

## Text S1

### Evolution of KaiC-dependent timekeepers: a proto-circadian timing mechanism confers adaptive fitness in the purple bacterium *Rhodospseudomonas palustris*

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#### Supplemental Methods

##### Construction of *kaiC<sup>Rp</sup>* expression strains in *S. elongatus*

The *kaiC<sup>Rp</sup>* gene was cloned from genomic DNA of *R. palustris* TIE-1 strain and ligated with the plasmid P<sub>trc</sub>-NSII by *Nde*I (Xu et al., 2013). The resulting plasmid, P<sub>trc</sub>-NSII-*kaiC<sup>Rp</sup>*, was transformed into the wild-type reporter strain AMC149 (Kondo et al., 1993). By transformation, the *kaiC<sup>Rp</sup>* gene was incorporated into the Neutral Site II of the genome of AMC149 under the control of an IPTG-inducible promoter *trc* {P<sub>trc</sub>, (Xu et al., 2013)}, resulting in the corresponding *kaiC<sup>Rp</sup>* overexpression strain, AMC149ox*kaiC<sup>Rp</sup>*. The *kaiC<sup>Rp</sup>* overexpression strain harbors the *psbA1p::luxAB* luminescence reporter located at the Neutral Site I of AMC149 (Kondo et al., 1993; Xu et al., 2000).

##### Measuring luminescence rhythms in cyanobacterial strains

The *S. elongatus kaiC<sup>Rp</sup>* overexpressing strain AMC149ox*kaiC<sup>Rp</sup>* was cultured on BG-11 solid medium supplemented with appropriate antibiotics (Kondo et al., 1993; Xu et al., 2000). Before measuring luminescence rhythms, toothpick colonies of these strains were grown at 30°C for two LD cycles. After the cells were released to LL, the inducer IPTG was added to final concentrations of 0, 5, 10, 100, 250, 500 and 1000  $\mu$ M to induce the expression of *kaiC<sup>Rp</sup>*. Then the agar plates containing these strains were placed in a custom luminescence monitoring apparatus (Ishiura et al., 1998) to measure the luminescence rhythms for 5-7 d at 30°C and constant light conditions (40-50  $\mu$ E m<sup>-2</sup>s<sup>-1</sup>). AMC149 colonies were also included on the agar plates as controls.

#### Supplemental References

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Xu Y, Mori T, and Johnson CH. Circadian clock-protein expression in cyanobacteria: rhythms and phase setting. *EMBO J*. 2000; 19: 3349-3357.

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