CMLS, Cell. Mol. Life Sci. 60 (2003) 1990–1997
1420-682X/03/091990-08
DOI 10.1007/s00018-003-3090-z

CMLS Cellular and Molecular Life Sciences

Research Article

Differential regulation of the Sir2 histone deacetylase gene family by inhibitors of class I and II histone deacetylases

S. Kyrylenko [a], O. Kyrylenko [a], T. Suuronen [a] and A. Salminen [b,*]

[a] Department of Neuroscience and Neurology, University of Kuopio, P.O. Box 1627, 70211 Kuopio (Finland)
[b] Department of Neurology, University Hospital of Kuopio, P.O. Box 1777, 70211 Kuopio (Finland),
e-mail: antero.salminen@uku.fi

Received 28 February 2003; received after revision 19 June 2003; accepted 24 June 2003

Abstract. The Sir2 histone deacetylase gene family consists of seven mammalian sirtuins (SIRTs) which are NAD-dependent histone/protein deacetylases. Sir2 proteins regulate, for instance, genome stability by chromatin silencing in yeast. In mammals, their function is still largely unknown. Due to the NAD^+ dependency, Sir2 might be the link between metabolic activity and histone/protein acetylation. Regulation of gene expression also seems to play an important role in Sir2 functions, since increasing the dosage of Sir2 genes increases genome stability in yeast and *Caenorhabditis elegans*. We observed that the modification of histone/protein acetylation status by several class I and II histone deacetylase (HDAC) inhibitors induces differential changes in gene expression profiles of seven SIRT mRNAs in cultured neuronal cells. SIRT2, SIRT4 and SIRT7 were upregulated, whereas SIRT1, SIRT5 and SIRT6 were downregulated by trichostatin A (TSA) and n-butyrate. The upregulation of SIRT mRNAs was inhibited by actinomycin D. Interestingly, the regulation of SIRT mRNAs was highly similar both in mouse Neuro-2a neuroblastoma cells and post-mitotic rat primary hippocampal and cerebellar granule neurons. Using a chromatin immunoprecipitation technique, we showed that the upregulation of SIRT2 expression with TSA is related to the hyperacetylation of DNA-bound histone H4 within the first 500 bp upstream of the transcription start site of the SIRT2 gene. Chemically different types of HDAC inhibitors, such as TSA, apicidin, SAHA, M344 and n-butyrate induced remarkably similar responses in SIRT1-7 mRNA expression patterns. Differential responses in SIRT mRNA expression profiles indicate that the expression of the Sir2 family of genes is selectively regulated and dependent on histone/protein acetylation status.

Key words. Histone acetylation; aging; epigenetics; hippocampus; trichostatin A; n-butyrate; SIRT; HDAC.

Mammalian histone deacetylases (HDACs) can be separated into three classes on the basis of their similarity to yeast histone deacetylases [1]. Class I and II HDACs are homologous to yeast Rpd3 and Hda1-like proteins, respectively, and can be inhibited by trichostatin A (TSA) and n-butyrate. The third class is homologous to the silent information regulator 2 (Sir2) protein family which contains NAD-dependent deacetylases insensitive to both TSA and n-butyrate [2–4]. Sir2 family members are conserved during evolution and in humans there are seven members of Sir2 proteins, named sirtuins SIRT1 to SIRT7 [5]. To date, there is no classification of various sirtuins in different species. Here we identify different mouse and rat sirtuin homologs according to the current classification of human sirtuin genes (SIRT1 to SIRT7 homologs).

Sir2 proteins mediate gene silencing of repeated DNA sequences at telomeres, ribosomal DNA and mating type

* Corresponding author.

loci [6]. Recent observations have shown that Sir2 proteins are HDACs which repress transcription by opposing the acetylation of lysines at histone H3 and H4 amino tails [2, 4]. Furthermore, Sir2 proteins also deacetylate non-histone proteins, such as p53 protein [7, 8] by SIRT1 and α-tubulin by SIRT2 [9]. Whether these activities are the only function of SIRT1 and SIRT2 is not currently known.

Several reports have recently verified that Sir2 proteins are NAD-dependent HDACs [4, 10–12]. Sir2 enzymes couple protein substrate deacetylation and beta-NAD^+ cleavage to produce 2′- and 3′-O-acetyl-ADP-ribose [13], a novel product whose function is unknown. NADH and NADP do not activate Sir2 deacetylases. This property of the Sir2 type of HDACs provides an interesting link between metabolism and chromatin silencing. Guarente [3] has proposed that Sir2 might be the link between metabolic activity, genomic stability and, finally, aging.

We have examined whether a variety of signaling pathways and metabolic conditions regulate the expression of mammalian SIRT mRNAs in neuronal cells. Interestingly, we observed that the modification of histone/protein acetylation status by two well-known class I and II HDAC inhibitors, TSA and n-butyrate [14, 15], induced strong, diverse responses in the expression levels of seven SIRT mRNAs. SIRT2, SIRT4 and SIRT7 were upregulated, whereas SIRT1, SIRT5 and SIRT6 were downregulated. The upregulation of mRNA expression was inhibited by actinomycin D. Interestingly, the differential regulation of SIRT mRNAs was highly similar in cancerous neuroblastoma cells and post-mitotic primary neurons. Furthermore, chemically different HDAC inhibitors, such as TSA, apicidin, SAHA, M344 and n-butyrate, induced similar responses in SIRT1–7 mRNA expression profiles. The chromatin immunoprecipitation assay followed by semi-quantitative PCR showed that the human SIRT2 promoter contains elements responsive to the inhibitors of class I and II HDACs. We thus postulate that sirtuin expression may be regulated directly by HDACs. These observations suggest that the expression of the Sir2 family of genes can be selectively regulated and possibly modulatable by drugs.

Materials and methods

Riboprobes

Gene-specific fragments were generated by PCR, cloned into pGEM-T Easy vector (Promega) and verified by sequencing. The probes used in this study correspond to nucleotides 454–1094 on the sequences with GenBank accession number NM_019812.1 for SIRT1, 149–667 on NM_022432.1 for SIRT2, rat sequence homologous to nucleotides 379–905 on NM_022433 for SIRT3, 186–746 on NM_012240.1 for SIRT4, 681–1359 on NM_012241.1 for SIRT5, 256–748 on AK013316.1 for SIRT6, 438–856 on XM_126741.1 for SIRT7 and 963–1108 on M11188 for 18S rRNA. Templates for in vitro transcription were prepared by PCR on recombinant plasmids with gene-specific sense primers and modified M13/pUC universal (5′-ggttttcccagtcacgacg) or reverse (5′-cacacaggaaacagctatgac) primers, depending on the orientation of the insert, and used for in vitro transcription after QIAquick PCR purification (Qiagen). ^{32}P-labeled riboprobes were generated with the Strip-EZ kit (Ambion) and used without further purification.

Northern blot analysis

Total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Total RNA (4 µg) was resolved in 1% agarose (Sigma) in MOPS buffer with 0.6 M formaldehyde for 1.5 h with a 3.3 V/cm voltage gradient. RNA was transferred to Magna Graph nylon membrane (Osmonics) by a downward capillary process, fixed by UV irradiation at 72 mJ/cm^2, dried at 55 °C for an hour, and stained with methylene blue to check transfer efficiency. Hybridization with ^{32}P-labeled riboprobes was performed in the modified high-stringency Church buffer [16] with 250 mM Na^+ , 125 mM PO_4^{2-}, 7% SDS, 1% BSA, 1 mM EDTA, 50% formamide, pH 7.2 at 52–62 °C (depending on the GC content of a probe). After hybridization, membranes were rinsed with 1 × SSC, 0.2% SDS and washed for 30 min in the same solution at 68 °C followed by the final wash at 68 °C in 0.1 × SSC, 0.2% SDS for 0.5–1 h. Signals were visualized with Storm 860 PhosphorImager (Molecular Dynamics). Filters were reprobed with ^{32}P-labeled 18S riboprobe in the presence of cold 18S in vitro transcripts (about tenfold excess relative to 18S rRNA fixed on membranes). Pixel volumes of specific bands were calculated with ImageQuaNT 4.2a software (Molecular Dynamics). For normalization and standardization, the intensities of specific bands were divided by intensities of 18S bands of corresponding lines.

Cell culture

Mouse Neuro-2a neuroblastoma cells as well as human SH-SY-5Y and Sk-N-As neuroblastoma cell lines were obtained from the American Type Culture Collection (CCL 131). Cells were cultured in DMEM medium (Sigma) supplemented with 10% FBS (Gibco), 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. Hippocampal neurons were isolated from 17-day-old Wistar rat embryos and cultured as described by Brewer et al. [17]. Cerebellar granule cells were isolated from the cerebella of 7-day-old Wistar rats and cultured as described by Schousboe et al. [18]. Proliferation of astrocytes was inhibited by Ara-C [18]. We earlier calculated the number of astrocytes in neuronal cultures using

these techniques [19]. It was 1.7% for cerebellar granule cell cultures and 8.5% for hippocampal cultures.

Exposure to HDAC inhibitors

Several different HDAC inhibitors were studied to compare their effects on the expression profile of SIRT mRNAs. TSA and n-butyrate were obtained from Sigma, apicidin and SAHA were from Alexis Corp. M344 was kindly provided by Dr. M. Jung (Westfalische Wilhelms-Universitat Munster, Munster, Germany). Neuro-2a cells were exposed to HDAC inhibitors 24 h after plating at the exponential growth phase. Cultured post-mitotic primary neurons were exposed to HDAC inhibitors after the differentiation phase of 7–8 days. We earlier studied HDAC inhibitor-induced apoptotic responses in these cell culture models [20]. Concentrations of HDAC inhibitors used in this study were lower than those inducing apoptotic parameters, such as caspase-3 activation.

Chromatin immunoprecipitation assay

The procedure was done using SH-SY-5Y and Sk-N-As human neuroblastoma cell lines as human genomic DNA sequences upstream of sirtuin-coding parts have been sequenced and deposited in public data banks. The chromatin immunoprecipitation (Chip) assay was performed according to the protocol of the Chromatin Immunoprecipitation Assay Kit (Upstate) with rabbit polyclonal antibody to histone H4-acetyl K5, K8, K12, K16 (Abcam). For semi-quantitative PCR, the amount of precipitated input DNA was first equalized in amplification with primers from the QuantumRNA Classic II 18S Internal Standard Kit (Ambion). The proximal (with respect to the transcription start site) part of the human SIRT2 promoter was amplified with primers S2/600-A, 5′-gcagcctaaggttcactcacg-3′ and S2/600-B, 5′-gcagcctaaggttcactcacg (636-bp fragment); and distal part with primers S2/1200-A, 5′-gcagcctaaggttcactcacg and S2/1200-B, 5′-tcactgaggatggggacacg (623-bp fragment). The primers amplified two adjacent promoter regions similar in size. The proximal fragment (region –600) included the transcription start site (TSS) of the SIRT2 gene plus 125 bp downstream, and about 500 bp of the promoter region upstream of the TSS. The distal fragment (region –1200) contained the upstream promoter region from –500 bp to –1100 from the TSS. The PCRs were run for the number of cycles ensuring a linear range of amplification with ^{33}P-dCTP (NEN) spiked into the PCR mix as described in the protocol for the QuantumRNA Classic II 18S Internal Standard Kit (Ambion). The PCR fragments were resolved in non-denaturing 6% PAAG and visualized with a Storm 860 PhosphorImager (Molecular Dynamics). The results are presented as ratios of the intensities of the fragments amplified from immunoprecipitated DNA of the treated cell culture to the corresponding fragments amplified from control (non-treated) cell culture.

Results

Differential regulation of SIRT1–7 mRNA expression by HDAC inhibitors

Our purpose was to find out whether class I and II HDAC inhibitors, TSA and n-butyrate, affect the mRNA expression levels and whether there is a differential regulation among the members of the Sir2 gene family. Neuro-2a cells were selected because all seven SIRTs were expressed in these cells (fig. 1) and, moreover, we had earlier screened the toxicity and apoptotic sensitivity of Neuro-2a cells to TSA and n-butyrate [20]. Interestingly, TSA and n-butyrate treatments induced diverse responses in the expression levels of seven SIRT mRNAs.

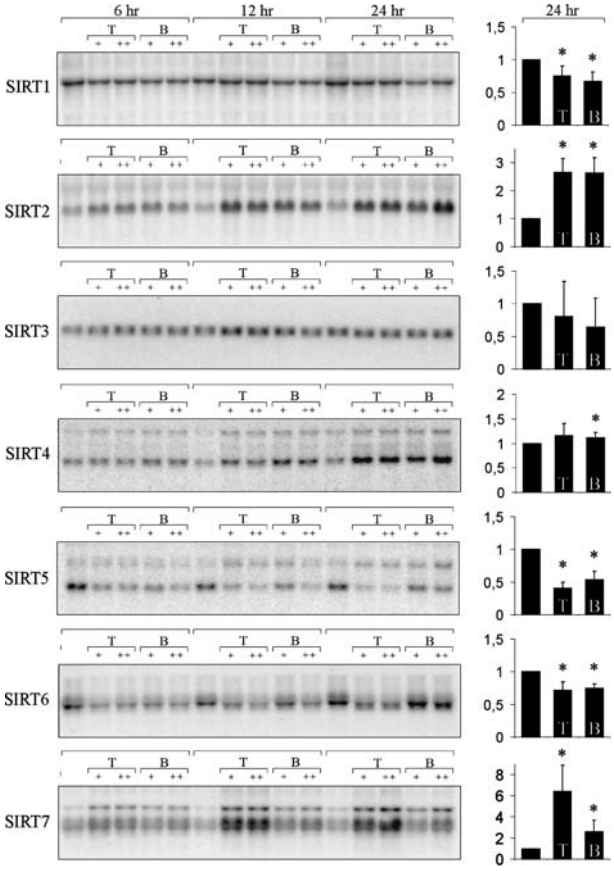

Figure 1. Northern blots show that different sirtuin homologs (SIRT1–7) undergo different changes in their expression in response to class I and II HDAC inhibitors in a neuroblastoma cell line. The Neuro-2a mouse neuroblastoma cell line was treated with TSA and n-butyrate for the time indicated. Total RNA was extracted and analyzed by Northern assay as described in Materials and methods. All experiments were repeated at least three times. Typical Northern pictures for different mouse sirtuin homologs (SIRT1–SIRT7) are shown on the left. T+, TSA 0.5 μM; T++, TSA 1 μM; B+, n-butyrate 2 mM; B++, n-butyrate 5 mM. The quantified values of specific bands normalized to 18S rRNA are shown on the right for the 24-h time point with ++ concentrations of drugs (1 μM for TSA and 5 mM for n-butyrate), *Mann-Whitney U test, $p \le 0.03$, $n = 4$.

SIRT2, SIRT4 and SIRT7 showed statistically significant upregulation, whereas SIRT1, SIRT5 and SIRT6 were downregulated and SIRT3 was unaffected (fig. 1). The responses in SIRT expression were very similar after TSA and n-butyrate treatments; only SIRT7 was more responsive to TSA than to n-butyrate. Most of the mRNA responses, especially the downregulation of SIRT5, were already present after 6 h, but most of the differences were more prominent after 12 and 24 h (fig. 1). The concentrations which induced strong responses in mRNA levels were considerably lower than those which induced apoptotic changes in Neuro-2a cells observed earlier [20].

Similar changes in expression profiles of SIRT1–7 mRNAs in neuroblastoma cells and post-mitotic primary neurons

HDAC inhibitors are potent differentiation and apoptotic inducers in a variety of cancer cells [21–23]. Therefore, we wanted to study whether HDAC inhibitors also induce similar changes in cultured primary post-mitotic neurons and whether the expression changes in the Sir2 family are related to differentiation of Neuro-2a cells. Surprisingly, TSA and n-butyrate induced changes in mature post-mitotic cerebellar granule neurons and hippocampal pyramidal neurons very similar to those in Neuro-2a neuroblastoma cells (fig. 2). The expression of SIRT2, SIRT4 and SIRT7 was upregulated, whereas that of SIRT1, SIRT5 and SIRT6 was downregulated (fig. 2). The expression levels of SIRT mRNAs were similar both in cerebellar granule neurons and hippocampal pyramidal neurons. Class I and II HDAC inhibitors seem to induce a differential response profile in the expression of Sir2 family members general to different species and in both transformed and primary post-mitotic neuronal cells.

Actinomycin D blocks the induction of SIRT2 mRNA expression

Next, we studied whether the SIRT responses induced by HDAC inhibitors are related to changes in transcriptional regulation or to mRNA stabilization. Figure 3A shows that actinomycin D totally blocked the TSA- and n-butyrate-induced increase in SIRT2 mRNA level in Neuro-2a cells. Furthermore, actinomycin D did not revert HDAC inhibitor-induced SIRT5 mRNA downregulation. These results indicate that HDAC inhibitors regulate SIRT2 gene expression at the transcriptional level. The protein synthesis inhibitor cycloheximide can cause transcriptional superinduction of some genes [24]. Figures 3B and 3C show that cycloheximide did not modulate the upregulation of SIRT2 or the downregulation of SIRT5 induced by HDAC inhibitors.

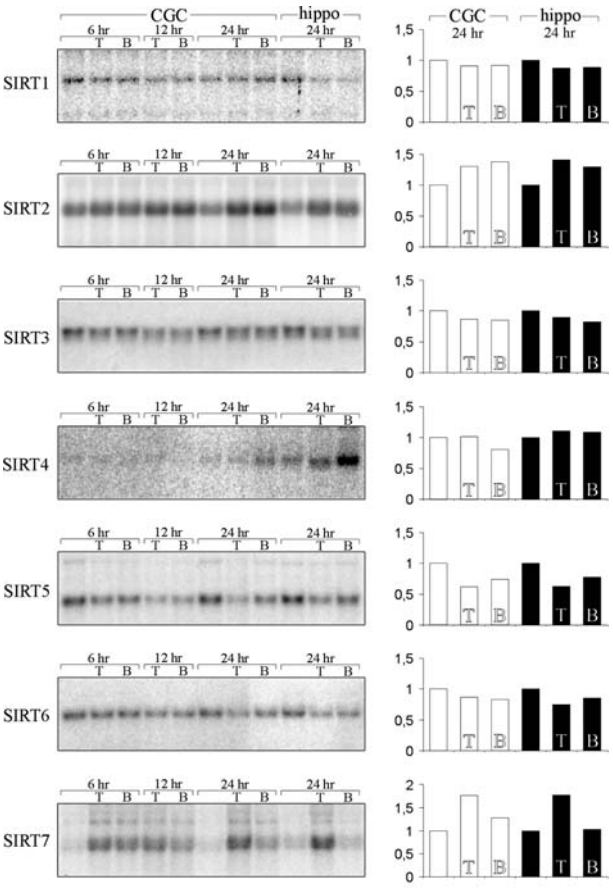

Figure 2. Northern blots showing that inhibition of class I and II HDACs in rat post-mitotic primary neuronal cells evokes diverse responses in SIRT1–7 gene expression which are similar to the expression changes in the mouse neuroblastoma cell line. Primary rat cerebellar granule cells and rat hippocampal neurons were treated as described in Materials and methods with 2 µM TSA and 5 mM n-butyrate for the time indicated. Total RNA was extracted and analyzed by Northern assay. Typical Northern pictures for different rat sirtuin homologs (SIRT1–SIRT7) are shown on the left. The quantified values of specific bands normalized to 18S rRNA are shown on the right for the 24-h time point. CGC, cerebellar granule cells; hippo, hippocampal neuronal cells; T, TSA; B, n-butyrate.

Similar changes in SIRT expression profile by different types of HDAC inhibitor

There are chemically different types of HDAC inhibitors which are effective against class I and II HDACs but not against Sir2 types of HDAC [15, 21, 23]. At present, all known Sir2 inhibitors, such as sirtinol, are effective at high micromolar levels (e.g. IC_{50} inhibition of SIRT2 is 38 µM) [25]. However, sirtinol turned out to be very toxic for Neuro-2a cells at this concentration (data not shown). We compared the effects of apicidin, n-butyrate, M344, SAHA and TSA on the expression profile of SIRT1–7 mRNAs. Interestingly, all chemically distinct HDAC inhibitors induced similar responses in SIRT1–7 mRNA expression in Neuro-2a cells (fig. 4). This indicates that the differential regulation of the Sir2 HDAC family is

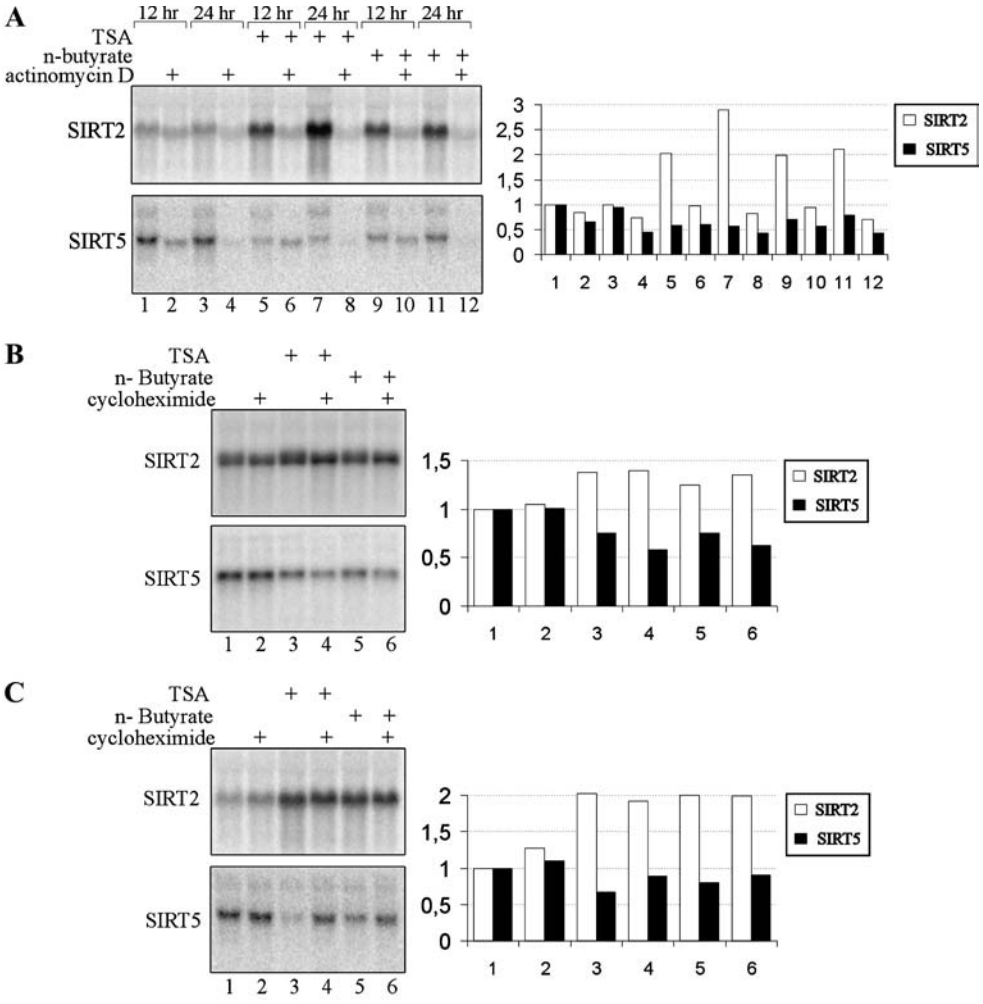

Figure 3. The induction of SIRT2 mRNA expression is reverted by the RNA synthesis inhibitor actinomycin D. (*A*) Mouse Neuro2a cells were treated with 1 µg/ml actinomycin D together with 0.5 µM TSA or 2 mM n-butyrate for the time indicated. (*B*) The protein synthesis inhibitor cycloheximide does not induce superinduction of SIRT mRNAs. Seven-day-old rat hippocampal neurons were treated with 0.2 µg/ml cycloheximide together with 0.75 µM TSA or 5 mM n-butyrate for 17 h. (*C*) Mouse Neuro-2a neuroblastoma cells were treated with 0.2 µg/ml cycloheximide together with 0.5 µM TSA or 2 mM n-butyrate for 10 h. Total RNA was extracted and analyzed by Northern assay. (*A–C*) Typical Northern pictures for SIRT2 and SIRT5 homologs are shown on the left. The quantified values of specific bands normalized to 18S rRNA are shown on the right (numbers below correspond to lane numbering on the Northern pictures).

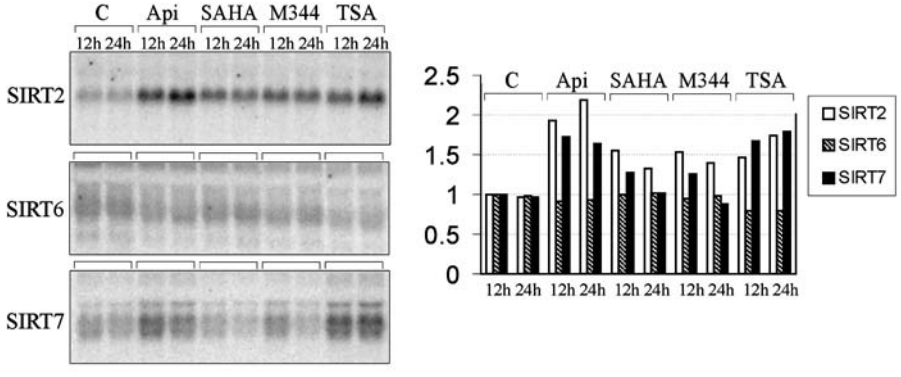

Figure 4. Various inhibitors of class I and II HDACs evoke similar effects on the expression profiles of different SIRTs. Mouse Neuro-2a neuroblastoma cells were treated with 1.0 µM apicidin (Api), 1.0 µM SAHA, 1.0 µM M344 or 0.5 µM TSA for the time indicated. Total RNA was extracted and analyzed by Northern assay. Typical Northern pictures for SIRT2, SIRT6 and SIRT7 homologs are shown on the left. The quantified values of specific bands normalized to 18S rRNA are shown on the right.

specifically induced by HDAC inhibition and not by unspecific effects.

Upregulation of SIRT2 expression is related to the hyperacetylation of H4 at the SIRT2 promoter region

Chip assay results showed (fig. 5) that the proximal part of the SIRT2 promoter (from TSS to –500 bp) is responsive to the histone acetylation status in two different human cell lines. In both SH-SY-5Y and Sk-N-As human neuroblastoma cells, treatment with the HDAC inhibitor TSA led to the hyperacetylation of DNA-bound histones in the region within the first 500 bp upstream of the TSS of the SIRT2 gene. Consequently, the fraction of DNA containing the proximal part of the promoter is increased in the population of shared histone-bound DNA, precipitated with the antibodies specific to acetylated histone H4. In semi-quantitative PCR, the proximal (with respect to the TSS) fragment is significantly more abundant in TSA-treated cells versus non-treated cells. Both the adjacent distal region of the same promoter and 18S housekeeping internal standard did not show significant differences between treated and non-treated cells. Therefore, we postulate that the SIRT2 promoter contains elements responsive to the inhibitors of class I and II HDACs, and sirtuin gene expression may be subjected to regulation directly by class I and II HDACs.

Discussion

Sir2 proteins are NAD-dependent HDACs [10–12] which couple the deacetylation of substrate proteins and beta-NAD^+ cleavage to produce 2′- and 3′-O-acetyl-ADP-ribose [13]. This property of the Sir2 type (class III) of HDACs provides an interesting link between energy metabolism, gene expression and chromatin stability. For class I and II HDACs, this kind of link to metabolic activity has not yet been described. Furthermore, there are differences in catalytic properties between three different classes of HDACs, and the inhibitors of class I and II HDACs do not inhibit Sir2 types of HDACs [26]. At present, there are several, chemically different inhibitors for class I and II HDACs [15, 21–23, 26]. However, no effective inhibitors for Sir2 enzymes have been developed, although some molecules have been identified, such as sirtinol [25] and splitomicin [27].

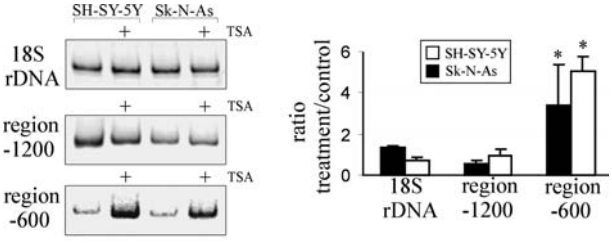

Figure 5. Chip assay results show that the proximal part of the SIRT2 promoter (from the TSS to –500 bp) is responsive to the histone acetylation status in two different human cell lines. SH-SY-5Y and Sk-N-As human neuroblastoma cells were treated with TSA at 1 μM for 9 h. The formaldehyde-cross-linked DNA-histone complexes were immunoprecipitated with antibodies specific to acetylated histone H4. The recovered DNA was used as a template for semi-quantitative PCR with addition of ^{33}P-dCTP. The 18S-specific primer pair was used as an internal (housekeeping) standard. Each experiment was repeated at least three times. A typical PCR is shown on the left. The fragment lengths were 324 bp for the 18S-specific band, 623 bp for the distal promoter part (region –1200) and 636 bp for the proximal promoter part (region –600). On the left, the results are presented as ratios of the intensities of the fragments amplified from the treated cell culture to the corresponding fragments amplified from immunoprecipitated DNA of control (non-treated) cell culture. * Mann-Whitney U test, $p \leq 0.03$, $n = 3$.

HDAC inhibitors can be divided into several different structural classes [15, 21]. TSA, a hydroxamic acid-based compound, is the most studied, potent inhibitor of mammalian HDACs [14, 15, 21]. TSA induces the hyperacetylation of histones and regulates the expression of several genes, either activating or repressing transcription [23]. SAHA is another HDAC inhibitor of the hydroxamic acid class [15, 21]. Short-chain fatty acids, such as butyrate and valproic acid, are also weak HDAC inhibitors and regulate the transcription of several genes [23]. Cyclic tetrapeptides, such as apicidin and trapoxin A, are the third class of HDAC inhibitor [15, 21, 23].

HDAC inhibitors induce a variety of biological responses, such as cell cycle arrest, differentiation and apoptotic cell death [14, 21, 23]. Transformed cells are especially sensitive to HDAC inhibitors, and hence these inhibitors are promising new cancer drugs. Several HDAC inhibitors are already in clinical trials in patients with cancer [21]. HDAC inhibitors affect gene expression selectively, either inducing upregulation or downregulation [21, 23]. TSA, for example, alters the expression of about 2% of the genes expressed in cultured tumor cells [28]. Furthermore, the transcription profiles of the yeast deletion mutants of *rpd3*, *hda1* and *sir2* showed differences from wild type only in a small fraction of genes [29].

We were interested to study whether the inhibition of class I and II HDACs induces feedback responses in the transcription of seven mammalian SIRTs of the Sir2 family (class III HDACs). TSA and n-butyrate are known to induce differential transcriptional responses among class I and II members of HDACs [30]. Furthermore, in HDAC1-deficient cells, the expression of HDAC2 and HDAC3 is induced, which might show the feedback regulation [31]. Genomewide studies in yeast showed that three classes of HDACs have overlapping regulatory roles [32]. Functional expression profiles also showed that Sir2p and Hda1 (class II HDACs) may have functional commonality whereas Sir2p and Rpd3 exert opposite effects. Interestingly, we observed that TSA and n-butyrate, inhibitors of class I and II HDACs, induced strong but

highly diverse responses in the espression profiles of SIRT mRNAs. The expression of SIRT2, SIRT4 and SIRT7 were significantly upregulated, whereas those of SIRT1, SIRT5 and SIRT6 were downregulated. These effects may be direct transcriptional effects or secondary responses, mediated, for example, by the acetylation of signaling molecules. The differences in direct transcriptional regulation of SIRTs might be due to HDAC repression either through Sin3 and Nurd complexes or DNA methylation [32, 33]. Inhibition of HDACs could induce a fast upregulation of SIRT2, SIRT4 and SIRT7 mRNAs. However, transcriptional characteristics of SIRT genes are currently mostly unknown. Here, we studied whether TSA induces histone H4 acetylation in the promoter regions of the SIRT2 gene which would be related to the upregulation of SIRT2 expression. Our results show that the SIRT2 promoter contains elements responsive to the inhibitors of class I and II HDACs, and SIRT2 gene expression may be subjected to direct regulation by class I and II HDACs.

HDAC inhibitors are potent differentiation and apoptotic inducers in transformed cells [21–23]. Hence, the observed changes in SIRT expression profile could be related to differentiation and apoptosis of Neuro-2a cells. This seems unlikely since very similar profile changes were observed both in transformed neuroblastoma cells and post-mitotic primary hippocampal and cerebellar granule neurons. Moreover, TSA and n-butyrate concentrations used were lower than the concentrations which induced apoptotic changes in these cells [20]. Class I and II HDACs seem to selectively regulate the transcription of SIRTs not only in transformed cells but also in primary post-mitotic cells. Likewise, SIRTs may regulate the transcription of other HDACs, but the verification is still waiting for effective Sir2 inhibitors.

HDACs comprise a functionally diverse group of deacetylating enzymes. A typical characteristic feature for class I HDACs is complex formation with Sin3 and NuRD multiprotein repressors. For class II HDACs it may be the nucleocytoplasmic shuttling, and linking energy metabolism to protein/histone deacetylation for class III SIRTs [26]. There are substantial differences also within class I and II HDACs. Our results indicate that there is also a prominent functional diversity among SIRTs. Based on transcriptional regulation, upregulated SIRT2, SIRT4 and SIRT7 might form one functional group and downregulated SIRT1, SIRT5 and SIRT6 the second group. Currently, there is not enough molecular data for more exact transcriptional characterization of SIRT genes. However, our data show that the expression of Sir2 types of HDACs can be regulated at the level of transcription and histone H4 acetylation is involved in the upregulation of SIRT2 expression by TSA. Differential responses in SIRT mRNA expression profiles indicate that the expression of the Sir2 family of genes is selectively regulated and dependent on histone/protein acetylation status.

Acknowledgements. We would like to thank S. Vaisanen for his technical advice about the Chip assay. This study was supported by grants from the Academy of Finland (A. S.) and the University of Kuopio, Finland (S. K., O. K., T. S.).

1 Gray S. G. and Ekström T. J. (2001) The human histone deacetylase family. Exp. Cell Res. **262:** 75–83
2 Shore D. (2000) The Sir2 protein family: a novel deacetylase for gene silencing and more. Proc. Natl. Acad. Sci. USA **97:** 14030–14032
3 Guarente L. (2000) Sir2 links chromatin silencing, metabolism, and aging. Genes Dev. **14:** 1021–1026
4 Dutnall R. N. and Pillus L. (2001) Deciphering NAD-dependent deacetylases. Cell **105:** 161–164
5 Frye R. A. (2000) Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochem. Biophys. Res. Commun. **273:** 793–798
6 Gasser S. M. and Cockell M. M. (2001) The molecular biology of the SIR proteins. Gene **279:** 1–16
7 Luo J., Nikolaev A. Y., Imai S., Chen D., Su F., Shiloh A. et al. (2001) Negative control of p53 by Sir2α promotes cell survival under stress. Cell **107:** 137–148
8 Vaziri H., Dessai S. K., Ng Eaton E., Imai S. I., Frye R. A., Padita T. K. et al. (2001) hSIR2^{SIRT1} functions as an NAD-dependent p53 deacetylase. Cell **107:** 149–159
9 North B. J., Marshall B. L., Borra M. T., Denu J. M. and Verdin E. (2003) The human Sir2 ortholog, SIRT2, is an NAD-dependent tubulin deacetylase. Mol. Cell **11:** 437–444
10 Min J., Landry J., Sternglanz R. and Xu R. M. (2001) Crystal structure of a SIR2 homolog-NAD complex. Cell **105:** 269– 279
11 Finnin M. S., Donigian J. R. and Pavletich N. P. (2001) Structure of the histone deacetylase SIRT2. Nat. Struct. Biol. **8:** 621–625
12 Sauve A. A., Celic I., Avalos J., Deng H., Boeke J. D. and Schramm V. L. (2001) Chemistry of gene silencing: the mechanism of NAD+-dependent deacetylation reactions. Biochemistry **40:** 15456–15463
13 Jackson M. D. and Denu J. M. (2002) Structural identification of 2′- and 3′-O-acetyl-ADP-ribose as novel metabolites derived from the Sir2 family of beta-NAD+-dependent histone/protein deacetylases. J. Biol. Chem. **277:** 18535–18544
14 Yoshida M., Horinouchi S. and Beppu T. (1995) Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. Bioessays **17:** 423–430
15 Jung M. (2001) Inhibitors of histone deacetylase as new anticancer agents. Curr. Med. Chem. **8:** 1505–1511
16 Church G. M. and Gilbert W. (1984) Genomic sequencing. Proc. Natl. Acad. Sci. USA **81:** 1991–1995
17 Brewer G. J., Torricelli J. R., Evege E. K. and Price P. J. (1993) Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. J. Neurosci. Res. **35:** 567– 576
18 Schousboe A., Meier E., Drejer J. and Hertz L. (1989) Preparation of primary cultures of mouse (rat) cerebellar granule cells. In: A Dissection and Tissue Culture Manual of the Nervous System, pp. 203–206, Shahar A., Vellis J. de, Vernadalis A. and Haber B. (eds), Liss, New York
19 Suuronen T., Kolehmainen P. and Salminen A. (2000) Protective effect of L-deprenyl against apoptosis induced by okadaic acid in cultured neuronal cells. Biochem. Pharmacol. **59:** 1589–1595
20 Salminen A., Tapiola T., Korhonen P. and Suuronen T. (1998) Neuronal apoptosis induced by histone deacetylase inhibitors. Mol. Brain Res. **61:** 203–206

21 Marks P. A., Richon V. M., Breslow R. and Rifkind R. A. (2001) Histone deacetylase inhibitors as new cancer drugs. Curr. Opin. Oncol. **13:** 477–483
22 Grozinger C. M. and Schreiber S. L. (2002) Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. Chem. Biol. **9:** 3–16
23 Johnstone R. W. (2002) Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. Nat. Rev. Drug Disc. **1:** 287–299
24 Ma Q., Renzelli A. J., Baldwin K. T. and Antonini J. M (2000) Superinduction of CYP1A1 gene expression: regulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced degradation of Ah receptor by cycloheximide. J. Biol. Chem. **275:** 12676–12683
25 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
26 Knochbin S., Verdel A., Lemercier C. and Seigneurin-Berny D. (2001) Functional significance of histone deacetylase diversity. Curr. Opin. Genet. Dev. **11:** 162–166
27 Bedalov A., Gatbonton T., Irvine W. P., Gottschling D. E. and Simon J. A. (2001) Identification of a small molecule inhibitor of Sir2p. Proc. Natl. Acad. Sci. USA **98:** 15113–15118
28 Marks P. A., Richon V. M. and Rifkind R. A. (2000) Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. J. Natl. Cancer Inst. **92:** 1210–1216
29 Bernstein B. E., Tong J. K. and Schreiber S. L. (2000) Genomewide studies of histone deacetylase function in yeast. Proc. Natl. Acad. Sci. USA **97:** 13708–13713
30 Dangond F. and Gullans S. R. (1998) Differential expression of human histone deacetylase mRNAs in response to immune cell apoptosis induction by trichostatin A and butyrate. Biochem. Biophys. Res. Commun. **247:** 833–837
31 Lagger G., O'Carroll D., Rembold M., Khier H., Tischler J., Weitzer G. et al. (2002) Essential function of histone deacetylase 1 in proliferation control and CDK inhibitor repression. EMBO J. **21:** 2672–2681
32 Ayer D. E. (1999) Histone deacetylases: transcriptional repression with SINers and NuRDs.Trends Cell Biol. **9:** 193–198
33 Razin A. (1998) CpG methylation, chromatin structure and gene silencing – a three-way connection. EMBO J. **17:** 4905–4908