Coupled Models of the Cell Cycle and Circadian Clock for Chronotherapy Optimization

François Fages

Inria Saclay

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

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

ANR HYCLOCK (2014-18) F. Delaunay,

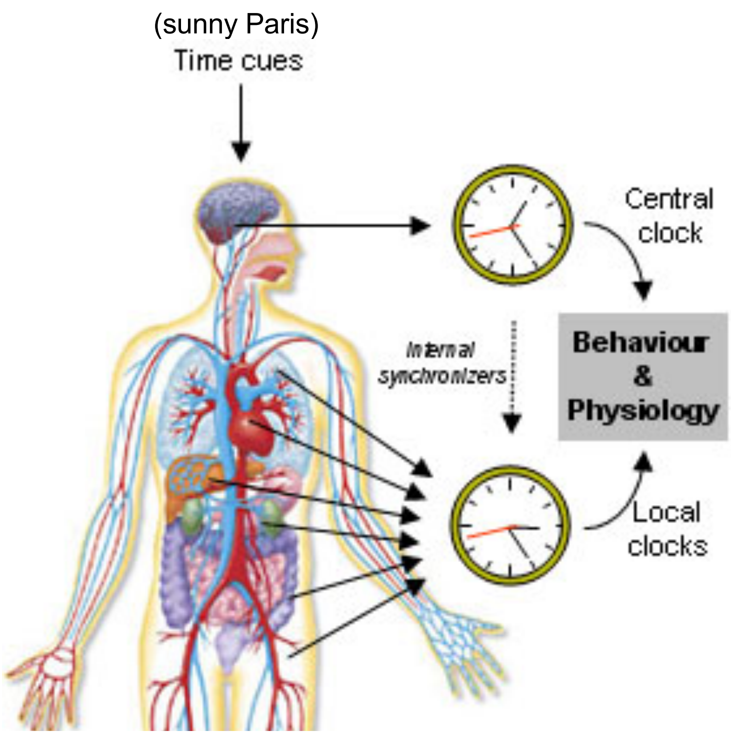
EraNet SysBio C5SYS (2010-2013) F. Lévi, D. Rand,

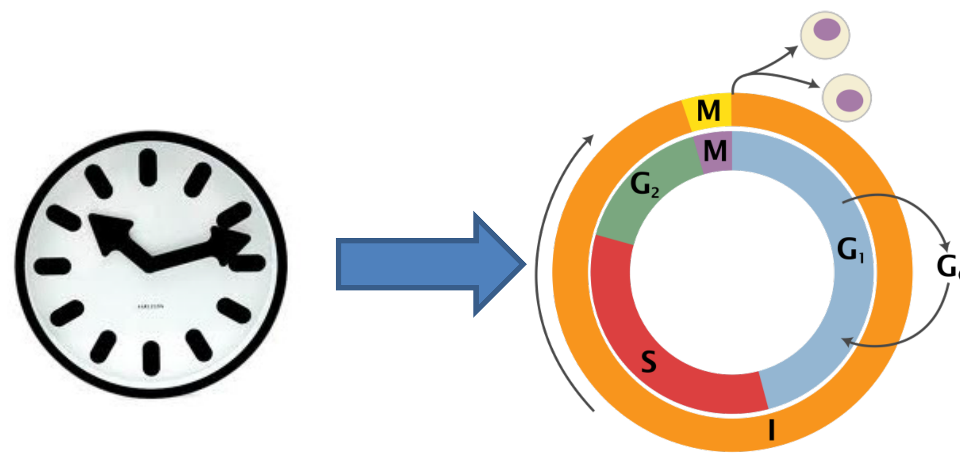
EU FP6 TEMPO (2006-2009) F. Lévi

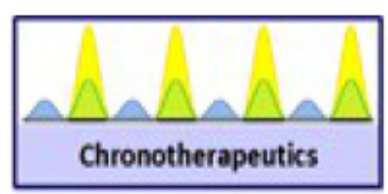
Inria

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)



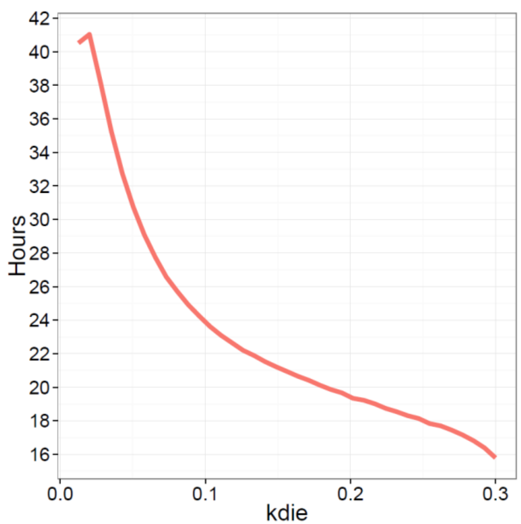




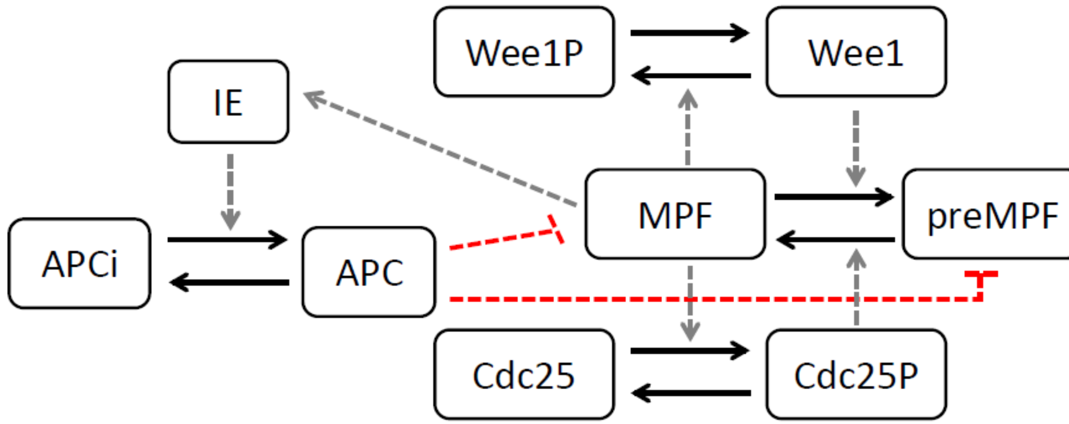
Mormont MC, Levi F.
Cancer chronotherapy: principles, applications, and perspectives.
Cancer, 2003.

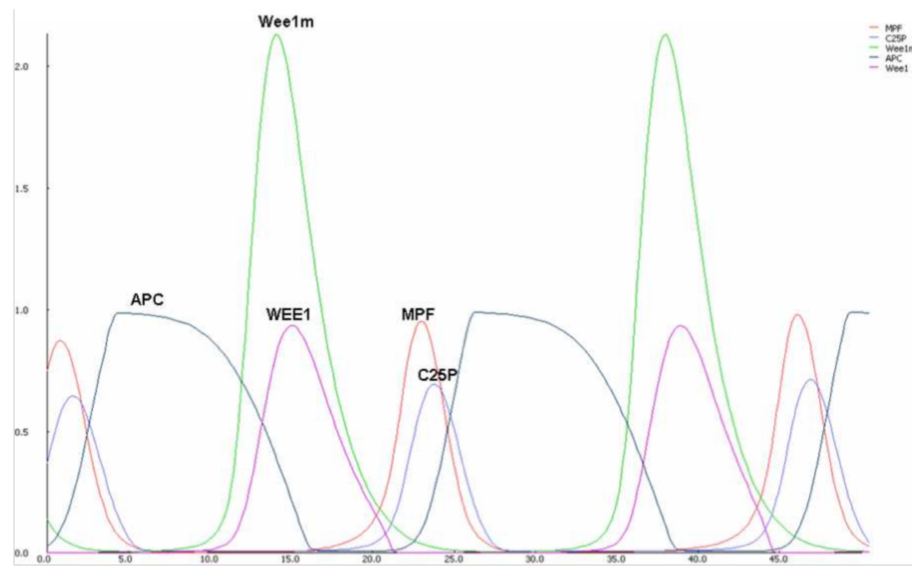
Cell Cycle Model [Qu-McLellan-Weiss Model 2003]

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



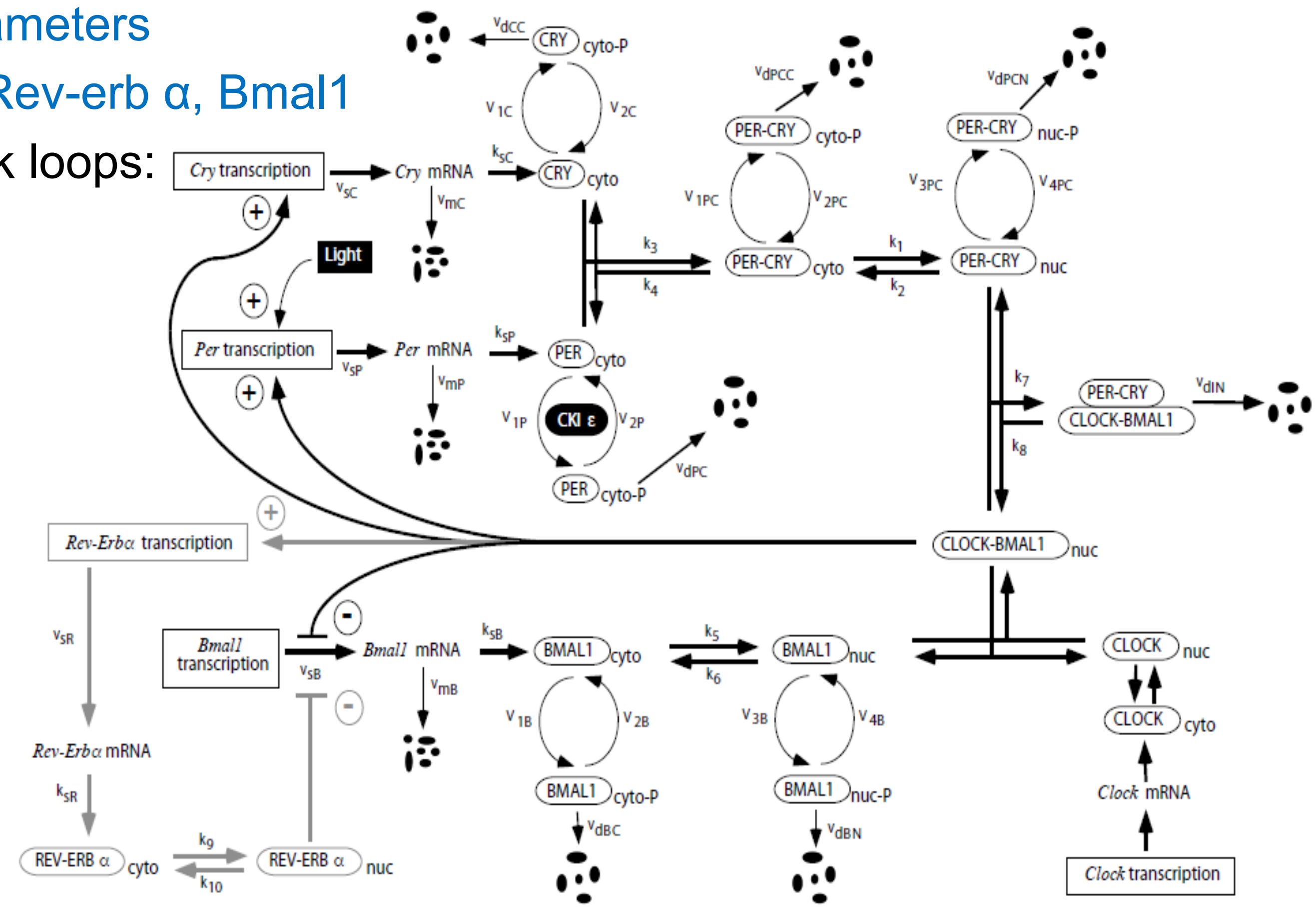
Variation of the cell cycle free period by *kdie* degradation rate constant (important in growing G1 phase)



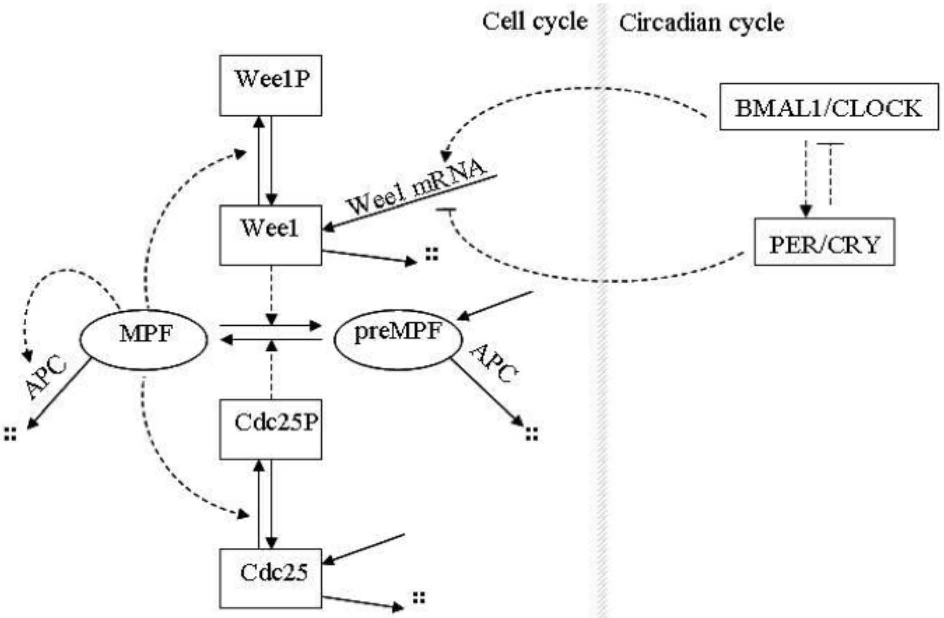


Circadian Clock Model [Leloup Goldbeter 03]

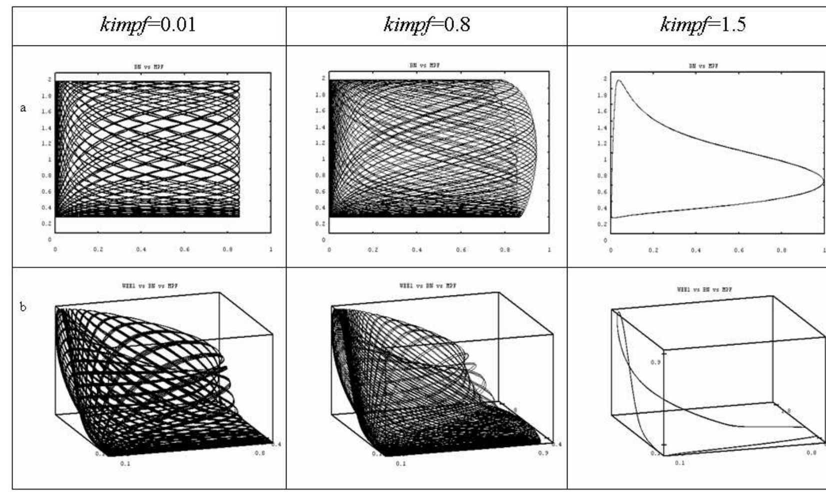
- 19 species, 70 parameters
- 4 genes: Per, Cry, Rev-erb α, Bmal1
- 2 negative feedback loops:
  - Per-Cry
  - Rev-erb α



Coupled Cell Cycle ← Circadian Clock Model



[Matsuo et al 2003]



Entrainment conditions (limit cycle) on parameter values

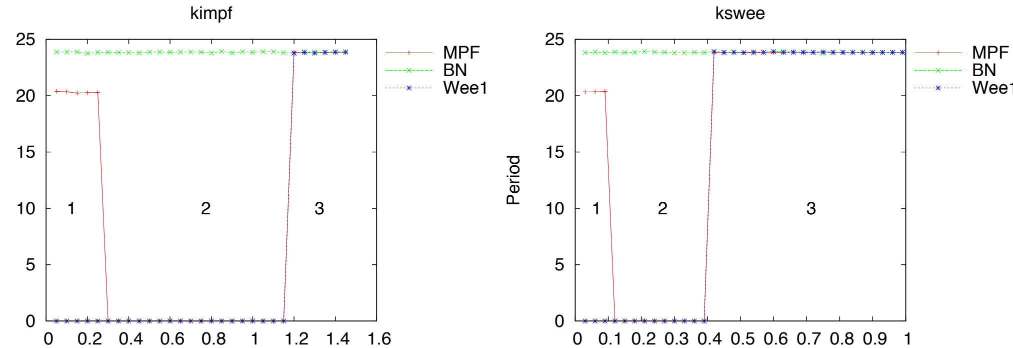
[Calzone Soliman 2006]

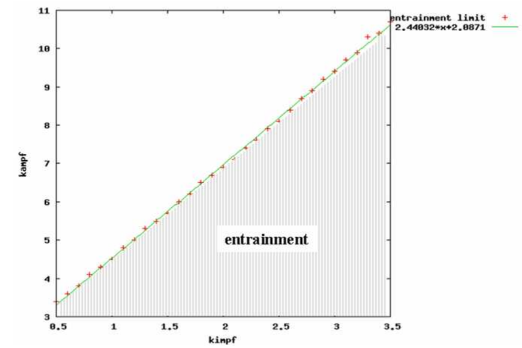
Coupling synthesis reaction of Wee1activated by Bmal1 repressed by Per-Cry

```
(ksweemp+ksweem*[Bmal1])/(Kweem+kwpcn*[PC]) for _ => mWee1
```

Conditions of Entrainment

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

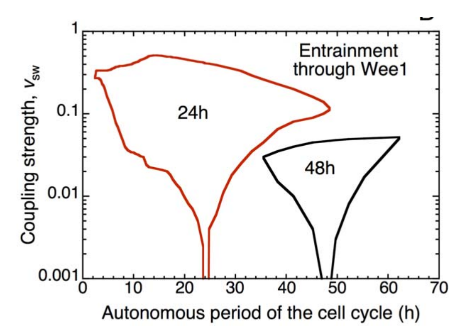




- Conditions of entrainment on Bmal1-Wee1 and MPF activation parameters

- Period doubling (24h, 48h) phenomena

  [Gerard Goldbeter PLOS 2012]



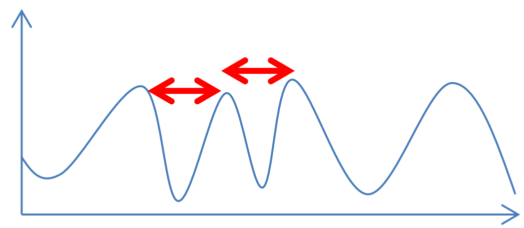
Formal Behavior Specification in Temporal Logic

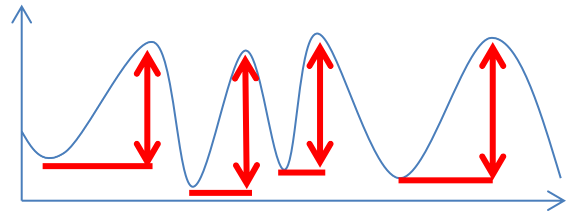
- Linear Time Temporal logic (LTL) extends classical logic with time operators
  X: next, **F**: finally, **G**: globally, **U**: until
  – Reachability of a stable set of states **FG**(s)
- First-order LTL with linear constraints, FO-LTL(R_{lin}), express quantitative properties about concentrations:

  – Reachability of threshold **F**(x>c)
  – Maximum value **G**(x<v)

  – Distance between successive peaks
  – Amplitude of next peak

  – Period constraints
  – Phase constraints …



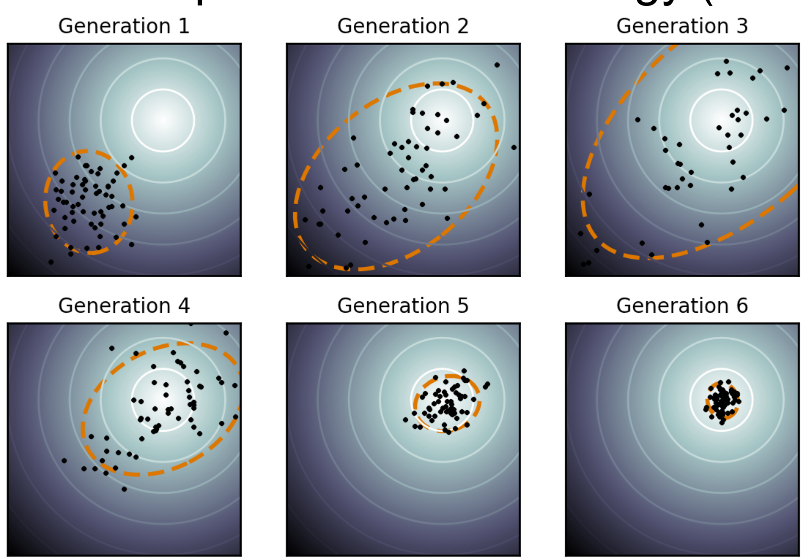


- Implemented in our modeling software BIOCHAM (Biochemical Asbtract Machine) http://lifeware.inria.fr/biocham4

Parameter Fitting and Parameter Optimisation

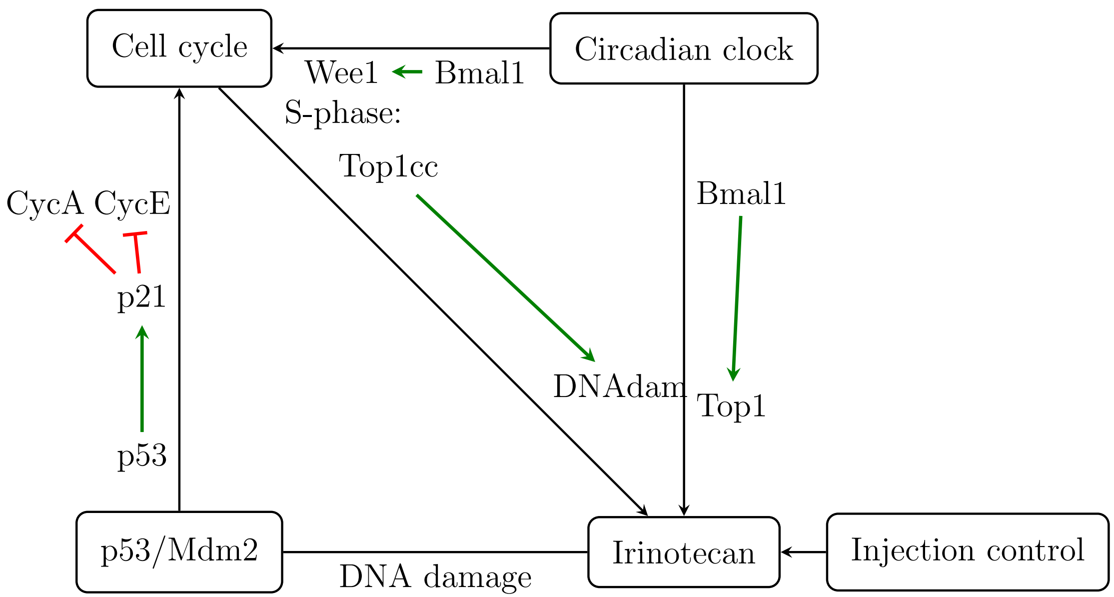
- Algorithm for computing the validity domain of free variables on a trace
- Continuous satisfaction degree in [0,1] of an FO-LTL(Rlin) formula with objective values for its free variables from distance to validity domain
- Measure of robustness of FO-LTL(Rlin) property as mean satisfaction degree
-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

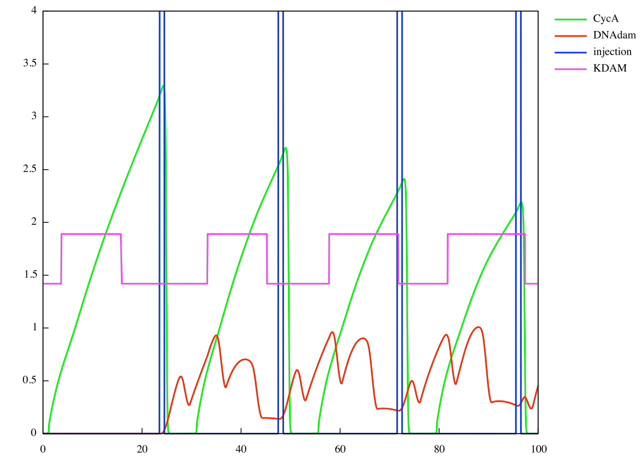
Covariance matrix adaptive evolution strategy (CMAES) [Hansen 01-]

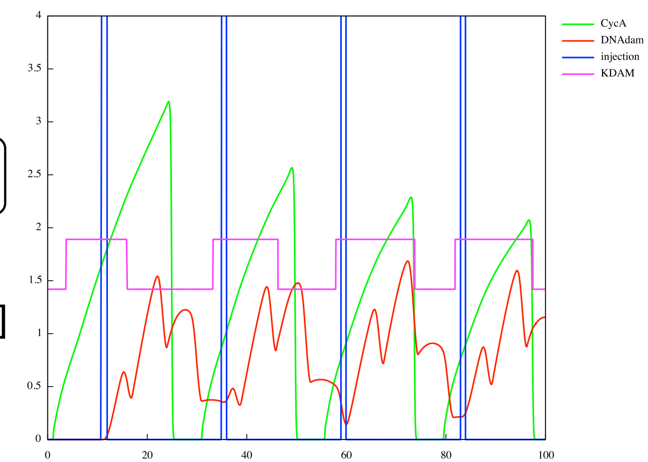


Irinotecan Exposure Chronotherapy Model

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

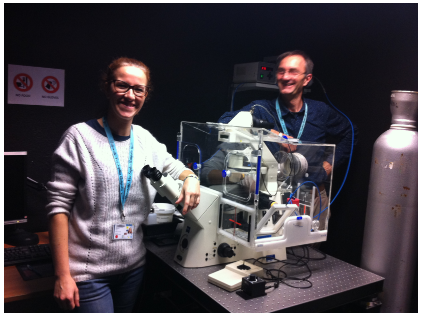
Whole body PK/PD drug injection model [Ballesta et al PlosCB 2011]

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

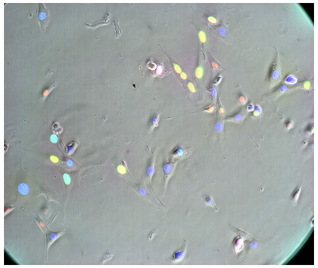
Time series data in individual mice fibroblasts [Feillet Delaunay 2012]

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

Medium with various concentrations of serum (FBS)



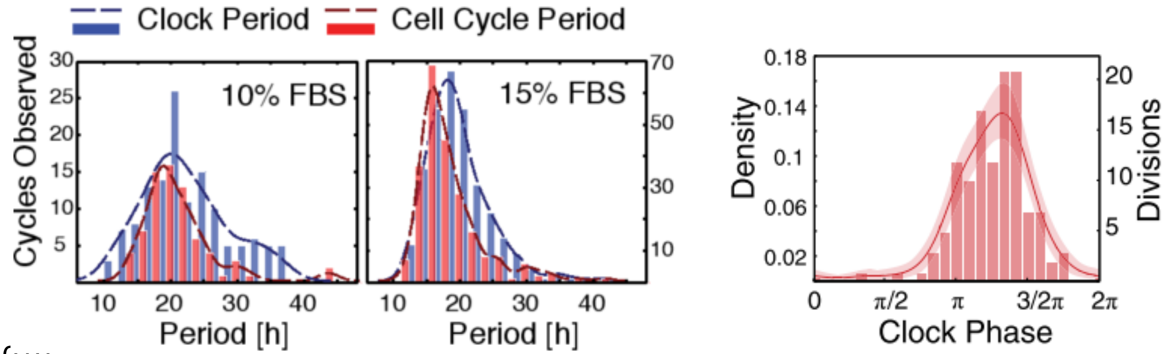
- FBS modulates the cell cycle frequency
- No observed time gating for mitosis
- But observed acceleration of the circadian clock
  in fastly dividing cells ! and not in confluent cells (24h)



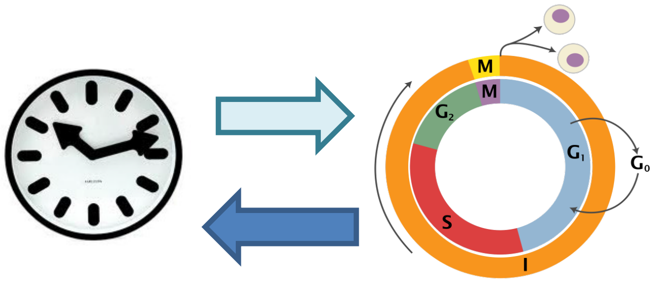
FBS 10% → Cell cycle 22h → Circadian clock 22h, phase 7h

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

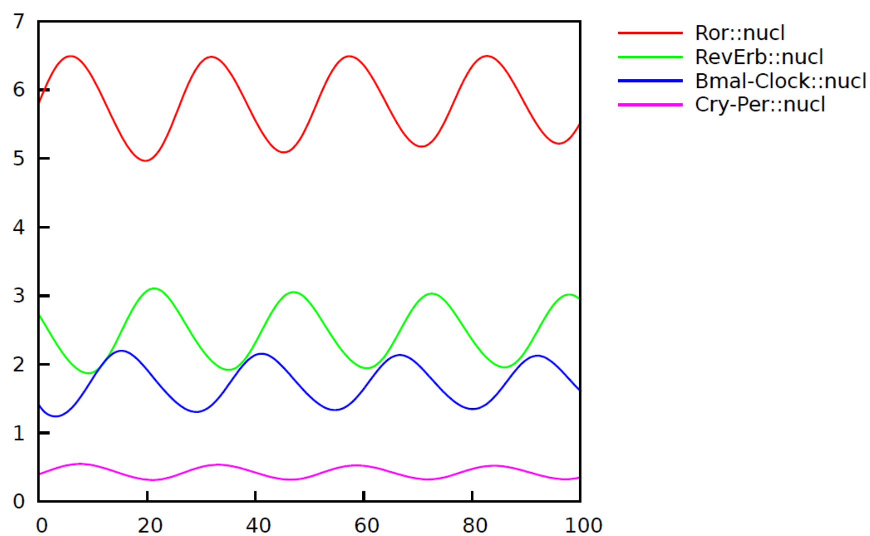
- Entrainment in period and phase fitted to experimental data
- Prediction of reverb up-regulation during mitosis (or Bmal1 down)

[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.



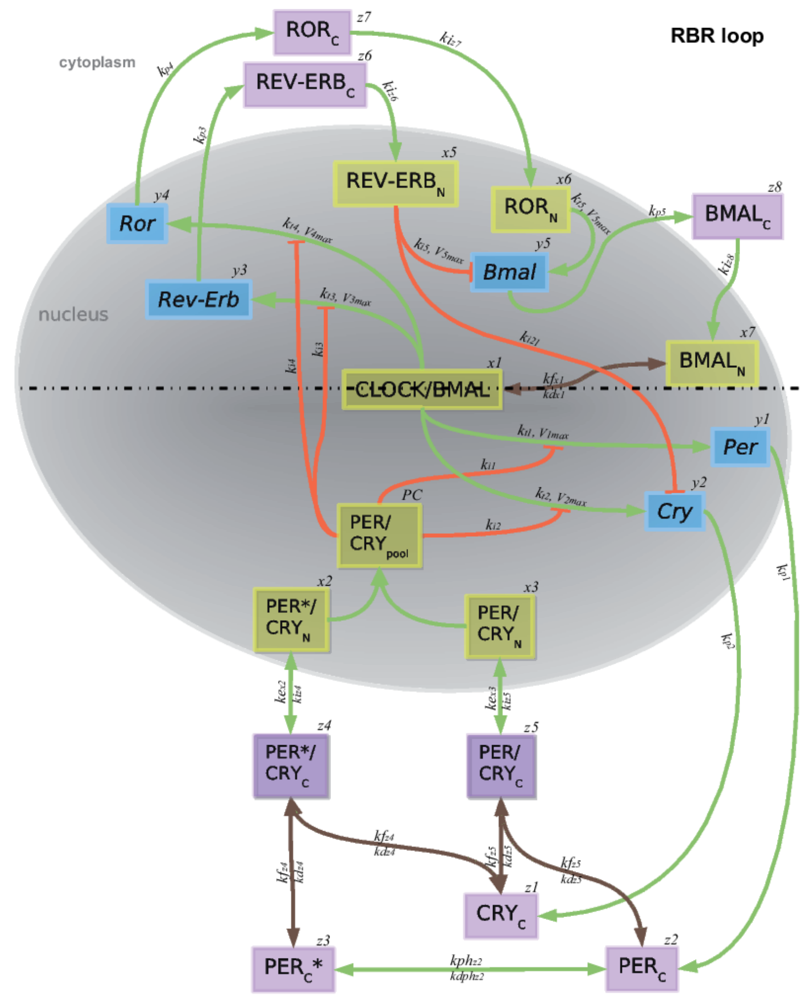
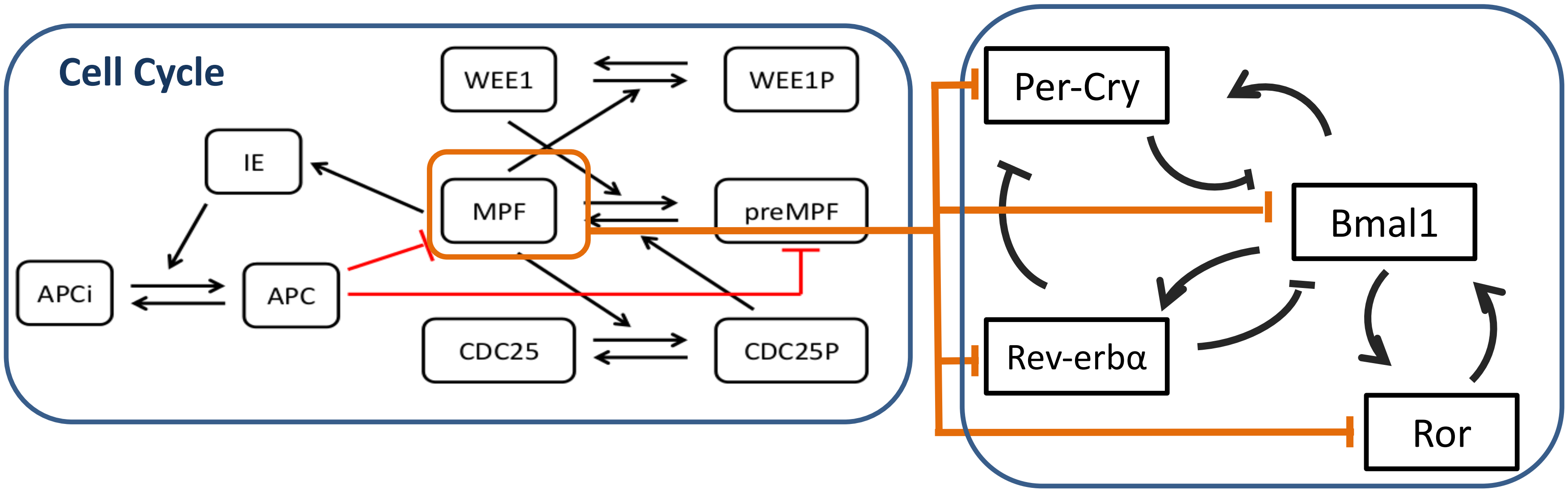
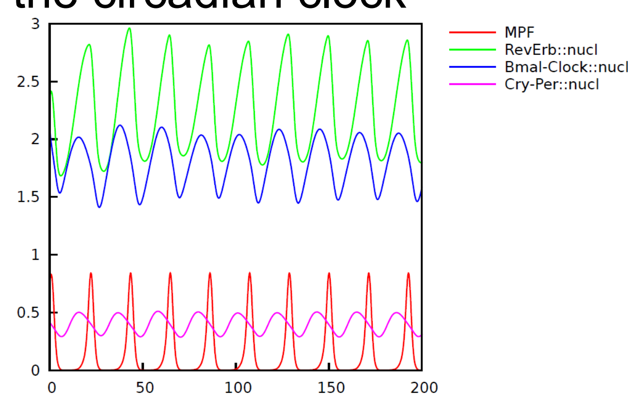


Figure S1

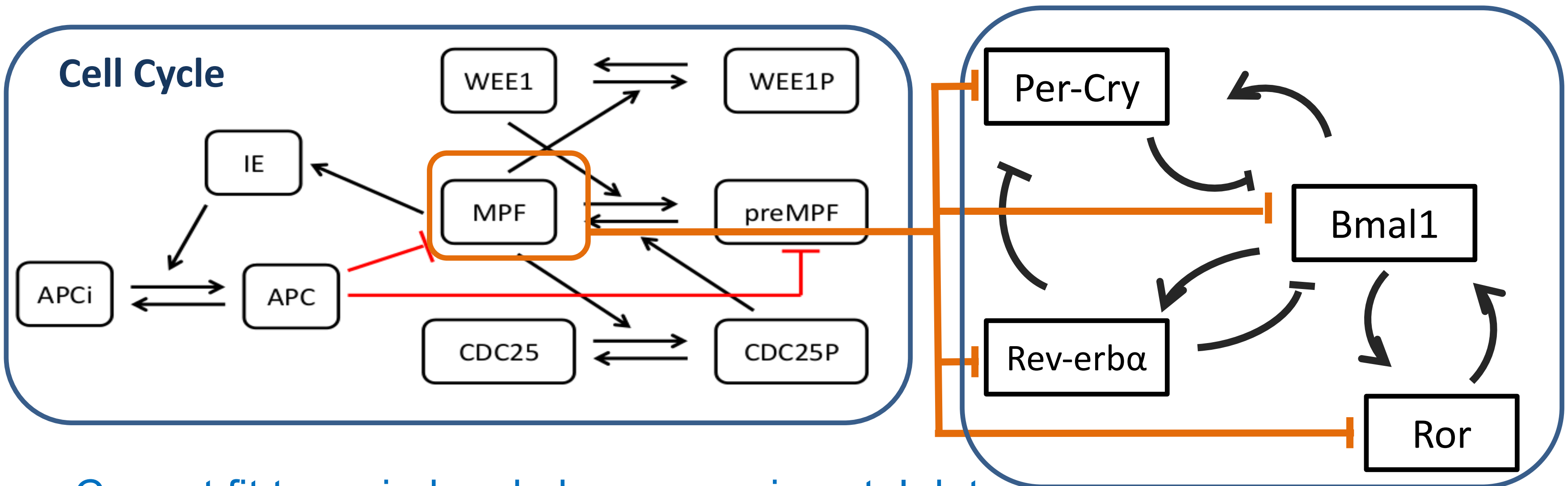
Hypothesis 1: Uniform Inhibition of Transcription during Mitosis [Kang et al. 2008]



- 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:

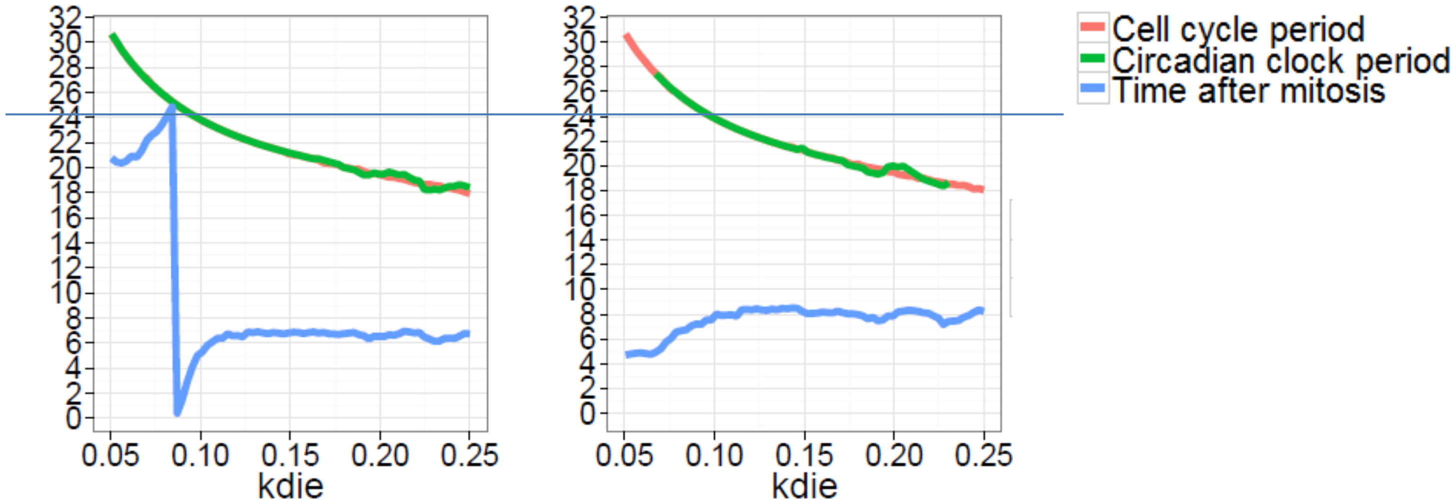
either down-regulation of Bmal1
or up-regulation of RevErbα
during mitosis

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

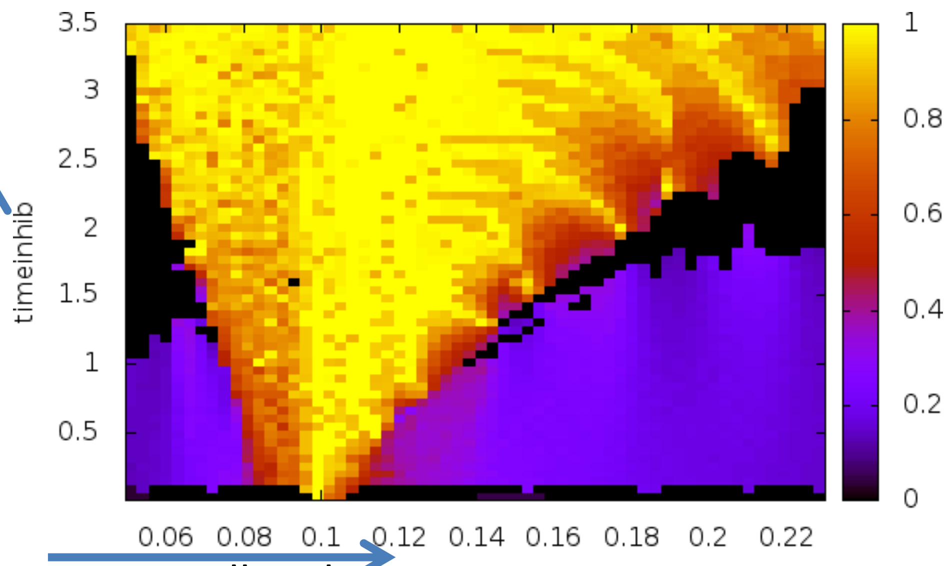
Hypothesis 2: Predictions

Results:

Inhibition of Bmal1 or **Activation of RevErb**



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



Stronger control of the clock by the divisions

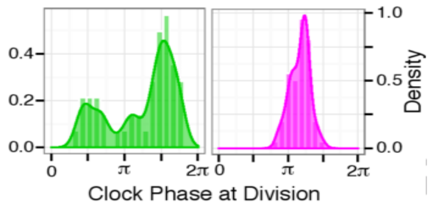
Faster cell cycle

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%

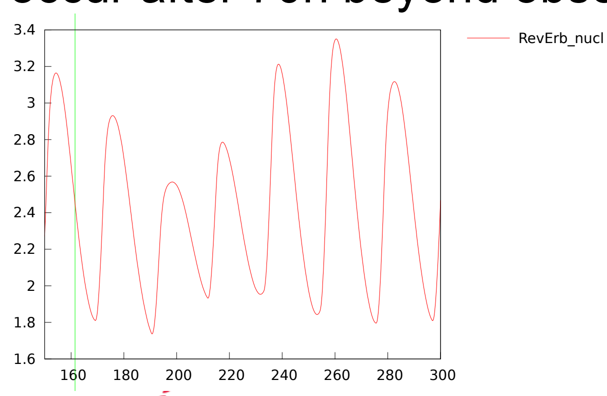
[C. Feillet et al. Phase locking and multiple oscillating attractors for the coupled mammalian clock and cell cycle., PNAS 2014]

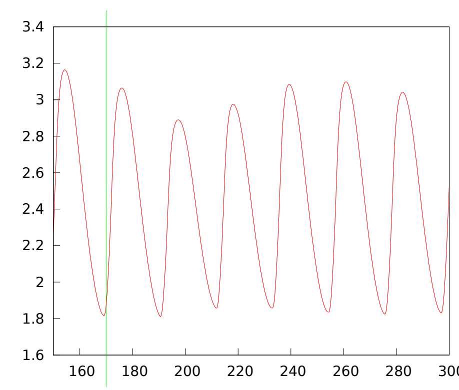
- In our model, Dex pulse modeled by induction of high level of Per.

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





Summary

- Analysis of NIH3T3 embryonic fibroblast single cell time series data
  - Cell division time and Rev-Erbα peak time at FBS =15%, 10%
  - Model-based prediction of Rev-Erbα up-regulation during mitosis
  - (model-based predictions at 5% FBS in favor of up RevErbα vs down Bmal1)
  - Uniform inhibition of transcription during mitosis could not fit phase data

- Different interpretations 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

- Big picture: bi-directional coupling through two mechanisms
  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)