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An Essential Switch in Subunit Composition of a Chromatin Remodeling Complex during Neural Development

Julie Lessard[1,5], **Jiang I. Wu**[1,5], **Jeffrey A. Ranish**[2], **Mimi Wan**[3], **Monte M. Winslow**[1], **Brett T. Staahl**[1], **Hai Wu**[1], **Ruedi Aebersold**[4], **Isabella A. Graef**[1], and **Gerald R. Crabtree**[1,6]

1*Howard Hughes Medical Institute and Departments of Developmental Biology and Pathology, 279 Campus Drive, Stanford University, Stanford, CA 94305 USA*

2*Institute for Systems Biology, 1441 North 34th Street, Seattle, WA 98103 USA*

SUMMARY

Mammalian neural stem cells (NSCs) have the capacity to both self-renew and to generate all the neuronal and glial cell-types of the adult nervous system. Global chromatin changes accompany the transition from proliferating NSCs to committed neuronal lineages, but the mechanisms involved have been unclear. Using a proteomics approach, we show that a switch in subunit composition of neural, ATP-dependent SWI/SNF-like chromatin remodeling complexes accompanies this developmental transition. Proliferating neural stem and progenitor cells express complexes in which BAF45a, a novel Krüppel/PHD domain protein and the actin-related protein BAF53a are quantitatively associated with the SWI2/SNF2-like ATPases, Brg and Brm. As neural progenitors exit the cell cycle, these subunits are replaced by the homologous BAF45b, BAF45c and BAF53b. BAF45a/53a subunits are necessary and sufficient for neural progenitor proliferation. Preventing the subunit switch impairs neuronal differentiation, indicating that this molecular event is essential for the transition from neural stem/progenitors to post-mitotic neurons. More broadly, these studies suggest that SWI/SNF-like complexes in vertebrates achieve biological specificity by combinatorial assembly of their subunits.

INTRODUCTION

The array of specialized neuronal and glial cell-types that characterize the adult central nervous system (CNS) originate from multipotent neuroepithelial (NE) precursor cells that line the ventricles of the developing spinal cord and brain (Gage, 2000; Roegiers and Jan, 2004; Temple, 2001). The decision of NE precursor cells to either self-renew or differentiate is regulated by both extrinsic and intrinsic mechanisms including environmental signals, cell-cell interactions and transcriptional events mediated, at least in part, by bHLH, STAT/Smad proteins and nuclear receptors (reviewed in Bertrand et al., 2002; Gotz and Huttner, 2005). Rapid nuclear reorganization and global changes in gene expression in newly-born neurons suggest that epigenetic changes at the level of chromatin structure might also be involved

6Correspondence: E-mail: crabtree@stanford.edu; phone (650)723-8391; fax (650)723-5158.
3Present address: Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06520 USA
4Present address: Institute for Molecular Systems Biology, ETH Zurich and Faculty of Natural Sciences, University of Zurich, Switzerland
5These authors contributed equally

(Hsieh and Gage, 2005; Kondo, 2006). Epigenetic regulatory mechanisms, such as histone modification, polyADP-ribosylation, DNA methylation, regulatory noncoding RNAs and even retrotransposition have been linked with changes in neural plasticity and long-term memory (reviewed in Hsieh and Gage, 2005; Kandel, 2001). However, the role of chromatin-based epigenetic mechanisms in early neural development remains poorly understood.

The mammalian genome encodes 29 different SWI2/SNF2-like ATPases, several of which are assembled into mammalian SWI/SNF-like complexes suggesting functional diversification within this large family of complexes. Two of these, Brg and Brahma(Brm) are interchangeable subunits in a subfamily of 2 MDa chromatin remodeling complexes termed BAF (Brg/Brm associated factor) or mSWI/SNF (Khavari et al., 1993; Kwon et al., 1994; Lemon et al., 2001; Wang et al., 1996b). BAF complexes contain 10 core subunits, some of which are similar to those in yeast SWI/SNF, while others, such as BAF57 and BAF53, are similar to subunits of the yeast SWR1 and INO80 complexes (Mizuguchi et al., 2004; Shen et al., 2003). The subunits of BAF complexes are tightly associated with the Brg/Brm ATPases and have no detectable rate of exchange *in vitro* when challenged with purified subunits (Zhao et al., 1998). *In vitro* studies indicate that BAF complexes facilitate nucleosome exchange between chromosomal templates, increase the accessibility of DNA to sequence-specific transcription factors and nucleate the polymerization of actin filaments from β-actin, which is a core component of these complexes (reviewed in Cairns, 2005; Martens and Winston, 2003).

Evidence that mSWI/SNF (BAF) subunits might have non-redundant and dosage-sensitive roles in neural development has come from several observations. Mice heterozygous for either *Brg* or *BAF155/Srg3* are predisposed to exencephaly, possibly by generation of too few neurons (Bultman et al., 2000; Kim et al., 2001). Similarly, the *C. elegans psa-1/Brg* and *psa-4/BAF155* genes are required for asymmetric neurogenic divisions. Temperature-sensitive (ts) alleles of these genes in worms generate fewer neurons and favor non-neuronal cell types (Sawa et al., 2000). In vertebrates, BAF complexes in post-mitotic neurons are different from those in all other cell-types in that they contain the BAF53b Arp-4 like protein instead of BAF53a (Olave et al., 2002). Studies in *Xenopus* indicated that Brg is required for neuronal differentiation by mediating the transcriptional activities of the proneural bHLH Neurogenin or NeuroD proteins (Seo et al., 2005b). While *Xenopus* Brg was reported not to be required for neural stem cell (NSC) function, recent studies suggested that mouse Brg is essential for the repression of neuronal commitment in NSCs, as a means of permitting glial cell differentiation in response to gliogenic signals (Matsumoto et al., 2006). The discrepancy between these studies might be related to functional evolutionary diversification of these complexes or perhaps different roles of Brg/Brm at different times during neurogenesis. Indeed, the finding that post-mitotic neurons express complexes with BAF53b, rather than BAF53a, which is found in all other cell-types (Olave et al., 2002), suggests that the function of these complexes might change during neural development.

A distinctive characteristic of the vertebrate BAF complexes, when compared to the yeast SWI/SNF and *Drosophila* BAP complexes, is their combinatorial assembly from the products of gene families encoding their 10 core subunits (Lemon et al., 2001;Wang et al., 1996b). The position of the subunits within the complex can be occupied by one and only one family member (Wang et al., 1996a; Zhao et al., 1998). While earlier studies have suggested that combinatorial assembly of vertebrate BAF complexes might contribute to specificity (Bultman et al., 2000; Olave et al., 2002; Reyes et al., 1998), others have suggested that gene family members might be partially or fully redundant (Lickert et al., 2004; Strobeck et al., 2002; Yamamichi-Nishina et al., 2003). One interesting possibility is that structurally distinct BAF complexes create distinct patterns of chromatin accessibility; thereby imparting specialized regulatory functions to ubiquitously expressed transcriptional regulators.

We have investigated the biological significance of combinatorial assembly of mammalian ATP-dependent chromatin remodeling complexes using a proteomics approach in the developing nervous system. Purification of Brg and Brm-associated proteins revealed a new family of four stoichiometric subunits of mSWI/SNF or BAF complexes that we termed BAF45. Remarkably, we show that the transition from proliferating neural stem/progenitor cells to post-mitotic neurons requires a switch in subunit composition of these complexes. At this developmental transition, functional specificity of BAF complexes appears to arise from the combinatorial use of the family members of the 10 subunits like letters in a 10-letter word. Self-renewal and proliferative activities of neural stem/progenitor cells require npBAF complexes containing BAF45a and BAF53a and assembled on the Brg/Brm ATPases. The dynamic exchange of these subunits for the alternative BAF45b, BAF45c and BAF53b subunits in post-mitotic neurons orchestrates cell cycle withdrawal and the acquisition of neuronal properties.

RESULTS

Purification of ATP-Dependent Chromatin Remodeling Complexes from the Developing Murine CNS Reveals a New Family of Subunits

To understand the role of ATP-dependent chromatin remodeling complexes in neural development, we purified the endogenous complexes based on the Brg and Brm ATPases from newborn (P0) mouse brains using a combination of biochemical and affinity steps. We first generated affinity purified antibodies that recognized only Brg and Brm (Figure 1A). Affinity purification of complexes with these antibodies under stringent conditions (Figure 1B and 1C and supplemental experimental procedures) revealed a highly specific pattern of co-purifying proteins (Figure 1C). Brg/Brm associated proteins were subjected to proteolytic digestion, analyzed by multi-dimensional liquid chromatography Electrospray Ionization-Tandem Mass Spectrometry (ESI-MS/MS; Figure 1A) (Link et al., 1999) and identified using SEQUEST (Eng and McCormack, 1994). The purification based on the anti-Brg/Brm antisera yielded 47 unambiguously identified proteins with a minimal *ProteinProphet* probability of 0.9 (Nesvizhskii et al., 2003), including most (14 out of 15) family members of the 10 core subunits of BAF complexes (Table 1, Figure 1D–1F and unpublished results). No peptides from the BAF60b subunit were obtained, while ten peptides were identified from BAF60a and seven from BAF60c, suggesting that neural BAF complexes lack BAF60b (Table 1). In contrast, only 3 protein identifications were obtained from sequencing of the IgG eluates (minimal probability score of 0.8; hnRNPA3 (n=1 peptide), kininogen precursor (n=1 peptide) and α-1-antitrypsin precursor (n=6 peptides) (data not shown)).

Several peptides corresponding to 4 homologous proteins, not previously known to be part of SWI/SNF-like complexes were obtained. We referred to them as Brg/Brm associated factor of 45 kDa or BAF45a, b, c and d (Figure 1D–1F and Figure S1). Five different tryptic peptides were found from the previously uncharacterized open reading frame (ORF) of mouse BAF45a (15% mass coverage), twenty-two peptides from BAF45b (43% mass coverage), seven peptides from BAF45c (27% mass coverage) and eight peptides from BAF45d (30% coverage, Figure 1D and Table 1). BLAST searches identified substantial similarity to potential ORFs in several eukaryotic organisms including the worm, fly, pufferfish, zebrafish, frog, chicken, rodent and human (Figure S1 for BAF45a and data not shown). The 4 family members have a distinctive organization with a novel N-terminal domain of unknown function, a central C_2H_2-type Krüppel zinc finger motif with potential nucleic acid binding activity and a C-terminal d4 domain consisting of two tandem PHD fingers, with potential histone H3 trimethyl-lysine binding activity (Wysocka et al., 2006) (Figure 1E and Figure S1).

BAF45a, BAF45b and BAF45c are Largely Confined to 2 MDa BAF Complexes in Neural Cells

To determine whether the BAF45 proteins were simply associated with Brg/Brm-containing complexes in neural cells or were novel core subunits, we generated polyclonal rabbit antisera specific for each family member. Co-immunoprecipitation experiments confirmed their interaction with Brg and other core components of the complex under stringent conditions in neural cells (Figure 2A, Figure S2 and data not shown). BAF45 proteins are largely dedicated to neural BAF complexes since the affinity purified anti-Brg/Brm antibody depleted them from mouse brain (P0) nuclear extracts (Figure 2B). Glycerol gradient co-sedimentations of brain (E15.5) nuclear extracts demonstrated that virtually all of BAF45a, b and c co-fractionate with known subunits of BAF complexes such as Brg, BAF170 and the actin-related protein (Arp) BAF53b (Figure 2C) (Wang et al., 1996b). In contrast, the Polybromo-1/BAF180 protein, which is part of both P-BAF (Lemon et al., 2001) and kinetocore complexes (Nie et al., 2000), fractionated in two distinct molecular weight complexes in neural cells (E15.5; Figure 2C). The difference in the peak of mobility of the complexes with BAF45a, 45b and Brg probably reflects the combinatorial assembly of this polymorphic group of complexes in the E15.5 mouse brain, which consists of a mixture of stem/progenitor cells and differentiated neurons (Figure 2C). Low affinity biochemical interactions due to the relatively mild cell lysis and differential centrifugation conditions might also contribute to the size differences in the mobility of the complexes (see supplemental experimental procedures). As the BAF45 and BAF53 family proteins are near quantitatively associated with the neural Brg/Brm-containing complexes, we conclude that they are stoichiometric subunits of BAF complexes in neural cells and not simply associated proteins.

Mutually Exclusive Expression of the BAF45a/ BAF53a and BAF45b-45c/BAF53b Subunits in Neural Stem/Progenitor Cells and Post-Mitotic Neurons

Neural differentiation occurs by an anatomically ordered process in which progenitors for both neurons and glia originate in a proliferative zone lining the ventricles and migrate laterally as they exit mitosis and undergo differentiation (Gilbert, 2003; Noctor et al., 2004). Western blot analyses of mouse brain nuclear extracts isolated at different developmental stages (E10.5 to P3) demonstrated a switch in expression of BAF45 and BAF53 family proteins during neurogenesis. E10.5–E11.5 neural cells predominantly express the BAF45a and BAF53a subunits while BAF45b, BAF45c and BAF53b expression becomes detectable from E13.5 onwards (Figure 2D). In the developing spinal cord (E10.5 to E16.5), BAF45a and BAF53a are specifically expressed in proliferating neural progenitors of the ventricular zone, which are positive for LeX/CD15 and Ki-67 (Figure 2E and data not shown). In differentiated neurons of the post-mitotic zone, which express the neuron-specific type III β-tubulin (TuJ1) and NeuN markers, expression of the BAF45a and BAF53a subunits is replaced by the alternative homologous BAF45b, BAF45c and BAF53b subunits (Figure 2E and Figure S3) (Olave et al., 2002). In the developing forebrain and cerebellar primordium, BAF45a and BAF53a expression is also restricted to proliferating neuroepithelial progenitors and cerebellar granule precursors, while BAF45b, BAF45c and BAF53b are strictly expressed in post-mitotic neurons (Figures S4–S6 and data not shown). Reciprocal and mutually exclusive expression of *BAF45a* and *BAF53a* in the progenitor zone and *BAF45b*, *BAF45c* and *BAF53b* in the post-mitotic zone of the developing spinal cord was also observed by *in situ* hybridization (E10.5–E13.5; Figure S7 and data not shown). Although *BAF45a* and *BAF53a* are expressed selectively in neural progenitors of the developing neural tube, (Figure 2E and Figure S2), they are expressed in most adult tissues (data not shown) (Olave et al., 2002). In contrast, *BAF250a*, *180*, *170*, *155*, *60a*, *57*, *47*, *45d* and *ARID2* were expressed ubiquitously throughout the developing spinal cord, brain and other embryonic tissues at E10.5 to E16.5 (Table 1 and data not shown). Since BAF45a/53a and BAF45b/BAF53b are quantitatively associated with Brg (Figure 2B and 2C) in neural cells, these observations demonstrate that SWI/SNF-like BAF complexes switch subunits during neural development. For clarity, we refer to the SWI/SNF-

like BAF complexes expressed in neural stem/progenitors cells and post-mitotic neurons as npBAF and nBAF, respectively.

BAF45a and BAF53a are Required for Proliferation of Neural Stem and Progenitor Cells

The studies described above indicated that proliferating neural stem/progenitor cells express 2 MDa npBAF complexes containing BAF45a/53a, but lacking BAF60b, which is expressed in all other cell types examined thus far (Wang et al., 1996b). To understand the roles of the npBAF complexes in neural progenitor function, we engineered shRNA constructs against the progenitor-specific BAF45a and BAF53a subunits (Figure 3A). Knockdown of both subunits ($BAF45a^{kd}$, $BAF53a^{kd}$) either individually or simultaneously in E13.5 cortical cultures specifically reduced their levels of expression by about 50 to 80% (Figure 3A). This reduction in protein levels significantly impaired neural stem/progenitor proliferation, as evidenced by a commensurate reduction in their rate of BrdU (5-bromodeoxyuridine) incorporation relative to controls (Figure 3A). These effects cannot be due to off-site targets of RNAi since either construct reduced the rate of neural stem/progenitor cell proliferation (Figure 3A). The decrease in neural stem/progenitor proliferation observed upon reducing BAF45a and BAF53a levels is not due to precocious and/or enhanced terminal differentiation as the percentage of transfected (GFP^+) $TuJ1^+$ cells was similar between the knockdown and control cultures (data not shown). Inactivation of both BAF45a and BAF53a in these cells did not affect their survival, as demonstrated by Annexin V and propidium iodide (PI) staining (data not shown). Together, these studies indicated that BAF45a/BAF53a and thereby the npBAF complexes with which they quantitatively associate are required for neural progenitor proliferation. The finding that a reduction in BAF45a/53a concentration produces a significant effect upon neural progenitor proliferation is consistent with the genetically dominant role of Brg in neural tube closure (Bultman et al., 2000).

BAF45a but not BAF45b is Sufficient to Enhance Neural Progenitor Proliferation

To further study the role of the newly identified progenitor-specific BAF45a subunit in regulating neural stem/progenitor function, we generated transgenic mice expressing *BAF45a* or the homologous *BAF45b* under the control of the neural-specific *Nestin* promoter (Chenn and Walsh, 2002). To facilitate detection of the cells expressing the transgenes, *BAF45a* and *BAF45b* cDNAs were linked to an internal ribosomal entry site (IRES) and *GFP* (Figure 3B). Using this strategy, we obtained high BAF45a or BAF45b expression (together with GFP) in neural stem/progenitor cells and differentiating neurons (although at relatively lower levels; Figures S5 and S6). As evidenced by anti-phosphorylated histone 3 (Ser10) reactivity (an M-phase marker), E13.5 *BAF45a* transgenic brains displayed a 2- to 4-fold increase in the number of mitotically active neural stem/progenitors compared to control littermates (Figure 3C and 3D). These proliferating ($H3P^+$) progenitors were mostly confined to the apical/ventricular zone of the developing cortex and midbrain of the *BAF45a* transgenics, although many mitotic cells were observed outside the periventricular progenitor domains (i.e ganglionic eminences; Figure 3C and 3D). In the developing cerebellum, *BAF45a* transgenic mice showed a significant increase in the number of mitotically active granule cell progenitors in the external granular layer (EGL) relative to controls (E14.5; Figure 4B and 4C). M-phase $H3P^+$ cells were also found in the internal differentiating zone of the developing cerebellum of *BAF45a* transgenics (Figure 4C). BrdU pulse-labeling experiments performed *in vivo* also revealed a marked increase in neural stem/progenitor cell proliferation in the midbrain of *BAF45a* transgenics (E14.5; Figure 4C). This increase in proliferation cannot be explained by a block to terminal differentiation or an alteration in cell death (Figures S8 and S9 and data not shown). Importantly, 3 transient transgenic lines expressing the homologous nBAF subunit BAF45b, which is normally expressed only in post-mitotic neurons, had normal numbers of M-phase ($H3P^+$) neural stem/progenitor cells (Figure 3C and 3D) and their rate of BrdU incorporation was similar to that of controls (E14.5; data not shown). Possible explanations

for this lack of effect is that BAF45b needs to be expressed in the context of the other post-mitotic specific BAF subunits (e.g. BAF53b) to assume its function and/or needs to be post-translationally modified by enzymatic moieties that are not present or active in the neural stem/progenitor compartment. Together, these results indicate that extended expression of one specific component of the neural progenitor (npBAF) complexes, BAF45a, is sufficient to extend the proliferative phase of neural stem/progenitor and cerebellar precursor cells in the developing CNS.

The N-terminal and Krüppel-like Domains Direct BAF45a to Neural Stem/Progenitor Proliferation in Transgenic Mice

The finding that BAF45a but not the homologous BAF45b subunit is sufficient to enhance neural stem/progenitor cell proliferation prompted us to define the regions of BAF45a that distinguish it from BAF45b in mediating this function. BAF45a and BAF45b are highly similar throughout the C-terminal double PHD (d4) domain (46% identity and 63% similarity at the amino acid level), however their N-terminal and C_2H_2-type Krüppel-like zinc finger domains are less conserved (Figure 1E and 1F). Based on these observations, we generated BAF45a deletion mutants (i.e. the N-terminal, Krüppel-like and d4 domains, Figure 4A) and tested their ability to drive neural stem/progenitor cell proliferation in transgenic mice. The constructs were FLAG-tagged and expressed under the control of the *Nestin* promoter with an IRES-*GFP* marker to allow comparison of transgene expression levels (Figure 4A and Figure S10). Each of the FLAG-tagged BAF45a deletion mutants incorporated as efficiently as the wild-type protein into npBAF complexes (Figure 4A). *BAF45a* transgenic mice expressing a deletion of the highly conserved d4 domain (Δd4; deletion aa 286–409) showed elevated rates of BrdU incorporation similar to the wild-type protein in neural progenitors of the midbrain and cerebellum (E14.5; Figure 4B and 4C). The number of dividing M-phase $H3P^+$ progenitors was also increased in the ventricular zone of the midbrain of these mice relative to controls (Figure 4C). In the developing cerebellum, mitotic ($H3P^+$) cells were observed within the internal differentiating zone (Figure 4C). In contrast, BAF45a mutants encoding either a deletion of the N-terminal (ΔN-term; deletion aa 1–97) or the Krüppel-like domain (ΔKrüppel-like; deletion aa 204–246) did not induce the proliferation of neural stem/progenitor cells (E14.5; Figure 4B and 4C). Together, these studies indicate that the N-terminal and Krüppel-like domains of the progenitor-specific BAF45a subunit are essential for its ability to induce neural stem/progenitor cell proliferation (Figure 4B and 4C). As these deletion mutant proteins associate with endogenous npBAF complexes (Figure 4A), the N-terminal and Krüppel-like domains of BAF45a likely direct the activity of the complex to a specific program of progenitor proliferation. Interestingly, these domains of BAF45a are the most divergent from the BAF45b and BAF45c neuronal-specific subunits (Figure 1E and Figure 3C), which lack the ability to induce neural stem/progenitor cell proliferation.

Brg is Essential for Neural Progenitor Development

Previous studies reported conflicting results concerning the role of the Brg ATPase subunit in the neural stem cell (NSC) population. In *Xenopus*, Brg was suggested not to be required for NSC function (Seo et al., 2005a, 2005b) while murine Brg was reported to be necessary for the expression of NSC markers but could not be detected in the developing neural tube before E14 (Matsumoto et al., 2006). In contrast, we detected Brg in periventricular stem/progenitor cells of the developing neural tube from E10.5 onwards using Brg-specific antibodies (H-88 and G-7; Figure 2 and Figure 5B and data not shown). Since Brg is quantitatively associated with BAF45a and BAF53a in neural stem/progenitor cells (Figure 2), we reasoned that we could more thoroughly evaluate the role of the npBAF complexes by Cre-mediated deletion of *Brg* specifically in neural stem/progenitor cells. To this aim, we bred mice containing a LoxP-flanked allele of *Brg* (*Brg^{flox}*) to mice expressing *Cre* recombinase under the control of the *Nestin* promoter (Figure 5A) (Sumi-Ichinose et al., 1997; Tronche et al., 1999).

In *Brg*$^{f/f}$*;Nestin-Cre* mice, the *Brg* gene is deleted at E10.5 and protein levels are undetectable by immunostaining in neural stem/progenitors from E12.5 onwards (Figure 5B and data not shown). Most other npBAF subunits (as well as Brm) are expressed at normal levels in the absence of Brg (Figure 5A) and probably incorporated into Brm-based complexes. All *Brg*$^{f/f}$*;Nestin-Cre* mice died at birth without breathing. We found reduced brain size, severe thinning of the neocortex and midbrain and nearly complete absence of the cerebellum in *Brg*$^{f/f}$*;Nestin-Cre* embryos (Figure 5C). Exencephaly was also observed in 6 of 43 *Brg* heterozygotes at E12.5 (data not shown), indicating that Brg and the family of npBAF complexes that contain Brg have non-redundant and dosage-sensitive roles in neural development.

Brg is Essential for the Self-Renewal/Maintenance of Neural Stem/Progenitor Cells

The cortical thinning observed in *Brg*$^{f/f}$*;Nestin-Cre* mice suggested that the multipotent neural stem/progenitor cells were unable to supply sufficient neurons and/or glial cells to the developing CNS. At E12.5, 12–24 hours after the disappearance of Brg protein, we observed a mild increase in the number of H3P^{+} M-phase progenitors in the ventricular zone of the *Brg*-deficient cortices relative to controls (Figure 5D, left panel). This increase in mitotic figures was not due to an M-phase arrest since the percentage of LeX^{+} cells with a 4N DNA content was not increased in *Brg*-deficient cultures (data not shown). However, by E15.5, the number of M-phase progenitors in the periventricular zone of *Brg*$^{f/f}$*;Nestin-Cre* cortices was clearly reduced and by E17.5, neural progenitors were virtually absent, as evidenced by absence of H3P and Nestin staining (Figure 5D, right panel). E17.5 *Brg* mutant cortices were thinner than controls and consisted mostly of post-mitotic differentiated (TuJ1^{+}) neurons (Figure 5D, right panel). A modest increase in the number of apoptotic cells in the ventricular zones of *Brg*-deficient cortices was also noted at both E12.5 and E15.5 relative to controls (Figure S11). We also observed a significant reduction in the number of GFAP^{+} astrocytes in *Brg* mutant cortices at late embryonic stages (i.e. E17.5 and later; data not shown), suggesting a defect at the level of the multipotent neural stem cell population to both neurons and glia. Together, these studies suggest that Brg and the npBAF complexes are initially dispensable for the division of the multipotent neural stem cells (NSCs), but are essential for their self-renewal and/or maintenance.

To directly examine the role of *Brg* and by inference npBAF complexes in regulating the self-renewal and proliferative capacities of neural stem cells (NSCs), we studied neurosphere formation from single NSCs. Strikingly, when analyzed after 7 days *in vitro* (div), both the number and size of the neurospheres were severely reduced in *Brg*-deficient cultures compared to controls, indicating that the proliferative potential of the neurosphere forming-NSC (nf-NSC) is impaired in the absence of Brg (E13.5; Figure 5E, left panels). The number of secondary neurospheres generated by subcloning single primary neurospheres is considered an estimate of self-renewal (expansionary) divisions of the nf-NSC (Reynolds and Weiss, 1996). To determine whether the ability of the *Brg*-deficient nf-NSCs to self-renew was impaired, individual primary colonies were dissociated and observed for their ability to form secondary neurospheres. In contrast to controls, secondary colonies were rarely generated from the *Brg*-deficient subcultures (Figure 5E, right panels). These observations are consistent with the progressive depletion of neural progenitors observed in *Brg*$^{f/f}$*;Nestin-Cre* mice and indicate that the Brg-containing npBAF complexes are essential for the self-renewal/proliferative capacity of the multipotent neural stem cells.

To determine if Brg is required for neural stem/progenitor cell proliferation, we measured the rate of BrdU incorporation (3 hrs pulse) in *Brg*-deficient E14.5 cortical cultures. The percentage of BrdU^{+} LeX^{+} neural stem/progenitor cells was significantly reduced in *Brg*-deficient cultures compared to controls (1 div; Figure 5F). BrdU pulse-labeling experiments performed *in vivo*

provided similar results (data not shown). This indicated that the impaired self-renewal potential of *Brg*-deficient NSCs might be partially explained by a slower cell cycle. These findings are surprising since both Brg and Brm were previously shown to restrain cellular proliferation by activating $p21^{Cip1/Waf1}$ and $p15^{INK4b}$ and repressing *cyclin* gene expression (Coisy et al., 2004; Dunaief et al., 1994; Kang et al., 2004; Strobeck et al., 2000).

To determine if Brg had a specific role in regulating neural stem/progenitor cell proliferation, we analyzed the proliferation rate of cultured *Brg*-deficient astrocytes ($GFAP^+$) under similar culture conditions as those for neural stem/progenitor cells (E16.5 cortical cultures at 3 div; 4-hour pulse of BrdU). Surprisingly, *Brg*-deficient astrocytes proliferated normally, even though these cells are the direct descendants of the neural stem/progenitor cells that require Brg for their proliferation (Figure 5G). This suggests that npBAF complexes selectively regulate the self-renewal/proliferation of neural progenitors, but have little role in the proliferation of glia after they have committed to this lineage. In other studies, we find that glia express BAF complexes with a different subunit composition (Figure S4 and GRC and JL, unpublished observation). This specialized function of Brg in neural stem/progenitors is likely mediated through its interaction with the progenitor-specific BAF45a and BAF53a subunits of npBAF complexes, which are both necessary and sufficient for neural stem/progenitor cell proliferation.

Brg Directly Regulates the Expression of Components of the Notch and Sonic Hedgehog Pathways Involved in Neural Development

To understand the molecular mechanism by which Brg and the 2MDa npBAF complexes regulate the self-renewal/proliferation of the NSCs, we defined Brg-dependent genes 12–24 hours after its disappearance, when neural stem and progenitor cells are the predominant cell-types in the nervous system (Figure 6A). RNA from three sets of E12.5 $Brg^{f/f}$*;Nestin-Cre* mutant and littermate control telencephalons were prepared and hybridized to transcript arrays. Student's t-test comparison yielded 440 transcripts with more than 2-fold difference in expression levels and a p value <0.01 (253 genes increased and 187 genes decreased out of 39,000 spots; Figure 6A and data not shown).

This analysis revealed that Brg is necessary for the normal expression of several components of the Notch signaling pathway, which are essential for the maintenance of the stem/progenitor cell population in the nervous system (Henrique et al., 1997; Hitoshi et al., 2002) (Figure 6A and 6B). Brg is also necessary for the repression of several components as well as downstream targets of the Sonic Hedgehog (SHH) pathway involved in neural development (Figure 6A and 6B) (Chiang et al., 1996; Rowitch et al., 1999). Finally, the expression of several transcription factors involved in neurogenesis, such as *NeuroD4, Foxg1,* and *Emx2,* was perturbed in *Brg*-deficient progenitors (Figure 6A and 6B).

Chromatin immunoprecipitation followed by quantitative polymerase chain reaction (PCR) experiments indicated that Brg, BAF45a, BAF53a and by inference npBAF complexes specifically occupy the promoters of several components of the SHH and Notch signaling pathways including *Patched, Olig1* and *Jagged1* but not that of the *CD4* gene, a direct and functional transcriptional target of BAF complexes in T cells (Chi et al., 2002) (E13.5; P1 regions; Figure 6C and data not shown). Binding was specific since regions only 5 kb further upstream provided a far weaker signal (P2 regions; Figure 6C). These results suggest that Brg-containing npBAF complexes may promote neural stem cell self-renewal/proliferation by enhancing Notch-dependent proliferative signals, while concurrently making the NSC insensitive to SHH-dependent differentiating cues.

Switching Subunits of ATP-Dependent Chromatin Remodeling Complexes is Essential for Neural Development

To determine if the exchange of npBAF and nBAF components is essential for neural development, we extended the expression of the progenitor-specific subunits past the time at which they are normally repressed. This was done by directing the expression of *BAF45a* and *BAF53a* (with IRES-*GFP*) under the control of the β-*actin* promoter in the developing chicken neural tube by *in ovo* electroporation. The differentiation outcome of multipotent electroporated (GFP^+) neural progenitors was analyzed in Hamburger-Hamilton (HH) stage 22–24 embryos (48–50 hrs post-electroporation). Immunoprecipitation experiments revealed that the transfected FLAG-tagged subunits were readily incorporated into endogenous BAF complexes (data not shown). Extending the expression of the BAF45a and BAF53a progenitor-specific subunits after cell-cycle exit was incompatible with the differentiation of specific classes of neurons since GFP-positive cells rarely expressed the MNR and Islet1 determinants of motor neuron differentiation (n= 9 of 10 embryos; Figure 7A). This block of neuronal differentiation was specific to the progenitor subunits since embryos expressing the neuronal subunits, BAF45b and BAF53b had similar numbers of GFP^+ MNR and Islet 1 post-mitotic neurons (n=10 of 10 embryos; Figure 7A). The failure of appearance of specific classes of neurons was paralleled by a reduction in the size of the spinal cord on the transfected side of the BAF45a/BAF53a embryos (n= 9/10 embryos), but not of the BAF45b/BAF53b embryos (n=10/10 embryos; Figure 7A and Figure S12). The number of GFP^+ cells coexpressing the TuJ1 and NeuN pan-neuronal markers was not significantly affected by ectopic expression of BAF45a and BAF53a in post-mitotic neurons (data not shown). TUNEL assays indicated that these changes cannot be explained by increased cell death (Figure S12). Therefore, suppression of the progenitor-specific BAF45a and BAF53a subunits after cell cycle exit is essential for differentiation of specific classes of neurons in the chick neural tube (Figure 7A).

DISCUSSION

An Epigenetic Switch Controlling Neural Development: the Lexicon of Chromatin Remodeling

Our studies define a previously unknown, but essential epigenetic mechanism underlying the transition from proliferating neural stem/progenitor cells to post-mitotic neurons. We show that the progenitors to both neurons and glia are maintained in a proliferative state by a specialized SWI/SNF-like npBAF complex that contains both BAF45a and BAF53a (Figure 7B, 1). The transition from proliferative to neurogenic divisions requires the exchange of these subunits for the homologous BAF45b or BAF45c and BAF53b proteins (Figure 7B, 2). Remarkably BAF45a, but not the related BAF45b, is sufficient to direct progenitor proliferation within the context of the developing murine nervous system. Extending expression of BAF45a and BAF53a in the neuronal lineage prevents the differentiation of specific classes of neurons, while expression of the homologous BAF45b and 53b subunits in progenitors is compatible with neuronal differentiation. Our studies also show that the developing neural tube lacks BAF60b, which is present in all other tissues examined to date. These findings are consistent with the concept that a specific combination of subunits (i.e. the “word”) rather than the simple presence of a specific subunit (i.e. the “letter”) provides functional specificity to neural progenitor (npBAF) and neuronal (nBAF) complexes based on the Brg ATPase in the developing nervous system.

The npBAF Complex is Essential for Neural Stem Cell Self-Renewal

Neural stem cells (NSCs) are characterized by their ability to generate, in a controlled fashion, all neuronal and glial cell lineages and maintain their original pool through self-renewal. The process of stem cell self-renewal is probably distinguished from conventional proliferation by the need to replicate the genome in a multipotent epigenetic state. Our studies indicate that a

chromatin remodeling complex containing Brg, BAF45a and BAF53a (npBAF complex) is essential for the inheritance and maintenance of this unique multipotent epigenetic state. Deletion of the Brg ATPase which is quantitatively associated with BAF45a and BAF53a in neural stem/progenitor cells results in a progressive depletion of the NSC population and subsequent reduction in the number of both neurons and glia. The role of Brg and the npBAF complexes in regulating the self-renewal and proliferative activity of the multipotent NSCs is also illustrated by the reduced number and size of primary neurospheres in *Brg*-deficient cortical cultures and their inability to form secondary neuropheres when replated. Interestingly, Brg is not a general regulator of cellular proliferation (e.g. glia derived from NSCs proliferate normally in its absence as do mouse embryonic fibroblasts (MEFs) (Bultman et al., 2000)) and has a tumor suppressive role in many cell types (Bultman et al., 2000; Dunaief et al., 1994; Hendricks et al., 2004). The specific role of Brg in regulating the proliferation of neural stem/progenitor cells is likely due to the presence of BAF45a and BAF53a in the complexes, which are both necessary and sufficient for their proliferation (Figure 3).

How might npBAF complexes specifically promote neural stem cell self-renewal and proliferation? We find that the ability of the progenitor-specific BAF45a subunit to induce neural progenitor proliferation requires the Krüppel-like domain and the N-terminus, but not the PHD (d4) domains. These essential domains are the most divergent with the neuronal-specific BAF45b subunit which, although highly homologous, lacks this ability. The Krüppel-like domain of BAF45a probably does not bind DNA, since residues previously shown to be critical for DNA binding are not conserved. BAF53a, the other progenitor subunit, is an actin-related protein (Arp) and is also unlikely to contact DNA (Wang et al., 1996b). Thus, we favor a mechanism in which BAF45a and BAF53a function to allow association of the npBAF complexes with key regulators of neural stem cell self-renewal/proliferation. *Bmi-1*, a member of the *Polycomb-group* gene (*PcG*) chromatin-remodeling family was recently shown to be essential for the long-term maintenance/self-renewal of both neural (Molofsky et al., 2003) and hemopoietic stem cells (Lessard and Sauvageau, 2003; Park et al., 2003). An interesting possibility is that Bmi1-based complexes and npBAF complexes cooperate to establish a chromatin landscape that favors self-renewal and ensures identical multipotency and proliferative capacities to progeny cells.

The function of npBAF complexes in neural stem cell self-renewal/proliferation is likely related to a direct role of these complexes in the coordinated control of cell cycle regulators as well as components of the Notch and Sonic hedgehog (SHH) signaling pathways. Work by Kroll and colleagues indicated that *Xenopus* Geminin maintains the undifferentiated neural progenitor state by antagonizing Brg's function in mediating the transcriptional activity of the proneural bHLH Neurogenin (Ngn) and NeuroD proteins (Seo et al., 2005a, 2005b). However, our high stringency purification of the neural BAF complexes did not reveal Geminin, NeuroD or Ngn peptides (data not shown), suggesting either a weak/transient interaction or evolutionary differences between the frog and mouse neural BAF complexes. Alternatively, our studies suggest that Notch signaling, which is essential for the self-renewal/proliferation of stem cells in the nervous system (Henrique et al., 1997; Hitoshi et al., 2002), is altered in $Brg^{-/-}$ multipotent neural progenitors (Figure 6). Moreover, our observation that several components and targets of Sonic Hedgehog signaling are directly repressed by Brg in E12.5 telencephalic cells suggests that npBAF complexes might prevent neural stem cells from sensing SHH signals that would otherwise induce neuronal differentiation and patterning (Chiang et al., 1996; Rowitch et al., 1999).

One of the goals of modern developmental biology is to recapitulate pathways of development for the regeneration of tissues and cell-types. We have demonstrated that a switch from a stem/progenitor to a post-mitotic chromatin remodeling mechanism occurs as neurons exit the cell cycle and become committed to their adult state. This switch is driven by chromatin remodeling

complexes with distinctive and specialized roles in neural progenitors (this study) and post-mitotic neurons (Wu et al., submitted). These findings raise the possibility that the switch could be reversed by activating progenitor subunits (BAF45a or 53a) or inactivating neuronal subunits (BAF45b or 53b) in adult neurons, thereby allowing them to develop stem/progenitor cell characteristics.

EXPERIMENTAL PROCEDURES

Antibody Production

Polyclonal antibodies against Brg/Brm (J1) and BAF45 (a, b and c) proteins were raised in rabbits using MBP fusion proteins encoding amino acids (aa) 1257 to 1338 of hBrg, aa 96 to 207 or aa 180 to 282 (M2–34) of mBAF45a (NP_077212), aa 95 to 187 of mBAF45b (NP_038902) and aa 95 to 188 of mBAF45c (NP_478119). BAF45a mouse monoclonal antibodies (clones 46 and 75) were raised against aa 180 to 282 of mBAF45a.

Cortical Cell Cultures

E13.5 to E16.5 mouse cortices were trypsinized and dissociated to single cells. Serum-free media (DMEM supplemented with B-27, N2, NAC) was used in neurosphere cultures (with 20 ng/ml FGF2). Media was supplemented with 5% serum for the neural progenitor and astrocyte proliferation assays. Astrocyte differentiation was induced with 100 ng/ml CNTF for 3 days.

Immunohistochemistry and TUNEL Assays

Timed mouse pregnancies were determined by plugging date as day 0.5. Paraffin or frozen sections were stained with anti-Brg (G7 or H-88, Santa Cruz), anti-Nestin (BD; clone rat 401), TuJ1 (Covance), anti-phosphorylated Histone 3 (Ser 10; Upstate), Ki-67 (BD) and anti-NeuN (Chemicon) antibodies and visualized using a Leica SP2 AOBS confocal microscope. TUNEL assays were performed using an *In situ* Cell Death Detection kit (Roche).

Chicken *in ovo* Electroporation

Supercoiled plasmid DNA was injected into the lumen of the neural tube of Hamburger-Hamilton (HH) stage 10–12 chicken embryos at 1.0 μg/μl with 50 ng/μl Fast-Green. Electrodes were placed flanking the neural tube and pulsed 5 times at 21V for 50.6 msec. using an ECM 830 Electro Square electroporator (BTX Genetronics). Embryos were harvested 48–50 hrs later (HH stage 22–24), fixed in 4% PFA, incubated in PBS for 15 hrs, cryoprotected in 30% sucrose and embedded in O.C.T compound. 20 μm thick sections were stained using: anti-MNR2 and anti-Islet (Developmental Studies Hybridoma Bank) and Alexa Fluor-594 (Molecular Probes) secondary antibodies. For BrdU incorporation assays, 100 μM BrdU was applied to chick embryos *in ovo* and incubated 4 hrs at 38.3°C.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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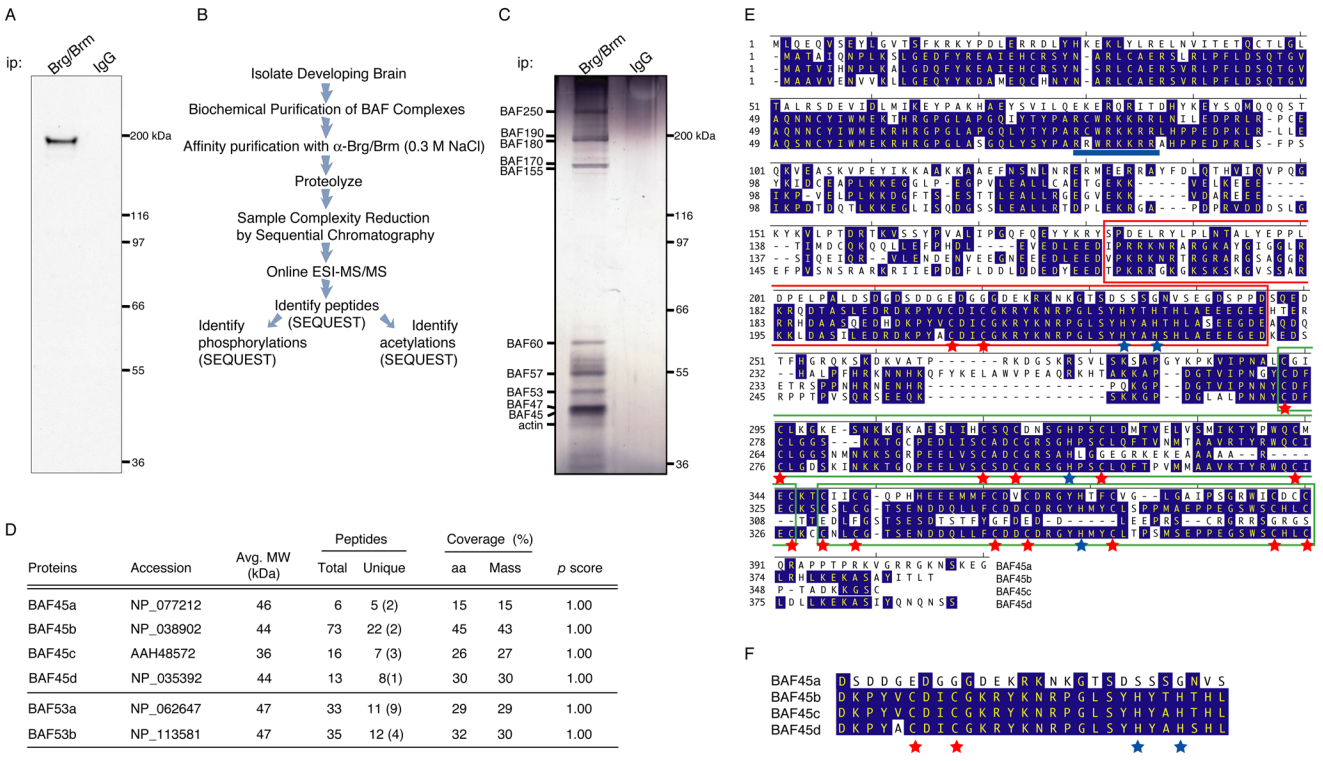

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Proteins	Accession	Avg. MW (kDa)	Peptides		Coverage (%)		*p* score
			Total	Unique	aa	Mass	
BAF45a	NP_077212	46	6	5 (2)	15	15	1.00
BAF45b	NP_038902	44	73	22 (2)	45	43	1.00
BAF45c	AAH48572	36	16	7 (3)	26	27	1.00
BAF45d	NP_035392	44	13	8(1)	30	30	1.00
BAF53a	NP_062647	47	33	11 (9)	29	29	1.00
BAF53b	NP_113581	47	35	12 (4)	32	30	1.00

Figure 1. BAF45 family proteins are subunits of neural SWI/SNF-like BAF complexes

A. Immunoprecipitation and Western blot analysis of Brg/Brm proteins in P0 mouse nuclear extracts using anti-Brg/Brm affinity purified antibodies. kDa, kilodaltons.

B. Strategy for purification and sequencing of neural SWI/SNF-like BAF complexes. Highly stringent conditions were used to isolate only the core subunits of the complexes plus tightly associated proteins (see supplemental experimental procedures). ESI-MS/MS, Electrospray Ionization- Tandem Mass Spectrometry.

C. Silver-stain analysis of immunopurified (anti-Brg/Brm) endogenous neural BAF complexes in P0 mouse brain nuclear extracts.

D. Peptides identified from the BAF45 and BAF53 families of subunits by mass spectrometry. The numbers in parentheses are the numbers of peptides corresponding to unique protein entries. MW, calculated molecular weight; aa, amino acids.

E. Conservation and divergence among mouse BAF45 family members. Conserved amino acids are highlighted in blue. The C_2H_2-type Krüppel-like domains are boxed in red and the PHD domains of the d4 domain are boxed in green. Red and blue stars indicate the conserved cysteine and histidine residues, respectively. The putative nuclear localization sequence is underlined in blue and is displaced 28 aa C-terminal in BAF45a. Note that only the first C_3H motif of the PHD1 domain is conserved in BAF45c.

F. The C^2H_2-type Krüppel-like zinc finger motif is not conserved in BAF45a. The conserved cysteine and histidine residues are labeled with red and blue stars, respectively. Note their absence in BAF45a protein.

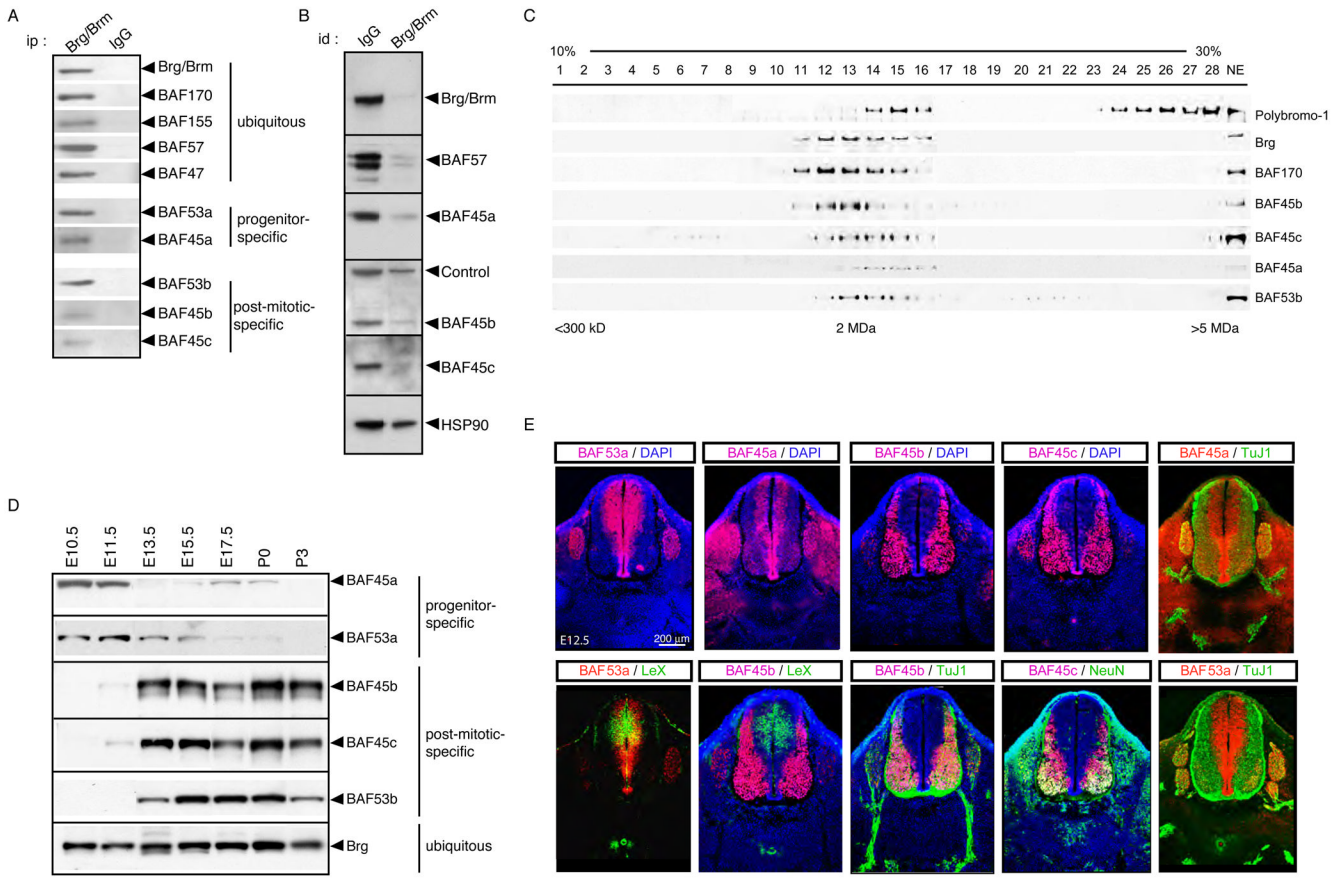

Figure 2. The subunit composition of neural BAF complexes changes as cells develop from neural progenitors to post-mitotic neurons

A. Western blot analyses of immunopurified (anti-Brg/Brm) neural BAF complexes in P0 mouse brain nuclear extracts.

B. BAF45a, BAF45b and BAF45c are dedicated to neural BAF complexes. Serial immunodepletions were performed on anti-Brg/Brm columns in P0 brain nuclear extracts. Supernatants were blotted with anti-BAF antibodies. Hsp90, heat shock protein 90; id, immunodepletion.

C. BAF45a, BAF45b and BAF45c co-sediment with other components of neural BAF complexes. E15.5 brain nuclear extracts were separated by centrifugation on glycerol gradients (10 to 30 %) and isolated fractions analyzed by Western blotting.

D. Sequential expression of BAF45a, BAF53a and BAF45b, BAF45c and BAF53b during neurogenesis. Brain nuclear extracts were isolated at different developmental stages and blotted with BAF45, BAF53 and Brg specific antibodies.

E. BAF45a and BAF53a are expressed specifically in neural progenitors and are replaced by the BAF45b/c and BAF53b homologous subunits in post-mitotic neurons. Immunostaining on cross sections of E12.5 spinal cords together with progenitor-(LeX) and neuronal-specific (TuJ1 and NeuN) markers. TuJ1, antibody against β-tubulin type III; DAPI, 4′,6-Diamidino-2-phenylindole.

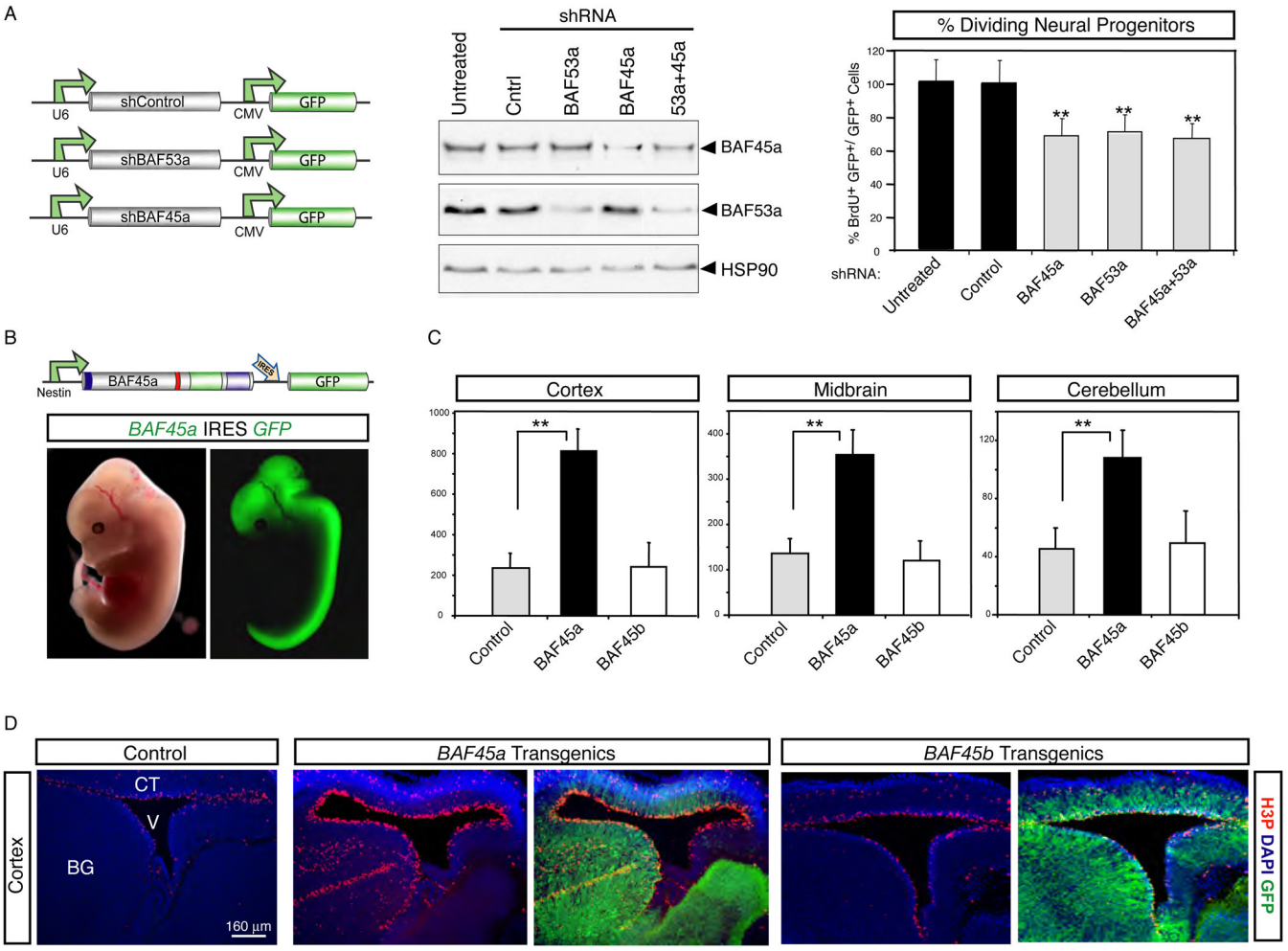

Figure 3. BAF45a and BAF53a are necessary and sufficient for the proliferation of neural progenitors

A. shRNA-mediated knockdown of BAF45a and/or BAF53a impairs the proliferative activity of neural progenitors in E14.5 adherent cortical cultures. Western blot analyses of protein extracts from these cultures (3 div) are shown. Hsp90 is a loading control. E14.5 cortical cells electroporated (Amaxa Inc, MD) with shRNA or control vectors were cultured in serum-containing media (4 div), stained with anti-LeX or anti-nestin and BrdU antibodies and analyzed by flow cytometry. BrdU was added 2 hrs before analysis. The percentage of dividing GFP^+ $BrdU^+$ neural progenitors in these cultures (n=3 independent experiments) is shown. Results are expressed as mean ±SD. **, $p<0.01$. See supplemental information for shRNA sequences. CMV, cytomegalovirus immediate early enhancer/promoter.

B. *Nestin*-driven *BAF45a* IRES-*GFP* expression in mouse E13.5 transient transgenics. Nestin, nestin-gene regulatory elements; IRES, internal ribosomal entry site. Note that the BAF45a and BAF45b expression patterns and levels were similar in all the *BAF45a* and *BAF45b* transient transgenic mice analyzed.

C. Quantification of the number of proliferating ($H3P^+$) neural progenitors in the cortex, midbrain and cerebellum of *BAF45a* and *BAF45b* transient transgenic mice (n=5 and n=3 embryos, respectively) relative to control littermates. $H3P^+$ cells (red) were manually counted using digitalized pictures of comparative brain sagittal sections stained with anti-H3P antibody

(n>6 pictures per embryo). Results are expressed as mean ± SD. **, $p<0.01$. H3P, anti-phosphorylated Histone 3 (Ser10).

D. Increased number of proliferating ($H3P^+$) neural progenitors in the developing cortices and basal ganglia of E13.5 *BAF45a* transgenic embryos relative to *BAF45b* transgenic and control embryos. Comparable sagittal sections were stained with anti-H3P antibodies (red) and DAPI (blue). Representative images are shown. BG, basal ganglia; CT, cortex; V, ventricle.

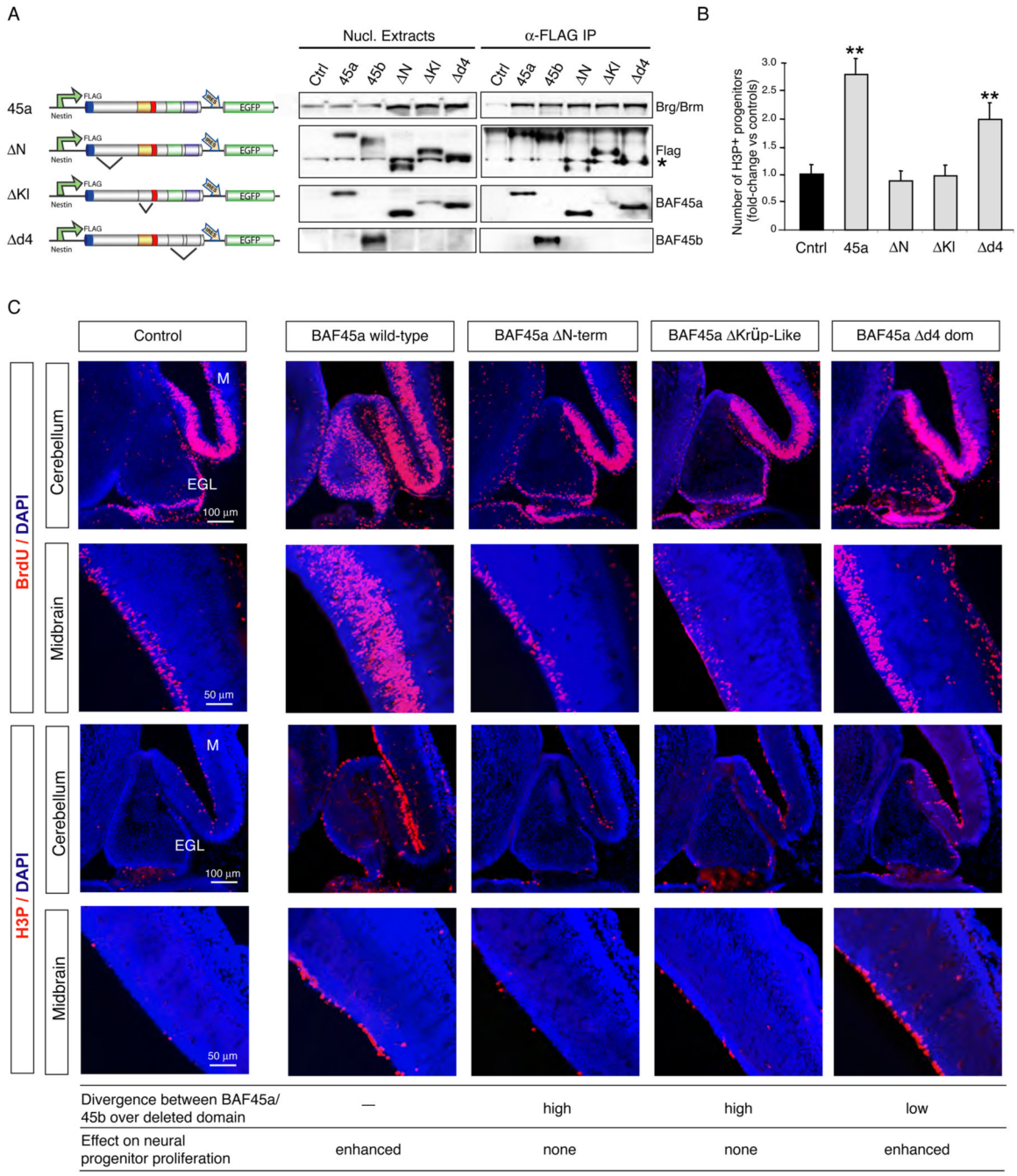

Figure 4. The N-terminus and Krüppel-like domains of BAF45a are essential to induce neural progenitor proliferation

A. The N-terminal, Krüppel-like and d4 domains of BAF45a are not required for its assembly within npBAF complexes in P19 cells. Schematic representations of the transgenes are shown. Nuclear extracts of transfected P19 cells expressing similar levels of the FLAG-tagged proteins (β-*actin* promoter) were immunoprecipitated with M2 (anti-FLAG) agarose beads and analyzed by Western blotting. Note the lower detection level of the BAF45a ΔKrüppel-like protein (deletion aa 204–246) with the α-BAF45a antibody (raised against aa 180 to 282). *; non-specific band recognized by the FLAG antibody.

B. Number of M-phase ($H3P^+$) cerebellar progenitors in E14.5 Nestin-driven *BAF45a* (n=3), *BAF45a ΔN-terminal* (n=2), *BAF45a ΔKrüppel-like* (n=3) and *BAF45a Δd4* domain (n=3) transient transgenic mice relative to control littermates. $H3P^+$ cells (red) were manually counted using digitalized pictures of comparative brain sagittal sections stained with anti-H3P antibody (n>6 pictures per embryo). Results are expressed as mean ± SD. **, $p<0.01$
C. Dividing ($BrdU^+$ and $H3P^+$) neural progenitors in the developing cerebellums and midbrains of *Nestin*-driven *BAF45a* and *BAF45a* deletion mutant transgenic mice (E14.5, 1 hr BrdU pulse). Comparable sagittal sections were stained with anti-BrdU (red) or anti-H3P (red) antibodies and DAPI (blue). Number of independent transgenic lines: *BAF45a wild-type* (n= 3), *BAF45a ΔN-terminal* (n= 2), *BAF45a ΔKrüppel-like* (n= 3) and *BAF45a Δd4* domain (n=3). Representative images are shown. EGL, external granular layer; M, midbrain.

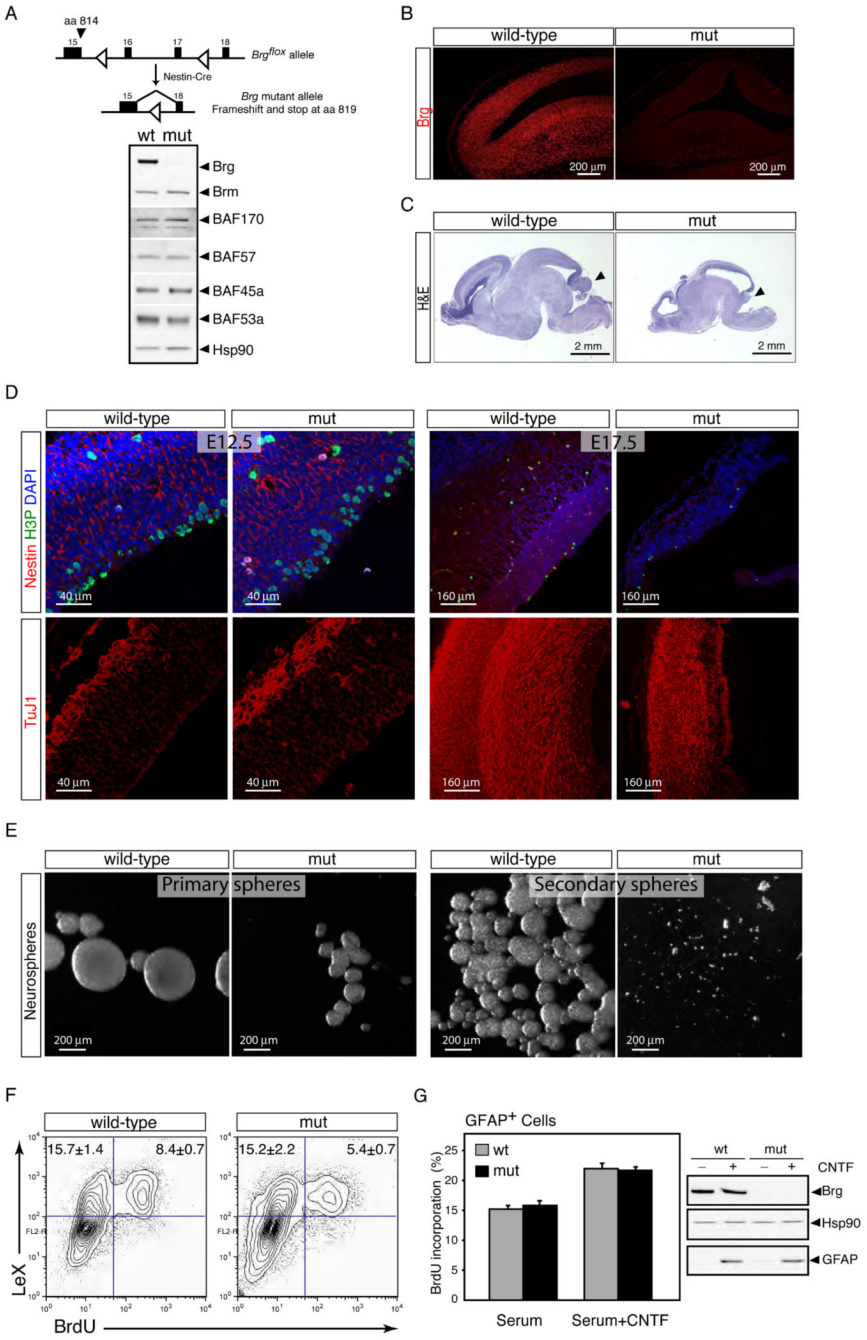

Figure 5. Brg is essential for the self-renewal and maintenance of neural stem/progenitor cells

A. Brg deletion does not affect the expression of Brm and other npBAF subunits. Strategy for deletion of *Brg* in neural progenitors. Genomic structure of the floxed *Brg* allele from exon 15 through 18 encoding part of the conserved ATPase domain (Sumi-Ichinose et al., 1997). Two *LoxP* sites (empty arrowheads) were inserted in introns 15 and 17. The Cre recombinase expressed in neural progenitors by the *Nestin-Cre* transgene recombines the two *LoxP* sites and results in the deletion of exons 16 and 17. If exon 15 splices to exon 18, translation of the Brg protein stops at aa 819. Western blot analysis of BAF subunits in cellular extracts from E13.5 control and *Brg* mutant cortices. Hsp90 is a loading control.

B. *Brg* is deleted in neural stem/progenitor cells by E12.5. Sagittal sections of E12.5 control (wt) and *Brg* mutant frontal cortices were immunostained with specific anti-Brg antibodies (red). mut, mutant.

C. *Brg* mutant brains are smaller and lack a cerebellum (arrows). Hematoxylin and eosin (H&E) staining of sagittal sections of E18.5 wt and *Brg* mutant brains.

D. Progressive depletion of cortical neural progenitors in the $Brg^{f/f}$*;Nestin-Cre* mice during embryogenesis. Upper panels: comparable sections of E12.5 and E17.5 cortices stained with anti-Nestin (red), anti-H3P (green) antibodies and DAPI (blue). Note the near absence of $Nestin^{+}$ cells in E17.5 *Brg* mutant cortices. Lower panels: similar cortical regions stained with TuJ1 antibody (red).

E. Defective neurosphere formation by *Brg*-deficient cortical cells. Primary neurospheres were cultured from dissociated E13.5 control and mutant cortices for 7 div. Secondary spheres were cultured from dissociated primary spheres (2 wt neurospheres and 20 *Brg* mutant neurospheres) for another 4 div.

F. *Brg*-deficient progenitors proliferate slower than wild-type progenitors. *Brg* mutant and control E14.5 cortical cells were cultured in serum-containing media for 15 hrs (1 div), stained with anti-LeX and BrdU antibodies and analyzed by flow cytometry. BrdU was added 3 hrs before the analysis. Representative images of n=3 independent experiments performed in duplicate are shown.

G. Brg is not required for glia proliferation *in vitro*. *Brg* mutant and control E16.5 cortical cells were cultured in serum-containing media (3 div) with or without CNTF (100 ng/ml), stained with anti-GFAP and BrdU antibodies and analyzed by flow cytometry. Results are expressed as mean ± SD. Western blot analyses of Brg (H-88) and GFAP protein levels in these cultures are shown. CNTF, ciliary neurotropic factor; GFAP, glial fibrillary acidic protein.

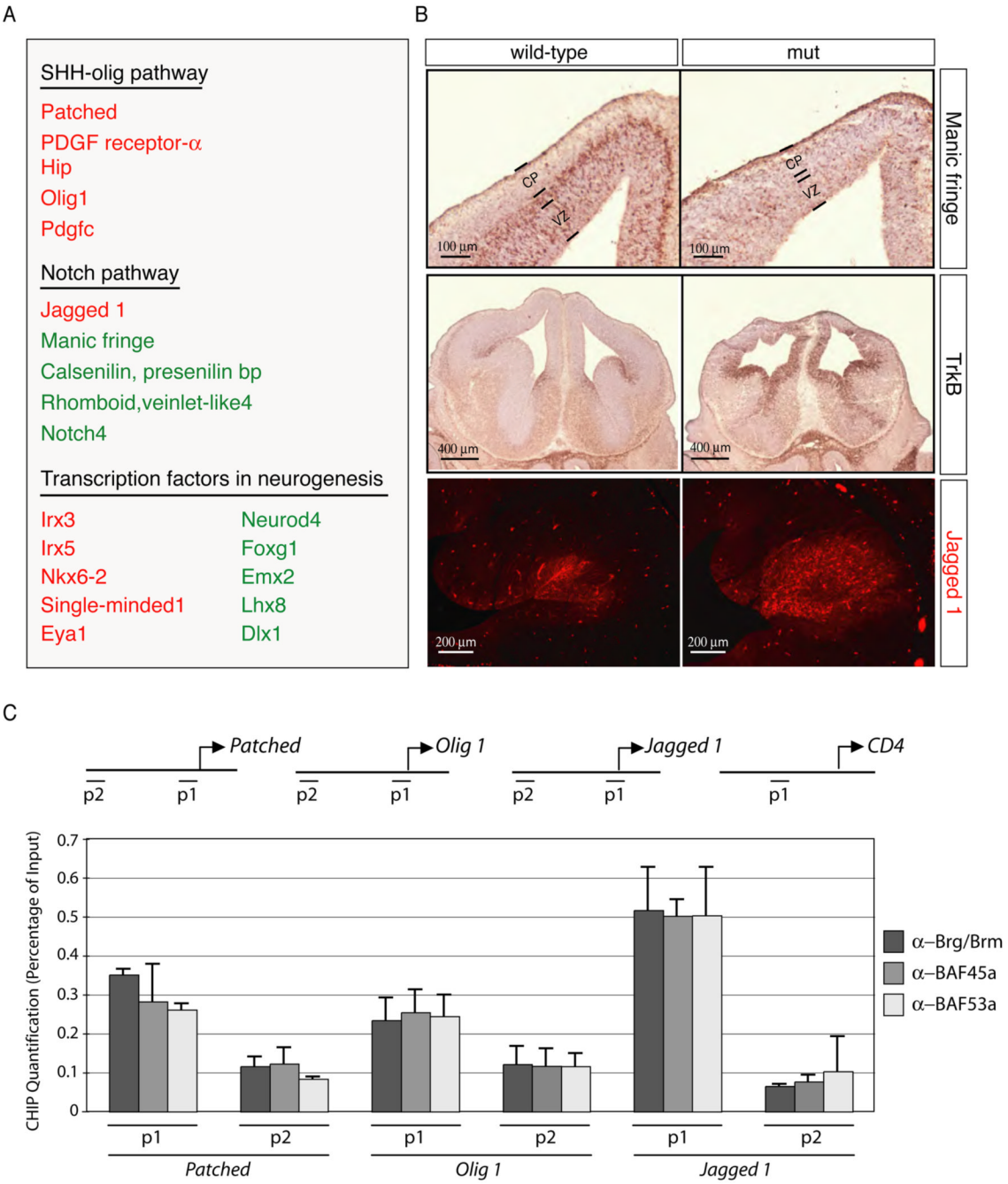

Figure 6. Brg-containing npBAF complexes regulate the transcription of Sonic Hedgehog (SHH) and Notch signaling components in neural development

A. Microarrays (Mouse 430 2.0 arrays (Affymetrix) containing 39,000 transcripts) were probed with cRNA generated from RNAs isolated from 3 control and 3 *Brg*-deficient E12.5 telencephalons. Default settings were used to normalize the data and for comparison. Selected genes changed more than two-fold ($p<0.01$) are shown. Genes that are downregulated in the absence of Brg appear in green and genes that are up-regulated are in red.

B. *In situ* hybridization and immunostaining analyses of transcriptional targets of npBAF complexes in the E13.5 mouse brain. Positive cells are dark brown (coronal sections). Upper panel: Expression of *Manic fringe* in newly-born neurons requires Brg. VZ: ventricular zone;

CP: cortical plate. Middle panel: Repression of *TrkB* expression in neural progenitors requires Brg. Lower panel: Increased Jagged1 protein levels in *Brg*-deficient basal forebrain.

C. BAF45a, BAF53a, Brg and npBAF complexes are present at the promoters of components of the SHH and Notch signaling pathways (P1). P2 regions are ~5 kb upstream. Anti-Brg/Brm, BAF45a and BAF53a antibodies were used for chromatin immunoprecipitation from E13.5 mouse forebrain lysates followed by quantitative PCR. A genomic element of the murine CD4 gene was used as a negative binding control (Chi et al., 2002). The relative binding abundance of each region is presented as percentage of the input DNA material. The data were normalized to the relative abundances of the CD4 P1 region and DNA precipitated by an anti-GFP antibody (negative control) (n=3 independent experiments). Primer sequences are available upon request.

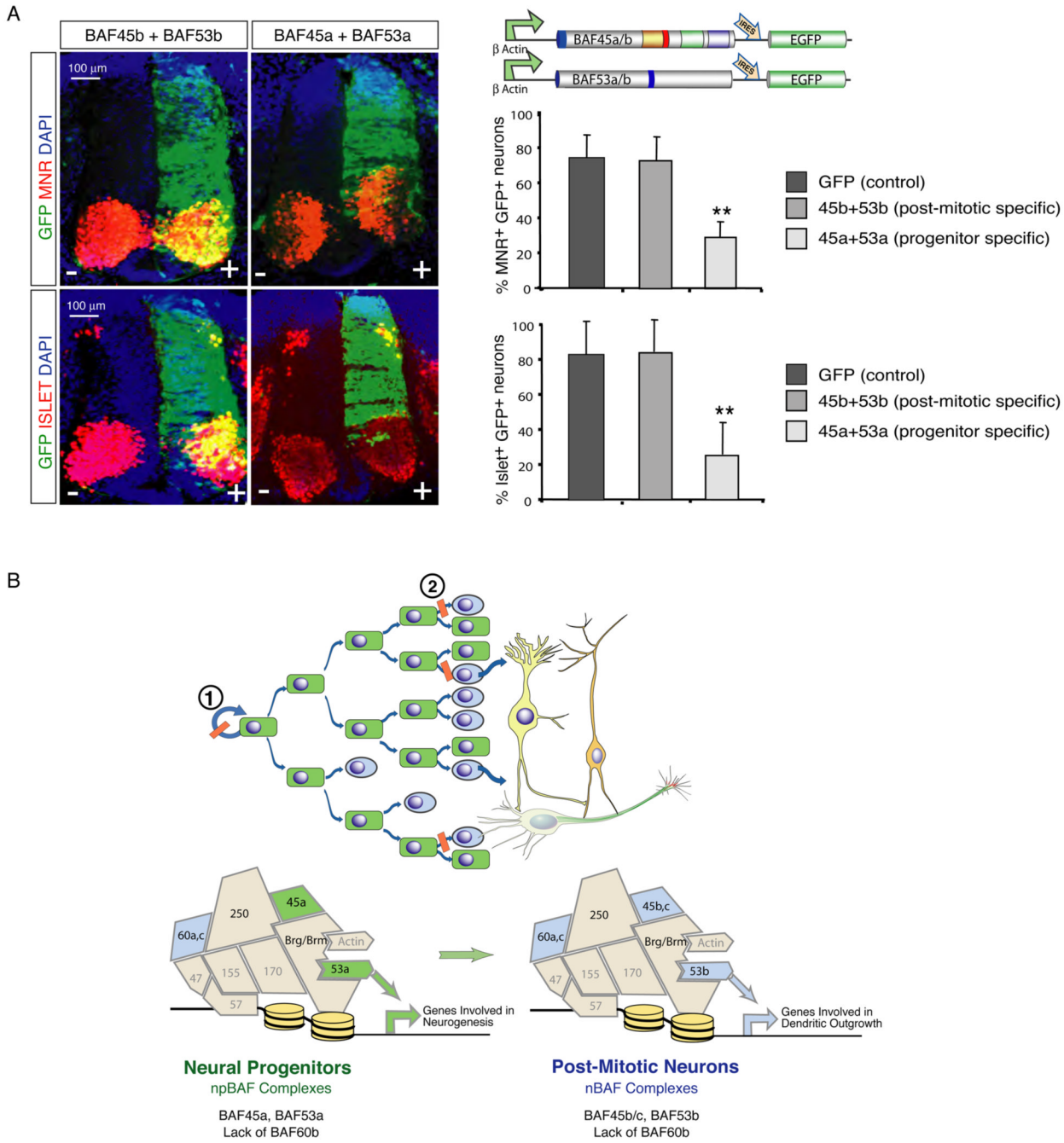

Figure 7. Repression of BAF45a and BAF53a is essential for neuronal differentiation

A. Left panels: expression of the post-mitotic-specific BAF53b and BAF45b subunits in neural progenitors of the embryonic chicken spinal cord does not interfere with neuronal differentiation. Representative images are shown. Note the high number of (yellow) double positive GFP$^+$ and MNR$^+$ or Islet$^+$ neurons. Right panels: forced expression of the npBAF progenitor-specific BAF45a and BAF53a subunits in post-mitotic neurons is incompatible with neuronal differentiation. Note the low number of (yellow) double-positive GFP$^+$ and MNR$^+$ or Islet$^+$ neurons and the reduced size of the spinal cord on the transfected side. MNR, Motor neuron reactive cells; Isl, Islet-1/2 reactive cells. A quantification of GFP$^+$ /MNR$^+$ or Islet$^+$ double positive neurons is shown. Results are expressed as mean ± SD. **, p<0.01.

B. A switch in subunit composition of SWI/SNF-like complexes underlies vertebrate neural development. We propose that Brg and npBAF complexes are required for neural stem cell self-renewal and proliferation (1). The exchange of npBAF and nBAF components is essential for the transition from proliferating neural stem/progenitor cells to post-mitotic neurons (2). BAF complexes are drawn in jigsaw puzzle configuration to denote the apparent fit of the subunits within the complexes. For example, BAF53 and actin directly interact with the ATPase domain of Brg (Zhao et al., 1998). However, the position of the other BAF subunits within the complexes has not been experimentally defined.

Table 1.

Core Subunits of the Neural BAF SWI/SNF-like ATP-dependent Chromatin Remodeling Complexes as Identified by a Proteomics Approach

BAF subunits	# peptides different[a] (unique[b])	Coverage (% mass)	Accessions	*p* scores
BAF250a/b;ARID1a/b/Osa1	75 (17)	-	[c]	1.00
ARID2	13 (8)	15.4	[d]	1.00
BAF190/Brm/Smarca2	10 (4)	17.5	NP_035546	1.00
BAF190/Brg/Smarca4	50 (0)	23.1	NP_035547	1.00
BAF180/polybromo-1	18 (4)	20.9	NP_240329[e]	1.00
BAF170/Smarcc2	34 (2)	31.3	NP_937803	1.00
BAF155/Srg3/Smarcc1	15 (0)	14.0	NP_033237	1.00
BAF60a/Smarcd1	10 (1)	17.9	NP_114030	1.00
BAF60c/Smarcd3	7 (1)	18.1	NP_080167	1.00
BAF57/Smarce1	15 (0)	49.6	NP_065643	1.00
BAF53a/Actl6a	11 (9)	29.3	NP_062647	1.00
BAF53b/Actl6b	12 (4)	30.5	NP_113581	1.00
BAF45a/PHF10	5 (2)	15.0	NP_077212	1.00
BAF45b/DPF1	22 (2)	43.0	NP_038902	1.00
BAF45c/DPF3	7 (3)	27.1	AAH48572	1.00
BAF45d/DPF2	8 (1)	30.2	NP_035392	1.00
BAF47/Ini1/mSnf5/Smarcb1	8 (0)	28.6	NP_035548	1.00
BAF42/actin[f]	13 (4)	-	-	-

[a] Number of different peptides for each protein entry.

[b] Number of peptides corresponding to a unique protein entry in the IPI human/mouse databases (Nesvizhskii et al., 2003).

[c] *Mus musculus* BAF250a/ARID1a/Osa, NP_291044; BAF250b/ARID1b, XP_139711.

[d] The protein sequence of mouse ARID2 (AT-rich interactive domain 2) will be submitted to NCBI. ARID2 is an evolutionarily conserved protein of 1161 amino acids with a typical ARID DNA-binding domain characteristic of a protein family that includes *Drosophila* OSA/eyelid, yeast Swi1p, yeast Rsc9 and mammalian BAF250a/ARID1a and BAF250b/ARID1b. *Drosophila* BAP170, a recently identified subunit of the SWI/SNF-like BAP complex is 43% similar and 26% identical to human ARID2 and most likely represents its orthologue in flies. From an independent purification, human ARID2 was identified and termed BAF200 (Yan et al., 2005).

[e] *Rattus norvegicus* Pb-1 protein sequence.

[f] α-, β- and γ-actin family of proteins (J.L. and G.R.C, unpublished results).