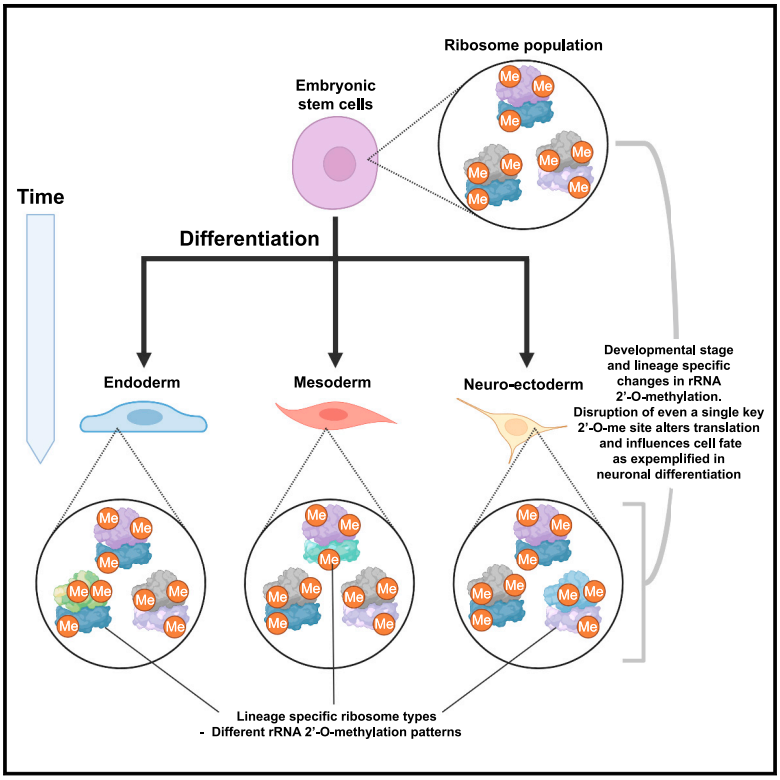
Article

Developmental Cell

Ribosomal RNA 2′-O-methylation dynamics impact cell fate decisions

Graphical abstract



Highlights

- rRNA modifications change during mouse brain development and hESC differentiation
- Changes to the rRNA methylation profile occur during germ layer specification
- Abrogation of methylation at 28S:U3904 impacts the translation of WNT pathway members
- Lack of methylation at 28S:U3904 shifts hESCs toward a neurectoderm fate

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In brief

Ribosomes produce one of the main building blocks of living beings—proteins—and exhibit structural variations, such as RNA methylations. Häfner et al. show that some methylations behave dynamically during cell differentiation and that their manipulation impacts cell fate decision-making.

Häfner et al., 2023, Developmental Cell *58*, 1593–1609
September 11, 2023
https://doi.org/10.1016/j.devcel.2023.06.007

CellPress

Developmental Cell

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Ribosomal RNA 2′-O-methylation dynamics impact cell fate decisions

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https://doi.org/10.1016/j.devcel.2023.06.007

SUMMARY

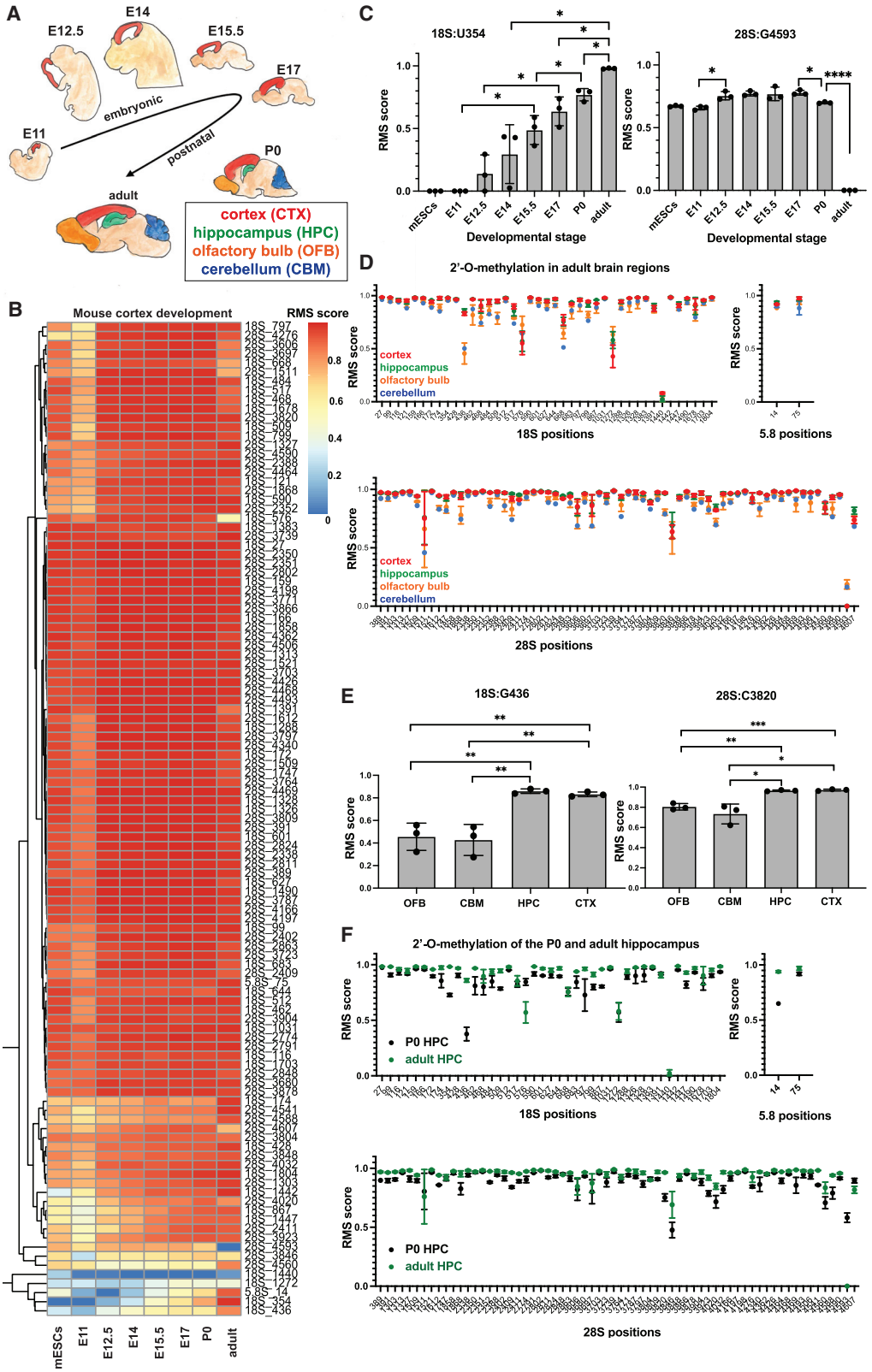
Translational regulation impacts both pluripotency maintenance and cell differentiation. To what degree the ribosome exerts control over this process remains unanswered. Accumulating evidence has demonstrated heterogeneity in ribosome composition in various organisms. 2′-O-methylation (2′-O-me) of rRNA represents an important source of heterogeneity, where site-specific alteration of methylation levels can modulate translation. Here, we examine changes in rRNA 2′-O-me during mouse brain development and tri-lineage differentiation of human embryonic stem cells (hESCs). We find distinct alterations between brain regions, as well as clear dynamics during cortex development and germ layer differentiation. We identify a methylation site impacting neuronal differentiation. Modulation of its methylation levels affects ribosome association of the fragile X mental retardation protein (FMRP) and is accompanied by an altered translation of WNT pathway-related mRNAs. Together, these data identify ribosome heterogeneity through rRNA 2′-O-me during early development and differentiation and suggest a direct role for ribosomes in regulating translation during cell fate acquisition.

INTRODUCTION

Embryonic development requires specific and accurately dosed protein subsets with utmost spatiotemporal precision, often paralleled by profound changes in cell proliferation and overall protein synthesis rates.[1–4] In particular, the formation of the mammalian nervous system entails an exceptionally fine-tuned protein homeostasis to generate and connect hundreds of neural subtypes,[5–8] and any failure of the translation machinery derails normal brain development and function.[7,9–11] Although transcriptional, post-transcriptional, and post-translational regulations have been studied in many developmental model systems,[12–14] the role of translation, and specifically the intrinsic regulatory potential of modifications to the ribosome itself, remains understudied.[5,15]

In eukaryotes, a highly controlled and energy-consuming ribosome biosynthesis pathway ensures the correct assembly of this huge macromolecular complex made of ribosomal RNA (rRNA) and proteins (RPs).[16–18] Beyond the core ribosome, a large number of associated factors have been identified in different organisms.[19] Further complexity arises through post-translational modification of RPs and the large number of different rRNA modifications.[20] The two most abundant rRNA modifications are pseudouridines (Ψ) and 2′-O-methylations (2′-O-me).[20] Both modifications are added to specific rRNA nucleotides by generic enzymes guided by small nucleolar RNAs (snoRNAs) via complementary base-pairing interactions.

Despite the inherent complexity of the ribosome, investigation into the mechanisms by which translation is controlled has mainly focused on mRNA abundance, sequence, and secondary structure, as well as regulation by initiation and elongation factors.[21,22] However, over recent years, evidence has accumulated, suggesting that ribosomes are not generic machines but come with a considerable amount of natural and pathologic variations.[20] As such, several studies have reported variation in the RP composition through the incorporation of RP paralogs or alterations in RP stoichiometry,[23–27] and their post-translational modifications.[19] Likewise, ribosome variation can stem from changes in the rRNA post-transcriptional modification profiles.[28–31] The establishment of ribosome heterogeneity has led to the hypothesis of functional ribosome specialization, where alternating core protein composition as well as protein or rRNA



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modifications could confer additional layers of regulation to the translation process by influencing translation speed and fidelity or promoting the translation of specific mRNA subsets.[20,28,32]

Using RiboMeth-seq (RMS),[33,34] we have previously observed that about a third of the 111 rRNA positions known to carry 2′-O-me in humans are fractionally methylated, i.e., not all ribosomes of a given cell or tissue carry a modification at one of these positions.[35,36] These findings have been corroborated by studies demonstrating variation of the rRNA 2′-O-me-profile during normal development in zebrafish and mice[37,38] and in pathologies such as diffuse large B cell lymphoma[39] and breast cancer.[40] Together, the data suggest the existence of ribosome subtypes characterized by different 2′-O-me modification patterns. Emerging experimental evidence supports the notion of 2′-O-me sites facilitating ribosome specialization. For instance, we have recently shown that expression of the MYC oncogene results in specific alterations of the ribosome 2′-O-me pattern in human cells, particularly at 18S:C174, which in turn impacts the translation of distinct mRNAs depending on their codon composition.[35]

Here, we aim to understand the importance of ribosomal 2′-O-me for cell fate establishment in early embryonic development and during neuronal specification. We show that the rRNA 2′-O-me profile undergoes significant and profound changes during mouse embryonic and postnatal brain development. Tracing development back to germ layer specification, we demonstrate that the directed differentiation of human embryonic stem cells (hESCs) into the three embryonic germ layers triggers significant differentiation type-specific 2′-O-me dynamics. The importance of these dynamics is highlighted by our finding that the removal of a single, dynamic 2′-O-me modification push cell fate toward the neurectoderm. This is mediated at least partially through an altered translation of WNT signaling pathway members and differential association of the translational regulator fragile X mental retardation protein (FMRP) in the vicinity of the modulated 2′-O-me site.

Together, these data indicate that rRNA modification constitutes a previously unrecognized and essential regulatory mechanism in regulating mammalian gene expression and establishing cellular identity.

RESULTS

Temporal and regional rRNA 2′-O-me dynamics during mouse brain development

Previously, we demonstrated the existence of 2′-O-me dynamics in cell culture models.[35] To investigate whether heterogeneity and dynamics of 2′-O-me exist *in vivo* during the transition from multipotent stem cells to differentiation, we focused on a developmental system with a tightly timed sequence of neurogenesis. We performed microdissection of mouse brain neocortex (CTX) during embryonic windows just prior to neurogenesis (E11), throughout neurogenesis (E12.5, E14, E15.5, and E17), and in the postnatal period after neurogenesis is complete (P0 and adult) (Figure 1A). Subsequently, RMS quantification of the 109 known 2′-O-me sites was performed on all samples in biological triplicates.

We detect pronounced changes to rRNA 2′-O-me patterns over the course of cortex development (Figure 1B). In accordance with previous observations,[35,36] sub-stoichiometric methylation is detected at a subset of sites only, and significant changes in the degree of 2′-O-me are seen at 43 sites (Table S1). Among the variable sites, most display an increase in 2′-O-me levels over the course of neocortex development. Some positions transit from undetectable to fully methylated (such as 18S:U354), whereas other sites display a late but substantial drop in methylation levels at the adult stage, for example, at 28S:G4593 (Figure 1C). We observe hypo-methylation at embryonic stage (E11) when the neuroepithelium is yet to commit to a more restricted neural stem cell lineage at E12.5, giving rise to pyramidal neurons throughout subsequent embryonic stages. The 2′-O-me profile of mouse embryonic stem cells (mESCs) cultured *in vitro* more closely resembles the multipotent E11 neuroepithelium (Figure 1B). Interestingly, most changes in the maturing neocortex are sequential and progressive over time, perhaps indicating a role for rRNA methylation dynamics in the stepwise acquisition of mature neuronal fate (Figure 1B).

We next asked whether the neocortex rRNA methylation profile is aligned with other brain regions in the postnatal period. Toward that, we additionally microdissected hippocampus (HPC), cerebellum (CBM), and olfactory bulb (OFB) tissue from the same neonates and adult animals used for the cortex development analysis and performed RMS (Figures 1D and S1A). We identified 9 positions with significant differences between at least two brain regions at the P0 stage and 8 positions in the adult (Table S1). Two examples in the adult, 18S:G436 and 28S:C3820, are shown in Figure 1E. Both positions displayed higher methylation levels in the cortex and HPC compared with the CBM and the OFB. More generally, the 2′-O-me profiles of the cortex and HPC and those of the CBM and OFB, respectively, form two separate clusters, consistent with their divergent neurodevelopmental origins (Figure S1B).

Subsequently, we extended our comparison to the 2′-O-me profiles of the same four brain regions between neonates (P0)

Figure 1. rRNA 2′-O-me dynamics in the developing mouse brain

(A) Murine model system. Cortex from 7 developmental stages. Hippocampus, olfactory bulb, and cerebellum from neonates (P0) and adult.

(B) rRNA 2′-O-me levels in the developing mouse cortex and a mESC line measured by RiboMeth-seq (RMS scores). Columns: developmental stages, rows: all rRNA positions known to be potentially 2′-O-methylated from the 28S, 18S, and 5.8S rRNA. Color scale (blue low, red high) indicates the mean RMS score from three biological replicates.

(C) RMS scores for two examples of rRNA sites displaying 2′-O-me dynamics during mouse cortex development, position 18S:U354 and 28S:G4593. Columns indicate mean RMS score for each condition of n = 3 sequenced libraries from different animals, points denote each value separately. Error bars represent ±SD. *p $\leq$ 0.05, **p $\leq$ 0.01, ***p $\leq$ 0.001, ****p $\leq$ 0.0001 (Welch's unpaired t test).

(D) Comparison of RMS scores between four brain regions from adult mice. Known methylated positions from the 28S, 18S, and 5.8S rRNA are depicted on separate graphs on the x axis. y axis: average RMS score. Points represent mean RMS scores of n = 3 sequenced libraries from different animals. Error bars represent ±SD.

(E) As (C), for RMS scores at 18S:G436 in different brain regions (OFB, olfactory bulb; CBM, cerebellum; HPC, hippocampus; CTX, cortex) at the adult stage.

(F) As (D), for hippocampus of neonates (P0) (black) and adult mice (green).