論文の内容の要旨

Post-translational modifications of mouse CRYs are essential for circadian oscillation of the central clock.

（時計タンパク質 CRY の翻訳後修飾によるマウス体内時計の 24 時間リズム形成）

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Circadian rhythms with a period of approximately 24 hr are generated by an internal timekeeping mechanism referred to as the circadian clock. The mammalian circadian clocks are driven by a transcription-translation-based negative feedback loop. Among the clock proteins, CRY1 and CRY2 act as key players through their strong repressive activities on the E-box-mediated transcription. The previous study reported that CRY2 is phosphorylated at Ser557 in a circadian manner in the mouse SCN and liver. The priming phosphorylation of CRY2 at Ser557 allows subsequent phosphorylation at Ser553, and the two-step phosphorylation of CRY2 leads to its proteasomal degradation. On the other hand, FBXL3, an F-box-type E3 ubiquitin ligase, ubiquitinates CRYs and mediates their degradation. Two point mutations in mouse *Fbxl3*, *i.e., After-hour (Afh)* and *Overtime (Ovtm),* each causes remarkable lengthening of the free-running period of the mouse behavioral rhythms. In the present study, 1) I investigated the physiological role of Ser557 phosphorylation of

CRY2 by using mutant mice carrying a mutation at Ser557. Furthermore, 2) I focused on FBXL21, a homologous protein of FBXL3, and examined its regulatory mechanism of CRY stability and the circadian clock. These analyses revealed a complex network of CRY regulation by posttranslational modification and their critical role in the circadian clock.

1). S557A-CRY2 knock-in mice exhibited significantly longer circadian periods of wheel-running activity rhythm as compared to the wild-type mice. In the mutant mice liver, CRY2 protein levels were abnormally increased, resulting in downregulation of E-box-mediated transcription. Collectively, I concluded that Ser557 phosphorylation-dependent degradation of CRY2 inhibited the abnormal accumulation of CRY2 proteins and is required for maintaining circadian periods in both the central and peripheral clocks.

2). I found that FBXL21-catalyzed ubiquitination stabilized CRY proteins and antagonized FBXL3-mediated destabilization by conjugating different type of ubiquitin chains. *Fbxl21*-null mice exhibited normal periodicity of behavioral rhythms with compromised organization of daily activities, while an extremely long period of *Fbxl3* null mice was compensated in *Fbxl3*/*Fbxl21* double-knockout mice. The double knockout destabilized the behavioral rhythms progressively in constant darkness and sometimes elicited arrhythmicity. These results emphasize the physiological importance of antagonizing actions between FBXL21 and FBXL3 on CRYs, and their combined actions at different subcellular locations provide stable oscillation of the circadian clock.