

From Logical Regulatory Graphs to Standard Petri Nets: Dynamical Roles and Functionality of Feedback Circuits

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Abstract. Logical modelling and Petri nets constitute two complementary approaches for the dynamical modelling of biological regulatory networks. Leaning on a translation of logical models into standard Petri nets, we propose a formalisation of the notion of circuit functionality in the Petri net framework. This approach is illustrated with the modelling and analysis of a molecular regulatory network involved in the control of Th-lymphocyte differentiation.

Keywords: genetic regulatory graphs, Petri nets, feedback circuit, discrete dynamics, qualitative analysis.

1 Introduction

Regulatory networks are found at the core of all biological functions, from biochemical pathways, to gene regulation mechanisms, and intercellular 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. The modelling of biological regulatory networks has been addressed using a large variety of formal approaches, from ordinary or partial differential systems, to sets of stochastic equations (for a recent review, see [10]). However, as precise, quantitative information about the shape of regulatory functions, or the values of involved parameters is generally lacking, qualitative approaches are usually more easily deployed.

Our work relies on a qualitative approach which consists in modelling regulatory networks in terms of logical equations, using either Boolean or multi-level variables (see [12], [33] and references therein). The development of logical models for various biological networks has already led to interesting insight into the

relationships between the regulatory network structure (*i.e.*, presence of regulatory feedback circuits) and the corresponding dynamical properties [33]. The generalised logical approach of R. Thomas has been recently implemented in a software tool, GINsim, which enables the biologist to specify a regulatory model and check the qualitative evolution of the system for given initial states [9,19,38]. However, as the number of qualitative states grows exponentially with the number of elements involved in the regulatory network, there is a need for proper mathematical methods to cope with the analysis of larger regulatory networks.

At this stage, it appears interesting to articulate the logical approach with another qualitative approach, namely the Petri net modelling [22,28]. Indeed, Petri nets (PN) offer a mathematical framework to model, analyse and simulate the dynamical behaviour of large systems. As a first step in this direction, we have recently proposed a translation of logical regulatory models into specific *regulatory Petri nets*, focusing on the Boolean case [8]; this has been extended to the multilevel case in [7]. This bridge between the two formalisms should help us to simultaneously exploit the corresponding analytical and simulation tools.

Petri nets have been successfully applied to the modelling and the analysis of metabolic networks [24,16,18,15]. As emphasised in [36], one can draw extensive relationships between the traditional biochemical modelling and Petri net theory. In particular, the stoichiometry matrix of a metabolic network corresponds to the Petri net incidence matrix. One can identify clear correspondences between qualitative properties of the dynamics of biological networks and classical PN behavioural properties. For example, a dead marking represents a stable state of the system, while transition invariants correspond to cyclical trajectories.

Although the kinetic parameters are generally not precisely accessible, most of the works related to Petri net approaches for the modelling of biological networks concentrate on quantitative aspects. Leaning on simulations, these works refer to several extensions of Petri nets, including hybrid PN, where places and transitions are either discrete, or continuous [2,13,6,20,11].

In the case of genetic regulatory networks, the PN representation is not so natural because the semantics associated with the interactions between components varies. Furthermore, regulators are usually not consumed during the regulatory processes (while reactants are transformed into products by chemical reactions). In this paper, regulatory interactions are considered at a qualitative abstraction level, where the details of the regulation processes are not taken into account.

Amid the promising applications of Petri net theory to biological systems, we have proposed a systematic rewriting of Boolean regulatory graphs into a Petri net formalism in [8], and then extended this procedure to the multivalued case in [7].

The paper is organised as follows. First, we define the *Boolean Regulatory Petri Nets* (BRPN) which correspond to Boolean regulatory models. Then, after recalling the properties of isolated regulatory circuits, we introduce a formal definition of the notion of regulatory feedback circuit functionality. Next, these definitions are applied to a regulatory network involved in the control of T-lymphocyte differentiation. Finally, conclusions and prospects are proposed.

2 Boolean Regulatory Petri Nets

In this section, we recall the definition of the Petri net corresponding to a Boolean regulatory graph, *i.e.*, the PN whose dynamics simulates the dynamical behaviour of the underlying genetic regulatory network (for further detail, see [8]).

First, we briefly describe logical regulatory graphs in the Boolean case: a gene can be ON or OFF (for more details on the formalism in the multivalued case, see [9]).

A *regulatory graph* is a directed graph representing interactions between genes g_1, \dots, g_n . Each interaction involves two genes (or other kinds of molecular components), the source and the target. As one gene can be the target of several interactions, we define, for each gene g_i , the set $\mathcal{I}(i)$, called *input of g_i* , which contains the source genes of all interactions targeting g_i . For each gene g_i , a *parameter* $K_i(X)$ is defined for each subset X of $\mathcal{I}(i)$. The value of this parameter gives the level to which g_i tends when X is the set of the sources of the incoming interactions which are operating (we consider that an interaction is operating when its source gene is ON). In the Boolean case, these parameters take their values in $\{0, 1\}$.

More formally, a regulatory graph is a triple $(\mathcal{G}, \mathcal{A}, \mathcal{K})$ which consists in:

- a set of nodes $\mathcal{G} = \{g_1, \dots, g_n\}$,
- a set of arcs \mathcal{A} , which leads to the specification of the sets $\mathcal{I}(i)$, defining the sources of interactions towards g_i , $\forall i \in \{1, \dots, n\}$,
- a set of parameters $\mathcal{K} = \{K_i(X), i = 1, \dots, n, X \subseteq \mathcal{I}(i)\}$.

For a given regulatory graph of n genes, we can now address its dynamical behaviour. A *state* of the system is defined as the n -dimensional vector of the expression levels of the n genes. We further define a *state transition graph* where nodes represent states and arcs represent transitions between states. For a specific initial state, the corresponding state transition graph defines all the possible trajectories of the system from the selected initial conditions. We can also consider the *whole* state transition graph consisting of all the 2^n states. We thus face a classical combinatorial explosion problem: the size of the whole state transition graph exponentially increases with the number of genes.

In the sequel, we briefly describe our translation of Boolean regulatory graphs into standard Petri nets.

Basic Definitions and Properties: Consider a Boolean regulatory graph $\mathcal{R} = (\mathcal{G}, \mathcal{A}, \mathcal{K})$. We shall define the corresponding Petri net with the following properties:

- To each gene correspond two places g_i, \bar{g}_i , $i \in \{1, \dots, n\}$, such that the sum of tokens in places g_i and \bar{g}_i equals 1 (they are then complementary). The position of the token in g_i or \bar{g}_i indicates whether the gene is ON or OFF. The set of places P thus contains $2n$ elements: $P = \mathcal{G} \cup \bar{\mathcal{G}}$, with $\bar{\mathcal{G}} \triangleq \{\bar{g}_1, \dots, \bar{g}_n\}$.
- To each parameter $K_i(X)$, where $i \in \{1, \dots, n\}, X \subseteq \mathcal{I}(i)$, corresponds a transition t_X^i . The transition t_X^i is enabled as soon as all places of the set X

AND all complementary places of the set $\mathcal{I}(i) \setminus X$, the complementary set of X in $\mathcal{I}(i)$, are marked.

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

- $P = \mathcal{G} \cup \overline{\mathcal{G}} = \{g_1, \overline{g_1}, \dots, g_n, \overline{g_n}\}$ is the set of places.
- $T = \{t_X^i, i = 1, \dots, n, X \subseteq \mathcal{I}(i)\}$ is the set of transitions.
- $Pre : P \times T \rightarrow \{0, 1\}$ is the mapping defining arcs between places and transitions (Pre-conditions).
- $Post : T \times P \rightarrow \{0, 1\}$ 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_X^i , the only terms to be defined (i.e., all other terms equal zero) are:

$$Pre(g_i, t_X^i) = Post(t_X^i, \overline{g_i}) = 1 - K_i(X), \quad (1)$$

$$Pre(\overline{g_i}, t_X^i) = Post(t_X^i, g_i) = K_i(X), \quad (2)$$

$$Pre(g_j, t_X^i) = Post(t_X^i, g_j) = 1 \quad \forall g_j \in X, \quad (3)$$

$$Pre(\overline{g_j}, t_X^i) = Post(t_X^i, \overline{g_j}) = 1 \quad \forall g_j \in \mathcal{I}(i) \setminus X. \quad (4)$$

2. Case $g_i \in \mathcal{I}(i)$ (g_i is a self-regulator). For a given transition t_X^i ,
 - 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:

$$Pre(g_i, t_X^i) = Post(t_X^i, \overline{g_i}) = 1, \quad (5)$$

$$Pre(g_j, t_X^i) = Post(t_X^i, g_j) = 1 \quad \forall g_j \in X, g_j \neq g_i, \quad (6)$$

$$Pre(\overline{g_j}, t_X^i) = Post(t_X^i, \overline{g_j}) = 1 \quad \forall g_j \in \mathcal{I}(i) \setminus X. \quad (7)$$

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

$$Pre(\overline{g_i}, t_X^i) = Post(t_X^i, g_i) = 1, \quad (8)$$

$$Pre(g_j, t_X^i) = Post(t_X^i, g_j) = 1 \quad \forall g_j \in X, \quad (9)$$

$$Pre(\overline{g_j}, t_X^i) = Post(t_X^i, \overline{g_j}) = 1 \quad \forall g_j \in \mathcal{I}(i) \setminus X, g_j \neq g_i. \quad (10)$$

Equations (1)-(2) state that if the parameter $K_i(X)$ equals 1, g_i is an input and $\overline{g_i}$ an output of the corresponding transition t_X^i . In other words, there can be a decrease of the level of g_i if it is already present. Symmetrically, if $K_i(X) = 0$, then $\overline{g_i}$ is an input and g_i is an output of the corresponding transition t_X^i . Equations (3)-(4), (6)-(7) and (9)-(10) state that the elements contributing to the combination of interactions involved in $K_i(X)$ (i.e., which are in X) constitute “side conditions” of the corresponding transitions (represented by “test arcs”).

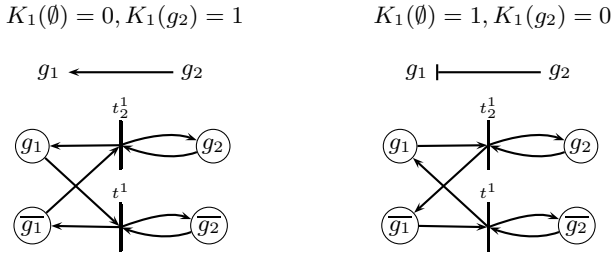


Fig. 1. Modelling of the activation of g_1 by g_2 (left) and of the inhibition of g_1 by g_2 (right). **Top:** regulatory graph representation and logical parameters (K 's) for each type of regulation. $K_1(\emptyset)$ represents the basal expression of gene g_1 , *i.e.*, in the absence of the regulatory product of gene g_2 , whereas $K_1(g_2)$ represents the expression of g_1 in the presence of the regulatory product. **Bottom:** corresponding Petri net representation, with two places for each gene. Transition t^1 corresponds to parameter $K_1(\emptyset)$, while t^1_2 corresponds to $K_1(g_2)$.

As an illustration, let us consider the simplest case where a gene g_2 regulates a gene g_1 . The two interesting situations occur when a change of the level of g_2 leads to a change of the level of g_1 ; this interaction is called *activation* if the presence of g_2 implies the presence of g_1 and conversely the absence of g_2 implies the absence of g_1 . The opposite situation corresponds to an *inhibition*.

Figure 1 illustrates the BRPNs corresponding to an activation (left) and to an inhibition (right) between two genes, respectively (to simplify the notation, we have denoted $K_i(\{g_j\})$ by $K_i(g_j)$ and $t^i_{\{g_j, \dots, g_k\}}$ by $t^i_{j, \dots, k}$).

Given a regulatory graph involving n genes, the corresponding BRPN has exactly $2n$ places and up to $\sum_{i=1, \dots, n} 2^{|\mathcal{I}(i)|}$ transitions. This number of transitions can be lowered applying two kinds of reductions. Remark 1 deals with the first reduction related to self-regulations, already considered in Definition 1, case 2. The second class of reductions regards the possible simplification of the logical formulæ associated to each gene (see Remark 2).

Remark 1. Let g_i be a self-regulator; there should be one transition t^i_X for each parameter $K_i(X)$. But two cases do not lead to any change on g_i : when $g_i \notin X$ (*i.e.*, g_i is absent) and $K_i(X) = 0$, and when $g_i \in X$ (*i.e.*, g_i is present) and $K_i(X) = 1$. The resulting transitions are never enabled and are therefore omitted.

Remark 2. Each set $X \subseteq \mathcal{I}(i)$, $i = 1, \dots, n$ defines a logical formula which is a conjunction of literals $[x_j = 1]$ for all $g_j \in \mathcal{I}(i)$ and $\neg[x_j = 1]$ for all $g_j \notin \mathcal{I}(i)$. Now, for a given gene g_i , consider all the logical parameters having the same value x (0 or 1). They define a disjunction of conditions (the corresponding sets $X \in \mathcal{I}(i)$) under which g_i should tend to its level x . This formula is a disjunctive normal form (DNF, *i.e.*, a disjunction of conjunctions of literals). Such DNF can often be simplified, resulting in a reduction of the number of transitions to consider in the corresponding Petri net. One approach to simplify DNF uses

ordered binary decision diagrams as introduced in [5]. An illustration of this type of reduction is provided in Section 4.

Although the graphical representation of a BRPN is more complex than the corresponding regulatory graph (indeed the BRPN represents the regulatory graph together with its parameterisation), several analytical tools available for the standard PN framework should be useful in our context.

Let us introduce the following notations for all $i \in \{1, \dots, n\}$:

- $\widehat{K}_i(X) \triangleq 2K_i(X) - 1$;
- d_i the number of transitions for g_i ($d_i \leq 2^{\#\mathcal{I}(i)}$).
- X_i^j the j th subset of $\mathcal{I}(i)$ (for an arbitrary numbering).

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

$$C = \begin{pmatrix} \boxed{D_1} & 0 & \dots & 0 \\ 0 & \boxed{D_2} & \dots & 0 \\ \dots & \dots & \dots & \dots \\ \dots & \dots & \dots & \dots \\ 0 & 0 & \dots & \boxed{D_n} \end{pmatrix} \quad \text{with} \quad D_i \triangleq \begin{pmatrix} \widehat{K}_i(X_i^1) & \dots & \widehat{K}_i(X_i^{d_i}) \\ -\widehat{K}_i(X_i^1) & \dots & -\widehat{K}_i(X_i^{d_i}) \end{pmatrix}.$$

Note that matrix C does not totally reflect the incidence relations of the BRPN which is not pure (it contains a number of test arcs).

Definition 2. Given a regulatory Petri net $\mathbf{N}(\mathcal{R}) = (P, T, Pre, Post)$, a **valid marking** $M : P \rightarrow \{0, 1\}$ corresponds to a state of the Boolean regulatory graph \mathcal{R} which verifies: $\forall g_i \in \mathcal{G}, M(g_i) = 1 - M(\overline{g_i})$.

In the sequel, we will only consider valid markings.

The *Boolean state transition graph* is isomorphic to the *reachability graph* of the corresponding BRPN. Given a regulatory graph with n nodes, a state S in the state transition graph is a n -dimensional vector giving the state of each gene (expressed or not), while a valid marking in the corresponding BRPN is a $2n$ -dimensional vector.

There exists an edge from state S_1 to state S_2 in the whole state transition graph related to a Boolean regulatory graph $\mathcal{R} = (\mathcal{G}, \mathcal{A}, \mathcal{K})$, iff there exists an enabled transition t in the associated BRPN such that M_1 verifies $M_1[t]M_2$ (t is enabled by M_1 and its firing leads to the marking M_2) with, for all $i = 1, \dots, n$,

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

Note that usually, in the PN formalism, the (reachability) marking graph is defined for a specific initial marking. Here, we consider the *whole* marking graph of a Petri net corresponding to the whole state transition graph associated to a regulatory graph (containing all possible states).

3 Dynamical Role of Regulatory Circuits

For complex regulatory networks, R. Thomas has enounced rules binding the dynamical behaviour to the presence of specific types of circuits. More precisely, he has conjectured that a necessary condition for multistationarity is the presence of a positive circuit (*i.e.*, containing an even number of inhibitions), whereas a necessary condition for homeostasis and/or sustained, stable oscillations is the presence of a negative circuit (with an odd number of inhibitions) (cf. [33] and references therein). These rules have already been formally stated and partly demonstrated whithin different formalisms [32,14,30,3,31,26,27].

In what follows, we first derive a general Petri net formulation for these two classes of isolated circuits and check that their dynamical properties depend on their signs (section 3.1). Then, we focus on the notion of functionality of circuits (section 3.2). Indeed, the presence of a circuit is not sufficient to give rise to the corresponding dynamical property, it has to be moreover *functional*. We propose here a formal definition of this notion (which has been introduced in [29] in the multilevel degenerated case), and an algorithm to test if a circuit is functional in specific regions of the Boolean state space.

3.1 Isolated Regulatory Circuits

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 , the length of the circuit, *i.e.*, $i + n = i$).

Let $\mathcal{C} = (\mathcal{G}, \mathcal{K})$ be a regulatory circuit, with $\mathcal{G} = \{g_1, \dots, g_n\}$ and $\mathcal{K} = \{K_i(\emptyset), K_i(g_{i-1})\}_{i=1, \dots, n}$, recalling that $\mathcal{I}(i) = \{g_{i-1}\}$ (cf. [25] for more details). In this simpler context, when we consider the interaction from g_i to g_{i+1} , the values of parameters for which the circuit is *functional* (see Section 3.2) are $K_{i+1}(\emptyset) = 0$ and $K_{i+1}(g_i) = 1$ (we say that this interaction is an activation), or $K_{i+1}(\emptyset) = 1$ and $K_{i+1}(g_i) = 0$ (the interaction is an inhibition).

The corresponding regulatory Petri nets $\mathbf{N}(\mathcal{C})$ have a well defined structure, see Figure 2.

In [25], we have proved that an isolated functional positive circuit generates two stable states, which are *mirroring* each other (a component is ON in one state iff it is OFF in the other state), and 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. These results can be restated in the Petri net formalism (see [8]):

- Property 1.* – Let $\mathbf{N}(\mathcal{R})$ 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 other valid marking.
- Let $\mathbf{N}(\mathcal{R})$ be a regulatory Petri net corresponding to a (functional) negative regulatory circuit and E be the set of all valid markings which enable exactly one transition.

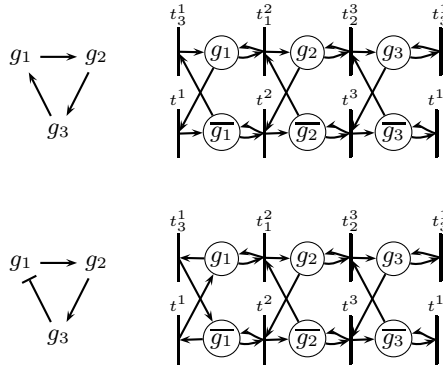


Fig. 2. Three-element regulatory circuits and the corresponding Petri nets. Upper row: positive circuit. Lower row: negative circuit. Note that transitions t^1 and t_3^1 are repeated, illustrating the ring structure of the net.

1. Any valid marking of the net is not dead.
2. E has $2n$ elements and is organised as a cycle (defining a livelock).
3. Each marking in E is reachable from any valid marking.

3.2 Functionality of Regulatory Circuits

Thomas’ conjectures only refer to necessary conditions. Indeed, in many cases, although circuits do exist in the regulatory graph, we do not observe the expected dynamical properties, or at least not everywhere in the phase space.

Consider the following example:

$$\mathcal{R} = (\mathcal{G}, \mathcal{A}, \mathcal{K}) \text{ with } \mathcal{G} = \{x, y, z\}, \mathcal{A} = \{(x, y), (y, x), (z, y)\}, \text{ and}$$

$$\mathcal{K} = \{K_x(\emptyset) = 0, K_x(y) = 1, K_y(\emptyset) = 0, K_y(x) = K_y(z) = K_y(x, z) = 1\}.$$

The circuit $x \rightleftharpoons y$ could be embedded in a larger graph, but, for the sake of conciseness, we represent the external influence upon this circuit by the input variable z , which acts here only on y (cf. Figure 3).

For the parameter values selected, when the variable z is set to 0, multistationarity appears, but when z is set to 1, the dynamical behaviour is degenerated (see Figure 3). Indeed, when z is present, the state of x (its presence or absence) has no influence on y ($K_y(z) = K_y(x, z)$). We say that the interaction $x \rightarrow y$ is not functional when z is present (and, *a fortiori*, the circuit $x \rightleftharpoons y$ is not functional).

In the sequel, we present a formal definition of the notion of functionality, for one interaction and, more generally, for a circuit. This definition is local, *i.e.*, functionality is defined *within a specific context*, since the functionality of a circuit embedded in a more complex regulatory network may depend on the effect of some combination of incoming interactions. These definitions are proposed here in the Petri net framework, but they easily translate into other discrete modelling frameworks.

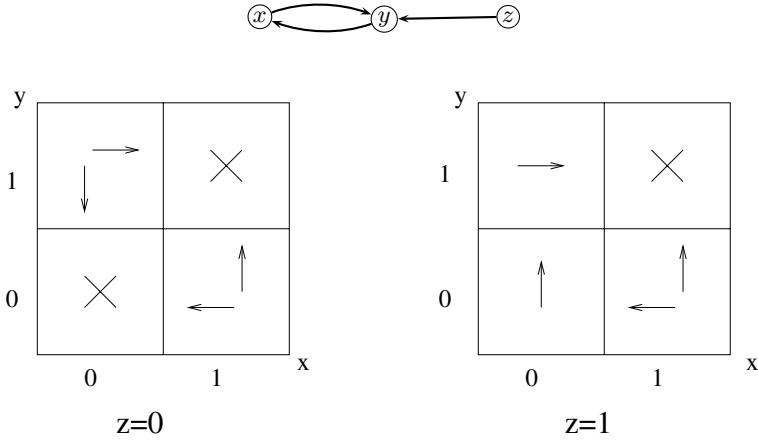


Fig. 3. Top: Two-element regulatory circuit $x \rightleftharpoons y$ submitted to an external influence represented by node z . Bottom: Dynamical behaviour of the regulatory circuit depending on the value of the input z . When z is OFF (left), the system reaches two stable states (boxes [00] and [11]); when z is ON (right), the system exhibits a unique stable state (box [11]).

Definition 3. Functionality of an interaction Let $\mathcal{R} = (\mathcal{G}, \mathcal{A}, \mathcal{K})$ be a Boolean regulatory graph, and $\mathbf{N}(\mathcal{R}) = (P, T, Pre, Post)$ its associated Boolean regulatory Petri net. Let us consider an interaction $A : g_i \longrightarrow g_j$ of the graph \mathcal{R} , and (X, X') a partition of $\mathcal{I}(j) \setminus \{g_i\}$ into two sets, possibly empty, of g_j regulators. When

$$Post(t_X^i, g_j) \neq Post(t_{X \cup \{g_i\}}^i, g_j),$$

we say that A is **functional in the context** (X, X') . Then, its **functionality marking set** is denoted $\mathcal{S}_A \subseteq \{M : P \rightarrow \{0, 1\}\}$, and contains all the valid markings for which A is functional:

$$\mathcal{S}_A = \bigcup_{(X, X')} \{M : P \rightarrow \{0, 1\} \mid M(g) = 1 \ \forall g \in X, \text{ and } M(g) = 0 \ \forall g \in X'\},$$

where the union is taken over all the partitions (X, X') of $\mathcal{I}(j) \setminus \{g_i\}$.

Therefore, an interaction is said to be functional within some context if the modification of the level of expression of its source leads to a change of the level of its target. The functionality of a circuit thus depends on the existence of a context for which all the transitions of this circuit are functional.

Definition 4. Functionality of a circuit A marking M belongs to the **functionality marking set** of a circuit \mathcal{C} if it belongs to the intersection $\mathcal{S}_{\mathcal{C}}$ of the functionality marking sets of all interactions of \mathcal{C} .

If such a marking exists ($\mathcal{S}_{\mathcal{C}} \neq \emptyset$), then $\mathcal{S}_{\mathcal{C}}$ is the **functionality marking set** of the circuit \mathcal{C} .

Note that if $\mathcal{S}_{\mathcal{C}} = \emptyset$, we say that the circuit is not functional.

We propose hereafter an algorithm to determine the functionality marking set of a given circuit \mathcal{C} embedded in a Boolean regulatory graph \mathcal{R} .

Let $\mathbf{N}(\mathcal{R}) = (P, T, Pre, Post)$ be the BRPN associated to \mathcal{R} . Assume that \mathcal{C} consists in p interactions $I^i : g_i \rightarrow g_{i+1}$, for $i = 1, \dots, p$ (notation *modulo* p). For each i , apply the following procedure:

1. Determine $\text{Trans}(g_{i+1})$, the set of all transitions t_X^{i+1} , for all $X \subseteq \mathcal{I}(i+1)$:
 $\text{Trans}(g_{i+1}) = \{t \in T, C(g_{i+1}, t) = 1 \text{ or } C(\overline{g_{i+1}}, t) = 1\}$, where C is the incidence matrix of $\mathbf{N}(\mathcal{R})$.
2. Determine the pairs of transitions $(t, t') \in \text{Trans}(g_{i+1})$ such that: $Pre(g_i, t) \neq Pre(g_i, t')$, and for all g different from g_i and g_{i+1} , $Pre(g, t) = Pre(g, t')$.
3. For each such pair (t, t') , test whether the interaction is functional, *i.e.*, $Post(t, g_{i+1}) \neq Post(t', g_{i+1})$. In this case,
 - The context of functionality (X, X') is given by:

$$X = \{g \in \mathcal{I}(i+1) \setminus \{g_i\} \text{ s.t. } Pre(g, t) = Pre(g, t') = 1\}$$

$$X' = \{g \in \mathcal{I}(i+1) \setminus \{g_i\} \text{ s.t. } Pre(g, t) = Pre(g, t') = 0\}.$$
 - We define the set $\mathcal{S}_{t, t'}$ of markings M such that for each $g \in X$, $M(g) = 1$ and for each $g \in X'$, $M(g) = 0$.
4. Define \mathcal{S}_i to be the union of all the $\mathcal{S}_{t, t'}$.

Finally, the functionality marking set of \mathcal{C} is the intersection of the functionality marking sets \mathcal{S}_i , $i = 1, \dots, p$.

In conclusion, the functionality of a circuit has to be considered for all different possible contexts. This relates to the notion of "local graph" derived from the Jacobian matrix in the case of ODE systems (see [31] and [26] for the Boolean case).

4 Application to the Modelling of Th-Lymphocyte Differentiation

4.1 Introducing Th-Cell Differentiation

The vertebrate immune system contains diverse cell populations, like antigen presenting cells, natural killer cells, and B and T lymphocytes. Among the latter, CD4+ T helper lymphocytes (Th), upon receiving an appropriate antigenic stimulus, can further differentiate into T-helper 1 (Th1) or Th2 cells, which enable cell mediated immunity and humoral responses, respectively. Th1 and Th2 cells can be distinguished according to their pattern of cytokine secretion. Immune responses biased towards the Th1 phenotype may result in autoimmune diseases, while enhanced Th2 responses can lead to allergic reactions [1,23]. Various mathematical models have been proposed for the differentiation, activation and proliferation of Th-lymphocytes, but most of these models focus on interactions between immunological cell populations at a macroscopic level [4,35]. Other model analyses aim at understanding the mechanism of the generation of antibody and T-cell receptors diversity, as well as the dynamical properties of the large networks defined by the interactions between cytokines [17] or between immunoglobulins (see *e.g.* [34]).

The Boolean model considered hereafter is a simplification of the multi-valued logical model defined and analysed in [21], where the biological justification of

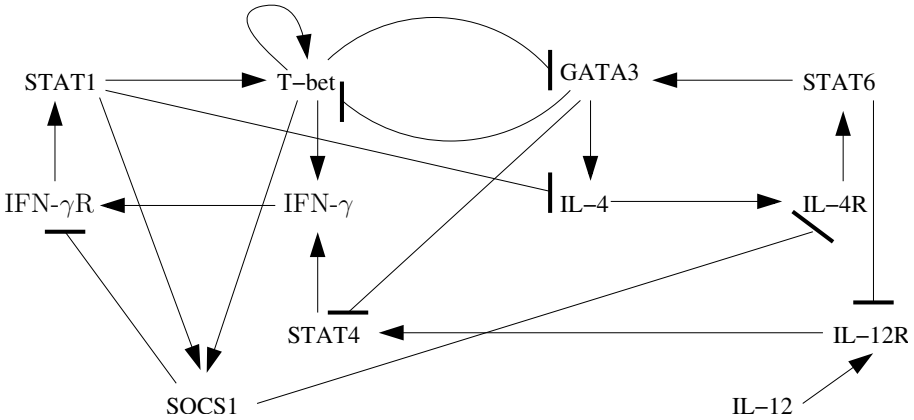


Fig. 4. Regulatory graph of the network controlling Th lymphocyte differentiation. The nodes represent transcription regulatory factors (T-bet, GATA-3), signaling transduction factors (STAT1, STAT4, STAT6, SOCS-1), lymphokines (IFN- γ , IL-4, IL-12) and receptors (IFN- γ R, IL-4R, IL-12R), whereas the edges represent activations (arrows) or inhibitions (blunt arrows) between these components.

the set of interactions is given, accompanied with an extensive list of references to the immunological literature.

Here, we focus on the delineation of the multi-stability properties of the network as a result of the functionality of specific positive regulatory circuits found in the corresponding regulatory graph. Furthermore, we show that this type of feedback circuit analysis can be processed directly in the context of the Petri net formalism, relying on the sole analysis of the *Pre* and *Post* matrices.

Given the regulatory graph in Figure 4, and the set of parameters in Table 1, we construct the associated BRPN $(P, T, Pre, Post)$ with:

- the set of places $P = \{g_1, \overline{g_1}, \dots, g_{12}, \overline{g_{12}}\}$ (the numbering of the places is defined in Table 1),
- the set of transitions $T = \{t_X^i, i = 1 \dots 12, X \subset \mathcal{I}(i)\}$ (where t_X^i denotes the transition corresponding to parameter $K_i(X)$).

The complete definition of the BRPN corresponding to the regulatory graph is defined through the matrices *Pre* (of size (24×39)) and *Post* (of size (39×24)) (not shown). Applying the reduction rules described in Remark 1 and Remark 2, the number of transitions can be reduced to 31. To illustrate the simplification of the resulting BRPN, let consider the case of IFN- γ , we have $K_1(9) = K_1(11) = K_1(9, 11) = 1$. These parameters lead to the definition of 3 transitions. In this case, it is easy to simplify the Boolean function: IFN- γ is called to increase if one of its activator is present, whatever the state of the other regulator; therefore, 2 transitions can account for this situation. Let now consider the case of T-bet, which is a self-regulator and have 2 other regulators, namely STAT1 and GATA3. As T-bet is subject to 3 interactions, $8 (= 2^3)$ parameters are to be defined, 5 of them are zero (see Table 1). First, applying Remark 1 for the non-zero

Table 1. For each gene of the regulatory graph presented in Figure 4: the non-zero parameters and the corresponding expression level in each stable state (S_1, S_2, S_3). As $IL - 12$ acts as an input, the only parameter to be defined is its *base value* ($K_{12}(\emptyset)$ which is set to 0).

Genes	Non-zero parameters	Stable states		
		S_1	S_2	S_3
IFN- γ (1)	$K_1(9) K_1(11) K_1(9, 11)$	0	0	1
IL-4 (2)	$K_2(12)$	0	1	0
IL-12 (3)		0	0	0
IFN- γ R (4)	$K_4(1)$	0	0	0
IL-4R (5)	$K_5(2)$	0	1	0
IL-12R (6)	$K_6(3)$	0	0	0
STAT1 (7)	$K_7(4)$	0	0	0
STAT6 (8)	$K_8(5)$	0	1	0
STAT4 (9)	$K_9(6)$	0	0	0
SOCS1 (10)	$K_{10}(7) K_{10}(11) K_{10}(7, 11)$	0	0	1
T-bet (11)	$K_{11}(7) K_{11}(11) K_{11}(7, 11)$	0	0	1
GATA-3 (12)	$K_{12}(8)$	0	1	0

parameters, we can dismiss t_{11}^{11} and $t_{7,11}^{11}$. Then, for the situations conducing to a decrease of the level of T-bet, again, we can dismiss all transitions corresponding to conditions where T-bet is absent (Remark 1).

The regulatory graph of Figure 4 contains 18 regulatory circuits (15 positive, three negative), involving from one to ten elements. Using the logical formalism, it can be shown that nine of these circuits (seven positive, two negative) are functional. Still in the context of the logical approach, three stable states are found. In what follows, we show that these three stable states actually correspond to “dead markings” in the corresponding BRPN, and we apply the procedure described in Section 3.2 to one particular circuit.

4.2 Stable States and Their Biological Interpretation

Using the logical approach, it can be shown that the system encompasses the three stable states included in Table 1. The first stable state (S_1) corresponds to the virgin Th cells, whereas the second and third stable states correspond to Th2 and Th1 differentiated lymphocytes, respectively. We can easily check that these stable states correspond to dead markings for the corresponding BRPN, verifying that: $\forall t \in T, \exists p \in P$ s.t. $Pre(p, t) = 1$ and $M(p) = 0$. We will only give here some hint of the proof:

- For S_1 , where all genes are OFF ($S_1(g_i) = 0, i = 1, \dots, 12$), it is clear that no transition is enabled because of the matrix Pre which verifies: $\forall t \in T, \exists g$ s.t. $Pre(g, t) = 1$.
- For S_2 , $S_2(g_2) = S_2(g_4) = S_2(g_8) = S_2(g_{12}) = 1$ whereas $S_2(g_i) = 0$ for the other genes. $S_2(g_2) = 1$ could enable $t^2, t_7^2, t_{7,12}^2, t_2^5$ and $t_{2,10}^5$, but:

$$\begin{aligned}
Pre(\overline{g_{12}}, t^2) &= Pre(\overline{g_{12}}, t_7^2) = 1 \quad \text{and} \quad S_2(g_{12}) = 1 \quad (\Leftrightarrow S_2(\overline{g_{12}}) = 0), \\
Pre(g_7, t_7^2) &= 1 \quad \text{and} \quad S_2(g_7) = 0, \\
Pre(g_5, t_2^5) &= 1 \quad \text{and} \quad S_2(g_5) = 0, \\
Pre(g_{10}, t_{2,10}^5) &= 1 \quad \text{and} \quad S_2(g_{10}) = 0.
\end{aligned}$$

Consequently, no transition is enabled under the marking S_2 .

- A similar reasoning can be developed for S_3 .

The logical approach is well suited to determine the set of stable states of a regulatory network (generating the "whole" state transitions graph when it is feasible, or using a constraint-programming approach). In the PN framework, we may be also able to determine the set of dead markings, independently of the initial conditions, using model-checking or pure algebraic approaches.

Once, the dead markings are determined, it is necessary to check their reachability from specific initial conditions. We have performed such analyses with INA (Integrated Net Analyzer [37] and obtained meaningful trajectories (see [21]). From an initial marking corresponding to a virgin cell state (S_1) but with IL-4 present (simulating the effect of adding IL4 to the extracellular medium), the resulting marking graph contains 14 markings including the two reachable dead markings S_1 and S_3 . Now, with an initial state corresponding to a virgin cell state but with IFN- γ present, the marking graph contains 24 markings, including the two reachable dead markings S_1 and S_2 .

4.3 Regulatory Circuits: Dynamical Roles and Functionality Constraints

To illustrate the procedure defined in Section 3.2, let us consider the following positive circuit: $\mathcal{C} = [\text{IL-4}, \text{IL-4R}, \text{STAT6}, \text{GATA-3}]$. In order to determine the functionality marking set of this circuit, we perform the first step of the procedure, on each of the four interactions defining \mathcal{C} , successively. This leads to the selection of the following submatrices of *Pre* (Table 2) and *Post* (Table 3).

Note that the set of genes to be considered encompasses not only the four genes of \mathcal{C} (IL-4, IL-4R, STAT6, GATA-3), but also the genes exerting inputs on these genes (STAT1, SOCS1, T-bet).

Following the procedure, we now need to apply steps 1-4 to each interaction of the circuit \mathcal{C} (we denote \mathcal{S}_i the functionality marking set of the interaction targeting the node i). We limit ourselves here to their applications to the interaction from IL-4 (2) to IL-4R (5). Step 1 of the procedure leads to the definition of the set $\text{Trans}(\text{IL} - 4\text{R}) = \{t^5, t_2^5, t_{10}^5, t_{2,10}^5\}$. Then, step 2 results in the selection of the pairs (t^5, t_2^5) and $(t_{10}^5, t_{2,10}^5)$. Proceeding with steps 3 and 4:

- For the pair (t^5, t_2^5) , we have $Post(t^5, g_5) \neq Post(t_2^5, g_5)$, and the interaction is thus functional in the context $X = \emptyset$ and $X' = \{g_{10}\}$. The corresponding functionality marking set is $\mathcal{S}_2 = \{M : P \rightarrow \{0, 1\} \text{ s.t. } M(g_{10}) = 0\}$.
- For the second pair, we have $Post(t_{10}^5, g_5) = Post(t_{2,10}^5, g_5)$. Consequently, these transitions add no further marking to the current functionality marking set.

Table 2. Sub-matrix Pre for the circuit $\mathcal{C} = [\text{IL-4}, \text{IL-4R}, \text{STAT6}, \text{GATA-3}]$ and its inputs

	t^2	t_7^2	t_{12}^2	$t_{7,12}^2$	t^5	t_2^5	t_{10}^5	$t_{2,10}^5$	t^8	t_5^8	t^{12}	t_8^{12}	t_{11}^{12}	$t_{8,11}^{12}$
IL-4 (2)	1	1	0	1	0	1	0	1	0	0	0	0	0	0
	0	0	1	0	1	0	1	0	0	0	0	0	0	0
IL-4R (5)	0	0	0	0	1	0	1	1	0	1	0	0	0	0
	0	0	0	0	0	1	0	0	1	0	0	0	0	0
STAT1 (7)	0	1	0	1	0	0	0	0	0	0	0	0	0	0
	1	0	1	0	0	0	0	0	0	0	0	0	0	0
STAT6 (8)	0	0	0	0	0	0	0	0	1	0	0	1	0	1
	0	0	0	0	0	0	0	0	0	1	1	0	1	0
SOCS1 (10)	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	0	0	0	0	1	1	0	0	0	0	0	0	0	0
T-bet (11)	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	0	0	0	0	0	0	0	0	0	0	1	1	0	0
GATA-3 (12)	0	0	1	1	0	0	0	0	0	0	1	0	1	1
	1	1	0	0	0	0	0	0	0	0	0	1	0	0

Table 3. Sub-matrix $Post^T$ for the circuit $\mathcal{C} = [\text{IL-4}, \text{IL-4R}, \text{STAT6}, \text{GATA-3}]$ and its inputs

	t^2	t_7^2	t_{12}^2	$t_{7,12}^2$	t^5	t_2^5	t_{10}^5	$t_{2,10}^5$	t^8	t_5^8	t^{12}	t_8^{12}	t_{11}^{12}	$t_{8,11}^{12}$
IL-4 (2)	0	0	1	0	0	1	0	1	0	0	0	0	0	0
	1	1	0	1	1	0	1	0	0	0	0	0	0	0
IL-4R (5)	0	0	0	0	0	1	0	0	0	1	0	0	0	0
	0	0	0	0	1	0	1	1	1	0	0	0	0	0
STAT1 (7)	0	1	0	1	0	0	0	0	0	0	0	0	0	0
	1	0	1	0	0	0	0	0	0	0	0	0	0	0
STAT6 (8)	0	0	0	0	0	0	0	0	0	1	0	1	0	1
	0	0	0	0	0	0	0	0	1	0	1	0	1	0
SOCS1 (10)	0	0	0	0	0	0	1	1	0	0	0	0	0	0
	0	0	0	0	1	1	0	0	0	0	0	0	0	0
T-bet (11)	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	0	0	0	0	0	0	0	0	0	0	1	1	0	0
GATA-3 (12)	0	0	1	1	0	0	0	0	0	0	0	1	0	0
	1	1	0	0	0	0	0	0	0	0	1	0	1	1

The same reasoning can be followed for the three other interactions of the circuit \mathcal{C} , leading to: $\mathcal{S}_5 = \{M : P \rightarrow \{0, 1\}\}$ (all possible valid markings), $\mathcal{S}_8 = \{M : P \rightarrow \{0, 1\} \text{ s.t. } M(g_{11}) = 0\}$, and $\mathcal{S}_{12} = \{M : P \rightarrow \{0, 1\} \text{ s.t. } M(g_7) = 0\}$.

Finally, the functionality marking set of the circuit \mathcal{C} is the intersection of the four resulting marking sets: $\mathcal{S}_{\mathcal{C}} = \mathcal{S}_2 \cap \mathcal{S}_5 \cap \mathcal{S}_8 \cap \mathcal{S}_{12} = \{M : P \rightarrow \{0, 1\} \text{ s.t. } M(g_7) = M(g_{10}) = M(g_{11}) = 0\}$.

In conclusion, the positive circuit \mathcal{C} is functional provided that the products STAT1 (g_7), SOCS1 (g_{10}) and T-bet (g_{11}) are absent. It is interesting to note that, if we switch the value of the parameter $K_2\{12\}$ to zero, this positive circuit

loses its functionality. This loss of functionality results in the loss of the stable state Th2 (S_2).

5 Conclusions and Prospects

In this paper, we have described a systematic way to translate Boolean models of genetic regulatory networks into standard Petri nets (see also [8]). We have particularly focused on the delineation of a procedure to determine the marking sets insuring the functionality of the feedback circuits found in genetic regulatory graphs. This procedure leads to interesting insights into the temporal behaviour of the modelled system, as it is well established that regulatory circuits are found at the origin of fundamental dynamical properties, such as multistability or homeostasis. The functionality marking set of a circuit defines the sub-region of the phase space where this circuit generates the corresponding dynamical property (*e.g.* the presence of a separatrix in the positive circuit case).

To illustrate our approach, we have delineated the Petri net translation of a Boolean model for the molecular regulatory network controlling Th-lymphocyte differentiation [21]. On the basis of this translation, we have shown that all three logical stable states correspond to dead markings in the Petri net model. These states represent specific lymphocyte populations: the virgin Th population, and the differentiated Th1 *versus* Th2 populations, corresponding respectively to the enhancement of cellular *versus* humoral immune response. Focusing on one functional positive circuit, we have further illustrated how our formal procedure can be applied to the Th-lymphocyte network model in order to delineate the corresponding functionality marking set. Moreover, we have shown that the contradiction of the functionality constraints (*e.g.* by changing the value of a logical parameter in the logical formalism, or the incidence matrix in the Petri net model) leads to the loss of one of the stable states.

To cover the full expression power of the generalised logical formalism [33], we have recently proposed a generalisation of our rewriting rules to encompass multilevel logical models in [7]. However, the feedback circuit analysis presented in Section 3. has still to be generalised to the resulting *Multilevel Regulatory Petri Nets* (MRPN).

Along with theoretical studies, our aim is to provide an integrated software suite which allows the biologist to specify regulatory networks, develop tentative models, and obtain qualitative results. We have already developed a software which implements our logical modelling approach (GINsim, [38]). We are currently developing a module to automate the translation of logical regulatory networks into Petri nets, allowing the analysis of the reachability graph using existing tools such as INA (Integrated Net Analyzer, [37]). Furthermore, as many of the crucial qualitative dynamical properties can be expressed in temporal logic (*e.g.* CTL), we are looking forward the application of model-checking techniques developed in the Petri nets framework.

Finally, we are considering the use of colored Petri nets to generate graphically simpler and more readable nets. The BRPN would then constitute the reference unfolding to perform different types of analysis.

Logical modelling and Petri nets constitute two complementary approaches for the dynamical modelling of biological regulatory networks. Their combination opens new prospects for the analysis of complex regulatory networks. In particular, our rewriting rules for genetic regulatory interactions should ease the modelling and analysis of mixed metabolic-genetic networks. The PN translation of logical regulatory models further constitutes a promising intermediate step for the development of more quantitative models, e.g. using hybrid or stochastic extensions of PN framework.

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