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Low temperature nullifies the circadian clock in cyanobacteria through Hopf bifurcation

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Supplementary Figure Legends

Fig. S1. Low temperature limit of the in vitro KaiC phosphorylation rhythm. (A) Analysis of a preparation of recombinant Kai proteins different from that described in Fig. 2A and B. The mixture of Kai proteins was incubated at 4°C for 24 h and then transferred to the respective temperatures (10–29.9°C) for 96 h as described in Fig. 2A and B. Aliquots of the mixtures were collected every 4 h, and the ratios of phosphorylated KaiC (P-KaiC) to total KaiC were plotted vs incubation time. (B) Consistency of the period and decrease in amplitude. Period and amplitude were estimated by fitting to a sinusoidal function with a linear component as well as Fig. 2B.

Fig. S2. Low temperature limit of the bioluminescence rhythm in cyanobacteria. Cells were cultured using a continuous culturing system to maintain a constant optical density. After two light-dark cycles at 30°C to synchronize the clock, the cells were released into LL conditions at 18–30°C. Bioluminescence in a flow cell was measured using a photomultiplier tube-based monitoring system. The inserts show a magnified view of the data from 67 to 120 h.

Fig. S3. Recovery from low temperature-induced arrhythmia. (A) The mixture of Kai proteins was incubated at 16°C for 48 h and then transferred to 20°C (green), 22°C (yellow), 25°C (red), and 30°C (black). KaiC phosphorylation was monitored and plotted against incubation time. (B–D) Model for the recovery from low temperature-induced arrhythmia. The phases of the rhythms of the Kai solutions resumed after exposure to different temperature were not equivalent (Fig. 2D). In addition, the phases of in vitro rhythms resumed by incubation at different temperatures were not equivalent (Fig. S3A). We interpret these observations as transition from a damped oscillator to a self-sustained oscillator, according to the findings described in the Results. We hypothesize that the location of the fixed point generated in a phase plane depends on temperature. (B) Diagram of the transition from the limit cycle to a damped

oscillator. The generated stable fixed points are indicated by black and blue dots, respectively. (C) Limit cycles and the fixed points depicted in Fig. S3B redrawn on a phase plane. The dotted lines represent hypothetical isochrons for the yellow limit cycles. The stable fixed points localize to the isochrons CT 0 and CT 3, suggesting that the phase of the resumed rhythm can differ (Fig. 2D) if the equilibrium state of the system depends on the pre-incubation temperature. (D) The limit cycles and the fixed points are identical to those shown in Fig. S3C. The hypothetical isochron is drawn for the pink limit cycle. The blue fixed point localizes to CT 3 and CT 0 for the yellow limit and pink limit cycles, respectively, suggesting that the phase of resumed rhythm depends as well on the elevated temperature (Fig. S3A).

Fig. S4. Effect of 30°C pulse on KaiC phosphorylation. (A) After 24 h incubation at 4°C, samples were subjected to 30°C pulse for 12 h and then transferred to the respective temperature (10–21.3°C). KaiC phosphorylation after the pulse was monitored for 108 h and the data were plotted against incubation time. The time courses of KaiC phosphorylation were fitting to a damped sinusoidal function $A + Bt + C\exp(-Dt)\sin(\frac{2\pi}{T}t + E)$, through Levenberg-Marquardt method with fitting parameters A, B, C, D, E, and T. The fitted function is represented by dotted red line. (B) The estimated period of the damped rhythm, T, plotted against temperature (black circle) with asymptotic standard error. The period shown in Fig. 2B is superimposed by the gray circles.

Fig. S5. Forced oscillation of KaiC phosphorylation during temperature cycles of 16.7 and 18.7°C. Reaction mixtures of Kai proteins were incubated at 18.7°C for 24 h and then subjected to symmetrical temperature cycles (durations of the 16.7°C and 18.7°C pulses were equal) with a period of T h (T = 6–48 h). KaiC phosphorylation was monitored for 104 h (incubation time 24–128 h). To estimate the amplitude of KaiC phosphorylation, we chose two peaks (pink dots) and

two troughs (blue dots), and then connected the peaks and troughs with a line. Amplitude was defined as the distance between two lines after 100 h.

Fig. S6. Temperature cycle-induced resonance of the Hatakeyama–Kaneko model. (A) Bifurcation diagram for temperature as a function of temperature. Maximum and minimum ratios of phosphorylated KaiC at steady state are plotted as a function of the inverse temperature, β. As β is increased from 3.4, the amplitude of the oscillation diminishes and rhythmicity disappears above 3.5. We chose two values of β for simulation of exposing temperature cycles, indicated by arrows. (B) Trajectory of the Hatakeyama–Kaneko model during temperature cycles with a period T = 20, 31, 53, 59.5, 65, and 89.5 h. The gray dots in each panel represent equilibrium state under where the β values are fixed to the value chosen in Fig. S6A. The closed orbits surround the equilibrium states. (C) Amplitude of phosphorylated KaiC during temperature cycles with a period of T h. The amplitude is defined as the difference between maximum and minimum levels of phosphorylated KaiC at steady state.

Fig. S7. Resonance of Stuart–Landau oscillator. (A–D) Numerical simulation of periodic switching between two damped Stuart–Landau equations where the location of the stable fixed points differ between modes (See Material and Methods). The parameters in the model were $\mu = -0.4, a = 3$. The orbits of the oscillator in modes I (A) and II (B) eventually approach α, the origin and (0.5, 0.5) on a complex plane of Z, respectively. (C) Closed orbits during periodic switching among the modes with a period of T. (D) Resonance of the Stuart–Landau oscillator. The amplitude was defined as the difference between maximum and minimum of $\mathrm{Re}(Z)$ at steady state. (E–H) The Stuart–Landau model can exhibit overdamping when $\mu = -4$. The overdamped system with periodic switching between modes III (E) and IV (F), where the locations of the stable fixed points are different. (G) Smaller closed orbits of the overdamped system during periodic mode switching. (H) Absence of resonance in the overdamped system.

(I-L) The modified Stuart-Landau model, $\dot{Z} = (\mu + ia - |Z|^2)Z + ie$ does not exhibit resonance. This system shows SNIC bifurcation at $\mu = 1, a = e = 0.2,$ and possesses a pair of unstable and stable fixed points when $e > 0.2$ (ref. 29 in the main text). We chose $e = 0.3$ (mode V, I) and 0.5 (mode IV, J) where the system settles down to a unique stable fixed point. (K) Closed orbits during switching the modes with a period of T. (L) Absence of resonance in the system.

Fig. S8. Apparatus for temperature gradient. (A) Side view, (B) top view, and (C) diagram of the temperature-gradient apparatus. Two heating baths were bridged by an aluminum block. By setting an arbitrary temperature of the heating baths, desirable temperatures gradient was generated. To enhance accuracy, the aluminum block was covered with heat insulator and was connected by a double side adhesive thermal conductive tape (HT-09, Ainex) with heating bath. Furthermore, the apparatus was put in walk in temperature-controlled chamber. (D) Accuracy of temperature control. Temperature on the aluminum block was monitored at two points during the experiment and was kept with an accuracy of ±0.1°C.