Formal modeling of biological cyclic behavior with checkpoints: the cell cycle regulation

PhD Defense of Déborah Boyenval

Supervised by : Gilles Bernot, Franck Delaunay and Jean-Paul Comet

15 Décembre 2022

Outline

The regulation of the mammalian cell cycle

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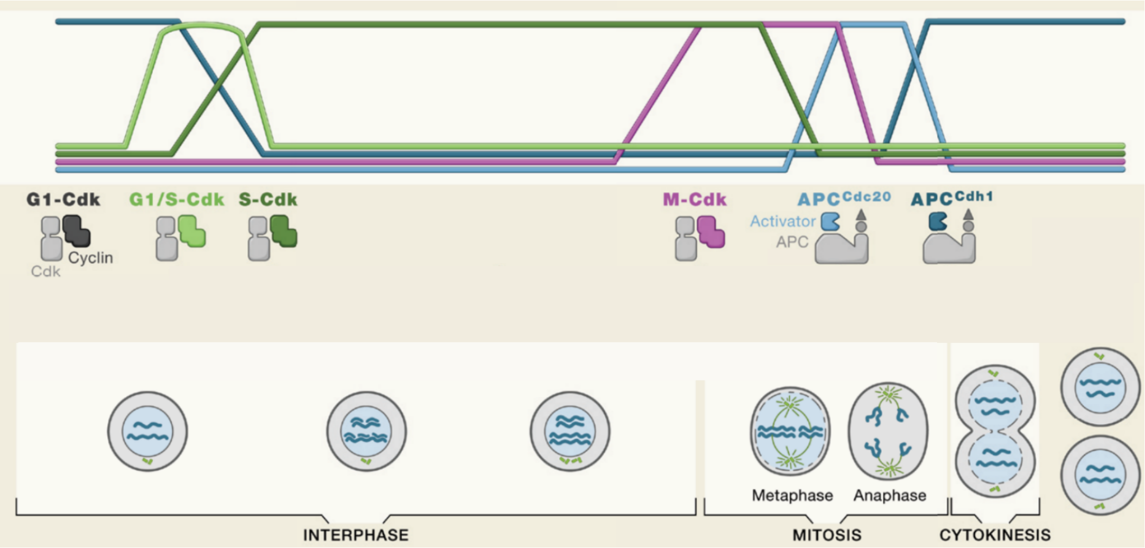

Cell-Cycle Regulators I, Cell, David O. Morgan, 2008, Cell SnapShot (Figure adaptée).

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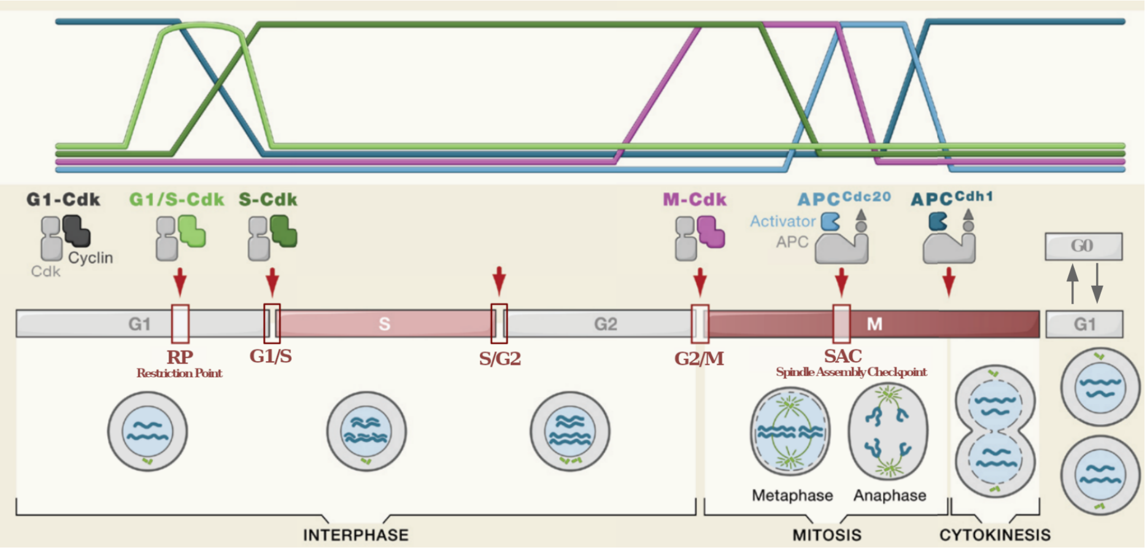

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Nature and types of checkpoints

1. Intrinsic checkpoints:
   - Temporal separation of cell cycle phases
   - Irreversibility of phase transitions

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2. Extrinsic checkpoints:
   - Inhibition of an event required for the completion of a phase

Intrinsic cell cycle checkpoint $\iff$ (property 1 $\wedge$ property 2)

Purpose of the thesis: focus on the modeling of intrinsic checkpoints

Property 1: Temporal separation of cell cycle phases

The phase onset is blocked as long as all the characteristic events of the previous phase have not taken place

Property 2 : Irreversibility of phase transitions

There is no path between two adjacent phases that goes to a third phase

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- Prove that the notion of checkpoint is purely discrete
- Define (yet) a(nother) cell cycle model
    - up-to-date
    - at a correct level of abstraction

Discrete formalisation of biological networks

- Automata networks
- Boolean networks and their extensions
- Petri nets
- Boolean/multivalued semantics of BioChAM
- Biological regulatory networks with multiplexes
  R. Thomas, H. Snoussi, G. Bernot, J-P. Comet (2008)

Contribution of key cell cycle models to the thesis

Model (formalism)	Phase?	Checkpoint?
Tyson et Novak, 2004/8 *Gerard et Goldbeter 2009* (ODEs)		
Traynard et al. 2016 (multivalued network)		
Diop et al. 2019 (boolean network)		
Deritei et al. 2019 (boolean network)		

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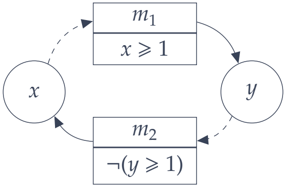
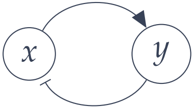
Key concepts introduced into the thesis: regulatory event, phase domain, temporal separation

Outline

1. Introduction
2. René Thomas' framework for modeling biological regulation networks
3. Modeling of the cell cycle and formalisation of its phases
4. Proof of concept: formalisation and formal verification of cell cycle checkpoints
5. Conclusion

Biological Regulation graph with Multiplexes (BRGM)





- Triple $\mathscr{G} = (V, M, E)$
- Literals : $(x \geqslant 1)$, $\neg(y \geqslant 1)$

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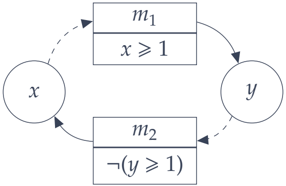

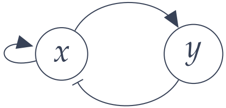

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- Formula φ_m associated with a multiplex

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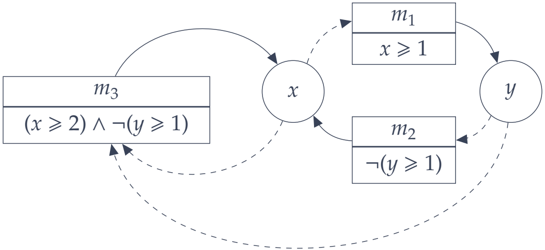
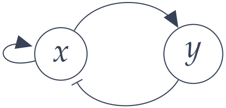
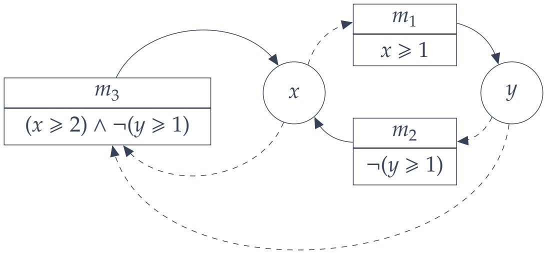




- Triple $\mathscr{G} = (V, M, E)$
- Literals : $(x \geqslant 1)$, $\neg(y \geqslant 1)$ et $(x \geqslant 2)$
- Formula φ_m associated with a multiplex
- The inhibition of y overrides the self-activation of x.

Transition graph



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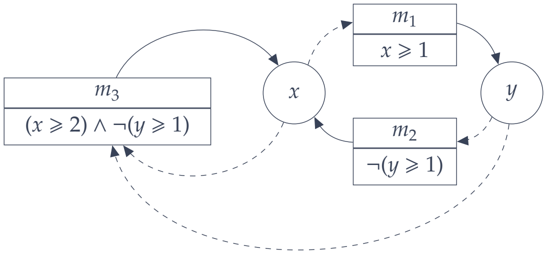
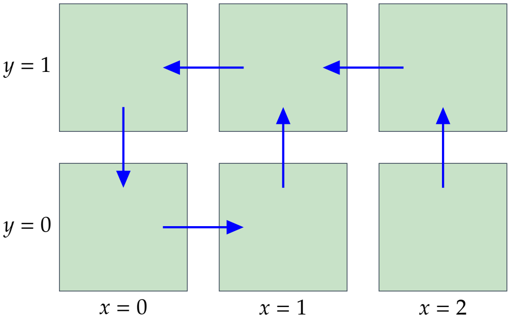
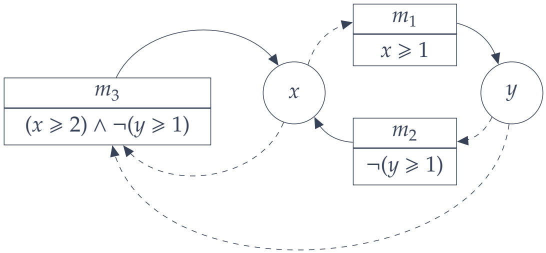
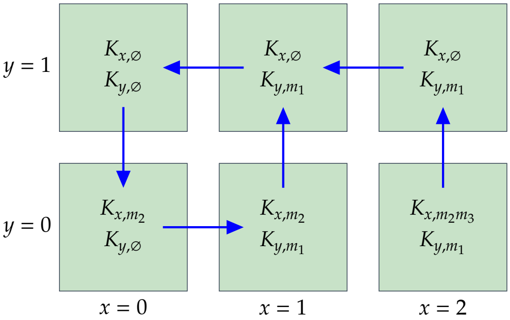




- State $\eta \ : \ V \to \mathbb{N}$ with $\eta(v) \in [\![0, b_v]\!]$
- Parameter $K_{v,\omega} \mid \omega \subseteq \{E^{-1}(v)\}$
- Resource $\omega : \{m \mid \eta \vDash \varphi_m\}$ (fixed state)

Transition graph





- State η : $V \to \mathbb{N}$ with $\eta(v) \in [\![0, b_v]\!]$
- Parameter $K_{v,\omega}$ | $\omega \subseteq \{E^{-1}(v)\}$
- Resource $\omega : \{m \mid \eta \vDash \varphi_m\}$ (fixed state)
- Parameterisation σ : $K_{v,\omega} \to \mathbb{N}$ with $\sigma(K_{v,\omega}) \in [\![0, b_v]\!]$

Computer-aided discrete modeling of regulatory networks

- Model-checking of CTL formula (`SMBioNet`, Adrien Richard)

  Bernot *et al.*, 2004, JTB

- Genetically modified Hoare logic (`HoareFol`, Maxime Folschette)

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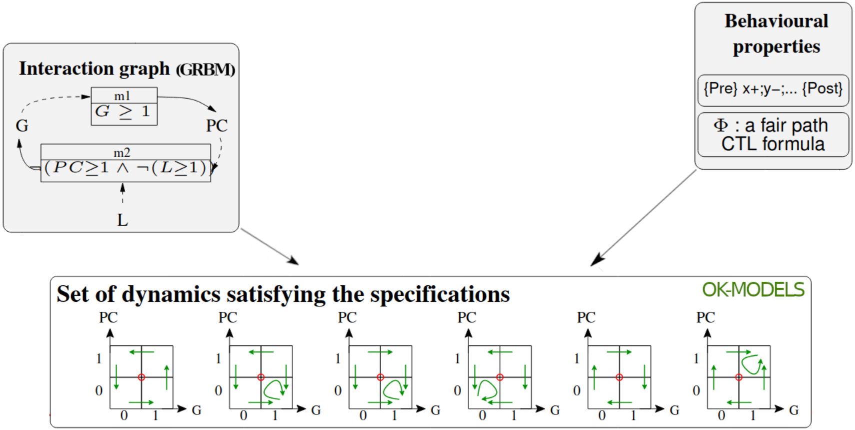
$$\{Precondition\}\ \ path\ \ \{Postcondition\}$$

($path$: succession of events of the form $v+$ or $v-$)

TotemBioNet: Software platform for Rene Thomas' model design



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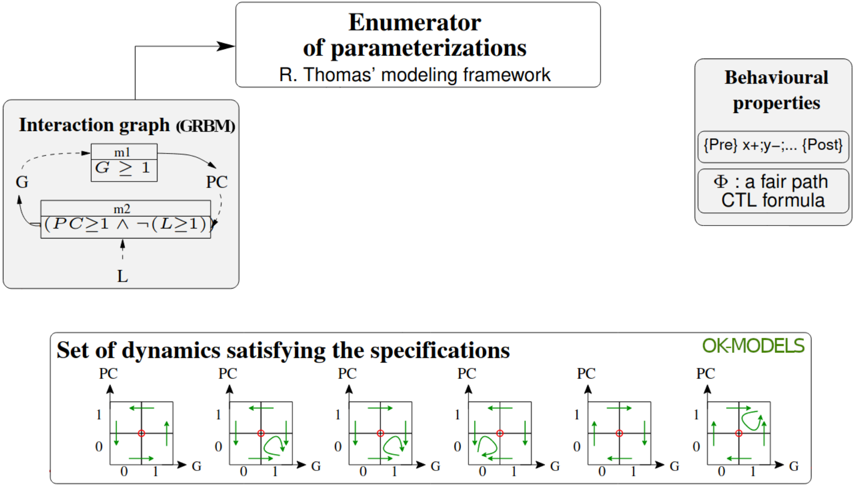

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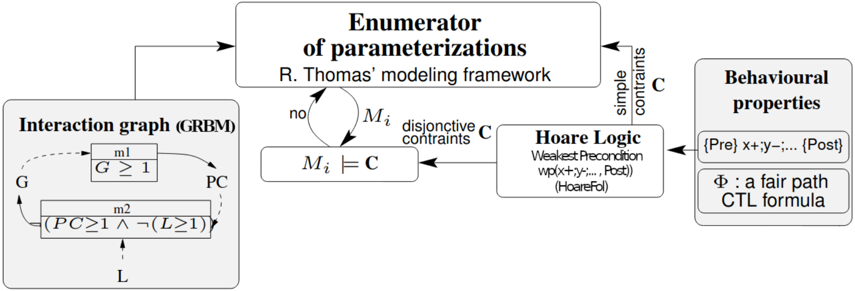

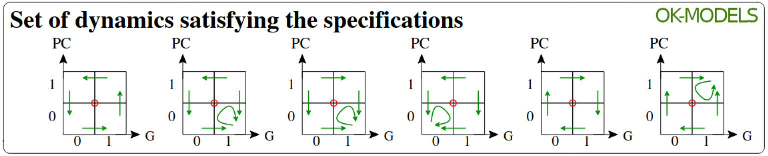

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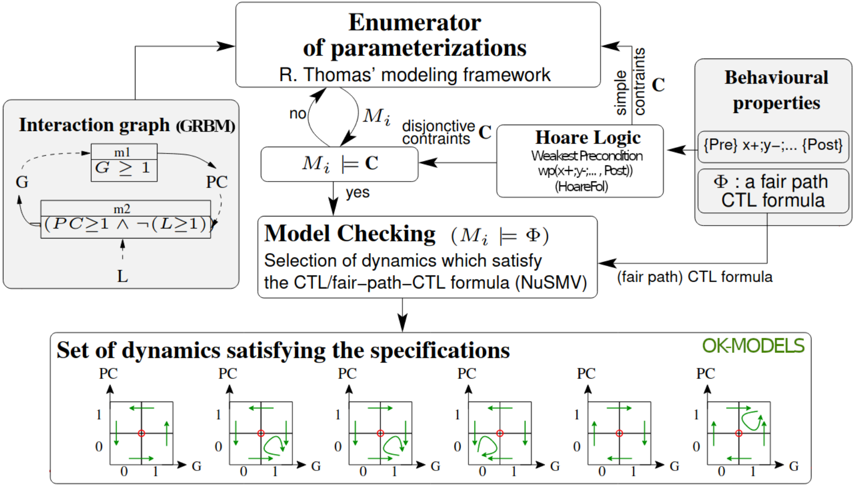
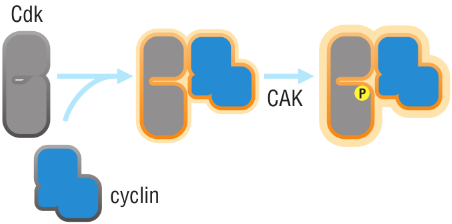


Déborah Boyenval, Gilles Bernot, Hélène Collavizza, Jean-Paul Comet,
What is a cell cycle checkpoint ? The TotemBioNet answer.
Computational Methods in Systems Biology 2020

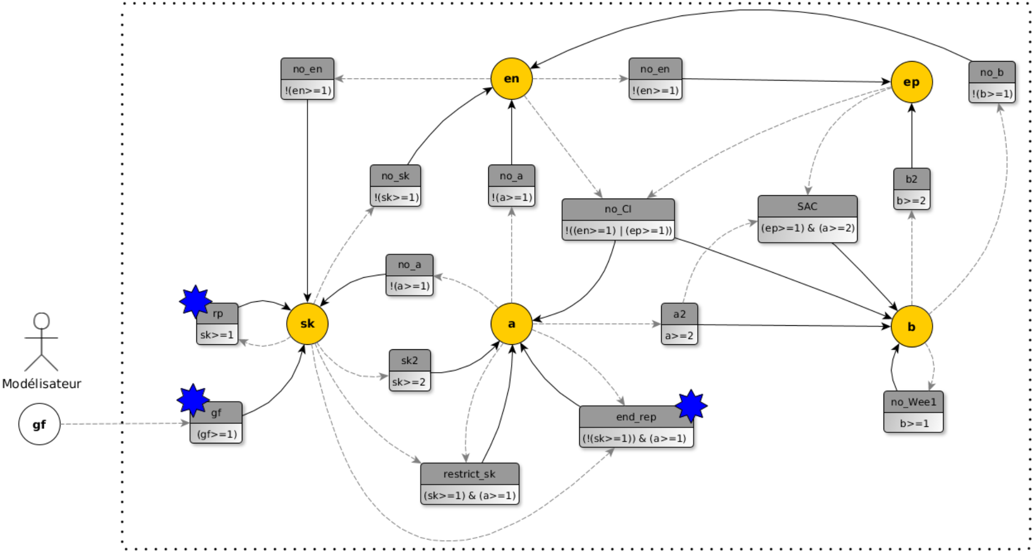
Outline

Our cell cycle BRGM ($\mathscr{G}_c$)

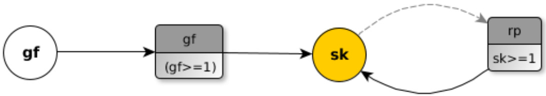


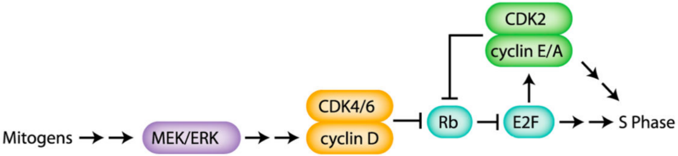
Variable	Terminology	Set of molecules	Domain
sk	"Starting kinase"	cyclinE/cdk2, CAK	$[\![0, 2]\!]$
a	"Cyclin A"	cyclinA/cdk1, cyclinA/cdk2, CAK	$[\![0, 2]\!]$
b	"Cyclin B"	cyclinB/cdk1, CAK	$[\![0, 2]\!]$
en	"Enemies"	APC-cdh1, P21, P27, PP1/2A	$[\![0, 1]\!]$
ep	"Exit protein"	APC-cdc20	$[\![0, 1]\!]$
gf	Growth factors	EGF, FGF, PDGF, IGF, TGFβ	$[\![0, 1]\!]$

Three notable multiplexes in $\mathscr{G}_c$



Mechanistic multiplexes: example of `gf`



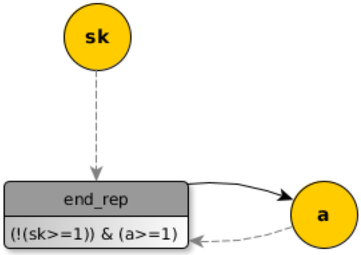


The Proliferation-Quiescence Decision Is Controlled by a Bifurcation in CDK2 Activity at Mitotic Exit, Spencer et al., 2013, Cell

REACTOME

Phenomenological multiplexes: example of `end_rep`

Regulation:



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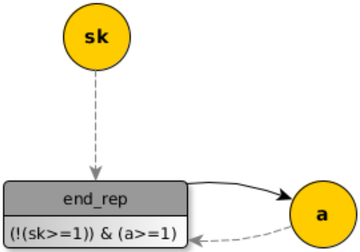
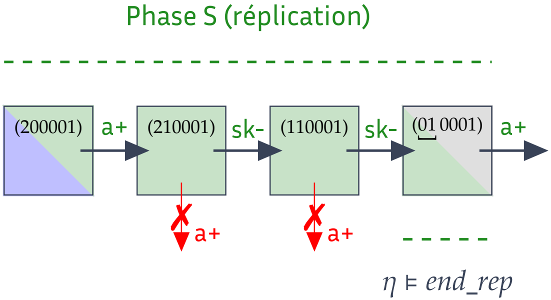


Dynamics:



Phenomenological multiplexes: example of `end_rep`

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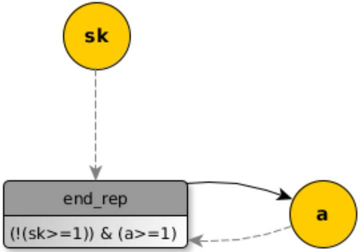
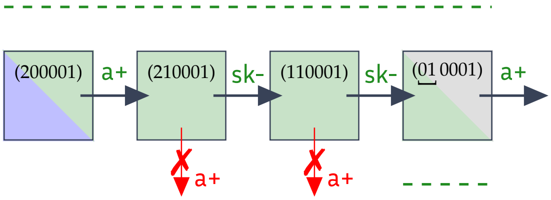


Dynamics:

Phase S (réplication)



$\eta \models end\_rep$

$\varphi_{end\_rep} \equiv (\neg(sk \geqslant 1) \wedge (a \geqslant 1))$ (no mechanistic explanation)

An intrisic S/G2 checkpoint enforced by ATR, Salvidar et al., 2019, Science

The canonical trace of the cell cycle and its phases

$$P_{G1} \equiv (sk = 0 \wedge a = 0 \wedge b = 0 \wedge ep = 0 \wedge en = 1 \wedge gf = 1)$$

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$$\{P_{G1}\}\ \overbrace{sk+; en-; sk+;}^{p_{G1}} \overbrace{a+; sk-; sk-;}^{p_S} \overbrace{a+; b+;}^{p_{G2}} \overbrace{b+; ep+; a-; a-; b-; b-; ep-; en+}^{p_M}\ \{P_{G1}\}$$

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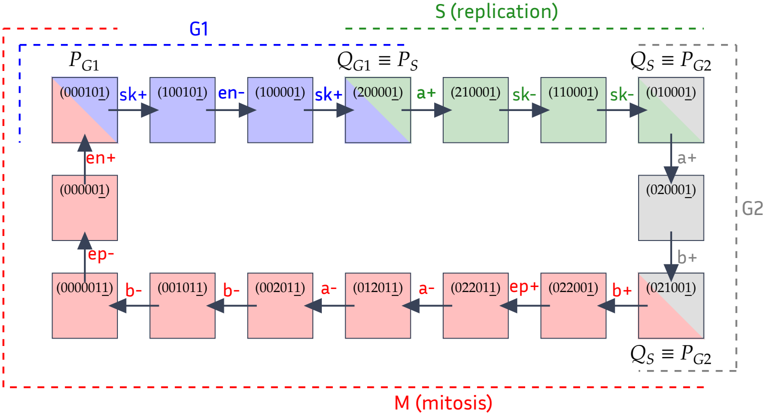


Pre-screening of relevant models before formalising the phases

Elementary Hoare triple of the canonical cell cycle

Insensitivity of part of the cell cycle to growth factors

Stabilité de la phase de quiescence

Pre-screening of relevant models before formalising the phases

Elementary Hoare triple of the canonical cell cycle

$$\{P_{G1}\}\ \underbrace{sk+\ ;\ en-\ ;\ sk+}_{p_{G1}};\ \underbrace{a+\ ;\ sk-\ ;\ sk-}_{p_S};\ \underbrace{a+\ ;\ b+}_{p_{G2}};\ \underbrace{b+\ ;\ ep+\ ;\ a-\ ;\ a-\ ;\ b-\ ;\ b-\ ;\ ep-\ ;\ en+}_{p_M}\ \{P_{G1}\}$$

PRP

ADN Pol α processivity

CPR activation

CPR inactivation

Mitotic substrates activation

Mitosis exit activation

Mitotic substrates inactivation

SAC inhibition (M/A)

Insensitivity of part of the cell cycle to growth factors

$$\left\{P_{G1}\left[\begin{array}{l} gf \leftarrow gf-1 \\ sk \leftarrow sk+1 \end{array}\right]\right\}\ \underbrace{en-\ ;\ sk+}_{p_{G1_{tardif}}};\ \underbrace{a+\ ;\ sk-\ ;\ sk-}_{p_S};\ \underbrace{a+\ ;\ b+}_{p_{G2}};\ \underbrace{b+\ ;\ ep+\ ;\ a-\ ;\ a-\ ;\ b-\ ;\ b-\ ;\ ep-\ ;\ en+}_{p_M}\ \{P_{G0}\}$$

Stabilité de la phase de quiescence

$$P_{G0} \equiv P_{G1}[gf \leftarrow gf-1] \quad \text{et} \quad \mathtt{AG}(gf=0) \implies \mathtt{AF}(P_{G0})$$

Pre-screening of relevant models before formalising the phases

Elementary Hoare triple of the canonical cell cycle

and

Insensitivity of part of the cell cycle to growth factors

and

Stability of the quiescent phase

↓

16 pre-selected models / $\approx 10^{20}$ models

TotemBioNet: $\approx 850ms$

Pre-screening of relevant models before formalising the phases

Elementary Hoare triple of the canonical cell cycle

and

Insensitivity of part of the cell cycle to growth factors

and

Stability of the quiescent phase

$\downarrow$

Heuristics:

- Snoussi's condition
- Constraints on the $K_{v,\omega}$ domain

16 pre-selected models / $\approx 10^{20}$ models

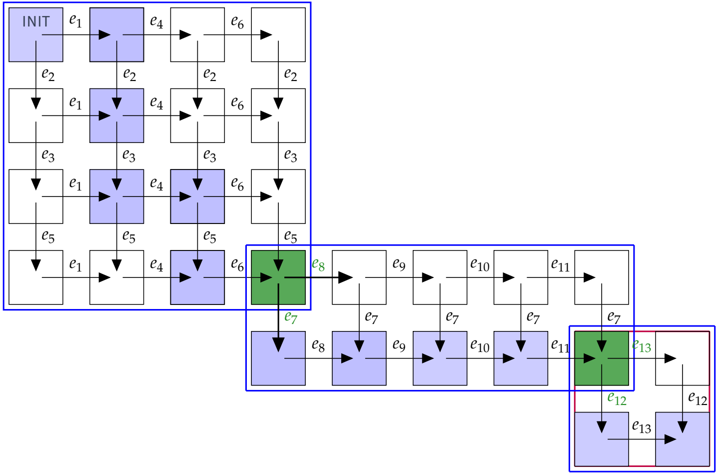
TotemBioNet : $\approx 850ms$

Our key concepts: toy example of three phases and two variables

π_1

π_2

π_3



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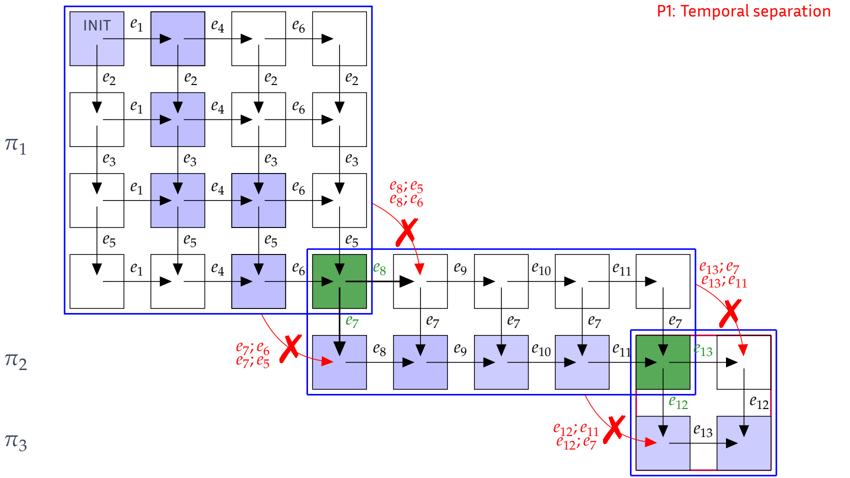

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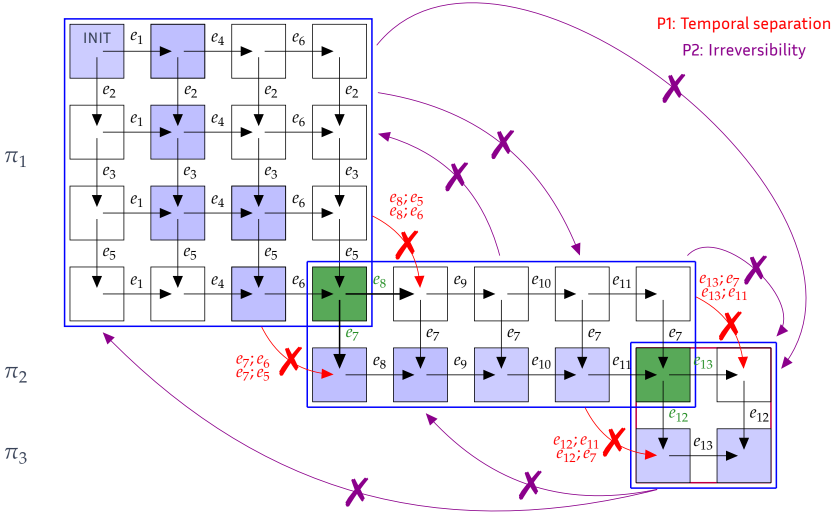

The domain of a cell cycle phase

$$\frac{\text{Hyper-pavement of } H \equiv \{P\}\, p\, \{Q\}}{p \;:\; v+ \;\; or\; v-}$$

e.g. $H_{G1} \equiv \{P_{G1}\}\, sk+; en-; sk+ \; \{Q_{G1}\}$

The domain of a cell cycle phase

Hyper-pavement of $H \equiv \{P\}\, p\, \{Q\}$

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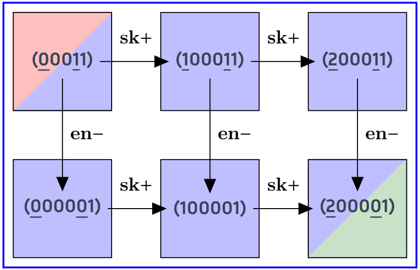
$\eta_{P_{G1}}$



$\eta_{Q_{G1}}$(Checkpoint state)

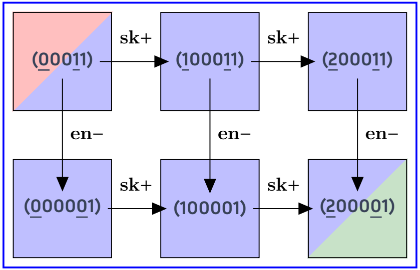
The domain of a cell cycle phase

Hyper-pavement of $H \equiv \{P\}\, p\, \{Q\}$

$p\ :\ v+\ or\ v-$

e.g. $H_{G1} \equiv \{P_{G1}\}\, sk+; en-; sk+\ \{Q_{G1}\}$

$\eta_{P_{G1}}$



$\eta_{Q_{G1}}$ (Checkpoint state)

Counting and bounding:

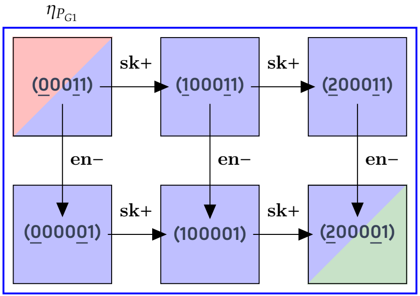
- $min_v^H = max(0, \eta(v) - \sharp_v^-)$
- $max_v^H = min(b_v, \eta(v) + \sharp_v^+)$

The domain of a cell cycle phase

Hyper-pavement of $H \equiv \{P\}\, p\, \{Q\}$

$p\ :\ v+\ or\ v-$

e.g. $H_{G1} \equiv \{P_{G1}\}\, sk+; en-; sk+\ \{Q_{G1}\}$



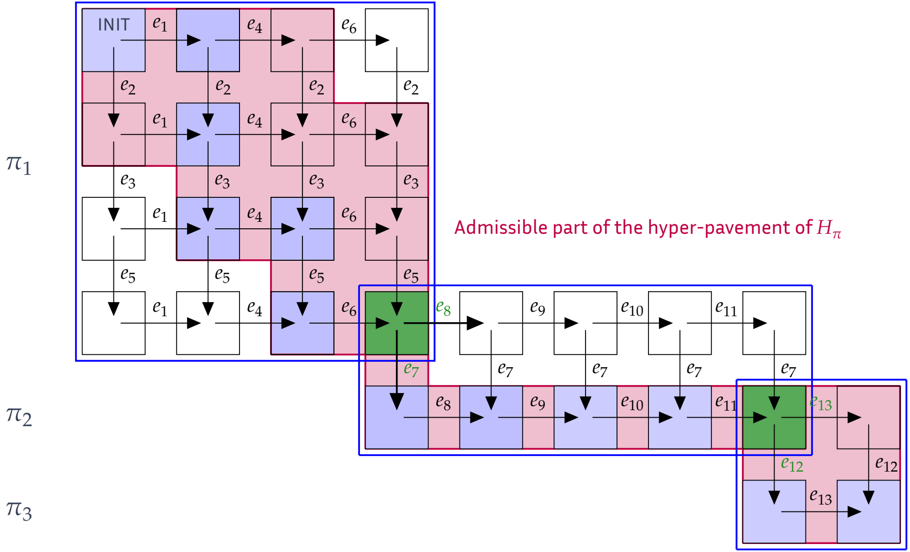
Counting and bounding:

- $min_v^H\ = max(0, \eta(v) - \sharp_v^-)$
- $max_v^H\ = min(b_v, \eta(v) + \sharp_v^+)$

Characteristic property of the hyper-pavement of H:

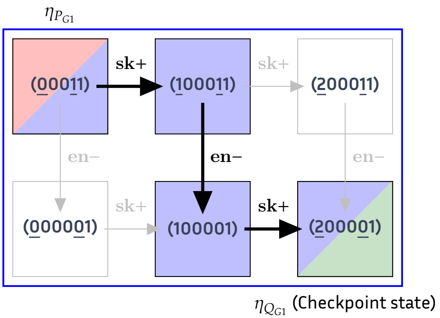
- $\psi_H \equiv \bigwedge_{v \in V} (v \geqslant min_v^H\ \wedge v \leqslant max_v^H)$
- $\psi_{H_{G1}} \equiv (sk \geqslant 0 \wedge sk \leqslant 2) \wedge (en \geqslant 0 \wedge en \leqslant 1)$
  $\wedge\ (a = 0 \wedge b = 0 \wedge ep = 0 \wedge gf\ = 1)$

Our key concepts: toy example of three phases and two variables



The domain of a cell cycle phase

Part of H_{G1} admissible by $\mathscr{G}_c$ and σ



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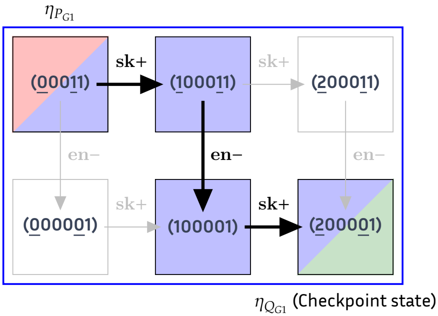

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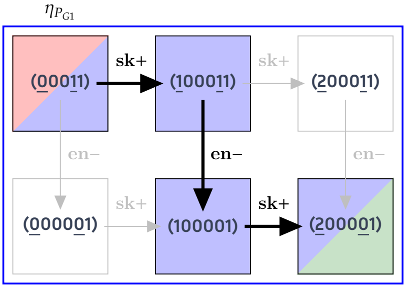
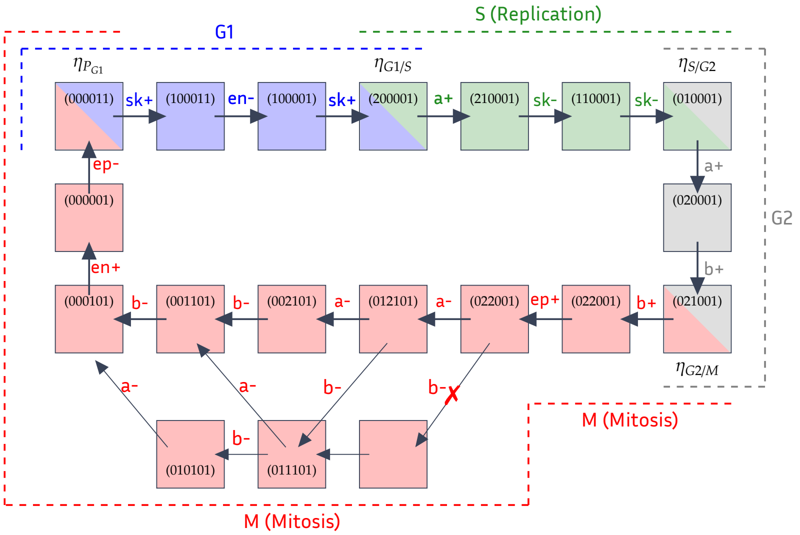


$\eta_{Q_{G1}}$ (Checkpoint state)

$$p' \in permutations(p) \quad and \quad \sigma \vDash \underbrace{wp(p', Q)}$$

$$(P_{G1}) \wedge$$
$$\underbrace{(K_{sk,\{no\_a,restrict\_gf\}} > 0)}_{sk+} \wedge$$
$$\underbrace{(K_{en,\{no\_a,no\_b\}} < 1)}_{en-} \wedge$$
$$\underbrace{(K_{sk,\{no\_a,restrict\_gf,no\_en\}} > 1)}_{sk+}$$

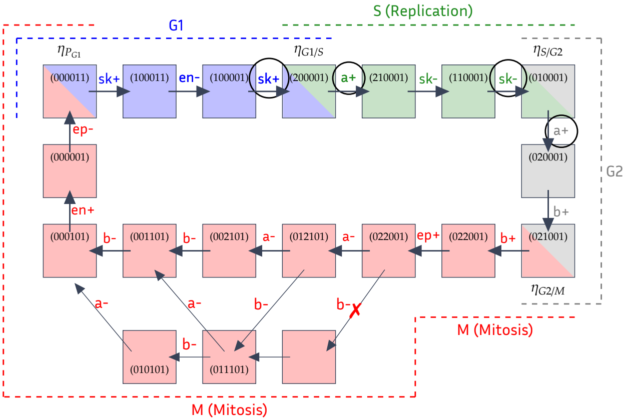
The admissible part of the hyperpavements of our canonical phases



Outline

Property 1: Temporal separation of cell cycle phases

The phase onset is blocked as long as the set of characteristic events of the previous phase has not taken place



The *temporal – separation* predicate

Given:

- $\mathscr{G}$: a BRGM
- π_i and π_{i+1}: two (portions of) adjacent canonical phases
- Σ: a set of parameterisations σ

$$\underbrace{canEnd_\sigma(E, \pi_i)} \quad \wedge \quad \underbrace{canStart_\sigma(E', \pi_{i+1})} \implies isRequired_\sigma(E, E')$$

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$$\forall i \in [\![1,5]\!],\quad temporal - separation(\pi_i, \pi_{i+1}) \quad \Rightarrow \quad \exists \sigma \in \Sigma \mid \forall E, \forall E',$$

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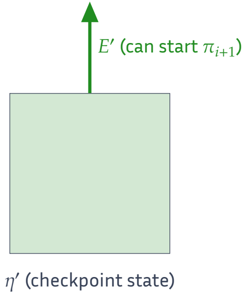
Constraint for the temporal separation between two phases



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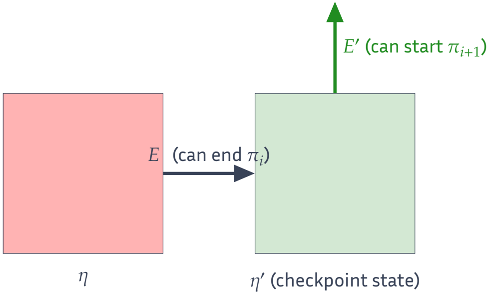

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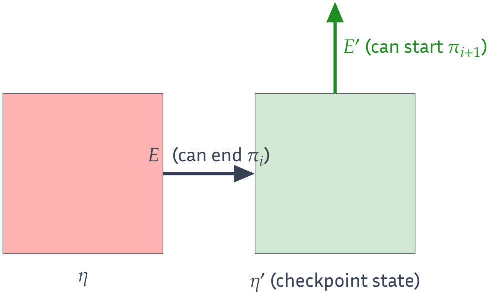

$$isRequired_{\sigma}(E, E') \ :- \qquad \underbrace{[\sigma(K_{v',\omega'}) - \eta'(v')]}_{E' \text{ is admitted from } \eta'.}$$

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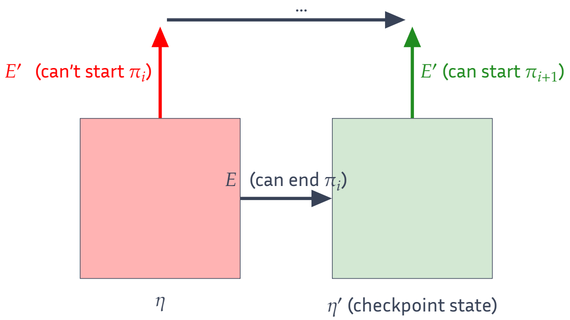

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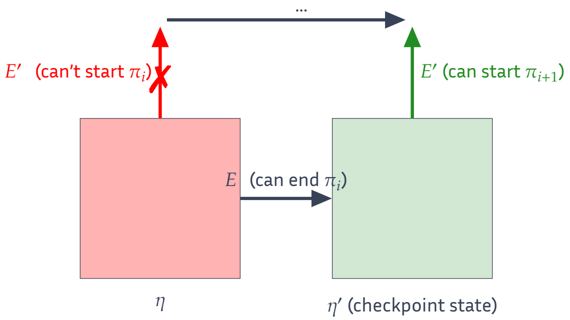

$$isRequired_\sigma(E, E') \ :- \ \underbrace{[\sigma(K_{v',\omega}) - \eta(v')]}_{E' \text{ is not admitted from } \eta} \qquad \underbrace{[\sigma(K_{v',\omega'}) - \eta'(v')]}_{E' \text{ is admitted from } \eta'.}$$

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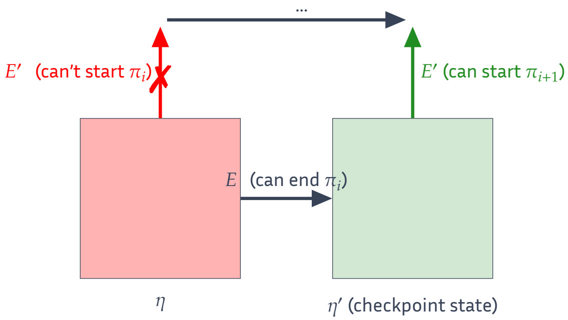

$$isRequired_{\sigma}(E, E') \;:-\; \underbrace{[\sigma(K_{v',\omega}) - \eta(v')]}_{E' \text{ is not admitted from } \eta} \times \underbrace{[\sigma(K_{v',\omega'}) - \eta'(v')]}_{E' \text{ is admitted from } \eta'.} \leqslant 0$$

The resulting Horn clause can be implemented straight in `Prolog`

$$temporal - separation(\mathscr{G}, \Sigma, H_{\pi_i}, H_{\pi_{i+1}}, E, E') :-$$

$$canEnd(\mathscr{G}, \Sigma, H_{\pi_i}, E),\ canStart(\mathscr{G}, \Sigma, H_{\pi_{i+1}}, E'), isRequired(\mathscr{G}, \Sigma, H_{\pi_i}, H_{\pi_{i+1}}, E, E').$$

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Predicate arguments:

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$$\boxed{findall([E, E'],\ temporal-separation(\mathscr{G}, \Sigma, H_{\pi_i}, H_{\pi_{i+1}}, E, E'))}$$

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avec :

$$peutInitier(\mathscr{G},\Sigma,H,E)\ :-\ \underbrace{isAdmitted(\mathscr{G},\Sigma,H,p')}_{\texttt{TotemBioNet : }|\texttt{OK-models}|>0}\ ,\ premier(p',E).$$

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First part of our proof of concept

π_i/π_{i+1}	E	E'	$\lvert\sigma\rvert \vDash temporal - separation(\pi_i, \pi_{i+1})$	Computation time
$G1/S$	$\{sk+\}$	$\{a+\}$	16/16	2.1 sec
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$G2/M$	$\{b+\}$	$\{b+\}$	16/16	1h12min

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A value of a parameter $K_{v,\omega}$ (admitted by G_c) has been discarded

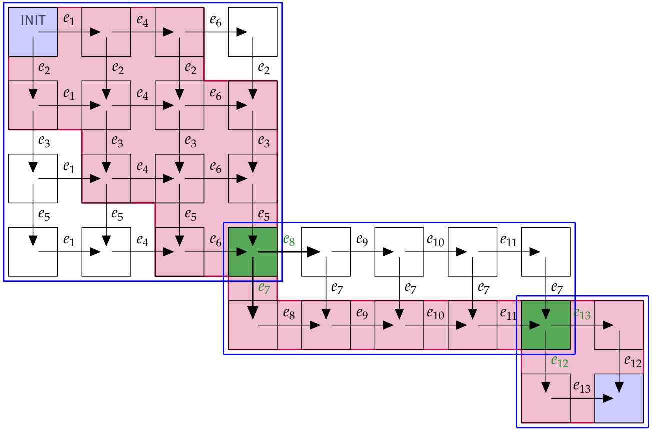
Property 2: Irreversibility of phase transitions

There is no path between two adjacent phases that goes to a third phase

π_1

π_2

π_3



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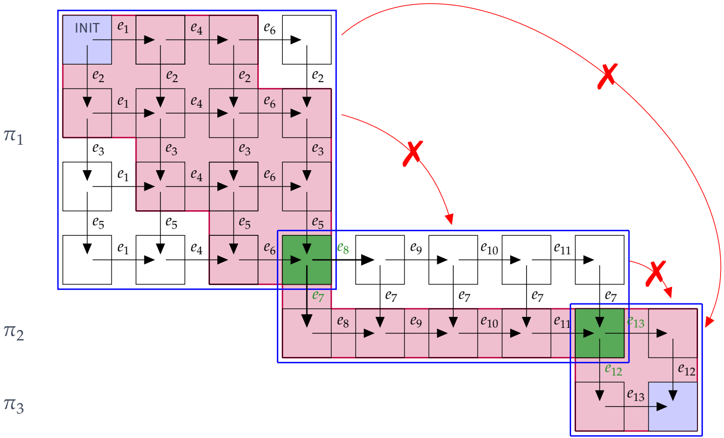

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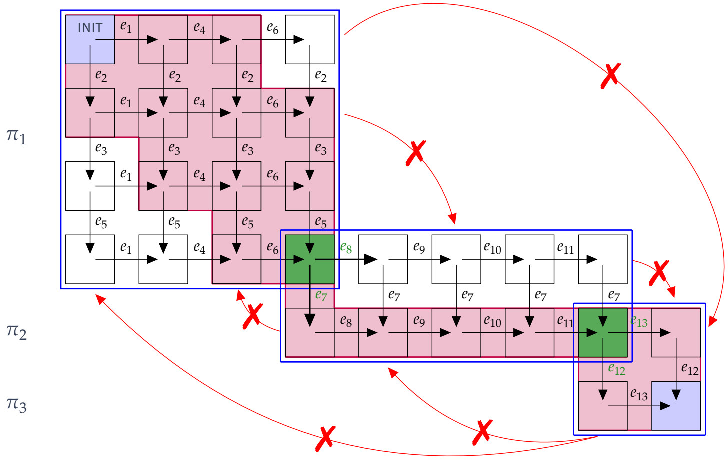

Second part and thus validation of our proof of concept

The checkpoints of the cell cycle are satisfiable iff : $\forall i \in [\![1,3]\!],\ \exists \sigma \in \Sigma \mid$

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∗ A feedback loop of conditionally stable circuits drives the cell cycle from checkpoint to checkpoint, Deritei et al., 2019, Nature

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8/8 models

Two premises:

$\mathrm{AG}(gf = 1)$ ou

$\mathrm{AG}(gf = 0)$

* *A feedback loop of conditionally stable circuits drives the cell cycle from checkpoint to checkpoint, Deritei et al., 2019, Nature*

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- Extrinsic checkpoints and metabolic checkpoints (anti-cancer therapeutic strategies)

End of the talk

Thank you for your attention!

Appendices

Parameterisations of a BRGM $\mathscr{G}$

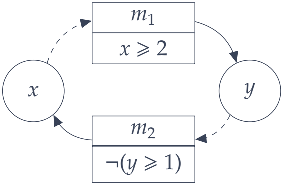
State		Resources		Parametrisations	
x	y	ω_x	ω_y	$\sigma(K_{x,\omega_x})$	$\sigma(K_{y,\omega_y})$
0	0	m_2	$\emptyset$	1	0
0	1	$\emptyset$	$\emptyset$	0	0
1	0	m_2	m_1	1	1
1	1	$\emptyset$	m_1	0	1

Wink (Boolean network):

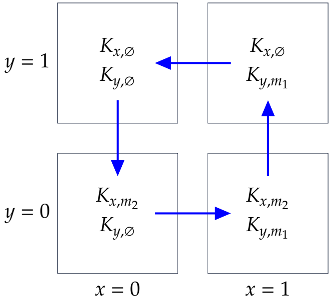
$$f_y : x$$

$$f_x : \neg y$$

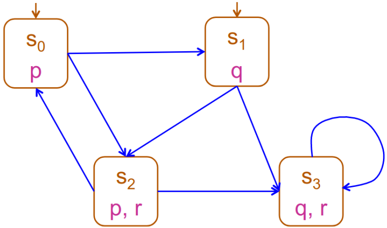
Here, a model is a couple $(\mathscr{G}, \sigma)$

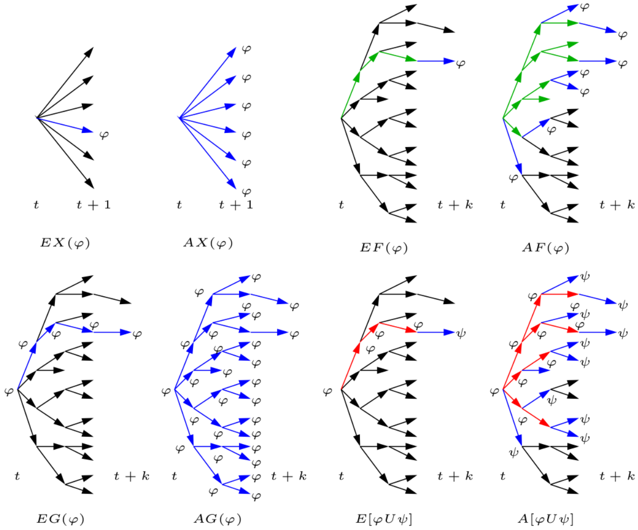


$\mathscr{K}$ and σ →

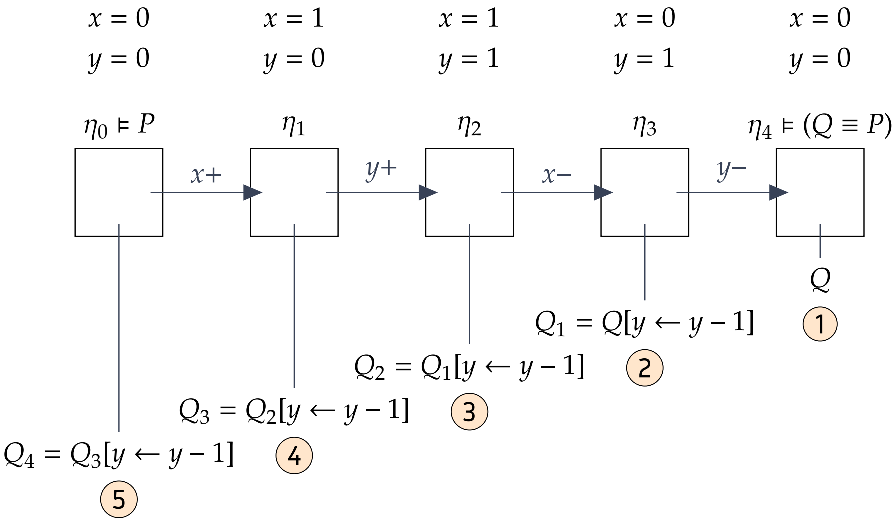


Model-checking of CTL formulas



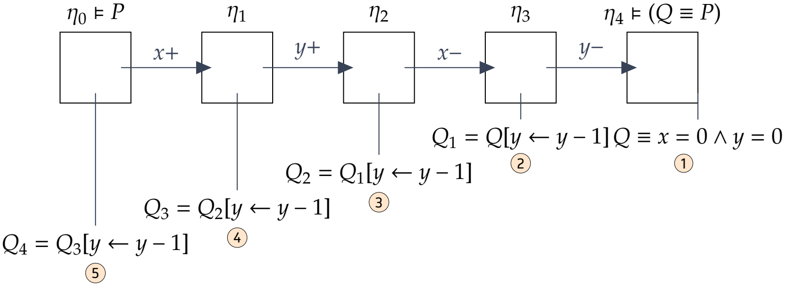


Hoare logic (Tony Hoare and Edsger Dijkstra)



Elementary Hoare triple: $\{P\}\ p\ \{Q\}$, axioms of path language: $v+$ and $v-$

Hoare's genetically modified logic



2 $x = 0 \wedge y = 1 \wedge K_{y,\omega\ y_{\eta_3}} < 1$

3 $x = 1 \wedge y = 1 \wedge (K_{y,\omega\ y_{\eta_3}} < 1) \wedge (K_{x,\omega\ x_{\eta_2}} < 1)$

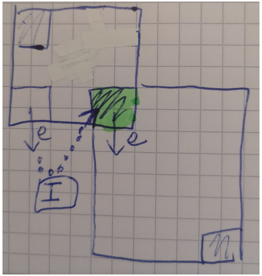
4 $x = 1 \wedge y = 0 \wedge (K_{y,\omega\ y_{\eta_3}} < 1) \wedge (K_{x,\omega\ x_{\eta_2}} < 1) \wedge (K_{y,\omega\ y_{\eta_1}} > 1)$

5 $\underbrace{x = 1 \wedge y = 0 \wedge (K_{y,\omega\ y_{\eta_3}} < 1) \wedge (K_{x,\omega\ x_{\eta_2}} < 1) \wedge (K_{y,\omega\ y_{\eta_1}} > 1) \wedge (K_{x,\omega\ x_{\eta_0}} > 1)}_{wp(p,Q)}$

Axioms of incrementing and decrementing, constraint extension ($\wedge$)

The irreversibility of transitions between two phases does not imply their temporal separation

Counter-example approach:



Furthermore, if the temporal separation is false then the irreversibility of the phase transitions is false

Introduction of priorities in the René Thomas formalism

- **Multi-system modeling**: particularly relevant for two-speed systems

- **Example**: the coupling between the cell cycle and the metabolism regarding the metabolic chekpoints
  - Frequency of ATP and NADH oscillations?
  - Frequency of oscillations of cyc/cdk? complexes (Jonathan Behaegel's PhD)

- Fauré *et.al.*, 2006, Dynamical analysis of a generic boolean model for the control of the mammalian cell cycle, Bioinformatics

- Fauré *et.al.*, 2009, Modular logical modelling of the budding yeast cell cycle, Mol. BioSyst.

Introduction of priorities in the René Thomas formalism

A biological regulatory graph enriched in multiplexes and priorities (BRGMP) is a quadruplet $\mathscr{G}_p = (V, M, E, R)$ where:

- V is a finite set of variables v with a bound $b_v \in \mathbb{N}$.
- M is a finite set of symbols m with a formula φ_m on the language $\mathscr{L}$ defined inductively by :
  - for all variable v ∈ V and for all integer n ∈ $\mathbb{N}$ such that n $\leqslant b_v$, $(v \geqslant n) \in \mathscr{L}$,
  - if $(\varphi_1, \varphi_2) \in \mathscr{L}^2$ then $\neg\varphi_1$ and $(\varphi_1 \wedge \varphi_2) \in \mathscr{L}$.
- E is the set of edges where E is included in $M \times V$. Let $m \to v$ be an element of E and $E^{-1}(v) = \{m \in M \mid (m \to v)\}$ the set of predecessors of v.
- R is a set of priority rules of the form $\omega_1, \neg\omega_2 \to v$ where v ∈ V and ω_1 and ω_2 are disjoint subsets of $E^{-1}(v)$.

Consequences on dynamics? Pseudo-asynchronism

Introduction of priorities in the René Thomas formalism

A picture is worth a thousand words:

Poster Priorité Modelife