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Positive circuits and *d*-dimensional spatial differentiation: Application to the formation of sense organs in *Drosophila*

Anne Crumière [a,*], Mathieu Sablik [b]

[a] *Institut de Mathématiques de Luminy, Campus de Luminy, Case 907, 13288 Marseille Cedex 9, France*
[b] *Unité de Mathématiques Pures et Appliquées, ENS Lyon, 46 allée d'Italie, 69364 Lyon Cedex 07, France*

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ABSTRACT

We discuss a rule proposed by the biologist Thomas according to which the possibility for a genetic network (represented by a signed directed graph called a regulatory graph) to have several stable states implies the existence of a positive circuit. This result is already known for different models, differential or discrete formalism, but always with a network of genes contained in a single cell. Thus, we can ask about the validity of this rule for a system containing several cells and with intercellular genetic interactions. In this paper, we consider the genetic interactions between several cells located on a *d*-dimensional lattice, i.e., each point of lattice represents a cell to which we associate the expression level of *n* genes contained in this cell. With this configuration, 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. We then illustrate this theorem through the example of the formation of sense organs in *Drosophila*.

1. Introduction

Proteins synthesised by genes play an essential part in many cellular processes: they can bind to DNA and regulate the transcription of specific genes. This regulation of transcription is a very complex mechanism. A gene, when expressed, leads to the production of proteins that can either activate or inhibit the expression of other genes. Therefore the activity of a gene in a cell is measured by the concentration of the transcribed RNA in the cell, a quantity called the *expression level of the gene*. Genetic interactions form a genetic regulatory network, from which one can draw a *regulatory graph*. It is a signed directed graph: the vertices are the genes, the edges are 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 graphs and their dynamical properties. The biologist Thomas has enounced the following general rule (Thomas, 1981): *a necessary condition for multistability* (*i.e., the existence of several stable fixed points in the dynamic*) *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 dynamic of a single cell, and it has given rise to mathematical statements and proofs in a differential dynamical formalism (Plahte et al., 1995; Snoussi, 1998; Gouzé, 1998; Soulé, 2003), and more recently in a discrete formalism (Remy et al., submitted for publication, 2006; Richard, 2006). This result in a discrete framework is recalled in Section 3.1. Thus in this paper we try to extend 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. Positive circuits are often associated to spatial differentiation: see, e.g., González et al. (2006) for a study of dorsal–ventral boundary in the *Drosophila* wing. On the other hand, Soulé raises this question in Soulé (2006).

In a first paper (Crumière and Ruet, 2006), we consider as a starting point the case of fixed cells located on a one-dimensional-infinite grid: more simply each cell is represented by an integer. This is a simplification which has the advantage of emphasing the basic formalism. Intercellular communication is assumed to be local: a gene may interact only with genes in its own cell at the position $m \in \mathbb{Z}$ and neighbouring cells, left or right, $m-1$ or $m+1$. This assumption, which is biologically reasonable is standard and at the basis of cellular automata (von Neumann, 1966).

In this present paper, we generalize in Section 2 the model above. We study an intercellular genetic network: the location of cells is done by a lattice, i.e., a discrete subgroup of $\mathbb{R}^d$, the expression level of genes is multivaluated and intercellular communication is extended to some neighbourhood. With this general framework, we obtain the Thomas' rule with a spatial condition on stable states. This theorem is the purpose of Section 3. Each notion is illustrated

* Corresponding author.
E-mail addresses: crumiere@iml.univ-mrs.fr (A. Crumière), msablik@umpa.ens-lyon.fr, sablik@latp.univ-mrs.fr (M. Sablik).

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by an example in two-dimensional: more precisely cells are located on a hexagonal two-dimensional grid. The choice of hexagons was followed on from a discussion with biologists in order to be in agreement with the biological reality. This layout implies that a cell has six neighbouring cells. We then apply our model through the example of the *Drosophila* in Section 4, in particular the formation of sense organs as modelled in an article from Ghysen and Thomas (2003).

2. Intercellular Case: The Formalism

In this paper, we shall consider cells with a fixed location on an arbitrary lattice. To illustrate our formalism, we choose to consider hexagonal cells which tile the plane. Thus cells are localized on a hexagonal lattice. This correspond to the realistic biological model of the formation of sense organs in *Drosophila* which is developed in Section 4.

2.1. Global Dynamic

Now we shall be interested in the evolution of the system composed of cells which contain in each cell the same collection of genes chosen in a finite set I. For a gene $i \in I$, $\mathcal{A}_i = [0, k_i]$ denotes the expression level of gene i. A state of a cell is an element $a = (a_i)_{i\in I} \in \mathcal{A}$ where $\mathcal{A}$ is the Cartesian product of Card(I) finite intervals of integers, i.e., $\mathcal{A} = \prod_{i\in I} A_i$.

Generally, a biological system is made up of several cells. We can assume that cells laid regularly and of discrete way in the space $\mathbb{R}^d$. So we can consider that cells are located on a lattice $\mathbb{M}$, i.e., a discrete subgroup of $\mathbb{R}^d$ endowed with the operation $+$. Each cell is in state of the *alphabet* $\mathcal{A}$. A *state of this system* or a *configuration* is a sequence of elements of $\mathcal{A}$ indexed by $\mathbb{M}$, i.e., an element of $\mathcal{A}^{\mathbb{M}}$. For all $x \in \mathcal{A}^{\mathbb{M}}$ and $\mathbb{U} \subset \mathbb{M}$, $x_{\mathbb{U}}$ denotes the restriction of x to $\mathbb{U}$. Furthermore, $x_{(i,m)}$ denotes the expression level of gene $i \in I$ in the cell number $m \in \mathbb{M}$.

Now we shall be interested in the evolution of the expression level of a gene in a cell. Intercellular communication is local: a cell can only interact with its neighbourhood. Thus, the expression level of a gene in a cell evolves in the time according to the expression level of genes in this cell and also in the neighbouring cells. Moreover, each cell interact uniformly in the space. A modelling of this phenomena is given by cellular automaton. We consider a finite set $\mathbb{V} \subset \mathbb{M}$ called *neighbourhood* and a *local function* $\bar{F} : \mathcal{A}^{\mathbb{V}} \to \mathcal{A}$. The *global dynamics* of the system is given by the *cellular automaton* $F : \mathcal{A}^{\mathbb{M}} \to \mathcal{A}^{\mathbb{M}}$ defined by

$$F(x)_m = \bar{F}((x_{m+v})_{v\in\mathbb{V}}) \quad \text{for all } x \in \mathcal{A}^{\mathbb{M}} \text{ and } m \in \mathbb{M}.$$

The definition of F from $\bar{F}$ corresponds to the assumption that cells interact locally, uniformly and synchronously.

Remark 1. In Remy et al. (submitted for publication, 2006) and Richard (2006), we consider the intracellular case which corresponds to the case where $\mathbb{M} = \{0\}$.

Example 1 *(Hexagonal lattice).* In this example, there is two genes per cells, i.e. $I = \{a, b\}$, which have two expression levels, thus $\mathcal{A} = \{0, 1\} \times \{0, 1\}$.

Moreover cells are located on a plane, i.e. $\mathbb{R}^2$. For biological reasons, cells are represented by hexagonals which tile the plane. Thus, we use the *hexagonal lattice* $\mathbb{M}$ generated by the vectors $\mathbf{e_1}$ and $\mathbf{e_2}$, i.e. $\mathbb{M} = \mathbb{Z}\mathbf{e_1} + \mathbb{Z}\mathbf{e_2}$. As usually used, for $m_1, m_2 \in \mathbb{Z}$, (m_1, m_2) denotes the coordinates of a cell $m \in \mathbb{M}$ in the lattice according to the base $(\mathbf{e_1}, \mathbf{e_2})$ and an arbitrary origin O (see Fig. 1).

Since cells are by hypothesis laid in hexagonal configurations, the neighbourhood $\mathbb{V}$ of the cell $(0, 0)$ is the set consisting in the six cells surrounding the cell $(0, 0)$ and the cell itself, that is to say $\mathbb{V} = \{(0, 0), (0, 1), (1, 1), (-1, 0), (1, 0), (-1, -1), (0, -1)\}$.

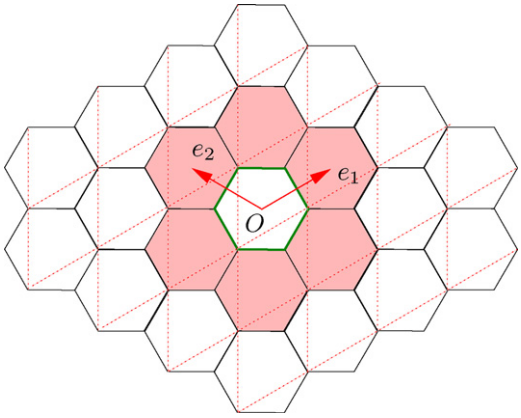


Fig. 1. Localisation.

Thus, by regularity of the lattice, the neighbourhood of a cell $m = (m_1, m_2)$ is $m + \mathbb{V} = \{(m_1, m_2), (m_1, m_2 + 1), (m_1 + 1, m_2 + 1), (m_1 - 1, m_2), (m_1 + 1, m_2), (m_1 - 1, m_2 - 1), (m_1, m_2 - 1)\}$. For $x \in \mathcal{A}^{\mathbb{M}}$ we write $x_{m+\mathbb{V}}$ as a 3×3 matrix with two holes:

$$x_{m+\mathbb{V}} = \begin{pmatrix} & x_{m_1,m_2+1} & x_{m_1+1,m_2+1} \\ x_{m_1-1,m_2} & x_{m_1,m_2} & x_{m_1+1,m_2} \\ x_{m_1-1,m_2-1} & x_{m_1,m_2-1} & \end{pmatrix}$$

For example, the local state $x_{\mathbb{V}}$ in Fig. 2 is composed of seven cells represented by hexagons. The two numbers in each cell are the expression levels of two genes. This state is mathematically represented by the matrix:

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

where each pair gives the expression levels of the two genes a and b inside the corresponding cell.

To simplify this example afterwards, we assume that there is just a gene, the gene A per cell, i.e. $I = \{A\}$, moreover this gene as just two levels of expression, i.e. $\mathcal{A}_A = \{0, 1\}$. One example of partial local dynamic for this gene is done in Fig. 3.

2.2. Asynchronous Dynamic

The synchronous assumption is not biologically acceptable. Indeed, it does not take into account explicit delays. In particular, no difference is made between intracellular regulation process on the one hand, and on the other hand the regulation due to diffusion, which occurs in general via transmembrane signaling, hence faster than regulation. That why Thomas (1981) describes the phenomena in the intracellular case with an asynchronous dynamic starting from a global dynamic. In our case, it is possible to describe the asynchronous dynamic in the intercellular case starting from the global dynamic.

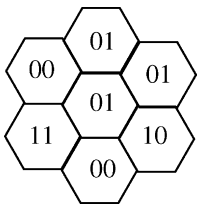


Fig. 2. $x_{\mathbb{V}}$.

$\overline{F}\begin{pmatrix} 0&0\\1&0&1\\0&0\end{pmatrix}=0$	$\overline{F}\begin{pmatrix} 0&0\\0&1&0\\0&0\end{pmatrix}=1$	$\overline{F}\begin{pmatrix} 0&0\\1&1&1\\1&0\end{pmatrix}=1$
$\overline{F}\begin{pmatrix} 0&0\\0&1&1\\1&1\end{pmatrix}=0$	$\overline{F}\begin{pmatrix} 0&0\\0&1&0\\1&1\end{pmatrix}=0$	$\overline{F}\begin{pmatrix} 0&1\\0&0&0\\0&1\end{pmatrix}=0$
$\overline{F}\begin{pmatrix} 0&1\\1&1&0\\1&1\end{pmatrix}=1$	$\overline{F}\begin{pmatrix} 0&1\\0&1&1\\1&1\end{pmatrix}=1$	$\overline{F}\begin{pmatrix} 0&1\\0&0&0\\1&1\end{pmatrix}=1$
$\overline{F}\begin{pmatrix} 0&1\\0&1&0\\0&1\end{pmatrix}=0$	$\overline{F}\begin{pmatrix} 0&1\\0&1&0\\1&0\end{pmatrix}=0$	$\overline{F}\begin{pmatrix} 0&1\\0&1&0\\1&1\end{pmatrix}=1$
$\overline{F}\begin{pmatrix} 0&1\\1&1&1\\1&1\end{pmatrix}=0$	$\overline{F}\begin{pmatrix} 0&1\\0&0&1\\1&1\end{pmatrix}=1$	$\overline{F}\begin{pmatrix} 0&1\\0&1&0\\1&1\end{pmatrix}=1$
$\overline{F}\begin{pmatrix} 0&1\\0&1&1\\0&1\end{pmatrix}=1$	$\overline{F}\begin{pmatrix} 0&1\\0&1&1\\1&0\end{pmatrix}=1$	$\overline{F}\begin{pmatrix} 0&1\\0&1&0\\0&1\end{pmatrix}=1$
$\overline{F}\begin{pmatrix} 0&1\\1&1&1\\0&0\end{pmatrix}=1$	$\overline{F}\begin{pmatrix} 0&1\\1&0&1\\0&1\end{pmatrix}=0$	$\overline{F}\begin{pmatrix} 0&1\\0&1&1\\1&1\end{pmatrix}=0$
$\overline{F}\begin{pmatrix} 1&0\\0&0&0\\1&0\end{pmatrix}=0$	$\overline{F}\begin{pmatrix} 1&0\\1&1&1\\0&1\end{pmatrix}=1$	$\overline{F}\begin{pmatrix} 1&0\\0&1&0\\1&0\end{pmatrix}=1$
$\overline{F}\begin{pmatrix} 1&0\\1&0&1\\1&0\end{pmatrix}=0$	$\overline{F}\begin{pmatrix} 1&1\\0&1&1\\1&1\end{pmatrix}=1$	$\overline{F}\begin{pmatrix} 1&1\\1&0&0\\1&1\end{pmatrix}=0$
$\overline{F}\begin{pmatrix} 1&1\\0&1&0\\1&1\end{pmatrix}=1$	$\overline{F}\begin{pmatrix} 1&1\\0&1&0\\1&1\end{pmatrix}=1$	

Fig. 3. Local dynamics.

For $t\in\mathbb{Z}$, define $\mathrm{sig}(t)=0$ if $t=0$, $\mathrm{sig}(t)=+1$ if $t>0$ and $\mathrm{sig}(t)=-1$ if $t<0$. For all $x,y\in\mathcal{A}^{\mathbb{M}}$ and $(i,m)\in I\times\mathbb{M}$, define $x^{(i,m)\lhd y}$ by for all $(j,n)\in I\times\mathbb{M}$:

$$x^{(i,m)\lhd y}_{(j,n)}=\begin{cases}x_{(j,n)} & \text{for all } (j,n)\neq(i,m)\\ x_{(i,m)}+\mathrm{sig}\,(y_{(i,m)}-x_{(i,m)}) & \text{if } (j,n)=(i,m).\end{cases}$$

Given such a cellular automaton $F:\mathcal{A}^{\mathbb{M}}\to\mathcal{A}^{\mathbb{M}}$, the nondeterministic *asynchronous dynamic* is a graph, denoted $GTA(F)$, defined by

- the vertex set which is $\mathcal{A}^{\mathbb{M}}$;
- the set of oriented edges, denoted $\mathrm{TA}(F)$, where there is an edge from x to y, i.e. $(x,y)\in\mathrm{TA}(F)$, when there exist $i\in I$ and $m\in\mathbb{M}$ such that

$$y=x^{(i,m)\lhd F(x)}.$$

The system evolves from a state $x\in\mathcal{A}^{\mathbb{M}}$ to another state $y\in\mathcal{A}^{\mathbb{M}}$ following the edges of $\mathrm{GTA}(F)$, i.e. $(x,y)\in\mathrm{TA}(F)$. Thus, $F(x)_{(i,m)}$ denotes the value to which the expression level of gene i in cell number m could tend when the system is in state x. Consequently, the expression level of at most one gene is updated at each step in at most one cell. Other dynamics can be considered, like the (deterministic) synchronous dynamics where all the expression level of genes are simultaneously updated in one step. But as argued in Remy et al. (submitted for publication), the asynchronous dynamic is more realistic.

Remark 2. The principal property studied in this paper is the presence of fixed points, which is independent from any reasonable assumption on the dynamics: synchronous, asynchronous, with delays.

2.3. Notion of Stability

In this article, we consider a general notion of stability according to a hyperrectangle of $\mathcal{A}^{\mathbb{M}}$. A *hyperrectangle* is a Cartesian product $\mathcal{P}=\prod_{(i,m)\in I\times\mathbb{M}}\mathcal{P}_{(i,m)}$ where each $\mathcal{P}_{(i,m)}$ is an interval of $\mathcal{A}_i$. For all $x,y\in\mathcal{A}^{\mathbb{M}}$ and $\mathbb{U}\subset\mathbb{M}$, the smallest hyperrectangle which contains $x_{\mathbb{U}}$ and $y_{\mathbb{U}}$ is denoted by $\pi(x_{\mathbb{U}},y_{\mathbb{U}})=\prod_{(i,m)\in I\times\mathbb{U}}[\min(x_{(i,m)},y_{(i,m)}),\max(x_{(i,m)},y_{(i,m)})]$.

Definition 1. Let $F:\mathcal{A}^{\mathbb{M}}\to\mathcal{A}^{\mathbb{M}}$ be a cellular automaton and $\mathcal{P}$ be a hyperrectangle of $\mathcal{A}^{\mathbb{M}}$, a state $x\in\mathcal{P}$ is $\mathcal{P}$-stable if for all $y\in\mathcal{A}$ such that $(x,y)\in TA(F)$, then $y\notin\mathcal{P}$.

It is easy to verify that a point $x\in\mathcal{A}^{\mathbb{M}}$ which is $\mathcal{A}^{\mathbb{M}}$-stable is a fixed point of F, i.e. $F(x)=x$. Thus, in this case, we obtain the classical framework of the Thomas' conjecture (Thomas, 1981).

2.4. Regulatory Graphs

Generally, we observe the variations of expression level of a gene when the other genes interact with itself. These variations are highlighted in the calculation of the discrete Jacobian of the global dynamic and represented by the regulatory graph. There exists a formalism in the intracellular case which can be generalized in the intercellular case. We just add the position of the cell in the lattice $\mathbb{M}$.

Let $x,y\in\mathcal{A}^{\mathbb{M}}$, $\mathbb{U}\subset\mathbb{M}$ and $(i,m),(j,n)\in I\times\mathbb{U}$, we define the *discrete Jacobian* of F in $x_{\mathbb{U}}\in\mathcal{A}^{\mathbb{U}}$ towards $y_{\mathbb{U}}\in\mathcal{A}^{\mathbb{U}}$ by $\partial_{(i,m),(j,n)}F(x,y)=$

- $\mathrm{sig}(y_{(j,n)}-x_{(j,n)})\mathrm{sig}(F(x^{(j,n)\lhd y})_{(i,m)}-F(x)_{(i,m)})$ if $F(x)_{(i,m)}$ and $F(x^{(j,n)\lhd y})_{(i,m)}$ are on both sides of $x_{(i,m)}+\mathrm{sig}(y_{(i,m)}-x_{(i,m)})/2$,
- 0 otherwise.

Remark 3. When we consider the discrete Jacobian in one point, it is interesting to calculate it according to the direction which involves the maximum of variation. With the multilevel formalism, the variation of the expression level of one gene in the same time is likely to generate two possibilities: crease or decrease. In Remy et al. (submitted for publication, 2006), Crumière and Ruet (2006) and Crumière (2007), the different authors use the Boolean formalism which is a particular case of the multilevel formalism. With the Boolean formalism, at each time, the variation of the expression level of one gene has just one possibility of variation. That is why, in this case, we consider $\partial F(x,\bar{x})$ where $\bar{x}_{(i,m)}=0$ if $x_{(i,m)}=1$ and $\bar{x}_{(i,m)}=1$ if $x_{(i,m)}=0$ for all $(i,m)\in I\times\mathbb{M}$.

To represent the action of one gene in one cell on another gene in the same cell or other cells in a region $\mathbb{U}\subset\mathbb{M}$, we define the *regulatory graph* of $\partial F(x_{\mathbb{U}},y_{\mathbb{U}})$, denoted $G(\partial F(x_{\mathbb{U}},y_{\mathbb{U}}))$. The regulatory graph is a signed directed graph, i.e. a directed graph with a sign, $+1$ or -1, attached to each edge and it is defined by:

- the set of vertices $I\times\mathbb{U}$ which represents the genes of each cell of the region $\mathbb{U}$;
- there is an edge from the gene j in the cell n (i.e. the vertex $(j,n)\in I\times\mathbb{U}$), to the gene i in the cell m (i.e. the vertex $(i,m)\in I\times\mathbb{U}$) if $\partial_{(i,m),(j,n)}F(x_{\mathbb{U}},y_{\mathbb{U}})\neq 0$. The sign of $\partial_{(i,m),(j,n)}F(x,y)$ determines the sign of the edge from (j,n) to (i,m).

In this paper we link the multistationarity to the following property of regulatory graph.

Definition 2. A regulatory graph $G(\partial F(x_{\mathbb{U}}, y_{\mathbb{U}}))$ has an *elementary positive circuit* if there exist $L \in \mathbb{N}$ and $(i_l, m_l) \in I \times \mathbb{M}$, with $l \in [0, L]$, all distincts, such that $\prod_{l=0}^{L} \partial_{(i_l,m_l),(i_{l+1},m_{l+1})} F(x_{\mathbb{U}}, y_{\mathbb{U}}) > 0$with the convention$(i_{L+1}, m_{L+1}) = (i_0, m_0)$.

Example 2 *(Hexagonal lattice).* Consider the dynamic given in Example 1 with one gene per cell.

The regulatory graph

$$G\left(\partial F\left(\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 0 \\ 1 & 1 & \end{pmatrix}\begin{pmatrix} & 1 & 0 \\ 1 & 0 & 1 \\ 0 & 0 & \end{pmatrix}\right)\right)$$

contains three edges, a positive edge from the high right cell to the central cell, because:

$$\bar{F}\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 0 \\ 1 & 1 & \end{pmatrix} \neq \bar{F}\begin{pmatrix} & 0 & 0 \\ 0 & 1 & 0 \\ 1 & 1 & \end{pmatrix} \quad \text{and} \quad \bar{F}\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 0 \\ 1 & 1 & \end{pmatrix} = 1,$$

a negative edge from the right cell to the central cell, because:

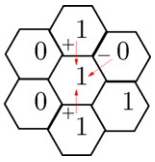
$$\bar{F}\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 0 \\ 1 & 1 & \end{pmatrix} \neq \bar{F}\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 1 \\ 1 & 1 & \end{pmatrix} \quad \text{and} \quad \bar{F}\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 0 \\ 1 & 1 & \end{pmatrix} \neq 0,$$

and a positive edge from the low cell to the central cell, because:

$$\bar{F}\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 0 \\ 1 & 1 & \end{pmatrix} \neq \bar{F}\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 0 \\ 1 & 0 & \end{pmatrix} \quad \text{and} \quad \bar{F}\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 0 \\ 1 & 1 & \end{pmatrix} = 1,$$

Thus:

$$G\left(\partial F\left(\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 0 \\ 1 & 1 & \end{pmatrix}, \begin{pmatrix} & 1 & 0 \\ 1 & 0 & 1 \\ 0 & 0 & \end{pmatrix}\right)\right) =$$

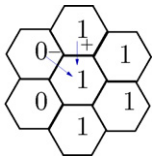


In the same way, the regulatory graph

$$G\left(\partial F\left(\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 1 \\ 1 & 1 & \end{pmatrix}, \begin{pmatrix} & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & \end{pmatrix}\right)\right)$$

contains two edges, a negative edge from the high cell to the central cell and a positive edge from the high right cell to the central cell. Thus:

$$G\left(\partial F\left(\begin{pmatrix} & 0 & 1 \\ 0 & 1 & 1 \\ 1 & 1 & \end{pmatrix}, \begin{pmatrix} & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & \end{pmatrix}\right)\right) =$$



Thus, we build the regulatory graph

$$G\left(\partial F\left(\begin{pmatrix} & 0 & 1 \\ \mathbf{0} & \mathbf{1} & 1 \\ 0 & \mathbf{1} & \mathbf{1} \\ 1 & 1 & \end{pmatrix}, \begin{pmatrix} & 1 & 0 \\ \mathbf{1} & \mathbf{0} & 0 \\ 1 & \mathbf{0} & \mathbf{0} \\ 0 & 0 & \end{pmatrix}\right)\right)$$

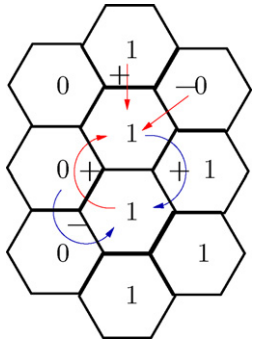
which is by definition the union of

$$G\left(\partial F\left(\begin{pmatrix} & 0 & 1 \\ \mathbf{0} & \mathbf{1} & 0 \\ \mathbf{1} & \mathbf{1} & \end{pmatrix}, \begin{pmatrix} & 1 & 0 \\ \mathbf{1} & \mathbf{0} & 1 \\ \mathbf{0} & \mathbf{0} & \end{pmatrix}\right)\right)$$

and

$$G\left(\partial F\left(\begin{pmatrix} & \mathbf{0} & \mathbf{1} \\ 0 & \mathbf{1} & \mathbf{1} \\ 1 & 1 & \end{pmatrix}, \begin{pmatrix} & \mathbf{1} & \mathbf{0} \\ 1 & \mathbf{0} & \mathbf{0} \\ 0 & 0 & \end{pmatrix}\right)\right)$$

which contains an elementary positive circuit:



3. Necessary Condition for Multistationarity: Positive Circuit

3.1. 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 in the multilevel framework (Remy et al., submitted for publication; Richard, 2006). With our formalism, the lattice considered is just $\mathbb{M} = \{0\}$ and the theorem obtained by Richard (2006) can be expressed by:

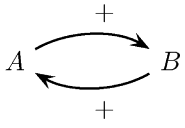
Theorem 1 (Richard, 2006). *Let $F : \mathcal{A}^{\{0\}} \to \mathcal{A}^{\{0\}}$ be a global dynamic and $\mathcal{P}$ be a hyperrectangle of $\mathcal{A}$. If two distinct states $x, y \in \mathcal{P}$ are $\mathcal{P}$-stables, then there exists $z \in \pi(x, y)$ such that $G(\partial F(z, y))$ contains an elementary positive circuit.*

Example 3 *(Intracellular example).* In order to illustrate this theorem, we present a very simple example of a system of two genes, $I = \{A, B\}$, and each gene has just two expression levels. Thus $\mathcal{A} = \{0, 1\} \times \{0, 1\}$. We assume that the system has the following dynamic: $F(x, y) = (y, x)$ with $x, y \in \mathcal{A}$.

We have:

$$\partial_{A,A}F((0,0),(1,1)) = \mathrm{sig}(F(1,0)_A - F(0,0)_A) = 0$$
$$\partial_{A,B}F((0,0),(1,1)) = \mathrm{sig}(F(0,1)_A - F(0,0)_A) = 1$$
$$\partial_{B,A}F((0,0),(1,1)) = \mathrm{sig}(F(1,0)_B - F(0,0)_B) = 1$$
$$\partial_{B,B}F((0,0),(1,1)) = \mathrm{sig}(F(0,1)_B - F(0,0)_B) = 0$$

So, the regulatory graph $G(\partial F((0,0),(1,1)))$ contains two edges: an edge from gene A to gene B and one from gene B to gene A. Moreover, the interactions between genes A and B are positive (activations). Thus:

$G(\partial F((0,0),(1,1))) =$ 

F has two fixed points, i.e. two $\mathcal{A}$-stable points, $(0, 0)$ and $(1, 1)$. In accordance with Theorem 5, the regulatory graph $G(\partial F((0,0),(1,1)))$ contains a positive circuit between gene A and gene B.

3.2. The Intercellular Case

We recall that $\mathbb{V}$ is the neighbourhood of the global function $F : \mathcal{A}^{\mathbb{M}} \to \mathcal{A}^{\mathbb{M}}$. For $\mathbb{U}'$ and $\mathbb{U}''$ two subsets of $\mathbb{M}$, denote $\mathbb{U}' + \mathbb{U}'' = \{u' + u'' : u \in \mathbb{U}' \text{and} u'' \in \mathbb{U}''\}$. For $\mathbb{U} \subset \mathbb{M}$ finite, define $\partial\mathbb{U} = (\mathbb{U} + \mathbb{V}) \setminus \mathbb{U}$ the *boundary* of $\mathbb{U}$ and $\bar{\mathbb{U}} = \mathbb{U} + \mathbb{V}$ the *closure* of $\mathbb{U}$ by $\mathbb{V}$.

Theorem 2. *Let $F : \mathcal{A}^{\mathbb{M}} \to \mathcal{A}^{\mathbb{M}}$ be a global function, $\mathcal{P} = \prod_{(i,m)\in I\times\mathbb{M}} \mathcal{P}_{i,m}$ be a hyperrectangle of $\mathcal{A}^{\mathbb{M}}$ and $\mathbb{U} \subset \mathbb{M}$ be a finite subset. If $x, y \in \mathcal{A}^{\mathbb{M}}$ are two $\mathcal{P}$ - stable points which verify $x_{\partial\mathbb{U}} = y_{\partial\mathbb{U}}$ and $x_{\mathbb{U}} \neq y_{\mathbb{U}}$, then there exists $z \in \pi(x, y)$ such that $G(\partial F(z, y))$ has an elementary positive circuit.*

Proof. Define $\hat{F} : \mathcal{A}^{\bar{\mathbb{U}}} \to \mathcal{A}^{\bar{\mathbb{U}}}$ such that

$$\hat{F}((a_u)_{u\in\bar{U}})_{u'} = \begin{cases} \bar{F}((a_{u'+\upsilon})_{\upsilon\in\mathbb{V}}) & \text{for all } u' \in \mathbb{U}, \\ F(y)_{u'} & \text{for all } u' \in \partial\mathbb{U}. \end{cases}$$

The intuition is that this dynamics $\hat{F}$ preserves the local dynamics of intercellular communication, i.e. a gene can only interact with a gene of the same cell or with a gene in the neighbourhood of the cell, and let invariant the boundary.

It is easy to verify that $x_{\bar{\mathbb{U}}}$ and $y_{\bar{\mathbb{U}}}$ are $\mathcal{P}_{\bar{\mathbb{U}}}$-stables where $\mathcal{P}_{\bar{\mathbb{U}}} = \prod_{(i,m)\in I\times\bar{\mathbb{U}}} \mathcal{P}_{i,m}$. Applying Theorem 1 at the intracellular dynamics $\hat{F} : (\mathcal{A}^{\bar{\mathbb{U}}})^{\{0\}} \to (\mathcal{A}^{\bar{\mathbb{U}}})^{\{0\}}$, there exists $z_{\bar{\mathbb{U}}} \in \pi(x_{\bar{\mathbb{U}}}, y_{\bar{\mathbb{U}}})$ such that $G(\partial\hat{F}(z_{\bar{\mathbb{U}}}, y_{\bar{\mathbb{U}}}))$ has an elementary positive circuit. That is to say, there exist $L \in \mathbb{N}$ and $(i_l, m_l) \in I \times \bar{\mathbb{U}}$, for all $l \in [0, L]$, all distincts, such that

$$\prod_{l=0}^{L} \partial_{(i_l,m_l),(i_{l+1},m_{l+1})} \hat{F}(z_{\bar{\mathbb{U}}}, y_{\bar{\mathbb{U}}}) > 0$$

with $(i_{L+1}, m_{L+1}) = (i_0, m_0)$.

Since $x_{\partial\mathbb{U}} = y_{\partial\mathbb{U}}$ and $z_{\bar{\mathbb{U}}} \in \pi(x_{\bar{\mathbb{U}}}, y_{\bar{\mathbb{U}}})$, one has $z_{\partial\mathbb{U}} = y_{\partial\mathbb{U}}$. Thus $\partial_{(i,m),(j,n)}\hat{F}(z, y) = 0$ for all $n \in \partial\mathbb{U}$. One deduces that $m_l \notin \partial\mathbb{U}$ for all $l \in [0, L]$. Put $z_{\mathbb{M}\setminus\mathbb{U}} = y_{\mathbb{M}\setminus\mathbb{U}}$. Since F and $\hat{F}$ are defined locally by $\bar{F}$, for all $m, n \in \mathbb{U}$ and $i, j \in I$, one deduces that $\partial_{(i,m),(j,n)}\hat{F}(y_{\bar{\mathbb{U}}}, z_{\bar{\mathbb{U}}}) = \partial_{(i,m),(j,n)}F(y, z)$. The theorem follows. □

Remark 4. Since $G(\partial F(z, y))$ contains an elementary positive circuit and $z_{\mathbb{M}\setminus\mathbb{U}} = y_{\mathbb{M}\setminus\mathbb{U}}$, the positive circuit is located on $\mathbb{U}$.

3.3. Case of Periodic Configurations

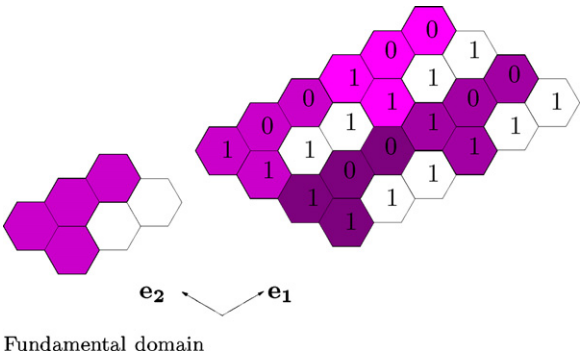
In Crumière and Ruet (2006) and Crumière (2007), we study periodic configurations in the space. This is usual in some biological setting.

With our formalism, a configuration x is $\mathbb{M}'$-*periodic* according to a sub-lattice $\mathbb{M}'$ of $\mathbb{M}$ if $x_{m+m'} = x_m$ for all $m \in \mathbb{M}$ and $m' \in \mathbb{M}'$. If the sub-group quotient $\mathbb{M}/\mathbb{M}'$ is finite (i.e. $\mathbb{M}$ and $\mathbb{M}'$ have the same dimension), there exists at least a finite subset $\mathbb{U}$ such that $\mathbb{U} + \mathbb{M}' = \mathbb{M}$. Such sets are called *f*undamental domains of $\mathbb{M}'$. Of course, we have $\text{Card}(\mathbb{U}) = \text{Card}(\mathbb{M}/\mathbb{M}')$. In this case, the assumptions on the boundary of Theorem 7 could be simplified.

Corollary 1. *Let $F : \mathcal{A}^{\mathbb{M}} \to \mathcal{A}^{\mathbb{M}}$ be a global function and $\mathcal{P} = \prod_{(i,m)\in I\times\mathbb{M}} \mathcal{P}_{i,m}$ be a hyperrectangle of $\mathcal{A}^{\mathbb{M}}$. Let $\mathbb{M}'$ be a sub-lattice of $\mathbb{M}$ and $\mathbb{U} \subset \mathbb{M}$ be a fundamental domain. Let $\mathbb{C} \subset \mathbb{U}$ such that $\partial(\mathbb{U} \setminus \mathbb{C}) \subset \mathbb{C} + \mathbb{M}'$, we say that $\mathbb{C}$ verifies the spatial condition.*

If $x, y \in \mathcal{A}^{\mathbb{M}}$ are two $\mathcal{P}$ -stables $\mathbb{M}'$ -periodic points which verify $x_{\mathbb{C}} = y_{\mathbb{C}}$ and $x_{\mathbb{U}} \neq y_{\mathbb{U}}$, then there exists $z \in \pi(x, y)$ such that $G(\partial F(z, y))$ contains an elementary positive circuit.

Proof. Let $\mathbb{W} = \mathbb{U} \setminus \mathbb{C}$. Since $\mathbb{C}$ satisfies the spatial condition, by $\mathbb{M}'$-periodicity, we have $x_{\partial\mathbb{W}} = y_{\partial\mathbb{W}}$. Then we apply Theorem 7 with $\mathbb{W}$ in the role of $\mathbb{U}$. □



Fundamental domain

Fig. 4. Spatial condition for a $\mathbb{M}'$-periodic configuration.

Example 4 *(One-dimensional lattice).* In the framework of Crumière and Ruet (2006), we have $\mathbb{M} = \mathbb{Z}$. A sub-group of $\mathbb{M}$ is $n\mathbb{Z}$ with $n \in \mathbb{N}$. We consider the global dynamic $F : \mathcal{A}^{\mathbb{Z}} \to \mathcal{A}^{\mathbb{Z}}$ of neighbourhood $\mathbb{V} = \{-1, 0, 1\}$, that is to say, a cell can interact just itself or with the neighbouring cells on the left or on the right. Let $x, y \in \mathcal{A}^{\mathbb{Z}}$ two fixed periodic points of period respectively k and k' such that $x_0 = y_0$. The points x and y are $\mathcal{A}^{\mathbb{Z}}$-stables and $d\mathbb{Z}$-periodics, where $d = gcd(k, k')$ is the biggest common divisor of k and k'. Thus, it is possible to apply Corollary 8 with $\mathbb{C} = \{0\}$ and $\mathbb{U} = \{0, \ldots, d-1\}$. We obtain again Theorem 4.1 of Crumière and Ruet (2006).

Example 5 *(Hexagonal lattice).* Following the setting of Examples 1 and 4. Assume that in our model the configurations are periodics according to the sub-lattice $\mathbb{M}' = 3\mathbf{e_1}\mathbb{Z} + 2\mathbf{e_2}\mathbb{Z}$. A fundamental domain is $\mathbb{U} = \{(0, 0), (1, 0), (2, 0), (0, 1), (1, 1), (2, 1)\}$ and the subset $\mathbb{C} = \{(0, 0), (0, 1), (1, 1), (2, 1)\}$ satisfies the spatial condition. In Fig. 4, we represent a fundamental domain of $\mathbb{M}'$ and the coloured part correspond to $\mathbb{C}$. We present also a $\mathbb{M}'$-periodic configuration.

3.4. Boundary Effects

Sometimes, some points of the lattice are not occupied by a cell. For example, when the biological system does not occupy all the space. In this case, with some modifications, Theorem 7 still holds. For that, we add to the alphabet $\mathcal{A}$ a *neutral state*, denoted q. Let $\mathcal{A}_q = \mathcal{A} \cup \{q\}$ and we extend the local function $\bar{F}$ to a local function $\overline{F_q} : \mathcal{A}_q^{\mathbb{V}} \to \mathcal{A}_q$, such that:

- the restriction of $\overline{F_q}$ at $\mathcal{A}^{\mathbb{V}}$ is $\bar{F}$;
- if $\upsilon \in \mathcal{A}_q^{\mathbb{V}}$ verifies $\upsilon_0 = q$ then $\overline{F_q}(\upsilon) = q$, i.e. a neutral state stays neutral;
- boundary effects are correctly defined, i.e. if $\upsilon \in \mathcal{A}_q^{\mathbb{V}}$ such that there exists $m \in \mathbb{V} \setminus \{0\}$ which verifies $\upsilon_m = q$ then $\bar{F}_q(\upsilon)$ is chosen according to the more natural biological behaviour.

In this case, Theorem 7 holds with the global function $F_q : \mathcal{A}_q^{\mathbb{M}} \to \mathcal{A}_q^{\mathbb{M}}$.

Generally, $\bar{F}$ is *isotropic*, i.e., does not depend on the position of neighbouring cells. In this case, it is easy to consider boundary effects. Indeed, if a cell q appear in the neighbourhood, the central cell where we apply the local function $\bar{F}_q$ is just influenced by other cells. We are going to see that in the following example.

Example 6 *(Isotropic local function).* Let $\bar{F} : \mathcal{A} \to \mathcal{A}$ be a local function such that $\bar{F}((a_\upsilon)_{\upsilon\in\mathbb{V}}) = f(a_0, (\max\{a_{(i,\upsilon)} : \upsilon \in \mathbb{V} \setminus \{0\}\})_{i\in I})$, where $f : \mathcal{A} \times \mathcal{A} \to \mathcal{A}$ is a function. Biologically, this means that the level of expression of one gene just depends on the level of expression of the genes of this cell and the maximum level of expression of genes of neighbouring cells. In this case, when $\overline{F_q}$ consider a cell

in the state q, it is natural to assume that all level of expression of genes of this cell is 0. This means that a cell in the state q can not have an influence on our system.

4. Application

This application deals with a genetic network leading to the formation of individual sense organs in *Drosophila*. A logical approach has been proposed in Ghysen and Thomas (2003).

The formation of *Drosophila*'s sense organs depends on the activity of proneural genes. Proneural activity is controlled by 4 types of genes:

- prepattern genes, which activate the proneural genes in clusters of cells at defined regions of the ectoderm;
- neurogenic genes, noted B, which mediate a process of lateral inhibition whereby the competence is restricted to a single cell in each group;
- "cell cycle" genes, noted C, which control the entry in mitosis;
- proneural genes, noted A.

The expression of proneural genes depends on local combinations of activating and repressing prepattern genes. Prepattern genes must be considered as simple entries: we assume that the expression level of prepattern genes is at a sufficient level to allow the expression of proneural genes. Moreover the different cells are located on a plane according to the hexagonal lattice $\mathbb{M}$ as in Example 1.

Thus, prepattern genes will not be considered inside our genetic network and each cell will be composed by only 3 types of genes: A, B and C, i.e. $I = \{A, B, C\}$. Variables a_m, b_m and c_m represent the expression levels concentration of genes A,B and C, respectively in the cell number m. Variable c_m is a Boolean variable, i.e., the expression level of gene C is assumed to be either 0 (gene not expressed) or 1 (expressed). While variables a_m and b_m are ternary variables, two thresholds will be distinguished for each gene: the value of the variable will be 0 when the concentration of the product will be underneath the first threshold, 1 between the two threshold and 2 above the second threshold. Thus $\mathcal{A}_A = \{0, 1, 2\}$, $\mathcal{A}_B = \{0, 1, 2\}$ and $\mathcal{A}_C = \{0, 1\}$, so $\mathcal{A} = \mathcal{A}_A \times \mathcal{A}_B \times \mathcal{A}_C$. We can now examine closely the model of the system.

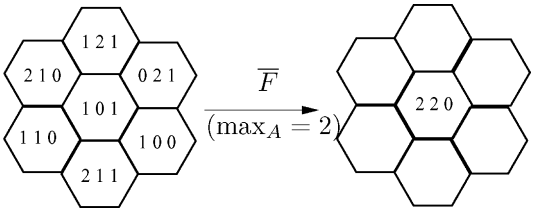
The cells are located on the hexagonal lattice and each cell interacts with the six neighbouring cells. Thus, as in Example 1 about the hexagonal lattice, the neighbourhood of the local function $\bar{F}$ which defines the dynamic is $\mathbb{V} = \{(0, 0), (0, 1), (1, 1), (-1, 0), (1, 0), (-1, -1), (0, -1)\}$.

In this application, we make this assumption that not all the parameters (expression levels of genes) of the neighbouring cells are taken into account. To be more precise, only the expression level of gene A of the six neighbouring cells might have an influence on the expression level of gene of central cell at next step. Let $\max_A(m)$ denote the maximum expression level of gene A in the six neighbouring cells of the cell $m \in \mathbb{M}$: variable $\max_A(m)$ will thus be a ternary variable. For example, if $\max_A(m) = 0$, it means that the value of expression level of gene A in each neighbouring cell of the studied cell is 0. As in Example 6, the local function can be define as: $\bar{F}((a_\upsilon, b_\upsilon, c_\upsilon)_{\upsilon \in \mathbb{V}}) = f((a_0, b_0, c_0), \max(a_\upsilon : \upsilon \in \mathbb{V} \setminus \{0\})) = f((a_0, b_0, c_0), \max_A(0))$, where $f : \mathcal{A} \times \mathcal{A}_A \to \mathcal{A}$ is given in Fig. 5. The expression level of genes of a cell are naturally represented by three numbers because there are three genes in each cell and each number is the expression level of one gene in the cell.

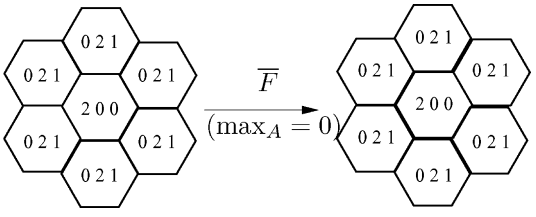
for $\max_A = 0$		*for* $\max_A = 1$		*for* $\max_A = 2$	
abc	$f(abc, 0)$	abc	$f(abc, 1)$	abc	$f(abc, 2)$
000	101	000	111	000	121
001	111	001	111	001	121
010	101	010	111	010	121
011	111	011	111	011	121
020	001	020	011	020	021
021	011	021	011	**021**	**021**
100	200	100	210	100	220
101	210	101	210	101	220
110	100	**110**	**110**	110	120
111	110	111	110	111	120
120	000	120	010	120	020
121	010	121	010	121	020
200	**200**	200	210	200	220
201	210	201	210	201	220
210	200	**210**	**210**	210	220
211	210	211	210	211	220
220	100	220	110	220	120
221	110	221	110	221	120

Fig. 5. Definition of $f : \mathcal{A} \times \mathcal{A}_A \to \mathcal{A}$.

For example, here is a state and its image by $\bar{F}$ at next step (we have $\max_A = 2$ and the table gives $f(101, 2) = 220$):



We can notice that the local state $x_{\mathbb{V}} \in \mathcal{A}^{\mathbb{V}}$ centred on $\mathbb{V}$ below is steady:



Moreover, $\mathbb{V}$ is a fundamental domain of the sub-lattice $\mathbb{M}' = (2\mathbf{e_1} - \mathbf{e_2})\mathbb{Z} + (\mathbf{e_1} + 3\mathbf{e_2})\mathbb{Z}$ where $(\mathbf{e_1}, \mathbf{e_2})$ is the base of the hexagonal lattice introduced in Example 1. Observe that the state generated by the previous local state, denoted $x_{\mathbb{V}}$ composed by seven cells, allows one to obtain a fixed point ($F(x) = x$), or a $\mathcal{A}^{\mathbb{M}}$-stable state, $x \in \mathcal{A}^{\mathbb{M}}$ such that $x_{m+\mathbb{V}} = x_{\mathbb{V}}$ for all $m \in \mathbb{M}$ (Fig. 6).

In the same way, the state generated by this following hexagonal local state $y_{\mathbb{V}} \in \mathcal{A}^{\mathbb{V}}$ allows to obtain a new stable state $y \in \mathcal{A}^{\mathbb{M}}$ such

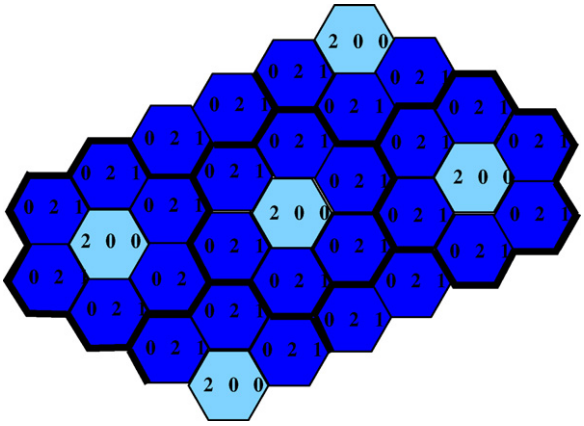


Fig. 6. Fixed point x.

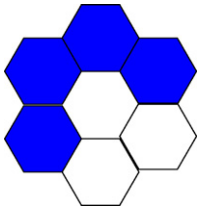


Fig. 7. Fundamental domain and spatial condition.

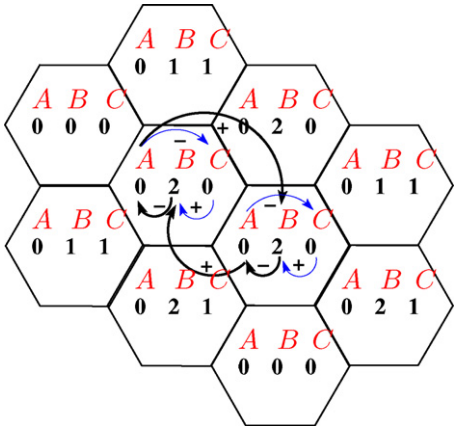
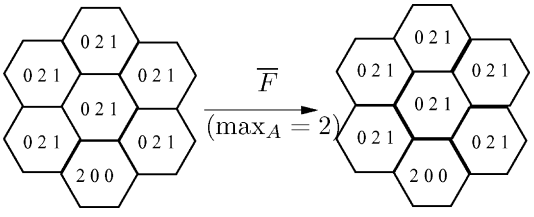


Fig. 8. Example of positive circuit.

that $y_{m+\mathbb{V}} = y_{\mathbb{V}}$ for all $m \in \mathbb{M}$.



Given the fundamental domain $\mathbb{V}$ of $\mathbb{M}'$ composed by seven cells. Let

$$\mathbb{C} = \{(-1, 0), (1, 0), (1, 1), (1, 0)\},$$

it is the coloured set of the fundamental domain in Fig. 7.

Part $\mathbb{C}$ satisfies the spatial condition of Corollary 8. We thus have our two fixed states x and y which enjoy the conditions of Corollary 8. The two local state x and y have the same expression level of genes on this coloured set. Then, there is a state z such as $G(\partial F(z, y))$ has a positive circuit. Here is one example (Fig. 8):

There are an intracellular positive circuit and an intercellular positive circuit. This last circuit is on two cells, the genes A and B in the two cells interact. Let us remark that a single fixed periodic state with a certain regularity suffices to imply the existence of a positive circuit. Since the local state y is just obtained by translation of x.

5. Perspectives

This article presents a discrete model of intercellular genetic networks and generalizes Thomas' rule with a spatial condition on stable states. The principal result, Theorem 7, is not yet optimum.

Indeed, in the present work, the size of the positive circuit is controlled by the size of the tile: the genes involved in the circuit are localised on the cells contained in the tile, more precisely in $\mathbb{U}$. We may conjecture a stronger location constraints on the positive circuit which must exist in Theorem 7. We can expect a bound independent of the dynamic: for instance the circuits will be on at most three or four neighbouring cells.

Moreover, cells are located on a lattice which gives a rigid structure at the biological system. We can imagine that biological system are not such regular. An idea in order to study no regular configurations is to use quasi-periodic structures where the most famous is the Penrose's tiling.

Another possibility would be to focus on spatial properties in a differential framework.

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