Developmental Biology 385 (2014) 230–241

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

CBP regulates the differentiation of interneurons from ventral forebrain neural precursors during murine development

David Tsui [a,c], Anastassia Voronova [a,b], Denis Gallagher [a,b], David R. Kaplan [b,c,d], Freda D. Miller [a,c,d,e,*], Jing Wang [a,b,**]

[a] Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, Canada M5G 1L7
[b] Cell Biology, Hospital for Sick Children, Toronto, Canada M5G 1L7
[c] Institute for Medical Sciences, University of Toronto, Toronto, Canada M5G 1X5
[d] Department of Molecular Genetics, University of Toronto, Toronto, Canada M5G 1X5
[e] Department of Physiology, University of Toronto, Toronto, Canada M5G 1X5

ARTICLE INFO

Article history:
Received 18 July 2013
Received in revised form
31 October 2013
Accepted 7 November 2013
Available online 15 November 2013

Keywords:
Neural stem cell
CBP
Interneuron
Ganglionic eminence
Neurogenesis
Cortical development
Rubinstein–Taybi syndrome
Epilepsy

ABSTRACT

The mechanisms that regulate appropriate genesis and differentiation of interneurons in the developing mammalian brain are of significant interest not only because interneurons play key roles in the establishment of neural circuitry, but also because when they are deficient, this can cause epilepsy. In this regard, one genetic syndrome that is associated with deficits in neural development and epilepsy is Rubinstein–Taybi Syndrome (RTS), where the transcriptional activator and histone acetyltransferase CBP is mutated and haploinsufficient. Here, we have asked whether CBP is necessary for the appropriate genesis and differentiation of interneurons in the murine forebrain, since this could provide an explanation for the epilepsy that is associated with RTS. We show that CBP is expressed in neural precursors within the embryonic medial ganglionic eminence (MGE), an area that generates the vast majority of interneurons for the cortex. Using primary cultures of MGE precursors, we show that knockdown of CBP causes deficits in differentiation of these precursors into interneurons and oligodendrocytes, and that overexpression of CBP is by itself sufficient to enhance interneuron genesis. Moreover, we show that levels of the neurotransmitter synthesis enzyme GAD67, which is expressed in inhibitory interneurons, are decreased in the dorsal and ventral forebrain of neonatal $CBP^{+/-}$ mice, indicating that CBP plays a role in regulating interneuron development *in vivo*. Thus, CBP normally acts to ensure the differentiation of appropriate numbers of forebrain interneurons, and when its levels are decreased, this causes deficits in interneuron development, providing a potential explanation for the epilepsy seen in individuals with RTS.

Introduction

The mammalian cerebral cortex consists of two major classes of neurons: glutamatergic, excitatory projection neurons, and GABAergic, inhibitory interneurons. While projection neurons are generated by radial precursors within the developing cortex (Malatesta et al., 2000; Noctor et al., 2001), interneurons are generated in the medial ganglionic eminence (MGE) before migrating along the superficial and deep tangential migratory streams to enter the cortex (Lavdas et al., 1999; Xu et al., 2008). A clonal analysis study recently showed that the process by which interneurons are generated is very similar to the radial units in the cerebral cortex, with a radial glial precursor cell giving rise to several subtypes of interneurons which then use these radial glial precursors as scaffolds for migration (Brown et al., 2011). In addition to interneurons, MGE precursors also give rise to oligodendrocytes, making them bipotent *in vivo* (He et al., 2001; Yung et al., 2002; Petryniak et al., 2007).

Rubinstein–Taybi Syndrome (RTS) is a genetic neurodevelopmental disorder that is caused, in most cases, by mutation of a single allele of the gene encoding CREB-binding protein (CBP) (Petrij et al., 1995). One of the major phenotypes observed in individuals with RTS is moderate to severe intellectual disability (Wiley et al., 2003), which often occurs at birth. CBP is a transcriptional co-activator that binds to many different transcription factors, and is thought to activate gene transcription by acting as a physical bridge to the transcriptional apparatus, and via its histone acetyltransferase activity (Bannister and Kouzarides, 1996). In this regard, we previously asked how CBP haploinsufficiency in RTS might cause early intellectual disability, focusing upon the embryonic cerebral cortex (Wang et al., 2010). In that study, we showed that CBP promotes differentiation of embryonic cortical

* Corresponding author at: Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, Canada M5G 1L7.
** Corresponding author. Current address: Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, Canada K1H 8L6.
E-mail addresses: fredam@sickkids.ca (F.D. Miller), jiwang@toh.on.ca (J. Wang).

0012-1606/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ydbio.2013.11.005

precursors by modifying chromatin at the promoters of neural genes and thereby enhancing transcription of those genes (Wang et al., 2010). As a consequence, when CBP was haploinsufficient in a mouse model of RTS, this caused decreased genesis of both glia and cortical excitatory projection neurons, and behavioral perturbations during early postnatal life (Wang et al., 2010).

Intriguingly, in addition to intellectual disability, a meta-analysis of 732 cases of RTS found that approximately 58% of these individuals displayed EEG anomalies and 27% exhibited epilepsy (Cantani and Gagliesi, 1998). A similar seizure phenotype was seen in a small proportion of mice carrying a human CBP mutation that results in expression of a truncated protein (Oike et al., 1999). Since seizures in both mice (Kash et al., 1997; Cobos et al., 2005) and humans (Kitamura et al., 2002) are frequently due to dysfunction in the generation of GABAergic interneurons and/or the inhibitory neurotransmitter GABA, then these findings suggest that CBP might be important for the genesis of interneurons. Here we have tested this idea, focusing on the medial ganglionic eminence, which generates the large majority of interneurons for the cerebral cortex. We provide evidence that CBP is required for the appropriate differentiation of MGE derived interneurons and that in mice that are haploinsufficient for CBP, there are deficits in forebrain interneurons in early postnatal life, providing one explanation for the epilepsy phenotype that is associated with RTS.

Materials and experimental procedures

Animals

All animal use was approved by the Animal Care Committee of the Hospital for Sick Children in accordance with the Canadian Council of Animal Care policies. CD1 mice were obtained from Charles River Laboratory and were used for all experiments except the Western blot analyses. B6.126S6-Crebbp^{tm1Dli} mice were used for the Western blot analyses, and were obtained from The Jackson Laboratory (Kung et al., 2000) and maintained in the same genetic background, as previously described (Wang et al., 2010, 2012).

Primers and plasmids

Two primer sets were used for the RT-PCR analysis of CBP mRNA. The first set was forward-1 (5′-CTGAGCCTGAACCTACTGAATC-3′), reverse-1 (5′-AGGAGATGTTGATTGTGAGGC-3′), and the second was forward-2 (5′-AGCAAATGGAGAGGTTCGAG-3′), reverse-2 (5′-CTTAAGGAAGTGGCATTCTGTTG-3′). The nuclear EGFP expression plasmid was driven from the *Ef1*α (Mouse Genome Informatics) promoter (pEF-EGFP) and has been previously described (Barnabé-Heider et al., 2005). The CBP expression plasmid has been previously described (Wang et al., 2010). For CBP knockdown, the ON-TARGETplus SMARTpool Crebbp siRNAs (proprietary mixture, Dharmacon) and the ON-TARGETplus Non-targeting pool siRNA (Dharmacon) were previously characterized (Wang et al., 2010).

Medial ganglionic eminence precursor cell cultures

Neural precursor cells from the medial ganglionic eminence (MGE) were cultured as we have previously done for cortical precursor cell cultures (Barnabé-Heider et al., 2005; Tsui et al., 2013). Briefly, the MGE was dissected from embryonic day 12 CD1 mouse embryos in ice-cold HBSS (Invitrogen) and transferred to Neurobasal medium (Invitrogen) containing 500 μM L-glutamine (Cambrex Biosciences), 2% B27 supplement (Invitrogen), 1% penicillin–streptomycin (Invitrogen), and 40 ng/ml FGF2 (BD Biosciences). The tissue was mechanically triturated with a plastic pipette and plated onto four-well chamber slides (Nunc) precoated with 2% laminin (BD Biosciences) and 1% poly-D-lysine (Sigma). Cells were plated at a density of 150,000 cells per well. For CBP knockdown experiments, 1–2 h after plating, 1 μg of pEF-EGFP plasmid and 80 pmol siRNA were mixed with 1 μl of Plus™ (Invitrogen) in 100 μl of Opti-MEM medium (Invitrogen) and incubated for 5 min at room temperature, followed by addition of 1.5 μl of lipofectamine™ LTX (Invitrogen) into the mixture for another 30 min incubation at room temperature. Then, the mixtures were added to the cultures. For CBP overexpression studies, 1 μg total DNA (comprised of 0.3 μg of pEF-EGFP plasmid plus 0.7 μg of wild type-CBP expression plasmid or 0.7 μg of empty vector) and 2 μl of lipofectamine 2000 (Invitrogen) were mixed with 100 μl of Opti-MEM (Invitrogen), incubated at room temperature for 30 min and added to the cultures. These transfection methods resulted in a transfection of, at most, 1–3% of cells. To ensure that these cotransfections were efficacious, we cotransfected EGFP and RFP expression plasmids into MGE precursors under similar conditions, and then analyzed these cells immunocytochemically. This analysis showed that all RFP-positive cells also coexpressed EGFP. For trichostatin A (TSA) experiments, MGE precursors were treated with 20 nM TSA (Calbiochem) dissolved in 0.1% DMSO 12 h after transfection.

Immunocytochemistry and histological analysis

Immunocytochemistry was performed as previously described (Barnabé-Heider et al., 2005; Wang et al., 2010; Tsui et al., 2013). Briefly, cultured cells were fixed in 4% paraformaldehyde for 10 min, and then permeabilized with 0.2% NP40 in PBS. Cells were then blocked with 0.5% BSA and 6% normal goat serum diluted in PBS. Primary antibodies were diluted in 0.25% BSA and 3% normal goat serum in PBS, and incubated overnight. Secondary antibodies were diluted in the same blocking solution as primary antibodies and incubated for 1 h. Hoechst 33258 was diluted in PBS. For embryonic brain sections, coronal cryosections were fixed in 4% paraformaldehyde for 10 min, and then permeabilized and blocked with 5% BSA and 0.3% Triton-X in phosphate-buffered saline (PBS) for 1 h. Primary antibodies were diluted in the same permeabilization and blocking buffer and incubated at 4 °C overnight. Secondary antibodies and Hoechst 33258 were diluted in PBS. Sections were washed three times for 5 min with PBS between each step. The primary antibodies used were mouse anti-GFP (1:1000; Invitrogen), rabbit anti-GFP (1:5000; Abcam), chicken anti-GFP (1:2000; Abcam), mouse anti-βIII-tubulin (1:1000; Covance), rabbit anti-βIII-tubulin (1:1000; Covance), mouse anti-Sox2 (1:400; Millipore), rabbit anti-Olig2 (1:5000; Millipore), mouse anti-GAD67 (1:400; Millipore), rabbit anti-GFAP (1:2000; Abcam), mouse anti-O4 (1:400; Millipore), rat anti-MBP (1:400; Serotec). rabbit anti-CBP (1:1000; Santa-Cruz), mouse anti-nestin (1:400; Millipore), mouse anti-Ki67 (1:400; BD), rabbit anti-Sox6 (1:500; Abcam), rabbit anti-Nkx2.1 (1:100; Abcam), and rat anti-SST (1:1000; Chemicon), The secondary antibodies used were Alexa Fluor 555-, Alexa Fluor 488- and Alexa Fluor 647-conjugated goat antibodies to mouse, rabbit or rat IgG or mouse IgM (1:1000; Invitrogen). Nuclear staining was performed with Hoechst 33258 (Sigma).

Western blot analysis

Western blots were performed as described previously (Barnabé-Heider et al., 2005; Wang et al., 2010). Briefly, forebrain tissue was harvested and incubated for 30 min at 4 °C in RIPA lysis buffer supplemented with 1 mM of PMSF, 1 mM Na_3VO_4, 10 μg/ml aprotenin and 10 μg/ml leupeptin, 20 mM sodium fluoride. Lysates

were then centrifuged at 13000 RPM for 10 min and the supernatants were collected. Protein concentration was measured with the BCA Protein Assay Kit (Thermo Scientific). Lysates containing 50–100 μg were loaded onto each lane of a 10% SDS gel. Protein was then transferred to nitrocellulose membranes (Bio-Rad) at 250 mA at 4 °C overnight (Bio-Rad). Membranes were blocked with 5% skim milk in TBST for 1 h, and incubated with primary antibodies diluted in 5% skim milk in TBST at 4 °C overnight. Secondary antibodies were also diluted in 5% skim milk in TBST and incubated for 1 h at room temperature. Three 5 min washes were applied between every step. To develop the blots, membranes were incubated in either ECL for 1 min or ECL-prime (GE Healthcare) for 5 min and then exposed for varying periods of time. The primary antibodies used were mouse anti-GAD67 (1:2000; Millipore), and rabbit anti-ERK1 K23 (1:5000; Santa Cruz Biotechnology). Secondary antibodies were HRP-conjugated goat anti-mouse or anti-rabbit IgG (1:5000; Boehringer Mannheim). To quantify the Western blots, films were scanned with the Canoscan 4200F scanner and densitometry analysis was performed using the ImageJ program. We measured the relative GAD67 level to total ERK1 protein level in each sample and normalized these numbers to one wildtype sample as 100%.

RT-PCR

The MGE was dissected from E12 embryos and RNA was isolated using Trizol (Invitrogen) according to the manufacturer's protocol. RNA was treated with DNase (Fermentas) to remove any contaminating genomic DNA. Reverse transcription was performed using RevertAid H Minus M-MuLV Reverse Transcriptase (Fermentas) primed with random hexamers, according to the manufacturer's instructions. All reactions were subjected to the following PCR protocol: 94 °C for 2 min, 35 cycles of 94 °C for 15 s, 58 °C for 30 s and 72 °C for 30 s, and a final elongation at 72 °C for 2 min. Products were then resolved on a 2% agarose gel.

Microscopy and quantification

For quantification, over 200 cells per condition per experiment were counted and analyzed using a Zeiss Axioplan2 upright microscope equipped with fluorescence optics. Digital image acquisition was performed with Northern Eclipse software (Empix, Mississauga, Ontario, Canada) using a Sony (Tokyo, Japan) XC-75CE CCD video camera.

Statistics

All data were expressed as the mean plus or minus the standard error of the mean (SEM), and were tested for statistical significance with two-tailed Student's *t*-tests unless otherwise indicated. Differences were considered significant if $p < 0.05$.

Results

CBP is expressed in embryonic precursors of the ganglionic eminence

To ask if CBP is expressed in embryonic precursors of the medial ganglionic eminence (MGE), we performed RT-PCR with RNA isolated from the murine MGE at embryonic day 12 (E12) using two different sets of primers. This analysis demonstrated that CBP mRNA is expressed at the MGE in this early timepoint (Fig. 1A). To ask whether CBP protein is also expressed, we immunostained sections through the E12 ventral forebrain with an antibody for nestin, a general marker for neural precursors, together with a CBP antibody that we have previously characterized and used to identify expression of CBP in precursors of the dorsal forebrain (Wang et al., 2010). CBP immunoreactivity was detected in nuclei of cells throughout the embryonic forebrain, including nestin-positive MGE precursors lining the lateral ventricle (Fig. 1B).

To ask about the function of CBP in ventral forebrain precursors, we developed a culture system for MGE precursors. Specifically, we dissected the murine MGE at embryonic day 12 (E12), dissociated the cells mechanically and plated them in neurobasal medium supplemented with B27, FGF2 and L-glutamine, conditions we use for culturing embryonic cortical precursors (Wang et al., 2010, 2012). Immunostaining and quantification demonstrated that, 1 day after plating, approximately 70% of the cells in these cultures expressed the precursor marker Sox2 and the proliferation marker Ki67 (Fig. 1C and D). Moreover, immunostaining for CBP demonstrated that all of these proliferating precursors were CBP-positive (Fig. 1C), as predicted by the *in vivo* data. The other 30% of cells in these cultures were neurons, as demonstrated by immunostaining for the newborn neuron marker βIII-tubulin (Fig. 1D and E). These neurons were also CBP-positive (data not shown).

To characterize differentiation in these cultures, we maintained the cells for 3 or 7 days, and immunostained them for Sox2 to identify precursors, βIII-tubulin for neurons, O4 as a marker for oligodendrocyte precursors, or GFAP as a marker for astrocytes (Fig. 1E). Quantification at 3 days in culture showed that approximately equal numbers of neurons and precursors were present, with no glial cells (Fig. 1D). By 7 days, all four cell types were present; precursors had decreased to approximately 33%, while differentiated cell types comprised the remainder of the culture, with neurons at approximately 56%, oligodendrocyte precursors at approximately 10%, and a very small number (around 2%) of GFAP-positive astrocytes (Fig. 1D). These results are consistent with previously-published work showing that MGE precursors start to differentiate into neurons at around E11 (Marin et al., 2000), and that they generate predominantly neurons and oligodendrocytes (He et al., 2001; Yung et al., 2002; Petryniak et al., 2007).

We then asked whether the neurons that were produced in these cultures were interneurons, as would be predicted by the known differentiation potential of these cells *in vivo*. Initially, we immunostained the cultures at 3 or 7 days for CBP and for GAD67, which is positive in all interneurons, or somatostatin, which is expressed in many interneurons generated by MGE precursors. This analysis showed that at 3 days, many cells with the morphology of neurons were positive for both GAD67 and CBP (Fig. 1F). By 7 days we detected somatostatin-positive cells that also coexpressed CBP (Fig. 1F). These somatostatin-positive cells also expressed GAD67 (Fig. 1F), consistent with an interneuron phenotype. To confirm that these were indeed interneurons, we double-labelled cultures for GAD67 and Sox6, a marker for GE-derived interneurons and their parent progenitors (Azim et al., 2009; Batista-Brito et al., 2009). This analysis (Fig. 1G) showed that all GAD67-positive neurons also expressed Sox6. In addition, some GAD67-negative cells also expressed Sox6, presumably interneuron progenitors and/or newborn interneurons that did not yet express GAD67.

To determine the time-course of acquisition of the interneuron phenotype, we cultured MGE cells for 1, 3 or 7 days, and double-labelled them for GAD67 and βIII-tubulin. At 1 day, 30% of the cells were βIII-tubulin-positive, but only a very small fraction of these, approximately 5%, expressed GAD67 (Fig. 1H). By 3 days, approximately half of the βIII-tubulin-positive neurons (which at this point comprise about 50% of the cells in the cultures), were GAD67-positive (Fig. 1H). By 7 days, the proportion of newborn neurons expressing GAD67 had increased to almost 70% of the total βIII-tubulin-positive population (Fig. 1H). Thus, acquisition of

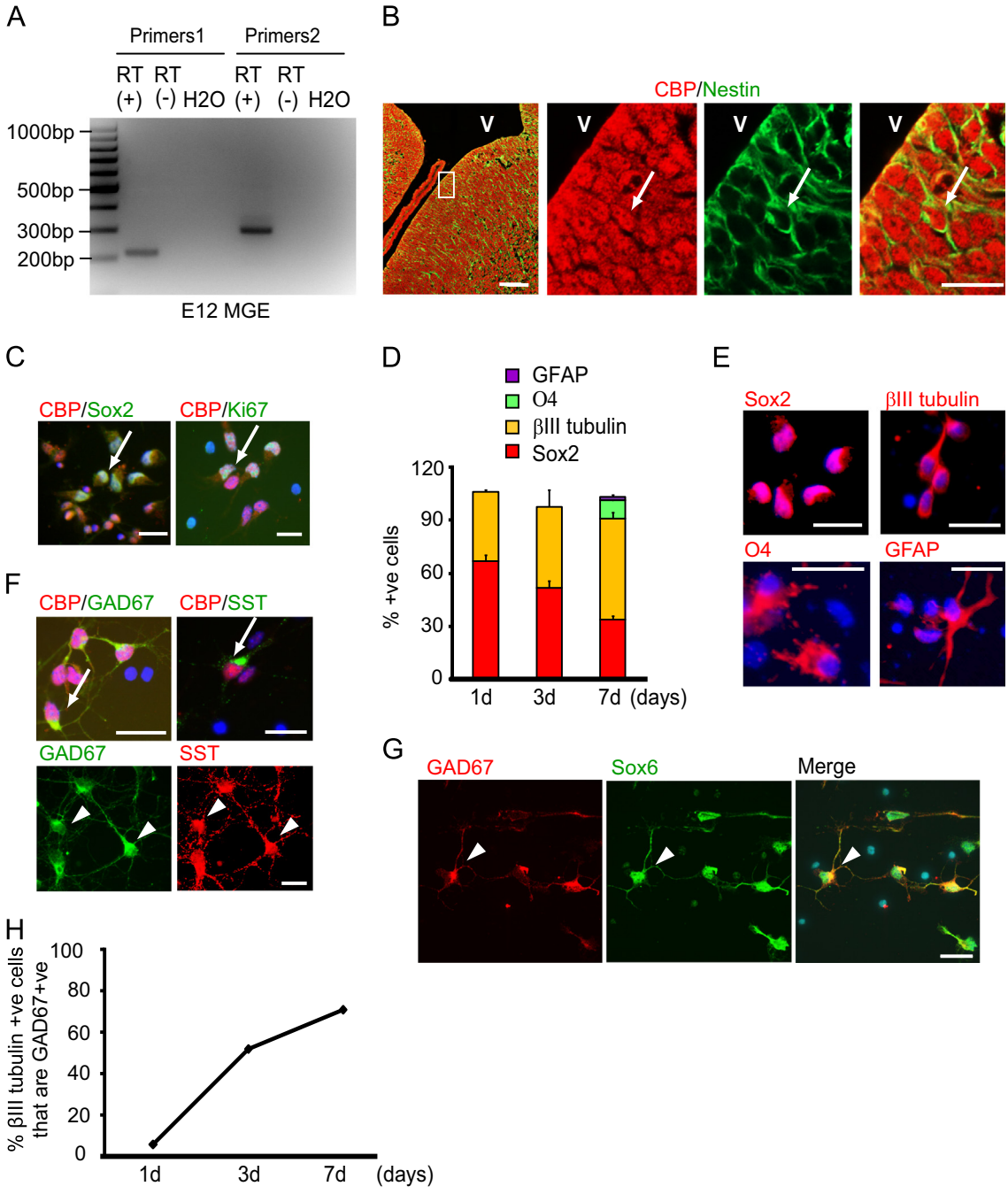

Fig. 1. Expression of CBP in MGE precursors that generate interneurons and oligodendrocytes in culture. (A) RT-PCR analysis of CBP mRNA in total RNA isolated from the E12 murine MGE, amplified using two different primer sets. RT(−) refers to the same samples without the addition of reverse transcriptase. H_2O refers to the water control. Molecular weight markers are shown to the left of the panel. (B) Fluorescence micrographs of a coronal section through the E12 murine ventral forebrain, immunostained for CBP (red) and nestin (green). The left panel shows a lower magnification micrograph, while the right three panels show higher magnification images focussed on the MGE of the same section. Arrows denote a double-labelled cell, and V indicates the ventricle. Scale bars = 100 μm (left-most panel) and 20 μm (right three panels). (C) Fluorescence photomicrographs of E12 MGE precursors cultured for 3 days and immunostained for CBP (red) and either Sox2 (green, left panel) or Ki67 (green, right panel). Cells were also counterstained with Hoechst 33258 (blue). Arrows denote double-labelled cells. Scale bar = 20 μm. (D) E12 MGE precursors were transfected with an EGFP plasmid and immunostained for EGFP, to identify transfected cells, and the precursor marker Sox2, newborn neuron marker βIII-tubulin, oligodendrocyte precursor marker O4, or astrocyte marker GFAP at different timepoints indicated on the *x* axis. The bars represent the quantification of the percentage of the four cell types over total transfected cells. Graph shows combined data from 3 independent experiments. Note the percentage of Sox2-positive precursors decreased over time while the percentage of βIII tubulin-positive neurons increased over time. O4-positive oligodendrocytes and GFAP-positive astrocytes only appeared at 7 days. ($n = 3$ independent experiments). (E) Fluorescence photomicrographs of E12 MGE precursors cultured for 3 days and immunostained for Sox2 or βIII-tubulin, or cultured for 7 days and immunostained for O4 or GFAP (all red), and counterstained with Hoechst 33258 (blue). Scale bar = 20 μm. (F) Fluorescence photomicrographs of E12 MGE precursors cultured for 7 days, and immunostained for CBP (red, top two panels) and GAD67 (green, top left) or somatostatin (green; top right, SST), or for GAD67 (green, bottom left) and somatostatin (red, bottom right). The two bottom panels show the same field. Arrows denote double-labelled cells, and arrowheads indicate cells positive for both GAD67 and SST. Cells in the top panels were also counterstained with Hoechst 33258 (blue). Scale bar = 20 μm. (G) Fluorescence photomicrographs of E12 MGE precursors cultured for 3 days and immunostained for GAD67 (red, left panel) and Sox6 (green, center panel). The merge is shown in the right panel (counterstained with Hoechst 33258 in blue). Arrowheads denote a double-labelled neuron. Scale bar = 90 μm. (H) E12 MGE precursors were plated and immunostained for βIII-tubulin and GAD67 at timepoints indicated on the *x* axis, and the proportion of βIII-tubulin-positive cells that also expressed GAD67 was plotted over time.

an inhibitory neurotransmitter phenotype lags several days behind the first neuron-specific gene expression in these cultures.

CBP knockdown increases the proportion of MGE precursor cells

To ask whether CBP plays an essential role in MGE precursor cell development, we used previously-characterized CBP siRNAs (Wang et al., 2010, 2012) to knock it down in culture. Initially, we assessed the efficacy of siRNA-mediated CBP knockdown; cultured E12 MGE precursors were cotransfected with a plasmid encoding nuclear-targeted EGFP together with either scrambled or CBP siRNAs. Three days later, cells were immunostained for EGFP and CBP (Fig. 2A) and the level of CBP immunofluorescence in EGFP-positive cells was categorized as high or low/undetectable. Quantification showed that while approximately 70% of scrambled siRNA-transfected cells expressed high levels of CBP, only 30% of CBP siRNA-transfected cells expressed similarly high levels (Fig. 2A and B).

Having established the efficacy of the CBP siRNA in MGE precursors, we asked whether CBP knockdown affected their survival or proliferation. We cultured E12 MGE precursor cells and transfected them with a plasmid encoding EGFP together with either scrambled or CBP siRNA. Two days later, we assessed cell death by quantifying the proportion of EGFP-positive cells with condensed, apoptotic nuclei as visualized by Hoechst 33258 staining (Fig. 2C). Quantification showed that approximately 10% of cells were apoptotic, and that this was similar with scrambled *versus* CBP siRNAs (Fig. 2D). We then assessed proliferation at 2 days by quantifying the percentage of EGFP-positive cells that were positive for Ki67 (Fig. 2E). Quantification showed that CBP knockdown had no effect on the proportion of proliferating cells (Fig. 2F) at 2 days. Thus, CBP knockdown does not affect MGE precursor survival or proliferation at 2 days in culture.

We next asked whether CBP knockdown had any effect on precursor number. To do this, we examined two different precursor markers, the pan-precursor marker Sox2 and Olig2, which labels multipotent neural precursors in the ventral forebrain (Miyoshi et al., 2007; Petryniak et al., 2007). Double-labeling for these two precursor markers demonstrated that virtually all Sox2-positive cells in these cultures were also positive for Olig2 at 3 days (Fig. 2G). We therefore cotransfected MGE cultures with an EGFP plasmid plus CBP or scrambled siRNAs in the first few hours following plating, and determined the proportion of Sox2 or Olig2-positive transfected cells 3 or 7 days later (Fig. 2H). Quantification showed that CBP knockdown significantly increased the proportion of EGFP-positive cells expressing Sox2 or Olig2 at both 3 and 7 days (Fig. 2J and K). We also asked about interneuron precursors, analyzing similar experiments for the transcription factor Nkx2.1, which is downregulated in cortical interneurons shortly after cell cycle exit (Marin et al., 2000). Quantification at 3 days showed that CBP knockdown significantly increased the proportion of EGFP-positive cells expressing Nkx2.1 and that the magnitude of this effect was similar to that seen with the other precursor markers (Fig. 2I and L).

CBP regulates the genesis of oligodendrocytes from MGE precursor cells

One explanation for this increase in the relative proportion of precursors seen following CBP knockdown is that CBP is important for precursor differentiation. In support of this idea, we previously showed that CBP activation enhances the differentiation of neurons and glial cells from dorsal cortical precursors (Wang et al., 2010, 2012). We therefore asked whether CBP knockdown decreases the genesis of oligodendrocytes from MGE precursors. Specifically, we transfected MGE precursors in the first few hours following plating with either CBP or scrambled siRNAs together with an EGFP plasmid and then immunostained these cultures after 7 days for O4, a marker for oligodendrocyte precursors, or myelin basic protein (MBP), a marker for differentiated oligodendrocytes (Fig. 3A). Quantification demonstrated that CBP knockdown significantly reduced differentiation into the oligodendrocyte lineage, as monitored by either marker (Fig. 3B and C). We also asked about astrocytes in these cultures by performing similar experiments and immunostaining for GFAP (Fig. 3D). Quantification showed that only a very small number of astrocytes were generated in these cultures, even after 7 days, and that this was not statistically altered by CBP knockdown (Fig. 3E).

CBP regulates MGE interneuron genesis and differentiation

These data indicate that CBP is important for MGE precursors to differentiate into oligodendrocytes. We therefore asked whether CBP also regulates the genesis and/or maturation of interneurons, as might be predicted by the high incidence of epilepsy in individuals carrying a CBP mutation (Cantani and Gagliesi, 1998). To do this, we knocked down CBP in MGE precursor cells by cotransfecting them with either scrambled or CBP siRNAs together with an EGFP plasmid. We then immunostained these cultures for EGFP, βIII-tubulin and GAD67 (Fig. 4A). Quantification showed that after 3 days, the percentage of EGFP-positive, βIII-tubulin-positive newborn neurons was significantly decreased in CBP siRNA treated cultures (Fig. 4B). CBP knockdown also reduced the proportion of EGFP-positive, GAD67-positive interneurons, but the magnitude of this decrease, almost 50% (Fig. 4C), was larger than that seen with βIII-tubulin-positive newborn neurons. These findings indicate that CBP inhibits the genesis of interneurons, and suggest that it might also delay or inhibit the acquisition of a GABAergic phenotype. To directly test this latter idea, we determined the proportion of transfected βIII-tubulin-positive neurons that also expressed GAD67 at 3 days. This analysis demonstrated that, in scrambled siRNA-treated cultures, approximately 50% of the βIII-tubulin-positive cells also expressed GAD67 (Fig. 4D). In contrast, this percentage was significantly reduced to approximately 38% in cultures transfected with CBP siRNA (Fig. 4D).

To ask if these effects persisted, we performed similar experiments at 7 days in culture, analyzing cells that expressed βIII-tubulin and GAD67. We also assessed expression of somatostatin, since this relatively late interneuron marker is turned on by 7 days in these cultures. Immunostaining and quantification for these various markers demonstrated that even at this later timepoint, CBP knockdown caused a modest but significant reduction in the proportion of EGFP positive, βIII-tubulin positive newborn neurons (Fig. 4E) and GAD67-positive interneurons (Fig. 4F). Moreover, somatostatin-positive interneurons were also reduced (Fig. 4G), indicating that normal levels of CBP are required for the genesis of appropriate numbers of interneurons. However, in contrast to results at 3 days, at this later timepoint the proportion of βIII-tubulin-positive neurons that coexpressed GAD67 was similar for scrambled and CBP siRNAs (Fig. 4H), indicating that CBP knockdown delays but does not inhibit acquisition of an interneuron phenotype.

These data indicate that normal levels of CBP are necessary for MGE precursors to generate appropriate numbers of interneurons. In this regard, previous work on CBP indicated that it mediates its effects on cortical precursor differentiation via its histone acetyltransferase activity (Wang et al., 2010). We therefore asked whether we could rescue the interneuron deficit caused by CBP knockdown by treating MGE precursors with the HDAC inhibitor trichostatin A (TSA) to increase histone acetylation. To do this, we cotransfected E12 MGE precursors with a plasmid encoding EGFP together with scrambled or CBP siRNAs, and treated the cells the

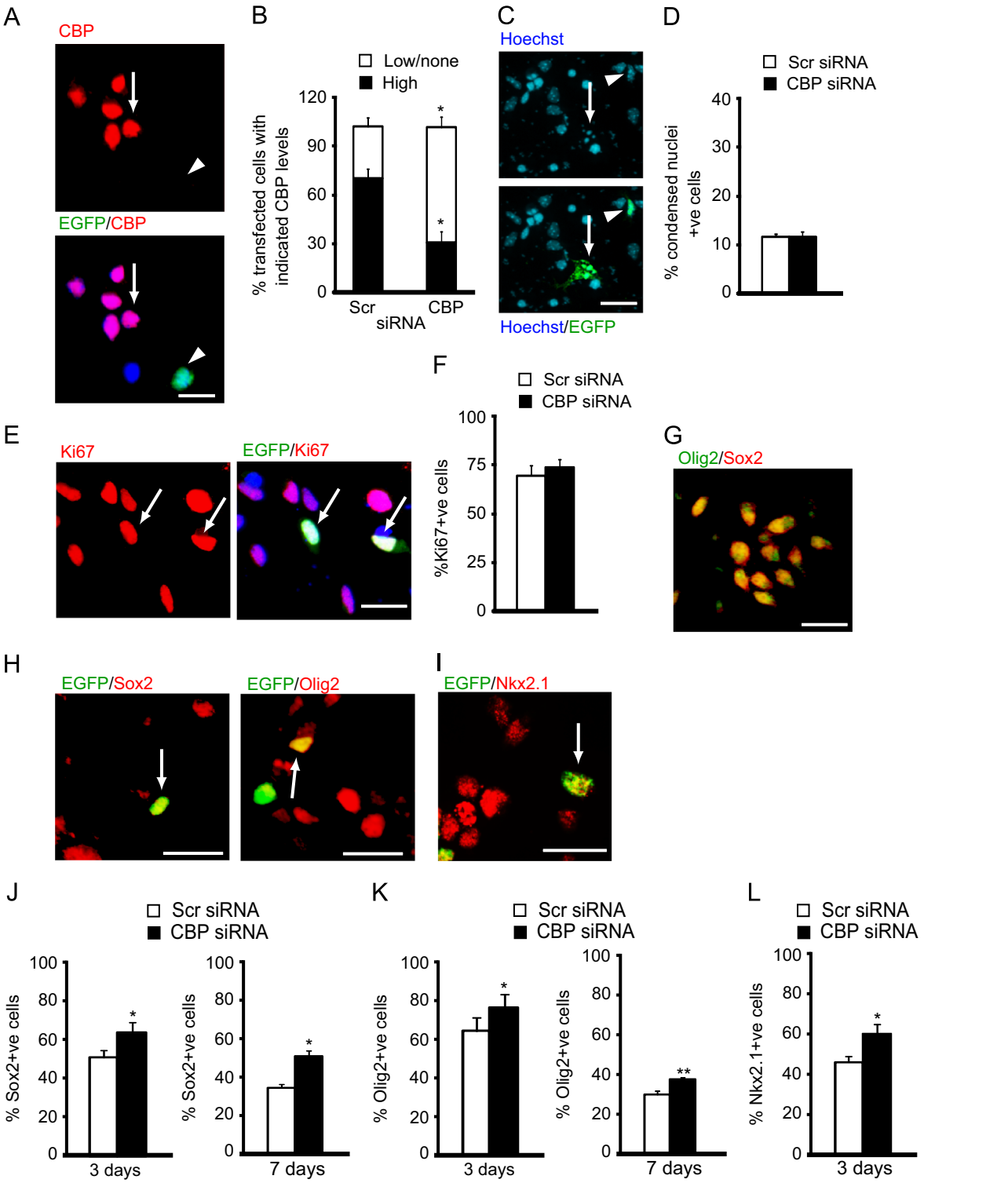

Fig. 2. CBP knockdown increases the proportion of MGE precursor cells. E12 MGE precursors were cotransfected with a nuclear EGFP plasmid, to identify transfected cells, and scrambled or CBP siRNAs. (A) Fluorescence photomicrographs of MGE precursors transfected with CBP siRNA and immunostained 2 days later for EGFP (green) and CBP (red; cells were also counterstained with Hoechst 33258 in blue). Arrows denote a control cell that expresses high levels of CBP, while the arrowheads denote a transfected cell that expresses no detectable CBP. Scale bar = 20 μm. (B) Quantification of the percentage of transfected cells that expressed high levels of CBP *versus* low/undetectable levels of CBP in experiments similar to that shown in A. The graph represents combined data from 3 independent experiments. $^{*}p < 0.05$ relative to the scrambled siRNA-transfected cultures. (C and D) MGE precursors were cultured for 2 days, immunostained for EGFP and counterstained with Hoechst 33258 (C) and the percentage of transfected cells with condensed, fragmented apoptotic nuclei was quantified (D). The arrowheads and arrows in C denote transfected cells with control and apoptotic nuclei, respectively. Results are pooled data from 3 independent experiments. Scale bar = 25 μm. (E) Fluorescence photomicrographs of MGE precursors immunostained at 2 days in culture for EGFP (green) and Ki67 (red), and counterstained with Hoechst 33258 (blue). Arrows denote double-labelled cells. Scale bar = 20 μm. (F) Quantification of the percentage of EGFP-positive, Ki67-positive cells in experiments similar to that shown in (E). The graph shows pooled data from 3 independent experiments. (G) Fluorescence photomicrographs of MGE precursors cultured for 3 days, and immunostained for Sox2 (red) and Olig2 (green). Scale bar = 20 μm. (H) Fluorescence photomicrographs of cultured MGE precursors at 3 days immunostained for EGFP (green) and Sox2 (red, left panel), or Olig2 (red, right panel). Arrows denote double-labelled cells. Scale bar = 25 μm. (I) Fluorescence photomicrograph of MGE precursors cultured for 3 days, and immunostained for EGFP (green) and Nkx2.1 (red). Arrow denotes a double-labelled cell. Scale bar = 20 μm. (J) Quantification of the percentage of EGFP-positive cells that also expressed Sox2 in MGE precursors cultured for 3 and 7 days in experiments similar to that shown in (H). Graphs show pooled data from 3 to 4 independent experiments at each timepoint. $^{*}p < 0.05$ relative to scrambled siRNA-transfected cultures. (K) Quantification of the percentage of EGFP-positive, Olig2-positive cells in MGE precursors cultured for 3 and 7 days in experiments similar to that shown in (H). Results are pooled data from 3 to 4 independent experiments at each timepoint. $^{*}p < 0.05$, $^{**}p < 0.01$ relative to scrambled siRNA-transfected cultures. (L) Quantification of the percentage of EGFP-positive, Nkx2.1-positive cells in MGE precursors cultured for 3 days in experiments similar to that shown in (I). $^{*}p < 0.05$ relative to scrambled siRNA-transfected cultures. Error bars denote S.E.M.

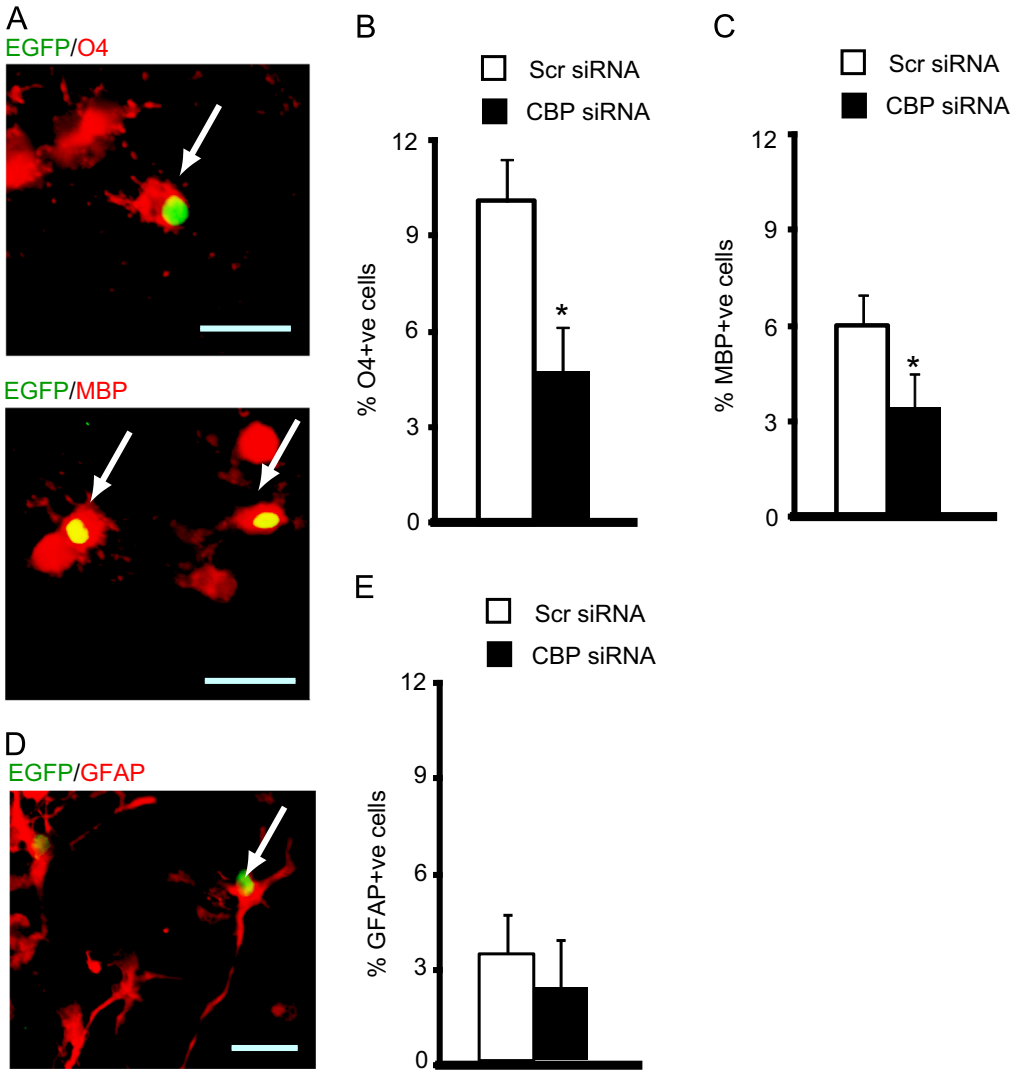

Fig. 3. CBP regulates the genesis of oligodendrocytes from MGE precursors. E12 MGE precursors were cotransfected with a nuclear EGFP plasmid, to label transfected cells, and either scrambled or CBP siRNAs, and analyzed 7 days later. (A) Fluorescence photomicrographs of transfected cells immunostained for EGFP (green) and O4 (red, top panel) or MBP (red, bottom panel). Arrows denote EGFP-positive cells that are also positive for the oligodendrocyte markers. Scale bar = 20 μm. (B and C) Quantification of the percentage of EGFP-positive, O4-positive cells (B), or EGFP-positive, MBP-positive cells (C) in experiments similar to those shown in A. Graphs show pooled data from 3 independent experiments each. $^{*}p < 0.05$ relative to scrambled siRNA-transfected cultures. (D) Fluorescence micrographs of transfected cells immunostained for EGFP (green) and GFAP (red). Arrow denotes a double-labelled cell. Scale bar = 20 μm. (E) Quantification of the percentage of transfected, GFAP-positive cells in experiments similar to that shown in (D). Graph shows pooled data from 3 independent experiments. Error bars denote S.E.M.

next day with either DMSO or 20 nM TSA, a concentration we have previously shown to increase H3K9/K14 acetylation in cortical precursors (Wang et al., 2010). We then analyzed these cultures 3 days later by immunostaining for GAD67. Quantification demonstrated that TSA had no effect on the number of GAD67-positive cells that were generated by cells transfected with the scrambled siRNA, but that it completely rescued the decreased numbers of interneurons seen with the CBP siRNAs (Fig. 4I). Thus, CBP likely promotes interneuron maturation by promoting histone acetylation.

These data indicate that CBP is necessary for MGE precursors to generate interneurons. To ask if CBP was also sufficient to enhance interneuron genesis, we overexpressed wildtype murine CBP in these precursors. Specifically, we cotransfected MGE precursors with a CBP expression plasmid that we characterized previously (Wang et al., 2010) along with an EGFP plasmid. Three days later, we immunostained these cultures for EGFP and GAD67. This analysis demonstrated that CBP overexpression caused an almost two-fold increase in the proportion of transfected GAD67-positive cells in these cultures (Fig. 4J).

Haploinsufficiency for CBP causes a deficit in interneurons in the neonatal forebrain

These results indicate that CBP is both necessary and sufficient for the genesis of appropriate numbers of interneurons from MGE precursors in culture. To ask if CBP is also important for forebrain interneurons *in vivo*, we studied $CBP^{+/-}$ mice. Specifically, we dissected the dorsal cortex and ventral forebrain from $CBP^{+/-}$ and $CBP^{+/+}$ littermates at postnatal day 3–4. We then performed Western blot analysis for GAD67, analyzing each mouse individually. This analysis demonstrated that there was a reduction in the amount of GAD67 protein in both the dorsal cortex and ventral forebrain of $CBP^{+/-}$ mice (Fig. 5A and B). This reduction was consistent between different littermates of the same genotype, and between litters from different parents. To quantify these differences, we reprobed the same blots for ERK protein as a loading control and performed scanning densitometry. This quantification showed that relative to ERK1 protein, GAD67 levels were significantly decreased in both the ventral forebrain and cortex of P4 $CBP^{+/-}$ mice (Fig. 5C).

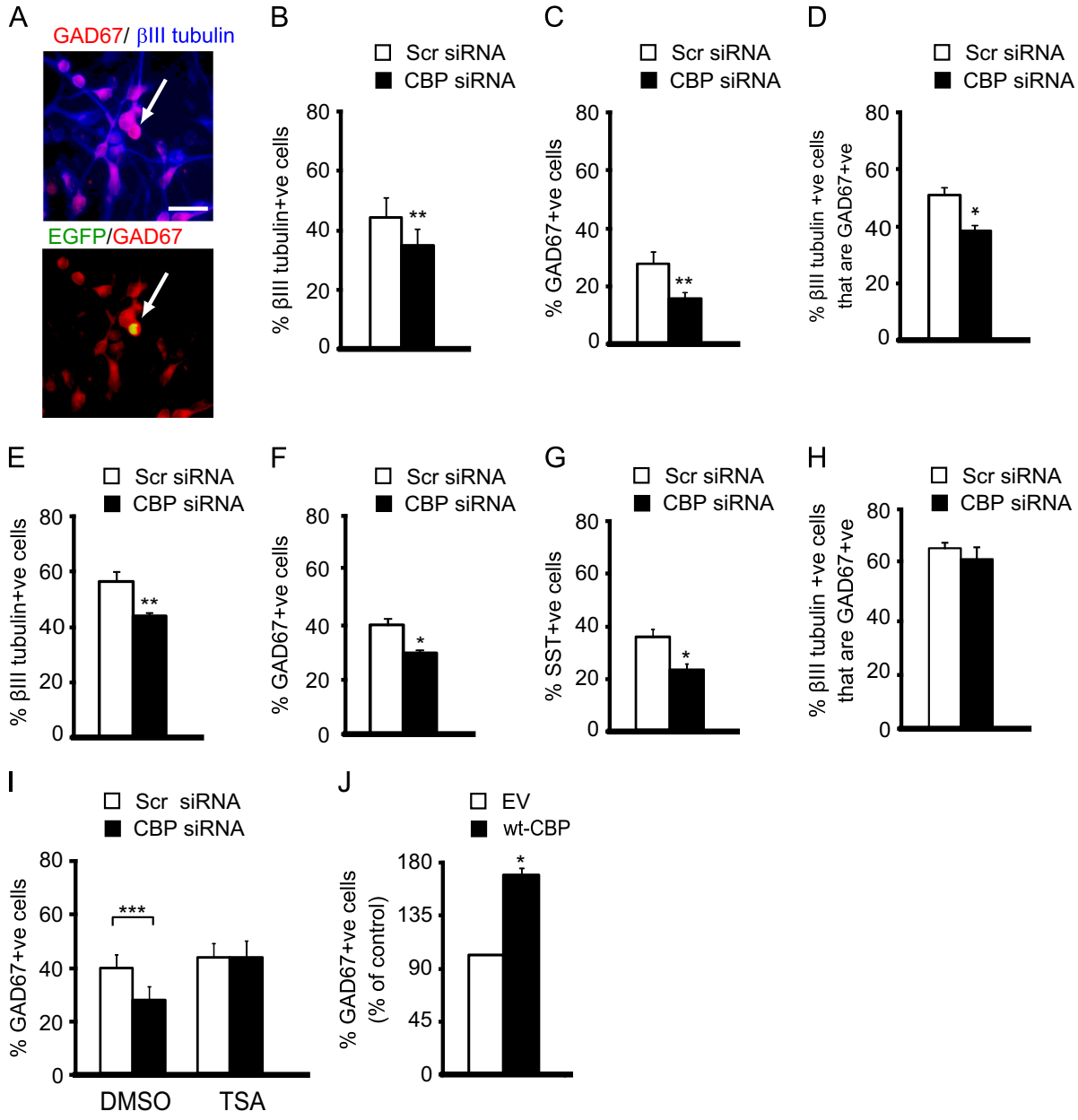

Fig. 4. CBP regulates MGE interneuron genesis and differentiation in culture. (A–H) E12 MGE precursors were cotransfected with a nuclear EGFP plasmid to identify transfected cells, and either scrambled or CBP siRNAs, and analyzed 3 days (A–D) or 7 days (E–H) later. (A) Fluorescence photomicrographs of the same field of MGE precursors cultured for 3 days, and immunostained for EGFP (green), GAD67 (red) and βIII tubulin (blue). Arrows denote an EGFP-positive cell that is positive for both βIII-tubulin (top panel) and GAD67 (top and bottom panels). Scale bar = 20 μm. (B) Quantification of the percentage of EGFP-positive cells that were also βIII-tubulin-positive at 3 days in experiments similar to that shown in A. Results are pooled data from 4 independent experiments. $^{**}p < 0.01$ relative to scrambled siRNA-transfected cultures. (C) Quantification of the percentage of EGFP-positive, GAD67-positive cells at 3 days in experiments similar to that shown in A. Graph shows pooled data from 4 independent experiments. $^{**}p < 0.01$ relative to scrambled siRNA-transfected cultures. (D) Quantification of the percentage of EGFP-positive, βIII-tubulin-positive cells that were also GAD67-positive at 3 days in experiments similar to that shown in A. Graph shows pooled data from 4 independent experiments. $^{*}p < 0.05$ relative to scrambled siRNA-transfected culture. (E) Quantification of the percentage of EGFP-positive, βIII-tubulin-positive cells at 7 days in experiments similar to that shown in A. Graph shows combined data from 3 independent experiments. $^{**}p < 0.01$ relative to scrambled siRNA-transfected cultures. (F) Quantification of the percentage of EGFP-positive, GAD67-positive cells at 7 days in experiments similar to that shown in A. Graph shows pooled data from 3 independent experiments. $^{*}p < 0.05$ relative to scrambled siRNA-transfected cultures. (G) Quantification of the percentage of transfected cells that were also positive for somatostatin at 7 days. Graph shows pooled data from 3 independent experiments. $*p < 0.05$ relative to scrambled siRNA-transfected cultures. (H) Quantification of the percentage of EGFP-positive, βIII-tubulin-positive cells that were also GAD67-positive at 7 days in experiments similar to that shown in A. Graph shows pooled data from 3 independent experiments. $p > 0.05$. (I) E12 MGE precursors were cotransfected with an EGFP plasmid and scrambled or CBP siRNAs in the presence of either 0.1% DMSO or 20 nM TSA in 0.1% DMSO. Cultures were immunostained 3 days later for EGFP and GAD67, and the proportion of transfected cells that were GAD67-positive was determined. The graph shows pooled data from 3 independent experiments. $^{***}p < 0.001$ relative to scrambled siRNA-transfected cultures. (J) E12 MGE precursors were cotransfected with an EGFP plasmid and either a wildtype CBP (wt-CBP) expression plasmid or the empty vector (EV), and analyzed 3 days later by immunostaining for EGFP and GAD67. The graph shows the proportion of transfected, GAD67-positive cells, normalized to sister cultures that were transfected with the empty vector (considered as 100%). Graphs represent combined data from 3 independent experiments. $^{*}p < 0.05$ relative to scrambled siRNA transfected cultures. Error bars denote S.E.M.

To ask if this deficit in GAD67 expression persisted into adulthood, we performed a similar analysis of the dorsal cortex in mice at 6 weeks of age. Western blot analysis of equal amounts of protein from cortices of $CBP^{+/-}$ *versus* $CBP^{+/+}$ mice demonstrated that similar levels of GAD67 protein were present in all mice, regardless of genotype (Fig. 5D). This conclusion was confirmed by reprobing the same blots for ERK protein as a loading control and performing scanning densitometry (Fig. 5D

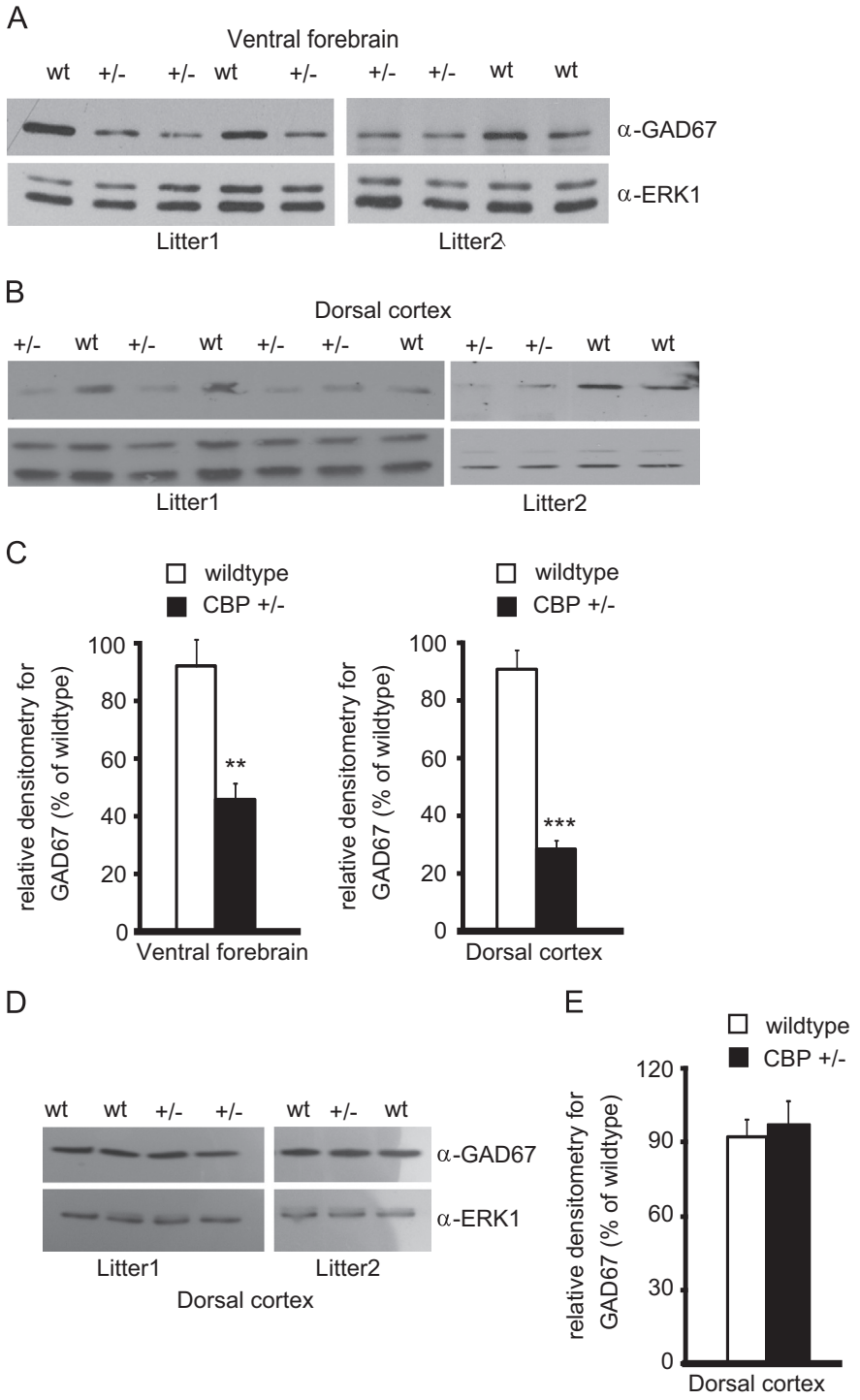

Fig. 5. Haploinsufficiency for CBP causes a deficit in levels of the interneuron neurotransmitter synthesis enzyme GAD67 in the neonatal but not adult murine forebrain. (A and B) Western blot analysis for GAD67 in equal amounts of lysates from the dorsal cortex and ventral forebrain of postnatal day 3–4 $CBP^{+/+}$ and $CBP^{+/-}$ mice, analyzed individually. Two independent litters (Litter 1 and Litter 2) were analyzed. Blots were reprobed with an antibody for ERK protein as a loading control. (C) Quantification by scanning densitometry of Western blots similar to those in (A and B), with the relative levels of GAD67 normalized to ERK levels for each independent sample, with the data normalized to one of the wildtype samples on the same gel as 100%. $^{**}p < 0.01$; $^{***}p < 0.001$, relative to wildtype samples ($n = 5$ $CBP^{+/+}$ and 6 $CBP^{+/-}$ analyzed in 2 independent experiments). (D) Western blot analysis for GAD67 in equal amounts of lysates from the dorsal cortex of 6 week old $CBP^{+/+}$ and $CBP^{+/-}$ mice, analyzed individually. Two independent litters (Litter 1 and Litter 2) were analyzed. Blots were reprobed with an antibody for ERK protein as a loading control. (E) Quantification by scanning densitometry of Western blots similar to those in (D) with the relative levels of GAD67 normalized to the ERK levels for each independent sample, and with the data normalized to one of the wildtype samples on the same gel as 100%. $p > 0.05$. ($n = 4$ $CBP^{+/+}$ and 3 $CBP^{+/-}$). Error bars denote S.E.M.

and E). Thus, as monitored by GAD67 protein levels, haploinsufficiency for CBP caused a delay, but not a long-lasting deficit, in the genesis and/or maturation of GAD67-positive interneurons in the developing forebrain.

Discussion

The findings presented here support four major conclusions. First, we show that ventral forebrain MGE precursors can be

cultured, and will differentiate appropriately into interneurons and oligodendrocytes in culture, as they do *in vivo* (He et al., 2001; Yung et al., 2002; Petryniak et al., 2007). We then use this culture system to show that normal levels of CBP are required for appropriate oligodendrogenesis, as we have previously observed with embryonic cortical precursors (Wang et al., 2010). Second, we demonstrate that CBP is both necessary and sufficient for the genesis of appropriate numbers of interneurons from MGE precursors. More specifically, we show that genetic knockdown of CBP in cultured MGE precursors decreases the number of interneurons that are generated while overexpression of CBP is sufficient to cause an increase in the numbers of interneurons that are generated. The decrease in interneuron number observed following CBP knockdown is accompanied by an increase in the number of precursors, arguing that CBP acts to enhance the differentiation of precursors into interneurons. Third, we provide data suggesting that CBP plays a role in the maturation of interneurons, since CBP knockdown delays the acquisition of a GABAergic phenotype in newborn neurons in the MGE cultures. Fourth, we show that levels of GAD67, an enzyme that regulates synthesis of the inhibitory neurotransmitter GABA, are decreased in the early postnatal cortex and ventral forebrain of $CBP^{+/-}$ mice, indicating that normal levels of CBP are also important for appropriate interneuron development *in vivo*. However, levels of GAD67 are normal in the cortex of adult $CBP^{+/-}$ mice, suggesting that, as seen in culture, the acquisition of a GABAergic phenotype is delayed but not inhibited *in vivo*. Thus, CBP acts to ensure the appropriate genesis and differentiation of interneurons in the developing forebrain, thereby providing a potential explanation for the association between epilepsy and RTS, where CBP is haploinsufficient.

The MGE is the primary source of GABAergic interneurons for the cortex (Tamamaki et al., 1997; Lavdas et al., 1999; Wichterle et al., 1999; Nery et al., 2002; Xu et al., 2004, Welagen and Anderson, 2010). These ventral precursors generate interneurons starting around E11–12 (Marin et al., 2000), and these newborn interneurons initially migrate tangentially along the superficial and deep migratory streams into the cortex (Lavdas et al., 1999; Xu et al., 2008), and then change direction to enter the cortical plate by following a radial or an oblique path (Kriegstein and Noctor, 2004). Damage or loss of these inhibitory GABAergic interneurons is associated with impaired inhibitory control of cortical pyramidal neurons, leading to hyperexcitability and epileptogenesis (Valencia et al., 2006; Bouilleret et al., 2000; Sakakibara et al., 2012). Intriguingly, an accumulating body of evidence demonstrates that developmental abnormalities can cause aberrant interneuron development and seizure phenotypes, leading to the concept that epilepsy is a neurodevelopmental disorder (Bozzi et al., 2012). In this regard, RTS is also associated with EEG abnormalities and epilepsy (Cantani and Gagliesi, 1998), raising the possibility that CBP regulates appropriate interneuron development, and that the epilepsy seen in RTS might be due to inappropriate interneuron function.

Here, we established an embryonic MGE culture system to directly address the possibility that decreases in CBP might dysregulate interneuron development. We show that these primary cultures mimic ventral forebrain development, with the MGE precursors generating interneurons first and oligodendrocytes second, with very few astrocytes generated, as is seen *in vivo* (He et al., 2001; Yung et al., 2002; Petryniak et al., 2007). We then used this culture system to ask about the effects of CBP knockdown, and showed that this perturbation led to a decrease in the numbers of interneurons that were generated by MGE precursors. What is the molecular explanation for this decrease? We posit that CBP acts as a prodifferentiation switch, enhancing the genesis of interneurons via its histone acetyltransferase activity. In support of this idea, we show (a) that MGE precursors are increased concomitant with the decrease in interneurons, (b) that CBP overexpression is sufficient to increase the number of interneurons that are generated, and (c) that the deficit in interneuron numbers following CBP knockdown can be rescued by the HDAC inhibitor TSA. Additional support for this idea comes from our previous study of CBP and cortical precursor differentiation, where activation of an aPKC-CBP pathway caused cortical precursors to differentiate down all three neural cell lineages (Wang et al., 2010, 2012). Presumably CBP mediates this prodifferentiation activity in MGE precursors by binding to transcription factors that are important for interneuron differentiation. As one example of such a potential mechanism, retinoic acid is important for genesis of GE-derived interneurons *in vivo* (Chatzi et al., 2011), and CBP is a well-known cofactor for nuclear receptor-mediated transcription (Chakravarti et al., 1996) that is thought to be essential for retinoic acid-mediated differentiation (Kawasaki et al., 1998).

An alternative explanation for the decrease that we observe in interneuron genesis is that when CBP is knocked down, MGE precursors start to make neural cell types other than GABAergic interneurons. Our data argue against this second explanation, since the numbers of both neurons and oligodendrocytes are decreased by CBP knockdown (astrocytes, which are very few in number, are unaltered), and since we found no evidence for the genesis of excitatory neurons in these cultures under any conditions (data not shown).

In addition to a decrease in the numbers of interneurons that are generated, we provide evidence that CBP knockdown delays the acquisition of an interneuron phenotype as monitored by GAD67 expression. These findings raise the possibility that CBP might directly regulate expression of genes important for interneuron maturation. Indeed, one way it could do this is by association with CREB since various late interneuron markers are known to be regulated by CREB signaling. For example, the somatostatin gene is regulated by a cAMP–protein kinase A–CREB pathway (Gonzalez and Montminy, 1989) and CREB has been shown to bind to the cAMP responsive element (CRE) in the promoter of the somatostatin gene in cortical neurons (Cha-Molstad et al., 2004). GAD65 expression has also been shown to be regulated via the Ras–ERK–CREB pathway in cortical interneurons (Sanchez-Huertas and Rico, 2011). In particular, that study found that CREB binds the CRE in the promoter of GAD65 gene and directly regulates its expression. While a CRE has not been definitively identified in the GAD67 promoter, the GAD67 promoter region does contain a motif with high homology to CREB binding sites (Kobori and Dash, 2006). Given that it is thought that CBP recruitment via phosphorylation of Ser-133 of the CREB protein is required for cAMP-mediated transcription (Nakajima et al., 1997), it is plausible that CBP itself is crucial for the expression of these interneuron specific genes. With specific regard to the culture studies reported here, it is likely that exogenous FGF2 activates this same Ras–ERK–CREB pathway in MGE precursors (Pende et al., 1997), thereby potentially priming these cultured cells to respond to CBP with transcription of genes important for acquisition of an interneuron phenotype.

Our study also suggests that histone acetylation plays an important role in interneuron development. In particular, we demonstrate that an HDAC inhibitor, TSA, can rescue the deficit in interneuron maturation caused by CBP knockdown, suggesting that CBP regulates interneuron maturation either directly by its own HAT activity, or indirectly by its interaction with other HATs. This finding is reminiscent of our previous study showing that CBP regulates cortical precursor differentiation via its histone acetyltransferase function (Wang et al., 2010). Intriguingly, mice that are homozygous for a mutation in the *querkopf* gene, which encodes a MYST family histone acetyltransferase, have reduced GAD67-positive interneurons in the

cerebral cortex (Thomas et al., 2000). The hypothesis that histone acetylation plays important roles in interneuron development is further strengthened by the observation that the promoters of interneuron genes are epigenetically regulated by histone modification. For example, various HDAC inhibitors have been shown to up-regulate GAD67 expression either directly or indirectly through histone hyperacetylation (Dong et al., 2007; Kundakovic et al., 2009). In NIH3T3 cells, recruitment of the CBP homolog p300 to the somatostatin promoter leads to acetylation of histone H4 and subsequent transcription of a reporter construct (Asahara et al., 2001).

Together, these findings demonstrate that CBP is required for appropriate interneuron development, regulating the initial genesis of these neurons from embryonic neural precursors, and potentially regulating their later maturation. Moreover, our data showing a decrease in GAD67 levels in the cortex and dorsal forebrain of neonatal *CBP*$^{+/-}$ mice argue that heterozygosity for CBP is sufficient to deregulate interneuron development, albeit only transiently. Thus, our findings support a model where CBP haploinsufficiency in humans perturbs interneuron development in early life, thereby upsetting the normal balance of inhibitory *versus* excitatory signaling, and setting the stage for the later development of epilepsy in individuals with RTS.

Acknowledgments

This work was supported by Canadian Institutes of Health Research (CIHR) Grant MOP-125945 to F.D.M. and D.R.K. F.D.M. and D.R.K. are Canada Research Chairs, and F.D.M. is a Howard Hughes Medical Institute Senior International Research Scholar. J.W. was supported by fellowships from the Multiple Sclerosis Society of Canada and the Three to Be Foundation, D.T. by a CIHR Michael Smith Studentship, and A.V. by an HSC Restracomp Fellowship, and D.G. by McEwen/McMurrich Fellowship and by the Three to Be Foundation. We thank Dennis Aquino, and members of the Kaplan/Miller laboratory for advice and assistance.

References

Asahara, H., Santoso, B., Guzman, E., Du, K., Cole, P.A., Davidson, I., Montminy, M., 2001. Chromatin-dependent cooperativity between constitutive and inducible activation domains in CREB. Mol. Cell Biol. 21, 7892–7900.
Azim, E., Jabaudon, D., Fame, R.M., Macklis, J.D., 2009. SOX6 controls dorsal progenitor identity and interneuron diversity during neocortical development. Nat. Neurosci. 12, 1238–1247.
Bannister, A.J., Kouzarides, T., 1996. The CBP co-activator is a histone acetyltransferase. Nature 384, 641–643.
Barnabé-Heider, F., Wasylnka, J.A., Fernandes, K.J., Porsche, C., Sendtner, M., Kaplan, D.R., Miller, F.D., 2005. Evidence that embryonic neurons regulate the onset of cortical gliogenesis via cardiotrophin-1. Neuron 48, 253–265.
Batista-Brito, R., Rossignol, E., Hjerling-Leffler, J., Denaxa, M., Wegner, M., Lefebvre, V., Pachnis, V., Fishell, G., 2009. The cell-intrinsic requirement of Sox6 for cortical interneuron development. Neuron 63, 466–481.
Bouilleret, V., Loup, F., Kiener, T., Marescaux, C., Fritschy, J.M., 2000. Early loss of interneurons and delayed subunit-specific changes in GABA(A)-receptor expression in a mouse model of mesial temporal lobe epilepsy. Hippocampus 10, 305–324.
Bozzi, Y., Casarosa, S., Caleo, M., 2012. Epilepsy as a neurodevelopmental disorders. Front. Psychiatr. 3, 19.
Brown, K.N., Chen, S., Han, Z., Lu, C.-H., Tan, X., Zhang, X.-J., Ding, L., Lopez-Cruz, A., Saur, D., Anderson, S.A., Huang, K., Shi, S.-H., 2011. Clonal production and organization of inhibitory interneurons in the neocortex. Science 334, 480–486.
Cantani, A., Gagliesi, D., 1998. Rubinstein–Taybi syndrome. Review of 732 cases and analysis of the typical traits. Eur. Rev. Med. Pharmacol. Sci. 2, 81–87.
Chakravarti, D., LaMorte, V.J., Neson, M.C., Nakajima, T., Schulman, I.G., Juguilon, H., Montminy, M., Evans, R.M., 1996. Role of CBP/P300 in nuclear receptor signalling. Nature 383, 99–103.
Cha-Molstad, H., Keller, D.M., Yochum, G.S., Impey, S., Goodman, R.H., 2004. Cell-type-specific binding of the transcription factor CREB to the cAMP-response element. Proc. Natl. Acad. Sci. USA 101, 13572–13577.
Chatzi, C., Brade, T., Duester, G., 2011. Retinoic acid functions as a key GABAergic differentiation signal in the basal ganglia. PLoS Biol. 9, e1000609.
Cobos, I., Calcagnotto, M.E., Vilaythong, A.J., Thwin, M.T., Noebels, J.L., Baraban, S.C., Rubenstein, J.L.R., 2005. Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. Nat. Neurosci. 8, 1059–1068.
Dong, E., Guidotti, A., Grayson, D.R., Costa, E., 2007. Histone hyperacetylation induces demethylation of reelin and 67-kDa glutamic acid decarboxylase promoters. Proc. Natl. Acad. Sci. USA 104, 4676–4681.
Gonzalez, G.A., Montminy, M.R., 1989. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell 59, 675–680.
He, W., Ingraham, C., Rising, L., Goderie, S., Temple, S., 2001. Multipotent stem cells from the mouse basal forebrain contribute GABAergic neurons and oligodendrocytes to the cerebral cortex during embryogenesis. J. Neurosci. 21, 8854–8862.
Kash, S.F., Johnson, R.S., Tecott, L.H., Noebels, J.L., Mayfield, R.D., Hanahan, D., Baekkeskov, S., 1997. Epilepsy in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. Proc. Natl. Acad. Sci. USA 94, 14060–14065.
Kawasaki, H., Eckner, R., Yao, T.P., Taira, K., Chiu, R., Livingston, D.M., Yokoyama, K.K., 1998. distinct roles of the co-activators p300 and CBP in retinoic-acid-induced F9-cell differentiation. Nature 393, 284–289.
Kitamura, K., Yanazawa, M., Sugiyama, N., Miura, H., Iizuka-Kogo, A., Kusaka, M., Omichi, K., Suzuki, R., Kato-Fukui, Y., Kamiirisa, K., Matsuo, M., Kamijo, S.-i., Kasahara, M., Yoshioka, H., Ogata, T., Fukuda, T., Kondo, I., Kato, M., Dobyns, W. B., Yokoyama, M., Morohashi, K.-i., 2002. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. Nat. Genet. 32, 359–369.
Kobori, N., Dash, P.K., 2006. Reversal of brain injury-induced prefrontal glutamic acid decarboxylase expression and working memory deficits by D1 receptor antagonism. J. Neurosci. 26, 4236–4246.
Kriegstein, A.R., Noctor, S.C., 2004. Patterns of neuronal migration in the embryonic cortex. Trends in Neurosci. 27, 392–399.
Kundakovic, M., Chen, Y., Guidotti, A., Grayson, D.R., 2009. The reelin and GAD67 promoters are activated by epigenetic drugs that facilitate the disruption of local repressor complexes. Mol. Pharmacol. 75, 342–354.
Kung, A.L., Rebel, V.I., Bronson, R.T., Chang, L.E., Sieff, C.A., Livingston, D.M., Yao, T.P., 2000. Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. Genes Dev. 14, 272–277.
Lavdas, A.A., Grigoriou, M., Pachnis, V., Parnavelas, J.G., 1999. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J. Neurosci. 19, 7881–7888.
Malatesta, P., Hartfuss, E., Gotz, M., 2000. Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. Development 127, 5253–5263.
Marin, O., Anderson, S.A., Rubenstein, J.L.R., 2000. Origin and molecular specification of striatal interneurons. J. Neurosci. 20, 6063–6076.
Miyoshi, G., Butt, S.G.B., Takebayashi, H., Fishell., G., 2007. Physiologically distinct temporal cohorts of cortical interneurons arise from telencephalic *Olig2*-expressing precursors. J. Neurosci. 27, 7786–7798.
Nakajima, T., Uchida, C., Anderson, S.F., Parvin, J.D., Montminy, M., 1997. Analysis of a cAMP-responsive activator reveals a two-component mechanism for transcriptional induction via signal-dependent factors. Genes. Dev. 11, 738–747.
Nery, S., Fishell, G., Corbin, J.G., 2002. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. Nat. Neurosci. 5, 1279–1287.
Noctor, S.C., Flint, A.C., Weissman, T.A., Dammerman, R.S., Kriegstein, A.R., 2001. Neurons derived from radial glial cells establish radial units in neocortex. Nature 409, 714–720.
Oike, Y., Hata, A., Mamiya, T., Kaname, T., Noda, Y., Suzuki, M., Yasue, H., Nabeshima, T., Araki, K., Yamamura, K., 1999. Truncated CBP protein leads to classical Rubinstein–Taybi syndrome phenotypes in mice: implications for a dominant-negative mechanism. Hum. Mol. Genet. 8, 387–396.
Pende, M., Fisher, T.L., Simpson, P.B., Russell, J.T., Blenis, J., Gallo, V., 1997. Neurotransmitter- and growth factor-induced cAMP response element binding protein phosphorylation in glial cell progenitors: role of calcium ions, protein kinase C, and mitogen-activated protein kinase/ribosomal S6 kinase pathway. J. Neurosci. 17, 1291–1301.
Petrij, F., Giles, R.H., Dauwerse, H.G., Saris, J.J., Hennekam, R.C., Masuno, M., Tommerup, N., van Ommen, G.J., Goodman, R.H., Peters, D.J., 1995. Rubinstein–Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature 376, 348–351.
Petryniak, M.A., Potter, G.B., Rowitch, D.H., Rubenstein, J.L.R., 2007. Dlx1 and Dlx2 control neuronal *versus* oligodendroglial cell fate acquisition in the developing forebrain. Neuron 55, 417–433.
Sakakibara, T., Sukigara, S., Otsuki, T., Takahashi, A., Kaneko, Y., Kaido, T., Saito, Y., Sato, N., Nakagawa, E., Sugai, K., Sassaki, M., Goto, Y., Itoh, M., 2012. Imbalance of interneuron distribution between neocortex and basal ganglia: consideration of epileptogenesis of focal cortical dysplasia. J. Neurol. Sci. 323, 128–133.
Sanchez-Huertas, C., Rico, B., 2011. CREB-dependent regulation of GAD65 transcription by BDNF/TrkB in cortical interneurons. Cereb. Cortex 21, 777–788.
Tamamaki, N., Fujimori, K.E., Takauji, R., 1997. Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. J. Neurosci. 17, 8313–8323.
Thomas, T., Voss, A.K., Chowdhury, K., Gruss, P., 2000. Querkopf, a MYST family histone acetyltransferase, is required for normal cerebral cortex development. Development (Cambridge, England) 127, 2537–2548.
Tsui, D., Vessey, J.P., Tomita, H., Kaplan, D.R., Miller, F.D., 2013. FoxP2 regulates neurogenesis during embryonic cortical development. J. Neurosci. 33 244–258

Valencia, I., Legido, A., Yelin, K., Khurana, D., Kothare, S.V., Katsetos, C.D., 2006. Anomalous inhibitory circuits in cortical tubers of human tuberous sclerosis complex associated with refractory epilepsy: aberrant expression of parvalbumin and calbindin-D28k in dysplastic cortex. J. Child Neurol. 21, 1058–1063.
Wang, J., Weaver, I.C., Gauthier-Fisher, A., Wang, H., He, L., Yeomans, J., Wondisford, F., Kaplan, D.R., Miller, F.D., 2010. CBP histone acetyltransferase activity regulates embryonic neural differentiation in the normal and Rubinstein–Taybi syndrome brain. Dev. Cell 18, 114–125.
Wang, J., Gallagher, D., Devito, L.M., Cancino, G.I., Tsui, D., He, L., Keller, G.M., Frankland, P.W., Kaplan, D.R., Miller, F.D., 2012. Metformin activates an atypical PKC-CBP pathway to promote neurogenesis and enhance spatial memory formation. Cell Stem Cell 11, 23–35.
Welagen, J., Anderson, S., 2010. Origins of neocortical interneurons in mice. Dev. Neurobiol. 71, 10–17.
Wichterle, H., Garcia-Verdugo, J.M., Herrera, D.G., Alvarez-Buylla, A., 1999. Young neurons from medial ganglionic eminence disperse in adult and embryonic brain. Nat. Neurosci. 2, 461–466.
Wiley, S., Swayne, S., Rubinstein, J.H., Lanphear, N.E., Stevens, C.A., 2003. Rubinstein–Taybi syndrome medical guidelines. Am. J. Med. Genet. A 119A, 101–110.
Xu, Q., Cobos, I., De La Cruz, E., Rubenstein, J.L., Anderson, S.A., 2004. Origins of cortical interneuron subtypes. J. Neurosci. 24, 2612–2622.
Xu, Q., Tam, M., Anderson, S.A., 2008. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. J. Comp. Neurol. 506, 16–29.
Yung, S.-Y., Gokhan, S., Jurcsak, J., Molero, A.E., Abrajano, J.J., Mehler, M.F., 2002. Differential modulation of BMP signaling promotes the elaboration of cerebral cortical GABAergic neurons or oligodendrocytes from a common sonic hedgehog-responsive ventral forebrain progenitor species. Proc. Natl. Acad. Sci. USA 99, 16273–16278.