Expression of Chromatin Remodeling Factors during Neural Differentiation[1]

Yuichi Machida, Kiyohito Murai, Katsuhide Miyake,[2] and Shinji Iijima

Department of Biotechnology, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603

Received July 17, 2000; accepted October 19, 2000

The yeast SWI/SNF complex is involved in remodeling of chromatin structure during transcriptional modulation. One of the key subunits of this complex, called SWI2/SNF2, has a DNA-dependent ATPase activity. Two different types of mammalian homolog of yeast SWI2/SNF2, called BRM and BRG1, were recently identified. They are closely similar in structure but have distinct functions. We investigated the expression of BRM and BRG1 during differentiation of neural precursor cells (NPCs) cultured *in vitro*. The expression of BRM was very low in NPCs and was induced to a high level during differentiation to neurons and astrocytes. In contrast, BRG1 was constantly expressed throughout differentiation. These phenomena were also observed in differentiation of P19 embryonal carcinoma cells to neural cells. Immunocytochemical analyses revealed that the expression of BRM started even in the undifferentiated nestin-positive cells. These results indicate that BRM may have an important role in neural cell differentiation.

Key words: BRG1, BRM, chromatin remodeling factor, differentiation, neural precursor cell.

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The modification of chromatin structure is increasingly recognized to be an important factor in transcriptional regulation (*1*). So far, it is known that the state of chromatin compaction is controlled by two major mechanisms (*2*, *3*). One is a histone acetyltransferase and deacetylase system (*4*), the other a regulatory system containing chromatin-remodeling complexes (*5*). The former enzymes acetylate or deacetylate N-terminal tail structures of histones and control chromatin opening and compaction (*4*). The chromatin-remodeling complex was characterized first in *Saccharomyces cerevisiae* as a multi-protein complex that controls mating type switch (SWI) and sucrose non-fermenting (SNF) genes. ATP-dependent chromatin remodeling activity of the complex was recently identified (*6*). This SWI/SNF complex has an ATPase subunit called SWI2/SNF2, and its activity is essential for chromatin remodeling (*6*). The homologs of SWI2/SNF2 have been identified in higher eukaryotes, and they also form multi-protein complexes of chromatin-remodeling factors (*1*).

Mammalian SWI/SNF complexes are composed of more than eight subunits (*7*). Of these, two ATPase subunits have been identified and named BRM and BRG1 (*8*, *9*). These two proteins are mutually exclusive in the SWI/SNF complex. Recently, it was reported that BRM or BRG1 alone shows chromatin-remodeling activity, and other subunits of the complex, such as INI1, BAF155, and BAF170, increase the activity (*7*). The SWI/SNF complex containing either BRG1 or BRM shows chromatin-remodeling activity and activates or inactivates gene expression (*10–16*). BRM and BRG1 have high sequence homology to each other and were reported to have both common and distinct roles. For instance, both of them bind retinoblastoma (RB) protein (*17*, *18*), cyclin E (*19*), several nuclear hormone receptors (*10–12*), and transcription factors (*13*, *14*, *16*). Since both of them bind RB protein, they seem to be important for cell cycle control. In fact, forced expression of either BRM or BRG1 results in growth arrest (*18–20*). In spite of these common features, only BRM is down-regulated by *ras*-transformation and up-regulated by growth arrest induced by serum starvation (*20*). Sumi-Ichinose *et al.* recently reported that inactivation of one of two *brg1* genes in F9 embryonal carcinoma cells alters their capacity for proliferation but not for differentiation (*21*). They also reported that inactivation of both *brg1* alleles severely affects F9 cell viability. In contrast, BRM knockout mice survive with almost normal phenotypes except for increase in body weight; and in this regard, cells isolated from the knockout animals showed increased growth ability (*22*). From these results, Yaniv *et al.* speculated that the BRM-containing complex may be important for the expression of genes relating to quiescence and terminal differentiation (*20*), but functions of BRM in cell differentiation are poorly understood.

In this article, we analyzed BRM expression during neural differentiation of NPCs and P19 embryonal carcinoma cells to study its role in neural differentiation.

[1] This work was supported partly by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists (to Y.M.).

[2] To whom correspondence should be addressed. Tel: +81-52-789-4278, Fax: +81-52-789-3221, E-mail: miyake@proc.nubio.nagoya-u.ac.jp

Abbreviations: DMSO, dimethyl sulfoxide; GFAP, glial fibrillary acidic protein; GST, glutathione *S*-transferase; MAP2, microtubule-associated protein 2; NPC, neural precursor cell; PBS, phosphate buffered saline; RA, retinoic acid; RB, retinoblastoma; RT-PCR, reverse transcription–polymerase chain reaction.

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MATERIALS AND METHODS

Cell Culture—Mouse NPCs were obtained from cerebrums of E10 ddY mouse embryos based on the method described by Kitani *et al.* (*23*). DMEM/F12 (Life Technologies, Rockville, MD) supplemented with 0.03 M $NaHCO_3$, 1% fetal bovine serum, 10 μg/ml insulin (Wako Pure Chemical Industries, Osaka), 10 μg/ml transferrin (Wako), 10 ng/ml cholera toxin (Sigma Chemical, St. Louis, MO), 0.01 μM sodium selenite, 80 μg/ml penicillin, and 90 μg/ml streptomycin was used as culture medium. NPCs were plated at a density of approximately 0.5×10^7 cells per 100-mm plastic dish coated with poly-D-lysine (Sigma). Primary neurons were obtained from cerebrums of E17 ddY mouse embryos. Cerebrums were cut into small pieces and washed with phosphate buffered saline (PBS). Cells were dissociated by the treatment with PBS containing 0.25% trypsin, 0.5% glucose, and 100 μg/ml DNase I at 37°C for 20 min. After washing, cells were plated on poly-D-lysine–coated 100-mm tissue culture dishes at a density of 2×10^7 cells per plate and cultured in DMEM/F12 supplemented with 10% fetal bovine serum for 2 days. In order to concentrate neurons, the medium was replaced with fresh medium containing 10 μM cytosine arabinoside (Sigma) and cells were cultured for an additional 2 days. By microscopic observation, more than 80% of cells were neurons as judged by their morphology. Primary astrocytes were obtained from cerebrums of newborn ddY mice as described previously (*24*) and cultured in DMEM (Life Technologies) supplemented with 10% fetal bovine serum. When astrocytes were matured by treatment with dibutyryl-cAMP (*25*) or forskolin (*26*), the serum was withdrawn from the medium. Isolated astrocytes were almost subconfluent after 7 days of culture. Confluent culture was achieved after 14 days. Cell growth was analyzed by [^{3}H]thymidine incorporation.

P19 embryonal carcinoma cells were maintained in αMEM (Life Technologies) containing 10% fetal bovine serum as described previously (*27*). In monolayer culture, cells were plated at a density of 1×10^6 cells per 100-mm plastic dish and differentiated into endodermal and mesodermal cells by adding 0.5 μM all-*trans* retinoic acid (RA) (Wako). For differentiation into neural or muscle cells, cells were grown in the medium containing 0.5 μM all-*trans* RA or 1% dimethyl sulfoxide (DMSO) (Wako), respectively, at a density of 1×10^6 cells per 100-mm bacteriological-grade petri dishes. After 4 days, cell aggregates were transferred into tissue culture dishes and cultured in the medium without inducers for an additional 6 days. All cultures were maintained at 37°C in humidified 5% CO_2.

Antiserum and Western Blot Analyses—Anti-BRM antibody was produced by use of a glutathione *S*-transferase (GST)–fusion protein expressed in *Escherichia coli*. An expression plasmid was constructed by inserting the cDNA fragment of mouse BRM (encoding amino acid residues 50–214 in the corresponding human sequence) into pGEX-5X-2 (Amersham Pharmacia Biotech, Buckinghamshire). The cDNA was prepared by reverse transcription–polymerase chain reaction (RT-PCR) by using primers designed on the corresponding sequence obtained from GenBank/EMBL/DDBJ database. The fusion protein was expressed in *E. coli* BL21 and purified on a glutathione-Sepharose 4B column (Amersham Pharmacia Biotech). For production of anti-BRG1 antibody, GST-BRG1 fusion protein was produced in a similar way with the cDNA fragment of mouse BRG1 encoding amino acid residues 49–330. The sequences of cloned DNA fragments were confirmed by using dye terminator cycle sequencing kit (Amersham Pharmacia Biotech). These fusion proteins were used as immunogens for production of polyclonal antibodies by rabbits. Antibodies were affinity purified by using their respective antigens immobilized on CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech). These two antibodies were raised against N-terminal portions of BRM and BRG1, which show lower homology than other domains (*28*). Nevertheless, each antibody showed a low cross-reactivity to the other's antigen. The cross-reactivity to the BRG1 antigen was removed from anti-BRM antibody by using a BRG1-immobilized column. The anti-BRG1 antibody was similarly treated by use of a BRM-immobilized column.

Brains were isolated from E10, E15, newborn, and adult (8 weeks) mice. After removing meninges, cells were dissociated by use of a homogenizer and sonically disrupted. Whole cell extracts were prepared as described previously (*20*). Western blotting was performed by the standard procedure. In addition to anti-BRM and anti-BRG1 antibodies, mouse monoclonal anti–rat nestin (Pharmingen, San Diego, CA), mouse monoclonal anti–bovine microtubule–associated protein 2 (MAP2) (Leinco Technologies, St. Louis, MO) and rabbit polyclonal anti–human glial fibrillary acidic protein (GFAP) (Biomeda, Foster City, CA) were used as primary antibodies. Immunoreactivity was determined with horseradish peroxidase–conjugated anti–rabbit IgG antibody, and detected by using enhanced chemiluminescence reagents (Amersham Pharmacia Biotech).

RNA Isolation, RT-PCR, and Northern Blotting—Total RNA was isolated by use of an RNeasy Midi Kit (Qiagen, Hilden) according to the manufacturer's instructions. After DNase I (Sigma) treatment, 5 μg of total RNA was primed with oligo (dT) primers and reverse-transcribed by using ReverTra Ace (Toyobo, Osaka). Control experiments were done without reverse transcriptase. Cycle parameters for PCR were 30 s at 94°C, 1 min at 55°C, and 1 min at 72°C for 25 cycles. RedTaq DNA polymerase was purchased from Sigma and used for PCR. The following primers were designed from the DNA sequences obtained from GenBank/EMBL/DDBJ database (BRM, BRG1, Etl-1, and β-actin) and from the previous report (CHD-1) (*29*): mouse BRM sense (5′-GACCTCCCAGCGTCTCACAT-3′) and antisense (5′-CAGGCAAGGTCCTTTTTCCC-3′), mouse BRG1 sense (5′-GACCTCCTTCAGCAGGAC-3′) and antisense (5′-TCTGCGGTGGCATTACA-3′), mouse CHD-1 sense (5′-AGTTACAGACCCGTGCAGAC-3′) and antisense (5′-TGATGGCGGTCCTTGTGATG-3′), mouse Etl-1 sense (5′-GCTTAAGGAGCATGAGTGGA-3′) and antisense (5′-TCCCGGTGAGCATCGTAACT-3′), mouse β-actin sense (5′-TGAGACCTTCAACACCCCAG-3′) and antisense (5′-GAGCCAGAGCAGTAATCTCC-3′). Since the cDNA sequence of mouse SNF2h has not been reported, the EST database was searched by using the human SNF2h cDNA sequence and yielded two sequences (AA407249 and AA190286) that showed close similarity to the human sequence. Primers were designed based on these sequences: mouse SNF2h sense (5′-ATCTTCAGGCTATGGACCG-3′) and antisense (5′-TCTCCTTTTCTTCTAGTTCC-3′). For detection of mRNA level, all PCR products were visualized on a 1.0% agarose gel.

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For Northern blot analyses, 30 μg of total RNA samples were fractionated by electrophoresis on a 1.0% agarose gel containing 6.0% formaldehyde and transferred to Hybond-C pure nitrocellulose membranes (Amersham Pharmacia Biotech). The cDNA fragment of mouse BRM encoding amino acid residues 50–214 in the corresponding human sequence was used as the probe. The mouse β-actin probe was obtained by RT-PCR. These probes were labeled with [α-^{32}P]dCTP (Amersham Pharmacia Biotech) by using a *Bca*BEST labeling kit (Takara, Tokyo).

Immunocytochemical Analyses—NPCs cultured on coverslips were rinsed with cold PBS and fixed with freshly prepared 3.7% formaldehyde for 10 min at room temperature. Cells were then washed in PBS and permeabilized with PBS containing 1% Triton X-100 for 5 min at room temperature. After washing with PBS again, cells were incubated overnight at 4°C in PBS containing 5% goat serum and 1% bovine serum albumin. The coverslips were rinsed with PBS and dipped in primary antibodies in PBS for 2 h at room temperature. Primary antibodies used were affinity-purified anti-BRM and anti-BRG1 polyclonal antibodies, mouse monoclonal anti–rat nestin and mouse monoclonal anti–bovine MAP2. After rinsing with cold PBS three times, cells were incubated with goat anti–mouse IgG coupled to tetramethylrhodamin-isothiocyanate (Leinco Technologies) and goat anti–rabbit IgG coupled to fluorescein isothiocyanate (Zymed Laboratories, Southern San Francisco, CA) for 30 min at room temperature. After washing with PBS, the coverslips were mounted onto slide glasses with 90% glycerol and 10% PBS. Samples were analyzed by using a confocal fluorescence microscope (MRC-1024, Bio Rad, Hercules, CA).

RESULTS

The Level of BRM but Not BRG1 Is Up-Regulated during Differentiation of NPCs—Since SWI2/SNF2 family subunits such as BRM and BRG1 have an ATPase activity and a helicase motif, they are believed to be key subunits for remodeling activity (*7*). Messenger RNA levels of various SWI2/SNF2-related proteins in cultured NPCs were studied by RT-PCR in order to detect changes in mammalian chromatin-remodeling complexes during neural development. Under our culture conditions, NPCs gradually differentiated to neurons and glial cells. As shown in Fig. 1, BRM mRNA gradually increased, while mRNA levels of other SWI2/SNF2 homologs such as BRG1, SNF2h (*30*), CHD-1 (*29*), and Etl-1 (*31*) remained constant. By Northern blotting, the expression level of BRM was very low on day 1 when NPCs were dominant, but the mRNA level increased on day 5 (Fig. 2A). On the other hand, the expression of BRG1 was almost constant throughout the culture (data

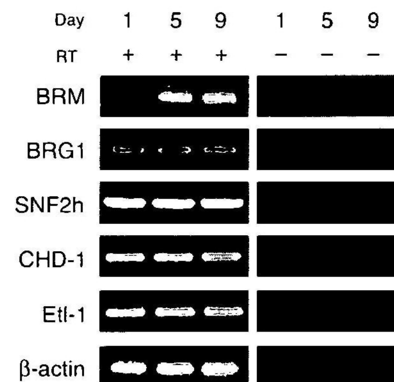

Fig. 1. **Expression of several mammalian ATPase subunits of chromatin-remodeling factors relating to SWI2/SNF2.** Total RNA was prepared from NPCs cultured for the indicated number of days and analyzed by RT-PCR with suitable primers in the presence (+) or absence (−) of reverse transcriptase. Since the amount of amplified β-actin cDNA increased colinearly up to PCR cycle number 30, the cycle number of PCR reaction was set at 25.

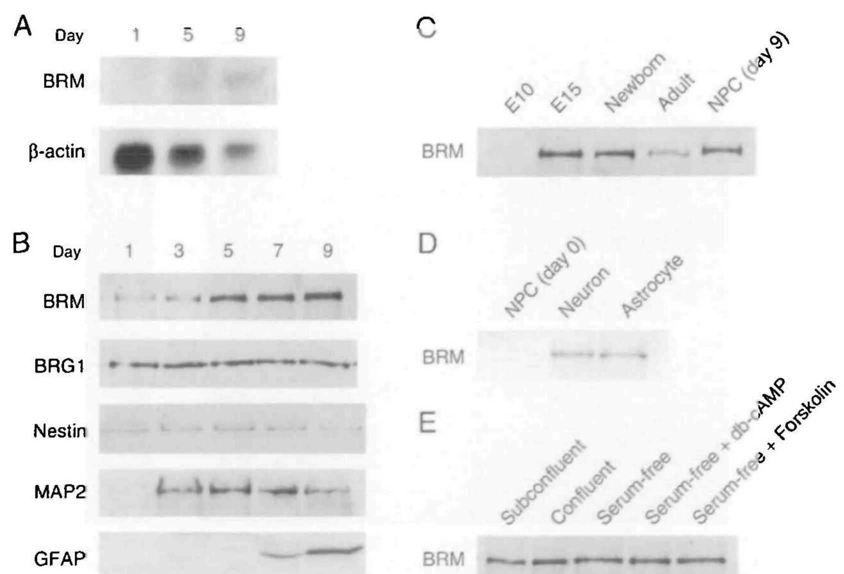

Fig. 2. **Expression of BRM and BRG1 during neural differentiation.** (A) Northern blot analysis of BRM mRNA in cultured NPCs. Total RNA was prepared from NPCs cultured for the indicated number of days, and 30-μg samples of mRNA were fractionated by gel electrophoresis. (B) Western blot analyses of BRM, BRG1, nestin, MAP2, and GFAP in cultured NPCs. Whole cell extracts were prepared from NPCs cultured for as above. (C) Western blot analysis of BRM during brain development. Whole cell extracts were prepared from brains of E10, E15, newborn, and adult mice and NPCs cultured for 9 days. (D) Western blot analysis of BRM-protein expression in NPCs without culture, neurons, and subconfluent astrocytes. Whole cell extracts were prepared from NPCs obtained from 10-day embryos, neurons from cerebrums of 17-day embryos and astrocytes from newborn mice. (E) Western blot analysis of BRM-protein expression in astrocytes. Confluent and subconfluent cells were cultured in the medium containing 10% fetal bovine serum. Alternatively, subconfluent cells were cultured in serum-free medium for 24 h, then medium was changed to serum-free medium containing 0.5 mM dibutyryl-cAMP (db-cAMP) or 10 μM forskolin. After 24 h, whole cell extracts were prepared. For Western blot analyses, samples containing 30 μg of protein were fractionated by SDS-PAGE (6% polyacrylamide).

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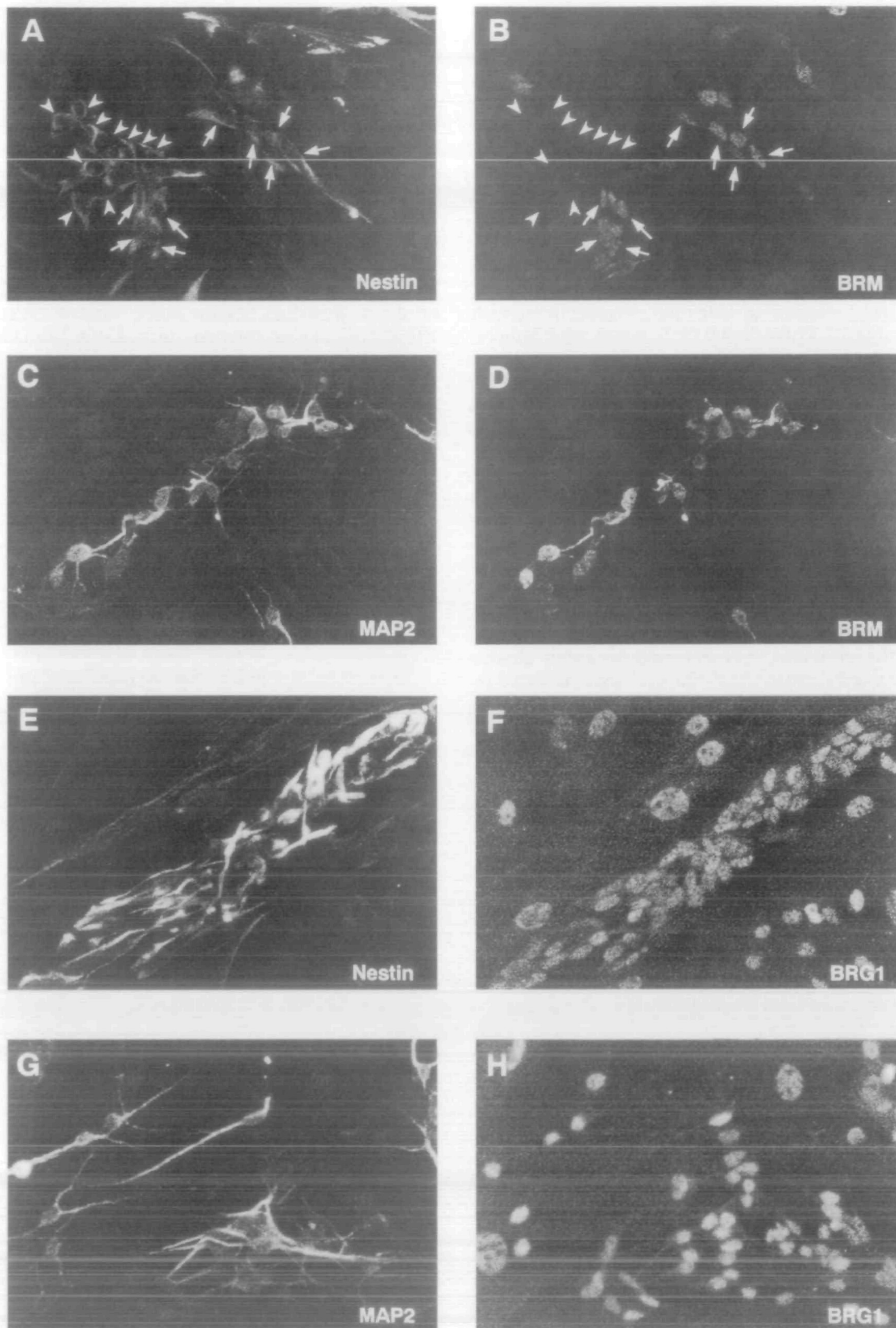

Fig. 3. **Immunocytochemical analyses of BRM and BRG1 in cultured NPCs on day 5.** NPCs cultured for 5 days were immunostained and observed with a confocal fluorescent microscope. (A) and (B), double staining with anti-nestin and anti-BRM. (C) and (D), double staining with anti-MAP2 and anti-BRM. (E) and (F), double staining with anti-nestin and anti-BRG1. (G) and (H), double staining with anti-MAP2 and anti-BRG1. Arrowheads and arrows in panels (A) and (B) indicate BRM-negative and BRM-positive cells expressing nestin, respectively.

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not shown). Western blotting by using anti-BRM and anti-BRG1 antibodies also confirmed these results (Fig. 2B); BRM expression level was low on day 1, increased thereafter and was constant after day 5.

To examine the expression of BRM *in vivo*, Western blotting was performed on protein samples of brains at several developmental stages (Fig. 2C). The BRM expression was induced between E10 and E15 and decreased in adult brain. This fact is consistent with induction of BRM during differentiation of NPCs isolated at E10. In addition, the level of BRM in NPCs cultured for 9 days was almost the same as that of *in vivo* NPCs.

NPCs were dominant in the original preparations of cells under the culture conditions, differentiation to neurons started from day 3, and astrocytes appeared on around day 7 as judged by the appearance of cell-type–specific markers (Fig. 2B). Nestin is a marker for proliferative NPCs, MAP2 for neurons and GFAP for astrocytes (*32*). To clarify whether up-regulation of BRM occurred in fetal neurons and astrocytes isolated from newborn mice, BRM expression level was studied by Western blotting. As shown in Fig. 2D, both cell types showed high level expression of BRM. Since astrocytes have growth ability, the expression of BRM in these cells is not consistent with the previous report that BRM is up-regulated by growth arrest (*20*). Therefore, we analyzed BRM expression in confluent astrocytes to examine whether it is up-regulated by growth arrest. Growth arrest was confirmed by [^{3}H]thymidine incorporation. Although we cultured growth-arrested confluent astrocytes for 3 days, BRM expression was the same as that of dividing subconfluent cells (Fig. 2E). Astrocytes stop growing and mature upon adding dibutyryl-cAMP (*25*) or forskolin (*26*) in serum-free medium. However, no change in the BRM expression level was observed after adding these reagents (Fig. 2E). We also confirmed BRM expression in astrocytes differentiated from NPCs (data not shown).

Expression of BRM Started in NPCs—To study the relation between neural cell differentiation and BRM expression, cultured NPCs were double-stained with anti-BRM and either anti-MAP2 or anti-nestin antibody. In 5-day NPC culture, both NPCs and neurons were observed, and a part of the population expressed nestin, while the remainder exhibited MAP2. As shown in Fig. 3, A and B, some nestin-positive cells showed BRM expression (arrows in Fig. 3, A and B) but others did not (arrowheads in Fig. 3, A and B). On the other hand, all the MAP2-positive cells expressed BRM (Fig. 3, C and D). NPCs isolated from E10 embryos did not express BRM protein at all (Fig. 2D) and BRM- and nestin-positive cells could not be detected in NPCs cultured for 12 h (data not shown). The heterogeneity of BRM expression in nestin-positive cells in 5-day culture seems to be due to unsynchronized differentiation. These results suggest that BRM expression begins in a subset of NPCs and is eventually detected in all mature MAP2-positive neurons. In contrast, double staining with anti-BRG1 and either anti-nestin or anti-MAP2 antibody showed that all the nestin- and MAP2-positive cells expressed BRG1 (Fig. 3, E–H).

Expression of BRM Was Induced during Differentiation of P19 Cells—To confirm the induction of expression of BRM in neural differentiation, P19 cells were also studied. Exposure of aggregated P19 cells to DMSO leads to the formation of cardiac and skeletal muscle cells. Alternatively, treatment of aggregated cells with all-*trans* RA results in differentiation to cells that resemble either neurons and glial cells, and treatment of monolayer cells with all-*trans* RA stimulates the formation of endodermal and mesodermal derivative cells (*33*).

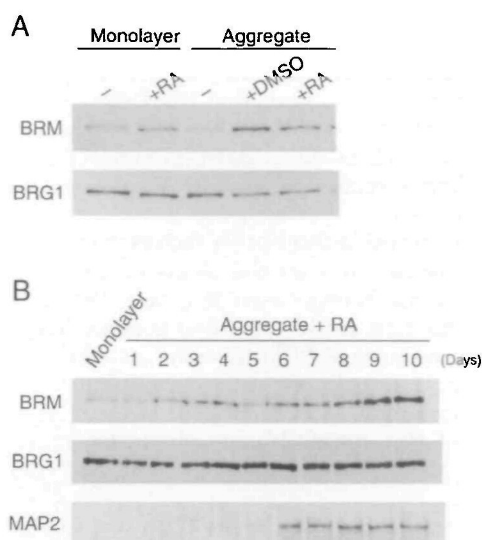

Fig. 4. **Expression of BRM and BRG1 during differentiation of P19 cells.** (A) P19 cells as monolayer culture were differentiated by all-*trans* RA to endodermal and mesodermal cells or as aggregate culture by all-*trans* RA to neural cells or by DMSO to muscle cells. Whole cell extracts (30 μg) were fractionated by SDS-PAGE (6% polyacrylamide) and probed with anti-BRM or anti-BRG1 antibodies. (B) Time course analyses of BRM, BRG1 and MAP2 expression during neural differentiation of P19 cells. On day 0, all-*trans* RA was added to aggregate cultures. Analyses were the same as in (A).

Figure 4A shows the BRM and BRG1 expression levels when P19 cells were differentiated to three different cell linages. Undifferentiated P19 cells showed a low level of BRM expression, but differentiated muscle, neural, and endodermal and mesodermal cells expressed higher levels of BRM independent of cell type. On the other hand, BRG1 was expressed constantly before and after differentiation. We analyzed the time course of BRM expression during neural differentiation of P19 cells. As shown in Fig. 4B, BRM expression started to increase on day 2, prior to the appearance of MAP2 on day 6. The induction of BRM observed in neural cells generated from P19 cells was in good accordance with the results of primary NPCs.

DISCUSSION

To assess the function of chromatin-remodeling factors in differentiation, cell systems that can be differentiated in *in vitro* culture were studied. We initially screened the mRNA levels of several ATPase subunits of remodeling factors in cultured NPCs. Among them, only BRM showed a change in expression level. Both in NPCs and P19 cells, expression of BRM increased during differentiation to neural cells. Furthermore, cytochemical analyses showed that a part of the nestin-positive cells started to express BRM protein. Since nestin-positive NPCs are committed to neural differentiation but still have growth ability (*32*), it is suggested

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that expression of BRM starts prior to final differentiation. Similarly, BRM expression started 3–4 days before the appearance of MAP2 in differentiation of P19 cells. These results support the hypothesis that BRM plays an important role in neural differentiation (*20*). Increased expression of BRM was also observed in differentiation of P19 cells to other cell types, suggesting that BRM may generally work in differentiation.

Because several transcription factors recruit BRM and BRG1 to specific sites on the chromosome, it is possible that BRM controls expression of genes relating to neural cell differentiation. In this regard, it is noteworthy that the *Drosophila* homolog of BRM (Brahma) activates expression of *Hox* genes. Recently, it was reported that the mammalian homolog of trithorax, named MLL/ALL-1, is a positive regulator of *Hox* genes (*34*) and physically interacts with SWI/SNF through INI1, as is the case with *Drosophila* (*35*).

Yaniv *et al.* proposed that BRM plays a role in growth arrest accompanied by differentiation (*20*). Our finding of increased BRM expression during differentiation of NPCs to neurons and of P19 cells to several cell types that ceased growing supports this hypothesis. However, the expression of BRM in glial cells (Fig. 2, D and E) suggests that growth arrest is not necessary for expression, since glial cells have strong growth ability. Conversely, the expression of BRM did not change when growth arrest was induced by contact inhibition or treatment with dibutyryl-cAMP or forskolin. These results indicate that induction of BRM expression does not directly lead to or result from growth arrest in glial cells.

Recently, it was reported that several SWI/SNF subunits, such as BAF155, BRG1, and BRM, directly interact with cyclin E, and cyclin E modulates the activity of the chromatin-remodeling factor (*19*). Zhang *et al.* reported that a histone deacetylase–RB–BRG1 triple complex persists in G1-arrested cells and an RB-BRG1 complex regulates exit from S phase (*36*). These results indicate that histone deacetylase, cyclin and SWI/SNF complex interact with RB, possibly with p107 and p130, and cell cycle progression may be controlled in a very complicated manner with these complexes. It is also possible that this regulation may be cell-type-specific, since the levels of these proteins are different depending on cell type. In this regard, it is necessary to study the complex formation of BRM in each cell type. If BRM plays a role in growth arrest of neuronal cells, it is possible that BRM could form a complex with RB and other proteins such as cyclin E. Our preliminary results showed that the levels of RB and p130 in primary neuron and glial cells were almost the same (Machida, Y., unpublished results). Now we are analyzing the complex formation both in neurons and glial cells to clarify whether BRM plays a role in growth arrest during differentiation.

REFERENCES

1. Björklund, S., Almouzni, G., Davidson, I., Nightingale, K.P., and Weiss, K. (1999) Global transcription regulators of eukaryotes. *Cell* **96**, 759–767
2. Gregory, P.D. and Hörz, W. (1998) Life with nucleosomes: chromatin remodelling in gene regulation. *Curr. Opin. Cell Biol.* **10**, 339–345
3. Biggar, S.R. and Crabtree, G.R. (1999) Continuous and widespread roles for the Swi-Snf complex in transcription. *EMBO J.* **18**, 2254–2264
4. Kuo, M.-H. and Allis, C.D. (1998) Roles of histone acetyltransferases and deacetylases in gene regulation. *BioEssays* **20**, 615–626
5. Muchardt, C. and Yaniv, M. (1999) ATP-dependent chromatin remodelling: SWI/SNF and Co. are on the job. *J. Mol. Biol.* **293**, 187–198
6. Côté, J., Quinn, J., Workman, J.L., and Peterson, C.L. (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265**, 53–60
7. Phelan, M.L., Sif, S., Narlikar, G.J., and Kingston, R.E. (1999) Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. *Mol. Cell* **3**, 247–253
8. Muchardt, C. and Yaniv, M. (1993) A human homologue of *Saccharomyces cerevisiae SNF2/SWI2* and *Drosophila brm* genes potentiates transcriptional activation by the glucocorticoid receptor. *EMBO J.* **12**, 4279–4290
9. Khavari, P.A., Peterson, C.L., Tamkun, J.W., Mendel, D.B., and Crabtree, G.R. (1993) BRG1 contains a conserved domain of the *SWI2/SNF2* family necessary for normal mitotic growth and transcription. *Nature* **366**, 170–174
10. Chiba, H., Muramatsu, M., Nomoto, A., and Kato, H. (1994) Two human homologues of *Saccharomyces cerevisiae SWI2/SNF2* and *Drosophila brahma* are transcriptional activators cooperating with the estrogen receptor and the retinoic acid receptor. *Nucleic Acids Res.* **22**, 1815–1820
11. Singh, P., Coe, J., and Hong, W. (1995) A role for retinoblastoma protein in potentiating transcriptional activation by the glucocorticoid receptor. *Nature* **374**, 562–565
12. Ichinose, H., Garnier, J.-M., Chambon, P., and Losson, R. (1997) Ligand-dependent interaction between the estrogen receptor and the human homologues of SWI2/SNF2. *Gene* **188**, 95–100
13. Trouche, D., Chalony, C.L., Muchardt, C., Yaniv, M., and Kouzarides, T. (1997) RB and hbrm cooperate to repress the activation functions of E2F1. *Proc. Natl. Acad. Sci. USA* **94**, 11268–11273
14. Armstrong, J.A., Bieker, J.J., and Emerson, B.M. (1998) A SWI/SNF-related chromatin remodeling complex, E-RC1, is required for tissue-specific transcriptional regulation by EKLF in vitro. *Cell* **95**, 93–104
15. Murphy, D.J., Hardy, S., and Engel, D.A. (1999) Human SWI-SNF component BRG1 represses transcription of the c-*fos* gene. *Mol. Cell. Biol.* **19**, 2724–2733
16. Kowenz-Leuts, E. and Leutz, A. (1999) A C/EBPβ isoform recruits the SWI/SNF complex to activate myeloid genes. *Mol. Cell* **4**, 735–743
17. Strober, B.E., Dunaief, J.L., Guha, S., and Goff, S.P. (1996) Functional interactions between the hBRM/hBRG1 transcriptional activators and the pRB family of proteins. *Mol. Cell. Biol.* **16**, 1576–1583
18. Dunaief, J.L., Strober, B.E., Guha, S., Khavari, P.A., Ålin, K., Luban, J., Begemann, M., Crabtree, G.R., and Goff, S.P. (1994) The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. *Cell* **79**, 119–130
19. Shanahan, F., Seghezzi, W., Parry, D., Mahony, D., and Lees, E. (1999) Cyclin E associates with BAF155 and BRG1, components of the mammalian SWI-SNF complex, and alters the ability of BRG1 to induce growth arrest. *Mol. Cell. Biol.* **19**, 1460–1469
20. Muchardt, C., Bourachot, B., Reyes, J.-C., and Yaniv, M. (1998) *ras* transformation is associated with decreased expression of the brm/SNF2α ATPase from the mammalian SWI-SNF complex. *EMBO J.* **17**, 223–231
21. Sumi-Ichinose, C., Ichinose, H., Metzger, D., and Chambon, P. (1997) SNF2β-BRG1 is essential for the viability of F9 murine embryonal carcinoma cells. *Mol. Cell. Biol.* **17**, 5976–5986
22. Reyes, J.C., Barra, J., Muchardt, C., Camus, A., Babinet, C., and Yaniv, M. (1998) Altered control of cellular proliferation in the absence of mammalian brahma (SNF2α). *EMBO J.* **17**, 6979–6991
23. Kitani, H., Shiurba, R., Sakakura, T., and Tomooka, Y. (1991) Isolation and characterization of mouse neural precursor cells in primary culture. *In Vitro Cell Dev. Biol.* **27A**, 615–624

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24. McCarthy, K.D. and de Vellis, J. (1980) Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. *J. Cell Biol.* **85**, 890–902
25. Moonen, G., Cam, Y., Sensenbrenner, M., and Mandel, P. (1975) Variability of the effects of serum-free medium, dibutyryl-cyclic AMP or theophylline on the morphology of cultured new-born rat astroblasts. *Cell Tissue Res.* **163**, 365–372
26. Ramakers, G.J.A. and Moolenaar, W.H. (1998) Regulation of astrocyte morphology by RhoA and Lysophosphatidic acid. *Exp. Cell Res.* **245**, 252–262
27. Rudnicki, M.A. and McBurney, M.W. (1987) Cell culture methods and induction of differentiation of embryonal carcinoma cell lines in *Teratocarcinomas and Embryonic Stem Cells, a Practical Approach* (Robertson, E.J., ed.) pp. 19–49, IRL Press, Oxford
28. Muchardt, C., Reyes, J.-C., Bourachot, B., Legouy, E., and Yaniv, M. (1996) The hbrm and BRG-1 proteins, components of the human SNF/SWI complex, are phosphorylated and excluded from the condensed chromosomes during mitosis. *EMBO J.* **15**, 3394–3402
29. Delmas, V., Stokes, D.G., and Perry, R.P. (1993) A mammalian DNA-binding protein that contains a chromodomain and an SNF2/SWI2-like helicase domain. *Proc. Natl. Acad. Sci. USA* **90**, 2414–2418
30. LeRoy, G., Orphanides, G., Lane, W.S., and Reinberg, D. (1998) Requirement of RSF and FACT for transcription of chromatin templates in vitro. *Science* **282**, 1900–1904
31. Schoor, M., Schuster-Gossler, K., and Gossler, A. (1993) The *Etl-1* gene encodes a nuclear protein differentially expressed during early mouse development. *Dev. Dynamics* **197**, 227–237
32. Torii, M., Matsuzaki, F., Osumi, N., Kaibuchi, K., Nakamura, S., Casarosa, S., Guillemot, F., and Nakafuku, M. (1999) Transcription factors Mash-1 and Prox-1 delineate early steps in differentiation of neural stem cells in the developing central nervous system. *Development* **126**, 443–456
33. Bain, G., Ray, W.J., Yao, M., and Gottlieb, D.I. (1994) From embryonal carcinoma cells to neurons: the P19 pathway. *BioEssays* **16**, 343–348
34. Yu, B.D., Hanson, R.D., Hess, J.L., Horning, S.E., and Korsmeyer, S.J. (1998) Mll, a mammalian *trithorax*-group gene, functions as a transcriptional maintenance factor in morphogenesis. *Proc. Natl. Acad. Sci. USA* **95**, 10632–10636
35. Rozenblatt-Rosen, O., Rozovskaia, T., Burakov, D., Sedkov, Y., Tillib, S., Blechman, J., Nakamura, T., Croce, C.M., Mazo, A., and Canaani, E. (1998) The C-terminal SET domains of ALL-1 and TRITHORAX interact with the INI1 and SNR1 proteins, components of the SWI-SNF complex. *Proc. Natl. Acad. Sci. USA* **95**, 4152–4157
36. Zhang, H.S., Gavin, M., Dahiya, A., Postigo, A.A., Ma, D., Luo, R.X., Harbour, J.W., and Dean, D.C. (2000) Exit from G1 and S phase of the cell cycle is regulated by repressor complexes containing HDAC-Rb-hSWI/SNF and Rb-hSWI/SNF. *Cell* **101**, 79–89

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