平成 29 年度　徳島大学先端酵素学研究所「共同利用・共同研究」報告書

1）［申請者］

氏名：本庶　佑　所属：京都大学　職位：客員教授

紹介先：京都大学大学院医学研究科寄附講座「免疫ゲノム医学」

2）［共同研究項目］

共同研究: B-4. 疾患プロテオミクス研究分野（担当：谷口寿章 hisatan@tokushima-u.ac.jp）

3）［共同研究題目］

AID による DNA 切断メカニズムの解明に向けた微量核タンパク質のプロテオミクス解析

4）［共同研究組織］

研究代表者：本庶　佑（京都大学医学研究科）共同研究者：谷口　寿章（徳島大学疾患酵素学研究センター）

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5）［共同研究の概要］

Main project:

Chromatin Regulation of Antibody Class Switch Recombination (CSR)

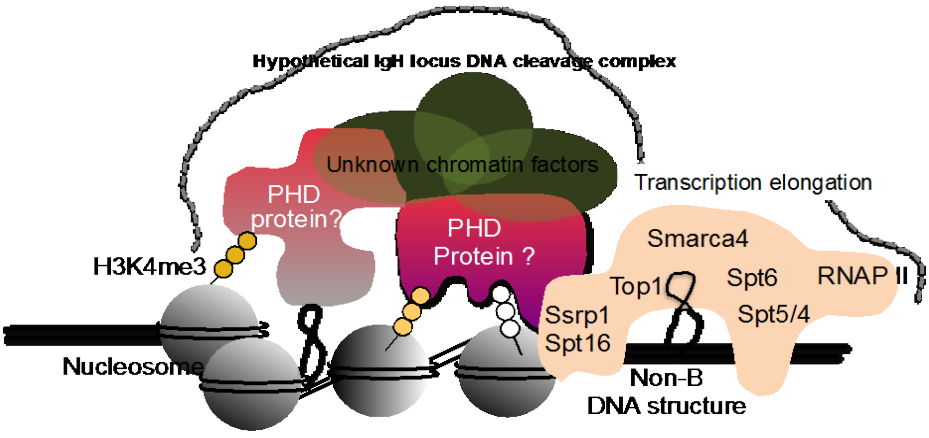
Specific Aim-1: **Identification of Top1 associated proteins from B cell**

Status : Completed

Publication: Chromatin remodeler SMARCA4 recruits topoisomerase 1 and suppresses transcription associated genomic instability. Husain, A. Begum, N. A., Taniguchi, T., Taniguchi, H., Kobayashi, M. and Honjo, T. Nature Commun. 7, 10549 (2016)

Specific Aim-2: **Proteomics of a PHD finger protein involved in CSR**

In order to identify regulatory molecules for CSR, we applied candidate gene targeting approach. During screening chromatin proteins, we identified a small PHD protein that seemed to have strong regulatory activity on CSR .



PHD (Plant Homeo Domain): recognizes H3K4me3

siRNA screening identifies of a novel PHD protein as a CSR co-factor

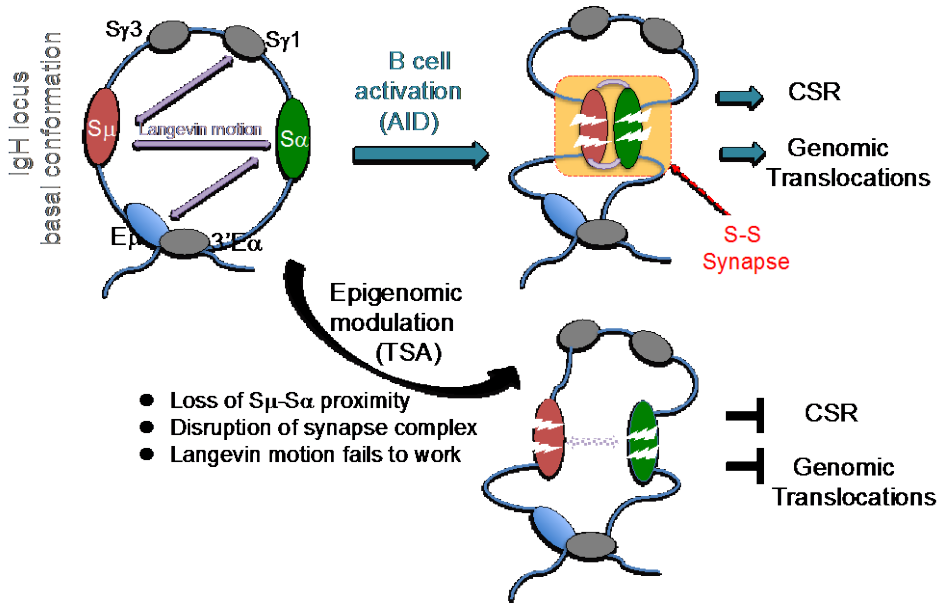
Although the identified protein was reported to associate with spliceosome, its function is poorly understood. As a PHD protein, it is expected to have histone modification(H3K4m3) specific binding and chromatin associated function. In collaboration with Prof. Taniguchi at Tokushima university, we aimed to identify its nuclear interacting partners. Myc-Flag epitope tagged PHD protein was expressed in a B cell line and following anti-Flag IP, co-IPed proteins were subjected to MS analysis. We not only detected spliceosomal subunits but also interesting chromatin proteins including histones and its variants.

The result of　MS analysis was very helpful to investigate the function of the identified PHD protein. Eventually, we revealed a novel mode of chromatin-linked repair function of the protein, which explained its strong connection with AID induced CSR and non-IgH locus associated genomic instability.

Status: We just completed this work.

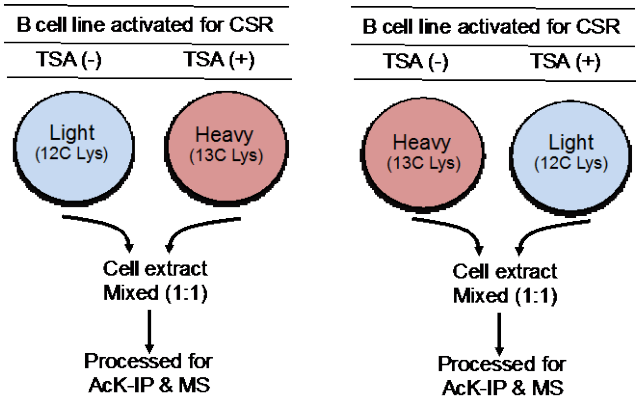
Publication: A manuscript is under preparation.

Specific Aim-3: **Investigation of proteins supporting S-S synapse in CSR**



Donor and acceptor switch(s) regions come into proximity during CSR to from S-S synapse, which facilitates recombination to take place at the site. We found that the HDAC inhibitor (TSA) causes a conformational defect in the IgH locus, which leads to impaired synapse formation and complete block of AID induced genomic instability.

In addition to gene expression profiling, we are interested to identify proteins that undergo TSA induced acetylation. We therefore planned to conduct acetylome analysis of the activated B cell with and without TSA treatment.



As we had no experience in the acetylome work, we faced various technical difficulties associated with SILAC labeling efficiency, protein digestion and contaminant free sample preparation for MS after Acetyl-Lysine(Anti-AcK) IP. We are greatly indebted to Prof. Taniguchi for his advice and analysis support in this work.

Current status: A list of acetylated proteins were identified, but it was only from one batch of successful IP. It may be necessary to repeat the experiment. Meanwhile, we are examining some of the interesting candidate proteins at the context of CSR.