A discrete modelling study devoted to the formalization and verification of mammalian cell cycle checkpoints.

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Public Lifeware Seminar

March 29, 2022

Context: pluridisciplinary research project

DIGITAL SYSTEMS FOR HUMANS
GRADUATE SCHOOL AND RESEARCH

UNIVERSITÉ CÔTE D'AZUR

Understanding the interactions between oscillating biological systems

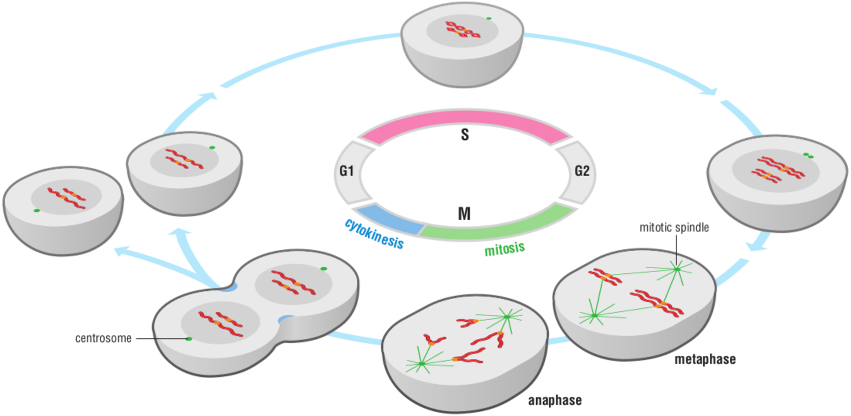
i3S sophia antipolis

SPARKS Team
Gilles Bernot and Jean-Paul Comet

institut Valrose Biologie

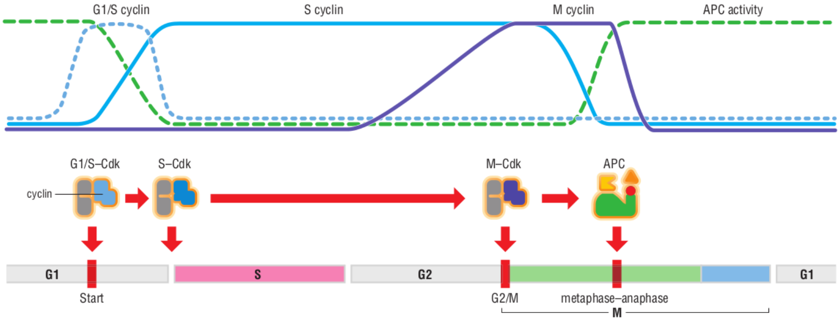
Franck Delaunay Team

Mammalian cell cycle



The Cell Cycle
Principles of Control - D. Morgan - Primers in Biology. 2006.

Molecular regulators of the cell cycle



The Cell Cycle - Principles of Control - D. Morgan - Primers in Biology. 2006.

- **G1/S cyclin/Cdk**: cycD/Cdk4-6, cycE/Cdk2
- **S cyclin/Cdk**: cycA/Cdk2-1
- **M cyclin/Cdk**: cycB/Cdk2-1
- **APC**: APC-cdh1, APC-cdc20

What is a cell cycle phase?

A phase π_i

- is a sequence of events of the form $e_1, \ldots, e_n$
- which connects an initial η_i and a final state η_f

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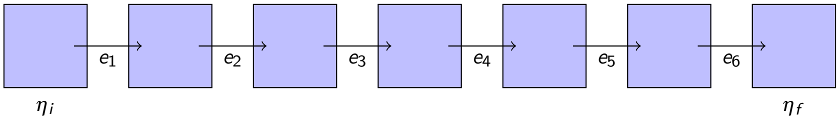

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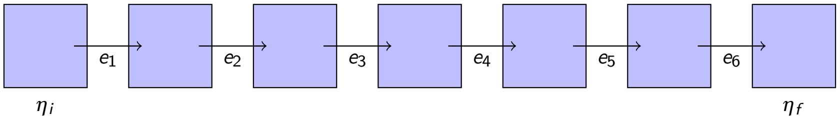

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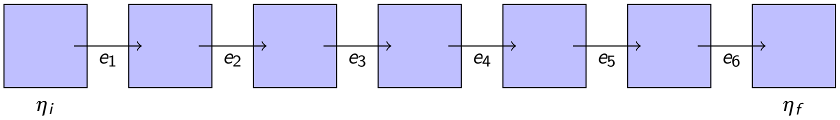

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2. **Its hyper-rectangle**: set of all permutations of the canonical sequence

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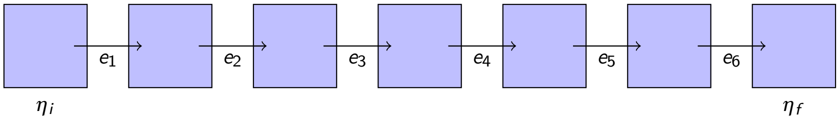
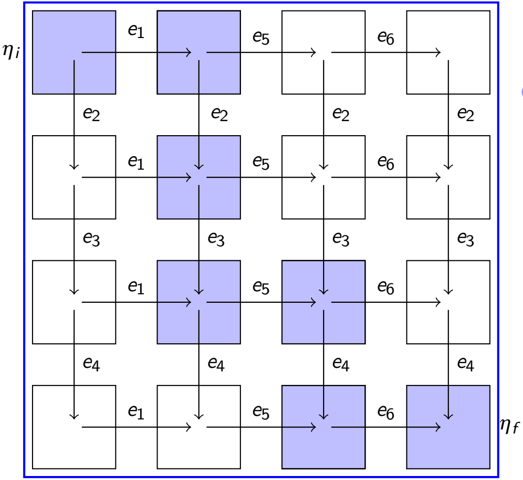


1. **Canonical phase**: a given *consensus* sequence of events
2. **Its hyper-rectangle**: set of all permutations of the canonical sequence
3. **Admissible subset**: permutations observed within the *biological* systems (formalized by a *mathematical* model)

A canonical phase and its hyper-rectangle



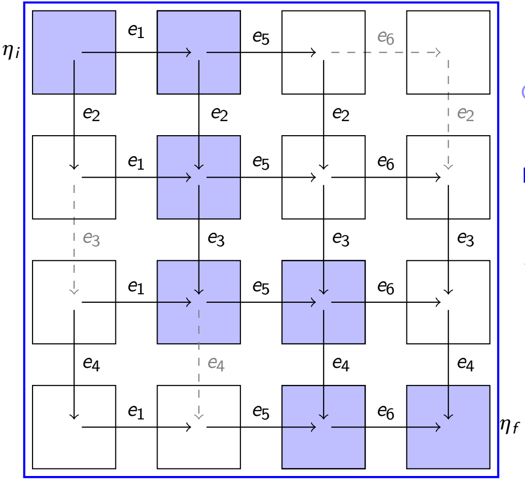
Canonical phase

Hyper-rectangle

$3 \rightarrow$: e_1, e_5, e_6

$3 \downarrow$: e_2, e_3, e_4

If some events are not biologically admissible ...



Canonical phase

Hyper-rectangle

$3 \rightarrow \; : \; e_1, e_5, e_6$

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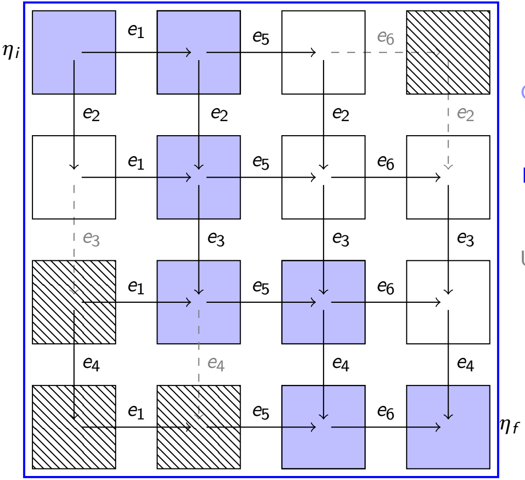
Thus some states are unreachable from the initial state of the phase

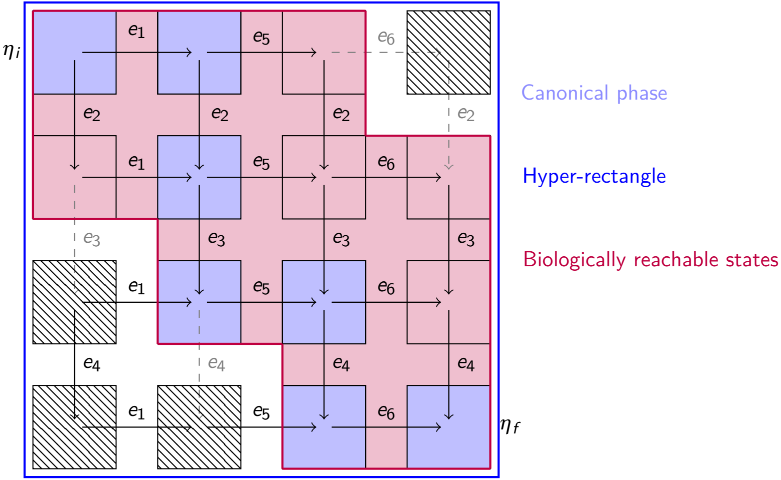


Canonical phase

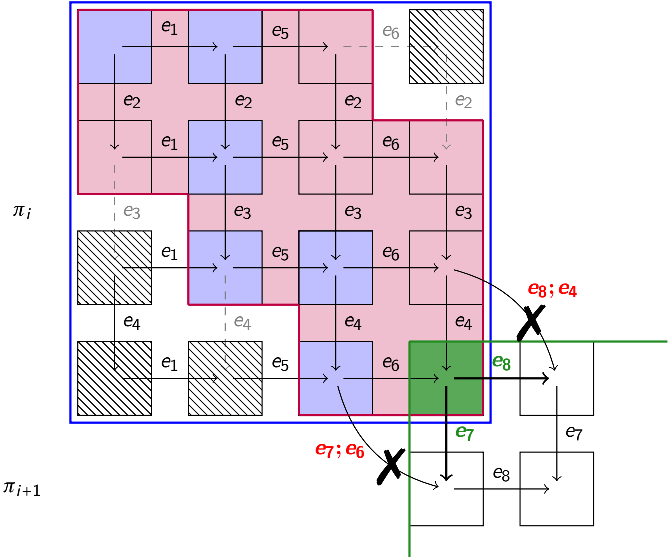
Hyper-rectangle

Unreachable states from η_i

Admissible subset



Checkpoint between two adjacent phases



Formalization of a checkpoint between two-adjacent phase

1. No permutation allowed: events that *canEnd* π_i and those that *canStart* π_{i+1}
2. Permutations of events admitted by the *cell cycle model*

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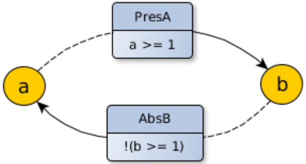
René Thomas modelling framework and Genetically modified Hoare logic

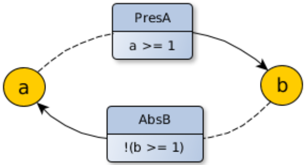
The René Thomas formalism



Biological Regulatory Graph with Multiplex $\mathcal{G} = (V, M, E)$

- V: a finite set of variables v together with a bound $b_v \in \mathbb{N}^*$
- M: a finite set of multiplexes m labelled by a propositinal formula φ_m (atoms: $v \geq n$ where $n \in [\![0, b_v]\!]$).
- E: a set of edges where $E \subseteq M \times V$

$E^{-1}(v)$: the set of predecessors of v



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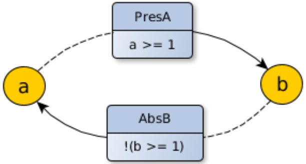
State η

substitution $\eta : V \to \mathbb{N}$ such that
$\forall v \in V,\ \eta(v) \in [0, b_v]$



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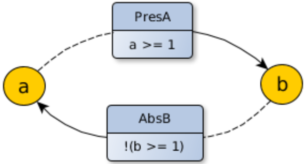
Set of resources ω of a variable v within a state η

Given a state η, ω is the set resources of v if
$\eta \models \Phi_v^\omega$ where:

$$\Phi_v^\omega \equiv \left(\bigwedge_{m \in \omega} \varphi_m \right) \wedge \left(\bigwedge_{m \in (E^{-1}(v) \setminus \omega)} \neg \varphi_m \right)$$



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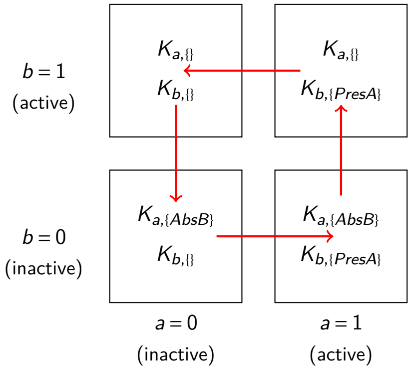
$K_{v,\omega}$ symbolizes a dynamical parameter

Parameterization σ

substitution $\sigma : \mathcal{K} \to \mathbb{N}$ such that
$\forall K_{v,\omega} \in \mathcal{K},\ \sigma(K_{v,\omega}) \in [0, b_v]$

The René Thomas' formalism



$$K_{a,\{\}} = 0, \quad K_{a,\{AbsB\}} = 1,$$
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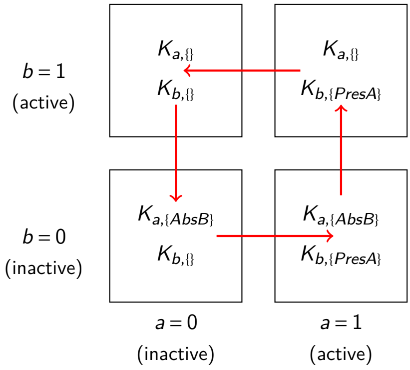

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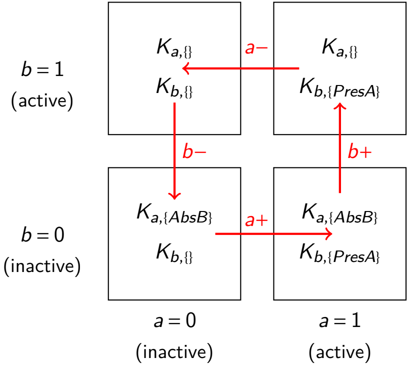
Asynchronous transitions graph

Given a parameterization σ,

- Set of vertices: set of possible states,
- there is a transition $\eta \rightarrow \eta'$ if $\exists v \in V$ such that $\sigma(K_{v,\omega}) \neq \eta(v)$ and:
  1. $\eta'(v) = \eta(v) + 1$ if $\sigma(K_{v,\omega}) > \eta(v)$
  2. $\eta'(v) = \eta(v) - 1$ if $\sigma(K_{v,\omega}) < \eta(v)$
  3. $\forall v' \in V, v' \neq v \quad \Rightarrow \quad \eta'(v') = \eta(v')$

The René Thomas' formalism



René Thomas: syntax and semantic of an event

- Change of value of a variable: $v+$ or $v-$.
- Transition between two adjacent states $\eta \rightarrow \eta'$

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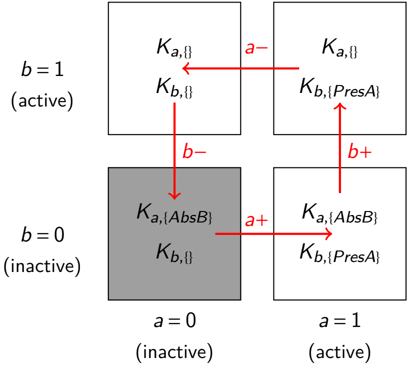

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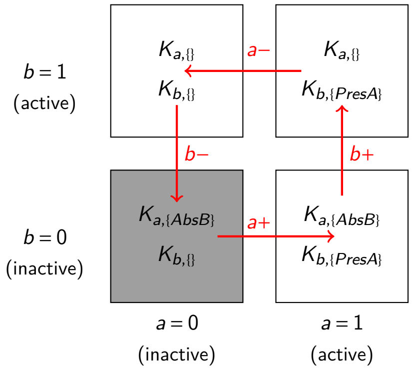

Syntax and semantic of an event

- Change of value of a variable: $v+$ or $v-$.
- Transition between two adjacent states $\eta \rightarrow \eta'$
- $a+; b+; a-, b-$

The genetically modified Hoare logic: syntax and semantics of a trace

	precondition P		path p		postcondition Q
$syntax:$	$\left\{\begin{array}{l} a=0 \\ b=0 \end{array}\right\}$ $a+$	; $b+$	;	$a-$;	$b-$ $\left\{\begin{array}{l} a=0 \\ b=0 \end{array}\right\}$

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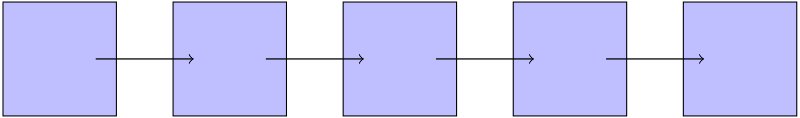

$\eta_P \models P$ $\eta_Q \models Q$

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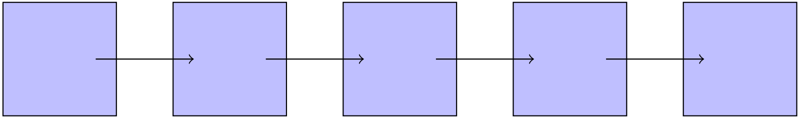

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To prove that the formalized biological trace is *admitted* by a Thomas model:

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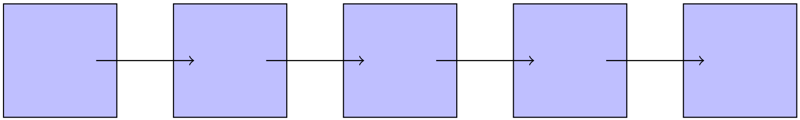

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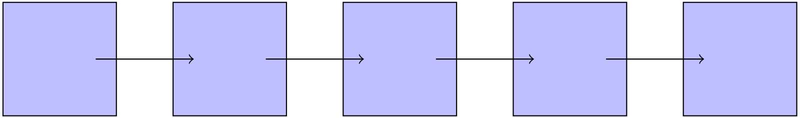

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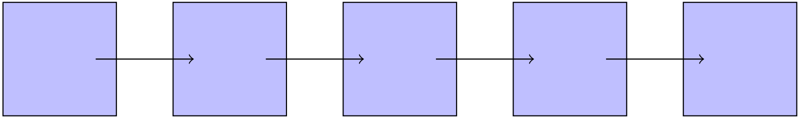

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To prove that the formalized biological trace is *admitted* by a Thomas model:

- Hoare logic proves $\{P\}\ p\ \{Q\}$ correctness
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The genetically modified Hoare logic: syntax and semantics of a trace

precondition P — *path p* — *postcondition Q*

syntax : $\left\{ \begin{array}{c} a = 0 \\ b = 0 \end{array} \right\} \quad a+ \quad ; \quad b+ \quad ; \quad a- \quad ; \quad b- \quad \left\{ \begin{array}{c} a = 0 \\ b = 0 \end{array} \right\}$

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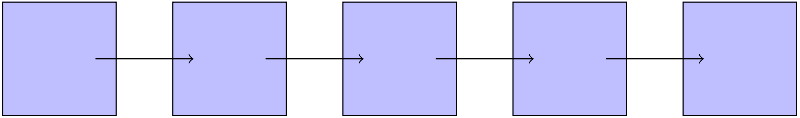

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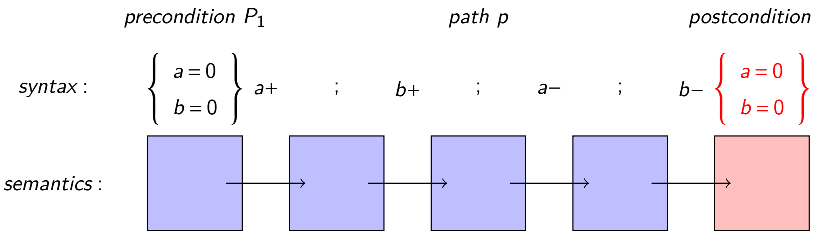
The genetically modified Hoare logic: inference rules

Hoare logic sequential composition rules

$$\frac{\{P_1\}\ p_1\ \{P_2\} \qquad \{P_2\}\ p_2\ \{Q\}}{\{P_1\}\ p_1;p_2\ \{Q\}}$$



Dijkstra: $\texttt{wp(a+;b+;a-;b-,}\ Q\texttt{)}$

The genetically modified Hoare logic: inference rules

Hoare logic sequential composition rules

$$\frac{\{P_1\}\ p_1\ \{P_2\} \qquad \{P_2\}\ p_2\ \{Q\}}{\{P_1\}\ p_1 ; p_2\ \{Q\}}$$

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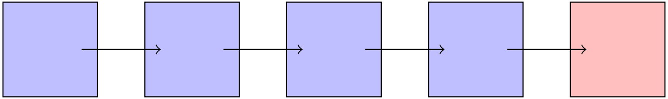

Dijkstra: $\mathtt{wp}(\mathtt{a+;b+;a-;b-},Q) = \mathtt{wp}(\mathtt{a+;b+;a-},\underline{\mathtt{wp}(\mathtt{b-},Q)})$

The genetically modified Hoare logic: inference rules

Decrementation rules

$$\frac{}{\{\Phi_v^- \ \wedge \ Q[v \leftarrow v-1]\} \quad v- \quad \{Q\}}$$

Constraint on parametrization

$$\Phi_v^- = \bigwedge_{\omega \in E^{-1}(v)} (\Phi_v^\omega \Rightarrow \underline{K_{v,\omega} < v})$$

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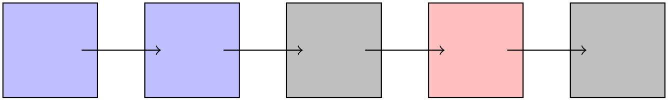
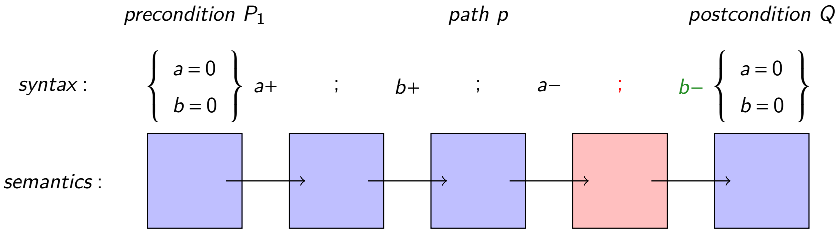


$$\texttt{wp(a+;b+;a-;b-},Q) \ = \ \texttt{wp(a+;b+;a-,}\texttt{wp(b-},Q))$$

The genetically modified Hoare logic: inference rules



$$\mathtt{wp}(\mathtt{a+};\mathtt{b+};\mathtt{a-};\mathtt{b-}, Q) = \mathtt{wp}(\mathtt{a+};\mathtt{b+};\mathtt{a-}, \{a=0 \wedge b=1 \wedge K_{b,\{\}<1}\})$$

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precondition P_1 | *path* p | *postcondition* Q

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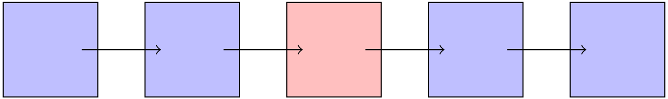

$$\mathtt{wp}(\mathtt{a+;b+;a-;b-}, Q) \quad = \quad \mathtt{wp}(\mathtt{a+;b+}, \{a=1 \wedge b=1 \wedge K_{b,\{\}<1} \wedge K_{a,\{AbsB\}<1}\})$$

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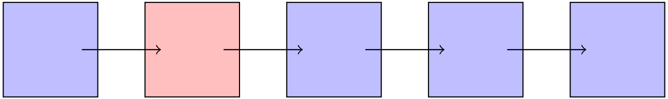

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precondition P_1 — *path* p — *postcondition* Q

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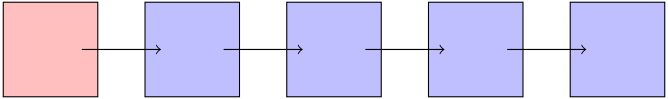

- $\texttt{wp}(p, Q) \quad = \quad \{a = 0 \land b = 0 \land K_{b,\{\}<1} \land K_{a,\{AbsB\}<1} \land K_{b,\{PresA\}>1} \land K_{a,\{\}>1}\}$

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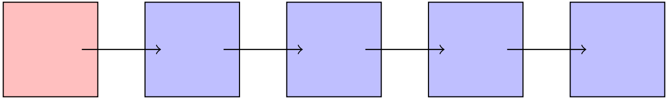
syntax : $\left\{ \begin{array}{c} a = 0 \\ b = 0 \end{array} \right\}$ $a+$; $b+$; $a-$; $b-$ $\left\{ \begin{array}{c} a = 0 \\ b = 0 \end{array} \right\}$

semantics :



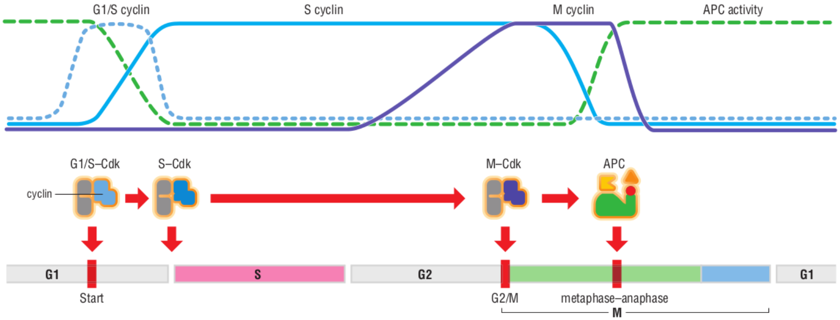
- $\mathtt{wp}(p, Q) \quad = \quad \{a = 0 \wedge b = 0 \wedge K_{b,\{\}<1} \wedge K_{a,\{AbsB\}<1} \wedge K_{b,\{PresA\}>1} \wedge K_{a,\{\}>1}\}$
- Bernot *et al.* CMSB 2015, Bernot *et al.* TCS 2019
- **HoareFol** (Folschette 2019), **TotemBioNet** (Boyenval *et al.* CMSB 2020)
- Model selection: $\sigma \quad | \quad \forall \eta_P \models P, \quad \sigma, \eta_P \models \mathtt{wp}(p, Q)$

Formalization of a checkpoint between two-adjacent phase

1. No permutation allowed: events that *canEnd* π_i and those that *canStart* π_{i+1}
2. Permutations of events admitted by the *cell cycle model*

Proof of concept

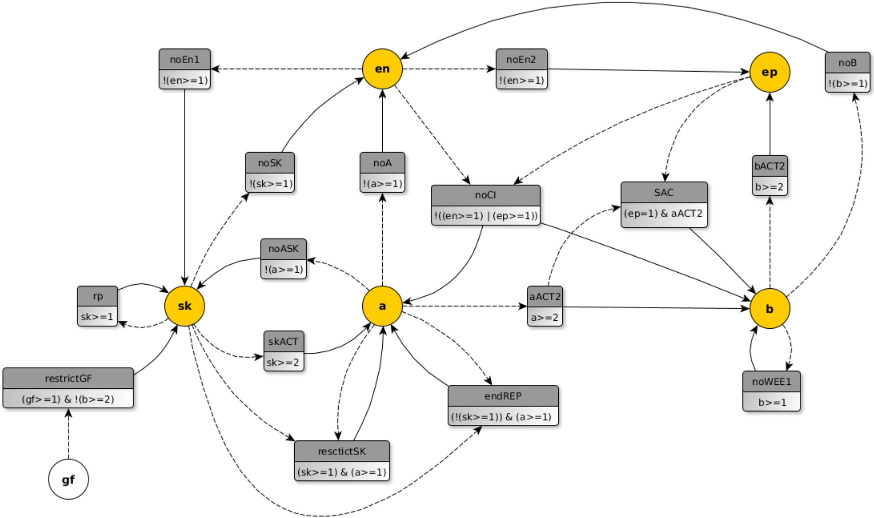
Molecular regulators of the cell cycle



The Cell Cycle - Principles of Control - D. Morgan - Primers in Biology. 2006.

- **G1/S cyclin/Cdk**: cycD/Cdk4-6, cycE/Cdk2 (variable *sk*)
- **S cyclin/Cdk**: cycA/Cdk2-1 (variable *a*)
- **M cyclin/Cdk**: cycB/Cdk2-1 (variable *b*)
- **APC**: APC-cdh1 (variable *en*), APC-cdc20 (variable *ep*).

Our updated cell cycle model reflects checkpoints

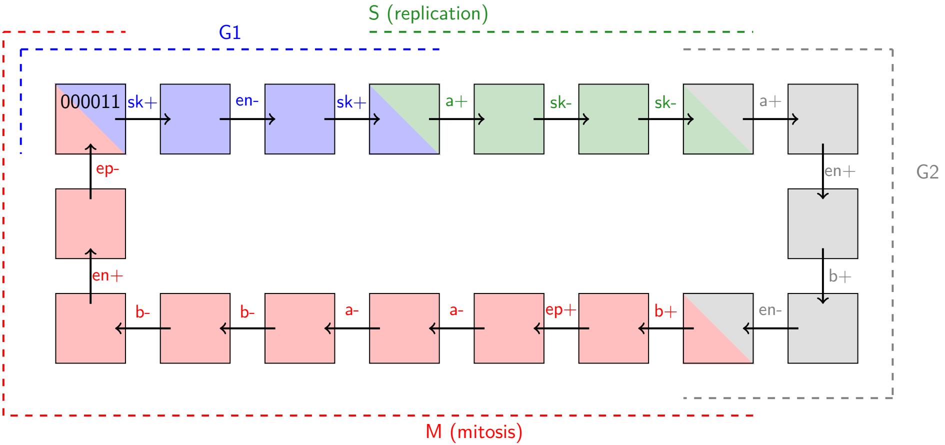


The canonical cell cycle and its phases

$$\{P_{G1}\}\quad \underbrace{sk+;en-;sk+}_{G1};\underbrace{a+;sk-;sk-}_{S};\underbrace{a+;en+;b+;en-}_{G2};\underbrace{b+;ep+;a-;a-;b-;b-;ep-;en+}_{M}\quad \{P_{G1}\}$$

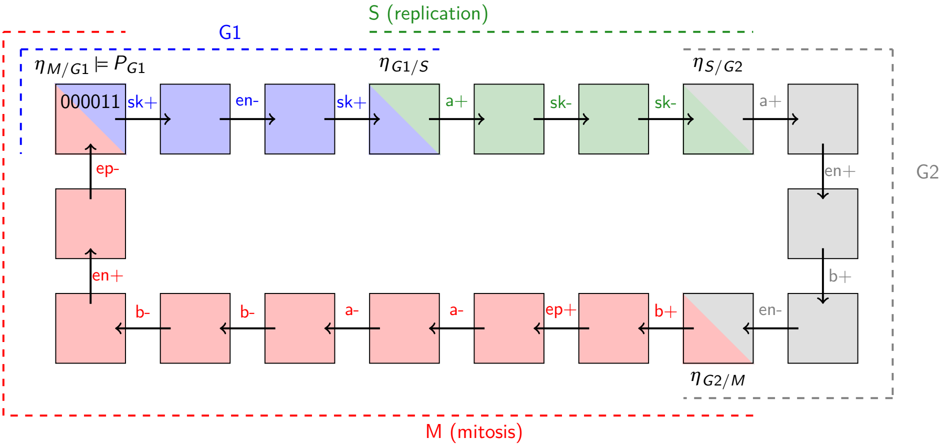
The canonical cell cycle and its phases

$\{P_{G1}\}$ $\underbrace{sk+; en-; sk+;}_{G1}$ $\underbrace{a+; sk-; sk-;}_{S}$ $\underbrace{a+; en+; b+; en-;}_{G2}$ $\underbrace{b+; ep+; a-; a-; b-; b-; ep-; en+}_{M}$ $\{P_{G1}\}$



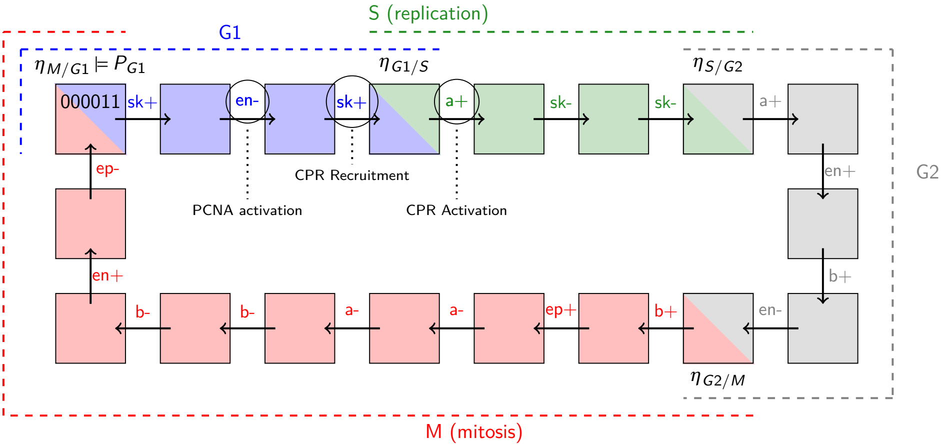
The canonical cell cycle and its phases

$$\{P_{G1}\}\quad \underbrace{sk+; en-; sk+;}_{G1}\ \underbrace{a+; sk-; sk-;}_{S}\ \underbrace{a+; en+; b+; en-;}_{G2}\ \underbrace{b+; ep+; a-; a-; b-; b-; ep-; en+}_{M}\quad \{P_{G1}\}$$

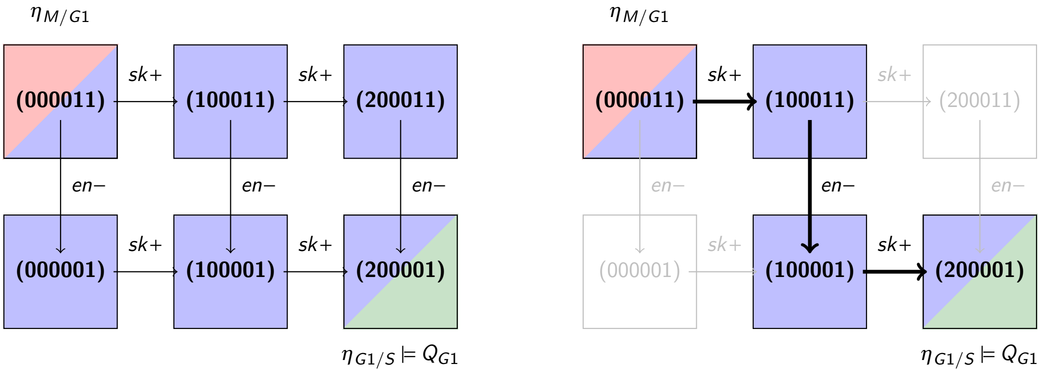


The canonical cell cycle and its phases

$$\{P_{G1}\} \quad \underbrace{sk+;en-;sk+;}_{G1}\underbrace{a+;sk-;sk-;}_{S}\underbrace{a+;en+;b+;en-;}_{G2}\underbrace{b+;ep+;a-;a-;b-;b-;ep-;en+}_{M} \quad \{P_{G1}\}$$

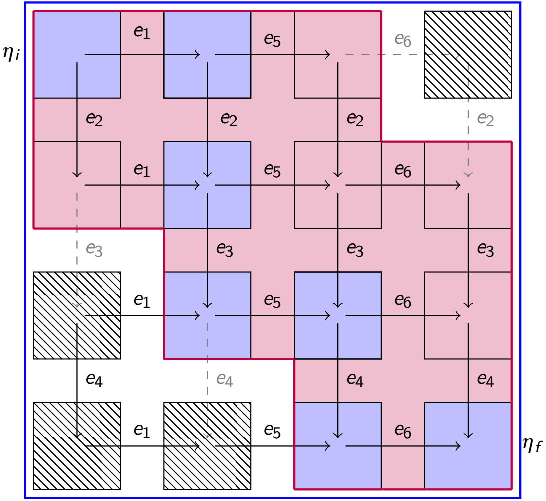


Admitted subset of the G1 hyper-rectangle



- Only the canonical path is admitted by our cell cycle model
- $\mathtt{wp}(p, Q_{G1})$ is unsatisfiable for all $p \in \{(sk+; sk+; en-), (en-; sk+; sk+)\}$

The admitted subpart of the hyper-rectangle by the model



Canonical phase

Hyper-rectangle

Biologically admitted states

canEnd and *canStart* predicates

1. Canonical phase π_i: $\{P\}\ p\ \{Q\}$
2. Hyper-rectangle
3. Admitted paths within the hyper-rectangle

canEnd and *canStart* predicates

1. Canonical phase π_i: $\{P\}\ p\ \{Q\}$
2. Hyper-rectangle
3. Admitted paths within the hyper-rectangle

$$\boldsymbol{canEnd_\sigma}(\boldsymbol{E},\boldsymbol{\pi_i}) \iff \exists\ p' \in \boldsymbol{permutations}(p) \mid (\sigma(\boldsymbol{wp}(p', Q)) \ \wedge\ E = \boldsymbol{last}(p'))$$

$$\boldsymbol{canStart_\sigma}(\boldsymbol{S},\boldsymbol{\pi_i}) \iff \exists\ p' \in \boldsymbol{permutations}(p) \mid (\sigma(\boldsymbol{wp}(p', Q)) \ \wedge\ S = \boldsymbol{first}(p'))$$

SWI Prolog

Boyenval et al. 2020, CMSB
Tool paper about TotemBioNet
`gitlab.com/totembionet/`

isRequired predicate and finally a first *checkpoint* predicate

$checkpoint(\pi_i, \pi_{i+1})$

$$\underline{\exists \sigma}$$

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$checkpoint(\pi_i, \pi_{i+1})$

$$\underline{\exists \sigma} \quad | \quad \underline{\forall \pi_i} \in [G1, S, G2, M]$$

isRequired predicate and finally a first *checkpoint* predicate

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$$\underline{\exists \sigma} \quad | \quad \underline{\forall \pi_i} \in [G1, S, G2, M], \quad \forall S, \forall E$$

isRequired predicate and finally a first *checkpoint* predicate

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$$\underline{\exists \sigma} \quad | \quad \underline{\forall \pi_i} \in [G1, S, G2, M], \quad \forall S, \forall E \ ,$$

$$\textbf{canEnd}_\sigma(E, \pi_i) \ \wedge \ \textbf{canStart}_\sigma(S, \pi_{i+1}) \Longrightarrow \textbf{isRequired}_\sigma(E, S)$$

SWI Prolog

isRequired predicate and finally a first *checkpoint* predicate

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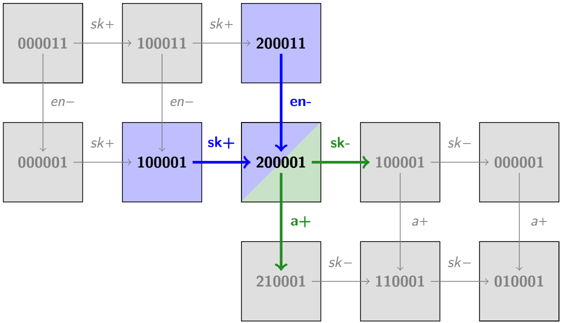
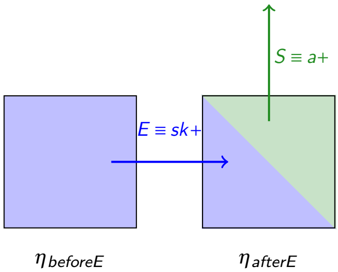
SWI Prolog



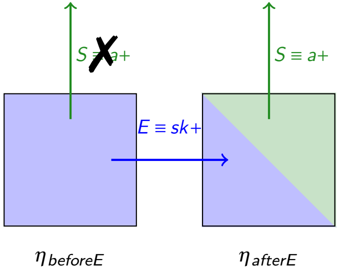
isRequired predicate and finally a first *checkpoint* predicate

$$\boldsymbol{isRequired_{\sigma}(E, S)}$$



isRequired predicate and finally a first *checkpoint* predicate

$$\boldsymbol{isRequired_\sigma(E,S)} \Leftrightarrow (\sigma(K_{v_s,\omega_{beforeE}}) - \eta_{beforeE}(v_s)) \times (\sigma(K_{v_s,\omega_{afterE}}) - \eta_{afterE}(v_s)) \leq 0$$



Does our cell cycle model reflect checkpoints? The answer

π	p_π: canonical path	P_π: precondition	Q_π: postcondition
G1	sk+,en-,sk+	$sk=0 \wedge ep=0 \wedge a=0 \wedge b=0 \wedge en=1$	P_S
S	a+,sk-,sk-	$sk=2 \wedge ep=0 \wedge a=0 \wedge b=0 \wedge en=0$	P_{G2}
G2	a+,en+,b+,en-	$sk=0 \wedge ep=0 \wedge a=1 \wedge b=0 \wedge en=0$	P_M
M	b+,ep+,a-,a-,b-,b-,en+,ep-	$sk=0 \wedge ep=0 \wedge a=2 \wedge b=1 \wedge en=0$	P_{G1}

Inputs

- Cell cycle regulations graph
- Set of parametrizations σ
- $\{P_\pi\}\ p_\pi\ \{Q_\pi\}$ $\forall \pi \in \{G1, S, G2, M\}$

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M	`b+,ep+,a-,a-,b-,b-,en+,ep-`	$sk=0 \land ep=0 \land a=2 \land b=1 \land en=0$	P_{G1}

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$\rightarrow$ `checkpoint.pl` $\rightarrow$

SWI Prolog

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Outputs

- $\sigma \models wp(p_{\pi_i}, Q_{\pi_i})$

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- Cell cycle regulations graph
- Set of parametrizations σ
- $\{P_\pi\}\ p_\pi\ \{Q_\pi\}$ $\forall \pi \in \{G1, S, G2, M\}$

→ `checkpoint.pl` →

SWI Prolog

Outputs

- $\sigma \models wp(p_{\pi_i}, Q_{\pi_i}) \wedge checkpoint(\pi_i, \pi_{i+1})$

Does our cell cycle model reflect checkpoints? The answer

π	p_π: canonical path	P_π: precondition	Q_π: postcondition
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Inputs

- Cell cycle regulations graph
- Set of parametrizations σ
- $\{P_\pi\}\ p_\pi\ \{Q_\pi\}$ $\forall \pi \in \{G1, S, G2, M\}$

→ `checkpoint.pl` →

SWI Prolog

TotemBionet

Outputs

- $\sigma \models wp(p_{\pi_i}, Q_{\pi_i}) \wedge checkpoint(\pi_i, \pi_{i+1})$
- For each σ and π:
  1. $E \mid canEnd(E, \pi)$
  2. $S \mid canStart(S, \pi)$

Does our cell cycle model reflect checkpoints? Outputs

Checkpoint	**Eval**	$\mid \sigma \models$ *checkpoint* $\mid$
G1/S	True	16/32
S/G2	True	32/32
G2/M	True	32/32
M/G1	True	32/32

Does our cell cycle model reflect checkpoints? Outputs

Checkpoint	**Eval**	$\mid \sigma \models checkpoint \mid$
G1/S	True	16/32
S/G2	True	32/32
G2/M	True	32/32
M/G1	True	32/32

π	$canStart(S,\pi)$	$canEnd(E,\pi)$
G1	S=[sk+]	E=[sk+]
S	S=[a+]	E=[sk-]
G2	S=[a+]	E=[en-]
M	S=[b+]	E=[a-,b-,en+,ep-]

Does our cell cycle model reflect checkpoints? Outputs

Checkpoint	Eval	$\mid \sigma \models checkpoint \mid$
G1/S	True	16/32
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S	S=[a+]	E=[sk-]
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0/32? Not yet!

Conclusion

Proof of concept: formalization of a checkpoint between two adjacent phases

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Formalization of a checkpoint in the case of a phase exit

1. *By excess* formalization of a phase domain using its hyper-rectangle
2. Negation of the checkpoint bypass

Enrich the approach with additional properties on cell cycle checkpoints

- Any event in S and M already realized cannot be undone
- Integration of DNA damage response pathways

Conclusion

Proof of concept: formalization of a checkpoint between two adjacent phases

Formalization of a checkpoint in the case of a phase exit

1. *By excess* formalization of a phase domain using its hyper-rectangle
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Enrich the approach with additional properties on cell cycle checkpoints

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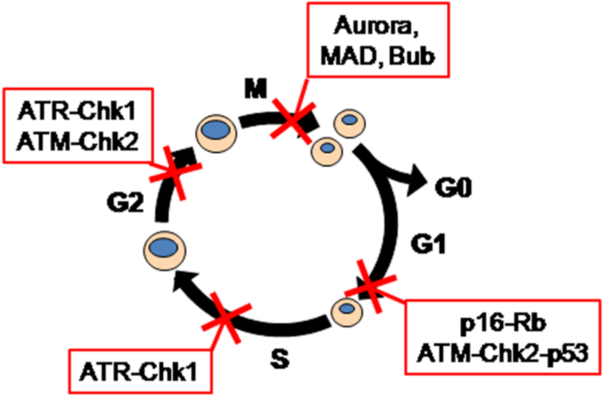
Thank you !

Integration of DNA damage response pathways



Gabrielli et al. 2012

Negation of checkpoint bypass

Tunnel phase

Given $\pi \in [G1, S, G2, M]$, the initial (resp. final) state of a phase π described by the a precondition P_π (resp. Q_π):

$$isTunnel(\pi) \iff P_\pi \Rightarrow \mathrm{A}\Big(\psi_{H_\pi} \vee \neg(\bigvee_{\pi' \neq \pi} \psi_{H_{\pi'}}) \; \mathrm{U} \; Q_\pi\Big)$$

where ψ_H is the characteristic formula of the hyper-rectangle H