（様式２）

令和３年度研究助成（参加費助成）研究成果報告書

2021 年　6 月　29 日

公益財団法人遺伝学普及会 代表理事　殿

貴財団より助成のありました研究の成果を下記のとおり報告します。

氏名 川瀬雅貴

出席学会等名称　FASEB The Mobile DNA Conference

開 催 場 所　オンライン

開 催 期 間　令和 3 年 6 月 8 日 ～ 令和 3 年 6 月 9 日

研 究 成 果 の 概 要

この度，私は FASEB The Mobil DNA Conference に参加し，ポスター形式の研究報告を行った．なお本会は新型コロナウイルス感染拡大を受けオンラインで行われた．

本会は全米実験生物科学連合が主催する転移因子に関する研究発表の場である．転移因子は多くの生物種のゲノム内に存在する反復配列の一種であり，ゲノム内を転移する能力を持つ物の総称である．とりわけレトロトランスポゾンと呼ばれる転写を介した転移をする因子はヒトやマウスのゲノムの約 40%を占める配列であり，生物種のゲノム情報の破壊や進化を促進させる要因として着目されている．私は本会にて，生殖細胞におけるレトロトランスポゾンの制御システムに，抑制性エピジェネティック修飾であるヒストン H3 リジン 9 トリメチル化（H3K9me3）と DNA のシトシン残基のメチル化（DNA メチル化）がどのように関わっているかを報告した．報告内容の概要を以下に記す．

ヒストン H3K9 のトリメチル化酵素 SETDB1 の生殖細胞特異的な欠損マウスを使い，トランスクリプトームやエピゲノム解析を行った．*Setdb1* 欠損の減数分裂期の細胞では MMERVK10C など一部のレトロトランスポゾンの脱抑制が観察され，脱抑制したそれらは H3K9me3 の減少が伴っていた．しかし DNA メチル化の変化は観察されなかった．他方で DNA メチル化に関わる *Dnmt3L* 欠損体の減数分裂細胞で，MMERVK10C は DNA メチル化の減少とともに脱抑制し，H3K9me3 の変化は見られなかった．これらのことから MERVK10C は H3K9me3 と DNA メチル化の両者によって抑制されているが，二つの修飾は独立に働いていると考えられた．さらに解析を進めると，L1Md_A と呼ばれるレトロトランスポゾンは，いずれの欠損体の減数分裂細胞でも H3K9me3 が減少しているにも関わらず，DNA メチル化が減少した時（*Dnmt3L* 欠損細胞）でのみ脱抑制することから，このレトロトランスポゾンは DNA メチル化の制御下にあると示唆された．

この報告の結果，オーディエンスからはこの制御機構の差がどこで生まれるかなど，私の研究を今後さらに発展させるようなサジェスションもあり，非常に有意義な発表となったと感じている．

また本会にはアメリカだけでなく，フランスやドイツなどのヨーロッパの研究者も参加しており，この分野の世界的トップランナーたちが集合していた．日本国内ではなかなか聞けない，彼らの講演は非常に刺激的であった．転移因子に対する多角的なアプローチや転移因子を利用した解析手法など，現在の私の研究だけではフォローできない広い知見を肌身に感じることができ，転移因子を取り巻く研究の奥深さを感じることができた．そして，この経験を今後の研究の糧にし，より研究を推し進めようとさらに強く思うようになった．

以上のように，非常に刺激的で今後の研究の発展に繋がる経験を得ることが出来たのは，遺伝普及会のご支援あってのものであります．この場を借りてお礼を申し上げます．誠にありがとうございました．

Abstract

Mammalian germ cells have a unique role: transferring the genomic information to next generations. Therefore, the integrity of their genomic information needs to be maintained, implying that the mobility of retrotransposons is restricted in the germline. Whereas transcription of retrotransposons is repressed by epigenetic modification including DNA methylation and histone modifications in somatic cells, the amounts of these modifications in the nucleus change dynamically during germ cell development. For example, DNA methylation and H3K27me3 decreased dramatically in primordial germ cells then they increase later, whereas H3K9me2 disappears at pachynema in spermatocytes. It is unclear how cells regulate the retrotransposon activity with these epigenetic waves during germ cell development.

In this study, we have focused on SETDB1, an enzyme catalyzing trimethylation of H3K9. To investigate its function in meiotic germ cells (spermatocytes), conditional knockout (cKO) mice were used, in which *Setdb1* is disrupted specifically in pre-meiotic germ-cells. The development of *Setdb1* cKO germ cells aborted at late zygotene stage of meiosis, indicating the importance of H3K9me3 for meiotic progression. Our mRNA-seq analysis on FACS-sorted *Setdb1* cKO spermatocytes revealed derepression of several retrotransposons, such as RLTR10C and MMERVK10C. Consistently, ChIP-seq analysis revealed a decrease in H3K9me3 in these retrotransposons in the mutant cells. The whole-genome bisulfite sequencing analysis of the *Setdb1* cKO spermatocytes revealed no decrease in DNA methylation in these retrotransposons, suggesting that their upregulation is due to a decrease in H3K9me3. The *Dnmt3l* KO spermatocytes also showed upregulation of RLTR10C and MMERVK10C with a decrease in DNA methylation but not in H3K9me3. Thus, H3K9me3 and DNA methylation independently work to repress RLTR10C and MMERVK10C. On the other hand, derepression was not observed for L1 families in the *Setdb1* cKO spermatocytes, consistent with that H3K9me3 and DNA methylation were not affected in L1 promoter regions. The L1 families are shown to be derepressed in the *Dnmt3l* KO spermatocytes, where their DNA methylation level is decreased. ChIP-seq analysis revealed that their H3K9me3 level was also decreased in the *Dnmt3l* KO spermatocytes, arguing in favor that H3K9me3 deposition and/or maintenance at L1 promoters depends on the presence of DNA methylation. Taken, together, our data suggest that the hosts have different strategies, such as H3K9me3, DNA methylation, and piRNAs, to repress different retrotransposons during meiosis. The integrity of the host genome in the germline is therefore maintained by cooperation of these epigenetic regulatory systems.

The role of SETDB1 in repression of retrotransposons in mouse germ cells

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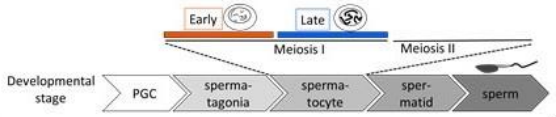
Introduction

The germ cells are the sole type of cells that convey the genomic information to the next generation in mammals. Since active transposition of retrotransposons introduces mutations, it is important to restrict their activity in germ cells to maintain the genomic integrity of a species.

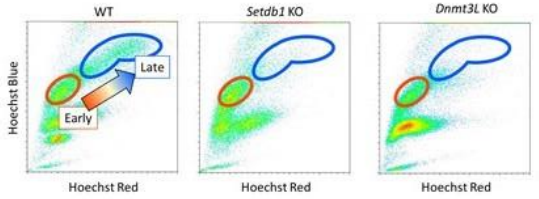
It is known that, in ES cells and the embryo, transcription of evolutionarily young retrotransposons, such as L1 and IAP, is repressed by DNA methylation. Moreover, histone H3 lysine-9 trimethylation (H3K9me3) is involved in repression of different families of retrotransposons in ES cells, such as endogenous retrovirus (ERVs) of the MMERVK10 and RLTR1 families. Studies using *Dnmt3L* knockout (KO) mice, where DNA methylation is severely decreased in male germ cells, have showed that DNA methylation represses retrotransposons in developing germ cells as well, and that a failure in *de novo* DNA methylation results in male infertility. piRNAs are also involved in retrotransposon repression, and a deficiency of piRNA biogenesis results in loss of DNA methylation and H3K9me3 in the promoter of young L1 families, and consequently increases their transcription.

However, the actual role for H3K9me3 in retrotransposon repression in germ cells remains elusive. To elucidate this, we analyzed conditional KO mice in which *Setdb1*, encoding an H3K9 methyltransferase, is disrupted specifically in developing germ cells. The mutant showed a failure in meiosis. Then, transcription, DNA methylation, and H3K9me3 were analyzed by NGS, which together suggest that the H3K9me3 mark represses some of ERVs, especially MMERVK10C, in developing germ cells in a manner that is independent of DNA methylation.



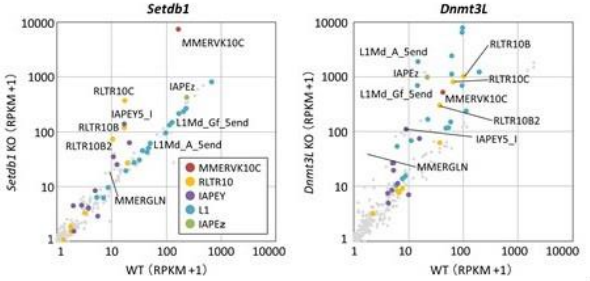
Result1
Setdb1 deficiency caused a developmental arrest of germ cells

We generated *Setdb1* cKO male mice, which have a flox and a deleted alleles of *Setdb1* (*Setdb1*$^{flox/\Delta}$) and a *Cre* gene under the control of the *Stra8* promoter (*Stra8-Cre*). In these mice, the flox allele becomes deleted specifically in spermatogonia, pre-meiotic male germ cells. Flow cytometric analysis of testicular cells (1 month old) stained with Hoechst revealed the absence of germ cells at late meiosis I (MI) stages, MII, and spermatids in *Setdb1* cKO mice, very similar to *Dnmt3L* KO mice. These results suggest that the *Setdb1* deficiency causes meiotic arrest at an early MI stage (called zygonema). To study the effect of *Setdb1* depletion in retrotransposon repression, we collected these early MI cells from wild-type and cKO mice by FACS to perform mRNA-seq, ChIP-seq and whole-genome bisulfite sequencing. We previously studied the same stage of *Dnmt3L* KO spermatocytes[1] showing a severe decrease in DNA methylation; therefore, these two mutant data were compared.



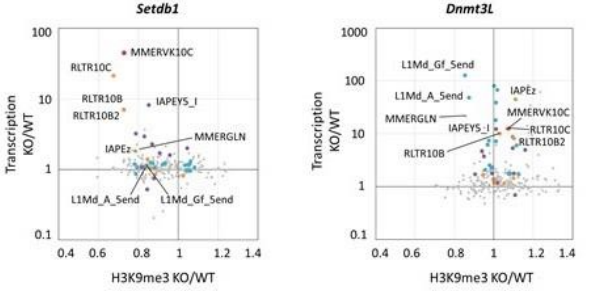
Result 2
Several retrotransposons were derepressed in *Setdb1* KO spermatocytes

To analyze the transcriptome of retrotransposons, mRNA-seq reads were mapped to consensus sequences of mouse-specific retrotransposons. In *Setdb1* KO spermatocytes, expression of 13 retrotransposons were increased by >2-fold. These included MMERVK10C (45 fold, red), RLTR10 families (7-21 fold, orange), and IAPEY families (2-8 fold, purple). Interestingly, derepression of the MMERVK10C and RLTR10 families were observed in *Dnmt3L* KO spermatocytes as well. In contrast, whereas the expression of several L1 subfamilies (light blue) were increased in *Dnmt3L* KO spermatocytes by about 100-fold, these were not derepressed in *Setdb1* KO spermatocytes.



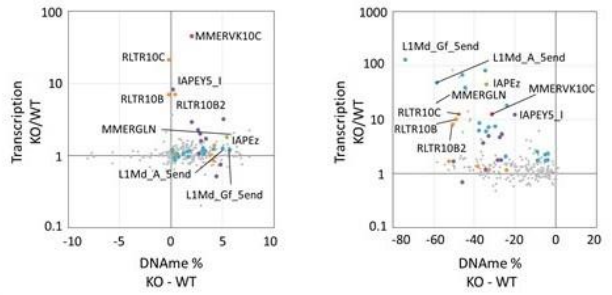
Result 3
H3K9me3 was decreased in the derepressed retrotransposons in *Setdb1* KO spermatocytes

Since SETDB1 is an H3K9 methyltransferase, we analyzed the H3K9me3 levels in *Setdb1* KO spermatocytes using published ChIP-seq data[2]. The amount of H3K9me3 in MMERVK10C and RLTR10 families was decreased by about 30% in *Setdb1* KO, consistent with the increase in mRNA expression of these retrotransposons. On the other hand, H3K9me3 in L1Md_A and L1Md_Gf was only slightly decreased. For comparison, we performed ChIP-seq with H3K9me3 for *Dnmt3L* KO spermatocytes, which revealed that H3K9me3 was not decreased in the MMERVK10C and RLTR10 families despite their increased expression by about 10-fold. H3K9me3 in L1Md_A and _Gf was slightly decreased. The level of the decrease was similar to that in *Setdb1* KO, suggesting that the slight decrease in H3K9me3 in *Dnmt3L* KO spermatocytes is not a major cause for the L1 derepression.



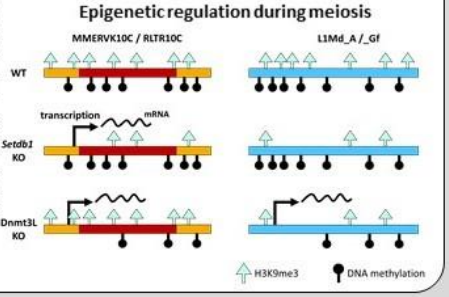
Result 4
DNA methylation was not decreased in the derepressed retrotransposons in *Setdb1* KO spermatocytes

Since the MMERVK10C and RLTR10 families were derepressed by a decrease in DNA methylation in *Dnmt3L* KO spermatocytes, we performed whole genome bisulfite sequencing (WGBS) to analyze DNA methylation levels in *Setdb1* KO and WT spermatocytes. The results showed that the *Setdb1* deficiency and accompanied decrease in H3K9me3 did not reduce DNA methylation in the MMERVK10C and RLTR10 families in *Setdb1* KO spermatocytes. Moreover, regardless of loss or gain of H3K9me3, almost all retrotransposons did not show a decrease in DNA methylation in *Setdb1* KO spermatocytes.



Discussion

This study revealed that the *Setdb1* gene and likely proper methylation of H3K9 are essential for the meiotic progression (result 1), especially at the stage to enter late meiosis I (called pachynema). We observed derepression of 13 retrotransposons in *Setdb1* KO, eight of which were also derepressed in *Dnmt3L* KO (result 2). In the eight retrotransposons, including MMERVK10C and its LTR sequence, RLTR10C, H3K9me3 was reduced while DNA methylation was unaffected in *Setdb1* KO (results 3 & 4). Likewise in *Dnmt3L* KO, DNA methylation was reduced in these retrotransposons but H3K9me3 was not. The results suggest that these retrotransposons are repressed by two independent mechanisms, DNA methylation and H3K9me3. On the other hand, the loss of the *Setdb1* function and a concomitant small loss of H3K9me3 did not liberate the L1 expression, whereas a loss of DNA methylation significantly induced the L1 expression. Thus, L1 families are likely repressed by DNA methylation, whereas H3K9me3 plays a minor role if any, although this histone modification is enriched in the L1 promoters in the wild-type spermatocytes. In comparison to the situation in ES cells, where MMERGLN, RLTR1, IAPEz, and some other retrotransposons are regulated by H3K9me3, these retrotransposons were not derepressed in *Setdb1* KO spermatocytes whereas MMERGLN and IAPEz were highly depressed in *Dnmt3L*. Therefore, spermatocytes seem to be more dependent on DNA methylation for retrotransposon regulation than H3K9me3. This may be related to the facts that histone variant composition changes dynamically around the onset of meiosis, and that the histone amount gradually declines after the cells complete meiosis.



Acknowledgment: We thank Dr. Yoichi Shinkai for providing the *Setdb1 flox* mouse

Reference : [1] Inoue et al. PLoS Genet. (2017) 13, e1006926 [2] Hirota et al. Dev. Cell (2018) 47, 645-659