Transcription Factor Positive Regulatory Domain 4 (PRDM4) recruits Protein Arginine Methyltransferase 5 (PRMT5) to mediate histone arginine methylation and control neural stem cell proliferation and differentiation.

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***Running Title:** PRDM4 controls NSC differentiation

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Background: Neural stem cells generate all the cell types of the central nervous system.
Results: Transcription factor, PRDM4, recruits protein arginine methyltransferase 5 (PRMT5) to control the timing of neurogenesis.
Conclusions: PRDM4 and PRMT5-mediated histone arginine methylation controls neural stem cell proliferation and differentiation.
Significance: Histone arginine methylation is a novel epigenetic mechanism which regulates neural stem cell reprogramming.

SUMMARY

During development of the cerebral cortex, neural stem cells (NSCs) undergo a temporal switch from proliferative (symmetric) to neuron-generating (asymmetric) divisions. We investigated the role of Schwann cell factor 1 (SC1/PRDM4), a member of the PRDM family of transcription factors, in this critical transition. We discovered that SC1 recruits the chromatin modifier PRMT5, an arginine methyltransferase that catalyzes symmetric dimethylation of histone H4 arginine 3 (H4R3me2s), and that this modification is preferentially associated with undifferentiated cortical NSCs. Over-expressing SC1 in embryonic NSCs led to an increase in the number of Nestin-expressing precursors; mutational analysis of SC1 showed that this was dependent on recruitment of PRMT5. We found that SC1 protein levels are down-regulated at the onset of neurogenesis and that experimental knock-down of SC1 in primary NSCs triggers precocious neuronal differentiation. We propose that SC1 and PRMT5 are components of an epigenetic regulatory complex that maintains the - cellular state of the NSC by preserving their proliferative capacity and modulating their cell cycle progression. Our findings provide evidence that histone arginine methylation regulates NSC differentiation.

INTRODUCTION

During central nervous system (CNS) development, embryonic neural stem cells (NSCs) in the ventricular zone (VZ) of the brain and spinal cord first proliferate symmetrically to increase NSC

boiled at 95°C for 5 min in Laemmli buffer (60 mM Tis-HCl, pH6.8, 10% [v/v] glycerol, 2% [w/v] sodium dodecyl sulphate, 5% [v/v] β3

subsequently mounted in OCT (Tissue-Tek) on dry ice. Mounted embryos were sectioned at 10 µm using a Leica cryostat and air-dried for at least 1 hour. Sections were permeabilized for 3 times in PBS, incubated in sodium citrate buffer (10mM sodium citrate, 0.05% Tween-20, pH 6.0) at 95°C for 30 minutes for antigen retrieval, allowed to cool to room temperature, rinsed three times in PBS, then incubated for 1 hour at 20-25°C in blocking solution, following incubation with the primary antibody in blocking solution overnight at 4°C. Sections were washed three times in PBS at 20-25°C and incubated with fluorescent secondary antibodies and Hoechst dye (to visualize cell nuclei) for 1 hour at 20-25°C, rinsed three times in PBS, once in water and mounted using DAKO mounting medium. Fluorescence images were

(Fig. 1C), but not in GFAP-expressing differentiated astrocytes (A.C., unpublished). Thus, high levels of SC1 appeared to be expressed preferentially in mitotically active cells (NSCs and OLPs) and down-regulated in differentiated neurons and astroglia. This suggested that down-regulation of SC1 might be involved in, and possibly required for, the smrequS

precursor cell differentiation and changes in the cell cycle parameters are likely to regulate various aspects of the responsiveness of these cells to extracellular signals. Moreover, the observation that both SC1 and PRMT5 are

where PRMT5 has been shown to be necessary for OLP differentiation (this paper and (17) further underscores the principle of utilizing the same transcription factors in a graded manned throughout development to direct different developmental outcomes.

We observe that SC1 and PRMT5 complex is involved in the down-regulation of *cycB* and *Bub1b* genes. The observation is of interest as previous investigation of the mechanisms responsible for asymmetric partitioning of cell fate determinants during neuroblast divisions in *D. melanogaster* has identified the cdc2/cycB complex as instrumental in regulating this

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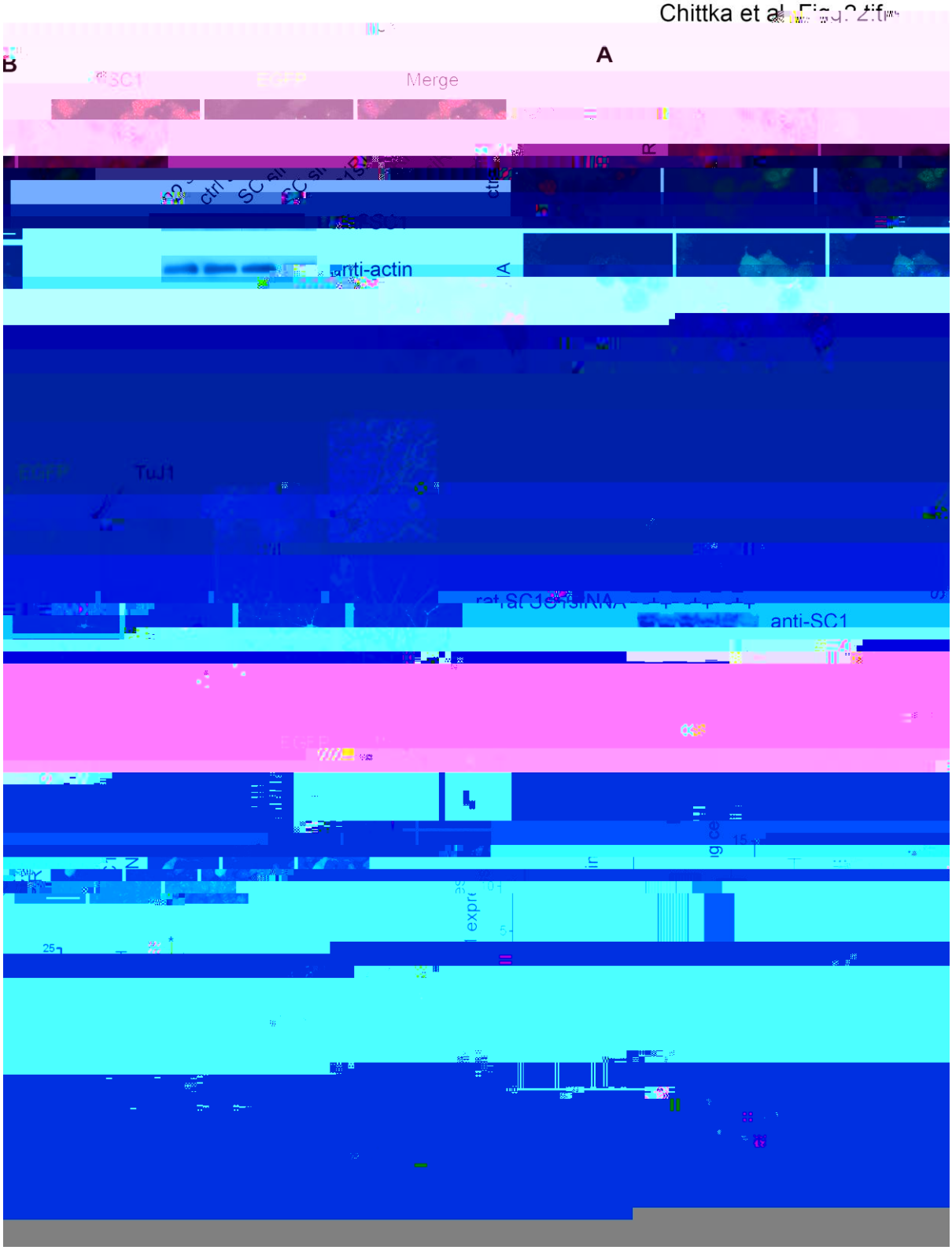
the same blot probed with anti-pan Lysine antibody and (Panel 4) the same membrane stained with Coomassie brilliant blue (cbb).

FIGURE 4. SC1 recruits PRMT5 to direct H4 methylation. (A) mycSC1 or empty vector was expressed in HEK293T cells as indicated on the panel. Anti-Myc IPs from transfected cells were analyzed using anti-PRMT5 antibody. Endogenous PRMT5 is found in the complex (*arrow*, top) with IPed mycSC1. Total protein inputs and Iped mycSC1 are shown (two middle and bottom panels, respectively). (B) Endogenous PRMT5 was IPed from HEK293T cells transfected with mycSC1 or empty vector and the IPs were analyzed by Western blot with anti-Myc (*arrow*, MycSC1 protein); two middle and bottom panels show the input mycSC1 and PRMT5 and IPed PRMT5, respectively. **(**C) Diagram of the deletion constructs of Myc-tagged SC1 used to map the domains of interaction with PRMT5. (D) The NH-terminus and to a lesser extent PR/SET domain of SC1 bind PRMT5. Indicated Myc-tagged SC1 full length or deleted constructs and HA/Flag-tagged PRMT5 were co-expressed in HEK293T cells. Anti-HA IPs (PRMT5) were probed on Western blots with anti-Myc antibody to detect co-IPed myc-tagged SC1 constructs. Inputs were analyzed using anti-Flag antibody for PRMT5 and anti-Myc antibody for SC1. Anti-tubulin antibody was used as a loading control. mycSC1 proteins that co-IP with PRMT5 are highlighted by asterisks on the top panel of the Western blot. (E) mycSC1FL, mycSC1dNH or empty vector were transfected into HEK293T cells. Anti-Myc IPs were used for in vitro HMTase assays. (Above) fluorogram (F) of histones methylated by the indicated IPed complexes, (below) Western blot of IPed proteins used for the in vitro methylation reactions, probed with anti-Myc.

FIGURE 5. PRMT5, SC1 and H4R3me2s are expressed in developing NSCs and (

protein loading. Normalised protein levels of SC1 are shown in the graph. (D) Mouse cortex from E13.5 embryos was immunolabelled for TuJ1 and PRMT5. Moderate levels of PRMT5 protein were detected in the cortex at E13.5. TuJ1 staining is towards the pial surface in all panels. (E) Western blot analysis of cycB1 protein expression in the developing cortex. Protein homogenates from embryonic cortices of indicated ages were analysed by probing with anti-cycB1 antibodies (top panel) and anti-actin (bottom panel) antibodies to control for protein loading. Data (in C) are shown as mean ± s.d from three independent western blot quantifications [n=3, $p<0.05$(*)]. Scale bar, 75μm (top panels), 25 μm (bottom panels).



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