How CRY Regulates the Clock: Structural Studies of a Dynamic Mammalian Circadian Complex

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Circadian oscillators are molecular pathways that confer rhythmic signaling with a period that approximates the day/night cycle. In the mammalian system, the transcription factor CLOCK: BMAL1 forms the core of a transcriptional/translational negative feedback loop that drives the rhythmic expression of large swaths of the genome, including the genes that encode the circadian repressors, CRY1/2 and PER1/2. While repression by CRY and PER is essential to generate circadian rhythms, a mechanistic understanding of how CRY and PER work together to repress CLOCK: BMAL1 has remained elusive, in part through the difficulty in obtaining crystallographic data of circadian protein complexes. Our work combines structural data from single particle cryo-EM and small angle X-ray scattering with computational methods to generate an integrative model of CRY-mediated repression in the clock. We show that CRY1 interacts directly with the PAS-B domain of CLOCK through the secondary pocket of its photolyase homology region (PHR) domain, recruiting PER to the transcription factor and sequestering the transactivation domain of BMAL1 from transcriptional co-activators to repress CLOCK:BMAL1 activity. We also describe how PER2 binding to the PHR domains regulates CRY1/2 in an isoform-specific manner by remodeling a dynamic loop at the secondary pocket in CRY2 that results in CRY1-like affinity for CLOCK:BMAL1, serving as a molecular equalizer.