

## R. Thomas' Modeling of Biological Regulatory Networks: Introduction of Singular states in the Qualitative Dynamics

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**Abstract.** In the field of biological regulation, models extracted from experimental works are usually complex networks comprising intertwined feedback circuits. The overall behavior is difficult to grasp and the development of formal methods is needed in order to model and simulate biological regulatory networks. To model the behavior of such systems, R. Thomas and coworkers developed a qualitative approach in which the dynamics is described by a state transition system. Even if all steady states of the system can be detected in this formalism, some of them, the singular ones, are not formally included in the transition system. Consequently, temporal properties in which singular states have to be described, cannot be checked against the transition system. However, steady singular states play an essential role in the dynamics since they can induce homeostasis or multistationnarity and sometimes are associated to biological phenotypes.

These observations motivated our interest for developing an extension of Thomas formalism in which all singular states are represented, allowing us to check temporal properties concerning singular states. We easily demonstrate in our formalism the previously demonstrated theorems giving the conditions for the steadiness of singular states. We also prove that our formalism is coherent with the Thomas one since all paths of the Thomas transition system are preserved in our one, which in addition includes singular states.

**Keywords:** biological networks, feedback circuits, singular states, steady states.

### 1. Introduction

It is now becoming clear for a lot of researchers that to elucidate the fundamental principles that govern how genomic information translates into organismal complexity, one has to overcome the current habit of ad hoc explanations and instead embrace novel and formal concepts that will involve computer modeling[9]. These new approaches form the *systems biology*[32] which tends to deal with functioning of modular circuits, including their robustness, design and manipulation[12, 10, 8]. Computational systems biology addresses questions fundamental to our understanding of life. For this, we need to establish methods and techniques that enable us to understand biological systems as systems, which means to understand: the structure of the system, such as

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gene/metabolic/signal transduction networks and physical structures, the dynamics of such systems, methods to control systems, and methods to design and modify systems to generate desired properties[11].

Biological regulatory systems often are complex networks comprising several intertwined feedback circuits. The behavior of such systems is extremely anti-intuitive and cannot be solved without adequate formalization. Most modeling approaches deal with simulation of a recreated living system with computer in which are put as much as possible details. Traditionally, biochemical systems are modeled with kinetics differential equations using the mathematical language of dynamic systems [29] in a quantitative simulator. Biological systems are also accurately described by non-linear ordinary differential equations which, however, cannot be solved analytically. To model the behavior of such systems, R. Thomas and coworkers developed a qualitative approach in which the dynamics is described by a state transition system depending on some parameters [24, 23]. To capture all steady states, Snoussi and Thomas introduced the concept of circuit-characteristic states which are particular singular states. They play an essential role in the dynamics since they can induce homeostasis or multistationnarity and sometimes are associated to biological phenotypes [18, 28, 21, 26, 27]. Then available biological knowledge on homeostasis, multistationarity or on observed experimental behavior can be used for constraining the underlying parameters [2]. Even if all steady states of the system can be detected in this formalism, some of them, the characteristic ones, are not formally included in the transition system. Consequently, temporal properties in which singular states have to be described, cannot be checked against the transition system. These observations motivated our interest for developing an extension of Thomas formalism in which all singular states are included in the dynamics. Such an extension, which allows us to check temporal properties concerning singular states, is indeed essential for describing in a coherent and precise way all possible transitions and all possible attractors, including both regular and singular states.

This paper presents an extension of R. Thomas' modeling which includes the singular states in the transition system. In section 2 we present the continuous dynamics of biological regulatory networks based on ordinary differential equations which constitute the common grounds of our and R. Thomas' qualitative approaches. Then a discretization map is introduced leading to the definition of all qualitative states of our modeling. Section 3 treats of the discretization of the continuous dynamics used to define the transition system. We also introduce the resources and the qualitative parameters which allows us to define the qualitative dynamics independently of the continuous system. In section 4 we revisit the definition of the characteristic states of feedback circuits in a qualitative and formal manner. The conditions for the steadiness of characteristic states, which make the corresponding circuits functional (see [27] for example), are shown to be the same than in the Thomas' modeling. After having given some comparisons between both modelings, we prove that the R. Thomas' state graph is in a certain sense included in our transition system. Finally in section 5 perspectives are presented.

## 2. Qualitative values and qualitative regulatory networks

Interactions between biological entities, often macromolecules or genes, are classically represented by labeled directed graphs, where vertices abstract biological entities and arcs their interactions. If the interaction is an inhibition (resp. activation), the label is  $-$  (resp.  $+$ ). This static representation is formally defined as following.

### Definition 2.1. (Regulatory network)

A regulatory network is a labeled directed graph  $G = (V, E)$  where:

- each vertex  $v$  of  $V$ , called *variable*, represents a biological entity,
- each arc  $(u \rightarrow v)$  of  $E$  is labeled with the sign of the interaction  $\alpha_{uv} \in \{+, -\}$ .

In the sequel we denote, for each vertex  $v \in V$ , the set of predecessors (resp. successors) of  $v$  by  $G^-(v)$  (resp.  $G^+(v)$ ) and the cardinal of a set  $S$  by  $\#S$ .

We now present the continuous dynamics of such systems based on ordinary differential equations. This approach has been fruitfully applied to different systems [31, 20, 3, 30] and constitutes the grounds of the qualitative approach first introduced by Thomas. To each variable  $v$  is associated a continuous value  $x_v \in \mathbb{R}^+$  which represents its concentration. At a given time the vector  $x = (x_v)_{v \in V}$ , composed of all concentrations, defines the state of the regulatory network. The evolution of the system is generally given by the following system of ordinary differential equations:

$$\frac{dx_v}{dt} = \mathcal{S}_v(x) - \lambda_v x_v, \quad \forall v \in V \quad (1)$$

where  $\mathcal{S}_v(x)$  and  $\lambda_v > 0$  represent respectively the synthesis rate and the degradation coefficient of the variable  $v$ . The synthesis rate  $\mathcal{S}_v : \mathbb{R}_+^{\#V} \rightarrow \mathbb{R}_+$  is often defined by:

$$\mathcal{S}_v(x) = \sum_{u \in G^-(v)} \mathcal{I}^{\alpha_{uv}}(x_u, \theta_{uv}) \quad (2)$$

where the function  $\mathcal{I}^{\alpha_{uv}} : \mathbb{R}_+^2 \rightarrow \mathbb{R}_+$  describes the influence of a regulator  $u$  on the synthesis rate of  $v$ .  $\alpha_{uv}$  and  $\theta_{uv}$  are respectively the sign and the threshold of the interaction  $u \rightarrow v$ . Indeed for the majority of the biological interactions, under a certain threshold  $\theta_{uv}$  of the concentration of  $u$ , the interaction  $u \rightarrow v$  has a quasi null effect on  $v$ , and a saturated effect over it. More precisely the function  $\mathcal{I}^{\alpha_{uv}}$  is near 0 on one side of the threshold and near the saturation effect  $k_{uv} > 0$  on the other, it can be represented by a sigmoid as a Hill function (see figure 1). In such a case, the threshold is the inflexion point of the Hill function.

With such non linear interactions the system 1 has no analytical solution. The solution can be numerically approximated but the precision may be misleading [27] because the parameters are most often unknown. Thomas proposed to estimate the sigmoid function  $\mathcal{I}^{\alpha_{uv}}$  by the step function  $\tilde{\mathcal{I}}^{\alpha_{uv}}$  (figure 1) defined by:

$$\tilde{\mathcal{I}}^+(x_u, \theta_{uv}) = \begin{cases} 0 & \text{if } x_u < \theta_{uv} \\ k_{uv} & \text{if } x_u > \theta_{uv} \end{cases} \quad \tilde{\mathcal{I}}^-(x_u, \theta_{uv}) = \begin{cases} k_{uv} & \text{if } x_u < \theta_{uv} \\ 0 & \text{if } x_u > \theta_{uv} \end{cases}$$

In such a case the system 1 has an analytic solution on each domain where the synthesis rates are constant. But with this approximation,  $\tilde{\mathcal{I}}^{\alpha_{uv}}$  is undefined when  $x_u = \theta_{uv}$ . A state in which there is at least one variable on a threshold is thus called a *singular state*. To define the differential equation system 1 for the singular states Snoussi and Thomas [18] represent the *uncertain* influence of  $u$  on  $v$  when  $x_u = \theta_{uv}$  by an open interval:

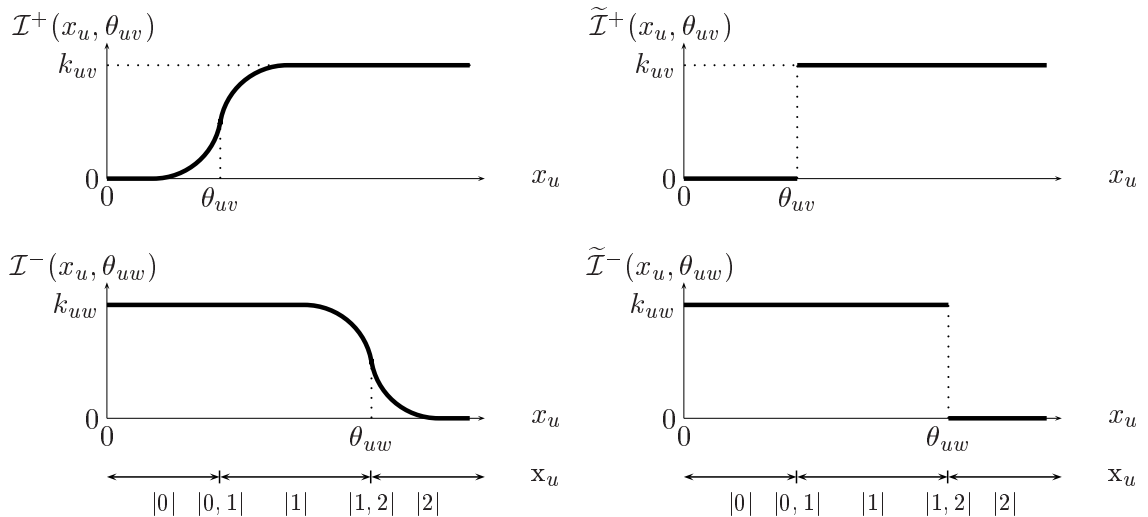


Figure 1. Approximation of sigmoids by step functions and discretization

$\tilde{\mathcal{I}}^{\alpha_{uv}}(\theta_{uv}, \theta_{uv}) = ]0, k_{uv}[$ . This interval represents the set of possible influences of  $u$  on  $v$  strictly included between the case where  $u$  acts on  $v$  ( $x_u > \theta_{uv}$ ) and the case where it does not ( $x_u < \theta_{uv}$ ). Then the system 1 has to be seen as a system of differential inclusions [5]:

$$\frac{dx_v}{dt} \in \mathcal{S}_v(x) - \lambda_v x_v, \quad \forall v \in V, \quad \text{with} \quad \mathcal{S}_v(x) = \sum_{u \in G^-(v)} \tilde{\mathcal{I}}^{\alpha_{uv}}(x_u, \theta_{uv}). \quad (3)$$

To introduce our qualitative approach we define the *qualitative values* used to abstract the concentration of a variable even if it is on a threshold.

**Definition 2.2. (Qualitative Values)**

- A qualitative value, noted  $|a, b|$  is a couple of integers ( $|a, b| \in \mathbb{N}^2$ ) where  $a \leq b$ . The set of qualitative values is noted  $Q$ .
- The relations  $=, <, >, \subseteq$  are defined for 2 qualitative values  $|a, b|$  and  $|c, d|$ :
  - $|a, b| = |c, d|$  if  $(a = c)$  and  $(b = d)$ .
  - $|a, b| < |c, d|$  if  $(b < c)$  or  $(b = c$  and  $(a < b$  or  $c < d))$
  - $|a, b| > |c, d|$  if  $|c, d| < |a, b|$
  - $|a, b| \subseteq |c, d|$  if  $\begin{cases} |a, b| = |c, d| \text{ or} \\ (a = b) \text{ and } (c < a) \text{ and } (b < d) \text{ or} \\ (a < b) \text{ and } (c \leq a) \text{ and } (b \leq d). \end{cases}$

Intuitively, qualitative values has to be seen as intervals. On one hand, if  $a < b$  then  $|a, b|$  represents the open interval  $]a, b[$ . On the other hand, if  $a = b$ , the qualitative value is similar to the close interval  $[a, b]$  which contains only the value  $a$ . Then two open intervals are comparable if they are not overlapping:  $]a, b[ < ]c, d[$  if  $b < c$ . The relation  $\subseteq$  is simply the inclusion relation on intervals. The previous definition leads to two kinds of qualitative values: a qualitative value  $|a, b|$  is said *regular* if  $a = b$  and *singular* otherwise. The notation  $|a|$  represents the regular qualitative value  $|a, a|$ .

Let us now introduce for each variable  $u$  the set of out-thresholds defined by  $\Theta_u = \{\theta_{uv} \mid v \in G^+(u)\}$ . The thresholds of  $\Theta_u$  are ranked from the smallest to the largest:  $\theta_u^1 < \theta_u^2 < \dots < \theta_u^{b_u}$  where  $\theta_u^i$  is the  $i$ -th smallest value of  $\Theta_u$  and  $b_u$  is the cardinal of  $\Theta_u$ .

**Definition 2.3. (Discretization map)**

The discretization map  $d_u : \mathbb{R}_+ \rightarrow Q$  which associates a qualitative value to each concentration of variable  $u$ , is defined as follows:

$$x_u = d_u(x_u) = \begin{cases} |q| & \text{if } \theta_u^q < x_u < \theta_u^{q+1} \\ |q-1, q| & \text{if } x_u = \theta_u^q \end{cases} \quad \text{where } \theta_u^0 = -\infty \quad \text{and} \quad \theta_u^{b_u+1} = +\infty.$$

**Property 1.** The map  $d_v$  is an increasing function.

The proof is straightforward from the definition 2.2.

To understand why  $d_u$  represents the qualitative behavior of  $u$ , let us consider a regulatory network in which  $u$  acts positively on  $v$  and negatively on  $w$ . Let us suppose that  $\theta_{uv} < \theta_{uw}$  (figure 1).  $x_u = |0|$  means that  $u$  does not act neither on  $v$  nor on  $w$ ,  $x_u = |0, 1|$  means that  $u$  does not act on  $w$  and acts uncertainly on  $v$ ,  $x_u = |1|$  means that  $u$  acts only on  $v$ ,  $x_u = |1, 2|$  means that  $u$  acts on  $v$  and acts uncertainly on  $w$  and finally  $x_u = |2|$  means that  $u$  acts on both.

Following Snoussi [16], we suppose that all out-thresholds are different, in other words that the number  $b_u$  of out-thresholds of  $u$  is the cardinal of  $G^+(u)$ . The qualitative value  $x_u$  can have  $2b_u + 1$  different values:  $b_u + 1$  regular qualitative values  $x_u = |q|, q \in \{0, \dots, b_u\}$  which indicate that  $u$  effectively acts on the  $q$  targets  $t$  for which we have  $\theta_{ut} \leq \theta_u^q$ , and  $b_u$  singular qualitative values  $x_u = |q, q + 1|, q \in \{0, \dots, b_u - 1\}$ , which indicate that  $u$  effectively acts on the same targets  $t$  and that the regulation is uncertain for the target  $t'$  such that  $\theta_{ut'} = \theta_u^{q+1}$ . In the sequel,  $b_u$  denotes the number of targets of  $u$ .

**Definition 2.4. (Qualitative regulatory network)**

A qualitative regulatory network, denoted by QR is a regulatory network  $G = (V, E)$  in which each interaction  $u \rightarrow v \in E$  is labeled by a couple  $(\alpha_{uv}, q_{uv}) \in \{-, +\} \times \{1, \dots, b_u\}$ , such that for all  $v' \in G^+(v) \setminus \{v\}$ ,  $q_{uv'} \neq q_{uv}$ .  $q_{uv}$  is called the threshold rank and the qualitative threshold  $s_{uv}$  of the interaction  $u \rightarrow v$  is defined by  $s_{uv} = |q_{uv} - 1, q_{uv}|$ .

For a given regulatory network  $G = (V, E)$ , there is a finite number of qualitative regulatory networks. One can remark that for each regulation  $u \rightarrow v$  labeled by  $q_{uv}$ , we have  $s_{uv} = d_u(\theta_{uv})$  if  $\theta_{uv} = \theta_u^{q_{uv}}$ .

**Definition 2.5. (Qualitative states, regular and singular states)**

Let QR be a qualitative regulatory network built on  $G = (V, E)$ .

- The set of possible values of  $x_u$  is  $Q_u = \{|0|, |0, 1|, |1|, \dots, |q - 1|, |q - 1, q|, |q|, \dots, |b_u|\}$ .
- The qualitative state of the network is the vector  $x = (x_v)_{v \in V}$  composed by all qualitative concentrations. It belongs to the finite space of qualitative states  $Q_{QR} = \prod_{v \in V} Q_v$ .
- A state is *singular* if one of its coordinates is singular, otherwise it is *regular*.

To illustrate the different concepts of our modeling, we take as running example the mucus production in *Pseudomonas aeruginosa*. These bacteria are commonly present in the environment and secrete mucus only in lungs affected by cystic fibrosis. As this mucus increases the respiratory deficiency of the patient, it is the major cause of mortality. The regulatory network of the mucus production system has been widely studied [13, 7]. The main regulator for the mucus production, AlgU, supervises an operon which is made of 4 genes among which one codes for a protein that is a repressor of AlgU. Moreover AlgU favors its own synthesis. The regulatory system can then be sketched into the qualitative regulatory network of Fig. 2-a, where variable  $u$  represents AlgU, and variable  $v$  its repressor. The order of thresholds  $q_{uv}$  and  $q_{uu}$  is not deductible from biological knowledge and in fact both orderings have to be considered. Figure 2 assumes that  $q_{uv} < q_{uu}$ .

Figure 2-b gives the associated qualitative state space which is composed of 15 states: 6 regular states and 9 singular ones. The 6 regular states correspond to 6 open domains in the continuous space, and the 9 others to 7 segments and two points.

In the modeling of R. Thomas, the qualitative concentration of a variable is an integer given by the following discretization function  $d_v^{RT} : \mathbb{R}_+ \setminus \Theta_v \rightarrow \mathbb{N}$  such that  $d_v^{RT}(x_v) = q$  iff  $\theta_v^q < x_v < \theta_v^{q+1}$  with  $\theta_v^0 = -\infty$  and  $\theta_v^{b_v+1} = +\infty$ . Thus regular states of the Thomas model correspond to our regular states. In other words if  $x_v \notin \Theta_v$  then  $d_v(x_v) = |d_v^{RT}(x_v)|$ .

### 3. Dynamics of regulatory networks

In this section we show how the discretization map can be used to extract the essential qualitative features of the continuous dynamics. We first present briefly the analytic solution of the differential equation system when the interactions are approximated by step functions. In such a case the different thresholds define domains in which

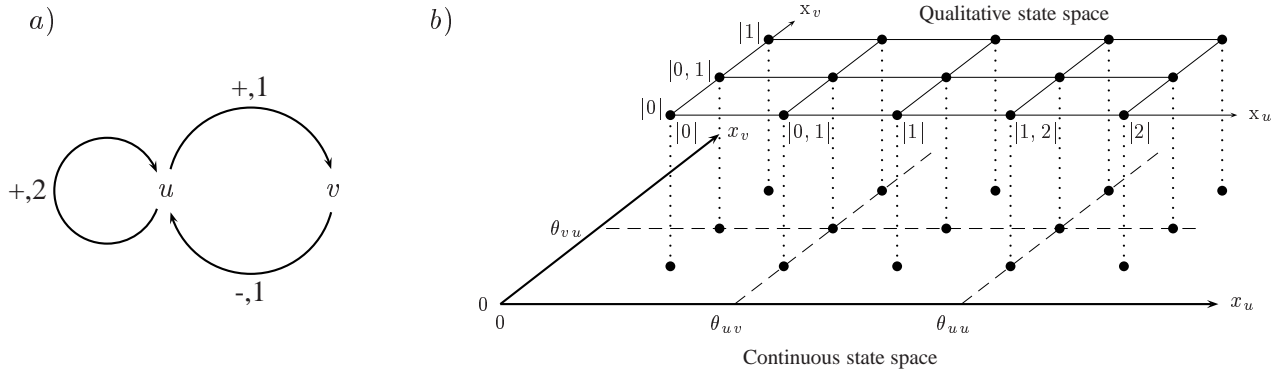


Figure 2. Qualitative regulatory network (a) and its qualitative state space (b)

the synthesis rates are constant. Let us introduce two functions  $\mathcal{D}_v : Q_v \rightarrow \mathcal{P}(\mathbb{R}_+)$  and  $\mathcal{D} : Q_{QR} \rightarrow \mathcal{P}(\mathbb{R}_+)^{\#V}$  defined by:

$$\mathcal{D}_v(x_v) = \{x_v \in \mathbb{R}_+ \mid d_v(x_v) = x_v\} \quad \text{and} \quad \mathcal{D}(x) = (\mathcal{D}_v(x_v))_{v \in V}$$

where  $\mathcal{P}(\mathcal{E})$  denotes the power set of the set  $\mathcal{E}$ . These functions give respectively the set of continuous concentrations and the set of continuous states for which the discretization corresponds to the qualitative value  $x_u$  and to the qualitative state  $x$ .  $\mathcal{D}_v(x_v)$  and  $\mathcal{D}(x)$  are called the *domains* of  $x_v$  and  $x$ .

If  $x$  is a qualitative regular state,  $\forall x_u \in \mathcal{D}_u(x_u)$  and  $\forall v \in G^+(u)$ ,  $\tilde{\mathcal{I}}^{\alpha_{uv}}(x_u, \theta_{uv}) \in \{0, k_{uv}\}$ . Thus we can deduce that  $\forall v \in V$ , the synthesis rate  $\mathcal{S}_v(x)$  is constant in  $\mathcal{D}(x)$ . Since the degradation  $\lambda_v x_v$  of  $v$  is linear, the differential equation system 1 has one solution on  $\mathcal{D}(x)$ . If the initial state is  $x^0 \in \mathcal{D}(x)$ , the solution is :

$$x_v(t) = \mathcal{X}_v(x^0) - (\mathcal{X}_v(x^0) - x_v^0)e^{-\lambda_v t}, \quad \forall v \in V \quad \text{where} \quad \mathcal{X}_v(x) = \frac{\mathcal{S}_v(x)}{\lambda_v}. \quad (4)$$

Thus all continuous states of the domain  $\mathcal{D}(x)$  tend to the same constant state  $\mathcal{X}(x^0) = (\mathcal{X}_v(x^0))_{v \in V}$  that is called the *attractor* of the domain  $\mathcal{D}(x)$ . If  $\mathcal{X}(x^0) \in \mathcal{D}(x)$ , all trajectories starting in  $\mathcal{D}(x)$  will never leave the domain  $\mathcal{D}(x)$  and they will reach (in  $+\infty$ ) the continuous steady state  $\mathcal{X}(x^0)$ . Otherwise if  $\mathcal{X}(x^0) \notin \mathcal{D}(x)$ , then a trajectory starting in  $\mathcal{D}(x)$  goes towards  $\mathcal{X}_v(x^0)$  up to leave the domain  $\mathcal{D}(x)$ . If additionally we suppose that the parameters  $k$  and  $\lambda$  are taken such that the attractor of domain of each regular state is regular, the trajectory leaves in a finite time  $\mathcal{D}(x)$  by reaching a threshold hyperplane. Outside the domain, the solution of the system is not the same and the attractor can be different. Thus the state  $\mathcal{X}_v(x^0)$  can never be reached. For a regular qualitative state  $x$ , we define naturally the qualitative attractor of  $v$  as the discretization of the attractor  $\mathcal{X}_v(x)$ :

$$X_v(x) = d_v(\mathcal{X}_v(x)), \quad \forall x \in \mathcal{D}(x). \quad (5)$$

If  $x$  is a singular qualitative state, there is at least one variable  $u$  such that for all continuous states  $x \in \mathcal{D}(x)$  we have  $x_u \in \Theta_u$  and there exists  $v$  such that  $x_u = \theta_{uv}$ . Then  $\tilde{\mathcal{I}}^{\alpha_{uv}}(x_u, \theta_{uv}) = ]0, k_{uv}[$  so  $\mathcal{X}_v(x)$  is an interval (see equations 4 and 3). Thus for generalizing the qualitative attractor of equation 5 to a singular qualitative state we define the discretization of an interval  $]a, b[$  where  $a$  and  $b$  are not in  $\Theta_v$  by

$$d_v(]a, b[) = ]q_a, q_b[ \quad \text{where } q_a \text{ and } q_b \text{ are the integers such that } |q_a| = d_v(a) \text{ and } |q_b| = d_v(b). \quad (6)$$

We can note that  $q_a \leq q_b$  because  $a \leq b$  and  $d_v$  is an increasing function. The attractors are now defined for all qualitative states.

The attractor of a variable  $v$  at the qualitative state  $x$  does not give the value at the next step, but only the tendency. If  $x_v < X_v(x)$  the variable tends to increase, if  $x_v > X_v(x)$  the variable tends to decrease and finally,

if  $x_v \subseteq X_v(x)$ , the variable  $v$  is said steady. Then one considers that a state  $x$  is steady if all variables are steady. The following theorem shows that this inclusion is reduced to the equality if  $x_v$  is regular.

**Theorem 3.1.** Let QR be a qualitative regulatory network built on  $G$  and  $x$  be a steady qualitative state. If  $x_v$  is regular, then  $x_v = X_v(x)$ .

**Proof:** Reasoning by contradiction, let us suppose that there exists a variable  $v$  such that  $x_v \subset X_v(x)$  with  $x_v$  regular. We have  $X_v(x) = ]a, b[$  with  $a < b$ .  $X_v(x) = d_v(\mathcal{X}_v(x))$ ,  $x \in \mathcal{D}_v(x)$ . Since  $X_v(x)$  is a singular value, then  $\mathcal{X}_v(x)$  is an interval<sup>1</sup> and there exists  $u_1 \in G^-(v)$  which regulates  $v$  uncertainly:  $x_{u_1} = \theta_{u_1 v} \Leftrightarrow x_{u_1} = s_{u_1 v}$ .  $u_1$  is different than  $v$  because  $x_{u_1}$  is singular and  $x_v$  is regular.

Since  $x$  is steady,  $x_{u_1} \subseteq X_{u_1}(x)$  and  $X_{u_1}(x)$  is singular. With the same reasoning we deduce that there exists  $u_2 \in G^-(u_1)$  which regulates  $u_1$  uncertainly:  $x_{u_2} = \theta_{u_2 u_1} \Leftrightarrow x_{u_2} = s_{u_2 u_1}$ . Moreover, as  $x_{u_2}$  is singular,  $u_2 \neq v$ .

By iteration, one constructs a sequence  $u_0, u_1, u_2 \dots u_n$ , where  $u_0 = v$ , such that  $u_i \neq u_0$  and  $x_{u_i} = s_{u_i u_{i-1}}$  for all  $i > 0$ . Since the set of variables  $V$  is finite, there exists  $l$  such that the variables  $u_0, u_1, u_2, \dots, u_{l-1}$  are different and that  $u_l = u_k$  with  $k \in \{1, 2, \dots, l-1\}$ . We have  $x_{u_l} = s_{u_l u_{l-1}}$  and  $x_{u_k} = s_{u_k u_{k-1}}$ . By setting  $u = u_l = u_k$ ,  $x_u = s_{u u_{l-1}} = s_{u u_{k-1}}$ . Since  $u_{l-1} \neq u_{k-1}$ ,  $u$  regulates two different variables with the same threshold, contradicting our assumption that all out-thresholds are different.  $\square$

Now it is possible to state the following theorem which proves that the discretization preserves the steady states.

**Theorem 3.2.** A qualitative state  $x$  is steady iff there is a continuous steady state in  $\mathcal{D}(x)$ .

**Proof:** Let QR be a qualitative regulatory network built on  $G = (V, E)$ ,  $x$  a qualitative state and  $x \in \mathcal{D}(x)$  a continuous one. According to the definition of the steadiness given in [18]  $x$  is steady if:

$$x_v = \mathcal{X}_v(x) \text{ if } x_v \notin \Theta_v \quad \text{and} \quad x_v \in \mathcal{X}_v(x) \text{ if } x_v \in \Theta_v, \quad \forall v \in V.$$

If  $x$  is steady then  $x$  is steady because for all  $v \in V$ :

1. If  $x_v \notin \Theta_v$ , we have  $x_v = \mathcal{X}_v(x)$  so  $d_v(x_v) = d_v(\mathcal{X}_v(x))$  which is equivalent to  $x_v = X_v(x)$ .
2. If  $x_v \in \Theta_v$ ,  $x_v = d_v(x_v)$  is a singular qualitative value and we have  $x_v \in \mathcal{X}_v(x) = ]a, b[$ . Since the function  $d_v$  is increasing,  $d_v(a) \leq d_v(x) \leq d_v(b)$ . Then  $d_v(x_v) \subseteq ]d_v(a), d_v(b)[$  (see definition 2.2) that is  $d_v(x_v) \subseteq d_v(]a, b[)$  which is equivalent to  $x_v \subseteq X_v(x)$ .

If  $x$  is steady, there is one continuous steady state in  $\mathcal{D}(x)$  because for all  $v$  in  $V$ :

1. if  $x_v$  is regular then following theorem 3.1  $x_v = X_v(x) \iff d_v(x_v) = d_v(\mathcal{X}_v(x))$  for all  $x \in \mathcal{D}(x)$ , in other words,  $x_v$  and  $\mathcal{X}_v(x)$  are in the same domain  $\mathcal{D}(x)$ . As  $x_v \notin \Theta_v$  and  $\mathcal{X}_v(x) \in \mathcal{D}_v(x_v)$ , the attractor  $\mathcal{X}_v(x)$  is steady.
2. Let us suppose now that  $x_v = ]q-1, q[$ . Since  $x_v \subseteq X_v(x)$ , then  $X_v(x)$  is a singular value denoted by  $]a, b[$  (with  $a < b$ ). For each  $x \in \mathcal{D}(x)$ , the attractor  $\mathcal{X}_v(x)$  is a non empty interval  $] \alpha, \beta [$  such that  $d_v(\alpha) = ]a, b[$  and  $d_v(\beta) = ]q-1, q[$ . Since  $]q-1, q[ \subseteq ]a, b[$ , we have

$$\begin{aligned} |a| < |q-1, q| < |b| &\iff d_v(\alpha) < d_v(x_v) < d_v(\beta) \\ &\iff x_v \in ] \alpha, \beta [ \\ &\iff x_v \in \mathcal{X}_v(x). \end{aligned}$$

<sup>1</sup>We have excluded the cases where the attractors are equals to some thresholds.



□

Since the function  $X_v : \mathbb{Q}_{\text{QR}} \rightarrow \mathbb{Q}_v$  gives the qualitative attractor of variable  $v$  at the qualitative state  $\mathbf{x}$ , it would be more suitable to express  $X_v$  as a function of the qualitative state  $\mathbf{x}$  independently of the continuous state  $x \in \mathcal{D}(\mathbf{x})$ . In this perspective let us define the regular/singular resources and the qualitative parameters.

**Definition 3.1. (Regular/singular resources)**

Let QR be a qualitative regulatory network built on  $G = (V, E)$  and  $v \in V$ .

- The set of regular resources  $R_v(\mathbf{x})$  of  $v$  at the state  $\mathbf{x}$  is the set of predecessors of  $v$  which acts positively on  $v$  (effective activators or non effective inhibitors):

$$R_v(\mathbf{x}) = \{u \in G^-(v) \mid (x_u > s_{uv} \text{ and } \alpha_{uv} = +) \text{ or } (x_u < s_{uv} \text{ and } \alpha_{uv} = -)\}.$$

- The set of singular resources  $S_v(\mathbf{x})$  of  $v$  at the state  $\mathbf{x}$  is the set of predecessors of  $v$  which acts uncertainly on  $v$ :

$$S_v(\mathbf{x}) = \{u \in G^-(v) \mid x_u = s_{uv}\}.$$

Note that  $u \in R_v(\mathbf{x})$  iff  $\tilde{\mathcal{I}}^{\alpha_{uv}}(x_u, \theta_{uv}) = k_{uv}$  and  $u \in S_v(\mathbf{x})$  iff  $\tilde{\mathcal{I}}^{\alpha_{uv}}(x_u, \theta_{uv}) = ]0, k_{uv}[$  for all  $x \in \mathcal{D}(\mathbf{x})$ .

**Definition 3.2. (Qualitative parameters)**

Let QR be a qualitative regulatory network built on  $G = (V, E)$ . The qualitative parameters  $K = \{K_{v,\omega}\}$  is a family of integers indexed by couples  $(v, \omega)$  such that:

- $v$  belongs to  $V$  and  $\omega$  is a subset of  $G^-(v)$ ,
- $K_{v,\omega} = 0$  if  $\omega = \emptyset$  and  $K_{v,\omega} \in \{0, \dots, b_v\}$  otherwise,
- $\omega \subseteq \omega' \implies K_{v,\omega} \leq K_{v,\omega'}$ .

An instantiation of these qualitative parameters is called in the remainder, a *model* of the qualitative regulatory network. For example of figure 2-a, four among six qualitative parameters have to be instantiated:  $K_{u,v}$ ,  $K_{u,u}$ ,  $K_{u,uv}$  and  $K_{v,u}$  since  $K_{u,\emptyset} = K_{v,\emptyset} = 0$  by definition.

Definition 3.2 allows us to set down  $|K_{v,\omega}| = d_v(\frac{1}{\lambda_v} \sum_{u \in \omega} k_{uv})$ , because

1. if  $\omega = \emptyset$  then<sup>2</sup>  $d_v(\frac{1}{\lambda_v} \sum_{u \in \omega} k_{uv}) = d_v(0) = |0|$  else  $d_v(\frac{1}{\lambda_v} \sum_{u \in \omega} k_{uv}) \in \{|0|, |1|, \dots, |b_v|\}$  under the hypothesis that  $(\frac{1}{\lambda_v} \sum_{u \in \omega} k_{uv}) \notin \Theta_v$ ,
2. if  $\omega \subseteq \omega'$  then  $(\frac{1}{\lambda_v} \sum_{u \in \omega} k_{uv}) \leq (\frac{1}{\lambda_v} \sum_{u \in \omega'} k_{uv})$ , and since  $d_v$  is an increasing function,  $d_v(\frac{1}{\lambda_v} \sum_{u \in \omega} k_{uv}) \leq d_v(\frac{1}{\lambda_v} \sum_{u \in \omega'} k_{uv})$ .

Then it is possible to define the function  $X_v : \mathbb{Q}_{\text{QR}} \rightarrow \mathbb{Q}_v$  as a function of regular/singular resources and of the qualitative parameters  $\{K_{v,\omega}\}$ .

**Theorem 3.3.** Setting  $|K_{v,\omega}| = d_v(\frac{1}{\lambda_v} \sum_{u \in \omega} k_{uv})$  the function  $X_v$  is given by  $X_v(\mathbf{x}) = |K_{v,R_v(\mathbf{x})}, K_{v,R_v(\mathbf{x}) \cup S_v(\mathbf{x})}|$ .

<sup>2</sup>With the convention  $\sum_{u \in \emptyset} a = 0$ .



**Proof:** Using equations 2 and 4, we have  $\mathcal{X}_v(x) = \frac{1}{\lambda_v} \sum_{u \in G^-(v)} \tilde{\mathcal{I}}^{\alpha_{uv}}(x_u, \theta_{uv})$ . The contribution of all predecessors which are not resources (regular or singular), is null. So we can write, for all  $x \in \mathcal{D}(x)$ ,  $\mathcal{X}_v(x) = (\frac{1}{\lambda_v} \sum_{u \in R_v(x)} k_{uv}) + \frac{1}{\lambda_v} \sum_{u \in S_v(x)} ]0, k_{uv}[$  with the convention  $\sum_{u \in \emptyset} a = 0$ . If  $S_v(x)$  is empty, then  $\mathcal{X}_v(x) = \frac{1}{\lambda_v} \sum_{u \in R_v(x)} k_{uv}$ . Thus  $X_v(x) = d_v(\frac{1}{\lambda_v} \sum_{u \in R_v(x)} k_{uv}) = |K_{v,R_v(x)}| = |K_{v,R_v(x)}, K_{v,R_v(x) \cup S_v(x)}|$ . On the other hand, if  $S_v(x)$  is not empty,  $\mathcal{X}_v(x) = (\frac{1}{\lambda_v} \sum_{u \in R_v(x)} k_{uv}) + ]0, \frac{1}{\lambda_v} \sum_{u \in S_v(x)} k_{uv}[$ . This can be written as  $\mathcal{X}_v(x) = ]\frac{1}{\lambda_v} \sum_{u \in R_v(x)} k_{uv}, \frac{1}{\lambda_v} \sum_{u \in R_v(x) \cup S_v(x)} k_{uv}[$ . Thus

$$X_v(x) = d_v \left( \left[ \frac{1}{\lambda_v} \sum_{u \in R_v(x)} k_{uv}, \frac{1}{\lambda_v} \sum_{u \in R_v(x) \cup S_v(x)} k_{uv} \right] \right).$$

As  $d_v(\frac{1}{\lambda_v} \sum_{u \in R_v(x)} k_{uv}) = |K_{v,R_v(x)}|$  and  $d_v(\frac{1}{\lambda_v} \sum_{u \in R_v(x) \cup S_v(x)} k_{uv}) = |K_{v,R_v(x) \cup S_v(x)}|$ , we have  $X_v(x) = |K_{v,R_v(x)}, K_{v,R_v(x) \cup S_v(x)}|$  according to 6.  $\square$

With this theorem, the qualitative parameters are sufficient to define the attractors of each state for a given qualitative regulatory network. For the running example, table of figure 3 gives the symbolic attractors expressed as the vector of the qualitative values  $X_v(x) = |K_{v,R_v(x)}, K_{v,R_v(x) \cup S_v(x)}|, v \in V$ . Then the attractors are explicitly computed for an instantiation of qualitative parameters. We deduce from these attractors the tendency of each variable which allows us to define a state graph, or transition system, representing the dynamics of the network.

### Definition 3.3. (Asynchronous state graph)

Let QR be a qualitative regulatory network built on  $G = (V, E)$ . The asynchronous state graph (or state graph for short) SG associated to a model of QR, is a directed graph  $SG = (S, T)$  where S is the set of qualitative states of QR, and T is the set of transitions between qualitative states such that:

1.  $x \rightarrow x' \in T$  if  $x$  is steady,
2.  $x \rightarrow x' \in T$  if  $\exists v \in V$  such that  $\begin{cases} x'_v = \Delta_v^+(x_v) \text{ and } x_v < X_v(x) \\ \text{or} \\ x'_v = \Delta_v^-(x_v) \text{ and } x_v > X_v(x) \end{cases}$  and  $x'_u = x_u \forall u \in V \setminus \{v\}$

where  $\Delta_v^+$  and  $\Delta_v^-$  are the evolution operators defined as following:

$$\Delta_v^+(x_v) = \begin{cases} |q, q+1| \text{ if } x_v = |q| \\ |q| \text{ if } x_v = |q-1, q| \end{cases} \quad \text{and} \quad \Delta_v^-(x_v) = \begin{cases} |q-1, q| \text{ if } x_v = |q| \text{ and } q > 0 \\ |q| \text{ if } x_v = |q, q+1| \end{cases}$$

To explain this definition we have to notice that the attractors define the state towards which the system tends to evolve. We consider that two variables cannot evolve simultaneously, that is why the state graph is said asynchronous. When several variables tend to evolve at a given state, additional information is needed to select which one first changes. In fact the values of time delays associated to each variation of variables, are to be known to define which one effectively evolves first [27]. As we have no information about time delays, all possible variations are considered. As a consequence a state for which  $n$  variables tend to evolve, has  $n$  successors.

Practically to build a state graph associated to a qualitative regulatory network built on  $G = (V, E)$ , we have to instantiate the qualitative parameters  $K = \{K_{v,\omega} \mid v \in V, \omega \subseteq G^-(v)\}$ . Note that there is a finite number of models associated to a qualitative regulatory network, since the number of possible instantiations of parameters is itself finite. Thus, the qualitative approach allows with a finite number of models to study the qualitative features of the infinity of continuous dynamics associated to a regulatory network.

In the Thomas' approach, the logical parameters are defined in the same way. Thus the models associated to a qualitative regulatory network are the same in both approaches (in particular there is the same number of

models), but the state graphs deduced from these models are different. Indeed, the attractor of a variable  $v$  at the state  $x$  can be written with our notation by  $X_v^{RT}(x) = K_{v,R_v}(\tilde{x})$  where  $x$  is a state of  $R$ . Thomas (a vector of integers) and where  $\tilde{x}$  is the qualitative regular state identifiable to  $x$  ( $\tilde{x}_v = |x_v|$  for all  $v \in V$ ). Thus the attractors of the states of Thomas' approach are the attractors of our regular states:  $X_v(\tilde{x}) = |X_v^{RT}(x)|$ . The  $R$ . Thomas' state graph contains only transitions between regular states and similarly to the previous definition,  $x \rightarrow x'$  is a transition if  $x$  is steady and  $x \rightarrow x'$  is a transition if:

$$\exists v \in V \text{ such that } \begin{cases} x'_v = x_v + 1 \text{ and } x_v < X_v^{RT}(x) \\ \text{or} \\ x'_v = x_v - 1 \text{ and } x_v > X_v^{RT}(x) \end{cases} \quad \text{and} \quad x'_u = x_u \quad \forall u \in V \setminus \{v\}.$$

Figure 3 uses definition 3.3 to construct the asynchronous state graph associated to the running example with particular values for qualitative parameters. It is compared to the  $R$ . Thomas' state graph, for which only one of the three continuous steady states is abstracted by a qualitative one.

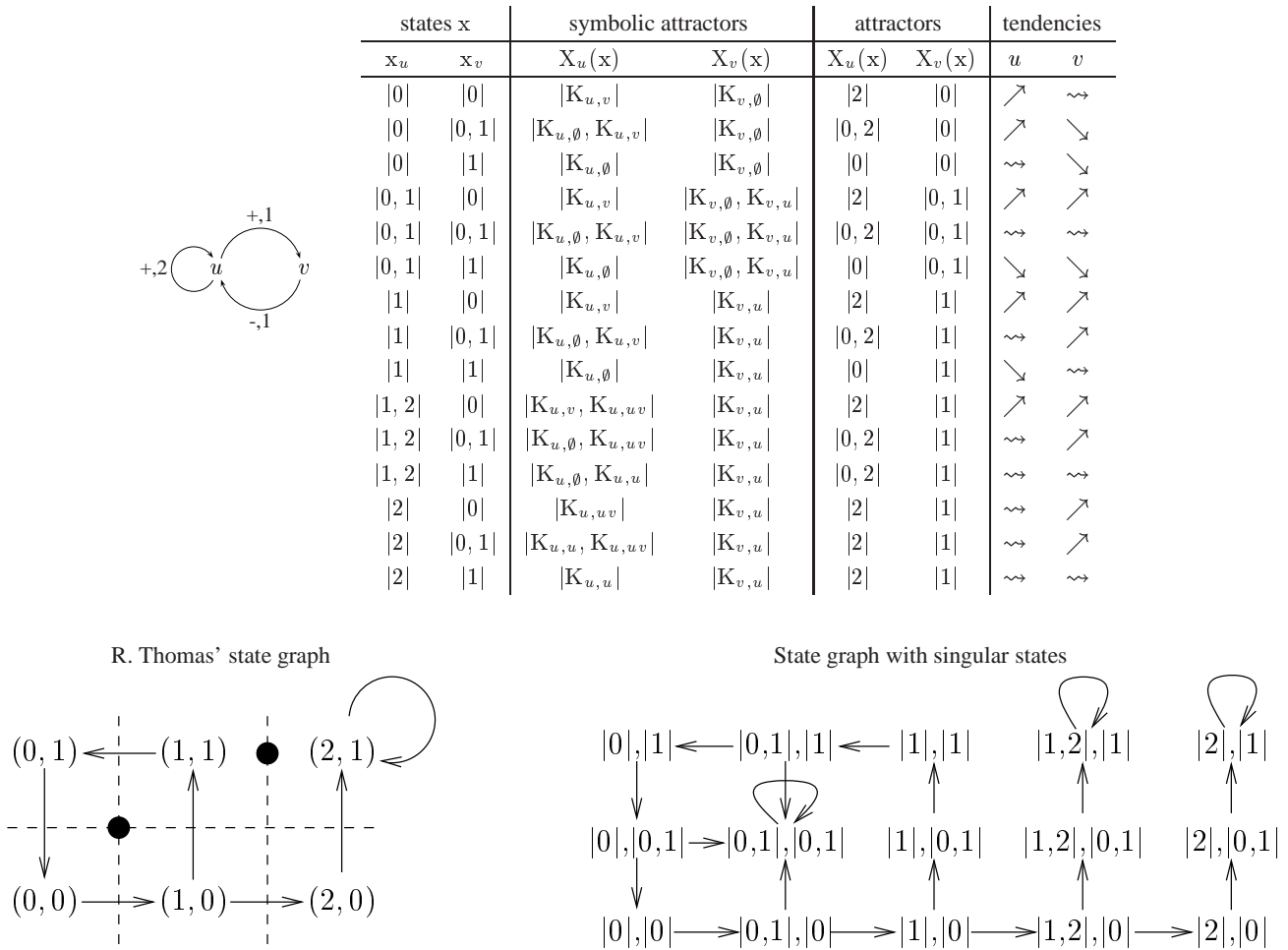


Figure 3. State graphs deduced from the running example for the qualitative parameters:  $K_{u,\emptyset} = 0$ ,  $K_{u,v} = 2$ ,  $K_{u,u} = 2$ ,  $K_{u,uv} = 2$ ,  $K_{v,\emptyset} = 0$  and  $K_{v,u} = 1$ .

## 4. Functionality of feedback circuits

Most often, there is a huge number of models associated to a regulatory network since the number of parameters  $K_{v,\omega}$  associated to each  $v \in V$  is exponential according to the number of its predecessors. These parameters are often unknown and thus, the major issue of the modeling activity is to select the suitable set of parameters which give a qualitative behavior coherent with the experimental knowledge on the system. Three different kinds of dynamic properties are often used to aid the selection of suitable models: steady states, multistationarity and homeostasis. The selection of models which presents a given set of steady states remains a simple application of definition of steady states (see section 3). On the other hand the two other dynamic properties are not directly expressed in term of parameters, and their detection in a given model is not trivial. Hopefully the feedback circuits theory [28] allows us to link multistationarity and homeostasis to parameters and to select appropriate models.

In a feedback circuit, each variable has an influence on its target but also an indirect effect on all following elements including itself. A feedback circuit is said *positive* (resp. *negative*) if each variable has a positive (resp. negative) influence on itself. The sign of the circuit is determined by the number of negative interactions: the circuit is negative if the number of negative interactions is odd, otherwise it is positive. It has been shown that it is possible to associate to a feedback circuit a typical dynamic behavior: in a **negative circuit**, a high concentration of a variable tends to make decrease itself (and reversely). Thus the circuit makes the concentration of each variable to tend to (or oscillate around) an equilibrium concentration. It then generates homeostasis. In a **positive circuit**, a high (resp. low) concentration of a variable tends to make it increase (resp. decrease). Thus each variable can stay at a low or high concentration and the positive circuit generates multistationarity. A feedback circuit, which presents a typical dynamic behavior is said 'functional'. Several other properties on the behavior of feedback circuits have been proved: at least one positive regulatory circuit is necessary to generate multistationarity whereas at least one negative circuit is necessary to generate a stable oscillatory behavior[28, 22, 14, 6, 17, 19]. One can then demonstrate that  $m$  functional disjointed positive feedback circuits generate  $3^m$  steady states among which  $2^m$  are regular.

In the sequel we first introduce the notion of characteristic states of a feedback circuit which play a fundamental role: the steadiness of one of them leads to the functionality of the circuit. We then prove in our framework as Snoussi and Thomas did in the context of R. Thomas theory [18], that among singular states only characteristic states can be steady. The condition of steadiness of a characteristic state can be rewritten in term of constraints on parameters. The proof of these conditions by Snoussi and Thomas is quite technical in their modeling because they do not formally express the qualitative attractors of singular states. After comparison of both state graphs for a model which verifies the condition of functionality, we give a theorem which shows that the dynamics of Thomas are preserved in our modeling.

### 4.1. Characteristic states

A circuit can be described by the finite set of arcs which compose it. A characteristic state of a circuit [18] is defined as a state in which  $u$  is a singular resource of  $v$  iff  $(u \rightarrow v)$  is an arc of the circuit. This notion of characteristic state can be extended to the unions of disjointed circuits<sup>3</sup>.

#### Definition 4.1. (Characteristic state of a union of circuits)

Let QR be a qualitative regulatory network built on  $G = (V, E)$  and let  $C$  a union of disjointed circuits. The state  $x$  is characteristic of  $C$  if we have:  $u$  is a singular resource of  $v$  iff  $u \rightarrow v$  is an arc of the union of circuits, that is if  $C = \{u \rightarrow v \in E \mid u \in S_v(x)\}$ .

Note that a characteristic state is singular and that there are several characteristic states associated to a union of circuits if it does not contain all variables of the network. Some examples of unions of disjointed circuits with their characteristic states are given in Figure 4. It is possible to demonstrate the following useful property: a

<sup>3</sup>Circuits are disjointed if there is no vertex which belongs to more than one circuit.

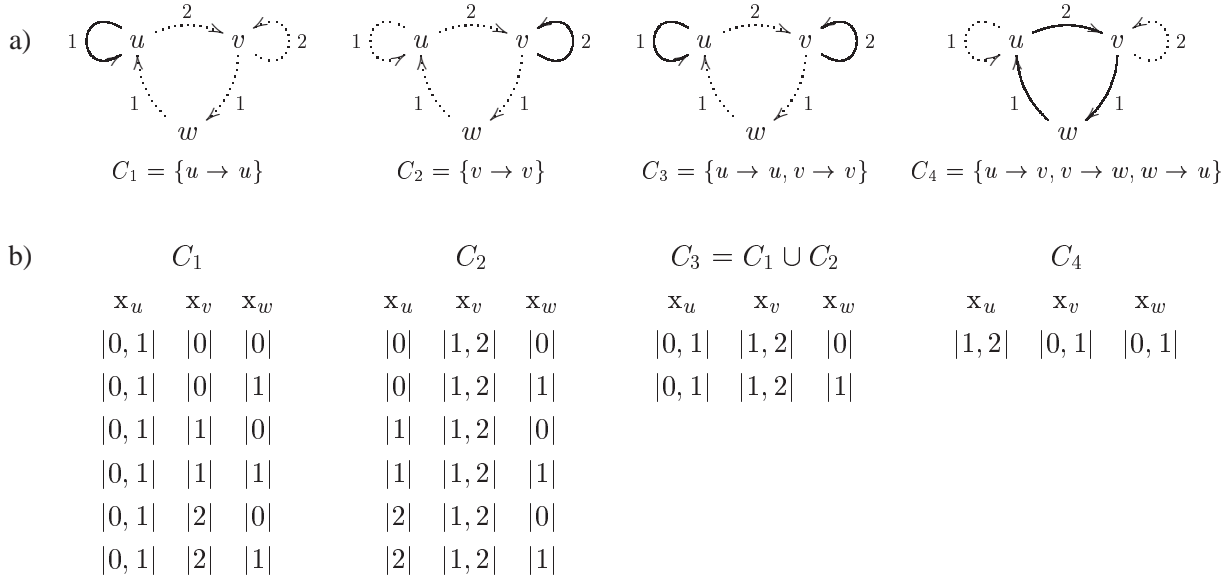


Figure 4. Unions of disjointed circuits of a regulatory network (a) and their characteristics states (b).

singular state such that each variable with a singular qualitative value is uncertainly regulated by another variable, is a characteristic state.

**Property 2.** Among singular states, only characteristic states can be steady.

**Proof:** Let QR be a qualitative regulatory network built on  $G = (V, E)$  and let  $x$  a non characteristic singular state. One can deduce from the previous remark that there is a variable  $v \in V$  such that  $x_v$  is singular and  $S_v(x) = \emptyset$ . We deduce that the attractor of  $v$  at this state is a regular qualitative value  $X_v(x) = |K_{v, R_v(x)}|$ . Since a singular value cannot be contained in a regular qualitative value,  $x_v \not\subseteq X_v(x)$ , and  $x$  cannot be steady.  $\square$

## 4.2. Constraints for functionality of feedback circuits

To select suitable models, it can be useful to translate functionality of feedback circuits in terms of constraints on parameters. Thomas realized that the functionality of feedback circuits is directly linked to the stationnarity of characteristic states: a circuit is functional if one associated characteristic state is steady [18]. For a functional negative circuit, the steady characteristic state acts as an attractor since for all variables implicated in the circuit, if the concentration is above (resp. below) the out-threshold in the circuit, the negative effect on itself tends to make it decrease (resp. increase). On the other hand, for a functional positive circuit, the steady characteristic state is unstable, because for each variable of the circuit the slightest departure from the threshold is sufficient to make topple down the variable under or over its threshold.

In our modeling, the constraints for steadiness of characteristic states can be expressed as constraints on parameters, which give thus the constraints for functionality of associated circuit: let  $x$  be a characteristic state of a circuit  $C$ . It is steady iff for each variable  $v$ ,  $x_v \subseteq X_v(x)$ .

- For each variable  $u$  not implicated in  $C$ ,  $S_u(x) = \emptyset$  and  $x_u \subseteq X_u(x) \iff x_u = X_u(x) \iff x_u = |K_{u, R_u(x)}|$ .

- For each variable  $u$  implicated in  $C$ , we have  $S_u(x) \neq \emptyset$  and  $x_u = s_{uv} = |q_{uv} - 1, q_{uv}|$  where  $q_{uv}$  is the threshold rank labeling  $u \rightarrow v \in C$ , then

$$\begin{aligned} x_u \subseteq X_u(x) &\iff |q_{uv} - 1, q_{uv}| \subseteq |K_{u, R_u(x)}, K_{u, R_u(x) \cup S_u(x)}| \\ &\iff K_{u, R_u(x)} \leq q_{uv} - 1 \quad \text{and} \quad q_{uv} \leq K_{u, R_u(x) \cup S_u(x)} \\ &\iff K_{u, R_u(x)} < q_{uv} \leq K_{u, R_u(x) \cup S_u(x)}. \end{aligned}$$

Snoussi and Thomas deduced similar constraints in their modeling which are given in the following theorem.

**Theorem 4.1.** [18] For a given model, there exists a steady characteristic state of a circuit  $C$  if there are two regular qualitative states (in the Thomas model)  $x^+$  and  $x^-$  such that:

- for each variable  $u$  not implicated in  $C$ ,  $x_u^- = x_u^+ = X_u^{RT}(x^+) = X_u^{RT}(x^-)$
- for each variable  $u$  implicated in  $C$  whose the successor in  $C$  is  $v$ ,

$$\left\{ \begin{array}{l} \text{if } \alpha_{uv} = + \text{ then} \\ \text{if } \alpha_{uv} = - \text{ then} \end{array} \right\} \begin{cases} x_u^+ = q_{uv} \\ x_u^- = q_{uv} - 1 \\ x_u^+ = q_{uv} - 1 \\ x_u^- = q_{uv} \end{cases} \quad \text{and} \quad X_u^{RT}(x^-) < q_{uv} \leq X_u^{RT}(x^+)$$

where  $q_{uv}$  is the threshold rank of  $u \rightarrow v$ . The states  $x^+$  and  $x^-$  are called the adjacent regular states of the characteristic state of the circuit and they give respectively the minimal and maximal attractors.

The notion of resources permits us to develop the constraints of the previous theorem in terms of parameters. We assimilate both  $x^+$  and  $x^-$  in the modeling of Thomas to the corresponding qualitative regular states in our modeling. The characteristic state  $x$  which has  $x^+$  and  $x^-$  for adjacent states, is defined as the only characteristic state of  $C$  which verifies  $x_u = x_u^- = x_u^+$  for all  $u$  not implicated in the circuit. By definition, the resources of each variable of the circuit at the state  $x^-$  are not in the circuit. Since for all  $u$  not implicated in the circuit we have  $x_u = x_u^-$ , then  $R_u(x^-) = R_u(x)$ . In contrast, by definition of the state  $x^+$ , each variable implicated in the circuit is a resource of its successor in the circuit. So we have for all  $u$ ,  $R_u(x^+) = R_u(x) \cup S_u(x)$  (with  $S_u(x) \neq \emptyset$  if  $u$  is implicated in the circuit and  $S_u(x) = \emptyset$  otherwise). Thus:

- for each variable  $u$  not implicated in  $C$ , we have  $S_u(x) = \emptyset$  and

$$x_u^\epsilon = X_u^{RT}(x^\epsilon) \iff x_u^\epsilon = |K_{u, R_u(x^\epsilon)}| \iff x_u = |K_{u, R_u(x)}| \quad \text{with } \epsilon \in \{+, -\}$$

- for each variable  $u$  implicated in  $C$  whose the successor in  $C$  is  $v$ , we have  $S_u(x) \neq \emptyset$  and

$$X_u^{RT}(x^-) < q_{uv} \leq X_u^{RT}(x^+) \iff K_{u, R_u(x^-)} < q_{uv} \leq K_{u, R_u(x^+)} \iff K_{u, R_u(x)} < q_{uv} \leq K_{u, R_u(x) \cup S_u(x)}$$

Thus the constraints elaborated for the Thomas' modeling are translated in our modeling in terms of constraints on parameters.

### 4.3. Examples and comparison with R. Thomas modeling

In the previous subsections, we highlighted that characteristic states play a central role for the circuit functionality and we showed that the conditions for functionality are the same in the R. Thomas modeling and in our one. Now we compare the dynamics of models for which some circuits are functional. Let us consider first the example which contains a unique vertex which acts on itself positively or negatively:

$$L_- : v \overset{\ominus}{\circlearrowleft} -1 \quad L_+ : v \overset{\oplus}{\circlearrowleft} +1$$

We deduce from these two loops the following symbolic attractors:

$$L_- : \begin{array}{c|c} x_v & X_v(x) \\ \hline |0| & |K_{v,v}| \\ |0, 1| & |K_{v,\emptyset}, K_{v,v}| \leftarrow \text{characteristic state} \rightarrow \\ |1| & |K_{v,\emptyset}| \end{array} \quad L_+ : \begin{array}{c|c} x_v & X_v(x) \\ \hline |0| & |K_{v,\emptyset}| \\ |0, 1| & |K_{v,\emptyset}, K_{v,v}| \\ |1| & |K_{v,v}| \end{array}$$

The loops are functional if the associated characteristic state  $|0, 1|$  is steady, that is if

$$|0, 1| \subseteq X_v(|0, 1|) \iff K_{v,\emptyset} < 1 \leq K_{v,v}.$$

Thus,  $K_{v,\emptyset} = 0$  and  $K_{v,v} = 1$  is the only one possible instantiation of parameters satisfying these constraints. The attractors and the tendencies for both models become:

$$L_- : \begin{array}{c|c|c} x_v & X_v(x) & \text{Tendencies} \\ \hline |0| & |1| & \nearrow \\ |0, 1| & |0, 1| & \rightsquigarrow \leftarrow \text{steady characteristic state} \rightarrow \\ |1| & |0| & \searrow \end{array} \quad L_+ : \begin{array}{c|c|c} x_v & X_v(x) & \text{Tendencies} \\ \hline |0| & |0| & \rightsquigarrow \\ |0, 1| & |0, 1| & \rightsquigarrow \\ |1| & |1| & \rightsquigarrow \end{array}$$

The state graphs can then be deduced:

|         | R. Thomas state graph | state graph with singular states |
|---------|-----------------------|----------------------------------|
| $L_- :$ |                       |                                  |
| $L_+ :$ |                       |                                  |

In the 4 state graphs, homeostasis or multistationarity induced by the loop functionality is present. The greatest difference concerns the negative circuit:

1. The paths of the Thomas' state graph do not correspond to paths between regular states in our state graph. When a characteristic state of a negative loop  $v \rightarrow v$  is steady, then the Thomas' state graph is not "contained" in the state graph with singular states (see property 3 for details).
2. The state graph reflects a damped oscillation towards the characteristic state in our modeling and a sustained oscillation for Thomas modeling.

In the Thomas modeling it is not possible to represent the softening which can be generated by the functionality of negative circuits. That can infer a confusion about the interpretation of the circuit functionality. Let us consider

now the qualitative regulatory network of figure 3 containing 2 variables with a negative circuit of length 2 and a positive loop. The following table gives the constraints for steadiness of each characteristic state.

|           | characteristic states |       | Symbolic attractors          |                              | Constraints for functionality      |                                    |
|-----------|-----------------------|-------|------------------------------|------------------------------|------------------------------------|------------------------------------|
|           | $x_u$                 | $x_v$ | $X_u(x)$                     | $X_v(x)$                     |                                    |                                    |
| Circuit - | 0, 1                  | 0, 1  | $ K_{u,\emptyset}, K_{u,v} $ | $ K_{v,\emptyset}, K_{v,u} $ | $K_{u,\emptyset} < 1 \leq K_{u,v}$ | $K_{v,\emptyset} < 1 \leq K_{v,u}$ |
| Circuit + | 1, 2                  | 0     | $ K_{u,v}, K_{u,uv} $        | $ K_{v,u} $                  | $K_{u,v} < 2 \leq K_{u,uv}$        | $K_{v,u} = 0$                      |
| Circuit + | 1, 2                  | 1     | $ K_{u,\emptyset}, K_{u,u} $ | $ K_{v,u} $                  | $K_{u,\emptyset} < 2 \leq K_{u,u}$ | $K_{v,u} = 1$                      |

The necessary conditions for functionality of both circuits are compatible in the case of steadiness of the second characteristic state of the positive circuit. The instantiation of parameters of figure 3 makes functional both circuits and multistationarity is predicted (functional positive loop). This multistationarity is important because the network of the running example is supposed to control an epigenetic switch<sup>4</sup> [2]. Then models which present a multistationarity have to be considered. In the Thomas' state graph there is only one steady state (the two others are singular) and from each state it is possible to reach it. In our modeling the multistationarity is represented in the dynamics since it contains three steady states.

However, the paths between states in the Thomas modeling are coherent with our modeling. Indeed, each transition  $x^1 \rightarrow x^2$  of the Thomas' state graph corresponds to a path  $x^a \rightarrow x^s \rightarrow x^b$  where  $x^a$  and  $x^b$  are identifiable to  $x^1$  and  $x^2$  and where  $x^s$  is a singular state. The following property gives more details about the coherence between both modelings.

### Property 3.

- Let  $x^1 \rightarrow x^2$  (with  $x^1$  distinct from  $x^2$ ) be a transition of the state graph of R. Thomas deduced from a model of a qualitative regulatory network QR built on  $G = (V, E)$ ,
- let  $u$  be the only variable which evolves during the transition ( $x_u^1 \neq x_u^2$ ),
- let  $x^a$  and  $x^b$  be the qualitative regular states identifiable to  $x^1$  and  $x^2$  ( $x_v^a = |x_v^1|$  and  $x_v^b = |x_v^2|$  for all  $v$  in  $V$ ), and finally let  $x^s$  be the qualitative singular state between  $x^a$  and  $x^b$ .

Then the qualitative state graph deduced from the same model contains the path  $x^a \rightarrow x^s \rightarrow x^b$  if  $x_u^s$  is not steady ( $x_u^s$  is steady imposes that  $x^s$  is a characteristic state of the negative loops  $u \rightarrow u$ ).

**Proof:** Let us set down  $x_u^a = |q|$ . Since  $x^1 \rightarrow x^2$  is a transition of the state graph of R. Thomas and since  $x^1 \neq x^2$ ,  $x_u^1 \neq X_u^{RT}(x^1) \iff x_u^a \neq |K_{u,R_u(x^a)}| \iff |q| \neq |K_{u,R_u(x^a)}|$ .

- If  $|q| < |K_{u,R_u(x^a)}|$  then  $x_u^s = |q, q + 1|$ ,  $x_u^b = |q + 1|$ , so  $x^a \rightarrow x^s$  is a transition of our state graph, and  $|q + 1| \leq |K_{u,R_u(x^a)}|$ .
  - If  $x^s$  is not a characteristic state then either  $(u \rightarrow u) \notin E$  or the threshold of the auto-regulation  $(u \rightarrow u)$  is not equal to  $x_u^s = |q, q + 1|$ . So  $S_u(x^s) = \emptyset$  and  $X_u(x^s) = |K_{u,R_u(x^s)}| = |K_{u,R_u(x^a)}|$ . So  $x_u^s = |q, q + 1| < |K_{u,R_u(x^a)}|$  and  $x^s \rightarrow x^b$  is a transition of our state graph.
  - If  $x^s$  is a characteristic state then  $(u \rightarrow u) \in E$  and  $s_{uu} = |q, q + 1|$ . Thus  $S_u(x^s) = \{u\}$ .
    - If the sign of  $u \rightarrow u$  is positive,  $\alpha_{uu} = +$ , then  $X_u(x^s) = |K_{u,R_u(x^s)}, K_{u,R_u(x^s) \cup \{u\}}| = |K_{u,R_u(x^a)}, K_{u,R_u(x^a) \cup \{u\}}|$ . So  $x_u^s = |q, q + 1| < |K_{u,R_u(x^a)}, K_{u,R_u(x^a) \cup \{u\}}|$  and  $x^s \rightarrow x^b$  is a transition of our state graph.

<sup>4</sup>stable change of phenotype without mutation



- If  $\alpha_{uu} = -$  then  $X_u(x^s) = |K_{u,R_u(x^s)}, K_{u,R_u(x^s) \cup \{u\}}| = |K_{u,R_u(x^a) \setminus \{u\}}, K_{u,R_u(x^a)}|$ . So, if  $x_u^s$  is not steady we have  $x_u^s = |q, q + 1| \not\subseteq |K_{u,R_u(x^a) \setminus \{u\}}, R_u(x^a)| \implies q < K_{u,R_u(x^a) \setminus \{u\}} \iff q + 1 \leq K_{u,R_u(x^a) \setminus \{u\}}$ . So  $|q, q + 1| < |K_{u,R_u(x^a) \setminus \{u\}}, K_{u,R_u(x^a)}|$  and  $x^s \rightarrow x^b$  is a transition of our state graph.
- If  $|q| > |K_{u,R_u(x^a)}|$  then  $x_u^s = |q - 1, q|$ ,  $x_u^b = |q - 1|$ , so  $x^a \rightarrow x^s$  is a transition of our state graph, and  $|q - 1| \geq |K_{u,R_u(x^a)}|$ .
  - If  $x^s$  is not a characteristic state then the demonstration is similar.
  - If  $x^s$  is a characteristic state, then  $(u \rightarrow u) \in E$  and  $s_{uu} = |q - 1, q|$ . Thus  $S_u(x^s) = \{u\}$ .
    - If  $\alpha_{uu} = +$  then  $X_u(x^s) = |K_{u,R_u(x^s)}, K_{u,R_u(x^s) \cup \{u\}}| = |K_{u,R_u(x^a) \setminus \{u\}}, K_{u,R_u(x^a)}|$ . So  $x_u^s = |q - 1, q| > |K_{u,R_u(x^a) \setminus \{u\}}, K_{u,R_u(x^a)}|$  and  $x^s \rightarrow x^b$  is a transition of our state graph.
    - If  $\alpha_{uu} = -$  then  $X_u(x^s) = |K_{u,R_u(x^s)}, K_{u,R_u(x^s) \cup \{u\}}| = |K_{u,R_u(x^a)}, K_{u,R_u(x^a) \cup \{u\}}|$ . So, if  $x_u^s$  is not steady we have  $x_u^s = |q - 1, q| \not\subseteq |K_{u,R_u(x^a)}, K_{u,R_u(x^a) \cup \{u\}}| \implies q > K_{u,R_u(x^a) \cup \{u\}} \iff q - 1 \geq K_{u,R_u(x^a) \cup \{u\}}$ . So  $|q - 1, q| > |K_{u,R_u(x^a)}, K_{u,R_u(x^a) \cup \{u\}}|$  and  $x^s \rightarrow x^b$  is a transition of our state graph.

□

Application of the previous property to the running example of *Pseudomonas aeruginosa* is straightforward: since the network does not contain negative loop (circuit of length 1) then, for each model, the R. Thomas' state graph is "contained" in our one.

## 5. Conclusion and perspectives

In this paper we present a new qualitative modeling based on the R. Thomas works which allows us to represent the singular states in the dynamics. Both modelings are based on a discretization of piecewise-linear differential equations system. However through our discretization map, all the steady states of the continuous dynamics are preserved in our state graph. Moreover:

- the increase in the number of states does not imply an increase in the number of models associated to a network,
- the state graph reflects the softening of the negative functional circuits,
- the dynamics do not contradict the R. Thomas' ones, and
- finally, the theorems of the functionality of feedback circuits have been extended to our modeling, using the notion of singular/regular resources.

The formal modeling of regulatory networks allows us to take advantage of the corpus of formal methods to confront the models to biological knowledge. Now that singular states, which can play an important biological role, are included in trajectories, temporal properties concerning them can be automatically checked on the transition system. This leads to a more extended set of checkable properties which can be involved in the modeling process. Indeed we want to select models which are coherent not only with the static conditions (functionality of feedback circuits) but also with some known dynamic properties extracted from biological experiments or hypothesis. We have already implemented a software, SMBioNet [2, 15] (Selection of Models of Biological Networks), which allows, in the context of Thomas modeling, the selection of models for a given regulatory network satisfying specified temporal properties. The software takes as input a qualitative regulatory network,

some temporal properties expressed as a CTL formula and a set of functional circuits. Then it gives as output the models which satisfy the specified temporal properties (using the NuSMV model checker [4]). The global methodology of this computer aided modeling process can be extended to the formalism presented in this paper. For a large amount of biological systems, this refinement would be useful to be more coherent with the biology.

More generally the formal methods can be applied in the field of biological regulatory networks in order to explicit some behaviors or to model some other biological knowledge. Let us mention for example that the introduction of transitions in the regulatory network could help to specify how the different regulators cooperate for inducing or repressing their common target [1]. One can also take into account time delays[25] between the beginning of the activation order and the synthesis of the product and conversely for the turn-off delays. These constitute ongoing or future works of our genopole<sup>®</sup> research groups.

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