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Homeostasis of the period of post-translational biochemical oscillators

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ABSTRACT

Generally, circadian clocks or biological oscillations are resistant to external conditions such as temperature and nutrient concentration. We propose that enzyme-limited competition provides a general mechanism of homeostasis of the period of post-translational oscillators based on protein modifications, and demonstrate it by nutrient compensation in a theoretical model of cyanobacterial circadian clock. The rate change by nutrient concentration is counterbalanced by the amount of available free enzyme, which occurs because of the competition among the various substrates for the limited enzyme. The temperature and nutrient compensation are determined by the postulate that the catalytic modification reactions are rate limiting.

1. Introduction

Biochemical oscillations are commonly observed from cells to humans and provide the basic functions for the organism. Such oscillations are thought to work as pacemakers, and the maintenance of their characteristic period to a constant value against changes in external conditions is important. To date, it is known that some of such oscillators show homeostasis against external changes [1,2].

Indeed, circadian clocks, one of the most important biological oscillators, are known to be resistant against various types of stimuli. The most prominent example is temperature compensation, i.e., robustness of the period against temperature changes. In addition to temperature compensation, circadian clocks show robustness against changes in trophic conditions, which is called nutrient compensation [3–5]. Such homeostatic features (i.e., temperature and nutrient compensation) are known to be universally conserved across a wide range of organisms, and their mechanisms have been investigated. Moreover, the relationship among different homeostatic responses of clocks have been also discussed [6,7]. However, the question how various homeostatic responses are achieved simultaneously remains unsolved.

Here, we propose a general mechanism and suggest a condition for homeostasis of the period of post-translational biochemical oscillation against various environmental changes. The post-translational oscillation, which consists of modification and demodification reactions of proteins without *de novo* transcription and translation, is one of the basic mechanisms of circadian clocks. Indeed, the Kai-protein system, which constitutes a cyanobacterial post-translational clock, has been investigated in depth as a basic model. Kondo and his colleagues succeeded in the *in vitro* reconstruction of the cyanobacterial clock by mixing three proteins, KaiA, KaiB, and KaiC, with adenosine triphosphate (ATP) as the energy source [8]. KaiC has a hexameric structure with six monomers, each of which has two phosphorylation sites [9]. Furthermore, it has both auto-phosphorylation and auto-dephosphorylation activities; however, since the auto-dephosphorylation activity is usually stronger, the protein is spontaneously dephosphorylated [10,11]. The binding of the dimer of KaiA [9] to KaiC enhances KaiC's auto-phosphorylation activity, leading to its phosphorylation [11,12], while KaiB inhibits the activity of KaiA [11,13]. This phosphorylation/dephosphorylation process of KaiC constitutes a circadian rhythm.

In a previous study focused on a model of the KaiC system, we showed that the temperature compensation of biochemical oscillators is achieved by enzyme-limited competition (ELC) [14]. The model consisted of catalytic phosphorylation reactions and non-catalytic dephosphorylation reactions for KaiC modification sites, with KaiA as the catalyst. Each phosphorylation reaction competes for the limited abundance of the catalyst. When the external temperature is increased, the competition for the catalyst among the substrates increases, leading to a decrease of the amount of free catalysts. We showed that the decrease of the amount of free catalyst, indeed, could counterbalance the temperature-induced increase in the rate of elementary reactions, when two conditions are met:

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1. The amount of the catalyst is not high.
2. The activation energies of the catalytic reactions are higher than those of the non-catalytic reactions.

The first condition is required to limit the amount of enzyme, while the second condition is needed for the catalytic reactions to be rate limiting. Hence, the speed of a catalytic process is more sensitive than that of a non-catalytic process, because the rate constant depends on the temperature according to the Arrhenius's law: $k \propto \exp(-E_\alpha/kT)$ where E_α is the activation energy and T is the temperature.

Although the temperature is one of the key factors that alter the speeds of biochemical reactions, other factors can also play a role. For example, rate constants depend on the changes in the concentrations of donor substrates (e.g., ATP for phosphorylation, S-adenosyl methionine for methylation, and acetyl-CoA for acetylation) following mass-action kinetics or Michaelis–Menten type kinetics. Osmotic pressure and pH also affect the rate constants because of protein denaturalization. However, it is unknown whether there is a mechanism that commonly compensates the period against such changes in the reaction speeds.

Here, we show that the ELC can work as a general framework for homeostasis of the period of biochemical oscillators. As an example, we show that the nutrient compensation of the period is achieved by ELC. Because the nutrient compensation at the level of organisms may involve more complicated mechanisms, it is important to first understand the mechanism at the molecular level. At the molecular level, changes in nutrient conditions lead to drastic changes in the concentration of the donor substrates through metabolic networks and, thus, alter the speed of modification reactions. In spite of such changes, circadian clocks tick with almost the same period. Indeed, the reconstructed Kai system showed homeostasis of the period against changes in the concentration of ATP [3,5]. Therefore, to understand the mechanism of nutrient compensation at the molecular level, we should consider the homeostatic response against changes in the concentration of donor substrates. Here, we will show that such nutrient compensation can be achieved by ELC at low metabolic condition. Moreover, such homeostasis of the period is demonstrated to be robust against not only the changes in parameter values but also in the components and a structure of the reaction network.

2. Models and methods

We adopted the KaiC allosteric model previously reported [14,15]. In this model, each KaiC monomer has two states (i.e., active and inactive) that are allosterically regulated. Moreover, KaiC hexamers in the active state can be phosphorylated, whereas those in the inactive state can be dephosphorylated. According to the concerted Monod–Wyman–Changeux (MWC) model, a phosphorylated KaiC monomer energetically prefers the inactive state, whereas a dephosphorylated KaiC has the opposite tendency [16]. Here, the flip–flop transition between active and inactive states occurs only from the fully phosphorylated or fully dephosphorylated states. No inter mediate states are assumed. Hence, the reaction process exhibits a cyclic structure. Note that KaiB was not explicitly included here, because changes in the concentration of KaiB only slightly affect the period [17].

Furthermore, KaiA facilitates the phosphorylation of active KaiC with an affinity that depends on the number of phosphorylated residues on each KaiC hexamer. KaiCs with a smaller phosphorylation number have stronger affinity to KaiA and are phosphorylated faster. This assumption is necessary for generating stable oscillations [15] and partially verified by the recent experiment [18]. Then, the reactions are given by

$$C_6 \xrightarrow{f} \widetilde{C}_6,\ \widetilde{C}_0 \xrightarrow{b} C_0,\ \widetilde{C}_i \xrightarrow{k_{dp}} \widetilde{C}_{i-1} \tag{1}$$

$$C_i + A \underset{k_i^{Ab}}{\overset{k^{Af}}{\rightleftarrows}} AC_i \xrightarrow{k_p} C_{i+1} + A \tag{2}$$

Here C_i and $\widetilde{C}_i$ denote active KaiC and inactive KaiC, respectively, with i phosphorylated sites; A denotes free KaiA dimer and AC_i denotes the KaiA–KaiC complex with i phosphorylation sites.

Here, the formation and dissociation of KaiA–KaiC complexes occur at much faster rates than other reactions do and, therefore, are adiabatically eliminated. Then, ordinary differential equations for such reactions are given by

$$\frac{d[C_i]}{dt} = (1-\delta_{i,0})\frac{k_p[A]_{free}[C_{i-1}]}{K_{i-1}+[A]_{free}} - (1-\delta_{i,6})\frac{k_p[A]_{free}[C_i]}{K_i+[A]_{free}} + \delta_{i,0}b[\widetilde{C}_i] - \delta_{i,6}f[C_i] \tag{3}$$

$$\frac{d[\widetilde{C}_i]}{dt} = k_{dp}((1-\delta_{i,6})[\widetilde{C}_{i+1}] - (1-\delta_{i,0})[\widetilde{C}_i]) - \delta_{i,0}b[\widetilde{C}_i] + \delta_{i,6}f[C_i] \tag{4}$$

where $\delta_{i,j}$ is Kronecker delta ($\delta_{i,j} = 1$ when $i = j$, $\delta_{i,j} = 0$ otherwise), $[x]$ indicates the concentration of x, and $K_i(=k_i^{Ab}/k^{Af})$ are the dissociation constants. The change in the concentration of free A (which will also be denoted as $[A]_{free}$ for clarity later) is given by

$$[A]_{total} = [A]_{free} + \sum_{i=0}^{5}\frac{[A]_{free}[C_i]}{K_i+[A]_{free}} \tag{5}$$

Considering the increase in affinity for KaiA with the number of phosphorylated sites, we set $K_i = K_0\alpha^i$ ($\alpha > 1.0$).

The rates of phosphorylation and dephosphorylation depend on external factors such as temperature and nutrient conditions. The temperature dependence of phosphorylation and dephosphorylation reactions follows the Arrhenius kinetics as $k_p \propto \exp(-\beta E_p)$ and $k_{dp} \propto \exp(-\beta E_{dp})$, where β is the inverse of the temperature by taking the unit of the Boltzmann constant as unity and E_p and E_{dp} are the activation energies of the phosphorylation and dephosphorylation reactions, respectively. The model is known to re produce the circadian rhythm of the phosphorylation level [15], as well as the temperature compensation of its period [14].

Here, the dependence of a reaction speed on the concentration of ATP is explicitly considered. We chose two forms of reactions, mass-action kinetics and the Michaelis–Menten kinetics. The former assumes that the phosphorylation reactions are immediately completed when ATP binds to the KaiA–KaiC complex, and the latter assumes that the binding reaction between ATP and the KaiA–KaiC complex is faster than the phosphorylation reaction, so that the ratio of [KaiA–KaiC] and [KaiA–KaiC–ATP] is immediately equilibrated. The speed of the modification reaction is given as $k_p = c_p\exp(-\beta E_p)[ATP]$ for the mass-action kinetics and as $k_p = c_p\exp(-\beta E_p)[ATP]/([ATP]+K_{ATP})$ for the Michaelis–Menten kinetics, where c_p is a constant independent of the temperature and ATP concentration. Note that we assumed that only the phosphorylation reaction rate depends on the concentration of ATP, because the dephosphorylation reaction does not need ATP as the donor substrate. Thus, the speed of the demodification reaction is given as $k_{dp} = c_{dp}\exp(-\beta E_{dp})$, where c_{dp} is a temperature-independent constant.

3. Results

3.1. The biochemical oscillator shows nutrient compensation

First, we analyzed the Kai protein model following the mass-action kinetics. This model shows oscillations in KaiC phosphorylation levels over a wide range of ATP concentration (Fig. 1). Since

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