SW13 Cells Can Transition between Two Distinct Subtypes by Switching Expression of BRG1 and Brm Genes at the Post-transcriptional Level

Received for publication, August 19, 2002, and in revised form, November 21, 2002
Published, JBC Papers in Press, December 17, 2002, DOI 10.1074/jbc.M208458200

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The human adrenal carcinoma cell line, SW13, has been reported to be deficient in both BRG1 and Brm expression and therefore is considered to lack a functional SWI/SNF complex. We found that the original cell line of SW13 is composed of two subtypes, one that expresses neither BRG1 nor Brm (SW13(vim)) and the other, which expresses both (SW13(vim+)). The presence of BRG1 and Brm in SW13 correlates completely with the cellular ability to express such genes as vimentin, collagenase, c-met, and CD44 that were under the control of a transcription factor, AP-1, which was shown previously to require a functional SWI/SNF complex for its transactivating activity. Transient treatment with inhibitors of histone deacetylase induced a stable transition of SW13(vim−) to a cell type indistinguishable from SW13(vim+), suggesting that these two subtypes are epigenetically different. Run-on analysis indicated that, unlike these four genes driven by AP-1, transcription of the BRG1 and Brm genes in SW13(vim−) are initiated at a frequency comparable with SW13(vim+). In both SW13(vim−) and SW13(vim+) cells, the BRG1 and Brm genes were transcribed through the entire gene at a similar efficiency, indicating that their expression was completely suppressed at the post-transcriptional level in SW13(vim−) cells. We would like to propose that SW13 can spontaneously transition between two subtypes by switching expression of BRG1 and Brm at the post-transcriptional level.

During development, differential gene expression generates the cellular diversity required to establish pattern formation and organogenesis. Although the cellular specificity involved in development has been described well, the mechanisms that ensure long term cellular memory, by which the developmental fate of a particular cell is passed on to its descendents, are largely unknown. In both Drosophila and mammals regulatory mechanisms that allow gene expression patterns to be maintained have been shown to be supported by the products of two classes of regulatory genes, the Polycomb group (Pc-G) and the trithorax group (trx-G). The proteins required to maintain a repressed state are contained in the Pc-G complex, whereas those required for persistence of expression are in the trx-G complex. The Pc-G and trx-G protein complexes counteract each other to repair previously established chromosomal domains of specific genes throughout development.

Insight into the role of the trx-G protein in transcriptional regulation has come from studies of the Brahma gene (belonging to trx-G) in Drosophila and its mammalian homologues, Brm and BRG1. Brm and BRG1 have DNA-dependent ATPase activity and are the catalytic subunits of the mammalian SWI/SNF chromatin remodeling complex. This complex contains either Brm or BRG1, but not both, and recent reports on the glucocorticoid receptor (5), c-Myc (6), C/EBPβ (7), estrogen receptor (8), AP-1 (9), and p53 (10) support a model in which transcription factors recruit the SWI/SNF complex to target genes (11, 12), providing mechanistic links between epigenetic transcriptional regulation and chromatin remodeling.

The SW13 cell line derived from human adrenal adenocarcinoma was reported to express neither BRG1 nor Brm and has been used in many experiments because of this unique property (9, 14–20). In our previous analysis on AP-1 (21) (composed of Fos/Jun dimers), we found that a SWI/SNF complex subunit, BAF60a, binds to different Fos/Jun dimers with distinct affinities by interacting with two interfaces for c-Fos and c-Jun, respectively, and further showed that the SWI/SNF complex is a major determinant of the transactivation potential of Fos/Jun dimers. In SW13 cells, which lack a functional SWI/SNF complex, the transactivating activity of Fos/Jun dimers is kept at basal levels when assayed by transient transfection experiments. However, transactivation by the c-Fos/c-Jun heterodimer (having the highest affinity to BAF60a) was specifically enhanced by cotransfecting Brm or BRG1 into SW13 to recover the functional SWI/SNF complex (9).

Considering that the SWI/SNF complex is composed of important trx-G proteins, functional loss of the SWI/SNF complex in SW13 might affect long term cellular memory, leading to an obvious question whether the SW13 cells are epigenetically stable. We are especially interested in previous observations (22) that SW13 cells express vimentin in a mosaic pattern. There are two cellular subtypes in SW13 culture, the subtype that does not express vimentin protein (designated as SW13(vim−)), and the other, which does (designated as SW13(vim+)) (23). We examined the molecular basis for the presence of these two subtypes and addressed whether this
phenomenon was related to the deficiency in BRG1 and Brm expression in this cell line.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—Human tumor cell lines SW13 (adrenocortical carcinoma), MDA-MB435 (breast duct carcinoma), and Ptg-S2 (preparing cell line for retrovirus vector production) were maintained in high glucose Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum and incubated at 37 °C. CHAP31 (24) (gifts from the Japan Energy Corp., Saitama, Japan) at 2–8 nm, 50–200 nm triacetin A (TSA; Wako Pure Chemicals, Tokyo, Japan), 0.5–2 μM 5-azacytidine (Sigma), 0.5–2 μM 5-azacytidine (Sigma), and 100 nm 12-O-tetradecanoylphorbol-13-aceta (TPA, Sigma) were added to the culture medium.

**Immunocytochemistry**—SW13 cells were fixed with 4% paraformaldehyde, treated with anti-vimentin mouse monoclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Antibody binding was visualized with Alexa Fluor 488 conjugated anti-mouse IgG antibody (Molecular Probes Inc., Eugene, OR) for immunofluorescence microscopy or detected with biotinated anti-mouse IgG antibody (Chemicon International, Temecula, CA) using the VECTASTAIN ABC kit (Vector Laboratories Inc., Burlingame, CA).

**Isolation of SW13(vim−) and SW13(vim+) by End-point Dilution.**—As previously (22, 23), SW13 cells express vimentin protein in a mosaic pattern when observed under immunofluorescence microscope (Eclipse 800, Nikon). SW13 cells were subcloned by end-point dilution using 96-well plastic plates (Corning Glass Inc., Corning, NY). Fifty subclones were obtained and examined for human vimentin fibers by immunofluorescence microscopy; 31 subclones were completely negative for vimentin expression (SW13(vim−)), whereas 19 subclones were positive (SW13(vim+)). Two subclones of SW13(vim−) were selected and designated as SW13(vim−) #1 and #2, respectively, and two subclones of SW13(vim+) were selected and designated as SW13(vim+) #1 and #2, respectively.

**Plasmid Constructions**—pCR2.1-BRG1(1), -Brm(F), -β-actin, -collagenase and -c-met were generated by inserting PCR products from the corresponding mRNAs into pCR2.1 (Invitrogen), a TA cloning vector designed to directly clone PCR products. The RT-PCR primer pairs used were as follows: BRC1(1), 5′-atggcagggactcattggctgc-3′ and 5′-ctctgaggtctcagggg-3′; Brm(F), 5′-caggagaaagggggtcaggctgctgc-3′ and 5′-gctgcctctgctggtctgeaacaaac-3′; β-actin, 5′-atggcagggactcattggctgc-3′ and 5′-ctctgaggtctcagggg-3′; and c-met, 5′-tttctgtactctgggtgactcagaggg-3′ and 5′-tttctgtactctgggtgactcagaggg-3′. First, the corresponding mRNA into pCR2.1 (Invitrogen), a TA cloning vector.

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**Probe Preparation**—Digoxigenin-labeled probes for in situ hybridization were synthesized from linearized plasmids using the digoxigenin RNA labeling kit (Roche Molecular Biochemicals). To synthesize the BRG1 and Brm antisense riboprobes, pBluescriptSK(+)-BRG1(1) and pBluescriptSK(+)-Brm(1) were hybridized with digoxigenin-labeled antisense riboprobes, pBluescriptSK(+)-vimentin and pBluescriptSK(+)-vimentin were linearized with EcoRI and transcribed by T7 RNA polymerase. To synthesize the vimentin antisense riboprobe, pBluescriptSK(+)-vimentin was linearized with EcoRI and transcribed by T7 RNA polymerase.

**Results**—SW13(vim−) Does Not Express Either BRG1 or Brm but SW13(vim+) Expresses Both—Subclones of SW13 cells, either expressing or not expressing vimentin, were selected previously by limiting dilution from the parent cell line SW13, on the basis of the presence or absence of a vimentin filament detectable by immunofluorescence microscopy. Using the same protocol, we have cloned two SW13(vim−) subclones, #1, #2, and two SW13(vim+) subclones, #21, #22. For each clone, the expression levels of vimentin were determined both by semiquantitative RT-PCR of total RNA (Fig. 1A) and by Western blot analysis of the total cellular lysate (Fig. 1B) and were compared with those of a control cell line, MDA-MB435, that expresses vimentin. Vimentin mRNA and vimentin protein were clearly detectable in SW13(vim+) #21 and #22, whereas they are completely undetectable in SW13(vim−) #1 and #2. The generation times of clone SW13(vim−) #1 and #2 and SW13(vim+) #21 and #22 cultures were 32–34 h, indicating that SW13(vim−)
Fig. 1. Comparison in gene expression patterns between SW13(vim−) and SW13(vim+). A, expression levels of the endogenous vimentin, BRG1, and Brm genes, as well as a housekeeping gene, GAPDH, were measured by semi-quantitative RT-PCR of total RNA isolated from SW13(vim−) #1 and #2 and SW13(vim+) #21 and #22, as well as a control human cell line, MDA-MB435. Expression levels in SW13(vim+) #21 were taken as 1.0, and columns are shown as the average ratio obtained by three independent experiments. The bars represent the S.D. B, expression of vimentin, BRG1, and Brm protein was analyzed by Western blot of total cellular lysates (20 μg per lane) prepared from SW13(vim−) #1 and #2, SW13(vim+) #21 and #22, and MDA-MB435. Arrows indicated the position of vimentin (55 kDa), BRG1 (190 kDa), and Brm (190 kDa). C, expression levels of the corresponding mRNA were determined by Northern blot of the poly(A) RNA (20 μg per lane). Position of single strand RNA markers (RNA Ladder; Invitrogen) are shown on the left.

and SW13(vim+) had no significant difference in their growth rates in monolayer culture.

The vimentin gene has been reported to be one of the representative genes that are under the control of transcription factor AP-1. Tandem AP-1 binding sites that mediate serum inducibility were identified in its promoter region (28). We have reported previously that transactivating activity of AP-1 depends on the presence of a functional SWI/SNF complex and that AP-1 function in SW13 cells was strongly attenuated, because the cells are deficient in the expression of both catalytic subunits of the SWI/SNF complex. Considering these prior observations, we hypothesized that the expression of BRG1 and Brm might be recovered in SW13(vim+). Therefore, we have determined the expression levels of BRG1 and Brm in both SW13(vim−) and SW13(vim+). SW13(vim−) #1 and #2 have no detectable levels of both BRG1 and Brm protein (Fig. 1B), as judged by Western blot and as has been reported for the original cell line of SW13. When BRG1 and Brm mRNA levels in these clones were assessed by RT-PCR, they were also negative (Fig. 1A). In SW13(vim+) #21 and #22, however, mRNA and the protein product of either BRG1 or Brm genes were clearly detectable (Fig. 1, A and B) like in MDA-MB435, which is competent for the SWI/SNF complex (15). Despite the clear contrast in the gene expression patterns of vimentin, BRG1, and Brm, SW13(vim−) #1 and #2 and SW13(vim+) #21 and #22 expressed almost the same mRNA levels of a housekeeping gene, GAPDH.

By Northern blot analysis of poly(A) RNA prepared from SW13(vim−) #1 or SW13(vim+) #21, SW13(vim+) did not express either full-length mRNA of BRG1 and Brm or any aberrant transcript when detected by specific probes that covered entire coding regions (Fig. 1C). These results are consistent with our hypothesis that BRG1 and Brm are expressed in SW13(vim+) and further suggest that this subtype of SW13 has a functional SWI/SNF complex, which supports the expression of several endogenous genes, including vimentin.

To examine whether the clear difference in the expression patterns observed between the two subtypes of SW13 can be stably maintained in the cloned culture, we monitored the expression of BRG1, Brm, and vimentin mRNA at single cell resolution (Fig. 2). After in situ hybridization with specific probes (red), each monolayer culture was counterstained with TOTO-1 to detect cellular nuclei. The entire population of SW13(vim−) #1 (or #2) failed to express these three genes, whereas all the cells in SW13(vim+) #21 (or #22) cultures expressed them at a significant level, as shown for SW13(vim+) #1 and SW13(vim+) #21 (Fig. 2). These results indicate that each subtype maintains its phenotypes stably after cloning by end-point dilution (corresponding to about 20 cycles of cell division).

Several Genes under the Control of AP-1 Are Expressed Specifically in SW13(vim+) Because of the Presence of Functional BRG1 and Brm—We have reported previously that collagenase (intracisternal collagenase, MMP1) and c-met genes could be induced after DNA transfection of expression vectors carrying the BRG1 or Brm gene, together with c-Fos and c-Jun expres-
tion vectors, into SW13 cells (9). The CD44 gene is another representative gene that is under the control of AP-1 (29) and was recently reported to be induced by either BRG1 or Brm supplied exogenously into SW13 cells (16, 17, 30). We therefore examined the expression status of three other genes, collagenase, c-met, and CD44, in SW13(vim−) #1 and #2 and SW13(vim+) #21 and #22. Like the vimentin gene, expression of collagenase, c-met, and CD44 genes were completely undetectable in SW13(vim−) #1 and #2, whereas they are expressed in SW13(vim+) #21 and #22 (Fig. 3) at a significant level. These results clearly reveal that several genes under the control of AP-1 are expressed specifically in SW13(vim−) because of the presence of functional BRG1 and Brm. In none of these biochemical and cytological analyses of the expression levels (see Figs. 1–3) (data not shown) did we detect any significant differences between cellular clones #1 and #2 or between #21 and #22. Therefore, we used clones #1 and #21 for SW13(vim−) and SW13(vim+), respectively, for further analyses.

Introduction of a Physiological Level of Exogenous BRG1 or Brm in SW13(vim−) Switches Expression of vimentin, collagenase, c-met, and CD44 Genes—Next we used retrovirus vectors to examine whether SW13(vim−) can induce vimentin, collagenase, c-met, and CD44 by exogenous expression of BRG1 or Brm at a physiological level. Three days after infection, we detected clear induction of the mRNA of these four genes in SW13(vim−) at levels comparable with those observed in SW13(vim+) (Fig. 4). These results indicate that exogenous expression of the BRG1 or Brm gene in SW13(vim−) switches the expression of several genes, driven by such transcriptional factors as AP-1, in an all or none manner. In SW13(vim−), endogenous expression of both the c-fos and c-jun genes was readily detectable by RT-PCR at levels similar to those in SW13(vim+), indicating that the amount of c fos/c Jun dimer, which contributed most strongly to the recruitment of the SWI/SNF complex by AP-1, would not be rate-limiting (data not shown).

SW13(vim−) Can Transition to a Cell Type Indistinguishable from SW13(vim+) by Adding HDAC Inhibitors—Complete loss of the BRG1 and Brm genes in SW13(vim−) could represent either genetic defects or epigenetical suppression. If the BRG1 and Brm genes are not mutated at all in SW13(vim−), and if their expression is epigenetically suppressed in SW13(vim−), we might artificially relax the suppression of BRG1 and Brm expression in SW13(vim−) cultures by adding some reagents that modulate the epigenetical regulation. In such a case, BRG1 and Brm induced in SW13(vim−) could reconstitute the functional SWI/SNF complex, and the cells would recover the ability to express vimentin. We screened several reagents for an ability to efficiently induce vimentin expression by detecting the presence of vimentin protein using immunocytochemical staining. The reagents tested included a protein kinase C inhibitor (TPA), HDAC inhibitors (CHAP31 and trichostatin A), and DNA methyltransferase inhibitors (5-azacytidine and 5-azadeoxycytidine). We also tested conditioned medium recovered from SW13(vim+) overnight cultures, considering that SW13(vim+) might secrete some inducing factors that trigger the transition. We found that HDAC inhibitors CHAP31 (4–8 nM) and TSA (200 nM) caused efficient induction of vimentin protein in SW13(vim−) within 4 days (Fig. 5). Because 200 nM TSA had cytotoxic effects on SW13(vim−) cells 1 day after the treatment, we hereafter show only the results obtained by 4 nM CHAP31 as the HDAC inhibitor, but essentially the same results were obtained by using 200 nM TSA instead. 5-azadC (0.5 μM) treatment also caused vimentin induction in a lesser extent, but 5-azacytidine (2 μM) induced vimentin only marginally. Neither TPA treatment nor exchanges with the conditioned medium of SW13(vim+) had any effect (data not shown).

We next analyzed mRNA expression 3 days after the treatment with CHAP31 or 5-azadC by in situ hybridization using the BRG1, Brm, and vimentin probes, respectively. As shown for BRG1 (Fig. 6), all the populations of SW13(vim−) treated with either CHAP31 or 5-azadC were induced at a similar level of BRG1 mRNA to that observed in SW13(vim+). Similar results were obtained when a Brm or vimentin probe was used instead (data not shown), indicating that a clear phenotypic transition occurred in the entire population by these treatments.

We next measured the mRNA levels of BRG1 and Brm, as well as vimentin, collagenase, c-met, and CD44 in SW13(vim−) treated for 3 days with CHAP31 or 5-azadC and compared
them with mRNA levels in untreated SW13(vim−) or SW13(vim+) (Fig. 7). In the case of CHAP31 treatment, BRG1, Brm, vimentin, collagenase, c-met, and CD44 mRNA became detectable in SW13(vim−), and their expression levels were very close to the levels in SW13(vim+) (Fig. 7, +CHAP31/day0). Induction of BRG1, Brm, and vimentin protein was also confirmed by Western blot analysis (data not shown). After this 3-day treatment with CHAP31, the cells were subsequently grown for 7 days in the absence of this HDAC inhibitor and analyzed for the expression levels of the same series of genes (Fig. 7, +CHAP31/day7). The expression levels were unchanged after the subsequent growth, indicating that SW13(vim−) stably altered its phenotype to a cell type that was indistinguishable from SW13(vim+). After the SW13(vim−) culture was treated for 6 days with 4 nM CHAP31, we found that the cellular phenotype was stably maintained for at least 2 months when judged from the expression patterns of these six genes (data not shown).

In the case of 5-azadC treatment, the induced BRG1 mRNA level was close to the level in SW13(vim+), but the expression level of Brm mRNA was much lower than that in SW13(vim+). Expression levels of the four AP-1-driven genes were also significantly lower compared with those in SW13(vim+) (Fig. 7). When these 5-azadC-treated cells were kept for 7 days in the absence of this DNA methyltransferase inhibitor, expression of CD44 and collagenase mRNAs became undetectable (Fig. 7, +5-azadC/day7). These observations are reminiscent of the previous report that cells do not express the CD44 gene even in the presence of BRG1 protein in Brm null mice (18). These results show that 5-azadC can not establish stable transition of SW13(vim−) to SW13(vim+), although SW13(vim−) can transiently assume SW13(vim+) like phenotype after this treatment. Although underlying mechanisms here are largely unclear at this moment, we think a balance between BRG1 and Brm proteins would be important to maintain the SW13(vim+) phenotype.

SW13(vim−) Cells Initiate BRG1 or Brm Transcription at a Similar Frequency as Do SW13(vim+) Cells—Because the phenotypes of these two subtypes of SW13, SW13(vim−) and SW13(vim+), are likely to be regulated primarily by the BRG1 and Brm expression, we are interested in the molecular mechanisms involved in the lack of expression of these two genes in SW13(vim−). We prepared cellular nuclei from SW13(vim−), SW13(vim+) and MDA-MB435 and their transcriptional activity was measured by a nuclear run-on assay (Fig. 8). Surprisingly, nuclear extracts of either SW13(vim−) or SW13(vim+) synthesized mRNA of BRG1 and Brm to a similar extent. Because the DNA probes used to monitor the BRG1 and Brm transcripts (Fig. 9) include the promoter-proximal region covering the first (5’-noncoding) exon and the subsequent intron of these genes, these results indicate that SW13(vim−) cells initiate BRG1 or Brm transcription at a similar frequency as do SW13(vim+) and MDA-MB435 cells. However, no transcription of vimentin, collagenase, c-met, and CD44 genes was detected in the nuclear extracts of SW13(vim−) at all even by the slots containing 5-μg DNA probes, whereas they were readily detectable in SW13(vim+) by the slots of 1-μg DNA probes. These results confirm that these four genes are regulated at the
transcriptional level in an all or none manner by the presence or absence of functional SWI/SNF complex in the cells.

**Expression of the BRG1 and Brm Genes Is Completely Suppressed by Post-transcriptional Regulation Operating in SW13(vim–) Cells**—A growing list of genes transcribed by RNA polymerase II are regulated at the level of transcriptional elongation in the promoter-proximal region (31). These genes include such important genes as c-myc, hsp70, c-fos, and MAP kinase phosphatase-1 (32–36). In the case of c-myc, quiescent or differentiated cells reveal significant blocks to elongation during transcription through the first exon or first intron sequences, whereas RNA polymerase II in proliferating cells transcribes through the c-myc gene much more efficiently (32–34). As shown in the genomic structure of the BRG1 and Brm genes (Fig. 9), both genes possess a non-coding exon like the c-myc or N-myc genes. We therefore tested whether blocks of the transcriptional elongation of BRG1 and Brm are operating in SW13(vim–). For detailed run-on analysis in these genes, we prepared additional sets of DNA probes that detect exons covering the entire BRG1 gene (probe B, C, and D) and the entire Brm gene (probe F, G, and H), respectively. Relative band densities of probes B-D (or probes F-H) to that of probe A (or probe E) were determined (Fig. 9) for each cell. The results clearly indicated that the relative densities were unchanged between SW13(vim–) and SW13(vim+) and were close to those of MDA-MB435 cells, which produce high levels of BRG1 and Brm proteins. Thus we do not detect any specific blocks in transcriptional elongation in SW13(vim–) cells. These results indicate that the loss of mature mRNA of BRG1 and Brm in SW13(vim–) cells cannot be explained by premature transcriptional termination observed in such genes as c-myc and N-myc but are rather caused by the post-transcriptional suppression that is specifically operating in SW13(vim–) cells.

To examine how the HDAC inhibitor released the post-transcriptional suppression observed in SW13(vim–), cellular nuclei were isolated from a SW13(vim–) culture treated with CHAP31 for 3 days and analyzed by the run-on assay. As shown in Fig. 9, the HDAC inhibitor did not affect either the frequency of transcriptional initiation of BRG1 (probe A) and Brm (probe E) or the rate of transcriptional elongation of these genes (probes B–D and F–H). From these results, it is clear that the BRG1 and Brm genes are not the direct targets of CHAP31. Therefore we think that the HDAC treatment activates transcription of certain genes that are essential to counteract the post-transcriptional suppression operating in SW13(vim+).

**Co-culture with SW13(vim–) Causes Suppression of BRG1, Brm, and vimentin Expression in SW13(vim+)**—SW13 has been reported to be deficient in both BRG1 and Brm expression, but, as described above, we have presented evidence that the original cell line of SW13 is composed of two subtypes, one that does not express either BRG1 or Brm (SW13(vim–)) and the other subtype that does express both of them at a significant level (SW13(vim+)). Because SW13(vim+) clones were isolated frequently (19 of 50 clones described from the original cell line of SW13; see “Experimental Procedures”), this rather high frequency of SW13(vim+) isolation was unexpected, because many groups, including ours, have failed to detect any BRG1 and Brm expression in the original cell line of SW13.

**Fig. 7. Induction of BRG1 and Brm mRNA, as well as vimentin, collagenase, c-met, and CD44 mRNA, in SW13(vim–) by treatment with CHAP31 or 5-azadC.** SW13(vim–) #1 was treated with either CHAP31 (4 nM) or 5-azadC (0.5 μM) for 3 days. Total RNA was prepared immediately after treatment (day 0) or after subsequent growth for 7 days in the absence of the reagent (day 7). Expression levels in untreated SW13(vim+) #21 were taken as 1.0, and each column shows the average ratio obtained by three independent experiments. The bars represent the S.D.

**Fig. 8. Nuclear run-on transcription assay of several genes using SW13(vim–), SW13(vim+), and MDA-MB435.** Cellular nuclei were prepared from SW13(vim–) #1, SW13(vim+) #21, and MDA-MB435. After transcriptional elongation in the presence of [α-32P]UTP, the nuclear extracts were hybridized to the DNA plasmids slot-blotted on the filter (1 or 5 μg). Control plasmid (control) is the empty vector pCR2.1, which was used for cloning of most of the genes examined here. Another empty vector, pBluescriptSK(+), which was used for cloning vimentin and CD44, gave the same result.
Indeed, BRG1, Brm, and vimentin mRNA was nondetectable when the original cell line of SW13 that was used to isolate SW13(vim−) #1 and #2 and SW13(vim+) #21 and #22 was analyzed using the same RT-PCR procedure as shown in Fig. 1A.

To examine whether BRG1 and Brm mRNA expression in SW13(vim+) might be affected in the presence of SW13(vim−) in cultures, we prepared a series of mixed cultures composed of these two subclones. Four days after the initiation of mixed cultures, RNA was prepared, and mRNA expression levels of BRG1 and Brm were measured by RT-PCR. In a parallel experiment, we prepared a series of mixtures of RNA isolated from pure cultures of SW13(vim−) and SW13(vim+). In both BRG1 and Brm, mRNA levels of the mixtures of RNA samples increased linearly by elevating the percentage of the RNA sample isolated from pure SW13(vim+) culture. mRNA levels in the mixed cultures were significantly lower than those of mixture of the RNA samples at the corresponding percentage (Fig. 10). For example, when the equal cell number of SW13(vim−) and SW13(vim+) were co-cultured (50% of SW13(vim+)), the mRNA levels of BRG1, Brm, and vimentin were about one-fourth of those of the mixture of the two RNA samples. In the co-culture containing SW13(vim+) at 10%, none of these mRNA was detectable at all (Fig. 10). mRNA levels of collagenase, c-met, and CD44 (data not shown) were also significantly reduced in the co-cultures. The mRNA level of GAPDH was not affected at all by the co-cultivation (Fig. 10). These results indicate that expression of the BRG1 and Brm genes in SW13(vim+) are efficiently suppressed when co-cultured with SW13(vim−), suggesting that SW13(vim−) can somehow transfer its suppressive effects on SW13(vim+).

The next important point would be whether the reduction of this gene expression in SW13(vim−) is reversible. To discriminate these two possibilities, we isolated cellular clones by end-point dilution from a co-culture containing SW13(vim+) at 50%, which reduced vimentin mRNA to one-fourth during 4 days of co-culture as described above. The 15 isolated clones were analyzed for their expression levels of vimentin mRNA using RT-PCR. Among them, seven cellular clones expressed vimentin mRNA at the same level as the pure culture of SW13(vim+) #21, whereas the other eight clones expressed no detectable vimentin mRNA, like the original SW13(vim−) #1. These results clearly indicate that SW13(vim+) cells transiently reduce expression of vimentin gene after the co-culture but recover the full expression level of vimentin upon re-cloning. This phenomenon will explain why BRG1 and Brm expression in SW13 were not detected previously.

**Discussion**

The human adrenal carcinoma cell line, SW13, has been reported to be deficient in both BRG1 and Brm expression. It has been used for many experiments to analyze the function of the SWI/SNF complex, because the complex completely loses its catalytic subunits. In this work, however, we present evidence that the original cell line of SW13 is composed of two subtypes, one that does not express BRG1 and Brm (SW13(vim−)) and another that does express both genes at a significant level (SW13(vim+)). Both subtypes maintain their own phenotypes after cloning by end-point dilution and subsequent cell growth for more than one month. The presence of BRG1 and Brm in SW13 has an exact correlation to the cellular ability to express genes such as vimentin, collagenase, c-met, and CD44 (see Figs. 3 and 4). Transcription of these four genes has been known to be dependent upon either BRG1/Brm or AP-1, a transcription factor that requires the SWI/SNF complex for its transactivating activity. The clear switching of these four genes in an all or none manner implies that chromatin remodeling by a functional SWI/SNF complex is essential for their transcription under physiological conditions.

We intended to liberate SW13(vim−) from suppression and screened several reagents that induce the transition. Transient treatment with inhibitors of HDAC such as CHAP31 and TSA, supports the induction of BRG1 and Brm expression (see Figs. 6 and 7), which probably triggers the subsequent expression of the four genes driven by AP-1. These results indicate that, like
SW13 Subtypes Transitioned by Post-transcriptional Regulation

SW13(vim+) and SW13(vim−) retain the functional BRG1 and Brm genes, but their expression is especially suppressed. Cellular transition is relatively stable and produced a cellular phenotype that is indistinguishable from that of SW13(vim+) (Fig. 9). The transition is maintained for at least 2 months in the absence of CHAP31. Our nuclear run-on analysis indicated that, unlike the four genes driven by AP-1, transcription of the BRG1 and Brm genes are initiated and elongated through the entire gene in SW13(vim−) at a similar efficiency to SW13(vim+). These results indicate that the loss of mature mRNA of BRG1 and Brm in SW13(vim−) cells can not be explained by premature transcriptional termination observed in such genes as c-myc and N-myc but caused by the post-transcriptional suppression. Some specific steps during mRNA maturation from the primary transcript would be inhibited.

Because the run-on experiments indicated that the HDAC inhibitor did not affect either the frequency of transcriptional initiation or the rate of transcriptional elongation in SW13(vim−) cells (Fig. 9), it is clear that the transcriptional initiation of the BRG1 and Brm genes are not the direct target of CHAP31. Therefore we think that the HDAC treatment activates transcription of certain genes that are essential to counteract the post-transcriptional suppression. From our observations, we propose that SW13 can transit between two subtypes, either of which has distinct epigenetical stability, by switching expression of the BRG1 and Brm genes at the post-transcriptional level. We further suggest that the transition could occur spontaneously in a monolayer culture as a rare event.

We have shown previously that murine leukemia virus-based retrovirus vector transgene transcription is rapidly suppressed in human tumor cell lines lacking expression of Brm, such as C33A, SW13, Saos-2, and G401, even though these vectors can successfully enter, integrate, and initiate transcription (15). We further presented evidence that retroviral gene transcription was maintained by the couteraction between the trx-G protein complex (Brm-containing SWI/SNF subfamily) and a Pc-G protein complex (containing Y1 and HDACs) through a chromatin structure and histone acetylation in the promoter region of provirus, 5′-long terminal repeat (15, 37). In these experiments, we used SW13(vim−) as SW13, and the extent of the retroviral silencing in SW13(vim−) was high when we measured it by the "mosaic colony ratio" (81%) (15). When we measured the mosaic colony ratio of the SW13(vim+), it was 43%, indicating significant recovery from the gene silencing. Therefore like genes driven by AP-1, maintenance of retroviral transcription is enhanced by the presence of the Brm protein in SW13(vim+). The clear difference between provirus and the genes under the control of AP-1 examined here (vimentin, collagenase, c-met, and CD44) was that BRG1 had no effect to maintain retroviral gene expression (15).

At this moment, the molecular mechanisms involved in the post-transcriptional regulation of BRG1 and Brm remain largely unsolved. Sequence similarity between BRG1 and Brm might suggest that similar molecular mechanisms are operating in the regulation of these two genes, whereas all the direct target genes of post-transcriptional suppression might not be fully described. Therefore, we cannot exclude the possibility that the retroviral gene silencing observed in SW13(vim−) was partly enhanced by the post-transcriptional suppression mechanisms we detected.

It is also noteworthy that expression of the BRG1 and Brm genes in SW13(vim−) is efficiently suppressed when co-cultured with SW13(vim−) (Fig. 10). This observation explains why expression of these two genes had been undetected in the original SW13 cell line until now. By culturing SW13(vim+) in co-culture with SW13(vim−), every 12 h, we did not detect any phenotypic change in SW13(vim+). The apparent transfer of suppressive signals might involve cell-cell interaction or paracrine secretion from SW13(vim−), but the molecular mechanisms involved in it remain unsolved.

Acknowledgments—We thank Japan Energy, Corp. for supplying CHAP31 and N. Hashimoto and K. Takeda for assistance in preparing this manuscript. We are grateful to Dr. S. Minoguchi for critical reading of this paper. SW13 cells were obtained from the Japanese Collection of Research Bioresources and from Dr. Y. Miyaji (Toho University). We are grateful to A. Watanabe for the preparation of retrovirus vectors.

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