

Simple network motifs can capture key characteristics of the floral transition in *Arabidopsis*

Nick Pullen¹, Katja E Jaeger², Philip A Wigge², and Richard J Morris^{1,*}

¹Computational and Systems Biology; John Innes Centre; Norwich Research Park; Norwich UK; ²Sainsbury Laboratory; Cambridge University; Cambridge UK

Keywords: flowering time, floral transition, *Arabidopsis*, network motifs, mathematical modelling

Abbreviations: FT, Flowering Locus T; FD, Flowering Locus D; TFL1, Terminal Flower 1; API, Apetala 1; LFY, Leafy; ODE, Ordinary differential equation

*Correspondence to: Richard J Morris;
Email: richard.morris@jic.ac.uk

Submitted: 06/26/2013

Accepted: 08/14/2013

Citation: Pullen N, Jaeger KE, Wigge PA, Morris RJ. Simple network motifs can capture key characteristics of the floral transition in *Arabidopsis*. *Plant Signaling & Behavior* 2013; 8:e26149; PMID: 23989666; <http://dx.doi.org/10.4161/psb.26149>

Addendum to: Jaeger KE, Pullen N, Lamzin S, Morris RJ, Wigge PA. Interlocking feedback loops govern the dynamic behavior of the floral transition in *Arabidopsis*. *Plant Cell* 2013; 25:820–33; PMID:23543784; <http://dx.doi.org/10.1105/tpc.113.109355>

The floral transition is a key decision during plant development. While different species have evolved diverse pathways to respond to different environmental cues to flower in the correct season, key properties such as irreversibility and robustness to fluctuating signals appear to be conserved. We have used mathematical modeling to demonstrate how minimal regulatory networks of core components are sufficient to capture these behaviors. Simplified models inevitably miss finer details of the biological system, yet they provide a tractable route to understanding the overall system behavior. We combined models with experimental data to qualitatively reproduce characteristics of the floral transition and to quantitatively scale the network to fit with available leaf numbers. Our study highlights the value of pursuing an iterative approach combining modeling with experimental work to capture key features of complex systems.

Global approaches to understand the regulatory transcriptional network involved in controlling the floral transition have revealed that hundreds of transcripts are specifically affected in their expression in the apex upon floral induction.^{1–4} Modeling such large genetic regulatory networks is a challenging task as the determination of all relevant parameters is rarely experimentally feasible. The limited knowledge of component concentrations and kinetic interactions can result in a mathematically highly underdetermined problem. This means that the available data are not sufficient to uniquely determine the underlying parameters in the model, something that with typical biological data and models is rarely achieved.⁵ Methods have been

developed, such as Boolean networks or systems identification, which simplify the description, thereby reducing the numbers of parameters. Boolean networks aim to capture the underlying biology in that the gene network structure is maintained but the complexity of the interactions is reduced, whereas systems identification (“black box”) focuses on capturing the overall behavior, typically employing small systems of linear equations that do not map well onto the underlying biological mechanisms.^{6,7} Another approach is to reduce the gene network while preserving its core structure. Following this strategy,⁸ we sought to simplify the large regulatory network that controls flowering down to a core set of regulatory activities.⁹ As shown in Jaeger et al. (2013), a fairly simple network of core flowering time hubs¹⁰ is able to capture important characteristics of the floral transition.⁸ We approximated the effect of the various regulatory pathways that govern the floral transition by assuming they converge on the key regulator of flowering in higher plants, *FT*.^{11–15} *FT* expression increases under inductive conditions, and together with the FD transcription factor,^{16,17} *FT* activates key floral meristem identity genes such as *API*. For a number of species, homologs of the *Arabidopsis* master regulator *FT* are a core element of the photoperiod pathway.^{18,19} We use *API* hub levels as a proxy for the flowering state. Rosette leaves, cauline leaves, or flowers are produced based on the levels of *API* in the model. Increasing *FT* signals promote flowering time, noisy input signals are filtered out, and once initiated, the transition is irreversible. Although the degree to which this behavior manifests itself is very

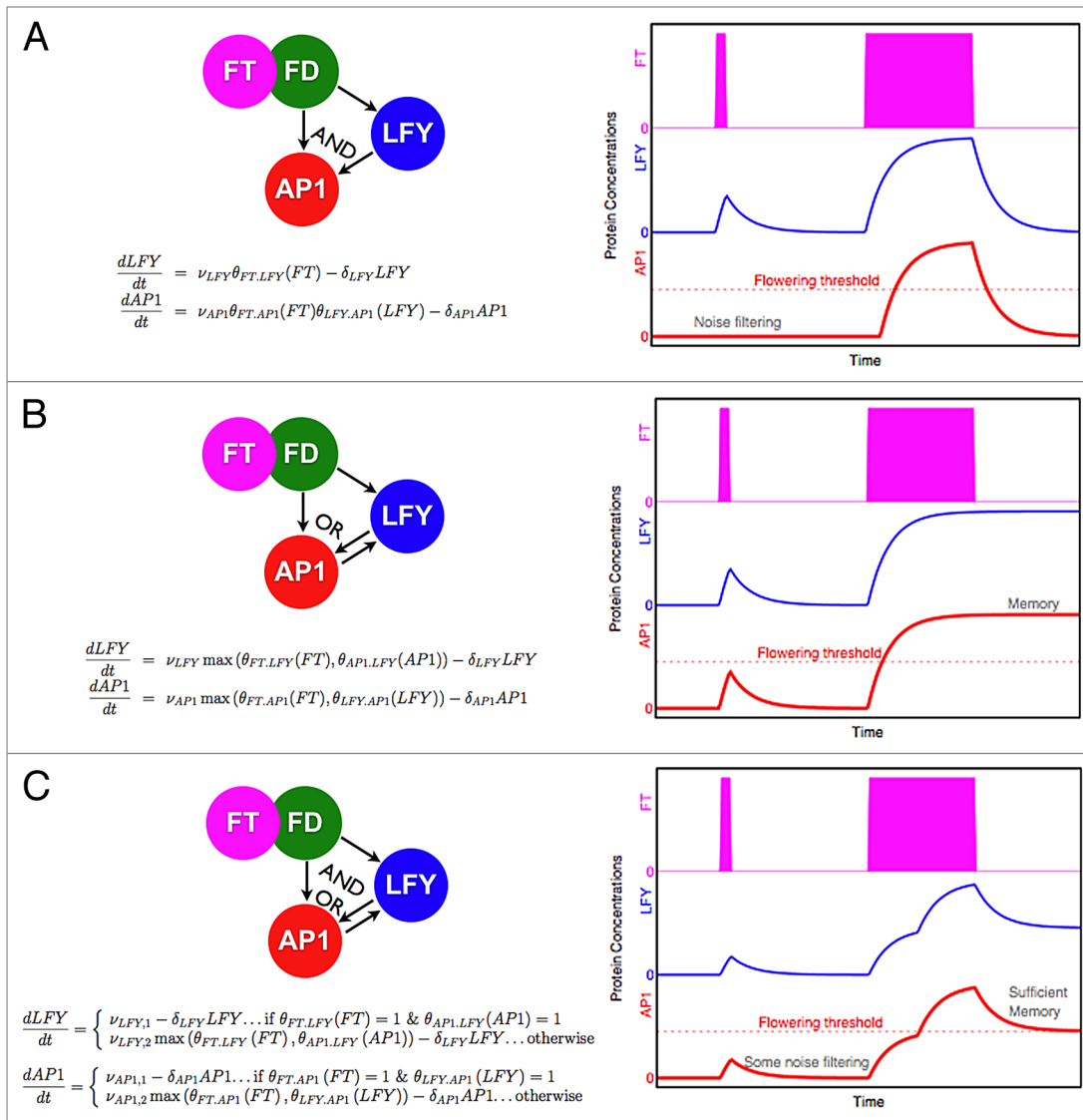


Figure 1. Simple network motifs can capture characteristics of the floral transition. On the left hand side of the figure, 3 simple networks are shown. The nodes consist of the complex FT with FD, and the proteins LFY and AP1. On the right hand side, we show the responses of LFY (blue) and AP1 (red) to a short and a long incoming FT signal (magenta). The model uses a set of ordinary differential equations to describe the dynamic behavior of the system. We used step functions for the transcriptional activation of genes and AND, OR, and AND/OR gating, depending on the network. In **(A)** a coherent feed-forward loop²⁰ using an AND gate at AP1 is shown. This network motif has been described previously^{20,26} and has been shown to exhibit noise filtering properties for short bursts of the incoming signal that are below the delay time through the different routes in the pathway. In **(B)** we show a regulated feed-forward loop with an OR gate at AP1. Once LFY reaches a concentration level that can activate AP1, this interaction is sufficient to maintain AP1 production even in the absence of the incoming signal FT. The network therefore shows a memory effect and irreversibility.²⁶ In **(C)** we combine the key features of both networks. The logic gating uses OR for transcriptional activation at a reduced level but requires AND for maximal activation. This gives rise to compromised characteristics for the individual properties but through the introduction of a flowering threshold for AP1 it is possible to capture a level of robustness to noise and partial memory that, depending on the threshold choice and parameters of the model, can give rise to irreversibility. These networks are reductions of the simple network presented in Jaeger et al. (2013) that included additional nodes with connections and Hill type gene activation.¹⁰ The ordinary differential equations governing the behavior of LFY and AP1 are given below the network motifs. All initial conditions are 0. The FT signal is modeled as a step function active at time points given in the supplement. An IPython notebook²⁷ to enable full reproducibility of this work can be found as supplemental material and is also available from Nick Pullen (nick.pullen@jic.ac.uk).

much parameter dependent, at a qualitative level the model is consistent with the experimental observations. Furthermore,

the model could be scaled to available leaf number data for a number of mutant genotypes. The modeling suggested how *TFL1*

and an IPython notebook.²⁷ As the correct timing of the floral transition is crucial, it is

signals contribute to the molecular basis for a non-flowering phenotype that has not been understood until now. For an extended network we found that, for some parameter values, initial levels of *LFY* and *TFL1* seem to control the final determinacy of the cell. Cells with higher initial *TFL1* levels are able to repress *LFY*, and thus also *API*, to remain in a vegetative state, or high initial *LFY* levels can cause a floral fate as specified by *API* levels.

Here we demonstrate how 2 important properties of the floral transition, namely noise-filtering and irreversibility, can be implemented by 3-node networks in feed-forward loops. The coherent feed-forward loop is a network motif that is commonly found in signaling networks.²⁰ As a major floral pathway integrator, we have placed the active *FT/FD* complex^{16,17} at the start of the transcriptional feed-forward loop, upregulating another integrator, the *LFY* hub (which includes the functional effect of *SOC1*), which both activate the floral initiator hub *API*,^{21,22} (Fig. 1A). If the joint regulation is with AND logic, this simple network has persistence detection and thus is able to be used as a noise filter that removes small blips in a signal.²⁰ The equations are shown in Figure 1 and explained in the supplemental information along with the parameters

important that the system is not incorrectly activated by noise. Another similar network, called regulated feedback, that uses an OR gating can exhibit irreversibility. With the same 3-node set up, an extra activating connection between the two targets, *LFY* and *API*, of the first transcription factor, *FT/FD*, will result in the targets being stably on once activated, (Fig. 1B). *API* mutually activates *LFY* in a positive feedback loop, thus creating the important memory element that is responsible for irreversibility of a plant committed to flowering. So while these simple network motifs capture separate characteristics of the floral transition, they are a crude approximation to the larger system.^{23,24} Both some level of noise filtering and irreversibility as well as the qualitative fit to *ft-10* and *lfy-12* mutants can be achieved by introducing 2 transcription rates,¹⁰ a low rate that can be activated by either *FT* or *LFY* and a higher rate that requires the presence of both *FT* and *LFY*, (Fig. 1C).

In summary, we have sought to show how simple regulatory networks can capture important properties of the floral transition. Genes with similar effects can be grouped into distinct hubs (denoted by underlining) or functional modules.²⁵ Such a reduced network that represents the core structure underlying the floral transition can be mapped to the simple feed-forward loops discussed above. Such a simple 3-node system, as presented here, can give intuitive understanding to a complex biological system. We point out that even for these simple networks, the available data was not sufficient to provide good estimates of the parameters. However, predictions¹⁰ can be made without precise knowledge of all the parameters.⁵ Adding further hubs to this network, for example including the floral repressor *TFL1*, is relatively straightforward.¹⁰ To begin modeling a new pathway, looking for the basic properties of simple networks that exhibit the desired behavior may be a good first step. As with all simplifications, this approach inevitably cannot account for the full spectrum of interacting pathways and variables seen in nature, but it may be a useful entry point for an iterative modeling-experimental cycle.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by the European Research Council, the Biotechnology and Biological Science Research Council (BB/J004553/1), and the John Innes Foundation.

Supplemental Materials

Supplemental materials may be found here: <http://www.landesbioscience.com/journals/psb/article/26149/>

References

- Simpson GG, Dean C. *Arabidopsis*, the Rosetta stone of flowering time? *Science* 2002; 296:285-9; PMID:11951029; <http://dx.doi.org/10.1126/science.296.5566.285>
- Wilczek AM, Roe JL, Knapp MC, Cooper MD, Lopez-Gallego C, Martin LJ, Muir CD, Sim S, Walker A, Anderson J, et al. Effects of genetic perturbation on seasonal life history plasticity. *Science* 2009; 323:930-4; PMID:19150810; <http://dx.doi.org/10.1126/science.1165826>
- Wellmer F, Alves-Ferreira M, Dubois A, Riechmann JL, Meyerowitz EM. Genome-wide analysis of gene expression during early *Arabidopsis* flower development. *PLoS Genet* 2006; 2:e117; PMID:16789830; <http://dx.doi.org/10.1371/journal.pgen.0020117>
- Schmid M, Uhlenhaut NH, Godard F, Demar M, Bressan R, Weigel D, Lohmann JU. Dissection of floral induction pathways using global expression analysis. *Development* 2003; 130:6001-12; PMID:14573523; <http://dx.doi.org/10.1242/dev.00842>
- Gutenkunst RN, Waterfall JJ, Casey FP, Brown KS, Myers CR, Sethna JP. Universally sloppy parameter sensitivities in systems biology models. *PLoS Comput Biol* 2007; 3:1871-8; PMID:17922568; <http://dx.doi.org/10.1371/journal.pcbi.0030189>
- Bornholdt S. Boolean network models of cellular regulation: prospects and limitations. *J R Soc Interface* 2008; 5(Suppl 1):S85-94; PMID:18508746; <http://dx.doi.org/10.1098/rsif.2008.0132.focus>
- Dalchau N. Understanding biological timing using mechanistic and black-box models. *New Phytol* 2012; 193:852-8; PMID:22212235; <http://dx.doi.org/10.1111/j.1469-8137.2011.04004.x>
- Thornley JH. A model for a biochemical switch, and its application to flower initiation. *Ann Bot (Lond)* 1972; 36:861-71
- Salazar JD, Saithong T, Brown PE, Foreman J, Locke JC, Halliday KJ, Carré IA, Rand DA, Millar AJ. Prediction of photoperiodic regulators from quantitative gene circuit models. *Cell* 2009; 139:1170-9; PMID:20005809; <http://dx.doi.org/10.1016/j.cell.2009.11.029>
- Jaeger KE, Pullen N, Lamzin S, Morris RJ, Wigge PA. Interlocking feedback loops govern the dynamic behavior of the floral transition in *Arabidopsis*. *Plant Cell* 2013; 25:820-33; PMID:23543784; <http://dx.doi.org/10.1105/tpc.113.109355>
- Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T. A pair of related genes with antagonistic roles in mediating flowering signals. *Science* 1999; 286:1960-2; PMID:10583960; <http://dx.doi.org/10.1126/science.286.5446.1960>
- Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D. Activation tagging of the floral inducer *FT*. *Science* 1999; 286:1962-5; PMID:10583961; <http://dx.doi.org/10.1126/science.286.5446.1962>

- Jaeger KE, Wigge PA. *FT* protein acts as a long-range signal in *Arabidopsis*. *Curr Biol* 2007; 17:1050-4; PMID:17540569; <http://dx.doi.org/10.1016/j.cub.2007.05.008>
- Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, et al. *FT* protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science* 2007; 316:1030-3; PMID:17446353; <http://dx.doi.org/10.1126/science.1141752>
- Mathieu J, Warthmann N, Küttner F, Schmid M. Export of *FT* protein from phloem companion cells is sufficient for floral induction in *Arabidopsis*. *Curr Biol* 2007; 17:1055-60; PMID:17540570; <http://dx.doi.org/10.1016/j.cub.2007.05.009>
- Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D. Integration of spatial and temporal information during floral induction in *Arabidopsis*. *Science* 2005; 309:1056-9; PMID:16099980; <http://dx.doi.org/10.1126/science.1114358>
- Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T. *FD*, a bZIP protein mediating signals from the floral pathway integrator *FT* at the shoot apex. *Science* 2005; 309:1052-6; PMID:16099979; <http://dx.doi.org/10.1126/science.1115983>
- Higgins JA, Bailey PC, Laurie DA. Comparative genomics of flowering time pathways using *Brachypodium distachyon* as a model for the temperate grasses. *PLoS One* 2010; 5:e10065; PMID:20419097; <http://dx.doi.org/10.1371/journal.pone.0010065>
- Ratcliffe OJ, Amaya I, Vincent CA, Rothstein S, Carpenter R, Coen ES, Bradley DJ. A common mechanism controls the life cycle and architecture of plants. *Development* 1998; 125:1609-15; PMID:9521899
- Mangan S, Alon U. Structure and function of the feed-forward loop network motif. *Proc Natl Acad Sci U S A* 2003; 100:11980-5; PMID:14530388; <http://dx.doi.org/10.1073/pnas.2133841100>
- Weigel D, Alvarez J, Smyth DR, Yanofsky MF, Meyerowitz EM. *LEAFY* controls floral meristem identity in *Arabidopsis*. *Cell* 1992; 69:843-59; PMID:1350515; [http://dx.doi.org/10.1016/0092-8674\(92\)90295-N](http://dx.doi.org/10.1016/0092-8674(92)90295-N)
- Blázquez MA, Soowal LN, Lee I, Weigel D. *LEAFY* expression and flower initiation in *Arabidopsis*. *Development* 1997; 124:3835-44; PMID:9367439
- Welch SM, Roe JL, Dong ZH. A genetic neural network model of flowering time control in *Arabidopsis thaliana*. *Agron J* 2003; 95:71-81; <http://dx.doi.org/10.2134/agronj2003.0071>
- Espinosa-Soto C, Padilla-Longoria P, Alvarez-Buylla ER. 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* 2004; 16:2923-39; PMID:15486106; <http://dx.doi.org/10.1105/tpc.104.021725>
- Milo R, Shen-Orr S, Itzkovitz S, Kashtan N, Chklovskii D, Alon U. Network motifs: simple building blocks of complex networks. *Science* 2002; 298:824-7; PMID:12399590; <http://dx.doi.org/10.1126/science.298.5594.824>
- Alon U. An introduction to systems biology: design principles of biological circuits. Boca Raton: Chapman & Hall/CRC, 2007.
- Pérez F, Granger BE. Ipython: a system for interactive scientific computing. *Comput Sci Eng* 2007; 9:21-9; <http://dx.doi.org/10.1109/MCSE.2007.53>; <http://ipython.org>.