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Research report

Change in nuclear-cytoplasmic localization of a double-bromodomain protein during proliferation and differentiation of mouse spinal cord and dorsal root ganglia

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Abstract

The human *Brd2* (*Bromodomain-containing 2*) gene codes for a double-bromodomain protein that associates with the cell cycle-driving transcription factors E2F-1 and E2F-2. Expression of mouse *Brd2* has been shown previously to be expressed in specific patterns in proliferating cells in the developing alveoli in the mammary gland. In the present study, in situ hybridization and immunohistochemical analyses were used to examine expression of *Brd2* in developing neural tissues. *Brd2* mRNA was detected in brain vesicles, neural tube, spinal cord and dorsal root ganglia (DRG). Immunostaining proved that the message is translated in these tissues and further revealed that Brd2 protein localizes to the nucleus in proliferating cells, but is cytoplasmic in differentiated neurons that are no longer cycling. Brd2 protein in the nuclei of the proliferating neuronal precursors is excluded from the heterochromatin. These observations are consistent with our previous finding that nuclear localization of Brd2 protein correlates with an active cell cycle in mouse mammary alveoli during the reproductive cycle, and similar results from others in cultured fibroblasts. Our findings are also consistent with the cell cycle progression/transcription coactivator function suggested by the association of Brd2 with E2F-1 and E2F-2.

Theme: Development and regeneration
Topic: Genesis of neurons and glia

Keywords: Bromodomain; Transcription coactivator; Nuclear-cytoplasmic shuttling; Neuronal differentiation; Motor neuron; Dorsal root ganglia

1. Introduction

There is increasing evidence suggesting a cell cycle-linked, Pol II transcription-regulation function for the double-bromodomain protein encoded by the mammalian *Brd2* (*Bromodomain-containing 2*) gene.[2] The Brd2 protein is

Abbreviations: ASC-1, Activating Signal Cointegrator 1; BDF, Bromodomain Factor; *Brd2*, *Bromodomain-containing 2*; CIITA, Class II Transactivator; DAPI, 4′,6-diamidino-2-phenylindole; *dhfr*, *dihydrofolate reductase*; DRG, dorsal root ganglion; E2F, E2 promoter-binding factor; EST, Expressed Sequence Tag; *fs(1)h*, *female sterile(1) homeotic*; *Fsrg1*, *female sterile homeotic-related gene 1*; NGF, Nerve Growth Factor; Pol II, RNA polymerase II; *RING3*, *Really Interesting New Gene 3*; SAGE, Serial Analysis of Gene Expression; SCG, superior cervical ganglion

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[2] The human gene is designated *BRD2* by the HUGO Gene Nomenclature Committee while the mouse gene is designated *Brd2* by the Mouse Genome Nomenclature Committee. For convenience, *Brd2* is used to refer to either the human or mouse gene in this article. *Brd2* was previously known as *Female sterile homeotic-related gene 1* (*Fsrg1)* in mouse [2,26] and *Really Interesting New Gene 3 (RING3)* in man [3,7].

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specifically localized to the nucleus of mouse mammary epithelial (alveolar) cells that proliferate during pregnancy, but is only detected in the cytoplasm after the cells exit the cycle and differentiate at the onset of lactation [2]. A similar observation was made for endogenous and transfected Brd2 in BALB/3T3 mouse fibroblast cells [7]. That is, there was exclusively nuclear localization in proliferating cells, but a patchy distribution throughout both cytoplasm and nucleus in serum-starved, quiescent cells.

The bromodomain is typically found in chromatin-remodeling and transcription coactivator/corepressor proteins and has been shown to bind acetylated lysine side-chains in histone H3 and H4 peptides [5,11]. The presence of two of these motifs in Brd2 suggests that it may also have coactivator/corepressor function. Several types of evidence consistent with such a function have been obtained. When present in the nucleus, Brd2 is localized to the transcribed portion (euchromatin) of the genome [2]. The addition of acetylated histone peptides to nuclear extracts stimulated the association of Brd2 with the sequence-specific DNA-binding transcription factor E2F-2. A complex containing E2F-1 and E2F-2, purified from HeLa cell nuclei, also contained Brd2 [3]. Finally, expression of exogenous Brd2 from a transfected construct in NIH3T3 cells stimulated transcription of several cell cycle-driving genes. The link between Brd2 function in the nucleus and cell cycle progression is bolstered by the observation that the transcription factors with which it associates, E2F-1 and E2F-2, specifically activate transcription of cell cycle progression genes. In addition, mutation of the E2F-binding site in the *cis*-regulatory region of the *dhfr* gene inhibits Brd2-enhanced transcription activation in NIH/3T3 cells [3].

Studies of the regulation of expression of the *Brd2* mRNA are also consistent with a function during cell proliferation. In mouse, the mammary alveolar cells that proliferate during pregnancy express *Brd2* message at a much higher level than the fully expanded, secreting alveolar cells of the lactating gland [2]. SAGE analysis and quantitation of EST frequency in normal and cancerous tissues has revealed increased transcription of *Brd2* during oncogenesis in mouse mammary tissue and in several human tumors ([17], National Cancer Institute, Cancer Genome Anatomy Project; http://cgap.nci.nih.gov). The increase in mRNA abundance in cancerous tissue relative to healthy tissue was 5-fold in mouse mammary gland and between 2.6- and 9-fold in human whole brain, cerebellum, eye, muscle, pancreas, bone marrow and vascular tissue. Bayesian analysis was used to determine the "posterior probability" that the observed increases in expression reflect an actual biological phenomenon and are not due to sampling error. The confidence value is 96% or greater for the examples cited above. Evidence consistent with repression of *Brd2* transcription in cells that have exited the cycle is provided by the observation that E2F-4 (an E2F isotype that, unlike E2F-1 and 2, *represses* transcription) binds to the *cis*-regulatory region of *Brd2* in quiescent human fibroblast cells [24].

Brd2 is a member of a family of four related genes in mammals ([2,4,10]; E. Shang, G. Salazar, T. E. Crowley, X. Wang, R.A. Lopez, X.Y. Wang, D. J. Wolgemuth, in preparation). A single homolog, *female sterile (1) homeotic* [*fs(1)h*], is present in the *Drosophila* genome and yeast has two homologs, *Bromodomain Factor 1* and *2* (*BDF1* and *BDF2*) [6]. Studies of BDF1 and BDF2 have demonstrated a role in transcription regulation consistent with the data described above for Brd2 [16,20].

During mouse embryonic development at 9–12 days postcoitum (E9–12), the neural tube differentiates into spinal cord. Variation in the timing of the differentiation (and therefore exit from the cell cycle) of neuronal precursors is evident along both the ventrodorsal and lateral–medial axes in transverse sections of developing spinal cord. Precursors migrate laterally from the ventricular zone that surrounds the lumen as they prepare for differentiation. This differentiation occurs earlier in the ventral region, where both motor neurons and interneurons form, than in the dorsal region where only interneurons arise. There is also a temporal gradient of differentiation along the rostrocaudal axis such that neuronal birthdays occur earlier in more rostral positions. In mouse, the relevant timepoints at the lumbar (caudal) position are: E11 for the ventral region and E13–14 for the dorsal region [21,22,27]. As the mouse dorsal root ganglia (DRG) develop, the stage at which the precursor cells exit the cycle to become neurons also varies along the rostrocaudal axis: E9.5–13.5 in the cervical region, but E10.5–14.5 in the lumbar region [18,19]. During development of the cerebellum, precursors exit the cycle and differentiate into granule and Purkinje neurons

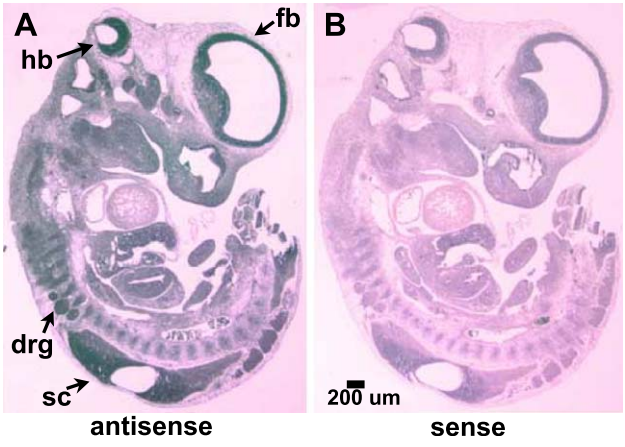

Fig. 1. *Brd2* mRNA is expressed in developing neural tissues during mouse embryogenesis. A radiolabeled antisense probe (derived from a cDNA fragment) was hybridized to an E11.5 left-lateral sagittal section and signal detected with a combination of brightfield and fluorescence microscopy (A). The green fluorescent signal indicates hybridization on the neuroepithelia of the forebrain (fb) and hindbrain (hb) vesicles, the DRG and the spinal cord (sc). For a negative control, a radiolabeled sense strand, derived from the same cDNA fragment used to produce the antisense probe, was hybridized to an equivalent section (B). No signal was detected on any tissue with the sense-strand probe. The nuclear counterstain was hematoxylin (blue) while the cytoplasmic counterstain was eosin (pink).

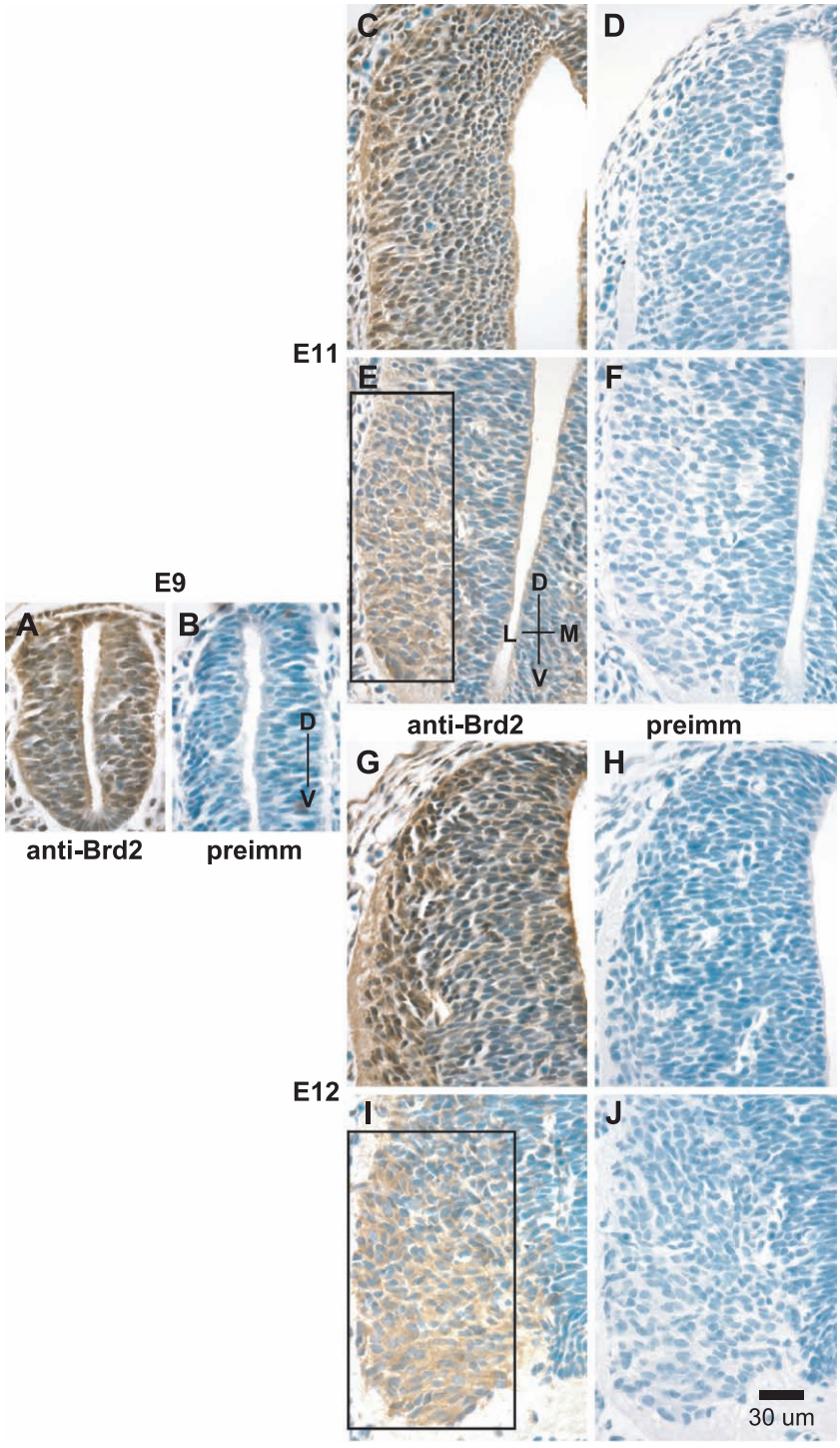

Fig. 2. Change in localization of Brd2 protein from the nucleus to the cytoplasm as precursors exit the cycle and differentiate into neurons in the developing spinal cord. Immunostaining of transverse sections of mouse embryos showed nuclear localization of Brd2 (orange–brown signal) throughout the neural tube at E9 (A) and continued nuclear localization in the dorsal region of the differentiating spinal cord at E11–12 (C and G) as these interneuron precursors continue to proliferate (nuclear counterstain: hematoxylin, blue). However, in the ventrolateral region of the developing spinal cord, localization of Brd2 shifts from nuclear to cytoplasmic during E11–12 (outlined area in E and I) as cells exit the cycle and differentiate into interneurons and motor neurons. (Quantitation in Table 1.) In nuclei positive for Brd2, the orange–brown signal masks the blue counterstain. Most of the cells in the more medial portion of the ventral region shown in E and I did not show signal for Brd2. The dorsal–ventral orientation of the E9 sections is shown in panel B, while the dorsal–ventral and lateral–medial orientations for the E11 and E12 sections are shown in panel E. Similar sections from each timepoint were stained with preimmune serum plus hematoxylin, and in these samples, only the blue nuclear counterstain signal is seen (B, D, F, H and J).

Table 1
Quantitation of nuclear versus cytoplasmic localization of Brd2 protein in sections of neural tube, spinal cord and DRG from the lumbar region

Stage	Region	Number of cells			Percentage			Figure panel used for counts
		N	N&C	C	N	N&C	C	
E9	neural tube	166	12	1	92.7	6.7	0.6	2A
E11	dorsal spinal cord[a]	466	19	0	96.1	3.9	0	2C
E11	ventrolateral spinal cord[a]	3	10	229	1.2	4.2	94.6	2E
E12	dorsal spinal cord[a]	375	46	0	89.1	10.9	0	2G
E12	ventrolateral spinal cord[a]	5	26	200	2.2	11.3	86.6	2I
E11.5	DRG	47	131	0	26	74	0	3A
E15	DRG	0	8	155	0	5	95	3C

For the ventrolateral regions at these stages, only the cells within the rectangle in panels E and I were counted. N: only nuclear, N&C: nuclear and cytoplasmic, C: only cytoplasmic.

[a] For the dorsal portion of the E11 and E12 spinal cord, all cells shown in panels C and G of Fig. 2 were counted.

after E13 [9]. Therefore, the hindbrain neuroepithelium revealed by an E11.5 sagittal section provides a sample in which all the cells are expected to be proliferating. The proliferation of precursor cells, the eventual exit from the cycle and the subsequent differentiation of neurons that occurs during development of spinal cord, sympathetic ganglia and brain vesicles in mammals provides an excellent system for examining the function and regulation of cell cycle progression factors.

2. Materials and methods

2.1. Source of tissues

CD1 mice were obtained from Charles River Laboratories (Wilmington, DE). Timed matings were set up with day of the vaginal plug as E0.5. Embryos were collected at the gestational ages indicated in the figure legends. To minimize pain and discomfort, all mouse manipulations were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH pub 80-23), rev. 1996.

2.2. Production and purification of anti-Brd2 antibody

The construct used to express the Brd2 protein in bacteria to produce antigen has been described previously [26]. Production of antiserum in rabbit, affinity purification of the serum and assessment of specificity have also been described previously [2]. The antigen consisted of amino acids 51–651, which includes both bromodomains, but not the ET domain. This antibody had previously been shown to

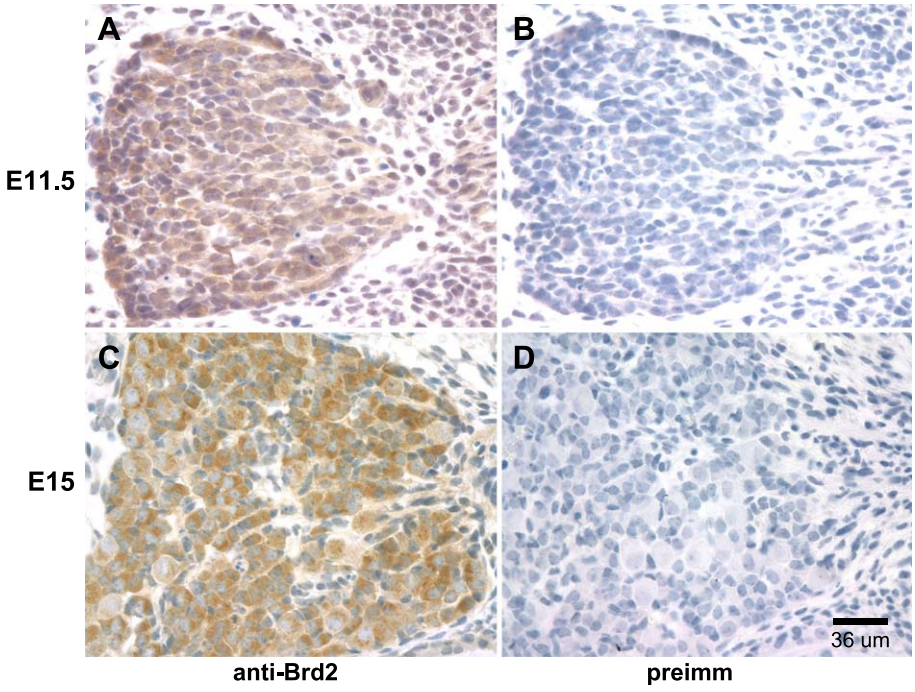

Fig. 3. Change in localization of Brd2 protein from nucleus to cytoplasm as precursors exit the cycle and differentiate into sensory neurons in the developing dorsal root ganglia. A strong signal for Brd2 (orange–brown) is detected in the nuclei of precursor cells at E11.5 (A). (Some E11.5 cells also show cytoplasmic signal.) After these cells leave the cycle and differentiate, the nuclear signal disappears in most cells while a strong cytoplasmic Brd2 signal becomes apparent (stage E15, panel C). (Quantitation in Table 1.) The nuclear counterstain is hematoxylin (blue). In nuclei positive for Brd2, the orange–brown signal masks the blue counterstain. Similar sections from each timepoint were stained with preimmune serum plus hematoxylin, and in these samples only the blue nuclear counterstain signal is seen (B, D).

be effective for immunostaining of mouse mammary epithelia [2]. For the negative control stainings, preimmune serum from the rabbit used for anti-Brd2 production was used. This serum was put through the same purification procedure as the anti-Brd2 serum.

2.3. Immunostaining

For conventional immunostaining, preparation of sections, staining with antibody and detection of signal was performed as described previously [30]. The affinity-purified anti-Brd2 was applied at dilutions ranging from 1:60 to 1:200. The goat-anti-rabbit secondary antibody was applied at a dilution of 1:100. The orange–brown signal was generated with the peroxidase/diaminobenzidine system (Vectastain ABC Kit, Vector Labs, Burlingame, CA). The blue nuclear counterstain was hematoxylin. For each staining experiment, an equivalent section was stained with preimmune serum, prepared as described above, as a negative control.

Immunofluorescent staining was performed in the same manner with the following alterations. The anti-Brd2 antibody was detected with an Alexa Fluor 594 (red)-conjugated, goat-anti-rabbit secondary antibody (Molecular Probes, Eugene, OR), applied at a 1:100 dilution. DAPI (blue), applied at 300 nM, was used as the nuclear counterstain.

2.4. In situ hybridization

Preparation of tissue sections, synthesis of ^{35}S-UTP-labeled single-strand RNA probes, hybridization of the probes to sections and generation of the signal by exposure to a photographic emulsion were performed as previously described [25]. The cDNA fragment used for probe synthesis was a portion of the protein-coding sequence between the two bromodomains, corresponding to nucleotides 1698–2340 of the previously reported sequence [26]. This probe recognizes both somatic *Brd2* transcripts. The antisense probe was synthesized with T7 polymerase, while the negative control sense probe was made with T3 polymerase. Hybridized slides were coated with photographic emulsion and exposed for 12 days.

2.5. Microscopy and imaging

Tissue sections with DAB and hematoxylin staining were viewed with a Nikon Eclipse E800 microscope using white light and Differential Interference Contrast optics. Immunofluorescent-stained preparations were viewed with the same microscope using a mercury lamp and the following filter combinations. For Alexa Fluor 594: excitation = 555–560 nm and barrier = 645–675 nm. For DAPI: excitation = 330–380 nm and barrier = 420 nm. The same microscope was

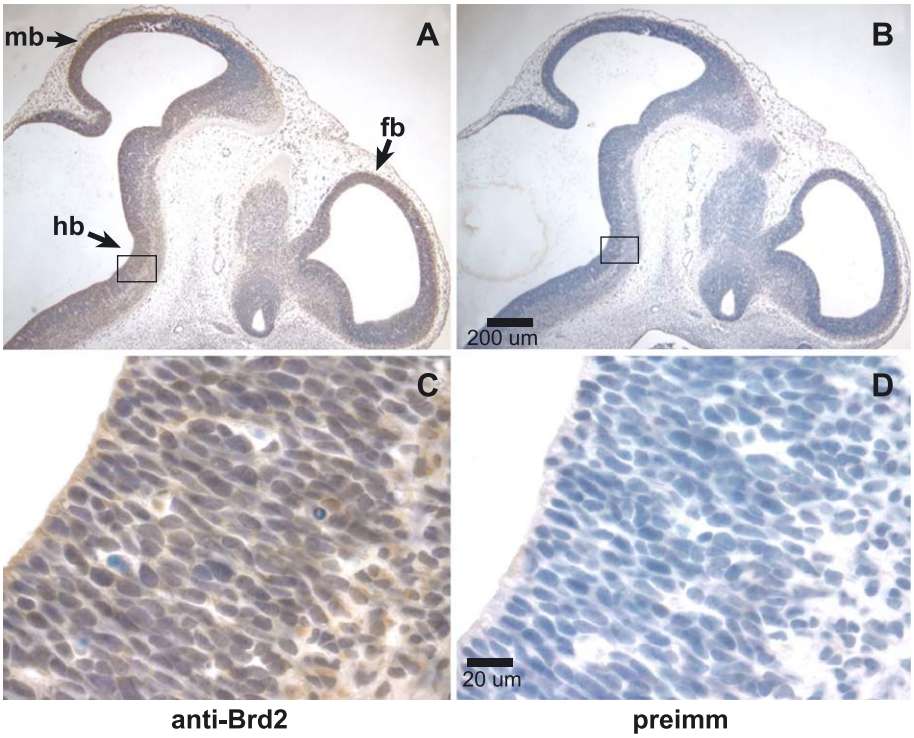

Fig. 4. Nuclear localization of Brd2 protein in the proliferating embryonic hindbrain neuroepithelium. Low (A) and high (C) magnification images of an E11.5, right-lateral sagittal section stained with anti-Brd2. The rectangle in panel A outlines the area included in panel C. The anti-Brd2 signal is orange–brown and the nuclear counterstain is hematoxylin (blue). Brd2 is detected in the nucleus throughout the neuroepithelium of the hindbrain (C). In nuclei positive for Brd2, the orange–brown signal masks the blue counterstain. A similar section was stained with preimmune serum plus hematoxylin and only the blue nuclear counterstain signal was seen in this sample (B and D). (fb, forebrain; mb, midbrain; hb, hindbrain).

used for viewing in situ hybridizations, however, a combination of brightfield and fluorescence microscopy (with an IGS filter that passes only green light, 535–550 nm, from the mercury lamp) was employed for these slides. Images were obtained with a Diagnostics Instruments SPOT 1.4.0 digital camera. The conversion of the blue DAPI signal to green, and the merge of the DAPI and Alexa Fluor 594 signals (Fig. 5C), were done with Adobe Photoshop.

3. Results

To determine if the *Brd2* gene is transcribed in the developing neural tissues of the mouse embryo, in situ hybridization analysis was performed using E11.5 left-lateral sagittal sections and a probe derived from between the two bromodomains. (*Brd2* produces two transcripts during embryogenesis, 4.0 and 4.6 kb, but only one version of the protein-coding sequence has been observed in cDNAs [26]. Thus, an interbromodomain probe is likely to give an equal signal for both transcripts.) A strong signal for the *Brd2* mRNA was detected on the neuroepithelia of the forebrain and hindbrain vesicles (the midbrain vesicle is typically not included in this type of section [13]). Other neural structures also strongly expressed *Brd2*, including the DRG and the spinal cord (Fig. 1A). Immunostaining of similar sagittal sections with anti-Brd2 antibody revealed that the *Brd2* message is translated in these tissues (data not shown).

The possibility that nucleocytoplasmic localization of this protein might be regulated in developing neural tissues, in a manner similar to that observed in mammary epithelia during the reproductive cycle [2], was addressed by immunostaining transverse sections of neural tube and spinal cord from the lumbar region of stage E9–12 embryos. Observation of these sections revealed a predominantly nuclear localization for Brd2 in the E9 neural tube, where cells are known to be proliferating [21,22,27] (Fig. 2A, Table 1). However, as the tube differentiates into spinal cord, the intracellular localization of Brd2 varies between the dorsal region where most of the interneuron precursors continue to

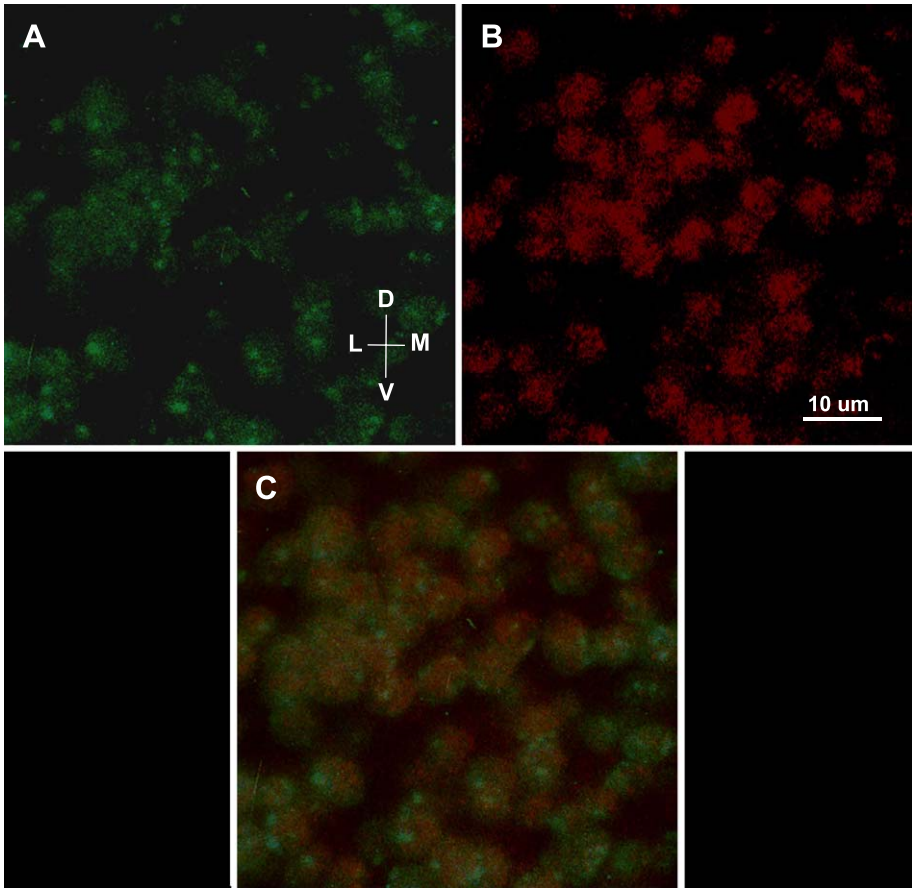

Fig. 5. The distribution of Brd2 protein in the nucleus of proliferating interneuron precursors is consistent with euchromatic localization. The dorsal portion of the spinal cord in an E10 transverse section (similar to those shown in Fig. 2C, D, G and H), stained with DAPI and anti-Brd2, is shown. Dorsal–ventral and lateral–medial orientations are indicated and are the same as in Fig. 2, panels C–J. DAPI binds DNA, concentrating on the heterochromatin, and gives a blue–white fluorescent signal (converted to green for better contrast in the merged image). A single piece of tissue was imaged with filter combinations producing only the DAPI signal (A) or only the anti-Brd2 (B), and a merge of the two signals was made with Adobe Photoshop (C). The 1–5 intense DAPI spots in each nucleus represent the heterochromatin, while the anti-Brd2 signal is more dispersed. Very little overlap of the signals, which would produce yellow, is visible in the merged image.

proliferate, and the ventrolateral region where differentiation produces some interneurons and all of the motor neurons. At E11 and E12, Brd2 is nuclear in the majority of the dorsal region cells (Fig. 2C and G, Table 1) but cytoplasmic in most ventrolateral region cells (Fig. 2E and I, Table 1). The ventromedial region in the E11 and E12 sections (Fig. 2E and I), which includes glial precursors ([22] and Samuel Pfaff, personal communication), did not show signal for Brd2 protein. Some of the precursor cells in the E9 neural tube and dorsal interneuron precursors at E11 and 12 showed a weak cytoplasmic signal, but these cells also showed a prominent nuclear signal. The total amount of staining per cell is somewhat reduced in the ventrolateral region relative to the dorsal region at E11 and E12. Sections of embryonic DRG, taken from the lumbar region of stages E11.5 and E15, were examined in the same manner. Brd2 protein was detected in the nuclei of all the proliferating neuronal precursors in the E11.5 DRG (Fig. 3A, Table 1). A weak cytoplasmic signal was seen in some cells, somewhat more intense than that observed in the cytoplasm of precursors in the E9 neural tube and dorsal interneuron precursors at E11 and 12. However, immunostaining of E15 DRG (Fig. 3C) revealed that in the majority of the neurons, that are presumably postmitotic, Brd2 was only in the cytoplasm (Table 1).

The nuclear localization of Brd2 protein in neuronal precursors known to be proliferating in the neural tube/spinal cord [21,22,27] and DRG [18,19] suggested that this protein has an essential function in the nucleus at this stage of differentiation. To obtain more evidence to support this conclusion, E11.5 sagittal sections that included the proliferating hindbrain neuroepithelium were immunostained with anti-Brd2 antibody. Brd2 was detected in virtually all the nuclei throughout this tissue, with only weak cytoplasmic staining (Fig. 4A and C). In this experiment, the Brd2 protein was also detected in the midbrain and forebrain (Fig. 4A) although the signal in the forebrain was weaker than that in the other regions.

Euchromatin is the transcribed portion of the genome and in mammals consists of approximately 40% GC base pairs. Heterochromatin is enriched in AT base pairs and is not transcriptionally active. The fluorescent DNA stain DAPI binds preferentially to AT pairs thereby concentrating in heterochromatin [15]. Immunofluorescent staining for a Pol II transcription coactivator in interphase cells is expected to produce a signal that does not overlap the most intense DAPI signal in the nucleus. Such a localization of Brd2 protein was observed in transverse sections of the dorsal region of an E10 spinal cord that is known to contain proliferating interneuron precursors (Fig. 5).

4. Discussion

There are many examples of the regulation of nuclear localization of transcription-regulatory proteins by ligand association/disassociation or posttranslational modification. Most relevant to the current study is the regulation of intracellular localization of known Pol II transcription coactivator/corepressor proteins such as Class II Transactivator (CIITA) and Activating Signal Cointegrator 1 (ASC-1). CIITA has histone acetyltransferase activity, and is believed to contribute to transcription activation by remodeling chromatin on target gene promoters [23]. These targets include MHC classes I and II genes. The intracellular distribution of CIITA is regulated by nuclear export control: GTP binding blocks export causing accumulation in the nucleus. ASC-1 contains a zinc finger motif and functions as a coactivator for DNA-binding nuclear receptor transcription factors including the retinoic acid receptor [14]. The regulation of nucleocytoplasmic distribution of ASC-1 in cultured cells was similar to that observed for Brd2: nuclear in cells proliferating in serum but cytoplasmic after serum was withdrawn and cells exited the cycle.

Nuclear localization of Brd2 protein in the proliferating hindbrain neuroepithelium at E11.5 suggests a possible role in cell cycle progression. Although the *Brd2* mRNA appeared to be equally abundant in the hindbrain and forebrain at E11.5, immunostaining produced a stronger signal on hindbrain. It is not yet known if this is due to a reduced rate of protein synthesis or an increased rate of degradation in the forebrain. The nonheterochromatic distribution of Brd2 protein in the nuclei of the proliferating neuronal precursors is consistent with the previous observation that Brd2 colocalizes with the transcription mediator subunit cyclin-dependent kinase 8, distinct from heterochromatin, in the nuclei of mammary epithelial cells [2]. This observation is also consistent with the hypothesis that this protein functions as a transcription coactivator of cell cycle-driving genes. The validity of this conclusion is strengthened by the timing of the change in Brd2 localization from nucleus to cytoplasm in the ventrolateral region of the developing spinal cord and throughout the DRG. This transition occurred between E9 and 12 in the ventrolateral portion of the cord at the lumbar rostrocaudal position where some of the interneurons and all of the motor neurons are known to differentiate at E11 [21,22,27]. The weaker total signal per cell in the ventrolateral relative to dorsal region at E11–12 may be due to decreased mRNA level resulting in less protein synthesis or reduced protein stability in the cytoplasm. This change in localization of Brd2 was also observed between E11.5 and 15 in the DRG at the lumbar position where neuronal differentiation occurs between E10.5 and 14.5 [18,19]. In the E9 neural tube and E11–12 dorsal spinal cord, a weak cytoplasmic signal was observed in some cells in addition to the strong nuclear signal in spite of the fact that these cells should still be proliferating. The cytoplasmic signal in the E11.5 DRG, in addition to the strong nuclear signal, was somewhat more noticeable than the cytoplasmic signal in the proliferating cells of the neural tube and spinal cord. Possibly this is because differentiation in the developing DRG occurs

gradually, beginning at E10.5, such that some cells have already exited the cycle at E11.5. The regulation of nucleocytoplasmic localization for CIITA and ASC-1 mentioned above, the previously published data for Brd2 [2,3,7] and the results presented here for Brd2 suggest that this type of regulation may control the activity of many coactivator/corepressor proteins. In particular, the correlation of cell cycle progression and nuclear localization found for ASC-1 and Brd2 may indicate that many of these proteins are needed in the nucleus in proliferating cells, but must be excluded to allow transition to the G_0 state.

The presence of Brd2 in the cytoplasm of differentiated neurons is consistent with the previous observation that this protein is present in the cytoplasm of G_0 mammary epithelial cells during lactation in mouse. Brd2 was found to localize again to the nucleus at the end of the lactation period as the epithelial cells are initiating programmed cell death (apoptosis), suggesting that it has a second function at this stage [2]. In mammary epithelial cells, Brd2 may function as a coactivator of genes that trigger apoptosis, or as a corepressor that contributes to silencing of genes that maintain the G_0 state. An indication that this second function may be relevant to neuronal development is the increased rate of *Brd2* transcription in the rat superior cervical ganglia (SCG) in which apoptosis had been induced by injection of anti-NGF [28]. Apoptosis is a natural part of SCG development in mammals; e.g. in rat, those neurons that fail to form a synapse with another neuron (33%) are lost between postnatal days 3 and 7 [29]. A similar phenomenon occurs among the motor neurons in the ventral region of the developing spinal cord, although in this case, 50% die [12]. Brd2 may have a role in an apoptosis-inducing mechanism in the developing spinal cord and DRG in mouse. A dual function in both cell cycle progression and apoptosis has been observed for a number of regulatory proteins in various cell types. For example, cyclin D1 has a fundamental role in cycle progression in all mammalian cell types and has been proven to function in apoptosis in mammary epithelial cells in culture [1,8]. In addition, transcription of *cyclin D1* increased in the rat SCG during apoptosis-induction in the same experiment described above for *Brd2* [28]. The possibility that Brd2 protein might function as a coactivator on the *cis*-regulatory region of *cyclin D1* in neurons entering apoptosis is suggested by the observed stimulation of *cyclin D1* transcription by transfected Brd2 in proliferating NIH/3T3 cells [3].

It has been shown that a basic region of Brd2 that matches the consensus sequence for a nuclear localization signal is necessary for nuclear import in response to mitogenic stimulation in BALB/3T3 cells [7]. Fusion of an 11-amino acid peptide including this sequence to the green fluorescent protein stimulated translocation to the nucleus in similar culture conditions. Thus, it is also sufficient for nuclear import. The mechanism by which Brd2 is restricted to the cytoplasm in differentiated mammary alveolar cells and neurons, e.g. inhibition of nuclear import, tethering to a cytoplasmic structure or an increased rate of export from the nucleus, is not known.

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