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extending Thomas' logical approach with temporal logic

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A Fruitful Application of Formal Methods to Biological Regulatory Networks

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Abstract : Based on the discrete definition of biological regulatory networks developped by R. Thomas, we provide a computer science formal approach to treat temporal properties. Our approach is illustrated with the mucus production in *Pseudomonas aeruginosa*. This first application of formal methods from computer science to biological regulatory networks should open the way to many other fruitful applications.

Keywords : biological regulatory networks, formal methods, temporal logic, model checking.

1 Introduction

To elucidate the principles that govern biological complexity, computer modeling has to overcome *ad hoc* explanations in order to make emerge novel and abstract concepts[4]. Computational *systems biology*[17] tries to establish methods and techniques that enable us to understand biological systems as systems, including their robustness, design and manipulation[7, 3]. It means to understand : the structure of the system, such as gene/metabolic/signal transduction networks, the dynamics of such systems, methods to control, design and modify systems to cope with desired properties[8].

Biological regulatory networks place the discussion at a biological level instead of a biochemical level, that allows one to study behaviours at an upper level. They modelize interactions between biological entities, often macromolecules or genes. They are statically represented by graphs : vertices abstract the biological entities, and edges abstract their interactions. Moreover at a given time each vertex has a numerical value to describe the level of concentration of the corresponding entity. Temporal evolutions of these levels constitute the dynamics of the system. The first approaches used differential equation systems to represent the dynamics. To face the combinatorial explosure of the parameters, R. Thomas introduced in the 70's a boolean approach for regulatory networks to capture the qualitative nature of the dynamics. He proved its usefulness in the context of immunity in bacteriophages[14, 13]. Later on, he generalized it to multivalued levels of concentration, so called "generalized logical" approach. Moreover the vertices of R. Thomas' regulatory networks are abstracted into "variables" allowing the cohabitation of heterogeneous informations (e.g. adding environmental variables to genetic ones).
Discrete and continuous modelings are coherent. The R. Thomas boolean approach has been justified as a discretization of the continuous differential equation system[11], then has been contronted to the more classical analysis in terms of differential equations[6]. Taking into account "singular states" , Thomas and Snoussi showed that all steady states can be found *via* the discrete approach[12]. More recently Thomas and Kaufman have shown that the discrete description provides a qualitative fit of the differential equations with a small number of possible combinations of values for the parameters[15].
Circuits in the graph are indeed the key concepts. A direct or indirect influence of a gene on itself corresponds to a closed oriented path which constitutes a feedback circuit. Feedback circuits are fundamental

because they decide the existence of steady states of the dynamics : it has been stated that at least one positive regulatory circuit is necessary to generate multistationarity whereas at least one negative circuit is necessary to obtain a homeostasy or a stable oscillatory behaviour[16], see Section 2.

In [10] we desccribed a very preliminary work on the application of model checking to biological regulatory networks. In this article we run the machinery of formal methods to revisit R. Thomas' regulatory networks. Formal methods impose detailled definitions which are introduced in the following sections. Section 2 defines biological regulatory graphs which describe the interactions between biological entities. Section 3 introduces the parameters which pilot the behaviours of the system. Section 4 defines the dynamics. Being in the domain of formal methods, we inherite from computer science the whole collection of validation and verification tools. Model checking tools are particularly suited as described in Section 5.

We take as running example the mucus production in *Pseudomonas aeruginosa*[2]. This bacteria secretes mucus in lungs affected by cystic fibrosis increasing the respiratory deficiency of the patient. In healthy lungs no mucus production arises. Taking a population of bacteria from diseased lungs, and after having selected a population which produces mucus in a stable way, one observes a mutation (elimination of an anti-sigma gene).

- *Does it mean that the mutation is the cause of the passage to the mucoid state ?*
- Or despite this, *could the mucoidy be induced by an epigenetic phenomenon ?* (stable change of phenotype without mutation). In this case the observed mutation could be, for example, favoured later on by the mucous environment.

Modeling can answer this last question. Formally the question becomes : *is it possible to find at least one model of the wild bacteria, which is compatible with the known biological results and that has a bi-stationarity where one stable state produces mucus and the other one does not ?* In Section 5 model checking gives a positive answer.
We started from the following biological results. The main regulator of mucus production is an alginate `AlgU`. It supervises an operon which is made up of 4 genes among which one gene codes for an anti-sigma, which is a membrane protein that plays a repressor role on `AlgU`. Moreover `AlgU` favors its own synthesis. This sketch of model (Figure 1) is rather simple but sufficient to illustrate each definition introduced in the sequel.

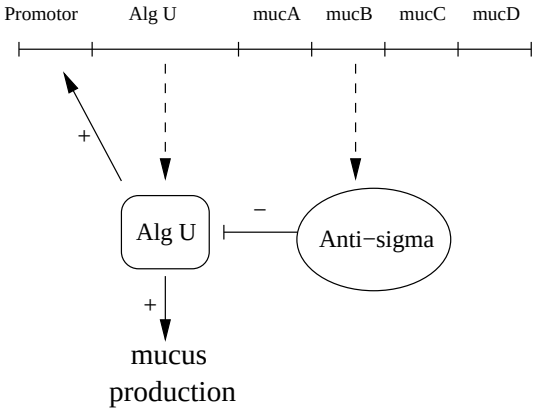

FIG. 1 – Main regularory genes of mucus production in *Pseudomonas aeruginosa*.

2 Biological regulatory graphs

Figure 2 assumes that x is a variable (for example a gene) which acts positively on y and negatively on z, each curve being the concentration rate of y (resp. z) with respect to the concentration rate of x after a sufficient delay for x to act on y (resp. z). Obviously three regions are interesting in the different rates of concentration of x.

– In the first region x acts neither on y nor on z.
– In the second region, x acts on y but it still does not act on z.
– In the last region, x acts both on y and z.

The sigmoid nature of the interactions shown in Figure 2 is almost allways verified and is necessary to justify this discretisation of the concentration rates of x : three abstract levels (0, 1 and 2) emerge corresponding to the three previous regions and constitute the only relevant information from a qualitative point of view. More generally as shown in Figure 3, if a variable acts on n variables, at most n+1 abstract regions are relevant (possibly less because two thresholds for two different target variables can be equal).

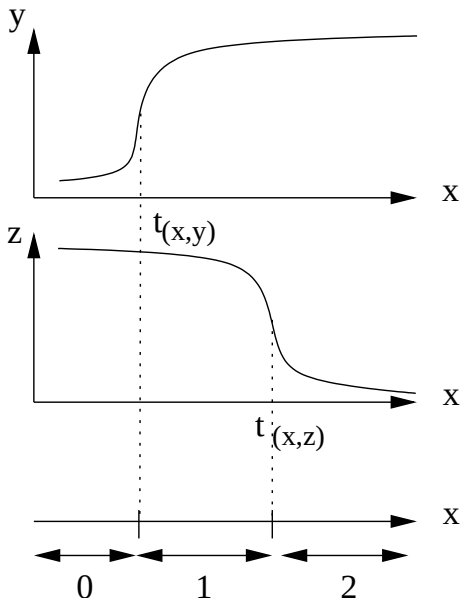

FIG. 2 – Definition of the abstract concentration levels

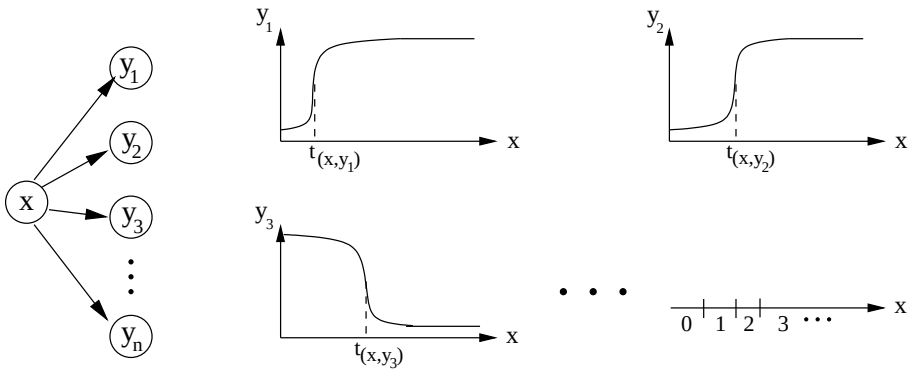

FIG. 3 – Successors of x in the graph determine the abstract levels.

Now, to formally define a biological regulatory network we use a labelled directed graph. A vertex represents a variable (gene, protein,...) and has a boundary which is the maximal value of its discrete concentration level. A directed edge (x, y) is labelled with a threshold and a sign + (resp. −) if x activates (resp. inhibits) y.

Definition 1 : A *biological regulatory graph* is a labelled directed graph $G = (V, A)$ where :
– each vertex v of V, called *variable*, is provided with a *boundary* $b_v \in \mathbb{N}^*$ less or equal to the out-degree of v in G, except when the out-degree is 0 where $b_v = 1$
– each edge $(v_1 \to v_2)$ of A is labelled with a couple (t, ε) where t, called *threshold*, is an integer between 1 and b_{v_1} and $\varepsilon \in \{-, +\}$.

The biological regulatory graph of Figure 4, deduced from Figure 1, contains two "classical" vertices x (AlgU) and y (anti-sigma) and an abstract "mucus" variable. The edges depict :

- $x \to x$: self-maintenance of AlgU,
- $y \to x$: inhibitor effect on AlgU,
- $x \to y$: positive influence of AlgU on its own inhibitors.

Since y acts on one variable, $b_y = 1$ and the threshold of edge $y \to x$ can only be 1. A question is to determine the thresholds of the other edges. It has been shown [2] that mucus production occurs when x is over its second threshold (x=2), but we do not know if x acts on y at a lower threshold than it acts on x or conversely. In other words, $b_x = 2$ and there are two possible biological regulatory graphs (Figures 4-a & 4-b).

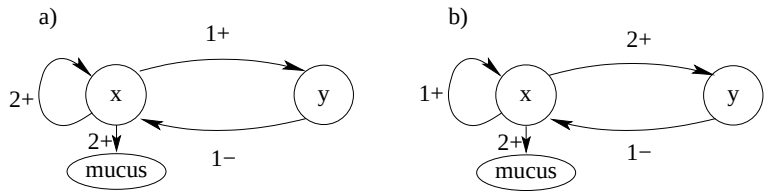

FIG. 4 – Two possible regulatory graphs for mucus production in *Pseudomonas aeruginosa*.

When successive interactions constitute a circuit, a variable has a feedback on itself. If the variable tends to favor (resp. decrease) its production *via* this circuit, the feedback circuit is said positive (resp. negative). A feedback circuit is positive (resp. negative) iff its number of negative arrows is even (resp. odd).

In Figure 4, x belongs to a positive circuit : $x \to x$. It also belongs to a negative circuit *via* $x \to y \to x$, as well as y for the same reason.

3 Biological regulatory networks

In Figure 3 we introduced the abstract concentration levels on the "horizontal" x axis. It remains to consider the "vertical" y axes : assuming that $x_1 \cdots x_n$ have an influence on y (entering arrows $x_i \to y$), toward which concentration level is y attracted? The set of all possible regulators of a variable is simply the set of its predecessors in the graph.

Notation : Let G a biological regulatory graph and v a variable, $G^{-1}(v)$ is the set of all predecessors of v, i.e the set of variables u such that $(u \to v)$ in an edge of G.

But regulatory variables are not allways active. At a given time, only some of them pass the threshold. Thus a variable v can be regulated by different subsets ω of its inhibitors/activators and we denote by $k_{v,\omega}$ the concentration level toward which v is attracted. Biological regulatory networks are biological regulatory graph (Definition 1) together with those parameters $k_{v,\omega}$.

Definition 2 : A *biological regulatory network* is a couple $\mathcal{R} = (G, \mathcal{K})$ where $G = (V, A)$ is a biological regulatory graph and $\mathcal{K} = \{k_{v,\omega}\}$ is a family of natural numbers indexed by the set of couples (v, ω) such that

- v belongs to V,
- ω is a subset of $G^{-1}(v)$ and will be called a set of resources of v,
- $k_{v,\omega} \leq b_v$.

For example to turn the biological regulatory graphs of Figure 4 into regulatory networks, six parameters have to be given $\mathcal{K} = \{k_{x,\{\}}, k_{x,\{y\}}, k_{x,\{x\}}, k_{x,\{x,y\}}, k_{y,\{\}}, k_{y,\{x\}}\}$. Because $b_x = 2$ and $b_y = 1$, each $k_{x,\ldots}$ can take the value 0, 1 or 2 and similarly for each $k_{y,\ldots}$. So $3^4 \times 2^2$ different networks can be *a priori* associated with each graph of Figure 4, which makes 648 different possible models.

Unfortunately in general the parameters of $\mathcal{K}$ are not measurable *in vivo*. Consequently, additional properties deduced from biological experiments are needed to eliminate the models which do not satisfy

them. This requires to study the dynamic behaviours of models.

4 Dynamics of biological regulatory networks

At a given time, each variable of a regulatory network has a unique concentration level. The collection of all these concentration levels is the *state* of the system.

Definition 3 : A *state* of a biological regulatory network is a tuple $(n_{v_1}, ..., n_{v_p})$ where p is the number of variables, such that for each variable v_i, $n_{v_i} \in \mathbb{N}$ and $n_{v_i} \leq b_{v_i}$.

From a given state, the set of resources of any variable is deduced from the threshold of each edge.

Definition 4 : Given a biological regulatory network, a state $(n_{v_1}, ..., n_{v_p})$ and an edge $(v_i \to v_j)$ labelled with (t, ϵ), the variable v_i is a *resource* of v_j iff :
- $n_{v_i} \geq t$ if $\epsilon = +$
- $n_{v_i} < t$ if $\epsilon = -$

This defines the set of resources ω of a variable v with respect to a state as a subset of $G^{-1}(v)$.

Note the algebraic trick : a resource is either the presence of an activator or the *absence* of an inhibitor (whose concentration level does not reach the threshold).

The parameters of $\mathcal{K}$ define in a simple way an automaton, called synchronous, which is an intermediate technical step to define the dynamics.

Definition 5 : Let $\mathcal{R} = ((V, A), \mathcal{K})$ be a regulatory network, its *synchronous state graph* $\mathcal{S} = (S, T)$ is defined as follow :
- the set of vertices S contains all possible states, i.e. $\prod_{v \in V} [0, b_v]$.
- T is the set of edges of the form : $(n_{v_1}, \cdots, n_{v_p}) \to (k_{v_1,\omega_1}, \cdots, k_{v_p,\omega_p})$ where for all i, ω_i is the set of resources of v_i for the state $(n_{v_1}, \cdots, n_{v_p})$.

The out-degree of each vertex is exactly one in the synchronous state graph, thus it can be represented by a table which gives for each state the next state. Table 1 characterizes the synchronous state graphs

x	y	X	Y
0	0	$k^a_{x,\{y\}}$	$k^a_{y,\{\}}$
0	1	$k^a_{x,\{\}}$	$k^a_{y,\{\}}$
1	0	$k^a_{x,\{y\}}$	$k^a_{y,\{x\}}$
1	1	$k^a_{x,\{\}}$	$k^a_{y,\{x\}}$
2	0	$k^a_{x,\{x,y\}}$	$k^a_{y,\{x\}}$
2	1	$k^a_{x,\{x\}}$	$k^a_{y,\{x\}}$

x	y	X	Y
0	0	$k^b_{x,\{y\}}$	$k^b_{y,\{\}}$
0	1	$k^b_{x,\{\}}$	$k^b_{y,\{\}}$
1	0	$k^b_{x,\{x,y\}}$	$k^b_{y,\{\}}$
1	1	$k^b_{x,\{x\}}$	$k^b_{y,\{\}}$
2	0	$k^b_{x,\{x,y\}}$	$k^b_{y,\{x\}}$
2	1	$k^b_{x,\{x\}}$	$k^b_{y,\{x\}}$

TAB. 1 – State tables deduced from Figures 4-a and 4-b respectively.

respectively associated to Figures 4-a & 4-b. The indices of the parameters $k_{v,\omega}$ are uniquely determined by the column of the table for v, and by the thresholds of the underlying regulatory graph for ω (according to Definition 4). Each instantiation of the parameters in $\mathcal{K}$ defines an *a priori* different synchronous state graph.

An instantiation of $\mathcal{K}$ being given we can draw the synchronous state graph in a grid of dimension p : in Figure 5 the mucus node has been ignored in order to get a 2-D grid.

Terminology : One calls *transition* an edge between two states of a state graph.

We build the dynamics of a regulatory network from the synchronous state graph according to two main ideas[15] :
- A diagonal arrow in the synchronous state graph is a transition that changes *simultaneously* the concentration level of two or more variables. The probability that both variables pass through their

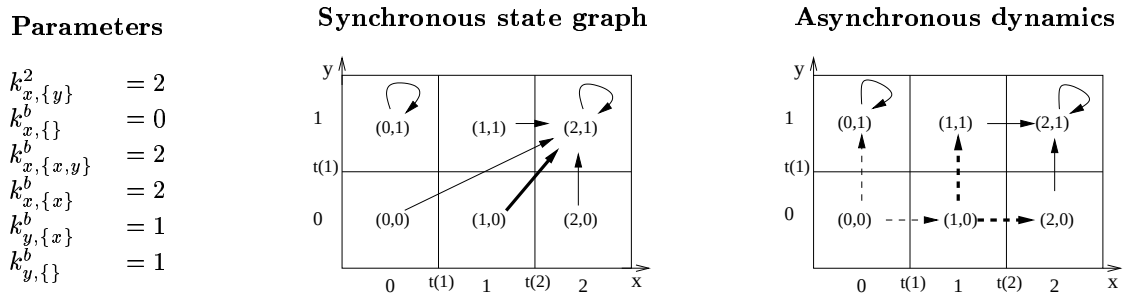

FIG. 5 – Deducing dynamics from parameter values.

respective thresholds at the same time is negligible *in vivo*, but we do not know which one will be passed first. Accordingly we replace any diagonal transition by the collection of the transitions which modify only one of the involved variables at a time. For example, $(1,0) \to (2,1)$ is replaced by the transitions $(1,0) \to (2,0)$ and $(1,0) \to (1,1)$ in the asynchronous dynamics of Figure 5 (bold arrows).

– An arrow of length greater or equal to 2 implies a variable which increases its concentration level abruptly and jumps several thresholds. Since we modelize a continuous phenomenon, the target of the synchronous transition is indeed only an attractor and real transitions should only address neighbor states. For example in Figure 5, $(0,0) \to (2,1)$ gives rise to $(0,0) \to (1,0)$ instead of $(0,0) \to (2,0)$.

Definition 6 : Let τ a transition $(n_{v_1}, \cdots, n_{v_p}) \to (n'_{v_1}, \cdots, n'_{v_p})$. A *desynchronization* of τ is a transition of the form

$$(n_{v_1}, \cdots, n_{v_{i-1}}, n_{v_i}, n_{v_{i+1}}, \cdots, n_{v_p}) \quad \to \quad (n_{v_1}, \cdots, n_{v_{i-1}}, n_{v_i} + \delta, n_{v_{i+1}}, \cdots, n_{v_p})$$

where :
– i is such that $n_{v_i} \neq n'_{v_i}$
– $\delta = 1$ if $n_{v_i} < n'_{v_i}$, else $\delta = -1$.

The dynamics of a regulatory network is defined by desynchronizing the synchronous state graph.

Definition 7 : Let $\mathcal{R}$ be a regulatory network, its *asynchronous state graph* is defined as follows :
– the set of vertices is the set of states $\prod_{v \in V} [0, b_v]$
– the set of transitions is the set of all the desynchronizations of all the transitions of the synchronous state graph of $\mathcal{R}$.

When several transitions start from the same state, they are concurrent and any of them can be randomly chosen state. The attractors of the synchronous state graph remain the attractors of the asynchronous one, but the paths to them differ and can change the behaviour from a given initial state. For example in Figure 5, $(0,0)$ can reach $(0,1)$.

5 Computational Tree Logic and Model Checking

Parameters of $\mathcal{K}$ play a major role on the dynamics of the model. Unfortunately most often they are not experimentaly measurable. Consequently finding suitable classes of those parameters constitutes a major issue of the modeling activity. Since R. Thomas' approach is a discretization of continuous differential equation system, parameters $k_{v,\omega}$ reflect a discretization of sums of ratios of positive constants [10]. So it's necessary to focus on models which verify the following constraint :

$$k_{v,\emptyset} = 0 \text{ and } \omega \subseteq \omega' \Rightarrow k_{v,\omega} \leq k_{v,\omega'}$$

For the *Pseudomonas aeruginosa* example, this constraint allows to select, amongst the 648 initial models, 56 of them (28 for each regulatory graph of figure 4) which lead to 42 different asynchonous state graphs (16 for the first regulatory graph and 26 for the second).

Biological knowledge about behaviour can also be used as undirect criteria to constrain $\mathcal{K}$. For example homeostasy (resp. multistationarity) is experimentaly observable and as mentioned in Section 1 it indicates that a negative (resp. positive) circuit is functional. Some necessary conditions for functionality, extracted from the static regulatory graph can be used to constrain $\mathcal{K}$ (notion of characteristic states in [16]). For the running example, if the *mucoidy* could be explained by an epigenetic phenomenon, a bi-stationnarity is necessary leading to the functionnality of the unique positive circuit $x \to x$ [16]. This selection remains 9 + 10 models for 7 + 10 asynchonous state graphs. But some of remaining models propose a dynamics which allows one to go from a behaviour to the other (see fig. 5).

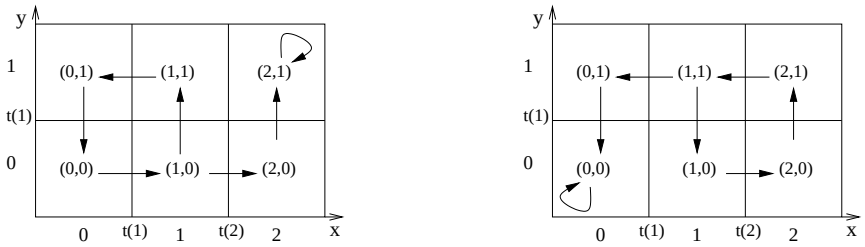

FIG. 6 – Two asynchonous state graphs which allows one to go from a behaviour to the second.

To go further such static conditions must be reinforced by properties (biological knowledge or hypotheses) on the dynamics, namely temporal properties. Since numerous models (families of parameters $\mathcal{K}$) have to be checked against those properties, a formal language (temporal logic [1]) is needed to perform the checkings by computer. The temporal logic chosen here is CTL (**C**omputation **T**ree **L**ogic). Time has a tree structure which can handle several futures for each state, precisely as in the asynchronous state graphs of Definition 7.

Definition 8 : A CTL formula on a regulatory network $\mathcal{R}$ is inductively defined by :
- atomic formulae are $\top$, $\bot$ or of the form $(v = n)$ where v is a variable of $\mathcal{R}$ and $n \in [0, b_v]$
- if ϕ and ψ are formulae, then $(\neg\phi)$, $(\phi \wedge \psi)$, $(\phi \vee \psi)$, $(\phi \Rightarrow \psi)$, $AX\phi$, $EX\phi$, $A[\phi U \psi]$, $E[\phi U \psi]$, $AG\phi$, $EG\phi$, $AF\phi$, $EF\phi$ are formulae.

$\top$ is the always true formula ; $\bot$ is the always false formula ; $(v = n)$ is true iff the concentration level of the variable v is equal to n in the current state ; $\neg, \wedge, \vee, \Rightarrow$ are the usual connectives (respectively *not*, *and*, *or*, *implication*). All the temporal connectives are pairs of symbols : the first element of the pair is A or E followed by X, F, G or U whose meanings are given in the next table.

A	for **A**ll paths choices	X	ne**X**t state
E	for at least one path choice (**E**xist)	F	some **F**uture state
		G	all future states (**G**lobally)
		U	**U**ntil

For example $AX\,(y = 1)$ means that in all next states accessible from the current state in the asynchronous state graph, the concentration level of y is 1. $EG(x = 0)$ means that there exists at least one path starting from the current state where the concentration level of x is constantly equal to 0. And so on.

To test the epigenetic hypothesis described in Section 1 "*to find a model of the wild bacteria, which has a bi-stationarity where one stable state produces mucus and the other one does not*", we have proved first that for any model "*produces mucus*" is equivalent to the fact that the concentration level of x is repeatedly equal to 2. Thus recurrence of mucus production can be expressed as follows :

$$(x = 2) \quad \Rightarrow \quad AX\ AF(x = 2) \tag{1}$$

where $AX\ AF(\phi)$ means that for all possible futures (excluding the present) ϕ will be satisfied at a given time. Moreover we know that the wild bacteria never produces mucus by itself when starting from a basal state (second stable state) :

$$(x = 0) \quad \Rightarrow \quad AG(\neg(x = 2)) \tag{2}$$

With respect to mucus production, $x = 0$ is the basal state and the AG statement says that mucus is never produced.

On the one hand proposing a method independent of the example to formally express a biological hypothesis remains a difficult open problem. Here the major key to overcome the problem is our lemma about the relationship between $x = 2$ and the mucus production. On the other hand the CTL formulae being given, one can automatically extract the compatible models, i.e. the compatible families of parameters $\mathcal{K}$. Given a state graph, *model checking* very efficiently computes all the states which satisfy a set of formulae [5]. If all the states satisfy the formulae, one says that the model satisfies them.

We have implemented a software, `SMBioNet` (Selection of Models of Biological Networks), which allows one to select models of a given regulatory graph according to their temporal properties. The software takes as input a biological regulatory graph (with a graphical interface), a CTL formula and a set of functional loops. It generates, from the graph, all the biological regulatory networks ; it selects all models which satisfies the functionality of the specified loops and returns the models and associated asynchronous state graphs which satisfy the CTL formula (using the SMV model checker [9]). Applied to the 19 models mentioned before with the two previous formulae, it gave us a positive answer to the epigenetic question : the set of remaining models is non empty, both for Figure 4-a and for Figure 4-b : 4 models leading to 4 different asynchronous state graphs for each regulatory graph.

If *Pseudomonas aeruginosa* is actually compatible with one of these remaining models, it could open new therapeutics in prospects. We know that *Pseudomonas aeruginosa* satisfies Formula 2 (non mucoid stationary state). Formula 1 constitutes the corner stone of the problem. Its logical structure suggests an experiment plan : pulse x up to saturation by an external signal, and *after the transitory phase* due to the pulse, check if the mucus production persists. By the way, automatic tools dedicated to software testing can generate this experiment plan (the initial pulse comes from the left handside atomic formula ($x = 2$), and so on). We have also proved that the success of this experiment plan (prepared at the university hospital center of Grenoble in France) is sufficient to validate the epigenetic hypothesis.

6 Conclusion and perspectives

We have defined a *formal* description of biological regulatory networks with the semantics of the discrete model of R. Thomas. This modeling takes place at a biological level instead of a biochemical level, that allows one to study behaviours at an upper level. Our approach allows us to take advantage of the whole corpus of formal methods, in particular temporal properties can be checked against models using CTL. Model checking is a first application of the formalization of biological regulatory networks. Formal methods from computer science open a large horizon of research perspectives. Let us mention for instance,

- To extract the specificities of the biological application domain for temporal logics (patterns of formulae to express functionality, etc).
- To take into account time delays[15] between the beginning of the activation order and the synthesis of the product and conversely for the turn-off delays.
- Automatic generation of experiment plans.
- Preservation of properties when a regulatory network is embedded in another one, including the systematic treatment of knock-out mutants as well as the structuration of huge regulatory networks.
- Useful extensions of the R. Thomas' framework such as specific treatments for activators and inhibitors, offering a language to control transitions, taking into account populations of networks whose states are not synchronized...

These constitute ongoing or future works of our genopole® research group.

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Références

[1] E.A. Emerson. *Handbook of theoretical computer science, Volume B : formal models and semantics*, chapter Temporal and modal logic, pages 995–1072. MIT Press, 1990.

[2] J. Guespin-Michel and M. Kaufman. Positive feedback circuits and adaptive regulations in bacteria. *Acta Biotheor.*, 49(4) :207–18, 2001.

[3] J. Hasty, D. McMillen, and J.J. Collins. Engineered gene circuits. *Nature*, 420(6912) :224–30, 2002.

[4] S. Huang. Genomics, complexity and drug discovery : insights from boolean network models of cellular regulation. *Pharmacogenomics.*, 2(3) :203–22, 2001.

[5] M. Huth and M. Ryan. *Logic in Computer Science : Modelling and reasoning about systems.* Cambridge University Press, 2000.

[6] M. Kaufman and R. Thomas. Model analysis of the bases of multistationarity in the humoral immune response. *J. Theor. Biol.*, 129(2) :141–62, 1987.

[7] H. Kitano. Computational systems biology. *Nature*, 420(6912) :206–10, 2002.

[8] H. Kitano. Looking beyond the details : a rise in system-oriented approaches in genetics and molecular biology. *Curr. Genet.*, 41(1) :1–10, 2002.

[9] K. McMillan. *Symbolic Model Checking.* Kluwer Academic Publishers, 1993.

[10] S. Pérès and J.-P. Comet. Contribution of computation tree logic to biological regulatory networks : example from pseudomonas aeruginosa. In C. Priami, editor, *Proceedings of the 1st Intern. Workshop CMSB'2003*, LNCS 2602, pages 47–56. Springer-Verlag, 2003.

[11] E.H Snoussi. Qualitative dynamics of a piecewise-linear differential equations : a discrete mapping approach. *Dynamics and stability of Systems*, 4 :189–207, 1989.

[12] E.H. Snoussi and R. Thomas. Logical identification of all steady states : the concept of feedback loop caracteristic states. *Bull. Math. Biol.*, 55(5) :973–991, 1993.

[13] R. Thomas. Logical analysis of systems comprising feedback loops. *J. Theor. Biol.*, 73(4) :631–56, 1978.

[14] R. Thomas, A.M. Gathoye, and L. Lambert. A complex control circuit. regulation of immunity in temperate bacteriophages. *Eur. J. Biochem.*, 71(1) :211–27, 1976.

[15] R. Thomas and M. Kaufman. Multistationarity, the basis of cell differentiation and memory. I. & II. *Chaos*, 11 :170–195, 2001.

[16] R. Thomas, D. Thieffry, and M. Kaufman. Dynamical behaviour of biological regulatory networks - I. *Bull. Math. Biol.*, 57(2) :247–76, 1995.

[17] O. Wolkenhauer. Systems biology : the reincarnation of systems theory applied in biology ? *Brief Bioinform.*, 2(3) :258–70, 2001.