**DNA methylome analysis identifies transcription factor-based epigenomic signatures of multi-lineage competence in neural stem/progenitor cells**

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Epigenetic regulation during *in vivo* specification of brain stem cells is still poorly understood. Here we report DNA methylome analyses of directly sampled cortical neural stem and progenitor cells (NS/PCs) at different development stages, as well as those of terminally differentiated cortical neurons, astrocytes and oligodendrocytes. We found that sequential specification of cortical NS/PCs is regulated by two successive waves of demethylation at early and late development stages, which are responsible for the establishment of neuron- and glia-specific low methylated regions (LMRs), respectively. The regulatory role of demethylation of the gliogenic genes was substantiated by enrichment of Nuclear factor I (NFI)-binding sites. We provide evidence that *de novo* DNA methylation of neuron-specific LMRs establishes glia-specific epigenotypes, essentially by silencing neuronal genes. Our data highlight the *in vivo* implications of DNA methylation dynamics in shaping epigenomic features that confer the differentiation potential of NS/PCs sequentially during development