

Cryptochromes highly expressed in the ovary of *Xenopus tropicalis*

Yoko Kubo¹, Takahiro Takeuchi¹, Keiko Okano, and Toshiyuki Okano^{1,2}

¹Department of Electrical Engineering and Bioscience, Waseda University, Japan, ²PRESTO, Japan Science and Technology Agency (JST), Japan.

Cryptochromes (CRYs) are flavoproteins that possess various functions such as photoreception and circadian clock regulation. However, functions of some CRY family proteins have been obscure. To better understand the functional diversity of lower vertebrate CRYs, we focused on the western clawed frog *Xenopus tropicalis*. We extracted RNA from *X. tropicalis* tadpoles and identified cDNA encoding three *Cry* genes, *XtCry1*, *XtCry2* and *XtCry4*. Examination of tissue specificity of these mRNA expressions by quantitative RT-PCR analysis revealed that all of *XtCrys* showed extremely high expression in the ovary. Expression of *XtCry4* was also examined by *in situ* hybridization, which revealed high *XtCry4* transcription in early stage of developing oocytes. For functional analysis of XtCRYs, we cloned cDNA for XtCLOCK and XtBMAL1, factors constituting the core transcription loop in vertebrate circadian clock along with CRYs, and found that both XtCRY1 and XtCRY2 but not XtCRY4 repressed transactivation from *XtPer1* E-box related transcriptional activation by XtCLOCK/XtBMAL1. These results were consistent with the cellular localization of GFP-tagged XtCRYs: GFP-XtCRY1 and GFP-XtCRY2 localized in the nucleus, while GFP-XtCRY4 localized in the cytoplasm. These results suggest that XtCRY4 has a function distinct from XtCRY1 and XtCRY2, which operate as circadian negative regulators in the *Xenopus* circadian clock.