ACADEMIC PRESS

Biochemical and Biophysical Research Communications 294 (2002) 63–70

BBRC

www.academicpress.com

# Induction of murine H-rev107 gene expression by growth arrest and histone acetylation: involvement of an Sp1/Sp3-binding GC-box☆

Karim Roder, Kee-Hong Kim, and Hei Sook Sul*

*Department of Nutritional Sciences and Toxicology, University of California, Berkeley, CA 94720, USA*

Received 17 April 2002

**Abstract**

H-rev107 is downregulated in many carcinomas and tumor cell lines. Using postconfluent NIH3T3 cells, we demonstrated that growth arrest caused by contact inhibition, but not serum deprivation, increased H-rev107 expression. Furthermore, histone deacetylase inhibitors induced H-rev107 expression in NIH3T3 cells and allowed its reexpression in H-rev107-deficient WEHI 7.1 lymphoma cells. In contrast, no effect of the postconfluent stage or histone deacetylase inhibitors on H-rev107 levels was observed in tumorigenic H-rev107-expressing cell lines, HepG2, HeLa, and SKBR3. Transfections showed that TSA treatment increased luciferase activity 20-fold in NIH3T3 cells. We found that the GC-box at −83/−75 is a key element for H-rev107 induction by TSA and growth arrest, although there were no changes in the pattern and intensity of Sp1/Sp3-binding after induction. These data suggest that contact inhibition of growth and growth arrest caused by histone deacetylase inhibitors probably use the same mechanism to stimulate H-rev107 expression via histone acetylation in NIH3T3 cells and this might contribute to the development of drugs that can induce H-rev107 expression in certain tumors. 

*Keywords:* H-rev107; Promoter; Histone acetylation; Sp1/Sp3; TSA

The class II tumor suppressor H-rev107 is a ubiquitously expressed gene encoding a 16 kDa protein localized in both the cytoplasm and cell membrane [1]. Recently, it became evident that H-rev107 is the member of a novel family of proteins involved in the control of cell proliferation. This protein family includes A-C1, predominantly expressed in skeletal muscle and TIG3/RIG1/hH-Rev107-2, a retinoid-induced gene in keratinocytes [2–5]. H-rev107 has been cloned as a gene highly expressed in a revertant cell line isolated from H-ras transformed rat fibroblasts [1,6]. While H-rev107 overexpression in H-Ras-expressing cell lines resulted in reduction of colony formation and attenuated tumor growth in nude mice implying its tumor-suppressing activity [7], Siegrist et al. [8] demonstrated a downregulation of H-rev107 expression in testicular germ cell tumors. Its anti-proliferative activity was also indicated by the fact that cells overexpressing H-rev107 could not be maintained in culture for an extended period of time [1,7]. Similarly, the H-rev107-related proteins, A-C1 and TIG3, significantly inhibited cell proliferation of H-ras-transformed NIH3T3 and 293 cells, respectively [2,3]. Therefore, we set out to examine the molecular mechanism of H-rev107 induction by growth arrest. In this study, we demonstrate that transcriptional activity of H-rev107 is induced in NIH3T3 fibroblasts by contact inhibition or by treatment with histone deacetylase inhibitors. In both cases, we show an involvement of the promoter-proximal Sp1/Sp3-binding GC-box, which implicates that contact inhibition and growth arrest caused by HDAC inhibitors use the same mechanism in stimulating H-rev107 expression. Furthermore, we also find that HDACs are involved in the repression of H-rev107 expression in H-rev107-deficient WEHI 7.1 lymphoma cells.

## Materials and methods

*RNA isolation and reverse transcription-PCR.* RNA samples were reverse transcribed using Superscript II (Invitrogen) and oligo(dT).

☆ *Abbreviations:* H-rev107, H-ras revertant protein 107.

* Corresponding author. Fax: +1-510-642-0535.
*E-mail address:* hsul@nature.berkeley.edu (H. Sook Sul).

0006-291X/02/$ - see front matter 
PII: S0006-291X(02)00440-0

PCR conditions were 94 °C for 2 min, then 23–27 cycles (murine H-rev107: 5′-GGTTGGAGAGTTTTTTTCTGGGAC-3′, 5′-TCCGAGGAACTCCATAGCGTAG-3′; human H-rev107: 5′-GGAGACCTGATTGAGATTTTTCGC-3′, 5′-CATTCACAAAGTGCTCGCAGTTC -3′; β-actin: 5′-TCCTATGTGGGTGACGAGGC-3′, 5′-CATGGCTGGGGTGTTGAAGG-3′; murine p21: 5′-AGATCCACAGCGATATCCAGAC-3′, 5′-ACACACAGAGTGAGGGCTAAGG-3′) of 94 °C for 60 s, 55 °C for 60 s, 72 °C for 60 s, and finally 4 min at 72 °C. The products were resolved on a 1.5% agarose gel. Generally, two different preparations of RNA were used for RT-PCR.

*Plasmid constructs.* The construction of H-rev107-luciferase reporter constructs used in this study has been described previously [9]. The cDNA of Sp1 was released by an *Xho*I digest from pPacSp1 [10] and cloned into pcDNA3.1/HisC (Invitrogen) to construct pcDNA3.1-His-Sp1. Similarly, pcDNA3.1-His-DNSp1 expressing an Sp1 construct in which the transactivation domain has been deleted was created by releasing the cDNA coding for the DNA binding domain of Sp1 (amino acids 592–778) by a *Bam*HI/*Xho*I digest from pPacSp1 and cloning into pcDNA3.1/HisB.

*Cell culture.* Murine WEHI 7.1 lymphoma cells [11], NIH3T3 fibroblasts as well as human hepatoma HepG2 cells and cervix carcinoma HeLa cells were cultured in DMEM supplemented with 10% fetal calf serum (FCS). Human SKBR3 adenocarcinoma cells were grown in Mac Coy's 5a medium supplemented with 10% fetal calf serum. Cells were treated with TSA (10–200 ng/ml), ethanol (control), or butyrate (5 mM) for 12–24 h. For serum deprivation experiments, cells were incubated overnight in complete medium, then washed twice in phosphate-buffered saline and subsequently cultured in DMEM supplemented with 0.5% FCS for 24 h.

*Transient and stable transfections.* Transient transfections were exactly performed as described previously [9]. TSA and butyrate treatments were started 24 h after transfection and continued for 24 h. For stable transfections, exponentially growing NIH3T3 cells were transfected with 12 μg of pGL3-H-rev107(−2106/+30) or pGL3-H-rev107(−2106/+30)-GC-box-mut and 1 μg of pcDNA6/V5-His C (Invitrogen) conferring resistance to blasticidin. After 48 h, cells were split at a ratio of 1:10 and stable transfectants were selected in 10 μg/ml blasticidin S (Invitrogen) for one week. Resultant colonies were pooled for further analysis to compensate for positional effects on promoter activity. Luciferase activities were analyzed and normalized to protein concentration.

*Electrophoretic mobility shift assay (EMSA).* EMSA using nuclear extracts from NIH3T3 was exactly performed as described previously [9]. The following double stranded oligonucleotide containing *Xba*I sites at its ends was used as probe: GC-box$_{H-rev107}$ (5′-CTAGCGAGGAGGGGCGGGGTGATCG-3′, 3′-GCTCCTCCCCGCCCCACTAGCGATC-5′) [9].

*Fixation of cells with glutaraldehyde.* WEHI 7.1 cells were fixed with glutaraldehyde as described previously [12]. Cells were resuspended in DMEM/10% FCS and added to sparsely seeded NIH3T3 cells.

## Results and discussion

### *Increase in H-rev107 mRNA levels by contact inhibition of NIH3T3 cells*

Our observation that overexpression of murine H-rev107 significantly inhibited growth of NIH3T3 cells (K.-H.K., unpublished data) is in agreement with previous reports on growth inhibition by overexpression of rat H-rev107 and the related murine AC-1 in different cell lines [1,2,7]. Therefore, we set out to examine H-rev107 mRNA levels in proliferating and postconfluent, growth arrested fibroblasts. After seeding NIH3T3 cells onto plates, confluence was generally reached on day 4. The H-rev107 mRNA level as determined by RT-PCR was increased several-fold in the postconfluent stage

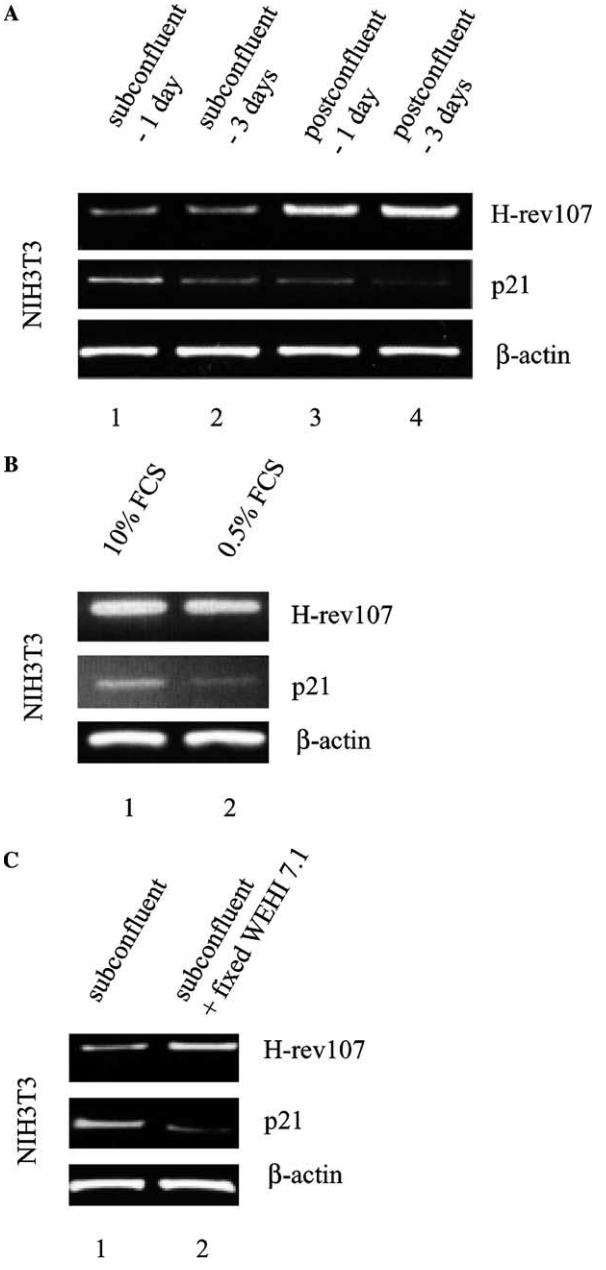


Fig. 1. Growth arrest induces H-rev107 expression. (A) Effect of cellular confluence on expression of H-rev107 in NIH3T3 cells. Cells were grown for 7 days and RNA was prepared at the indicated time points. The mRNA levels for H-rev107, p21, and β-actin as control were examined by RT-PCR. (B) Effect of serum deprivation on expression of H-rev107. Proliferating cells were incubated for 24 h in DMEM containing 0.5% or 10% FCS and their expression of H-rev107 analyzed. (C) Effect of contact inhibition induced by glutaraldehyde-fixed cells on H-rev107 mRNA levels. Contact inhibition was induced by the addition of a 10-fold excess of glutaraldehyde-fixed WEHI 7.1 cells to sparsely seeded NIH3T3 fibroblasts. Cells were incubated for 48 h and mRNA levels detected by RT-PCR.

(Fig. 1A), while the level of β-actin mRNA did not change after growth arrest. On the other hand, p21 mRNA levels dropped significantly after reaching confluency, as reported previously [13]. This suggests that p21 cannot be responsible for induction of growth arrest in murine fibroblasts. Both cell–cell contact and growth factor withdrawal can induce growth arrest of many cell types by inducing a $G_0$ state ([14] and references therein). To investigate the influence of deprivation of nutrients and growth factors on H-rev107 expression, we serum-starved subconfluent NIH3T3 cells for 24 h. Growth factor deprivation, however, did not have any significant effect on the H-rev107 mRNA level (Fig. 1B). Wiesler and colleagues [12,15] have previously shown that imitating cell–cell contacts by the addition of glutaraldehyde-fixed cells mimicks the physiological situation occurring in confluent cultures of human fibroblasts leading to arrest in late $G_1$-phase. Using the same approach, we cultured sparsely seeded NIH3T3 fibroblasts in 10% FCS and induced contact inhibition and resulting growth arrest by the simultaneous addition of fixed H-rev107-deficient lymphoma cells. H-rev107 mRNA levels were increased several-fold in proliferation-inhibited NIH3T3 cells (Fig. 1C). This implicates that contact-dependent growth inhibition is indeed the cause for elevated H-rev107 mRNA levels, which is in agreement with a previous study that showed elevated H-rev107 protein levels in growth arrested 208F rat fibroblasts [1].

*Increase in H-rev107 transcription by TSA treatment of NIH3T3 cells*

Histone deacetylase inhibitors generally cause arrest of the cell cycle, suggesting that histone (de)acetylation is involved in cell cycle control [16]. For that purpose, we studied the effect of TSA, a specific inhibitor of histone deacetylases, on H-rev107 expression in NIH3T3 cells. RT-PCR demonstrated significantly increased levels of H-rev107 mRNA in cells treated with 30 or 300 ng/ml TSA for 12–24 h (Fig. 2A). Increased mRNA levels were detectable as early as 3 h and up to 48 h after TSA treatment (data not shown). Furthermore, treatment of NIH3T3 cells with sodium butyrate, another growth arrest inducer and histone deacetylase inhibitor, caused increased H-rev107 mRNA levels (Fig. 2B) underscoring the importance of histone acetylation in H-rev107 and indicating that the elevation of H-rev107 expression by growth arrest is probably caused by histone acetylation. To investigate whether the increase of H-rev107 mRNA by TSA was due to an increase in the rate of H-rev107 mRNA synthesis or an enhancement of its stability, the transcriptional inhibitor actinomycin D (Act D) was used. Pretreatment with 4 μM ActD for 30 min completely abolished TSA-dependent induction of both H-rev107 expression (Fig. 2C), thus indicating that induction of both genes by TSA is dependent on transcriptional activity. We also tested whether H-rev107 was induced by an immediate drug response mechanism. As shown in Fig. 2D, the induction of H-rev107 was not affected by the simultaneous treatment with cycloheximide, indicating that de novo protein synthesis was not necessary for H-rev107 expression induced by TSA.

*Effect of cell density and TSA treatment on H-rev107 mRNA levels in tumor cells*

Since transformed cells overcome contact inhibition, which is a prerequisite for the neoplastic phenotype, H-rev107 expression in tumor cells should not be induced by the postconfluent stage or by treatment with histone deacetylase inhibitors. To prove our hypothesis, we examined the effects of cell density and histone deacetylase inhibitors on H-rev107 expression employing several tumor cell lines where H-rev107 expression is very low. As expected, we could not see any increase in H-rev107 mRNA levels in several tumorigenic cell lines, HeLa, HepG2, and SKBR3 grown to the postconfluent stage (Fig. 3A). While TSA and butyrate treatment caused H-rev107 reexpression in H-rev107-deficient WEHI 7.1 lymphoma cells (Fig. 3B), TSA/butyrate had no effect on mRNA levels in HeLa, HepG2, and SKBR3 cells (Fig. 3B and data not shown). Interestingly, we could not see any effect of cell density and HDAC inhibitor treatment on H-rev107 expression in non-transformed rat REF-52 fibroblasts. However, this H-ras resistant cell line has already high mRNA levels of endogenous H-rev107 at the subconfluent stage [1]. The data obtained with WEHI 7.1 cells implicate inactive chromatin mediated by histone deacetylation as a critical component of H-rev107 gene silencing in certain tumors and tumor cell lines, and this scenario might provide a target for drugs inducing H-rev107 expression and thereby inhibiting cell growth.

*TSA and growth arrest stimulate the H-rev107 promoter via its proximal GC-box*

To define what region of the H-rev107 promoter is responsive to histone acetylation, we used transient transfection and luciferase assays with a series of 5′-promoter deletions (Fig. 4A). Following a 24 h exposure of NIH3T3 cells to TSA or vehicle, the luciferase activities of the 5′-deletions, −7631, −2106, −516, and −113, were increased approximately 20-fold when compared with the untreated controls. We also observed that TSA-induction of H-rev107 expression and promoter activity is cell density-dependent with high inductions found only in fibroblasts cultured at low cell densities (data not shown). A possible explanation for this finding could be that certain HDACs that are involved in the inhibition of H-rev107 expression are

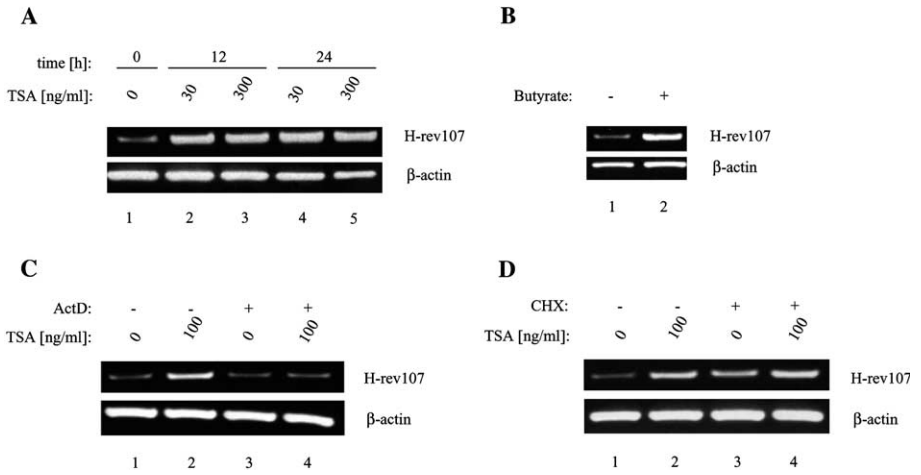


Fig. 2. TSA activates transcription of H-rev107. (A) Dose response and time-course for H-rev107 expression induced by TSA. NIH3T3 cells were treated with 30 or 300 ng/ml TSA for 12 and 24 h, and mRNA levels for H-rev107 and β-actin as control were examined by RT-PCR. (B) Effect of butyrate on H-rev107 mRNA levels in NIH3T3 cells. Cells were treated with 5 mM butyrate for 24 h, and mRNA levels for H-rev107 and β-actin as control were examined by RT-PCR. Effect of actinomycin D (C) and cycloheximide (D) on H-rev107 induction by TSA. NIH3T3 cells were pretreated with 4 μM ActD or 10 μg/ml CHX for 30 or 60 min, respectively. As control, cells were pretreated with vehicle only. TSA treatment followed for 12 h.

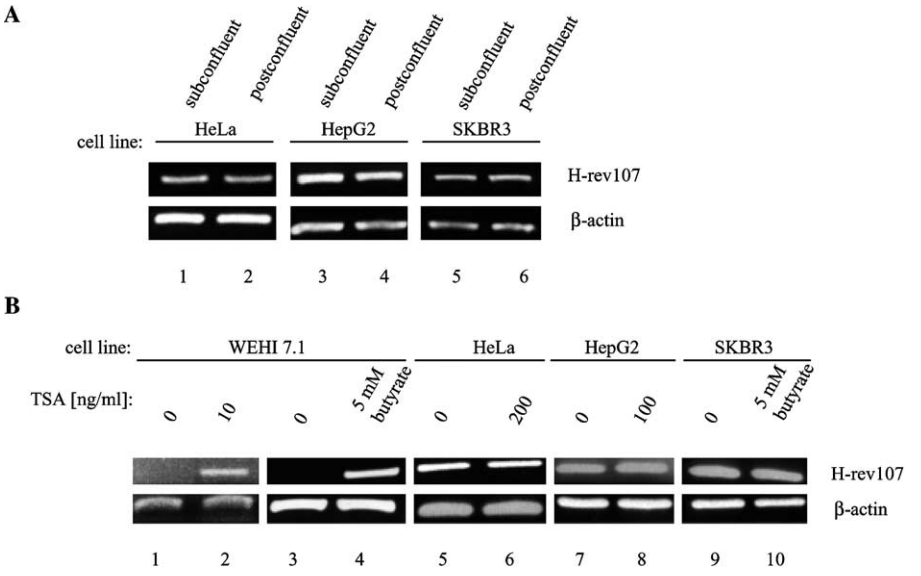


Fig. 3. Effect of cell density and TSA/butyrate on H-rev107 mRNA levels in different tumor cell lines. (A) No effect of cellular confluence on expression of H-rev107 in tumor cells. H-rev107 and β-actin expression profiles from subconfluent and postconfluent cells as indicated were examined by RT-PCR. RT-PCR reactions have been separately performed for each cell line. (B) Effect of TSA or butyrate on H-rev107 and β-actin mRNA levels in miscellaneous cell lines. RT-PCR reactions have been separately performed for each cell line.

downregulated at higher cell densities. This is supported by a recent report on HDAC1 showing that trichostatin A and low cell density upregulated its expression [17]. A further deletion of 39 bp of promoter sequence markedly decreased TSA response indicating that the region from −113 to −74 of the H-rev107 gene, probably the GC-box at −83/−75, is required for activation by TSA. Indeed, a mutation of the GC-box decreased TSA-induction of H-rev107 promoter activity to ap- proximately 7-fold (pGL3-H-rev107(−2106/+30)-GC-box-mut) and 5-fold (pGL3-H-rev107(−113/+30)-GC-box-mut), respectively (Fig. 4B). Similar results have been obtained using sodium butyrate as inducer (Fig. 4B). Since the mutation of the proximal GC-box did not completely abolish TSA/butyrate induction, a minor role of other 3′-DNA-binding sites is imaginable. To clarify the functional relationship between TSA treatment and transcriptional activity in a genomic context, we established a pool of stably transfected NIH3T3 cells having integrated copies of pGL3-H-rev107(−2106/+30) and

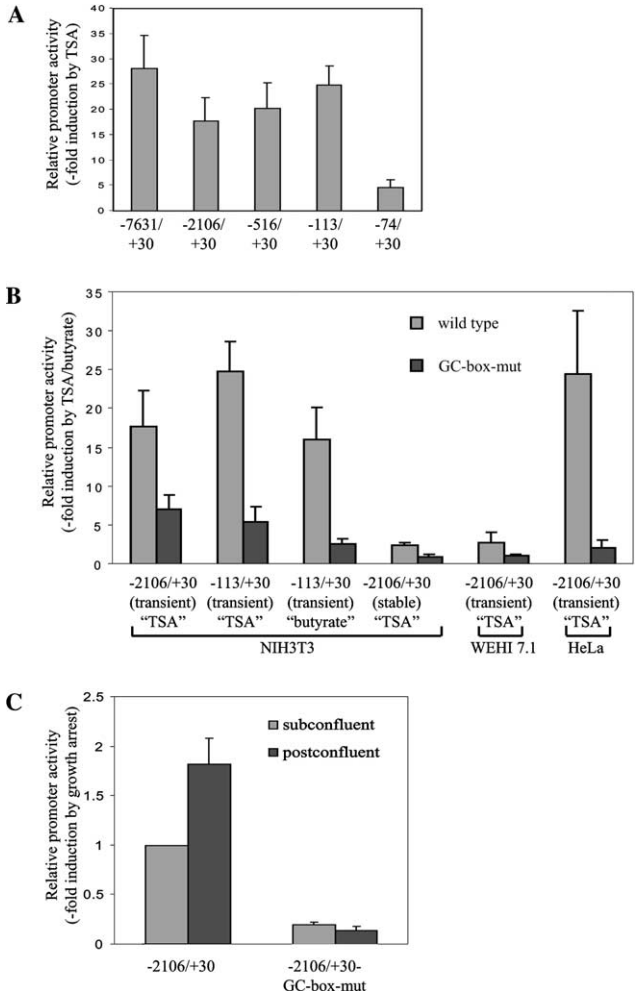


Fig. 4. TSA and growth arrest activate the H-rev107 promoter via the promoter-proximal GC-box. (A) TSA-induced promoter activity of the mouse H-rev107 gene in NIH3T3 cells. A series of H-rev107 promoter deletion constructs fused to the firefly luciferase reporter gene was transfected into NIH3T3. For each construct, the relative promoter activity refers to the ratio of normalized luciferase activity obtained with TSA relative to the untreated control. Values and their SD are the average of two to five independent experiments performed in duplicate. (B) TSA increases H-rev107 promoter activity in a variety of cell lines. NIH3T3 cells were transiently or stably transfected, while WEHI 7.1 and HeLa cells were transiently transfected, using pGL3-H-rev107(−2106/+30), pGL3-H-rev107(−113/+30), and their derivatives, pGL3-H-rev107(−2106/+30)-GC-box-mut and pGL3-H-rev107(−113/+30)-GC-box-mut, containing a mutation in the GC-box as the respective constructs. Pools of NIH3T3 clones and transiently transfected NIH3T3, WEHI 7.1, and HeLa cells were grown in the presence of TSA (NIH3T3: 100 ng/ml; WEHI 7.1: 10 ng/ml and HeLa: 200 ng/ml) or 5 μM butyrate for 24 h. (C) Growth arrest-induced H-rev107 promoter activity in NIH3T3 cells. NIH3T3 cells grown in 100 mm dishes were transiently transfected with pGL3-H-rev107 (−2106/+30) or pGL3-H-rev107(−2106/+30)-GC-box-mut at a confluency of 50%. Twenty-four hours later, confluent cells were plated into 6-well dishes at low (six 6-wells: approx. $6 \times 10^4$ cells/6-well) or high density (six 6-wells: approx. $7 \times 10^5$ cells/6-well) and grown for 48 h. The relative promoter activity of pGL3-H-rev107(−2106/+30)-transfected, subconfluent cells was arbitrarily set at 1.0. Values and their SD are the average of two independent experiments performed in triplicate. Luciferase values were normalized to protein concentration.

pGL3-H-rev107(−2106/+30)-GC-box-mut, respectively. Surprisingly, TSA treatment caused only a 2.5-fold induction of H-rev107 promoter activity (Fig. 4B) that is considerably lower than TSA inductions obtained with transiently transfected cells. It is tempting to speculate that a partly chromatinized plasmid structure might serve as a better target for HDACs than a genomic environment. As expected, the GC-box mutation completely abolished TSA-responsiveness of the H-rev107 promoter in stably transfected cells (Fig. 4B). We also performed transient transfections with wild type and GC-box-mutated promoter constructs using tumorigenic WEHI 7.1 and HeLa cells (Fig. 4B). While TSA induced promoter activity in WEHI 7.1 cells is 2.9-fold, its effect was almost completely eliminated by the GC-box mutation. Interestingly, the H-rev107 promoter was activated by TSA (Fig. 4B) and butyrate (data not shown) more than 20-fold in a GC-box-dependent manner in HeLa cells, although the endogenous gene was not upregulated by this treatment (Fig. 3B). This indicates the importance of a genomic context in comparison to a partly chromatinized plasmid.

We also tested whether the proximal GC-box in the H-rev107 promoter is essential for the growth arrest-dependent increase of H-rev107 promoter activity. For this purpose, transient transfections of pGL3-H-rev107(−2106/+30) and pGL3-H-rev107(−2106/+30)-GC-box-mut into NIH3T3 cells were employed (Fig. 4C). The luciferase activity of the wild type construct was modestly stimulated in postconfluent cells when compared to that in sparse cultures suggesting that transcription is somehow involved in upregulating H-rev107 mRNA levels during growth arrest. The discrepancies between inductions of mRNA levels (Fig. 1A) and promoter activities (Fig. 4C) by growth arrest could be explained by a possible involvement of H-rev107 mRNA stability. Nevertheless, the GC-box mutation completely abolished the growth arrest-dependent positive effect on promoter activity, suggesting that the GC-box plays a role during growth arrest induction of H-rev107. This finding has been supported by data obtained from transient transfections employing a larger construct, pGL3-H-rev107(−7631/+30), and by stable transfections (data not shown), and implies that growth arrest and HDAC inhibitors probably activate H-rev107 expression via the same mechanism.

### *Sp1/Sp3-binding to the major TSA-responsive element*

Recently, we have demonstrated the binding of Sp1 and Sp3 to the GC-box at −83/−75 [9]. To see whether TSA treatment has an effect on in vitro binding of Sp1 and Sp3 to the H-rev107 GC-box, EMSAs were performed using nuclear extracts from subconfluent mock- and TSA-treated NIH3T3 cells and an oligonucleotide encompassing the promoter region between −88

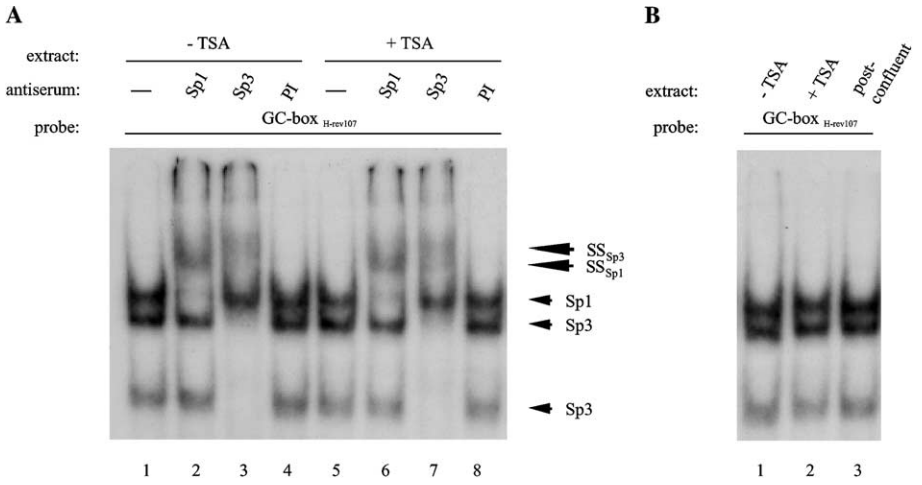


Fig. 5. In vitro analysis of the GC-box by EMSA. (A) Sp1 and Sp3 binding to the putative TSA-responsive element. EMSA was performed using nuclear extracts from control (−TSA) and TSA-treated NIH3T3 cells (+TSA) and labeled GC-box$_{H-rev107}$ oligonucleotide. Cells were treated with 100 ng/ml TSA for 24 h. For supershift analysis, antisera against Sp1 (lanes 2 and 6), Sp3 (lanes 3 and 7), or rabbit preimmune serum (PI; lanes 4 and 8) were included. Sp1 and Sp3 indicate DNA/protein complexes, while $SS_{Sp1}$, and $SS_{Sp3}$ show supershifted complexes. (B) The pattern and intensity of Sp1 and Sp3 binding is not changed during growth arrest. EMSA was performed using nuclear extracts from untreated (−TSA), TSA-treated (+TSA) subconfluent, and postconfluent NIH3T3 cells.

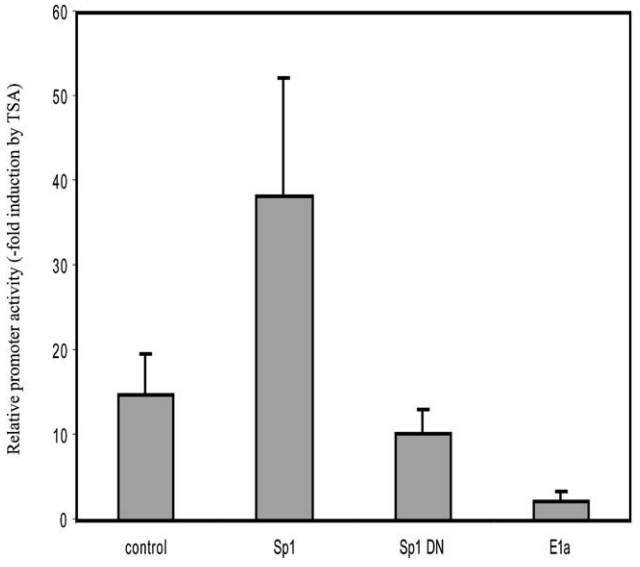


Fig. 6. Effect of Sp1 and E1a on TSA-induced H-rev107 promoter activity. NIH3T3 cells grown in 6-wells were transiently transfected with 1 μg pGL3-H-rev107(−113/+30) and 5 μg pcDNA3.1/HisC (control), pcDNA3.1-His-Sp1, pcDNA3.1-His-DNSp1, pBJ9ω (control), and pBJ9ω-E1A12S. Luciferase activity was measured following incubation for 24 h with or without 100 ng/ml TSA and normalized to protein concentration. The relative promoter activity refers to the ratio of normalized luciferase activity obtained with TSA and the co-transfected expression plasmid relative to the untreated control with the co-transfected expression plasmid.

and −70, GC-box$_{H-rev107}$ (Fig. 5A). However, no differences in the pattern and intensity of binding could be observed (cf. lanes 1 and 5), comparing the two extracts. Supershift experiments demonstrated the binding of Sp1 (cf. lanes 2 and 6) and Sp3 (cf. lanes 3 and 7) as reported previously [9]. As shown in Fig. 5B, nuclear extract from growth-arrested, postconfluent NIH3T3 caused the same binding pattern as nuclear extract from subconfluent mock- or TSA-treated cells. Since no changes in binding pattern and intensities were observed, binding of coactivators to Sp1/Sp3 after TSA treatment could be responsible for the induction of H-rev107 expression. In the case of the human IGFBP-3 promoter, Walker et al. [18] identified a multiprotein complex containing DNA-bound Sp1 and Sp3 together with histone deacetylase-1 and the histone acetyltransferase, p300, using a DNA affinity precipitation assay. After butyrate induction of breast cancer cells, p300 accumulated in this multiprotein complex. Similarly, Park et al. [19] presented data suggesting that the activation of the TGF-β type II receptor promoter by HDAC inhibitors is due to the recruitment of P/CAF to NF-Y that interacts with an inverted CCAAT box. Alternatively, Sp1/Sp3 could repress transcription by tethering HDACs to the promoter. Such a scenario has been described for the thymidine kinase promoter [20]. However, to evaluate the importance of HDACs, CBP/p300, or other coactivators, e.g. P/CAF, for the TSA-dependent stimulation of H-rev107 expression, chromatin immunoprecipitation assays need to be performed in the future.

### *Functional role of Sp1 in the TSA-activation of the H-rev107 promoter*

We next examined the possible involvement of Sp1 in the TSA-dependent activation of H-rev107 promoter activity by co-transfecting expression vectors for Sp1 and a dominant negative form of Sp1 with the H-rev107 minimal promoter-luciferase fusion plasmid, pGL3-H-rev107(−113/+30) (Fig. 6). Not unexpectedly,

Sp1 enhanced TSA-induced H-rev107 promoter activity approximately 2-fold, while the dominant negative form without the transactivation domain inhibited TSA-induced promoter activity slightly (20%). Similarly, Sp1 and Sp3 have been shown to mediate the histone deacetylase inhibitor-dependent upregulation of p21 [21], hTERT [22], adenine nucleotide translocase 2 [23], and IGFBP-3 [18]. TSA activation appeared to be due to the involvement of a coactivator, since the expression of E1A, which binds to multiple regulatory proteins such as the p300/CBP coactivator and proteins belonging to the family of the retinoblastoma gene product [24], reduced the expected potentiation (Fig. 6). Recently, Xiao et al. [25] presented co-transfection data indicating the involvement of p300 in the upregulation of p21 transcription by TSA in HeLa cells. However, our studies did not reveal any effect of a co-transfected expression vector for a dominant negative mutant of p300 [26] on the TSA-dependent H-rev107 promoter activation in NIH3T3, though the basal H-rev107 promoter activity was significantly reduced (data not shown). Similar experiments performed in HeLa cells showed both suppression of TSA-induction and a decrease in basal H-rev107 promoter activity implying a cell type-specific involvement of p300 in the TSA-dependent induction of H-rev107 promoter activity (data not shown).

Overall, our results demonstrate a positive role for histone acetylation in upregulating the transcriptional activity of the murine H-rev107 gene in non-transformed fibroblasts with low levels of H-rev107 expression. The importance of histone acetylation for H-rev107 expression is also supported by the fact that HDAC inhibitors allow reexpression of H-rev107 in WEHI 7.1 lymphoma cells. This may contribute to the design of drugs that increases H-rev107 expression and thereby exerting an antiproliferative effect in certain tumors.

## Acknowledgments

The authors are grateful to Drs. G. Suske (University of Marburg, Germany) for pPacO, R. Tjian (UC Berkeley, CA) for pPacSp1, T. Kouzarides (Cambridge University, UK) for pBJ9ω and pBJ9ω-E1A12S, A. Giordano (Thomas Jefferson University, PA) for pGAL4p300 and pGAL4p300/1514-1922, B. Diaz (UC Berkeley, CA) for NIH3T3 cells, K. Luo and Q. Zhu (UC Berkeley, CA) for HeLa cells. This work was supported by the National Institute of Health Research Grant DK50828 to H.S.S.

## References

[1] A. Hajnal, R. Klemenz, R. Schäfer, Subtraction cloning of H-rev107, a gene specifically expressed in H-ras resistant fibroblasts, Oncogene 9 (1994) 479–490.

[2] H. Akiyama, Y. Hiraki, M. Noda, C. Shigeno, H. Ito, T. Nakamura, Molecular cloning and biological activity of a novel Ha-Ras suppressor gene predominantly expressed in skeletal muscle, heart, brain, and bone marrow by differential display using clonal mouse EC cells, ATDC5, J. Biol. Chem. 274 (1999) 32192–32197.

[3] D. DiSepio, C. Ghosn, R.L. Eckert, A. Deucher, N. Robinson, M. Duvic, R.A.S. Chandraratna, S. Nagpal, Identification and characterization of a retinoid-induced class II tumor suppressor/growth regulatory gene, Proc. Natl. Acad. Sci. USA 95 (1998) 14811–14815.

[4] S.-L. Huang, R.-Y. Shyu, M.-Y. Yeh, S.-Y. Jiang, Cloning and characterization of a novel retinoid-inducible gene 1 (RIG1) deriving from human gastric cancer cells, Mol. Cell. Endocrinol. 159 (2000) 15–24.

[5] K. Husmann, C. Sers, E. Fietze, A. Mincheva, P. Lichter, R. Schäfer, Transcriptional and translational downregulation of H-REV107, a class II tumor suppressor gene located on human chromosome 11q11-12, Oncogene 10 (1998) 1305–1312.

[6] R. Schäfer, J. Iyer, E. Iten, A.C. Nirkko, Partial reversion of the transformed phenotype in HRAS-transfected tumorigenic cells by transfer of a human gene, Proc. Natl. Acad. Sci. USA 85 (1988) 1590–1594.

[7] C. Sers, U. Emmenegger, K. Husmann, K. Bucher, A.C. Andres, R. Schäfer, Growth-inhibitory activity and downregulation of the class II tumor-suppressor gene H-rev107 in tumor cell lines and experimental tumors, J. Cell. Biol. 136 (1997) 935–944.

[8] S. Siegrist, C. Feral, M. Chami, B. Solhonne, M.G. Mattei, E. Rajpert-De Meyts, G. Guellaen, F. Bulle, hH-Rev107, a class II tumor suppressor gene, is expressed by post-meiotic testicular germ cells and CIS cells but not by human testicular germ cell tumors, Oncogene 20 (2001) 5155–5163.

[9] K. Roder, M.-J. Latasa, H.S. Sul, Murine H-rev107 gene encoding a class II tumor suppressor: gene organization and identification of an Sp1/Sp3-binding GC-box required for its transcription, Biochem. Biophys. Res. Commun. 293 (2002) 793–799.

[10] A.J. Courey, R. Tjian, Analysis of Sp1 in vivo reveals multiple transcriptional domains, including a novel glutamine-rich activation motif, Cell 55 (1988) 887–898.

[11] K. Roder, M.-J. Latasa, H.S. Sul, Silencing of the mouse H-rev107 gene encoding a class II tumor suppressor by CpG methylation, submitted.

[12] G. Gradl, D. Faust, F. Oesch, R. Wieser, Density-dependent regulation of cell growth by contactinhibin and the contactinhibin receptor, Curr. Biol. 5 (1995) 526–535.

[13] K. Yanagisawa, A. Kosaka, H. Iwahana, H. Nakanishi, S.-i. Tominaga, Opposite regulation of the expression of cyclin-dependent kinase inhibitors during contact inhibition, J. Biochem. (Tokyo) 125 (1999) 36–40.

[14] J.F. Modiano, M.G. Ritt, J. Wojcieszyn, R. Smith III, Growth arrest of melanoma cells is differentially regulated by contact inhibition and serum deprivation, DNA Cell Biol. 18 (1999) 357–367.

[15] F. Oesch, B. Janik-Schmitt, G. Ludewig, H. Glatt, R.J. Wieser, Glutaraldehyde-fixed transformed and non-transformed cells induce contact-dependent inhibition of growth in non-transformed C3H/10T1/2 mouse fibroblasts, but not in 3-methylcholanthrene-transformed cells, Eur. J. Cell Biol. 43 (1987) 403–407.

[16] P.A. Marks, V.M. Richon, R. Breslow, R.A. Rifkind, Histone deacetylase inhibitors as new cancer drugs, Curr. Opin. Oncol. 13 (2001) 477–483.

[17] F. Dangond, M. Henriksson, G. Zardo, P. Caiafa, T.J. Ekström, S.G. Gray, Differential expression of class I HDACs: roles of cell density and cell cycle, Int. J. Oncol. 19 (2001) 773–777.

[18] G.E. Walker, E.M. Wilson, D. Powell, Y. Oh, Butyrate, a histone deacetylase inhibitor, activates the human IGF binding protein-3 promoter in breast cancer cells: molecular mechanism involves an

Sp1/Sp3 multiprotein complex, Endocrinology 142 (2001) 3817–3827.

[19] S.H. Park, S.R. Lee, B.C. Kim, E.A. Cho, S.P. Patel, H.B. Kang, E.A. Sausville, O. Nakanishi, J.B. Trepel, B.I. Lee, S.J. Kim, Transcriptional regulation of the TGF-beta type II receptor gene by histone acetyltransferase and deacetylase is mediated by NF-Y in human breast cancer cells, J. Biol. Chem. 277 (2002) 5168–5174.

[20] A. Doetzlhofer, H. Rotheneder, G. Lagger, M. Koranda, V. Kurtev, G. Brosch, E. Wintersberger, C. Seiser, Histone deacetylase 1 can repress transcription by binding to Sp1, Mol. Cell. Biol. 19 (1999) 5504–5511.

[21] H. Xiao, T. Hasegawa, K. Isobe, Both Sp1 and Sp3 are responsible for $p21^{waf1}$ promoter activity induced by histone deacetylase inhibitor in NIH3T3 cells, J. Cell. Biochem. 73 (1999) 291–302.

[22] M. Takakura, S. Kyo, Y. Sowa, Z. Wang, N. Yatabe, Y. Maida, M. Tanaka, M. Inoue, Telomerase activation by histone deacetylase inhibitor in normal cells, Nucleic Acids Res. 29 (2001) 3006–3011.

[23] Z. Hodny, R. Li, P. Barath, B.D. Nelson, Sp1 and chromatin environment are important contributors to the formation of repressive chromatin structures on the transfected human adenine nucleotide translocase-2 promoter, Biochem. J. 346 (2000) 93–97.

[24] G. Condorelli, A. Giordano, Synergistic role of E1A-binding proteins and tissue-specific transcription factors in differentiation, J. Cell. Biochem. 67 (1997) 423–431.

[25] H. Xiao, T. Hasegawa, K. Isobe, p300 collaborates with Sp1 and Sp3 in $p21^{waf1/cip1}$ promoter activation induced by histone deacetylase inhibitor, J. Biol. Chem. 275 (2000) 1371–1376.

[26] M.L. Avantaggiati, V. Ogryzko, K. Gardner, A. Giordano, A.S. Levine, K. Kelly, Recruitment of p300/CBP in p53-dependent signal pathways, Cell 89 (1997) 1175–1184.