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研究課題名（和文）Functional analysis of an RNA methyltransferase in regulation of neural stem cell fate

研究課題名（英文）Functional analysis of an RNA methyltransferase in regulation of neural stem cell fate

研究代表者

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研究成果の概要（和文）：動物の発生は、時間的にも空間的にも高度に制御された過程であり、臓器形成の時間的制御には、時計機構が関与しています。しかし、組織や組織幹細胞の時間的な状態は、主にゲノム全体のエピジェネティックな状態や転写の状態によって規定されることが示唆されているが、何が時計を動かしているのかについてはまだ明らかになっていません。本研究では、大脳皮質の発生過程における神経幹細胞（NSCs）の時間特異性の変化に着目し、大脳皮質におけるエピジェネティック修飾因子の翻訳効率を制御することで、rRNAのメチルトランスフェラーゼFibrillarinがこの時計を駆動することを明らかにした。

研究成果の学術的意義や社会的意義
In this study, we have identified the existence of a clock system in neural stem cells during brain development. By elucidating this mechanism, we can contribute to the understanding of the mechanisms of ageing in animals.

研究成果の概要（英文）：Animal development is a highly coordinated temporal and spatial process. A clock mechanism has been often implicated in the temporal control of organ development. Accumulating evidence suggests the temporal state of tissues or tissue stem cells is mainly defined by the genome-wide epigenetic and transcriptional state as the clock, while little is known about what drives the postulated clock. In this work, we focused on temporal changes in embryonic neural stem cells (NSCs) identity during neocortical development (which sequentially generates different types of neurons and is considered a typical example of a developmental clock) and found that a methyltransferase of rRNA: Fibrillarin drives this clock through controlling translational efficiencies of epigenetic modifiers in the cerebral cortex primordium.

研究分野：発生生物学

キーワード：neural stem cell　rRNA methylation　epigenetics

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１．研究開始当初の背景

The cerebral cortex, consisting of diverse types of neurons and glia, is generated from a small population of neural stem cells (NSCs) in a highly orchestrated order. In the initial proliferative stage, NSCs divide symmetrically to expand their pool. In the subsequent neurogenic stage, they divide asymmetrically to produce one stem cell and one neuron or an intermediate progenitor that mostly divides once before terminal differentiation. The competence of NSCs to produce different types of progenies changes as neurogenesis proceeds; NSCs initially produce deep-layer (early-born) neurons, followed by upper-layer (late-born) neurons and glia Thus, the identity of NSCs is temporally patterned. However, how this process is precisely regulated remains elusive, although transcriptional and epigenetic regulation have been suggested as major underlying mechanisms. I expected factors promoting the temporal pattern to show monotonic changes of expression. Therefore, we compared our single cell transcriptome data from embryonic (E) 11 and E14 NSCs and defined early- and late-onset genes as those highly expressed in E11 and E14 NSCs, respectively. Using weighted correlation network analysis (WGCNA), we identified a gene module showing higher expression in E11 than in E14 NSCs, highly enriched in genes whose products are located in nuclear regions essential for rDNA transcription and pre-rRNA processing, such as nucleolar part and fibrillar centre. Among these genes, we focused on *Fbl* (also known as *Fibrillarin*). *Fbl* was initially reported as an rRNA methyltransferase and is essential for the translational regulation of some mRNAs. However, it is unknown how Fbl impacts the temporal fate transition of NSCs.

２．研究の目的

In this study, I tried to clarify the functions of Fbl during brain development and uncover the mechanism that how a methyltransferase affects cell fate.

３．研究の方法

To examine *Fbl* function in NSCs, we conditionally deleted it in the developing dorsal cortex from around E9.5 using *Emx1-Cre*. Because knockout of Fbl led to apoptosis of NSCs. We obtained double-knockout mice with genotype *Fbl flox/flox, Trp53-/-, Emx1-Cre/+* (DKO hereafter). Using these mice, we performed single cell analysis, ribosome profiling and chromatin immunoprecipitation and sequencing (ChIP-seq) to analyse the

function of Fbl in brain development.

４．研究成果

Knockout of Fbl disrupts brain development independently of apoptosis

To determine whether Trp53-dependent apoptosis is responsible for microcephaly, we crossed Fbl CKO and Trp53-/- mice. As Trp53 knockout did not affect brain size, nor neuron number compared to wild type, we used wild type or heterozygous mice for Fbl as controls (designated as Fbl+/+or FblΔ/+). We obtained double-knockout mice with genotype Fbl flox/flox, Trp53-/-, Emx1-Cre/+ (DKO) in which we confirmed the loss of Fbl by immunohistochemistry. DKO brains were smaller than control brains, although apoptosis was completely suppressed, indicating that microcephaly could not be explained by NSC apoptosis alone (Figure 1).

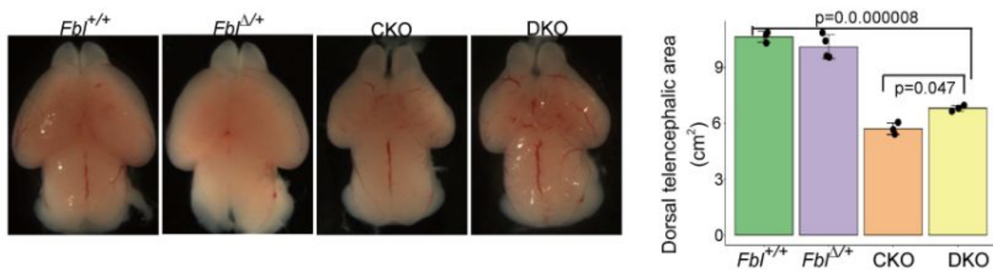

Figure 1. Fbl is essential for brain development. Whole-mount brain image at E17 showing microcephaly after Fbl knockout.

Analysis of temporal identity of Fbl-mutant NSCs at single cell level

To clarify the possible mechanisms leading to defective brain development in DKO, we performed transcriptome analysis at the single-cell level for different genotypes along the developmental timeline. We then asked whether Fbl promotes or suppresses the progression of NSCs along these time axes. To answer this question, we again constructed a continuous trajectory of all cells with Monocle (Figure 2A). The cells were thus

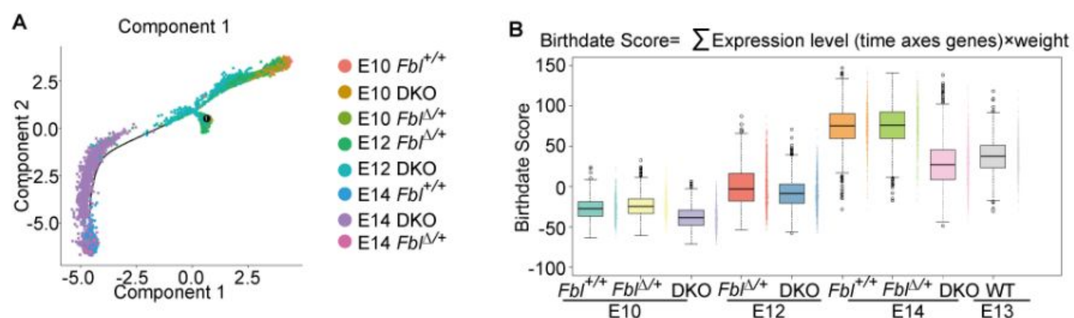

Figure2 Single cell transcriptome analysis of temporal patterning in NSCs. (A) Pseudo-time alignment of NSCs via Monocle. (B) Scoring single-cell identity with a mathematical model.

deposited along the temporal axes. DKO cells from E10 and E12 could not be distinguished from controls at the same stages, E14 DKO cells were closer to E12 FblΔ/+ than to E14 Fbl+/+or FblΔ/+ cells, implying delayed temporal identity transition(Figure 2A).To further confirm our results, we introduced a simple mathematical model to estimate the developmental time of each NSC. We defined the birthdate score of each cell as a weighted linear combination of specific temporal-axis genes. These scores are likely a faithful representation of each cell, as birthdate scores increased from E10 to E14. The birthdate scores of E14 DKO NSCs were lower than those of E14 Fbl+/+or FblΔ/+ NSCs, but similar to those of E13 Fbl+/+ NSCs (Figure 2B). These results strongly suggest that Fbl is required for the proper temporal patterning of NCSs.

Fbl is essential for translation of epigenetic modifiers

To investigate whether Fbl affects the translation of selected mRNAs, we performed ribosome profiling and RNA-seq using FblΔ/+ and DKO brains. Translational efficiency (TE) can be calculated by comparing the levels of translating mRNA (Ribo-seq) and total mRNA (RNA-seq). After analyzing the qualities of data according to several criteria, we detected 299 and 541 genes with an increased and decreased TE (q-value < 0.01) in DKO brains, respectively. Strikingly, chromatin-related genes were highly enriched among those with a decreased TE in DKO, consistent with the roles of epigenetic modifications in temporal patterning (Figure 3A). Among these highly enriched genes were Ezh2 and Kdm6b, which encode a methyltransferase and a demethylase, respectively, of H3K27me3 (Figure 3B).

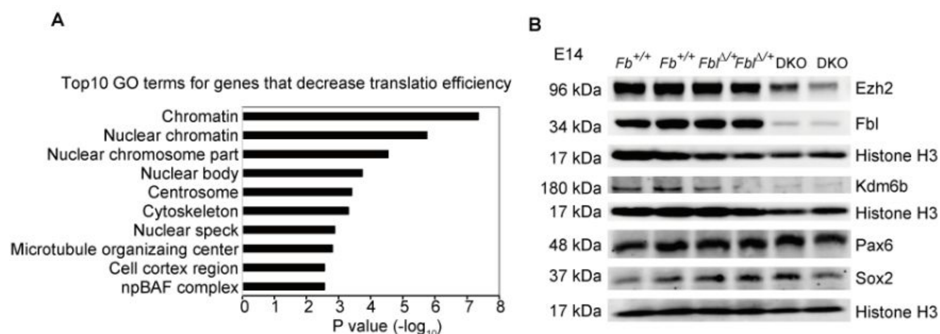

Figure 3 Fbl selectively regulates the translation of genes involved in H3K27me3 modification. (A) Top 10 GO terms of transcripts showing reduced translational efficency after knockout of Fbl. (B) Western blotting of the indicated genes, showing reduced protein levels of Ezh2 and Kdm6b in DKO brains at E14.

Fbl affects temporal patterning though H3K27m3 modification

We investigated histone modification changes upon Fbl deletion in DKO and control samples and demonstrated alterations of H3K27me3 and H3K4me3 modifications at 669 and 0 sites (q-value < 0.05), respectively, indicating significant defects in H3K27me3

marks. Moreover, the abundance of H3K27me3 peaks on early-onset genes did not show a clear difference between control and DKO samples (Figure 4A). In contrast, compared with control samples, the abundance of H3K27me3 peaks on early-onset genes (especially on transcription start sites) was higher in DKO samples, indicating the expression of these genes was repressed by H3K27me3 (Figure 4B).

Fbl control of both Ezh2 and Kdm6b led us to questioning the role of modification turnover during temporal patterning. To this end, we inhibited both methyltransferase and demethylase using the specific inhibitors GSK-343 and GSK-J4, respectively, investigated gene expression changes involved in birthdate, and calculated the birthdate scores after RNA-seq. Inhibition of H3K27me3 methyltransferase or demethylase alone slightly affected birthdate scores. In contrast, the simultaneous suppression of methyltransferase and demethylase reduced birthdate scores to much lower levels, indicating delayed temporal fate progression (Figure 4C). Therefore, we concluded that both writing and erasing of H3K27me3 are essential for temporal pattering, and that Fbl facilitates these processes by controlling the translation of key enzymes.

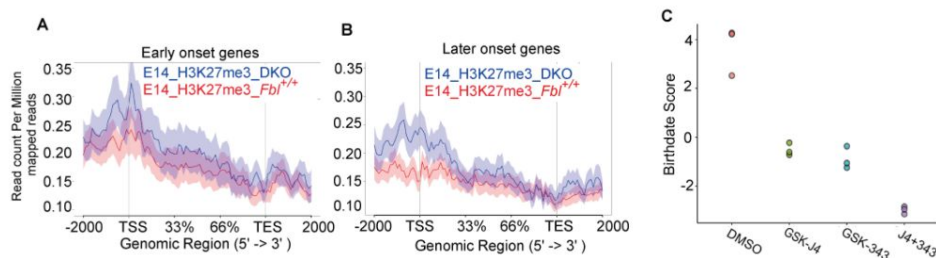

Figure 4 Fbl regulates H3K27me3 pattern in NSCs. (A, B) Read-density profiling of H3K27m3 at early-onset (A) and late-onset genes (B) in E14 control and DKO samples.(C) Birthdate scoring after simultaneous inhibition of H3K27me3 methyltransferase and demethylase, showing delayed temporal progression.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　2件）

1．著者名 Quan Wu, Yuichi Shichino, Takaya Abe, Taeko Suetsugu, Ayaka Omori, Hiroshi Kiyonari, Shintaro Iwasaki, Fumio Matsuzaki	4．巻 0
2．論文標題 Selective translation of epigenetic modifiers drives the developmental clock of neural stem cells.	5．発行年 2020年
3．雑誌名 BioRxiv	6．最初と最後の頁 1-21
掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/2020.10.08.330852	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

1．著者名 5.Shun Mase, Atsunori Shitamukai, Quan Wu, Mitsuru Morimoto, Thomas Gridley, Fumio Matsuzaki	4．巻 0
2．論文標題 Notch1 and Notch2 collaboratively maintain radial glial cells in mouse neurogenesis	5．発行年 2020年
3．雑誌名 Neuroscience Research	6．最初と最後の頁 1-11
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.neures.2020.11.007	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　3件）

1．発表者名 Quan Wu
2．発表標題 Fbl regulates temporal pattering in neural stem cells
3．学会等名 German-Japanese Developmental Neuroscience Meeting（国際学会）
4．発表年 2020年

1．発表者名 Quan Wu and Fumio Matsuzaki
2．発表標題 An rRNA methyltransferase: Fibrillarin is essential for brain development
3．学会等名 22nd Biennial Meeting of the International Society of Developmental Neuroscience（国際学会）
4．発表年 2018年

1．発表者名 Quan Wu, Yuichi Shichino, Shintaro Iwasaki and Fumio Matsuzaki
2．発表標題 A methyltransferase Fibrillarin is essential for fate transition of neural stem cell
3．学会等名 EMBO workshop（国際学会）
4．発表年 2018年

1．発表者名 Quan Wu, Yuichi Shichino, Shintaro Iwasaki and Fumio Matsuzaki
2．発表標題 A methyltransferase Fibrillarin is essential for fate transition of neural stem cell
3．学会等名 RNA frontier meeting
4．発表年 2018年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関