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主　論　文　の　要　旨

論文題目　Re-evaluation of the circadian clock model in mammals
哺乳類の概日時計モデルの再検討に関する研究

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論　文　内　容　の　要　旨

Circadian rhythms are biological oscillations of physical, mental, and behavioral activities with a period of approximately 24 hours driven by an endogenous cell-autonomous timing system called the circadian clock. The current molecular models of the mammalian circadian clocks are based on a molecular mechanism regulated by a transcriptional-translational negative feedback loop (TTFL) in which the translational products of clock genes repress transcription of their own mRNA. Consistent with this model, transcriptional and translational products of *Period*, *Cryptochrome*, and *Bmal1* show circadian rhythm in their accumulation. However, several studies have revealed that constitutively expressed clock genes effectively restore circadian oscillations. To understand this point more quantitatively, I expressed *Bmal1* from a doxycycline (DOX)-inducible promoter in *Bmal1*-disrupted U2OS cells containing a luciferase reporter under the control of the *Bmal1* promoter (P_{Bmal1}), and followed the P_{Bmal1} and P_{Per2} promoter activities. In the presence of 0.1 and 1 μg/mL of DOX, constitutively expressed BMAL1 restored circadian oscillation in P_{Bmal1} and P_{Per2} promoter activities as well as the antiphase relationship between P_{Bmal1} and P_{Per2} oscillations, although the level of BMAL1 and other clock proteins, REV-ERBα and CLOCK showed no clear rhythmicity. I applied a transient response analysis to P_{Bmal1} luminescence data in the presence of various concentrations of doxycycline and found that a slightly damped linear oscillator system can reproduce P_{Bmal1} promoter activity. The oscillation parameters were not dramatically impacted by the levels of *Bmal1* expression, however, the behavior of the baseline of oscillations was greatly impacted. Based on the obtained

transfer functions, this study suggests that BMAL1 is not directly involved in the oscillatory process but modulates the robustness of the oscillations by regulating the basal activity of the clock gene promoter.