Analyzing various models of Circadian Clock and Cell Cycle coupling

(Extended Abstract)

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The daily rhythm can influence the proliferation rate of many cell types [1]. In the mammalian system the transcription of the cell cycle regulatory protein *Wee1* is controlled by the circadian clock [2]. In [3], Zámborszky et al. present a computational model of the cell cycle and circadian rhythm coupling, showing that the coupling can lead to multimodal cell cycle time distributions. Biological data points to additional couplings, including a link back from the cell cycle to the circadian clock (e.g. [4]); this requires a more detailed description of both parts of the model. Hence, we main at further extending and analyzing the model presented in [3] using various modelling and computational techniques. First, we focus on the use of the CoSBi Lab[1], before taking advantage of two other modelling/simulation environments, namely BIOCHAM[2] and GINsim[3] tools.

Following the ideas presented in [8, 5], we translate the ODEs coupled

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model of the cell cycle and the circadian rhythm [3, 9] into BlenX [6, 7], a new stochastic programming language explicitly designed to represent biological entities and their interactions; the main difference w.r.t. the model presented in [3] is that our translated model is fully stochastic and discrete, i.e., deals with molecule numbers. We run 5000 stochastic simulations of the translated original model (OM) over different Mass Doubling Times (MDTs) of cell growth and observe the cell cycle time distribution, obtaining also in our case the multimodal distributions observed in [3]. We also analyze the simulation results using a method based on the Fourier analysis [10]. Analyses of the frequencies of the time courses of different species highlight the presence of multimodal cell cycle distributions and provide insights in the frequency components that characterize species time courses. Moreover, the comparison of Fourier analysis results for the coupled and uncoupled OM shows that the coupled OM is more noisy than the uncoupled one. This result holds for the comparison of the two models over different MDTs.

Next, we extend the OM model with a first unpacking of the mechanism regulating the phosphorylation of the *retinoblastoma* protein (Rb) and its inhibitory effect on the *E2F* transcriptional activation protein. In OM, this mechanism is implicitly described through a function used by the ODEs system. Following [11], we extend OM, obtaining an unpacked model (UM) in which this mechanism is explicitly described in terms of reactions. We run 5000 stochastic simulations over different Mass Doubling Times (MDTs) and observe the cell cycle time distribution. As expected, the results are in accordance (up-to noise) with the results of the OM. Also the Fourier analysis shows results similar to those obtained for the original model. Moreover, the comparison of the coupled OM and UM, over different MDTs, shows that the level of noise in the two models is equivalent. These results support the idea that the two models provides a trusty description of the same system at two different levels of abstractions. Moreover, this shows how the compositional nature of BlenX allows, in an effective way, the refinement of complex coarse-grain models.

In UM, the mechanism through which *Rb* is phosphorylated is still not completely unpacked. Indeed, as described in [12], *Rb* is subject to a multisite phosphorylation process that is not yet understood. In [12], different possible multisite phosphorylation scenarios are considered, each describing the effect of *Cdk/cyclin* complexes and their inhibition (through phosphorylation) of *Rb* activity using different mechanisms. Multisite phosphorylation is a source of combinatorial complexity, i.e., the number of combinations of

protein modifications tends to increase exponentially, and its modelling can be difficult or even impossible. Our BlenX model is general enough to cover, with simple modifications in the quantitative parameters, all the different multisite phosphorylation order scenarios presented in [12] (and even more). By providing an *educated guessing* of the missing quantitative parameters, we can cover different multisite phosphorylation mechanism through a function in UM. For example, this enables us to recover the nonlinear effect of *Rb* on the expression of *E2F*. More generally, this shows how BlenX can be use to cover, in a simple and effective way, scenarios governed by a combinatorial complexity.

As a further step, we try to extend the circadian rhythm model and fit it to experimental measurements. We add the transcription and degradation of *BMAL1* mRNA and refine the model (following ideas presented in [15, 14]) by adding different direct feedbacks (a positive, a negative and a combination of them) from the translated *BMAL1* on the transcription of its mRNA. Comparisons of the resulting models with the experimental data presented in [13] lead to inconsistencies in the oscillations of *BMAL1* and its mRNA. Hence, we further try to infer reaction rates from the experimental data using KInfer [16], a tool for estimating rate constants of biochemical network models from concentration data measured, with error, at discrete time points. This analysis results in sets of parameters impeding oscillations. Consequently, we conclude that there is a disagreement between the model and the experiments. This leads us to look for other revision or extensions of our models, in particular by considering in the feedbacks effect of *BMAL1* by the presence of intermediate proteins like *ROR* and *RevErb* [15, 14].

Still in the spirit of model extension, we initiate a network analysis of the OM. Using the *STRING* database [17], we build a protein-protein interaction network of our OM (25 nodes) and calculate measures such as the highest degree (connectedness) and highest betweenness (relative importance) of the proteins in the network, which further emphasize the importance of *Wee1*. We then extend the network including the proteins involved in the DNA damage pathway (29 nodes) and proteins connected with them, reaching a network with a size of 49 nodes. The highest betweenness of the resulting network suggests a possible direction for extending our OM by including *p53* and *CKll* proteins. These proteins, indeed, result extremely important in the coupling between the cell cycle and the circadian clock [4].

Our work continues trying to integrate our CoSBi Lab with BIOCHAM

and GINsim tools. By exporting the BlenX model in SBML[4] [18] we are able to provide an input file for BIOCHAM, thereby enabling the use of its model checking capabilities. BIOCHAM models are then exported, via the generation of an influence graph, in a format readable from the GINsim tool. This influence graph is finally processed to obtain a regulatory graph within GINsim, thereby enabling logical simulations and analyses of our model. Altogether, the different processing steps we consider in this work lead to the delineation of the following PIPELINE:

$$\text{ODEs} \rightarrow \text{BlenX} \rightarrow \text{Reactions} \rightarrow \text{Logical regulatory graphs}$$

This pipeline greatly eases reasoning on the same model from different perspectives, taking advantage of the complementary capabilities of different platforms. The analysis of the resulting Boolean model leads to novel insights and a better understanding of the gating mechanism (phase locking) emerging from the coupling of the cell cycle and the circadian clock.

The results of this work suggest directions for future experimental and computational investigations, in particular, the consideration of additional couplings (e.g. *p21*, M-phase transcription inhibition), and the use GINsim and BIOCHAM to design new experiments to run and analyse in the CoSBi Lab. At longer term, this model analysis could provide useful indications to define chrono-therapeutic protocols to treat diseases such as cancer [20].

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