Biological Regulatory Networks: Logical Description and Model Checking

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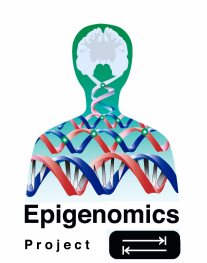
Programme ÉPIGÉNOMIQUE, Genopole®

Special thanks to J. Guespin, J-P. Comet & the Observability group



Menu

1. Modelling biological regulatory networks
2. Formal framework for biological regulatory networks
3. Temporal logic and Model Checking
4. Computer aided elaboration of formal models
5. Example: mucus production in *Pseudomonas aeruginosa*

Molecular Biology & Causality

Heaviness of "causality networks"

Causality loops

Counter-intuitive resulting behaviours

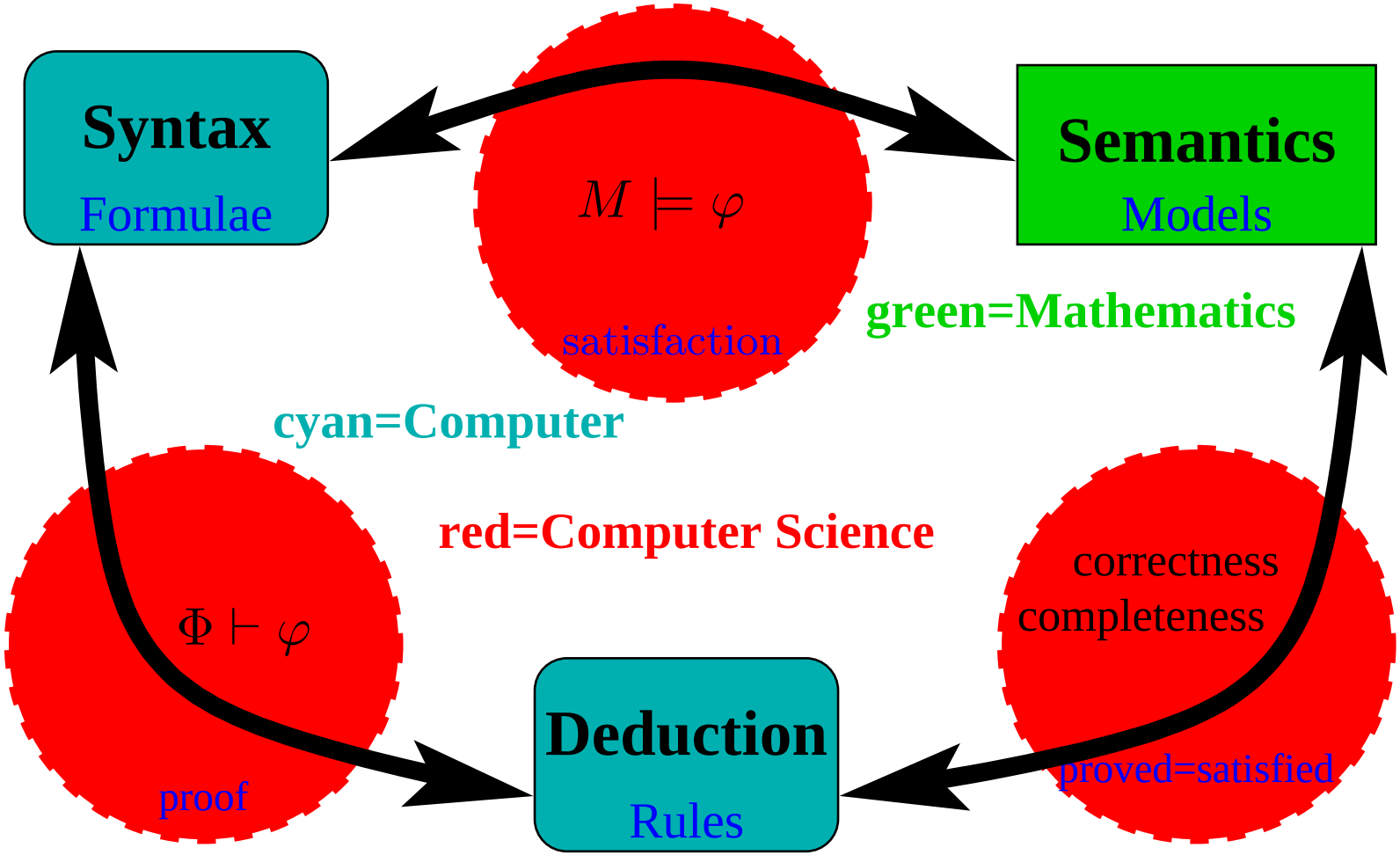
Predicting dynamics from models

Computer aided modelling methodologies

- quantitative approaches → e.g. differential equations
- qualitative approaches → logic & computer science can help
- mixed approaches → cf. *Marcelline Kaufman*

Biological questions are often of qualitative nature

Formal Logic: syntax/semantics/deduction



Regulatory Networks

To model *direct* or *indirect* regulations between biological objects (e.g. gene, macromolecule, signal, ...)

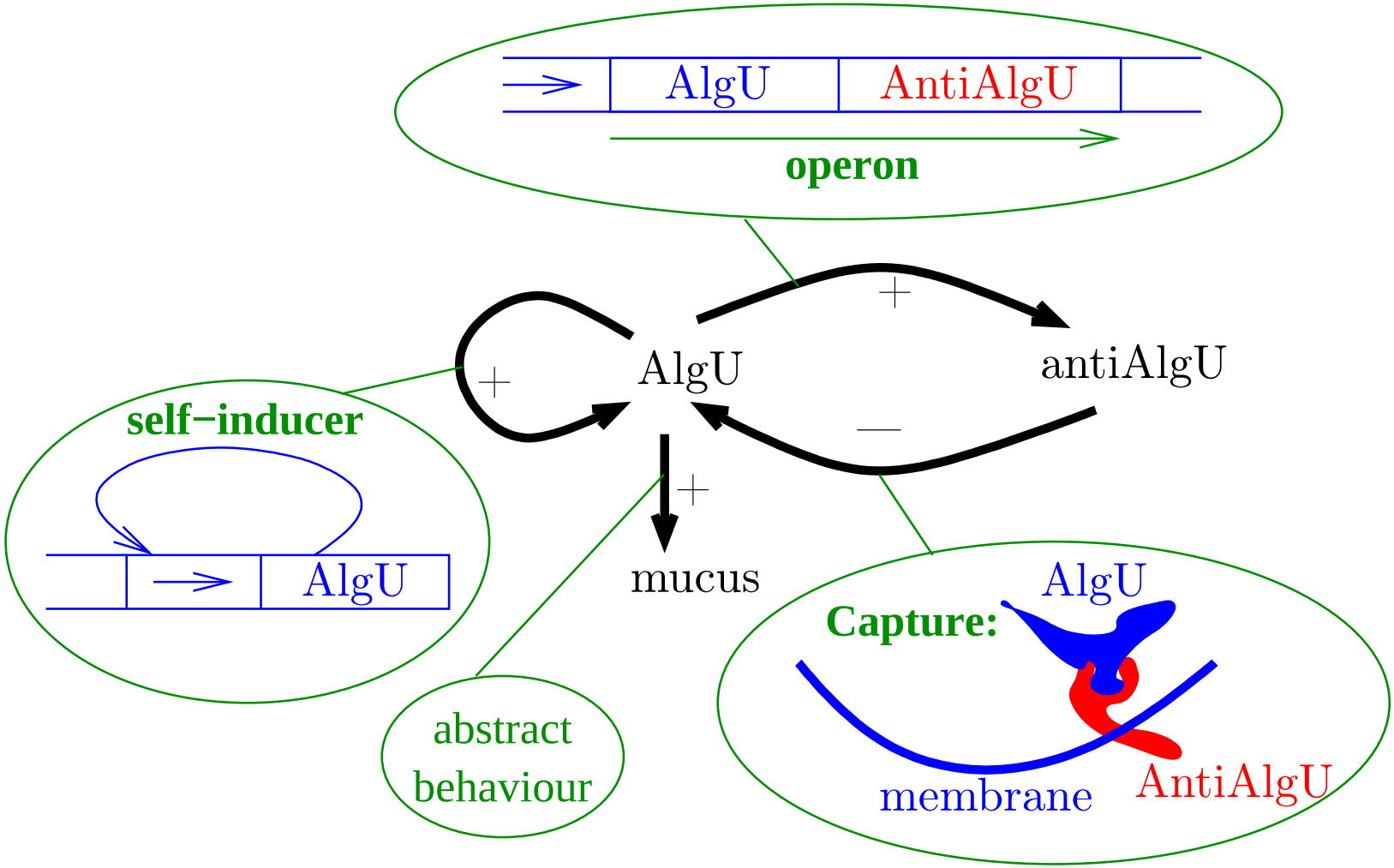
Direct: transcription factor, operon, repressor, ...

Indirect: cascade of events, capture of macromolecules, ...

x induces y : $x \xrightarrow{+} y$

x inhibits y : $x \xrightarrow{-} y$

Mucus Production in *P. aeruginosa*

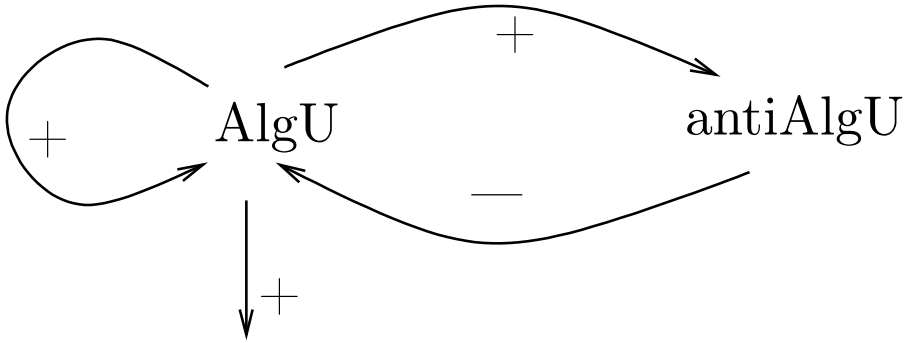


Static Graph & Dynamic Behaviour

Difficulty to predict the result of combined regulations

Difficulty to measure the strength of a given regulation

Example of "competitor" circuits



Positive *v.s.* Negative circuits

Even *v.s.* Odd number of "—" signs

Multistationarity *v.s.* Homeostasy

René Thomas, Snoussi, . . . , Soulé

Functional circuits "pilot" the behaviour

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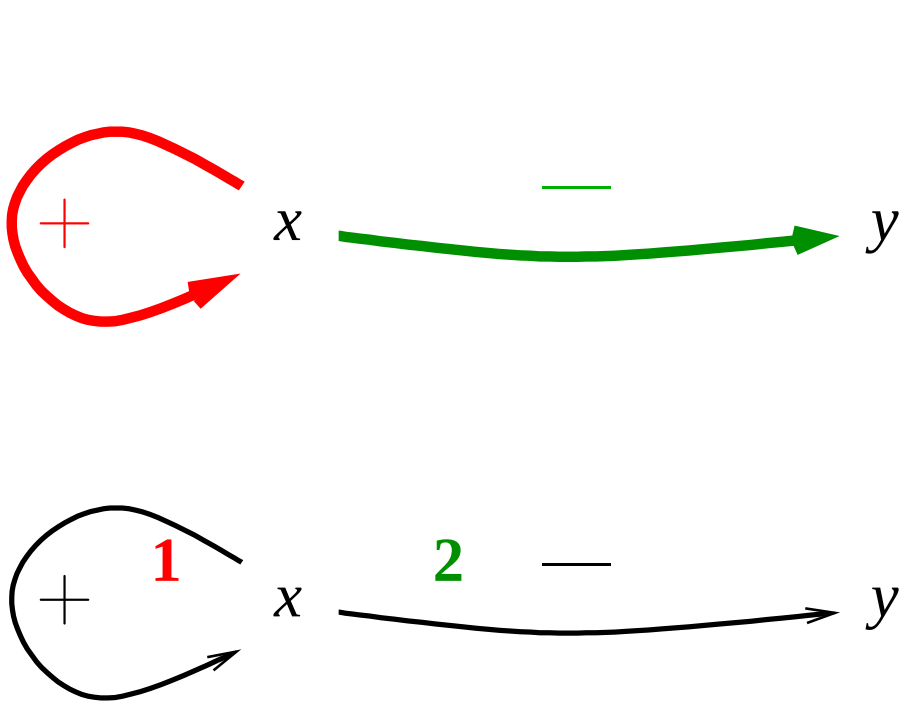
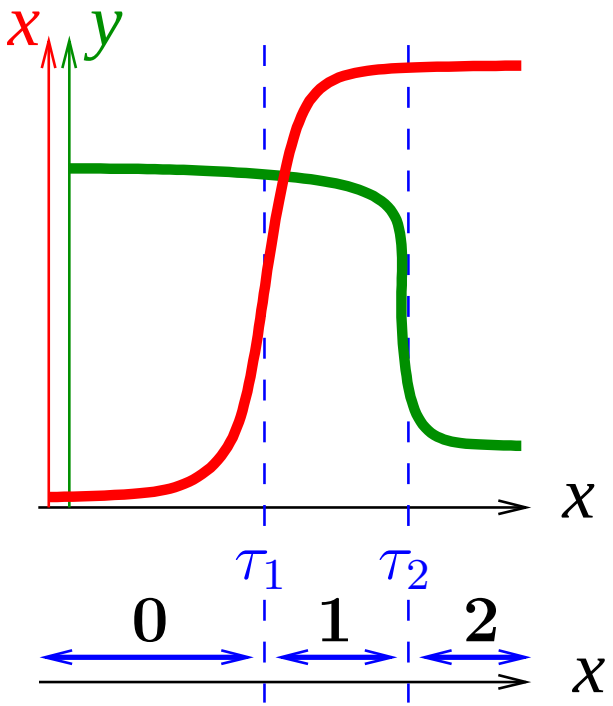
Multivalued Regulatory Graphs





Definition of Regulatory Graphs

A labelled directed graph $(\mathcal{V}, \mathcal{E})$

– each node of $\mathcal{V}$ is a variable x with a boundary $b_x \in I\!N$,
less or equal to the out-degree of x.

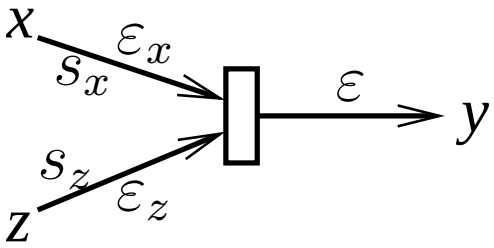
– each edge $x \to y$ of $\mathcal{E}$ is labelled by $\varepsilon \in \{+, -\}$ and
by $s \in [0 \cdots b_x]$.

Variant: bipartite graph

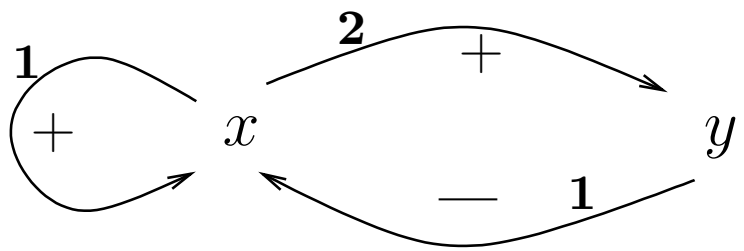
– complexation of two proteins

– inhibition of a regulation

– external conditions...



Regulatory Networks (R. Thomas)



Basal level : K_x K_y

x helps : $K_{x,x}$ $K_{y,x}$

Absent y helps : $K_{x,\overline{y}}$

Both : $K_{x,x\overline{y}}$

(x,y)	*Image*
(0,0)	$(K_{x,\overline{y}}, K_y)$
(0,1)	(K_x, K_y)
(1,0)	$(K_{x,x\overline{y}}, K_y)$
(1,1)	$(K_{x,x}, K_y)$
(2,0)	$(K_{x,x\overline{y}}, K_{y,x})$
(2,1)	$(K_{x,x}, K_{y,x})$

Resources in a Regulatory Network

States:

$\eta : \mathcal{V} \to I\!N$ ($\approx$ vector of integers)
$\eta(x) =$ *abstract concentration level of* x

Variant: *singular* states (values can be the thresholds τ_1, τ_2,. . .)

Resources:

For each $x \xrightarrow{+,s} y$, x is a resource of y iff $\eta(x) > s$

For each $x \xrightarrow{-,s} y$, x is a resource of y iff $\eta(x) \leq s$

Parameters:

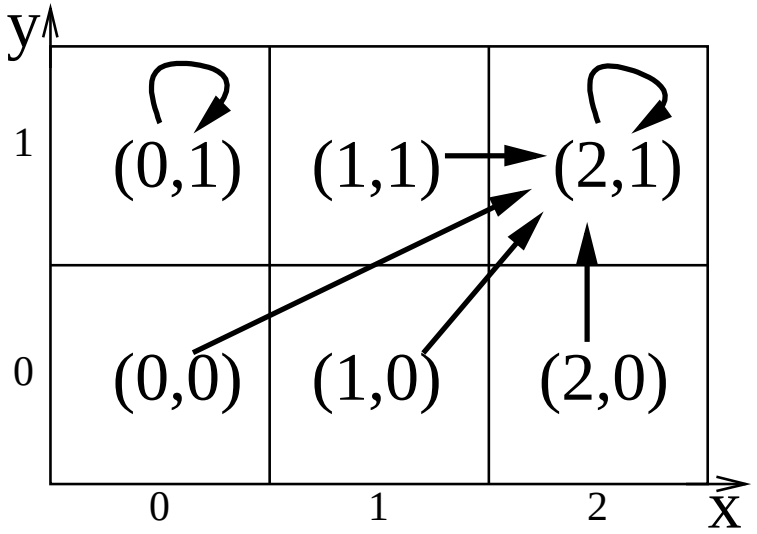
Partial function $K : \mathcal{V} \times \mathcal{P}(\mathcal{V}) \to I\!N$

Image:

Vector of the $K(y, \omega)$ where ω is the set of resources of y

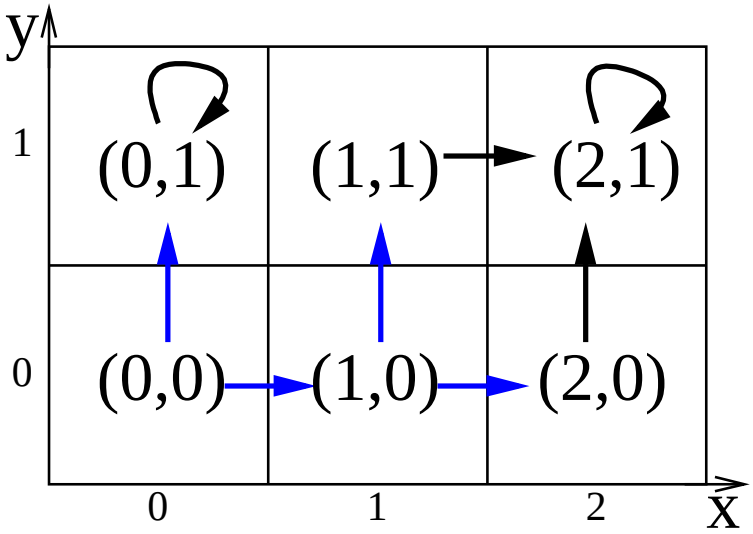
State Graphs

(x,y)	*Image*
(0,0)	$(K_{x,\overline{y}}, K_y)$=(2,1)
(0,1)	(K_x, K_y)=(0,1)
(1,0)	$(K_{x,x\overline{y}}, K_y)$=(2,1)
(1,1)	$(K_{x,x}, K_y)$=(2,1)
(2,0)	$(K_{x,x\overline{y}}, K_{y,x})$=(2,1)
(2,1)	$(K_{x,x}, K_{y,x})$=(2,1)

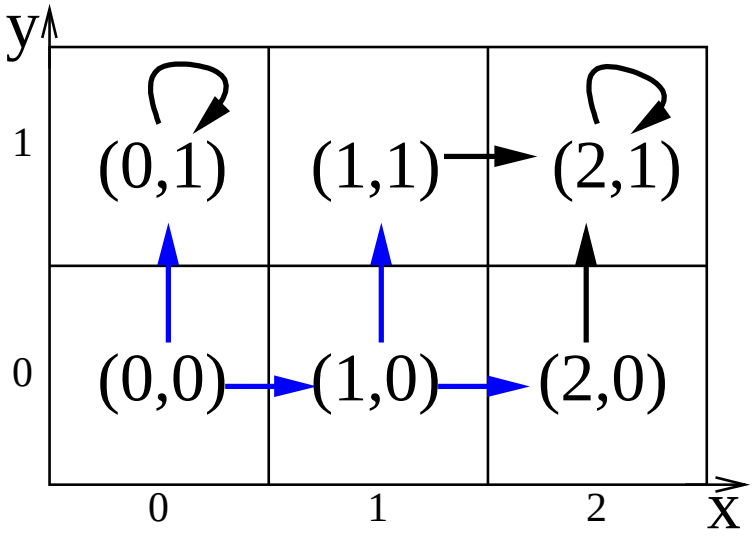


"desynchronization" $\longrightarrow$

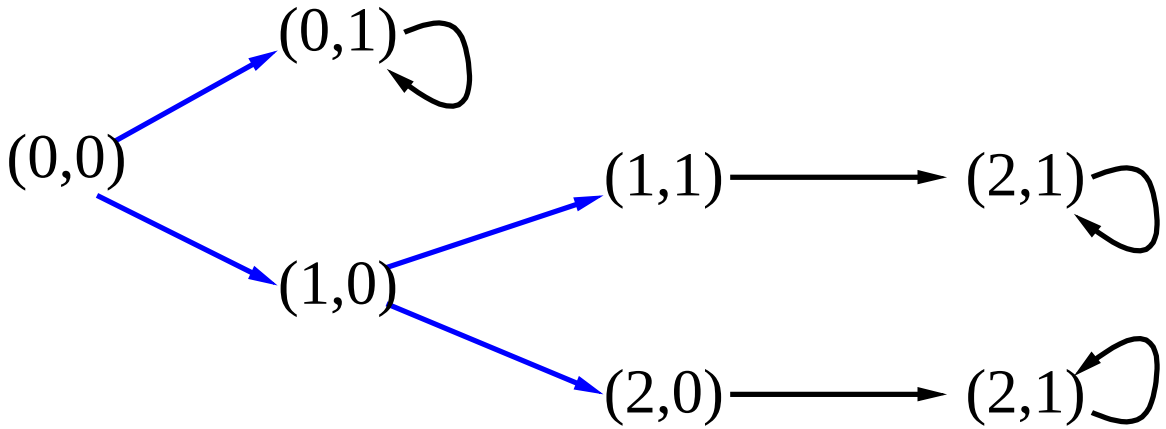
by units of Manhattan distance



Time has a tree structure

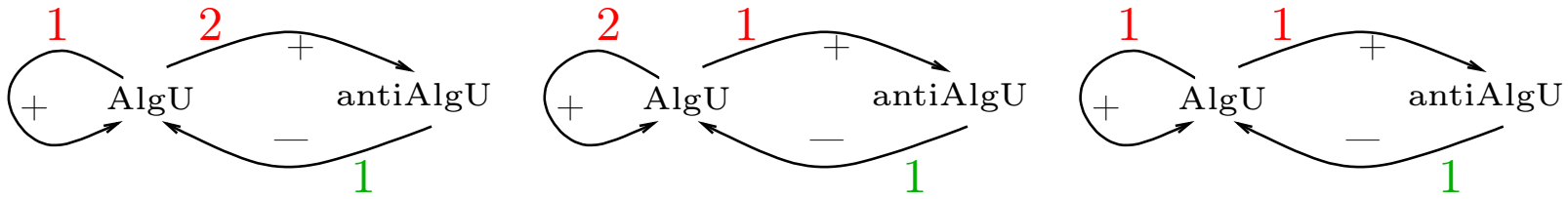


From an initial state:



Parameters & thresholds: often unknown

Thresholds for AlgU in *P.aeruginosa* are unknown:



and parameters are unknown:

$$3^4 \times 2^2 \qquad 3^4 \times 2^2 \qquad 2^4 \times 2^2$$

712 possible models

Some criteria exist to reduce the number of models,

but formal logic is needed to go further automatically

Note: some models are observably equivalent

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CTL = Computation Tree Logic

Atoms = comparaisons : (x=2) (y>0) ...

Logical connectives: $(\varphi_1 \wedge \varphi_2)$ $(\varphi_1 \Longrightarrow \varphi_2)$ $\cdots$

Temporal connectives: made of 2 characters

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

$AX(y = 1)$: the concentration level of y belongs to the interval 1 in all states directly following the considered initial state.

$EG(x = 0)$: there exists at least one path from the considered initial state where x always belongs to its lower interval.

Temporal Connectives of CTL

neXt state:

$EX\varphi$: φ can be satisfied in a next state

$AX\varphi$: φ is always satisfied in the next states

eventually in the Future:

$EF\varphi$: φ can be satisfied in the future

$AF\varphi$: φ will be satisfied at some state in the future

Globally:

$EG\varphi$: φ can be an invariant in the future

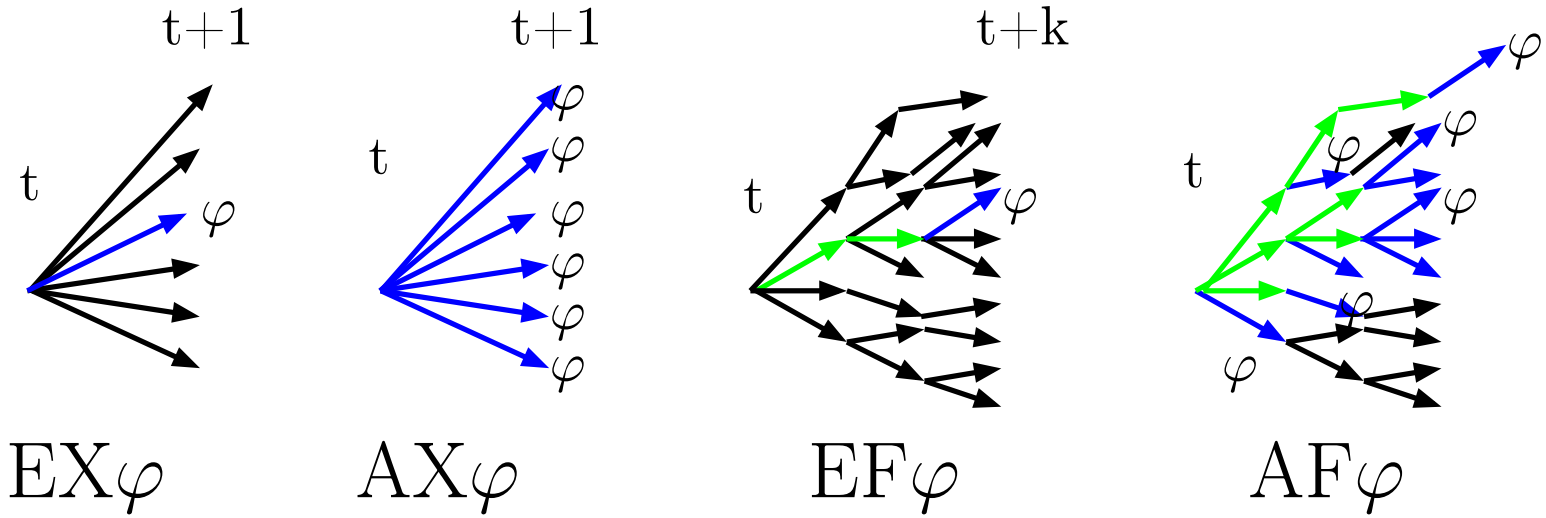
$AG\varphi$: φ is necessarilly an invariant in the future

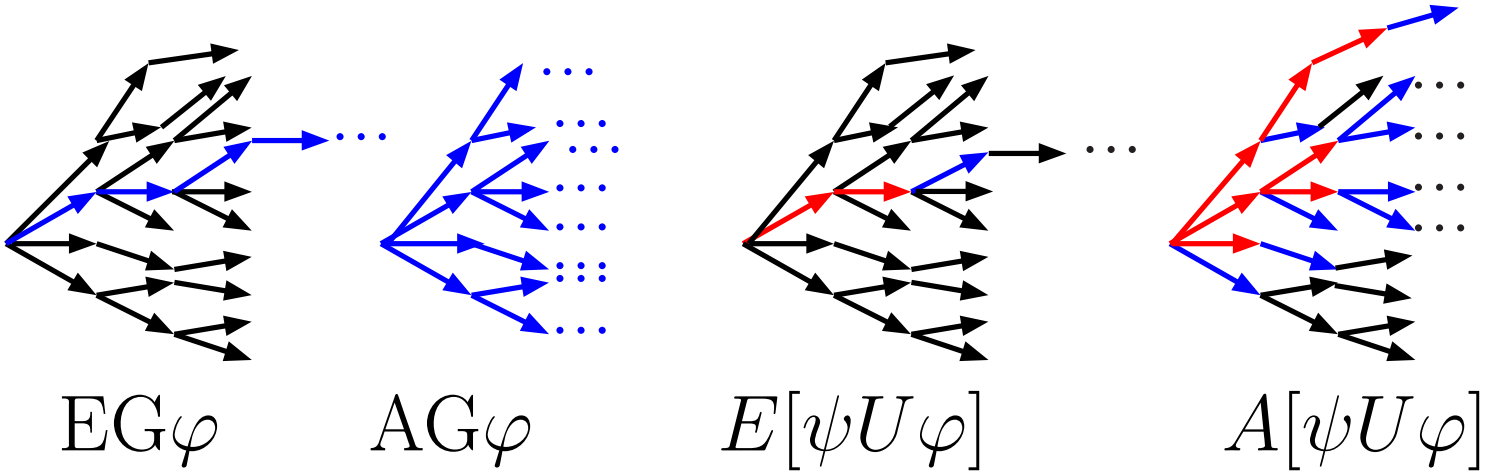
Until:

$E[\psi U\varphi]$: there exist a path where ψ is satisfied until a state where φ is satisfied

$A[\psi U\varphi]$: ψ is always satisfied until some state where φ is satisfied

Semantics of Temporal Connectives

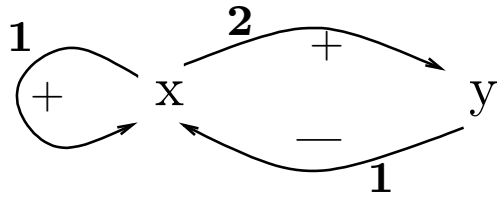




CTL to encode Biological Properties

Common properties:

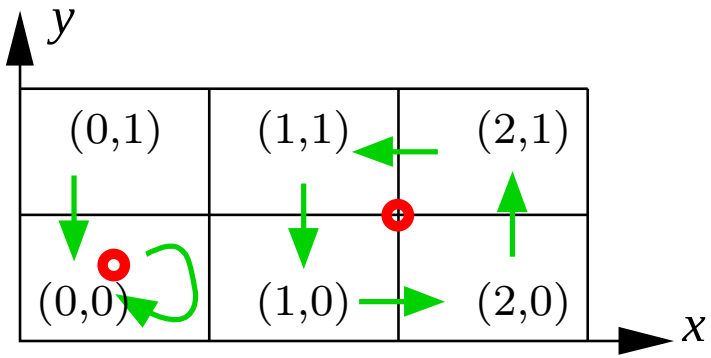
"functionality" of a sub-graph

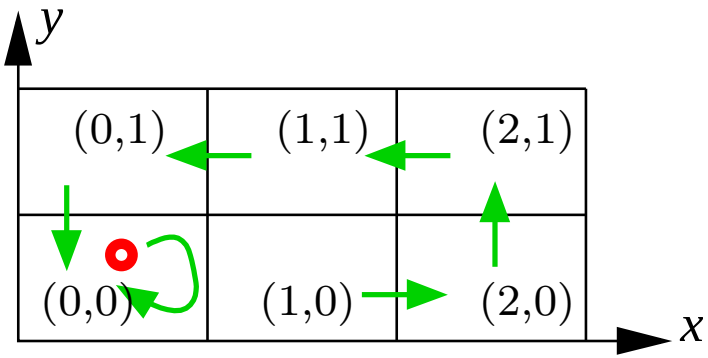


Special role of "feedback loops"

– positive: *multistationnarity* (even number of —)

– negative: *homeostasy* (odd number of —)





Characteristic properties:

$$(x = 2) \Longrightarrow AG(\neg(x = 0))$$

and

$$(x = 0) \Longrightarrow AG(\neg(x = 2))$$

They express *"the positive feedback loop is functional"*

(satisfaction of these formulae relies on the parameters $K_{...}$)

Theoretical Models $\leftrightarrow$ Experiments

CTL formulae are satisfied (or refuted) w.r.t. a set of paths from a given initial state

- They can be tested against the possible paths of the theoretical models ($M \models_{\eta} \varphi$)
- They can be tested against the biological experiments ($Biological\_Object \models_{experiment} \varphi$)

CTL formulae link theoretical models and biological objects together

Model Checking

Computes all the states of a theoretical model which satisfy a given formula: $\{ \eta \mid M \models_\eta \varphi \}$.

Idea 1: work on the state graph instead of the path trees.

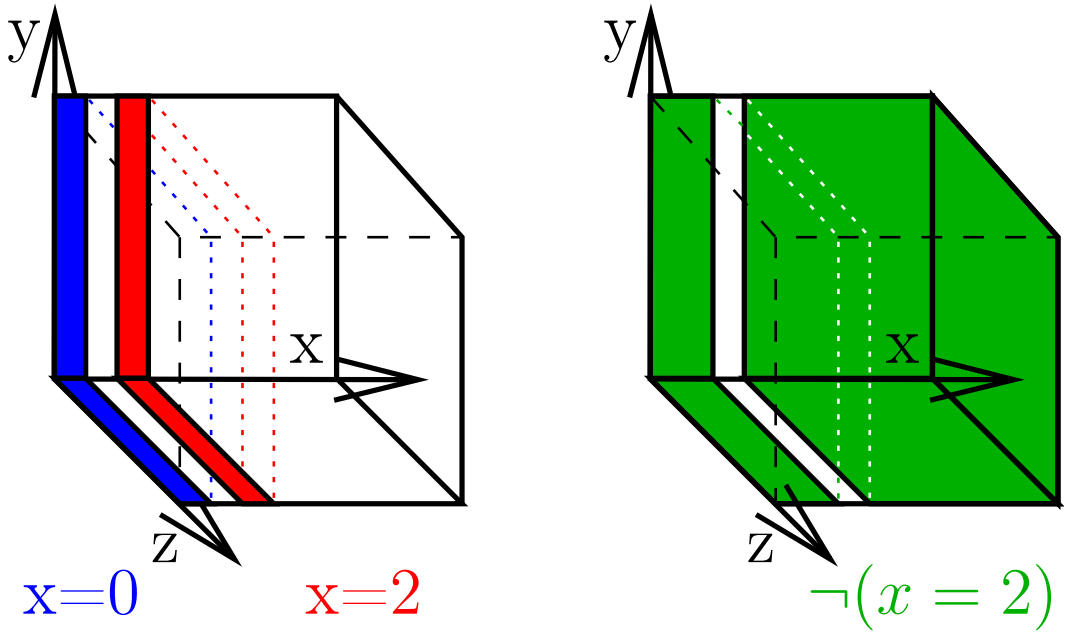
Idea 2: check first the atoms of φ and then check the connectives of φ with a bottom-up computation strategy.

Idea 3: (computational optimization) group some cases together using BDDs (Binary Decision Diagrams).

Example: $(x = 0) \implies AG(\neg(x = 2))$

Obsession: *travel the state graph as less as possible*

$$(x = 0) \quad \Longrightarrow \quad AG(\neg(x = 2))$$



x=0 x=2 $\neg(x = 2)$ and $AG(\neg(x = 2))$?

. . . one should **travel <u>all</u>** the paths from any green box and check if successive boxes are green: *too many boxes to visit.*

Trick: $AG(\neg(x = 2))$ is equivalent to $\neg EF(x = 2)$
start from the red boxes and follow the transitions backward.

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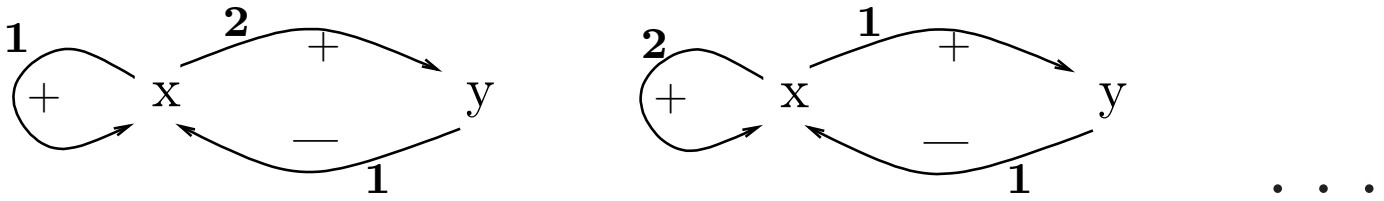
Computer Aided Elaboration of Models

From biological knowledge and/or biological hypotheses, it comes:

- properties:

  "Without stimulus, if gene x has its basal expression level, then it remains at this level."

- model schemas:

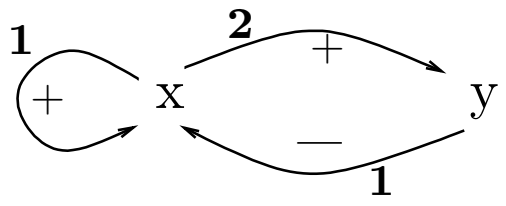


. . .

Formal logic and formal models allow us to:

- verify hypotheses and check consistency
- elaborate more precise models incrementally
- suggest new biological experiments to efficiently reduce the number of potential models

The Two Questions

$\Phi = \{\varphi_1, \varphi_2, \cdots, \varphi_n\}$ and $\mathcal{M} =$ 

. . .

1. Is it possible that Φ and $\mathcal{M}$?
   Consistency of knowledge and hypotheses. Means to select models belonging to the schemas that satisfy Φ.
   $(\exists ?\ M \in \mathcal{M} \ \mid \ M \models \varphi)$
2. If so, is it true *in vivo* that Φ and $\mathcal{M}$?
   Compatibility of one of the selected models with the biological object. Require to propose experiments to **validate** (or refute) the selected model(s).

$\rightarrow$ Computer aided *proofs* and *validations*

Question 1 = Consistency

1. Draw all the sensible regulatory graphs with all the sensible threshold allocations. It defines $\mathcal{M}$.
2. Express in CTL the known behavioural properties as well as the considered biological hypotheses. It defines Φ.
3. Automatically generate all the possible regulatory networks derived from $\mathcal{M}$ according to all possible parameters $K_{...}$.
   Our software plateform SMBioNet handles this automatically.
4. Check each of these models against Φ.
   SMBioNet uses model checking to perform this step.
5. If no model survive to the previous step, then reconsider the hypotheses and perhaps extend model schemas...
6. If at least one model survives, then the biological hypotheses are consistent. Possible parameters $K_{...}$ have been indirectly established. Now Question 2 has to be addressed.

Question 2 = Validation

1. Among all possible formulae, some are "observable" i.e., they express a possible result of a possible biological experiment.
   Let Obs be the set of all observable formulae.
2. Let Λ be the set of theorems of Φ and $\mathcal{M}$.
   $\Lambda \cap Obs$ is the set of experiments able to validate the survivors of Question 1. Unfortunately it is infinite in general.
3. Testing frameworks from computer science aim at selecting a finite subsets of these observable formulae, which maximize the chance to refute the survivors.
4. These subsets are often too big but in some cases, these testing frameworks can be applied to regulatory networks.
   It has been the case of the mucus production of *P.aeruginosa*.

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Mutation, Epigenesis, Adaptation

Terminology about phenotype modification:

genetic modification: inheritable and not reversible (mutation)

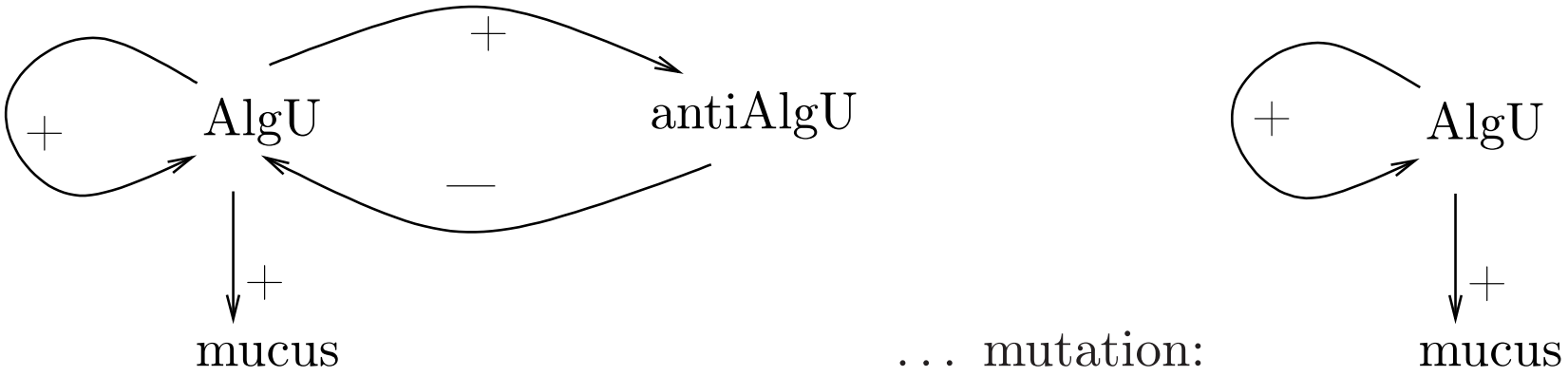
epigenetic modification: inheritable and reversible

adaptation: not inheritable and reversible

The biological questions (Janine Guespin):
are **mucus production** and/or **cytotoxicity** in *Pseudomonas aeruginosa* due to an epigenetic switch ?
[→ cystic fibrosis]

Mucus production in *P. aeruginosa*

Pseudomonas aeruginosa: (J.Guespin, M.Kaufman)



Epigenetic Hypothesis (without mutation) =

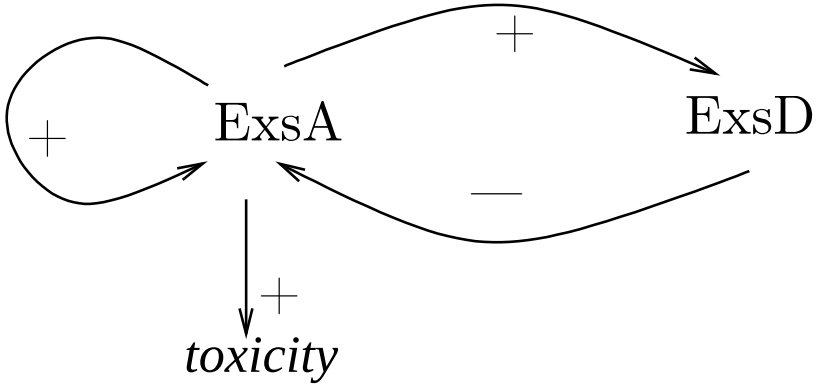
→ The positive feedback circuit is functional, with a mucoid stable state and another non mucoid stable state.

→ An external signal (in the cystic fibrosis' lungs) could switch AlgU from its lower stable state to the higher one.

→ The mutation could be favored later because the inhibitor complex is toxic for the bacteria. ⟹ New possible therapy.

Cytotoxicity in *P. aeruginosa*

(Janine Guespin)

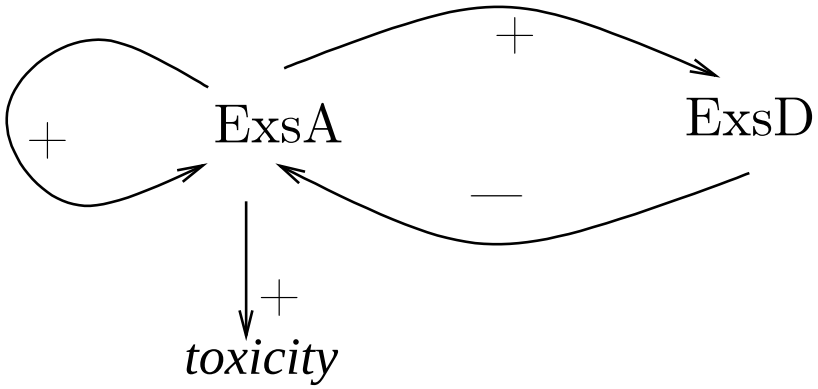


Epigenetic hypothesis =

→ The positive feedback circuit is functional, with a cytotoxic stable state and the other one is not cytotoxic.

→ An external signal (in the cystic fibrosis' lungs) could switch ExsA from its lower stable state to the higher one.

Consistency of the Hypothesis



One CTL formula for each stable state:

$$(\text{ExsA} = 2) \Longrightarrow AXAF(\text{ExsA} = 2)$$
$$(\text{ExsA} = 0) \Longrightarrow AG(\neg(\text{ExsA} = 2))$$

Question 1, consistency: proved by *Model Checking*

$\rightarrow$ 10 models among the 712 models are extracted by SMBioNet

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

Validation of the epigenetic hypothesis

Question 2 = to validate bistationnarity *in vivo*

Non cytotoxic state: $(\text{ExsA} = 0) \Longrightarrow AG(\neg(\text{ExsA} = 2))$

P. aeruginosa, with a basal level for ExsA *does not become spontaneously cytotoxic*: actually validated

Cytotoxic state: $(\text{ExsA} = 2) \Longrightarrow AXAF(\text{ExsA} = 2)$

Experimental limitation:

ExsA can be saturated but it cannot be measured.

Experiment:

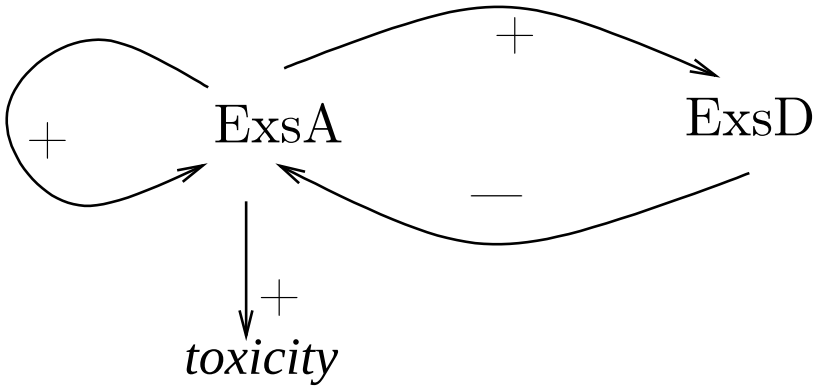
to pulse ExsA *and then to test if toxin production remain.*

($\Longleftrightarrow$ to verify a hysteresis)

This experiment can be generated automatically

To test $(\text{ExsA}{=}2) \Longrightarrow AXAF(\text{ExsA}{=}2)$

ExsA = 2 cannot be directly verified but $toxicity = 1$ can be verified.



Lemma: $AXAF(\text{ExsA} = 2) \Longleftrightarrow AXAF(toxicity = 1)$

(... formal proof by computer ...)

→ To test: $(\text{ExsA} = 2) \Longrightarrow AXAF(toxicity = 1)$

$$(\text{ExsA} = 2) \Longrightarrow AXAF(toxicity = 1)$$

Karl Popper:

to validate = to try to refute

thus A=false is useless

experiments must begin with a pulse

$A \Longrightarrow B$	*true*	*false*
true	true	false
false	true	true

The pulse forces the bacteria to reach the initial state ExsA = 2.

If the state were not directly controlable we had to prove lemmas:

$$(\text{ExsA} = 2) \Longleftarrow (something\ reachable)$$

General form of a test:

$$(something\ \underline{reachable}) \Longrightarrow (something\ \underline{observable})$$

Concluding Slogans

- Behavioural *properties* (Φ) are as much important as models ($\mathcal{M}$) for the modelling activity
- Modelling is significant only with respect to the considered experimental *reachability* and *observability* (*Obs*)
- The bigger is the risk of *refutation*, the better are the "surviving" models (Popper), thus models should be "simple" with few non observable parameters (Occam)

Formal methods (*syntax/semantics/proofs*) facilitate *abstraction* and consequently they simplify models

- They ensure *consistency* of the modelling activity
- They allow us to perform computer aided *validations* of models
- They take benefit of 30 years of researches in computer sciences