SUPPLEMENTAL DATA

The roles of the dimeric and tetrameric structures of the clock protein KaiB in the generation of circadian oscillations in cyanobacteria

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EXPERIMENTAL PROCEDURES

Cy3-maleimide fluorescent dye esters (GE Healthcare) were covalently coupled to the cystein residues of SasA. SasA (4 μM) was incubated with 40 μM Cy3-maleimide ester in gel filtration buffer at 4 °C for 2 h. After labeling, the reaction mixtures were loaded on a PD MidiTrap^{TM} G-25 column (GE Healthcare) to remove free unreacted dye from the labeled protein (Cy3-SasA). The amount of Cy3 introduced into SasA was calculated to be 0.62 ± 0.02 (n = 3) molecules of Cy3 per subunit of SasA from the absorbance of Cy3-SasA at 552 nm (A_{552}).

FIGURE LEGENDS

FIGURE S1. KaiC-induced changes in the diffusion time of Cy3-KaiB$_{1\text{-}94}$. Cy3-KaiB$_{1\text{-}94}$ (0.2 μM) was incubated with 0.2 μM KaiC$_{WT}$, KaiC$_{AA}$ or KaiC$_{DD}$ in HEPES reaction buffer at 25 °C for 18 h, and then the diffusion time of Cy3-KaiB$_{1\text{-}94}$ was measured by FCS analysis. The diffusion time in the presence of both 0.2 μM KaiA and KaiC$_{WT}$ was also measured. Values shown are the means ± SD from quadruple measurements.

FIGURE S2. KaiB-induced changes in the diffusion time of Cy3-SasA in the presence of KaiC$_{WT}$. Reaction mixtures containing 0.2 μM Cy3-SasA and 0.2 μM KaiC$_{WT}$ were incubated in the presence or absence of 1.0 μM or 2.0 μM KaiB$_{1\text{-}94}$ or KaiB$_{WT}$ in HEPES reaction buffer containing 0.1 mM DTT at 25 ˚C for 18 h, and then the diffusion time of Cy3-SasA was measured. The diffusion time in the absence of KaiC$_{WT}$ was also measured as a control. Values shown are the means ± SD from triplicate measurements.

FIGURE S3. Amino acid sequence alignment of KaiCs from 7 cyanobacterial strains and the sites of the conserved acidic residues among the 7 strains in the crystal structure of *Synechococcus* KaiC (37; PDB code: 2GBL). *A*, amino acid sequence alignment. The acidic residues of KaiCs are shown in red. Cyanobacterial strains: *T. elongatus*, *Thermosynechococcus*

elongatus; *Synechococcus*, *Synechococcus* sp. strain PCC 7942; *Synechocystis*, *Synechocystis* sp. strain PCC 6803; WH8102, *Synechococus* sp. strain WH 8102; *A. variabilis*, *Anabaena* sp. strain PCC 7937; *Anabaena*, *Anabaena* sp. strain PCC 7120; *Prochlorococcus*, *Prochlorococcus marinus*. Other conditions were the same as described in the Fig. 6 legend. *B*, sites of the conserved and similar acidic residues on the N-terminal domains of the KaiC (top view of the KaiC hexamer from the N-terminal domains). The conserved and similar acidic residues (D83, D108, D112, D114 [E113], E117, D123, E129, D156, E164, and D187 [E186] of *T. elongatus* KaiC; The corresponding residues of *Synechococcus* KaiC are shown in brackets) shown in red are located on an area around the pore opening and inside the pore of KaiC N-terminal domain. C, sites of the conserved and similar acidic residues on the interfaces between two adjacent homosubunits in the KaiC hexamer (side view). The conserved acidic residues (E78, E79, D83, D146, E184, E188, and E215 of *T. elongatus* KaiC) shown in red are located on an area around the intersubunit interface of one of two adjacent KaiC N-terminal domains. The ATP bound to the N-terminal domain of KaiC is shown in blue.