Project number 27

Elucidation of the role of FSXN5 in the regulation of circadian rhythm and pancreatic β-cell function

[1] Research group

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Expenditure report of research funds :

Consumables 130, 000 YEN

[2] Research setup

This research program has been advanced by a multidisciplinary team across the Tohoku region within the framework of the "Cryptochrome and Circadian Gene Research Team," under the long-standing leadership of the principal investigator (S.O.). The overarching aim of this program is to elucidate how disruption of the molecular clock influences pancreatic β-cell identity, fate, and disease progression.

To investigate the functional significance of zinc coordination in cryptochrome biology, we generated a CRY1 mutant ($CRY1^{C414A}$) in which a conserved zinc-coordinating cysteine residue was substituted with alanine. On the basis of this molecular design, we established transgenic mouse and complementary human cell systems overexpressing $CRY1^{C414A}$, thereby creating a mechanistically defined experimental platform for examining the consequences of altered circadian repression in β-cells. Functional characterization has shown that this mutant exerts stronger repressor activity than wild-type CRY1, accompanied by reduced circadian amplitude and prolonged periodicity, thus providing a valuable model of sustained clock dysregulation (Fig. 1).

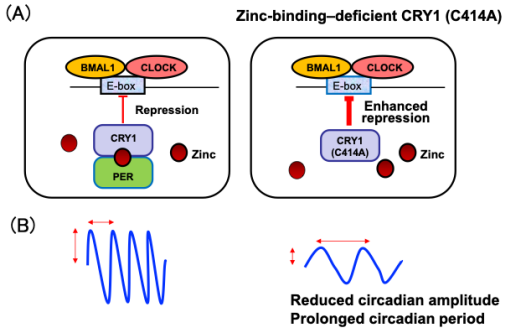

Figure 1. Molecular clock mechanism and the impact of zinc-binding–deficient CRY1 ($CRY1^{C414A}$).

CRY1 (C414A) enhances repression, resulting in reduced circadian amplitude and a prolonged period.

Mice overexpressing the mutant develop diabetes primarily in association with progressive β-cell loss, accompanied by impaired insulin production, islet architectural remodeling, and senescence-like alterations (Okano S. J Diabetes Res. 2016;2016:3459246). One particularly notable feature of this model is the frequent emergence of intra-islet ductal cells (IIDCs), the biological significance of which remains to be fully clarified in the setting of β-cell decline and circadian disruption (Okano S. et al, J Diabetes Res. 2019; 2019:7234549). A central objective of the present study is therefore to determine whether these lesions reflect dedifferentiation, lineage plasticity, compensatory remodeling, or a combination of these processes.

Transcriptomic analysis of transgenic islets identified Sfxn5 as a gene significantly downregulated relative to wild-type controls. SFXN5 is a mitochondrial protein implicated in metabolic regulation; however, its role in β-cell differentiation and maintenance remains insufficiently understood. The present project therefore seeks to clarify whether SFXN5 serves as a mechanistic link between mitochondrial metabolism and circadian regulatory pathways in β-cells.

An overview of the research activities conducted during the current fiscal year is provided below. The principal investigator visited the Institute of Development, Aging and Cancer to conduct research discussions with Professor Emeritus Akira Yasui and Dr. Kanno. Additional meetings with collaborators were held in person and at academic conferences. Professor Emeritus Kiyoshi Hayasaka, senior advisor to the research team (Former Professor, Department of Pediatrics, Yamagata University), has maintained close communication primarily via email, providing high-level strategic support and contributing to the flexible and stable operation of the research organization.

[3] Research outcomes

(3－1) Results

We have employed a depolarization-based model in MIN6 cells, in which sustained membrane depolarization is induced by 40 mM KCl. This system reproducibly causes intracellular zinc depletion and β-cell dedifferentiation-like changes. Our previous studies first demonstrated, by immunohistochemical analysis of mouse pancreas, that SFXN5 is expressed in pancreatic β-cells, and subsequently showed using a MIN6-based experimental system that Sfxn5 expression is upregulated under depolarizing conditions, supporting its association with β-cell immaturity (reported in the collaborative research reports of the Institute of Development, Aging and Cancer, Tohoku University, and available through its official website; Project No. 20, FY2023; Project No. 27, FY2024).

To further characterize this process, we examined its temporal dynamics and reversibility. KCl-induced depolarization rapidly triggered transcriptional changes consistent with β-cell dedifferentiation, which were partially reversed upon removal of KCl, indicating intrinsic transcriptional plasticity. Although detailed circadian gene analyses are not included here, these data will be presented in an e-poster at the American Diabetes Association (ADA) Scientific Sessions 2026 (Okano S. et al., "*Temporal Dynamics and Reversibility of Depolarization-Induced β-Cell Dedifferentiation and Its Association with Circadian Gene Expression*").Notably, Sfxn5 expression closely paralleled that of β-cell disallowed genes, suggesting a role in maintaining an undifferentiated or progenitor-like state.

In parallel, analysis of transgenic (TG) mice overexpressing zinc-binding-deficient CRY1 revealed mechanisms potentially underlying α-cell neogenesis associated with intra-islet ductal structures (IIDs). Metabolic profiling indicated increased hepatic amino acid flux and activation of the liver-α-cell axis, suggesting that metabolic alterations driven by insulin deficiency and relative glucagon dominance may promote α-cell expansion within IIDs. These results will also be presented at the ADA Scientific Sessions 2026 (Okano S. et al., "*Hepatic Metabolic Alterations and Alpha-Cell Hyperplasia in Mice Overexpressing a Zinc-Binding-Deficient CRY1 Mutant*").

(3－2) Future perspectives

These findings demonstrate that depolarization-induced β-cell dedifferentiation is at least partially reversible, highlighting the intrinsic plasticity of β-cell identity. Sfxn5 emerges as a candidate regulator of progenitor-like states, linking mitochondrial metabolism to maintenance of β-cell identity.

Moreover, the emergence of α-cells from intra-islet ductal structures and the formation of endocrine cell clusters in TG mice suggest a novel mechanism of endocrine cell regeneration that may contribute to in vivo replenishment of β-cell mass.

Collectively, this study provides new insights into β-cell plasticity, regeneration, and metabolic adaptation, and may inform future strategies for restoring β-cell function in diabetes.

[4] List of research achievements

1. Responses of Regulator of G Protein Signaling Proteins and Circadian Clock Components to Sustained Depolarization-Induced Dedifferentiation in MIN6 β-Cells. Okano S. et al., Exp Clin Endocrinol Diabetes. 2025;133:541-547.
2. Spasmolytic Polypeptide-Expressing Metaplasia-Like Features of Intraislet Ducts in Zinc-Binding Site-Mutated CRY1-Expressing Diabetic Mice. Okano S. et al., *Diabetes* 2025;74(Supplement_1):1805-P.