

Glial Cell-Specific Regulation of the JC Virus Early Promoter by Histone Deacetylase Inhibitors

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The human polyomavirus JC virus is the etiologic agent of the fatal disease demyelinating progressive multifocal leukoencephalopathy. Although multiple transcription factors have been shown to interact with the JC virus promoter and regulate transcriptional activity, their relevance to cell specificity remains elusive. To investigate whether chromatin structure controls glial cell-specific expression of JC virus early genes, glial and nonglial cells were transfected with a reporter plasmid containing the JC virus early promoter and then treated with the histone deacetylase (HDAC) inhibitors trichostatin A (TSA) and sodium butyrate. TSA and butyrate induced 20- to 30-fold activation of the JC virus promoter in nonglial cells, whereas less than 2-fold induction was observed in glial cells. These results indicate that the JC virus early promoter might be highly suppressed in nonglial cells by hypoacetylated chromatin and activated by hyperacetylation. In support of this, chromatin immunoprecipitation assays demonstrated acetylation of the JC virus promoter region in U87MG cells but no acetylation in HeLa cells. In addition, treatment of HeLa cells with TSA induced hyperacetylation of the JC virus promoter, whereas minimal induction was seen in U87MG cells. Deletional and site-directed mutational analyses revealed that the enhancer region and Sp1 binding site upstream of the TATA box were important for TSA-mediated activation. We confirmed TSA-mediated activation of the JC virus promoter in the context of natural chromatin structure in stable cell lines. Thus, it appears that chromatin structure may control JC virus transcription in a cell-specific manner.

Progressive multifocal leukoencephalopathy is a fatal demyelinating disease that results from oligodendrocyte infection by JC virus. JC virus selectively destroys oligodendrocytes, leading to multiple areas of demyelination and attendant loss of brain function (3, 31). Once a rare condition, progressive multifocal leukoencephalopathy is no longer infrequent, occurring in 5% of individuals with AIDS (4). JC virus infection exists in a persistent state in kidney tissue and peripheral blood lymphocytes throughout the life of healthy individuals. In the setting of immunodeficiency, the virus infects and destroys oligodendrocytes, producing patches of myelin loss in subcortical white matter (19). These neuropathological features suggest that reactivated JC virus infection is specific for glial cells.

JC virus is a 5-kb circular double-stranded DNA virus classified as a human polyomavirus. The viral genome is divided into early and late gene coding regions, between which lies a regulatory region containing a bidirectional promoter and the viral origin of replication. The JC virus early promoter directs cell-specific expression of the large T antigen, which is required for viral replication, and thus transcriptional regulation constitutes a major mechanism of glial tropism of progressive mul-

tifocal leukoencephalopathy (15). Many studies have identified transcription factors regulating JC virus early gene expression. However, relevance to cell specificity has not been clearly demonstrated.

Recently, the study of transcriptional regulation by chromatin has come under intensive study. The molecules involved in transcriptional regulation by chromatin include promoter DNA, histones, and nonhistone proteins. Polyomavirus DNA is assembled into a set of approximately 21 nucleosomes, both in the virion and in the infected cell, and the viral chromosome in the cell is structurally indistinguishable from host cell chromatin (22). Thus, we thought it possible that histone acetylation and deacetylation, which modulate chromatin structure, may play an important role in transcriptional regulation of glial cell-specific JC virus transcription. It has been reported that the simian virus 40 (SV40) promoter, which has structural similarity to that of JC virus, is controlled by chromatin structure and that the enhancer region plays an important role in this regulation (7, 8, 21).

In this study, we investigated whether the JC virus early promoter is regulated by chromatin structure and characterized regulation by histone acetylation and deacetylation. We found that histone deacetylase (HDAC) inhibitors induced very strong activation of the JC virus early promoter in nonglial cells. In contrast, only a minor increase was observed in glial cells. By using chromatin immunoprecipitation assays, we detected acetylation of the JC virus promoter in U87MG glioma cells but no acetylation in HeLa cells. In addition, trichostatin A (TSA) treatment dramatically increased acetyl histone H3

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binding to JC virus promoter in HeLa cells, whereas only a modest increase in binding was observed in U87MG cells. We further analyzed important elements for TSA-induced activation by deletional and site-directed mutagenesis and found that the enhancer region and Sp1 binding site upstream of the TATA box are critical for TSA-mediated activation. We also confirmed that TSA activates JC virus transcription in the context of host chromatin structure. Our data strongly support the hypothesis that chromatin structure surrounding the JC virus enhancer/promoter modulates JC virus transcription in a cell-specific manner.

MATERIALS AND METHODS

Cell culture and transient-transfection assays. U87MG and U373MG human glioma, HeLa human cervical carcinoma, and SK-HEP1 human hepatoma cell lines were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (HyClone), streptomycin, and penicillin. Transfection was performed by a standard calcium phosphate method. Cells (2×10^5 in 60-mm-diameter dishes) were transfected with 4 μ g of the reporter construct, 1 μ g of pRSV- β -gal, and pUC19 plasmid to a total of 10 μ g of DNA. Plasmids used for transient-transfection assays were prepared by using QIAGEN (Santa Clarita, Calif.) columns. After 48 h, cells were harvested and luciferase assays were performed as previously described (16).

For treatments with HDAC inhibitors, cells were treated with 5 mM sodium butyrate or 150 ng of TSA (Sigma, St. Louis, Mo.) per ml for 24 h prior to harvesting cells for luciferase assays. To correct for differences in transfection efficiencies among different DNA precipitates, luciferase activity was normalized to that of β -galactosidase activity, determined by an *o*-nitrophenyl- β -D-galactopyranoside (ONPG) assay. Within HDAC inhibitor treatment experiments, luciferase values were normalized by protein concentrations as determined by a protein assay reagent from Bio-Rad because HDAC inhibitors regulate the promoters commonly used for internal controls (e.g., Rous sarcoma virus promoter). All transfection assays were performed at least three times in duplicate.

Plasmids. The pMH1long-luc reporter construct contains the 408-bp sequence upstream of the JC virus large T antigen gene fused to the firefly luciferase gene (9). Base substitutions or deletions in the promoter region of the JC virus were generated in the context of the 408-bp upstream sequence, using a QuickChange PCR-based site-directed mutagenesis kit (Stratagene) according to the manufacturer's procedure. The oligonucleotide sequences for generating mutants of pentanucleotide, TATA, and Sp1 sites were described previously (11, 16).

To generate the deletion mutants of the enhancer sequences, oligonucleotide 5'-GCTTCCACTTCCCCTTGCTCCCTACCTTCCCTTT-3' was used for the sense strand primer and oligonucleotide 5'-AAAGGGAAGGTAGGGAGCAA GGGGAAGTGGAAAGC-3' was used for the antisense strand primer. For site-directed mutagenesis of NF-1 or AP1 binding sites in the enhancer region, the following oligonucleotides were used: 5'-GAGCTCATGCTCTAATCC AGC CATCCA-3' and 5'-CTGGATGGCTGGAGTTAGAGCATGAGCT-3' for the NF-1 mutant, 5'-CCTAGGTATGAGCTTGTGCTTGGCTGG-3' and 5'-GCCAGCCAAGCACAAAGCT CATACCTAG-3' for the AP1 mutant. The first set of primers represent coding-strand sequences of the promoter containing the desired mutations (italic bases), and the second set of primers represent the corresponding noncoding-strand sequences. Constructs with desired mutations were screened by restriction enzyme digestion and sequencing analysis.

Stable transfection. SK-HEP1 or HeLa cells were stably transfected with pMH1long-luc by the calcium phosphate method. Since pMH1long-luc does not carry a selectable marker in mammalian cells, pMH1long-luc was cotransfected with pRc-CMV (Invitrogen) at a molar ratio of 10:1, and stable clones were selected in 400 μ g of G418 per ml. To obtain clones derived from single cells, stable transfectants bearing pMH1long-luc were cloned by the dilution method (18). The luciferase activity of individual clones was measured in the absence and presence of TSA. To confirm JC virus promoter integration, Southern blotting of purified genomic DNA was performed with JC virus promoter DNA labeled as a probe.

Chromatin immunoprecipitation assay. HeLa or U87MG cells were transfected with pMH1long-luc by the calcium phosphate method and treated with 150 ng of TSA per ml for 24 h prior to harvesting cells. Cross-linking by addition of formaldehyde (to 1% final concentration) was allowed to proceed at 37°C for 10 min and terminated with glycine. After brief sonication, lysates were cleared by centrifugation and then chromatin was precleared with protein A-Sepharose at 4°C for 2 h. Precleared chromatin was incubated with 2 μ g of anti-acetyl

histone H3 antibody at 4°C overnight. Immune complexes were collected by protein A-Sepharose beads at 4°C for 4 h. At the end of the incubation, the precipitates were sequentially washed once with buffer A (20 mM Tris-HCl, 2 mM EDTA, 0.1% sodium dodecyl sulfate) containing 150 or 500 mM NaCl and once with buffer A containing 0.25 M LiCl. After the final wash, pellets were resuspended in 300 μ l of Tris-EDTA (TE) and incubated at 65°C overnight.

Samples were extracted with phenol-chloroform and ethanol precipitated. Pellets were resuspended in 10 μ l of H₂O and assayed by PCR. Thirty cycles of PCR were performed in a total volume of 20 μ l with 5 μ l of immunoprecipitated material, 0.4 pmol of each primer, and 1 U of *Taq* polymerase. The JC virus promoter was amplified with the primer pair JC56S (5'-GGCTGCTTTCCACT TCCCCTT-3') and JC342A (5'-GCCTCCACGCCCTTACTACTTCTG-3'). The resulting PCR product encompasses 277 bp of the enhancer and promoter region. The band was visualized by ethidium bromide staining after agarose gel electrophoresis.

RESULTS

Histone deacetylase inhibitors stimulate JC virus early promoter in a cell-specific manner. To address whether the JC virus early promoter is regulated by chromatin structure, JC virus expression was compared after treatment with the histone deacetylase (HDAC) inhibitors TSA and butyrate. TSA is known to be a potent and specific inhibitor of HDAC activity (30), whereas butyrate is a less specific inhibitor of HDAC (17, 23). Two glial and two nonglial cell lines were used for transient-transfection assays. As shown in Fig. 1, HDAC inhibitors increased JC virus promoter activity about 20- to 30-fold in nonglial SK-HEP1 and HeLa cells, and thus final activity became comparable to that in glial cells. In contrast, a less than twofold increase in JC virus expression was observed in glial U87MG and U373MG cells. The inhibitors did not influence the activity of a control backbone plasmid, pA₃PLUC (data not shown) (20). These data suggest that histone acetylation and deacetylation may be an important regulatory factor for glial cell-specific expression of JC virus.

To further investigate JC virus early promoter regulation by HDAC inhibitors, reporter plasmids containing the Mad-1 JC virus or SV40 promoter were transfected into U87MG and SK-HEP1 cells, and HDAC inducibility was compared with the MH1 JC virus early promoter. Both MH1 and Mad-1 JC virus promoters showed much larger induction by HDAC inhibitors in SK-HEP1 cells than in U87MG cells. In contrast, the SV40 promoter was induced to the same extent (about fivefold) in both U87MG and SK-HEP1 cells by the inhibitors (Fig. 2). These results support the hypothesis that HDAC inhibitors and HDAC control JC virus transcription in a glial cell-specific fashion.

Chromatin immunoprecipitation assays revealed acetylation of the JC virus promoter in U87MG cells but not in HeLa cells and TSA-induced hyperacetylation in HeLa cells. Treatment of cells with TSA blocks the activity of HDAC proteins, resulting in hyperacetylation of histones (2, 28). In order to determine if TSA treatment caused hyperacetylation of histones bound to the JC virus promoter, chromatin immunoprecipitation assays were performed on untreated cells or cells treated with TSA with an anti-acetyl histone H3 antibody. In Fig. 3, amplification of the input DNA from the JC virus promoter used in the chromatin immunoprecipitation assay and the DNA after immunoprecipitation with the anti-acetyl histone H3 antibody are shown. Acetylation of histones attached to the JC virus promoter region was seen in U87MG

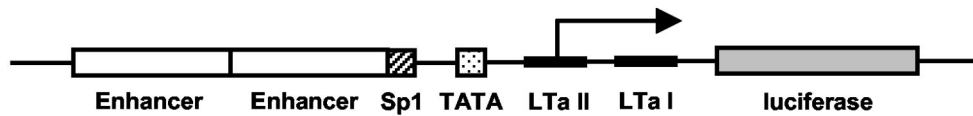
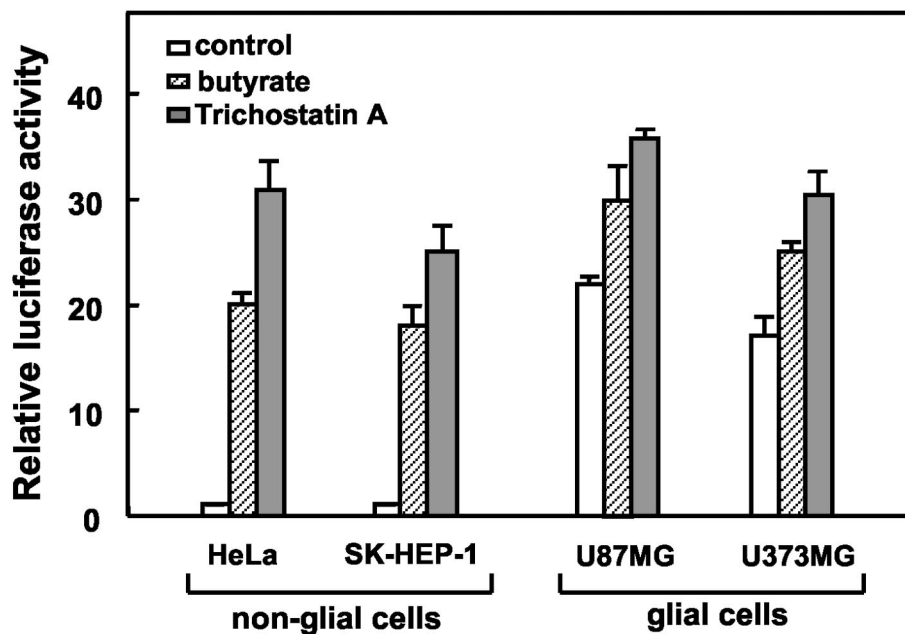
A**Reporter plasmid : pMH1long-luc****B**

FIG. 1. Cell-specific activation of MH1 JC virus promoter by HDAC inhibitors. (A) Schematic of the reporter plasmid containing the MH1 JC virus early promoter. The promoter is fused to the firefly luciferase gene. Open boxes indicate direct tandem repeats of the enhancer, dotted boxes represent TATA homologies, and striped boxes represent Sp1 binding sites upstream of the TATA sequence. Binding sites for the viral protein large T antigen (black boxes) are also indicated. (B) Two nonglial and two glial cell lines were transfected with pMH1long-luc plasmid DNA and treated with 150 ng of TSA per ml or 5 mM butyrate for 24 h prior to harvesting cells. Luciferase assays were performed with the cell extracts, and relative activities are shown. In nonglial cells, HDAC inhibitors caused 20- to 30-fold increases in JC virus transcription, whereas a <2-fold increase was observed in glial U87MG and U373MG cells.

cells, whereas no acetylation was observed in HeLa cells. Treatment of cells with TSA resulted in hyperacetylation of histones bound to the JC virus promoter region in HeLa cells. The effect of TSA on HeLa cells was much larger than on U87MG cells, where only a twofold increase was observed. These results indicate that histones bound to the JC virus promoter exist in an acetylated state in glial cells, whereas they are deacetylated in nonglial cells. HDAC inhibitors induced early JC virus transcription and also induced hyperacetylation of histone H3. Thus, histone acetylation/deacetylation may be important factors determining the cell specificity of JC virus.

Enhancer region and Sp1 binding site upstream of TATA are important for TSA-mediated activation. To determine the location of the transcription factor binding site(s) regulated by

HDAC inhibitors, deletional and site-directed mutagenesis was performed, and inductions by HDAC inhibitors was compared with that of the wild-type promoter in HeLa and SK-Hep1 cells (Fig. 4). Mutation of the pentanucleotide or TATA sequence, which are important for T antigen-mediated activation (16), did not produce significant changes in the increase by HDAC inhibitors. Alteration of two Sp1 binding sites downstream of the TATA box (mSp1-2 and mSp1-3) also did not produce a change. However, mutation of the Sp1 site upstream of the TATA box (mSp1-1) reduced induction sixfold. These results suggest that the proximal Sp1 binding site located upstream of the transcription initiation site is important for the regulation of JC virus expression by HDAC inhibitors. Interestingly, when one repeat of the enhancer was deleted, induc-

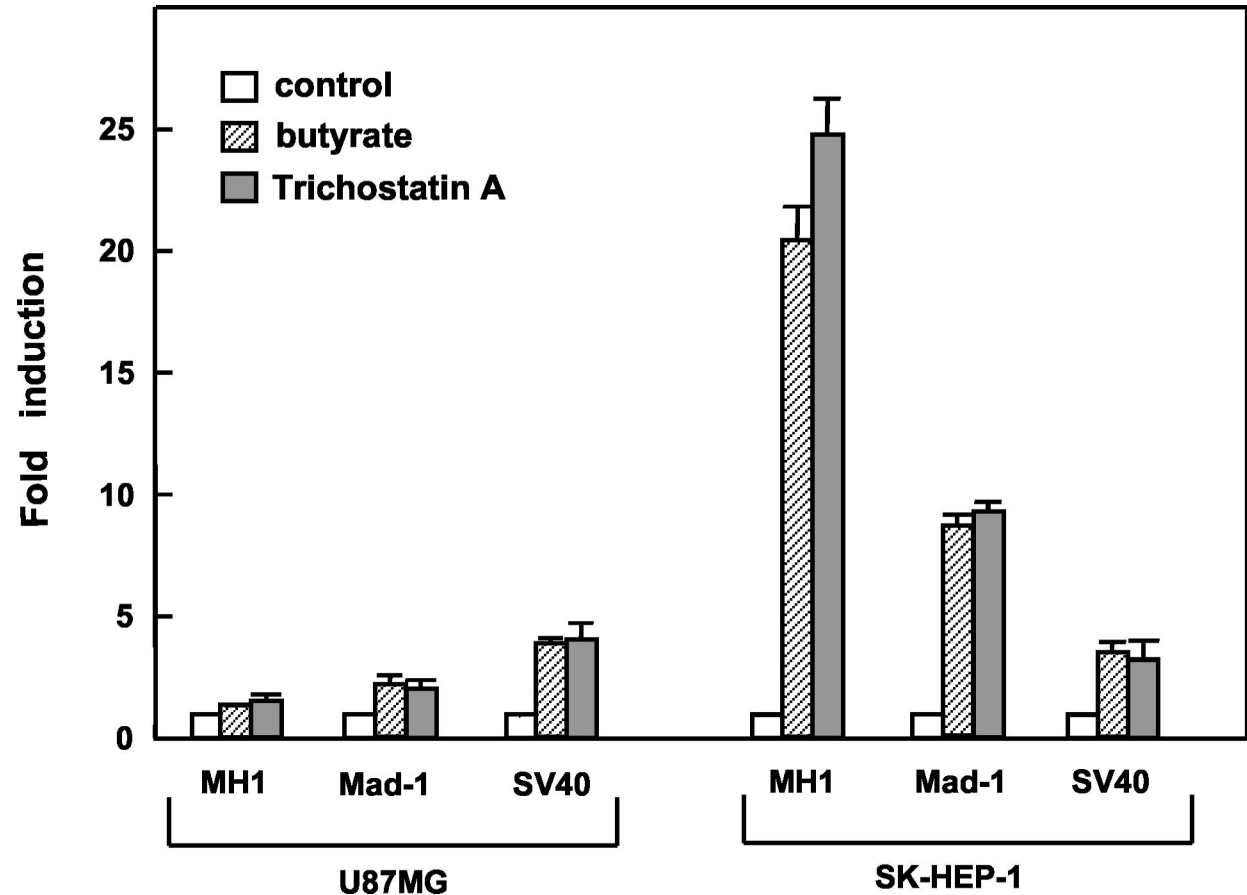


FIG. 2. Comparison of TSA-mediated inducibility of MH1 and Mad-1 JC virus promoters and SV40 promoter in U87MG and SK-HEP1 cells. Two types of JC virus promoters (MH1 and Mad-1) and SV40 promoter reporter plasmids were transfected into U87MG and SK-HEP1 cells, which were then treated with 5 mM butyrate or 150 ng of TSA per ml for 24 h prior to harvesting cells. Luciferase activity without TSA or butyrate treatment was set at 1.0 in each cell line, and inductions by the inhibitors is presented in the graph. Both of the JC virus promoters were more strongly induced in SK-HEP1 cells than in U87MG cells by the inhibitors. The SV40 promoter was induced in both cell lines to the same extent.

tion was also decreased about sixfold compared to the wild type. Moreover, deletion of both enhancer repeats completely abolished TSA-mediated activation, indicating that the enhancer region and Sp1 binding site upstream of TATA are important for TSA-mediated activation (Fig. 4A). Identical results were observed in HeLa and SK-HEP1 cells.

To further dissect the enhancer region, site-directed mutagenesis was employed to alter the NF-1 and AP1 binding sites (1, 27). Mutation of either of these sites did not alter induction compared to wild-type sequences (Fig. 4B). These results suggest that other transcription factor binding sites might be required for this activation or that nucleosome structure surrounding the enhancer region might mediate this inducibility independent of transcription factor binding.

JC virus promoter is stimulated by TSA after integration into the genome. Although nucleosomes are formed on DNA templates introduced into cells by transient transfection, these nucleosomes may be in a less repressive state than those formed on stably integrated DNA (25). To confirm JC virus promoter regulation by histone deacetylase in the context of natural chromatin structure, stable transfectants of SK-HEP1 and HeLa cells containing pMH1long-luc were generated. Seventeen independent single clones were selected from SK-

HEP1 transfectants, and five clones were selected from HeLa transfectants, and TSA-inducibility was tested for each clone. Integration of the JC virus promoter into host chromosomal DNA was confirmed by Southern blotting for all of these clones (data not shown). Induction varied considerably between the clones of SK-HEP1, ranging from 2- to 7,250-fold (Table 1). Five HeLa clones showed 20- to 144-fold induction. These results show that TSA induced strong activation of both a transiently and a stably integrated JC virus early promoter reporter equally well.

Because large differences were observed in the induction of activation in stable transfectants, we checked the gene dosage of each clone. There was no correlation between the degree of induction and the copy number of the integrated plasmid. Thus, expression of the stably integrated JC virus promoter region appeared to be strongly influenced by the chromosomal environment.

DISCUSSION

This study characterized glial cell-specific regulation of JC virus transcription by chromatin structure. We found that HDAC inhibitors increased JC virus early transcription more

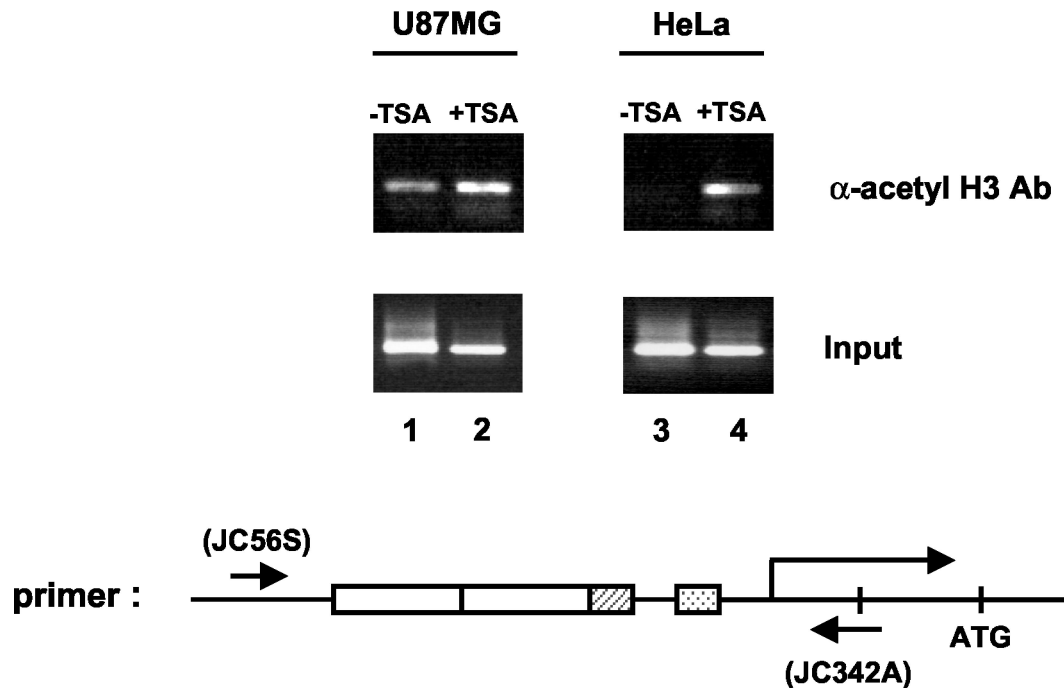


FIG. 3. Histone acetylation on MH1 JC virus promoter. To investigate direct evidence for regulation of the JC virus promoter by histone acetylation and deacetylation, chromatin immunoprecipitation assays were performed. After transfection of pMH1long-luc, DNA-histone complexes were immunoprecipitated with anti-acetyl histone H3 antibodies (Ab), and the eluted DNAs were amplified with primers spanning the enhancer and promoter regions. The primers used are indicated as JC56S for the 5' primer and JC342A for the 3' primer. The lower panels represent input DNAs before addition of anti-acetyl histone H3 antibody. The upper panels represent the DNAs bound to acetyl histone H3. Lanes 1 and 3, DNAs extracted from cells without TSA treatment. Lanes 2 and 4, DNAs extracted from TSA-treated cells. JC virus promoter-histone complexes exist in an acetylated state in U87MG glial cells but not in nonglial HeLa cells, while treatment with TSA dramatically increased histone acetylation in HeLa cells.

robustly in nonglial cells than in glial cells and that hyperacetylation was induced in nonglial cells by the inhibitors. The enhancer region and Sp1 binding site upstream of the TATA box were identified as being critical for this induction. By generating stable cell lines transformed with JC virus reporter plasmid, TSA-mediated activation was confirmed in the context of host chromatin structure.

In addition to the direct regulation by transcription factors, gene expression is also controlled by molecular and structural modifications of promoter DNA. Chromatin packing and DNA methylation are two such mechanisms. As for regulation by chromatin structure, it has become increasingly apparent that the equilibrium of histone acetylation and deacetylation plays an important role. Histone deacetylation occurs at lysine residues on the N-terminal tails of the histones, increasing their affinity for DNA. As a consequence, histone deacetylation alters nucleosomal conformation, which decreases the accessibility of transcriptional regulatory proteins to chromatin templates. Polyomavirus DNA is assembled into a set of approximately 21 nucleosomes, both in the virion and in the infected cell, with each nucleosome consisting of an octamer containing two copies of histone H2A, H2B, H3, and H4. In the infected nucleus, it appears that histone H1 is associated with at least some of the minichromosomes (5), and in fact the viral chromosome in the cell is structurally indistinguishable from host cell chromatin (22). Thus, to investigate the regulation of the JC virus promoter by histone acetylation/deacety-

lation, luciferase activities were measured in the cells before and after treatment with TSA. We found that HDAC inhibitors induced strong activation of the JC virus early promoter in nonglial cells and that the enhancer region plays an important role in this activation. These findings can be explained as follows: TSA/butyrate-induced modifications allow conformational changes in chromatin which increase the accessibility of regulatory proteins, examples of which could be RNA polymerases or other proteins that may bind to the enhancer region.

To confirm cell-specific regulation of JC virus promoter by HDAC inhibitors, the induction of two types of JC virus promoter, MH1 and Mad-1, and SV40 promoters was compared. Two JC virus promoters were more strongly activated by TSA in nonglial cells than in glial cells, whereas the SV40 promoter was induced by the same amount irrespective of cell type. These results indicate that HDAC inhibitors stimulate the JC virus promoter in a cell-specific manner, at least in glial and nonglial cells.

Direct evidence that histone acetylation/deacetylation targeted to the JC virus promoter was obtained by chromatin immunoprecipitation assays. Histone acetylation on the JC virus promoter was detected in U87MG glioma cells but was not detected in HeLa cells. In addition, TSA treatment caused a dramatic increase in acetylation on the JC virus promoter in HeLa cells, and the resulting acetylation approached the levels detected in U87MG cells. These results strongly support the

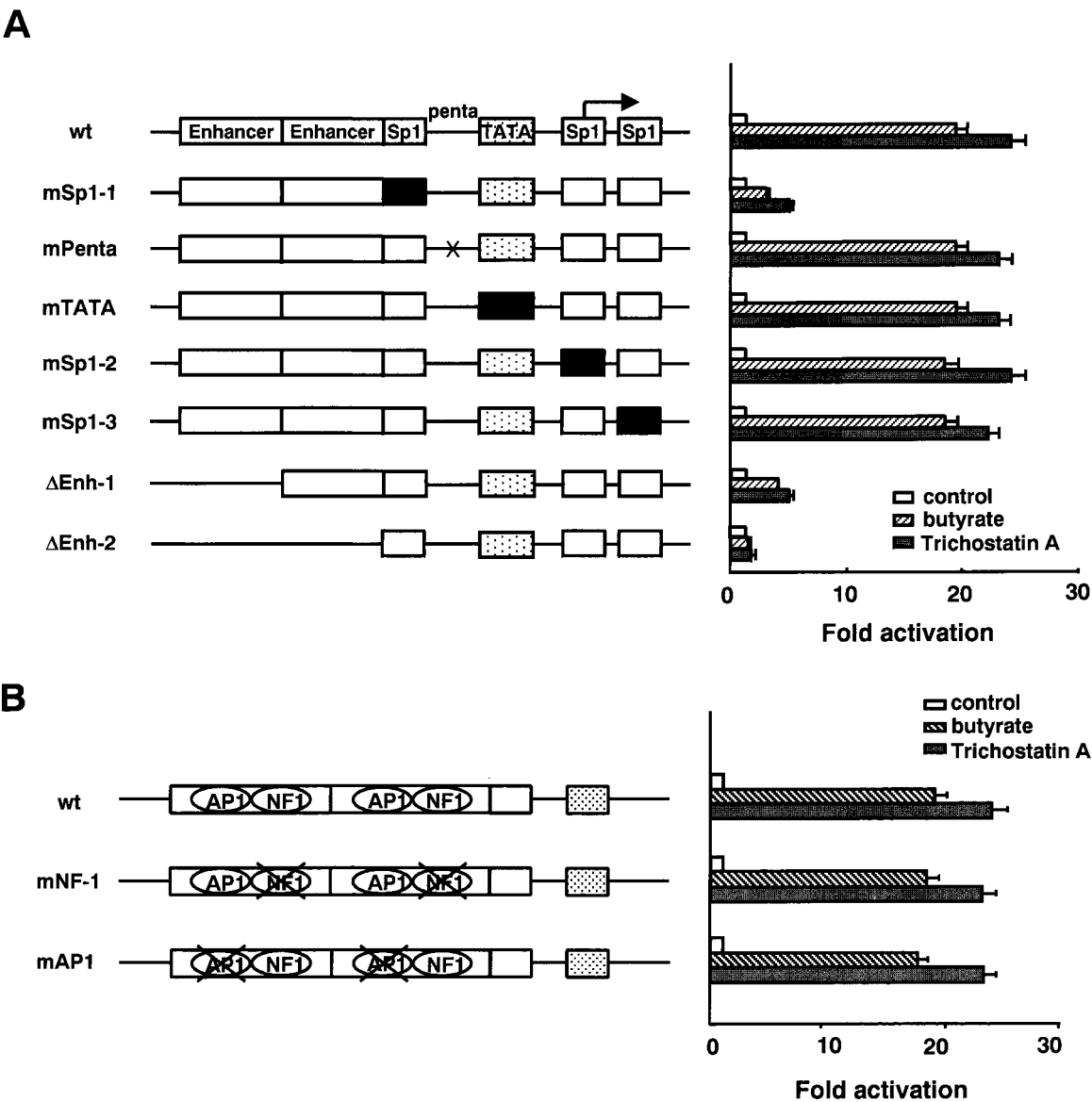


FIG. 4. Enhancer region and Sp1 binding site upstream of the TATA box are necessary for TSA-mediated activation of JC virus promoter. HeLa cells were transfected with pMH1long-luc plasmid DNA carrying mutations or deletions, as shown on the left. The experimental conditions were the same as in Fig. 1. The data were normalized to protein content and are expressed as activity obtained with butyrate or TSA treatment relative to that of the untreated control. All experimental points were run in duplicate. (A) Constructs with mutations in the basal promoter region or deletions in the enhancer region are indicated. Alteration of the Sp1 site upstream of the TATA box or deletion of the enhancer region significantly affected induction of the JC virus promoter, while mutations of other sites did not alter TSA-mediated activation. wt, wild type. (B) Mutations of AP1 and NF-1 binding sites are indicated. Mutation of AP1 or NF-1 binding sites did not alter activation.

idea that histone acetylation/deacetylation plays an important role in regulating JC virus transcription in a cell-specific fashion. Previous data demonstrated that butyrate activated the SV40 promoter, which has very close structural similarity to that of MH1 JC virus, and that the SV40 enhancer region was very important for this activation (7, 8, 21). Work done with SV40 minichromosomes has shown that an extended SV40 origin region, including the 72-bp repeated enhancer region, has a nucleosomal phasing pattern that differs from that of the remainder of the viral genome (14). This region, and the analogous region in other polyomaviruses, is also hypersensitive

to DNase I (12, 24). The host range and specificity of some polyomavirus mutants have been correlated with changes in the levels of histone hyperacetylation in these regions (12). In addition to the enhancer region, the Sp1 binding site upstream of the TATA box was shown to be important for TSA-mediated activation of the JC virus promoter. We have previously demonstrated Sp1 binding to this site and shown that Sp1 plays an important role in regulation of JC virus transcription (9, 10). HDAC1 was recently shown to form a complex directly with Sp1 (6), raising the possibility that Sp1 might target deacetylases to specific promoters, leading to hypoacetylation and decreased gene expression. Besides the JC

TABLE 1. JC virus promoter is stimulated by TSA after integration into the genome^a

Cells	Clone no.	Basal activity (U)	Activation by TSA (fold)
SK-HEP-1	1	188	62
	2	61	353
	3	34	70
	4	10	2,175
	5	116	16
	6	3,859	9
	7	23	13
	8	33	2
	9	64	789
	10	728	7,256
	11	9,846	7
	12	201	59
	13	126	22
	14	2,212	108
	15	38	1,688
	16	4,522	61
	17	301	908
HeLa	1	597	89
	2	442	38
	3	41	144
	4	9,673	20
	5	1,711	52

^a pMH1long-luc was integrated into the genome of SK-Hep1 or HeLa cells, and single-cell-derived clones were isolated. Individual clones were plated and grown for 48 h, after which they were grown for an additional 24 h in either the presence or absence of 150 ng of TSA per ml. Mean basal activity (luciferase expression in the absence of inhibitor, in arbitrary light units) and the activation induced by TSA for each clone are indicated.

virus promoter, several promoters have been reported to be activated by HDAC inhibitors specifically through Sp1 sites, including the human adenine nucleotide translocase-2 promoter (13), human *COX-1* promoter (28), mouse and human p21^{waf/cip1} promoters (26, 29), and the mouse thymidine kinase promoter (6). It is of interest that all these promoters are activated by deacetylase inhibitors via Sp1 sites located in similar positions relative to the transcription start site. The HDAC inhibitors also activate the SV40 early promoter (8), which has a similar promoter organization. Thus, it is tempting to speculate that Sp1 bound to specifically positioned GC elements might play a role in directing chromatin-dependent gene suppression.

To further analyze important factors directly involved in TSA-mediated activation, site-directed mutagenesis was performed in the binding sites of NF-1 and AP1, which are well-known transcription factors that bind to the enhancer region. Mutation of either of these sites did not alter induction compared to the wild type. These results suggest that other transcription factors interacting with the enhancer region may be involved in activation. Alternatively, nucleosome structure surrounding the enhancer region could mediate inducibility independent of transcription factors.

By constructing stable cell lines, we confirmed TSA-mediated induction of the JC virus promoter in the context of natural chromatin structure. When the basal and TSA-induced luciferase activities of individual clones were compared, they showed variations in responsiveness between clones, likely due to the different chromosomal environments where the promoter gene was integrated.

In conclusion, we have identified a new mechanism regulating JC virus transcription. We have provided evidence that histone acetylation/deacetylation may play an important role in glial cell-specific regulation of JC virus. The regulation of JC virus expression by histone acetylation occurs via the enhancer and the Sp1-binding site located upstream of the transcription initiation site. Thus, chromatin-modifying enzymes modulate cell-specific expression of JC virus.

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