Evidence, based on a variety of approaches, suggests that different cell types in the vertebrate retina are generated by multi-potential progenitors in response to interactions between cell intrinsic and cell extrinsic factors. The identity of some of the cellular determinants that mediate such interactions has emerged, shedding light on mechanisms underlying cell differentiation. For example, we know now that Notch signaling mediates the influence of the microenvironment on progenitors’ states of commitment by activating transcriptional repressors. Cell intrinsic factors such as the proneural basic helix-loop-helix and homeodomain transcription factors regulate a network of genes necessary for cell differentiation and maturation. What is missing from this picture is the role of developmental chromatin remodeling in coordinating the expression of disparate classes of genes for the differentiation of retinal progenitors. Here, we describe the role of Brm, an ATPase in the SWI/SNF chromatin remodeling complex, in the differentiation of retinal progenitors into retinal ganglion cells. Using the perturbation of expression and function analyses, we demonstrate that Brm promotes retinal ganglion cell differentiation by facilitating the expression and function of a key regulator of retinal ganglion cells, Brn3b, and the inhibition of Notch signaling. In addition, we demonstrate that Brm promotes cell cycle exit during retinal ganglion cell differentiation. Together, our results suggest that Brm represents one of the nexus where diverse information of cell differentiation is integrated during cell differentiation.

While the identification of cell intrinsic and cell extrinsic factors has helped our understanding of the mechanisms that regulate cell fate specification and subsequent cell differentiation in the nervous system are orchestrated and finessed by interplay between cell intrinsic and cell extrinsic factors. This process is exemplified during the development of the retina, an excellent model of the central nervous system (CNS). Recent evidence suggests that disparate transcription factors belonging to basic helix-loop-helix (bHLH), homeo-domain (HD) and zinc finger (ZF) classes cooperate toward lineage specification and differentiation (1-6). For example, the bHLH transcription factor, Math5, and the HD transcription factor, Pax6, have been shown to cooperate during the specification of retinal progenitors into retinal ganglion cells (RGCs) (2,7-9). As progenitors are committed along RGC lineage, Wt1, a zinc finger transcription factor and Brn3b, a HD transcription factor of POU class, are expressed and ultimately promote the differentiation and maturation of the specified progenitors into RGCs (10). The progress in the identification of intrinsic factors has been paralleled by the characterization of extrinsic factors and intercellular signaling pathways, e.g., Notch pathway, that mediates the regulatory influence of the microenvironment on retinal progenitors’ maintenance and their differentiation into RGCs (11-15).
the differentiation of retinal cell types, particularly that of RGCs, it is not known as to how the remodeling of chromatin, which is necessary for eukaryotic gene expression, is recruited towards the coordinated regulation of genes during RGC differentiation. There are two major classes of chromatin remodeling complexes, which are associated with specific enzymes that remodel chromatin by changing nucleosome structure or position (16,17). The first class includes complexes consisting of histone acetyltransferase (HAT) or histone deacetylase (HDAC) that covalently modify chromatin by adding and removing acetyl groups from the amino terminus of the four core histones, respectively. The second class includes SWI/SNF complexes that utilize the energy obtained from ATP hydrolysis to disrupt nucleosomal structure or position. The mammalian SWI/SNF complexes consist of 10-12 proteins, including the homologous but mutually exclusive ATPases, Brahma (Brm) or Brm related gene 1 (Brg1) (16,18).

Though both major classes of chromatin remodeling complexes are likely to contribute to developmental processes, including those in the CNS (19-21), evidence is emerging that the SWI/SNF chromatin remodeling complexes play an important role in the differentiation of specific cell types (22). For example, these complexes have been shown to facilitate the differentiation of a variety of cell types such as erythrocytes (7,23), macrophages (24), myeloid cells (25), adipocytes (26), myoblasts (27), osteoblasts (28), neurons (29,30) and glia (31). Here, we demonstrate the role of Brm in the differentiation of retinal progenitors into RGCs. Brm is expressed in the developing rat retina and its temporal and spatial patterns of expression correlate with retinal histogenesis. Using perturbation of expression and function analyses, we demonstrate that Brm influences the differentiation of retinal progenitors into RGCs by facilitating Brn3b expression and function, and inhibiting Notch signaling. In addition, we demonstrate that Brm may influence differentiation generically by promoting cell cycle exit. Together, our results suggest that chromatin remodeling by Brm may represent one of the nexus where cell intrinsic and cell extrinsic influence may be integrated towards the differentiation of retinal progenitors.

**EXPERIMENTAL PROCEDURES**

**Progenitor cell culture:** Timed-pregnant (E14) Sprague Dawley rats were obtained from Sasco (Wilmington, MA). The gestation day was confirmed by the morphological examination of embryos (32). Fertilized hen eggs were incubated in a humidified chamber at 38°C, and embryos were staged according to Hamburger and Hamilton (33). Embryos were harvested at appropriate gestation periods, and eyes were enucleated. Retina were dissected out and dissociated as previously described (34). Cells were cultured in RCM [DMEM:F12, 1X N2 supplement (Invitrogen, San Diego, CA), 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin] containing FGF2 (10 ng/ml), EGF (20 ng/ml) and 0.1% FBS for 5 days to generate clonal neurospheres. 5-Bromo-2-deoxyuridine (BrdU) (10 µM) was added to the culture for the final 24 hr. For co-culture, neurospheres were collected, washed extensively to remove BrdU, and plated on poly-D-lysine- and laminin-coated glass cover slips with E3 chick/PN1 rat retinal cells in 1% FBS. For RT-PCR analysis co-culture was performed across a 0.4 µm membrane (Millipore, Bedford, MA). Cells were either frozen for RNA extraction or fixed with 4% Para formaldehyde for 15 min at 4°C for immunofluorescence analysis.

**Immunofluorescence analysis:** Immunocytochemical analysis for detection of cell specific markers and BrdU was performed as previously described (35). Briefly, Para formaldehyde-fixed cells/sections were incubated in 1X PBS containing 5% NGS and 0.2–0.4% Triton X-100 followed by an overnight incubation in appropriate dilutions of antibodies against Brm (Santa Cruz biotechnology), Notch1 (Santa Cruz Biotechnology), Shh (Developmental Studies Hybridoma Bank), Brn3b (Covance), RPFl (36) and BrdU (Accurate chem. and Sci. Corp.) at 4°C. Cells/sections were examined for epifluorescence after incubation with IgG, conjugated to Cy3/FITC. Images were captured using a CCD camera (Princeton Instruments,
Trenton, NJ) and Openlab software (Improvision, Lexington, MA).

**Cell cycle analysis**: The DNA content of the retinal cells was measured by flow cytometry using propidium iodide as described (37). Cell cycle analysis was done using a FACStar flow cytometer (Becton Dickinson).

**Semi-quantitative RT-PCR**: Total RNA was isolated from frozen cells or explants using QIagen RNA isolation kit (Valencia, CA), and cDNA synthesis was performed as previously described using ~5µg of total RNA (38). Specific transcripts were amplified with gene specific forward and reverse primers using a step-cycle program on a Robocycler (Stratagene, La Jolla, CA) for less than 30 cycles to keep the amplification within the range of linearity. The gene specific primers used for RT-PCR are described in Table 1.

**Northern Analysis**: Northern analysis was carried out to detect rBrm transcripts as previously described (39). Briefly, ~2 µg of poly A RNA, isolated from adult retina, was electrophoresed on a 1.2% formaldehyde gel and transferred to Nytran Plus. Hybridization was carried out using a 32P-labeled rBrm cDNA probe overnight at 65°C. Blot was washed sequentially with 2X SSC, 0.1% SDS at room temperature for 20min, then twice with 1X SSC, 0.1% SDS at 65°C for 15min and finally with 0.1X SSC, 0.1% SDS at 65°C for 10min, followed by autoradiography.

**siRNA electroporation**: Brm siRNA sequence was cloned into pSuper vector (Oligogene) according to vendor’s protocol. Electroporation was carried out according to modified protocol of Matsuda and Cepko (40). Briefly, E14 retinas were dissected out and collected in HBSS containing pSuper-Brm and pEGFP-C3. The explants were placed in wells made in a 2% agarose gel. Electroporation in agarose gel reduced the shock, and thereby cell death. It also prevented aggregation of explants with one another. Electroporation was carried out by applying 5 pulses at 40V for 50ms durations with 950 ms intervals using a gold-plated electrodes and an Electro Square Porator (BTX Inc.). The efficiency of electroporation was monitored by GFP epifluorescence and the explants were cultured in RCM containing 5% FBS for 4 days. The effect of siRNA was ascertained by analyzing the Brm/ β-actin protein and mRNA levels by Western and RT-PCR analyses, respectively.

**Recombinant virus preparation and infection**: Brm (pBabe-Brm), dominant negative Brm (pBabe-dnBrm) and empty (pBabe) retrovirus were made in BOSC-23 cells using calcium phosphate method, as previously described (27). Neurospheres/RGC-5 cells, at ~60% confluency, were exposed to retrovirus containing medium for 24hrs. After infection, the medium was replaced with fresh culture medium.

**Reporter assay**: RGC-5/293T cells, transduced with pBabe/pBabe-Brm/pBabe-dnBrm were transfected with pGL2-Brm3b-luciferase (RGC-5 cells) and pGV-B-Hes1/Hes5 luciferase-constructs (293T cells) using lipofectamine (Invitrogen). Transfection efficiency was examined by co-transfecting cells with pGFP-C3 (Clonetech). For luciferase assay, cells were lysed in 1X reporter lysis buffer (Promega) and 100µl of lysate was diluted five times using assay reagent (Promega). Diluted samples (100µl) were analyzed for luciferase activities using a luminometer (Pharmingen).

**Co-immunoprecipitation**: Total protein was extracted from RGC-5 cells using a M-PER protein extraction kit (Pierce). Five hundred microgram of total protein in 1ml RIPA buffer (50mM Tris-HCl, pH 7.4; 15mM NaCl; 1% Triton-X 100; 0.1% SDS; 1mM EDTA; 1% sodium deoxycholate) was incubated with 5µg of antibody for overnight at 4°C. Protein-antibody complex was precipitated by incubating with protein A/G sepharose for 1 hour at 4°C. The protein-antibody-sepharose complex was precipitated by centrifuging at 1000 rpm for 1min and precipitates were washed three times with RIPA buffer and final pellet was resuspended in appropriate volume of loading buffer. The mix was boiled to dissociate the complex and electrophoresed in a 7-9% denaturing polyacrylamide gel. Negative
controls included reactions carried out without the antibody and with IgG.

**Western blot analysis:** Western blot analysis was done as previously described (39). Samples from Co-IP or protein isolated from siRNA-transfected retinas were electroblotted onto PVDF membranes, following electrophoresis. Membranes were blocked for 1 hour in 5% non-fat dry milk in TBST and incubated with anti-Brm/anti-Notch (Santacruz Biotechnology), diluted 1:500 in TBST overnight at 4°C with shaking. After incubating with anti-mouse HRP, the blots were washed with TBST and immunoreactive bands were detected using ECL Western blotting detection reagents (Amersham, RPN 2108). The blots were then exposed to X-ray film to visualize immunoreactive bands.

**Chromatin immunoprecipitation:** Chromatin immunoprecipitation (ChIP) assay was done using a modified procedure from Upstate Biotechnology. Briefly, transduced (pBabe/pBabe-Brm) or transfected [pSuper-Brm siRNA/Wt1 (KTS)] RGC5 cells were grown until they reached confluency and histones were cross-linked to DNA by adding formaldehyde directly to culture medium to a final concentration of 1% and incubating for 10 min at room temperature on a rocking platform. Cells were washed three times with ice-cold PBS containing protease inhibitors. Cell pellets were re-suspended in pre-warmed SDS lysis buffer (1% SDS, 10mM EDTA, 50mM Tris, pH 8.1). To reduce the non-specific background, the samples were pre-cleared using 80µl salmon-sperm DNA/protein A agarose slurry at 4ºC for 30 minutes. Samples were centrifuged at 100 rpm for 1 min at 4°C. Supernatants were transferred to a new tube and the immunoprecipitating antibody was added and incubation was carried out overnight at 4°C on a rocking platform. For a negative control, we used no antibody or IgG. The histone-antibody complex was precipitated using 60µl salmon sperm DNA/protein A agarose slurry at 4°C for 30 minutes. Samples were centrifuged at 100 rpm for 1 min at 4°C. Supernatants were transferred to a new tube and the immunoprecipitating antibody was added and incubation was carried out overnight at 4°C on a rocking platform. For a negative control, we used no antibody or IgG. The histone-antibody complex was precipitated using 60µl salmon sperm DNA/protein A agarose slurry at 4°C for 1 hour at 4°C. Precipitates were washed sequentially at room temperature for 5 min, once with low salt immune complex wash buffer (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-Cl, pH 8.1, 500mM NaCl), and high salt immune complex wash buffer (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-Cl, pH 8.1, 500mM NaCl) and lithium salt immune complex wash buffer (2.25M LiCl, 1% IGEPAL-CA630, 1% deoxycholic acid -sodium salt, 1mM EDTA, 10mM Tris, pH 8.1), and twice with TE buffer (10mM Tris-Cl, 1mM EDTA, pH 8.0). After completely removing the TE buffer, the precipitate was resuspended and extracted twice in 250µl freshly prepared elution buffer (1% SDS, 0.1M NaHCO3). To reverse the histone-DNA cross-linking, samples were heated at 65°C for 4 hours. 200µl of initial sonicated sample was reverse cross-linked and used as an input. After removing the antibodies by protease digestion of the samples, DNA was recovered and column-purified. PCRs were performed using gene-specific primers.

**Restriction enzyme accessibility assay:** Restriction enzyme accessibility using LM-PCR assay was carried out as previously described (41). Briefly, 5x10^6 nuclei from pBabe/pBabe-Brm transduced RGC5 cells were subjected to BglII (75-100 units) restriction enzyme digest for 30 minutes at 37°C. First strand synthesis of genomic DNA (100ng) was carried out using pfu DNA polymerase (Stratagene) and gene-specific forward primer (5'ACCCACGTCTTTCTGCACTAG3'). Following linker ligation, using T4 DNA ligase overnight at 17°C, PCR was carried out on the ligated products using the gene-specific forward primer and linker-specific reverse primer (5'CCGGGAGATCTGAATTCGAT3') for 25 cycles at an annealing temperature at 65°C. The PCR products were resolved on 1.2% agarose gel followed by Southern analysis using a PCR product corresponding to sequence between the second taq1 site and the BglIII site in proximal Brn3b promoter (Figure 6D).

**Statistics:** Results were expressed as mean±SEM of at least 3 separate experiments. Statistical analyses were done using Student’s t test to determine the significance of the differences between the various conditions.

**RESULTS**

**Expression of Brm in the developing retina:** Emerging evidence suggests that Brm plays an
important role in cell differentiation (27,42). To ascertain whether or not Brm has a similar role in retinal development, we examined the temporal patterns of Brm expression during early (E12-E18) and late (E18-PN6) retinal histogenesis by RT-PCR analysis. Retinal cells are born in an evolutionarily conserved temporal sequence; RGCs, cone photoreceptors, horizontal cells and majority of amacrine cells are born during early histogenesis while rod photoreceptors, bipolar cells and Müller glia are generated during late histogenesis (43,44). We observed that levels of Brm transcripts increased at E14 stage, compared to those at E12 stage (Figure 1, A and B). The stages between E12 and E14 represent the period of active neurogenesis when the majority of RGCs, horizontal cells and cone photoreceptors are born (45). The decrease in the expression of Brm at the stage E16 is likely due to the fact that E16 represents a stage of relative quiescence during early histogenesis (12). Similarly, during late histogenesis, there was a significant increase in levels of Brm transcripts at PN1 and PN3, compared to those at E18 when the majority of rod photoreceptors, which constitute ~80% of total cells in rodent retina, are born (45). The Brm expression persisted through postnatal stages and its levels were highest in adult retina, compared to all stages examined. In addition, the specificity of Brm expression was determined by sequencing PCR products and Northern analysis of RNA from the adult retina, which revealed a 5.8kb band corresponding to full length Brm transcripts (46) (Figure 1C). Together, these results suggested that Brm expression is associated with retinal histogenesis. To obtain further insight into the involvement of Brm with the process of cell differentiation in retina, we examined the cell-specific expression of Brm in the retina of E14 and E18 embryos. We observed that Brn immunoreactivities were distributed towards the scleral and ventricular sides in the developing retina (Figure 1, D-I). Both in E14 and E18 retina, Brm immunoreactivities were predominantly localized towards the scleral side where differentiating precursors are located. As previously reported, Brm immunoreactivities had nuclear as well as cytoplasmic localization (47). Next, to test whether or not Brm expression is associated with differentiating precursor population, we examined the cellular distribution of Brm immunoreactivities in the retina of E14 and E18 embryos, pre-exposed to BrdU in utero to identify proliferating (BrdU+ cells) (Figure 1J). We observed that Brm immunoreactivities were associated with both BrdU- and BrdU+ cells. However, in the total cell population at E14, the proportion of BrdU-Brm- cells (6.8±0.23) was significantly higher than those of BrdU+Brm+ cells (4.09±0.23), suggesting that Brm expression was predominantly associated with post-mitotic cells (Figure 1L). A comparison of different classes of cells at the two different stages revealed that the proportion of BrdU+Brm+ cells increased ~2-fold in E18 retina relative to that in E14 retina (12.43±0.75 Vs 6.8±0.23, p <0.05), demonstrating a progressive association of Brm with post mitotic cells as development progressed. Together, these observations suggested that the expression of Brm correlated with the process of differentiation of retinal stem cells/progenitors.

Next, we examined the involvement of Brm expression and function in the differentiation of stem cells/progenitors along a specific lineage. Although it is likely that Brm is involved in the differentiation of multiple retinal cell types, we studied Brm in the context of RGC differentiation because these are the first cells born (15) and their regulators and markers (10) are well characterized, thus affording an unambiguous interpretation of results. To know whether or not there is a correlation between Brm expression and RGC differentiation, we examined the spatial and cellular distribution of Brm immunoreactivities in the developing retina. Immunohistochemical analysis of E14 retina revealed a spatial overlap of Brm immunoreactivities with that of Brn3b (Figure 2, A-D) and RPF1 (Figure 2, E-H). Immunocytochemical analysis of freshly dissociated E14 retinal cells revealed that Brm immunoreactivities are co-localized with that of regulators of RGCs, Brn3b, Wt1, RPF1 and Shh in a subset of cells (Figure 2, I-L). Together these observations demonstrated the association of Brm expression with nascent RGCs in E14 retina. To determine the temporal association of
Brm expression with RGC differentiation we carried out immunohistochemical analysis of retinal cell dissociates from embryos in different stages (E12, E14, E16 and E18 stages) of early histogenesis. We observed that a subset of Brm+ cells expressed Brn3b at all stages of early histogenesis, albeit at different proportions (Figure 2M). For example, compared to those at E12 stage, the proportion of Brm+Brn3b+ cells increased by ~2fold at E14, the peak of RGC generation (23.9±3.0 Vs 46.0±2.6, p<0.001). Subsequently, the number of Brm+Brn3b+ cells decreased with the time; compared to those at E14, the proportion of such cells decreased at E18 stage, by which time the majority of RGCs are differentiated (46.0±2.6 Vs 4.2±0.89, p<0.001). These results indicated towards the association of Brm with RGC differentiation in vivo; Brm is expressed in differentiating RGCs, and the expression is progressively extinguished from these cells as they complete their differentiation. To know whether Brm had a specific role in RGC differentiation required information regarding the dynamic patterns of Brm expression during RGC differentiation. Does Brm expression correlate with the process of RGC differentiation? This issue was addressed using neurosphere assay, which serves as a robust model of RGC differentiation (48). Retinal stem cells/progenitors in neurospheres utilize normal mechanism of RGC differentiation (38) and the resulting RGCs show target selectivity in terms of establishing contacts with cells in superior colliculus and not with those in inferior colliculus (49). Proliferating progenitors in E14 neurospheres were tagged with BrdU, before shifting them to conditions that promote RGC differentiation. This was to ensure that we accounted for the differentiation of only those cells that were proliferating before the onset of differentiation, and not of those that had spontaneously differentiated by asymmetrical division. Samples of neurospheres were assayed at specified times (0 hr, 6 hr, 12 hr, 24 hr and 48 hr) to determine the temporal proportion of Brm+ cells that were BrdU-tagged (BrdU+) or expressing Brn3b immunoreactivities. We observed that more Brm+ cells were BrdU-tagged in differentiating conditions and their proportions increased with the time, compared to those in proliferating conditions (0 hr), corroborating our earlier observation that the expression of Brm is tightly correlated with the process of differentiation (Figure 2N). That the increase in Brm expression is specifically correlated with the differentiation of RGCs was demonstrated by a coincidental increase in the proportion of Brm+ cells expressing Brn3b immunoreactivities, compared to those in proliferating conditions (0 hr). The difference in the proportion of Brm+Brn3b+ and Brm+BrdU+ cells is likely due to the presence of post-mitotic precursors, which did not incorporate BrdU, and proliferating progenitors, which failed to incorporate BrdU because they were not in S-phase of the cell cycle when exposed to Brdu.

Functional involvement of Brm in RGC differentiation: The correlation of Brm expression with RGC differentiation suggested that Brm influences the process of RGC differentiation. This premise was examined further by perturbing Brm expression and function in retinal stem cells/progenitors and examining the effects on RGC differentiation. First, we used the siRNA-mediated gene silencing approach to attenuate Brm expression. pSuper vector (Oligogene) containing Brm siRNA was co-electroporated with pEGFP-C3 plasmid in E14 rat retinal explants. Controls included explants, similarly electroporated with siRNA corresponding to scrambled Brm sequence. Since ~90% of cells in E14 retina are mitotic (50), the majority of cells electroporated with siRNA were likely to be proliferating progenitors. The efficiency of siRNA electroporation was demonstrated by GFP epifluorescence in explant sections (Figure 3A). The specificity of gene silencing was demonstrated by a decrease in levels of Brm protein and its corresponding transcripts in Brm siRNA-treated explants, compared to controls (Figure 3, B and H). As expected, the expression levels of non-targeted β-actin protein and its corresponding transcripts were similar in both groups. The effects on RGC differentiation were examined by changes in the proportion of cells expressing immunoreactivities corresponding to the RGC regulators and markers, Brn3b and RPF1 (36). Immunoreactivities corresponding to both RPF1 (C-F) and Brn3b (G-J) were...
decreased in siRNA-treated explants, compared to controls. The RPF1 immunoreactivities, displaying relatively discrete nuclear localization in explants than Brn3b, were used for the quantitation of the number of nascent RGCs. We observed a ~3-fold decrease in the proportion of RPF1<sup>+</sup> cells in Brm siRNA-treated retinal explants, compared to controls (RPF1<sup>+</sup> cells: 70.0±12.41 Vs 20±4.08, p<0.001), suggesting a decrease in RGC differentiation when Brm expression is attenuated (Figure 3K). RT-PCR analysis revealed a decrease in levels of regulators of RGCs, RPF1, Brn3b and Shh transcripts in Brm siRNA-treated explants, compared to controls, thus corroborating our immunohistochemical results.). Together, these results demonstrated that the attenuation of Brm expression adversely affected the number of cells expressing RGC-specific markers. Next, we determined whether or not Brm chromatin remodeling function was required for RGC differentiation. We over-expressed dominant negative Brm in retinal progenitors through retrovirus transduction (pBabe-dnBrm) of neurospheres culture. The dominant negative Brm contains a mutation in the ATPase domain and when over expressed it is expected to participate in the formation of non-functional ATPase chromatin remodeling complex, thus impairing activities of genes that require Brm (46,51). Controls included neurospheres transduced with wild type Brm (pBabe-Brm) and empty (pBabe) retrovirus. Following transduction, neurospheres were shifted from proliferating to differentiating conditions, and scored for the proportion of cells expressing RGC-specific markers. Since retroviral infection of retinal explants does not lead to a uniform transduction of retinal progenitors—which might lead to ambiguous results—we used E14 neurospheres instead. We observed a significant increase in the proportion of both Brm3b<sup>+</sup> and RPF1<sup>+</sup> cells in neurospheres infected with wild type Brm, compared to those infected with empty retrovirus (Brm3b<sup>+</sup> cells: 68.3±2.3 Vs 38.7±2.2, p<0.001; RPF1<sup>+</sup> cells: 56.0±2.4 Vs 37.3±4.2, p<0.001) (Figure 4, A-M). In contrast, there were significantly fewer cells expressing RGC markers in neurospheres infected with dominant negative Brm, compared to those infected with empty retrovirus (Brm3b<sup>+</sup> cells: 38.7±2.2 Vs 23.5±4.5, p<0.05; RPF1<sup>+</sup> cells: 37.3±4.2 Vs 11.7±0.3, p<0.005) (Figure 4M) suggesting the involvement of Brm-mediated chromatin remodeling in RGC differentiation. RT-PCR analysis showed an increase and a decrease in levels of transcripts corresponding to Brn3b, RPF1 and Shh in neurospheres infected with wild type Brm retrovirus and dominant negative Brm retrovirus, respectively, compared to controls (Figure 4N), corroborating the immunocytochemical results. There was no difference in Tunel-positive cells in control and experimental groups demonstrating that the observed difference in the number of RGCs was not due to Brm-induced survival of nascent RGCs (Figure 4, O-T). Next, we were interested in knowing whether the lack of Brm could lead to RGC phenotype in vivo. We carried out immunohistochemical analysis of retinal sections obtained from adult Brm<sup>−/−</sup> and wild type mice. The retina of Brm<sup>−/−</sup> mice was comparable to that of the wild type in terms of lamination and thickness. However, there were significantly fewer RPF1<sup>+</sup> and Brn3b<sup>+</sup> cells, observed more in the periphery than in the centre, in Brm<sup>−/−</sup> retina, compared to wild controls (Figure 5, A-E). Together, these observations suggested the involvement of Brm expression and its function in RGC differentiation.

**Influence of Brm on the regulation of Brn3b expression and function:** Next, we examined the mechanism of Brm-mediated RGC differentiation. Brn3b is a prominent RGC regulatory gene whose activation heralds phenotypic differentiation of RGC precursors through subsequent activation of downstream target genes such as Shh (10,52). We were interested in knowing whether Brm regulated RGC differentiation by facilitating the activation of Brn3b and Shh. The decrease in levels of Brn3b and Shh transcripts in response to siRNA-mediated attenuation of Brm expression (Figure 3) and over expression of dominant negative Brm (Figure 4), suggested that Brm positively regulated Brn3b and Shh expression in retinal progenitors. This notion was further supported by observations that the over-expression of wild type Brm led to an increase in levels of Brn3b
and Shh transcripts (Figure 4). To know if Brm directly activated Brn3b promoter, RGC-5 cells, a transformed RGC cell line (53,54), transduced with wild type Brm or empty retrovirus, were transiently transfected with Brn3b-Luc constructs (24). A 4.5-fold increase in reporter activities was observed in cells transduced with wild type Brm retrovirus, compared to those in controls, suggesting a direct influence of Brm on Brn3b promoter activities (Figure 6A). It has been demonstrated that ATPase chromatin remodeling complexes are recruited to specific promoters by cell-specific transcription factors where it remodels chromatin to facilitate promoters’ activities (55-57). To know if such a mechanism was involved in Brm-mediated facilitation of Brn3b expression, we examined whether or not Brm interacted with one of the upstream regulators of Brn3b, Wt1 (4,5). Co-immunoprecipitation analysis carried out on nuclear extracts from RGC5 cells revealed that Wt1 antibody precipitated protein complexes from the nuclear extract, which were immunoreactive to Brm antibody (Figure 6B). This observation suggested that Brm and Wt1 co-existed in protein complexes in RGC5 nuclei. To know whether Brm was recruited to endogenous Brn3b promoter and if the recruitment were influenced by Wt1, we carried out chromatin immunoprecipitation (ChIP) analysis on RGC5 cells. We observed that DNA-protein complex precipitated by Brm antibody contained sequences corresponding to Brn3b promoter, confirming the presence of Brm on Brn3b Promoter (Figure 6C). The specificity of Brm interactions with Brn3b promoter was demonstrated by increase and decrease in the levels of PCR products in pBabe-Brm transduced and Brm siRNA-treated RGC5 cells, respectively, compared to those in respective controls. To know whether or not Wt1 influenced interactions of Brm with Brn3b promoter, we carried out ChIP assay on RGC5 cells that were transfected with Wt1 expression constructs, following transduction with pBabe/pBabe-Brm retrovirus. We observed that the levels of amplified Brn3b promoter sequence increased when RGC5 cells over-expressed Wt1, compared to controls. Together, these observations suggested that Brm was recruited to Brn3b promoter by Wt1 and this step may constitute a mechanism to facilitate Brn3b expression. To know whether or not Brm expression is associated with nuclease accessibility at the endogenous Brn3b promoter, we examined the restriction enzyme accessibility at the BglII site, -613 bp upstream of the first ATG site in Brn3b promoter in RGC5 cells transduced with pBabe/pbabe-Brm retrovirus. In a LM-PCR assay, we observed a significant increase in the accessibility to the BglII sites in RGC5 cells transduced with pBabe-Brm, compared to those transduced with empty retrovirus (pBabe), suggesting a Brm-mediated change in chromatin configuration at Brn3b promoter (Figure 6D).

A similar mechanism could be invoked where the function of Brm3b in activating Shh is facilitated by Brm. To test this notion, RGC5 cells, transduced with wild type Brm or empty retrovirus were transiently transfected with luciferase reporter constructs in which a highly conserved Shh regulatory sequence containing a Brn3b binding site was cloned upstream of a prolactin minimal promoter (58). Controls included transfection of transduced RGC5 cells with reporter constructs containing only the minimal promoter. We observed a ~6-fold increase in reporter activities in Brm-transduced cells, transfected with reporter constructs containing the Shh regulatory sequence, compared to activities in those that were transduced with empty retrovirus (Figure 6E). Reporter activities were minimal in transduced cells, transfected with reporter constructs containing the minimal prolactin promoter, demonstrating the specificity of Brn3b binding site in Brm-mediated activation of reporter. To know if Brm interacted with Brn3b, the upstream regulator of Shh, we carried out co-immunoprecipitation analysis on nuclear extracts from RGC5 cells. We observed Brn3b antibody precipitated protein complexes that were immunoreactive to Brm antibody, suggesting interactions between Brm and Brn3b (Figure 6F). Next, to know whether Brm was recruited to endogenous Shh promoter, we carried out ChIP analysis on RGC5 cells. We observed that the DNA-protein complex precipitated by Brm antibody contained Shh regulatory sequences with Brn3b binding site.
The specificity of Brm interactions with Shh enhancer was demonstrated by increased and decreased levels of PCR products in pBabe-Brm transduced and Brm siRNA-treated RGC5 cells, respectively, compared to those in respective controls. Taken together, these results demonstrated that Brm facilitated the expression and function of Brn3b, a key regulator of RGC differentiation.

**Influence of Brm on Notch signaling:** One of the key regulators of retinal progenitors during RGC differentiation is Notch signaling. It is thought that attenuation of Notch signaling allows progenitors to commit along RGC lineage (11,59). Since it has been shown previously that Brm might interfere with Notch signaling by forming complexes with CSL (55), we were interested in knowing if Brm influenced Notch signaling during the differentiation of retinal progenitors into RGCs. For Brm to regulate Notch signaling, it should be co-expressed with Notch1 receptor in retinal progenitors. Immunocytochemical analysis of retinal progenitors in differentiating conditions revealed the co-localization of Brm and Notch1 immunoreactivities, suggesting their possible interactions (Figure 7, A and B). In order to understand the nature of interactions between Brm on Notch signaling, we first wanted to know if Brm affected the expression of Notch target genes, Hes1/Hes5 in retinal progenitors. We observed that, in response to over expression of dominant negative Brm, there was an increase in levels of transcripts corresponding to both Hes1 and Hes5, compared to those in control neurospheres, suggesting that Brm negatively regulated Notch signaling (Figure 7C). Next, we wanted to know the mechanism of Brm-mediated inhibition of Hes1 gene. Did Brm directly influence the activities of Hes1 promoter? To address this question, we transiently transfected Hes1-luc vectors in Notch intracellular domain (NICD) expressing 293T cells that were transduced with either wild type Brm retrovirus or empty retrovirus. Reporter activities were easily detected in cells transduced with Hes1-luc vectors and served as positive controls (Figure 7D). We observed a ~3 fold decrease in Hes1 promoter activities in cells transduced wild type Brm retrovirus, compared to controls, suggesting a direct negative effect of Brm on Hes1 promoter activities (Figure 7D).

Next, we wanted to know whether or not these effects on promoter activities involve interactions between CSL/NICD and Brm. CSL is a transcriptional repressor, which is converted to a transcriptional activator when NICD, cleaved upon the activation of Notch receptor, binds to it (60). To address this question, we carried out a co-immunoprecipitation assay, performed on nuclear extracts from cells, over-expressing Brm. We observed that protein complexes immunoprecipitated by CSL and NICD antibodies were immunoreactive to Brm, suggesting interactions between Brm and CSL (Figure 7E). To address the possibility that the faint the Brm immunoreactivity in the complex pulled down by NICD antibody was due to low levels Notch signaling, we carried out co-immunoprecipitation on 293T cells over expressing NICD. There was an increase in Brm immunoreactivity in complex precipitated by NICD antibody from cells overexpressing NICD, compared to controls, confirming the specificity of interactions between Brm and NICD. Next, to test whether or not Brm is recruited to Hes1 promoter, we carried out ChIP analysis in RGC5 cell lines. We observed that DNA-protein precipitated by Brm contained Hes1 promoter sequences suggesting the presence of Brm as a part of Hes1 transcriptional complex (Figure 7F). The specificity of Brm interactions with Hes1 promoter was demonstrated by increase and decrease in the levels of PCR products in pBabe-Brm transduced and Brm siRNA-treated RGC5 cells, respectively, compared to those in respective controls. Taken together, these observations suggested that Brm negatively regulated Notch signaling by facilitating the repression of Hes1.

**Influence of Brm on cell cycle:** Recent observations have demonstrated that ATPase chromatin remodeling complexes are involved in the facilitation of cell cycle exit (61-64). We were interested in knowing if Brm-mediated cell cycle exit could constitute another mechanism by which Brm promotes the differentiation of E14 progenitors into RGCs. We examined this possibility from different angles. We first determined if the proportion of proliferating
cells changed in response to perturbations in Brm expression and function in E14 neurospheres. Since retroviral infection of retinal explants does not lead to a uniform transduction of retinal progenitors, which might lead to ambiguous results we used E14 neurospheres, which are enriched for progenitors and transduced uniformly. E14 neurospheres were transduced with wild type Brm or dominant negative Brm retrovirus in proliferating conditions. Neurospheres were shifted to differentiating conditions, and collected at 4, 12, 24 and 48 hour time-points, after a 4 hours pulse with BrdU to gauge the temporal proportion of proliferating cells. We observed that in comparison to that at 4 hour, the proportion of BrdU+ cells decreased with the time in neurospheres, transduced with wild type Brm retrovirus, compared to controls (Figure 8A). In contrast, the proportion of BrdU+ cells increased with the time in neurospheres, transduced with dominant negative Brm retrovirus, suggesting a role of the endogenous Brm and its chromatin remodeling function in cell proliferation (Figure 8B). Next, we wanted to know, at which stage of the cell cycle Brm exerted its influence, so that we could speculate about the mechanism by which Brm promotes cell cycle exit. E14 neurospheres were transduced with wild type Brm retrovirus and maintained in proliferating condition for 48 hours. Cells were dissociated, stained with propidium iodide and subjected to cell cycle analysis by FACS. We observed that E14 neurospheres, transduced with the empty retrovirus, were enriched for cells that were in G2/M phase (Figure 8C). In contrast, the proportion of cells in G2/M phase had decreased and more cells were shifted to G1 phase in neurospheres transduced with wild type Brm retrovirus. These observations suggested that Brm influenced the G1-S transition. One of the mechanisms by which Brm may influence G1-S transition is by influencing the expression of cyclin E, the G1 phase cyclin that regulates G1 checkpoint (61). Therefore, we argued that the facilitation of RB-E2F-mediated repression of cyclin E, in particular, would ensure that committed precursors do not escape into S-phase. We also examined the expression of cyclin A because, like cyclin E, it is also regulated via Rb-E2F complex and therefore, a likely target of Brm-mediated repression. To test the notion, E14 neurospheres were transduced with wild type Brm/dominant negative Brm/empty retrovirus as described above and levels of transcripts corresponding cell cycle-related genes were examined by RT-PCR analysis. We observed that levels of transcripts corresponding to cyclin E, cyclin A, and Ki67, a cell cycle marker were decreased in neurospheres transduced with wild type Brm retrovirus, compared to controls (Figure 8D). In contrast, their levels increased in neurospheres transduced with dominant negative Brm retrovirus, compared to controls. Similarly, in a separate experiment that involved siRNA-mediated silencing of Brm in neurospheres, we observed an increase in levels of these transcripts in neurospheres treated with Brm siRNA, compared to controls (Figure 8E). Together, these observations suggested that Brm negatively regulated cell cycle during RGC differentiation, and the mechanism may involve inhibition of cyclin E and cyclin A expression. Since the expression of cyclin A is required for the entry into M phase, we surmise that a decrease in cyclin A expression may explain the persistence of cells in the S phase (61,64).

DISCUSSION

The existence of eukaryotic DNA as chromatin renders constraints on the accessibility of the regulatory sequence of genes to tissue-specific and basic transcription factors. The chromatin remodeling complexes, by relaxing (euchromatin) or compacting (heterochromatin) the chromatin organization, modulate the accessibility of these sequences to transcription factors and therefore, facilitate gene activation or repression. That SWI/SNF chromatin remodeling complexes have roles to play in neurogenesis was apparent from long known expression of Brg1 and Brm in the developing brain and retina (65,66). Brm and Brg1 are highly homologous ATPases, however, several lines of evidence suggest that they have different functions and they remodel chromatin in different cellular contexts (67). First, the expression of Brg1 is constitutive and associated with proliferating cells since early embryonic
stages while that of Brm strongly correlates with cell differentiation \textit{in vivo} (68,69) and \textit{in vitro} (70). Second, mice lacking Brg1 die at the pre-implantation stage (71) while those without Brm survive with an overt phenotype of weight gain (72). Third, SWI/SNF complexes containing Brg1 or Brm have different subunit compositions, and Brm-containing complexes appear to have lower chromatin remodeling activities than those with Brg1 (67,73,74). These distinct roles of Brg1 and Brm have emerged from the study of higher vertebrates, particularly mammals. In non-amniotic vertebrates, like frogs and fish, Brg1 has been observed to promote differentiation rather than the maintenance of stem cells/progenitors (29,30). A more recent study, using the conditional knockout strategy, has reaffirmed the earlier observations that Brg1 maintains stem cells, while Brm promotes their differentiation in mammals (31). Our study demonstrates a similar role for Brm in retinal progenitors.

Our results suggest that Brm-mediated chromatin remodeling affects three overlapping steps in RGC differentiation. First, Brm may promote RGC differentiation by facilitating transcriptional activation and function of \textit{Brn3b}. \textit{Brn3b} is a key RGC regulatory gene. Unlike \textit{Math5}, it is not required for RGC specification, but is essential for the normal differentiation and survival of RGCs (75-77). Therefore, it occupies a lower position than \textit{Math5} in the hierarchical regulatory gene network of RGC differentiation and is thought to be under the regulation of \textit{Math5} (76,78). Another important upstream regulator of \textit{Brn3b} during RGC differentiation is \textit{Wt1}. A recent study has demonstrated that \textit{Brn3b} is a direct target of \textit{Wt1} because its proximal promoter contains a \textit{Wt1} binding site, WRE, and \textit{Wt1} can directly activate \textit{Brn3b} promoter (5). Our results suggest that Brm interacts with \textit{Wt1} and gets recruited to \textit{Brn3b} promoter, where it may relax nucleosomal structure, facilitating transcriptional activities of \textit{Wt1}. In addition, we have demonstrated that a similar mechanism may be involved in promoting the function of \textit{Brn3b} in activating \textit{Shh} expression, thus facilitating a cascade of transcriptional activities needed for RGC differentiation. Our observations add to the evidence, emerging from other systems, that the recruitment of Brm to specific promoters by cell-specific transcription factors is a mechanism that provides cell-specificity to chromatin remodeling during differentiation (55). However, the observation that Brm interacts with \textit{Wt1} is at odds with a report that Brg1, and not Brm, interacts with zinc finger transcription factors (55). This discrepancy could be reconciled by the facts that SWI/SNF complexes consist of different subunits, which have tissue specific isoforms and act in tissue-specific manner (72,74,79). Such complex nature of interactions, with temporal and cellular contexts, may be the reason why interactions between Brg1 and \textit{β-catenin} (80), and Brg1 and bHLH transcription factors (30) observed by others in different systems, were not detected by Kadam and Emerson (55).

Second, Brm may influence RGC differentiation by attenuating Notch signaling, thereby promoting cell commitment. This notion is supported by the observation that levels of \textit{Hes1} and \textit{Hes5} increase when Brm expression and function are compromised. We are proposing that Brm influences Notch signaling by interacting with CSL (55). One of the consequences of such interactions could be the repression of \textit{Hes1}/\textit{Hes5}. For example, Brm may prevent NICD-CSL interactions by binding CSL and gets recruited to \textit{Hes1}/\textit{Hes5} promoter, where it could participate in CSL-mediated suppression of promoter activities as CSL without NICD acts as a transcriptional repressor. Additionally or alternatively, Brm may interact with NICD, accentuating the repressor function of CSL. In either case, Brm will inhibit Notch signaling by repressing \textit{Hes1}/\textit{Hes5}, thus promoting RGC differentiation. Repression of genes through Brm/Brg1-mediated chromatin remodeling complexes is not without precedence. For example, Brm/Brg1 forms a repressor complex with RB to inhibit the expression of E2F-mediated expression of \textit{cyclins} (77).

Third, Brm facilitates differentiation by ensuring that committed precursors do not make G1-S transition. The mammalian somatic cell cycle alternates between the S phase and the M phase with gaps, G1 and G2, between them
From the viewpoint of the maintenance of retinal progenitors, the G1 phase has a specific significance. A point that comes late in the G1 phase, when crossed, progenitors irreversibly enter the S phase (G1-S transition). That point is the G1 restriction/checkpoint. The G1 checkpoint is regulated by two types of cyclins, cyclin D and cyclin E that regulate activities of their respective cyclin-dependent kinases (cdks). D type cyclins (D1, D2, D3) are sensitive to growth factors (i.e., FGF2 and Wnts). The growth factor-mediated activation and accumulation of cyclin D-dependent kinases phosphorylate Rb, rendering it incapable of forming repression complex with HDAC and Brm complex. In the absence of Rb repression complex, E2F is able to activate cyclin E gene. The activation and accumulation of cyclin E-dependent kinases complete Rb phosphorylation and in addition, inactivates its own repressor, P27kip1, a member of Cip/Kip family of peptides that inhibit cyclin E- and cyclin A-dependent kinases. These two processes ensure G1-S transition, at which time cyclin E is degraded and replaced by cyclin A, the S-phase cyclin, whose expression is also E2F-dependent. Retinal neurogenesis is intricately linked to the cell cycle (83,84). Different cell cycle regulators, cyclin A, cyclin E, cyclin D1, cdk2 and P27kip1 and Rb, for example, are expressed in the developing retina. In addition, levels of their expression are different in stem cells, progenitors and precursor populations suggesting their modulation during retinal neurogenesis (83,84). Based on conditional and classical knockout experiments and perturbation of expression/function approaches, the roles some of these regulators play in retinal neurogenesis have begun to emerge. For example, cyclin D1 plays an essential role in progenitor populations because there is a remarkable decrease in retinal thickness in cyclin D1 knockout mice, due to compromised progenitor proliferation (85,86). Both Rb and P27kip1 play context dependent roles in the developing retina, i.e., depending on the cellular context they regulate either proliferation or differentiation, the later of Muller cells and the former of rod photoreceptors (87,88). We are proposing that Brm antagonizes G1-S transition by facilitating the inhibition of cyclin E and cyclin A. This notion is supported by our observations that both the position of cells in different phases of the cell cycle and the expression levels of cyclin E and cyclin A change in experiments involving the perturbation of expression and function of Brm. In one of the emerging mechanisms, based on a variety of approaches in transformed cell lines, Brm/Brg1 constitute an integral part of the repressor complex consisting of the retinoblastoma protein (Rb) and HDAC. This complex inhibits the E2F-mediated expression of cyclin E and cyclin A, thus preventing G1-S transition (61-64). A similar mechanism may be involved Brm-mediated repression of cyclin E and cyclin A during RGC differentiation. In summary, our observations suggest that developmental chromatin remodeling, mediated by Brm, may serve as the hub where diverse information for cell differentiation is integrated during neurogenesis. This mechanism is likely to be recruited reiteratively for temporal differentiation of retinal progenitors into different retinal cell types.
REFERENCES

1. Bertrand, N., Castro, D. S., and Guillemot, F. (2002) *Nat Rev Neurosci* **3**, 517-530.
2. Hatakeyama, J., and Kageyama, R. (2004) *Semin Cell Biol* **15**, 83-89.
3. Jessell, T. M. (2000) *Nat Rev Genet* **1**, 20-29.
4. Lee, S. K., and Pfaff, S. L. (2003) *Neuron* **38**, 731-745.
5. Wagner, K. D., Wagner, N., Schley, G., Theres, H., and Scholz, H. (2003) *Gene* **305**, 217-223.
6. Wagner, K. D., Wagner, N., Vidal, V. P., Schley, G., Wilhelm, D., Schedl, A., Englert, C., and Scholz, H. (2002) *Embo J* **21**, 1398-1405.
7. Brown, R. C., Pattison, S., van Ree, J., Coghill, E., Perkins, A., Jane, S. M., and Cunningham, J. M. (2002) *Mol Cell Biol* **22**, 161-170.
8. Kay, J. N., Finger-Baier, K. C., Roeser, T., Staub, W., and Baier, H. (2001) *Neuron* **30**, 725-736.
9. Marquardt, T., Ashery-Padan, R., Andrejewski, N., Scardigli, R., Guillemot, F., and Gruss, P. (2001) *Cell* **105**, 43-55.
10. Mu, X., and Klein, W. H. (2004) *Semin Cell Dev Biol* **15**, 115-123.
11. Ahmad, I., Dooley, C. M., and Polk, D. L. (1997) *Dev Biol* **185**, 92-103.
12. Austin, C. P., Feldman, D. E., Ida, J. A., Jr., and Cepko, C. L. (1995) *Development* **121**, 3637-3650.
13. James, J., Das, A. V., Rahnenführer, J., and Ahmad, I. (2004) *J Neurobiol* **61**, 359-376.
14. Perron, M., and Harris, W. A. (2000) *Cell Mol Life Sci* **57**, 215-223.
15. Rapaport, D. H., and Dorsky, R. I. (1998) *Semin Cell Dev Biol* **9**, 241-247.
16. Imbalzano, A. N., and Xiao, H. (2004) *Adv Protein Chem* **67**, 157-179.
17. Narlikar, G. J., Fan, H. Y., and Kingston, R. E. (2002) *Cell* **108**, 475-487.
18. Martens, J. A., and Winston, F. (2003) *Curr Opin Genet Dev* **13**, 136-142.
19. Hsieh, J., Nakashima, K., Kuwabara, T., Mejia, E., and Gage, F. H. (2004) *Proc Natl Acad Sci U S A* **101**, 16659-16664.
20. Kondo, T., and Raff, M. (2004) *Genes Dev* **18**, 2963-2972.
21. Song, M. R., and Ghosh, A. (2004) *Nat Neurosci* **7**, 229-235.
22. Marenda, D. R., Zraly, C. B., and Dingwall, A. K. (2004) *Dev Biol* **267**, 279-293.
23. Zhang, D., Johnson, M. M., Miller, C. P., Pircher, T. J., Geiger, J. N., and Wojchowski, D. M. (2001) *Exp Hematol* **29**, 1278-1288.
24. Liu, R., Liu, H., Chen, X., Kirby, M., Brown, P. O., and Zhao, K. (2001) *Cell* **106**, 309-318.
25. Kowenz-Leutz, E., and Leutz, A. (1999) *Mol Cell* **4**, 735-743.
26. Pedersen, T. A., Kowenz-Leutz, E., Leutz, A., and Nerlov, C. (2001) *Genes Dev* **15**, 3208-3216.
27. de la Serna, I. L., Carlson, K. A., and Imbalzano, A. N. (2001) *Nat Genet* **27**, 187-190.
28. Young, D. W., Pratap, J., Javed, A., Weiner, B., Ohkawa, Y., van Wijnen, A., Montecino, M., Stein, G. S., Stein, J. L., Imbalzano, A. N., and Lian, J. B. (2004) *J Cell Biochem* **90**, 282-292.
29. Gregg, R. G., Willer, G. B., Fadool, J. M., Dowling, J. E., and Link, B. A. (2003) *Proc Natl Acad Sci U S A* **100**, 6535-6540.
30. Seo, S., Richardson, G. A., and Kroll, K. L. (2005) *Development* **132**, 105-115.
31. Matsumoto, S., Banine, F., Struve, J., Xing, R., Adams, C., Liu, Y., Metzger, D., Chambon, P., Rao, M. S., and Sherman, L. S. (2006) *Dev Biol* **289**, 372-383.
32. Christie, G. A. (1964) *J Morphol* **114**, 263-283
33. Hamburger, V., and Hamilton, H. L. (1951) *J Morphol* **88**, 49-92
34. Ahmad, I., Dooley, C. M., Thoreson, W. B., Rogers, J. A., and Afia, S. (1999) *Brain Res* **831**, 1-10.
35. Das, A. V., Mallya, K. B., Zhao, X., Ahmad, F., Bhattacharya, S., Thoreson, W. B., Hegde, G. V., and Ahmad, I. (2006) *Dev Biol*
36. Zhou, H., Yoshioka, T., and Nathans, J. (1996) *J Neurosci* **16**, 2261-2274.
37. Ringel, J., Jesnowski, R., Moniaux, N., Luttiges, J., Choudhury, A., Batra, S. K., Kloppel, G., and Lohr, M. (2006) *Cancer Res* **66**, 9045-9053
38. James, J., Das, A. V., Bhattacharya, S., Chacko, D. M., Zhao, X., and Ahmad, I. (2003) *J Neurosci* **23**, 8193-8203
39. Dooley, C. M., James, J., Jane McGlade, C., and Ahmad, I. (2003) *J Neurobiol* **54**, 313-325.
40. Matsuda, T., and Cepko, C. L. (2004) *Proc Natl Acad Sci USA* **101**, 16-22
41. Hempel, W. M., and Ferrier, P. (2004) *Methods Mol Biol* **287**, 53-63
42. Roy, K., De La Serna, I. L., and Imbalzano, A. N. (2002) *J Biol Chem* **277**, 33818-33824.
43. Sidman, R. L. (1961) *Histogenesis of mouse retina studied with thymidine [3H]. Structure of the eye* (Smelser, G., Ed.), Academic, New York
44. Young, R. W. (1985) *Anat Rec* **212**, 199-205.
45. Rapaport, D. H., Wong, L. L., Wood, E. D., Yasumura, D., and LaVail, M. M. (2004) *J Comp Neurol* **474**, 304-324
46. Muchardt, C., and Yaniv, M. (1993) *Embo J* **12**, 4279-4290.
47. Fukuoka, J., Fujii, T., Shih, J. H., Dracheva, T., Mezerzman, D., Player, A., Hong, K., Settnes, S., Gupta, A., Buettow, K., Hewitt, S., Travis, W. D., and Jen, J. (2004) *Clin Cancer Res* **10**, 4314-4324
48. Hegde, G. V., James, J., Das, A. V., Zhao, X., Bhattacharya, S., and Ahmad, I. (2007) *Exp Eye Res* **84**, 577-590
49. Das, A. V., James, J., Edakkot, S., and Ahmad, I. (eds) (2005) *Retinal Stem Cells*, 2nd Ed. Stem Cells and CNS development. Edited by Rao, M., Humana Press
50. Alexiades, M. R., and Cepko, C. (1996) *Dev Dyn* **205**, 293-307
51. de La Serna, I. L., Carlson, K. A., Hill, D. A., Guidi, C. J., Stephenson, R. O., Sif, S., Kingston, R. E., and Imbalzano, A. N. (2000) *Mol Cell Biol* **20**, 2839-2851.
52. Mu, X., Fu, X., Sun, H., Beremand, P. D., Thomas, T. L., and Klein, W. H. (2005) *Dev Biol* **280**, 467-481
53. Frassetto, L. J., Schlieve, C. R., Lieven, C. J., Utter, A. A., Jones, M. V., Agarwal, N., and Levin, L. A. (2006) *Invest Ophthalmol Vis Sci* **47**, 427-438
54. Krishnamoorthy, R. R., Agarwal, P., Prasanna, G., Vopat, K., Lambert, W., Sheedlo, H. J., Pang, I. H., Shade, D., Wordinger, R. J., Yorio, T., Clark, A. F., and Agarwal, N. (2001) *Brain Res Mol Brain Res* **86**, 1-12.
55. Kadam, S., and Emerson, B. M. (2003) *Mol Cell* **11**, 377-389
56. Ohkawa, Y., Marfella, C. G., and Imbalzano, A. N. (2006) *Embo J* **25**, 490-501
57. Mu, X., Beremand, P. D., Zhao, S., Pershad, R., Sun, H., Scarpa, A., Liang, S., Thomas, T. L., and Klein, W. H. (2004) *Development* **131**, 1197-1210
59. Austin, T. W., Solar, G. P., Ziegler, F. C., Liem, L., and Matthews, W. (1997) *Blood* **89**, 3624-3635.

60. Mumm, J. S., and Kopan, R. (2000) *Dev Biol* **228**, 151-165.

61. Zhang, Z. K., Davies, K. P., Allen, J., Zhu, L., Pestell, R. G., Zagzag, D., and Kalpana, G. V. (2002) *Mol Cell Biol* **22**, 5975-5988.

62. Strobeck, M. W., Knudsen, K. E., Fribourg, A. F., DeCristofaro, M. F., Weissman, B. E., Imbalzano, A. N., and Knudsen, E. S. (2000) *Proc Natl Acad Sci U S A* **97**, 7748-7753.

63. Coisy, M., Roure, V., Ribot, M., Philips, A., Muchardt, C., Blanchard, J. M., and Dantonel, J. C. (2004) *Mol Cell* **15**, 43-56.

64. Zraly, C. B., Marenda, D. R., and Dingwall, A. K. (2004) *Genetics* **168**, 199-214.

65. Randazzo, F. M., Khavari, P., Crabtree, G., Tamkun, J., and Rossant, J. (1994) *Dev Biol* **161**, 229-242.

66. Schofield, J., Isaac, A., Golovleva, I., Crawley, A., Goodwin, G., Tickle, C., and Brickell, P. (1999) *Mech Dev* **80**, 115-118.

67. Wang, W. (2003) *Curr Top Microbiol Immunol* **274**, 143-169.

68. LeGouy, E., Thompson, E. M., Muchardt, C., and Renard, J. P. (1998) *Dev Dyn* **212**, 38-48.

69. Reisman, D. N., Sciarrotta, J., Bouldin, T. W., Weissman, B. E., and Funkhouser, W. K. (2005) *Appl Immunohistochem Mol Morphol* **13**, 66-74.

70. Machida, Y., Murai, K., Miyake, K., and Iijima, S. (2001) *J Biochem (Tokyo)* **129**, 43-49.

71. Bultman, S., Gebuhr, T., Yee, D., La Mantia, C., Nicholson, J., Gilliam, A., Randazzo, F., Metzger, D., Chambon, P., Crabtree, G., and Magnuson, T. (2000) *Mol Cell* **6**, 1287-1295.

72. Reyes, J. C., Barra, J., Muchardt, C., Camus, A., Babinet, C., and Yaniv, M. (1998) *Embo J* **17**, 6979-6991.

73. Sif, S., Saurin, A. J., Imbalzano, A. N., and Kingston, R. E. (2001) *Genes Dev* **15**, 603-618.

74. Wang, W., Cote, J., Xue, Y., Zhou, S., Khavari, P. A., Biggar, S. R., Muchardt, C., Kalpana, G. V., Goff, S. P., Yaniv, M., Workman, J. L., and Crabtree, G. R. (1996) *Embo J* **15**, 5370-5382.

75. Gan, L., Xiang, M., Zhou, L., Wagner, D. S., Klein, W. H., and Nathans, J. (1996) *Proc Natl Acad Sci U S A* **93**, 3920-3925.

76. Wang, S. W., Kim, B. S., Ding, K., Wang, H., Sun, D., Johnson, R. L., Klein, W. H., and Gan, L. (2001) *Genes Dev* **15**, 24-29.

77. Wang, S. W., Mu, X., Bowers, W. J., Kim, D. S., Plas, D. J., Crair, M. C., Federoff, H. J., Gan, L., and Klein, W. H. (2002) *Development* **129**, 467-477.

78. Brown, N. L., Patel, S., Brzezinski, J., and Glaser, T. (2001) *Development* **128**, 2497-2508.

79. Olave, I., Wang, W., Xue, Y., Kuo, A., and Crabtree, G. R. (2002) *Genes Dev* **16**, 2509-2517.

80. Barker, N., Hurlstone, A., Musisi, H., Miles, A., Bienz, M., and Clevers, H. (2001) *Embo J* **20**, 4935-4943.

81. Baek, S. H., Kioussi, C., Briata, P., Wang, D., Nguyen, H. D., Ohgi, K. A., Glass, C. K., Wynshaw-Boris, A., Rose, D. W., and Rosenfeld, M. G. (2003) *Proc Natl Acad Sci U S A* **100**, 3245-3250.

82. Sherr, C. J. (2000) *Cancer Res* **60**, 3689-3695.
83. Ohnuma, S., and Harris, W. A. (2003) *Neuron* **40**, 199-208
84. Ohnuma, S., Hopper, S., Wang, K. C., Philpott, A., and Harris, W. A. (2002) *Development* **129**, 2435-2446.
85. Fantl, V., Stamp, G., Andrews, A., Rosewell, I., and Dickson, C. (1995) *Genes Dev* **9**, 2364-2372
86. Sicinski, P., Donaher, J. L., Parker, S. B., Li, T., Fazeli, A., Gardner, H., Haslam, S. Z., Bronson, R. T., Elledge, S. J., and Weinberg, R. A. (1995) *Cell* **82**, 621-630
87. Dyer, M. A., and Cepko, C. L. (2001) *J Neurosci* **21**, 4259-4271.
88. Levine, E. M., Close, J., Fero, M., Ostrovsky, A., and Reh, T. A. (2000) *Dev Biol* **219**, 299-314.

**FOOTNOTES**

Thanks are due to Dr. Mengqing Xing for Brm promoter construct, Dr. William Klein for Shh enhancer construct, Dr. Jonathan Licht for Wt1 expression construct, Dr. Neeraj Agarwal for RGC5 cell line, Dr. Angie Rizzino for the ChIP assay protocol, Dr. Greg Bennett and Brittany Cody for a critical reading of the manuscript. This work was supported by Research to Prevent Blindness, Tobacco Fund for Biomedical Research, the Pearsons Foundation, and the Lincy Foundation.

**FIGURE LEGENDS**

**Figure 1. Temporal and spatial expression of Brm in rat retina.** Temporal analysis of Brm expression in the developing and adult retina by RT-PCR analysis reveals that levels of Brm transcripts increase with the onset of differentiation in both early and late stages of retinal histogenesis (A and B). Northern analysis, carried out using rat Brm cDNA as a probe, on mRNA isolated from adult retina, shows 5.8 Kb transcripts corresponding to full length Brm mRNA (C). Immunohistochemical analysis, carried out on E14 (D-G) and E18 (I-L) retina, obtained from embryos *in utero* treated with BrdU, reveals Brm immunoreactivities (arrows) in E14 retina, distributed both towards scleral (s) and ventricular (v) sides. In contrast, in E18 retina, Brm immunoreactivities (arrows) are predominantly localized towards the scleral side in the inner retina (IR). As expected, BrdU immunoreactivities are distributed throughout the width of E14 retina, whereas, they are confined in the outer neuroblastic retina (OR) in E18 retina, where proliferating progenitors are distributed. Immunocytochemical analysis, carried out on cell dissociates from BrdU-exposed E14 (M-P) and E18 retina demonstrate that Brm immunoreactivities are associated with both BrdU⁺ and BrdU⁻ cells (Q). However, the proportion of BrdU⁺ and Brm⁺ cells is significantly (p<.001) higher, compared to that of BrdU⁻ and Brm⁻ cells, suggesting a correlation of Brm expression with the process of retinal differentiation. Magnification, 200x.

**Figure 2. Correlation of Brm expression with the process of RGC differentiation.** Retina from E14 embryos were subjected to double immunohistochemical analysis to determine the spatial distribution of Brm immunoreactivities relative to that of Brn3b and RPF1. The distribution of Brm immunoreactivities overlapped with Brn3b (A-D) and RPF1 (E-H) near the scleral side, where nascent RGCs are located. To demonstrate that Brm is expressed in nascent RGCs, double immunocytochemical analysis was carried out on freshly dissociated E14 retinal cells. Brm immunoreactivities are co-localized with multiple RGC regulators, Wt1 (I), Brn3b (J), RPF1 (K) and Shh (L). To determine the temporal association of Brm expression with RGC differentiation, retina from E12, E14, E16 and E18 embryos were dissociated and subjected to double immunohistochemical analyses to determine the proportion of Brm-positive cells expressing Brn3b (M). Compared to that at E12, the proportion of Brm⁻ and Brn3b⁻ cells increased significantly (p<.001) at E14, the peak of the generation of RGCs. The proportion of Brm⁺ and Brn3b⁺
cells decreased significantly (p<.001) at E18 stage, when the majority of RGC have completed their differentiation. To determine the association of Brm with the dynamics of RGC differentiation, proliferating E14 progenitors, tagged with BrdU in a neurosphere assay, were shifted to differentiating conditions and double immunohistochemical analyses were carried out on aliquot of cells at different time points to determine the temporal proportion of BrdU+/Bnm− and Brm−/Bnm3b+ cells. The proportion of BrdU−/Bnm− cells increased steadily in differentiating conditions, compared to those in proliferating conditions (0 hour), suggesting that as BrdU-tagged progenitors differentiate, they express Brm (N). The proportion of Brm+/Bnm3b+ cells increased steadily in differentiating conditions, compared to those in proliferating conditions (0 hour), suggesting that, with the time, Brm+ cells differentiate into RGCs.

**Figure 3. Effects of perturbation of Brm expression on RGC differentiation.** To attenuate Brm expression during RGC differentiation, pSuper vector with Brm siRNA sequence was electroporated in E14 retinal explants with pGFP-C3 plasmid (A). After 4 days in culture, retinal explants were subjected to Western, immunofluorescence and RT-PCR analyses. Controls included explants electroporated with pSuper vector with scrambled Brm siRNA sequence. The specificity of siRNA-mediated silencing of Brm expression was demonstrated by a decrease in the levels of Brm protein, compared to controls and no change in levels of β-actin protein (B). There was a significant decrease (p<0.001) in the proportion of cells expressing RPF1 (C-F; K) and Brn3b (G-J) in Brm siRNA-treated explants, compared to controls. RT-PCR analysis showed a decrease in the levels of Brm transcripts along with that of the regulators of RGCs (Brn3b, RPF1 and Shh) in Brm siRNA-treated explants (lane 2), compared to controls (lane 1), corroborating immunohistochemical results (L). Magnification, 200x; *=p<0.05; **=p<0.001

**Figure 4. Effects of perturbation of Brm function on RGC differentiation.** To perturb Brm function during RGC differentiation, E14 neurospheres were transduced with dominant negative Brm (pBabe-dnBrm) and wild type Brm (pBabe-Brm) retrovirus, followed by immunocytochemical analyses of cells expressing RGC markers, Brn3b and RPF1. Controls included neurospheres transduced with empty (pBabe) retrovirus. Expression of wild type and dominant negative Brm led to a significant increase (p<0.05) and decrease (p<0.001) in the proportion of RPF1+ (E, F; I, J and M) and Brn3b+ (G, H; K, L and M) cells, respectively, compared to controls (A, B; C, D and M). RT-PCR analyses of transduced neurospheres revealed that expression of wild type Brm and dominant negative Brm led an increase and a decrease in levels of transcripts corresponding to Brn3b, RPF1 and Shh, respectively, compared to controls (N). Tunel analysis shows no significant difference in Tunel-positive cells in siRNA-treated and control explants (M).

**Figure 5. RGCs in the retina of Brm−/− mice.** To determine whether or not Brm−/− mice have RGC phenotype, immunohistochemical analysis was carried out on retina of 3 months old Brm−/− and wild type mice (n=3) (A-D). There was a significant decrease (p<0.001) in the proportion of RPF1+ (A-D; I) and Brn3b+ (E-H; I) cells in Brm−/− retina, compared to those in controls (E). X200 *=p<0.05; **=p<0.001

**Figure 6. Influence of Brm on the regulation of Brn3b and Shh expression.** To determine that Brm facilitates the activation of Brn3b expression, RGC5 cells, transduced with wild type Brm (pBabe-Brm) (+)/empty (pBabe) (-) retrovirus, were transiently transfected with Brn3b-luc (A). There was a significant increase in luciferase activities in cells over expressing Brm, compared to controls, suggesting a direct effect of Brm on Brn3b promoter activities. Co-immunoprecipitation analyses were carried out to determine interactions between Brm and Wt1. Complex immunoprecipitated with Wt1 antibody from the nuclear extract of RGC5 cells were immunoreactive to Brm antibody, suggesting a complex formation between Brm and Wt1 (B). DNA-protein complexes, immunoprecipitated with Brm antibody, in a ChIP assay on RGC5 cells, contained sequence corresponding to Brn3b promoter as demonstrated by the size and sequence specific PCR products, the levels of which were increased and decreased when RGC5 over-expressed Brm (lane 4) /over-expressed Wt1 (lane 5)/over-expressed Brm+Wt1 (lane 6) and treated
with Brm-siRNA (lane 8), respectively (C). Lane 2 (pbabe) and lane 7 (pSuper) represent controls for retrovirus mediated gene transduction and siRNA mediated attenuation of Brm expression, respectively. The analysis of input DNA (lane 1), ChIP assay with IgG (lane2) and the amplification of CD11 promoter sequence constituted non-specific controls. To examine the restriction enzyme accessibility at endogenous Brn3b promoter, we carried out LM-PCR assay on BglII digested nuclei of RGC5 cells, transduced with Brm (lane 2) or empty retrovirus (lane 1) (D). The levels of PCR products, amplified with gene-specific (forward arrow) and LM-PCR (reverse arrow) primers, were higher in cells over-expressing Brm, compared to those transduced with empty retrovirus. LM-PCR assay carried out on BglII digested RGC5 cell genomic DNA constituted controls (lane 3). To determine that Brm facilitates the activation of Shh, RGC5 cells, transduced with wild type Brm (pBabe-Brm) (+)/empty (pBabe) (-) retrovirus, were transiently transfected with luciferase reporter vectors (E). The luciferase reporter vectors contained the rat minimal prolactin promoter (“A”) or conserved 67 bp Shh sequence containing Brn3b binding site, upstream of the minimal prolactin promoter (“B”). There was a significant increase in luciferase activities in Brm-transduced cells, transfected with vector “b”, compared to those transfected with vector “a”. Co-immunoprecipitation analyses were carried out to determine the interactions between Brm and Brn3b. Complex immunoprecipitated with Brn3b antibody from the nuclear extract of RGC5 cells were immunoreactive to Brm antibody, suggesting a complex formation between Brm and Brn3b (F). DNA-protein complexes, immunoprecipitated with Brm antibody, in a ChIP assay on RGC5 cells, contained sequence corresponding to Shh enhancer as demonstrated by the size and sequence specific PCR products, the levels of which were increased and decreased when when RGC5 over-expressed Brm (lane 4) and treated with Brm-siRNA (lane 6), respectively (G). Controls are same as described in (C). *=p<0.05; **=p<0.001

**Figure 7. Interactions between Brm and Notch signaling.** Double immunocytochemical analysis carried out on E14 retinal cells shows the co-localization of Notch1 and Brm immunoreactivities, suggesting interactions between the two (A, B). Examination of the expression of Hes1 and Hes5 in E14 neurospheres transduced with dominant negative Brm (pBabe-dnBrm) and empty (pBabe) retrovirus revealed increase in levels of Hes1/Hes5 transcripts in the former, compared to latter, suggesting that Brm has a negative influence on Notch signaling (C). To determine the mode of the regulation of Hes1 by Brm, NICD expressing 293T cells, transduced with Brm (pBabe-Brm)/empty (pBabe) retrovirus, were transfected with luciferase reporter vectors, pGV-BHes1-Luc (positive control) / pGV-B-Luc (negative control) driven by Hes1 promoter (D). There was significant decrease in luciferase activities in Brm-transduced cells, compared to positive controls, suggesting a direct effect of Brm on Hes1 promoter activities(D). Complexes, immunoprecipitated with CSL and NICD antibodies from the nuclear extracts of cells transduced with wildtype Brm, were immunoreactive for Brm, suggesting interactions between Brm and CSL/NICD (E1). There was an increase in Brm immunoreactivity in complex immunoprecipitated by NICD antibody from cells transfected with NICD expression constructs (2), compared to those transfected with empty expression constructs (1) (E2). DNA-protein complexes, immunoprecipitated with Brm antibody, in a ChIP assay on RGC5 cells, contained sequence corresponding to Hes1 promoter as demonstrated by the size and sequence specific PCR products, the levels of which were increased and decreased when when RGC5 over-expressed Brm (lane 4) and treated with Brm-siRNA (lane 6), respectively (F). Controls are same as described in Figure 6C. Magnification, 200x *=p<0.05; **=p<0.001.

**Figure 8. Influence of Brm on cell proliferation during RGC differentiation.** To determine the influence of Brm on cell proliferation during RGC differentiation, temporal analysis of cell proliferation was carried out in response to perturbation of Brm expression and function. When E14 neurospheres were transduced with wild type Brm (pBabe-Brm) retrovirus and shifted to RGC differentiating conditions, the proportion of BrdU + cells decreased and increased with the time, compared to those transduced with empty retrovirus (A). In contrast, the proportion of BrdU + cells increased with the time in neurospheres transduced with the dominant negative Brm (pBabe-dnBrm), compared to those transduced with the
empty (pBabe) retrovirus (B). To know the influence of Brm on cell cycle regulation during RGC differentiation, E14 neurospheres, transduced with wild type Brm (pBabe-Brm)/empty (pBabe) retrovirus, were dissociated, labeled with propidium iodide and subjected to FACS analysis. The proportion of cells in G2/M phase decreased significantly (S phase, p<0.001; G2/M phase, p<0.05) and those in G1/G0 phase increased (p<0.05) in neurospheres transduced with wild type Brm retrovirus, compared to those in controls, suggesting Brm’s influence on G1-S check point (C). RT-PCR analysis revealed a decrease in the levels of transcripts corresponding to cyclin A, cyclin E and Ki67 in neurospheres transduced with wild type Brm, compared to controls (D). In contrast, levels of these transcripts were increased in neurospheres electroporated with Brm siRNA, compared to controls, suggesting a negative influence of Brm on cell cycle regulators during RGC differentiation (E). *=p<0.05; **=p<0.001
**Table 1:** List of primers and their respective sequences used for RT-PCR and ChIP analysis

| GENES          | PRIMER SEQUENCES                                                                 | PRODUCT SIZE (BP) | ACCESSION NUMBER | TEMP (°C) |
|----------------|----------------------------------------------------------------------------------|-------------------|------------------|-----------|
| β-actin        | Forward: 5'GTGGGGCGCCCCAGGCACCA 3' Reverse: 5' CTCCCTAAATGTCACGCGACGATTTTC 3'   | 548               | XM_O37235        | 50        |
| Hes1           | Forward: 5'GCTTTCTCATCCCCCAAG3' Reverse: 5'CGTATTATAGTGCACGCAAGAGAGAGAG 3'      | 224               | NM_024360        | 56        |
| Ki67           | Forward: 5'GAGCAGTTCAGGGGAACCGGAG3' Reverse: 5'CCTGACTTTTGTTGAGAGAGGCTG3'       | 262               | X82786           | 58        |
| Brn3b          | Forward: 5'GGCTGGAGGAAGCAGAGAGAGAATC 3' Reverse: 5'TTGGCTGGATGGCGAAAGTAG 3'     | 141               | AF390076141      | 60        |
| RPF1           | Forward: 5'TTCAGGGGATTTCTGCTGTGC 3' Reverse: 5'CGCTTTTTTGAGGGCTCAGTCTC 3'       | 359               | XM_344604        | 56        |
| Brm            | Forward: 5'TGCCCTGTTATCTCAGTGGA3' Reverse: 5'CTCCAGGCTCCAGGTACTTTAG 3'         | 180               | XM_005383        | 52        |
| Cyclin A       | Forward: 5'TACACACACAGAGGTAGTGACGCCAAG3' Reverse: 5'CACAAGCTTTTTCTCAGG 3'       | 307               | X60767           | 56        |
| Cyclin E       | Forward: 5'TGACAGACGTGGAAAGCAGCAG 3' Reverse: 5'AGAGAAACGCTCTCCTCCTC 3'         | 303               | D14015           | 59        |
| Shh            | Forward: 5'AGAGCGAGCACCACCAAAAAG 3' Reverse: 5'TTCACAGAGCAGCTGAGTAGG 3'         | 464               | NM_017221        | 56        |
| Hes5           | Forward: 5'TGGAGATGTGTCAGCTGCAAAG 3' Reverse: 5'GCTTTTGCTGCTTGACAGTGG 3'        | 199               | NM_024383        | 58        |
| Brn3b Promoter | Forward: 5'CAGCCCGCGAGGCATGTGTG 3' Reverse: 5'TCTGAAACCGGCGGAGGTCTC 3'         | 256               | NM_138944        | 58        |
| Shh Enhancer   | Forward: 5'GGAGGTCTTCAAGGAACACTTG 3' Reverse: 5'GGTGATGTGATGGAGGTTTG 3'         | 386               | AF098925         | 60        |
| Hes1 Promoter  | Forward: 5'TCTCTTTGCTCGATGGAATACTG 3' Reverse: 5'ATCTGCCCATTTCACCCCGAG 3'      | 398               | D16464           | 56        |
| CD11b Promoter | Forward: 5'GACCCAGGAGGGCTATGT 3' Reverse: 5'AAAGCAAGAAAGGGCCAGAAA 3'          | 122               | M84477           | 52        |
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7
Figure 8
SWI/SNF chromatin remodeling ATPase, BRM regulates the differentiation of early retinal stem cells/progenitors by influencing BRN3B expression and notch signaling

Ani V. Das, Jackson James, Sumitra Bhattacharya, Anthony N. Imbalzano, Marie Lue Antony, Ganapati Hegde, Xing Zhao, Kavita Mallya, Faraz Ahmad, Eric Knudsen and Iqbal Ahmad

J. Biol. Chem. published online September 11, 2007

Access the most updated version of this article at doi: 10.1074/jbc.M706742200

Alerts:

- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts