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

François Fages
Inria Saclay

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
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)

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 $k_{die}$ 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

Coupling synthesis reaction of Wee1 activated by Bmal1 repressed by Per-Cry

\[
\text{Coupling synthesis reaction of Wee1 activated by Bmal1 repressed by Per-Cry:} \quad (\text{kswemp} + \text{kswem} \ast [\text{Bmal1}]) / (\text{Kweem} + \text{kwpcn} \ast [\text{PC}]) \quad \text{for} \quad \_ \Rightarrow \text{mWee1}
\]

[Calzone Soliman 2006]
Conditions of Entrainment

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

- Period doubling (24h, 48h) phenomena

[Gerard Goldbeter PLOS 2012]
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(\(\mathbf{R}_{\text{lin}}\)), 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 Abstract Machine) [http://lifeware.inria.fr/biocham4](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

- Cell cycle
- Circadian clock
- Wee1 €\xrightarrow{\text{Bmal1}}$
- DNA damage
- S-phase:
  - Top1cc
  - Bmal1
- Top1
- p53
- p21
- CycA
- CycE
- p53/Mdm2
- Injection control
- Irinotecan

Optimal control of drug exposure [De Maria et al TCS 2011]
- Max pulses satisfying always $\text{DNAdam} < 1$
- with $\text{DNA damage} > 1$ on phase shifted cells

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

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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

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 RevErba during mitosis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>First set</th>
<th>Second set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesis coefficient for Per</td>
<td>0.66</td>
<td>2.40</td>
</tr>
<tr>
<td>Synthesis coefficient for Cry</td>
<td>2.30</td>
<td>0.67</td>
</tr>
<tr>
<td>Synthesis coefficient for RevErba-α</td>
<td>1.04</td>
<td>1.92</td>
</tr>
<tr>
<td>Synthesis coefficient for Ror</td>
<td>2.1</td>
<td>1.51</td>
</tr>
<tr>
<td>Synthesis coefficient for Bmal1</td>
<td>0</td>
<td>0.78</td>
</tr>
<tr>
<td>Duration</td>
<td>2.97h</td>
<td>2.81h</td>
</tr>
</tbody>
</table>
Hypothesis 2: Predictions

Results:

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

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

Stronger control of the clock by the divisions

Bioregul 2019
Complex Behaviors with High Variability observed after Treatment by Dexamethasone

- Dexamethasone synchronize cellular clocks, but complex dynamics observed

<table>
<thead>
<tr>
<th>Medium</th>
<th>Clock period</th>
<th>Division period</th>
<th>Mean delay</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS 10%</td>
<td>24.2 h ± 0.5 h</td>
<td>20.1 h ± 0.94 h</td>
<td>10.7 h</td>
</tr>
<tr>
<td>FBS 20%</td>
<td>21.25 h ± 0.36 h</td>
<td>19.5 h ± 0.42 h</td>
<td>8.3 h</td>
</tr>
<tr>
<td></td>
<td>29 h±1.05 h</td>
<td>16.05 h±0.48 h</td>
<td>6h/12h/22h</td>
</tr>
</tbody>
</table>

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)