Méthodes de modélisation informatique des processus cellulaires: cycle cellulaire et horloge circadienne

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EP LIFEWARE

http://lifeware.inria.fr/

BIOCHAM modeling and analysis software

http://lifeware.inria.fr/biocham4

From Biological Facts…

Biology is still mainly a **collection of facts**
Large amount of data but still few principles and processes really understood:

- Darwin's principle of **evolution and natural selection** in 1859
- Jakob and Monod's elucidation of **transcription and translation** in 1961
- **Multistability in gene networks** explaining cell differentiation, cell reprogramming
- **Oscillations** in gene or protein networks explaining cellular circadian clock, cell cycle, homeostasis, morphogenesis,…
- **Signaling** networks (e.g. MAPK) performing Analog-Digital signal conversion, noise filtering, ultrasensitivity and signal amplification

Need of formal methods to master the complexity of large interaction networks…

From Computer Science Methods…

Alan Turing created Computer Science in 1936 in the perspective of artificial intelligence:

> *« The behavior of a human doing calculations is at each instant determined by the symbols he observes and by his internal mental state »*

Church-Turing Thesis states that there is **one single notion of computation** and one notion of **universal machine**. Indeed

- Turing machines, Church's lambda calculus, Gödel's first-order logic
- Random Access Memory machines
- Fortran, C, Java, CAML, Python etc. programming languages

all have the same computational power.

According to this Thesis, any form of computation is thus necessarily representable by a program

… to Cell Processes as Computation

The behavior of a living cell is at each instant determined by the signals it observes and by its internal state.

By applying Church-Turing Thesis to biology, **cell compute**;
In the computational metaphor, a cell, a tissue, continuously compute.

The paradigm of « **Cells as Machines** » opens up the methods of Computer Science developped in the last decades for mastering the complexity of

- Circuits of trillions of transistors, programs of millions of instructions,
- World wide web of tenths of millions of web sites

to tackle the complexity of

- tenths of thousands of genes and proteins in a cell
- millions of cells.

Overview of the Course

1. **Rule-based modeling of biochemical reaction systems**
   - Syntax: Biocham notations (SBML compatible)
   - Semantics: Differential, Stochastic and Boolean interpretations of reactions
   - Examples of cell cycle control, circadian clock, gene expression

2. **Temporal Logic based formalization of biological properties**
   - *Quantitative properties* in Linear Time Logic LTL(R)
   - *Parameter search* in high dimension w.r.t. LTL(R) specifications
   - *Robustness and sensitivity analyses* w.r.t. LTL(R) specifications

3. **Coupled model of the cell cycle and circadian clock**
   - chronotherapy optimization

Computational Systems Biology

"Systems Biology aims at systems-level understanding which requires a set of principles and methodologies that links the behaviors of molecules to systems characteristics and functions."

H. Kitano, ICSB 2000

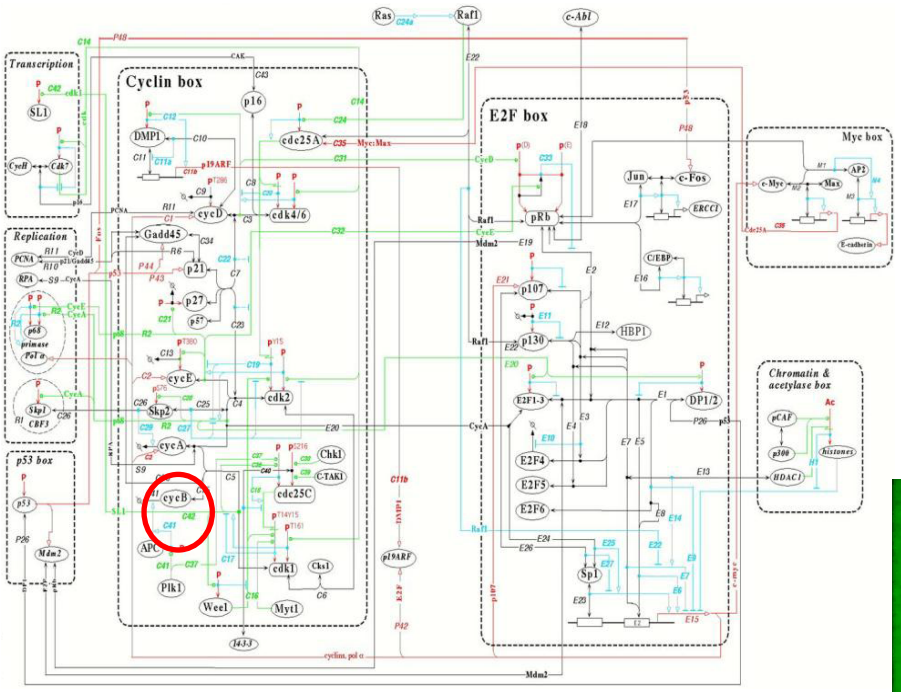
- Follow-up of the human genome project of the 90's
- Analyze post-genomic data produced with high-throughput technologies and made available in public databases like GO, KEGG, BioCyc, etc.;
- Integrate heterogeneous data about a specific problem;
- Predict the behavior of large networks of genes and proteins;
- Multi-scale models of cell processes, tissues, organisms, ecosystems…

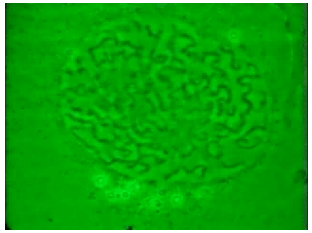
→ **Systems Biology Markup Language** (SBML): model exchange format

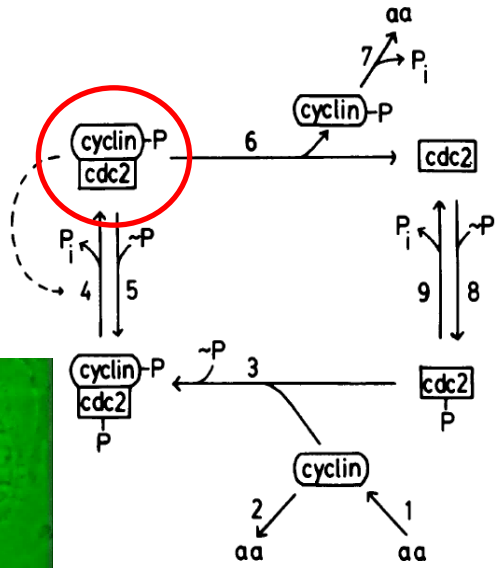
→ **SBML model repositories**: e.g. `biomodels.net` thousands of models

Biochemical reaction models

1) Models for representing knowledge : the more detailed the better
2) Models for answering questions : the more abstract the better







Mammalian cell cycle control interaction map [Kohn 1999]

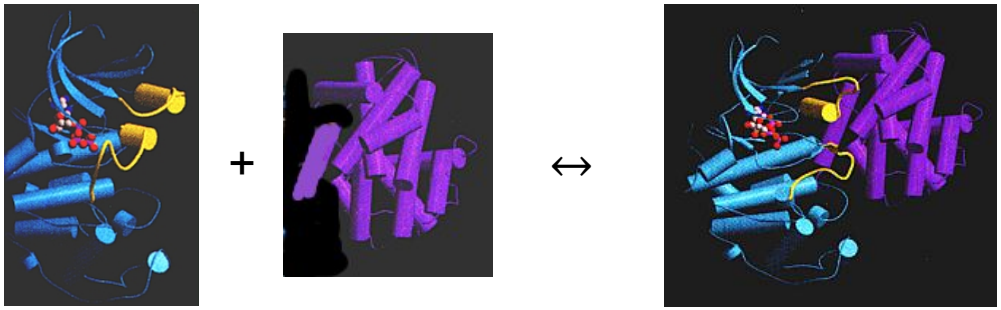
Simplified yeast cell cycle model [Tyson 1991]

Biochemical reactions

- Binding, complexation: $A + B \rightarrow C$

  $cdk1 + cycB \rightarrow cdk1cycB$

- Unbinding, decomplexation: $A \rightarrow B + C$



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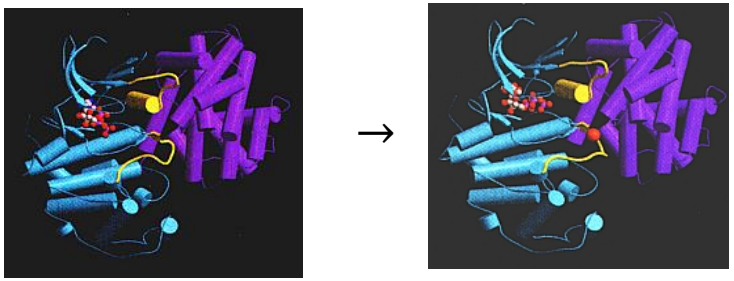
  $cdk1cycB \rightarrow cdk1cycBp$



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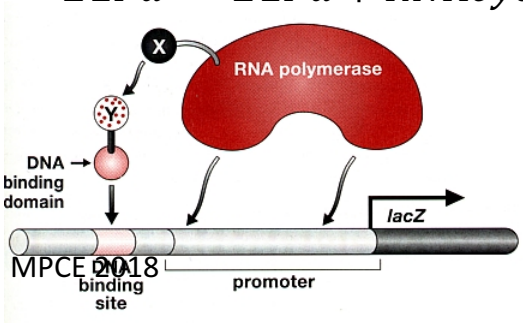
  $E2Fa \rightarrow E2Fa + RNAcycA$



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Biochemical reaction rates

Time matters

- Binding, complexation: $A + B \xrightarrow{k.A.B} C$

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- Unbinding, decomplexation: $A \xrightarrow{k.A} B + C$

- Transformation, phosphorylation, transport: $A \xrightarrow{v.A/(k+A)} B$

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Biochemical reaction rates

Time matters

Mass action law kinetics

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Michaelis Menten kinetics

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Hill sigmoid: switch like behavior

- Gene expression, synthesis:

  $E2Fa \rightarrow E2Fa + RNAcycA$

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- Degradation:

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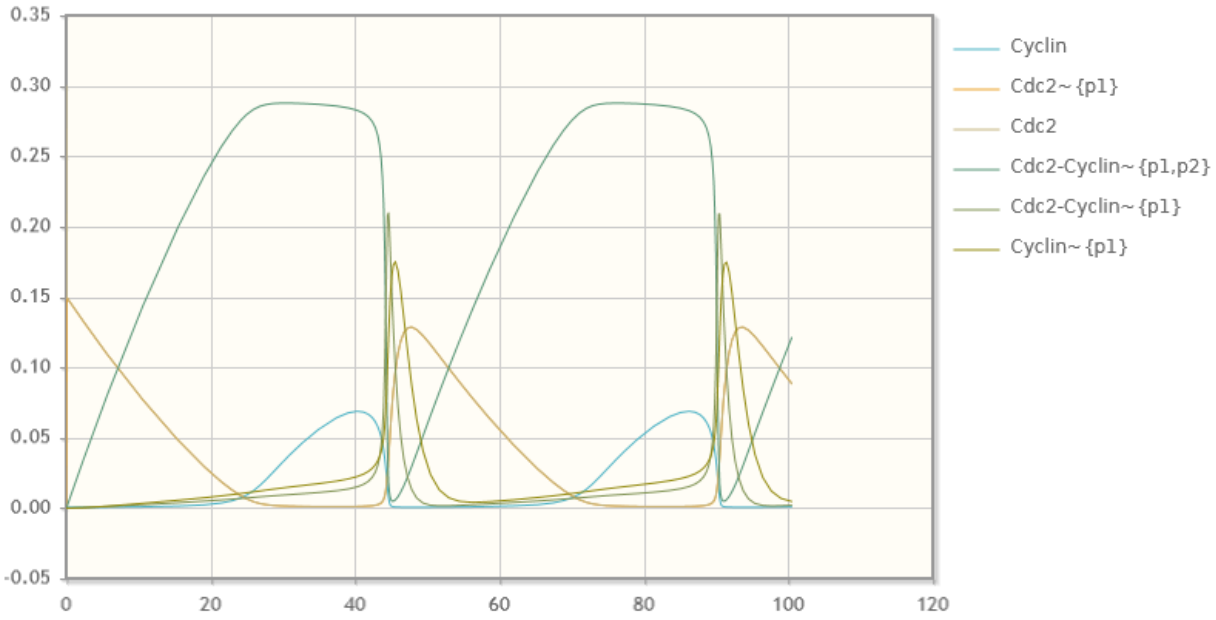
Semantics of Reaction Programs $A + B \xrightarrow{k.A.B} C$

Continuous semantics: concentrations, continuous time evolution

Ordinary differential equations (ODE) $\frac{dA}{dt} = -k.A.B \quad \frac{dB}{dt} = -k.A.B \quad \frac{dC}{dt} = k.A.B$



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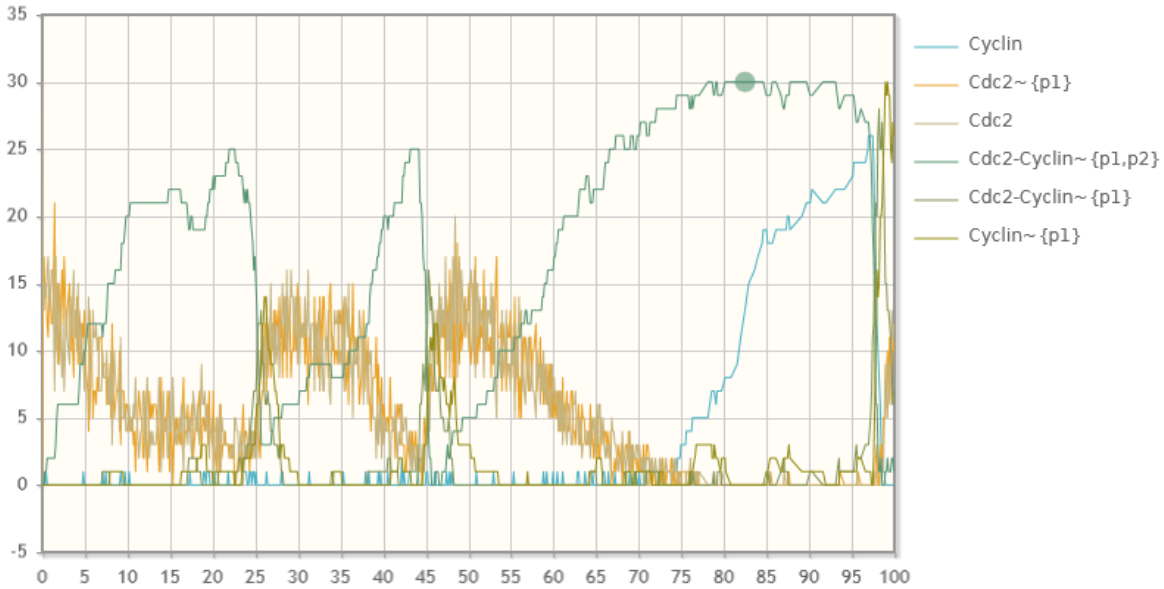
Stochastic semantics: numbers of molecules, probability and time of transition

Continuous Time Markov Chain (CTMC) $A , B \xrightarrow{p(S_i),\, t(Si)} C{+}{+}, A{-}{-}, B{-}{-}$



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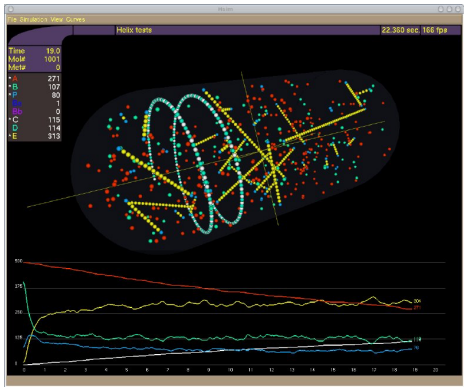
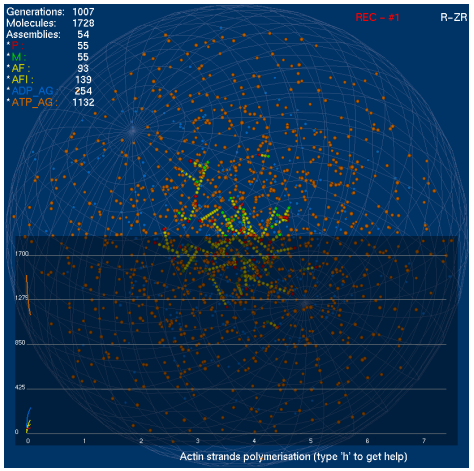
Multi-agent simulation: numbers of molecules, space, diffusion speed, affinity

Random walk (ex. Hsim simulator [Amar 04])





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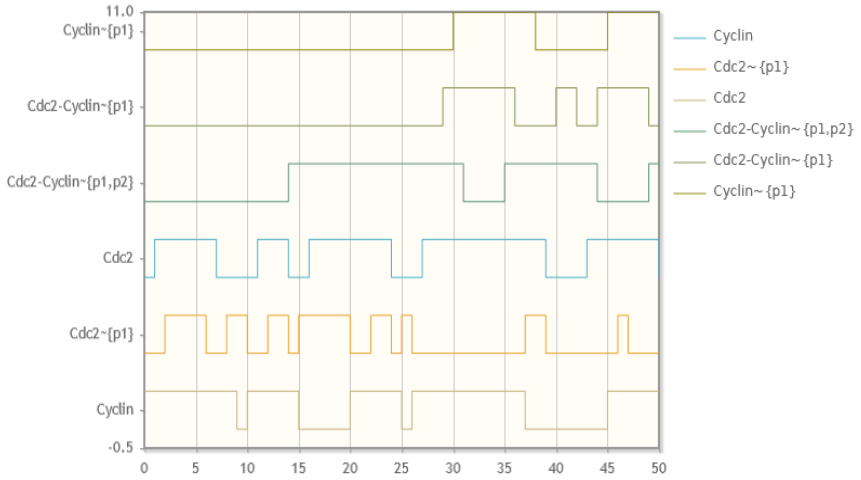
Petri net semantics: numbers of molecules $A , B \rightarrow C{+}{+}, A{-}{-}, B{-}{-}$

Multiset rewriting

CHAM [Berry Boudol 90] [Banatre Le Metayer 86]

P-systems [Paun 98]



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Multiset rewriting

CHAM [Berry Boudol 90] [Banatre Le Metayer 86]

Boolean semantics: presence/absence

$A \wedge B \rightarrow C \wedge \neg A \wedge \neg B$

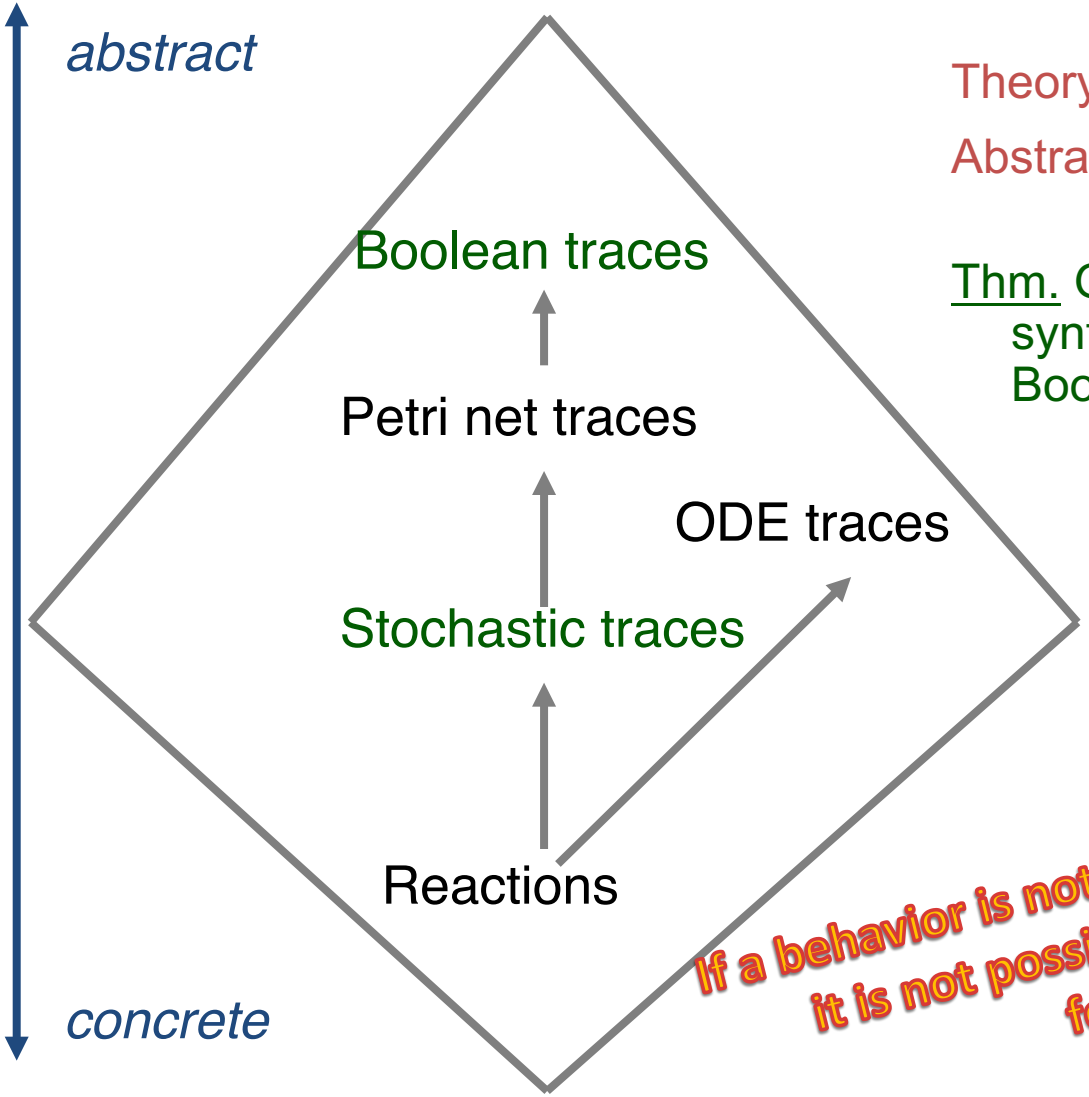
Asynchronous transition system

$A \wedge B \rightarrow C \wedge A \wedge \neg B$

$A \wedge B \rightarrow C \wedge \neg A \wedge B$

$A \wedge B \rightarrow C \wedge A \wedge B$

Hierarchy of Semantics: Abstractions



Theory of abstract Interpretation

Abstractions as Galois connections

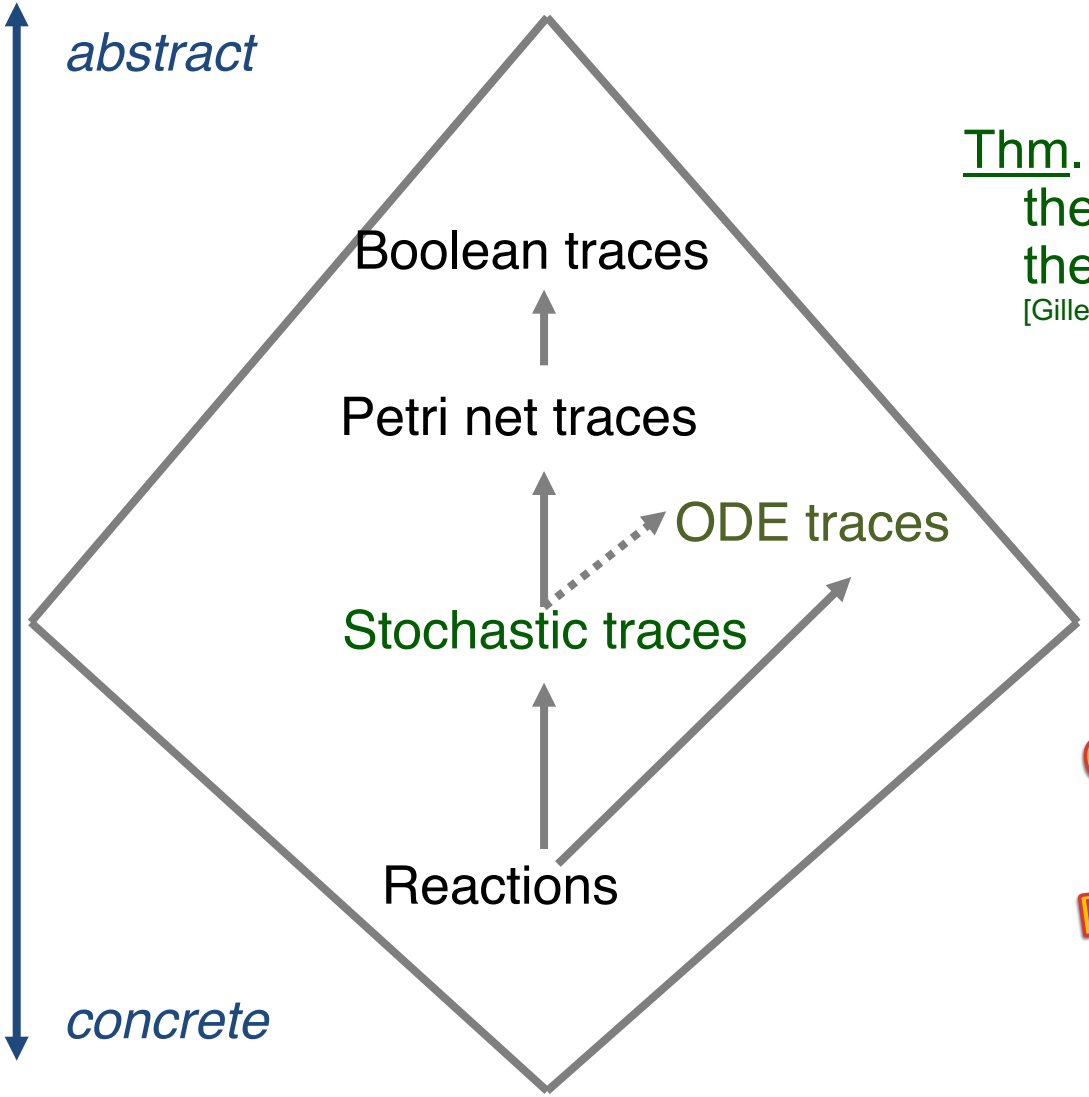
[Cousot Cousot POPL'77]

Thm. Galois connections between the syntactical, stochastic, Petri Net and Boolean semantics

[FF Soliman CMSB'06,TCS'08]

If a behavior is not possible in the Boolean semantics
it is not possible in the stochastic semantics
for any reaction rates

Hierarchy of Semantics: Approximations



Thm. Under large number conditions the ODE semantics approximates the mean stochastic behavior
[Gillespie 71]

Hot topic:
higher order moments
ODE for mean, variance,…

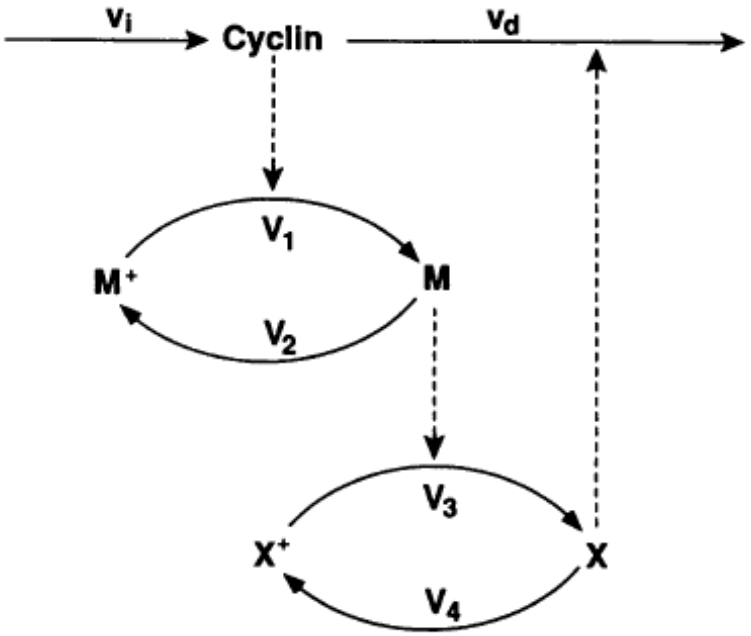
Model cell-to-cell variability
Intrinsic and extrinsic noise

Minimal Mitotic Oscillator [Goldbeter 91 PNAS]

Cyclin is synthesized at constant rate

Cyclin triggers the activation of Cdc2 kinase M (by dephosphorylation)

Cdc2 activates a protease X (by phosphorylation) that degrades the Cyclin



Demo Biocham Notebook

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Open MPCE file

Load oscillator.ipynb

Single Enzymatic Reaction

An enzyme E binds to a substrate S to catalyze the formation of product P:

$$E+S \rightarrow^{k1} C \rightarrow^{k2} E+P$$

$$E+S \leftarrow^{km1} C$$

Mass action law kinetics ODE:

$$dE/dt = -k_1ES+(k_2+k_{m1})C$$

$$dS/dt = -k_1ES+k_{m1}C$$

$$dC/dt = k_1ES-(k_2+k_{m1})C$$

$$dP/dt = k_2C$$

with two conservation laws: E+C=constant, S+C+P=constant,

Assuming $C_0=P_0=0$, we get $E=E_0-C$ and $S_0=S+C+P$

$$dS/dt = -k_1(E_0-C)S+k_{m1}C$$

$$dC/dt = k_1(E_0-C)S-(k_2+k_{m1})C$$

Quasi-Steady State Approximation

When $E<<S$, $k_1 >> k_2$ we have $dC/dt \sim 0 \sim dE/dt$

$C = E_0 S/(K_m+S)$ where $K_m=(k_2+k_{m1})/k_1$

$dP/dt = -dS/dt = V_m S / (K_m+S)$ where $V_m = k_2 E_0$

Leonor Michaelis Maud Menten 1913

Michaelis-Menten kinetics: **$V_m S /(K_m+S)$ for S => P**

Model reduction: the variables E and C are eliminated,
E is supposed constant and acting on only one substrate

Quasi-Steady State Approximation

When E<<S, k_1 >>k_2 we have dC/dt~0~dE/dt

C = $E_0S/(K_m+S)$ where $K_m=(k_2+k_{m1})/k_1$

K_m substrate concentration with half maximum velocity

dP/dt = -dS/dt = $V_mS / (K_m+S)$ where $V_m= k_2E_0$

V_m maximum velocity at saturing substrate concentration

Michaelis-Menten kinetics: $V_mS /(K_m+S)$ for S => P

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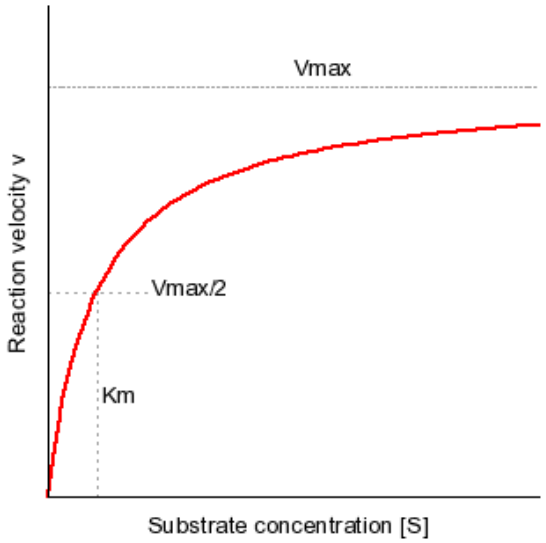
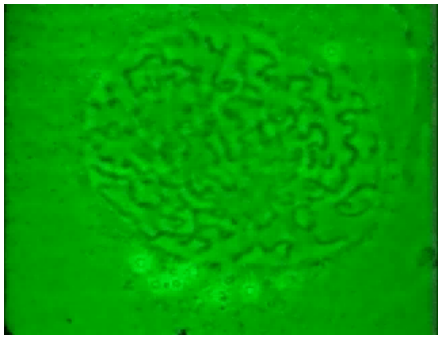
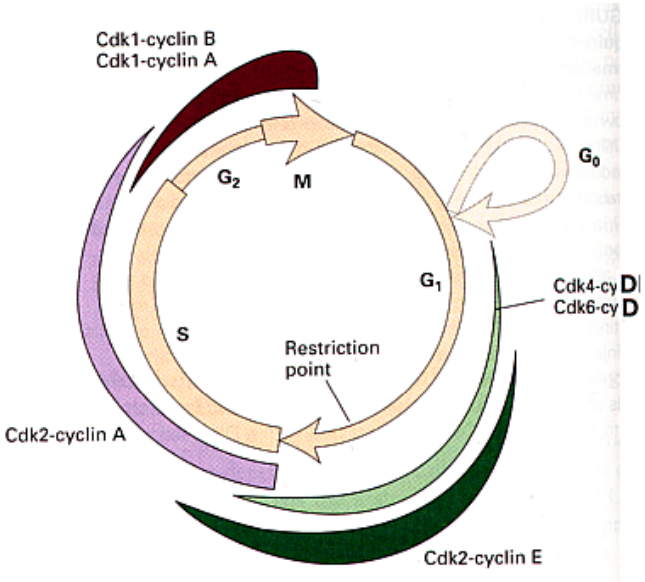


Cell Division Cycle



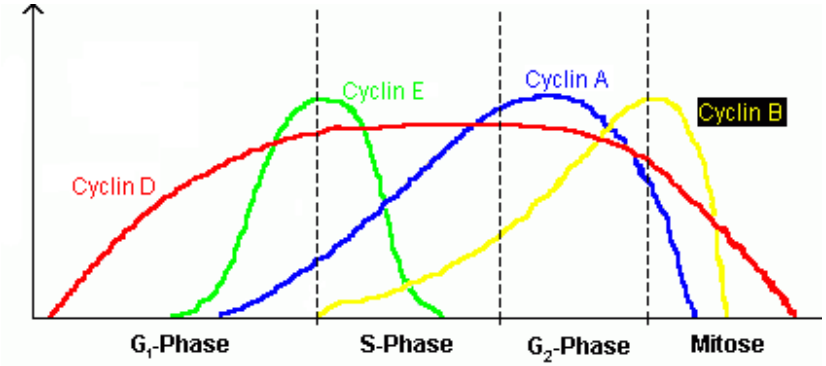


Sir Paul Nurse
Nobel prize 2001

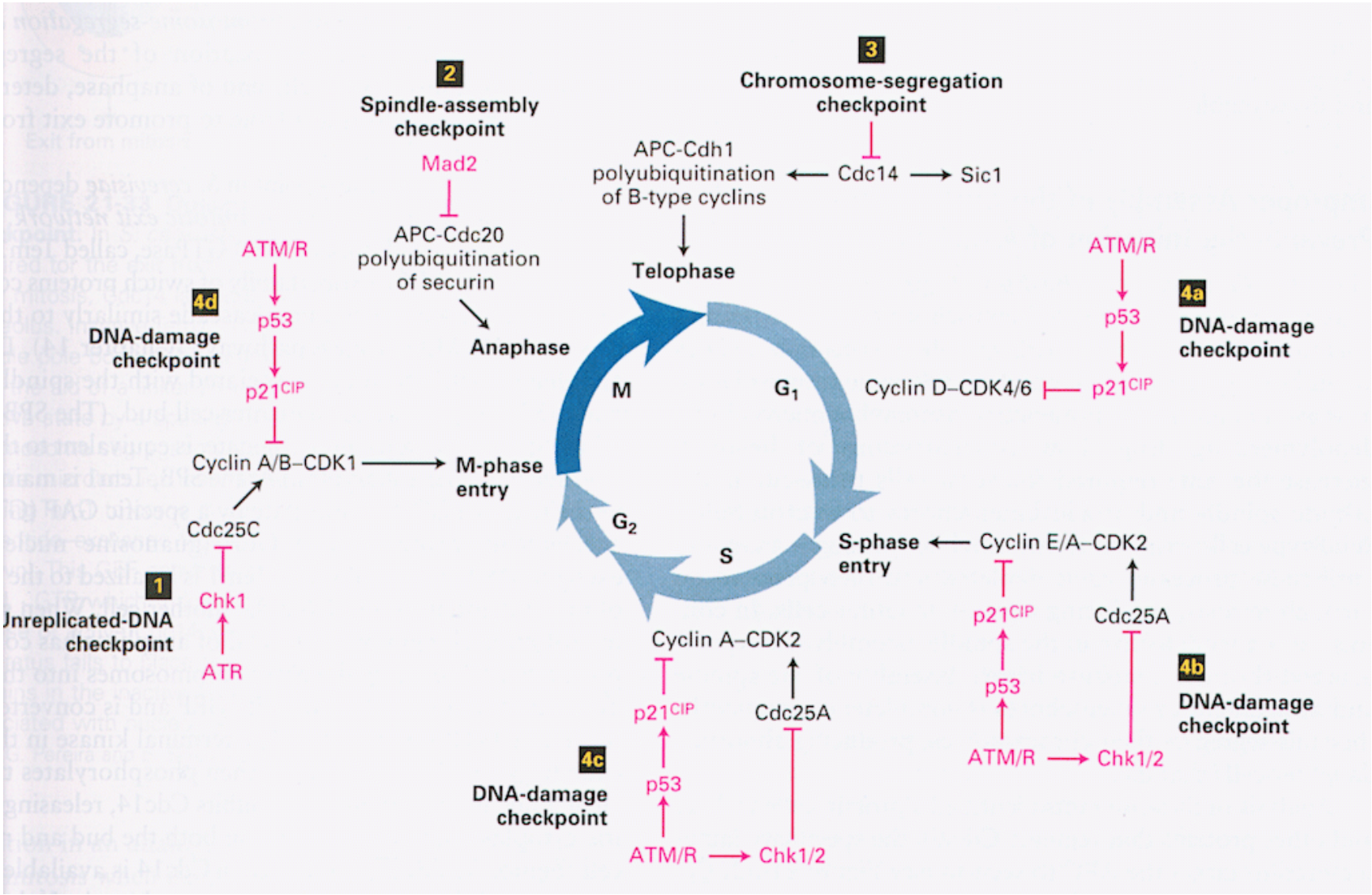
G1: CdK4-CycD
Cdk6-CycD
Cdk2-CycE

S: Cdk2-CycA

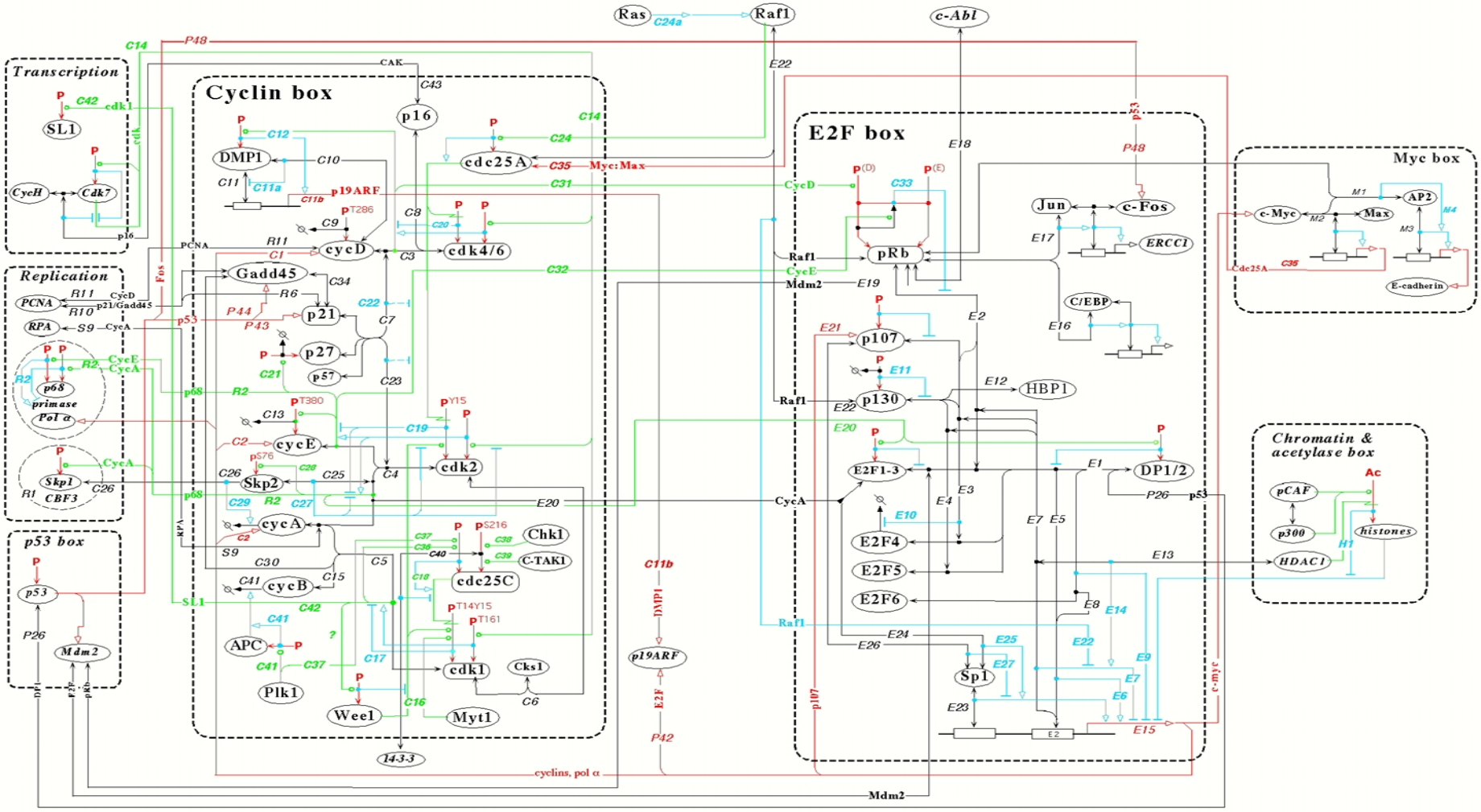
G2,M: Cdk1-CycA
Cdk1-CycB (MPF)



Cell Division Cycle Control

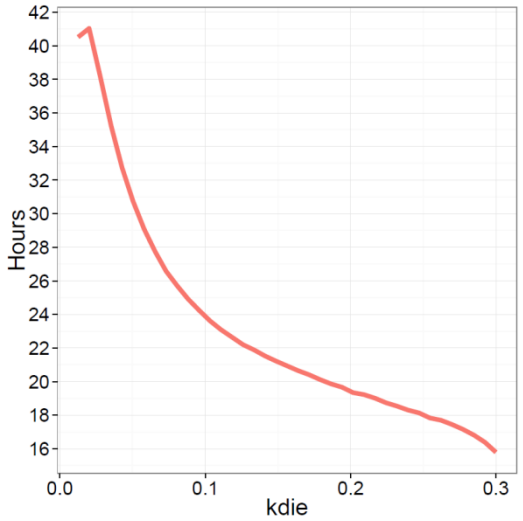


Mammalian Cell Cycle Control Map [Kohn 99]

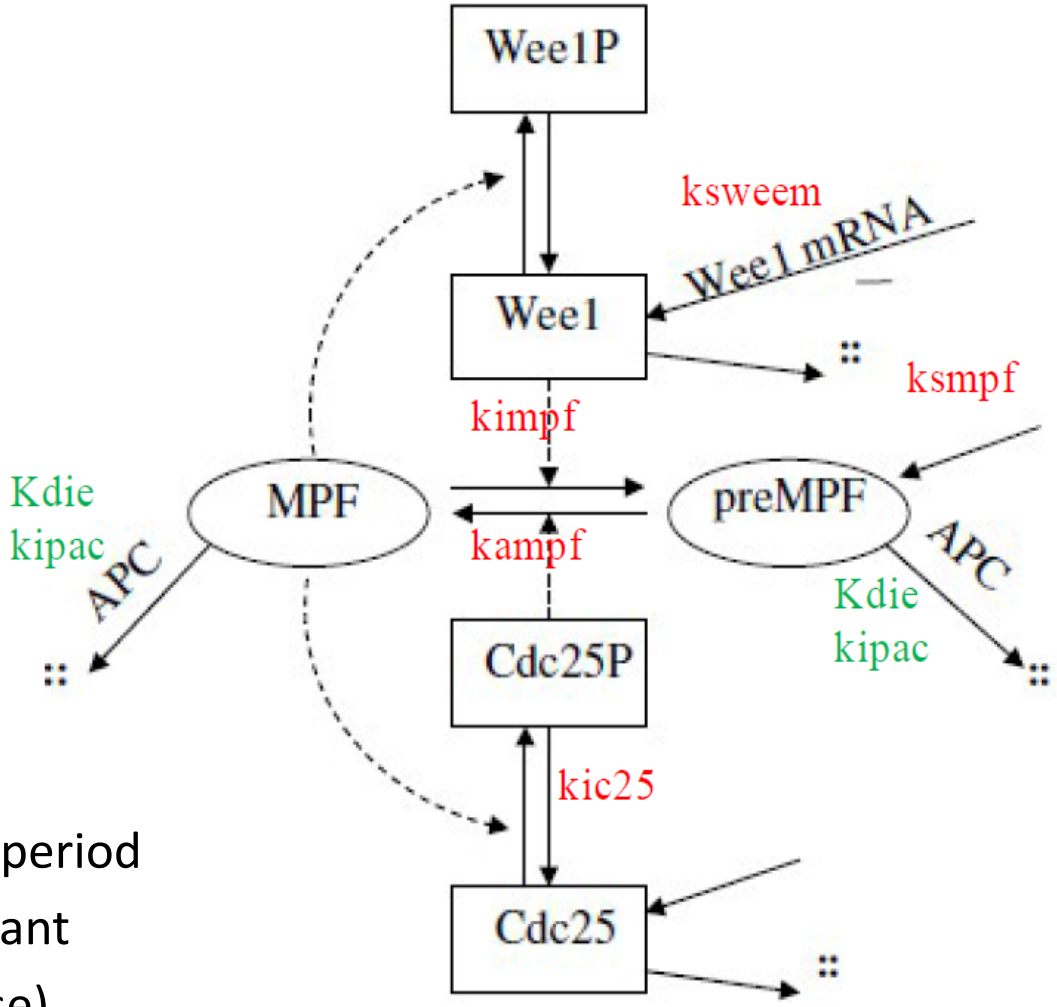


Cell Cycle Model [Qu McMillan Weiss 03]

- Focus on G2/M transition
- 10 molecular species
- 31 kinetic parameters



Variation of the cell cycle free period by *kdie* degradation rate constant (important in growing G1 phase)



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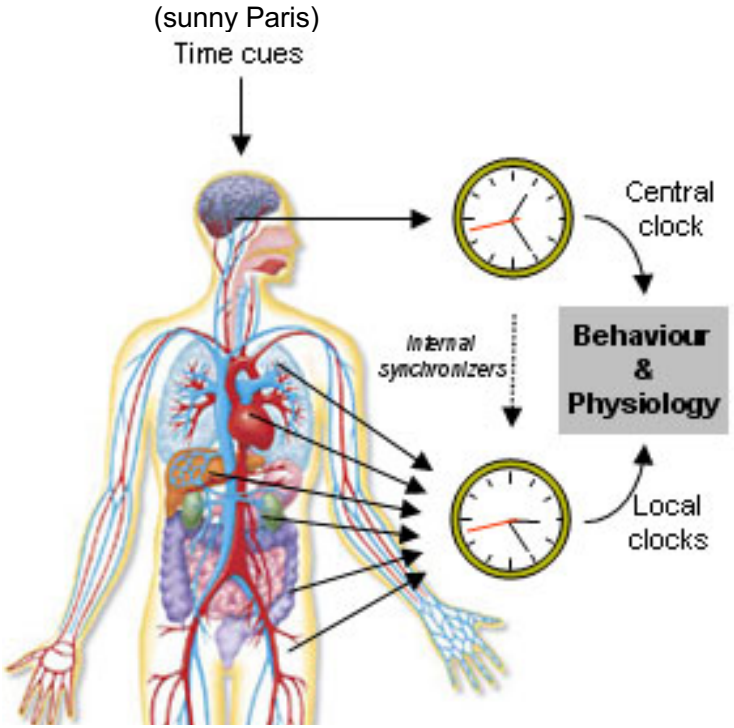
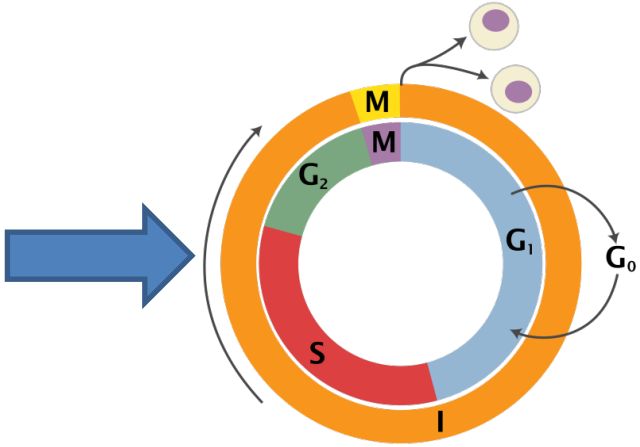
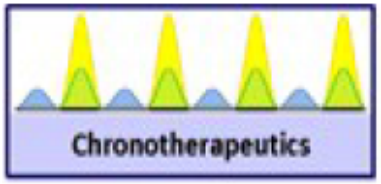
Control of the Cell Cycle by the Circadian CLock

- Time gating for mitosis by effects of clock genes on cell cycle genes
  inhibition of Wee1 synthesis by Clock-Bmal1 [Matsuo et al 2003]
- Model-based predictions on conditions of entrainment [Calzone Soliman 2006] and period doubling (24h, 48h) phenomena [Gerard Goldbeter 2012]
  (also repression of c-Myc by Clock-Bmal1 and inhibition of p21 by Reverb-α)



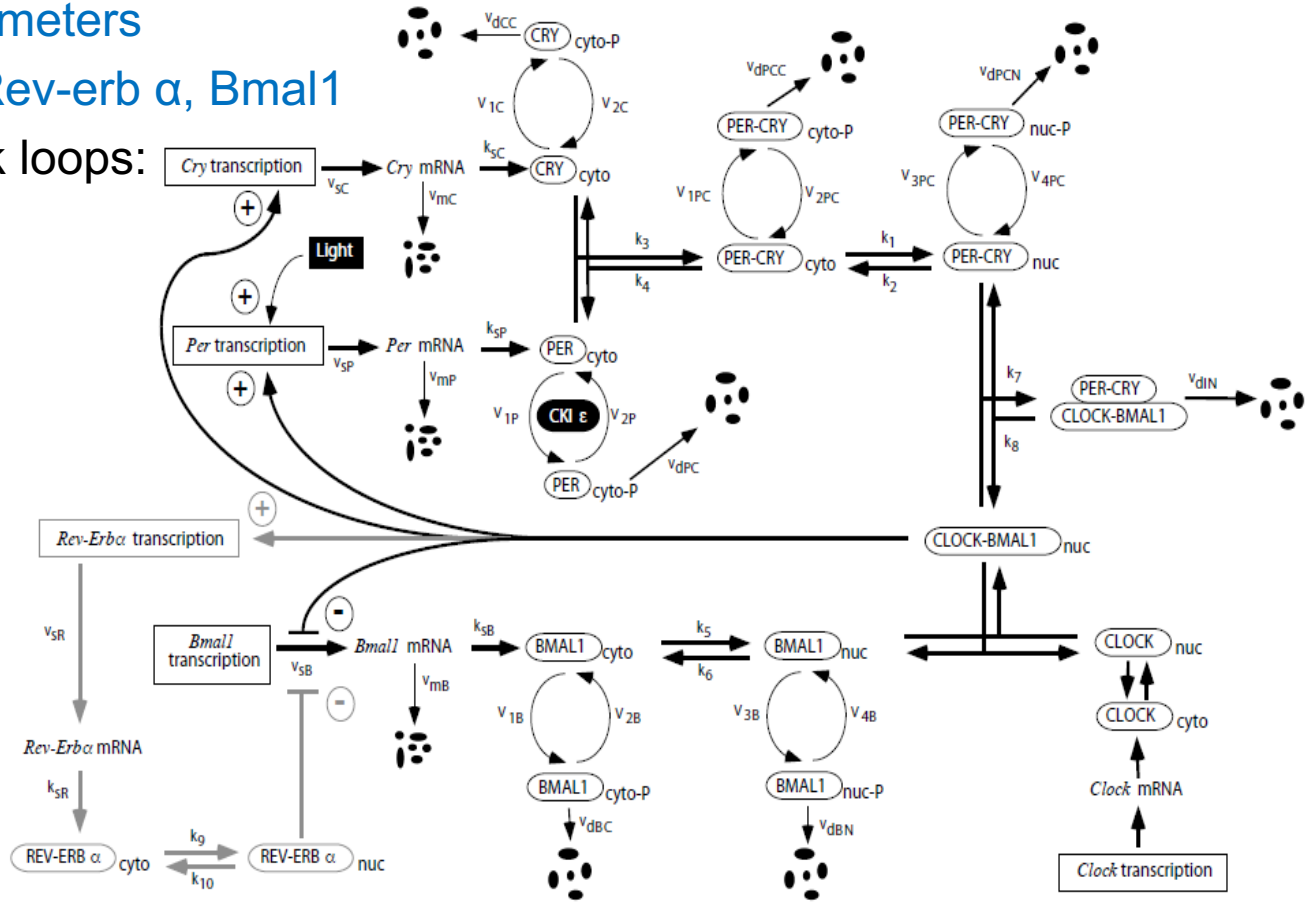




Mormont MC, Levi F.
Cancer chronotherapy: principles, applications, and perspectives.
Cancer, 2003.

Circadian Clock Model [Leloup Goldbeter 03]

- 19 species, 70 parameters
- 4 genes: Per, Cry, Rev-erb α, Bmal1
- 2 negative feedback loops:
  - Per-Cry
  - Rev-erb α



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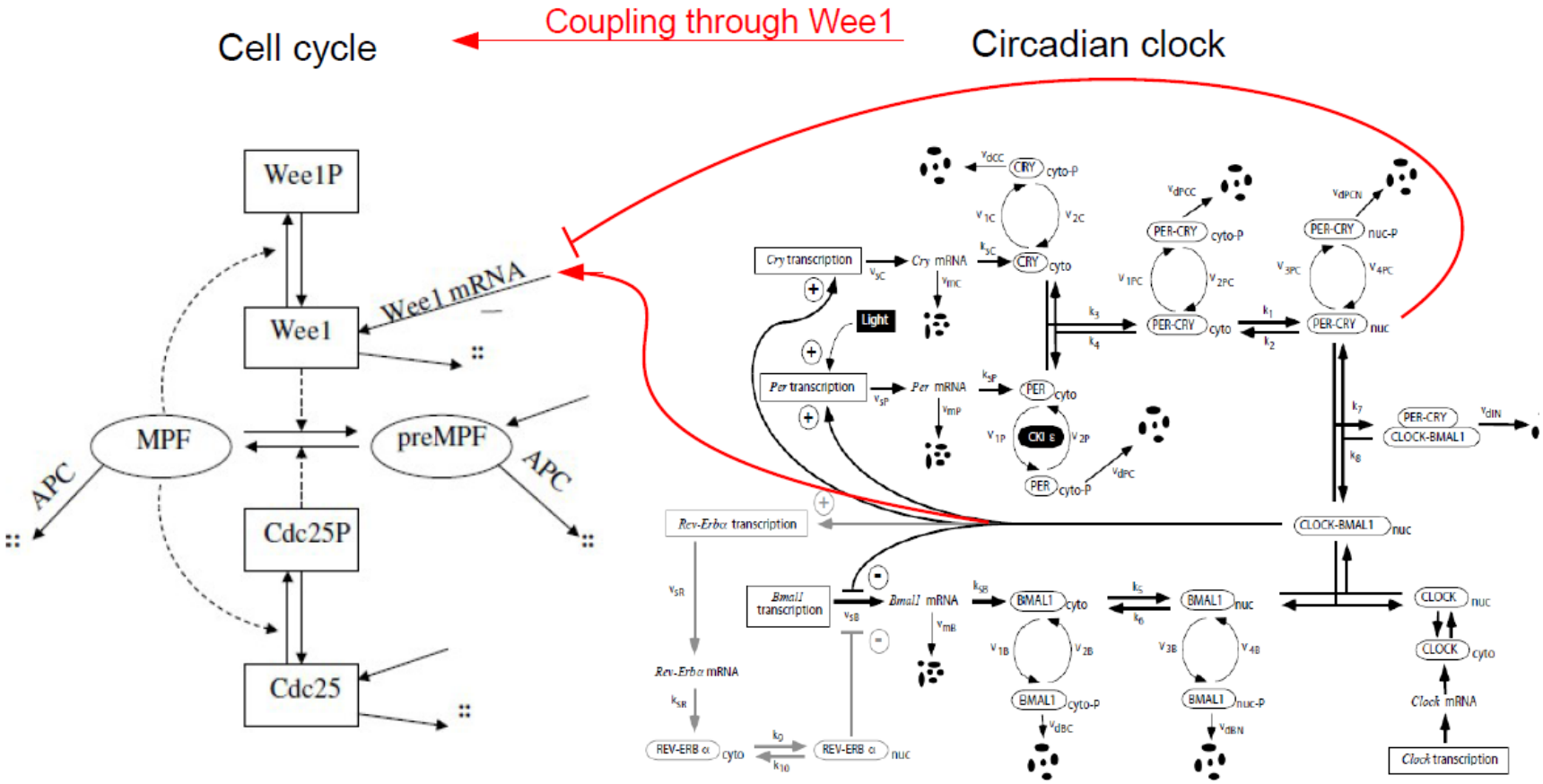
Load clock.ipynb

Coupled Model: time gating for mitosis

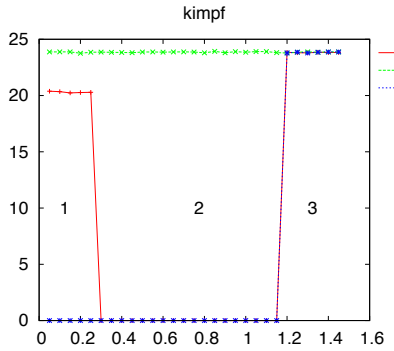


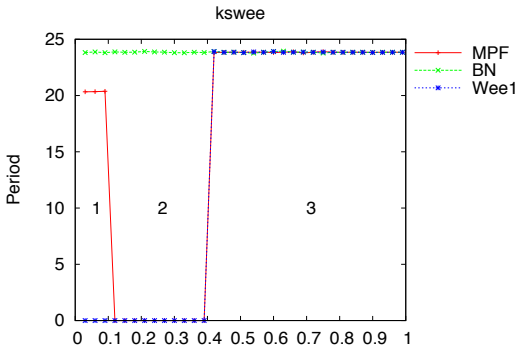
Coupling through Wee1 [Matsuo et al 2003] [Calzone Soliman 2006]

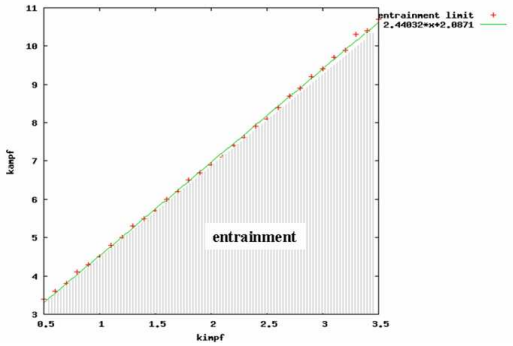
```
_ => mWee1 at rate (ksweemp+ksweem*[Bmal1N])/(Kweem+kwpcn*[PerCryN])
```

Conditions of Entrainment

$$
\begin{aligned}
\frac{d[preMPF]}{dt} &= ksmpf + kimpf \cdot [Wee1] \cdot [MPF] - kampf \cdot [C25P] \cdot [preMPF] \\
&\quad - kdmpf \cdot [APC] \cdot [preMPF] - kdmpfp \cdot [preMPF] \\
\frac{d[MPF]}{dt} &= kampf \cdot [C25P] \cdot [preMPF] - kimpf \cdot [Wee1] \cdot [MPF] \\
&\quad - kdmpf \cdot [APC] \cdot [MPF] - kdmpfp \cdot [MPF] \\
\frac{d[C25]}{dt} &= \frac{Vic \cdot [C25P]}{Kic25 + [C25P]} + ks25 - kd25 \cdot [C25] - \frac{Vapc + Vac \cdot [MPF] \cdot [C25]}{Kac25 + [C25]} \\
\frac{d[C25P]}{dt} &= \frac{Vapc + Vac \cdot [MPF] \cdot [C25]}{Kac25 + [C25]} - kd25 \cdot [C25P] - \frac{Vic \cdot [C25P]}{Kic25 + [C25P]} \\
\frac{d[Wee1]}{dt} &= \frac{Viw \cdot [Wee1P]}{Kiw + [Wee1P]} + kswee \cdot [Wee1m] \\
&\quad - kdwee \cdot [Wee1] - \frac{Vapw + Vaw \cdot [MPF] \cdot [Wee1]}{Kaw + [Wee1]} \\
\frac{d[Wee1P]}{dt} &= \frac{Vapw + Vaw \cdot [MPF] \cdot [Wee1]}{Kaw + [Wee1]} - kdwee \cdot [Wee1P] - \frac{Viw \cdot [Wee1P]}{Kiw + [Wee1P]} \\
\frac{d[APC]}{dt} &= \frac{kaapcp + kaapc \cdot [X] \cdot [APCi]}{Kapc + [APCi]} - \frac{kiapc \cdot [APC]}{Kapc + [APC]} \\
\frac{d[X]}{dt} &= ksx \cdot [MPF] - kdx \cdot [X] \\
\frac{d[Wee1m]}{dt} &= \frac{ksweemp + ksweem \cdot [BN]}{Kweem + kwpcn \cdot [PCN]} - kdweem \cdot [Wee1m] \\
\frac{d[APCi]}{dt} &= \frac{kiapc \cdot [APC]}{Kapc + [APC]} - \frac{kaapcp + kaapc \cdot [X] \cdot [APCi]}{Kapc + [APCi]}
\end{aligned}
$$



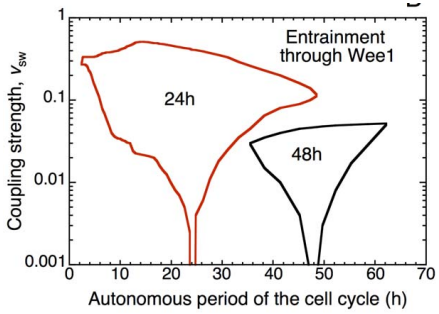




- Conditions of entrainment on Bmal1-Wee1 and MPF activation parameters

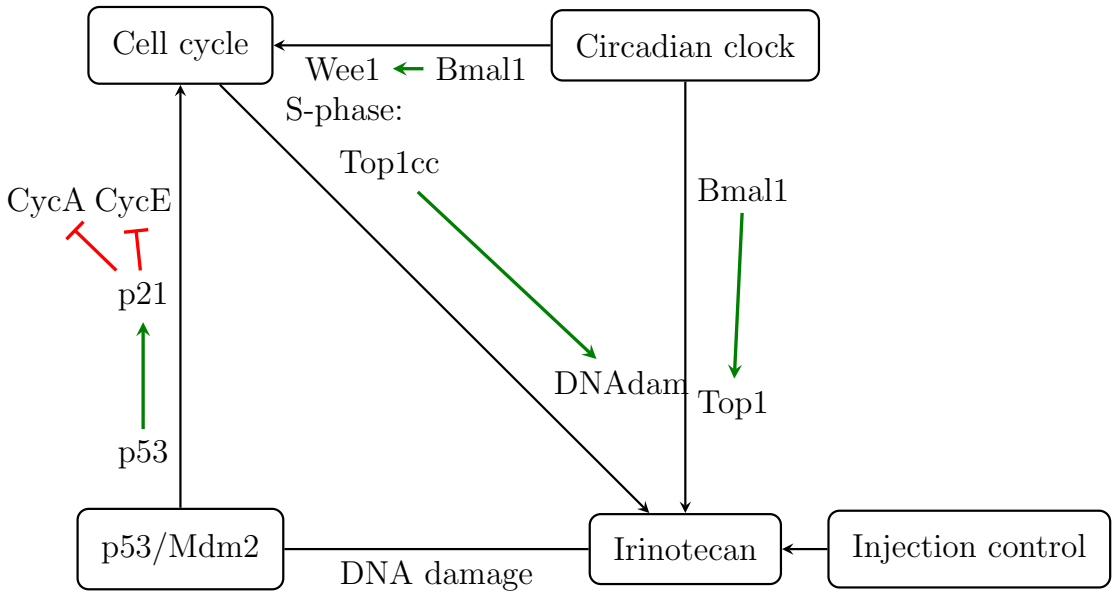
- Period doubling (24h, 48h) phenomena

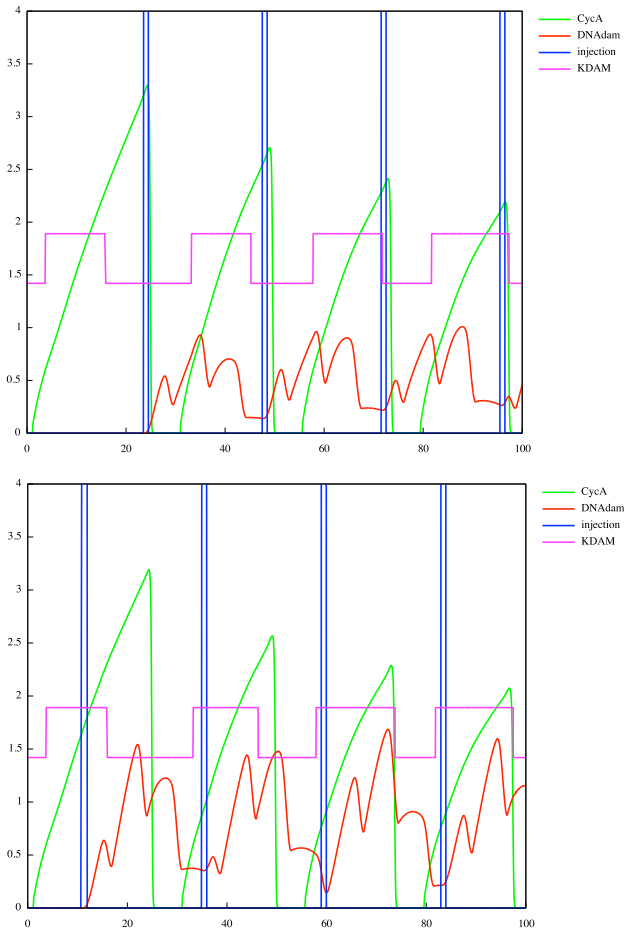
  [Gerard Goldbeter PLOS 2012]



Irinotecan Exposure Chronotherapy Model

Coupled cycle-clock-p53Mdm2-Irinotecan model





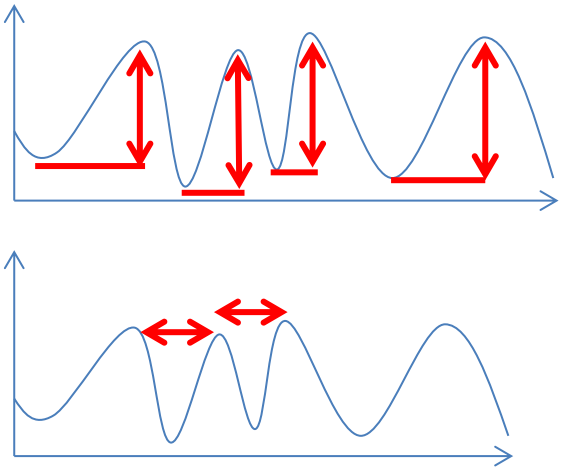
Optimal control of drug exposure [De Maria et al TCS 2011]

- Max pulses satisfying always DNAdam<1
- with DNA damage>1 on phase shifted cells

Whole body PK/PD drug injection model [Ballesta et al PlosCB 2011]

Specification of Temporal Behaviors

- Linear Time Temporal logic (LTL) extends classical logic with time operators
  X: next, **F**: finally, **G**: globally, **U**: until
- FO-LTL(R_{lin}), can express quantitative properties :
  - Stability **G**φ
  - Reachability **F**φ , thresholds **F**([A]>0.1),
  - Peaks of concentration **F**([A]<V ∧ **X**([A]=V ∧ **X**([A]<V))
  - Amplitude
  - Periods and phases as distance between peaks
  - …



- More flexible than curve fitting, can abstract from uncertain imprecise data

- Verification of reachability constraints w.r.t. qualitative observations
- Constraints for parameter search w.r.t. quantitative observations
  - Good fit → model-based predictions, control
  - No good fit → revisit structure → model-based contribution to biology

Naïve Parameter Scanning Algorithm

Input: an ODE model M(p) with n parameters p in range [pmin,pmax],
an LTL(R) specification ϕ

Output: parameter values v such that M(v) |= ϕ
or fail if no such values

1. Scan the parameter value space [pmin,pmax]^n with a fixed step
2. Test whether M(v) |= ϕ by trace-based model checking
3. Return the first value set v which satisfies ϕ

Exponential complexity in $O(s^n)$ where n is the number of parameters and s is the number of discrete values to try for each parameter

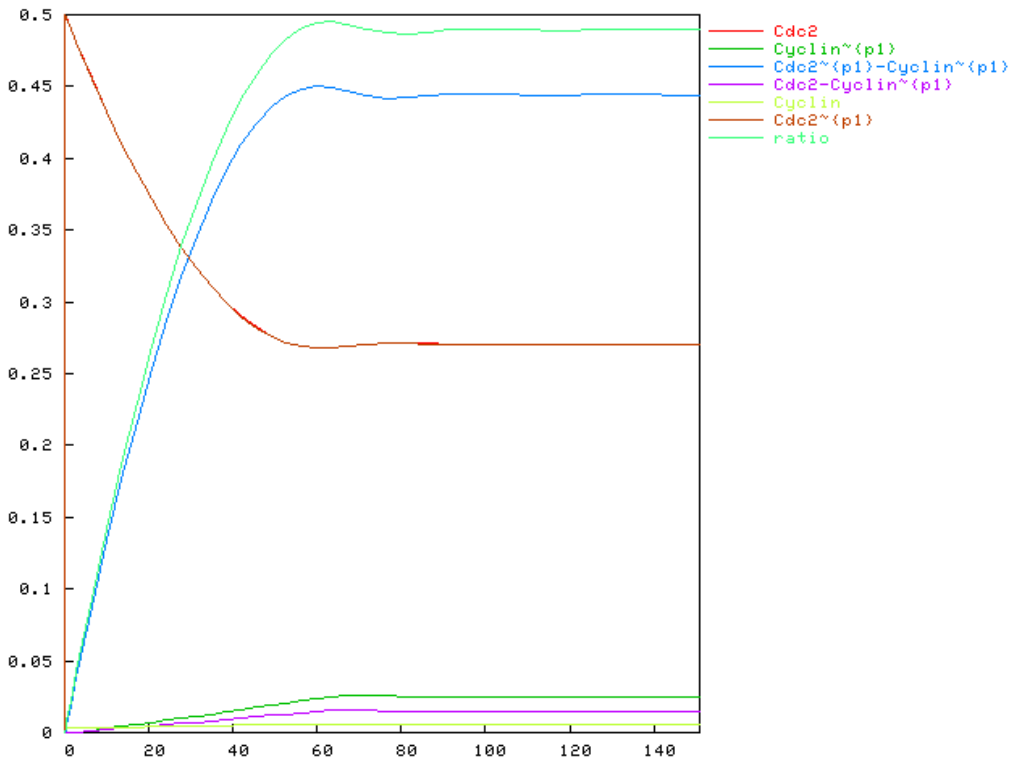
Parameter Scanning w.r.t. LTL(R) Specification

biocham: search_parameters([k3,k4],[(0,200),(0,200)],20,
oscil(Cdc2-Cyclin~{p1},3),150).

First values found :

parameter(k3,10).

parameter(k4,70).



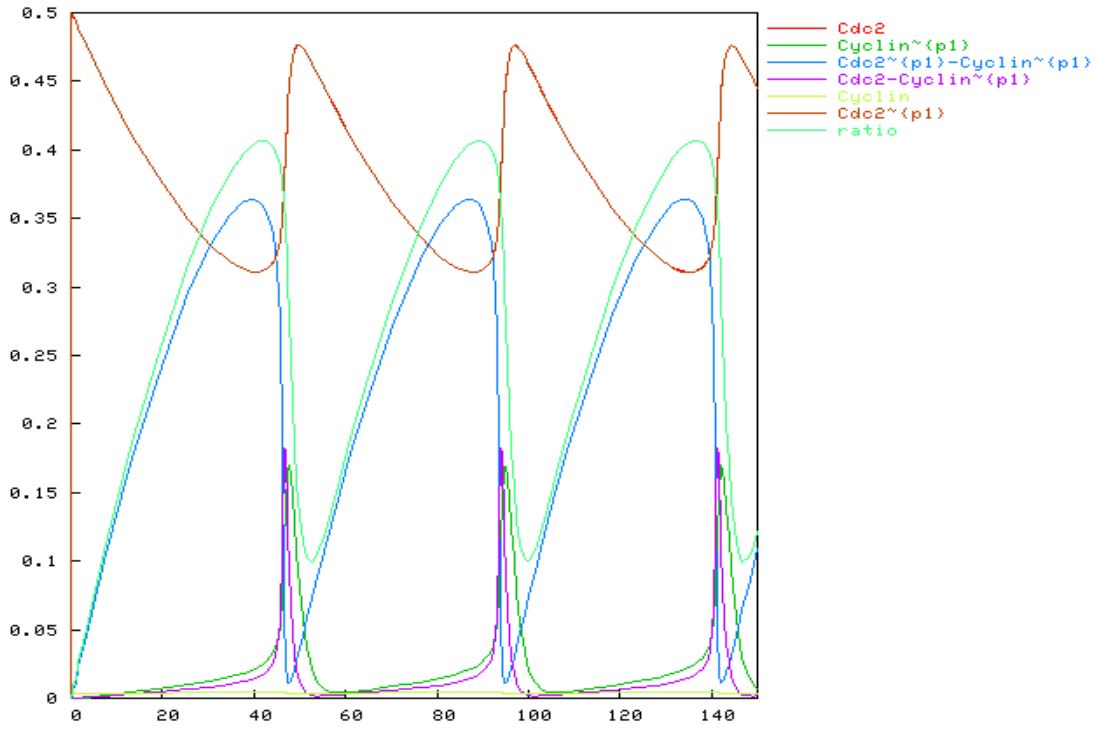
Parameter Scanning w.r.t. LTL(R) Specification

biocham: search_parameters([k3,k4],[(0,200),(0,200)],20,
oscil(Cdc2-Cyclin~{p1},3) & F([Cdc2-Cyclin~{p1}]>0.15), 150).

First values found :

parameter(k3,10).

parameter(k4,120).



Parameter Scanning w.r.t. LTL(R) Specification

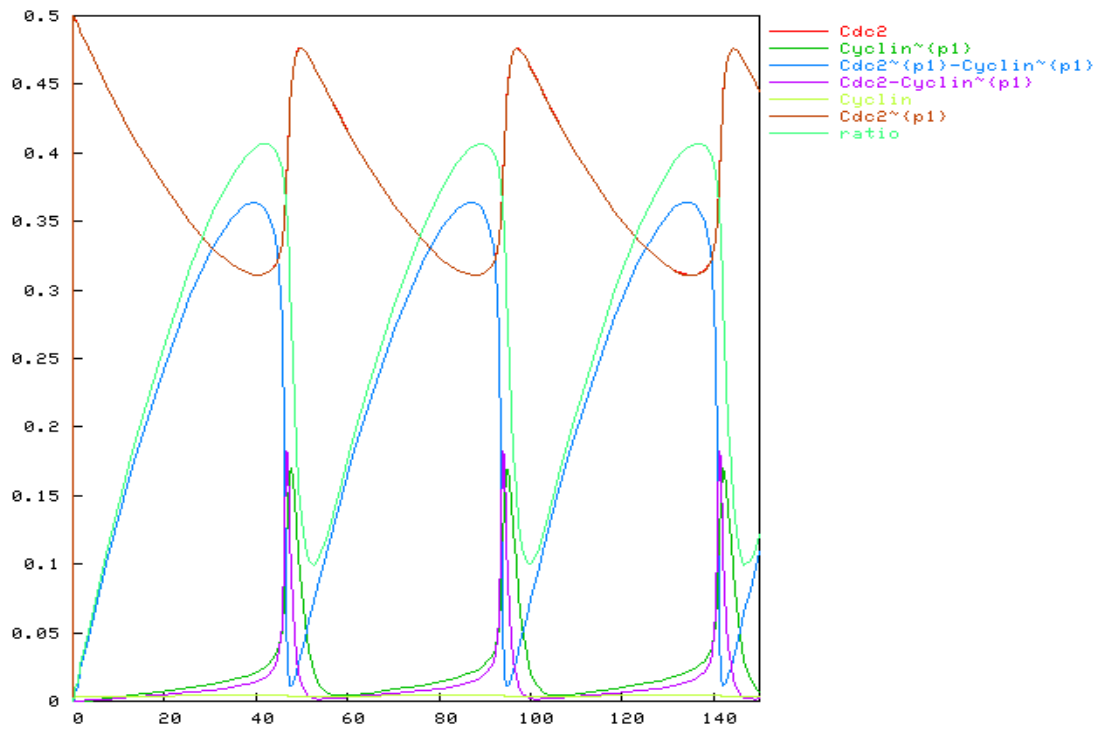
biocham: search_parameters([k3,k4],[(0,200),(0,200)],20,

oscil(Cdc2-Cyclin~{p1},3) & F([Cdc2-Cyclin~{p1}]>0.15), 150).

First values found :

parameter(k3,10).

parameter(k4,120).



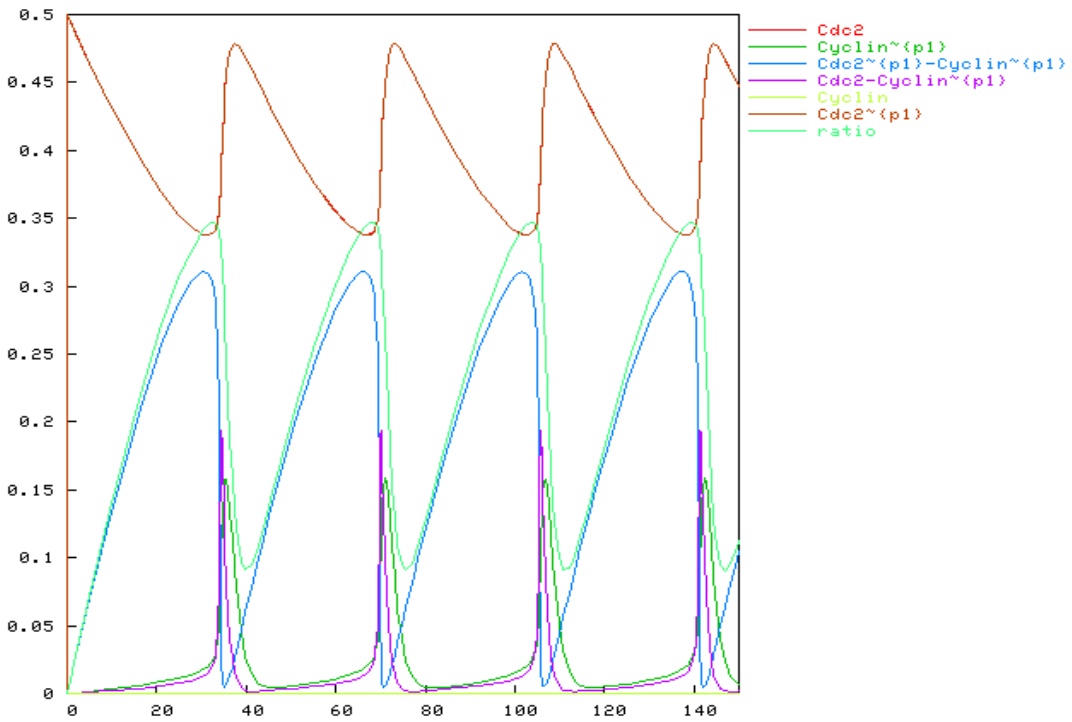
Parameter Scanning w.r.t. LTL(R) Specification

biocham: search_parameters([k3,k4],[(0,200),(0,200)],20,
period(Cdc2-Cyclin~{p1},35), 150).

First values found :

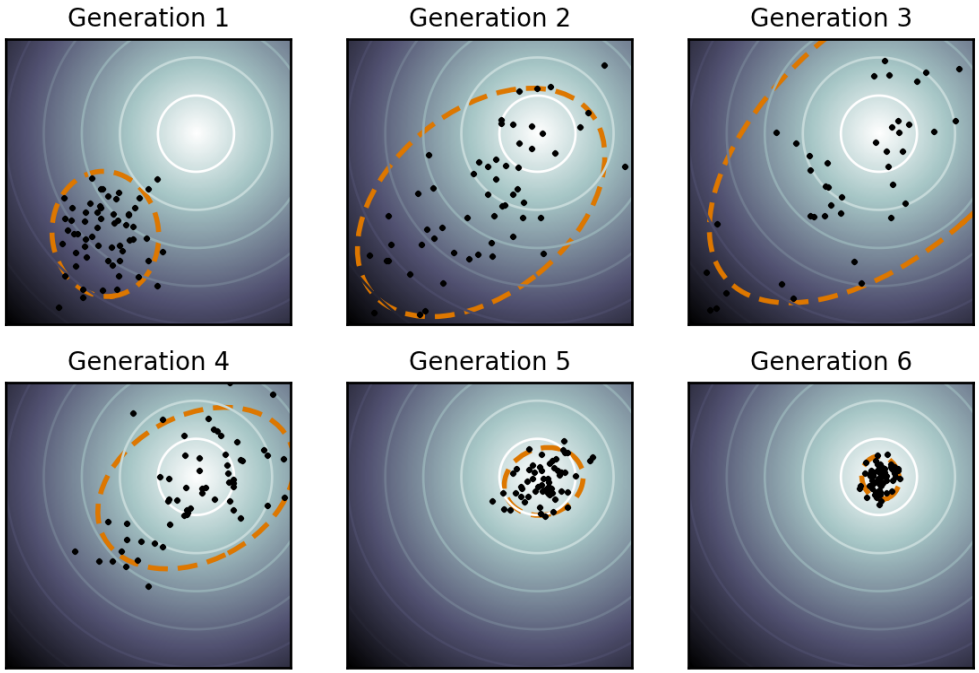
parameter(k3,10).

parameter(k4,180).



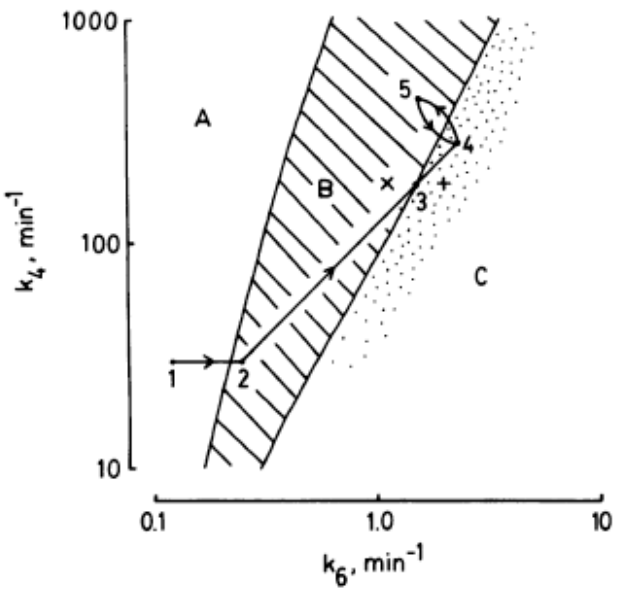
Parameter Optimization Evolutionary Algorithm

- Objective function: continuous satisfaction degree of LTL(R) formulae
- Covariance Matrix Adaptive Evolution Strategy (CMA-ES) [Hansen 01]

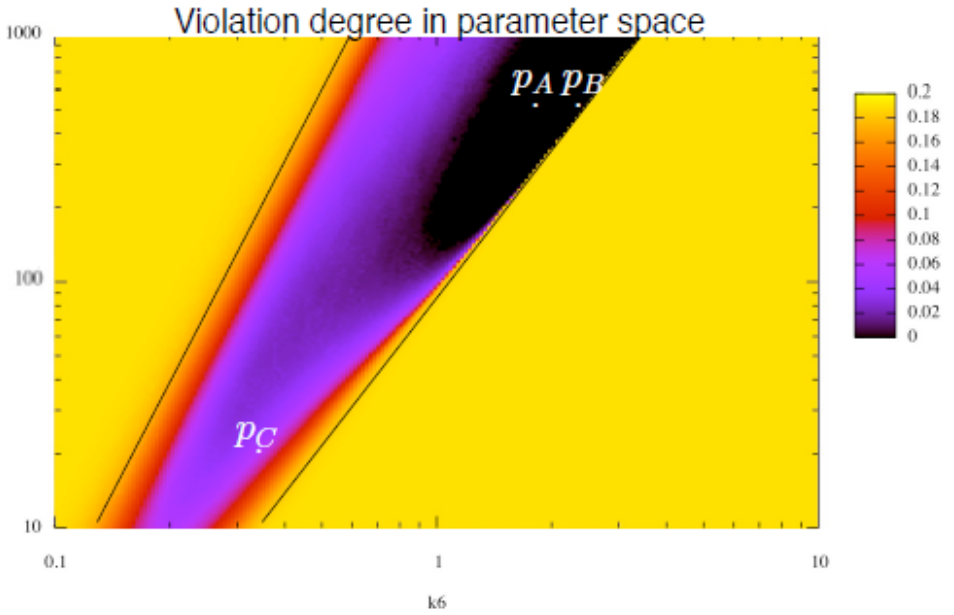


Landscape for Oscillation Amplitude Constraint

ϕ^*: **F**([A]>x $\wedge$ **F**([A]<y)); amplitude x-y$\geq$0.3



Bifurcation diagram



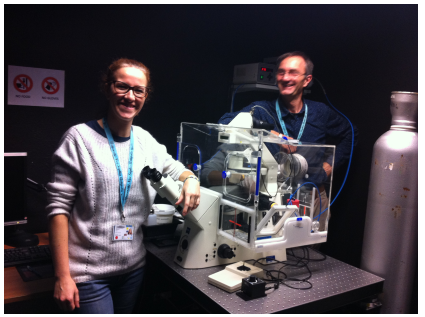
LTL satisfaction diagram

Unexpected Behavior of NIH3T3 Fibroblasts: Acceleration of the Clock at high FBS !

Time series data in individual mice fibroblasts [Feillet Delaunay 2012]

Fluorescent markers of the cell cycle and the circadian clock (RevErbα)

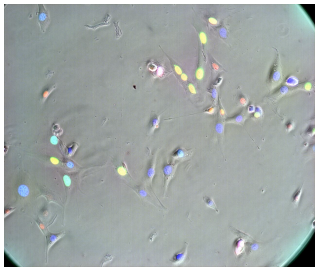
Medium with various concentrations of serum (FBS)



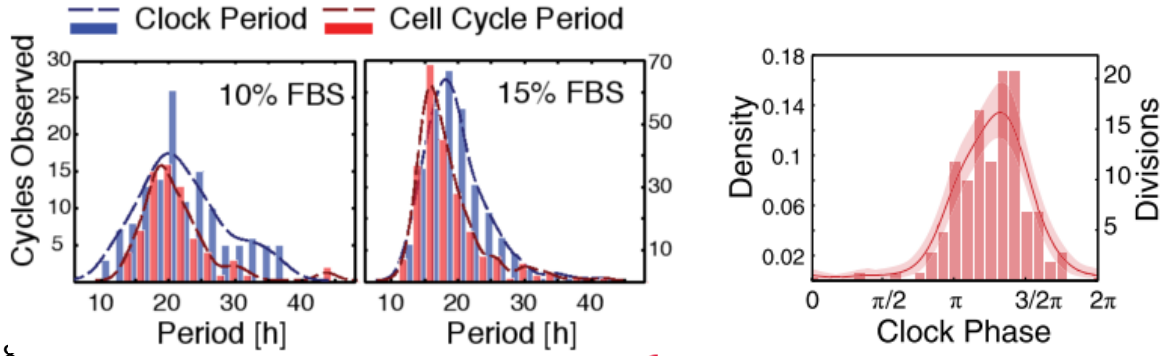
- FBS modulates the cell cycle frequency
- No observed time gating for mitosis
- But observed acceleration of the circadian clock in fastly dividing cells ! and not in confluent cells (24h)

FBS 10% → Cell cycle 22h → Circadian clock 22h, phase 7h

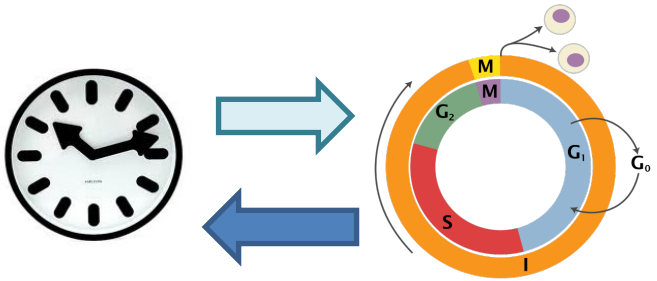
FBS 15% → Cell cycle 19h → Circadian clock 18h, phase 7h



Statistical model phase locking [Feillet et al Delaunay Rand PNAS 2014]



Reverse Effect Cell Cycle → Clock



Mechanistic model for this reverse effect ?

Hypothesis 1: Uniform inhibition of gene transcription during mitosis

- Entrainment in period
- No parameter values for correct entrainment in phase

Hypothesis 2: Selective regulation of clock genes during mitosis

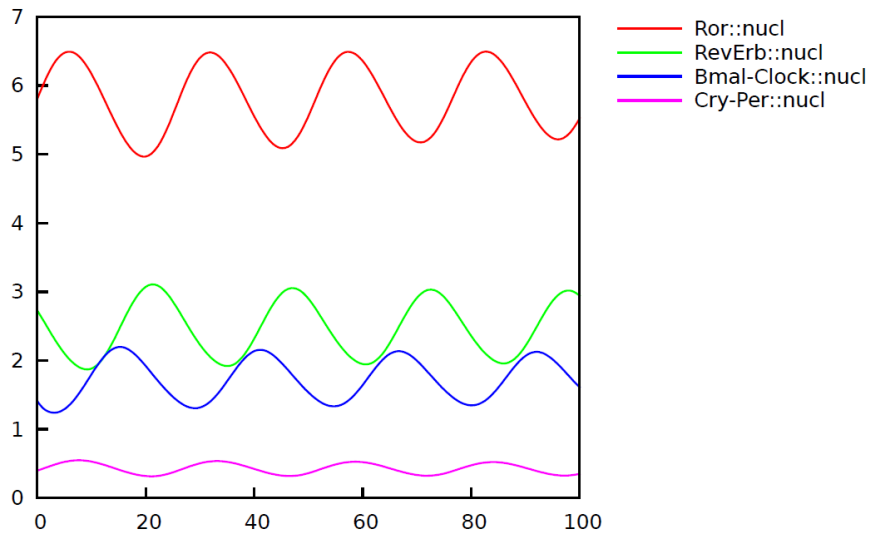
- Entrainment in period and phase fitted to experimental data
- Prediction of reverb up-regulation during mitosis (or Bmal1 down)

[Traynard, Feillet, Soliman, Delaunay, F., Biosystems 2016]

Relogio-Herzel Model of the Circadian Clock (2011)

- 20 species, 71 parameters
- 60 parameters fitted to liver cell data
  - amplitude, period and phase data
- Per, Cry, Reverb, Ror, Bmal genes

Relógio, A., Westermark, P. O., Wallach, T., Schellenberg, K., Kramer, A., & Herzel, H. (2011). Tuning the mammalian circadian clock: robust synergy of two loops. PLoS Computational Biology.



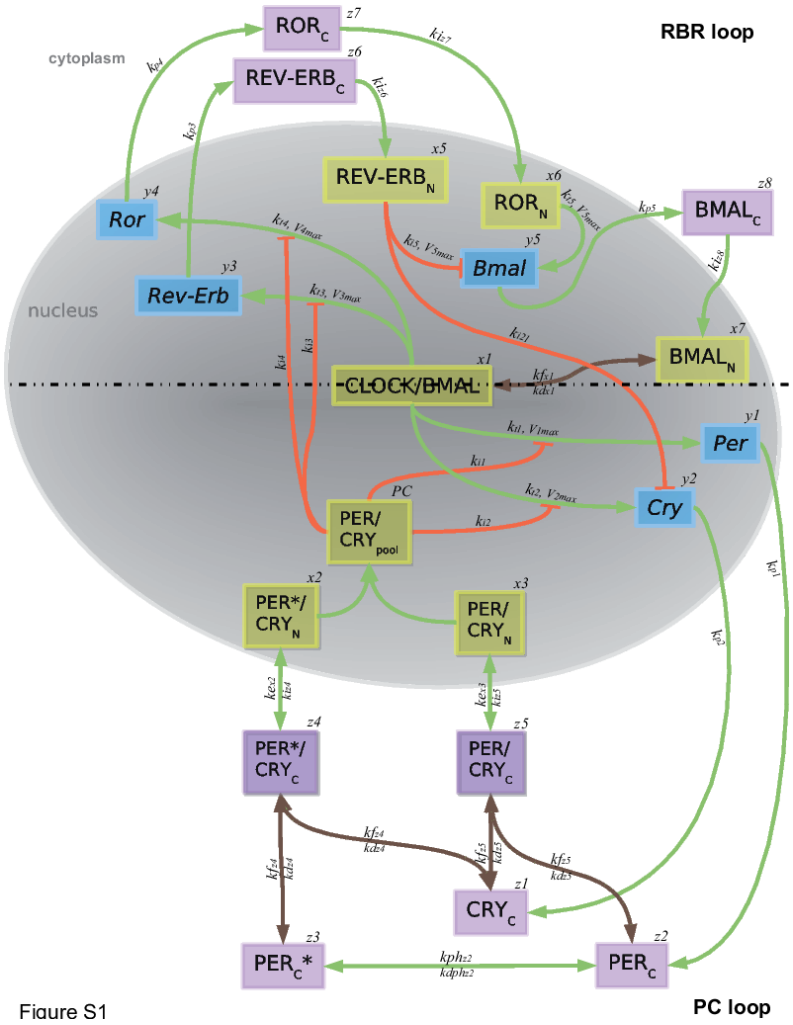
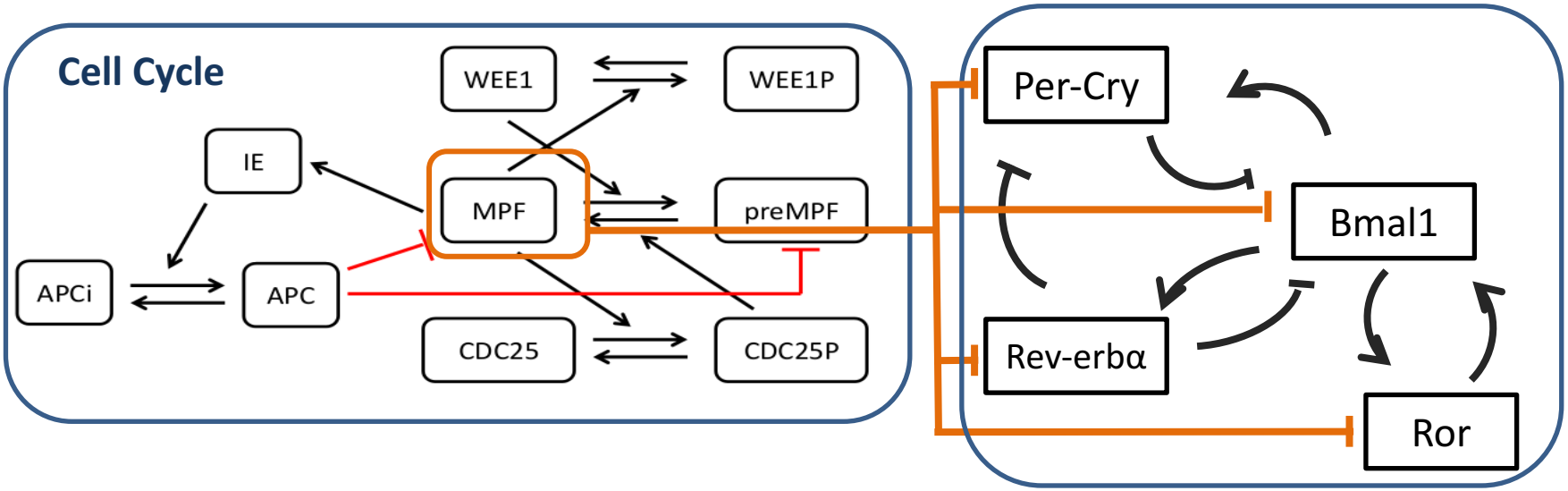
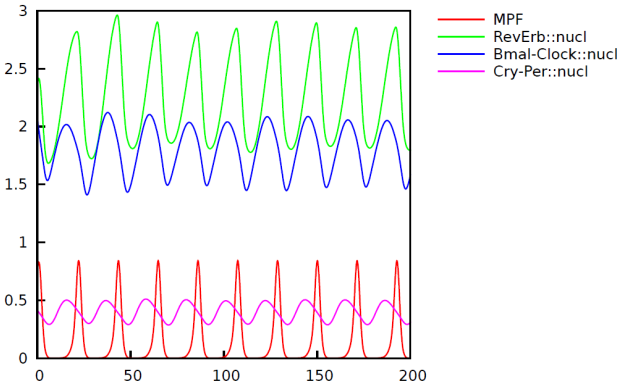


Figure S1

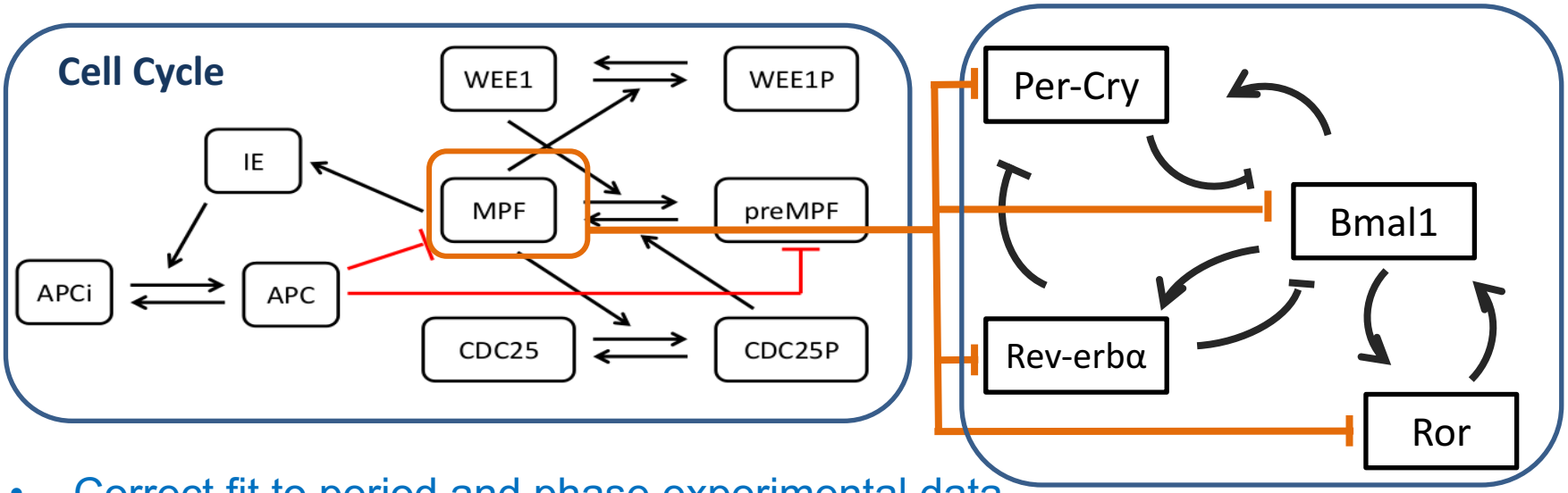
Hypothesis 1: Uniform Inhibition of Transcription during Mitosis [Kang et al. 2008]



- Correct acceleration of both the cell cycle and the circadian clock
- But impossible to fit experimental phase shift between cell division time and RevErb peak
  - Experimental phase: 7h
  - Model phase: 18h



Hypothesis 2: Selective Regulation of Clock Genes during Mitosis



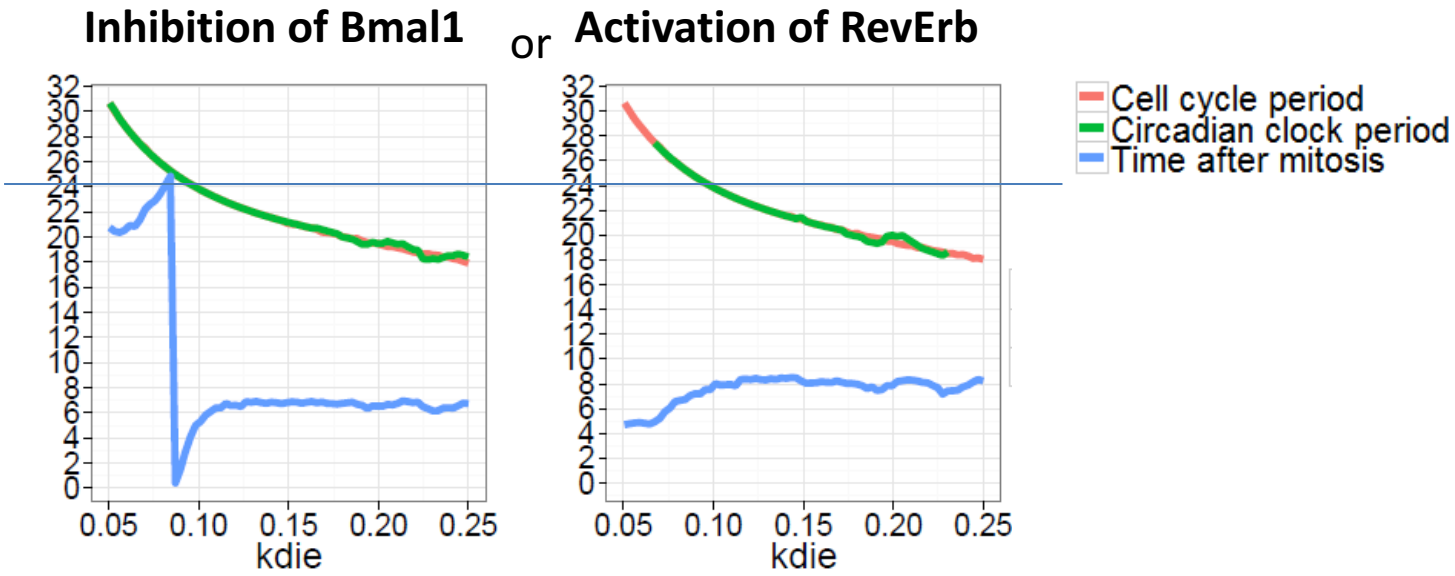
- Correct fit to period and phase experimental data
  (playing with only coupling strength regulation parameters)
- Two sets of parameter values fit the data:

either down-regulation of Bmal1
or up-regulation of RevErbα
during mitosis

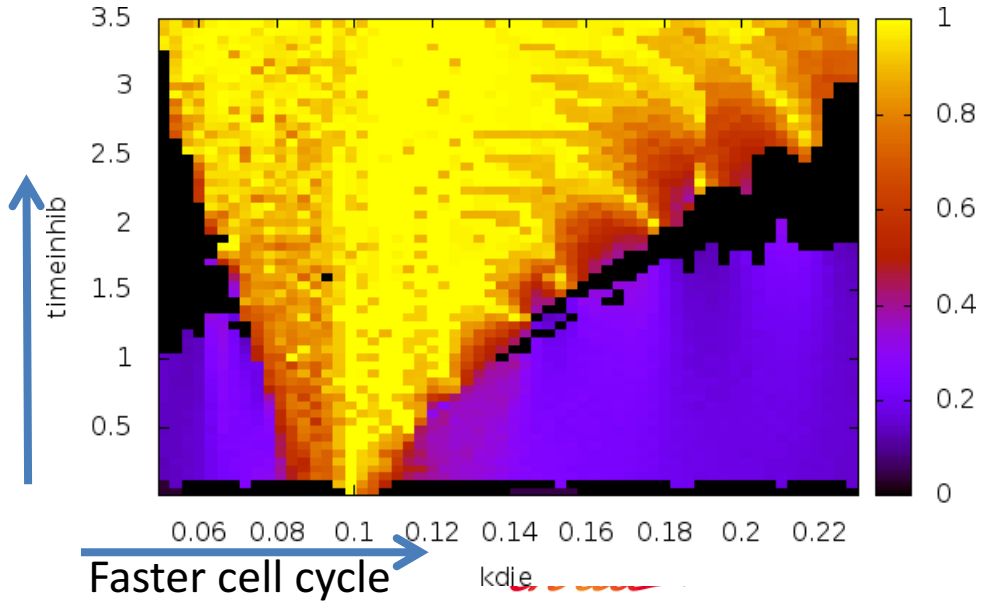
Parameters	First set	Second set
Synthesis coefficient for *Per*	0.66	2.40
Synthesis coefficient for *Cry*	2.30	0.67
Synthesis coefficient for *RevErb-α*	1.04	1.92
Synthesis coefficient for *Ror*	2.1	1.51
Synthesis coefficient for *Bmal1*	0	0.78
Duration	2.97h	2.81h

Hypothesis 2: Predictions

Results:



Prediction: different behaviors for a slow cell cycle (5% FBS)



Stronger control of the clock by the divisions

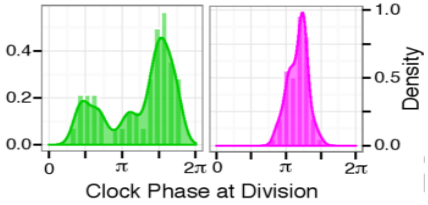
Faster cell cycle

Score for the property:
The cell cycle and the circadian clock have the **same period**

Complex Behaviors with High Variability observed after Treatment by Dexamethasone

- Dexamethasone synchronize cellular clocks, but complex dynamics observed

Medium	Clock period	Division period	Mean delay
FBS 10%	24.2 h ± 0.5 h	20.1 h ± 0.94 h	10.7 h
FBS 20%	21.25 h ±0.36 h	19.5 h ±0.42 h	8.3 h
	29 h±1.05 h	16.05 h±0.48 h	6h/12h/22h



Interpreted as 5:4 and 1:1 locking modes for 10% FBS and 3:2 and 1:1 for 15%

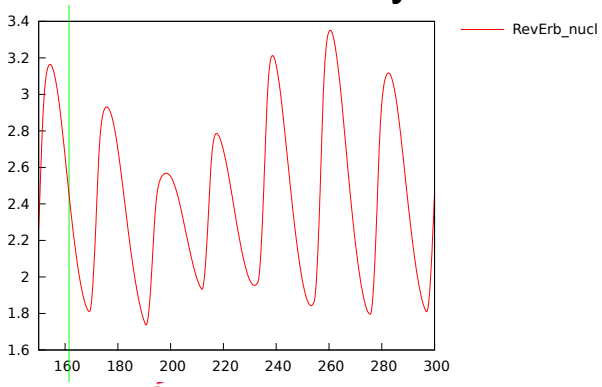
[C. Feillet et al. Phase locking and multiple oscillating attractors for the coupled mammalian clock and cell cycle., PNAS 2014]

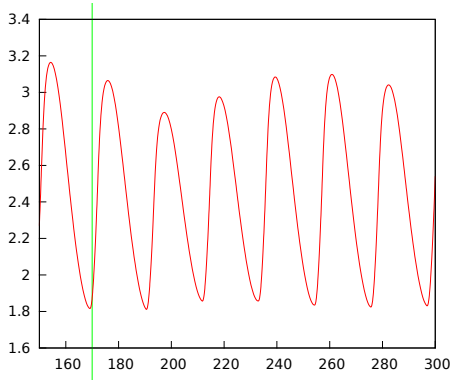
- In our model, Dex pulse modeled by induction of high level of Per.

Clock perturbation varies according to the time T of the pulse

Stabilization of the clock may occur after 70h beyond observed data…

peak-peak distance in
[18.8, 22.7] with T=162h
[20.9, 21.7] with T=170h





Wrap-up

- Bi-directional coupling of cell cycle and circadian clock through
  1. Regulation of cell cycle genes (Wee1, p21, Myc) by clock genes (Bmal1,Per,Rev)
  2. Regulation of clock genes by cell cycle (up regulation of Rev-Erbα during mitosis)

- Modeling of molecular interaction networks by formal reaction systems
  - Differential (ODE), stochastic (CTMC), Petri Net (PN) , Boolean semantics
  - Before simulation, static analysis and model-checking methods
- Modeling of temporal behaviors
  - quantitative temporal logic language FO-LTL
  - Sensitivity&robustness measures, parameter search w.r.t. FO-LTL specification
- BIOCHAM free software for modeling
  - reaction and influence systems (SBML compatible)
  - analysis and synthesis

- Same parameter search methods can be used for model-based control
  - Drug exposure optimization
  - External control of cell processes
  - Cell reprogramming