

Quantitative Modelling of the
Circadian Clock and Cell Cycle Coupling:
Prediction of RevErb- alpha Up-Regulation
during Mitosis in Mouse Embryonic Fibroblasts
Temporal Logic Constraints and Parameter Search in BIOCHAM

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Joint work with P. Traynard, C. Feuillet and F. Delaunay

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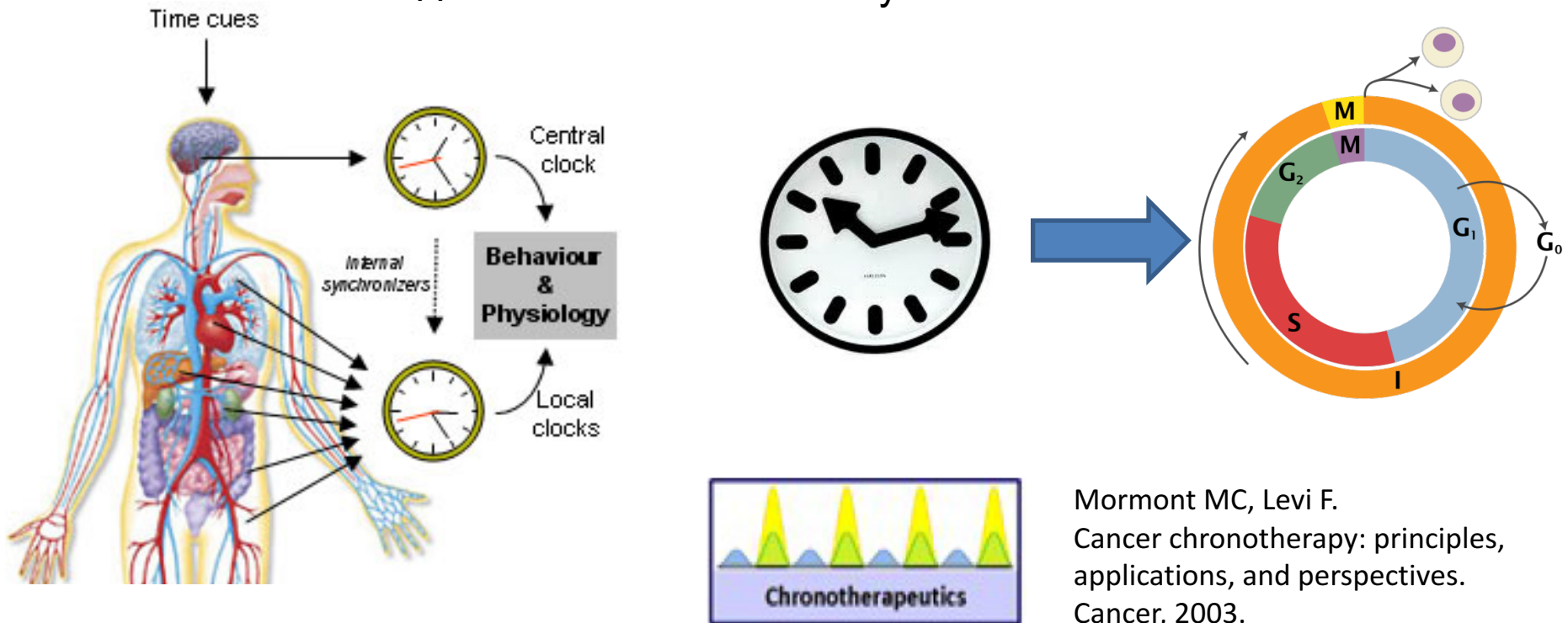
EP Lifeware, <http://lifeware.inria.fr/>

Dynamic Days, Szeged, June 2017

Control of the Cell Cycle by the Circadian Clock

Classical view:

- Time gating for mitosis by effects of clock genes on cell cycle genes (e.g. effect of Bmal1 on Wee1 transcription [Matsuo et al 2003])
- Period doubling phenomena for the cell cycle w.r.t. the circadian clock



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

Non Classical Behavior of NIH3T3 Fibroblasts: Acceleration of the Clock at high FBS

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

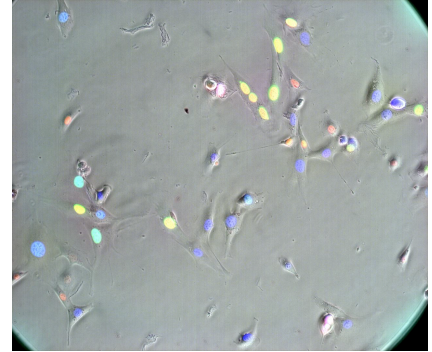
Fluorescent markers of the cell cycle and the circadian clock (RevErb α)

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)

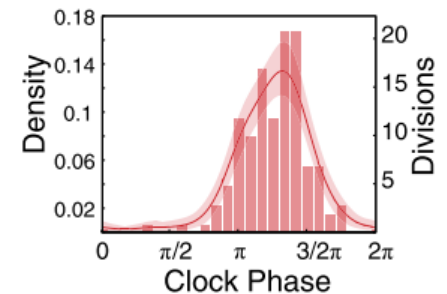
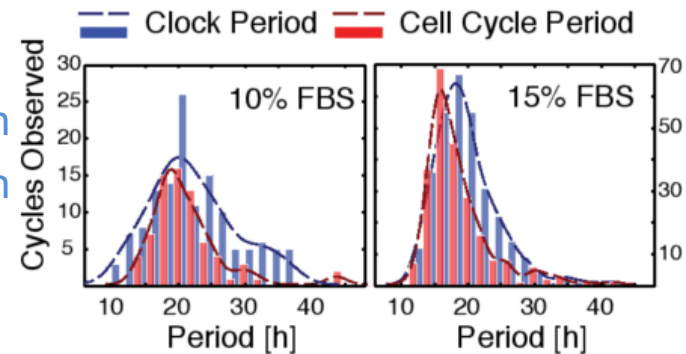
FBS 10% \rightarrow Cell cycle 22h \rightarrow Circadian clock 22h, phase 7h

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



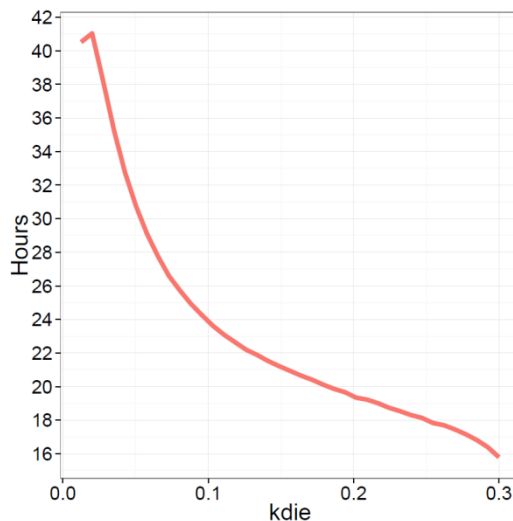
Reverse effect of the cell cycle on the circadian clock ?

- Hyp 1: Uniform inhibition of gene transcription during mitosis ?
- Hyp 2: Selective regulation of clock genes during mitosis ?

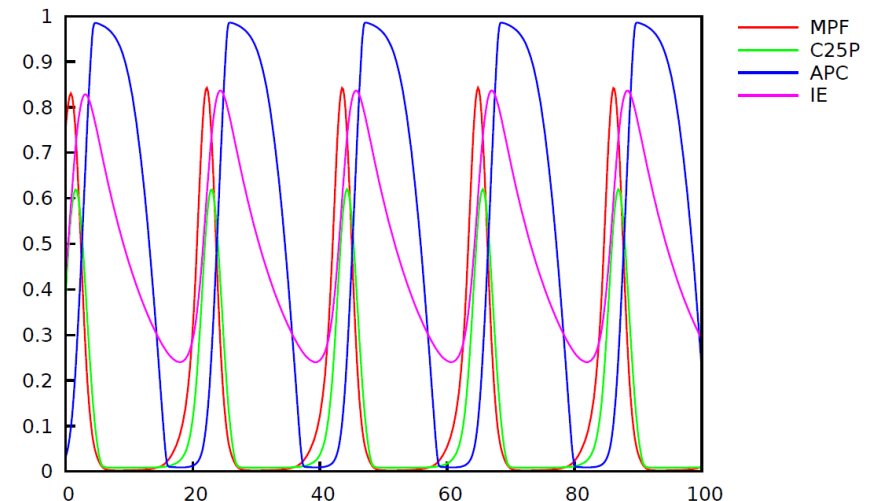
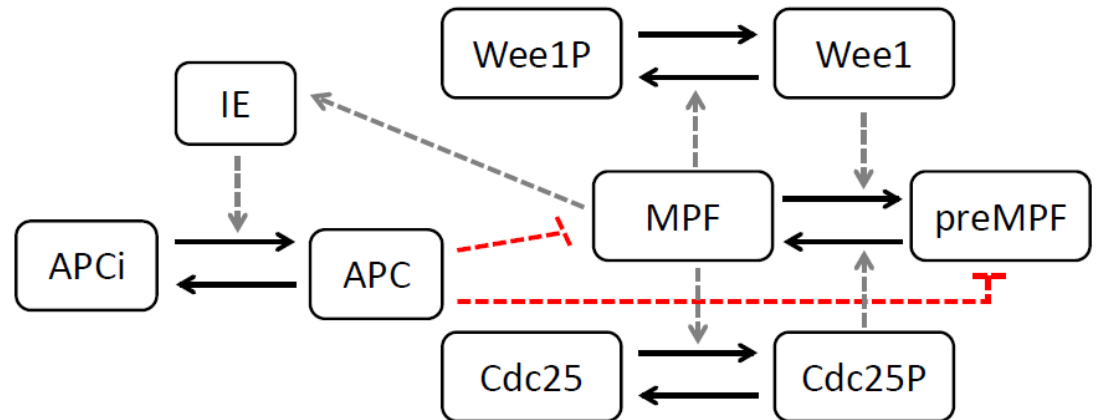


Qu-McLellan-Weiss Model of the Cell Cycle (2003)

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



FBS control of the cell cycle free period by k_{die} : IE degradation rate constant (important in G1/S phase)



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*.

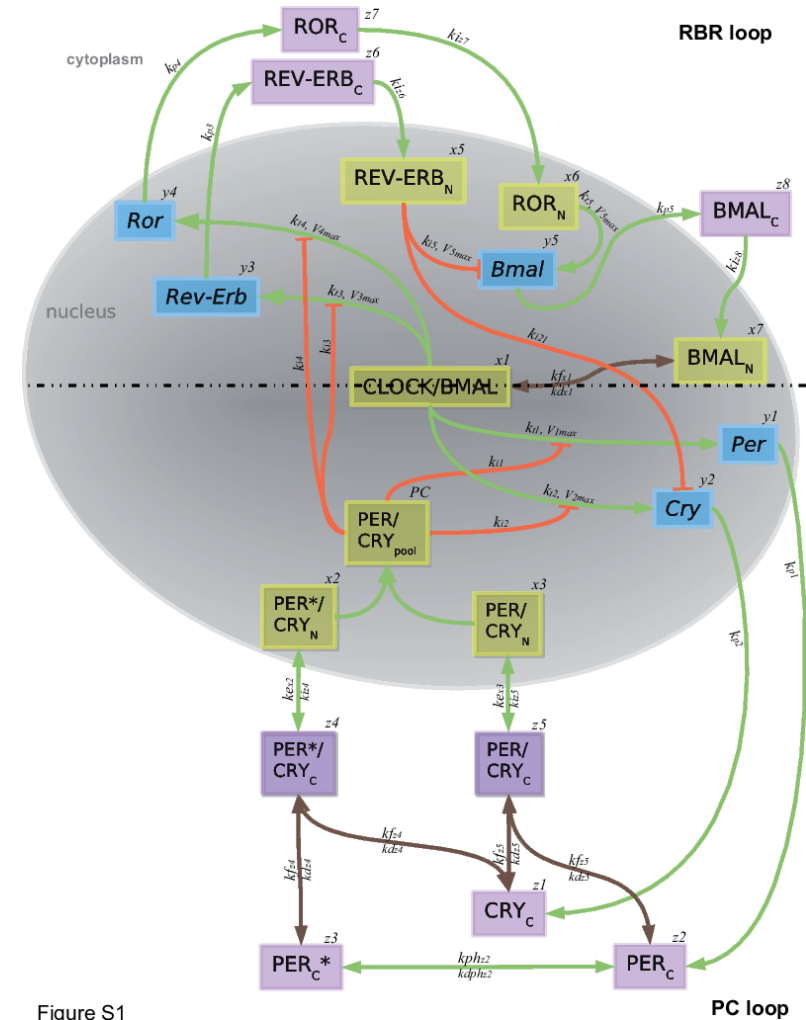
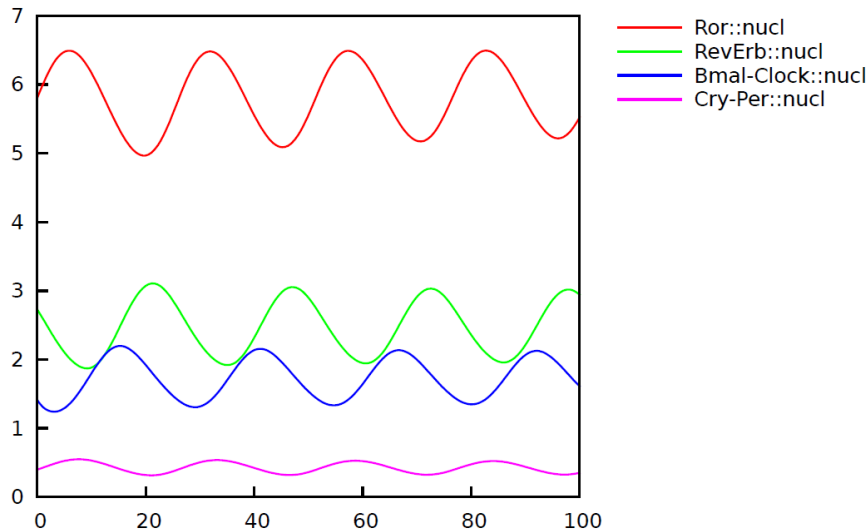
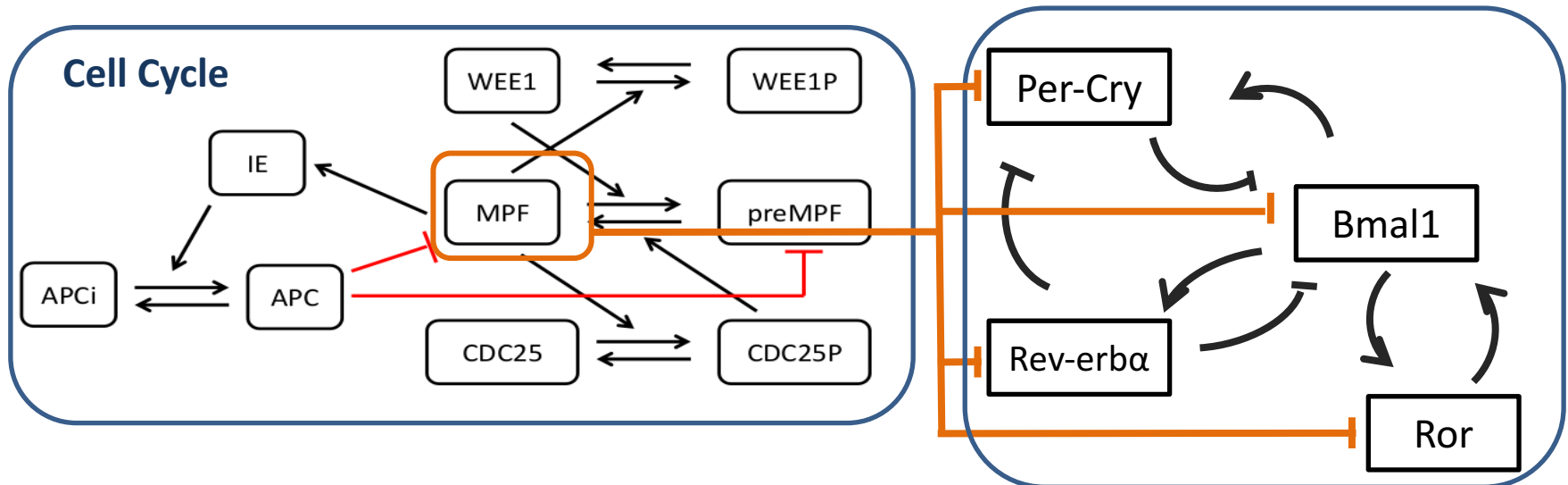


Figure S1

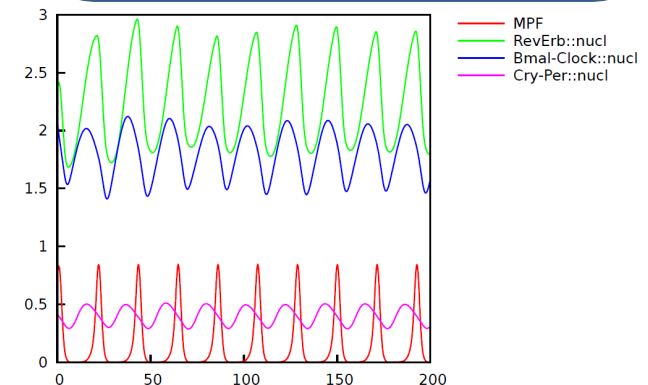
Hypothesis 1: Uniform Inhibition of Transcription during Mitosis [Kang et al. 2008]

- Correct acceleration of both the cell cycle and the circadian clock



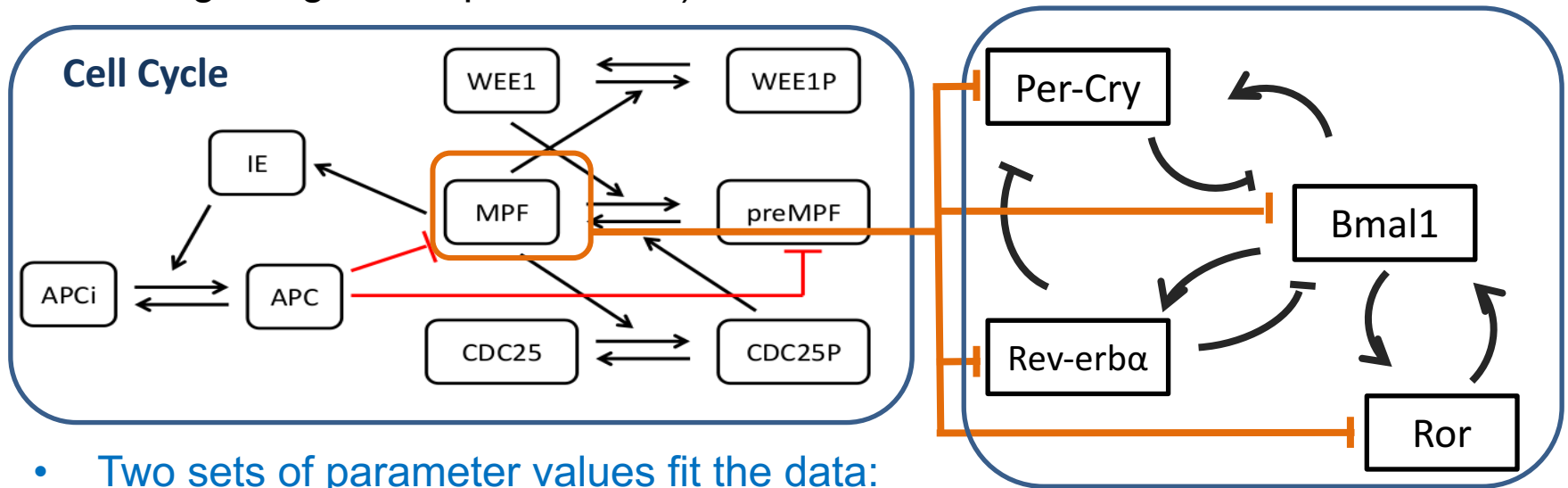
- But impossible to fit experimental phase shift between cell division time and RevErb peak

- 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:

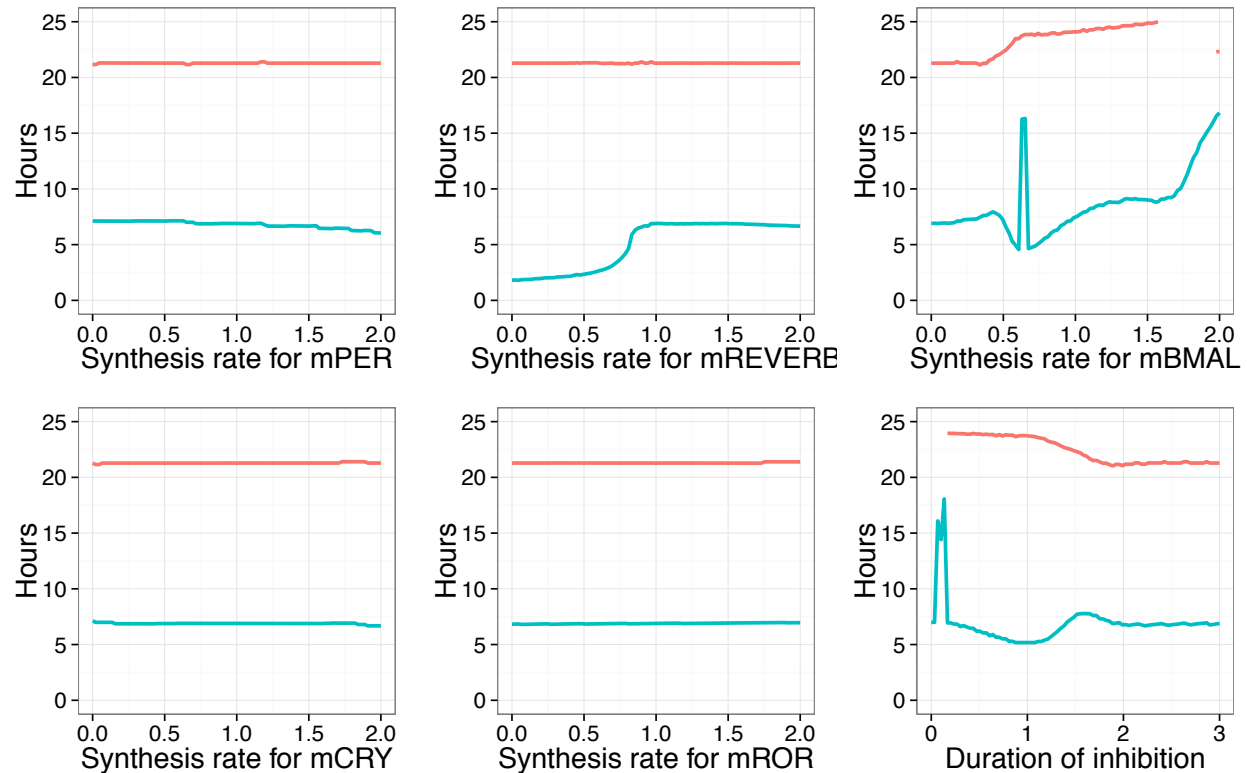
either down-regulation of *Bmal1* or up-regulation of *RevErb α* during mitosis

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

Hypothesis 2: Relevant Regulation Parameters

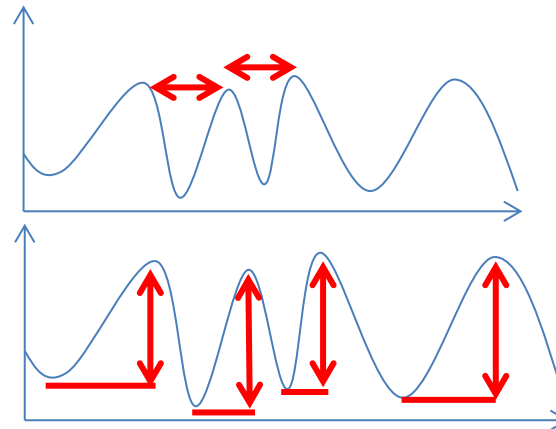
Effect of clock gene synthesis rate parameters and duration of regulation on the circadian clock period and phase shift between Reverb peak and cell division

— Circadian clock period
— Phase shift



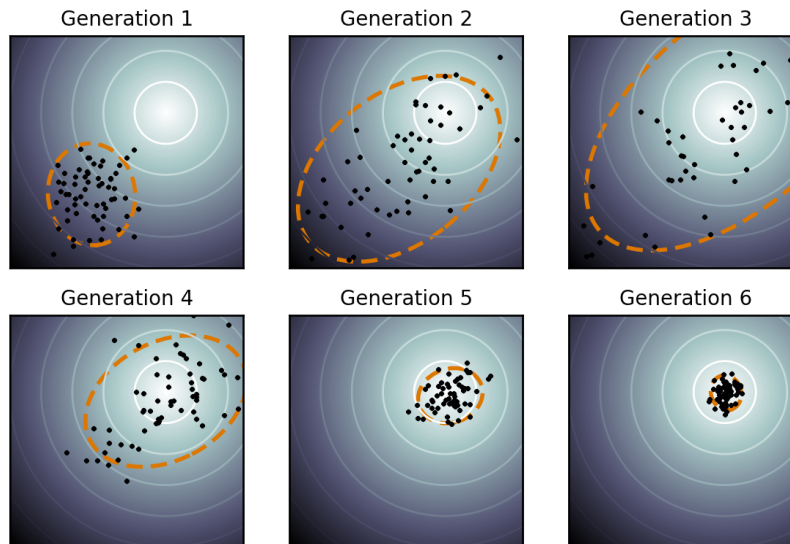
Temporal Logic Specification of Dynamical Properties

- Temporal logics introduced in Computer Science by Pnueli 1977 to reason about program execution traces
- Linear Time Temporal logic (LTL) extends Boolean propositional logic with **time operators** **X**: next, **F**: finally, **G**: globally, **U**: until
 - Reachability of a stable set of states **FG(s)**
- First-order LTL with linear constraints, FO-LTL(R_{lin}), express quantitative properties about concentrations:
 - Reachability of threshold **F(x>c)**
 - Minimum value **G(x>v)**
 - Distance between successive peaks
 - Amplitude of next peak
 - Period constraints
 - Phase constraints ...
- Implemented in our modeling free software BIOCHAM (Biochemical Abstract Machine) <http://lifeware.inria.fr/biocham>



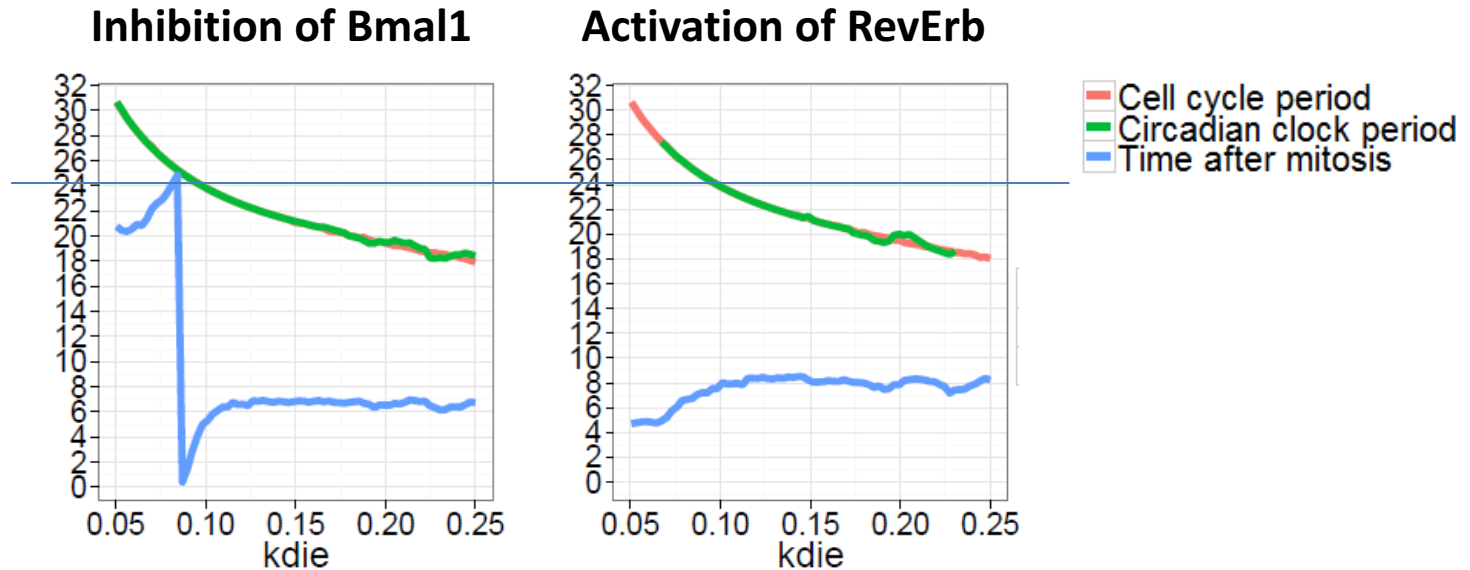
Continuous Satisfaction Degree of FO-LTL(Rlin) formulae and Parameter Search

- Algorithm for computing the **validity domain of free variables on a trace**
- Continuous satisfaction degree in $[0,1]$ of an FO-LTL(Rlin) formula with objective values for its free variables from **distance to validity domain**
- Measure of **robustness** of FO-LTL(Rlin) property as mean satisfaction degree
- **Sensitivity indices** w.r.t. FO-LTL(Rlin) property
- **Parameter search** maximizing satisfaction degree (up to 50-100 parameters)
Covariance matrix adaptive evolution strategy (CMAES) [Hansen 01-]

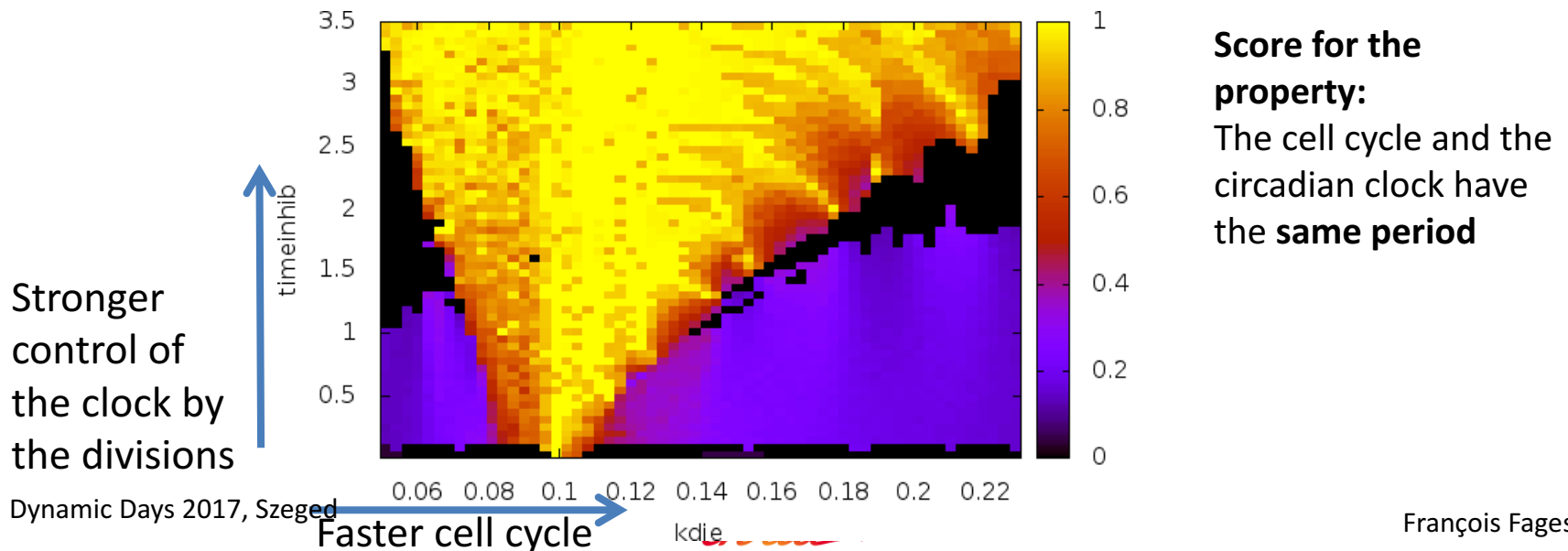


Hypothesis 2: correct entrainment in period and phase

Results:



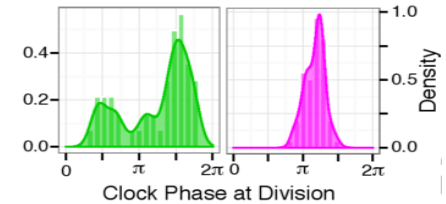
Prediction: different behaviors for a slow cell cycle (5% FBS)



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 \pm 0.5 h	20.1 h \pm 0.94 h	10.7 h
FBS 20%	21.25 h \pm 0.36 h	19.5 h \pm 0.42 h	8.3 h
	29 h \pm 1.05 h	16.05 h \pm 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]

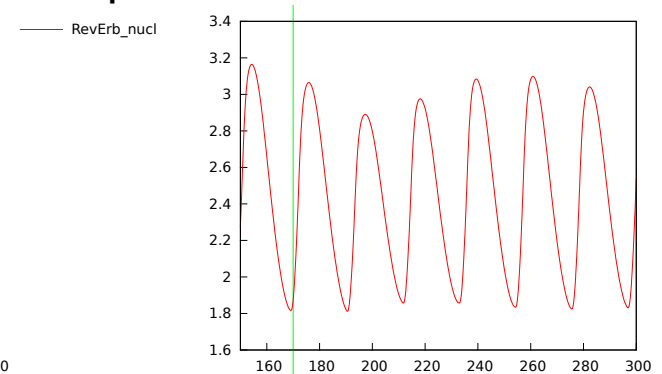
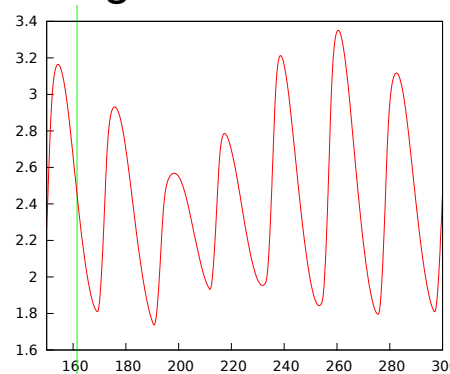
- In our model, Dex pulse modeled by induction of high level of Per. Stabilization of the clock occurs after 70h beyond observed data...

Clock perturbation varies according to the time T of the pulse

peak-peak distance in

[18.8, 22.7] with T=162h

[20.9, 21.7] with T=170h



Conclusion and Perspectives

- Analysis of NIH3T3 fibroblast time series
 - Cell division time and Rev-Erb α peak time at FBS =15%, 10%
- Model-based prediction of Rev-Erb α up-regulation during mitosis in NIH3T3
 - Coupled model of cell cycle and circadian clock
 - Fit to period and phase data (5% FBS exp. in favor of up RevErb α vs down Bmal1)
 - (uniform inhibition of transcription during mitosis cannot fit phase data)
- Different interpretation after treatment by Dexamethasone
 - Multiple attractors hypothesized interlock ratios 1:1 5:4 3:2 [Feillet et al. PNAS]
 - Variable transient states according to the clock time of the pulse
 - Chimera states interpretation to reconcile both interpretations ?
- Bi-directional coupled model through two mechanisms
 1. Regulation of cell cycle genes (Wee1) by clock genes (Bmal1)
 2. Regulation of clock genes by cell cycle (up regulation of Rev-Erb α during mitosis)
- BIOCHAM modeling tool for reaction systems and influence systems
 - Differential (ODE), stochastic (CTMC), Petri Net (PN) , Boolean semantics
 - Powerful quantitative temporal logic language (FO-LTL) for specifying behaviors
 - Sensitivity, robustness measure, parameter search w.r.t. FO-LTL specification

Lifeware Group



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