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Cyanobacterial circadian clock is nullified under low temperature via Hopf bifurcation

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Abstract: One of the key characteristics of all circadian rhythms is that the free-running period remains stable under a relatively broad range of ambient temperatures, referred to as "temperature compensation" of the period. Outside of the range of temperature compensation, circadian clocks stop running and are arrested at a certain phase. Based on bifurcation theory, Hopf bifurcation and saddle-node bifurcation are plausible scenarios of circadian arrhythmia at low temperature. We focused on a biochemical circadian oscillation, KaiC phosphorylation rhythms, which can be reconstituted in a test tube by mixing the three protines , KaiA, KaiB, and KaiC in the presence of ATP. The KaiC phosphorylation rhythm in vitro is the simplest circadian oscillation to observe directly and precisely dynamics of circadian oscillator. We found that the phenomena of nullification of KaiC phosphorylation rhythm by low temperature was explained by theory of Hopf bifurcation.

Keywords: circadian rhythms, cyanobacteria, Hopf bifurcation

1 INTRODUCTION

Biological rhythms are the physiological oscillation in living things. The oscillation with a 24 hours period is called circadian rhythms in biology and extensively studied. Awake-sleep cycle, nyctinasty movement of leaves, and periodical gene expressions are well examined in this research area.

One of the shared characteristics among all circadian rhythms is that the free-running period remains stable for a relatively broad range of ambient temperatures, referred to as "temperature compensation" of the period. Temperature at which temperature compensation is effective typically lies well within the physiological range, that is, the range permissive for growth. Interestingly, outside of the range of temperature compensation, circadian clocks stop running and are arrested at a certain phase [1, 2]. Although essentially identical results have been found in various organisms, it remains unclear if temperature stimuli affect the circadian clock directly or indirectly through such as metabolic changes.

In this presentation, we will focus on why circadian rhythms cannot be observed at low temperature conditions. Cyanobacterium, *Synechococcus elongatus* PCC 7942 is the simplest organism that exhibit circadian rhythms. The cyanobacterial circadian timing requires neither de novo transcription nor translation [3], and the post-translational oscillation can be reconstituted in a test tube using only three clock proteins, KaiA, KaiB, and KaiC [4]. The KaiC phosphorylation rhythm in vitro (hereafter referred to as "in vitro clock") satisfies the criteria of circadian rhythms [4,5] and disappears below a certain critical temperature (around 20 ºC: [6]).

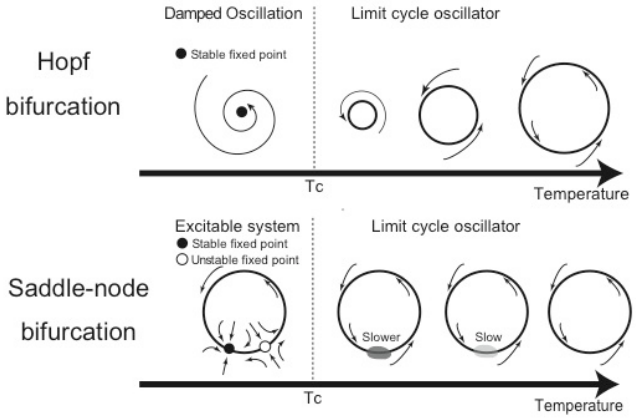

Fig.1 Two types of bifurcation for arrhythmia

2 BIFURCATION FOR ARRHYTHMIA

There are two typical scenarios for nullification of rhythmic phenomena according to bifurcation theory [7] (Fig.1, 2). One is called Hopf bifurcation and the other is called Saddle-node bifurcation on circle. The behavior of self-sustained oscillators (limit-cycle oscillators) depends on the

type of bifurcations. In the case of Hopf bifurcation, the amplitude of the oscillations can be altered and then the amplitude monotonically approach to 0 around bifurcation point. Below bifurcation point, self-sustained oscillator can not sustain its amplitude and is changed into a damping oscillator.

On the other hand, the amplitude does not change significantly in the case of Saddle-node bifurcation on circle. When a control parameter is altered, the period can be lengthened because the phase progress at a certain phase become s lower. The period of the oscillation approaches to infinity around the bifurcation point. Below critical point, stable and unstable fixed points are created on the circle.

Cell cycle is known as the famous example of the scenario of Saddle-node bifurcation on circle[8]. By inducing gene expressions involved in cell cycle (e.g. Cyclin), cell cycle can be arrested at a certain stable fixed point on circle. The fixed point is called "check point" in the research area of cell biology.

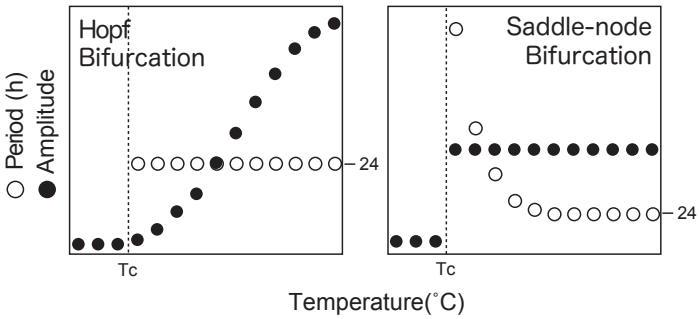

Fig.2 Amplitude and Period for Hopf and Saddle-node bifurcations

To distinguish the type of bifurcations of arrhythmia of in vitro clock, we prepared 15 samples mixing the three Kai proteins with ATP and incubated at various ambient temperatures. From 30 ºC to the critical temperature (ca. 19ºC), the amplitude of in vitro clock declined monotonically but its period remained relatively constant (Fig.3). This result indicates the rhythms can be nullified at low temperature conditions via Hopf bifurcation.

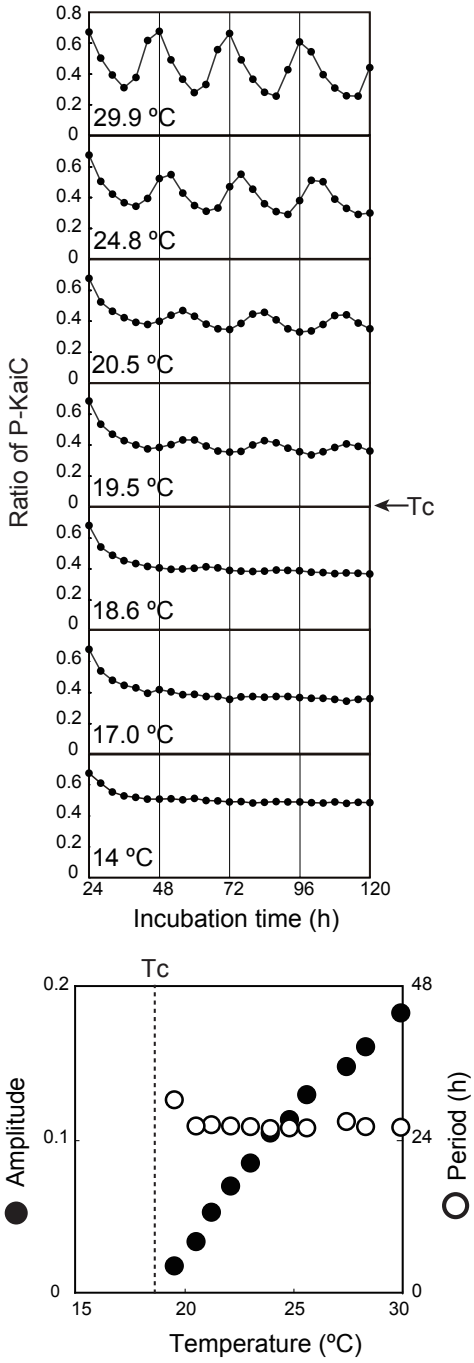

Fig3. Rhythms of in vitro clock at low temperature

3 DAMPING OSCILLATIONS BELOW CRITICAL TEMPERATURE

As stated at the previous section, the damping oscillation should be observed below critical point. We chilled samples at 4 ºC and exposed 30ºC pulse to them for 12 hours. Then we transferred the samples to below critical temperatures (10ºC – 19 ºC). The damping oscillator was kicked by high temperature pulse and the rapid damping rhythms were successively observed (Fig.3). Especially in

the case of the lowest conditions, we can not observe damping oscillation. Instead, no overshooting relaxation (over-damping) was observed. This result also suggests that circadian rhythms are abolished by low temperature through Hopf bifurcation.

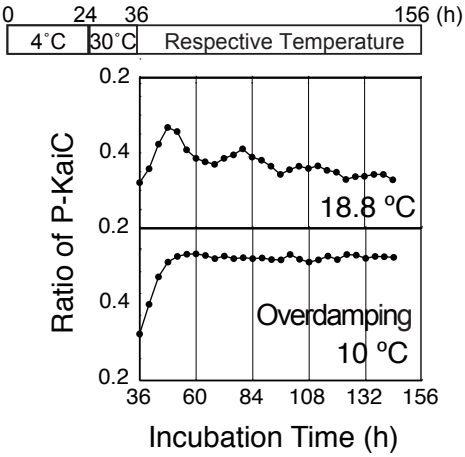

Fig.4 Damping oscillation of in vitro clock

4 RESONANCE BELOW CRITICAL TEMPERATURE

Under the critical point of Hopf bifurcation, the limit cycle oscillator can be changed into damping oscillator. Linear oscillator or damping oscillator under the external periodic environment can show resonance phenomena. When the damping oscillators is exposed to oscillatory external forces with the period of the natural frequency of the oscillator, the amplitude of the oscillator can grow significantly. In that sense, there is optimal frequency in external force, which can grow the amplitude of the oscillators.

We tested if there is optimal frequency of external force for the damping oscillation of in vitro clock below critical temperature. We observed in vitro clock under ambient temperature cycles of 16.7ºC / 18.7 ºC with a various period (6 hours – 36 hours). Both 16.7ºC and 18.7 ºC are below critical temperature.

We observed the forced oscillation with large amplitude when the period of external forces was 26 hours (Fig. 5). The observed resonant behavior might have a physiological meaning. Cyanobacteria populate most sea or lakes even near the north pole. The water temperature of cold area should be kept below critical temperature (19 ºC) for all day. However, diurnal change of water temperature might act as a periodical external force and grow the amplitude of KaiC phosphorylation rhythms in a cell. Thus, even under critical temperature, KaiC phosphorylation rhythms might function as a biological clock.

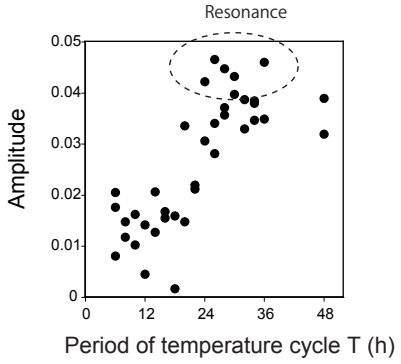

Fig. 5 Resonance of in vitro clock

5 A SIMPLE MODEAL FOR RESONANCE

To mathematically interpret the resonance phenomena, we performed a simple simulation. Consider Stuart-Landau oscillator,

$$\dot{z} = (a + i\omega - z^2)z$$

which is the generalized model near Hopf bifurcation point. a is a parameter specifying the distance from Hopf bifurcation and ω is the natural frequency (here, ω was fixed to 3). This model exhibits self- sustained oscillations if $a > 0$ and settles down at fixed point if $a \leq 0$. We chose parameter sets which can reproduce in vitro oscillation at 16 ºC and 18 ºC.

Under 16 ºC and 18ºC, in vitro clock exhibits damping oscillations. We hypothesized that the position of equilibrium points depends on the ambient temperature. Then, changing the ambient temperature corresponds to shifting the equilibrium point.

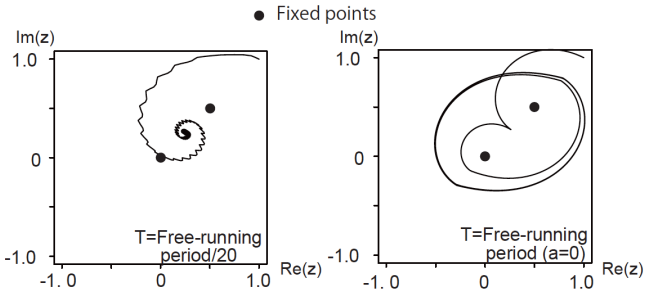

Fig. 6 Resonance of SL oscillator

The simulation can successively reproduce the resonance when the period of switching the equilibrium point (denoted as T) equals to the free-running period of the oscillator (Fig. 6). The periodic orbit on the phase plane surrounded the both equilibrium points. When much smaller T was chosen, the system settled down to a mid point between the two equilibrium points.

6 CONCLUSIONS

We have showed KaiC phosphorylation circadian rhythms in a test tube can be nullified via Hopf bifurcation. As bifurcation theory predicted, we succeeded in observing damping oscillation of the in vitro clock. We also succeeded in observing resonance of KaiC phosphorylation rhythms under low temperature cycles.

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