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B cell to plasma cell maturation is marked by the loss of MHC class II expression. This loss is due to the silencing of the MHC class II transcriptional coactivator CIITA. In this study, experiments to identify the molecular mechanism responsible for CIITA silencing were conducted. CIITA is expressed from four promoters in humans, of which promoter III (pIII) controls the majority of B cell-mediated expression. Chromatin immunoprecipitation assays were used to establish the histone code for pIII and determine the differences between B cells and plasma cells. Specific histone modifications associated with accessible promoters and transcriptionally active genes were observed at pIII in B cells but not in plasma cells. A reciprocal exchange of histone H3 lysine 9 acetylation to methylation was also observed between B cells and plasma cells. The lack of histone acetylation correlated with an absence of transcription factor binding to pIII, particularly that of Sp1, PU.1, CREB, and E47. Intriguingly, changes in chromatin architecture of the 13-kb region encompassing all CIITA promoters showed a remarkable deficit in histone H3 and H4 acetylation in plasma cells, suggesting that the mechanism of silencing is global. When primary B cells were differentiated ex vivo, most of the histone marks associated with pIII activation and expression were lost within 24 h. The results demonstrate that CIITA silencing occurs by controlling chromatin accessibility through a multistep mechanism that includes the loss of histone acetylation and transcription factor binding, and the acquisition of repressive histone methylation marks. The Journal of Immunology, 2006, 177: 3865–3873.

The MHC class II (MHC-II) molecule presents exogenously derived Ag to CD4+ T cells to initiate and regulate adaptive immune responses (1–3). MHC-II molecules are cell surface glycoproteins constitutively expressed on the surface of professional APCs, including macrophages, dendritic cells (4, 5), thymic epithelia, and B cells (6). By exposure to the immune cytokine IFN-γ, MHC-II expression can also be induced on most non-APCs, such as fibroblasts (7, 8). Expression of MHC-II genes is transcriptionally regulated by a group of transcription factors that bind a conserved regulatory region located 150 bp upstream of the transcription start site. These factors, including regulatory factor X (RFX) (9–12), CREB (13, 14), and NF-Y (15) are required for MHC-II gene expression. Although the binding of these factors to the MHC-II promoters is necessary, it is not sufficient for expression. CIITA, a non-DNA-binding transcriptional coactivator, is required for class II expression (16, 17). CIITA interacts directly with NF-Y, CREB, and RFX, while recruiting histone acetyltransferases such as CBP, as well as other basal transcriptional machinery to the promoters of MHC-II genes (reviewed in Refs. 18–20).

CIITA is transcriptionally regulated by four separate promoters (pI, pII, pIII, and pIV), each transcribing a unique first exon that will ultimately encode distinct CIITA isoforms (21). Differential expression of the various CIITA isoforms contributes to CIITA’s ability to regulate MHC-II expression in a cell type and developmentally specific manner. Promoter pI drives CIITA expression in splenic dendritic cells and macrophages (22, 23). Promoter pII is expressed at very low levels in humans, and little is known about its biological significance. Mouse, however, lack pII. Promoter pIV controls IFN-γ-inducible CIITA expression in most cell types (21, 24, 25). Finally, pIII primarily confers developmental and constitutive CIITA expression in B cells (26), as well as human CD4+ T cells (27, 28), monocytes, and plasmacytoid dendritic cells (29). Low levels of CIITA transcripts from pIV can also be detected in B cells (21, 24, 30).

MHC-II expression is developmentally regulated in B cells (31, 32) and can be observed as early as the pre-B cell stage (33–35). MHC-II expression increases upon B cell maturation and is constitutively expressed in mature B cells. During the B cell to plasma cell transition both MHC-II and CIITA gene expression are lost (6, 36–38). Transfection of CIITA into plasma cells can restore MHC-II expression (37), suggesting that silencing of MHC-II is solely due to the silencing of CIITA. Because B cell-specific expression is controlled by pIII, it is the regulation of expression at this promoter that is responsible for MHC-II silencing during this transition.

CIITA pII is regulated by a S’ promoter proximal regulatory region extending −322 bp upstream to + 124 bp downstream of the transcription start site (21, 26). In vivo genomic footprinting analyses identified several cis-regulatory elements termed site A, site B, site C, and activation response elements (ARE)1 and ARE2 (39). These elements were occupied in B cells but not in plasma cells, suggesting either the loss of the factors that interact with these elements or a loss in accessibility of this region to factor binding. Reporter assays and mutational studies indicated that ARE1 and ARE2 are critical for pII transcriptional activation in
both B cells and human T cells (39). An additional 5′UTR region, as well as an E box upstream of site C, are required for maximal transcriptional activity in B cells. The CREB protein has been shown to interact with ARE2 and the 5′UTR of pII. In addition, PU.1, IRF4, and E47 were shown to interact with site C, as well as two E box elements to potentially confer B cell-specific expression of pII. Although it is suggested that ARE1 binds a TEF-2-like factor in vitro, in vivo evidence for such factors has not been presented.

Originally identified as a B lymphocyte-induced maturation protein, Blimp1 (41) has been shown to function as a transcriptional repressor that controls in part the developmental fate of plasma cells (41, 42). The human ortholog PRD1-BF1 was originally identified due to its ability to bind and repress the IFN-β promoter through the PRD1 site (43). When ectopically expressed, Blimp1 can silence CIITA expression and was found associated with pIII DNA (44, 45). Blimp1 was also found to interact with members of the GROUCHO family of corepressors (46), as well as with G9a (47, 48). G9a catalyzes the dimethylation of histone H3 lysine 9 (49, 50), a mark associated with transcriptional silencing (reviewed in Ref. 51). Thus, one potential mechanism by which CIITA expression could be silenced during the transition between B cell to plasma cell is through the loss of epigenetic histone modifications associated with gene activation and the gain of silencing marks. Such changes would be predicted to have an impact on the accessibility of the pIII region to its cognate transcription factors. Chromatin remodeling has been previously shown to play an important role in CIITA expression during dendritic cell maturation (22) and may therefore play a role during B cell differentiation.

To investigate whether the above predictions with respect to chromatin accessibility and histone modification changes occur during the B cell to plasma cell transition, the histone code associated with CIITA promoters was examined in model human and murine cell lines. The results showed that in contrast to B cells, plasma cells displayed a general absence of gene activation marks across the entire CIITA upstream region encompassing all four human promoters. At the lymphocyte-specific promoter pIII, H3 lysine 9 acetylation, which was present in B cells, was replaced with a dimethyl mark in plasma cells, indicating a multistep mechanism of gene silencing. Chromatin inaccessibility prevented the binding of specific transcription factors to pIII in plasma cells, despite the fact that the factors were present at the same levels in both cell types. A time course of ex vivo B cell differentiation showed that the transcriptionally active histone marks are lost within 24 h of the process, indicating a rapid mechanism by which CIITA expression is silenced. These data provide evidence that the silencing of CIITA during this transition encompasses a mechanism that orchestrates the transition from an active chromatin state to a repressed and inaccessible state.

**Materials and Methods**

**Cell culture**

Raji B cells (CCL-86; American Type Culture Collection (ATCC)), a Burkett’s lymphoma-derived cell line, were grown in RPMI 1640 medium (Mediatech) supplemented with 5% FBS (HyClone Laboratories), 5% bovine calf serum (HyClone Laboratories), 100 U/ml penicillin, 100 U/ml streptomycin, and 0.25 mg/ml L-glutamine (Invitrogen Life Technologies). Raji B cells (CCL-1669; ATCC) were grown in RPMI 1640 medium with 2 mM L-glutamine, 0.05 mM L-2-ME and 10% heat-inactivated FBS. Primary spleenocytes were obtained from 5- to 6-week-old C57BL/6 mice. The use of animals for these experiments was approved by Emory University Institutional Animal Care and Use Committee. B lymphocytes were isolated by CD43 depletion using MACS beads (Miltenyi Biotec). Cells were treated for 5 days with 20 ng/ML IL-2 (Sigma-Aldrich or PeproTech), 10 ng/ML IL-5 (Sigma-Aldrich), and 20 μg LPS (Sigma-Aldrich) in RPMI 1640 medium with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate, 0.05 mM L-2-ME, and 10% heat-inactivated FBS. Primary spleenocytes were obtained from 5- to 6-week-old C57BL/6 mice. The use of animals for these experiments was approved by Emory University Institutional Animal Care and Use Committee. B lymphocytes were isolated by CD43 depletion using MACS beads (Miltenyi Biotec). Cells were treated for 5 days with 20 ng/ML IL-2 (Sigma-Aldrich or PeproTech), 10 ng/ML IL-5 (Sigma-Aldrich), and 20 μg LPS (Sigma-Aldrich) in RPMI 1640 medium with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate, 0.05 mM L-2-ME, and 10% heat-inactivated FBS. Primary spleenocytes were obtained from 5- to 6-week-old C57BL/6 mice. The use of animals for these experiments was approved by Emory University Institutional Animal Care and Use Committee. B lymphocytes were isolated by CD43 depletion using MACS beads (Miltenyi Biotec). Cells were treated for 5 days with 20 ng/ML IL-2 (Sigma-Aldrich or PeproTech), 10 ng/ML IL-5 (Sigma-Aldrich), and 20 μg LPS (Sigma-Aldrich) in RPMI 1640 medium with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate, 0.05 mM L-2-ME.

**Abs to transcription factors**

Abs to modified histone residues, as well as the Ab to Sp1, were purchased from Upstate Biotechnology. PU.1, IRF4, and E47 Abs were purchased from Santa Cruz Biotechnology. The rabbit Ab against CREB was previously described (14).

**Chromatin immunoprecipitation (ChIP) assays**

ChIP assays were preformed as previously described (52). One-tenth of the chromatin isolated from 4 × 10⁶ cells was used for each immunoprecipitation. The chromatin was preclearred with protein A beads to avoid non-specific binding. Immunoprecipitation of specific chromatin was performed overnight with 5 μg of each indicated Ab. The amount of immunoprecipitated ChIP DNA was determined by quantitative real-time PCR through comparison to a five point genomic DNA standard curve established during each experiment for each primer pair. To average samples between experiments and apply statistics, the ChIP immunoprecipitation DNA was normalized to the amount of the DNA input used in each assay. The data were then plotted as fold over the irrelevant Ab. In the transcription factor binding experiments, pIV primers were used as a negative control. In the histone acetylation studies, HLA-DRA promoter primers (52) served as a positive control for acetylation, whereas HLA-DRA promoter primers for the MCP1 gene (53), which is not expressed, was used as a negative control. Each ChIP analysis was repeated a minimum of three times from independent chromatin preparations. The Student’s t test was used to determine whether the obtained values were statistically significant. The primer sequences include: RealCIITApIII, 5′-TCACC AAATTCAGTCACAGTAAGG and 3′-GGCCCAAGGGCTGATTTC TC; RealtimepONE, 5′-GGTTGCTCCTGCTCGTGGTTTTC and 3′-AG TGTGCTAGTCGGCACTCGTTG; RealtimepTwo, 5′-CCGCTCTAGTGT CTCATCTATAAAGTG and 3′-TGGGCTCAGCACCTCG; RealtimepFOUR, 5′-CAGTGGAGGAAACCAGCTGAG and 3′-TGGAGGCAACCAAGCACCACCTACG.

**Real-time RT-PCR analysis**

RNA was prepared by using TRIzol Reagent (Invitrogen Life Technologies), and reverse transcription was conducted using the GeneAmp RNA PCR kit (Applied Biosystems) according to the manufacturer’s instructions. There was 2 μg of RNA used per sample. Each reaction contained a parallel control with no reverse transcriptase. One-tenth of a reverse transcriptase reaction mixture was used as a template in a real-time PCR. Real-time RT-PCR was performed as previously described (54). For each sample, a parallel PCR with primers for GAPDH transcripts was also performed as an input control. To compare samples, the results for all PCR assays were normalized to results obtained for the corresponding GAPDH RT-PCR assays, providing a relative quantitation value. The sequences of the primers used for real-time RT-PCR were as follows: HGAPDH, 5′-CCATGGGAAAGGTGATGGTGC and 3′-GGTTGGTGCAGGC ATTGCCTGATG; HCIITA, 5′-CTGAAGGATGTTGAACCTGTGGAAG AACG and 3′-GT CCCGGTACGTTTGGTCTACCTAC; HLA-DR, 5′-GAGTTTG TGATGCCCTAAGCTCTCCCA and 3′-CA GAGGCCTCTCCTCGGT CTGCTGCAAT; HSyndecan1, 5′-GAGCAGGACTTCACCTTTGA and 3′-TCCGGCTCCTCCTCCCTCTCC; Hemin, 5′-CA TCCATCTTATAAAGTG and 3′-TGGGCTCAGCACCTCG; RealtimepFOUR, 5′-CAGTGGAGGAAACCAGCTGAG and 3′-TGGAGGCAACCAAGCACCACCTACG.

For analysis of human markers, 10⁶ cells was used for each immunoprecipitation. The chromatin was preclearred with protein A beads to avoid non-specific binding. Immunoprecipitation of specific chromatin was performed overnight with 5 μg of each indicated Ab. The amount of immunoprecipitated ChIP DNA was determined by quantitative real-time PCR through comparison to a five point genomic DNA standard curve established during each experiment for each primer pair. To average samples between experiments and apply statistics, the ChIP immunoprecipitation DNA was normalized to the amount of the DNA input used in each assay. The data were then plotted as fold over the irrelevant Ab. In the transcription factor binding experiments, pIV primers were used as a negative control. In the histone acetylation studies, HLA-DRA promoter primers (52) served as a positive control for acetylation, whereas HLA-DRA promoter primers for the MCP1 gene (53), which is not expressed, was used as a negative control. Each ChIP analysis was repeated a minimum of three times from independent chromatin preparations. The Student’s t test was used to determine whether the obtained values were statistically significant. The primer sequences include: RealCIITApIII, 5′-TCACC AAATTCAGTCACAGTAAGG and 3′-GGCCCAAGGGCTGATTTC TC; RealtimepONE, 5′-GGTTGCTCCTGCTCGTGGTTTTC and 3′-AG TGTGCTAGTCGGCACTCGTTG; RealtimepTwo, 5′-CCGCTCTAGTGT CTCATCTATAAAGTG and 3′-TGGGCTCAGCACCTCG; RealtimepFOUR, 5′-CAGTGGAGGAAACCAGCTGAG and 3′-TGGAGGCAACCAAGCACCACCTACG.

**FACS analysis**

For analysis of human markers, 1 × 10⁶ cells were stained with anti-HLA-DR or anti-Syndecan-1 PE-conjugated Abs (BD Biosciences) and analyzed on a FACSCalibur apparatus. For analysis of mouse markers, 1 × 10⁶ cells were stained with anti-Ia/IE or anti-syndecan-1 PE-conjugated Abs (BD Biosciences) and analyzed on a FACS Calibur.
**EMSA analysis**

DNA protein interactions were analyzed by EMSA according to protocols previously described (13, 55). A DNA probe containing the ARE1 element of CIITA pIII was synthesized: ARE1, 5’-GGGGAGGGCTTAAAGGAGTGTGGTAAAAATTAGA-3’. Mutated probes were also used as competitors, including Mut1, 5’-GGGGAAATTATTAAAGGGAGTGTGGTAAATTAGA; Mut2, 5’-GGGGAGGGCTTAAATTGATGTGGTAAAATTAGA; and Mut3 5’-GGGGAGGGCTTAAGGGAGTAAACCAAAATTAGA. Ab supershift assays using anti-Sp1, Sp3, CIITA, AML1, p65 and p50 NF-κB, IκB, USF1, Stat1, IRF1, IRF4, CBP, cFos, cJun, AP2, ATF2, CBP, CREB, CREM, NF1, cMyc, cMyb, and RARα (Santa Cruz Biotechnology) were incubated with the DNA-binding reaction mixture for 20 min before electrophoresis.

**Results**

**Sp1 binds the ARE1 region of CIITA pIII in B cells**

CIITA and MHC-II expression is regulated by pIII in B lymphocytes (21, 26). In vivo genomic DNA footprinting and reporter assays have previously revealed several DNA-binding elements (Fig. 1A) that were occupied in B cells (our unpublished data and Ref. 28). Two regions, ARE1 and ARE2 were required for transcriptional activation of CIITA pIII (28, 39). Although CREB was identified as an ARE2-binding element (28), the factors interacting with ARE1 were not identified. To better understand the transcriptional regulation of CIITA pIII, it was necessary to first identify factors interacting with this critical region. EMSA to identify putative binding factors for the ARE1 region in vitro was performed using nuclear extracts prepared from the B cell line Raji. A specific protein-DNA complex was resolved with the ARE1 probe (Fig. 1B). EMSA analysis using Abs to various transcription factors identified the zinc finger transcription factor Sp1 as a potential candidate, as the Sp1 Ab supershifted the complex. Although a failure to supershift an EMSA product does not eliminate the possibility that the factor does bind the ARE1, a supershift was not seen by any of the other 16 Abs tested, including Sp3, a protein that shares sequence homology with Sp1. Additional EMSA analysis was conducted using labeled mutant DNA probes to demonstrate DNA binding specificity (Fig. 1, A and B). Of the mutant probes, only Mut2, a DNA probe containing mutations at the 5’ end of the ARE1 region, failed to bind the factor. Additional Ab...
supershifts demonstrated that Sp1 was the factor binding to the probes. These data indicate that Sp1 binds the ARE1 element in vitro.

To verify these in vitro findings, ChIP assays were conducted using Abs to an irrelevant Ab or Sp1. Real-time PCR analysis of the immunoprecipitated DNA with primers for CIITA pIII DNA indicated a significant level of Sp1 binding to pIII DNA (Fig. 1C). Sp1 binding was not observed at an irrelevant locus control (data not shown). This result indicates that Sp1 not only binds CIITA pIII in vitro, but also in vivo.

**CIITA and MHC-II expression in plasma cells**

To establish model cell lines and end points to monitor CIITA and MHC-II expression during the differentiation of B cells into plasma cells, the expression of CIITA and MHC-II genes were evaluated in human and murine B and plasma cell lines. Raji and BCL-1 were used as model mature human and murine B cell lines, respectively, and NCI-H929 and P3XAG were examined as human and murine plasma cell lines. Real-time RT-PCR conducted on RNA isolated from these cells showed that both MHC-II gene expression as well as CIITA expression was highly expressed in the B cell lines but silenced in the plasma cell lines (Fig. 2, A and B). CIITA expression in this case was assayed from pIII. Analysis by flow cytometry demonstrated a lack of MHC-II cell surface expression in the H929 plasma cell line as well (Fig. 2C), demonstrating that the cell lines provide a model system to investigate CIITA silencing.

**B cell factor occupancy is absent at CIITA pIII in plasma cells**

In addition to our identification of Sp1, CREB (56), Pu.1, IRF4, and E47 have been shown to interact with CIITA pIII in B cells (40). To determine whether the occupancy of these factors differs between B cells and plasma cells, ChIP assays for the presence of these factors were conducted on CIITA pIII in Raji and H929 cells. Sp1, CREB, PU.1, and E47 but not IRF4 were associated with CIITA pIII DNA by (Fig. 3A). Intriguingly, in H929 plasma cells the binding of Sp1, CREB, and E47 was the same as a nonspecific control Ab, and the binding of PU.1 was reduced by 3- to 4-fold. IRF4 also showed no binding. The failure to assemble at pIII may be due to the loss of expression of one or all of these factors. To determine whether this loss was the case, Western blot analysis for each of these proteins was conducted (Fig. 3B). As shown, CREB, Sp1, E47, and PU.1 were expressed similarly in both Raji and H929 cells. This finding suggests that a more active or alternative epigenetic mechanism may be at play to regulate the occupancy of pIII in plasma cells. These findings are in agreement with previous in vivo genomic footprinting comparisons between B cells and plasma cells (our unpublished observations) and (39).

**Changes in histone architecture at CIITA pIII**

One mechanism that may limit the accessibility of factors to CIITA pIII in plasma cells may be due to changes in the posttranslational modifications of the local histones that package the pIII DNA, as such modifications regulate the accessibility and transcriptional competence of a locus (reviewed in Refs. 57 and 58). To determine the histone code associated with pIII, as well as characterize changes in local chromatin architecture during B cell to plasma cell differentiation, ChIP analysis using Abs to acetylated histones H3 and H4 was performed on the model cell lines. In Raji and BCL-1 B cells, high levels of histone H3 and modest levels of histone H4 acetylation were observed (Fig. 4A). Intriguingly, in H929 plasma cells the binding of Sp1, CREB, and E47 but not IRF4 were associated with CIITA pIII DNA by (Fig. 3A). Sp1, CREB, PU.1, and E47 have been shown to interact with CIITA pIII in B cells (40). To determine whether the occupancy of these factors differs between B cells and plasma cells, ChIP assays for the presence of these factors were conducted on CIITA pIII in Raji and H929 cells. Sp1, CREB, PU.1, and E47 but not IRF4 were associated with CIITA pIII DNA by (Fig. 3A). Intriguingly, in H929 plasma cells the binding of Sp1, CREB, and E47 was the same as a nonspecific control Ab, and the binding of PU.1 was reduced by 3- to 4-fold. IRF4 also showed no binding. The failure to assemble at pIII may be due to the loss of expression of one or all of these factors. To determine whether this loss was the case, Western blot analysis for each of these proteins was conducted (Fig. 3B). As shown, CREB, Sp1, E47, and PU.1 were expressed similarly in both Raji and H929 cells. This finding suggests that a more active or alternative epigenetic mechanism may be at play to regulate the occupancy of pIII in plasma cells. These findings are in agreement with previous in vivo genomic footprinting comparisons between B cells and plasma cells (our unpublished observations) and (39).

Although the above ChIP analysis of H3 and H4 provides an overall indication of chromatin accessibility, it does not address the dynamics of specific modifications that may ultimately indicate

![FIGURE 2. CIITA and MHC-II expression in B cells and plasma cells.](image-url)

RNA was isolated from Raji and BCL-1 B cell lines or H929 and P3XAG plasma cell lines. mRNA levels of human or mouse CIITA (A) and MHC-II (B) were quantitated by real-time RT-PCR. The expression of each mRNA level is expressed relative to GAPDH control. C, Raji and H929 cells were incubated with PE-conjugated HLA-DR-specific Abs. Cell surface expression of MHC-II was then analyzed by flow cytometry.

![FIGURE 3. Transcription factor binding is lost in plasma cells, although all requisite factors are present.](image-url)

A, ChIP assays using Abs for Sp1, CREB, E47, PU.1, and IRF4 were used to compare factor binding at pIII in Raji and H929 cells. The DNA recovered from the ChIP assays was normalized to input chromatin and then normalized to an irrelevant Ab control. The mean raw value for the irrelevant Ab control was 2.8 ng ± 1.7. B, Western blot analysis was conducted on equal amounts of Raji or H929 whole cell extract and probed with the indicated antisera.
the factors that are regulating accessibility. To further characterize the pIII chromatin architecture, ChIP was conducted using Abs to specific histone acetylation of H3 lysines 9, 18, and 27, modifications associated with transcriptional activation. B cells displayed a high level of histone H3 acetylation at lysines 9, 18, and 27 (Fig. 4B). In the plasma cell lines, acetylation of lysine 9 and lysine 27 was absent or near absent, and acetylation of lysine 18 was reduced 8- to 10-fold when compared with the B cell lines. The levels of histone H3 dimethyl lysine 4, which indicate a region competent for transcription (59), and trimethyl lysine 4, which indicate a promoter region actively being transcribed (60), were also examined. Both human and canine B cells displayed high levels of H3 lysine 4 dimethylation and trimethylation (Fig. 4B). In the plasma cell lines, the level of H3 lysine 4 dimethylation was between two and four times lower than the comparable B cell line, suggesting that the region was less competent for transcription and less accessible. In addition, trimethylation of lysine 4, a mark for active transcription, was robust in both B cell lines and completely absent in the plasma cell lines. Importantly, both human and canine B cell systems provided near identical results, indicating that the modifications observed and the loss of these modifications likely occur through similar mechanisms.

Histone H3 lysine 9 modification has been suggested to represent a molecular switch between active and silenced chromatin. Although acetylation of this site is an indication of transcriptional activation (58), methylation is indicative of transcriptional silencing (61). As discussed, histone H3 lysine 9 acetylation was robust in the B cell lines and absent in the plasma cell lines. To determine whether the switch of modifications had occurred, ChIP assays for H3 dimethyl lysine 9 were conducted. Histone H3 lysine 9 dimethylation was not observed in Raji or BCL-1 B cells but was clearly observed in both plasma cell lines (Fig. 4C), indicating that there is an active mechanism to repress/silence CIITA expression.

Global changes in CIITA promoter histone architecture

The silencing of CIITA pIII as indicated by the loss of activating and gain of silencing epigenetic chromatin marks may not be limited to just pIII and may be more widespread and include the other CIITA promoter regions. To test this hypothesis, the nucleosome modifications at the other CIITA promoters, which span 13 kb of CIITA 5' DNA, were examined by ChIP (Fig. 5). Each promoter region displayed a significant level of acetylation of H3 lysine 9, lysine 18, and lysine 27 in Raji cells, albeit less than that seen at the constitutively active pIII. In H929, this acetylation was completely absent at pI and nearly absent at pIV. A residual level of lysine 18 acetylation was observed at pII. The levels of H3 lysine 4 dimethylation were also high at all three promoters in Raji cells and greatly reduced in the H929 plasma cells. In Raji cells, only pIV displayed trimethylation of H3 lysine 4, a marker for transcriptional activation. This result correlates with the fact that low levels of CIITA transcripts have been observed from pIV in B cells (30, 62). H3 lysine 4 trimethylation was absent in H929 cells. These data suggest that not only is the lymphocyte-specific promoter silenced, but the chromatin in the entire region is deacetylated and likely to be silenced in plasma cells.

CIITA pIII regulation in primary differentiated cells

Although model cell lines are invaluable tools for understanding molecular mechanisms, their immortalization may prevent an accurate representation of the primary cell situation. CIITA silencing in the context of a primary cell system was therefore examined.
CD43− splenic B cells were isolated from C57BL/6 mice. The cells were stimulated with IL-2, IL-5, and LPS to induce plasma cell differentiation. After 5 days of stimulation, mRNA levels of both CIITA and class II molecules were greatly reduced (Fig. 6A). In addition, CIITA protein expression was lost (Fig. 6B) and cell surface MHC-II expression was reduced significantly (data not shown). Increased mRNA levels of the plasma cell markers Blimp1, XBP1, and syndecan 1, as well as decreased levels of B cell-specific factors, Bcl-6 and Pax5 were also observed, verifying the plasma cell phenotype (data not shown).

ChIP was used to analyze factor binding and histone acetylation in these primary differentiated cells. After 5 days, a significant loss in the binding of Sp1, CREB, E47, and PU.1 to pIII DNA was observed (Fig. 6C). Acetylation and methylation were also examined in a time course following stimulation with IL-2, IL-5, and LPS. Whereas unstimulated cells displayed a high level of histone acetylation at pIII, differentiated cells showed marked decreases in histone acetylation over the time course (Fig. 6D). Examination of individual histone modifications showed a rapid decrease in acetylation at 12 h, with histone H3 acetylation of lysine 9 and lysine 27 approaching background levels (Fig. 6E). Histone H3 lysine 18 acetylation, although markedly decreased at 5 days, was delayed. Similarly, H3 lysine 4 dimethylation did not reach a plateau until after 24 h (Fig. 6E). In contrast, H3 lysine 4 trimethylation decreased rapidly. Taken together, these results suggest that histone deacetylation and loss of histone methylation plays a significant role in the silencing of CIITA pIII, not only in human but in mouse as well.
Discussion

The developmental control of MHC-II expression in the B lymphocyte lineage reflects the biology and function of B cells during the generation of humoral immune responses. MHC-II gene expression and subsequent Ag presentation in B lymphocytes is principally regulated by CIITA driven from pIII. As B cells differentiate into plasma cells where their terminal function is to secrete Ab, Ag presentation and MHC-II expression are no longer necessary. The loss of CIITA pIII expression is marked and controlled by changes in chromatin accessibility and transcriptional competence and can be divided into several stages based on our data.

Mature B cells express high levels of MHC-II and CIITA. pIII is highly acetylated at multiple histone residues, each serving as activation markers and substrates for additional chromatin remodeling enzymes and components of the transcriptional machinery. The chromatin is fully accessible to at least four transcription factors, Sp1, E47, PU.1, and CREB. Moreover histone methylation marks, associated with active transcription, are present. Additionally, low levels of transcripts have been observed from the downstream pIV region (21, 24, 30, 62), demonstrating that this region is also competent for transcription.

In the initial stage of B cell differentiation to plasma cell, histone H3 acetylation marks that are associated directly with transcriptional activation, such as lysine 9 and lysine 27, are lost, as is histone H3 lysine 4 trimethylation, a mark associated with actively transcribed genes. The loss of these marks occurs within the first 12 h of the ex vivo differentiation process. The loss of H3 lysine 9 acetylation can have profound effects as this mark is associated with the recruitment of components of the general transcription machinery to promoters (63). Acetylation marks can be rapidly removed by histone deacetylases (HDACs), suggesting that the first step may be the recruitment of a HDAC. Alternatively, removal of the acetylation marks can be mediated by the replacement of histones (reviewed in Ref. 64). Recently, H3 replacement with variant H3.3 has been shown to be associated with transcriptional activation of previously silenced genes (65, 66). It is possible that an exchange of modified H3 with histone variants (67) is associated with initial steps toward gene silencing. Also occurring at the same time is the loss of histone H3 lysine 4 trimethylation. The mechanism by which histones lose trimethylated lysine modifications is not known, although it is possible again that these histones are replaced/ exchanged with unmodified histones (reviewed in Ref. 64).

A second or a parallel event is the loss of acetylation of histone H3 lysine 18 and the decrease of dimethylated lysine 4. Lysine 18 deacetylation displayed slower kinetics than the loss of lysine 9 or lysine 27 acetylation, suggesting that it may be mediated by a different HDAC/complex or that the HDAC responsible has a lower affinity for lysine 18 acetyl residues. The delay in the loss of H3 dimethyl lysine 4 may indicate a stepwise process of lysine 4 dimethylation (i.e., tri- to di- to mono-). H3 dimethyl lysine 4 can be removed by the coREST complex containing the histone lysine-specific demethylase LSD1 (68, 69), suggesting a possible role for this factor in this process. This mark is associated with overall accessibility (59); thus, it is somewhat intriguing that some pIII regions still carry this mark. This finding was also true in the plasma cell lines, which are fully differentiated, possibly indicating some plasticity even at silenced genes. The loss of DNA binding factor occupancy likely occurs at a time point that is concordant with the loss in acetylation and accessibility. This timing naturally represents a significant step as these factors are likely responsible for the recruitment of the histone acetyltransferases to pIII.

The ARE1 binding element within pIII was identified previously as a critical region required for CIITA transcription in both B cells and human T cells (28, 39); however, no factors associated with this element were identified. Sp1 was found to bind to this site. Sp1 is ubiquitously expressed in both B cells and T cells (reviewed in Ref. 70) and belongs to a large family of factors known to bind GC-rich sequences. Sp1 as well as other Sp1 like molecules have been shown to interact with CBP/p300 coactivators (71, 72) as well as TFIID (73). In addition to Sp1, the factors CREB, PU.1, and E47 are also known to recruit CBP/p300 (74–76), basal transcriptional machinery (77), and RNA polymerase II (78) to promoters. CBP and p300 are responsible for modification of histone H3 lysine 18 in vivo (79). Thus, it is likely that the combination of these DNA binding factors mediate the recruitment of coactivators that are responsible for the histone code associated with the CIITA locus.

The last stage involved in CIITA silencing is the dimethylation of histone H3 lysine 9. Histone H3 lysine 9 dimethylation, a mark of transcriptional silencing, was observed in both human and murine plasma cell lines. Thus, the switch from a transcriptionally active to silent state is revealed by this lysine modification exchange. Despite several attempts, this mark was not observed in the primary differentiated mouse B cells. Several reasons could account for failure to detect this mark. The first is that the 5-day ex vivo culture may not be long enough to allow this complete change in chromatin structure to have occurred. A second possibility is that the ex vivo differentiation process lacks the necessary signaling events required to completely silence CIITA and therefore only shows the initial stages of the process. Because the endpoint stages of in vivo differentiation are revealed by the plasma cell lines, this possibility is favored. The dimethyl lysine 9 mark can be catalyzed by histone methyltransferases G9a, GLP, SUVar39h1, or SUVar9h2 (reviewed in Ref. 80) and can serve as a substrate for the heterochromatin protein-1 (HP1) (81). Minimal binding of HP1 was observed by ChIP in H929 cells (data not shown).

Previous studies have suggested that CIITA silencing is mediated by expression and binding of Blimp1 to pIII in plasma cells (44). Blimp1 is a transcriptional repressor responsible in part for initiating plasma cell differentiation (41, 82). Exogenously expressed tagged Blimp1 in a pre-B cell line or in IgM- and LPS-stimulated splenocytes down-modulated CIITA 4-fold, and was found associated with pIII, indicating a role for this factor in orchestrating CIITA silencing (44, 45). Experiments in which Blimp1 was exogenously expressed by either retrovirus or adenovirus infection in mature human or murine B cells displayed a modest increase in plasma cell markers but failed to ablate CIITA to the levels seen in our system (data not shown). Based on these results, we suggest that Blimp1 may be necessary but not sufficient to completely silence CIITA. It is intriguing that Blimp1 can interact with G9a in vitro (47). This interaction may serve as the mechanism to epigenetically mark CIITA chromatin in the silent state once differentiation is complete.

Epigenetic mechanisms are critical to B cell development. Treatment of cells with HDAC inhibitors (trichostatin and butyrate) alone can induce splenic B cell to plasma cell transition as indicated by increased expression of plasma cell specific genes, such as Blimp1, J chain, and syndecan-1, and CD43, and down-modulation of IgM, Pax5, and c-myc. In these experiments, however, MHC-II cell surface expression was not affected (83), suggesting that simple inhibition of deacetylases is not sufficient for MHC-II expression in splenic B cells. Conversely, trichostatin can induce endogenous CIITA pI and pIII CIITA expression in macrophage and dendritic cells and in plasmacytomas, respectively (data not shown) and (84). It remains unclear whether this re-expression of CIITA is due to specific effects on the HDACs targeted toward the promoters or results from more global effects.
CIITA expression from pl in dendritic cells is regulated by epige-
netic mechanisms as well (22). Immature dendritic cells express CIITA until exposed to TNF-α, LPS, or other maturation stimu-
lants. Stimulated dendritic cells down-regulate CIITA mRNA and pro-
tein levels. Unlike the B cell to plasma cell transition, analysis of the pl region did not show changes in the in vivo footprint region during this differentiation, but rather a global deacetylation of histones H3 and H4 across the entire 13 kb region encompassing the CIITA promoters (22). In this study, a similar change in global acetylation during the B cell to plasma cell transition was ob-
served. Together, these data suggest that histone deacetylation and global chromatin remodeling of the CIITA upstream regulatory region is a common mechanism for developmental silencing of CIITA among APCs.

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Disclosures

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References


10. Masternak, K., E. Barras, M. Zufferey, B. Conrad, G. Corthals, R. Aebersold,


16. Sartoris, S., G. Tosi, A. De Lernia Barbaro, T. Cestari, and R. S. Accolla. 1996. Active suppression of the class II transactivator-encoding AIR-I locus is respon-
17. Silacci, P., A. Mottet, V. Steimle, W. Reith, and B. Mach. 1994. Developmental extinction of major histocompatibility complex class II gene expression in plas-


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EPIGENETIC REGULATION OF CIITA


