

The time machine: structure-based elucidation of timekeeping mechanisms by the cyanobacterial circadian clock

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Circadian rhythms are an ancient evolutionary adaptation found across the domains of life, synchronizing behavior and physiology to the daily solar cycle for optimal fitness. In cyanobacteria, timekeeping is established by three Kai proteins (KaiA, B and C) that form distinct protein complexes throughout the day, comprising a molecular clock that measures ~24-hour timing. Remarkably, this protein-based clock exhibits accurate, temperature-compensated timekeeping in the presence of only ATP *in vitro*. The LiWang lab recently discovered that KaiB undergoes a rare transition from its tetrameric ground state to a rare fold-switched, monomeric signaling state that is needed to bind KaiC and advance the clock in its evening phase¹. To address the structural basis for this nighttime complex, we locked KaiB into its fold-switched state, allowing us to solve crystal and NMR structures of four key complexes that demonstrate how KaiB docks cooperatively on the 360 kDa KaiC hexamer to recruit KaiA and the output protein CikA². A new structure of the output protein SasA bound to KaiC illustrates how KaiB uses structural mimicry to compete with SasA binding at dusk. Collectively, these structures reveal how protein interactions regulate day/night transitions and highlight the essential role of fold-switching by KaiB for control of timekeeping and output signaling in the cyanobacterial circadian clock.

¹Chang, Y.G., Cohen, S.E., Phong, C., Myers, W.K., Kim, Y.I., Tseng, R., Lin, J., Zhang, L., Boyd, J.S., Lee, Y., Kang, S., Lee, D., Li, S., Britt, R.D., Rust, M.J., Golden, S.S., LiWang, A. (2015) Circadian Rhythms. A protein fold switch joins the circadian oscillator to clock output in cyanobacteria. *Science* 349:324-8.

²Tseng, R.* , Goularte, N.F.* , Chavan, A.* , Luu, J., Cohen, S.E., Chang, Y.G., Heisler, J., Li, S, Michael, A.K., Tripathi, S., Golden, S.S., LiWang, A., Partch, C.L. (2017) Structural basis of the day-night transition in a bacterial circadian clock. *Science*, 355:1174-1180.