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Examining the *S. elongatus* KaiBC Complex by CryoEM and MDFF

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The metabolisms of most living things are regulated by circadian rhythms, cycles of activity and biological processes with periods of 24 hours. These biological clocks exist in varying degrees of complexity. Humans have a complex biochemical clock, involving numerous components. In comparison, the cyanobacteria, *Synechococcus elongatus*, uses a clock composed of only three proteins. The overall pattern of phosphorylations and dephosphorylations of a population of KaiC hexamers represents the ticking of the clock, and this cycling is regulated by the binding of KaiA and KaiB to KaiC [1]. A diagram of this cycle is shown in Fig 1, in which a 24 cycle begins with KaiC binding to KaiA, leading to two sequential autophosphorylation events on KaiC. Once doubly phosphorylated, KaiC recruits KaiB, which sequesters KaiA. KaiC then sequentially dephosphorylates before KaiB dissociates, leading to completion of a single cycle. Understanding the protein-protein interactions and conformational changes of this molecular clock has proven difficult. While crystal structures exist for these three proteins [1], the complexes formed during the cycling of KaiC phosphorylation status are not characterized at high resolution.

CryoEM offers the opportunity to examine the structure of complexes in vitreous ice, which preserves a near-native environment. Particle images were collected on FEI Polara 300kV FEG electron microscope and image processing was performed with FREALIGN. We present a cryoEM structure of KaiB complexed with a C-terminal truncated form of KaiC

Molecular dynamics flexible fitting (MDFF) provides a way to examine the interacting surfaces of proteins guided by cryoEM density. In addition to the standard potential energy function, the density map provides a force field to guide conformational changes in the atomic structure [2]. We are using a moderate resolution cryoEM density map of the KaiBCΔ489 complex to flexibly fit crystal structures of a KaiC hexamer and KaiB dimers together. With this approach, we can predict the protein-protein interaction surfaces and important residues at the interface. A recent gold-labeling EM study identifies the CII ring of KaiC as the binding surface of KaiB [3]. Potential interfaces between KaiB and KaiC are evaluated by comparing the predicted nonbonded potential energies while varying the orientation of the KaiB dimer within the complex (Fig 2). Several residues of KaiC and KaiB are suggested as key for KaiB binding. This study offers insight into how KaiB may influence KaiC phosphorylation within the KaiC circadian oscillation cycle [4].

References

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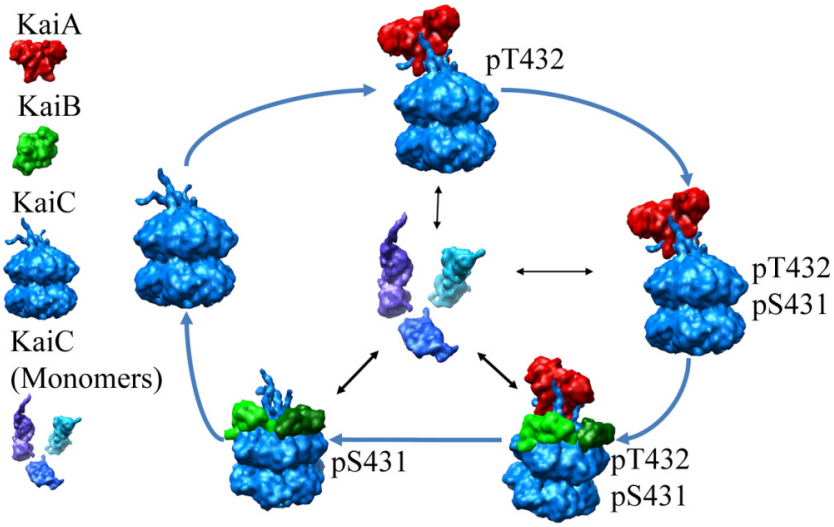

Figure 1. Cycle of KaiC phosphorylations. KaiA and KaiB interact with KaiC to create a cycle of autophosphorylation and subsequent dephosphorylation events on KaiC hexamers with a period of 24 hours. Initially, KaiA induces autophosphorylation of T432, and then S431. Doubly phosphorylated KaiC binds KaiB, which sequesters KaiA, and allows dephosphorylation of T432, and then S431. Robustness of this cycle is maintained through exchange of KaiC subunits with a population of monomers, as shown by black arrows, with rates of change indicated by the width of the arrows.

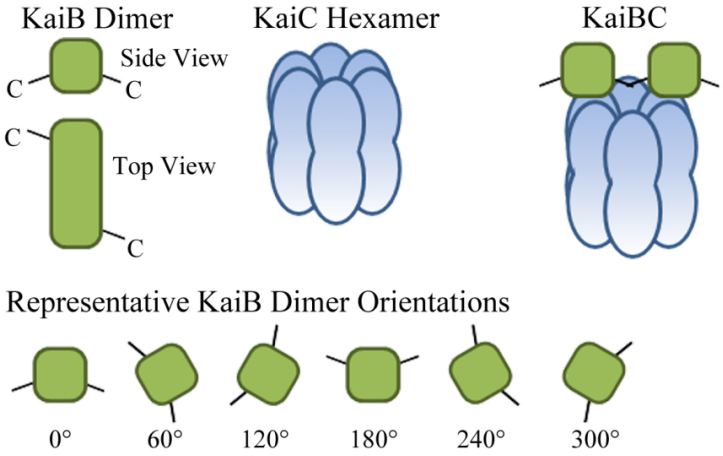

Figure 2. KaiBC Relative Orientations. Diagrams are shown for KaiB, KaiC, and KaiBC, along with representative KaiB dimer orientations that were evaluated by MDFF. Eighteen KaiB dimer rotations with respect to KaiC were tested with 20° spacings. The KaiB C-terminal tails are represented as black lines.

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