

On Circuit Functionality in Boolean Networks

Jean-Paul Comet · Mathilde Noual · Adrien Richard · Julio Aracena ·
Laurence Calzone · Jacques Demongeot · Marcelle Kaufman · Aurélien Naldi ·
El Houssine Snoussi · Denis Thieffry

Received: 24 February 2012 / Accepted: 27 February 2013
© Society for Mathematical Biology 2013

Abstract It has been proved, for several classes of continuous and discrete dynamical systems, that the presence of a positive (resp. negative) circuit in the interaction graph of a system is a necessary condition for the presence of multiple stable states

J.-P. Comet (✉) · M. Noual · A. Richard
Lab. I3S UMR CNRS 7271, Université Nice-Sophia Antipolis, 2000 Route des Lucioles,
06903 Sophia Antipolis, France
e-mail: comet@unice.fr

J. Aracena
CI2MA and Departamento de Ingeniería Matemática, Universidad de Concepción, Concepción,
Chile

L. Calzone
U900 INSERM, Mines ParisTech, Institut Curie, Paris, France

J. Demongeot
AGIM CNRS FRE 3405, Université Joseph Fourier-Grenoble 1, 38706 La Tronche, France

J. Demongeot
IXXI, Institut Rhône-Alpin des Systèmes Complexes, 69007 Lyon, France

M. Kaufman
Unit of Theoretical and Computational Biology, Université Libre de Bruxelles, C.P. 231,
1050 Brussels, Belgium

A. Naldi
Centre Intégréatif de Génomique, UNIL-Université de Lausanne, 1015 Lausanne, Switzerland

E.H. Snoussi
Université Mohammed V, Rabat, Morocco

D. Thieffry
Institut de Biology de l'ENS (IBENS) INSERM U1024 & CNRS UMR 8197, École Normale
Supérieure, Paris, France

(resp. a cyclic attractor). A positive (resp. negative) circuit is said to be functional when it “generates” several stable states (resp. a cyclic attractor). However, there are no definite mathematical frameworks translating the underlying meaning of “generates.” Focusing on Boolean networks, we recall and propose some definitions concerning the notion of functionality along with associated mathematical results.

Keywords Boolean network · Interaction graph · Feedback circuit · Fixed point · Multistability · Cyclic attractor

1 Introduction

Interactions between components of a dynamical system are often pictured by an interaction graph (also called influence, connection, or regulatory graph): vertices represent components, and arcs are signed in order to denote positive or negative influences between components. In this context, it is natural to study what kind of information on the dynamics of a system can be deduced from its interaction graph.

Thomas’ conjectures (Thomas 1981), stated in the context of gene networks, provide a very partial answer to this question: the presence of a positive (resp. negative) circuit is a necessary condition for the presence of multiple stable states (resp. a cyclic attractor); the sign of a circuit being defined as the product of the signs of its arcs. These conjectures have been proved for differential systems (Plathe et al. 1995; Snoussi 1998; Gouzé 1998; Cinquin and Demongeot 2002; Soulé 2003, 2006) and discrete systems (Aracena et al. 2004; Remy and Ruet 2006; Richard and Comet 2007; Aracena 2008; Remy et al. 2008; Richard 2010). They suggest that an essential role of circuits is to ensure the presence of multiple stable states (if positive) or cyclic attractors (if negative). In the sequel, we discuss this role only in the discrete case.

From a biological point of view, multistationarity and cyclic attractors are important dynamical properties used to explain differentiation and homeostasis or periodic phenomena. Practically, when a gene network controls such phenomena, it has to contain positive or negative circuits. This raises the question of what mechanisms underlying circuit interactions are sufficient or necessary to produce multistationarity or cyclic attractors. Primary studies in this direction have been done by Thomas and coworkers (Snoussi and Thomas 1993; Thomas et al. 1995; Thomas and Kaufman 2001). According to them, a positive (resp. negative) circuit is functional (or effective, operative) if it generates multistationarity (resp. a cyclic attractor). They also give conditions on mechanisms of interactions in circuits that are *sufficient* for this circuit to be functional (cf. conditions for the stationarity of a characteristic state). Unfortunately, these sufficient conditions are very strong and capture only a small part of the dynamical properties we are interested in. More recently, notable conditions on mechanisms of interactions in circuits have been proved to be *necessary* for the presence of multiple stable states or a cyclic attractor (Shih and Dong 2005; Remy et al. 2008). Practically, these necessary conditions allow one to restrict the set of systems to be considered, while encompassing all potentially interesting behaviors.

In this paper, we review and discuss different notions of circuit functionality leading to such necessary conditions. We start by introducing a natural definition of the

functionality of an arc along with the localization of this functionality in the phase space. Next, we propose different definitions of the functionality of a circuit based on where, in the phase space, the arcs composing the circuit are functional.

We focus on the class of asynchronous Boolean networks, which has been introduced by Thomas (1973) as a model for the dynamics of gene networks: On the one hand, these systems are elementary instances of complex systems and are largely used and, on the other hand, these systems constitute the reference for a large number of results about Thomas’ ideas.

This paper is organized as follows: Basic definitions are given in Sect. 2. In Sects. 3, 4, 5, and 6, we define different kinds of circuit functionality and state the corresponding mathematical results. Section 7 is devoted to discussion.

2 Preliminaries

Let $\mathbb{B} = \{0, 1\}$, and let I be a finite set. We denote by \mathbb{B}^I the set of functions from I to \mathbb{B} , seen as points of the $|I|$ -dimensional Boolean hypercube. For $i \in I$ and $x \in \mathbb{B}^I$, we write x_i instead of $x(i)$, and we denote by \bar{x}^i the point of \mathbb{B}^I such that $\bar{x}_i^i = 1 - x_i$ and $\bar{x}_j^i = x_j$ for all $j \neq i$. The Hamming distance d between two points x and y in \mathbb{B}^I is defined by $d(x, y) = \sum_{i \in I} |x_i - y_i|$.

A Boolean network is a function $f : \mathbb{B}^I \rightarrow \mathbb{B}^I$. The set I is the set of network components and \mathbb{B}^I is the set of possible states (or configurations). Hence, in a given state, each component is either present or absent. For all $i \in I$, we denote by f_i the function from \mathbb{B}^I to \mathbb{B} defined by $f_i(x) = f(x)_i$. We say that f is *nonexpansive* if $d(f(x), f(y)) \leq d(x, y)$ for all $x, y \in \mathbb{B}^I$. For all $i, j \in I$, the partial *discrete derivative* of f_i with respect to the j th variable is the function $f_{ij} : \mathbb{B}^I \rightarrow \{-1, 0, 1\}$ defined by

$$f_{ij}(x) = \frac{f_i(\bar{x}^j) - f_i(x)}{\bar{x}_j^j - x_j}.$$

The matrix of these partial derivatives at a given point may be seen as the Jacobian matrix of the system at this point. Hereafter, we use graphs instead of matrices to handle these partial derivatives.

An *interaction graph* G consists in a set of vertices V and a set of signed arcs $A \subseteq V \times \{+, -\} \times V$; the presence of both a positive and a negative arc from one vertex to another is thus possible. If G and H are two interaction graphs, we write $G \subseteq H$ to mean that G is a subgraph of H (i.e., each vertex of G is a vertex of H and each arc of G is an arc of H). A *circuit* of G is a subgraph of G that consists in a single directed cycle (with a unique arc from a vertex to the next one). A circuit of G is *positive* (resp. *negative*) if it contains an even (resp. odd) number of negative arcs.

Let $f : \mathbb{B}^I \rightarrow \mathbb{B}^I$ and $X \subseteq \mathbb{B}^I$. We denote by $Gf(X)$ the interaction graph whose vertex set is I and that contains a positive (resp. negative) arc from j to i if there exists $x \in X$ such that $f_{ij}(x) > 0$ (resp. $f_{ij}(x) < 0$). Clearly, if $X \subseteq Y$ then $Gf(X) \subseteq Gf(Y)$. For each $x \in \mathbb{B}^I$, we write $Gf(x)$ instead of $Gf(\{x\})$; this graph $Gf(x)$ is usually called the *local interaction graph* of f evaluated at point x . It contains the same information as the Jacobian matrix of f at point x . We use $G(f)$ as an

abbreviation of $Gf(\mathbb{B}^I)$; this graph $G(f)$ is usually called the *global interaction graph* of f .

The *asynchronous state transition graph* of f is the directed graph $\Gamma(f)$ defined as follows: The vertex set is \mathbb{B}^I , and for all $x, y \in \mathbb{B}^I$, there exists an arc from x to y if there exists $i \in I$ such that $y = \bar{x}^i$ and $f_i(x) \neq x_i$. The graph $\Gamma(f)$ can be seen as a (non-deterministic) dynamical system in which each transition of a trajectory changes a unique component. *Attractors* of $\Gamma(f)$ are defined as its terminal strongly connected components (i.e., strongly connected components without out-going arcs). Attractors of size 1 are called *stable states* and correspond to fixed points of f : $\{x\}$ is an attractor of $\Gamma(f)$ if and only if $f(x) = x$. Attractors of size at least 2 are called *cyclic attractors*.

3 Type-1 Functionality

If $G(f)$ has an arc from j to i then the function f_i depends on variable x_j and this dependency is visible only at points x where $f_{ij}(x) \neq 0$. It is natural to say that a positive (resp. negative) arc from j to i is *functional at point x* if $f_{ij}(x) > 0$ (resp. $f_{ij}(x) < 0$). The first type of functionality of a circuit we consider requires that all the arcs of a circuit C of $G(f)$ are functional at the same point.

Definition 1 Let C be a circuit of $G(f)$ and $x \in \mathbb{B}^I$. C is type-1 functional at x if $C \subseteq Gf(x)$. C is type-1 functional if it is type-1 functional for at least one $x \in \mathbb{B}^I$.

The following fundamental theorem is a slight extension of a theorem of Shih and Dong (2005). It shows that, in the absence of type-1 functional circuits, the asynchronous state transition graph of f is rather simple: It contains a unique attractor, which is a fixed point, reachable from any other point with at most $n - 1$ transitions. This suggests that type-1 functional circuits are necessary for the existence of “complex” behaviors.

Theorem 1 (Shih–Dong’s theorem (Shih and Dong 2005)) *If f has no type-1 functional circuits, then f has a unique fixed point x , and for all $y \in \mathbb{B}^I$, $\Gamma(f)$ has a path from y to x of length $d(x, y)$.*

Under the condition of Theorem 1, $\Gamma(f)$ may have a cycle so that some trajectories may never reach the fixed point. Thus, $\Gamma(f)$ only describes a *weak convergence* toward the fixed point.

According to Shih–Dong’s theorem, type-1 functional circuits are necessary for the presence of multiple fixed points or a cyclic attractor. In the light of Thomas’ conjectures, it is natural to ask whether Shih–Dong’s theorem may be refined by taking into account circuit signs, that is, if type-1 functional *positive* circuits are necessary for the presence of multiple fixed points, and if type-1 functional *negative* circuit are necessary for the presence of a cyclic attractor. The positive case is true.

Theorem 2 (Thomas’ rule—type-1 functional positive circuits (Remy et al. 2008; Richard and Comet 2007)) *If f has no type-1 functional positive circuits, then $\Gamma(f)$ has a unique attractor and in particular f has at most one fixed point.*

However, the negative case remains open.

Question 1 (Thomas’ rule—type-1 functional negative circuits) *Is it true that if f has no type-1 functional negative circuits then $\Gamma(f)$ has no cyclic attractors?*

Since the absence of cyclic attractors implies the existence of at least one fixed point, there is a weak version of this question that is interesting and also open: Is it true that if f has no type-1 functional negative circuits then f has at least one fixed point? This weak version has a positive answer in the nonexpansive case (Richard 2011), and from it one can deduce that question 1 has a positive answer, also. Indeed, suppose that f is not expansive and has no type-1 functional negative circuits. Then, by the weak form, f has at least one fixed point ξ . Let A be any attractor of $\Gamma(f)$, and let $x \in A$ be such that $d(x, \xi)$ is minimal. Then one can show that $d(x, \xi) \geq d(f(x), f(\xi)) = d(x, \xi) + d(x, f(x))$, thus $d(x, f(x)) = 0$. Hence, x is a fixed point and it follows that $A = \{x\}$. So, A is not cyclic.

4 Type-2 Functionality

For this type of functionality, we need additional definitions concerning functions derived from f by fixing some coordinates. Let $J \subseteq I$ and $z \in \mathbb{B}^{I \setminus J}$. For all $x \in \mathbb{B}^J$, we denote by $x \cup z$ the point $y \in \mathbb{B}^I$ defined by: $y_i = x_i$ if $i \in J$ and $y_i = z_i$ if $i \in I \setminus J$. The *subfunction* of f induced by z is the function $h : \mathbb{B}^J \rightarrow \mathbb{B}^J$ defined by

$$\forall x \in \mathbb{B}^J, \forall i \in J, \quad h_i(x) = f_i(x \cup z).$$

Hence, h is the function that we obtain from f by fixing to z_i the value of each component $i \in I \setminus J$. Note that for all $x \in \mathbb{B}^J$ and $i \in J$, $\Gamma(h)$ has an arc from x to \bar{x}^i if and only if $\Gamma(f)$ has an arc from $x \cup z$ to $\overline{x \cup z}^i$. Hence, $\Gamma(h)$ is isomorphic to the subgraph of $\Gamma(f)$ induced by the $|J|$ -dimensional subcube $\{x \cup z \mid x \in \mathbb{B}^J\}$ of \mathbb{B}^I (the isomorphism is $x \mapsto x \cup z$). Furthermore, $Gh(x)$ has a positive (resp. negative) arc from j to i if and only if this arc is in $Gf(x \cup z)$, that is: for all $x \in \mathbb{B}^J$ and $i, j \in J$, we have $h_{ij}(x) = f_{ij}(x \cup z)$. Hence, $Gh(x)$ is the subgraph of $Gf(x \cup z)$ induced by J . Thus, when h is a subfunction of f , the dynamics of h is contained in the dynamics of f and the interaction graph of h is contained in that of f .

Because circuits are crucial structural motifs, we are particularly interested in subfunctions whose interaction graphs are exactly circuits. Now, it is easy to see that given a circuit C there exists a unique function, denoted by h^C , whose interaction graph is C . We say that the circuit C is type-2 functional if h^C is a sub-function of f . This second type of functionality is equivalent to the definition of circuit functionality given in Naldi et al. (2007), when it is restricted to the Boolean case.

Definition 2 Let C be a circuit of $G(f)$ with vertex set J , and let $z \in \mathbb{B}^{I \setminus J}$. C is type-2 functional at z if h^C is the sub-function of f induced by z . C is type-2 functional if it is type-2 functional for at least one $z \in \mathbb{B}^{I \setminus J}$.

Functions of the form h^C have been thoroughly studied (Remy et al. 2003; Demongeot et al. 2011); in particular, it is well known that if C is positive, then h^C has exactly two fixed points, and that if C is negative, then h^C has no fixed points (hence, $\Gamma(h^C)$ has a cyclic attractor). In other words, h^C effectively generates two fixed points in the positive case and a cyclic attractor in the negative case, and the type-2 functionality of C allows f to behave locally as h^C (in some subcubes). Thus, type-2 functionality is a sufficient condition for the presence of either a “local bi-stability” or a “local cyclic attractor.” However, to make these local properties global, additional conditions are needed. For instance, if C is type-2 functional at $z \in \mathbb{B}^{I \setminus J}$, and if $f_i(x \cup z) = z_i$ for all $x \in \mathbb{B}^J$ and $i \in I \setminus J$, then f has at least two fixed points if C is positive and $\Gamma(f)$ has a cyclic attractor if C is negative. We refer the reader to Snoussi and Thomas (1993), Remy and Ruet (2008), Siebert (2009, 2011) for works on this kind of sufficient conditions.

The following theorem, proved in Richard (2013), shows that the type-2 functionality of a positive (resp. negative) circuit is, for some particular Boolean networks, a necessary condition for the presence of multiple fixed points (resp. a cyclic attractor).

Theorem 3 (Thomas’ rules—type-2 functional circuits—nonexpansive case) *Suppose that f is nonexpansive. If f has no type-2 functional positive circuits, then $\Gamma(f)$ has a unique attractor, and if f has no type-2 functional negative circuits, then $\Gamma(f)$ has no cyclic attractors.*

We now prove that type-2 functionality can be defined in terms of type-1 functionality.

Proposition 1 *Let C be a circuit of $G(f)$ with vertex set J , let $z \in \mathbb{B}^{I \setminus J}$. The next properties are equivalent:*

1. C is type-2 functional at z .
2. C is type-1 functional at $x \cup z$ for some $x \in \mathbb{B}^J$ and C has no chord in $Gf(\{x \cup z \mid x \in \mathbb{B}^J\})$ (a chord of C is an arc that is not in C and whose initial and terminal vertices are in C).
3. C is type-1 functional at $x \cup z$ for all $x \in \mathbb{B}^J$.

Proof Let us first prove $(1 \Rightarrow 2)$. Consider that C is type-2 functional at z . By definition, the subfunction of f induced by z is h^C , and thus $Gh^C(x) = G(h^C) = C$ for all $x \in \mathbb{B}^J$. Consequently, C equals the subgraph of $Gf(x \cup z)$ induced by J for all $x \in \mathbb{B}^J$ and we deduce property 2 (and also property 3).

Let us now prove $(2 \Rightarrow 3)$. Assume property 2 and let $x \in \mathbb{B}^J$ be such that C is type-1 functional at $x \cup z$. Let $i \in J$ and let us prove that C is type-1 functional at $\bar{x}^i \cup z$. If not, there exists an arc $k \rightarrow l$ of C , where $k \neq i$, which is not in $Gf(\bar{x}^i \cup z)$. So, the dependance of l according to k depends on i , thus l depends on i and we can prove that there exists an arc $i \rightarrow l$ either in $Gf(x \cup z)$ or $Gf(\bar{x}^k \cup z)$, contradicting hypothesis on chords of C . Thus, C is type-1 functional at $\bar{x}^i \cup z$, and repeating this argument, we deduce property 3.

Finally, let us prove $(3 \Rightarrow 1)$. By hypothesis, we have $C \subseteq Gf(x \cup z)$ for all $x \in \mathbb{B}^J$. Let h be the subfunction of f induced by z . If C has a positive arc from

j to i , then for all $x \in \mathbb{B}^J$, we have $f_{ij}(x \cup z) > 0$. We derive that $f_i(x \cup z) = x_j$ for all $x \in \mathbb{B}^J$, and thus $h_i(x) = x_j$ for all $x \in \mathbb{B}^J$. So, in $G(h)$, i has a unique predecessor j , and the arc from j to i is positive. Similarly, if C has a negative arc from j to i then, for all $x \in \mathbb{B}^J$, we have $f_{ij}(x \cup z) < 0$ so $f_i(x \cup z) = 1 - x_j$ and so $h_i(x) = 1 - x_j$. So, in $G(h)$, i has a unique predecessor j and the arc from j to i is negative. It follows that $G(h) = C$, i.e., $h = h^C$. Thus, C is type-2 functional at z . \square

According to this proposition, if a cycle C is type-1 functional and if C has no chord in $G(f)$ then C is type-2 functional. This has been previously proved by Remy and Ruet (2008).

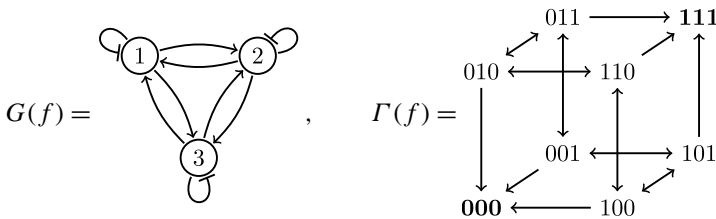
The two following examples show that type-1 functionality does not imply the one of type 2, and that Theorem 3 is false in the expansive case.

Remark In all examples that follow, I is an interval $\{1, 2, \dots, n\}$, and each point $x \in \mathbb{B}^I$ is seen as a string $x = x_1x_2 \dots x_n$ on the alphabet \mathbb{B} . Also, interaction graphs are represented with T-end arrows for negative arcs and normal arrows for positive ones.

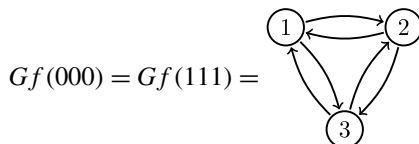
Example 1 $I = \{1, 2, 3\}$ and $f : \mathbb{B}^I \rightarrow \mathbb{B}^I$ is defined by:

$$\begin{cases} f_1(x) = (\overline{x_1} \wedge (x_2 \vee x_3)) \vee (x_2 \wedge x_3), \\ f_2(x) = (\overline{x_2} \wedge (x_3 \vee x_1)) \vee (x_3 \wedge x_1), \\ f_3(x) = (\overline{x_3} \wedge (x_1 \vee x_2)) \vee (x_1 \wedge x_2). \end{cases}$$

The global interaction graph of f and the asynchronous state transition graph of f are:



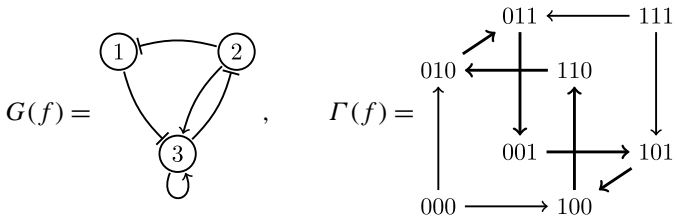
f has two fixed points, 000 and 111, but one can check that it has no type-2 functional positive circuits. According to Theorem 2, f has at least one type-1 functional positive circuit (for positive circuits, type-1 functionality does therefore not imply type-2 functionality). The only points for which the local interaction graph has a positive circuit are 000 and 111; for these two points the local interaction graph of f precisely has 5 positive circuits:



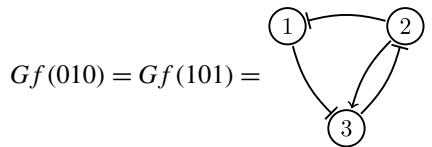
Example 2 $I = \{1, 2, 3\}$ and $f : \mathbb{B}^I \rightarrow \mathbb{B}^I$ is defined by:

$$\begin{cases} f_1(x) = \bar{x}_2, \\ f_2(x) = \bar{x}_3, \\ f_3(x) = (x_3 \wedge (\bar{x}_1 \vee x_2)) \vee (\bar{x}_1 \wedge x_2). \end{cases}$$

The global interaction graph of f and the asynchronous state transition graph of f are



$\Gamma(f)$ has a unique attractor, $\{010, 011, 001, 101, 100, 110\}$, which is cyclic, but one can check that f has no type-2 functional negative circuits. At each point, 011 and 100 excepted, the local interaction graph contains at least one negative circuit (for negative circuits, type-1 functionality does therefore not imply type-2 functionality). For instance, the local interaction graph has two negative circuits at points 010 and 101:



5 Type-3 Functionality

Recall that an arc from j to i is functional at point x if $f_{ij}(x) \neq 0$, that is, if $f_i(x) \neq f_i(\bar{x}^j)$. We then say that the arc $j \rightarrow i$ is visible between the adjacent points x and \bar{x}^j . Now, to each $X \subseteq \mathbb{B}^I$, we associate a new interaction graph $Gf[X]$ (slightly different from $Gf(X)$), which contains all visible arcs between adjacent points belonging to X .

Formally, for all $X \subseteq \mathbb{B}^I$, we denote by $Gf[X]$ the interaction graph defined as follows: The vertex set is I and there exists a positive (resp. negative) arc from j to i if there exists $x \in X$ such that $\bar{x}^j \in X$ and $f_{ij}(x)$ is positive (resp. negative). Clearly, $G(f) = Gf[\mathbb{B}^I]$, and if $Y \subseteq X$ then $Gf[Y] \subseteq Gf[X]$. Furthermore, because of the condition “ $\bar{x}^j \in X$ ”, $Gf[X] \subseteq Gf(X)$, and for all $x \in \mathbb{B}^I$, $Gf[x]$ has no arcs.

For all $x \in \mathbb{B}^I$, we denote by $\Gamma(f)[x]$ the reachability set of x , that is, the set of points $y \in \mathbb{B}^I$ such that $\Gamma(f)$ has a path from x to y (by convention, $x \in \Gamma(f)[x]$). Let us note that if X is an attractor of $\Gamma(f)$, then $\Gamma(f)[x] = X$ for all $x \in X$ (since X is strongly connected and has no outgoing arcs).

The third type of functionality of a circuit C is based on the visibility of each arc of C between two points that *both* belong to the reachability set of a particular point.

Definition 3 Let C be a circuit of $G(f)$, $x \in \mathbb{B}^I$ and $X = \Gamma(f)[x]$. C is *type-3 functional* at x if $C \subseteq Gf[X]$. C is *type-3 functional* if it is type-3 functional for at least one $x \in \mathbb{B}^I$.

The following theorem shows that type-3 functional negative circuits are necessary for the presence of cyclic attractors.

Theorem 4 (Thomas’ rule—type-3 functional negative circuits (Remy et al. 2008; Richard 2010)) *If X is a cyclic attractor of $\Gamma(f)$, then $Gf[X]$ has a negative circuit C which is type-3 functional at each point $x \in X$ (since $X = \Gamma(f)[x]$).*

As stated below, for negative circuits, type-2 functionality implies type-3 functionality.

Proposition 2 *Let C be a negative circuit of $G(f)$ with vertex set J , and let $z \in \mathbb{B}^{I \setminus J}$. If C is type-2 functional at z , then it is type-3 functional at $x \cup z$ for all $x \in \mathbb{B}^J$.*

Proof Suppose that C is type-2 functional at z and let h be the subfunction of f induced by z . Let $x \in \mathbb{B}^J$. As showed in Remy et al. (2003), for every $x \in \mathbb{B}^J$, $\Gamma(h)[x]$ contains a cyclic attractor. We derive from Theorem 4 that $C \subseteq Gh[\Gamma(h)[x]]$. Since $\Gamma(h)[x]$ is isomorphic to the subgraph of $\Gamma(f)$ induced by $X = \{y \cup z \mid y \in \Gamma(h)[x]\}$, we have $X \subseteq \Gamma(f)[x \cup z]$. Since $Gh(y)$ is a subgraph of $Gf(y \cup z)$ for all $y \in \mathbb{B}^J$, it follows that $C \subseteq Gh[\Gamma(h)[x]] \subseteq Gf[X] \subseteq Gf[\Gamma(f)[x \cup z]]$. Thus, C is type-3 functional. □

The next example shows that Proposition 2 does not hold for positive circuits, and that type-1 functionality does not imply type-3 functionality. It also shows that the type-3 functionality of a positive circuit is not necessary for the presence of multiple attractors.

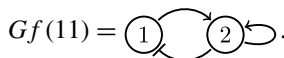
Example 3 $I = \{1, 2\}$ and $f : \mathbb{B}^I \rightarrow \mathbb{B}^I$ is defined by:

$$\begin{cases} f_1(x) = x_1 \wedge \overline{x_2}, \\ f_2(x) = x_1 \wedge x_2, \end{cases}$$

The global interaction graph of f and the asynchronous state transition graph of f are



Note that f has two fixed points. The local interaction graph of f at 11 is



Thus, f has a positive and a negative type-1 functional circuit. Furthermore, the sub-function of f induced by $x_1 = 1$ or by $x_2 = 0$ is the identity on \mathbb{B} . Since the

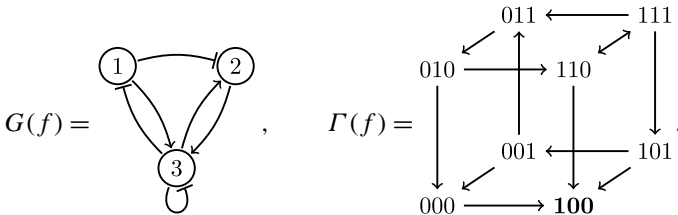
global interaction graph of the identity on \mathbb{B} is a positive circuit of length one, f has type-2 functional positive circuits. However, f has no type-3 functional circuits. Indeed, there are no arcs in the following three graphs: $Gf[\Gamma(f)[00]] = Gf[00]$, $Gf[\Gamma(f)[10]] = Gf[10]$ and $Gf[\Gamma(f)[01]] = Gf[\{01, 00\}]$. It follows that $Gf[\Gamma(f)[11]] = Gf[\{11, 01, 00\}]$ contains only an arc from vertex 1 to vertex 2. As a consequence, for positive and negative circuits, type-1 functionality does not imply type-3 functionality. For positive circuits, type-2 functionality does not imply type-3 functionality. Finally, type-3 functionality of a positive circuit is not necessary for the presence of multiple fixed points.

The following example shows that, in the positive case, type-3 functionality does not imply type-1 functionality (thus, it does not imply type-2 functionality either).

Example 4 $I = \{1, 2, 3\}$ and $f : \mathbb{B}^I \rightarrow \mathbb{B}^I$ is defined by:

$$\begin{cases} f_1(x) = \bar{x}_3, \\ f_2(x) = \bar{x}_1 \wedge x_3, \\ f_3(x) = x_1 \wedge x_2 \wedge \bar{x}_3. \end{cases}$$

The global interaction graph of f and the asynchronous state transition graph of f are:



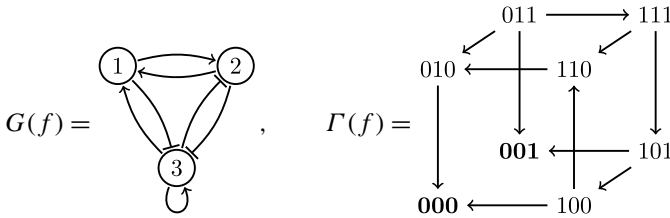
$G(f)$ has a positive circuit of length 2 and a positive circuit of length 3. The sole attractor of this Boolean network is the fixed point 100. If $x = 111$ or 110 then $\Gamma(f)[x] = \mathbb{B}^I$ so $Gf[\Gamma(f)[x]] = G(f)$. It follows that both positive circuits are type-3 functional. However, f has no type-1 functional positive circuits. Indeed, for all $x \in \mathbb{B}^I$, if $Gf(x)$ contains the arc from 2 to 3 (resp. from 3 to 2) then $x_1 = 1$ (resp. $x_1 = 0$) so $Gf(x)$ cannot contain these two arcs. Thus, the positive circuit of length 2 is not type-1 functional. Similarly, for all $x \in \mathbb{B}^I$, if $Gf(x)$ contains the arc from 1 to 2 (resp. from 2 to 3) then $x_3 = 1$ (resp. $x_3 = 0$), so $Gf(x)$ cannot contain these two arcs. Thus, the positive circuit of length 3 is not type-1 functional.

The following example gives the same conclusion for negative circuits.

Example 5 $I = \{1, 2, 3\}$ and $f : \mathbb{B}^I \rightarrow \mathbb{B}^I$ is defined by:

$$\begin{cases} f_1(x) = x_2 \wedge x_3, \\ f_2(x) = x_1 \wedge \bar{x}_3, \\ f_3(x) = \bar{x}_1 \wedge \bar{x}_2 \wedge x_3. \end{cases}$$

The global interaction graph of f and the asynchronous state transition graph of f are



$G(f)$ has a negative circuit of length 2 and a negative circuit of length 3. This Boolean network has two attractors which are the fixed points 000 and 001. If $x = 110$ then $\Gamma(f)[x] = \mathbb{B}^I$ so $Gf[\Gamma(f)[x]] = G(f)$. It follows that both negative circuits are type-3 functional. However, f has no type-1 functional negative circuits. Indeed, for all $x \in \mathbb{B}^I$, if $Gf(x)$ contains the arc from 1 to 3 (resp. from 3 to 1) then $x_2 = 0$ (resp. $x_2 = 1$) so $Gf(x)$ cannot contain these two arcs. Thus, the negative circuit of length 2 cannot be type-1 functional. Similarly, for all $x \in \mathbb{B}^I$, if $Gf(x)$ contains the arc from 1 to 2 (resp. from 2 to 3) then $x_3 = 0$ (resp. $x_3 = 1$), so $Gf(x)$ cannot contain these two arcs. Thus, the positive circuit of length 3 cannot be type-1 functional.

6 Type-4 Functionality

The last type of functionality considered here is a relaxation of type-3 functionality: it requires that each arc of a circuit C be visible between some two adjacent points such that *at least one* of them belong to the reachability set of a particular point.

Definition 4 Let C be a circuit of $G(f)$, $x \in \mathbb{B}^I$ and $X = \Gamma(f)[x]$. C is *type-4 functional at x* if $C \subseteq Gf(X)$. C is *type-4 functional* if it is type-4 functional for at least one $x \in \mathbb{B}^I$.

If $X = \Gamma(f)[x]$, then by definition, $Gf(x) \subseteq Gf(X)$ and $Gf[X] \subseteq Gf(X)$. Type-4 functionality is therefore a relaxation of both type-1 and type-3 functionalities.

Proposition 3 *If C is type-1 or type-3 functional at x , then C is type-4 functional at x .*

From this proposition, Theorem 2, and Theorem 4, we derive that for type-4 functionality, Thomas’ rules are valid in both the positive and negative cases.

Theorem 5 (Thomas’ rules–type-4 functional circuits) *If f has no type-4 functional positive circuits, then $\Gamma(f)$ has a unique attractor. And if f has no type-4 functional negative circuits, then $\Gamma(f)$ has no cyclic attractors.*

A positive answer to question 1 would provide a strong generalization of the second assertion of this theorem. Let us also note that Example 3 shows that in both the positive and negative cases, type-4 functionality does not imply type-3 functionality: in this example, f has type-1 functional positive and negative circuits, hence it has type-4 functional positive and negative circuits but no type-3 functional circuits. Finally, Examples 4 and 5 show that in the positive and negative cases, type-4 functionality implies neither type-1 functionality nor type-2 functionality: In Example 4 (resp. Example 5), f has type-3 functional positive (resp. negative) circuits so it has type-4 functional positive (resp. negative) circuits but no type-1 functional positive (resp. negative) circuits.

7 Discussion

Let us recall the starting point: A positive (resp. negative) circuit is said to be functional when it “generates” multiple attractors (resp. a cyclic attractor) but it is rather difficult to formalize the underlying meaning of “generate.” The approach presented here focuses on necessary conditions—on the functioning of the interactions of a circuit—for the presence of multiple attractors (positive case) or cyclic attractors (negative case). For instance, Theorem 2 states that in the absence of type-1 functional positive circuit, there are no multiple attractors. This way, the set of all type-1 functional positive circuits can be seen as being “responsible” for the presence of multiple attractors although this “responsibility” cannot be assigned to one particular circuit.

All the proposed notions of functionality are based on arc functionality: A positive (resp. negative) arc from j to i is said to be functional at point x if $f_{ij}(x)$ is positive (resp. negative); this functionality is then “visible” between the adjacent points x and \bar{x}^j . A circuit is type-1 functional when all its arcs are functional at the same point (this functionality may be called local or punctual) and it is type-2 functional if all its arcs are functional in all points of a sub-cube of \mathbb{B}^I (Proposition 1). A circuit is said to be type-4 functional if each arc is functional somewhere in the set of states that are reachable from a particular point. If in addition, the adjacent points revealing the functionality of each arc belong to this set, then the circuit is type-3 functional. Relationships between these definitions and the corresponding results are summarized in Fig. 1.

An “ideal” notion of functionality would correspond to conditions that are as strong as possible and that work in both the positive and negative cases (i.e., that are necessary for multiple attractors in the positive case and for cyclic attractors in the negative case). As shown by the previous diagram, the only functionality that works in both cases is that of Type 4. Unfortunately, it is the weakest type of functionality proposed here. Type 3 is stronger, but it works only in the negative case while Type 1, which is stronger too, has only been proved in the positive case (the negative case is still open). Type 2, which is the strongest, works in both cases only under very strong conditions on f (nonexpansivity).

Type 1 is the strongest working in the positive case. A positive answer to question 1 stating that this type of functionality works also in the negative case, would therefore

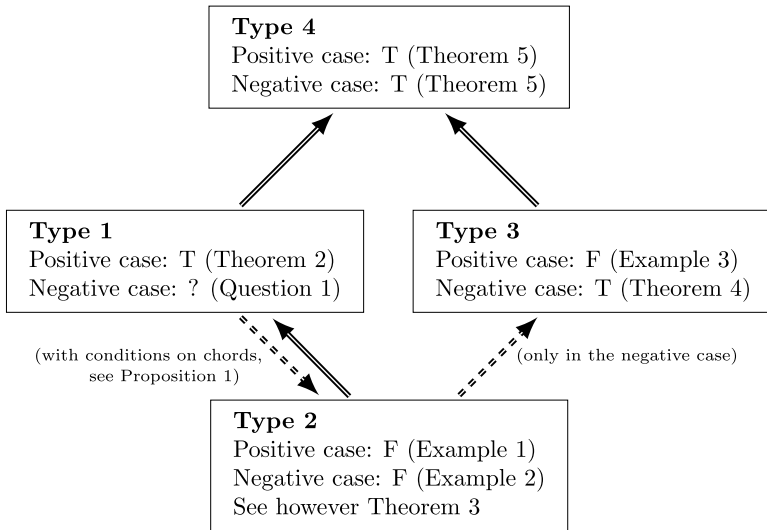


Fig. 1 In this diagram, there is an *arrow* from a “Type k ” box to a “Type l ” box if and only if type- k functionality of a circuit C implies type- l functionality of C ; the *arrow line is dashed* if and only if the implication holds only for negative circuits. In each “Type k ” box, “Positive case: T (resp. F)” means that type- k functionality of a positive circuit is necessary (resp. not necessary) for the presence of multiple attractors. “Negative case: T (resp. F)” means that type- k functionality of a negative circuit is necessary (resp. not necessary) for the presence of a cyclic attractor

lead to a satisfactory notion of functionality. However, while all the theorems presented here have natural extensions in the non-Boolean discrete case, question 1 has a negative answer in the non-Boolean discrete case (Richard 2010). Let us note that a positive answer to the question would also lead to a nice proof of Theorem 1: The uniqueness of the fixed point would be given by the positive case and the existence by the negative case.

In this article, we addressed the question of the dynamical roles of circuits by focusing on asynchronous Boolean networks and by associating positive circuits and multiple attractors on the one hand, and negative circuits and cyclic attractors on the other. We chose this setting because it leads to a large number of results in relation with Thomas’ ideas. Another way to address the functionality of a circuit would consist in looking for consequences of its elimination. This raises a series of questions with, a priori, no obvious answers: How are circuits to be destroyed? If they are to be destroyed by removing an arc, then which arc must that be? And what is the dynamical system resulting from the elimination of an arc?

Acknowledgements This paper results from a collective discussion that took place during the workshop *Logical formalism, gains, and challenges for the modeling of regulatory networks, held at Rabat, Morocco, from 12th to 15th of April 2011*. We are grateful to Mohamed Hedi Ben Amor, Claudine Chaouiya, Madalena Chaves, Eric Fanchon, Pedro Monteiro, Elisabeth Remy, Paul Ruet, Sylvain Sené, Christophe Soulé, Laurent Tournier, and Laurent Trilling for taking part to this discussion. This work is partially supported by FONDECYT project 1090549 and BASAL project CMM, Universidad de Chile, and also by the French National Agency for Research (ANR-10-BLANC-0218 BioTempo project).

References

- Aracena, J. (2008). Maximum number of fixed points in regulatory Boolean networks. *Bull. Math. Biol.*, *70*, 1398–1409.
- Aracena, J., Demongeot, J., & Goles, E. (2004). Positive and negative circuits in discrete neural networks. *IEEE Trans. Neural Netw.*, *15*, 77–83.
- Cinquin, O., & Demongeot, J. (2002). Positive and negative feedback: striking a balance between necessary antagonists. *J. Theor. Biol.*, *216*, 229–241.
- Demongeot, J., Noual, M., & Sené, S. (2011). Combinatorics of Boolean automata circuits dynamics. *Discrete Appl. Math.*, *160*, 398–415. 2012.
- Gouzé, J. (1998). Positive and negative circuits in dynamical systems. *J. Biol. Syst.*, *6*, 11–15.
- Naldi, A., Thieffry, D., & Chaouiya, C. (2007). Decision diagrams for the representation and analysis of logical models of genetic networks. In *LNCS: Vol. 4695. Computational methods in systems biology* (pp. 233–247). Berlin: Springer.
- Plathe, E., Mestl, T., & Omholt, S. (1995). Feedback loops, stability and multistationarity in dynamical systems. *J. Biol. Syst.*, *3*, 569–577.
- Remy, E., & Ruet, P. (2006). Positive and negative regulatory circuit inference from multilevel dynamics. In *LNCS: Vol. 341. Positive systems: theory and applications* (pp. 263–270). Berlin: Springer.
- Remy, E., & Ruet, P. (2008). From minimal signed circuits to the dynamics of Boolean regulatory networks. *Bioinformatics*, *24*, i220–i226.
- Remy, E., Mossé, B., Chaouiya, C., & Thieffry, D. (2003). A description of dynamical graphs associated to elementary regulatory circuits. *Bioinformatics*, *19*, 172–178.
- Remy, E., Ruet, P., & Thieffry, D. (2008). Graphic requirements for multistability and attractive cycles in a Boolean dynamical framework. *Adv. Appl. Math.*, *41*, 335–350.
- Richard, A. (2010). Negative circuits and sustained oscillations in asynchronous automata networks. *Adv. Appl. Math.*, *44*, 378–392.
- Richard, A. (2011). Local negative circuits and fixed points in non-expansive Boolean networks. *Discrete Appl. Math.*, *159*, 1085–1093.
- Richard, A. (2013). *Fixed point theorems for Boolean networks expressed in terms of forbidden subnetworks*. arXiv preprint.
- Richard, A., & Comet, J.-P. (2007). Necessary conditions for multistationarity in discrete dynamical systems. *Discrete Appl. Math.*, *155*, 2403–2413.
- Shih, M.-H., & Dong, J.-L. (2005). A combinatorial analogue of the Jacobian problem in automata networks. *Adv. Appl. Math.*, *34*, 30–46.
- Siebert, H. (2009). Deriving behavior of Boolean bioregulatory networks from subnetwork dynamics. *Math. Comput. Sci.*, *2*, 421–442.
- Siebert, H. (2011). Analysis of discrete bioregulatory networks using symbolic steady states. *Bull. Math. Biol.*, *73*, 873–898.
- Snoussi, E. (1998). Necessary conditions for multistationarity and stable periodicity. *J. Biol. Syst.*, *6*, 3–9.
- Snoussi, E., & Thomas, R. (1993). Logical identification of all steady states: the concept of feedback loop characteristic states. *Bull. Math. Biol.*, *55*, 973–991.
- Soulé, C. (2003). Graphical requirements for multistationarity. *Complexus*, *1*, 123–133.
- Soulé, C. (2006). Mathematical approaches to differentiation and gene regulation. *C. R. Biol.*, *329*, 13–20.
- Thomas, R. (1973). Boolean formalization of genetic control circuits. *J. Theor. Biol.*, *42*, 563–585.
- Thomas, R. (1981). On the relation between the logical structure of systems and their ability to generate multiple steady states or sustained oscillations. In *Springer series in synergies: Vol. 9. Numerical methods in the study of critical phenomena* (pp. 180–193). Berlin: Springer.
- Thomas, R., & Kaufman, M. (2001). Multistationarity, the basis of cell differentiation and memory. II. Logical analysis of regulatory networks in terms of feedback circuits. *Chaos*, *11*, 180–195.
- Thomas, R., Thieffry, D., & Kaufman, M. (1995). Dynamical behavior of biological regulatory networks—I. Biological role of feedback loops and practical use of the concept of the loop-characteristic state. *Bull. Math. Biol.*, *57*, 247–276.