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審査組織	グローバル教育院
学位論文題目	Functional characterization of testis-brain RNA-binding protein, TB-RBP/Translin, in mRNA regulation （mRNA制御におけるRNA結合タンパク質TB-RBP/Translinの機能解析）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Kanako Oyama describes the function of testis-brain RNA-binding protein, TB-RBP/Translin, in the translational regulation of mRNA. The summary is as follows:

（目的　Purpose）

Mammalian spermatogenesis is a highly specialized process of cellular differentiation, which is regulated at the transcriptional, post-transcriptional, and translational levels. In this thesis, the author analyzes the function of testis-brain RNA-binding protein, TB-RBP, an RNA/DNA-binding protein abundantly expressed in the testis and brain. RNA-binding proteins are known to bind specific target mRNAs, and to induce translational repression and/or mRNA degradation. Because the function of TB-RBP remains unclear, the author deciphered the roles of TB-RBP on RNA-binding, translation, and mRNA degradation.

（対象と方法　Materials and Methods）

To uncover the function of TB-RBP in translational repression, the author established TB-RBP-null HEK293T cell lines, using a CRISPR/Cas9 system. In addition, the author utilized analytical, biological approaches, including polysome fractionation, dual-luciferase reporter assays, RNA immunoprecipitation assays, m7GTP cap-agarose pull-down assays, and eCLIP (enhanced UV crosslinking and

immunoprecipitation) assays.

（結果　Results）

The author first analyzed the effects of TB-RBP on transcription and translation of reported target mRNAs. Based on the experimental results obtained, the author concluded that TB-RBP does not bind to well-known target mRNAs. The author also found that TB-RBP is involved in the degradation of mRNA in concert with Translin (TB-RBP) - associated factor X (TRAX), an endonuclease. Importantly, TRAX expression disappeared in TB-RBP-deficient cells, suggesting that TRAX is a genuine binding partner of TB-RBP. Indeed, TB-RBP possessed the domain necessary for repression of reporter gene expression and binding to TRAX. Moreover, the author conducted eCLIP assays and has identified several candidate RNAs of TB-RBP, some of which were up-regulated in TB-RBP-deficient cells, possibly owing to TB-RBP-mediated mRNA degradation. Finally, the author found mRNA levels of several genes important for cellular homeostasis are dysregulated by the loss of TB-RBP.

（考察　Discussion）

The results obtained shed light on the role of TB-RBP regarding translational regulation of mRNA, suggesting that the TB-RBP/TRAX RNA complex may be important for the maintenance of cellular homeostasis by modulating the levels of mRNAs through mRNA degradation.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The applicant proposes the novel roles of TB-RBP as a complex with TRAX in the translational regulation of mRNAs by using TB-RBP-knockout cells. Although the applicant has found very interesting functions of the TB-RBP/TRAX complex, the detailed mechanism at molecular level remains to be elucidated. Since the complex appears to affect mRNA degradation in a sequence-specific manner, it would be great if the applicant is able to demonstrate the change(s) of mRNA degradation in TB-RBP-knockout mice under physiological conditions.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on 8th March, 2021. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.