

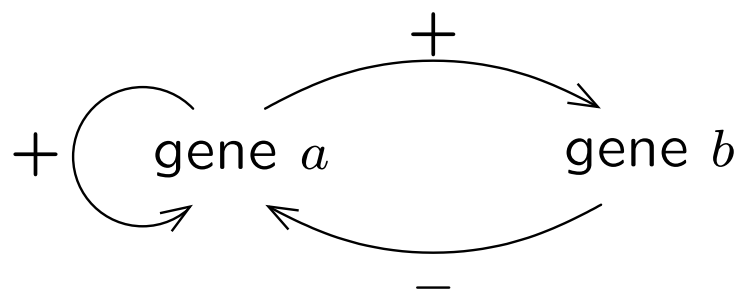
**A Discrete Approach
to Model Gene Regulatory Networks
and Use of Temporal Logic
to Propose Wet Experiments**

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Introduction

The structure of gene regulatory networks is often represented by an **interaction graph** G :



Because of the non-linearity of regulations and the presence of intertwined feedback circuits, **the dynamics is complex**.

⇒ Need of **mathematical models**.

Introduction

Mathematical models :



To specify the functioning of regulations, **these models use parameters whose value is most often unknown.**

⇒ The set of potential dynamics for a given interaction graph G is generally huge.

Introduction

In addition of an **interaction graph** G ,
from biological knowledge and/or biological hypothesis,
it comes some **dynamical properties** Φ :

*“If gene a has its basal expression level,
then the expression level of gene b oscillates”*

Main questions :

1. Is there dynamics for G which are consistent with Φ ?
2. If so, which are the wet experiments to perform in order to refute consistent dynamics ?

Introduction

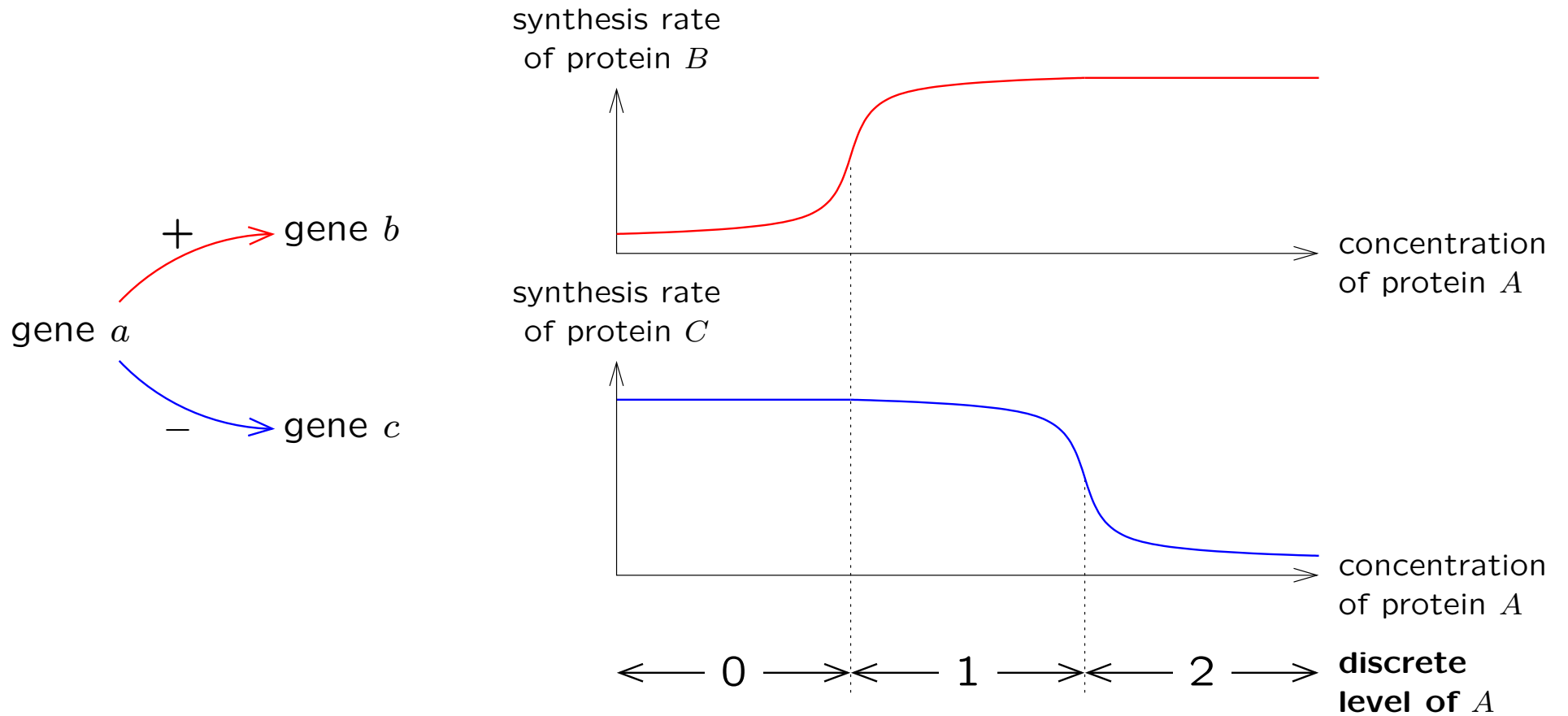
In this presentation,

we show that the *discrete networks of Thomas* and the *temporal logics* can offer computer environments which help to treat this questions.

Plan

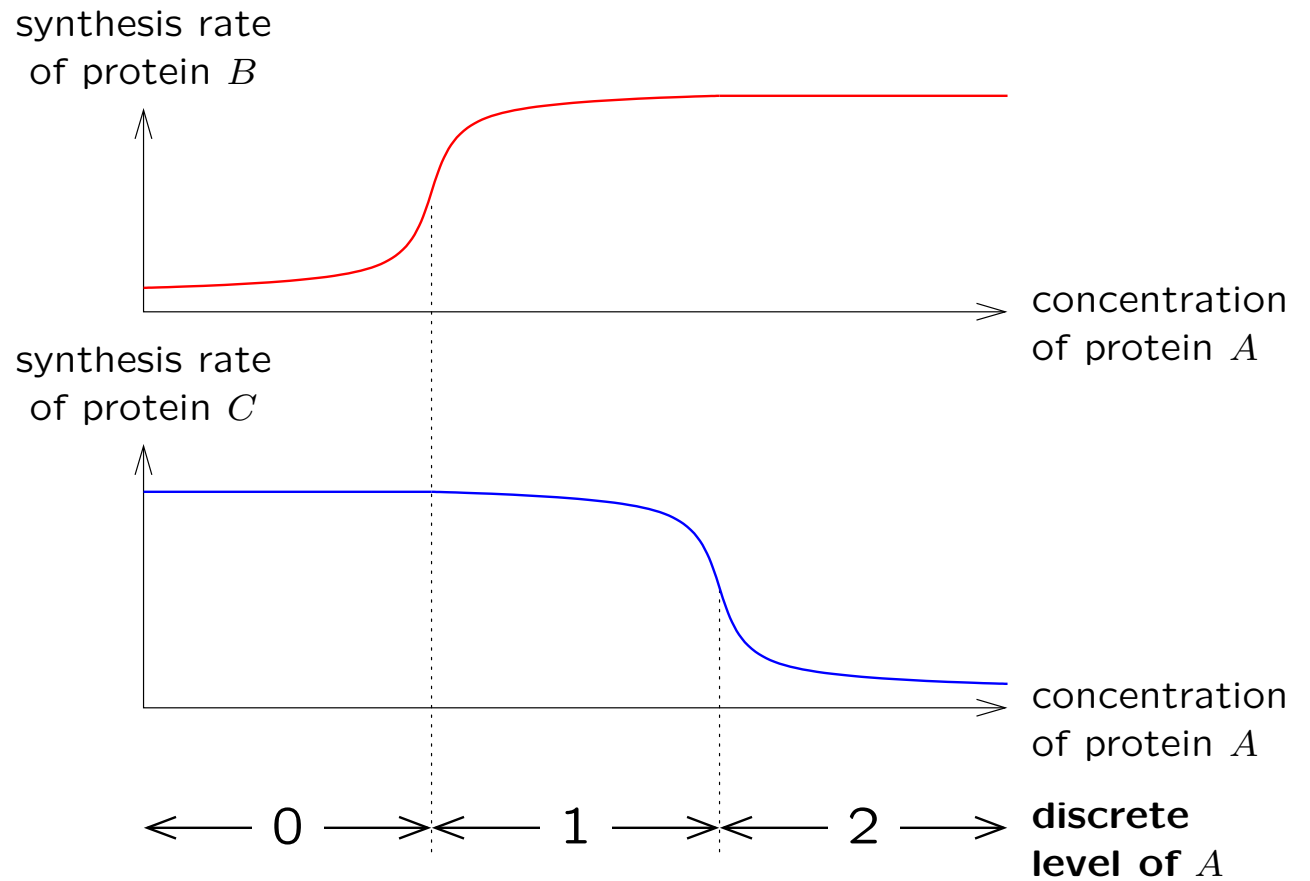
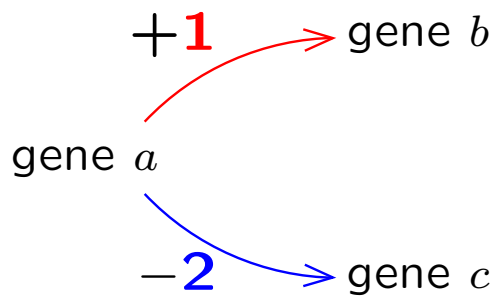
1. Introduction
- 2. Discrete networks of Thomas**
3. Temporal logic and model checking
4. Pedagogical example : *Pseudomonas aeruginosa*

Discretization of concentrations



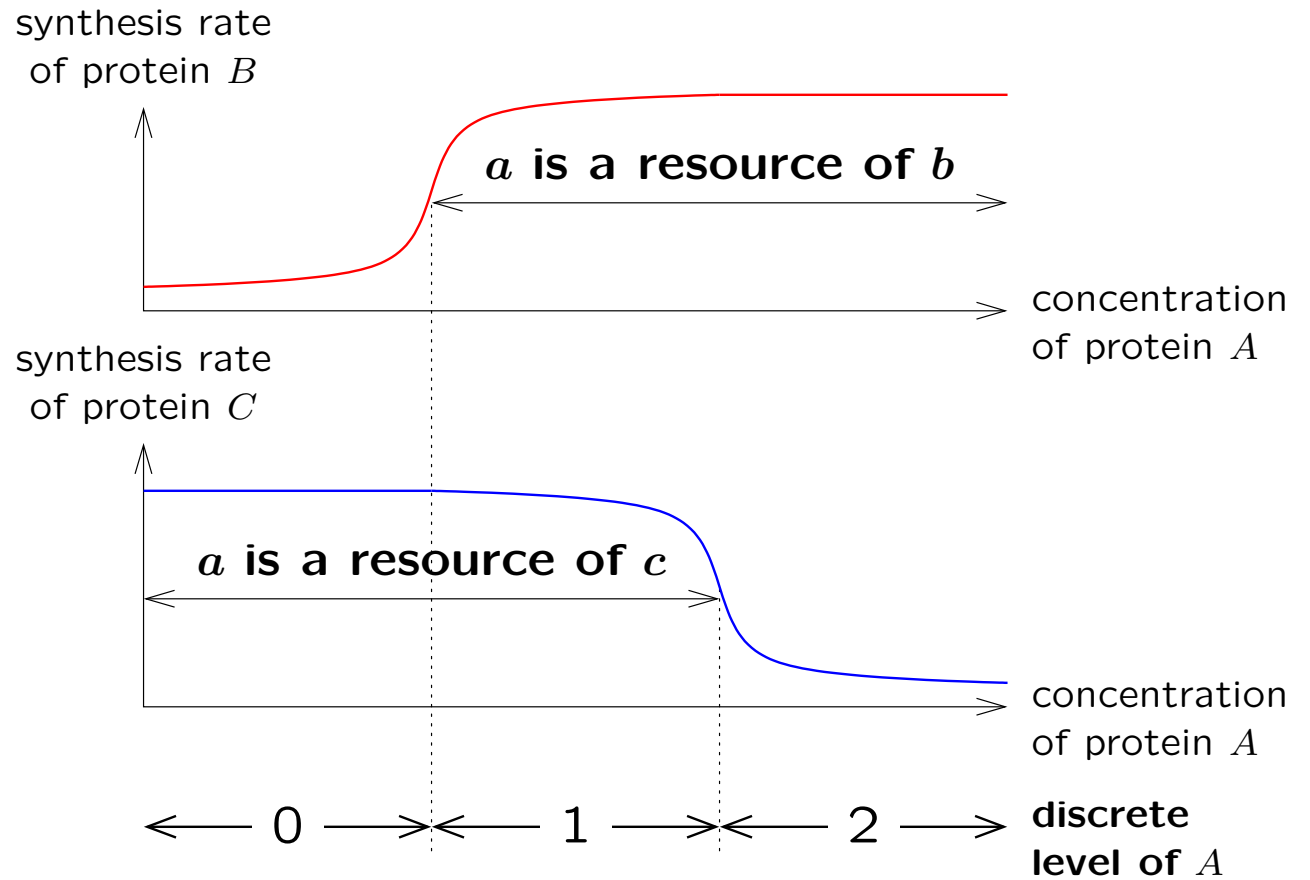
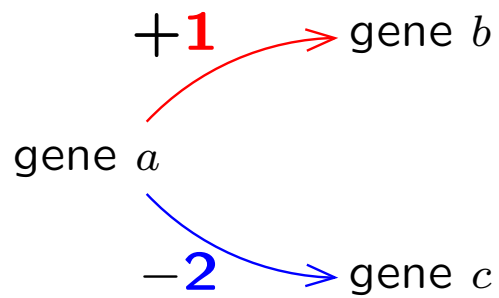
Discretization of concentrations

Addition of thresholds

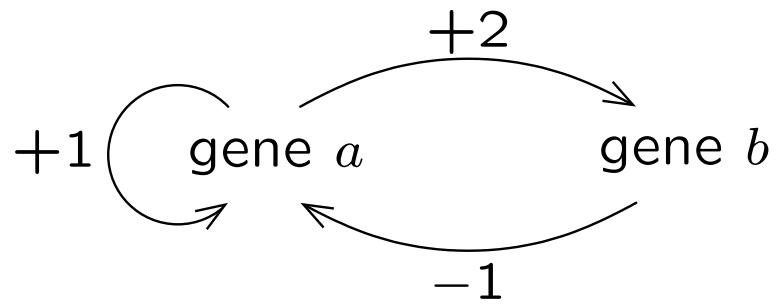


Discretization of concentrations

Addition of thresholds

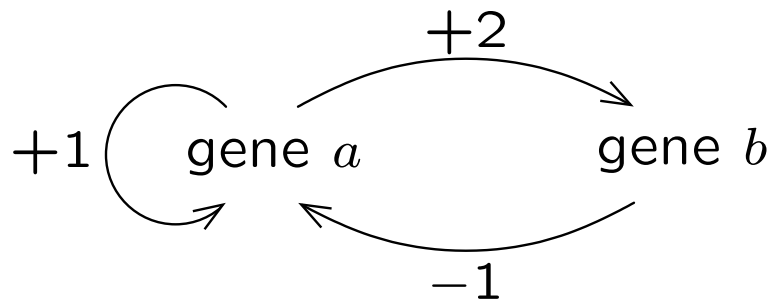


Resources



		resources	
<i>a</i>	<i>b</i>	of <i>a</i>	of <i>b</i>
0	0	{ <i>b</i> }	{}
0	1	{}	{}
1	0	{ <i>a</i> , <i>b</i> }	{}
1	1	{ <i>a</i> }	{}
2	0	{ <i>a</i> , <i>b</i> }	{ <i>a</i> }
2	1	{ <i>a</i> }	{ <i>a</i> }

Parameters K



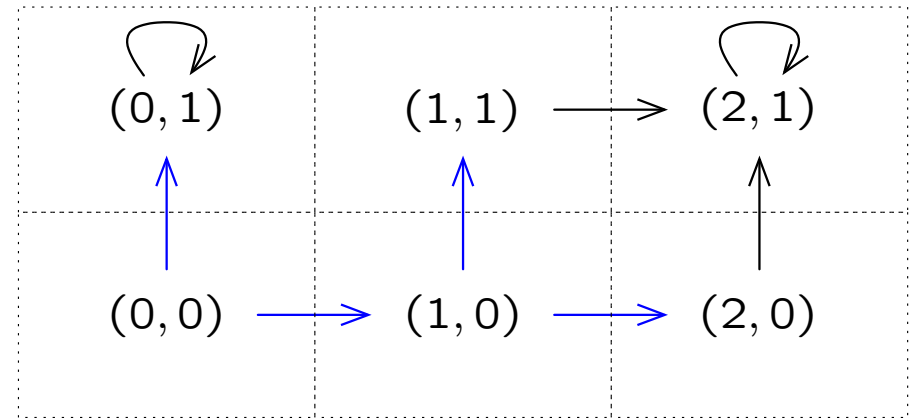
a	b	target levels	
0	0	$K_{a,\{b\}}$	$K_{b,\{\}}$
0	1	$K_{a,\{\}}$	$K_{b,\{\}}$
1	0	$K_{a,\{a,b\}}$	$K_{b,\{\}}$
1	1	$K_{a,\{a\}}$	$K_{b,\{\}}$
2	0	$K_{a,\{a,b\}}$	$K_{b,\{a\}}$
2	1	$K_{a,\{a\}}$	$K_{b,\{a\}}$

$K_{g,R}$ = level toward which gene g evolves when its resources are R

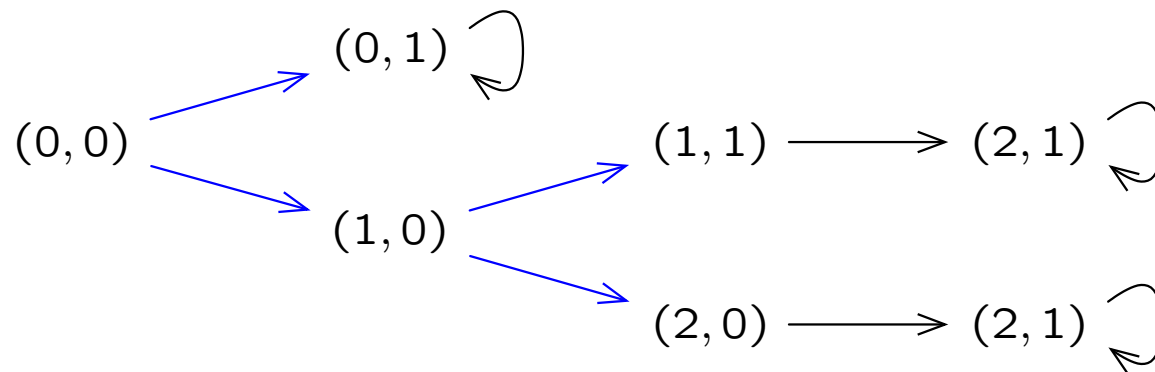
State transition graph

a	b	Target levels			
0	0	$K_{a,\{b\}} = 2$	$K_{b,\{\}} = 1$		
0	1	$K_{a,\{\}} = 0$	$K_{b,\{\}} = 1$		
1	0	$K_{a,\{a,b\}} = 2$	$K_{b,\{\}} = 1$		
1	1	$K_{a,\{a\}} = 2$	$K_{b,\{\}} = 1$		
2	0	$K_{a,\{a,b\}} = 2$	$K_{b,\{a\}} = 1$		
2	1	$K_{a,\{a\}} = 2$	$K_{b,\{a\}} = 1$		

State transition graph



Times has a tree structure :



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CTL = Computational Tree Logic

Atoms = comparisons : $(a = 2)$, $(b > 0)$...

Logical connectives : $(\varphi_1 \wedge \varphi_2)$, $(\varphi_1 \Rightarrow \varphi_2)$...

Temporal connectives : made of 2 characters

<u>first character</u>	<u>second character</u>
A = for A ll path choices	X = ne X t state
E = there E xist a choice	F = for some F uture state
	G = for all future states (G lobally)
	U = U ntil

$AX(a = 1)$: the level of a is 1 in **all** states **directly following** the considered initial state.

$EG(b = 0)$: there **exists** at least one path from the considered initial state where the level of b is **always** 0.

Question 1 : Consistency

1. Draw the interaction graphs G .
2. Express in CTL the known behavioral properties as well as the considered biological hypotheses. It formally defines Φ .
3. *Automatically generate all the possible dynamics* derived from the interaction graph G according to all the possible sensible threshold allocations and possible parameters K .
→ Our software SMBioNet handles this automatically.
4. *Check each generated models against Φ .*
→ SMBioNet uses model checking to perform this step.
5. If no model survive to the previous step,
then reconsider G and/or the behavioral properties Φ .
6. If at least one model survives, then biological knowledge and hypotheses are consistent, and possible thresholds/parameters have been indirectly established.

Question 2 : Theoretical models \leftrightarrow Experiments

1. Let Ψ be the set of CTL formulae satisfied by at least one selected models (predictions).
2. Among formulae of Ψ , some are “observable” : they express a possible result of a possible biological experiment.
3. Testing frameworks from computer science aim at selecting a finite subsets of these observable formulae, which maximize the chance to refute the selected models.
4. These subsets are often too big, nevertheless these testing frameworks can be suitably applied to regulatory networks.

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Mucus production in *P.aeruginosa*

Terminology about phenotype modification :

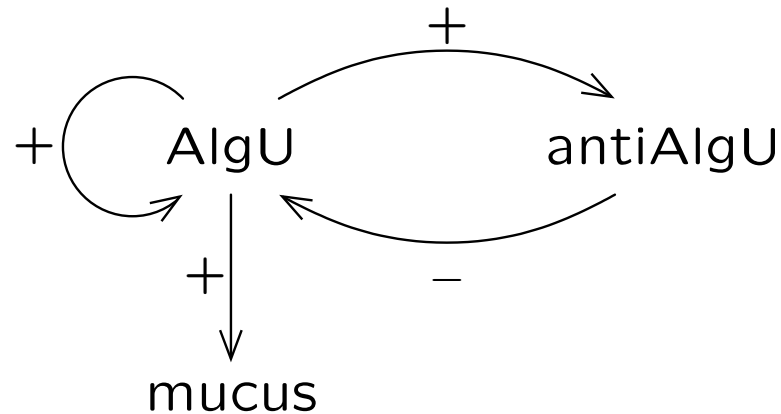
1. **Genetic modification** : inheritable and not reversible (mutation)
2. **Epigenetic switch** : inheritable and reversible

The biological question (Janine Guespin) :

Is **mucus production** in *Pseudomonas aeruginosa* due to an epigenetic switch ? \Rightarrow New possible therapy (cystic fibrosis)

Mucus production in *P.aeruginosa*

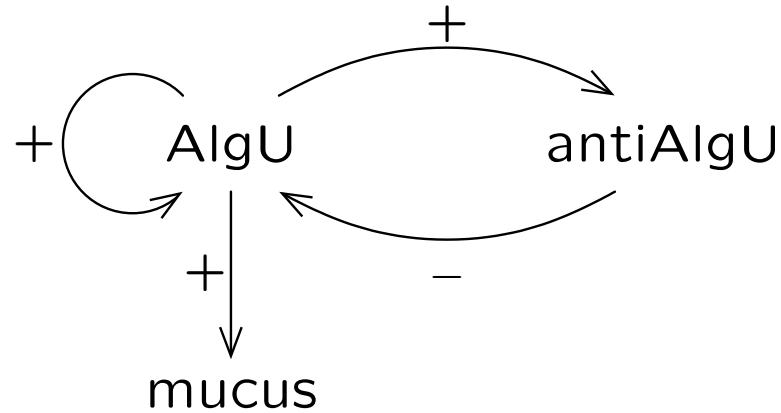
(Janine Guespin and Marceline Kaufman)



Epigenetic hypothesis :

- The positive feedback circuit induces 2 “stable states” : a cytotoxic stable state and the other one is not cytotoxic.
- An external signal (in the cystic fibrosis’ lungs) could switch AlgU from its lower stable state to the higher one.

Consistency of the hypothesis



One CTL formula for each stable state :

$(AlgU = 2) \Rightarrow AXAF(AlgU = 2)$

$(AlgU = 0) \Rightarrow AG(AlgU < 2)$

Question 1, Consistency : proved by *Model Checking* :

→ Thresholds and parameters K are unknown : 712 models.

→ 10 models are extracted by SMBioNet.

Question 2 : and *in vivo* ? ...

Validation of the epigenetic hypothesis

Question 2 = to validate bistationnarity *in vivo*

Non mucoid state : $(AlgU = 0) \Rightarrow AG(AlgU < 2)$

P. aeruginosa with a basal level for AlgU does not produce spontaneously mucus : actually validated

Mucoid state : $(AlgU = 2) \Rightarrow AXAF(AlgU = 2)$

Experimental limitation :

AlgU can be saturated but it cannot be measured.

Lemma (proved by computer) :

$$AXAF(AlgU = 2) \iff AXAF(mucus = 1)$$

Formula to test : $(AlgU = 2) \Rightarrow AXAF(mucus = 1)$.

Validation of the epigenetic hypothesis

To test $(AlgU = 2) \Rightarrow AXAF(mucus = 1)$ the experiment is :

To pulse AlgU and then to test if toxin production remains

More generally : if the hypothesis has the form

$$A \Rightarrow B$$

the experiments able to refute the hypothesis have the form

$$(something \text{ reachable}) \Rightarrow (something \text{ observable})$$

with (formal proof)

$$(something \text{ reachable}) \Rightarrow A \text{ and } B \Rightarrow (something \text{ observable})$$

Concluding comments

Behavioral *properties* are as much important as *models*.

Modeling is significant only with respect to the considered experimental *reachability* and *observability*.

Formal proofs can suggest wet experiments.

Current state of the art / promising proof oriented approaches :

1. *Timed Hybrid Petri Nets* [Sylvie Troncale, Gilles Bernot & Jean-Paul Comet (Product of automaton)]
2. *Hybrid models with delays* [Olivier Roux & al (HyTech), Heike Siebert & Alexander Bockmayr (product of automaton)]
3. *Constraint programming* [Laurent Trilling & Eric Fanchon]