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Abstract: The existence of links between the cell division cycle and the circadian clock has been recently discovered. In this research report, we perform a small *in silico* analysis of how mutations on the clock genes composing the mammalian circadian clock affect the phenotype of the cell cycle. For this purpose, we use a coupled model of the mammalian circadian clock and the mammalian cell cycle where the latter one is entrained by the former one via a common protein kinase WEE1. Our *in silico* experiments exploit the modeling environment BIOCHAM.

Key-words: Cell cycle, circadian clock, model coupling, mutations

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Résumé : L'existence de liens entre le cycle de division cellulaire et l'horloge circadienne a été mise au jour récemment. Dans ce rapport de recherche, nous menons une petite analyse *in silico* sur comment les mutations des gènes de l'horloge circadienne composant l'horloge circadienne des mammifères affectent le phénotype du cycle cellulaire. A cette fin, nous utilisons un modèle couplé de l'horloge circadienne et du cycle cellulaire des mammifères dans lequel ce dernier est entrainé par le premier via la protéine kinase WEE1. Nos expériences *in silico* exploitent l'environnement de modélisation BIOCHAM.

Mots-clés : Cycle cellulaire, horloge circadienne, couplage de models, mutations

1 Introduction

In this research report we aim at studying how mutations on the clock genes composing the mammalian circadian clock affect the phenotype of the cell cycle. For this purpose, we take into consideration the coupled model of the mammalian circadian cycle and the mammalian cell division cycle proposed by Calzone and Soliman in [1], following Matsuo et al. [2].

As for the circadian cycle, we refer to the model described by Leloup and Goldbeter in [3], that expresses the regulatory effects exerted on gene expression by the *PER* (period), *CRY* (cryptochrome), *BMAL1*, *CLOCK*, and *REV-ERB*α proteins [1] and post-translational regulation on these proteins by reversible phosphorylation. To account for entrainment of the circadian clock by light-dark cycles, the effect of the light is incorporated in the maximum rate of *PER* mRNA expression. The model gives rise to sustained circadian oscillations with a period close to 24 hours. *BMAL1* mRNA (*mBmal1*) oscillates in antiphase with *PER* and *CRY* mRNAs (*mPER* and *mCRY*, respectively). The proteins undergo similar oscillations and follow their mRNA by a few hours.

As for the cell division cycle, we consider a model based on that of Qu, MacLellan, and Weiss in [4], where a generic signaling module is used to represent either the G1/S or the G2/M transitions. The focus we consider here is on the G2/M transition, leading to a two-phase cell-cycle model with G1-S-G2 and M phases (see [1] for more details).

The kinetic differential equations present in the two papers allowed Calzone and Soliman to encode both models in the rule-based language of Biocham [2]. The link between the two models comes from the experiments of [2] and is reflected through a direct influence of CLOCK-BMAL1 (*Bmal1*) on the synthesis of WEE1 mRNA (*mWee1*), a kinase that delays or prevents entry into mitosis by phosphorylation of MPF.

We would like to underline that the models we use are not fitted to real data yet; however, they allow us to get an initial insight on the effects of circadian mutations on the cell cycle as a basis for discussion with biologists.

In Figure 1 there is a 100 hours-simulation of the coupled circadian/cell cycle model (entrainment by light-dark cycles is taken into account), where it is possible to observe that all the displayed compounds undergo oscillations whose period is approximately 23 hours (if the cell division cycle model is considered alone, the oscillation period is about 23 hours too).

The first three columns of Table 1 show the oscillation amplitude and period and the mean value of some selected compounds observed towards the end of a 650-hours simulation.

2 *In Silico* Circadian Clock Genes Knock-outs

Hereafter we describe how the cell cycle reacts to circadian gene/protein mutations in our coupled model. First we consider single mutations, then we study the combination of different mutations. As for single compound mutations, we

[1] For the sake of simplicity, we consider a version of the model where the role of *REV-ERB*α in the indirect negative feedback exerted by BMAL1 on the expression of the Bmal1 gene is not explicitly taken into account.

[2] For a review of Biocham syntax, which is very similar to SBML one, the reader can refer to [5].

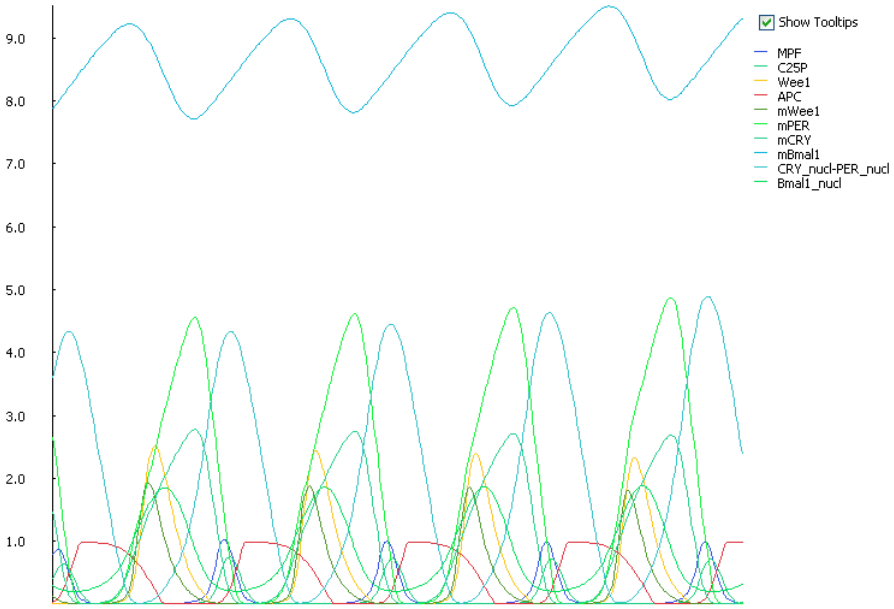

Figure 1: Simulation of the coupled model without mutations.

Compound	wild type			mPER=0			mBmal1=0		
	Amplitude	Period	Mean Value	Amplitude	Period	Mean Value	Amplitude	Period	Mean Value
MPF	1.015	24	0.5	-	∞	0.006	0.856	20.4	0.43
C25P	0.74	24	0.37	-	∞	0.009	0.627	20.4	0.32
Wee1	1.33	24	0.66	-	∞	4.02	0.005	20.4	0.003
APC	0.99	24	0.5	-	∞	0.0004	0.986	20.4	0.5
mWee1	0.99	24	0.5	-	∞	2.68	-	∞	0.005
mPER	5.83	24	2.9	-	∞	0	-	∞	0
mCRY	2.72	24	1.36	-	∞	6.22	-	∞	0
mBmal1	9.99	24	9.16	-	∞	5.88	-	∞	0
CRY_nucl-PER_nucl	6	24	3.04	-	∞	0	-	∞	0
Bmal1_nucl	1.9	24	1.04	-	∞	1.07	-	∞	0

Table 1: Oscillation amplitude and period and mean value towards the end of 650-hours simulations in the cases of no mutations, $mPER$=0, and $mBmal1$=0.

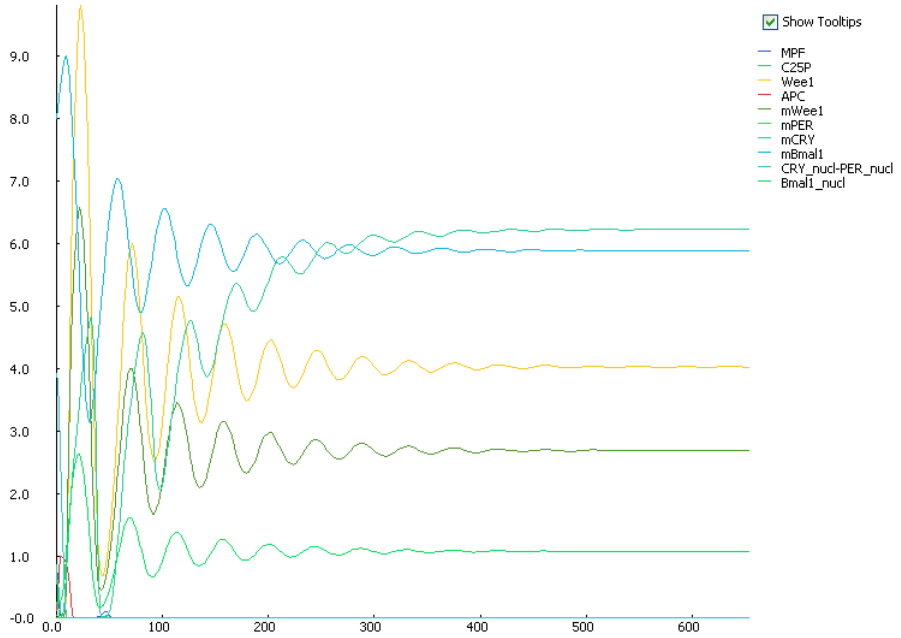

Figure 2: Simulation of the coupled model with *mPER=0*.

explore what happens when a given compound (for genes we will concentrate on the mRNA) is missing, that is, its concentration equals zero. To this aim, it is possible either to set the compound synthesis at zero, or to make the compound be absorbed by a "super-inhibitor" (e.g., the knock-out of a given compound C can be modeled by inserting in the model the rule $Inhibitor + C \rightarrow Inhibitor\text{-}C$, where the initial concentration of $Inhibitor$ is very high). As a matter of fact, both the alternatives have the same impact on the behavior of the coupled model. The mutations we take into consideration concern *mPER*, *mCRY*, *mBmal1*, and *Bmal1_nucl*. The simulations we provide in the following are up to 650 hours.

mPER=0. As graphically depicted in the plot of Figure 2, in this case the circadian rhythmicity is lost. The concentration of many compounds, including *mCRY*, *mBmal1*, *Wee1*, *mWee1*, and *Bmal1_nucl*, becomes constant after some oscillations of decreasing amplitude with a period of 40-50 hours lasting approximately 500 hours. *CRY_cyto* distinguishes itself because it reaches a constant value of 78.7. The other compounds, including *MPF*, *C25P*, *APC*, and *CRY_nucl-PER_nucl*, approach 0 after a few hours from the beginning of the simulation. A notable remark is that the mean value of the mitosis inhibitor *mWee1* (and of *Wee1*) is higher with respect to normal conditions.

More schematically, the amplitude, period, and mean value assumed by the mentioned compounds towards the end of a 650-hours simulation are reported in the third, fourth and fifth columns of Table 1. Since in this case there are no oscillations, the amplitude is omitted and the period is conventionally set to ∞.

mCRY=0. In this case the behavior of the model is approximately the same of the previous one (see Figure 3), that is, we can distinguish a first class

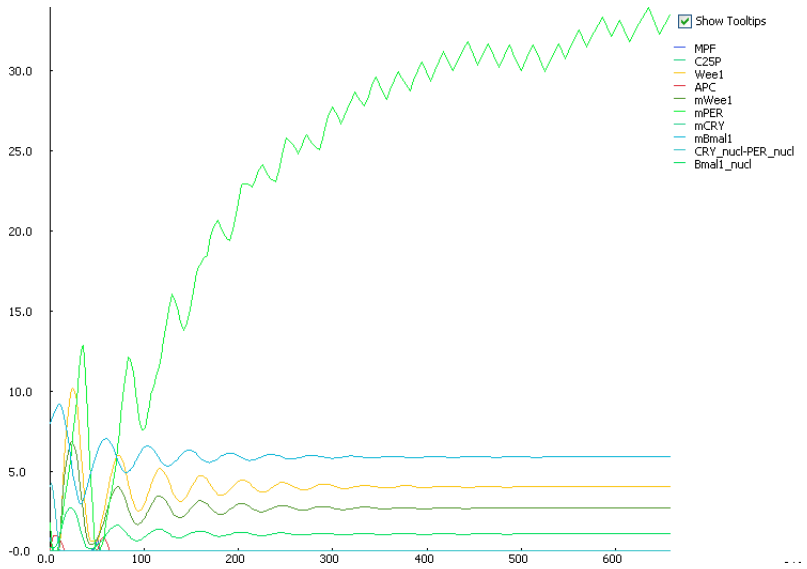

Figure 3: Simulation of the coupled model with *mCRY=0*.

of compounds whose concentration becomes constant after 500 hours, e.g., *mBmal1*, *Wee1*, *mWee1*, and *Bmal1_nucl*, and a second class of compounds whose concentration approaches 0 a few hours after the beginning of the simulation, e.g., *MPF*, *C25P*, *APC*, and *CRY_nucl-PER_nucl*. The difference is that setting *mCRY* value to 0 causes a big increase of *mPER*, whose value goes over 30, and a huge increase of PER_cyto, whose value goes over 1835 [3].

mBmal1=0. As the plot of Figure 4 shows, when *mBmal1* is not present it is possible to distinguish the following behaviors: all the cell cycle compounds except *mWee1* continue to oscillate with a period of approximately 20 hours (the amplitude of *Wee1* and *Wee1*$\sim \{p\}$ is very low, that is, 0.005 and 0.006, respectively), *mWee1* concentration stabilizes at 0.005, and all the circadian compounds approach 0 after a few hours from the beginning of the simulation (actually, *CRY_cyto*, *CRY_cyto-PER_cyto*, and *CRY_nucl-PER_nucl* approach 0 after an initial peak of amplitude greater than 5). The oscillation amplitude and period and the mean value of the compounds towards the end of a 650-hours simulation are reported in the last three columns of Table 1.

Bmal1_nucl=0. In this case the assumed behavior is the same that we get when *mBmal1=0*, that is, all the cell cycle compounds except *mWee1* oscillate intensely while *mWee1* and all the circadian compounds approach 0 after a few hours from the beginning of the simulation, with the difference that now *mBmal1* grows rapidly, getting over 20.

The simultaneous mutation of more than one gene is considered in the following.

mPER=mCRY=0. When both *mPER* and *mCRY* equal 0, we get the same behavior that we have when either *mPER* or *mCRY* equals 0.

[3] A similar behaviour is obtained when either *PER_cyto=0* (in this case, as well as in the case when *mPER*=0, *CRY_cyto* value goes over 78) or *CRY_cyto=0* (in this case, as well as in the case when *mCRY*=0, *PER_cyto* value goes over 1835).

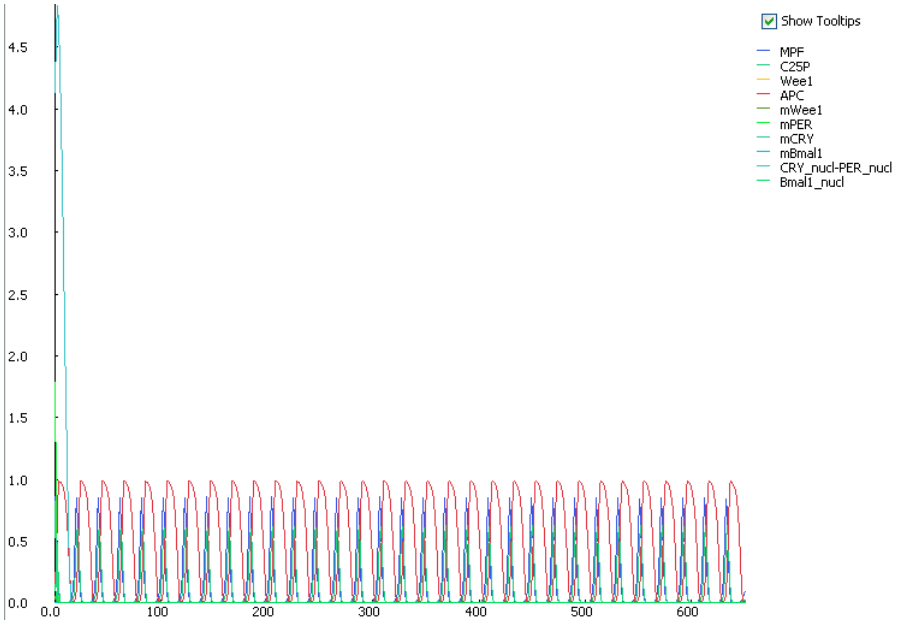

Figure 4: Simulation of the coupled model with *mBmal1=0*.

mPER=mBmal1=0. In this case, the model behaves as the model where only *mBmal1* is set to 0.

mCRY=mBmal1=0. As in the previous case, the model acts as the one where only *mBmal1* is set to 0.

mPER=mCRY=mBmal1=0. Again, the behavior of this model is assimilable to the one of the model where only *mBmal1* is set to 0.

3 Comparison with Literature about Circadian Clock Genes Mutations

In the following we itemize some facts we found in literature concerning the dependence of the cell division cycle on circadian rhythmicity/mutations and, when possible, we discuss the consistency with our results.

- Decreasing the level of *CRY* by halving the rate of *CRY* synthesis does not abolish the oscillations, but decreases the period (in continuous darkness) from 23.9 to 22.1 hours [3]. In our coupled model we obtain the same conclusion by halving the rate of *CRY* synthesis in continuous darkness.
- In absence of *PER* mRNA or *PER* protein it is possible to observe sustained oscillations in the circadian cycle [3]; according to our results it is only possible to observe oscillations up to 500 hours.
- The expression of several mammalian cell-cycle genes, including *c-myc*, *Cyclin-D1*, and *mWee1*, is regulated in a circadian manner [6].

- Overexpression of *PER1* leads to apoptosis whereas inhibition of *PER1* inhibits apoptosis. It appears that *PER1* antagonizes the cell cycle in an oscillatory fashion similar to the manner in which it antagonizes the function of Clock-Bmal1 [6]. According to our experiments, a *PER* inhibition produces an increase of *mWee1*, an thus a mitosis inhibition.
- *PER1* and *TIM* seem implicated on the DNA-damage response because both can be found complexed with the *ATM* and *ATR* kinases and the checkpoint kinases *Chk2* and *Chk1*, respectively [6].
- The double mutation of *PER1* and *PER2* may result in an alteration in the timing of cell division [6].
- The oscillatory expression of *c-myc* is abolished in *mPER2* mutant mice, which could then result in an alteration of the *p53* function [6].
- In *CRY* deficient cells, the circadian rhythmicity is lost [7], Wee1, overexpressed, and MPF, less active, loses rhythmicity [2]. According to our results, in case of a *CRY* knock-out the circadian compound concentrations become constant after some oscillations with a period of 40-50 hours lasting approximately 500 hours. The effect on *Wee1* and *MPF* are roughly consistent with data.

4 Conclusion

In particular, these are some of the conclusions in clear accordance with our *in silico* experiments:

- when either *mPER* or *mCRY* are missing, the cell cycle rhythm is slowed down;
- the absence of *mBmal1* causes an acceleration of the cell cycle;
- when either *mPER* or *mCRY* are missing, mitosis inhibition is stronger than in normal conditions;
- when *mBmal1* is not present, mitosis inhibition is very slight.

The confrontation of these results with more precise experimental data is the next step for us to fit our models and refine them where necessary.

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