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Regulation of Dendritic Development by Neuron-Specific Chromatin Remodeling Complexes

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SUMMARY

The diversity of dendritic patterns is one of the fundamental characteristics of neurons and is in part regulated by transcriptional programs initiated by electrical activity. We show that dendritic outgrowth requires a family of combinatorially assembled, neuron-specific chromatin remodeling complexes (nBAF complexes) distinguished by the actin-related protein BAF53b and based on the Brg/Brm ATPases. nBAF complexes bind tightly to the Ca^{2+}-responsive dendritic regulator CREST and directly regulate genes essential for dendritic outgrowth. BAF53b is not required for nBAF complex assembly or the interaction with CREST, yet is required for their recruitment to the promoters of specific target genes. The highly homologous BAF53a protein, which is a component of neural progenitor and nonneural BAF complexes, cannot replace BAF53b's role in dendritic development. Remarkably, we find that this functional specificity is conferred by the actin fold subdomain 2 of BAF53b. These studies suggest that the genes encoding the individual subunits of BAF complexes function like letters in a ten-letter word to produce biologically specific meanings (in this case dendritic outgrowth) by combinatorial assembly of their products.

INTRODUCTION

The elaboration of dendritic trees is required for both the establishment of precisely wired neuronal networks and the integration of synaptic inputs (Jan and Jan, 2003; Scott and Luo, 2001). Both environmental cues and intrinsic genetic factors are thought to act in concert to regulate dendritic growth and remodeling. Neuronal activity, which triggers Ca^{2+} influx through voltage-sensitive calcium channels (VSCCs) and N-methyl-D-aspartate receptors (NMDARs), initiates multiple signaling pathways mediated by intermediates such as the Rho GTPases, Ras/MAPK, and CaMK, which ultimately shape the dendritic cytoskeleton by affecting actin reorganization as well as microtubule assembly and stability (reviewed in Chen and Ghosh, 2005; Miller and Kaplan, 2003). Among others, a critical interplay between growth-inhibitory Rho and growth-activating Rac-mediated signaling events is emerging as a key regulatory mechanism of dendritic morphogenesis (Luo, 2000; Van Aelst and Cline, 2004).

While rapid changes in intracellular Ca^{2+} levels can have an impact on dendrite dynamics locally, they also globally regulate dendrite growth and remodeling through transcription-dependent mechanisms (Wong and Ghosh, 2002). Ca^{2+}-induced signaling pathways mediated by CaMK and Ras/MAPK have been shown to regulate the activity of several transcription factors involved in activity-dependent dendritic growth, such as cAMP responsive element binding protein (CREB), CREB binding protein (CBP), and the bHLH protein NeuroD (Gaudilliere et al., 2004; Redmond et al., 2002). Ghosh and colleagues recently identified CREST, a Ca^{2+}-responsive transcriptional coactivator, as a mediator of activity-dependent dendritic morphogenesis (Aizawa et al., 2004). *CREST* $^{-/-}$ mice are viable at birth but display defects in cortical and hippocampal dendrite development (Aizawa et al., 2004). However, CREST has no DNA-binding ability, and it remains unclear how it participates in Ca^{2+}-dependent transcriptional activation of genes involved in dendritic development (Aizawa et al., 2004).

The regulation of chromatin structures is emerging as an important level of transcriptional control during neural development (Hong et al., 2005; Hsieh and Gage, 2005). At least three processes govern the accessibility of DNA to regulatory proteins: DNA methylation, histone modification, and ATP-dependent chromatin remodeling (Goldberg et al., 2007). ATP-dependent chromatin remodeling complexes use energy derived from ATP-hydrolysis to regulate nucleosome mobility and chromatin accessibility

(Levine and Tjian, 2003; Schreiber and Bernstein, 2002). The mammalian genome encodes 29 SWI2/SNF2-like ATPases, two of which, Brg and Brm, are alternative subunits of 2 MDa BAF (Brg/Brm associated factor) complexes resembling the yeast SWI/SNF, INO80, and SWR1 complexes (Khavari et al., 1993; Mizuguchi et al., 2004; Wang et al., 1996a).

Unlike the corresponding SWI/SNF-like complexes in yeast, flies, and worms, most of the ten core subunits of mammalian BAF complexes are encoded by gene families, and the complexes are combinatorially assembled (Olave et al., 2002a; Wang et al., 1996b). Work over the past several years has discerned the rules of combinatorial assembly using antibodies specific to the different family members of each BAF subunit. The ten subunits are encoded by 25 genes. The position of each subunit within the complex appears to be occupied by only one family member. Conversely, each subunit can potentially associate with all members of the other subunit families (Olave et al., 2002a; Wang et al., 1996a, 1996b). BAF complexes are highly stable (resist dissociation in 2 M NaCl), and the subunits do not show an appreciable rate of exchange when challenged with in vitro synthesized subunits (Rando et al., 2002; Zhao et al., 1998; and our unpublished results). Thus, combinatorial assembly could potentially generate several hundred diverse and stably assembled BAF complexes. Although the biological significance of this diversity is still unclear, changes in complex composition might confer functional specificity by providing distinct polymorphic surfaces for interaction with regulatory elements or DNA-binding transcription factors. This is supported by the fact that Brg is required for early embryonic development and is haploinsufficient for T lymphocyte differentiation and neural tube closure (Bultman et al., 2000; Chi et al., 2002)—processes that are not affected by the absence of the alternative homologous Brm subunit (Reyes et al., 1998).

The purification and proteomic analysis of endogenous BAF complexes from the developing brain revealed an exchange of subunits accompanying the developmental transition from proliferating neural progenitors to postmitotic neurons (Lessard et al., 2007). *N*eural *p*rogenitors contain a specialized family of BAF complexes (npBAF) that include the actin-related protein (Arp) BAF53a and a newly identified double-PHD domain subunit, BAF45a. As neural progenitors exit mitosis and differentiate into neurons, these subunits are replaced by the homologous BAF53b and BAF45b subunits. BAF53b, found exclusively in neurons, is a stoichiometric component of *n*euron-specific BAF complexes (nBAF) (Olave et al., 2002a). We recently demonstrated that Brg and the npBAF subunits, BAF45a and BAF53a, are required for neural stem cell/progenitor self-renewal and proliferation (Lessard et al., 2007). However, the role of nBAF complexes and ATP-dependent chromatin remodeling in postmitotic neuronal development remains largely unexplored.

Here, we have assessed the function of nBAF complexes in the developing nervous system by preparing mice specifically lacking BAF53b. *BAF53b*$^{-/-}$ mice have lethal defects in neuronal development, including defects in activity-dependent dendritic outgrowth. Endogenous nBAF complexes copurify with CREST, a Ca^{2+}-dependent transcriptional coactivator known to regulate dendrite growth and arborization (Aizawa et al., 2004). Together with CREST, nBAF complexes regulate the activity of genes essential for dendrite growth. We show that BAF53b is required for targeting nBAF complexes and CREST to the promoters of several of these genes. Strikingly, the specific function of BAF53b in dendritic development cannot be replaced by its close family member and npBAF subunit BAF53a, indicating that combinatorial assembly provides functional specificity to ATP-dependent chromatin remodeling complexes.

RESULTS

Generation of Mice Lacking the Postmitotic Neuron-Specific Subunit BAF53b

To gain insights into the role of nBAF complexes in postmitotic neuronal development, we reasoned that we could specifically compromise nBAF function by deleting the *BAF53b* gene, which encodes one of the two dedicated neuron-specific subunits. Mice lacking exons 2 and 3 of *BAF53b* were generated by homologous recombination (Figure 1A). *BAF53b* mRNA was not detectable in the homozygous mice (data not shown), nor was there detectable protein (Figure 1B, right panel), indicating that the *BAF53b* mutant allele is a null. Mice lacking *BAF53b* develop normally in utero and appear morphologically indistinguishable from their littermates at birth. *BAF53b*$^{-/-}$ mice are born at the expected Mendelian ratio, but most die within 2 days, likely from a failure to nurse. In a mixed genetic background between 129/sv and C57BL/6 strains, ~25% of the *BAF53b*$^{-/-}$ mice survive beyond postnatal day 2 (P2), and ~12% survive to adulthood. *BAF53b*$^{-/-}$ mice that escape the perinatal lethality are extremely hyperactive, a phenotype that commonly points to neural developmental abnormalities. Consistent with the neuron-restricted pattern of *BAF53b* expression, we have not detected any nonneural phenotypes in the *BAF53b* null mice. *BAF53b* heterozygotes are indistinguishable from wild-type littermates, and both genotypes were used as controls in our studies.

BAF53b Is Not Essential for Assembly of nBAF Complexes

Understanding the function of other Arps in chromatin remodeling has been complicated by their requirement for complex assembly. For example, null mutations of the yeast Arp8 or Arp4 subunits give rise to INO80 and NuA4 complexes that are not fully assembled (Galarneau et al., 2000; Shen et al., 2003). Silver-staining of purified BAF complexes from the *BAF53b*$^{-/-}$ adult brain revealed the presence of all ten known core subunits except BAF53 (Figure 1C, compare lanes 1 and 2), indicating that BAF53b is not required for complex assembly. BAF53a, which is

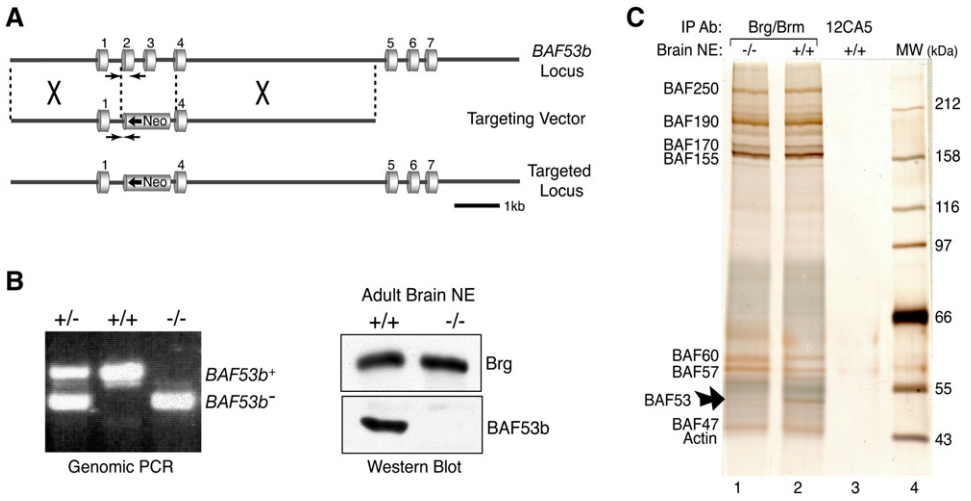

Figure 1. BAF53b Is Not Required for Assembly of SWI/SNF-like nBAF Complexes

(A) Production of mice lacking the neuron-specific *BAF53b* gene. The genomic structure of the *BAF53b* locus containing exons 1 through 7 is shown. The targeting vector contains 2 kb and 4.5 kb genomic fragments flanking exons 2 and 3, which were replaced by a Neo cassette in the recombined allele. The PCR primers for genotyping are indicated by opposing arrows.

(B) BAF53b protein is absent from *BAF53b*$^{-/-}$ brains. Left: PCR genotyping with primers shown in (A). Right: Western blot analysis of adult brain nuclear extracts with antibodies specific for BAF53b. Brg is a loading control.

(C) nBAF complexes are fully assembled without BAF53b. Silver-stained analyses of BAF complexes immunoprecipitated with anti-Brg/Brm antibody (J1) from adult wild-type and *BAF53b*$^{-/-}$ brain nuclear extracts (lanes 1 and 2). Note the absence of the BAF53 band and the presence of all other bands in the purified *BAF53b*$^{-/-}$ nBAF complex. Anti-HA antibody 12CA5 was used as a negative control (lane 3).

strictly expressed in neural stem cells and progenitors of the developing central nervous system (CNS) (Lessard et al., 2007), was not detectable in the BAF53b-deficient complexes purified from the adult brain, which contains mostly neurons and glia (Figure 1C and data not shown). Western blot and in situ hybridization analyses showed that BAF53a is not activated to compensate for BAF53b deficiency. As BAF53b is strictly expressed in neurons (Olave et al., 2002a), the phenotype of the *BAF53b*$^{-/-}$ mice strictly reflects the function of the neuronal BAF53b protein and the family of postmitotic neuronal complexes that contain it. Because BAF53a does not appear in the purified complexes from *BAF53b*$^{-/-}$ brains (Figure 1C), we conclude that glia have a different Brg/Brm-based complex. Thus, we use the term nBAF to designate the complexes specific to *n*eurons.

BAF53b$^{-/-}$ Neurons Are Defective in Activity-Dependent Dendrite Growth

The elaboration of dendrites and axons is one of the most characteristic and fundamental features of neurons. Morphologically, the brains of the P0 *BAF53b*$^{-/-}$ mice appear grossly normal (see Figure S1 in the Supplemental Data available with this article online and data not shown). Whole-mount neurofilament staining of E10.5 embryos indicated that initial neurite outgrowth is grossly normal in the absence of BAF53b (Figure S2A and data not shown). To better understand the basis of the defects in neural development of the *BAF53b*$^{-/-}$ mice, we cultured hippocampal neurons from mouse E18.5 brains. Synapse formation was severely impaired in long-term low-density cultures (16 days in vitro [DIV]) as shown by coincident staining of the presynaptic synapsin 1 and the postsynaptic PSD95 markers (data not shown). This reduction in synapse number might explain the perinatal lethality but appeared to be secondary to defects in dendritic arborization. Extensive analyses revealed that *BAF53b*$^{-/-}$ hippocampal neurons have significantly shorter and less complex dendritic trees than controls (Figure 2A). Measurement of MAP2-stained *BAF53b*$^{-/-}$ hippocampal neurons demonstrated an ~50% reduction of the total dendritic length relative to controls (Figure 2A, bar graph). The complexity of distal dendritic branches of *BAF53b*$^{-/-}$ neurons was also significantly reduced, as shown by standard Sholl analysis, suggesting a dendrite extension and/or nonprimary branching defect (Figure 2A, lower right).

Dendritic outgrowth occurs in two phases: activity independent and activity dependent (Van Aelst and Cline, 2004; Wong and Ghosh, 2002). Hence, reduction of dendritic length and complexity could be caused by an activity-dependent mechanism or an intrinsic, activity-independent outgrowth defect. To address this issue, we cultured hippocampal neurons for 5 days (before any synapses are formed) and used KCl-induced depolarization to mimic neuronal activity (Aizawa et al., 2004; Yu and Malenka, 2003). Although the dendritic growth of the nonstimulated *BAF53b*$^{-/-}$ neurons appeared normal, a significant reduction in activity-dependent dendritic outgrowth was observed in the *BAF53b*$^{-/-}$ cultures (Figure 2B). This defect cannot be attributed to a lack of Ca^{2+} influx

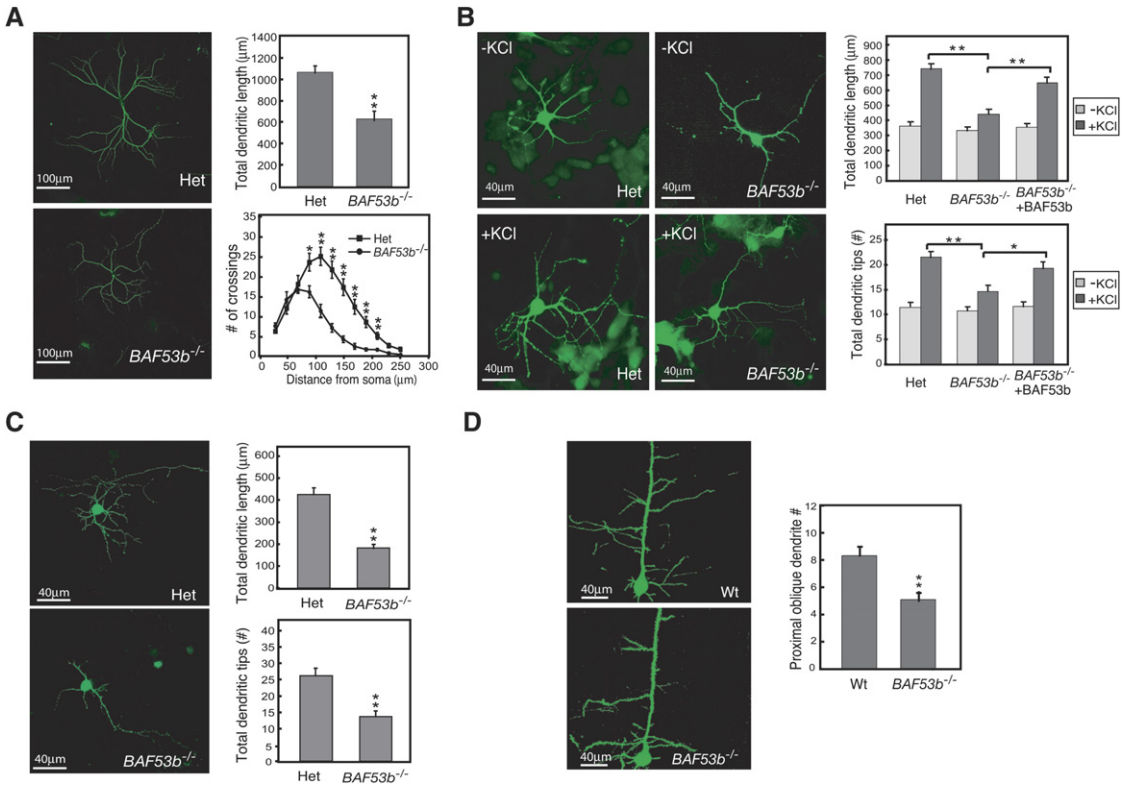

Figure 2. *BAF53b*$^{-/-}$ Neurons Are Defective in Activity-Dependent Dendritic Growth

(A) Long-term cultured *BAF53b*$^{-/-}$ hippocampal neurons have shorter and less complex dendrites. Left panels: Representative pictures of MAP2-stained hippocampal neurons cultured for 16 DIV. Upper right: Quantification of total dendritic length of neurons (n = 50). Bottom right: Standard Sholl analysis of neurons with more than three primary dendrites (n = 15).

(B) Activity-dependent dendrite development is impaired in cultured *BAF53b*$^{-/-}$ hippocampal neurons. Left panels: Representative images of GFP-transfected hippocampal neurons from control or *BAF53b*$^{-/-}$ mice cultured for 5 DIV with or without KCl treatment. Right panels: Comparison of the total dendritic length and total dendritic tip numbers of these neurons (n = 25). *BAF53b*$^{-/-}$ neurons transfected with a pBAF53b-IRES-GFP construct were labeled as *BAF53b*$^{-/-}$ +BAF53b.

(C) Defective dendrite growth of cultured *BAF53b*$^{-/-}$ cerebellar granule neurons. Left panels: Representative pictures of GFP-transfected P7 *BAF53b*$^{+/-}$ or *BAF53b*$^{-/-}$ cerebellar granule neurons cultured for 5 DIV with media containing 24.5 mM KCl. Right panels: Comparison of total dendritic length and total dendritic tip numbers of these neurons (n = 30).

(D) Fewer oblique dendrite branches in layer 5 pyramidal neurons in *BAF53b*$^{-/-}$ cortex. Left panels: Representative images of GFP-labeled cortical layer 5 pyramidal neurons from P18 GFPM *BAF53b*$^{+/+}$ and GFPM *BAF53b*$^{-/-}$ mice. Right panel: Quantification of the proximal oblique dendrite branch numbers of these neurons. The oblique branches of the apical dendrites located within 200 μm from the cell bodies were counted (n = 15).

*t test, $p < 0.05$; **t test, $p < 0.01$. All quantifications are presented as mean ± SEM.

via voltage-sensitive Ca^{2+} channels (VSCCs), as the activity-dependent phosphorylation of CREB at Ser 133 (Sheng et al., 1991) was comparable between *BAF53b*$^{-/-}$ and control neurons (data not shown). No significant increase in TUNEL staining was observed in the *BAF53b*$^{-/-}$ cultures, indicating that BAF53b is not required for neuron survival (Figure S3). Importantly, expression of exogenous BAF53b in *BAF53b*$^{-/-}$ neurons restored the activity-dependent dendrite growth defect (Figure 2B) indicative of a direct role for BAF53b in regulating this process. We also observed similar defects in cultured *BAF53b*$^{-/-}$ P7 cerebellar granule neurons and E14.5 cortical neurons (Figure 2C and data not shown), pointing toward a general role of BAF53b in activity-dependent dendritogenesis.

To examine the in vivo requirement of BAF53b in dendritic development, we crossed the *BAF53b*$^{-/-}$ mouse to a Thy1-GFP transgenic mouse strain (GFPM) (Feng et al., 2000). This allowed us to visualize the stereotypical dendrite pattern of cortical layer 5 pyramidal neurons in brain slices. *BAF53b*$^{-/-}$ apical dendrites showed a reduced number of oblique branches relative to controls (Figure 2D). The number of oblique dendrites of Golgi-stained *BAF53b*$^{-/-}$ neurons was also significantly reduced compared to controls (data not shown). Thus, BAF53b appears to be necessary for full dendrite complexity in vivo. Because in BAF53b-deficient nBAF complexes, BAF45b and the other subunits are still present (Figure 1C), the phenotypes of *BAF53b*$^{-/-}$ neurons might

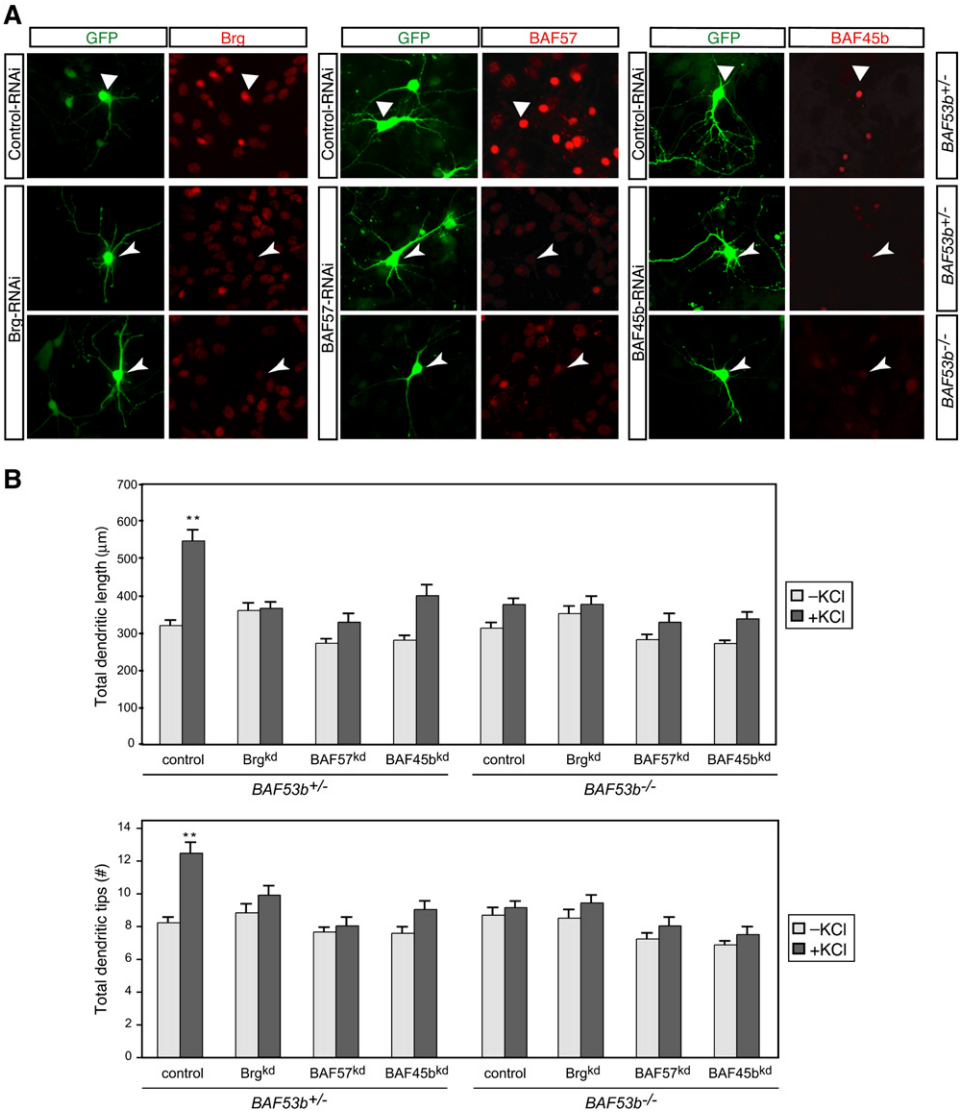

Figure 3. Neuron-Specific nBAF Complexes Are Required for Activity-Dependent Dendritic Outgrowth

(A) Representative images of heterozygous (control) or *BAF53b*$^{-/-}$ hippocampal neurons cotransfected with GFP and Brg RNAi constructs, BAF57 RNAi constructs, BAF45b shRNA oligos, or control RNAi constructs. Protein levels of Brg, BAF57, and BAF45b were examined by immunostaining. Brg and BAF57 are both highly expressed in neurons (triangle arrowhead), and their expression levels are substantially reduced in GFP^{+} Brg^{kd} and BAF57^{kd} neurons, respectively (arrows). Cocultured glial cells express low levels of Brg and BAF57 (dull nuclei). BAF45b is exclusively expressed in neurons.

(B) Comparison of the total dendritic length and total dendritic tip numbers of neurons transfected with either control or nBAF (Brg, BAF57, and BAF45b) RNAi constructs (n = 25–35 neurons per condition). Results are expressed as mean ± SEM.

result from compromise of the complexes rather than a full loss of activity.

nBAF Complexes Are Required for Activity-Dependent Dendritic Growth

BAF53a has been identified in a number of different chromatin remodeling complexes (Fuchs et al., 2001; Ikura et al., 2000). To determine whether the role of BAF53b in dendritic morphogenesis is mediated by nBAF complexes and whether BAF53b is essential for nBAF function, we used an RNAi strategy to specifically knock down the ATPase subunit Brg and other nBAF subunits in heterozygous (control) and *BAF53b*$^{-/-}$ neurons. The double-PHD domain subunit BAF45b is specifically expressed in postmitotic neurons and is a stoichiometric component of nBAF complexes (Figure 3A and Lessard et al., 2007). BAF57, an HMG domain-containing protein encoded by a single gene, is ubiquitously expressed and is a core component of BAF complexes in all cell-types (Wang et al., 1998). Past studies indicated that inactivation of

BAF57 produces phenotypes similar to the deletion of the Brg ATPase (Chi et al., 2002; G.R.C., unpublished data). Brg and BAF57 are highly expressed in cultured hippocampal neurons (Figure 3A, upper panels, bright nuclei) but at lower levels in cocultured glial cells (Figure 3A, upper panels, dull nuclei). Immunofluorescence and western blot analyses confirmed that Brg, BAF57, and BAF45b protein levels were substantially reduced in Brg^{kd}, $BAF57^{kd}$, and $BAF45b^{kd}$ neurons, respectively (5 days posttransfection; Figure 3A, lower panels and data not shown). Importantly, Brg^{kd}, $BAF57^{kd}$, and $BAF45b^{kd}$ neurons phenocopied the activity-dependent dendritic growth defects observed in *BAF53b*$^{-/-}$ neurons, in regard to both dendritic length and branch numbers (Figure 3B). An siRNA with limited homology to any known sequence in the mouse genome was used as a negative control and had little effect (Figure 3). Because BAF53b, BAF57, and BAF45b are stoichiometrically associated with Brg/Brm-containing nBAF complexes in postmitotic neurons (Lessard et al., 2007), these studies demonstrate that nBAF chromatin remodeling complexes are specifically required for activity-dependent dendritic growth.

RNAi knockdown of Brg, BAF57, and BAF45b in *BAF53b*$^{-/-}$ background did not increase the severity of the dendritic growth phenotype of *BAF53b*$^{-/-}$ neurons (Figures 3A and 3B), suggesting that the function of nBAF complexes in activity-dependent dendritic development is BAF53b dependent. Reducing Brg, BAF57, and BAF45b protein levels did not cause cell death in control neurons and only mildly increased cell death in *BAF53b*$^{-/-}$ neurons (TUNEL assays; Figure S3), indicating that nBAF complexes are not required for neuron survival. However, since BAF53b is only one of the two neuron-specific nBAF subunits and the BAF53b-deficient complexes are fully assembled, it remains possible that a genetic knockout of BAF45b or other nBAF core subunits in *BAF53b*$^{-/-}$ neurons would produce a more severe phenotype.

Deletion of BAF53b Leads to Abnormal Axonal Development

Grossly normal neurofilament staining of E10.5 *BAF53b*$^{-/-}$ embryos suggested that BAF53b is not required for initial neurite outgrowth and pathfinding (Figure S2A and data not shown). However, closer examination of the *BAF53b*$^{-/-}$ postnatal brains suggested that axonal myelination is impaired. Toluidine Blue staining of P18 hippocampal cross sections indicated a reduction in the size of the myelinated axon bundles that run through the hippocampal alveus in the *BAF53b*$^{-/-}$ mice (Figure S2B, upper panel, dark staining), suggestive of fewer axons and/or fewer myelinated axons. Electron microscopy also confirmed the size reduction of the myelinated axon bundles in the *BAF53b*$^{-/-}$ hippocampus (Figure S2B, middle panel) and indicated a 1.7-fold reduction in the percentage of the myelinated axons within these clusters (Figure S2B, lower panels). Therefore, the axons of *BAF53b*$^{-/-}$ neurons are undermyelinated. This was surprising, as BAF53b expression is undetectable by immunostaining in oligodendrocytes, which make myelin (Figure S2C). Thus, the myelination defect is likely secondary to a failure of neurons to produce a growth or differentiation factor for oligodendrocytes. Another possibility is that, in the absence of BAF53b, a reduction in axon formation and the subsequent defects in electrical activity hamper oligodendrocyte development and myelination (Barres and Raff, 1993). The latter would be consistent with the rather severe reduction in synapse formation by cultured *BAF53b*$^{-/-}$ neurons.

CREST, a Ca^{2+}-Dependent Regulator of Dendritic Development, Specifically Interacts with Postmitotic nBAF Complexes

Previous studies have shown that complexes containing only Brg, BAF155, and BAF47 but lacking BAF53 are sufficient for in vitro activities of these complexes, such as nucleosome mobility or remodeling (Phelan et al., 1999; Wang et al., 1998; and our unpublished studies), yet the other subunits are required for the biological activities. To understand the molecular mechanisms underlying BAF53b and nBAF function in vivo, we turned to a proteomics strategy and examined proteins that copurify with the endogenous complexes in the developing mouse brain (see Supplemental Experimental Procedures and Lessard et al., 2007). Under stringent purification conditions, we identified peptides from several transcriptional regulators, which represent putative candidates for mediating BAF53b and nBAF function in developing neurons (J.L. and G.R.C., unpublished data). Strikingly, one of these was the Ca^{2+}-responsive coactivator CREST, a known regulator of activity-dependent dendritic morphogenesis (Aizawa et al., 2004) (two independent peptides; Figures 4A and 4B). Cortical and hippocampal neurons from *CREST*$^{-/-}$ mice are compromised in dendritic development (Aizawa et al., 2004), a phenotype similar to that observed in *BAF53b*$^{-/-}$ mice. The interaction between CREST and nBAF complexes is specific, as endogenous CREST, Brg, and other components of nBAF complexes coimmunoprecipitated using antibodies directed to Brg in P0 brain nuclear extracts (Figure 4C). In contrast, CtBP, CREB, and SYT, another CREST family member, did not coimmunoprecipitate (Figure 4C and data not shown). Reciprocally, using antibodies against CREST, we found that endogenous Brg, BAF53b, and BAF170 were coimmunoprecipitated, indicating considerable dedication of CREST to nBAF complexes (Figure S4). BAF53b is dispensable for the interaction between CREST and nBAF complexes, as the interaction persists in *BAF53b*$^{-/-}$ neurons (Figure 4C). Thus, CREST may interact with nBAF complexes through other BAF subunits. Glycerol gradient fractionation experiments of E15.5 brain nuclear extracts indicated that a significant proportion of endogenous CREST protein cosediments at 2 MDa with Brg, BAF53b, and other core subunits of nBAF complexes (Figure 4D), suggesting substantial interactions between CREST and nBAF complexes in neurons. However, we do not consider CREST as a core subunit of nBAF complexes due to the relatively mild conditions required for coimmunoprecipitation

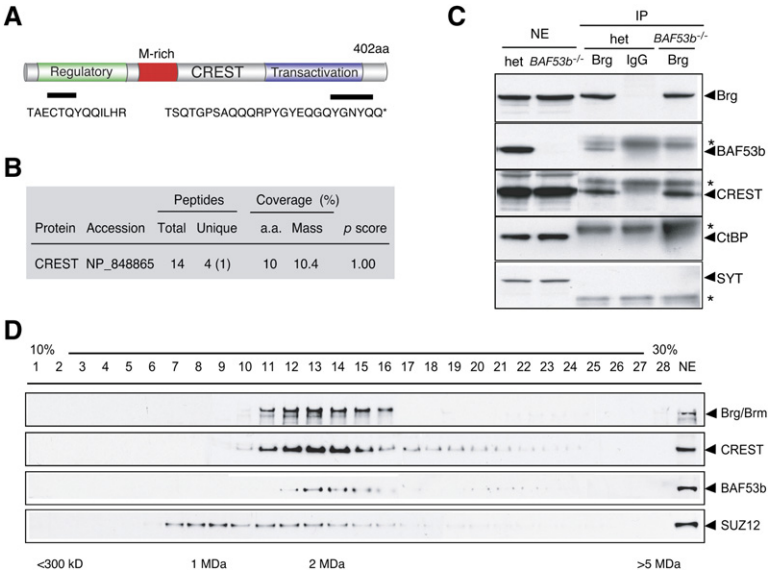

Figure 4. The Ca^{2+}-Responsive Dendritic Regulator, CREST, Interacts with Endogenous nBAF Complexes

(A) Peptides identified in the CREST protein by mass spectrometry analyses. *Peptide solely corresponding to CREST (Aizawa et al., 2004).

(B) Summary of CREST peptides and probability of identity. The numbers in parentheses are numbers of peptides corresponding to unique protein entries.

(C) Endogenous CREST specifically interacts with endogenous nBAF complexes. P0 control or *BAF53b*$^{-/-}$ brain nuclear extracts were immunoprecipitated with anti-Brg/Brm (J1) antibody and blotted with antibodies against Brg, BAF53b, CREST, CtBP, or SYT. Rabbit IgG was used as a negative control for IP. *Rabbit IgG heavy chain. Note that the BAF53b band runs just below the IgG heavy chain.

(D) CREST and BAF53b cosediment with nBAF complexes at 2 MD in E15.5 mouse brain nuclear extracts. The Polycomb protein SUZ12 of the PRC2 complex was blotted as a control and peaks in the ~1 MDa fractions.

(0.3 M NaCl and 0.2% NP40). In contrast, core subunits remain associated under highly stringent conditions (0.3 M NaCl, 1% NP40, 0.5% deoxycholate, and 0.1% SDS) (Lessard et al., 2007; Wang et al., 1996a; Zhao et al., 1998).

nBAF Complexes Regulate Genes Important for Neurite Outgrowth

To determine whether nBAF complexes control dendrite development by regulating a transcriptional program critical for dendritic growth, we defined the genes dependent upon BAF53b using RNA isolated from P16 heterozygous (control) or *BAF53b*$^{-/-}$ hippocampi. Student's t test comparison yielded 163 transcripts with significant difference in expression levels (106 genes increased and 57 genes decreased in *BAF53b*$^{-/-}$ hippocampi out of 39,000 spots; Figure S5 and data not shown). However, the changes were not large (mostly between 1.3- and 2.5-fold), possibly reflecting the fact that the *BAF53b*-deficient nBAF complexes are fully assembled, contain another neuron-specific subunit (BAF45b), and display full ATPase activity in vitro (data not shown).

mRNA levels of several essential regulators of neurite outgrowth (e.g., *GAP43*, *Stmn2*, *Rap1A*, and *Gprin1*) were altered in the absence of BAF53b, suggesting that BAF53b and nBAF complexes control a transcriptional program of dendritic development (Figure 5A and Figure S5). Importantly, several Rho family GTPase regulators were misregulated in *BAF53b*$^{-/-}$ neurons (Figure 5A). Rho-GTPases are important players in activity-dependent dendritic development (Luo, 2002; Van Aelst and Cline, 2004). Notably, the expression of *Ephexin1*, a *dbl* family Rho-GEF, was significantly decreased in both *BAF53b*$^{-/-}$ and *CREST*$^{-/-}$ neurons (Figure 5B, left panel). The protein levels of Ephexin1 as well as another BAF53b target gene, GAP43, were similarly reduced in *BAF53b*$^{-/-}$ neurons (Figure 5B, right panel). Ephexin1 plays important roles in both axon growth and Eph-dependent growth cone collapse, possibly by shifting the balance between the Rho and Rac/CDC42 signaling pathways (Sahin et al., 2005; Shamah et al., 2001). Hence, we reasoned that if Ephexin1 was a key nBAF/CREST target gene for activity-regulated dendrite patterning, increasing Ephexin1 levels should rescue the *BAF53b*$^{-/-}$ dendritic growth defect. Indeed, expression of exogenous Ephexin1 protein in *BAF53b*$^{-/-}$ neurons significantly corrected the dendritic extension and branching defects upon KCl activation, yet had little effect on untreated neurons (Figure 5C and Figure S6). *Ephexin1* overexpression in control neurons impaired activity-dependent dendrite growth (Figure 5C), consistent with Ephexin1's known role in regulating the balance between the inhibitory Rho and the activating Rac/CDC42 signaling pathways (Sahin et al., 2005; Shamah et al., 2001). However, Ephexin1 has four other closely related family members (Sahin et al., 2005). The phenotype of the *Ephexin1* null mouse is relatively mild and in one published study, *Ephexin1*$^{-/-}$ mice were not examined for a dendritic growth defect (Sahin et al., 2005). Hence, rescue of the dendritic phenotype could occur in the context of an already compromised state. Nevertheless, as *Ephexin1* expression is regulated by both CREST and nBAF complexes, *Ephexin1* is a suitable biological target to probe the mechanism of the CREST-nBAF interaction in dendritic patterning.

BAF53b Is Required to Target nBAF Complexes and CREST to Specific Promoters

To understand the significance of the interaction between CREST and nBAF complexes in neurons, we examined in vivo promoter occupancy of BAF53b-dependent genes that are essential for dendritic outgrowth. Using chromatin immunoprecipitation (ChIP) assays in P2 mouse forebrains or cultured E15.5 cortical neurons (that predominantly

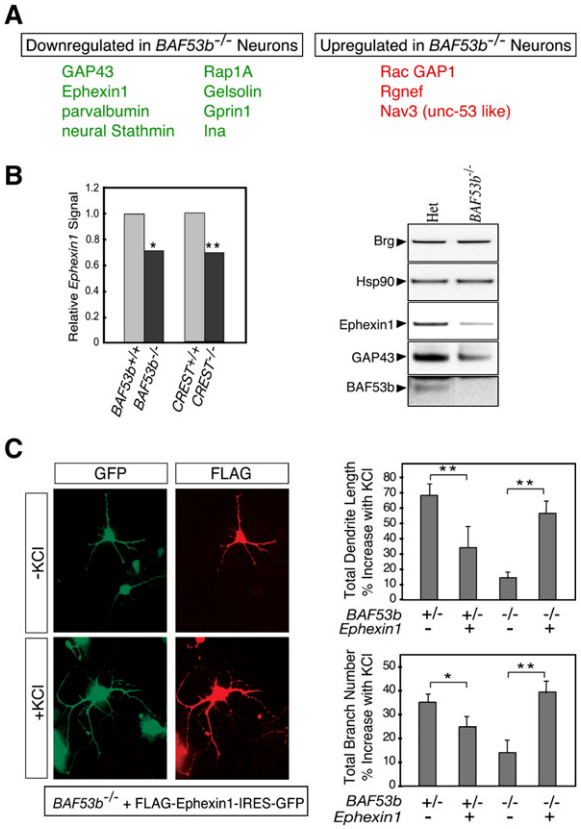

Figure 5. Regulation of *Ephexin1* Expression by nBAF Complexes Is Required for Activity-Dependent Dendritic Growth

(A) BAF53b regulates genes important for neurite outgrowth. List of genes: Genes known to function in neurite outgrowth that were misregulated in the *BAF53b*$^{-/-}$ hippocampal neurons detected by microarray analysis. Genes that were significantly increased in *BAF53b*$^{-/-}$ neurons are shown in red, genes decreased are in green (t test, $p < 0.05$).

(B) *Ephexin1* expression levels are reduced in *BAF53b*$^{-/-}$ neurons. Left panel: Relative *Ephexin1* microarray signals comparing *BAF53b*$^{-/-}$ or *CREST*$^{-/-}$ with wild-type neurons. Right panel: Western blot analyses of cultured E15.5 cortical neuron lysates from control and *BAF53b*$^{-/-}$ mice with antibodies against Brg, Hsp90, Ephexin1, and BAF53b are shown.

(C) Increasing Ephexin1 levels rescues the activity-dependent dendrite growth defect of *BAF53b*$^{-/-}$ neurons. Left panels: Representative images of *BAF53b*$^{-/-}$ hippocampal neurons transfected with a FLAG-Ephexin1-IRES-GFP construct and cultured for 5 days with or without KCl treatment. Right panels: Comparison of the total dendritic length and total dendritic tip numbers of control and *BAF53b*$^{-/-}$ neurons transfected with either GFP or Ephexin1 expression constructs (four experiments, n = 25–35 neurons each).

*t test, $p < 0.05$; **t test, $p < 0.01$. Results are presented as mean ± SEM.

contain nBAF complexes where BAF53b and BAF45b are stoichiometrically associated with Brg/Brm) and affinity-purified antibodies, we examined the *Ephexin1* gene locus and found that both CREST and the Brg/Brm-containing nBAF complexes directly bind the promoter region (i.e., 500 bp around the transcription initiation site; Figure 6 and data not shown). Interestingly, the binding of nBAF complexes to the *Ephexin1* promoter was significantly reduced in *BAF53b*$^{-/-}$ neurons, suggesting a BAF53b-dependent binding mechanism (Figures 6A and 6B, Ephexin1). CREST binding to the *Ephexin1* promoter was also significantly diminished (Figures 6A and 6B, Ephexin1), consistent with the hypothesis that CREST requires nBAF complexes to bind the *Ephexin1* promoter. Indeed, CREST has no DNA-binding ability (Aizawa et al., 2004) and might be recruited to promoter elements through direct interactions with other nBAF subunits such as BAF250 (Z.Q. and A.G., unpublished data). We found that expression of *GAP43*, a gene important for neurite outgrowth (Strittmatter et al., 1995), is also reduced in *BAF53b*$^{-/-}$ neurons (Figures 5B and 7D). Both CREST and nBAF complexes directly bound the *GAP43* promoter, and the occupancy was similarly dependent upon BAF53b (Figures 6A and 6B, GAP43). The reduction of *Ephexin1* and *GAP43* levels in *BAF53b*$^{-/-}$ neurons most likely results from the absence of CREST and nBAF complexes at the promoter.

Examination of other BAF53b target genes, either decreased or increased in *BAF53b*$^{-/-}$ neurons, indicated that promoter occupancy by both Brg/Brm and CREST is generally reduced in the absence of BAF53b (Figure 6B). One exception was the *RacGAP1* promoter, which is overexpressed in *BAF53b*$^{-/-}$ neurons (Figure 5A and data not shown) and whose binding by CREST and nBAF complexes was BAF53b independent (Figures 6A and 6B, RacGAP1). The observation that BAF53b is not necessary for binding of nBAF complexes to the *RacGAP1* promoter but is required for the activity of this promoter suggests that BAF53b's function probably extends beyond recruitment of the complexes to genetic loci (Lessard et al., 2007; Rando et al., 2002; Zhao et al., 1998). In contrast, several genes whose expression levels were not changed in *BAF53b*$^{-/-}$ neurons had similar levels of both Brg/Brm and CREST at their promoters (Figure 6B), suggestive of a BAF53b-independent recruitment mechanism. Notably, the expression levels of *patched*, a direct target of *n*eural *p*rogenitor BAF (npBAF) complexes, were not affected by the absence of *BAF53b*, and its promoter remained bound by both Brg/Brm and CREST in *BAF53b*$^{-/-}$ neurons. An intragenic region of the *CD4* gene, which is a target of BAF complexes in T lymphocytes (Chi et al., 2003), showed no detectable occupancy under comparable conditions and served as a negative control (Figure 6, CD4). Together, these studies suggest that BAF53b is specifically required for promoter occupancy of a group of target genes by nBAF complexes and CREST. The observation that BAF53b is generally required for promoter occupancy by CREST but is dispensable for the interaction of CREST with nBAF complexes further suggests that CREST is recruited to these genetic loci via an interaction with other nBAF subunits.

BAF53b Specifically Promotes Dendritic Outgrowth through Its Actin Fold Subdomain 2

A central question regarding the mechanisms of ATP-dependent chromatin remodeling is the degree to which

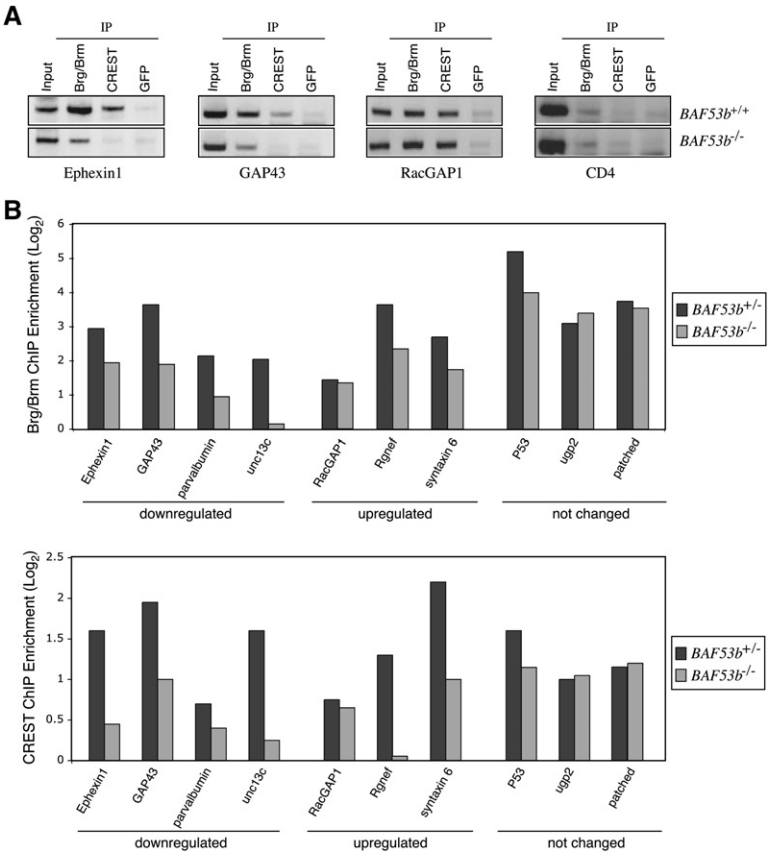

Figure 6. BAF53b Is Required for Promoter Occupancy of Specific Target Genes by nBAF Complexes and CREST

Chromatin immunoprecipitation (ChIP) assays of P2 mouse forebrains or cultured 5 DIV E15.5 wild-type and *BAF53b*$^{-/-}$ cortical neurons were carried out using the affinity purified J1 anti-Brg/Brm or anti-CREST antibodies. Anti-GFP antibody was used as a negative control. Primers were designed so that the PCR products are within 500 bp upstream of the transcription initiation site (promoter region).

(A) Representative pictures of ChIP products separated on agarose gels.

(B) A representative ChIP experiment (E15.5 cultured neurons) quantified by real-time PCR with the intragenic region of the murine CD4 gene as a negative control reference (Chi et al., 2002). We first determined the abundance of the promoter regions relative to the reference in the anti-Brg/Brm, anti-CREST, and anti-GFP antibodies precipitated materials. We then divided the abundance of the promoter region in the antibody-precipitated material by that in the anti-GFP control to calculate the "ChIP enrichment."

biological diversity is created by combinatorial assembly of the complex subunits (Olave et al., 2002a; Wang et al., 1996a, 1996b). To begin to address this question, we took advantage of the *BAF53b*$^{-/-}$ mouse and tested the ability of the highly homologous BAF53a protein (87% identical at the amino acid level) to rescue the perinatal lethality and neural developmental defects due to *BAF53b* deficiency. Expression of a *BAF53b* transgene under the control of a ubiquitous promoter significantly suppressed the lethal defects of the *BAF53b*$^{-/-}$ mice (Figure 7A). However, a *BAF53a* transgene expressed at similar levels (using the same promoter) failed to rescue the lethality of the *BAF53b*$^{-/-}$ mice, even though exogenous BAF53a was readily incorporated into the Brg/Brm-based nBAF complexes (Figure 7A and data not shown). Accordingly, expression of BAF53b in cultured *BAF53b*$^{-/-}$ hippocampal neurons rescued the activity-dependent dendritic growth defects and expression of target genes such as *Ephexin1* and *GAP43* (Figures 2B, 7C, and 7D), but BAF53a expression failed to repress the *BAF53b*$^{-/-}$ dendritic phenotype (Figure 7C and Figure S7). The failure of BAF53a to rescue the *BAF53b* mutant mice might be due to an obligate partnership between BAF53a and BAF45a, perhaps based on complementary interacting surfaces (see the jig-saw puzzle model in Figure 8). To determine whether BAF53a requires BAF45a to function and to rescue the defects in the *BAF53b*$^{-/-}$ neurons, we cotransfected BAF53a and BAF45a expression constructs both containing an IRES-GFP indicator (Lessard et al., 2007) into cultured *BAF53b*$^{-/-}$ hippocampal neurons and assayed for activity-dependent dendritic growth (Figure S8). Significantly, BAF45a/BAF53a failed to rescue the defect in the *BAF53b*$^{-/-}$ neurons. Together, these studies indicate that BAF53a- and BAF53b-containing BAF complexes are functionally distinct and that unique functional characteristics of BAF53b are required in dendritic development.

To better understand the molecular mechanism underlying the specific role of BAF53b in dendritic development, we defined the structural domains of the protein that are essential for mediating its function. Mammalian Arps (Arp1 to Arp11) form actin-like structures that can be divided into four structurally distinct subdomains (Muller et al., 2005 and Figure 7B). Their most divergent region is the subdomain 2, which in the case of actin, is involved in mediating protein-protein interactions (Muller et al., 2005). Sequence alignment between BAF53a and BAF53b revealed that their region of lowest conservation corresponds to the subdomain 2 of their actin fold (aa 39–82; Figure 7B). To determine whether this subdomain is essential for mediating BAF53b's function in dendritic development, we engineered chimeric proteins of BAF53a and BAF53b that specifically contained the subdomain 2 of the alternative family member (Figure 7B). To allow accurate quantification of the chimeric proteins, we made

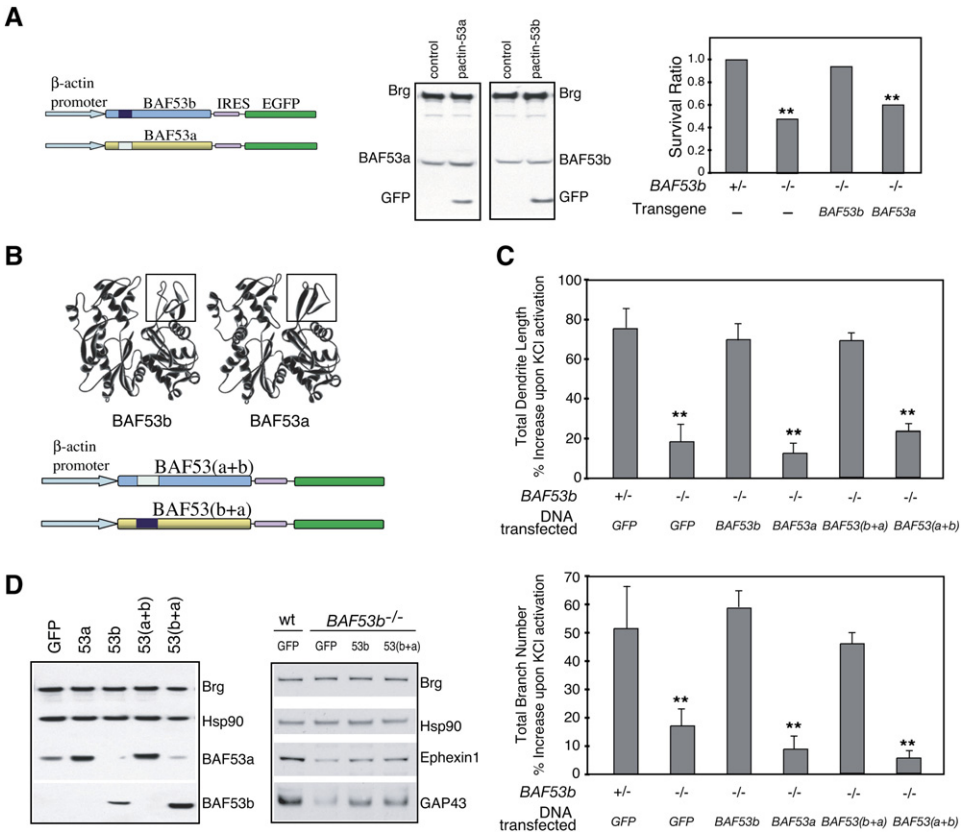

Figure 7. BAF53b Specifically Promotes Dendritic Outgrowth through Its Actin Fold Subdomain 2

(A) BAF53b but not BAF53a rescues lethality of the *BAF53b*$^{-/-}$ mouse. Left panel: Diagrams of the BAF53b and BAF53a transgenic constructs. The transgenes were expressed under the chicken β-actin promoter and followed by an IRES-GFP cassette. Middle panel: Western blot analyses of lysates isolated from P3 control or transgenic brains using antibodies against Brg, BAF53a, BAF53b, or GFP. Right panel: Relative survival ratio of P20 *BAF53b*$^{-/-}$ and *BAF53b*$^{-/-}$ + BAF53b or BAF53a transgenic mice compared to *BAF53b*$^{+/-}$ mice. **χ^2 test, $p < 0.01$ ($n > 20$ mice per genotype).

(B) Swapping the actin fold subdomain 2 of BAF53 proteins. Top panel: Predicted structures of BAF53a and BAF53b proteins (Oma et al., 2003) showing that subdomain 2 (shown in the box) is the most divergent region. Lower panel: Diagram of constructs expressing chimeric proteins that exchange subdomain 2 between BAF53a and BAF53b.

(C) The actin fold subdomain 2 provides specificity to BAF53b in regulating activity-dependent dendritic growth. Percentage of increase in total dendritic length and total dendritic tip numbers of neurons transfected with constructs expressing either GFP or the different BAF53 proteins upon KCl activation (three experiments, n = 25–35 neurons per condition per experiment). *t test, $p < 0.05$; **t test, $p < 0.01$. Results are presented as mean ± SEM.

(D) A BAF53a chimeric protein containing the actin fold subdomain 2 of BAF53b rescues the expression of BAF53b target genes. Left panel: Western blot analyses of lysates of 5 DIV cultured *BAF53b*$^{-/-}$ E15.5 cortical neurons transfected with constructs expressing GFP, BAF53a, BAF53b, or the chimeric BAF53 proteins using Brg, Hsp90, BAF53a, or BAF53b antibodies. The BAF53a and BAF53b antibodies were raised against their specific subdomain 2 regions and do not crossreact. Right panel: Western blot analyses of lysates of 5 DIV cultured control or *BAF53b*$^{-/-}$ E18.5 hippocampal neurons transfected with constructs expressing GFP, BAF53b, or the chimeric BAF53a protein with subdomain 2 of BAF53b using Brg, Hsp90, Ephexin1, or GAP43 antibodies.

antibodies specific to subdomain 2 of BAF53a and BAF53b that did not crossreact with the alternative wild-type and chimeric proteins (Figure 7D, left panel). Remarkably, expression of a BAF53a chimeric protein with subdomain 2 of BAF53b was sufficient to restore the dendritic outgrowth of *BAF53b*$^{-/-}$ neurons (Figure 7C and Figure S7) and the expression of BAF53b target genes such as *Ephexin1* and *GAP43* (Figure 7D). In contrast, the reciprocal chimeric protein (subdomain 2 of BAF53a swapped into BAF53b, Figure 7B) failed to rescue the defect (Figure 7C and Figure S7). In each case, the expression level of the chimera was similar to the wild-type protein (Figure 7D). Thus, the role of BAF53b in activity-dependent dendritic outgrowth is not related to its pattern of expression, but to a specific feature of its divergent subdomain 2 within the actin fold.

DISCUSSION

Postmitotic Neuron Development Requires a Family of Neuron-Specific Chromatin Remodeling BAF Complexes

During early neural development, neural progenitors divide along the ventricular surface. Upon cell cycle exit,

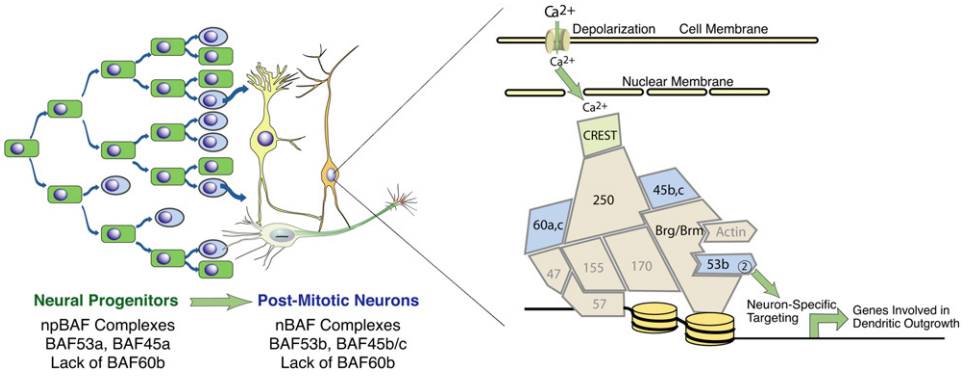

Figure 8. Model: nBAF Complexes and CREST Are Required for Postmitotic Neural Development and Dendritic Outgrowth

Left side: Subunit exchange within ATP-dependent BAF chromatin remodeling complexes accompanies the developmental transition from proliferating neural progenitors to postmitotic neurons. In neural progenitors, npBAF complexes contain BAF53a and BAF45a, which are exchanged for homologous alternative BAF53b and BAF45b subunits in neuron-specific complexes (nBAF) (Lessard et al., 2007). BAF60b was not found in either progenitor or neuronal complexes. Green cells represent neural stem cell/progenitors, while blue cells represent neurons. Right side: A postmitotic neuron is shown in which nBAF complexes together with CREST regulate specific features of neurons such as activity-dependent dendritic growth upon Ca^{2+} influx. nBAF complexes have six DNA-binding domains, two PHD domains, and one bromodomain, each of which can contribute to the localization of the complexes at promoters. Hence, multiple mechanisms could contribute to recruitment of these complexes, and the figure is not meant to specify a particular means of recruitment. Actin fold subdomain 2 is shown in BAF53b, which may contribute to neuronal-specific targeting. Subunits shown in blue are neuron specific, while the subunits in gray are ubiquitous. The relative position of Brg, actin, and BAF53 is adapted based on data from Zhao et al. (1998).

differentiating neurons develop axonal and dendritic processes and eventually participate in neural circuits that are in part dependent on specialized dendritic morphologies. Neural progenitors require a unique family of 2 MDa SWI/SNF-like npBAF chromatin-remodeling complexes containing BAF53a and BAF45a for their self-renewal and proliferative activities (Lessard et al., 2007). At mitotic exit, the BAF53a and BAF45a subunits are replaced by homologous BAF53b and BAF45b. In this study, we show that neurons require a family of specialized SWI/SNF-like nBAF complexes, characterized by neuron-specific BAF53b and BAF45b subunits, for postmitotic neuronal development (Figure 8). The dendritic growth defect observed in *BAF53b*$^{-/-}$ neurons likely reflects a role of nBAF complexes because RNAi-mediated knockdown of Brg, BAF57, or BAF45b produced similar defects in cultured neurons. The specific association of the Ca^{2+}-responsive, dendritic regulator CREST with nBAF complexes supports a mechanism of nBAF action in which synaptic activity or depolarization leads to Ca^{2+} influx, the activation of CREST, which in association with nBAF directly regulates genes necessary for dendritic development (Figure 8). These findings are consistent with a recent RNAi screen in which components of the *Drosophila* BAP complex, including BAP55 (the *Drosophila* homolog of BAF53) were found among 78 transcriptional regulators required for dendrite morphogenesis of sensory neurons (Parrish et al., 2006). Remarkably, homologs of other BAF subunits, such as Brg/Brm, BAF60, and BAF47 (Brm, BAP60, and Snr1, respectively, in *Drosophila*), were also detected in this screen (Parrish et al., 2006), implicating function of the entire BAP complex, and not just a single subunit, in dendritic morphogenesis. Although it remains unclear how BAP55 and the *Drosophila* BAP complex regulate dendritic development, these studies suggest a conserved role of nuclear actin-related proteins in an epigenetic program regulating dendritic outgrowth.

The Role of Actin-Related Proteins in Chromatin Remodeling

Genetic studies in yeast have directly implicated Act3p/Arp4, the yeast homolog of BAF53, in the transcriptional control of gene expression, where it sets the probability of an "on" or off" state for the *HIS4* and *LYS2* genes and probably others (Jiang and Stillman, 1996). Phylogenetic analyses revealed that at least 11 Arps are encoded in the yeast, plant, and mammalian genomes (Blessing et al., 2004). While cytoplasmic Arps (Arp1 to Arp3, Arp10, and Arp11) seem to be involved in the regulation of dynein motor activity and actin polymerization, the function of the nuclear Arps (Arp4 to Arp9) as components of chromatin remodeling complexes has remained elusive, mainly because of their requirement in complex assembly and/or stabilization (Blessing et al., 2004; Galarneau et al., 2000; Olave et al., 2002b; Shen et al., 2003). By analogy to the Arp2/3 complex, it has been proposed that nuclear Arps might have a role in the nucleation and branching of nuclear actin filaments, perhaps as part of a mechanism for targeting the complexes to transcriptionally active subnuclear domains (Blessing et al., 2004; Olave et al., 2002b; Rando et al., 2002; Zhao et al., 1998). In mammalian BAF complexes, Brg, actin, and BAF53 form a stable subcomplex that resists dissociation in 3 M urea (Zhao et al., 1998) (Figure 8), suggesting that BAF53 may play a role in the regulation of nuclear actin via its tight association.

The observation that the entire nBAF complex is assembled in the absence of BAF53b gave us the opportunity to study the role of an Arp in the biological activity of an ATP-dependent chromatin remodeling complex in vivo. In vitro nucleosome remodeling and ATPase activities of BAF complexes lacking BAF53, or even all core subunits but Brg, are normal, indicating that only Brg is necessary for these in vitro functions (Phelan et al., 1999; Wang et al., 1998). Our studies demonstrate that BAF53b is required for targeting nBAF complexes and the calcium-responsive transcriptional coactivator CREST to the promoter of genes essential for dendritic growth. Because BAF53b has no DNA-binding domain, we favor a model where BAF53b interacts with DNA via other DNA-bound nuclear factors and/or coactivators (Figure 8). The specificity of its function in dendritic development appears to arise from such interactions through its actin fold subdomain 2. Consistent with a role for BAF53b in targeting the complexes to chromatin, yeast Arp4 and Arp8 have been reported to bind to nucleosomal core histones (Downs et al., 2004; Harata et al., 1999; Shen et al., 2003). Thus, an interesting possibility is that BAF53b recognizes certain histone marks and acts as a molecular bridge between nBAF complexes and the chromatin.

Combinatorial Assembly of BAF Complexes during Neural Development

The finding that vertebrate SWI/SNF-like BAF complexes are combinatorially assembled into perhaps several hundred different complexes (Wang et al., 1996a, 1996b; Zhao et al., 1998) raised the question of whether combinatorial assembly produces biological specificity or simply redundancy. Recent studies of the vertebrate *Polycomb*-Group (*Pc*-G) complexes indicate that their subunits are encoded by gene families and hence might also be combinatorially assembled (Lund and van Lohuizen, 2004). While earlier studies have suggested that combinatorial assembly of SWI/SNF-like BAF complexes might contribute to specificity (Bultman et al., 2000; Kadam and Emerson, 2003; Wang et al., 2004), others have suggested that the genes encoding the subunits might be partially or fully redundant (Indra et al., 2005; Lickert et al., 2004). Lickert and colleagues demonstrated that BAF60c is essential for some aspects of early cardiac development, processes that could be rescued by transgenic expression of the alternative BAF60b subunit (Lickert et al., 2004). However, because of the small amount of tissue available and the lack of specific antibodies to these subunits, levels of expression of the rescuing protein could not be accurately quantified. The restricted expression pattern of BAF53b in postmitotic neurons allowed us to begin addressing the functional significance of combinatorial assembly by gene ablation and rescue. Our studies indicate that combinatorial assembly of BAF complexes generates functional specificity and underlies an epigenetic program required for postmitotic neuron development. This functional specificity of nBAF complexes appears to be dependent upon the unique actin subdomain 2 of BAF53b and CREST, which are essential to engage an activity- and Ca^{2+}-dependent regulatory pathway of dendritic development (Figure 8). Additional support for this conclusion comes from the comparison of the gene list regulated by BAF53b (thus nBAF complexes) in neurons and Brg/BAF53a-containing npBAF complexes in neural progenitors (Lessard et al., 2007). These microarray analyses revealed that nBAF complexes specifically regulate a genetic program of postmitotic neuron development, while npBAF complexes regulate a distinct set of target genes directed at neural stem/progenitor self-renewal and proliferation.

Of note, we did not detect peptides from the BAF60b protein in our proteomics analysis of the neural BAF complexes, although BAF60b is a known subunit of BAF complexes in several other nonneural tissues and cell types (Wang et al., 1996b). Hence, BAF60b exclusion might further distinguish the nBAF complexes and be significant for the specialized nBAF-dependent regulatory program of postmitotic neuronal development. Our studies suggest that the subunits of BAF complexes function like letters in a ten-letter word to produce specific biological meanings by combination.

A Signaling Pathway Involving Ca^{2+}, CREST, and nBAF Complexes Controlling Dendritic Morphogenesis

One of the most unique features of adult neurons is the remarkably diverse yet stereotypic morphologies of their dendritic trees. Dendritic geometry determines neural connectivity and underlies the distinctive ways that individual neurons receive and integrate synaptic inputs (Hausser and Mel, 2003; Jan and Jan, 2003). Although early dendritic growth is determined by intrinsic factors and environmental guidance cues, neuronal activity stimulates dendritic extension and branching: a process that requires transcription (Jan and Jan, 2003; Scott and Luo, 2001; Wong and Ghosh, 2002). Hippocampal neurons from *BAF53b*$^{-/-}$ mice display normal basal dendritic growth but extensive defects in dendritic morphogenesis induced by depolarization, indicating that the BAF53b-containing nBAF complexes participate in the transcriptional program underlying activity-dependent dendritic development. These defects could be corrected by exogenous expression of BAF53b in cultured neurons, indicating that they are primary and not related to earlier developmental abnormalities. BAF53b's function in activity-induced dendritic maturation likely reflects that of the entire nBAF complexes because reducing the levels of Brg, BAF57, or BAF45b, components of the postmitotic nBAF complexes, produces similar developmental defects.

Activity-dependent dendritic outgrowth is known to be activated by Ca^{2+} signaling and to require CREST, a Ca^{2+}-regulated transcriptional coactivator (Aizawa et al., 2004). The following lines of evidence support the notion that CREST and nBAF complexes participate in a signaling pathway beginning with Ca^{2+} influx and resulting in the epigenetic control of genes essential for dendritic

outgrowth (Figure 8). (1) Neurons from *CREST*$^{-/-}$ mice elaborate fewer and shorter dendritic branches in response to depolarizing stimuli (Aizawa et al., 2004). These defects resemble those seen in *BAF53b*$^{-/-}$ neurons from several regions of the brain, both in vitro and in vivo. (2) Endogenous nBAF complexes specifically copurify with endogenous CREST, and a significant proportion of CREST cosediments with Brg, BAF53b, and other components of nBAF complexes in nuclear extracts isolated from the brain. (3) Together with CREST, nBAF complexes directly regulate genes important for dendritic outgrowth. Finally, (4) nBAF complexes are involved in the recruitment of CREST to target genes through a BAF53b-dependent mechanism. Thus, our studies suggest that BAF53b and nBAF complexes directly interact with CREST to mediate a Ca^{2+}-dependent program of dendritic morphogenesis, most likely initiated through voltage-sensitive Ca^{2+} channels and NMDA receptors (Figure 8). However, it is worth noting that CREST is not a stable stoichiometric subunit of nBAF complexes, and BAF53b-containing nBAF complexes might perform CREST-independent functions in postmitotic neurons. Indeed, *BAF53b*$^{-/-}$ mice die earlier than *CREST*$^{-/-}$ mice, and our data indicate that axonal outgrowth is also likely to be compromised in *BAF53b*$^{-/-}$ mice (Figure S2B and data not shown). Therefore, besides activity-dependent dendritic growth, nBAF complexes may participate in other transcriptional programs required for postmitotic neuron development.

Why do neurons require a specialized mechanism of chromatin remodeling? One possibility might relate to the fact that the stable postmitotic state of neurons, with unique neurotransmitters, connectivities, and remarkably intricate morphologies, can persist for a century. In the nervous system, this stability is paradoxically accompanied by an underlying plasticity that allows learning to occur throughout life by mechanisms that require transcription (Kandel, 2001). It is possible that neurons require specialized mechanisms of chromatin remodeling not found in other cell types to maintain this state of dynamic plasticity. Our identification of specific subunits of these postmitotic neuronal complexes, with demonstrated functions and direct transcriptional targets, will make direct tests of this hypothesis possible.

EXPERIMENTAL PROCEDURES

Generation of *BAF53b*$^{-/-}$ Mice

BAF53b$^{-/-}$ mice were generated using standard homologous recombination methods. Two genomic DNA fragments (2 kb and 4.5 kb) flanking exons 2 and 3 were cloned into pPNT vector and electroporated into 129S6 ES cells. Positive clones were identified by PCR and Southern blot.

Image Analyses

Cultured neuron images were captured with a Leica DM5000 fluorescent microscope at 40×. Dendrite length and branch tip numbers were measured using ImageJ software (NIH). Sholl analysis was performed using Neurolucida software (MicroBrightField). In each condition, 25 to 35 randomly chosen healthy neurons were analyzed. Each experiment was repeated at least three times. In long-term hippocampal cultures, to reduce variation, only neurons with more than three primary dendrites were used for Sholl analysis (n = 15). Z-stack images of GFP-labeled neurons in Thy1-GFPM transgenic mice were captured from 100 μm vibratome sections using a Leica SP2 AOBS confocal microscope and reconstructed with the Volocity software (Improvision). All quantifications are presented as mean ± SEM.

See Supplemental Data for other experimental procedures.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/56/1/94/DC1/.

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