

Spatial Differentiation and Positive Circuits in a Discrete Framework

Anne Crumière¹ and Paul Ruet¹

*CNRS - Institut de Mathématiques de Luminy
Campus de Luminy, Case 907, 13288 Marseille Cedex 9 (France)*

Abstract

The biologist R. Thomas has enounced a rule relating multistationarity in a system of genes interacting in a single cell to the existence of a positive circuit in the regulatory graph of the system. In this paper, we address the question of a similar rule for spatial differentiation. We consider the interactions of genes in several biological cells located on a 1-dimensional infinite grid, and we assume that the expression levels of genes are Boolean. We show that the existence of a positive circuit is a necessary condition for a specific form of multistationarity, which naturally corresponds to spatial differentiation.

Keywords: Cell communication, Differentiation, Genetic regulatory graph, Positive circuit.

1 Introduction

Biologists often represent genetic interactions by means of graphs. In these *genetic regulatory graphs*, vertices represent genes or their regulatory products, whereas edges are directed and represent regulatory effects from one gene on another. Edges are often labelled with a sign, positive (+1) in the case of an activation and negative (−1) for an inhibition.

This paper deals with relationships between the structure of such regulatory graphs and their dynamical properties. The biologist R. Thomas has enounced the following general rule [14]: a necessary condition for multistability (i.e., the existence of several stable fixed points in the dynamics) is the presence of a positive circuit in the regulatory graph, the sign of a circuit being the product of the signs of its edges. Multistability corresponds to important biological phenomena, namely cell differentiation processes. This rule is about the dynamics of a single cell, and it has given rise to mathematical statements and proofs mostly in a differential dynamical

¹ Email: {crumiere,ruet} at iml.univ-mrs.fr

formalism [7,11,5,12], and more recently in the discrete formalism [8,10,9]. These results are recalled in Section 2.

The present paper aims at extending this rule to regulatory interactions spanning within cells (as in Thomas' rule) and between cells, by establishing connections between spatial differentiation and the existence of positive circuits. This is motivated by the importance of spatial aspects in most biological processes (morphogenesis, immunology, etc), and indeed, positive regulatory circuits are often associated with spatial differentiation: we refer the reader to, e.g., [4] for a study of dorsal-ventral boundary in the *Drosophila* wing. The question of relating spatial differentiation and positive circuits is formally raised by Soulé in [13].

In this paper, we consider as a starting point the case of fixed cells located on a 1-dimensional infinite grid, which we assume to be the set \mathbb{Z} of integers. This is a simplification which has the advantage of emphasising the basic formalism. The more biologically realistic situation of hexagonal 2-dimensional grids has been later studied in [1], with an application to the formation of sense organs in *Drosophila* as modelled in [3].

We further assume that intercellular communication is local, in the sense that a gene may interact only with genes in its own cell x and neighbouring cells $x-1, x+1$. This assumption, which is biologically reasonable but for polarised cells (typically neurons), is standard and at the basis of cellular automata [15]. We then prove that the existence of a positive circuit is necessary for the presence of several periodic fixed points having at least one cell with the same expression levels (Theorem 4.1). We then apply this result to show that a single non-constant periodic fixed point actually suffices to imply the existence of a positive circuit, as long as its smallest period has two cells with the same expression levels (Corollary 4.3).

These results are the topic of Sections 3 and 4, which concentrate on the Boolean case (the expression level of a gene is either 0 or 1), but we claim that they can be simply generalised to the discrete multilevel formalism of [10]. We then discuss further issues through several examples and counterexamples in Section 5.

A natural prospect would be to extend these results to other topological cellular configurations, like cyclic grids, other finite grids with boundaries, networks of cells, etc.

Acknowledgements. We wish to thank Claudine Chaouiya, Élisabeth Remy and Denis Thieffry for helpful discussions, as well as the anonymous referees for their remarks.

2 The intracellular case

In this section we recall the theorem relating multistationarity in the case of a single cell to the existence of a positive circuit [8].

Let us start with preliminary notations. For $\alpha \in \{0, 1\}$, we define $\bar{\alpha}$ by $\bar{0} = 1$ and $\bar{1} = 0$. For $s \in \{0, 1\}^n$ and $I \subseteq \{1, \dots, n\}$, $\bar{s}^I \in \{0, 1\}^n$ is defined by $(\bar{s}^I)_i = s_i$ for $i \notin I$ and $(\bar{s}^I)_i = \bar{s}_i$ for $i \in I$. When $I = \{i\}$ is a singleton, $\bar{s}^{\{i\}}$ is denoted by \bar{s}^i .

Let n be a positive integer. Genes are denoted by numbers $1, \dots, n$. A state s is a n -tuple $(s_1, \dots, s_n) \in \{0, 1\}^n$, where s_i denotes the expression level of gene i : either 1 when gene i is expressed, or 0 when gene i is not expressed.

The dynamics of the system consisting in the n genes is given by a map $f : \{0, 1\}^n \rightarrow \{0, 1\}^n$. For each $i = 1, \dots, n$, f_i is the i -th coordinate map $\{0, 1\}^n \rightarrow \{0, 1\}$. For each $s \in \{0, 1\}^n$ and $i = 1, \dots, n$, $f_i(s)$ denotes the value to which the expression level of gene i tends when the system is in state s . A fixed point for f is a state $s \in \{0, 1\}^n$ such that $f(s) = s$.

A *regulatory graph* is a signed directed graph, i.e., a directed graph with a sign, $+1$ or -1 , associated to each edge. To any $f : \{0, 1\}^n \rightarrow \{0, 1\}^n$ and $s \in \{0, 1\}^n$ is associated a regulatory graph $G(f)(s)$ as follows: its vertex set is $\{1, \dots, n\}$, and $G(f)(s)$ has an edge from i to j when

$$f_j(\bar{s}^i) \neq f_j(s),$$

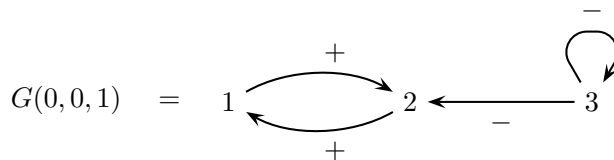
with positive sign when $s_i = f_j(s)$ and negative sign otherwise.

It may be important to note here that the approach we develop in this paper consists in deriving regulatory graphs from a given dynamics as in [12,8,9]. This is however not the most usual presentation of the correspondance between graphs and dynamics: indeed, biologists often use regulatory graphs as a basis to generate dynamical models, using either a differential framework or a discrete framework; see, e.g., [5,11]. However, the two approaches are clearly compatible, as it has been observed in [9] for instance.

As an example, the following table represents a possible dynamics for a system of three genes in a single cell:

| s | $(0, 0, 0)$ | $(0, 0, 1)$ | $(0, 1, 0)$ | $(0, 1, 1)$ | $(1, 0, 0)$ | $(1, 0, 1)$ | $(1, 1, 0)$ | $(1, 1, 1)$ |
|--------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| $f(s)$ | $(0, 1, 1)$ | $(0, 0, 0)$ | $(0, 1, 0)$ | $(1, 0, 0)$ | $(1, 0, 0)$ | $(0, 1, 0)$ | $(1, 0, 0)$ | $(1, 1, 0)$ |

The regulatory graph $G(f)(s)$ for $s = (0, 0, 1)$ thus contains four edges: a self-loop on gene 3, as well as edges from gene 1 to gene 2, from gene 2 to gene 1 and from gene 3 to gene 2. As $s_1 = f_2(s)$ and $s_2 = f_1(s)$, the interactions between genes 1 and 2 are positive (activations), whereas $s_3 \neq f_2(s)$ and $s_3 \neq f_3(s)$ hence the other two interactions are negative.



The sign of a circuit in a regulatory graph is the product of the signs of its edges. If $I \subseteq \{1, \dots, n\}$, an I -circuit is a circuit whose vertices are in I .

Theorem 2.1 (Remy-Ruet-Thieffry [8]) *If f has least two fixed points, then there is an $s \in \{0,1\}^n$ such that $G(f)(s)$ has a positive circuit. More precisely, if f has two fixed points a and b , and if I is such that $b = \bar{a}^I$, then there is an $s \in \{0,1\}^n$ such that $G(f)(s)$ has a positive I -circuit.*

In the previous example, f has two fixed points $a = (0,1,0)$ and $b = (1,0,0)$, thus $b = \bar{a}^{\{1,2\}}$. In accordance with Theorem 2.1, the regulatory graph $G(f)(0,0,1)$ contains a positive circuit between gene 1 and gene 2.

3 The intercellular case

We now turn to the case of several interacting cells. In this paper, we shall consider cells with a fixed location on an infinite 1-dimensional grid. We shall be interested in the evolution of the system consisting in the same collection of n genes $1, \dots, n$ in each cell.

3.1 Dynamics

A *state* is a map $s : \mathbb{Z} \rightarrow \{0,1\}^n$. The set of states is denoted by S . If $s \in S$, s_i denotes, for each $i = 1, \dots, n$, the i -th coordinate map $\mathbb{Z} \rightarrow \{0,1\}$. For $i = 1, \dots, n$ and $x \in \mathbb{Z}$, $s_i(x)$ denotes the expression level of gene i in cell number x . A *local state* is a map $s :]x, y[\rightarrow \{0,1\}^n$ for some open interval $]x, y[\subset \mathbb{Z}$. The restriction of a state s to an interval $]x, y[\subset \mathbb{Z}$ is a local state denoted by $s|_{]x, y[} :]x, y[\rightarrow \{0,1\}^n$.

Consider a map $f : \{0,1\}^{3n} \rightarrow \{0,1\}^n$. A map $F : S \rightarrow S$ is then defined by

$$F(s)(x) = f(s(x-1), s(x), s(x+1)).$$

For any s , x and $i = 1, \dots, n$, $F_i(s)(x)$ denotes the value to which the expression level of gene i in cell number x tends when the system is in state s . The definition of F from f corresponds to the assumption that cells interact locally, i.e., a cell can only interact with itself or its immediate neighbours. A fixed point for F is a state s such that for all $x \in \mathbb{Z}$, $f(s(x-1), s(x), s(x+1)) = s(x)$. By abuse of terminology, we shall say that s is then a fixed point for f too.

Now, given such a map f , the nondeterministic *asynchronous dynamics* is a graph with vertex set S , and with an edge from s to s' when there exist i and x such that $F_i(s)(x) \neq s_i(x)$ and s' is the state $\bar{s}^{i,x}$ defined by:

$$\begin{aligned} \bar{s}^{i,x}(x) &= s(x)^i \\ \bar{s}^{i,x}(y) &= s(y) \quad \text{for } y \neq x. \end{aligned}$$

Observe that f determines the dynamics only locally, i.e., inside a local state restricted to three cells, and the global dynamics F is obtained by gluing together these local pieces of dynamics.

The asynchrony assumption does not take into account explicit delays. In particular, no difference is made between intracellular regulation processes on the one

hand, and on the other hand the regulation due to diffusion, which occurs in general via transmembrane signaling. Despite of these limitations, it is worth observing that the main dynamical property we shall be investigating is the presence of fixed points, which is independent from any reasonable assumption on the dynamics: synchronous, asynchronous, with delays, parallel evolution of the cells as in Lindenmayer systems [6], etc. (Of course however, the synchronous dynamics has more periodic behaviours than the asynchronous one.)

3.2 Regulatory graphs

Let $f : \{0, 1\}^{3n} \rightarrow \{0, 1\}^n$, $s : \mathbb{Z} \rightarrow \{0, 1\}^n$ and $x \in \mathbb{Z}$. The regulatory graph $G(f)(s)(x)$ has as vertices pairs $(i, x - 1)$, (i, x) and $(i, x + 1)$ with $i \in \{1, \dots, n\}$. Its edges are of three types:

- $G(f)(s)(x)$ has an edge from $(i, x - 1)$ to (j, x) when

$$f_j(s(x - 1)^i, s(x), s(x + 1)) \neq f_j(s(x - 1), s(x), s(x + 1))$$

with positive sign when $s_i(x - 1) = f_j(s(x - 1), s(x), s(x + 1))$ and negative sign otherwise,

- $G(f)(s)(x)$ has an edge from (i, x) to (j, x) when

$$f_j(s(x - 1), s(x)^i, s(x + 1)) \neq f_j(s(x - 1), s(x), s(x + 1))$$

with positive sign when $s_i(x) = f_j(s(x - 1), s(x), s(x + 1))$ and negative sign otherwise,

- $G(f)(s)(x)$ has an edge from $(i, x + 1)$ to (j, x) when

$$f_j(s(x - 1), s(x), s(x + 1)^i) \neq f_j(s(x - 1), s(x), s(x + 1))$$

with positive sign when $s_i(x + 1) = f_j(s(x - 1), s(x), s(x + 1))$ and negative sign otherwise.

The union of two graphs here is simply the union of vertex sets and the union of edges sets. We define the regulatory graph $G(f)(s)$ associated to a state s as follows:

$$G(f)(s) = \bigcup_{x \in \mathbb{Z}} G(f)(s)(x).$$

The following Lemma is an immediate consequence of the definition of F from f as a local interaction between cells (Section 3.1).

Lemma 3.1 *Given F any map from S to S and $s \in S$, let $G(F)(s)$ be the graph with vertex set $\{1, \dots, n\} \times \mathbb{Z}$ and an edge from (i, x) to (j, y) when*

$$F_j(\bar{s}^{i,x})(y) \neq F_j(s)(y),$$

with positive sign when $s_i(x) = F_j(s)(y)$ and negative sign otherwise. If F arises from $f : \{0, 1\}^{3n} \rightarrow \{0, 1\}^n$ as in Section 3.1, then $G(F)(s) = G(f)(s)$.

Given an interval $]x, y[\subset \mathbb{Z}$, the regulatory graph $G(f)(s \upharpoonright_{]x, y[})$ on the restricted state $s \upharpoonright_{]x, y[}$ is defined by:

$$G(f)(s \upharpoonright_{]x, y[}) = \bigcup_{x < z < y} G(f)(s)(z).$$

In particular, $G(f)(s)(x) = G(f)(s \upharpoonright_{]x-1, x+1[})$.

3.3 Example

Consider for instance the following table defining a partial dynamics in the intercellular case with two genes in each cell (the two rows correspond to genes 1 and 2, and columns correspond to cells). As observed in Section 3.1, this dynamics is defined by giving the expression levels to which the two genes tend (an element of $\{0, 1\}^2$ in this case) for each tuple σ of expression levels in the neighbourhood. In this case, σ is a triple of elements of $\{0, 1\}^2$ corresponding to expression levels in the current cell and in the two surrounding ones. The following dynamics is partial in the sense that we do not consider here all 64 possible values for σ .

| | | | | | | | |
|-------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|
| σ | $\begin{pmatrix} 1, 0, 0 \\ 1, 0, 1 \end{pmatrix}$ | $\begin{pmatrix} 0, 0, 0 \\ 1, 0, 1 \end{pmatrix}$ | $\begin{pmatrix} 1, 1, 0 \\ 1, 0, 1 \end{pmatrix}$ | $\begin{pmatrix} 1, 0, 1 \\ 1, 0, 1 \end{pmatrix}$ | $\begin{pmatrix} 1, 0, 0 \\ 0, 0, 1 \end{pmatrix}$ | $\begin{pmatrix} 1, 0, 0 \\ 1, 1, 1 \end{pmatrix}$ | $\begin{pmatrix} 1, 0, 0 \\ 1, 0, 0 \end{pmatrix}$ |
| $f(\sigma)$ | $\begin{pmatrix} 1 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 0 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 1 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 0 \\ 0 \end{pmatrix}$ |
| σ | $\begin{pmatrix} 0, 0, 1 \\ 0, 1, 1 \end{pmatrix}$ | $\begin{pmatrix} 1, 0, 1 \\ 0, 1, 1 \end{pmatrix}$ | $\begin{pmatrix} 0, 1, 1 \\ 0, 1, 1 \end{pmatrix}$ | $\begin{pmatrix} 0, 0, 0 \\ 0, 1, 1 \end{pmatrix}$ | $\begin{pmatrix} 0, 0, 1 \\ 1, 1, 1 \end{pmatrix}$ | $\begin{pmatrix} 0, 0, 1 \\ 0, 0, 1 \end{pmatrix}$ | $\begin{pmatrix} 0, 0, 1 \\ 0, 1, 0 \end{pmatrix}$ |
| $f(\sigma)$ | $\begin{pmatrix} 1 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 1 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 0 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 0 \end{pmatrix}$ |
| | | σ | $\begin{pmatrix} 0, 0, 1 \\ 0, 0, 1 \end{pmatrix}$ | $\begin{pmatrix} 0, 1, 0 \\ 0, 1, 0 \end{pmatrix}$ | $\begin{pmatrix} 1, 1, 1 \\ 1, 1, 1 \end{pmatrix}$ | | |
| | | $f(\sigma)$ | $\begin{pmatrix} 0 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 1 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 1 \end{pmatrix}$ | | |

For $\sigma = \begin{pmatrix} 1, 0, 0 \\ 1, 0, 1 \end{pmatrix}$, let

$$\sigma^\ell = \begin{pmatrix} 1 \\ 1 \end{pmatrix}, \sigma^c = \begin{pmatrix} 0 \\ 0 \end{pmatrix}, \sigma^r = \begin{pmatrix} 0 \\ 1 \end{pmatrix}$$

denote its left, central and right columns. Then the regulatory graph $G(f)(\sigma)$ contains three edges, a positive edge from gene 1 in the left cell to gene 1 in the central cell, because

$$f_1 \begin{pmatrix} 1, 0, 0 \\ 1, 0, 1 \end{pmatrix} = 1 \neq 0 = f_1 \begin{pmatrix} 0, 0, 0 \\ 1, 0, 1 \end{pmatrix} \quad \text{and} \quad \sigma_1^\ell = 1 = f_1 \begin{pmatrix} 1, 0, 0 \\ 1, 0, 1 \end{pmatrix},$$

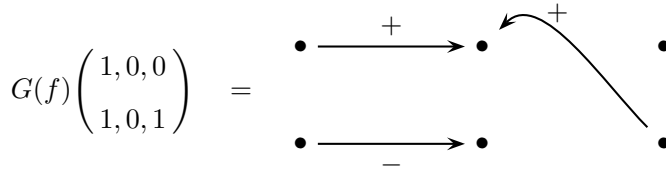
a negative edge from gene 2 in the left cell to gene 2 in the central cell,

$$f_2 \begin{pmatrix} 1, 0, 0 \\ 1, 0, 1 \end{pmatrix} \neq f_2 \begin{pmatrix} 1, 0, 0 \\ 0, 0, 1 \end{pmatrix} \quad \text{and} \quad \sigma_2^\ell \neq f_2 \begin{pmatrix} 1, 0, 0 \\ 1, 0, 1 \end{pmatrix},$$

and a positive edge from gene 2 in the right cell to gene 1 in the central cell, because

$$f_1 \begin{pmatrix} 1, 0, 0 \\ 1, 0, 1 \end{pmatrix} \neq f_1 \begin{pmatrix} 1, 0, 0 \\ 1, 0, 0 \end{pmatrix} \quad \text{and} \quad \sigma_2^r = f_1 \begin{pmatrix} 1, 0, 0 \\ 1, 0, 1 \end{pmatrix}.$$

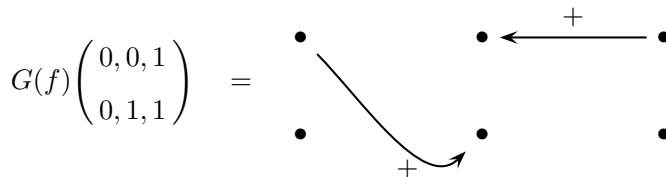
Therefore:



Where the first row corresponds to gene 1, the second row to gene 2, and columns correspond to cells. Similarly, the regulatory graph

$$G(f) \begin{pmatrix} 0, 0, 1 \\ 0, 1, 1 \end{pmatrix}$$

contains two edges, a positive edge from gene 1 of the right cell to gene 1 of the central cell and a positive edge from gene 1 of the left cell to gene 2 of the central cell.

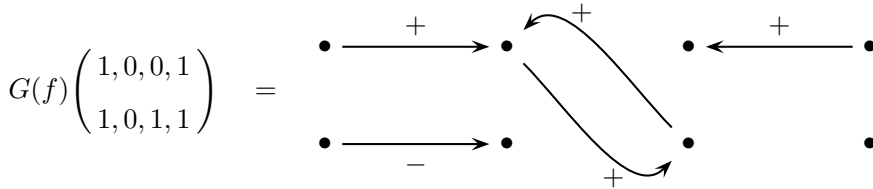


We may now build the regulatory graph

$$G(f) \begin{pmatrix} 1, 0, 0, 1 \\ 1, 0, 1, 1 \end{pmatrix}$$

which is by definition the union of

$$G(f) \begin{pmatrix} 1, 0, 0 \\ 1, 0, 1 \end{pmatrix} \quad \text{and} \quad G(f) \begin{pmatrix} 0, 0, 1 \\ 0, 1, 1 \end{pmatrix} :$$



4 Positive circuits

Consider the action of \mathbb{Z} on S given by $(z \cdot s)(x) = s(z + x)$. A state s is periodic when there exists some integer z such that $z \cdot s = s$; and z is then a period of s . Therefore, periodicity here means periodicity in space.

Two states s and s' are equivalent modulo translation when they are in the same orbit for this action, i.e., when there is a $z \in \mathbb{Z}$ such that $s' = z \cdot s$. On the other hand, let us say that two states s and s' have a common cell when there exist $x, x' \in \mathbb{Z}$ such that $s(x) = s'(x')$.

Theorem 4.1 *Let $f : \{0, 1\}^{3n} \rightarrow \{0, 1\}^n$. If f has two periodic fixed points which are not in the same orbit and have a common cell, then there is a state s such that $G(f)(s)$ has a positive circuit. More precisely, if f has two fixed points a and b with periods k and k' , then there is a state s such that $G(f)(s|_{]1, K+1[})$ has a positive circuit, where K is the least common multiple of k and k' .*

As in Section 2, the sign of a circuit is the product of the signs of its edges.

Proof. Let a and b be two fixed points of periods k and k' respectively. Since a and b have a common cell and the action of \mathbb{Z} clearly preserves the fact of being a fixed point, we may assume that $a(1) = b(1)$.

Let us introduce the following notation: if $c = (c_1, \dots, c_\ell) \in \{0, 1\}^{n\ell}$, let c^∞ denote the state which sends $y + k\ell \in \mathbb{Z}$ to

$$c^\infty(y + k\ell) = c_y, \quad 1 \leq y \leq \ell, k \in \mathbb{Z}.$$

With this notation, a and

$$(a(1), \dots, a(k))^\infty$$

are in the same orbit (under the above action), and similarly, b and $(b(1), \dots, b(k'))^\infty$ are in the same orbit.

Let K be the least common multiple of k and k' . We can define an imaginary intracellular dynamics with Kn genes as follows. Intracellular states in $\{0, 1\}^{Kn}$ are denoted as K -tuples (s^1, \dots, s^K) of n -tuples. Let \hat{f} be the map from $\{0, 1\}^{Kn}$ to $\{0, 1\}^{Kn}$ defined by:

$$\hat{f}(s^1, \dots, s^K) = \left(f(s^K, s^1, s^2), \dots, f(s^{x-1}, s^x, s^{x+1}), \dots, f(s^{K-1}, s^K, s^1) \right).$$

(Here, we have identified $\{0, 1\}^{Kn}$ with $(\{0, 1\}^n)^K$, i.e., the set of K tuples of n -tuples of 0's and 1's.) The intuition is that this dynamics \hat{f} preserves the local dynamics of intercellular communication, i.e., a gene i of a cell number x can only interact with genes in cells $x - 1$, x and $x + 1$: each gene has a field of activity restricted to a window of three cells. More precisely, as stated in Lemma 4.2 below, the restriction of $G(\hat{f})(s)$ to vertices in $\{1, \dots, n\} \times \{2, \dots, K\}$ equals $G(f)(s^\infty \upharpoonright_{2,K[})$.

Now, $a \upharpoonright_{]0, K+1[}$ and $b \upharpoonright_{]0, K+1[}$ are two different fixed points of \hat{f} such that

$$a \upharpoonright_{]0, K+1[}(1) = b \upharpoonright_{]0, K+1[}(1).$$

According to Theorem 2.1, there is therefore an $s \in \{0, 1\}^{Kn}$, such that $G(\hat{f})(s)$ has a positive I -circuit for $I = \{1, \dots, n\} \times \{2, \dots, K\}$. This circuit does not involve vertices of the form $(i, 1)$ (i.e., vertices corresponding to the first cell), hence by the previous paragraph, it is in $G(f)(s^\infty \upharpoonright_{2,K[})$ as well. \square

Lemma 4.2 *With the above notations, $G(f)(s^\infty \upharpoonright_{2,K[})$ equals the restriction of $G(\hat{f})(s)$ to vertices in $\{1, \dots, n\} \times \{2, \dots, K\}$.*

Proof. In this restriction of $G(\hat{f})(s)$, we have five types of edges to consider:

- (i) An edge from $(i, x - 1)$ to (j, x) for $x \in \{3, \dots, K - 1\}$ and $i, j = 1, \dots, n$ when

$$\hat{f}_j(s^1, \dots, s^{x-1}{}^i, s^x, \dots, s^K)(x) \neq \hat{f}_j(s^1, \dots, s^{x-1}, s^x, \dots, s^K)(x).$$

This is equivalent to $f_j(s^{x-1}{}^i, s^x, s^{x+1}) \neq f_j(s^{x-1}, s^x, s^{x+1})$ by definition of \hat{f} . By definition of the regulatory graph in the intercellular case, this is also equivalent to the existence of an edge from $(i, x - 1)$ to (j, x) in $G(f)(s^\infty \upharpoonright_{2,K[})$. The equality of the signs of the edges is immediate.

- (ii) An edge from $(i, K - 1)$ to (j, K) for $i, j = 1, \dots, n$ when

$$\hat{f}_j(s^1, \dots, s^{K-1}{}^i, s^K)(K) \neq \hat{f}_j(s^1, \dots, s^{K-1}, s^K)(K)$$

which is equivalent to $f_j(s^{K-1}{}^i, s^K, s^1) \neq f_j(s^{K-1}, s^K, s^1)$, hence there is an edge from $(i, K - 1)$ to (j, K) in $G(f)(s^\infty \upharpoonright_{2,K[})$ and conversely.

(iii) An edge from $(i, x+1)$ to (j, x) for $x \in \{2, \dots, K-1\}$ and $i, j = 1, \dots, n$ when

$$\widehat{f_j}(s^1, \dots, s^x, \overline{s^{x+1}}^i, \dots, s^K)(x) \neq \widehat{f_j}(s^1, \dots, s^x, s^{x+1}, \dots, s^K)(x)$$

which is equivalent to $f_j(s^{x-1}, s^x, \overline{s^{x+1}}^i) \neq f_j(s^{x-1}, s^x, s^{x+1})$, hence there is an edge from $(i, x+1)$ to (j, x) in $G(f)(s^\infty \upharpoonright_{[2, K[})$ and conversely.

(iv) An edge from (i, x) to (j, x) for $x \in \{2, \dots, K-1\}$ and $i, j = 1, \dots, n$ when

$$\widehat{f_j}(s^1, \dots, \overline{s^x}^i, \dots, s^K)(x) \neq \widehat{f_j}(s^1, \dots, s^x, \dots, s^K)(x)$$

which is equivalent to $f_j(s^{x-1}, \overline{s^x}^i, s^{x+1}) \neq f_j(s^{x-1}, s^x, s^{x+1})$, therefore there is an edge from (i, x) to (j, x) in $G(f)(s^\infty \upharpoonright_{[2, K[})$ and conversely.

(v) An edge from (i, K) to (j, K) for $i, j = 1, \dots, n$ when

$$\widehat{f_j}(s^1, \dots, \overline{s^K}^i)(K) \neq \widehat{f_j}(s^1, \dots, s^K)(K)$$

which is equivalent to $f_j(s^{K-1}, \overline{s^K}^i, s^1) \neq f_j(s^{K-1}, s^K, s^1)$, therefore there is an edge from (i, K) to (j, K) in $G(f)(s^\infty \upharpoonright_{[2, K[})$ and conversely.

□

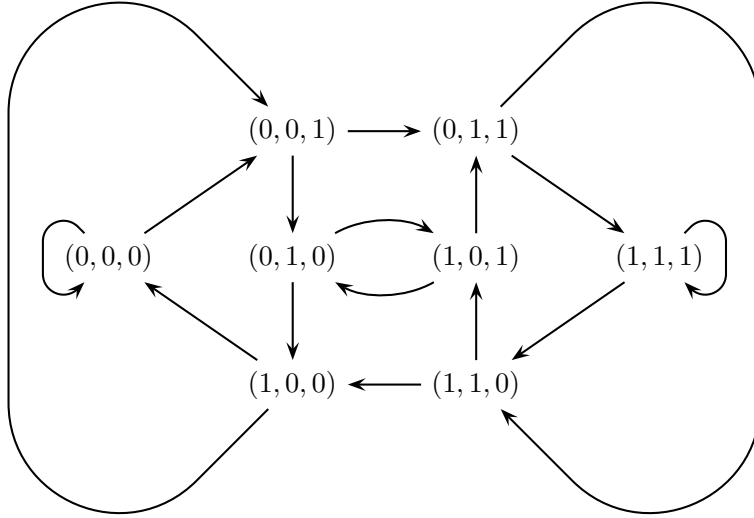
Remark that for a map $f : \{0, 1\}^{3n} \rightarrow \{0, 1\}^n$ which only depends on the expression levels in the central cell ($f(a, b, c)$ only depends on b), there is no communication between cells: there are only intracellular interactions and we recover the case of Theorem 2.1. In the general case with intercellular communication, Theorem 4.1 ensures that a positive circuit can be found in a neighbourhood of a most $K-1$ cells: there is thus an upper bound on the length of the predicted circuit. One may however conjecture a stronger, more local, result implying, under some reasonable hypothesis, a positive circuit in a neighbourhood of at most two cells, for instance.

It is also interesting to observe that a single periodic fixed point a suffices to imply the existence of a positive circuit, as long as a is non-constant and has two common cells in its (smallest) period ($a(\ell) = a(k)$ for some $k \neq \ell$). Indeed, we may then recover the hypothesis of Theorem 4.1 by shifting from k to ℓ .

Corollary 4.3 *Let $f : \{0, 1\}^{3n} \rightarrow \{0, 1\}^n$. If f has one periodic fixed point which has two common cells in its period, then there is a state s such that $G(f)(s)$ has a positive circuit.*

It is also worth observing that the existence of a fixed point ensures the presence

of a periodic one. Indeed, let B be the following de Bruijn graph [2]:



A state $s : \mathbb{Z} \rightarrow \{0, 1\}$ is the same as a doubly infinite path in B : for instance, given s , the path consists in all edges from $(s(x), s(x+1), s(x+2))$ to $(s(x+1), s(x+2), s(x+3))$ for $x \in \mathbb{Z}$, and conversely any path in B induces a state up to the \mathbb{Z} -action. Similarly, a state $s : \mathbb{Z} \rightarrow \{0, 1\}^n$ is the same as a doubly infinite path in the categorical product $B \times \cdots \times B$ of n copies of B . (Recall that the categorical product $G \times G'$ of two graphs G and G' has vertex set the Cartesian product of the vertex sets and has an edge from (x, x') to (y, y') for each edge from x to y in G and each edge from x' to y' in G' .) Now, a fixed point $s : \mathbb{Z} \rightarrow \{0, 1\}^n$ determines a path γ in $B \times \cdots \times B$, hence a sequence of vertices $(\gamma_x)_{x \in \mathbb{Z}}$ such that $\gamma_x = (\gamma_x^1, \gamma_x^2, \gamma_x^3)$ and $f(\gamma_x) = \gamma_x^2$. From this infinite sequence of vertices in a finite graph, it is possible to extract a cyclic one, therefore one corresponding to a periodic fixed point.

Finally, it is clearly not difficult to adapt Theorem 4.1 and Corollary 4.3 from our Boolean formalism to the multilevel formalism of [10].

5 Examples and discussion

5.1 Illustration of Theorem 4.1

Consider for instance the dynamics of Section 3.3. f has two fixed points modulo the \mathbb{Z} -action

$$\begin{pmatrix} 100 \\ 100 \end{pmatrix}^\infty \quad \text{and} \quad \begin{pmatrix} 1 \\ 1 \end{pmatrix}^\infty.$$

They are periodic with periods 3 and 1 respectively, are not in the same orbit and have a common cell

$$\begin{pmatrix} 1 \\ 1 \end{pmatrix}.$$

Thus according to Theorem 4.1, there is a state $s : \mathbb{Z} \rightarrow \{0,1\}^2$, such that $G(f)(s|_{\mathbb{J}_{1,4}[]})$ has a positive circuit. This is actually the case for

$$s = \begin{pmatrix} 100 \\ 101 \end{pmatrix}^\infty, \quad \text{since} \quad G(f)(s|_{\mathbb{J}_{1,4}[]}) = G(f) \begin{pmatrix} 1, 0, 0, 1 \\ 1, 0, 1, 1 \end{pmatrix}$$

for this choice of s . This graph has been computed at the end of Section 3.3. Another example is given by the following dynamics, with one gene in each cell:

| | | | | | | | | |
|--------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| s | $(0, 0, 0)$ | $(0, 0, 1)$ | $(0, 1, 0)$ | $(0, 1, 1)$ | $(1, 0, 0)$ | $(1, 0, 1)$ | $(1, 1, 0)$ | $(1, 1, 1)$ |
| $f(s)$ | (1) | (0) | (1) | (0) | (0) | (0) | (0) | (0) |

This dynamics corresponds to $f(a, b, c) = \bar{a} \wedge \bar{c}$ for $a, b, c \in \{0,1\}$, and has two non-constant fixed points $(0, 1)^\infty$ and $(1, 0, 0)^\infty$ with a common cell. By Theorem 4.1, there is a state s , such that $G(f)(s)$ has a positive circuit. This is the case for $s = (1, 0, 0)^\infty$, since $G(f)((1, 0, 0)^\infty)$ is the following disconnected graph (where the values attached to vertices correspond here to the states of cells):



Observe that for this dynamics, there is no state s such that $G(f)(s)$ has an intracellular circuit, simply because $f(a, b, c)$ does not depend on b .

5.2 Common cell

The common column allows one to avoid the edges between the left and right sides of the “window”, and thus to avoid wrong positive circuits. As an example to illustrate this point, consider the following dynamics in the intercellular case with one gene in each cell:

| | | | | | | | | |
|--------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| s | $(0, 0, 0)$ | $(0, 0, 1)$ | $(0, 1, 0)$ | $(0, 1, 1)$ | $(1, 0, 0)$ | $(1, 0, 1)$ | $(1, 1, 0)$ | $(1, 1, 1)$ |
| $f(s)$ | (0) | (0) | (0) | (0) | (1) | (1) | (1) | (1) |

This dynamics is simply given by $f(a, b, c) = a$ for $a, b, c \in \{0,1\}$, and corresponds to a positive influence from left to right. We can easily prove that for any state, the

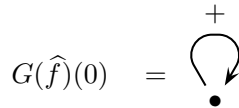
regulatory graph is:



and has no positive circuit. There are two fixed points, the two constants states $(0)^\infty$ and $(1)^\infty$ of period 1, which are not in the same orbit but have no common cell. However $\hat{f} : \{0, 1\} \rightarrow \{0, 1\}$ is given by the following table:

| s | $\hat{f}(s)$ |
|-----|--------------|
| 0 | 0 |
| 1 | 1 |

and $G(\hat{f})(0)$ has a positive autoregulation:



This positive loop is the result of “gluing” the two sides of the window. It is therefore conceivable that the common cell condition could be removed when the grid is finite and circular, but this is beyond the framework considered in this paper.

5.3 A unique (non-constant) fixed point

Consider the following intercellular dynamics with one gene in each cell:

| s | $(0, 0, 0)$ | $(0, 0, 1)$ | $(0, 1, 0)$ | $(0, 1, 1)$ | $(1, 0, 0)$ | $(1, 0, 1)$ | $(1, 1, 0)$ | $(1, 1, 1)$ |
|--------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| $f(s)$ | (1) | (1) | (1) | (1) | (0) | (0) | (0) | (0) |

This dynamics is given by $f(a, b, c) = \bar{a}$ for $a, b, c \in \{0, 1\}$, and just amounts to a negative influence from left to right. We can easily prove that for any state, the regulatory graph:



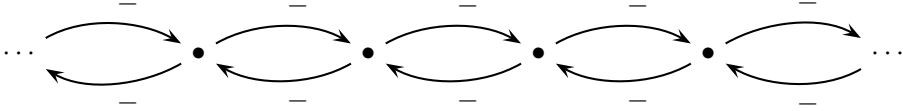
has no positive circuit, although f has a non-constant fixed point $(01)^\infty$.

Remark that if we change the values of $f(0, 1, 1)$ and $f(1, 0, 0)$, the fonction f is isotropic in the following sense: $f : \{0, 1\}^{3n} \rightarrow \{0, 1\}^n$ is *isotropic* when for all $a, b, c \in \{0, 1\}^n$,

$$f(a, b, c) = f(c, b, a).$$

The intuition for this condition is that communication is undirected, a situation which occurs among non-polarised cells.

Then f still has a non-constant fixed point $(01)^\infty$ and $G(f)((01)^\infty)$:



has a positive circuit. It is possible that the isotropy condition and the presence of a non-constant fixed point suffice for the presence of positive circuit, but we have no proof of this conjecture.

5.4 Symmetrically ultimately periodic states

A state s is *symmetrically ultimately periodic* when there exist two states $s_1 : I_1 \rightarrow \{0, 1\}^n$ and $s_2 : I_2 \rightarrow \{0, 1\}^n$ restricted to finite intervals $I_1, I_2 \subset \mathbb{Z}$ such that s is in the same orbit as $\cdots s_1 s_1 s_2 s_1 s_1 s_1 \cdots$, a state which we simply denote by $(s_1)^\infty s_2 (s_1)^\infty$.

Lemma 5.1 *Let $f : \{0, 1\}^{3n} \rightarrow \{0, 1\}^n$. If f has two symmetrically ultimately periodic fixed points which are not in the same orbit and have a common cell, then there is a state s such that $G(f)(s)$ has a positive circuit.*

Proof. Three cases are to be considered:

- (i) The common cell is in the periodic part of each state. From a symmetrically ultimately periodic fixed point, it is possible to extract a periodic fixed point: if $s = (s_1)^\infty s_2 (s_1)^\infty$ with $s_1 : I_1 \rightarrow \{0, 1\}^n$, $s_2 : I_2 \rightarrow \{0, 1\}^n$, then $(s_1)^\infty$ is a periodic fixed point for f . The result is therefore an immediate consequence of Theorem 4.1.
- (ii) The common cell is in the non-periodic part of each state. In order to apply Theorem 4.1, choose a window of length K sufficiently large to overlap the two periodic part of each state. More precisely, let s and s' be two such symmetrically ultimately periodic fixed points, who are respectively in the same orbit as

$$(s_1)^\infty s_2 (s_1)^\infty \quad \text{and} \quad (s'_1)^\infty s'_2 (s'_1)^\infty.$$

Here $s_1 : I_1 \rightarrow \{0, 1\}^n$, $s_2 : I_2 \rightarrow \{0, 1\}^n$, $s'_1 : I'_1 \rightarrow \{0, 1\}^n$, $s'_2 : I'_2 \rightarrow \{0, 1\}^n$, I_1, I_2, I'_1, I'_2 are of lengths k_1, k_2, k'_1, k'_2 respectively. It suffices to take for K the least common multiple of k, k' with $k = 2k_1 + k_2$ and $k' = 2k'_1 + k'_2$.

- (iii) The common cell is in the periodic part of a state and in the non-periodic part of the other one. This case follows from the two previous cases.

□

Consider for instance the following partial dynamics in the intercellular case with

two genes in each cell:

| | | | | | | | |
|--------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|
| s | $\begin{pmatrix} 1, 1, 1 \\ 1, 1, 1 \end{pmatrix}$ | $\begin{pmatrix} 1, 0, 1 \\ 1, 1, 1 \end{pmatrix}$ | $\begin{pmatrix} 0, 1, 0 \\ 1, 1, 0 \end{pmatrix}$ | $\begin{pmatrix} 1, 0, 0 \\ 1, 0, 1 \end{pmatrix}$ | $\begin{pmatrix} 0, 0, 1 \\ 0, 1, 1 \end{pmatrix}$ | $\begin{pmatrix} 1, 1, 1 \\ 0, 0, 0 \end{pmatrix}$ | $\begin{pmatrix} 1, 1, 0 \\ 0, 0, 0 \end{pmatrix}$ |
| $f(s)$ | $\begin{pmatrix} 1 \\ 1 \end{pmatrix}$ | $\begin{pmatrix} 0 \\ 1 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 1 \end{pmatrix}$ | $\begin{pmatrix} 0 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 0 \\ 1 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 0 \end{pmatrix}$ | $\begin{pmatrix} 1 \\ 0 \end{pmatrix}$ |

$$\begin{array}{c} s \\ f(s) \end{array} \left| \begin{array}{c} \begin{pmatrix} 0, 0, 1 \\ 0, 0, 0 \end{pmatrix} \\ \begin{pmatrix} 0 \\ 0 \end{pmatrix} \end{array} \right| \begin{array}{c} \begin{pmatrix} 0, 1, 1 \\ 0, 0, 0 \end{pmatrix} \\ \begin{pmatrix} 1 \\ 0 \end{pmatrix} \end{array}$$

f has two symmetrically ultimately periodic fixed points

$$\begin{pmatrix} 1 \\ 1 \end{pmatrix}^\infty \begin{pmatrix} 0100 \\ 1101 \end{pmatrix} \begin{pmatrix} 1 \\ 1 \end{pmatrix}^\infty \quad \text{and} \quad \begin{pmatrix} 1 \\ 0 \end{pmatrix}^\infty \begin{pmatrix} 00 \\ 00 \end{pmatrix} \begin{pmatrix} 1 \\ 0 \end{pmatrix}^\infty$$

with a common cell

$$\begin{pmatrix} 0 \\ 0 \end{pmatrix}.$$

According to Lemma 5.1, there is a state $s : \mathbb{Z} \rightarrow \{0, 1\}^2$, such that $G(f)(s)$ has a positive circuit. Actually, we have:

$$G(f) \begin{pmatrix} 1, 1, 1 \\ 1, 1, 1 \end{pmatrix} = \begin{array}{ccc} \bullet & \begin{array}{c} + \\ \curvearrowright \end{array} \bullet & \bullet \\ \bullet & \bullet & \bullet \end{array}$$

References

- [1] A. Crumière. Positive circuits and two-dimensional spatial differentiation. Application to the formation of sense organs in *Drosophila*. Preprint Institut de Mathématiques de Luminy 2007-6. Available at <http://iml.univ-mrs.fr/editions/preprint2007/preprint2007.html>. *Information Processing in Cells and Tissues*, 2007.
- [2] N. G. de Bruijn. A combinatorial problem. In *Nederlandse Academie van Wetenschappen*, volume 49, pages 758–764, 1946.

- [3] A. Ghysen and R. Thomas. The formation of sense organs in *Drosophila*: a logical approach. *BioEssays*, 25:802–807, 2003.
- [4] A. González, C. Chaouiya, and D. Thieffry. Dynamical analysis of the regulatory network defining the dorsal-ventral boundary of the *Drosophila* wing imaginal disc. *Genetics*, 2006.
- [5] J.-L. Gouzé. Positive and negative circuits in dynamical systems. *Journal of Biological Systems*, 6:11–15, 1998.
- [6] A. Lindenmayer. Mathematical models for cellular interaction in development I. filaments with one-sided inputs. *Journal of Theoretical Biology*, 18:280–289, 1968.
- [7] E. Plahte, T. Mestl, and S. W. Omholt. Feedback loops, stability and multistationarity in dynamical systems. *Journal Biological Systems*, 3:409–413, 1995.
- [8] É. Remy, P. Ruet, and D. Thieffry. Graphic requirements for multistability and attractive cycles in a Boolean dynamical framework. *Advances in Applied Mathematics*, 2008. To appear.
- [9] É. Remy, P. Ruet, and D. Thieffry. Positive or negative regulatory circuit inference from multilevel dynamics. In *Positive Systems: Theory and Applications*, volume 341 of *Lecture Notes in Control and Information Sciences*, pages 263–270. Springer, 2006.
- [10] A. Richard and J.-P. Comet. Necessary conditions for multistationarity in discrete dynamical systems. Technical Report 123-2005, Laboratoire de Méthodes Informatiques, Université d’Evry, 2005.
- [11] E. H. Snoussi. Necessary conditions for multistationarity and stable periodicity. *Journal of Biological Systems*, 6:3–9, 1998.
- [12] C. Soulé. Graphic requirements for multistationarity. *ComPlexUs*, 1:123–133, 2003.
- [13] C. Soulé. Mathematical approaches to gene regulation and differentiation. In *Comptes Rendus de l’Académie des Sciences*, volume 329 of *Biologies*, pages 13–20. Elsevier, 2006.
- [14] R. Thomas. On the relation between the logical structure of systems and their ability to generate multiple steady states and sustained oscillations. In *Series in Synergetics*, volume 9, pages 180–193. Springer, 1981.
- [15] J. von Neumann. *Theory of self-reproducing automata*. University of Illinois Press, 1966.