THE BULLETIN OF OKAYAMA UNIVERSITY OF SCIENCE No. 42 A pp. 39-46 (2006)

Effects of Histone Deacetylase Inhibitors on Transcription Promoter Activities of Bidirectional Gene Pairs in Mammalian Cells

Shogo IKEDA, Motohiro MIKI, Yoshihisa MATSUMOTO and Yuichi SEKI*

Department of Biochemistry, Faculty of Science, Okayama University of Science, 1-1 Ridaicho, Okayama 700-0005, Japan

**Chugoku Occupational Health Association, Okayama Medical Examination Center, 147-6 Obara, Tsuyama 708-0001, Japan*

(Received September 4, 2006; accepted November 6, 2006)

Gene pairs with a head-to-head configuration are frequently present in mammalian genomes. This arrangement of the gene pairs implies a coordinate regulation of the genes by a bidirectional promoter. In this study, we examined the effect of chromatin remodeling by histone acetylation and deacetylation on the regulation of transcription of the bidirectional gene pairs. An increase in the acetylation level of the histone protein by inhibition of histone deacetylase (HDAC) induced an open chromatin conformation in cells. Promoter activities of the bidirectional gene pairs including *Apex/Osgep*, *Nthl1/Tsc2*, and *Fen1/1810006K21Rik* genes were measured by a luciferase assay in the presence or absence of HDAC inhibitors. Three kinds of HDAC inhibitors, trichostatin A (TS-A), MS-275, and sirtinol, with a different target spectrum were administered to one human and 6 mouse cell lines. Although the supercoiled form of reporter plasmid demonstrated extremely high activity compared to the linear form of the same DNA in the luciferase assay, transcriptions from both supercoil and linear forms were activated to the same extent by TS-A. In many cell lines the promoter activity of the bidirectional genes was strongly activated by the addition of TS-A and MS-275. Transcription of each gene in bidirectional pairs was individually regulated by these HDAC inhibitors depending on the cell type. Sirtinol did not activate the transcription of any genes.

Keywords: bidirectional promoter; gene regulation; chromatin remodeling; histone deacetylase; HDAC inhibitor; luciferase assay.

1. Introduction

Recently human and mouse whole genome sequences were completed and as a result information concerning the density and distribution of genes in the genome has been clarified. Through Adachi and Lieber's report [1] the fact that two neighboring gene pairs with a head-to-head orientation exist in an unexpectedly large number became known. A report by Trinkelein *et al.* [2], which analyzed the distance between 23,752 human genes, clarified that there was a head-to-head orientation in 11.8% of the genes' transcription initiation sites in the space of 1 kb. Furthermore many genes that work in DNA repair have the same head-to-head orientation structure. The analysis that Adachi and Lieber [1] made of the 290 house-keeping genes showed that 87 genes (30%) had the same head-to-head genetic orientation while as many as 50 of the 120 DNA repair related genes (i.e. 42%) had this orientation. Furthermore 40 out of the 50 genes neighbored each other within a 300-bp tract. Trinklein *et al.* [2] clarified that the number of DNA repair related genes that are oriented head-to-head is 5 times more than house keeping genes. Genes that are oriented 5' to 5' have a common feature: they possess a CpG island and lack a typical TATA box in-between the gene pairs. It can be speculated that transcription of these genes might be regulated divergently by the bidirectional

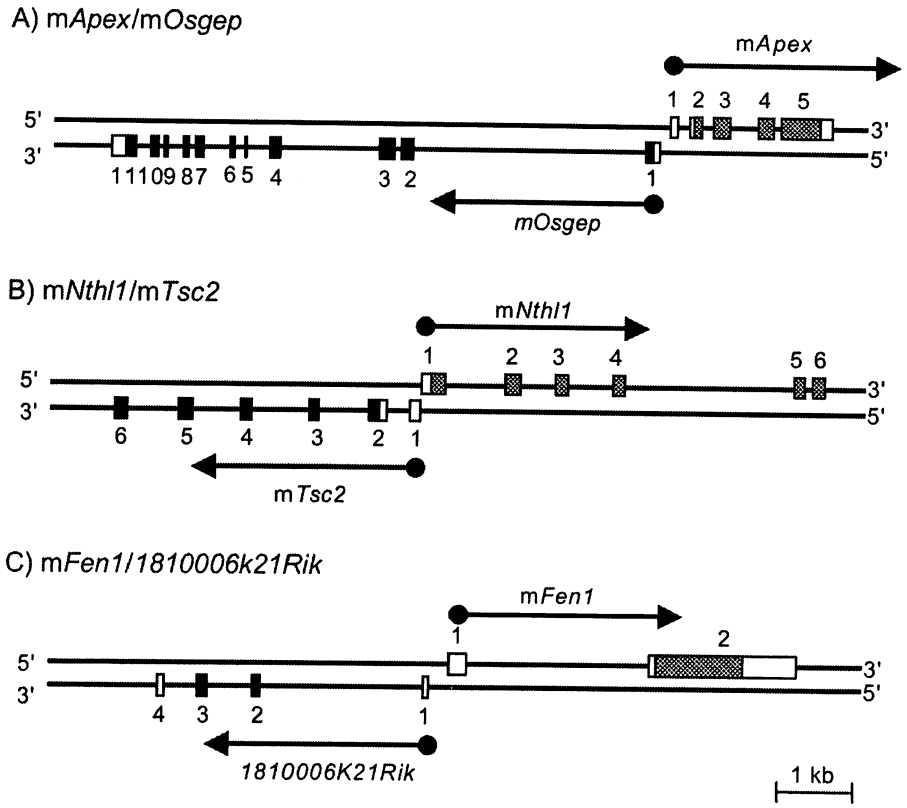

Fig. 1. Gene organization of mouse bidirectional gene pairs used in this study. A, *Apex/Osgep*; B, *Nthl1/Tsc2*; C, *Fen1/1810006K21Rik*. Introns are indicated by a solid line. In the exons, filled and open boxes represent translated and untranslated regions, respectively.

promoter that exists in the spacer sequence of each gene pair. In our laboratory we have been analyzing the structure of the DNA repair enzyme genes that are involved in base excision repair [3-6]. Among these genes we have clarified that APEX nuclease (*Apex*), thymine glycol DNA glycosylase (*Nth1*), and Flap endonuclease 1 (*Fen1*) are oriented head-to-head in a genomic structure and are regulated by a bidirectional promoter (Fig. 1).

Recently it was discovered that chromatin remodeling by histone modification plays an important role in the induction of transcription. DNA has a high dimensional structure, chromatin, which consists of nucleosome arrays. The nucleosome is made of a central protein complex (the histone octamer) and 1.65 turns of DNA (about 146 base pairs), which are wrapped around the histone octamer complex. Chromatin is normally kept in a deacetylated state and is tightly packed. With the action of transcription factors histone proteins are acetylated only when necessary followed by unwinding of the chromatin structure. Therefore at the place where active transcription occurs, histone is acetylated and histone acetyltransferase (HAT) and histone deacethylase (HDAC) play an increasingly relevant and important role. Proteins with HAT activity are well known among the transcription cofactors. Sometimes they form a complex other than the monomer and are involved in general transcription activation or hormonal transcriptional activation. On the other hand several HDACs are known and many of them form a complex with other factors and may play a role as a corepressor.

In this present research we investigated the effect of chromatin structure on bidirectional promoters using the gene pairs consisted by DNA repair genes as models. We transformed the chromatin region of DNA to an acetylated state by the addition of an HDAC inhibitor and investigated the effect of that inhibitor on the transcription of each gene using a

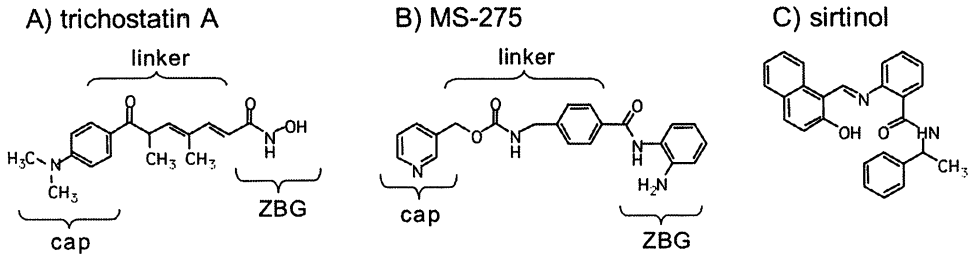

Fig. 2. Chemical structures of HDAC inhibitors. Systematic name of trichostatin A (TS-A) is 4,6-Dimethyl-7-[p-dimethylaminophenyl]-7-oxahepta-2,4-dienohydroxamic Acid. HDAC inhibitor I (MS-275) is N-(2-Aminophenyl)-4-[N-(pyridine-3-ylmethoxycarbonyl)aminomethyl]benzamide. Sir Two Inhibitor Naphthol (sirtinol) is 2-[(2-Hydroxynaphthalen-1-ylmethylene)amino]-N-(1-phenethyl)benzamide. TS-A and MS-275 consist of three parts; a long alkyl chain (liker), a zinc-binding group (ZBG) and a hydrophobic group (cap).

luciferase assay. The most profound result is that we were successful in clarifying the effect of chromatin structure on the transcriptional regulation of a bidirectional promoter.

2. Materials and Methods

2.1. Cells

The following single human cell line and 6 mouse cell lines were used for DNA transfection; HeLa (cell number, JCRB9004), BALB/3T3 (RCB0005), NIH/3T3 (JCRB0615), Hepa 1-6 (RCB1638), LLC (RCB0558), 707.fl (RCB0596), and FM3A (RCB0086). They were grown at 37°C in a humidified atmosphere of 5% CO_2. HeLa cells were cultured in Eagle's minimal essential medium (MEM, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% fetal calf serum. BALB/3T3 were grown in MEM supplemented with 10% calf serum, NIH/3T3 cells in Dulbecco's Modified MEM (DMEM, Nissui) supplemented with 10% calf serum, Hepa 1-6 cells in DMEM supplemented with 0.45% glucose and 10% fetal calf serum, LLC cells in DMEM supplemented with 10% fetal calf serum, and FM3A cells in RPMI 1640 medium (Nissui) supplemented with 10% fetal calf serum.

2.2. HDAC inhibitors

Torichostatin A (TS-A) and sir two inhibitor naphthol (sirtinol) were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). And HDAC Inhibitor I (MS-275) was from Calbiochem (San Diego, CA). The chemical structures of these compounds are shown in Fig. 2.

2.3. Construction of reporter plasmids

The DNA fragments used for the luciferase assay are shown in Fig. 3. They were amplified by PCR using *pfu* DNA polymerase (Stratagene, CA) using specific primer sets, and then inserted into the firefly luciferase vector pGV-B (TOYO B-Net Co. Ltd., Tokyo, Japan). The construction of reporter plasmids for *Apex/Osgep*, *Nthl1/Tsc2* and *Fen1/1810006K21 Rik* bidirectional gene pairs were previously described in the reports by Ikeda *et al.* [5], Ikeda *et al.* [3], and Emoto *et al.* [6], respectively.

2.4. DNA transfection and luciferase assay

The cells were inoculated into a culture dish (ϕ 5cm) at a cell density of 25%, and the following day the culture media were changed. After one day the cells were peeled off from the dish by trypsinization and 5×10^4 cells were then inoculated in each well of a 24-well plate. 18 to 20 h after subculture HeLa cells were transfected using FuGENE 6 transfection reagent (Roche Diagnostics Corp., IN) with the reporter plasmid (300 ng/well). Other cell lines except for HeLa cells were transfected using Lipofectamine 2000 (Invitrogen, CA) with the reporter plasmid (600 ng/well). 24 h after transfection, HDAC inhibitors were added into the culture media, and the cell cultures were continued for additional 24 h. The cell extracts were prepared with a lysis buffer (PicaGene Cell Culture Lysis Reagent Luc, TOYO B-Net). Luciferase activity was measured using PicaGene Luminescence Kit (TOYO B-Net) and an AB-2200-R luminometer (ATTO Corporation, Tokyo, Japan). Proteins in the extracts were quantitated by the dye-binding method (Bio-Rad Laboratories Inc.,

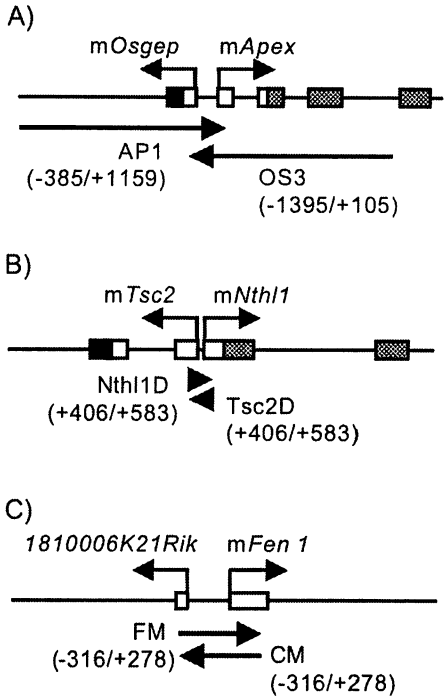

Fig. 3. DNA fragments used for luciferase assay. Promoter regions of the bidirectional gene pairs are illustrated with DNA fragments used for luciferase assay as a long bar with an arrowhead. Numbers in parentheses are the base position of either end of the fragment. Numbering of the nucleotide position of *Apex/Osgep*, *Nthl1/Tsc2* and *Fen1/1810006K21Rik* bidirectional gene pairs are derived from studies by Ikeda *et al.* [5], Ikeda *et al.* [3], and Emoto *et al.* [6], respectively.

CA) using bovine serum albumin as the standard. All values of luciferase activity were normalized to the amount of protein in the same extract.

3. Results and Discussion

3.1. Bidirectional promoters studied in this study

Thymine glycol DNA glycosylase and APEX nuclease are DNA repair enzymes involved in the initial steps of base excision repair [7]. Previously we showed that the APEX nuclease gene (*Apex*) lies immediately adjacent to the gene for *O*-sialoglycoprotein endopeptidase (*Osgep*) in a head-to-head orientation [4,5,8] (Fig. 1A). The spacer sequence between *Osgep* and *Apex* has bidirectional promoter activity, and the CCAAT box and Sp1-binding sequence proximal to the transcription initiation site of *Osgep* are involved in the transcription of both *Osgep* and *Apex* genes [5]. The gene of mammalian thymine glycol DNA glycosylase (*Nthl1*) also lies immediately adjacent to one of the tuberous sclerosis disease-determining genes *Tsc2* (Fig. 1B). We found that the short spacer sequence of 63 bp between the mouse *Nthl1* and *Tsc2* genes shows bidirectional promoter activity essential for the transcription of both genes [3]. The 63-bp sequence, which contains two Ets-transcription factor binding sites facing opposite directions, positively regulates the transcription of both *Nthl1* and *Tsc2* genes. Flap endonuclease 1 is a structure-specific nuclease involved in DNA replication and repair. The mouse *Fen1* gene is located immediately adjacent to the *1810006K21Rik* gene in a head-to-head orientation [6] (Fig. 1C). Transcription initiation sites of each gene are 274 bp apart in the mouse genome. A 594-bp fragment between the *Fen1* and *1810006K21Rik* genes functions as a bidirectional promoter (Fig. 3).

3.2. Properties of HDACs and their inhibitors

18 kinds of mammalian HDACs have been identified and classified into three classes on the basis of their amino acid sequences. Class I HDACs (1, 2, 3, and 8) are homologs of yeast RPD3 and localize to the nucleus [9]. Class II HDACs (4, 5, 6, 7, 9 and 10) are homologs of yeast Hda1 and are found in both the nucleus and cytoplasm [10-12]. HDAC-11 has properties of both class I and class II HDACs. Class III HDACs (Sirt1 to Sirt7) are homologs of yeast Sir2 and form a structurally distinct class of NAD-dependent enzymes found in both the nucleus and cytoplasm [13].

HDAC inhibitors (TS-A, MS-275 and sirtinol) employed in this study exhibited a different target spectrum. Conserved from yeast to human, HDAC classes I and II are inhibited by TS-A [14,15]. TS-A blocks cell cycle progression at the G1 phase in HeLa cells and induces a 12-fold increase in intracellular levels of gelsolin [16]. TS-A also induces the reversion of oncogenic *ras*-transformed NIH/3T3 cells to a normal morphology [17]. MS-275 mainly inhibits HDAC-1 and -3, but not HDAC-8 at all [18]. In *in vitro* and *in vivo*, MS-275 induces differentiation, transcription of growth factor b II receptor (TbRII), and inhibits the proliferation of pediatric solid tumors [19]. Sirtinol acts as a specific and direct inhibitor of the class III HDAC activity with no effect on human HDAC-1 [13]. Sirtinol

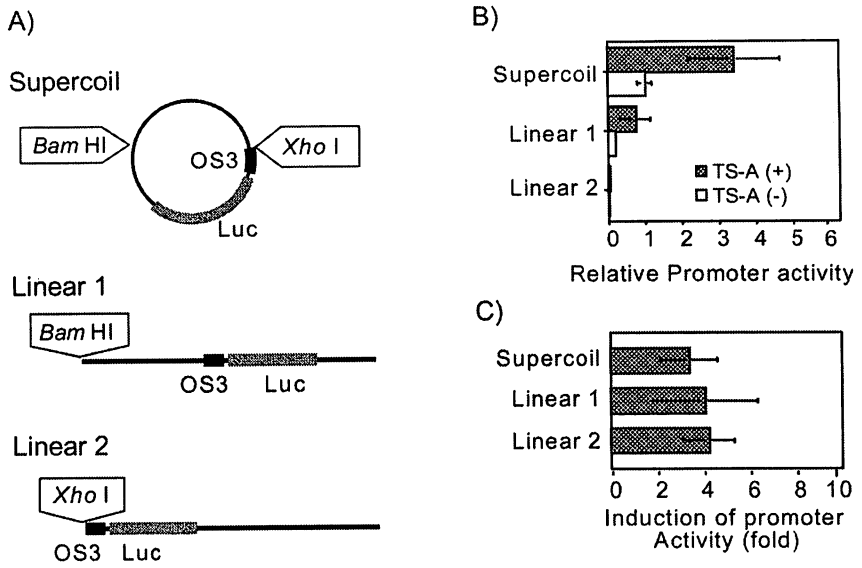

Fig. 4. Effect of DNA conformation on the promoter activity and the influence of HDAC inhibitor. A) The reporter plasmid carrying the *Osgep* promoter (OS3) was digested with *Bam* H1 or *Xho* I to linearize the DNA. Non-digested plasmid DNA (supercoil) and DNA digested with *Bam* H1 (linear 1) or with *Xho* I (linear 2) were transfected into NHI/3T3 cells, and then luciferase activity expressed from each DNA was measured. Closed boxes and gray boxes indicate the promoter sequence and luciferase reporter gene, respectively. B) The DNAs were transfcted to NIH/3T3 cells. Relative luciferase activity from each DNA conformation in the absence or presence of TS-A are shown in the bar graph. The activity of supercoiled DNA in the absence of TS-A was designated as 1. C) Induction of promoter activity by the addition of TS-A is shown in the case of each DNA conformation.

inhibits Sir2p transcriptional silencing activity *in vivo*, and NAD-dependent histone deacetylase activity of purified recombinant yeast Sir2p and human SIRT2 *in vitro* [20].

3.3. Measurement to quantify the effect of HDAC inhibitors on transcriptional activity

In order to investigate the effect of HDAC inhibitors on transcription from bidirectional promoters, we employed the luciferase assay. As test cell lines we used human HeLa cells derived from cervix carcinoma. We also used mouse cell lines BALB/3T3 (whole embryo), NIH/3T3 (whole embryo), Hepa1-6 (liver), 707.fl (spleen), LCC (lung) and FM3A (mammary) each of which is derived from a different mouse tissue. In the luciferase assay we inserted the promoter sequence to be tested into the multiple cloning sites of a reporter plasmid pGV-B and quantified the transcription of the promoter using luciferase activity (Fig. 3). Although this method is an artificial experimental system, it is possible to analyze the structure of important *cis*-elements in expression regulation by using point mutation or deletion analysis. Because transfected DNA also forms chromatin structure in the nucleus of the recipient cells, this method can be used to research the change in chromatin structure with an HDAC inhibitor addition. In a general experimental system we cotransfect another reporter plasmid ligated to the cytomegalovirus (CMV) promoter in order to normalize the cell number and transfection efficiency used for the assay. However in preliminary experiments we realized that the CMV promoter is also influenced by TS-A, an HDAC inhibitor. So in this present experiment we normalized luciferase activity by the protein amount of transfected cell extracts. And in each experiment we transfected to 3 wells of the 24-well plate in which cells were simultaneously grown and repeated the procedure 2 to 3 times with corresponding statistical treatment.

3.4. Effect of DNA conformation on transcriptional activity

We investigated the effect of DNA conformation

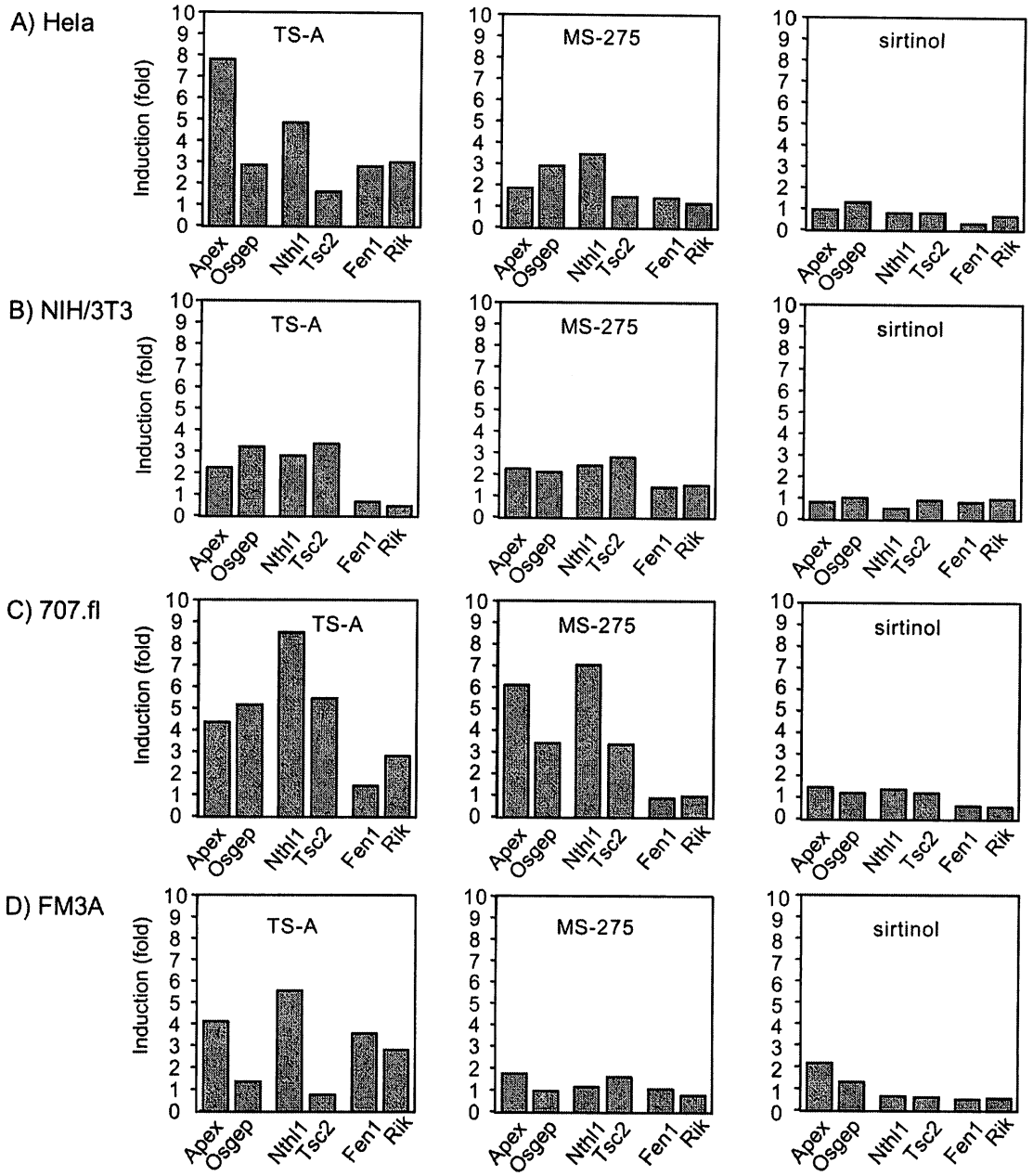

Fig. 5. Effect of HDAC inhibitors on promoter activity of bidirectional gene pairs in various cell lines. Promoter activity of the bidirectional gene pairs in the presence or absence of HDAC inhibitors (TS-A, MS-275, and sirtinol) were measured in HeLa (A), NIH/3T3 (B), 707.fl (C), and FM3A (D) cells. Induction of promoter activity by the addition of HDAC inhibitor is shown in each cell line.

and TS-A on transcriptional regulation. First the promoter region OS3 which drives the transcription of the *Osgep* gene was inserted upstream of the pGV-B luciferase gene (Fig. 3). We constructed supercoil and linear forms of DNA of the OS3 plasmid (Fig. 4A). We further constructed two linearforms with different upstream region distances from the promoter. Linear form 1 has a 2,705 bp region upstream of the promoter region OS3 constructed by digestion of the supercoiled DNA with *Bam* HI. So this plasmid contains a region about 4 kb upstream of the *Osgep* transcription initiation site. Linear form 2 was constructed by digestion of the *Xho* I site at the 5' terminal of the OS3 promoter,

so the DNA contains a region about 1.3 kb upstream of the *Osgep* transcription initiation site. Thereafter the transcriptional activity of each construct was compared in the presence or absence of the HDAC inhibitor. In NIH/3T3 cells the supercoiled form of DNA demonstrated extremely high luciferase activity compared to the linear form of DNA (Fig. 4B). It was concluded that a construct with a longer upstream region has a better transcription efficiency. However, constructs having both supercoil and linear forms were activated to the same extent by TS-A (Fig. 4C). This implies that three plasmid DNAs with different conformations contain a common sequence that interacts with the HDAC complex. Following the conformation of the chromatin structure in the nucleus, the HDAC inhibitor influenced these different DNAs equally, and transcription was subsequently activated. On the basis of this result we decided to use the supercoil form of DNA which produced the highest luciferase activity.

3.5. Activation of bidirectional promoter by HDAC inhibitors

We transfected each luciferase plasmid DNA, which contains a bidirectional promoter (shown in Fig. 3), to 6 kinds of mouse cell lines and 1 kind of human cell line and measured the extent of the induction of luciferase activity after the addition of HDAC inhibitors. The addition of HDAC inhibitors activated the transcriptional activity of most of the bidirectional promoters, but did not influence others (Fig. 5). In some cases activation levels were less than 1-fold, implying that HDAC inhibitors negatively control the transcription. Taking error into consideration genes which were activated more than 3-fold are further discussed. Transcriptional activity of the promoters was induced most frequently by TS-A, then by MS-275. However as far as we could ascertain no promoters were activated by sirtinol. Promoter activity of the HeLa and 707.fl cells were most activated by HDAC inhibitors (7- to 9-fold at most). NIH/3T3 and FM3A cells showed a relatively small response (about 3- to 6-fold). BALB/3T3, LLC and Hepa1-6 cells were almost not influenced (data not shown).

When examining the transcription activation by TS-A we compared the influence of TS-A on each representative gene pair. Concerning *Apex/Osgep* both genes were activated 2- to 3-fold in NIH3T3 cells and 4- to 5-fold in 707.fl cells. In HeLa cells, although *Apex* was activated about 8-fold *Osgep* was activated only about 2- to 3-fold. In FM3A cells *Apex* was activated 4-fold but *Osgep* was not influenced at all. Therefore it is possible that each gene transcribed by bidirectional promoters is regulated differently depending on the cell type. As for *Nthl1/Tsc2*, both genes were activated to almost the same extent (2- to 3-fold) in NIH/3T3 cells by TS-A. In HeLa cells *Nthl1* was influenced about 5-fold whereas no influence by the direction of *Tsc2* was observed. And in 707.fl cells *Nthl1* was activated about 9-fold and *Tsc2* about 5-fold. In FM3A cells *Nthl1* was activated about 6-fold but *Tsc2* was not influenced at all. Both genes of *Fen1/1810006K21Rik* in HeLa and FM3A cells were activated 3- to 4-fold by TS-A. The MS-275 transcription of *Apex* was activated about 6-fold in 707.fl cells. On the other hand about a 3-fold activation was observed in *Osgep*. In HeLa and NIH/3T3 cells both genes were activated about 2- to 3-fold by MS-275. Almost the same result was obtained with the *Nthl1/Tsc2* gene pair. *Fen1/1810006K21Rik* did not respond to MS-275 in any cells.

In summary, we demonstrated that chromatin structure influence the transcriptional regulation of a bidirectional promoter. And in every gene pair investigated promoters were regulated differently depending on the cell type. This indicates that HDAC molecules which were relevant to repression of gene expression also behaved differently.

References

1. Adachi, N., and Lieber, M.R. (2002) Bidirectional gene organization: a common architectural feature of the human genome. *Cell* **109**, 807-809.
2. Trinklein, N.D., Aldred, S.F., Hartman, S.J., Schroeder, D.I., Otillar, R.P., and Myers, R.M. (2004) An abundance of bidirectional promoters in the human genome. *Genome Res.* **14**, 62-66.
3. Ikeda. S., Mochizuki, A., Sarker, A.H., and Seki, S. (2000) Identification of functional elements in the bidirectional promoter of the mouse *Nthl1* and *Tsc2* genes. *Biochem. Biophys. Res. Comm.* **273**, 1063-1068.
4. Seki, Y., Ikeda, S., Kiyohara, H., Ayabe, H., Seki, T., and Matsui, H. (2002) Sequencing analysis of a putative human *O*-sialoglycoprotein endopeputidase gene (*OSGEP*) and analysis of a bidirectional promoter between the *OSGEP* and *APEX* genes. *Gene* **285**, 101-108.
5. Ikeda, S., Ayabe, H., Mori, K., Seki, Y., and Seki, S. (2002) Identification of the functional elements in the bidirectional

promoter of the mouse *O*-sialoglycoprotein endopeptidase (m*Osgep*) and APEX nuclease (m*Apex*) genes. *Biochem. Biophys. Res. Comm.* **296**, 785-791.

6. Emoto, M., Miki, M., Sarker, A.H., Nakamura, T., Seki, Y., Seki, S., and Ikeda, S. (2005) Structure and transcription promoter activity of mouse flap endonuclease 1 gene: alternative splicing and bidirectional promoter. *Gene* **357**, 47-54.
7. Ikeda, S., and Seki, S. (2001) Base excision repair: DNA glycosylase and AP endonuclease. *Tanpakushitsu Kakusan Koso* **46**, 916-923 (in Japanese).
8. Ikeda, S., Ayabe, H., Seki, Y., and Seki, S. (2003) Gene structure and transcription regulation of mammalian *O*-sialoglycoprotein endopeptidase gene. *Recent Res. Devel. Biophys. Biochem.* 3, 791-799.
9. Khochbin, S., Verdel, A., Lemercier, C., and Seigneurin-Berny, D. (2001) Functional significance of histone deacetylase diversity. *Curr. Opin. Genet. Dev.* **11**, 162-166.
10. Grozinger, C.M., Hassig, C.A., and Schreiber, S.L. (1999) Three proteins define a class of human histone deacetylases related to yeast Hda1p. *Proc. Natl. Acad. Sci. USA* **96**, 4868-4873.
11. Fischle, W., Emiliani, S., Hendzel, M.J., Nagase, T., Nomura, N., Voelter, W., Verdin, E. (1999) A new family of human histone deacetylases related to *Saccharomyces cerevisiae* HDA1p. *J. Biol. Chem.* **274**, 11713-11720.
12. Fischle, W., Kiermer, V., Dequiedt, F., and Verdin, E. (2001) The emerging role of class II histone deacetylases. *Biochem. Cell Biol.* **79**, 337-348.
13. Grozinger, C.M., Chao, E.D., Blackwell, H.E., Moazed, D., and Schreiber, S.L. (2001) Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening. *J. Biol. Chem.* **276**, 38837-38843.
14. Tsuji, N., Kobayashi, M., Nagashima, K., Wakisaka, Y., and Koizumi, K. (1976) A new antifungal antibiotic, trichostatin. *J. Antibiot.* **29**, 1-6.
15. Yoshida, M., Kijima, M., Akita, M., and Beppu, T. (1990) Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by trichostatin A. *J. Biol. Chem.* **265**, 17174-17179.
16. Hoshikawa, Y., Kwon, H.J., Yoshida, M., Horinouchi, S., and Beppu, T. (1994) Trichostatin A induces morphological changes and gelsolin expression by inhibiting histone deacetylase in human carcinoma cell lines. *Exp. Cell Res.* **214**, 189-197.
17. Futamura, M., Monden, Y., Okabe, T., Fujita-Yoshigaki, J., Yokoyama, S., and Nishimura, S. (1995) Trichostatin A inhibits both *ras*-induced neurite outgrowth of PC12 cells and morphological transformation of NIH3T3 cells. *Oncogene* **10**, 1119-1123.
18. Saito, A., Yamashita, T., Mariko, Y., Nosaka, Y., Tsuchiya, K., Ando, T., Suzuki, T., Tsuruo, T., and Nakanishi, O. (1999) A synthetic inhibitor of histone deacetylase, MS-27-275, with marked *in vivo* antitumor activity against human tumors. *Proc. Natl. Acad. Sci. USA* **96**, 4592-4597.
19. Jaboin, J., Wild, J., Hamidi, H., Khanna, C., Kim, C.J., Robey, R., Bates, S.E., and Thiele, C.J. (2002) MS-27-275, an inhibitor of histone deacetylase, has marked *in vitro* and *in vivo* antitumor activity against pediatric solid tumors. *Cancer Res.* **62**, 6108-6115.
20. Denu, J.M. (2005) The *Sir* 2 family of protein deacetylases. *Curr. Opin. Chem. Biol.* **9**, 431-440.