

Coordinated changes of histone modifications and HDAC mobilization regulate the induction of MHC class II genes by Trichostatin A

Manolis Gialitakis^{1,2}, Androniki Kretsovali¹, Charalampos Spilianakis³,
Lara Kravariti¹, Jörg Mages⁴, Reinhard Hoffmann⁵, Antonis K. Hatzopoulos^{6,7}
and Joseph Papamatheakis^{1,2,*}

¹Institute of Molecular Biology and Biotechnology, FORTH, Heraklion 71110, Greece, ²Department of Biology, University of Crete, Heraklion 71110, Greece, ³Section of Immunobiology, Yale University School of Medicine, New Haven, Connecticut 06520, USA, ⁴Technical University, Institute for Medical Microbiology, 81675 Munich, Germany, ⁵Department of Bacteriology, Max-von-Pettenkoffer Institute, 80336 Munich, Germany, ⁶Vanderbilt University Medical Center, Division of Cardiovascular Medicine, Nashville, Tennessee 37232-6300, USA and ⁷GSF-Research Center for Environment and Health, Institute for Clinical Molecular Biology and Tumor Genetics, 81377 Munich, Germany

Received October 4, 2005; Revised December 22, 2005; Accepted January 5, 2006

ABSTRACT

The deacetylase inhibitor Trichostatin A (TSA) induces the transcription of the Major Histocompatibility Class II (MHC II) DRA gene in a way independent of the master coactivator CIITA. To analyze the molecular mechanisms by which this epigenetic regulator stimulates MHC II expression, we used chromatin immunoprecipitation (ChIP) assays to monitor the alterations in histone modifications that correlate with DRA transcription after TSA treatment. We found that a dramatic increase in promoter linked histone acetylation is followed by an increase in Histone H3 lysine 4 methylation and a decrease of lysine 9 methylation. Fluorescence recovery after photobleaching (FRAP) experiments showed that TSA increases the mobility of HDAC while decreasing the mobility of the class II enhanceosome factor RFX5. These data, in combination with ChIP experiments, indicate that the TSA-mediated induction of DRA transcription involves HDAC relocation and enhanceosome stabilization. In order to gain a genome-wide view of the genes responding to inhibition of deacetylases, we compared the transcriptome of B cells before and after TSA treatment using Affymetrix microarrays. This analysis showed that in addition to the DRA gene, the entire MHC II family and the adjacent histone cluster that are located in

chromosome 6p21-22 locus are strongly induced by TSA. A complex pattern of gene reprogramming by TSA involves immune recognition, antiviral, apoptotic and inflammatory pathways and extends the rationale for using Histone Deacetylase Inhibitors (HDACi) to modulate the immune response.

INTRODUCTION

Epigenetic mechanisms of gene expression are important for cell function and homeostasis (1). Histone Deacetylase Inhibitors (HDACi) such as Trichostatin A (TSA), butyric acid and SAHA, are epigenetic chemicals that modulate gene expression and thus affect cell proliferation, differentiation and apoptosis. The above actions, in combination with their tumor-selectivity make HDACi promising molecules for cancer treatment that are currently under phase I and II trials.

Products of the Major Histocompatibility Class II (MHC II) genes present antigenic peptides to T cells and are critical for the specificity and efficiency of the immune response. Increasing evidence shows that MHC II products also contribute to the recognition of tumor cells by CD4+ T cells and anti-tumor immunity. MHC II expression (2) relies on the constitutive or cytokine induced synthesis of the master regulator Class II Transactivator (CIITA) (3).

Many reports have established the importance of histone acetylation as positive regulator of MHC II transcription (4–6). The interaction of CIITA with various Histone Acetylases (HATs) such as CBP (7,8), PCAF (9) and SRC-1 (10) was

*To whom correspondence should be addressed. Tel: +30 2810 391165; Fax: +30 2810 391101; Email: papamath@imbb.forth.gr

linked to MHC II gene activation. Interestingly, CIITA also interacts with HDACs and may act as a molecular switch between opposing chromatin regulators (11,12). CIITA deletions or epigenetic silencing during development and in tumors eliminate MHC II expression (13). A yet unexplored issue is the regulation of MHC II transcription without CIITA. CIITA knock out mice have residual MHC II expression in certain cell types (14,15) and CIITA independent induction of MHC II in epithelial cells was also reported (16).

To investigate if histone acetylation promotes MHC II transcription independently of CIITA, we used TSA to treat the CIITA mutant line RJ 225 (17) as reported previously (18). We show that the transcriptional activation of the MHC II DRA gene by TSA is linked to promoter hyperacetylation and an increase of H3 lysine 4 methylation and a decrease of lysine 9 methylation. TSA-mediated transcription correlates with increased mobility of HDACs and reduced mobility of the RFX5 enhanceosome factor. Gene profiling of RJ225 before and after TSA treatment using Affymetrix microarrays reveals diverse groups of genes that are regulated by TSA. Many members of the MHC II and the Hist1 loci, both located in human chromosome 6p21-22, are among the highly induced genes. These data, on both gene specific mechanisms and global transcriptome effects of HDACi in a B-lymphoid cell background indicate that HDACi -besides hindering tumor growth- can also boost MHC II expression strengthening the host's immune response. This information can have important implications in future therapeutic strategies.

MATERIALS AND METHODS

Cell culture and reagents

HeLa cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum. Raji and RJ2.2.5 cells were cultured in RPMI. IFN γ (R&D) was used at a final concentration of 100 U/ml. Antibodies against acetylated and methylated histones were from Upstate biotechnology; TBP, RNA polymerase II, CBP and GCN5 from Santa Cruz Biotechnology; and RFX5, NFY-A from Rockland Immunochemicals.

Plasmids, transfections and flow cytometry

The MHC II gene promoter was cloned upstream of the luciferase coding sequence in the pGL3-basic vector (Promega). Lymphoma cells were electroporated using the BioRad Gene Pulser electroporator at 0.23 kV and 960 μ F.

RT-PCR and ChIPs

RNA was prepared with the Trizol reagent (Gibco-BRL). RT reactions were performed with MMLV from Finnzymes. ChIPs were performed according to the protocol described previously (6). For real-time PCR, we used SYBR Green I and the Opticon Monitor system from MJ Research. Primers for RT-PCR and ChIP are indicated in the Supplementary data.

Detailed description of the FRAP and Affymetrix microarray analysis protocols are included in the Supplementary data.

RESULTS

The MHC II enhancer determines transcriptional induction by TSA

To study the mechanism of transcriptional activation of the MHC II DRA gene in a CIITA independent manner, we used the HDAC inhibitor TSA. Total RNA from RJ225 cells that are defective in CIITA synthesis (17) was analyzed using quantitative RT-PCR. Figure 1A (black bars) shows a significant increase of DRA gene transcription which occurs within 2 h after TSA addition and increases continuously up to 12 h post-treatment in an agreement with an earlier report (18). For comparison, we examined in parallel the expression of the p53 tumor suppressor gene shown to be suppressed by TSA (19). Quantitative RT-PCR analysis confirms that p53 is down regulated by TSA treatment in RJ225 but not in HeLa cells (Figure 1B). For comparison, the levels of GAPDH remained unaffected in both cell types (Figure 1C). To investigate whether induction of DRA was regulated at the transcriptional level, we applied TSA to RJ225 cells pre-treated with

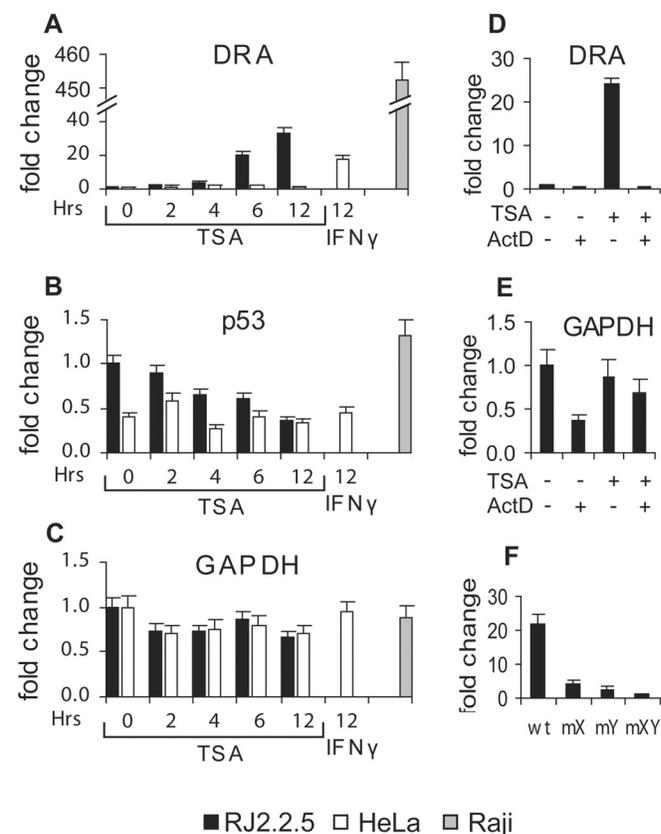


Figure 1. Effects of Trichostatin on gene expression. Real-time RT-PCR at various time points after 200 nM TSA treatment of HeLa and RJ225 cells shows induction of DRA (A) reduction of p53 (B) and no change of GAPDH (C) RNA. The expression levels at the zero hours time point is set to one. The RNA levels after 12 h of interferon- γ treatment in HeLa cells and wt Raji are shown for comparison. Actinomycin D reduces the GAPDH (E) RNA levels and blocks TSA induction of DRA (D). The X and Y box elements of the MHC II enhancer are important for TSA response (F). Cells were transiently transfected with the indicated wild type (wt) class II promoter-Luciferase fusion or its isogenic derivatives that carry a mutated X or Y box (mX, mY) or both (mXY). Data are averages of 3–5 experiments showing the ratio of TSA treated for 24 h over untreated RJ225 cells.

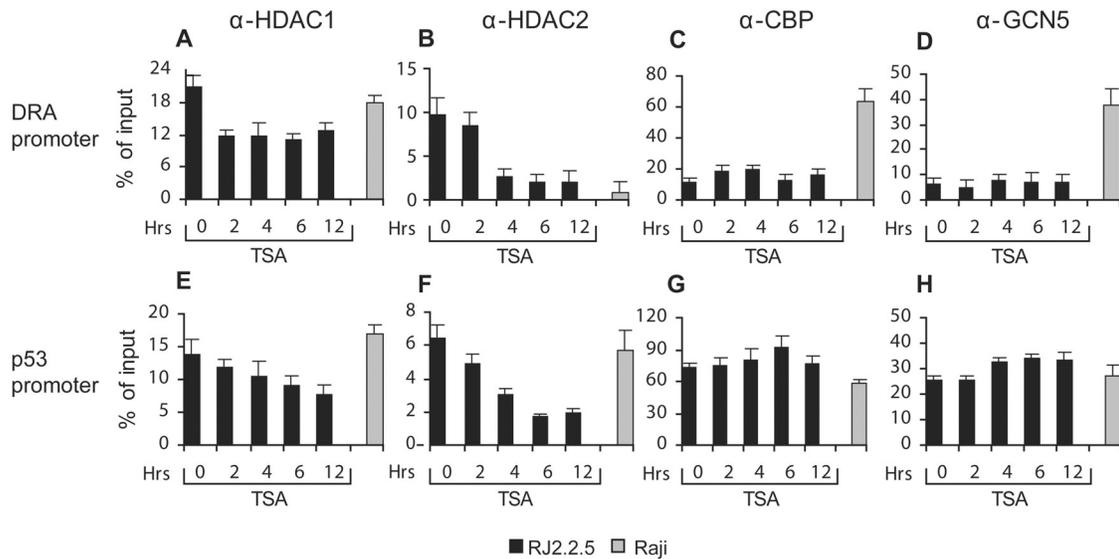


Figure 2. Promoter associated HATs and DACs on the DRA and p53 genes. Chromatin immunoprecipitates with anti-HDAC1 (A and E), anti-HDAC2 (B and F), anti-CBP (C and G) or anti-GCN5 (D and H) antibodies were subjected to real-time PCR using primers for DRA (A,B,C,D) or p53 (E,F,G,H) promoters. The levels of untreated Raji cells are shown for comparison. Results represent averages of 3 experiments with their error bars and are expressed as % of the input chromatin that was set at 5% of the amount used for immunoprecipitation.

actinomycin D, an inhibitor of RNA synthesis. Quantitative RT-PCR (Figure 1E) showed that TSA-mediated induction of DRA requires new RNA synthesis and does not involve post-transcriptional mechanisms (Figure 1D). To examine the cell type specificity of the TSA-mediated induction of MHC II transcription, we tested whether TSA could upregulate MHC II transcription in HeLa cells. In this cell type, MHC II genes are silent and can be induced by IFN γ treatment that stimulates CIITA synthesis. As opposed to RJ225 cells, DRA gene expression was not induced in HeLa cells (Figure 1A) at comparable or even higher TSA concentrations (400 nM or 1 μ M, data not shown). The functional significance of these results was further shown by demonstrating the ability of TSA to induce surface expression of DR protein as measured by flow cytometry (Supplementary Figure S1) in agreement with the RT-PCR data.

Both the constitutive and inducible expression of MHC II genes require the presence of an intact proximal enhancer that consists of three conserved *cis*-regulatory elements, the S, X and Y boxes (3). To determine the requirement of distinct MHC II *cis*-regulatory elements for TSA induction, we used MHC II promoter-luciferase gene fusions. Luciferase activity was determined in transient transfection experiments into RJ225 cells treated or untreated with TSA. Figure 1F shows that addition of 200 nM TSA strongly induced the activity of the promoter that carries an intact enhancer in RJ225. An enhancer with disrupted X box (mX) retained only 20% of the intact enhancer inducibility to TSA. Disruption of the Y box (mY) or elimination of both X and Y boxes (mXY) led to almost complete elimination of responsiveness to TSA (Figure 1F). The significance of the CCAAT or Y box in mediating a positive response to TSA has been shown in the case of Gdf11 gene (20). These results indicate that the effect of TSA on the DRA gene is mediated through both X and Y boxes that are required for the MHC II enhanceosome assembly. Overall these data demonstrate that TSA rescues MHC II

surface expression in a cell type and enhanceosome specific manner.

TSA-induced changes of histone modifications

TSA treatment induces high levels of bulk histone acetylations in RJ225, Raji and, to a lesser extent, HeLa cells (Supplementary Figure S2). To study the occupancy of the DRA Class II promoter by HDAC and HATs, we used ChIP assays. Figure 2A and B show that at the silent state, the DRA promoter binds HDAC1 and HDAC2. Two hours following TSA treatment, HDAC1 levels on the DRA promoter were reduced to 50% of the control and remained stable over the course of monitoring (Figure 2A) whereas HDAC2 showed even higher decrease to 20% of the control (Figure 2B). In order to determine which factors are involved in promoter recruitment of HDACs, we examined the interaction of HDAC1 with the enhanceosome factors. Supplementary Figure S3 shows that among the known seven class II enhanceosome factors RFX5 and RFX-AP strongly interact with HDAC1. RFX-ANK and CREB show weak interaction whereas the NFY subunits do not interact at all.

We further examined the recruitment of the HAT coactivators CBP and GCN5, known to be involved in the CIITA mediated DRA gene transcription (6). The DRA promoter contains low levels of CBP and GCN5 in RJ225 cells (Figure 2C and D) that do not significantly change after TSA. Therefore, we conclude that pre-existing HATs on the silent DRA promoter are sufficient to lead fast to hyperacetylation after inhibition of HDAC activity.

To monitor the status of histone modifications in the DRA promoter following TSA treatment, we analyzed the histone H3 and H4 acetylation levels using anti-acetylated H3 (acK9 and K14) and anti-acetylated H4 (acK5, K8, K12 and K16) antibodies. In RJ225 cells, the DRA promoter contains very low levels of acetylated H3 (Figure 3A) and acetylated H4

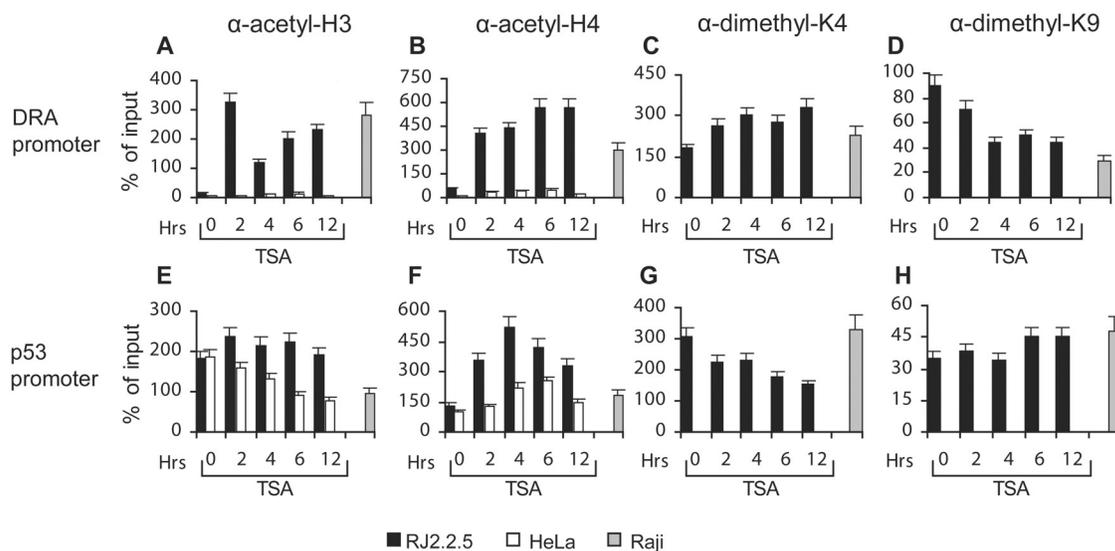


Figure 3. Promoter associated histone acetylation and methylation levels correlate with gene induction or repression. Chromatin from the indicated cells was immunoprecipitated with antibodies against acetyl H3 (A and E), acetyl H4 (B and F), anti-dimethyl H3-K4 (C and G) and anti-dimethyl H3-K9 (D and H) and amplified by real-time PCR with primers for the DRA promoter (A,B,C,D) and the p53 promoter (E, F, G, H). Experimental procedure and statistics are as in Figure 2.

(Figure 3B). As early as 2 h upon TSA treatment, high levels of histone acetylation (up to 30-fold) were detected in the DRA promoter at levels comparable to the parental Raji cells. The histone acetylation of the DRA promoter in HeLa cells was not influenced by TSA (Figure 3A). H4 acetylation of the DRA promoter showed a 6–9-fold increase in RJ225 cells upon TSA treatment (Figure 4B). Histone acetylation of the GAPDH promoter showed small gradual decrease over the time course of these experiments (data not shown). Thus TSA causes a dramatic increase in histone H3 acetylation and a lower increase in histone H4 acetylation of the DRA promoter in RJ22.5 cells.

To correlate the gene response to TSA with histone modification marks, we examined the dynamics of histone methylation following TSA treatment. Figure 3C shows that significant levels of histone H3 dimethylated at K4 (dimK4) reside at both the transcriptionally silent (RJ225 cells) and the active (Raji cells) DRA promoters. High histone H3-K4 dimethylation has been linked to a permissive chromatin state that is either active or potentially active (21,22). Accordingly, TSA treatment increased H3 dimK4 levels at the inducible DRA promoter as opposed to the situation observed on the repressed p53 promoter (Figure 3G). Conversely, histone H3-K9 dimethylation (dimK9), a modification that marks silent euchromatin (23,24), was increased in the transcriptionally silent DRA promoter in RJ225 cells relative to the transcriptionally active DRA promoter in Raji cells. This histone modification was progressively reduced during the course of TSA induction in RJ225 cells (Figure 3D).

Promoter recruitment of transcription factors by TSA

The RJ225 line contains a truncated form of CIITA that is detectable at the RNA level (17) but it is unclear whether any truncated protein is detectable in RJ225 cell extracts. Earlier studies with CHIP showed that such a truncated protein cannot be recruited to the promoter (5). To eliminate the possibility

that TSA may result to the recruitment of the truncated CIITA to the DRA promoter, we performed experiments outlined in Supplementary Figure S4.

To determine whether TSA affects the promoter bound MHC enhanceosome, we monitored the binding of the RFX5 and NFY-A factors. Figure 4C and D, show high occupancy by both RFX5 and NFY-A in TSA untreated RJ225 cells. Upon TSA treatment a 2-fold increase of the binding of those factors was observed in responder RJ225 but not in non-responder HeLa cells (Figure 4C). This effect is also observed in interferon γ treated HeLa cells (11), indicating that the TSA-mediated class II enhanceosome stabilization is functionally connected to its transcriptional action.

Finally, we examined the effect of TSA on the recruitment of the general transcription machinery (GTM). We observed an early increase of TBP recruitment (Figure 4A) and higher occupancy by RNA PolIII (Figure 4B) on the DRA promoter. Contrary to this, TSA reduced PolIII occupancy on the p53 promoter (Figure 4F).

Therefore, the TSA-induced changes of histone modification and the recruitment of the GTM correlate with the gene specific transcriptional effect.

TSA affects HDAC and RFX5 protein mobilities

To study whether promoter CHIP results correlate with the global properties of HDACs or the enhanceosome factors, we investigated the relative mobilities of HDAC1, HDAC2 and RFX5 in RJ225 cells using fluorescence recovery after photobleaching (FRAP) experiments. Figure 5 shows the recovery curves obtained after photobleaching of green fluorescent protein (GFP)-fused DACs or RFX5 in the nucleus. TSA treatment significantly increases the recovery rate of either HDAC1 (half recovery time 1 versus 3 s of the untreated) or HDAC2 (half recovery time 1.5 versus 3.7 s of the untreated) (Figure 5A and B) whereas that of RFX5 decreases (half recovery time 9 versus <1.5 s of the untreated)

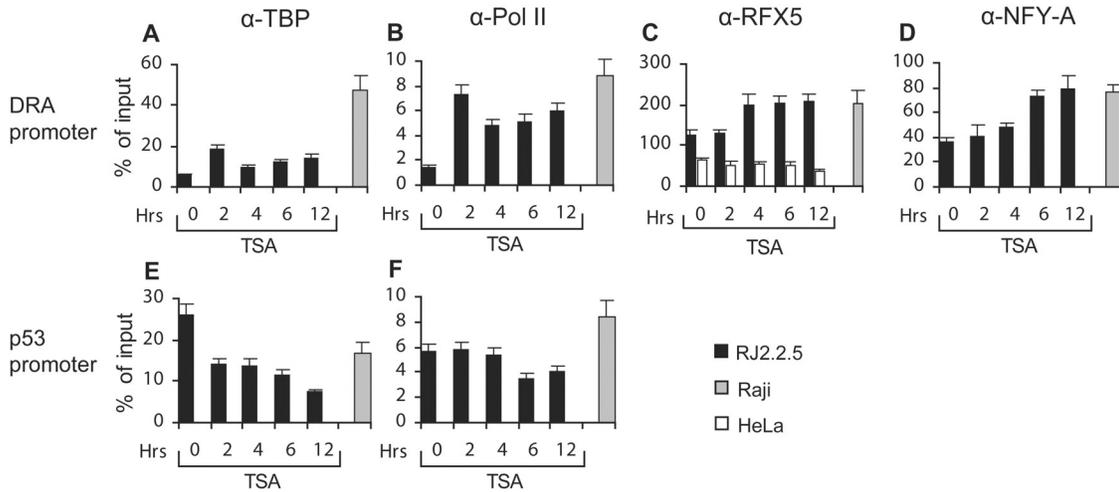


Figure 4. Enhanced recruitment of enhanceosome and general transcription components correlate with responsiveness to TSA. ChIP with antibodies against TBP (A and E) and the large subunit of Pol II (B and F) were amplified by real-time PCR with DRA (A and B), and p53 (E and F) primers from the indicated cells and time points as in Figure 3. The RFX5 in RJ2.2.5 and HeLa (C) and NFY-A (D) enhanceosome components monitored on the DRA promoter during the TSA time course.

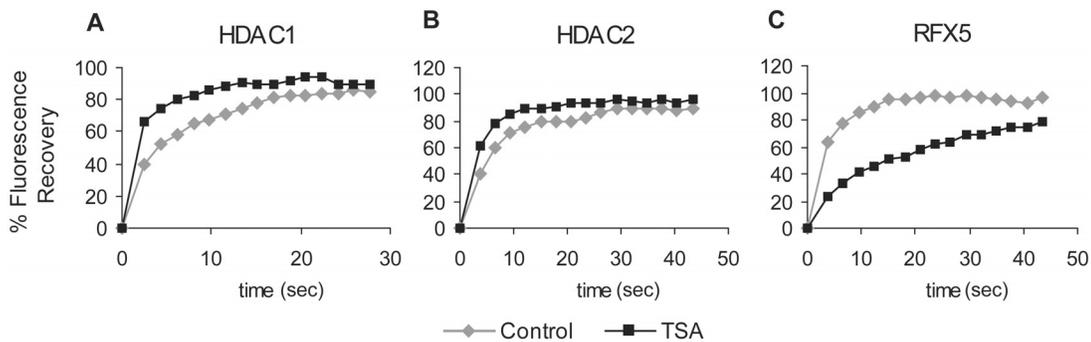


Figure 5. Divergent effects of TSA on the nuclear mobility of HDAC1, HDAC2 and RFX5. RJ225 cells were transfected with the corresponding GFP fusion constructs of HDAC1 (A), HDAC2 (B) and RFX5 (C) and cultured without or in the presence of 200 nM TSA for 18 h as indicated. The graphs show representative fluorescence recovery curves of the bleached areas in the nucleus over the shown time course (in seconds). The results are percentages of the postbleach over the prebleach intensities after correction for background and fluorescent decay during imaging. The error range was smaller than 20% of the mean values.

(Figure 5C). Thus the bulk nuclear behavior of the above factors correlates with that of the DRA promoter associated proteins. High mobility of HDACs after TSA may result in their reduced DRA promoter occupancy. Conversely, the decrease of the RFX5 mobility by TSA is consistent with stabilization of the enhanceosome on the DRA promoter.

The TSA-elicited gene expression program in RJ2.2.5

We next asked what are the genome-wide consequences of applying TSA to the RJ225 cells. This analysis aimed to: (i) detect how the other MHC locus members respond to epigenetic modifications; (ii) identify genes that are co-regulated with MHC II that might be involved in class II gene induction or function; and (iii) assess the transcriptome effects TSA besides rescue of MHC II gene expression. To this end, RJ225 cells untreated or treated with 200 nM TSA for 4 and 12 h were profiled by probing the human Affymetrix GeneChip U133A array containing ~22 000 probe sets. The complete list of TSA-regulated genes (Supplementary Table S1) along with relevant information are provided in the Supplementary data.

This analysis shows that 260 genes responded to TSA treatment in a positive (156 genes) or negative (104 genes) manner at the ~3-fold level. Many up-regulated genes fall into two major groups: genes involved in cell defence mechanisms (interferon or virus regulated, pro-inflammatory or apoptotic pathways, immune response) and genes of the histone family. Interestingly, the MHC class I and II genes and the great majority of the histone genes are located on the Chromosome 6p21-22 (Table 1) suggesting a clustering of HDAC-sensitive, common gene regulatory mechanisms and/or TSA-mediated long range chromatin changes. Figure 6 shows that the MHC class I genes were minimally up-regulated. Concerning MHC II genes, in addition to the highly induced DRA gene, the DRB and DPB genes showed also strong induction whereas the DQA, DQB, DPA, DPB, DMA and DMB were induced at a lower (2–4-fold) level. In contrast, the DO genes that produce molecules antagonistic to the action of DM for CLIP-antigenic peptide replacement (25) showed a distinct response: The DOA gene showed 2-fold increase whereas the DOB gene that has unusually high basal levels relative to the other MHC II members (26) was slightly reduced (70% of the control). Thus TSA enhances antigen presentation

Table 1. Genes on Chr.6p21 with modified expression upon TSA treatment

Gene names	Maximum fold change
HLA-DRA	11.7 ± 1.9
HLA-DRB1	5.9 ± 1.3
HLA-DPB1	4.4 ± 1.2
HLA-DRB3	4.0 ± 1
H3 member K	10.2 ± 0.6
H1 member 2	9.8 ± 1.3
H2B member A	17.1 ± 5.2
H2A member L	6.5 ± 1.7
H2A member P	6.5 ± 1.7
H2B member B	5.1 ± 0.2
H2B member J	5.1 ± 0.2
H2B member T	4.9 ± 0.3
H2B member L	4.8 ± 1.6
H2B member H	4.6 ± 0.3
H2B member G	3.7 ± 0.3
H2B member K	3.2 ± 0.3
Tubulin beta polypeptide	7.2 ± 1.6
Sialidase 1 (lysosomal sialidase)	7.2 ± 2.7
Immediate early response 3	4.4 ± 1.6
Tumor necrosis factor (TNF superfamily, member 2)	3.6 ± 0.1
Lymphotoxin alpha (TNF superfamily, member 1)	0.2 ± 0.1
Transcription factor EB	0.3 ± 0.04

Maximum fold change with standard deviation for the genes on chromosome 6p21 at either 4 or 12 h of TSA treatment relative to the untreated control are shown.

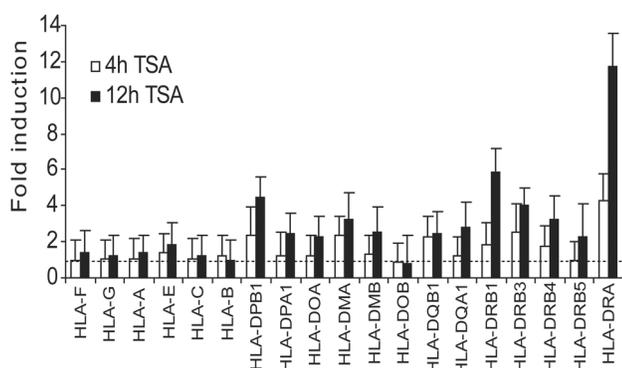


Figure 6. MHC locus response to TSA. Affymetrix analysis data from RJ225 cells cultured without or in the presence of 200 nM TSA for 4 and 12 h. The graph shows average fold induction with their corresponding error bars at 4 and 12 h after TSA of the various MHC genes represented in the array.

functions without affecting the expression of negatively acting non-classical MHC II genes.

DISCUSSION

HDAC inhibition is a strong modulator of the expression pattern of many cellular genes that has multiple therapeutic applications (27). In this report we have studied the molecular mechanism of gene induction by TSA at both a gene specific and genome-wide level. We determined the sequence of events that are required for the TSA-induced transcription of the MHC II gene DRA in the B cell type RJ225 that is lacking the master regulator CIITA.

We show that in RJ225 cells, the DRA gene is suppressed via repressive histone modifications such as histone H3 and H4

hypoacetylations and H3-K9 dimethylation. In the absence of the master coactivator CIITA, the class II enhanceosome is recruiting considerable amounts of HDACs and low amounts of HAT proteins. On the promoter of DRA gene TSA induces very rapid and strong histone acetylations on both H3 and H4 that are accompanied by an increase of histone H3 dimethylated K4 and a reduction of histone H3 dimethylated K9. A similar synergy in gene repression between histone deacetylation and K9 methylation was recently reported in the *Xenopus* oocyte system (28).

In correlation with the histone modifications, we found that TSA increases the intranuclear mobility of HDAC 1 and 2 in RJ225 cells. This effect can account for the reduction of the amount of HDACs residing on the promoter of DRA following TSA addition. As a consequence, acetylation of the promoter rises without an increase in the recruitment of HAT type cofactors. Contrary to its effects on HDACs, TSA affects the mobility of the class II enhanceosome factor RFX5 in the opposite way. This may be the basis of the higher DRA promoter occupancy observed by the ChIP experiments. We conclude that the effects of TSA on HDAC depletion and inhibition that permit chromatin hyperacetylation, in combination with enhanceosome stabilization, are sufficient to attract the transcription machinery and allow DRA expression.

We show that TSA induces DRA gene transcription in a B cell environment but not in HeLa cells. This selectivity of TSA-regulated transcription may reflect differences in chromatin environments. For instance, the DRA gene in B cells may be in a chromatin state permissive to the effect of TSA, whereas in HeLa cells the same gene may reside in a refractory chromatin structure. Restriction site accessibility studies using B-lymphoid and HeLa cells support this assumption (29).

Surprisingly, in spite of its non selective DAC inhibition, the TSA-elicited gene program in this B cell leukemia model has very little overlap with similar studies in other cell types, suggesting that its transcriptome-wide responses are highly cell type specific. In our system, TSA globally rescues MHC II expression in the absence of CIITA and upregulates many histone members that reside in the 6p21 chromosomal region. Many other up-regulated genes in this system are part of general and immune specific defences.

Inspection of the genes differentially regulated by CIITA (30) and TSA (this study) in RJ225 cells reveals that besides the MHC II, only two other genes, tubulin beta and PEG10 are in common. It is thus likely that tubulin beta and PEG10 share common regulatory mechanisms with MHC II genes.

Growing evidence positively correlates expression of MHC II with cancer cell differentiation or prognosis (31,32) and epigenetic inactivation of CIITA expression is found in tumor lines or cancer tissue. These results suggest that up-regulation of MHC II may be beneficial in cancer and are in agreement with the proposed importance of MHC II molecules in tumor-antigen recognition and the generation of an anti-tumor immune response. Indeed in recent studies exogenous CIITA has been used in a gene therapy approach to restore MHC II and/or tumor immune-resistance (33,34).

We show here that global up-regulation of MHC II genes in a CIITA deficient environment can also be achieved by an epigenetic modifier. Recent studies emphasize the cell cycle arrest and apoptotic properties of HDACi to justify their use in cancer therapy and ongoing clinical trials. Indeed recent

observations show that TSA induces the ability of antigen presentation and anti-tumor immunity (35–37). Our results extend the rational basis for using HDACi in cancer treatment by demonstrating the up-regulation of many host defence genes including MHC II genes that are positively involved in tumor-antigen immune presentation.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

We thank T. Makatounakis and G. Vrentzos for expert technical assistance and T. Agalioti for helpful comments on the manuscript. This work was supported by grants from the Hellenic Ministry of Education (Herakleitos KE1865 and EU Social Fund and National Resources—Pythagoras KA 1944), IMBB intramural funds to J.P. and the German National Genome Science Network (NGFN I) grant No. 01GS0113 to A.K.H. Funding to pay the Open Access publication charges for this article was provided by KE 1865 and IMBB.

Conflict of interest statement. None declared.

REFERENCES

- Fischle,W., Wang,Y. and Allis,C. (2003) Histone and chromatin cross-talk. *Curr. Opin. Cell Biol.*, **15**, 172–183.
- van den Elsen,P., Holling,T., Kuipers,H. and van der Stoep,N. (2004) Transcriptional regulation of antigen presentation. *Curr. Opin. Immunol.*, **16**, 67–75.
- Ting,J.P. and Trowsdale,J. (2002) Genetic control of MHC class II expression. *Cell*, **109**, S21–S33.
- Beresford,G.W. and Boss,J.M. (2001) CIITA coordinates multiple histone acetylation modifications at the HLA-DRA promoter. *Nature Immunol.*, **2**, 652–657.
- Masternak,K. and Reith,W. (2002) Promoter-specific functions of CIITA and the MHC class II enhanceosome in transcriptional activation. *EMBO J.*, **21**, 1379–1288.
- Spilianakis,C., Kretsovali,A., Agalioti,T., Makatounakis,T., Thanos,D. and Papamatheakis,J. (2003) CIITA regulates transcription onset via Ser5-phosphorylation of RNA Pol II. *EMBO J.*, **22**, 5125–5136.
- Kretsovali,A., Agalioti,T., Spilianakis,C., Tzortzakaki,E., Merika,M. and Papamatheakis,J. (1998) Involvement of CREB binding protein in expression of major histocompatibility complex class II genes via interaction with the class II transactivator. *Mol. Cell. Biol.*, **18**, 6777–6783.
- Fontes,J.D., Kanazawa,S., Jean,D. and Peterlin,B.M. (1999) Interactions between the Class II Transactivator and CREB Binding Protein increase transcription of Major Histocompatibility Complex Class II genes. *Mol. Cell. Biol.*, **19**, 941–947.
- Spilianakis,C., Papamatheakis,J. and Kretsovali,A. (2000) Acetylation by PCAF enhances CIITA nuclear accumulation and transactivation of Major Histocompatibility Complex Class II genes. *Mol. Cell. Biol.*, **20**, 8489–8498.
- Tzortzakaki,E., Spilianakis,C., Zika,E., Kretsovali,A. and Papamatheakis,J. (2003) Steroid receptor coactivator 1 links the steroid and interferon gamma response pathways. *Mol. Endocrinol.*, **17**, 2509–2518.
- Zika,E., Greer,S.F., Zhu,X.S. and Ting,J.P. (2003) Histone deacetylase 1/mSin3A disrupts gamma interferon-induced CIITA function and major histocompatibility complex class II enhanceosome formation. *Mol. Cell. Biol.*, **23**, 3091–3102.
- Zika,E. and Ting,J. (2005) Epigenetic control of MHC-II: interplay between CIITA and histone-modifying enzymes. *Curr. Opin. Immunol.*, **17**, 58–64.
- LeibundGut-Landmann,S., Waldburger,J., Krawczyk,M., Otten,L., Suter,T., Fontana,A., Acha-Orbea,H. and Reith,W. (2004) Specificity and expression of CIITA, the master regulator of MHC class II genes. *Eur. J. Immunol.*, **34**, 1513–1525.
- Chang,C., Guerder,S., Hong,S., van Ewijk,W. and Flavell,R. (1996) Mice lacking the MHC class II transactivator (CIITA) show tissue-specific impairment of MHC class II expression. *Immunity*, **4**, 167–178.
- Williams,G., Malin,M., Vremec,D., Chang,C., Boyd,R., Benoist,C. and Mathis,D. (1998) Mice lacking the transcription factor CIITA—a second look. *Int. Immunol.*, **10**, 1957–1967.
- Collinge,M., Pardi,R. and Bender,J. (1998) Class II transactivator-independent endothelial cell MHC class II gene activation induced by lymphocyte adhesion. *J. Immunol.*, **15**, 1589–1593.
- Brown,J., He,X., Westerheide,S. and Boss,J. (1996) Characterization of the expressed CIITA allele in the class II MHC transcriptional mutant RJ2.2.5. *Immunogenetics*, **43**, 88–91.
- Magner,W.J., Kazim,A.L., Stewart,C., Romano,M.A., Catalano,G., Grande,C., Keiser,N., Santaniello,F. and Tomasi,T.B. (2000) Activation of MHC class I, II, and CD40 gene expression by histone deacetylase inhibitors. *J. Immunol.*, **165**, 7017–7024.
- Peltonen,K., Kiviharju,T.M., Jarvinen,P.M., Ra,R. and Laiho,M. (2005) Melanoma cell lines are susceptible to histone deacetylase inhibitor TSA provoked cell cycle arrest and apoptosis. *Pigment Cell Res.*, **18**, 196–202.
- Zhang,X., Wharton,W., Yuan,Z., Tsai,S.C., Olshaw,N., Seto,E. and Lee,H. (2004) Activation of the growth-differentiation factor 11 gene by the histone deacetylase (HDAC) inhibitor trichostatin A and repression by HDAC3. *Mol. Cell. Biol.*, **24**, 5106–5118.
- Lachner,M. and Jenuwein,T. (2002) The many faces of histone lysine methylation. *Curr. Opin. Cell Biol.*, **14**, 286–298.
- Liang,G., Lin,J., Wei,V., Yoo,C., Cheng,J., Nguyen,C., Weisenberger,D., Egger,G., Takai,D., Gonzales,F. et al. (2004) Distinct localization of histone H3 acetylation and H3-K4 methylation to the transcription start sites in the human genome. *Proc. Natl Acad. Sci. USA*, **11**, 7357–7362.
- Peters,A., Kubicek,S., Mechtler,K., O’Sullivan,R., Derijck,A., Perez-Burgos,L., Kohlmaier,A., Opravil,S., Tachibana,M., Shinkai,Y. et al. (2003) Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol. Cell*, **12**, 1577–1589.
- Rice,J., Briggs,S., Ueberheide,B., Barber,C., Shabanowitz,J., Hunt,D., Shinkai,Y. and Allis,C. (2003) Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. *Mol. Cell*, **12**, 1591–1598.
- Alfonso,C. and Karlsson,L. (2000) Nonclassical MHC class II molecules. *Annu. Rev. Immunol.*, **18**, 113–142.
- Hake,S., Tobin,H., Steimle,V. and Denzin,L. (2003) Comparison of the transcriptional regulation of classical and non-classical MHC class II genes. *Eur. J. Immunol.*, **33**, 2361–2371.
- Marks,P., Richon,V., Miller,T. and Kelly,W. (2004) Histone deacetylase inhibitors. *Adv. Cancer Res.*, **91**, 137–168.
- Stewart,M., Li,J. and Wong,J. (2005) Relationship between histone H3 lysine 9 methylation, transcription repression, and heterochromatin protein 1 recruitment. *Mol. Cell. Biol.*, **25**, 2525–2538.
- Mudhasani,R. and Fontes,J. (2005) Multiple interactions between BRG1 and MHC class II promoter binding proteins. *Mol. Immunol.*, **42**, 673–682.
- Nagarajan,U., Bushey,A. and Boss,J. (2002) Modulation of gene expression by the MHC class II transactivator. *J. Immunol.*, **169**, 5078–5088.
- Rimsza,L., Roberts,R., Miller,T., Unger,J., LeBlanc,M., Braziel,R., Weisenberger,D., Chan,W., Muller-Hermelink,H., Jaffe,E. et al. (2004) Loss of MHC class II gene and protein expression in diffuse large B-cell lymphoma is related to decreased tumor immunosurveillance and poor patient survival regardless of other prognostic factors: a follow-up study from the Leukemia and Lymphoma Molecular Profiling Project. *Blood*, **103**, 4251–4258.
- Matoba,K., Iizuka,N., Gondo,T., Ishihara,T., Yamada-Okabe,H., Tamesa,T., Takemoto,N., Hashimoto,K., Sakamoto,K., Miyamoto,T. et al. (2005) Tumor HLA-DR expression linked to early intrahepatic recurrence of hepatocellular carcinoma. *Int. J. Cancer*, **114**, 231–240.

33. Hillman,G., Kallinteris,N., Lu,X., Wang,Y., Wright,J., Li,Y., Wu,S., Forman,J., Gulfo,J., Humphreys,R. *et al.* (2004) Turning tumor cells in situ into T-helper cell-stimulating, MHC class II tumor epitope-presenters: immuno-curing and immuno-consolidation. *Cancer Treat. Rev.*, **30**, 281–290.
34. Meazza,R., Comes,A., Orengo,A., Ferrini,S. and Accolla,R. (2003) Tumor rejection by gene transfer of the MHC class II transactivator in murine mammary adenocarcinoma cells. *Eur. J. Immunol.*, **33**, 1183–1192.
35. Chou,S.D., Khan,A.N., Magner,W.J. and Tomasi,T.B. (2005) Histone acetylation regulates the cell type specific CIITA promoters, MHC class II expression and antigen presentation in tumor cells. *Int. Immunol.*, **17**, 1483–1494.
36. Moldenhauer,A., Frank,R.C., Pinilla-Ibarz,J., Holland,G., Bocconi,P., Scheinberg,D.A., Salama,A., Seeger,K., Moore,M.A. and Nimer,S.D. (2004) Histone deacetylase inhibition improves dendritic cell differentiation of leukemic blasts with AML1-containing fusion proteins. *J. Leukoc. Biol.*, **76**, 623–633.
37. Khan,A.N., Magner,W.J. and Tomasi,T.B. (2004) An epigenetically altered tumor cell vaccine. *Cancer Immunol. Immunother.*, **53**, 748–754.