研究題目　Proteomics analysis of recombination complex that promotes site specific genomic instability in B cell

研究組織

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【1】研究の概要

[1-1] 本研究の目的・概要・ Purpose and outline

Antigen activated mature B cells undergo Ig gene diversity through a complex DNA break and recombination mechanism induced by Activation-Induced Cytidine Deaminase (AID). In order to understand the AID induced this recombination mechanism, we need to characterize (1) the RNA/DNA editing protein complex that associates with AID and its co-factor, and (2) the chromatin complex that accumulates at the Ig locus during antibody class switch recombination (CSR).

One of the AID's co-factor for CSR is hnRNPL, which interacts with AID and other unknown proteins during CSR. Recently, mutagenesis study identified critical hnRNPL mutants that are unable to support CSR. Therefore, the specific purpose is to know the proteins interacting with wild type-hnRPL but not with the CSR loss of function mutant-hnRNPL.

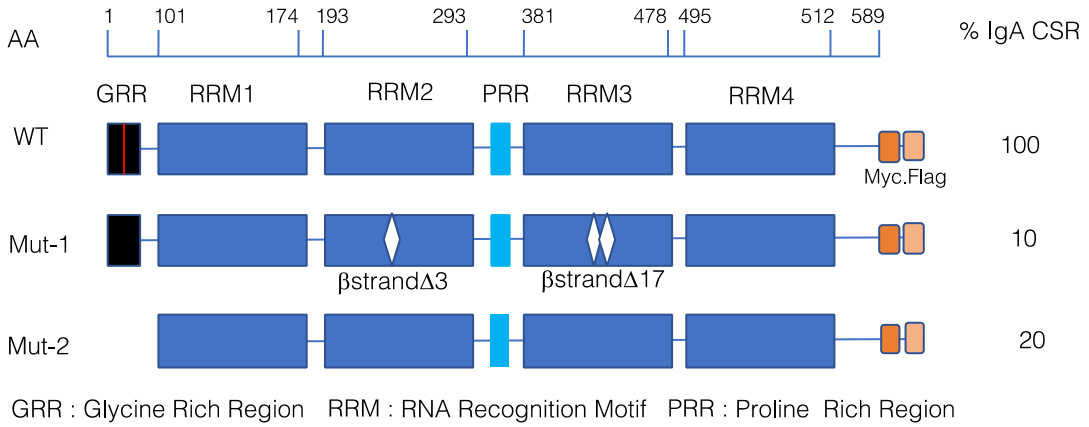
[1-2] 研究の方法・経過・Method

Emploing standard mutagenesis procedure, deletion and point mutants of hnRNPL were generated. CSR efficiency of various mutants was examined by monitoring IgM to IgA switching efficiency in a mouse B cell line. WT and mutant proteins were also examined for their interaction with AID in 293T cells. Since hnRNPL was tagged with FLAG epitope, we performed anti-FLAG immunprecipitation (IP) of WT and the mutant hnRNPL. IPed proteins were separated on TGX gradient gel and silver stained. The gels with promising

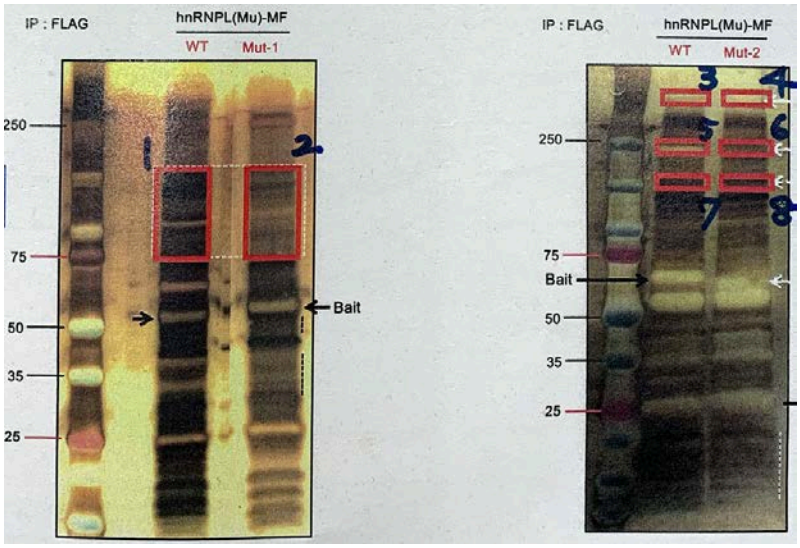
difference (WT versus Mutant) were sent to Prof. H. Kosako's laboratory at Tokushima University for MS analysis.

【2】研究成果

[2-1] 本共同研究で明らかになった研究成果・Result



➢ The schematic view shows the domain structure of wild type and two critical hnRNPL mutants. IgM to IgA switching efficiency was examined, which indicates that both the mutants are defective in CSR.



➢ Comparison of hnRNPL immunnoprecipitation on silver stained gel. Red rectangles indicate the area of interest, which have been subjected to MS analysis.

- Many proteins including known AID interacting proteins were identified from this initial trial. The Table below (comparison of WT vs. Mut-1) is an example, which shows that some proteins can be found only with WT but not Mutant-1.

Accession	Description	Molecular Function	Gene Symbol	Found in WT	Found in Mutant
Q8NDT2	Putative RNA-binding protein 15B	protein binding;RNA binding	RBM15B	High	Not Found
O60231	Pre-mRNA-splicing factor ATP-dependent RNA helicase	catalytic activity;nucleotide binding;protein binding;RNA binding	DHX16	High	Not Found
Q13769	THO complex subunit 5 homolog	protein binding;RNA binding	THOC5	High	Not Found
Q96SB4	SRSF protein kinase 1	catalytic activity;metal ion binding;nucleotide binding;protein binding;RNA binding	SRPK1	High	Not Found
O75400	Pre-mRNA-processing factor 40 homolog A	protein binding;RNA binding	PRPF40A	High	Not Found

[2-2] 本共同研究による波及効果及び今後の発展性・Discussion

It is surprising that some known AID interacting protein like DHX or THO was detected in the hnRNPL IP. Moreover, many interacting proteins appear to be RNA binding proteins. If this is the case, AID-hnRNPL could be a much bigger RNP (ribonucleoprotein) complex than we expected. Currently, little is known about the function of AID's RNP complex with hnRNPL. MS results showed some promising interacting molecule like RBM15B, because another RBM protein (RBM47) has been found as a RNA editing co-factor for Apobec1.

【3】主な発表論文等

[3-1] 論文発表

1）[雑誌]著者名.題名.雑誌名　巻：頁□頁，発行年. (publication in journals)

2）[書籍]著者名.題名.Ｉｎ：編集者名・編 書籍名.出版社,発行地，頁-頁,発行年 (publication in books)

[3-2] 学会発表

1）発表者名.題名.学会名，発表地，発表月日，発表年

(presentation in meetings)

[3-3] 成果資料等

【4】今後の課題等 (Future directions)

We need reproducible IP/MS result for hnRNPL from 293T and B cell system. As per Prof. Kosako's suggestion gel-free MS approach will be adopted for the faster progress, and also to obtain a comprehensive overview of the interacting proteins. Under identical condition, AID IP will be also necessary in the near future.