

# Logical modelling in biology through the case study of the cell cycle and its checkpoints

Déborah Boyenval  
iBV seminar

March 24, 2022

# Pluridisciplinary current research project



Team SPARKS  
(Gilles Bernot and Jean-Paul Comet)

- PhD student in bioinformatics
- Initial education in biology



Team Franck Delaunay

Understanding the interactions between biological oscillating systems





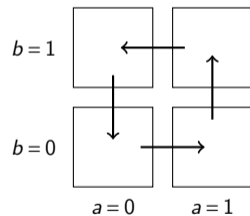
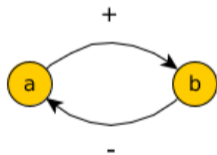




# The study of oscillating processes

## Logical modeling framework of biological regulatory network

- Formalizes the interactions between biological species using *logical formulas*
- Is an oscillatory behavior compatible with these formalized interactions?



- $K_{V,\omega}$  formalizes the influence of all the regulators of a variable on its activity

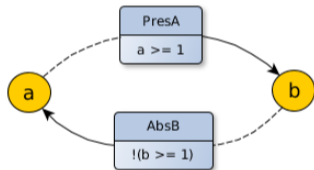
# Logical modeling framework of biological regulatory network

The value of  $K_{v,\omega}$  is the value towards which a variable  $v$  is attracted given a set of its resources  $\omega$

(1)

(2)

(3)



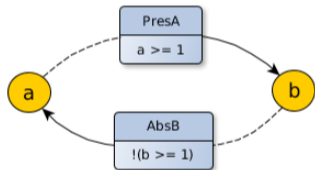
$$\left\{ \begin{array}{l} K_{a,\{\}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{\}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right\}$$



# Logical modeling framework of biological regulatory network

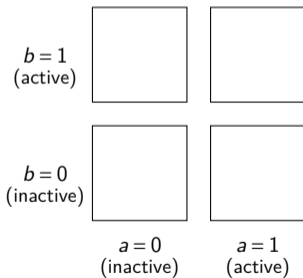
The value of  $K_{v,\omega}$  is the value towards which a variable  $v$  is attracted given a set of its resources  $\omega$

(1)



$$\left\{ \begin{array}{l} K_{a,\{\}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{\}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right\}$$

(2)

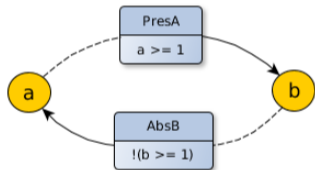


(3)

# Logical modeling framework of biological regulatory network

The value of  $K_{v,\omega}$  is the value towards which a variable  $v$  is attracted given a set of its resources  $\omega$

(1)



$$\left\{ \begin{array}{l} K_{a,\{\}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{\}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right\}$$

(2)

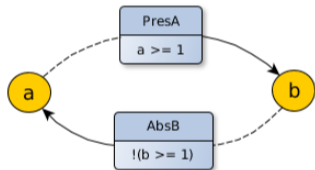
$b = 1$ (active)	$K_{a,\{\}}$	$K_{a,\{\}}$
$b = 0$ (inactive)		
	$a = 0$ (inactive)	$a = 1$ (active)

(3)

# Logical modeling framework of biological regulatory network

The value of  $K_{v,\omega}$  is the value towards which a variable  $v$  is attracted given a set of its resources  $\omega$

(1)



$$\left\{ \begin{array}{l} K_{a,\{\}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{\}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right\}$$

(2)

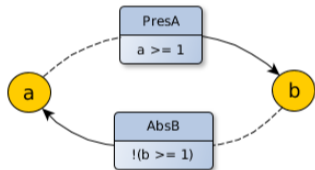
$b = 1$ (active)	$K_{a,\{\}}$	$K_{a,\{\}}$
$b = 0$ (inactive)	$K_{a,\{AbsB\}}$	$K_{a,\{AbsB\}}$
	$a = 0$ (inactive)	$a = 1$ (active)

(3)

# Logical modeling framework of biological regulatory network

The value of  $K_{v,\omega}$  is the value towards which a variable  $v$  is attracted given a set of its resources  $\omega$

(1)



$$\left\{ \begin{array}{l} K_{a,\{\}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{\}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right\}$$

(2)

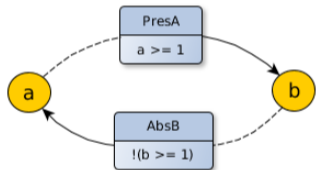
$b = 1$ (active)	$K_{a,\{\}}$ $K_{b,\{\}}$	$K_{a,\{\}}$
$b = 0$ (inactive)	$K_{a,\{AbsB\}}$ $K_{b,\{\}}$	$K_{a,\{AbsB\}}$
	$a = 0$ (inactive)	$a = 1$ (active)

(3)

# Logical modeling framework of biological regulatory network

The value of  $K_{v,\omega}$  is the value towards which a variable  $v$  is attracted given a set of its resources  $\omega$

(1)



$$\left\{ \begin{array}{l} K_{a,\{\}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{\}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right\}$$

(2)

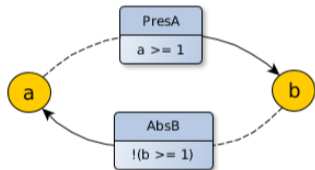
$b = 1$ (active)	$K_{a,\{\}}$ $K_{b,\{\}}$	$K_{a,\{\}}$ $K_{b,\{PresA\}}$
$b = 0$ (inactive)	$K_{a,\{AbsB\}}$ $K_{b,\{\}}$	$K_{a,\{AbsB\}}$ $K_{b,\{PresA\}}$
	$a = 0$ (inactive)	$a = 1$ (active)

(3)

# Logical modeling framework of biological regulatory network

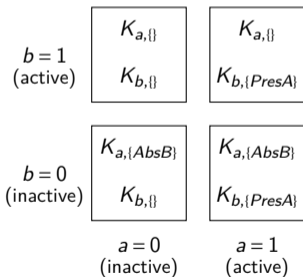
The value of  $K_{v,\omega}$  is the value towards which a variable  $v$  is attracted given a set of its resources  $\omega$

(1)

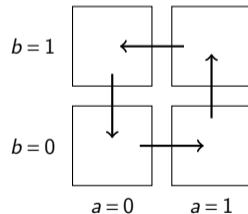


$$\left\{ \begin{array}{l} K_{a,\{\}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{\}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right\}$$

(2)



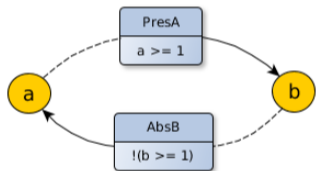
(3)



# Logical modeling framework of biological regulatory network

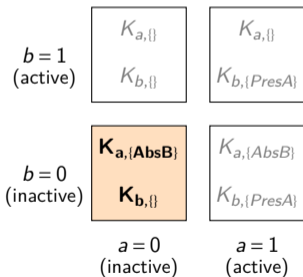
The value of  $K_{v,\omega}$  is the value towards which a variable  $v$  is attracted given a set of its resources  $\omega$

(1)

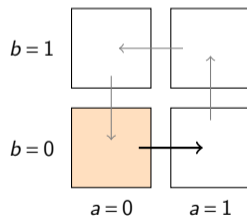


$$\left\{ \begin{array}{l} K_{a,\{\}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{\}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right\}$$

(2)



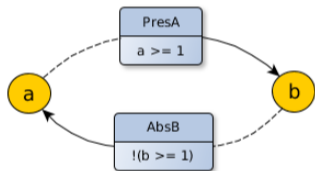
(3)



# Logical modeling framework of biological regulatory network

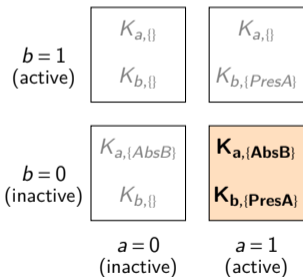
The value of  $K_{v,\omega}$  is the value towards which a variable  $v$  is attracted given a set of its resources  $\omega$

(1)

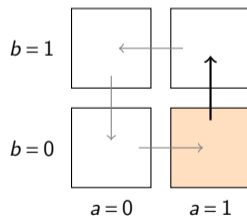


$$\left\{ \begin{array}{l} K_{a,\{\}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{\}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right.$$

(2)



(3)

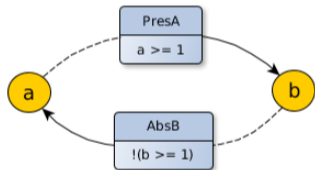




# Logical modeling framework of biological regulatory network

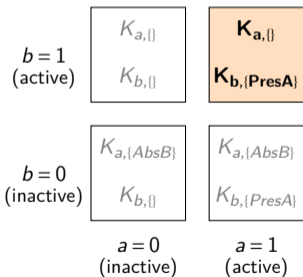
The value of  $K_{v,\omega}$  is the value towards which a variable  $v$  is attracted given a set of its resources  $\omega$

(1)

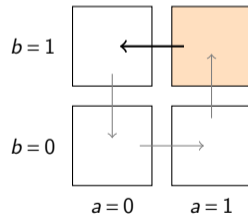


$$\left\{ \begin{array}{l} K_{a,\{\}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{\}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right\}$$

(2)



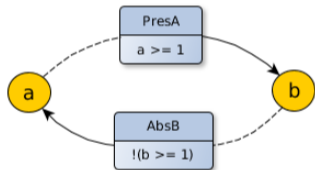
(3)



# Logical modeling framework of biological regulatory network

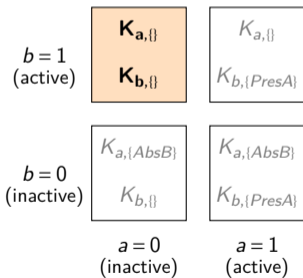
The value of  $K_{v,\omega}$  is the value towards which a variable  $v$  is attracted given a set of its resources  $\omega$

(1)

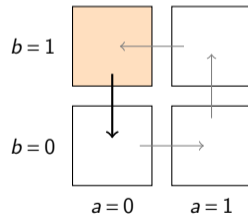


$$\left\{ \begin{array}{l} K_{a,\{\}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{\}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right\}$$

(2)

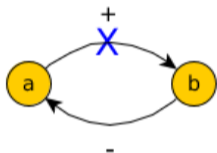


(3)

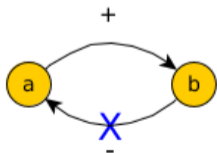
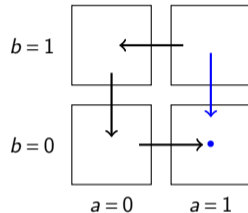


# What is a logical model of a biological system?

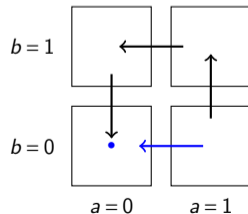
Interaction graph + One parametrization (transition graph)



$$\left\{ \begin{array}{l} K_{a,\{}} = 0 \\ K_{a,\{AbsB\}} = 1 \\ K_{b,\{}} = 0 \\ K_{b,\{PresA\}} = 0 \end{array} \right\}$$



$$\left\{ \begin{array}{l} K_{a,\{}} = 0 \\ K_{a,\{AbsB\}} = 0 \\ K_{b,\{}} = 0 \\ K_{b,\{PresA\}} = 1 \end{array} \right\}$$



These two models with a non-functional interaction (crossed edge)  $\Rightarrow$  No oscillation

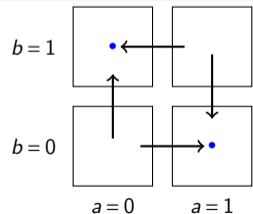
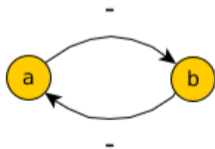
# What are the necessary conditions to observe certain behaviors?

## What are the models that produce oscillations?

- **Odd** number of inhibiting edges (*i.e.* a functional **negative cycle**)
- There is a mathematical proof! So it is generalized to any model!

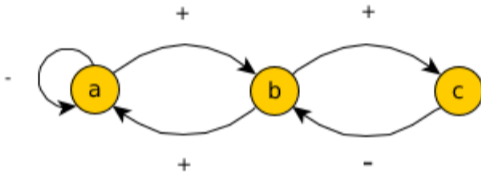
## What are the models that produce multiple steady states?

- **Even** number of inhibiting edges (*i.e.* a functional **positive cycle**)



This *interaction graph* will never produce oscillations, but can produce bistability !

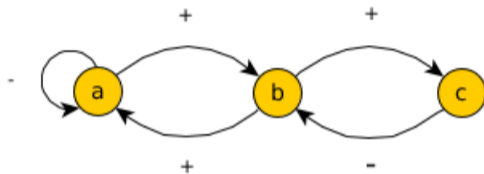
# Logical reasoning on higher dimensional model



Let's try to answer these questions:

- Does this biological interaction network produce oscillations?
- What are the key interactions to obtain an oscillatory behaviour?

# Logical reasoning on higher dimensional model



Let's try to answer these questions:

- Does this biological interaction network produce oscillations?
- What are the key interactions to obtain an oscillatory behaviour?



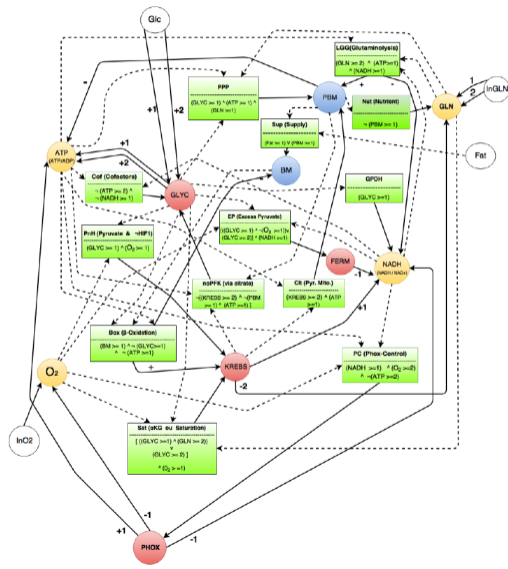
This is clearly non trivial !  
It depends on the functionality of negative cycles  
i.e. the parameterizations

# Logical reasoning on higher dimensional model

Automation of the logical reasoning (René Thomas' approach)

- Application of the logical modeling formalism manually: certainly not!

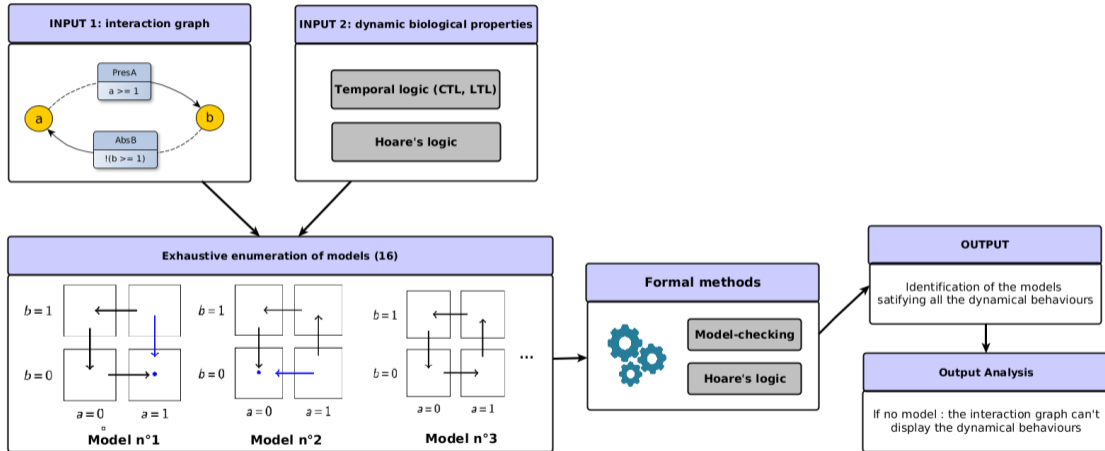
# A very complex interaction graph of the energy metabolism!



- 14 variables and 100 parameters
- More parametrizations than stars in the universe
- *Khoodeeram et al. 2017 - Advances in Systems and Synthetic Biology*

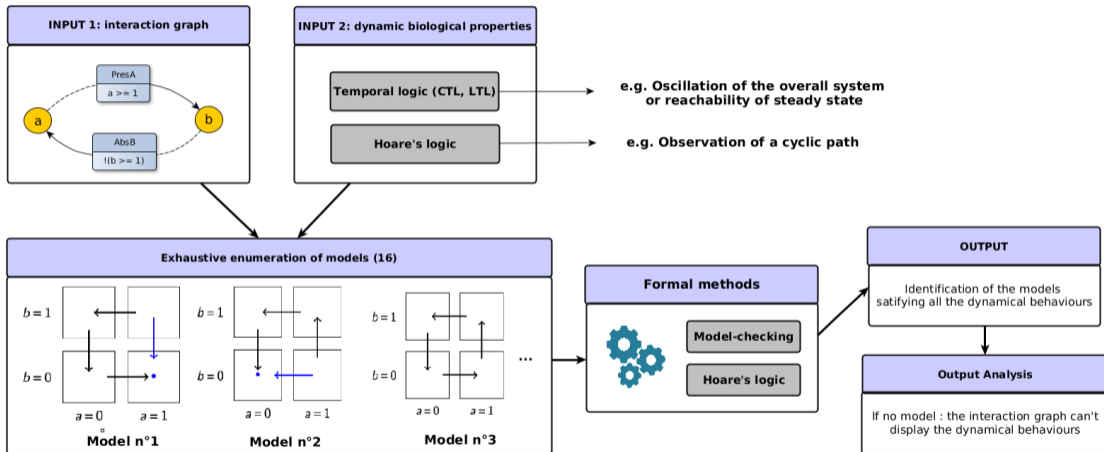


# Learning of parameterizations verifying given biological behaviors



The TotemBioNet tool  
 Boyenval *et al.* 2020 - CMSB

# Learning parameterizations verifying given biological behaviors

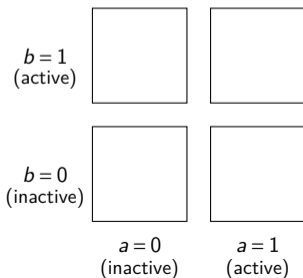


The TotemBioNet tool  
Boyenval *et al.* 2020 - CMSB

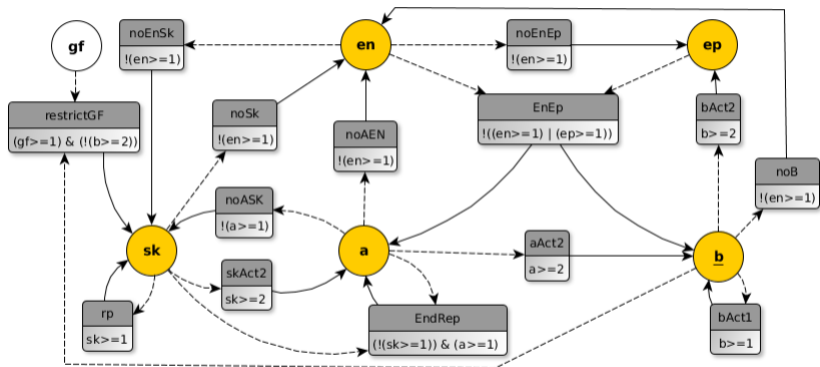
# Logical reasoning on higher dimensional model

## Qualitative dynamics (René Thomas' approach)

- Concentration space discretization
- Opens the door to formal methods in theoretical computer science
- No blind spot: all parameterizations (interaction graph dynamics) are calculated
- Unlike ODEs system for example



# The cell cycle study case

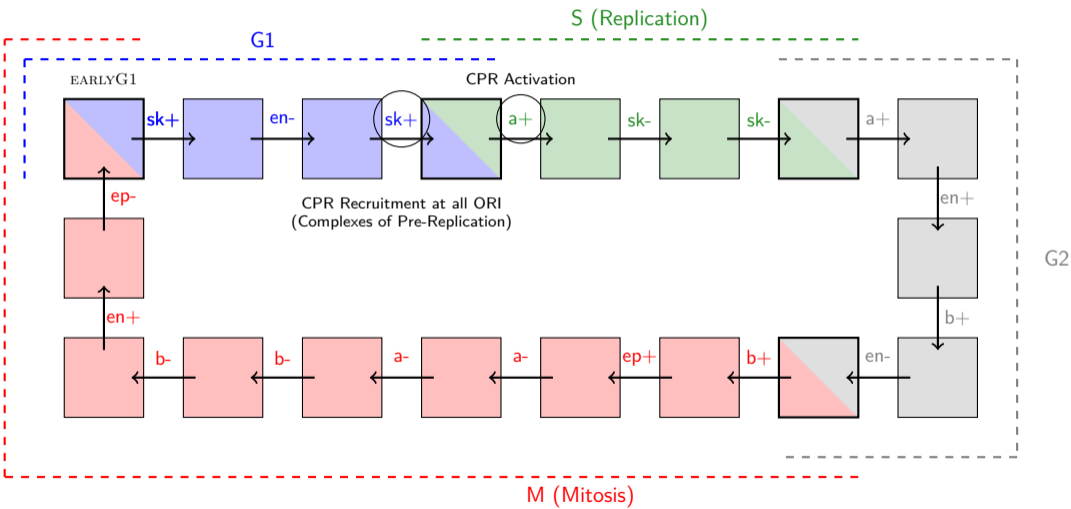


- **sk** (starting kinases) :  
CycE/Cdk2
- **a** : CycA/Cdk1 et  
CycA/Cdk2
- **b** : CycB/Cdk1
- **en** (enemies) :  
APC-cdh1, Wee1,  
p21, p27, PP1 et  
PP2
- **ep** (exit protein) :  
APC-cdc20
- **gf** (growth factors) :  
EGF, FGF, PDGF, ...

Interaction graph of the mammalian cell cycle progression  
(revised from Behaegel *et al.* 2015)



# The cell cycle itself: Observation of a canonical cyclic path

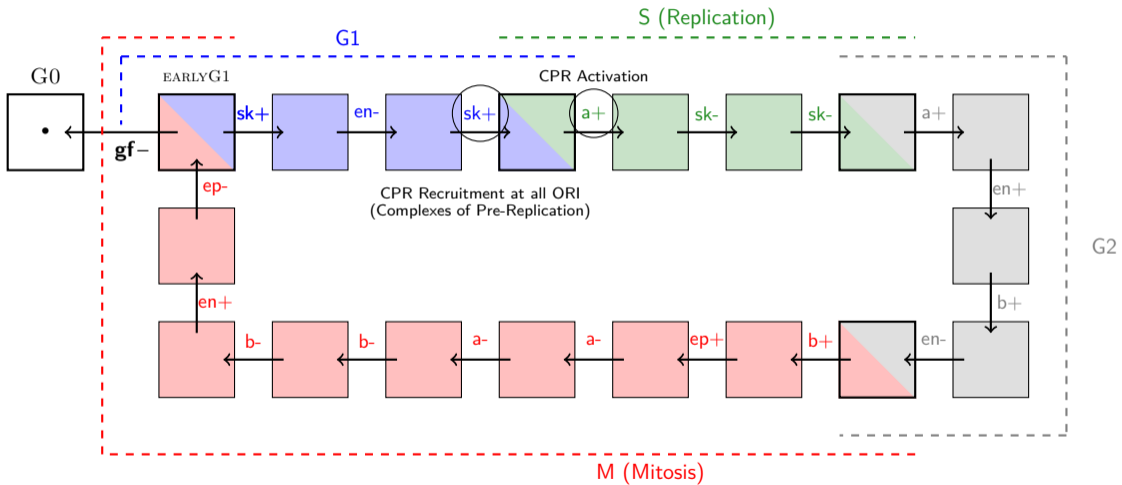


# What are the models that verify the major properties of the cell cycle?

The quiescence phase blocking in absence of growth factors (Temporal logics - CTL)

- $G0$  (another initial state) specified by  $sk = 0 \wedge a = 0 \wedge b = 0 \wedge ep = 0 \wedge en = 1 \wedge gf = 0$
- $AG(gf = 0) \implies AG(G0)$

# The quiescence phase blocking in absence of growth factors







# Checkpoints are intrinsically discrete mechanisms

## Cell cycle discrete phase in common language

- {EARLYG1}  $\underbrace{sk+; en-; sk+; a+; sk-; sk-; a+; en+; b+; en-}_{G1}; \underbrace{a+; sk-; sk-; a+; en+; b+; en-}_{S}; \underbrace{a+; en+; b+; en-}_{G2}; \underbrace{b+; ep+; a-; a-; b-; b-; ep-; en+}_{M}$  {EARLYG1}
- A phase is defined by an initial state and *unordered* set of events.

## Discrete definition of cell cycle checkpoints

A completion requirement of events of a given **phase** before the onset of the first event of next **phase**. If each checkpoint is satisfied then the cell ensures the transmission of intact DNA to its daughter cell.

## Hypothesis

- A checkpoint does not work on the basis of a regulation delay but on the basis of a chronology of events
- No chronometric modeling study (EDO systems or hybrid formalisms)



# Checkpoint formalization in 1<sup>st</sup> order logic

The generic predicate *checkpoint*

$$\underline{\exists \sigma} \mid \overline{\forall \pi_i} \in [G1, S, G2, M], \quad \forall X, \forall Y \text{ (two events of the form } v+ \mid v-),$$

$$\mathbf{canEnd}_{\sigma}(X, \pi_i) \wedge \mathbf{canStart}_{\sigma}(Y, \pi_{i+1}) \implies \mathbf{isRequired}_{\sigma}(X, Y)$$

The predicates *canEnd* and *canStart*

$$\mathbf{canEnd}_{\sigma}(X, \pi_i) \iff \exists p' \in \mathbf{permutations}(p) \mid (\sigma(\mathbf{wp}(\{\mathit{init}\}p'\{\mathit{final}\})) \wedge X = \mathbf{last}(p'))$$

$$\mathbf{canStart}_{\sigma}(Y, \pi_i) \iff \exists p' \in \mathbf{permutations}(p) \mid (\sigma(\mathbf{wp}(\{\mathit{init}\}p'\{\mathit{final}\})) \wedge Y = \mathbf{first}(p'))$$

The predicate *isRequired*

- $X = x+ \text{ or } x-, Y = y+ \text{ or } y-$
- $\mathbf{isRequired}(X, Y) \iff (\sigma(K_{y, \omega_{\text{before}}}) - \eta_{\text{before}}) \times (\sigma(K_{y, \omega_{\text{after}}}) - \eta_{\text{after}}) \leq 0$

# What are the models that verify the 4 cell cycle checkpoints?

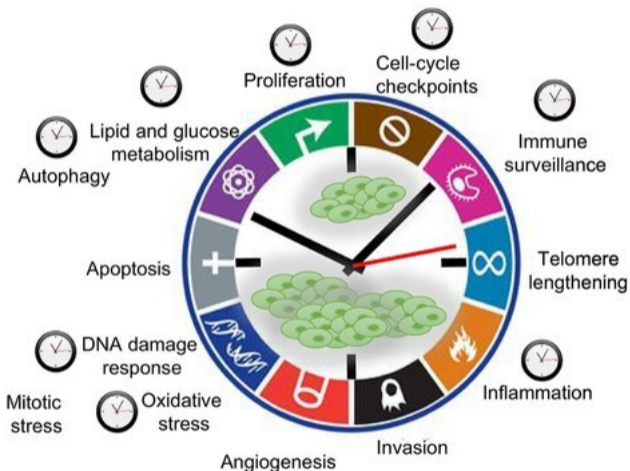
## Results after implementation in Prolog

- Conclusion: 3 of the 4 modelled checkpoints are satisfied.
- Not the mitosis exit checkpoint !
- Identification of a biological counter-example ... of the generic definition
- Towards a new definition of the mitosis exit checkpoint (work in progress)

Checkpoint	Eval	Output ( $ models \models \mathit{checkpoint} $ )	Computation time
G1/S	True	24/32	1.9s
S/G2	True	32/32	11s
G2/M	True	32/32	1h10min
Mitosis Exit	False	0/32	1h12min



# Cell cycle checkpoints and integration of oscillating processes



- Cellular processes are intrinsically interdependent
- Circadian oscillator is linked to many cellular processes
- Oscillators coupling understanding
- Anticancer chronotherapy

*Sulli et al. 2019 - Trends Cancer*





