Qualitative Modelling of Genetic Networks: From Logical Regulatory Graphs to Standard Petri Nets

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Abstract. In this paper, a systematic rewriting of logical genetic regulatory graphs in terms of standard Petri net models is proposed. We show that, in the Boolean case, the combination of the logical approach with the standard Petri net framework enables the analysis of isolated regulatory circuits, confirming their most fundamental dynamical properties. Furthermore, two more realistic applications are also presented, the first dealing with the control of the early cell cycles in the developing fly, the second dealing with flower morphogenesis.
The combination of logical and Petri net formalisms open new prospects for the delineation of specific relationships between the feedback structure and the dynamical properties of complex regulatory systems. Moreover, this approach should ease the definition of integrated models of networks encompassing various kinds of interactions: genetic or metabolic regulations, signal transduction cascades...
Keywords: regulatory graphs, gene regulation, discrete dynamics, qualitative analysis.

1 Introduction

Regulatory networks are found at the core of all biological functions, from biochemical pathways, to gene regulation, and cell communication processes. Their complexity often defies the intuition of the biologist and calls for the development of proper mathematical methods to model their structure and simulate their dynamical behaviour (for a recent review, see [3]). A large variety of formal approaches has already been applied to biological regulatory networks, from ordinary or partial differential systems, to sets of stochastic equations. However, the lack of precise, quantitative information about the shape of regulatory functions or about the values of involved parameters pleads for the development of qualitative approaches.

One qualitative approach consists in modelling regulatory networks in terms of logical equations (using either Boolean or multi-level discretisation) [4, 15]. The development of logical models for various biological networks has already led to interesting insight in network structures (in particular, regulatory feedback circuits) and the corresponding dynamical properties [16]. Relying on the **generalised logical approach** of R. Thomas, we have recently developed a software tool, GIN-sim, which enables the biologist to specify a regulatory model and check the qualitative temporal evolution of the system for given initial states [1]. However, as the number of qualitative (*i.e.* logical or discrete) states grows exponentially with the number of elements involved in the regulatory network, there is a pressing need for proper analytical approaches to cope with the rapid delineation of larger regulatory networks. In this respect, in a recent study, we have derived a series of analytical results concerning properties of the dynamics in the case of isolated regulatory circuits with arbitrary numbers of elements [13].

The Petri net (PN) formalism offers another, complementary framework to deal with the analysis of the dynamical properties of large systems, either from a qualitative or a quantitative point of view. Indeed, Petri nets have already been applied to various types of biological networks. In particular, metabolic networks, which are endowed with conservation laws, can be relatively easily represented into the PN framework [12, 6, 7]. Several applications of PN to genetic regulatory networks can also be found in the literature [5, 9]. However, these applications largely rely on simulations and consequently provide limited insights in the general properties of biological regulatory networks. Moreover, previous PN models of genetic networks largely rely on sophisticated (application-driven) representations rather than on the definition of a systematic method to represent genetic regulatory networks into standard PN.

At this stage, it should be interesting to articulate the logical generalised approach with the PN formalism, in order to combine the delineation of the dynamical roles of specific feedback structures, with the algebraic tools underlying the PN framework for the study of fundamental dynamical properties. In this paper, we propose a rigourous and systematic rewriting of logical regulatory models into specific PN, focusing on the Boolean case.

In the following section, the notion of logical regulatory graph is introduced. Next, we define the corresponding Petri net and prove some basic general properties. The case of isolated regulatory circuits is then analysed in more details, in order to illustrate the fact that their PN counterparts also enable the delineation of the fundamental properties of such circuits. Afterwards, referring to published logical models, we derive the Petri nets corresponding to two biological regulatory networks, the first involved in the control of the cell cycle during the early stages of *Drosophila melanogaster* development, the second involved in the control of flower morphogenesis in *Arabidopsis thaliana*. Finally, conclusions and prospects are proposed.

2 Logical Regulatory Graphs

In this section, we briefly describe regulatory graphs in the **Boolean** case, *i.e.* when the expression state of each gene takes its value in $\{0, 1\}$ (0 when the level of the regulatory product is negligible, 1 when the regulatory product is present at a "sufficient" level). For more details, see [1] where the formalism is described in the general multi-valued case.

A **regulatory graph** is a labelled directed graph which represents interactions between genes. Each interaction is oriented, and involves two genes: the source and the target. An interaction is said to be **operating** whenever the level of expression (or activity) of the source is sufficient (*i.e.* at level 1). An interaction is called an **activation** when its effect on the targeted gene tends to be positive, *i.e* to an increase of the level of expression of the target. It is called a **repression** (or inhibition) when its effect on the targeted gene tends to be negative *i.e* to a decrease of the level of expression. Note, however, that effective activatory or inhibitory effects generally depend on the presence or the absence of cofactors. Indeed, one gene can be the target of several interactions. For each gene g_j, we define the subset $\mathcal{I}(j)$, called *input of* g_j and containing the source genes of all incoming interactions on g_j. Note that as we consider here the Boolean case, there are no multi-arcs between genes and therefore the interactions are fully defined by their sources and targets. When the expression levels of the genes are given, we know which interactions are operating, and we represent their global effects through **logical parameters** defined as follows.

For each gene g_j, the application K_j, called **logical function**, associates a **parameter** $K_j(X)$ to each subset X of $\mathcal{I}(j)$. The value of this parameter defines the level to which g_j tends when X is the set of operating incoming interactions. As we consider here the Boolean case, these functions take their values in $\{0, 1\}$. Thus, for each gene, the corresponding logical function allows the qualitative specification of the effects of any combination of incoming interactions.

Consequently, we can describe a regulatory graph by three components:

- a set of nodes $\mathcal{G} = \{g_1, \ldots, g_n\}$,
- a set of arcs defined by the sets $\mathcal{I}(j), j = 1, \ldots, n$,
- a set of parameters $\mathcal{K} = \{K_j(X), j = 1, \ldots, n, X \subseteq \mathcal{I}(j)\}$.

In order to represent the (discrete) dynamics of the system, we define a second type of graphs, called **dynamical graphs**, where vertices represent states of the system (*i.e.* n-tuples giving the expression levels of the n genes), and edges represent transitions between states. In most applications, dynamical graphs are generated either on the basis of a fully synchronous assumption [19] or on the basis of a fully asynchronous approach [16].

3 Regulatory Petri Nets

Consider a Boolean regulatory graph $\mathcal{R} = (\mathcal{G}, \mathcal{I}, \mathcal{K})$. In this section, we define the Petri net corresponding to $\mathcal{R}$, *i.e.* whose dynamics simulates the dynamical behaviour of the genetic regulatory network.

3.1 Preliminary rewriting

The most natural way is to define a finite capacity Petri net, with inhibitor arcs (Figure 1):

- to each gene corresponds a place g_j, $j = 1, \ldots, n$,
- to each parameter $K_j(X)$, $j \in \{1, \ldots, n\}, X \subseteq \mathcal{I}(j)$ corresponds a transition $t_{g_j,X}$.

Transition $t_{g_j,X}$ is enabled as soon as all the places of the set X are marked AND all the places of the complementary set of X in $\mathcal{I}(j)$ (denoted $\mathcal{I}(j) \setminus X$) are empty. So the places of $\mathcal{I}(j)$ are the *input* places of the transition $t_{g_j,X}$, with the places of X connected to $t_{g_j,X}$ by standard arcs, and the places of $(\mathcal{I}(j) \setminus X)$ connected to $t_{g_j,X}$ by inhibitor arcs.

Place g_j is an output (*resp.* input) of $t_{g_j,X}$ if $K_j(X) = 1$ (*resp.* if $K_j(X) = 0$). We now need to ensure two constraints:

1. there is no "consumption" of the tokens in the places of X; this constraint is satisfied by adding *read arcs* for these places (self-loops); indeed, the present regulatory products of the input genes activate the transcription of the regulatees, but are not consumed;
2. the number of tokens in each place should be limited to 1 (Boolean case); this constraint can be satisfied by adding a capacity restriction for each place.

Figure 1 illustrates the PN representation of activation *versus* inhibition.

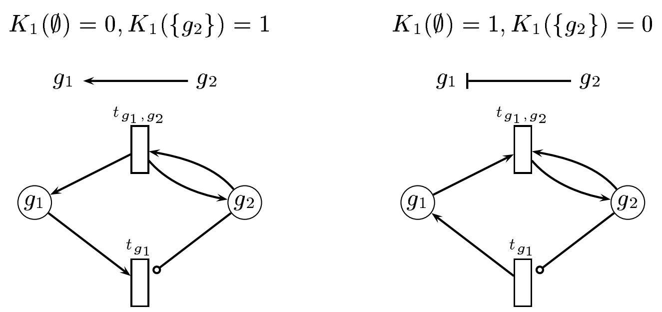


Fig. 1. Petri nets corresponding to the activation of g_1 by g_2 (left) and to the inhibition of g_1 by g_2 (right)

Remark 1. Note that the case of a self-regulation is slightly different. Indeed, if g_j is a self-regulator, there is one transition $t_{g_j,X}$ for each parameter $K_j(X)$, $j \in \{1, \ldots, n\}, X \subseteq \mathcal{I}(j)$ verifying: $K_j(X) = 0$ if $g_j \in X$ and $K_j(X) = 1$ if $g_j \notin X$ ($g_j \notin X$ means that g_j is absent, and $K_j(X) = 0$ does not lead to any change on g_j, and $g_j \in X$ means that g_j is present, and $K_j(X) = 1$ does not lead to any change on g_j). Consequently,

- when $g_j \in X$ ($K_j(X) = 0$), g_j is only an input of transition $t_{g_j,X}$.
- when $g_j \notin X$ ($K_j(X) = 1$), g_j is an input connected by an inhibitor arc, and a "standard" output of transition $t_{g_j,X}$.

Property 1. This Petri net is equivalent to the Boolean regulatory graph $\mathcal{R}$, *i.e.* $\mathcal{R}$ can be reconstructed from it.

3.2 Boolean Regulatory Petri Nets

Let us now consider the net obtained by a complementary-place transformation (cf [11], p. 543): a complementary place $\overline{g_i}$ is defined for each place g_i, such that the sum of tokens in places g_i and $\overline{g_i}$ equals 1 for each $i = 1, \ldots, n$ (Figure 2). The set of places P contains now $2n$ elements: $P = \mathcal{G} \cup \overline{\mathcal{G}}$, with $\overline{\mathcal{G}} = \{\overline{g_1}, \ldots, \overline{g_n}\}$. With this transformation, the capacity restriction is automatically satisfied, and inhibitory arcs are not further needed.

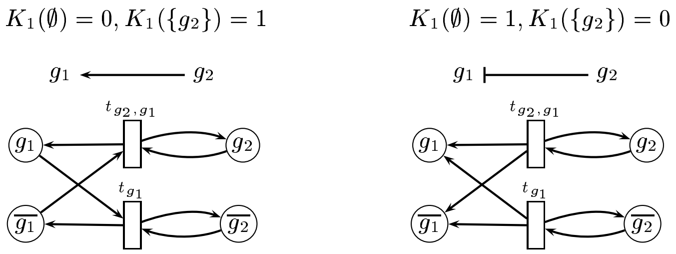


Fig. 2. Regulatory Petri net corresponding to the activation of g_1 by g_2 (left) and to the inhibition of g_1 by g_2 (right), respectively.

Definition 1. *Given a Boolean regulatory graph,* $\mathcal{R} = (\mathcal{G}, \mathcal{I}, \mathcal{K})$*, the associated **Boolean regulatory Petri net (BRPN)*** $\mathbf{N}(\mathcal{R}) = (P, T, Pre, Post)$ *is defined as follows:*

- $P = \mathcal{G} \cup \overline{\mathcal{G}} = \{g_1, \overline{g_1}, \ldots, g_n, \overline{g_n}\}$ *is the set of places,*
- $T = \{t_{g_i,X},\ i = 1, \ldots n,\ X \subseteq \mathcal{I}(i)\}$ *is the set of transitions,*
- $Pre : P \times T \to \{0, 1\}$ *is the mapping defining arcs between places and transitions (*Pre-conditions*).*
- $Post : T \times P \to \{0, 1\}$ *is the mapping defining arcs between transitions and places (*Post-conditions*).*

The functions Pre and Post are defined as follows:

1. *Case* $g_i \notin \mathcal{I}(i)$ *(*g_i *is not a self-regulator). For a given transition* $t_{g_i,X}$*, only the following terms have to be defined (all the other terms being equal to*

zero):

$$Pre(g_i, t_{g_i,X}) = Post(t_{g_i,X}, \overline{g_i}) = 1 - K_i(X)\,, \tag{1}$$
$$Pre(\overline{g_i}, t_{g_i,X}) = Post(t_{g_i,X}, g_i) = K_i(X)\,, \tag{2}$$
$$Pre(g_j, t_{g_i,X}) = Post(t_{g_i,X}, g_j) = 1 \qquad \forall g_j \in X\,, \tag{3}$$
$$Pre(\overline{g_j}, t_{g_i,X}) = Post(t_{g_i,X}, \overline{g_j}) = 1 \qquad \forall g_j \in \mathcal{I}(i) \setminus X\,. \tag{4}$$

2. *Case $g_i \in \mathcal{I}(i)$ (g_i is a self-regulator). Consider a given transition $t_{g_i,X}$.*
   - *if $g_i \in X$, the only case to be considered is $K_i(X) = 0$ (cf. Remark 1). Therefore, the only terms to be defined are:*

$$Pre(g_i, t_{g_i,X}) = Post(t_{g_i,X}, \overline{g_i}) = 1\,, \tag{5}$$
$$Pre(g_j, t_{g_i,X}) = Post(t_{g_i,X}, g_j) = 1 \qquad \forall g_j \in X\,, g_j \neq g_i\,, \tag{6}$$
$$Pre(\overline{g_j}, t_{g_i,X}) = Post(t_{g_i,X}, \overline{g_j}) = 1 \qquad \forall g_j \in \mathcal{I}(i) \setminus X\,. \tag{7}$$

   - *if $g_i \notin X$, the only case to be considered is $K_i(X) = 1$. Therefore, the only terms to be defined are:*

$$Pre(\overline{g_i}, t_{g_i,X}) = Post(t_{g_i,X}, g_i) = 1\,, \tag{8}$$
$$Pre(g_j, t_{g_i,X}) = Post(t_{g_i,X}, g_j) = 1 \qquad \forall g_j \in X\,, \tag{9}$$
$$Pre(\overline{g_j}, t_{g_i,X}) = Post(t_{g_i,X}, \overline{g_j}) = 1 \qquad \forall g_j \in \mathcal{I}(i) \setminus X\,, g_j \neq g_i \tag{10}$$

Equations (1)-(2) state that if the parameter $K_i(X)$ equals 1, g_i is an output and $\overline{g_i}$ an input of the corresponding transition $t_{g_i,X}$. In other words, there can be an increase of the level of the product of g_i if it is not already present. Symmetrically, if $K_i(X) = 0$, then $\overline{g_i}$ is an output and g_i is an input of the corresponding transition $t_{g_i,X}$.

In the case of a self-regulation, two situations are considered, both leading to a change of the value of g_i. Equation (5) states that if $g_i \in X$ (*i.e.* the product of g_i is present), then g_i is an input and $\overline{g_i}$ an output of the corresponding transition $t_{g_i,X}$. Conversely, equation (8) states that if $g_i \notin X$ (*i.e.* the product of g_i is absent) then $\overline{g_i}$ is an input and g_i an output of the corresponding transition $t_{g_i,X}$.

Equations (3)-(4), (6)-(7) and (9)-(10) state that the regulatory products contributing to the combination of interactions involved in $K_i(X)$ (*i.e.* which are in X) are the inputs of the corresponding transitions and are not consumed by these transitions.

Remark 2. Consider a BRPN $\mathbf{N}(\mathcal{R})$. Definition 1 gives a unique rewriting $\mathbf{N}(\mathcal{R})$. But if we consider two complementary places, p and $\overline{p}$, we have no way to determine which one corresponds to the presence of the regulatory product. Consequently, Property 1 does not hold anymore.

We shall use the following notation:

- for $i = 1, \ldots, n$: $\widehat{K}_i(X) = 2\,K_i(X) - 1\,;$

- d_i denotes the number of parameters for g_i. If $g_i \notin \mathcal{I}(i)$, then $d_i = 2^{\#\mathcal{I}(i)}$. Otherwise, if g_i is auto-regulated, then d_i is smaller and depends on the combined effect of incoming interactions together with the self-regulation (cf. Case 2 in Definition 1);
- $X_i^1, \ldots, X_i^{d_i}$ denote the subsets of $\mathcal{I}(i)$ which characterise the parameters for g_i. (The order between these subsets is a meaningless convention.)

Property 2. The **incidence matrix** $C = Post^T - Pre$ is a $2n \times (\sum_{i=1\ldots n} d_i)$ matrix. Its components take their values in $\{-1, 0, 1\}$, and C has the following structure:

$$C = \begin{pmatrix} \boxed{C_1} & 0 & \cdots & 0 \\ 0 & \boxed{C_2} & \cdots & 0 \\ 0 & 0 & \ddots & 0 \\ 0 & 0 & \cdots & \boxed{C_n} \end{pmatrix}, \quad \text{where} \quad C_i = \begin{pmatrix} \widehat{K}_{i,X_i^1} & \ldots & \widehat{K}_{i,X_i^{d_i}} \\ -\widehat{K}_{i,X_i^1} & \ldots & -\widehat{K}_{i,X_i^{d_i}} \end{pmatrix}.$$

It is important to point out that the incidence matrix of a **BRPN** does not correspond to the structure of the underlying graph, because of the *read arcs*.

Definition 2. *Given a regulatory Petri net $(P, T, Pre, Post)$, a* ***valid marking*** *$M : P \to \{0,1\}$ corresponds to a state of the Boolean regulatory graph $(\mathcal{G}, \mathcal{K})$ and verifies:*

$$\forall g_i \in \mathcal{G},\; M(g_i) = 1 - M(\overline{g_i}). \tag{11}$$

The following property is straightforward and ensures the conservation of the validity of the markings as the system evolves.

Property 3. Given a regulatory Petri net $(P, T, Pre, Post)$ with a valid initial marking, any reachable marking is still valid and therefore the BRPN is *1-safe* (*i.e.* the marking of any place is at most 1).

Property 4 shows the equivalence between the asynchronous dynamical graph associated with the Boolean regulatory graph and the reachability graph of the corresponding BRPN. A state in the dynamical graph is described by a valid marking, *i.e.* the current values associated to each element (1 if the regulatory product g_i is present at this state, 0 otherwise). Note that we only consider the fully asynchronous assumption, as it is generally the case in the Petri net framework, but we could also consider the fully synchronous assumption, modifying the firing rule (in this case, all enabled transitions should fire simultaneously).

Property 4. There exists a transition between two states S_1 and S_2 in the asynchronous dynamical graph related to a Boolean regulatory graph $\mathcal{R} = (\mathcal{G}, \mathcal{I}, \mathcal{K})$, iff there exists an enabled transition t in the associated BRPN such that M_1 verifies $M_1[t\rangle M_2$ (t is enabled by M_1 and its firing leads to the marking M_2) with, for all $i = 1, \ldots n$,

$$\begin{aligned} M_1(g_i) &= S_1(i) \quad & M_1(\overline{g_i}) &= 1 - S_1(i)\,, \\ M_2(g_i) &= S_2(i) \quad & M_2(\overline{g_i}) &= 1 - S_2(i)\,. \end{aligned}$$

4 Regulatory circuits

As mentioned in the introduction, in the context of the logical approach, several authors have emphasised the crucial dynamical roles of regulatory circuits. More specifically, **positive** circuits (*i.e.* involving an even number of inhibitions) have been associated to multi-stationary behaviour, whereas **negative** circuits (involving an odd number of inhibitions) can generate sustained periodic behaviour [16]. A circuit is said functional when it generates the corresponding dynamical property. In this section, we derive a general PN formulation for these two classes of regulatory circuits and check their dynamical properties.

In the case of isolated regulatory circuits, each gene g_i is the target of a unique interaction exerted by g_{i-1}, and is the source of a unique interaction towards g_{i+1} (here and in the sequel, indices are considered *modulo n*, *i.e.* $i+n=i$). Note that sets $\mathcal{I}$ are singleton sets, uniquely defined and therefore they will be omitted.

In this section, to simplify the notations, we will denote

$$\begin{array}{llll} K_i(\{g_{i-1}\}) & \text{by } K_i(g_{i-1}), & K_i(\emptyset) & \text{by } K_i, \\ t_{g_i,\{g_{i-1}\}} & \text{by } t_{i,i-1}, & t_{g_i,\emptyset} & \text{by } t_i. \end{array}$$

Let $\mathcal{C}=(\mathcal{G},\mathcal{K})$ be a regulatory circuit, with $\mathcal{G}=\{g_1,\ldots,g_n\}$ and $\mathcal{K}=\{K_i,\,K_i(g_{i-1})\}_{i=1,\ldots,n}$ ($\mathcal{I}(i)=\{g_{i-1}\}$; cf. [13] for a more detailed description). In this simpler context, the notion of activation *versus* inhibition (cf. Section 2) can be simply expressed depending on the values of the parameters. Consider the interaction from g_i to g_{i+1},

- if $K_{i+1}=0$ and $K_{i+1}(g_i)=1$, *i.e.* the presence of the regulatory product of g_i increases the expression level of g_{i+1}, then we say that this interaction is an activation; it is labelled with the sign $\varepsilon_i=+1$,
- if $K_{i+1}=1$ and $K_{i+1}(g_i)=0$, *i.e.* the presence of the regulatory product of g_i decreases the expression level of g_{i+1}, then we say that this interaction is an inhibition; it is labelled with the sign $\varepsilon_i=-1$.

The structure of the regulatory Petri net $\mathbf{N}(\mathcal{C})$ corresponding to a Boolean circuit is described by the following property (see also Figure 3):

Property 5. Consider a Boolean regulatory circuit $\mathcal{C}=(\mathcal{G},\mathcal{K})$. The corresponding regulatory Petri net $\mathbf{N}(\mathcal{C})$ is given by the following components:

- $P=\mathcal{G}\cup\overline{\mathcal{G}}=\{g_1,\overline{g_1},\ldots,g_n,\overline{g_n}\}$ is the set of places, with cardinal $2n$.
- $T=\{t_i,\,t_{i,i-1},\ i=1,\ldots n\}$ is the set of transitions, with cardinal $2n$.
- $Pre: P\times T\to\{0,1\}$ is a $2n\times 2n$ matrix. The only terms which may be non-zero are:

$$\begin{array}{llll} Pre(g_i,t_i) & = 1-K_i & Pre(\overline{g_i},t_i) & = K_i \\ Pre(g_i,t_{i,i-1}) & = 1-K_i(g_{i-1}) & Pre(\overline{g_i},t_{i,i-1}) & = K_i(g_{i-1}) \\ Pre(g_{i-1},t_{i,i-1}) & = 1 & Pre(\overline{g_{i-1}},t_i) & = 1. \end{array}$$

– $Post : T \times P \to \{0,1\}$ is a $2n \times 2n$ matrix. The only terms which may be non-zero are:

$$\begin{array}{llll} Post(t_i, \overline{g_i}) & = 1 - K_i & Post(t_i, g_i) & = K_i \\ Post(t_{i,i-1}, \overline{g_i}) & = 1 - K_i(g_{i-1}) & Post(t_{i,i-1}, g_i) & = K_i(g_{i-1}) \\ Post(t_{i,i-1}, g_{i-1}) & = 1 & Post(t_i, \overline{g_{i-1}}) & = 1. \end{array}$$

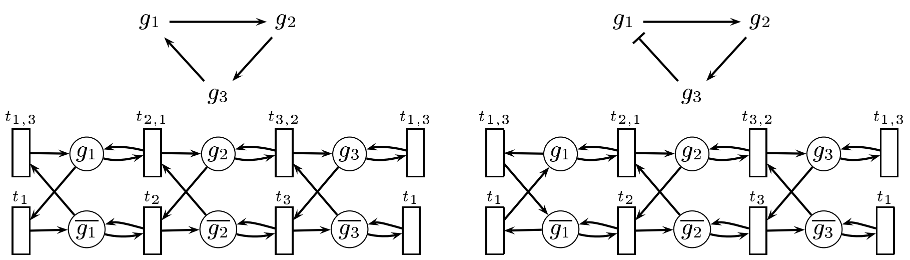


Fig. 3. Three-element regulatory circuits and the corresponding Petri nets. Left: positive circuit. Right: negative circuit. Note that transitions between g_1 and g_3 are repeated, illustrating the general structure of the net.

We use the following notation already introduced in 3.2, for $i = 1, \ldots, n$:

$$\widehat{K}_i = 2\,K_i - 1$$
$$\widehat{K}_{i,i-1} = 2\,K_i(g_{i-1}) - 1.$$

Using the Property 5, we can further characterize the regulatory Petri nets corresponding to Boolean circuits as follows:

Property 6. Let $\mathbf{N}(\mathcal{C}) = (\mathcal{G} \cup \overline{\mathcal{G}}, T, Pre, Post)$ be the regulatory Petri net corresponding to the Boolean regulatory circuit $\mathcal{C}$. The incidence matrix C has a block-diagonal $2n \times 2n$ form:

$$C = \begin{pmatrix} \boxed{C_1} & 0 & \cdots & 0 \\ 0 & \boxed{C_2} & \cdots & 0 \\ 0 & 0 & \ddots & 0 \\ 0 & 0 & \cdots & \boxed{C_n} \end{pmatrix},$$

where

$$C_i = \begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix} \quad \text{if } g_{i-1} \text{ is an activator for } g_i \text{, and}$$
$$C_i = \begin{pmatrix} 1 & -1 \\ -1 & 1 \end{pmatrix} \quad \text{if } g_{i-1} \text{ is an inhibitor for } g_i.$$

Proof. It follows from Property 2 that the incidence matrix C is block-diagonal in the case of a circuit. The blocks are:

$$C_i = \begin{pmatrix} \widehat{K}_i & \widehat{K}_{i,i-1} \\ -\widehat{K}_i & -\widehat{K}_{i,i-1} \end{pmatrix};$$

using Property 5, we further have $K_i = 0$, $K_i(g_{i-1}) = 1$ if g_{i-1} activates g_i, and $K_i = 1$, $K_i(g_{i-1}) = 0$ if g_{i-1} inhibits g_i. ■

4.1 Isomorphic Petri nets

For a given length n, all positive (resp. negative) circuits have the same type of dynamical properties. It should thus suffice to study one representative for each of these classes. This property is expressed through the notion of *isomorphic Petri nets.*

Definition 3. *Let $N = (P, T, Pre, Post)$ and $N' = (P', T', Pre', Post')$ be two Petri nets. N and N' are said to be* ***isomorphic Petri nets*** *when there exists a graph isomorphism $\phi : (P \cup T, Pre \cup Post) \to (P' \cup T', Pre' \cup Post')$ such that $\phi(p) \in P'$ for each $p \in P$ and $\phi(t) \in T'$ for each $t \in T$ (note that adjacency is preserved).*

For $n \in \mathbb{N}, n \geqslant 1$, let $\mathcal{C}_n^+$ be the regulatory circuit $g_1 \overset{+1}{\to} g_2 \overset{+1}{\to} \cdots \overset{+1}{\to} g_n \overset{+1}{\to} g_1$ with n genes and all interactions positive and let $\mathcal{C}_n^-$ be the regulatory circuit $g_1 \overset{-1}{\to} g_2 \overset{+1}{\to} \cdots \overset{+1}{\to} g_n \overset{+1}{\to} g_1$ with n genes and all interactions positive except the first one.

Property 7. Let $\mathcal{R}$ be a regulatory circuit $g_1 \overset{\varepsilon_1}{\to} g_2 \overset{\varepsilon_2}{\to} \cdots \overset{\varepsilon_{n-1}}{\to} g_n \overset{\varepsilon_n}{\to} g_1$, with $\varepsilon_i = +1$ or -1. If $\mathcal{R}$ is positive, then $\mathbf{N}(\mathcal{R})$ is isomorphic to $\mathbf{N}(\mathcal{C}_n^+)$. If $\mathcal{R}$ is negative, then $\mathbf{N}(\mathcal{R})$ is isomorphic to $\mathbf{N}(\mathcal{C}_n^-)$.

Proof. Let us assume $\mathcal{R}$ has at least 2 negative interactions.

Let $g_i \overset{-1}{\to} g_{i+1}$ and $g_j \overset{-1}{\to} g_{j+1}$ be two negative interactions, $i < j$. By exchanging in $\mathbf{N}(\mathcal{R})$ the transitions t_{k+1} and $t_{k+1,k}$, and the places g_k and $\overline{g_k}$ for each k, $i+1 \leqslant k \leqslant j$, one obtains an isomorphic Petri net N'. Now, N' is clearly the Petri net corresponding to the circuit obtained from $\mathcal{R}$ by replacing the sequence

$$g_i \overset{-1}{\to} g_{i+1} \xrightarrow{\varepsilon_{i+1}} \cdots \xrightarrow{\varepsilon_{j-1}} g_j \overset{-1}{\to} g_{j+1}$$

by

$$g_i \overset{+1}{\to} \overline{g_{i+1}} \xrightarrow{\varepsilon_{i+1}} \cdots \xrightarrow{\varepsilon_{j-1}} \overline{g_j} \overset{+1}{\to} g_{j+1}.$$

Hence, if the indices i and j correspond to two consecutive negative interactions (*i.e.* such that $\varepsilon_k = +1$ for each k, $i < k < j$), by iterating the above process, one obtains either $\mathcal{C}_n^+$ when $\mathcal{R}$ is positive, or a circuit with one negative interaction when $\mathcal{R}$ is negative. In the latter case, if $g_i \overset{-1}{\to} g_{i+1}$ is the negative interaction, then the "rotation" $\phi : g_k \mapsto g_{k+i-1}$, $\overline{g_k} \mapsto \overline{g_{k+i-1}}$, $t_{k+1} \mapsto t_{k+i}$, $t_{k+1,k} \mapsto t_{k+i,k+i-1}$ is an isomorphism between N' and $\mathbf{N}(\mathcal{C}_n^-)$. ■

Consequently, in the sequel, we restrict our study to $\mathcal{C}_n^+$ and $\mathcal{C}_n^-$.

4.2 Positive circuits

In [13], we have proved that an isolated *functional* positive circuit (*i.e.* for proper logical parameter values) generates two stable states, which are *mirroring* each other (a component is "on" in one state iff it is "off" in the other state).

Property 8. Let $\mathbf{N}(\mathcal{R}) = (P, T, Pre, Post)$ be a regulatory Petri net corresponding to an isolated functional positive regulatory circuit, then there are exactly two dead valid markings M_d^1 and M_d^2 which are mirroring each other. Each of these two markings is reachable from any valid marking.

Proof. Using Property 7, we can restrict the proof for $\mathbf{N}(\mathcal{C}_n^+)$. We will first prove that there exists two mirroring dead markings M_d^1 and M_d^2.

For any $i = 1, \ldots n$, two transitions have to be considered. Transition t_i (corresponding to parameter $K_i = 0$) has two input places which are g_i and $\overline{g_{i-1}}$. It is not enabled under a marking M satisfying: $M(g_i) = 0$ or $M(\overline{g_{i-1}}) = 0$. And transition $t_{i,i-1}$ (corresponding to parameter $K_i(g_{i-1}) = 1$) has two input places which are $\overline{g_i}$ and g_{i-1}. It is not enabled under a marking M satisfying: $M(\overline{g_i}) = 0$ or $M(g_{i-1}) = 0$. The conjunction of these two constraints leads to $M(g_i) = M(g_{i+1}), \forall i = 1, \ldots n$. The only two markings satisfying these constraints are M_d^1 such that $M_d^1(g_i) = 0, M_d^1(\overline{g_i}) = 1, \forall i$, and M_d^2 such that $M_d^2(g_i) = 1, M_d^1(\overline{g_i}) = 0, \forall i$.

Now, let M be a valid marking different from M_d^1 and M_d^2. Then for M, there exists an index i such that $M(g_i) \neq M(g_{i+1})$. Two cases have to be considered:

1. $M(g_i) = 1$ and $M(g_{i+1}) = 0$: $t_{i+1,i}$ is enabled and its firing preserves all the values of M except for $i+1$ (the token in $\overline{g_{i+1}}$ has been transferred to g_{i+1}). Thus the firing of $t_{i+1,i}$ leads to a marking with a smaller number of 0 values for places g (consequently a smaller number of places $\overline{g}$ marked).
2. $M(g_i) = 0$ and $M(g_{i+1}) = 1$: t_i is enabled and its firing preserves all the values of M except for $i+1$ (there is now no token in g_{i+1}). Thus the firing of t_i leads to a marking with a smaller number of 1 values for places g (consequently a smaller number of places $\overline{g}$ unmarked).

More generally, for M different from M_d^1 and M_d^2, there obviously exists (because it is a circuit) an index i for which $M(g_i) = 1, M(g_{i+1}) = 0$ and an index k for which $M(g_k) = 0, M(g_{k+1}) = 1$. Therefore, using the above argument, there exists an enabled sequence σ^1 such that $M[\sigma^1\rangle M_d^1$ (iteratively decreasing the number of places g marked), and a sequence σ^2 such that $M[\sigma^2\rangle M_d^2$ (decreasing the number of places $\overline{g}$ marked). This proves that M_d^1 and M_d^2 are reachable from any valid marking. ■

4.3 Negative circuits

As mentioned above, negative regulatory circuits typically generate periodic dynamical behaviour. In [13], we have shown that an isolated functional negative circuit leads to a dynamical graph where all states feed a specific dynamical circuit of length twice the number of elements in the circuit.

Property 9. Let $\mathbf{N}(\mathcal{R}) = (P, T, Pre, Post)$ be a regulatory Petri net corresponding to a negative regulatory circuit and E be the set of all the valid markings which enable exactly one transition.

1. No dead marking is reachable from any initial valid marking.
2. E has $2n$ elements and is organised as a cycle.
3. Each marking in E is reachable from any valid marking.
4. $\mathbf{N}(\mathcal{R})$ is live for any initial valid marking.

Proof. By Property 7, we can restrict the proof to the case of $\mathbf{N}(\mathcal{C}_n^-)$.

1. The argument is similar to that used in the positive case. If M is a dead marking reached from an initial valid marking, then it is valid by Property 3, and for any $i \neq 1$, the tuple $(M(g_i), M(\overline{g_i}), M(g_{i+1}), M(\overline{g_{i+1}}))$ is either $(0,1,0,1)$ or $(1,0,1,0)$, and the tuple $(M(1), M(2), M(3), M(4))$ is either C$(0,1,1,0)$ or $(1,0,0,1)$ because the first interaction is negative. These constraints are clearly incompatible: for instance, if the marking M starts with $(0,1,1,0)$, then M has to be $(0,1,1,0,1,0,\ldots,1,0)$, but then the tuple $(M(g_n), M(\overline{g_n}), M(g_1), M(\overline{g_1}))$ is $(1,0,0,1)$, whereas it should be either $(0,1,0,1)$ or $(1,0,1,0)$.
2. Let M be a valid marking. If $i \neq 1$, the interaction between g_i and g_{i+1} is positive, so

$$\begin{aligned} M[t_{i+1}\rangle &\Leftrightarrow M(\overline{g_i}) = M(g_{i+1}) = 1 && \text{(see Fig. 2)} \\ &\Leftrightarrow (M(g_i), M(\overline{g_i}), M(g_{i+1}), M(\overline{g_{i+1}})) = (0,1,1,0) && (M \text{ valid}). \end{aligned}$$

Similarly, $M[t_{i+1,i}\rangle \Leftrightarrow (M(g_i), M(\overline{g_i}), M(g_{i+1}), M(\overline{g_{i+1}})) = (1,0,0,1)$. Otherwise, the interaction between g_1 and g_2 is negative, and thus

$$\begin{aligned} M[t_2\rangle &\Leftrightarrow (M(g_1), M(\overline{g_1}), M(g_2), M(\overline{g_2})) = (0,1,0,1) \\ M[t_{2,1}\rangle &\Leftrightarrow (M(g_1), M(\overline{g_1}), M(g_2), M(\overline{g_2})) = (1,0,1,0)\,. \end{aligned}$$

Let us define:

$$\begin{aligned} M_{i+1} &= (1,0,0,1,\ldots,0,1,\underbrace{\overset{g_i}{0},\overset{\overline{g_i}}{1},1,0}_{t_{i+1}},\ldots,1,0) \quad \text{for } i \neq 1, \\ M_{i+1,i} &= (0,1,1,0,\ldots,1,0,\underbrace{\overset{g_i}{1},\overset{\overline{g_i}}{0},0,1}_{t_{i+1,i}},\ldots,0,1) \quad \text{for } i \neq 1, \\ M_2 &= (\underbrace{0,1,0,1}_{t_2},0,1,\ldots,0,1), \\ M_{2,1} &= (\underbrace{1,0,1,0}_{t_{2,1}},1,0,\ldots,1,0). \end{aligned}$$

Then, we have, for all $j = 1, \ldots n$:

$$\begin{aligned} M \in E \text{ and } M[t_j\rangle &\Longleftrightarrow M = M_j\,, \\ M \in E \text{ and } M[t_{j,j-1}\rangle &\Longleftrightarrow M = M_{j,j-1}\,. \end{aligned}$$

Thus, $E = \{M_j,\, M_{j,j-1},\ j = 1, \ldots n\}$ and has exactly $2\,n$ elements.
Clearly E is organised as a cycle since $M_i[t_i\rangle M_{i+1,i}[t_{i+1,i}\rangle M_{i+1}$ for any i.

3. Given a valid marking M, we denote $\#M$ the number of indices i such that $M(g_i) = 1$. Note that M_2 is the unique marking s.t. $\#M_2 = 0$ and $M_{2,1}$ the only one s.t. $\#M_{2,1} = n$.
   Let $M \notin E$ be a valid marking. Obviously, $0 < \#M < n$. Consider $J(M) = \{j \mid 1 \leqslant j \leqslant n, M(g_j) = 0 \text{ and } M(g_{j+1}) = 1\}$. In particular, an index $j \neq 1$ belongs to $J(M)$ iff t_{j+1} is enabled.
   Follow a marking path from M to E along which $\#M$ decreases: while $J(M) \neq \emptyset$ and $J(M) \neq \{1\}$, for all $j \in J(M)$ and $j \neq 1$, we have $M[t_{j+1}\rangle M'$, with $\#M' = \#M - 1$. Finally,
   - if $J(M) = \emptyset$ then $M = M_2 \in E$,
   - if $J(M) = \{1\}$ then $M = M_{3,2} \in E$.
4. The liveness is a straighforward consequence of 2 and 3. ■

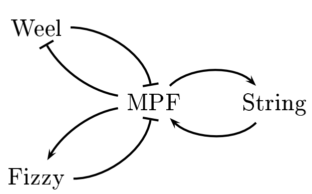
For regulatory Petri nets corresponding to negative circuits, it can also be proved that the number of enabled transitions at each valid marking is odd, and that firing one transition decreases this number either by 0 or 2.

5 Applications

In what follows, we present two biological illustrations of our rewriting of Boolean regulatory networks into Petri nets. The first application consists in a simplified model of the protein network controlling the cell cycle during the early stages of the development of the fly *Drosophila melanogaster*. The second application consists in a genetic regulatory network involved in the control of flower morphogenesis in the plant *Arabidopsis thaliana*. In both cases, the generalised logical approach (though still Boolean) led to interesting insight about the dynamical and biological roles of specific feedback circuits, as well as to the prediction of the behaviour of the system in new situations (mutations, perturbations) [18, 10].

5.1 Drosophila Cell Cycle

Extensive genetic and molecular data has been collected on the various components and individual interactions at the basis of the cell cycle and its properties. To integrate these diverse pieces of data, several authors have been working on the development of dynamical models [17]. Here, for the sake of simplicity, we consider a simple Boolean model involving the minimal number of components susceptible to generate the observed oscillatory behaviour during the early stages of Drosophila embryonic development [18]. At that time, the core of the cell cycle control network involves four main active regulatory compounds: the MPF complex (*i.e.*, the Mitosis Promoting Factor, made of the association of proteins Cyclin B and Cdc2), and the proteins Fizzy, Wee1, and String. Cross-interactions between these four compounds can be summarised in the form of



target	parameter
MPF	K_M
	$K_{M,WS}$
	$K_{M,S}$
	$K_{M,W}$
Fizzy	$K_{F,M}$
Wee1	K_W
String	$K_{S,M}$

Fig. 4. A simple logical model of the gene network controlling the first cell cycles during Drosophila embryogenesis. Left: Regulatory graph. Right: non-zero parameters for the four core regulators.

the regulatory graph shown in Figure 4. In the context of the logical approach, it proved possible to derive the parameter values enabling the generation of a unique periodic attractor, matching the periodic properties observed in the real system (see Figure 4 for the parameter values, and Figure 5 for the corresponding asynchronous dynamical graph). In order to illustrate the translation rules

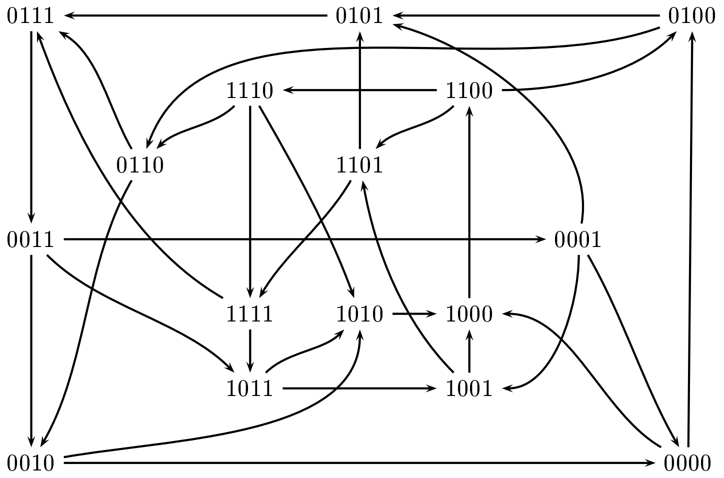


Fig. 5. Asynchronous dynamical graph encompassing all possible state transitions for the model defined in Figure 4.

described above, we derive hereafter the BRPN of this parameterised model and check its properties in reference to the Petri net analytical framework.

In the sequel, M will stand for MPF, F for $Fizzy$, W for $Wee1$ and S for $String$. The resulting BRPN can be defined by the following matrices:

$$
Pre = \begin{array}{c|cccccccccccccc}
 & t_M & t_{M,FWS} & t_{M,F} & t_{M,WS} & t_{M,FW} & t_{M,S} & t_{M,FS} & t_{M,W} & t_F & t_{F,M} & t_W & t_{W,M} & t_S & t_{S,M} \\
\hline
M & 0 & 1 & 1 & 0 & 1 & 0 & 0 & 1 & 0 & 1 & 0 & 1 & 0 & 1 \\
\overline{M} & 1 & 0 & 0 & 1 & 0 & 1 & 1 & 0 & 1 & 0 & 1 & 0 & 1 & 0 \\
F & 0 & 1 & 1 & 0 & 1 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\
\overline{F} & 1 & 0 & 0 & 1 & 0 & 1 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
W & 0 & 1 & 0 & 1 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
\overline{W} & 1 & 0 & 1 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 \\
S & 0 & 1 & 0 & 1 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 \\
\overline{S} & 1 & 0 & 1 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1
\end{array}
$$

$$
Post = \begin{array}{c|cccccccc}
 & M & \overline{M} & F & \overline{F} & W & \overline{W} & S & \overline{S} \\
\hline
t_M & 1 & 0 & 0 & 1 & 0 & 1 & 0 & 1 \\
t_{M,FWS} & 0 & 1 & 1 & 0 & 1 & 0 & 1 & 0 \\
t_{M,F} & 0 & 1 & 1 & 0 & 0 & 1 & 0 & 1 \\
t_{M,WS} & 1 & 0 & 0 & 1 & 1 & 0 & 1 & 0 \\
t_{M,FW} & 0 & 1 & 1 & 0 & 1 & 0 & 0 & 1 \\
t_{M,S} & 1 & 0 & 0 & 1 & 0 & 1 & 1 & 0 \\
t_{M,FS} & 0 & 1 & 1 & 0 & 0 & 1 & 1 & 0 \\
t_{M,W} & 1 & 0 & 0 & 1 & 1 & 0 & 0 & 1 \\
t_F & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\
t_{F,M} & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
t_W & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\
t_{W,M} & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
t_S & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \\
t_{S,M} & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0
\end{array}
$$

$$
Post^T - Pre = \begin{array}{c|cccccccccccccc}
 & t_M & t_{M,FWS} & t_{M,F} & t_{M,WS} & t_{M,FW} & t_{M,S} & t_{M,FS} & t_{M,W} & t_F & t_{F,M} & t_W & t_{W,M} & t_S & t_{S,M} \\
\hline
M & 1 & -1 & -1 & 1 & -1 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
\overline{M} & -1 & 1 & 1 & -1 & 1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
F & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\
\overline{F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\
W & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\
\overline{W} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 \\
S & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 \\
\overline{S} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1
\end{array}
$$

In order to demonstrate that this system has a cyclical attractor, we can refer to the absence of *deadlock* property.

Property 10. The system is deadlock-free.

Proof. We will prove that there is no marking such that no transition is enabled in the BRPN $\mathcal{C}$. In fact, such a marking should satisfy the following set of constraints; each constraint defines the condition under which the corresponding transition is not enabled, and is given by the relevant column of the Pre matrix:

$$
\begin{aligned}
t_M &: M_d(M) = 1 \vee M_d(F) = 1 \vee M_d(W) = 1 \vee M_d(S) = 1 \\
t_{M,FWS} &: M_d(M) = 0 \vee M_d(F) = 0 \vee M_d(W) = 0 \vee M_d(S) = 0 \\
t_{M,F} &: M_d(M) = 0 \vee M_d(F) = 0 \vee M_d(W) = 1 \vee M_d(S) = 1 \\
t_{M,WS} &: M_d(M) = 1 \vee M_d(F) = 1 \vee M_d(W) = 0 \vee M_d(S) = 0 \\
t_{M,FW} &: M_d(M) = 0 \vee M_d(F) = 0 \vee M_d(W) = 0 \vee M_d(S) = 1 \\
t_{M,S} &: M_d(M) = 1 \vee M_d(F) = 1 \vee M_d(W) = 1 \vee M_d(S) = 0 \\
t_{M,FS} &: M_d(M) = 0 \vee M_d(F) = 0 \vee M_d(W) = 1 \vee M_d(S) = 0 \\
t_{M,W} &: M_d(M) = 1 \vee M_d(F) = 1 \vee M_d(W) = 0 \vee M_d(S) = 1
\end{aligned}
$$

$$\begin{aligned}
t_F &: M_d(F) = 0 \vee M_d(M) = 1\\
t_{F,M} &: M_d(F) = 1 \vee M_d(M) = 0\\
t_W &: M_d(W) = 1 \vee M_d(M) = 1\\
t_{W,M} &: M_d(W) = 0 \vee M_d(M) = 0\\
t_S &: M_d(S) = 0 \vee M_d(M) = 1\\
t_{S,M} &: M_d(S) = 1 \vee M_d(M) = 0
\end{aligned}$$

Using the constraints on t_F and $t_{F,M}$, we obtain $M_d(F) = M_d(M)$. Using the constraints on t_W and $t_{W,M}$, we obtain $M_d(W) \neq M_d(M)$. Using the constraints on t_S and $t_{S,M}$, we obtain $M_d(S) = M_d(M)$. The only two markings that verify these constraints are (omitting the values for the complementary places):

$$\begin{bmatrix} M_d^1(M) = 0 \\ M_d^1(F) = 0 \\ M_d^1(W) = 1 \\ M_d^1(S) = 0 \end{bmatrix} \quad \text{and} \quad \begin{bmatrix} M_d^2(M) = 1 \\ M_d^2(F) = 1 \\ M_d^2(W) = 0 \\ M_d^2(S) = 1 \end{bmatrix}$$

The marking M_d^1 enables $t_{M,W}$ and M_d^2 enables $t_{M,FS}$. Therefore, there is no marking such that no transition is enabled. ■

We can further generate the complete reachability graph (for all the valid initial markings), which exactly matches the graph of Figure 5 (taking into account that we omit the marking of the complementary places).

Note that if the values of parameters K_F and $K_{F,M}$ are exchanged (changing the effect of gene MPF upon $Fizzy$, *i.e.* specifying an inhibition rather than an activation), then Property 10 does not hold anymore. One can easily check that markings $[1, 0, 0, 1]$ and $[0, 1, 1, 0]$ are indeed dead. This exchange of values corresponds to the transformation of the unique negative circuit into a third positive circuit in the regulatory graph (see Figure 4), consequently leading to the loss of the oscillatory behaviour associated to the negative circuit (cf Property 9).

5.2 Flowering in Arabidopsis

Let us now turn to a larger network involved in the control of flower morphogenesis in the model plant *Arabidopsis thaliana*. On the basis of a mutant analysis, plant geneticists have proposed an abstract combinatory model ('ABC model') to account for the differentiation of the flower meristem into the four typical flower organs: carpels, stamens, sepals and petals [2]. More recently, on the basis of new molecular genetic data, Mendoza *et al.* have proposed a Boolean regulatory model involving 10 genes cross-regulating each other [10]. The corresponding regulatory graph is shown in Figure 6. For proper parameter value sets, this model encompasses 6 stable states, four of them matching the qualitative gene expression patterns observed in the different flower organs, while the two last stable states correspond to non-flowering situations.

For sake of simplicity, we focus here on a subset of six genes which play a crucial role in the selection of specific flowering differentiative pathways, leaving aside the genes which can be treated as simple inputs (EMF1, UFO, LUG and SUP). A selection of parameter values is presented in Table 1 (Right). For this parameter set, the system has four stable states, each corresponding to a gene expression pattern associated with a specific flower organ.

Contrasting with the model of Drosophila cell cycle, we are now facing a network, whose biological role consists essentially in selecting specific differentiation pathways. Consequently, we do not expect to observe oscillatory behaviour here, but rather a specific set of dead markings, each representing a logical stable state, *i.e.* a specific gene expression state.

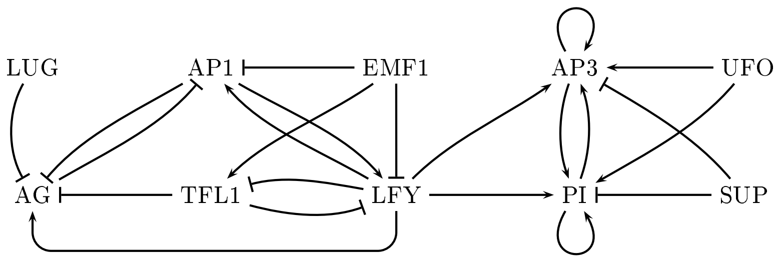


Fig. 6. Regulatory graph for the gene network controlling early flower morphogenesis in the plant *Arabidopsis thaliana*.

Gene name	Symbol	Variable
Terminal Flower 1	TFL1	T
LEAFY	LFY	L
APETALA1	AP1	A
AGAMOUS	AG	G
APETALA3	AP3	P
PISTILLATA	PI	I

T		L		A		G		P		I	
t_T	0	t_L	0	t_A	1	t_G	1	$t_{P,P}$	0	$t_{I,I}$	0
$t_{T,L}$	0	$t_{L,A}$	0	$t_{A,L}$	1	$t_{G,L}$	1	$t_{P,LP}$	0	$t_{I,LI}$	0
		$t_{L,T}$	0	$t_{A,G}$	0	$t_{G,A}$	0				
		$t_{L,AT}$	0	$t_{A,LG}$	0	$t_{G,T}$	0				
						$t_{G,LA}$	0				
						$t_{G,TA}$	0				
						$t_{G,TL}$	0				
						$t_{G,TLA}$	0				

Table 1. Left: Names, symbols and variables for the six core genes selected in Figure 6. Right: The transitions and associated parameter values for each of these six genes.

In the associated BRPN, we have 12 places (corresponding to the 6 genes and the complementary places) and 22 transitions. Note that in the case of P and I, which are auto-regulated, we consider only two parameters for each, as only $K_{P,P} = 0$ and $K_{P,LP} = 0$ (resp. $K_{I,I} = 0$ and $K_{I,LI} = 0$) are calling for a change of the value of P (resp. I).

The *Pre* and *Post* matrices of the BRPN associated (we give *Pre* transposed because of its size) are:

$$Pre^T = \left(\begin{array}{c|cccccccccccc}
 & T & \overline{T} & L & \overline{L} & A & \overline{A} & G & \overline{G} & P & \overline{P} & I & \overline{I} \\
t_T & 1&0&0&0&0&1&0&0&0&0&0&0 \\
t_{T,L} & 1&0&0&0&1&0&0&0&0&0&0&0 \\
t_L & 0&1&0&0&1&0&0&1&0&0&0&0 \\
t_{L,A} & 0&1&0&0&1&0&1&0&0&0&0&0 \\
t_{L,T} & 1&0&0&0&0&0&0&1&0&0&0&0 \\
t_{L,AT} & 1&0&0&0&1&0&1&0&0&0&0&0 \\
t_A & 0&0&0&0&1&0&0&1&0&1&0&0 \\
t_{A,L} & 0&0&0&0&0&1&1&0&1&0&0&0 \\
t_{A,G} & 0&0&0&0&0&1&1&0&1&0&0&0 \\
t_{A,LG} & 0&0&0&0&1&0&1&0&1&0&0&0 \\
t_G & 0&1&0&0&0&1&0&1&0&1&0&0 \\
t_{G,L} & 0&1&0&0&0&1&0&1&0&1&0&0 \\
t_{G,A} & 0&1&0&0&0&1&1&0&1&0&0&0 \\
t_{G,T} & 1&0&0&0&0&1&0&1&1&0&0&0 \\
t_{G,LA} & 0&1&0&0&1&0&1&0&1&0&0&0 \\
t_{G,TL} & 1&0&0&0&1&0&0&1&1&0&0&0 \\
t_{G,TA} & 1&0&0&0&0&1&1&0&1&0&0&0 \\
t_{G,TLA} & 1&0&0&0&1&0&1&0&1&0&0&0 \\
t_{P,P} & 0&0&0&1&0&1&0&0&0&0&1&0 \\
t_{P,LP} & 0&0&0&1&1&0&0&0&0&0&1&0 \\
t_{I,I} & 0&0&1&0&0&1&0&0&0&0&0&1 \\
t_{I,LI} & 0&0&1&0&1&0&0&0&0&0&0&1
\end{array}\right)$$

$$Post = \left(\begin{array}{c|cccccccccccc}
 & T & \overline{T} & L & \overline{L} & A & \overline{A} & G & \overline{G} & P & \overline{P} & I & \overline{I} \\
t_T & 0&1&0&0&0&1&0&0&0&0&0&0 \\
t_{T,L} & 0&1&0&0&1&0&0&0&0&0&0&0 \\
t_L & 0&1&0&0&0&1&0&1&0&0&0&0 \\
t_{L,A} & 0&1&0&0&0&1&1&0&0&0&0&0 \\
t_{L,T} & 1&0&0&0&0&0&0&1&0&0&0&0 \\
t_{L,AT} & 1&0&0&0&0&1&1&0&0&0&0&0 \\
t_A & 0&0&0&0&1&0&1&0&0&1&0&0 \\
t_{A,L} & 0&0&0&0&1&0&1&0&0&1&0&0 \\
t_{A,G} & 0&0&0&0&0&1&0&1&1&0&0&0 \\
t_{A,LG} & 0&0&0&0&1&0&0&1&1&0&0&0 \\
t_G & 0&1&0&0&0&1&0&1&1&0&0&0 \\
t_{G,L} & 0&1&0&0&0&1&0&1&1&0&0&0 \\
t_{G,A} & 0&1&0&0&0&1&1&0&0&1&0&0 \\
t_{G,T} & 1&0&0&0&0&1&0&1&0&1&0&0 \\
t_{G,LA} & 0&1&0&0&1&0&1&0&0&1&0&0 \\
t_{G,TL} & 1&0&0&0&1&0&0&1&0&1&0&0 \\
t_{G,TA} & 1&0&0&0&0&1&1&0&0&1&0&0 \\
t_{G,TLA} & 1&0&0&0&1&0&1&0&0&1&0&0 \\
t_{P,P} & 0&0&0&1&0&1&0&0&0&0&0&1 \\
t_{P,LP} & 0&0&0&1&1&0&0&0&0&0&0&1 \\
t_{I,I} & 0&0&0&1&0&1&0&0&0&0&0&1 \\
t_{I,LI} & 0&0&0&1&1&0&0&0&0&0&0&1
\end{array}\right)$$

As shown by Property 11, this PN gives rise to four dead marking matching the stable states, which correspond to the four flower organs.

Property 11. The system has four dead markings:

$$\begin{bmatrix} M_d^1(T) = 0 \\ M_d^1(L) = 0 \\ M_d^1(A) = 1 \\ M_d^1(G) = 0 \\ M_d^1(P) = 0 \\ M_d^1(I) \ = 0 \end{bmatrix} \begin{bmatrix} M_d^2(T) = 0 \\ M_d^2(L) = 0 \\ M_d^2(A) = 1 \\ M_d^2(G) = 0 \\ M_d^2(P) = 1 \\ M_d^2(I) \ = 1 \end{bmatrix} \begin{bmatrix} M_d^3(T) = 0 \\ M_d^3(L) = 0 \\ M_d^3(A) = 0 \\ M_d^3(G) = 1 \\ M_d^3(P) = 0 \\ M_d^3(I) \ = 0 \end{bmatrix} \begin{bmatrix} M_d^4(T) = 0 \\ M_d^4(L) = 0 \\ M_d^4(A) = 0 \\ M_d^4(G) = 1 \\ M_d^4(P) = 1 \\ M_d^4(I) \ = 1 \end{bmatrix}$$

$$Sepals \qquad Petals \qquad Carpels \qquad Stamens$$

The method for proving that these four markings are dead is very similar to that used to prove Property 10 and amounts to show that a set of Boolean constraints involving the Pre matrix is satisfied.

The four stable states corresponding to the four dead markings of Property 11 are reachable from an initial state with genes LEAFY (L) and APETALA 1 (A) ON, all the others being OFF. The reachability can be easily verified through the analysis of the reachability graph, but this method is not tractable for large networks. More generally, the question of the reachability of stable states is related to the notion of circuit functionality. We have shown that dead markings are reachable from any initial valid marking in the case of isolated positive circuits. However, when a positive circuit is embedded in a more complex regulatory network, this behaviour may only occur in specific regions of the logical state space, depending on the functionality domains of the corresponding circuit (work in progress). The general question of finding all the dead markings of a given regulatory Petri net goes well beyond the scope of the present paper and requires further investigation.

6 Conclusions

The combination of a logical approach with the standard Petri net framework offers a powerful set of analytical tools enabling the delineation of specific relationships between the feedback structure and the dynamical properties of complex regulatory systems. Our combined modelling approach encompasses two main steps:

1. A logical processing covers the model specification in terms of a generic regulatory graph, followed by its parameterisation, taking advantage of the flexibility of the definition of the logical parameters; the analysis of circuit functionality can be used here to select specific parameter constraints and/or to derive dynamical insights.
2. The PN corresponding to the resulting parameterised regulatory graph can then be systematically generated, allowing the application of existing algebraic methods to analytically evaluate dynamical properties such as the absence or the presence of deadlocks, the occurrence of specific paths in the reachability graph, etc.

In the case of isolated circuits, we have recovered the main results inferred from the logical framework. More specifically, an isolated functional positive circuit gives rise to two dead markings in the corresponding PN. On the other hand, a functional negative circuit leads to no dead marking but to a cyclical attractor in the corresponding reachability graph. Our approach has been further evaluated through the PN translation of two Boolean regulatory graphs involved in the control of Drosophila cell cycle and in the differentiation of Arabidopsis flower organs, respectively. Here again, we recovered the salient properties found in the original logical model analyses.

The results presented here are encouraging for the analysis of more complex regulatory networks, eventually combining genetic and metabolic interactions. They also point towards a systematic characterisation of the structure of the regulatory Petri nets, in order to derive specific theorems on induced dynamical properties such as liveness, reversibility or reachability. This approach should ease the analysis of large and complex regulatory systems which are difficult to explore through systematic simulations. However, the representation of complex regulatory networks requires the extension of our PN rewriting rules to encompass multi-level logical systems. Finally, it is important to note that the fully asynchronous approach used to generate the logical dynamical graphs covers in fact various (and often incompatible) temporal behaviours. In principle, the distinction between alternative temporal pathways can be forced through the delineation of specific assumptions on transition delays or on priority rules. In this context, the Generalised Stochastic Petri net approach [8] offers a framework enabling the representation of such assumptions taking into account experimental noise.

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