Approches logique, informatique et expérimentale de la multistationnarité chez Pseudomonas aeruginosa

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Logical, bio-computational and experimental approaches of multistationarity in Pseudomonas aeruginosa.

Bruxelles 30-31 Mai 2008

Summary

1) Hypothesis : Is the loss or acquisition of stable ability to become cytotoxic in *P. aeruginosa* in the lungs of cystic fibrosis patients an epignetic switch?

2) Looking for a **positive feedback loop**, and **modelling** (generalised logical analysis).

3) **Temporal logics** to test the coherence between model and hypothesis.

4) formal analysis to suggest experiments able to **validate or refute the hypothesis.**

5) **Experiments**

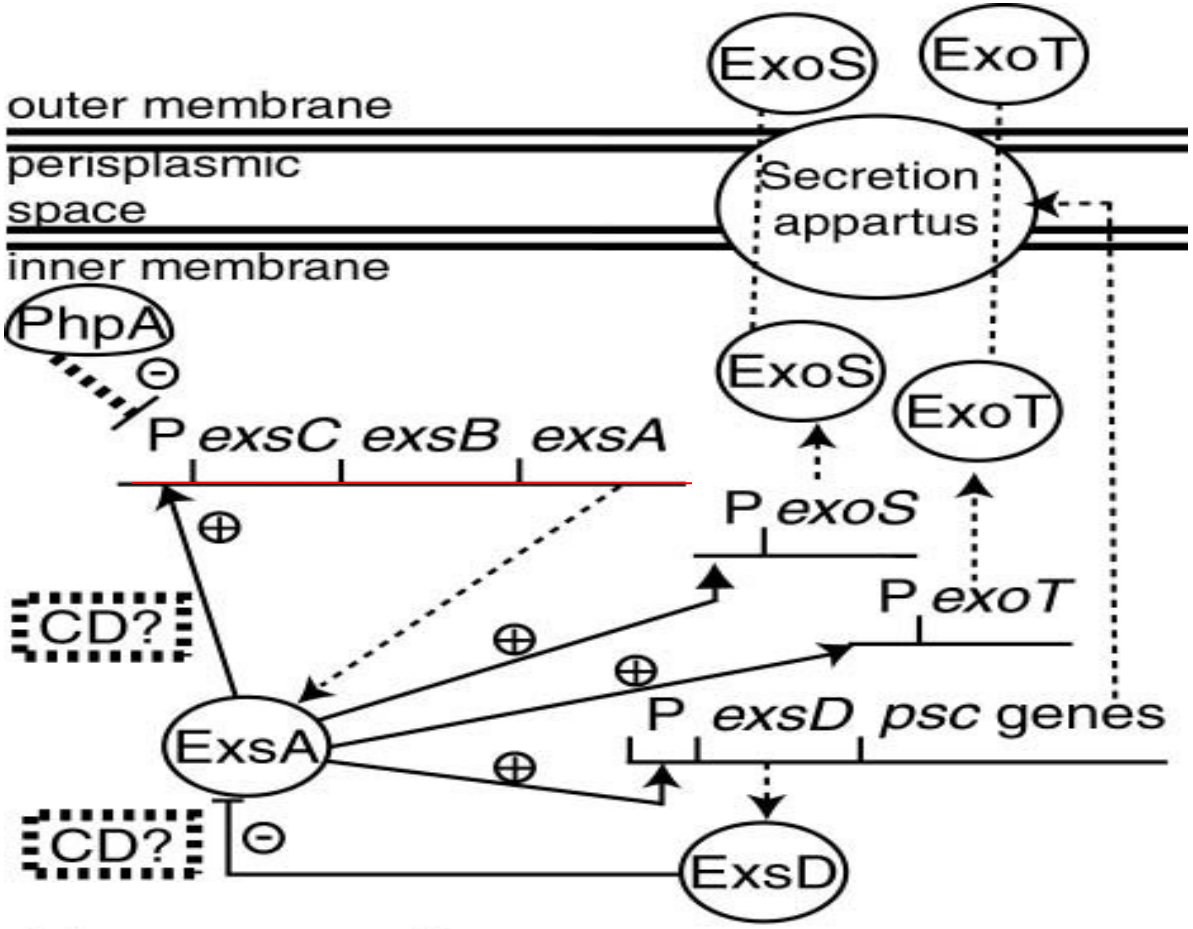
Pseudomonas aeruginosa is an opportunistic bacterium that turns out to be the main cause of lethality in cystic fibrosis disease. When it penetrates into the patient's lungs it must first multiply in spite of the host's defences. This is achieved through a cytotoxic mechanism (type III secretion) that kills the host defence cells. This ability is often lost when the infection is further established (mucoidy). Cytotoxic and non cytotoxic strains are stable when cultivated in the laboratory, but display no obvious genetic difference.

Hypothesis : cytotoxicity is due to an epigenetic switch.

This would mean that the bacterium displays two stable states, (presence or absence of the cytotoxic phenotype) which in turn implies that a **positive feedback circuit,** the necessary condition for such a **multitationnarity**, be present in the regulatory network of the cytotoxic process.

Regulatory transcriptional network of type III secretory apparatus

Toxin secretion as well as the construction of the secretory apparatus are under the same overall regulation. They take place in cytotoxic bacteria when the bacteria are in contact with the target cell, or when they are 'cheated' by a low Ca concentration in the medium.

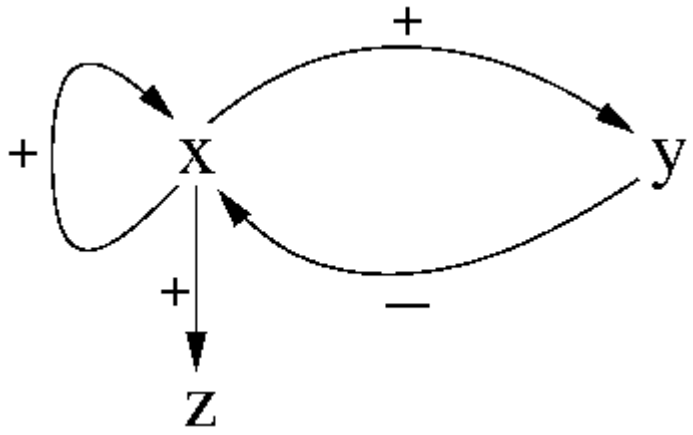


This network can be simplified as a positive feedback loop connected to a negative feed back circuit (2 models, according to the respective thresholds of the reactions driven by X).

X= ExsA

Y = ExsD

Z = secreton III and toxins



The same 2 graphs were obtained by Guespin-Michel and Kaufman (2001) when studying the regulation of mucoidy by the same bacterium.

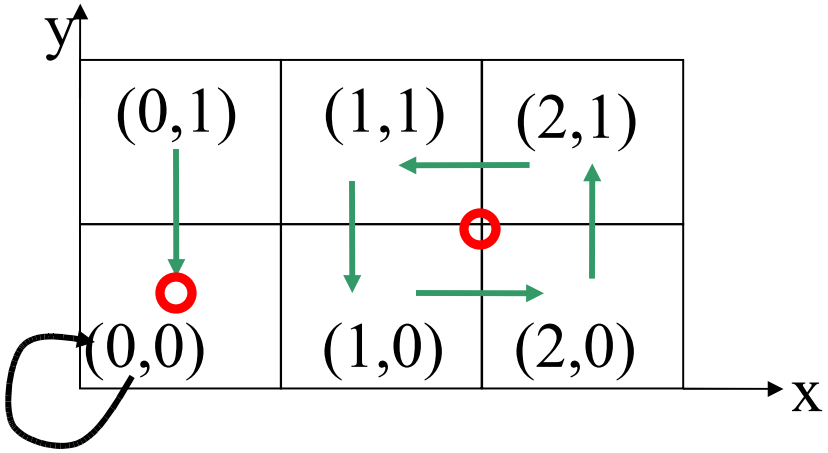
The dynamics of this model was studied by generalised logical analysis Which showed that each model could provide a set of parameters leading to bistability. Here only one of the two models is shown.

$$X = d_x[k_x + k_{x,x}\,{}^2x + k_{x,y}\,{}^1y]$$

$$Y = d_y[k_y + k_{y,x}\,{}^1x]$$

$$d\,[k_x] = k_x$$
$$d\,[k_x + k_{x,x}] = K_{x,x}$$
$$d\,[k_x + k_{x,y}] = K_{x,y}$$
$$d\,[k_x + k_{x,x} + k_{x,y}] = K_{x,xy}$$

$K_x=0$; $K_{x,x}=2$, $K_{x,y}=1$
$K_{x,xy}= 2$
$K_y=0$; $K_{y,x}= 1$



Thus there exists one set of parameters for each model that can lead to multistationarity, which supports the epigenetic hypothesis for mucoidy acquisition

Of course this is true whathever the nature of x and y.

Thus, our epigenetic hypothesis concerning the cytotoxicity is also consistent.

However it must be experimentally proven for two reasons:

The model does not necessarily reflect the real process;

dynamic behaviours, and multistationnarity are not yet a recognised paradigm among microbiologists.

Yet, if true, this hypothesis may have new important therapeutic consequences.

We started a cooperation with computer scientists with two complementary aims.

On my side, it was interesting to enhance the convincing strength of our arguments by having a formal method to prove the consistency of the hypothesis, and get the funding to do the experiments. In addition this formal method was able to propose experiments sufficient to validate the hypothesis.

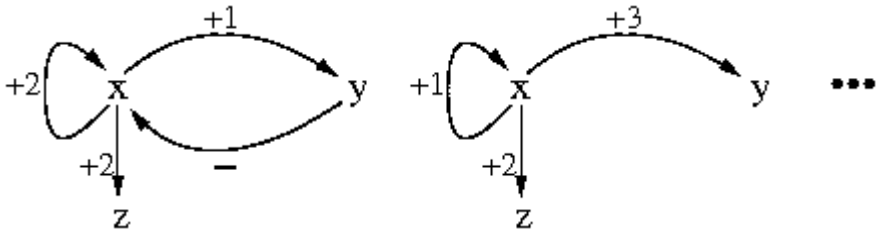
On Gilles Bernot's side it was interesting to start from this very simple complex system to create an automatic method for implementing logical analysis, and testing hypotheses.

Computer aided modelling

From biological knowledge & hypotheses, it comes :

• Properties Φ, e.g. *« without stimulus, if gene x has its basal expression level, then it remains at this level »*

•Model structures *M*, e.g.



It raises 2 questions :

•Consistency: is it possible that Φ and *M* ?

•Validation: if so, is it true *in vivo* that Φ and *M* ?

→ computer aided *proofs* and *test generation* (=experiments)

Temporal logic & Model Checking

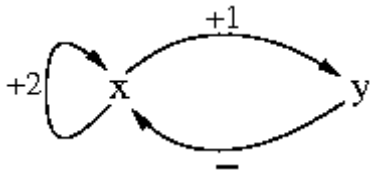
Atoms/Operators: (x=2) (y>0). . . $(\varphi_1 \wedge \varphi_2)$ $(\varphi_1 \Rightarrow \varphi_2)$ . . .

Time operators : made of 2 letters

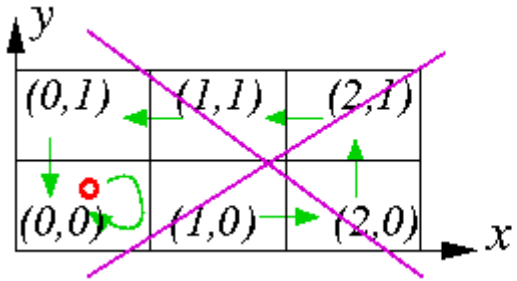
First letter	Second letter
A = for All path choices	X = neXt state
	F = for some Future state
E = there Exist a choice	G = for all future states (Globally)
	U = Until

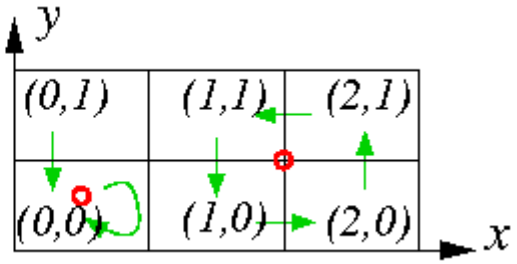
Φ: $(x=2) \Rightarrow AG(\neg(x=0))$
$(x=0) \Rightarrow AG(\neg(x=2))$

M:



Model checking:





Logic links theory & biology

Consistency:

- SMBioNet extracts 10 models from 712 via model checking
- 2 of them are those previously found manually

Validation:

- $(x=0) \Rightarrow AFAG(z=0)$ non mucoid state, actually valide
- $(x=2) \Rightarrow AFAG(z=1)$ Karl Popper:

to validate = to try to refute

thus $A=False$ is useless →

$A \Rightarrow B$	*True*	*False*
True	True	False
False	True	True

thus all experiments must begin with a pulse of x (i.e. $x=2$).

More generally test can be generated by formal methods.
General form of a test:

(something reachable) ⇒ (something observable)

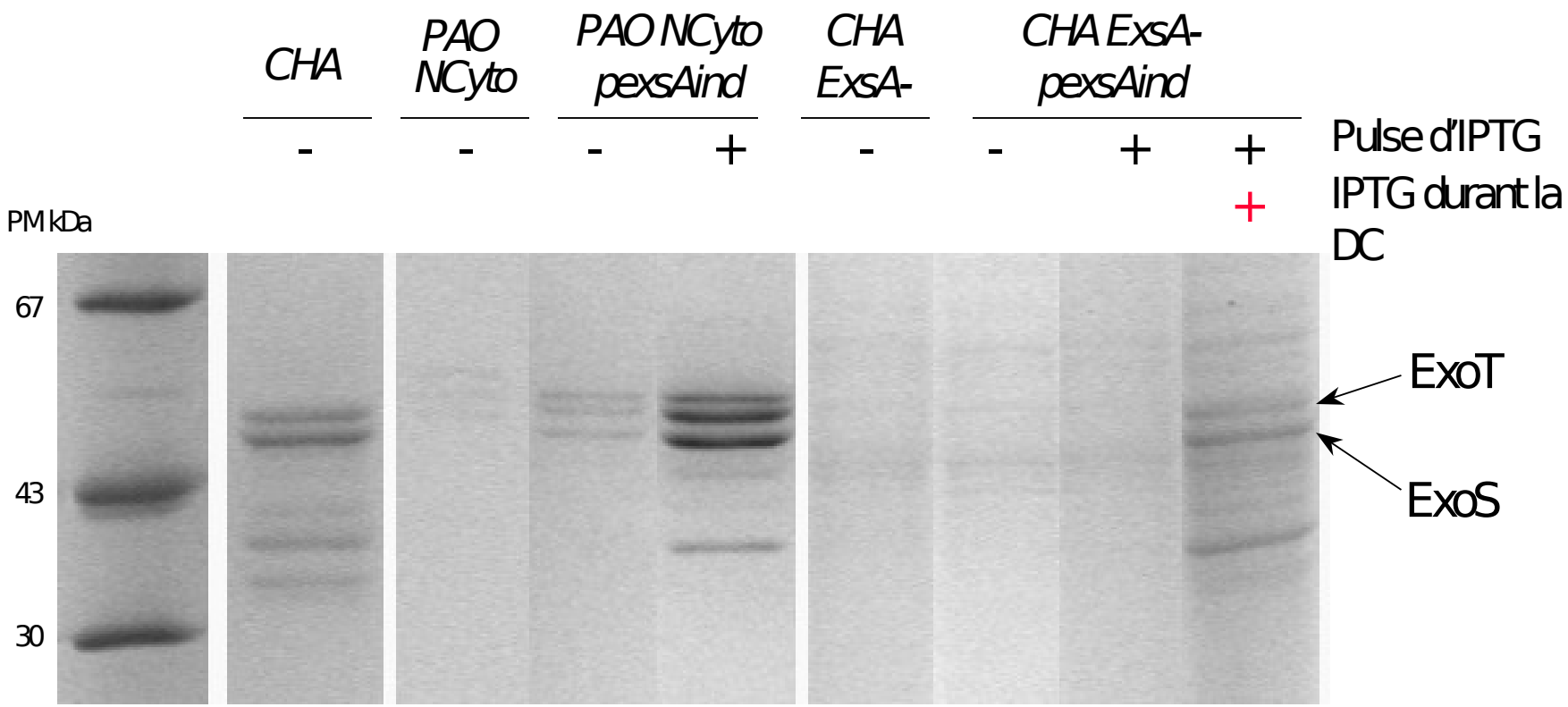
Experimental results

The experiment suggested required to increase temporarily the amount of ExsA protein in a wild type non cytotoxic bacterium, and to check whether this had stably restored cytotoxicity.

To increase temporarily ExsA we introduced gene *exsA* under the control of an inducible promotor in a non cytotoxic bacterium, and we gave it a short pulse of inducer.

To assay for cytotoxicity we detected extracellular toxins produced by the recombinant bacteria (and controls) several generations after the pulse.

Effet d'un pulse d'ExsA sur la sécrétion des Exoenzymes en déplétion calcique



➔ L'augmentation transitoire d'ExsA dans la souche PAONC la rend inductible en déplétion calcique ⇔ Passage d'un état à l'autre du système.

Q.E.D.

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Observability group in the *Epigenomics Project* of Genopole®-Evry

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