamical network models lead to formal mechanistic explanations of developmental mechanisms and, thus, of the way genetic interactions translate into phenotypic traits. This will enable novel predictions of the effects of specific perturbations of regulatory networks that in turn may form a basis for investigating how network architecture may constrain morphological evolutionary patterns (Salazar-Ciudad and Jernvall, 2002). Dynamic networks attain steady states that are formally referred to as attractors. In gene networks, each attractor corresponds to a combination of gene expression states that specifies a particular cell type (Kauffman, 1993). Hence, a single network architecture that functions as a dynamic circuit may lead to multiple cell types, each characterized by a different set of steady gene expression states. Network models have now been developed for cell-fate determination in *Arabidopsis thaliana* (e.g., Mendoza and Alvarez-Buylla, 1998, 2000) and segment-polarity determination in *Drosophila melanogaster* (e.g., Mjolsness et al., 1991; von Dassow et al., 2000; Albert and Othmer, 2003). Recent studies have also started to show that the dynamics of biological gene networks are robust to quantitative gene function alterations (von Dassow et al., 2000). Such robustness may be responsible in part for morphological pattern conservation.

A conserved floral plan is found among the vast majority of angiosperms with four main types of organs (sepals, petals,

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stamens, and carpels) and corresponding primordial cell types that appear in a stereotypical spatio-temporal pattern: sepals first in the outside, then petals, stamens, and last carpels in the center of the flower (Rudall, 1987). Studies at the molecular level are also suggesting that there is an overall conservation among genes and most of their interactions in angiosperm flower organ determination (Ferrario et al., 2004). We explore here if the Arabidopsis network of gene interactions of floral organ cell fate determination is a robust developmental module.

Experimental studies in Arabidopsis and *Antirrhinum majus* led to the ABC combinatorial model of gene expression states that predicts the identity of floral organ primordia (Coen and Meyerowitz, 1991) and has guided extensive experimental studies in many plant species (Ferrario et al., 2004). According to this model, A class genes (*APETALA1* [*AP1*] and *AP2*) specify sepal fate in the outer (first) floral whorl, A plus B genes (*AP3* and *PISTILLATA* [*PI*]) determine petal development in the second whorl, B plus C genes determine stamens in the third whorl, and the C gene (*AGAMOUS* [*AG*]) alone determines carpel fate in the central (fourth) whorl (Coen and Meyerowitz, 1991). The ABC model, however, does not provide a dynamical explanation of how the steady state pattern of gene expression characteristics of each primordial floral cell type is attained and maintained through interactions among ABC and non-ABC genes.

Here, we integrate data on ABC and non-ABC genes in a simple, dynamical network model for Arabidopsis that extends and improves our previous efforts (Mendoza and Alvarez-Buylla, 1998; Mendoza et al., 1999). The new model exploits a more complete understanding of genetic components and interactions, and in contrast with our previous model, we now obtain steady states that are coherent with experimental data. To achieve this, we developed logical rules grounded on experimental data to capture the qualitative information that appears to form the basis of this regulatory gene network. This new approach avoids the circular criteria used before to estimate the gene interaction parameters (Mendoza and Alvarez-Buylla, 1998).

The logical rules for each gene in the network were derived from molecular genetic experimental data (see references in logical rules section). The global dynamical behavior of the network was analyzed by obtaining all steady states of gene expression for all possible initial conditions. Interestingly, we found that the model is canalized to developmental pathways that converge only to a few documented gene expression patterns rather than reaching a diverse range of gene expression states depending on initial conditions. The model also attained expression profiles coherent with experimental data when loss and gain of function mutants were simulated. Hence, these qualitatively documented interactions are sufficient to recover expression states that have been experimentally observed in inflorescence meristem cells and in sepal, petal, stamen, and carpel primordial cells, suggesting that these result from the gene network architecture rather than from the parameters of the gene interactions. This was further verified directly by simulating perturbed networks.

Thus, the model integrates in a formal manner the available data on gene interactions underlying cell-fate determination during floral organ formation. This model is intended to be useful to test the coherence of experimental data and to hypothesize testable gene interactions that remain to be discovered. Several of such predictions are presented here. Finally, the model provides a tool for exploring the relative conservation of the basic structure of flowers among angiosperms, and we demonstrate that it can successfully predict the effects of evolutionary differences in the network architecture between Arabidopsis and *Petunia hybrida*.

RESULTS

The Model

We derived a discrete network model of cell-fate determination during floral organ primordia formation in Arabidopsis flower development. All parameters of the model are grounded on available experimental data for this species (see Methods; Figures 1 to 4). The updating of the gene's expression state depends on the tables of logical rules and on the states of the input genes in the previous time step. For each initial condition, the system attains an attractor after a number of iterations. Attractors can either be of type ''fixed point'' (steady state), in which the expression state of each gene is fixed, or ''limit cycles.'' In the latter, two or more gene activation combinations alternate periodically. The set of initial conditions that lead to each attractor is referred to as its basin of attraction.

Logical Rules for Each Network Node and Experimental Support

For all network nodes (genes) with inputs we provide a table of logical rules in Figures 1 to 3 and summarize the experimental evidence from which these rules were derived. Each table in Figures 1 to 3 lists the numerical values of the node's activity state as a function of the activity states that the nodes from which it receives input had in a previous time step. Eight of the nodes are Boolean with two possible outcomes: 0 (off) and 1 (on); but for the other seven nodes, experimental data enabled us to establish three levels of expression, 0, 1, and 2, with 1 representing an intermediate level of expression. The rationale that we used to codify the available experimental data into the logical rules is explained in Methods, but we summarize here the experimental evidence from which the rules were derived.

FLOWERING LOCUS T

Double *embryonic flower1* (*emf1*) *flowering locus t* (*ft*) mutants do not develop embryonic flowers typical of *emf1* single mutants (Haung and Yang, 1998), suggesting that the lack of *FT* activity suppresses the *emf1* phenotype because *EMF1* represses *FT*. *35S*:*LFY 35S*:*FT* double-transgenic plants flower immediately after germination (Kardailsky et al., 1999; Kobayashi et al., 1999), resembling the *emf1* phenotype and also supporting that *EMF1* represses *FT* (and *LEAFY* [*LFY*]; see below).

LFY

Double mutants of the weak *emf1-1* allele and *lfy-1* bear *lfy*-like flowers (Yang et al., 1995), suggesting that, for this trait, *lfy* is

A

EMF1	*FT*
0	1
1	0

B

AP1	*FUL*	*TFL1*	*EMF1*	*LFY*
X	X	0	0	2
0	0	0	1	1
0	0	1, 2	0	1
0	0	1, 2	1	0
0	1	1, 2	0	1
0	1	0	1	1
0	2	0, 1	0	2
0	2	0	1	1
0	2	2	0	1
0	2	1, 2	1	1*
0	1	1, 2	1	0
1	X	0, 1	0	2
1	X	2	1	0
1	X	0, 1	1	1
1	X	2	0	1
2	X	2	1	1
2	X	0, 1	X	2
2	X	X	0	2

C

AP1	*LFY*	*AP2*	*EMF1*	*TFL1*
X	X	X	0	0
2	X	X	1	0
0, 1	2	X	1	0
1	0, 1	1	1	0*
1	0, 1	0	1	1*
0	0, 1	X	1	2

D

LFY	*EMF1*
0	1
1, 2	0

E

TFL1	*SEP*
0	1
1, 2	0

Figure 1. Logical Rules for *FT*, *LFY*, *TFL1*, *EMF1*, and *SEP*.

The state of each network node (rightmost column in each table) depends on the combination of activity states of its input nodes (all other columns in each table). X represents any possible value. An asterisk denotes cases where subjective decisions had to be made and where we tested the alternative. The alternatives produced equivalent results to those obtained with the original values (see text). *FT* **(A)**, *LFY* **(B)**, *TFL1* **(C)**, *EMF1* **(D)**, and *SEP* **(E)**.

epistatic. These genes have antagonistic activities; hence, we infer that *LFY* is repressed by *EMF1*. The fact that in *terminal flower1* (*tfl1*) mutant plants *LFY* is ectopically expressed in the shoot apex (Ratcliffe et al., 1999) suggests that *TFL1* also represses *LFY*. We consider that when both *TFL1* and *EMF1* are inactive, *LFY* is fully active because *LFY* levels are highest during flower meristem determination when both former genes are inactive. In *ap1* and *ap1 cauliflower* (*cal*) double mutants, *LFY* expression is reduced (Piñeiro and Coupland, 1998), and *LFY* is turned on earlier in *35S:AP1* plants than in the wild type (Liljegren et al., 1999), suggesting that *LFY* rapidly achieves its highest activity when *AP1* is overexpressed. Finally, even though *LFY*:β-glucuronidase (*GUS*) expression is the same in the wild type and in *fruitfull* (*ful*) mutant plants, it is reduced in *ful ap1 cal* triple mutants relative to *ap1 cal* double mutant plants (Ferrándiz et al., 2000), suggesting that the role of *FUL* in *LFY* upregulation is important only when *AP1* is inactive.

TFL1

In *emf1-2 tfl1* double mutants, the *emf1-2* mutation is epistatic (Chen et al., 1997). As these two genes do not have opposite functions, this result suggests that EMF1 protein is needed for *TFL1* activity in wild-type Arabidopsis. In transgenic *35S:AP1* plants, the shoot apex is terminated in a flower resembling *tfl1* mutants (Mandel and Yanofsky, 1995a), whereas *TFL1* is severely diminished (Liljegren et al., 1999), and in *ap1 cal* double mutants *TFL1* is ectopically expressed (Liljegren et al., 1999), suggesting that *AP1* represses *TFL1*. The *35S:LFY* plants also resemble the *tfl1* mutant, and no *TFL1* expression is observed in these plants (Ratcliffe et al., 1999), whereas *TFL1* is ectopically expressed in *lfy* mutants (Liljegren et al., 1999). Finally, the *tfl1-1* mutation partially suppresses the *ap2-1 ap1-1* mutant phenotype (Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993), suggesting that both *AP1* and *AP2* repress *TFL1*. Hence, *TFL1* is repressed by *LFY*, *AP1*, and *AP2*.

EMF1

In double *emf1-2 lfy* mutants, *emf1* is epistatic to *lfy*, and a *35S:LFY* transgene enhances a weak *emf1* allele phenotype (*emf1-1*) (Chen et al., 1997). Together, these results suggest that *LFY* is a repressor of *EMF1*.

SEPALLATA1-3

The *SEPALLATA1-3* (*SEP1-3*) genes are fully expressed after floral induction (Savidge et al., 1995; Mandel and Yanofsky,

A

AG	*LFY*	*TFL1*	*FT*	*AP1*
2	X	X	X	0
0	X	X	2	2
1	X	X	2	1
0	*LFY≥TFL1*		0, 1	2
1	*LFY≥TFL1*		0, 1	1
0, 1	*LFY<TFL1*		0, 1	0

B

TFL1	*AP2*
0	1
1, 2	0

C

AP1	*TFL1*	*FUL*
X	1, 2	0
0	0	2
1	0	1
2	0	0

D

SEP	*LFY*	*UFO*	*AP3*	*PI*	*AG*	*AP1*	*AP3*
1	X	X	1, 2	1, 2	1, 2	X	2
1	X	X	1, 2	1, 2	X	1, 2	2
0	1, 2	1	X	X	X	X	1
X	1, 2	1	0	X	X	X	1
X	1, 2	1	X	0	X	X	1
X	1, 2	1	X	X	0	0	1
0	X	0	X	X	X	X	0
X	X	0	0	X	X	X	0
X	X	0	X	0	X	X	0
X	X	0	X	X	0	0	0
0	0	X	X	X	X	X	0
X	0	X	0	X	X	X	0
X	0	X	X	0	X	X	0
X	0	X	X	X	0	0	0

E

LFY	*SEP*	*AP3*	*PI*	*AG*	*AP1*	*PI*
X	X	0	X	0	X	0
X	1	1, 2	1, 2	1, 2	X	2
X	1	1, 2	1, 2	X	1, 2	2
1, 2	0	1, 2	1, 2	1, 2	X	1
1, 2	1	0	1, 2	1, 2	X	1
1, 2	1	1, 2	0	1, 2	X	1
1, 2	1	1, 2	1, 2	0	0	1
0	0	1, 2	1, 2	1, 2	X	0
0	1	0	1, 2	1, 2	X	0
0	1	1, 2	0	1, 2	X	0
0	1	1, 2	1, 2	0	0	0

Figure 2. Logical Rules for *AP1*, *AP2*, *FUL*, *AP3*, and *PI*.

The state of each network node (rightmost column in each table) depends on the combination of activity states of its input nodes (all other columns in each table). X represents any possible value. Comparative symbols (< and >) are used when the relative values are important to determine the state of activity of the target node. *AP1* **(A)**, *AP2* **(B)**, *FUL* **(C)**, *AP3* **(D)**, and *PI* **(E)**.

A

TFL1	*LFY*	*AP1*	*AG*	*WUS*	*AP2*	*SEP*	*LUG*	*CLF*	*AG*
TFL1≤*LFY*		X	X	X	0	X	X	X	2
TFL1>*LFY*		X	X	X	X	X	X	X	0
TFL1<*LFY*		X	X	1	1	X	X	X	2
2	2	X	X	1	1	X	X	X	2*
TFL1=*LFY*<2		X	X	X	1	X	X	X	0*
TFL1<*LFY*		X	0, 1	0	1	X	X	0	1
TFL1<*LFY*		X	2	0	1	0	X	0	1
TFL1<*LFY*		X	0, 1	0	1	X	0	X	1
TFL1<*LFY*		X	2	0	1	0	0	X	1
TFL1<*LFY*		0	0, 1	0	1	X	X	X	1
TFL1<*LFY*		0	2	0	1	0	X	X	1
2	2	X	0, 1	0	1	X	X	0	1
2	2	X	2	0	1	0	X	0	1
2	2	X	0, 1	0	1	X	0	X	1
2	2	X	2	0	1	0	0	X	1
2	2	0	0, 1	0	1	X	X	X	1
2	2	0	2	0	1	0	X	X	1
TFL1<*LFY*		1, 2	0, 1	0	1	X	1	1	0
TFL1<*LFY*		1, 2	2	0	1	0	1	1	0
2	2	1, 2	0, 1	0	1	X	1	1	0
2	2	1, 2	2	0	1	0	1	1	0
TFL1<*LFY*		X	2	0	1	1	X	X	2
2	2	X	2	0	1	1	X	X	2

B

WUS	*AG*	*SEP*	*WUS*
0	X	X	0
1	2	1	0
1	2	0	1
1	0, 1	X	1

Figure 3. Logical Rules for *AG* and *WUS*.

The state of each network node (rightmost column in each table) depends on the combination of activity states of its input nodes (all other columns in each table). X represents any possible value. Comparative symbols (< and >) are used when the relative values are important to determine the state of activity of the target node. Asterisks denote cases where subjective decisions had to be made and where we tested the alternative. The alternatives produced equivalent results to those obtained with the original values (see text). *AG* **(A)** and *WUS* **(B)**.

1998; Pelaz et al., 2000), but we are not aware of a direct inductor of these genes. Although the activator of these genes has not been discovered, we have considered that *TFL1*, which is a floral repressor, represses the *SEP* node (dotted t-bars in Figure 4). One or more undiscovered factors expressed in the inflorescence meristem and absent from the floral one or vice versa could play the role of *TFL1* in regulating *SEP* genes, and the network results would not be affected.

AP1

AP1 mRNA is ectopically expressed in the inner two whorls of *ag* mutant flowers, where *AG* is normally expressed in wild-type Arabidopsis (Gustafson-Brown et al., 1994). In *tfl1* mutants, *AP1* is ectopically expressed in the basal lateral meristems and in N-terminal flowers (Gustafson-Brown et al., 1994), suggesting that *TFL1* represses *AP1*. Also, *AP1* is expressed in *lfy* mutants but not in *35S*:*TFL1 lfy* transgenic plants (Ratcliffe et al., 1999), suggesting that *TFL1* repression over *AP1* is critical. The phenotypes of *tfl1 ap1*, *tfl1 lfy ap1*, and *tfl1 lfy* mutants further support the repression of *TFL1* over *AP1* (Shannon and Meeks-Wagner, 1993). It has been shown that *LFY* directly binds to the *AP1* promoter and activates it (Wagner et al., 1999). Furthermore, *AP1* expression is delayed in *lfy-6* null mutants and is ectopic in *35S*:*LFY* plants (Liljegren et al., 1999). In *ft lfy* double mutants, *AP1* mRNA is absent, whereas in the respective single mutants there is *AP1* expression, suggesting that at least one of these two genes needs to be present for *AP1* activation (Ruiz-García et al., 1997).

AP2

The absence of petals in *tfl1 ap2* double mutant flowers (Shannon and Meeks-Wagner, 1993) and the presence of these in *tfl1* single mutants suggest that there is ectopic *AP2* activity in the terminal flowers of *tfl1* single mutants and that *AP2* is repressed by *TFL1*.

FRUITFULL

FRUITFULL (*FUL*) is ectopically expressed in *ap1* mutants (Ferrándiz et al., 2000), suggesting that *FUL* expression is

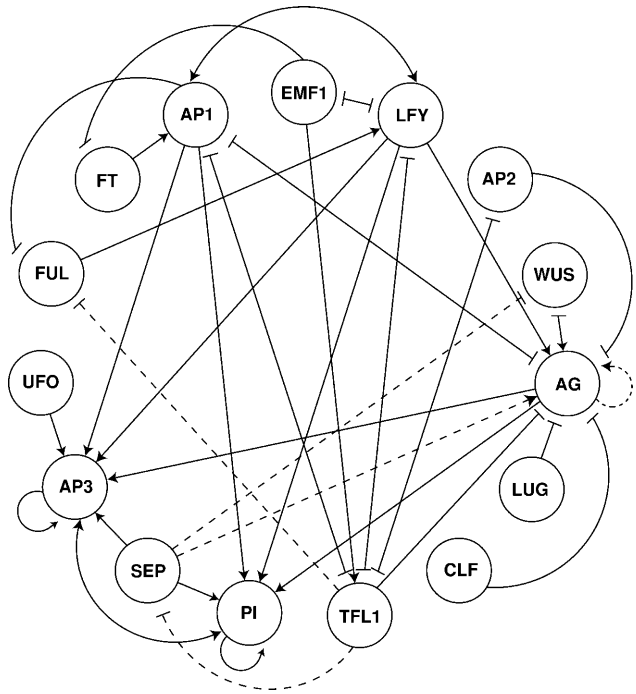

Figure 4. Gene Network Architecture for the Arabidopsis Floral Organ Fate Determination.

Network nodes represent active proteins of corresponding genes, and the edges represent the regulatory interactions between node pairs (arrows are positive, and blunt-end lines are negative). Dashed lines are hypothetical interactions for which there is no experimental support (see logical rules). The network includes F-box proteins (*UFO*), membrane bound signaling molecules (*TFL1* and *FT*), cofactors involved in transcriptional regulation (*EMF1* and *LUG*), chromatin remodeling proteins (*CLF*), and transcription factors (all others). Interactions have been confirmed to be direct transcriptional regulations in a few cases (*LFY* on *AG*, Busch et al., 1999; *LFY* on *AP1*, Wagner et al., 1999), and the rest can either be direct or indirect and can be transcriptional or other. See Results and Methods for model details.

repressed by *AP1*. Given that *FUL* has been shown to activate *LFY* (Ferrándiz et al., 2000), we would expect to find *LFY* in the tissues where *FUL* is expressed. However, whereas *FUL* is strongly expressed in inflorescence meristems, *LFY* is almost absent from there (Mandel and Yanofsky, 1995b; Ferrándiz et al., 2000). This suggests that there is an unidentified factor that impedes the activation of *LFY* by *FUL* in the inflorescence meristem and that the latter is nonfunctional in its capacity to activate *LFY* in this spatial domain. This interaction has to be at the posttranscriptional level because there is *FUL* mRNA in the inflorescence meristem. Here, we postulate that *TFL1* could be such an inhibitor. It also is possible that other factors play this posttranscriptional inhibitory role. In any case, such missing factors seem to be key for determining a steady state of gene expression characteristic of inflorescence meristematic cells because when the negative posttranscriptional regulation of *FUL* by *TFL1* is not considered, the nonfloral steady gene states disappear (see Supplemental Table 1 online). An alternative to postulating the negative regulation of *TFL1* over *FUL* would be to modify the logical rule of *LFY* by nullifying the activation of *LFY* by *FUL* when *TFL1* is on. Both approaches yield equivalent results (data not shown).

AP3

AP3 expression is reduced in *lfy* mutants (Weigel and Meyerowitz, 1993). In *lfy ap1* double mutants, *AP3* mRNA is not detected, but in *ap1* mutants, *AP3* is expressed as in wild-type plants (Hill et al., 1998). In *lfy-6* mutants, no stamens or petals are formed, but they develop if an inducible form of *LFY* is introduced and induced (Wagner et al., 1999). All these data suggest that *LFY* activates *AP3*. Both *LFY* and *UNUSUAL FLORAL ORGANS* (*UFO*) have to be overexpressed to cause ectopic expression of *AP3* (Parcy et al., 1998), also suggesting that *UFO* expression is needed for *AP3* activation by *LFY*. *LFY* and *UFO* are important for the onset of *AP3* expression, but *AP3*, as well as *PI*, expression is maintained through self-activation (Hill et al., 1998; Honma and Goto, 2000). In plants bearing a *35S*:*AP3*:*GR* construct, *AP3* is upregulated when induced with dexamethasone, supporting the notion that *AP3* self-activates (Honma and Goto, 2000). However, additional cofactors are needed for *AP3* self-activation.

The full activation of the B-function genes requires a tetramer with *AP1* or *AG*, as well as *SEP*, *PI*, and *AP3* proteins as shown by GUS activity in *AP3*:*GUS 35S*:*PI 35S*:*AP3 35S*:*AP1* plants (Honma and Goto, 2001) and because ectopic *PI*, *AP3*, *SEP*, and *AP1* expression is sufficient to transform leaves into petal-like organs (Pelaz et al., 2001). *AG* may substitute for *AP1* in maintaining *AP3* expression because cauline leaves of *35S*:*PI 35S*:*AP3 35S*:*SEP3 35S*:*AG* are converted into stamen-like organs (Honma and Goto, 2001). Moreover, there is weaker GUS expression in the third whorl of *ag-1 AP3*:*GUS* flowers than in wild-type plants (Hill et al., 1998), further supporting that *AG* activates *AP3*. The inclusion of other factors (such as *SEP*, *AP1*, and *AG*) required for the self-activation of B genes is sufficient to avoid the unrealistic steady state with B genes and floral repressors turned on at the same time that was found in an earlier study (Mendoza and Alvarez-Buylla, 1998; Mendoza et al., 1999).

PI

We assume that only when *AG* or *AP3* are active, *PI* expression is maintained because this gene is not active in sepals that are the only floral organs where both *AG* and *AP3* are inactive. However, direct experimental evidence is still lacking to support this interaction. The level of *PI* mRNA and its domain of expression are severely reduced in *lfy-6* null mutants, and there is no GUS expression in *lfy PI*:*GUS* early flowers (Honma and Goto, 2000). Moreover, *lfy-6* null mutants lack petals and stamens, but when *lfy-6 35S*:*LFY*:*GR* is induced to express *LFY* ectopically, the wild-type phenotype is rescued (Wagner et al., 1999), supporting *PI* activation by *LFY*. Finally, *PI* self-activation requires the same conditions needed for *AP3* self-activation (activity of *AP3*, *PI*, the *SEP* genes and either *AP1* or *AG*; Honma and Goto, 2000, 2001).

AG

In strong *ap2* mutant alleles, *AG* RNA accumulates in the four floral whorls, and in *ap2-2* and *ap2-9* alleles, medial 1st whorl organs are converted into carpels or carpelloid sepals, and 2nd whorl organs are absent (Deyholos and Sieburth, 2000), suggesting that *AP2* represses *AG*. All strong *wuschel* (*wus*) alleles lack carpels and most stamens (Laux et al., 1996), suggesting that *WUS* upregulates *AG*. Moreover, in *AP3*:*WUS* transgenic plants, 2nd whorl organs are carpelloid stamens instead of petals (Lenhard et al., 2001; Lohmann et al., 2001), whereas in *AP3*:*WUS ag* plants, 2nd and 3rd whorl organs do not differentiate into carpelloid stamens (Lenhard et al., 2001). There are data that support that *AG* is repressed by *LEUNIG* (*LUG*) and *CURLY LEAF* (*CLF*). First whorl sepals are frequently carpelloid, second whorl organs are staminoid petals in *lug* or *clf* mutants, and in the *lug ag* or *clf ag* double mutants, the *ag* mutation is epistatic (Liu and Meyerowitz, 1995; Goodrich et al., 1997). Also, the amount of *AG* RNA is greater in *lug* than in wild-type plants (Liu and Meyerowitz, 1995), and in *clf* mutants, *AG* mRNA is detected in sepals (Goodrich et al., 1997). Because of the constitutive expression of *LUG* and *CLF* (Goodrich et al., 1997; Conner and Liu, 2000), we considered these genes to be always active for the wild-type simulation. *AG* misexpression is increased by the *ap1* mutation in a *lug* or *ap2* background, and *AG* is occasionally expressed in the outer whorls of *ap1-1* mutants. Moreover, whorl 1 organs are sometimes carpelloid, and whorl 2 organs are staminoid in *ap1* mutants (Liu and Meyerowitz, 1995), suggesting that *AP1* also represses *AG*. The data above suggest that *WUS* activity is sufficient in Arabidopsis wild-type flowers to overcome the repression of *AG* by *LUG*, *CLF*, *AP1*, and *AP2* because in *AP3*:*WUS* lines there is *AG* activity in the second floral whorl where *LUG*, *CLF*, *AP1*, and *AP2* normally repress *AG*.

Normal stigmas and styles in terminal flowers in a *lfy ap1* background are rescued if the *tfl1* mutation is added (Shannon and Meeks-Wagner, 1993), supporting that *AG* is also repressed by *TFL1*. The *LFY* protein binds to an enhancer sequence in *AG*'s first intron (Busch et al., 1999). Also in *lfy-6* mutants, the onset of *AG* expression is delayed, and its final domain of expression is reduced (Weigel and Meyerowitz, 1993). In addition, the expression of a fusion of *LFY* to a strong activation domain produces increased and ectopic *AG* expression (Parcy et al., 1998). These facts indicate that *LFY* directly upregulates *AG*. Finally, we

assumed that *AG* activates itself either directly or indirectly and that this positive feedback loop requires full *AG* activity (*AG* = 2) and *SEP* activity.

WUS

It has been experimentally established that *AG* represses *WUS* (Lenhard et al., 2001; Lohmann et al., 2001). We infer that *SEP* activity is required for *WUS* downregulation by *AG* because *sep1 sep2 sep3* triple mutant plants bear indeterminate flowers (Pelaz et al., 2000). We also assume that *WUS* activity depends on *WUS* state of expression in a previous time step. We assumed this to avoid *WUS* activity in those system's states where *AG* is not active (e.g., the petals). *SHOOT MERISTEMLESS* may mediate *WUS* self-activation (Mayer et al., 1998).

The Arabidopsis Network Architecture: Novel Predictions on Gene Interactions

In Figure 4, we show the architecture of the floral organ cell fate determination network. Each node corresponds to the concentration of active or functional protein encoded by each gene. The edges represent the regulatory interactions between nodes. Arrows represent positive (activation) interactions, and blunt-end lines represent negative interactions (repression). In this article, when we refer to network architecture we are considering the connections among nodes, the sign of the regulatory interactions (both illustrated in Figure 4), and the relative importance of the input genes in determining the expression state of the target gene. The latter are precisely defined in the tables of logical rules given in Figures 1 to 3. Note that even though the network of gene interactions depicted in Figure 4 only shows either activation (positive) or repression (negative) regulatory interactions, the effect of these on the activation state of the downstream gene depends on the logical rules derived for each gene.

We have incorporated genes that codify for transcription factors (*AG*, *PI*, *AP3*, *WUS*, etc.; Coen and Meyerowitz, 1991; Mayer et al., 1998), F-box proteins such as UFO (Samach et al., 1999), likely membrane bound signaling molecules such as TFL1 and FT (Bradley et al., 1997; Simpson et al., 1999), positive or negative cofactors probably involved in transcription such as EMF1 (Aubert et al., 2001) and LUG (Conner and Liu, 2000), and CLF (Goodrich et al., 1997), which is a chromatin remodeling protein. To keep the coherence of available experimental data, we incorporated four interactions that have not been documented. These are indicated by dashed lines and constitute novel predictions of the model that should be tested experimentally.

The first assumption concerns the *SEP1-3* genes. These are fully expressed after floral induction (Savidge et al., 1995; Mandel and Yanofsky, 1998; Pelaz et al., 2000), but to our knowledge, there is no evidence of positive or negative regulators of these genes that could explain their expression pattern. Therefore, in the model, we have considered that the floral repressor *TFL1* negatively regulates these genes. However, the *SEP1-3* genes could be inhibited by any other floral repressor or activated by a flower meristem identity gene such as *LFY*, and results would still hold. The second assumption is that *FUL* is repressed posttranscriptionally by *TFL1* (Ferrándiz et al., 2000; see *FUL* logical rule). It would be equivalent to assuming that other meristem identity genes regulate *FUL*.

To recover the observed pattern of expression of *PI* in the three inner floral whorls, we assumed that *PI* expression is maintained only when *AG* or *AP3* are active because neither *AG* or AP3 are expressed in sepals. However, it is most probable that *PI* is regulated by one or more additional factors that are yet to be discovered.

The final assumption concerns *AG* and *WUS*. The repression of *WUS* by *AG* has been experimentally established (Lenhard et al., 2001; Lohmann et al., 2001). However, adding this interaction in the model, without assuming a mechanism that maintains the activity of *AG*, would make *AG* and *WUS* activities to cycle on and off because of the negative feedback loop. If we would have modeled this interaction as continuous, *WUS* and *AG* levels of activity could either cycle or attain an intermediary steady state in which both *AG* and *WUS* would not be fully active. This is unlikely because such intermediary *WUS* steady state should be lower than the in situ limit of detection because in wild-type flowers *WUS* mRNA is not detected after *AG* is turned on (Lenhard et al., 2001) and it should be lower than a threshold activity that would cause floral meristem determinacy. Furthermore, at the same time such intermediary levels of *WUS* activity would have to be strong enough to maintain *AG* level of activity and C function. Alternatively, one may predict that *AG* has a direct or indirect positive feedback loop that maintains its expression even in the absence of *WUS*. But such a loop has not been documented experimentally and thus constitutes a prediction of this model that should be tested experimentally.

The Steady States of the Network Model Coincide with Experimental Gene Expression Profiles

The network had 139,968 possible initial conditions, and it attained only 10 fixed-point attractors or steady gene expression states (see supplemental data online for complete basins of attraction). These steady gene states (Table 1) predicted by the model coincide with the gene expression profiles that have been documented experimentally in cells of wild-type Arabidopsis inflorescence meristems and floral organ primordia. For example, in the *Infl* steady states, floral meristem identity genes (*LFY*, *AP1*, and *AP2*) and floral organ identity genes (*AP1*, *AP2*, *AP3*, *PI*, *SEP*, and *AG*) are off, whereas the inflorescence identity genes (*EMF1* and *TFL1*) are on. Interestingly, all four possible combinations of *UFO* and *WUS* activity states determine the existence of the four *Infl* meristem attractors. These correspond to four regions observed in Arabidopsis wild-type meristems with the same combinations of *UFO* and *WUS* expression states (Laux et al., 1996; Mayer et al., 1998; Samach et al., 1999; Haecker and Laux, 2001; Brand et al., 2002).

The experimentally documented ABC gene expression profiles are reproduced in the steady states corresponding to cells of each organ type (Bowman et al., 1991; Coen and Meyerowitz, 1991; Jofuku et al., 1994). The same is true for all the non-ABC genes included in the network (for example, *SEP1-3*, Savidge et al., 1995; *CLF*, Goodrich et al., 1997; *UFO*, Samach et al., 1999; *LUG*, Conner and Liu, 2000).

Table 1. Gene Expression in Each of the 10 Steady Gene Activation States Attained for All Possible Initial Conditions (139,968) in the Wild-Type Arabidopsis Gene Network

FT	*EMF1*	*TFL1*	*LFY*	*FUL*	*AP1*	*AP3*	*PI*	*AG*	*UFO*	*WUS*	*AP2*	*SEP*	*LUG*	*CLF*	Cell Type
0	1	2	0	0	0	0	0	0	0	0	0	0	1	1	*Infl1*
0	1	2	0	0	0	0	0	0	1	0	0	0	1	1	*Infl2*
0	1	2	0	0	0	0	0	0	1	1	0	0	1	1	*Infl3*
0	1	2	0	0	0	0	0	0	0	1	0	0	1	1	*Infl4*
1	0	0	2	0	2	0	0	0	0	0	1	1	1	1	*Sep*
1	0	0	2	0	2	2	2	0	1	0	1	1	1	1	*Pe1*
1	0	0	2	0	2	2	2	0	0	0	1	1	1	1	*Pe2*
1	0	0	2	2	0	2	2	2	1	0	1	1	1	1	*St1*
1	0	0	2	2	0	2	2	2	0	0	1	1	1	1	*St2*
1	0	0	2	2	0	0	1	2	0	0	1	1	1	1	*Car*

Infl, inflorescence meristematic cells; Sep, sepal primordial cells; Pe, petal primordial cells; St, stamen primordial cells; Car, carpel primordial cells.

The model predicted two steady states for each petal and stamen cell type: one with and another one without *UFO* activity. This implies that the activity of this gene is not critical to maintain petal or stamen cell identity. This has been supported by experimental data because in *35S:AP3 35S:PI* double transgenic plants, in which *PI* and *AP3* are expressed in a constitutive manner, B gene determined organs are formed even in the first and fourth floral whorls (Krizek and Meyerowitz, 1996) where there is no *UFO* expression.

The basins of attraction of the attractors that correspond to inflorescence meristem cell identity (Table 2) indicate that to attain this steady state, in the initial conditions the floral meristem identity genes (e.g., *AP1* and *LFY*) should be off and the floral repressors (*TFL1* and *EMF1*) should be on. This also coincides with the experimental results obtained in Arabidopsis and other plant species, where gain-of-function transgenic plants for the floral meristem identity genes or loss-of-function mutants of the floral repressors (Shannon and Meeks-Wagner, 1993; Coupland, 1995; Mandel and Yanofsky, 1995a; Weigel and Nilsson, 1995; Ratcliffe et al., 1999) bear practically normal terminal flowers that consume the shoot apical meristem.

The size of the basins of attraction may indicate how stable each morphogenetic response is and which genes are critical to attain each cell fate (see supplemental data online). It is noteworthy that the ratio of the number of initial states leading to floral organ versus inflorescence primordial cells is ~36:1 (Table 3). This could explain why it has been so hard to find a single gene whose mutation determines a nonflowering phenotype (Koornneef et al., 1998) and why once flowering genes are turned on it is so hard to interrupt flower development. However, this result could be biased because many of the genes controlling the transition to flowering, such as *CONSTANS* (Simon et al., 1996), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (Samach et al., 2000), and *FLOWERING LOCUS C* (Michaels and Amasino, 1999) have not been included yet.

Interestingly, the sizes of the basins of attraction of stamens or carpels are much larger than those of sepals or petals, suggesting that the fates of the reproductive organs (stamens and carpels) are much more stable than the fates of the perianth organs (sepals and petals; Table 3; see also supplemental data online).

Simulations of Loss- and Gain-of-Function Mutants Recover Experimentally Observed Gene Expression Profiles

We simulated loss-of-function mutants (Tables 4, 5, and 6) by turning the node corresponding to the mutated gene permanently off. In all cases, the model recovered steady gene activation states that have been observed experimentally (see references in the tables). We also performed simulations of gain-of-function mutants (Tables 7 and 8) and recovered experimentally observed patterns in all cases. We found a minor discrepancy in the simulation of *35S:AP3* lines that lack carpels but have sepals in Arabidopsis, but in our simulation sepal identity was not recovered. This is because of the assumption made to recover *PI* expression in the inner three whorls (*AP3* is on in sepals and this turns *PI* on hereto because of the B gene positive feedback loop) and hence indicates that this might not be the way *PI* is regulated in Arabidopsis; instead, an additional network element has to be invoked.

As an additional mutational analysis to validate the model in relation to experimental evidence and to evaluate the role of the

Table 2. Sets of Initial Conditions That Lead to *Infl* Steady Activation States in the Arabidopsis Wild Type Network

FT	*EMF1*	*TFL1*	*LFY*	*FUL*	*AP1*	*AP3*	*PI*	*AG*	*UFO*	*WUS*	*AP2*	*SEP*	*LUG*	*CLF*
0	1	2	0	X	1	X	X	0, 1	X	X	0	X	X	X
X	1	2	0	X	1	X	X	2	X	X	0	X	X	X
0	1	1, 2	0	0, 1	0	X	X	0	X	X	X	X	X	X
X	1	1, 2	0	0, 1	0	X	X	1, 2	X	X	X	X	X	X

X represents any possible value.

Table 3. Size of the Basins of Attraction for the Arabidopsis Wild Type Gene Network

Steady Activation States	*N*	*P*	*M*
Infl1-4	3,744	1.639	3
Sep	4,026	3.575	5
Pe1	4,698	3.717	5
Pe2	672	2.748	4
St1	63,414	3.962	8
St2	3,024	2.674	4
Car	60,390	3.959	8

Mean (*P*) and maximum (*M*) number of iterations needed to attain each steady state. *N*, size of the basins of attraction.

network architecture in determining the steady states, we systematically removed each of the interactions (activation or inhibition arrows) postulated in the network model (see Methods) and found the new steady states and corresponding basins of attraction (see Supplemental Table 1 online). This procedure also enabled us to test for possible errors in the logical rules and allows for novel predictions on the effects of canceling the input of single interactions. For example, if the regulation of *AG* by *LFY* could be deleted without altering other functions of *LFY*, flowers with sepals and petals instead of carpels and stamens would be expected. More interestingly, when the activation of *AP1* by *LFY* is removed, the model recovers states of normal flowers, suggesting that this interaction is not necessary for floral meristem identity. However, other aspects of the function of both genes are critical because both *lfy* and *ap1* mutant flowers have flower meristem identity defects.

Results summarized in Supplemental Table 1 online clearly show that the network architecture determines the steady states recovered. To test if these are robust to alterations in the details of the laws governing the activation of each network component keeping the overall architecture intact, we performed in silico point perturbations of the outputs in the tables of logical rules.

The Network Steady States Are Robust to Small Alterations of Gene Function

We tested the robustness of the steady states predicted by the model with single in silico random point alterations of the outputs in the tables of the logical rules. At each run, one alteration was done in one of the outputs of a randomly selected network node. In the simulations, we had all possible states of input genes spelled out and altered the output states for only one of the combinations. The probability of altering any particular node was proportional to the number of different possible combinations of expression states of the genes that affect it. After each point alteration, the steady gene states were obtained for all possible initial conditions and compared with the steady states predicted by the wild-type network. This was repeated until the proportion of novel steady states recovered of all altered networks tested reached a plateau (see Supplemental Figure 1 online). The plateau was reached at 6000 trials and we stopped at 7500.

Only 67 (0.89%) of the 7500 altered networks had at least one initial condition that lead to a new steady state that was not obtained in the wild-type simulation. However, most of these 67 networks predicted very similar cell types to those obtained with the original network. For example, in 25 (0.33%) of these 67 networks, the novel steady activation states predicted after the point alteration were different to the original ones only by a change in a single gene activation state, and it usually affected the level of expression of genes with more than two states. In 16 (0.213%) other networks, no novel steady gene activation states were predicted. Instead, limit cycles in which one of the states was equal to a steady activation state from the original network were obtained (although intermittent, the expected gene expression pattern was maintained). Two (0.0266%) other networks corresponded to cases in which the *SEP* node, which represented three redundant genes (Pelaz et al., 2000) and hence implied a triple mutant, was altered. Hence, out of 7500 random networks generated by in silico point alterations in the logical rules, only 24 (<0.5%) yielded different steady gene activation states to the ones recovered with the original network.

Evolution of Development: Floral Network Alterations Explain the *green petals* Mutant Phenotype for Petunia

Deciphering network architectures underlying cell differentiation is a first step toward understanding the mechanisms that rule conservation and variation in morphological traits. Although most flowering species have an overall conserved plan of floral organ determination (Rudall, 1987), mutations have revealed some important variations. In the case of petunia, the overall network of cell fate determination during flower organ development seems to be conserved with respect to Arabidopsis (Ferrario

Table 4. Steady States Predicted by the Model When *ap2* Mutants Are Simulated and Relationship with Cell Types Found in Structures of the Mutant Arabidopsis Plants (Liu and Meyerowitz, 1995; Deyholos and Sieburth, 2000)

FT	*EMF1*	*TFL1*	*LFY*	*FUL*	*AP1*	*AP3*	*PI*	*AG*	*UFO*	*WUS*	*AP2*	*SEP*	*LUG*	*CLF*	Cell Type
0	1	2	0	0	0	0	0	0	0	0	0	0	1	1	*Infl1*
0	1	2	0	0	0	0	0	0	1	0	0	0	1	1	*Infl2*
0	1	2	0	0	0	0	0	0	1	1	0	0	1	1	*Infl3*
0	1	2	0	0	0	0	0	0	0	1	0	0	1	1	*Infl4*
1	0	0	2	2	0	2	2	2	1	0	0	1	1	1	*St1*
1	0	0	2	2	0	2	2	2	0	0	0	1	1	1	*St2*
1	0	0	2	2	0	0	1	2	0	0	0	1	1	1	*Car*

Infl, inflorescence meristematic cells; St, stamen primordial cells; Car, carpel primordial cells.

Table 5. Steady States Predicted by the Model When *lug* Mutants Are Simulated and Relationship with Cell Types Found in Structures of the Mutant Arabidopsis Plants (Liu and Meyerowitz, 1995)

FT	*EMF1*	*TFL1*	*LFY*	*FUL*	*AP1*	*AP3*	*PI*	*AG*	*UFO*	*WUS*	*AP2*	*SEP*	*LUG*	*CLF*	Cell Type
0	1	2	0	0	0	0	0	0	0	0	0	0	0	1	*Infl1*
0	1	2	0	0	0	0	0	0	1	0	0	0	0	1	*Infl2*
0	1	2	0	0	0	0	0	0	1	1	0	0	0	1	*Infl3*
0	1	2	0	0	0	0	0	0	0	1	0	0	0	1	*Infl4*
1	0	0	2	1	1	0	1	1	0	0	1	1	0	1	*CS*
1	0	0	2	1	1	2	2	1	1	0	1	1	0	1	*SP1*
1	0	0	2	1	1	2	2	1	0	0	1	1	0	1	*SP2*
1	0	0	2	2	0	2	2	2	1	0	1	1	0	1	*St1*
1	0	0	2	2	0	2	2	2	0	0	1	1	0	1	*St2*
1	0	0	2	2	0	0	1	2	0	0	1	1	0	1	*Car*

Infl, inflorescence meristematic cells; St, stamen primordial cells; Car, carpel primordial cells; CS, carpelloid sepal primordial cells; SP, staminoid petal primordial cells.

et al., 2004). However, mutant analyses have suggested some differences. For example, whereas in Arabidopsis the *AP3* mutation yields a homeotic flower with two whorls of sepals and two whorls of inner carpels, in petunia the mutation in the *AP3* ortholog results in a flower with two whorls of sepals, a whorl of unaltered stamens, and a whorl of carpels (Vandenbussche et al., 2004). Vandenbussche and collaborators (2004) have shown that another *AP3* duplicate upregulated by an *AG* ortholog in petunia could be partially substituting for the functions of the *AP3* ortholog in stamens. Our simulation results suggest that an architecture like the one proposed here for the Arabidopsis network that includes a duplicated *AP3* would yield the gene expression patterns observed for the wild type and a single *AP3*-like gene mutant and provides a prediction for the double loss-of-function mutations of *AP3*-like genes in petunia (Tables 9 to 11, Figure 5).

DISCUSSION

Theoretical studies (e.g., Kauffman, 1969, 1993; Thomas, 1991) and the more recent analyses of molecular networks using genomic data (e.g., Jeong et al., 2000; Wagner, 2001) have been fundamental contributions toward the understanding of molecular regulatory networks and cell differentiation. Initial models considered random networks in which all nodes were connected, on average, to the same number of other network nodes (Kauffman, 1993). But recent studies have shown that unequal connectivity among nodes can greatly influence network dynamics (Aldana and Cluzel, 2003), and biological networks at different levels are certainly not uniformly connected (Jeong et al., 2000).

Obtaining the repertoire of the architectures of relatively smaller networks from more detailed experimental data and studying their structures and dynamical behaviors (e.g., Mjolsness et al., 1991; McAdams and Shapiro, 1995; Mendoza and Alvarez-Buylla, 1998; Albert and Othmer, 2003) will be particularly relevant for understanding developmental mechanisms if small, structurally, and dynamically isolated networks of key regulatory genes determine fundamental and specific aspects of cell differentiation (Wagner, 1996; Hartwell et al., 1999). For such networks, the general conclusions derived from analyses of the statistical behavior of large networks may not hold. In this article, we have shown that a relatively small network, yet derived from thorough experimental data (see references in logical rules section above), is canalized to steady gene activation states that coincide with those observed experimentally in meristematic inflorescence cells and in primordial cells of sepals, petals, stamens, and carpels. This network can be the basis to elaborate explicit spatio-temporal dynamic models of cell specification during floral organ determination.

Table 6. Steady States Predicted by the Model when *ap3* Mutants Are Simulated and Relationship with Cell Types Found in Structures of the Mutant Arabidopsis Plants (Bowman et al., 1989)

FT	*EMF1*	*TFL1*	*LFY*	*FUL*	*AP1*	*AP3*	*PI*	*AG*	*UFO*	*WUS*	*AP2*	*SEP*	*LUG*	*CLF*	Cell Type
0	1	2	0	0	0	0	0	0	0	0	0	0	1	1	*Infl1*
0	1	2	0	0	0	0	0	0	1	0	0	0	1	1	*Infl2*
0	1	2	0	0	0	0	0	0	1	1	0	0	1	1	*Infl3*
0	1	2	0	0	0	0	0	0	0	1	0	0	1	1	*Infl4*
1	0	0	2	0	2	0	0	0	0	0	1	1	1	1	*Sep*
1	0	0	2	0	2	0	0	0	1	0	1	1	1	1	*Sep*
1	0	0	2	2	0	0	1	2	1	0	1	1	1	1	*Car*
1	0	0	2	2	0	0	1	2	0	0	1	1	1	1	*Car*

Infl, inflorescence meristematic cells; Sep, sepal primordial cells; Car, carpel primordial cells.

Table 7. Steady States Predicted by the Model When *35S:AG* Transgenic Lines Are Simulated and Relationship with Cell Types Found in Structures of the Transgenic Arabidopsis Plants (Mizukami and Ma, 1997)

FT	*EMF1*	*TFL1*	*LFY*	*FUL*	*AP1*	*AP3*	*PI*	*AG*	*UFO*	*WUS*	*AP2*	*SEP*	*LUG*	*CLF*	Cell Type
0	1	2	0	0	0	0	0	2	0	0	0	0	1	1	*Infl1*
0	1	2	0	0	0	0	0	2	1	0	0	0	1	1	*Infl2*
0	1	2	0	0	0	0	0	2	1	1	0	0	1	1	*Infl3*
0	1	2	0	0	0	0	0	2	0	1	0	0	1	1	*Infl4*
1	0	0	2	2	0	2	2	2	1	0	1	1	1	1	*St1*
1	0	0	2	2	0	2	2	2	0	0	1	1	1	1	*St2*
1	0	0	2	2	0	0	1	2	0	0	1	1	1	1	*Car*

Infl, inflorescence meristematic cells; St, stamen primordial cells; Car, carpel primordial cells.

A network may be considered a developmental module if its steady states are robust to initial conditions and small alterations of parameters defining gene interactions (von Dassow et al., 2000). However, this has been tested for very few gene networks grounded on experimental data (von Dassow et al., 2000). Our results strongly suggest that the network put forward here constitutes a robust developmental module for organ cell-fate determination that may partly underlie the conservation of overall floral plan among angiosperms (Ambrose et al., 2000; Ferrario et al., 2004). On the other hand, the fact that using the same overall network with a duplicated *AP3*-like gene we are able to recover the *green petals* phenotype of petunia (Vandenbussche et al., 2004) shows that this type of model can also be useful for exploring variations in developmental mechanisms along species lineages. Particularly, we were able to provide additional support for the prediction made by Vandenbussche and collaborators (2004) for the double mutant of the two *AP3*-like genes and possible regulatory interaction predictions for the other genes included in the network for petunia. This case is also a documented example for maintenance of overall network functionality and predicted steady states after an event of gene duplication and functional divergence. Furthermore, this event enables mutations in *AP3*-like genes that do not abolish stamen cell differentiation. Studies of the evolution of development in other eukaryotic systems have also started to explore the role of developmental mechanisms defined in terms of gene networks and how these constrain the patterns of morphological variation that emerge during evolution and thus limit the scope of natural selection (Salazar-Ciudad et al., 2000; Goodwin, 2001).

Additionally, the Arabidopsis network model may be used as a framework to find holes or inconsistencies in gene interactions documented experimentally or to test and/or refine hypotheses in silico that can then be tested experimentally in vivo (Eldar et al., 2002). Our simulations predict that (1) the *SEP* and *FUL* genes are positively regulated by a flower meristem identity gene or repressed by an inflorescence meristem identity gene. Note that *FUL* is off in the *Infl* steady state (Table 1), whereas its mRNA has been observed in cells of the inflorescence meristem. The regulators (positive or negative) of this gene have not been characterized experimentally. Here, we have considered that this gene is posttranscriptionally repressed by *TFL1* (see *FUL* logical rules). (2) A regulator of *PI* that restricts its domain of expression to petals, stamens, and carpels needs to be discovered. (3) *AG* self-activates either directly or indirectly. An alternative less parsimonious possibility would be that *WUS* activates a yet to be discovered gene that should persist in the absence of *WUS* (maybe through self-activation) and thus maintain *AG* expression when *WUS* is off. The possibility of *AG* self-regulation has been neglected because in *ag-1* plants the *AG* mRNA pattern of expression is as in wild-type Arabidopsis (Gustafson-Brown et al., 1994). However, this data could still be compatible with an *AG* positive feedback loop because in the *ag-1* background the nonactive AG protein is unable to repress *WUS* that in turn would permanently upregulate *AG*. To test the positive feedback

Table 8. Steady States Predicted by the Model When *35S:AP3* Transgenic Lines Are Simulated and Relationship with Cell Types Found in Structures of the Transgenic Arabidopsis Plants (Krizek and Meyerowitz, 1996)

FT	*EMF1*	*TFL1*	*LFY*	*FUL*	*AP1*	*AP3*	*PI*	*AG*	*UFO*	*WUS*	*AP2*	*SEP*	*LUG*	*CLF*	Cell Type
0	1	2	0	0	0	2	0	0	0	0	0	0	1	1	*Infl1*
0	1	2	0	0	0	2	0	0	1	0	0	0	1	1	*Infl2*
0	1	2	0	0	0	2	0	0	1	1	0	0	1	1	*Infl3*
0	1	2	0	0	0	2	0	0	0	1	0	0	1	1	*Infl4*
1	0	0	2	2	0	2	2	2	1	0	1	1	1	1	*St1*
1	0	0	2	2	0	2	2	2	0	0	1	1	1	1	*St2*
1	0	0	2	0	2	2	2	0	1	0	1	1	1	1	*Pe1*
1	0	0	2	0	2	2	2	0	0	0	1	1	1	1	*Pe2*

Infl, inflorescence meristematic cells; St, stamen primordial cells; Pe, petal primordial cells.

Table 9. Gene Expression in Each of the 10 Steady Gene Activation States Attained for All Possible Initial Conditions (419,904) in the Wild-Type Petunia Gene Network

FT	*EMF1*	*TFL1*	*LFY*	*FUL*	*FBP26*	*PhDEF*	*PhGL01,2*	*pMADS3*	*UFO*	*WUS*	*PhAP2A*	*FBP2,5*	*LUG*	*PhCLF1,2*	*PhTM6*	Cell Type
0	1	2	0	0	0	0	0	0	0	0	0	0	1	1	0	*Infl1*
0	1	2	0	0	0	0	0	0	1	0	0	0	1	1	0	*Infl2*
0	1	2	0	0	0	0	0	0	1	1	0	0	1	1	0	*Infl3*
0	1	2	0	0	0	0	0	0	0	1	0	0	1	1	0	*Infl4*
1	0	0	2	0	2	0	0	0	0	0	1	1	1	1	0	*Sep*
1	0	0	2	0	2	2	2	0	1	0	1	1	1	1	0	*Pe1*
1	0	0	2	0	2	2	2	0	0	0	1	1	1	1	0	*Pe2*
1	0	0	2	2	0	2	2	2	1	0	1	1	1	1	2	*St1*
1	0	0	2	2	0	2	2	2	0	0	1	1	1	1	2	*St2*
1	0	0	2	2	0	0	0	2	0	0	1	1	1	1	1	*Car*

Note that the names of the Arabidopsis genes are used in those cases in which no ortholog gene in petunia has been described (see Figure 5 for petunia gene names). Infl, inflorescence meristematic cells; Sep, sepal primordial cells; Pe, petal primordial cells; St, stamen primordial cells; Car, carpel primordial cells.

loop, we would need to show ectopic GUS staining in an *AG*:*GUS* × *35S*:*AG* cross. We are presently pursuing this experiment.

To test the dynamical consequences of the *AG* positive feedback loop, we ran all analyses presented here for a network that lacks the loop for *AG* as well as the inhibition of *WUS* by *AG* (see supplemental data online). Overall results are the same as those obtained for the network in Figure 4. There are, however, some differences that suggest that the network in Figure 4 is more robust than the one in supplemental data online. Also, although for the network of Figure 4 we did not find that steady states are most sensitive to changes at nodes that have the highest number of outgoing links (Albert et al., 2000), this was the pattern found in the same type of simulations for the network in the supplemental data online. These results suggest that the sensitivity of networks' steady states to alterations of genes with different degrees of connectivity may depend on the number and type of network motifs (Milo et al., 2002). It now becomes relevant to explore the presence and dynamic significance of motifs in networks of different sizes grounded on experimental data.

This network includes most published gene interactions during floral organ cell fate determination and recovers steady states that correspond to observed patterns of gene expression in wild-type and mutant backgrounds. In our previous effort to model cell fate determination during flower morphogenesis, we recovered steady states that did not match any observed gene expression patterns (Mendoza and Alvarez-Buylla, 1998), pointing to missing data. Additionally, in this article, we have derived logical rules grounded on Arabidopsis experimental data to estimate parameters of gene interactions and therefore avoided the circular criteria that we used previously. The tables of logical rules also provide a framework to systematically refine and expand them. Finally, in this article, we have achieved a robustness analysis and simulations that illustrate variations in developmental mechanisms among species.

The fact that wild-type and mutant simulations using the whole network recover states that are observed in wild-type and mutant plants suggests that the key cell-autonomous factors at play in Arabidopsis floral organ fate determination have been included. Even though the architecture of the network importantly relied on mutant analyses of the genes included, the global network behavior under wild-type and mutant assumptions cannot be predicted without formal analyses as the ones performed here, such as the robustness simulations. Although real gene networks are likely to be more complex than the one proposed here, it is

Table 10. Steady States Predicted by the Model When *phdef* Mutants Are Simulated and Relationship with Cell Types Found in Structures of Mutant Petunia Plants (Vandenbussche et al., 2004)

FT	*EMF1*	*TFL1*	*LFY*	*FUL*	*FBP26*	*PhDEF*	*PhGL01,2*	*pMADS3*	*UFO*	*WUS*	*PhAP2A*	*FBP2,5*	*LUG*	*PhCLF1,2*	*PhTM6*	Cell Type
0	1	2	0	0	0	0	0	0	0	0	0	0	1	1	0	*Infl1*
0	1	2	0	0	0	0	0	0	1	0	0	0	1	1	0	*Infl2*
0	1	2	0	0	0	0	0	0	1	1	0	0	1	1	0	*Infl3*
0	1	2	0	0	0	0	0	0	0	1	0	0	1	1	0	*Infl4*
1	0	0	2	0	2	0	0	0	0	0	1	1	1	1	0	*Sep*
1	0	0	2	0	2	0	1	0	1	0	1	1	1	1	0	*Sep2*
1	0	0	2	2	0	0	2	2	1	0	1	1	1	1	2	*St1*
1	0	0	2	2	0	0	2	2	0	0	1	1	1	1	2	*St2*
1	0	0	2	2	0	0	0	2	0	0	1	1	1	1	1	*Car*

Infl, inflorescence meristematic cells; Sep, sepal primordial cells; St, stamen primordial cells; Car, carpel primordial cells. Gene names as in Table 9 and Figure 5.

Table 11. Steady States Predicted by the Model When *phdef phtm6* Double Mutants Are Simulated and Predicted Relationship with Cell Types in Structures of the Mutant Petunia Plants (Vandenbussche et al., 2004)

FT	*EMF1*	*TFL1*	*LFY*	*FUL*	*FBP26*	*PhDEF*	*PhGL01,2*	*pMADS3*	*UFO*	*WUS*	*PhAP2A*	*FBP2,5*	*LUG*	*PhCLF1,2*	*PhTM6*	Cell Type
0	1	2	0	0	0	0	0	0	0	0	0	0	1	1	0	*Infl1*
0	1	2	0	0	0	0	0	0	1	0	0	0	1	1	0	*Infl2*
0	1	2	0	0	0	0	0	0	1	1	0	0	1	1	0	*Infl3*
0	1	2	0	0	0	0	0	0	0	1	0	0	1	1	0	*Infl4*
1	0	0	2	0	2	0	0	0	0	0	1	1	1	1	0	*Sep*
1	0	0	2	0	2	0	1	0	1	0	1	1	1	1	0	*Sep2*
1	0	0	2	2	0	0	0	2	0	0	1	1	1	1	0	*Car*
1	0	0	2	2	0	0	1	2	1	0	1	1	1	1	0	*Car2*

Infl, inflorescence meristematic cells; Sep, sepal primordial cells; Car, carpel primordial cells. See Table 9 and Figure 5 for gene names.

outstanding that of the many possible initial conditions and developmental routes, this network is canalized to the few steady state gene expression profiles observed upon floral organ primordia determination. This suggests that specific developmental routes starting on few initial conditions elicited by precise signaling mechanisms are not needed to attain proper cell identity. This indicates that the steady gene activation profiles of floral organ primordia are determined not only by previous gene activation states, but also by the overall network architecture and dynamics. Our analyses suggest also that the network steady states are robust to small alterations of gene function.

It is noteworthy that the basins of attraction of reproductive organ primordial cells (stamens and carpels) are much larger than those of perianth organs primordial cells (sepals and petals). This leads to the prediction that variants with altered or lacking perianth organs should be more common than those with affected reproductive whorls, and this pattern indeed coincides with some surveys (Meyer, 1966). Robustness of reproductive organs is advantageous because alterations of reproductive organs are likely to have greater impacts on fitness than perianth modifications. However, it is not straightforward that natural selection is directly responsible for the origin of this robustness. An alternative explanation could lie in the fact that cell fate determination mechanisms of reproductive organs are older. Molecular mechanisms of reproductive cell fate seem in fact to be conserved among gymnosperms and angiosperms (Winter et al., 1999), whereas the perianth originated later and is privative of angiosperms. Hence, it is reasonable to assume that the basins of attraction of the newly evolved attractors that correspond to perianth organs are relatively smaller if network functionality has to be preserved and the older cell types maintained. In this scenario, natural selection may have been important in preserving network functionality so that it continued to generate reproductive structures, but not in making certain states more robust (with larger basins of attraction) than others from a phase space that was originally equally partitioned among states. Nonetheless, the action of natural selection in directly achieving robustness of reproductive organs cannot be ruled out at this moment.

A very similar model to the one proposed here was recently proposed for the pair-rule genes in Drosophila (Albert and Othmer, 2003). Their discrete Boolean network reproduced the results from analyses of an earlier continuous model (von Dassow et al., 2000) and yielded patterns of gene expression coherent with experimental data. Therefore, they concluded that their network is a robust developmental module in which the dynamics of the system depends on the network architecture. Whether most gene networks are canalized and robust is still an unanswered question, but most networks underlying cell differentiation are not autonomous and cell–cell interaction mechanisms, such as protein and hormone diffusion, active transport, and ligand–receptor interactions, likely affect gene network behavior and transitions in cell identity. These mechanisms may be in part responsible for canalization as well. On the other hand, for patterns and/or processes that are more variable or even plastic among individuals of the same species, a discrete approach might not be adequate (e.g., Krakauer et al., 2002; Salazar-Ciudad and Jernvall, 2002). In such instances, specific parameter values of continuous functions may be responsible for alternative behaviors. In addition, cell division is also coupled to cell differentiation during morphogenesis, and the geometry of the spatial domains may constrain the spatial patterns of cell types that emerge (Murray, 1989; Barrio et al., 2004). Therefore, an explicit spatio-temporal framework must be considered to be able to explore the relative role of intercellular signaling mechanisms, patterns of cell division, geometry of the spatial domain, and the network of gene interactions during cell patterning and morphogenesis.

Recent studies have started to propose formal models with explicit spatio-temporal dynamics to explore the mechanisms underlying aerial meristem development in Arabidopsis. Jönsson and collaborators (2003) have considered a small network of genes responsible for stem cell identity and meristematic patterning and have coupled these network dynamics to cell proliferation to recover spatial patterns of gene expression.

Before incorporating spatio-temporal dynamics, we consider that it is important to first derive from experimental data gene network architectures that underlie cell differentiation. This may guarantee that no critical nodes or interactions are left out and avoid ending up with the wrong network and equations with adjusted ad hoc parameters to recover observed gene expression patterns. Once cell-fate determination networks are assembled, hypotheses on the role of cellular dynamics, geometry, growth, and intercellular communication mechanisms in pattern formation and morphogenesis can be tested in a formal framework.

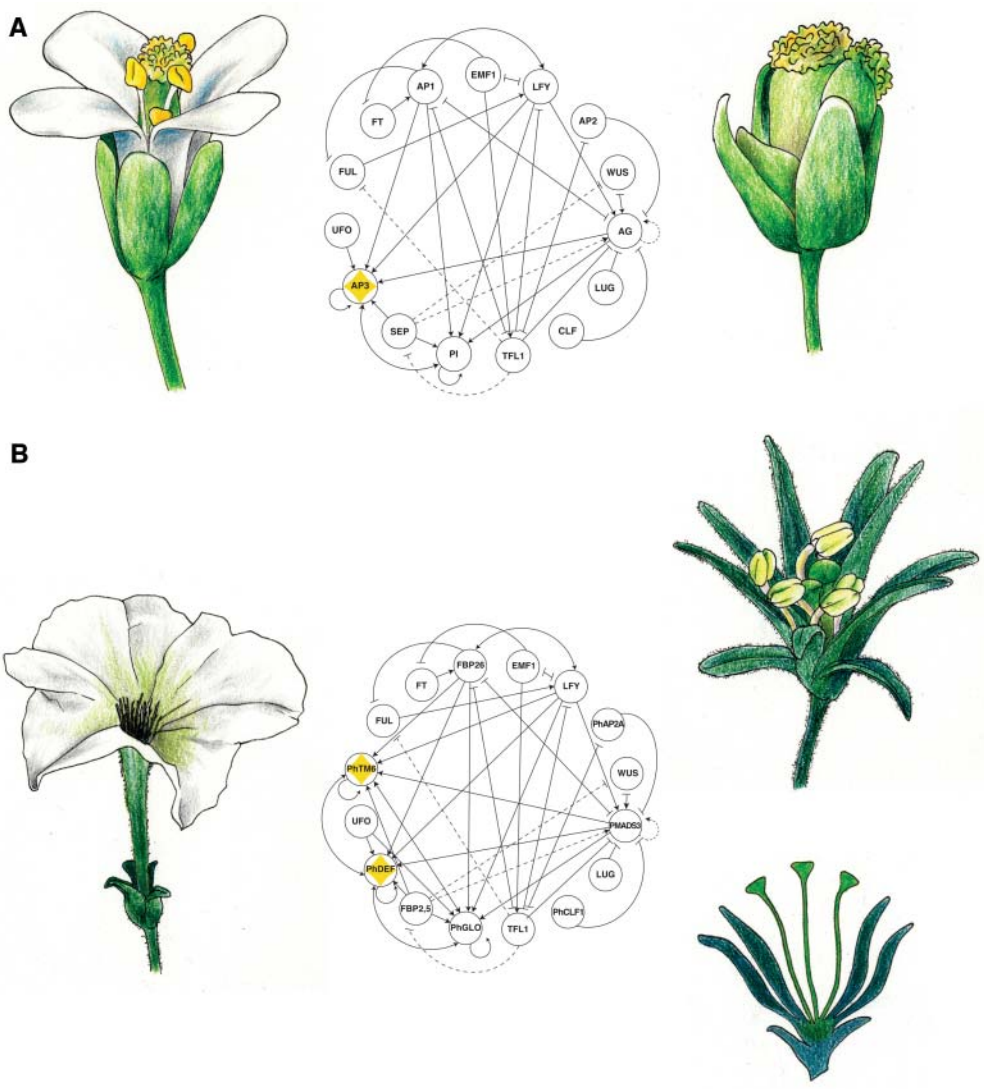

Figure 5. Arabidopsis and Petunia Wild Type (left), *ap3* Mutant (right) Flowers, and Corresponding Network Models.

Single petunia mutant for *PhDEF* is shown in the top part of **(B)** and a scheme of the predicted double mutant for *PhDEF* and *PhTM6* is shown below (see also prediction in Vandenbussche et al., 2004). Arabidopsis is shown in **(A)**. The networks indicate which nodes were turned off (yellow) to simulate mutants. Network architecture as in Figure 4 for Arabidopsis and as in Supplemental Figure 2 online for petunia. Steady states for wild type and mutant simulations found respectively in Tables 1 and 6 for Arabidopsis and Tables 9, 10 (single mutant for *PhDEF* gene), and 11 (double mutant for *PhDEF* and *PhTM6* genes) for petunia. Note that Arabidopsis gene names are used if the corresponding gene has not been characterized in petunia (also in Tables 9 to 11 and Supplemental Figure 2 online). The Arabidopsis orthologs of the cloned petunia genes are as follows: *FLORAL BINDING PROTEIN26* (*FBP26*) is an *AP1* ortholog (Immink et al., 1999), *PhDEF* (formerly known as *GREEN PETALS*) is an *AP3* and *DEFICIENS* (*DEF*; from *A. majus*) ortholog, *PhGLO1* (*FBP1*) and *PhGLO2* (*PETUNIA MADS BOX GENE2*; *pMADS2*) are *PI* and *GLOBOSA* (*GLO*; from *A. majus*) orthologs (Vandenbussche et al., 2004), *pMADS3* is an *AG* ortholog (Kapoor et al., 2002), *PhAP2A* is an *AP2* ortholog (Maes et al., 2001), *FBP2* and *FBP5* are *SEP* orthologs (Ferrario et al., 2003; Vandenbussche et al., 2003), *PhCLF1* and *PhCLF2* are *CLF* orthologs (Mayama et al., 2003), and *PETUNIA HYBRIDA TM6* (*PhTM6*) is a paleo*AP3* gene (Vandenbussche et al., 2004). Drawings are not to scale. Drawings based on photographs from http://www.weigelworld.org (**[A]**, left), http://www.salk.edu/LABS/pbio-w/gallery.html (**[A]**, right), and Van Tunen et al. (1994) (**[B]**, left and top right).

Conclusions

Careful experiments will be fundamental to continue unraveling the mechanistic details in gene transcriptional regulation and translation. Mechanistic details have already proven to be fundamental in being able to precisely understand the molecular nature of biological systems, and they will be critical for future quantitative descriptions and for designing convenient perturbations for clinical or agricultural applications (Hasty et al., 2002). However, the study presented here and other recent modeling approaches for gene control of development (e.g., Mendoza and

Alvarez-Buylla, 2000; Albert and Othmer, 2003) show that qualitative data of key elements of a complex biological system may be sufficient to organize genetic interactions into integrative mechanistic explanations that capture critical aspects of the biological systems' global dynamical behavior.

Moreover, only a formal dynamical approach can be used to explicitly address if a developmental network is canalized to particular steady states of gene activation and robust to perturbations of gene functions or not. These are also relevant issues for biotechnological applications of gene network knowledge but also for studies of evolution of development to explore the role of developmental mechanisms in restricting the range of phenotypic variation that may appear during evolution (Salazar-Ciudad et al., 2000) and the role of natural selection in determining network characteristics (Milo et al., 2002) and phenotypic evolution. Finally, once network architecture is validated for a particular study system, it is easier to make explicit predictions on the modifications that may underlie morphological or developmental mechanisms encountered in other systems, as is the case for petunia shown here.

METHODS

The model is discrete. If N is the number of genes involved in the network, then X_n is a vector with expression state for each gene in a space of N dimensions, representing the network state after n iterations as follows:

$$X_n = (x_1(n), x_2(n), \ldots x_N(n)), \quad (1)$$

where $x_i(n)$ represents the state of expression of the gene i at the iteration n. We then write:

$$X_{n+1} = g(X_n), \quad (2)$$

which indicates that the state at the iteration $(n + 1)$ is determined by the state at the previous iteration.

Each node, except *SEP* (redundant *SEP1*, *SEP2*, and *SEP3* genes), stands for the activity of a single gene involved in floral organ fate determination. Most nodes could assume three levels of expression (on, 1 or 2, and off, 0) to enable different activation thresholds when experimental data was available (Thomas, 1991). The system has a finite number of possible initial conditions equal to 139,968, and each one is represented by a vector of dimension 15 in which each column corresponds to the expression state of each network node at initial conditions in the following order: *FT EMF1 TFL1 LFY FUL AP1 AP3 PI AG UFO WUS AP2 SEP LUG CLF*. The vector of 15 entries that keeps track of the activity level of each node describes the system at each time point. We updated the state of each node synchronously. Starting on each initial condition we iterated the network until it reached an attractor. Thus, we determined the steady gene activation states described in Table 1. All attractors were fixed point attractors (Kauffman, 1993) in which the activity level of all genes remains the same as in the previous iteration. We also kept track of the number of iterations needed for a given initial condition to attain each steady state for future model developments. The set of initial conditions that lead to each of the system's steady states is the basin of attraction of each attractor. The model can be represented by a set of difference equations in which gene interactions are modeled according to logical rules.

Logical rules were grounded on published experimental evidence of gene interactions (see model section above). Expression data can readily be translated into an interaction edge with a particular sign. If a gene's loss-of-function mutation causes ectopic or diminished expression of a second gene, the mutated gene represses or activates the second gene, respectively, and vice versa for a gain-of-function mutation. A similar rationale was used for crosses of promoter:reporter transgenic lines crossed to loss- and gain-of-function mutants. In epistasis analysis, if we have two genes, X and Y, and their corresponding loss of function mutant alleles, x- and y-, when the double mutant (x-y-) has the same phenotype as y-, we say that Y is epistatic over X and that both genes are in the same pathway. If both genes function in the same direction, then we infer a positive regulatory interaction with Y upstream of X. This is the case for *emf1* and *tfl1* mutants, whose effect on the phenotype is not opposite with the other (Chen et al., 1997; see *TFL1* logical rules). However, if the phenotypes of the two gene mutations are opposite, (e.g., *ag lug* double mutants, in which *ag* is epistatic; Liu and Meyerowitz, 1995; see *AG* logical rules), the most parsimonious explanation for epistasis is that the epistatic gene (Y) is repressed by the other one (X) in wild-type plants.

The rationale exemplified above is sufficient to get the sign (inhibition or activation) of regulatory interactions; however, it is not enough to obtain the logical rules. We give here three examples. In the *TFL1* logical rule, we concluded from the epistasis data that if the epistatic gene (*EMF1*) is off, then *TFL1* is off, independently of other input states (first row of the logical rule table for *TFL1*). However, although *EMF1* is needed for *TFL1* activation, it is not sufficient because in plants that overexpress *LFY* with a wild-type *EMF1* allele, no *TFL1* mRNA is detected (Ratcliffe et al., 1999), suggesting that if *LFY* is fully active (*LFY* = 2), then *TFL1* is inactive even if *EMF1* is on. This also occurs in the background of *ap1* loss-of-function mutants, and it is also true for overexpression of *AP1* itself. Hence, we write the second and third rows of the table of logical rules for *TFL1*.

Another example of how we codified the effect of multiple inputs on the output of the target gene is for *AG*. *LUG*, *CLF*, and *AP2* repress *AG* in the two outer floral whorls (Liu and Meyerowitz, 1995; Goodrich et al., 1997; Deyholos and Sieburth, 2000), and *LFY* and *WUS* activate it in the two inner floral whorls (Lenhard et al., 2001; Lohmann et al., 2001). We considered that *WUS* and *LFY* activity is sufficient to overcome *AG* repression by *LUG*, *CLF*, and *AP2* because in *AP3*:*WUS* transgenic plants that express *WUS* in the second floral whorl where *LFY* is also expressed (Ferrándiz et al., 2000), there is ectopic *AG* activity there as well (Lenhard et al., 2001). Hence, we write the third and fourth rows of the table of *AG* logical rules.

Finally, we give an example for *AP3*. This gene was modeled as a three-state variable that reaches its highest threshold of activity (*AP3* = 2) only if *AP3*, *PI*, *SEP1*, *SEP2*, or *SEP3*, and either *AP1* (second row of table of *AP3* logical rules) or *AG* (first *AP3* logical rule) (Honma and Goto, 2001; Pelaz et al., 2001) are active (i.e., their activity must not be equal to 0). If this does not occur, the first threshold is surpassed (*AP3* = 1) only if *UFO* and *LFY* are both active (Parcy et al., 1998; third to sixth rows of table of *AP3* logical rules); otherwise, *AP3* = 0.

In most cases, the logical rules were objectively derived from experimental data. In the few cases that subjective decisions had to be made, we tested if the alternative rules could have significant impacts on the attractors predicted by running the model with the most contrasting rules. The latter are marked with an asterisk in the output value on the rightmost column of the table for the corresponding target node. In all such cases tested, the same attractors and very similar basins of attraction were recovered, suggesting that the different options are equivalent.

We systematically removed each interaction between pairs of network components and obtained the steady states and basins of attraction for each new altered network lacking each of the postulated interactions (see Supplemental Table 1 online). This was achieved by setting to zero the activation state of the input node when the activation level of the target node was being updated. After the updating of the target node, the activation level of the input node was set to its previous level. We simulated loss- and gain-of-function mutants by setting the activation state of the mutated gene to 0 in the first case or 1 or 2 in the second one during the entire simulation.

We performed robustness analyses by testing small changes in the logical rules. We performed random in silico point alterations of the outputs in the tables of logical rules and obtained the ratio of networks that attained steady states that were different to those predicted by the wild-type model and stopped the simulations when this ratio reached a plateau (see Supplemental Figure 1 online). After each alteration, the network was run again for all possible initial conditions to obtain the steady states and basins of attraction, and these were compared with those of the original network. All programs were written in C++ and are available upon request.

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REFERENCES

Albert, R., Jeong, H., and Barabási, A.-L. (2000). Error and attack tolerance of complex networks. Nature **406,** 378–382.

Albert, R., and Othmer, H.G. (2003). The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in *Drosophila melanogaster*. J. Theor. Biol. **223,** 1–18.

Aldana, M., and Cluzel, P. (2003). A natural class of robust networks. Proc. Natl. Acad. Sci. USA **100,** 8710–8714.

Ambrose, B.A., Lerner, D.R., Ciceri, P., Padilla, C.M., Yanofsky, M.F., and Schmidt, R.J. (2000). Molecular and genetic analyses of the *Silky1* gene reveal conservation of floral organ specification between Eudicots and Monocots. Mol. Cell **5,** 569–579.

Aubert, D., Chen, L., Moon, Y., Martin, D., Castle, L.A., Yang, C., and Sung, Z.R. (2001). EMF1, a novel protein involved in the control of shoot architecture and flowering in *Arabidopsis*. Plant Cell **13,** 1865–1875.

Barrio, R., Maini, P.K., Padilla, P., Plaza, R., and Sánchez-Garduño, F. (2004). The effect of growth and curvature on pattern formation. J. Dyn. Differ. Equ., in press.

Bowman, J., Smyth, D., and Meyerowitz, E. (1991). Genetic interactions among floral homeotic genes of *Arabidopsis*. Development **112,** 1–20.

Bowman, J.L., Smyth, D.R., and Meyerowitz, E.M. (1989). Genes directing flower development in Arabidopsis. Plant Cell **1,** 37–52.

Bradley, D., Ratcliffe, O., Vincent, C., Carpenter, R., and Coen, E.S. (1997). Inflorescence commitment and architecture in *Arabidopsis*. Science **275,** 80–83.

Brand, U., Grünewald, M., Hobe, M., and Simon, R. (2002). Regulation of *CLV3* expression by two homeobox genes in *Arabidopsis*. Plant Physiol. **129,** 565–575.

Busch, M.A., Bomblies, K., and Weigel, D. (1999). Activation of a floral homeotic gene in *Arabidopsis*. Science **285,** 585–587.

Chen, L., Cheng, J., Castle, L., and Sung, Z.R. (1997). *EMF* genes regulate Arabidopsis inflorescence development. Plant Cell **9,** 2011–2024.

Coen, E.S., and Meyerowitz, E. (1991). The war of the whorls: Genetic interactions controlling flower development. Nature **353,** 31–37.

Conner, J., and Liu, Z. (2000). *LEUNIG*, a putative transcriptional corepressor that regulates *AGAMOUS* expression during flower development. Proc. Natl. Acad. Sci. USA **97,** 12902–12907.

Coupland, G. (1995). Genetic and environmental control of flowering time in *Arabidopsis*. Trends Genet. **11,** 393–397.

Deyholos, M.K., and Sieburth, L.E. (2000). Separable whorl-specific expression and negative regulation by enhancer elements within the *AGAMOUS* second intron. Plant Cell **12,** 1799–1810.

Eldar, A., Dorfman, R., Weiss, D., Ashe, H., Shilo, B., and Barkai, N. (2002). Robustness of the BMP morphogen gradient in *Drosophila* embryonic patterning. Nature **419,** 304–308.

Ferrándiz, C., Gu, Q., Martienssen, R., and Yanofsky, M.F. (2000). Redundant regulation of meristem identity and plant architecture by *FRUITFULL*, *APETALA1* and *CAULIFLOWER*. Development **127,** 725–734.

Ferrario, S., Immink, R.G., and Angenent, G.C. (2004). Conservation and diversity in flower land. Curr. Opin. Plant Biol. **7,** 84–91.

Ferrario, S., Immink, R.G., Shchennikova, A., Busscher-Lange, J., and Angenent, G.C. (2003). The MADS box gene *FBP2* is required for *SEPALLATA* function in *Petunia*. Plant Cell **14,** 914–925.

Goodrich, J., Puangsomlee, P., Martin, M., Long, D., Meyerowitz, E.M., and Coupland, G. (1997). A Polycomb-group gene regulates homeotic expression in *Arabidopsis*. Nature **386,** 44–51.

Goodwin, B. (2001). How the Leopard Changed Its Spots: The Evolution of Complexity, 2nd ed. (Princeton, NJ: Princeton Science Library).

Gustafson-Brown, C., Savidge, B., and Yanofsky, M.F. (1994). Regulation of the *Arabidopsis* floral homeotic gene *AP1*. Cell **76,** 131–143.

Haecker, A., and Laux, T. (2001). Cell-cell signaling in the shoot meristem. Curr. Opin. Plant Biol. **4,** 441–446.

Hartwell, L., Hopfield, J.J., Leibler, S., and Murray, A.W. (1999). From molecular to modular cell biology. Nature **402,** C47–C52.

Hasty, J., McMillen, D., and Collins, J.J. (2002). Engineered gene circuits. Nature **420,** 224–230.

Haung, M., and Yang, C. (1998). *EMF* genes interact with late-flowering genes to regulate *Arabidopsis* shoot development. Plant Cell Physiol. **39,** 382–393.

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.

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.

Immink, R.G.H., Hannapel, D.J., Ferrario, S., Busscher, M., Franken, J., Lookeren Campagne, M.M., and Angenent, G.C. (1999). A *Petunia* MADS box gene involved in the transition from vegetative to reproductive development. Development **126,** 5117–5126.

Jeong, H., Tombor, B., Albert, R., Oltvai, Z.N., and Barabási, A.-L. (2000). The large scale organization of metabolic networks. Nature **407,** 651–654.

Jofuku, K.D., den Boer, B.G.W., Van Montagu, M., and Okamuro, J.K. (1994). Control of *Arabidopsis* flower and seed development by the homeotic gene *APETALA2*. Plant Cell **6,** 1211–1225.

Jönsson, H., Shapiro, B.E., Meyerowitz, E.M., and Mjolsness, E. (2003). Signaling in multicellular models of plant development. In On Growth, Form, and Computers, S. Kumar and P.J. Bentley, eds (London: Academic Press).

Kapoor, M., Tsuda, S., Tanaka, Y., Mayaman, T.M., Okuyama, Y., Tsuchimoto, S., and Takatsuji, H. (2002). Role of *Petunia pMADS3* in determination of floral organ and meristem identity, as revealed by its loss of function. Plant J. **32,** 115–127.

Kardailsky, I., Shukla, V.K., Ahn, J.H., Dagenais, N., Christensen, S.K., Nguyen, J.T., Chory, J., Harrison, M.J., and Weigel, D. (1999). Activation tagging of the floral inducer *FT*. Science **286,** 1962–1965.

Kauffman, S.A. (1969). Metabolic stability and epigenesis in randomly constructed genetic nets. J. Theor. Biol. **22,** 437–467.

Kauffman, S.A. (1993). The Origins of Order: Self-Organization and Selection in Evolution. (Oxford: Oxford University Press).

Kobayashi, Y., Kaya, H., Goto, K., Iwabuchi, M., and Araki, T. (1999). A pair of related genes with antagonistic roles in mediating flowering signals. Science **286,** 1960–1962.

Koornneef, M., Alonso-Blanco, C., Blankestijn-de Vries, H., Hanhart, C.J., and Peters, A.J.M. (1998). Genetic interactions among late-flowering mutants of *Arabidopsis*. Genetics **148,** 885–892.

Krakauer, D.C., Page, K.M., and Sealfon, S. (2002). Module dynamics of the GnRH signal transduction network. J. Theor. Biol. **218,** 457–470.

Krizek, B.A., and Meyerowitz, E.M. (1996). The *Arabidopsis* homeotic genes *APETALA3* and *PISTILLATA* are sufficient to provide the B class organ identity function. Development **122,** 11–22.

Laux, T., Mayer, K.F.X., Berger, J., and Jurgens, G. (1996). The *WUSCHEL* gene is required for shoot and floral meristem integrity *Arabidopsis*. Development **122,** 87–96.

Lenhard, M., Bonhert, A., Jurgens, G., and Laux, T. (2001). Termination of stem cell maintenance in *Arabidopsis* floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. Cell **105,** 805–808.

Liljegren, S.J., Gustafson-Brown, C., Pinyopich, A., Ditta, G.S., and Yanofsky, M.F. (1999). Interactions among *AP1*, *LFY*, and *TFL1* specify meristem fate. Plant Cell **11,** 1007–1018.

Liu, Z., and Meyerowitz, E.M. (1995). *LEUNIG* regulates *AGAMOUS* expression in *Arabidopsis* flowers. Development **121,** 975–991.

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.

Maes, T., Van de Steene, N., Zethof, J., Karimi, M., D'Hauw, M., Mares, G., Van Montagu, M., and Gerats, T. (2001). *Petunia AP2*-like genes and their role in flower and seed development. Plant Cell **13,** 229–244.

Mandel, A., and Yanofsky, M. (1995a). A gene triggering flower formation in *Arabidopsis*. Nature **377,** 522–525.

Mandel, A., and Yanofsky, M. (1995b). The *Arabidopsis AGL8* MADS-box gene is expressed in inflorescence meristems and negatively regulated by *APETALA1*. Plant Cell **7,** 1763–1771.

Mandel, A., and Yanofsky, M. (1998). The *Arabidopsis AGL9* MADS box gene is expressed in young flower primordia. Sex. Plant Reprod. **11,** 22–28.

Mayama, T., Ohtsubo, E., and Tsuchimoto, S. (2003). Isolation and expression analysis of *Petunia CURLY LEAF*-like genes. Plant Cell Physiol. **44,** 811–819.

Mayer, K.F.X., Schoof, H., Haecker, A., Lenhard, M., Jürgens, G., and Laux, T. (1998). Role of *WUSCHEL* in regulating stem cell fate in the *Arabidopsis* shoot meristem. Cell **95,** 805–815.

McAdams, H.H., and Shapiro, L. (1995). Circuit simulation of genetic networks. Science **269,** 650–656.

Mendoza, L., and Alvarez-Buylla, E.R. (1998). Dynamics of the genetic regulatory network of *Arabidopsis thaliana* flower morphogenesis. J. Theor. Biol. **193,** 307–319.

Mendoza, L., and Alvarez-Buylla, E.R. (2000). Genetic regulation of root hair development in *Arabidopsis thaliana*: A network model. J. Theor. Biol. **204,** 311–326.

Mendoza, L., Thieffry, D., and Alvarez-Buylla, E.R. (1999). Genetic control of flower morphogenesis in *Arabidopsis thaliana*: A logical analysis. Bioinformatics **15,** 593–606.

Meyer, V.G. (1966). Floral abnormalities. Bot. Rev. **32,** 165–195.

Michaels, S.D., and Amasino, M. (1999). *Flowering Locus C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell **11,** 949–956.

Milo, R., Shen-Orr, S., Itzkovitz, S., Kashtan, N., Chklovskii, D., and Alon, U. (2002). Network motifs: Simple building blocks of complex networks. Science **298,** 824–827.

Mizukami, Y., and Ma, H. (1997). Determination of *Arabidopsis* floral meristem identity by *AGAMOUS*. Plant Cell **9,** 393–408.

Mjolsness, E., Sharp, D.H., and Reinitz, J. (1991). A connectionist model of development. J. Theor. Biol. **152,** 429–453.

Monod, J., and Jacob, F. (1961). General conclusions: Telenomic mechanisms in cellular metabolism, growth, and differentiation. Cold Spring Harb. Symp. Quant. Biol. **26,** 389–401.

Murray, J.D. (1989). Mathematical Biology. (Berlin: Springer-Verlag).

Parcy, F., Nilsson, O., Busch, M.A., Lee, I., and Weigel, D. (1998). A genetic framework for floral patterning. Nature **395,** 561–566.

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., Tapia-López, R., Alvarez-Buylla, E.R., and Yanofsky, M.F. (2001). Conversion of leaves into petals in *Arabidopsis*. Curr. Biol. **11,** 182–184.

Piñeiro, M., and Coupland, G. (1998). The control of flowering time and floral identity in *Arabidopsis*. Plant Physiol. **117,** 1–8.

Ratcliffe, O.J., Bradley, D.J., and Coen, E.S. (1999). Separation of shoot and floral identity in *Arabidopsis*. Development **126,** 1109–1120.

Rudall, P. (1987). Anatomy of Flowering Plants: An Introduction to Structure and Development. (London: Edward Arnold).

Ruiz-García, L., Madueño, F., Wilkinson, M., Haughn, G., Salinas, J., and Martínez-Zapater, J.M. (1997). Different roles of flowering-time genes in the activation of floral initiation genes in *Arabidopsis*. Plant Cell **9,** 1921–1934.

Salazar-Ciudad, I., Garcia-Fernandez, J., and Solé, R.V. (2000). Gene networks capable of pattern formation: From induction to reaction-diffusion. J. Theor. Biol. **205,** 587–603.

Salazar-Ciudad, I., and Jernvall, J. (2002). A gene network model accounting for development and evolution of mammalian teeth. Proc. Natl. Acad. Sci. USA **99,** 8116–8120.

Samach, A., Klenz, J.E., Kohalmi, S.E., Risseeuw, E., Haughn, G.W., and Crosby, W.L. (1999). The *UNUSUAL FLORAL ORGANS* gene of *Arabidopsis thaliana* is an F-box protein required for normal patterning and growth in the floral meristem. Plant J. **20,** 433–445.

Samach, A., Onouchi, H., Gold, S.E., Ditta, G.S., Schwarz-Sommer, Z., Yanofsky, M.F., and Coupland, G. (2000). Distinct roles of *CONSTANS* target genes in reproductive development of *Arabidopsis*. Science **288,** 1613–1616.

Savidge, B., Rounsley, S.D., and Yanofsky, M.F. (1995). Temporal relationships between the transcription of two *Arabidopsis* MADS-box genes and the floral organ identity genes. Plant Cell **7,** 721–733.

Schultz, E.A., and Haughn, G.W. (1993). Genetic analyses of the floral initiation process (FLIP) in *Arabidopsis*. Development **119,** 745–765.

Shannon, S., and Meeks-Wagner, D.R. (1993). Genetic interactions that regulate inflorescence development in *Arabidopsis*. Plant Cell **5,** 639–655.

Simon, R., Igeno, M.I., and Coupland, G. (1996). Activation of floral meristem identity genes in *Arabidopsis*. Nature **384,** 59–62.

Simpson, G., Gendall, A., and Dean, C. (1999). When to switch to flowering. Annu. Rev. Cell Dev. Biol. **99,** 519–550.

Thomas, R. (1991). Regulatory networks seen as asynchronous automata: A logical description. J. Theor. Biol. **153,** 1–23.

Vandenbussche, M., Zethof, J., Royaert, S., Weterings, K., and Gerats, T. (2004). The duplicated B-class heterodimer model: Whorl-specific effects and complex genetic interactions in *Petunia hybrida* flower development. Plant Cell **16,** 741–754.

Vandenbussche, M., Zethof, J., Souer, E., Koes, R., Tornielli, G.B., Pezzotti, M., Ferrario, S., Angenent, G.C., and Gerats, T. (2003). Toward the analysis of the *Petunia* MADS box gene family by reverse and forward transposon insertion mutagenesis approaches: B, C, and D floral organ identity functions require *SEPALLATA*-like MADS box genes in *Petunia*. Plant Cell **15,** 2680–2693.

Van Tunen, A.J., Busscher, M., Colombo, L., Franken, J., and Angenent, G.C. (1994). Molecular control of floral organogenesis and plant reproduction in *Petunia hybrida*. In Molecular and Cellular Aspects of Plant Reproduction, R.J. Scott and A.D. Stead, eds (Cambridge, UK: Cambridge University Press), pp. 9–16.

von Dassow, G., Meir, E., Munro, E.M., and Odell, G.M. (2000). The segment polarity network is a robust developmental module. Nature **406,** 188–193.

Wagner, A. (2001). The yeast protein interaction network evolves rapidly and contains few redundant duplicate genes. Mol. Biol. Evol. **18,** 1283–1292.

Wagner, D., Sablowski, R.W.M., and Meyerowitz, E.M. (1999). Transcriptional activation of *APETALA1* by *LEAFY*. Science **285,** 582–584.

Wagner, G.P. (1996). Homologues, natural kinds and the evolution of modularity. Am. Zool. **36,** 36–43.

Weigel, D., and Meyerowitz, E.M. (1993). Activation of floral homeotic genes in *Arabidopsis*. Science **261,** 1723–1726.

Weigel, D., and Nilsson, O. (1995). A developmental switch sufficient for flower initiation in diverse plants. Nature **377,** 495–500.

Winter, K.U., Becker, A., Münster, T., Kim, J.T., Saedler, H., and Theissen, G. (1999). MADS-box genes reveal that genetophytes are more closely related to conifers than to flowering plants. Proc. Natl. Acad. Sci. USA **96,** 7342–7347.

Yang, C., Chen, L., and Sung, Z.R. (1995). Genetic regulation of shoot development in *Arabidopsis*: Role of the *EMF* genes. Dev. Biol. **169,** 421–435.