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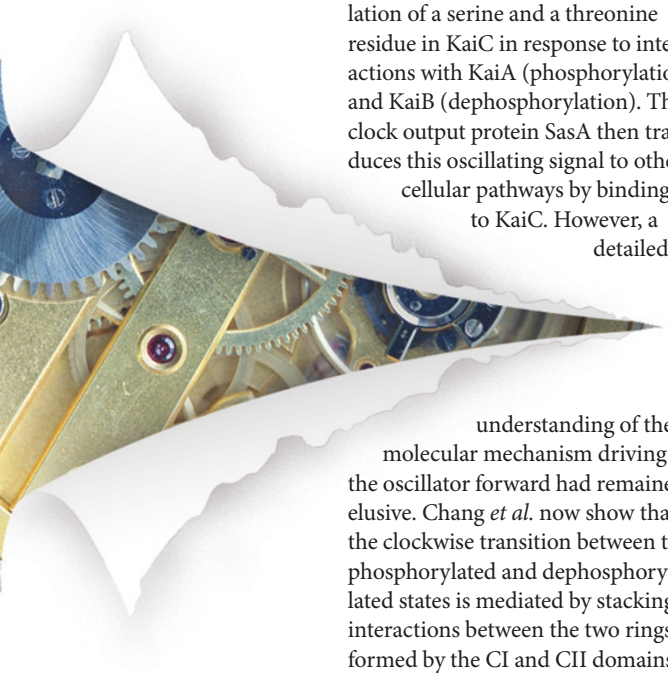
BACTERIAL PHYSIOLOGY

Uncovering the circadian clockwork



MACMILLAN AUSTRALIA

Circadian rhythms in cyanobacteria are temporally regulated by an oscillating system that depends on the phosphorylation and dephosphorylation of a serine and a threonine residue in KaiC in response to interactions with KaiA (phosphorylation) and KaiB (dephosphorylation). The clock output protein SasA then transduces this oscillating signal to other cellular pathways by binding to KaiC. However, a detailed understanding of the molecular mechanism driving the oscillator forward had remained elusive. Chang *et al.* now show that the clockwise transition between the phosphorylated and dephosphorylated states is mediated by stacking interactions between the two rings formed by the CI and CII domains of KaiC in the KaiC homohexamer.

Chang *et al*. used NMR spectroscopy and gel filtration to investigate the binding of *Thermosynechococcus elongatus* KaiB to hexameric S431E-KaiC, a phosphomimic of serine-phosphorylated KaiC, and also to hexameric complexes of isolated CI and S431E-CII domains. Whereas KaiB could bind S431E-KaiC, the authors observed no binding of KaiB to the isolated CI or S431E-CII rings individually, but binding was restored when the two domains were added together. When a FLAG epitope was added to the CI domain to weaken the interaction with CII, KaiB could not bind, suggesting that stacking of the hexameric rings formed by the KaiC CI and CII domains is important for the formation of the KaiB–KaiC complex.

Previous work had suggested that, in *Synechococcus elongatus,* KaiB interacts with the CII domain of KaiC. However, the authors found that, in *T. elongatus* at least, KaiB bound to the CI and not the CII domain, but only when CI had been dissociated from a hexameric ring to form monomers by increasing the ADP/ATP ratio in the solution. This suggests that the KaiB-binding site is normally hidden in the hexameric CI ring. Binding of KaiB to the KaiA–KaiC complex is thought to lead to the sequestration of KaiA at the CI domain, thereby terminating the phosphorylation phase of the oscillation cycle and promoting dephosphorylation. Accordingly, the authors found that addition of KaiA to a solution containing monomeric CI and KaiB led to the formation of a complex containing all three proteins.

The authors propose a model in which the interaction of KaiA and KaiC triggers phosphorylation of specific serine and threonine residues of KaiC, altering the stacking interactions between the CI and CII rings of the protein and, by doing so, revealing the KaiB-binding site of the CI domain. When bound, KaiB then induces the transition to the dephosphorylated state by recruiting KaiA from the CII domain to the CI domain of KaiC.

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ORIGINAL RESEARCH PAPER Chang, Y. *et al.* Rhythmic ring-ring stacking drives the circadian oscillator clockwise. *Proc. Natl Acad. Sci. USA* 11 Sep 2012 (doi:10.1073/pnas.1211508109)
FURTHER READING Lenz, P. & Søgaard-Andersen, L. Temporal and spatial oscillations in bacteria. *Nature Rev. Microbiol.* **9**, 565–577 (2011)