

Verification of *Bmal* and *Clock* Gene Functions in Circadian Rhythms by Simulation

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Abstract

We verified the role of CLOCK/BMAL complex as a transactivator for the genes including *Per* and *Cry* in terms of two criteria; “stability of oscillation” and “influence to oscillation period.” The computer simulations with hybrid functional Petri net implied that alternative activations of CLOCK/BMAL is preferable than constant activation for these genes.

Keywords: circadian rhythm, simulation, hybrid functional Petri net

1 Introduction

In recent years, trials to elucidate the function of gene mechanism with computer simulations become popular. Furthermore, it has been thought that this technique is effective to understand genetic mechanisms in circadian rhythms[1]. In this report, we verify roles of *Bmal* and *Clock* involved in the biological clock mechanism by using a technique for pathway modeling called Hybrid Functional Petri Net (HFNP)[2].

2 CLOCK/BMAL Behavior with Published Parameters

Figure 1 shows a mutual relationship of known clock genes. We paid attention to the protein complex CLOCK/BMAL in Figure 1. This complex promotes the transcription of *Per*, *Cry*, *Rev-Erb*, and *Ror* genes in the nucleus (These are expressed by the arrows of the dashed line in Fig.1). We have constructed an HFNP model of the genetic interaction based on the published parameters used in [3] and simulated this HFNP model(HFNP1) with Cell Illustrator(CI). The simulation result of HFNP1 shows the oscillation of CLOCK/BMAL that expresses in the level that always exceeds the threshold level for the activations of *Per*, *Cry*, *Rev-Erb*, and *Ror* genes. Since all genes in Fig. 1 exhibit these normal oscillations, we can see that periodic oscillation of CLOCK/BMAL is not necessary for the circadian genes to perform periodic oscillations.

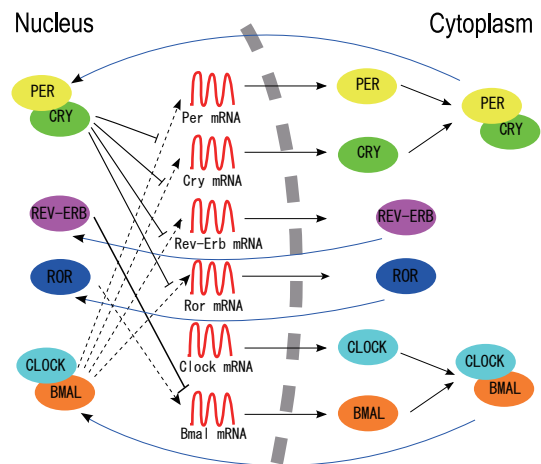


Figure 1: Established model.

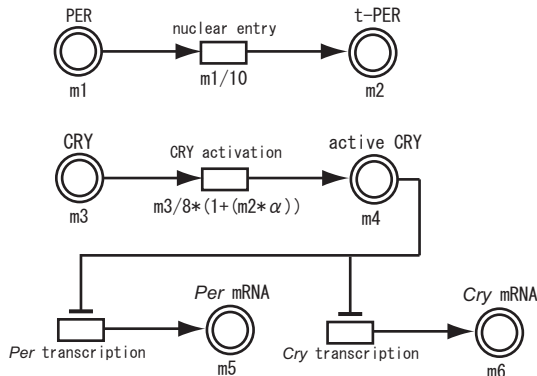


Figure 2: Obstruction of CRY by PER.

3 Interlock model

3.1 Stability of the circadian rhythm

In the case of HFPN1 in which only PER/CRY complex controls clock gene transcription the entire oscillation stops when some inconvenience happens in the feedback system of *Per* and *Cry*[4]. If activations and inactivations of clock genes *Per*, *Cry*, *Rev-Erb*, and *Ror* are alternatively happened by the swings of CLOCK/BMAL expression, CLOCK/BMAL works as transcription factors for these clock genes. In this case, the circadian clock can keep its oscillation even when either of two transcription factors PER/CRY or CLOCK/BMAL is disrupted. We call this model “interlock model” in this paper. The interlock model increases the stability of circadian clock mechanism because of two oscillation loops that compliment these oscillations each other. Consequently, the circadian clock keeps its oscillation even if either of these two loops stops its function.

3.2 Extension of the oscillation rhythm

The model of HFPN1 does not realize 24h oscillation. Since transcription and translation are normally ended in 2 or 3 hours, this HFPN1 model is insufficient for 24h oscillations. In the HFPN1 model, PER and CRY proteins inhibit the transcriptions after forming complex followed by translocation to the nucleus. There is a suggestion that circadian oscillation cycle can be prolonged by the function of PER that works as inhibitor for CRY that inhibits the transcriptions of other genes. We have constructed a new HFPN model (HFPN2) that reflects this suggestion in which the HFPN shown in Fig.2 is incorporated. Fig.3 shows simulation results of Cell Illustrator that illustrates changes in cycle length of circadian rhythms along with the value α in Fig.2. From this figure, we can see that although oscillation collapses in the published parameter model during 1.4 to 1.6 of α , cycle length of circadian oscillation keeps growing in the interlock parameter model. This implies that the biological suggestion mentioned above can be supported by the interlock model but not by the published parameter model.

References

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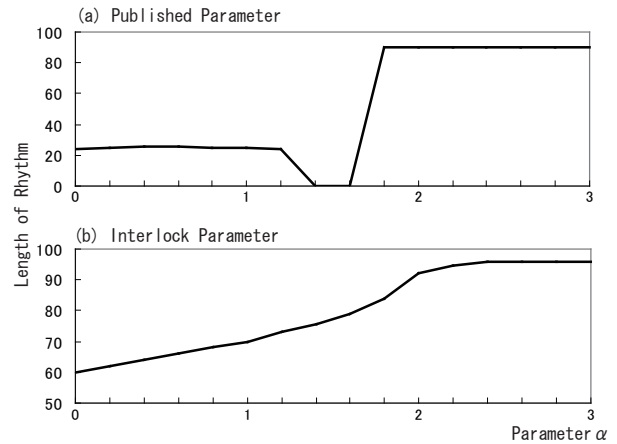


Figure 3: Comparing of two models.