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Differential regulation of chromatin structure of the murine 3′ IgH enhancer and IgG2b germline promoter in response to lipopolysaccharide and CD40 signaling

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Abstract

Class switch recombination (CSR) of murine immunoglobulin heavy chain (IgH) is controlled by germline transcription-coupled modification of the accessibility of the highly repetitive switch regions (S) located upstream of the constant region genes. Activation of the 3′ IgH enhancer (3′E) is believed to regulate CSR during B cell terminal differentiation, although the detailed molecular mechanism remains unclear. Here, we show that BAF57 and BRG1, two essential subunits of murine SWI/SNF complex, differentially associate with the DNase I hypersensitive region HS1/2 of 3′E and the IgG2b germline promoter in response to LPS activation or CD40 engagement. Both LPS and CD40 signaling cause SWI/SNF complex to dissociate from HS1/2 and associate with their responsive IgG2b germline promoter, suggesting the potential fluidity of chromatin structure and specific regulatory mode for the ATP-dependent chromatin remodeler during CSR. More interesting, increase in histone acetylation is either inverse or parallel with the action of SWI/SNF complex at HS1/2 enhancer or IgG2b germline promoter, respectively. Chromatin immunoprecipitation experiments show that alteration of histone H3 and H4 acetylation has overall similarities in response to LPS and CD40 signaling, with H3 hyperacetylated and H4 hypoacetylated at the HS1/2 enhancer and reversed modification patterns at the IgG2b germline promoter. Finally, the specificity of LPS and CD40 signaling in control of CSR could be partially coded by the specific acetylation marking of H3 and H4. Our results further strengthen the notion that chromatin remodeling plays a critical role in CSR.
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Keywords: Chromatin remodeling; Histone acetylation; IgH; Class switch recombination

1. Introduction

Upon encountering antigens, small resting B lymphocytes become activated and differentiate into plasma cells that have undergone CSR to secrete various classes of immunoglobulins. The genetic alterations during CSR involve the replacement of IgH constant region (C_H) gene Cμ with one of the downstream constant region genes (Cγ, Cε or Cα) by recombination between the donor and recipient S regions located upstream of the cognate C_H genes, without modifying the variable region gene segments (Stavnezer, 2000). This process is regulated by germline transcription-coupled modifications of the accessibility of the S regions and activation of 3′E located ∼4 kb downstream of Cα. The murine 3′ IgH enhancer spans ∼30 kb and consists of four DNase I hypersensitive segments, designated HS3a/Cα3′E, HS1/2, HS3b

Abbreviations: BAF, Brahma-associated factor; BRG, Brahma-related gene; ChIP, chromatin immunoprecipitation; CSR, class switch recombination; HDAC1, histone deacetylase 1; IgH, immunoglobulin heavy chain; LPS, lipopolysaccharide; SWI/SNF, switching defective/sucrose nonfermenting

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and HS4 (Khamlichi et al., 2000). Genetic and biochemical analyses indicate that HS1/2 is not active in resting B cells but can be activated via CD40 crosslinking (T cell-dependent signal, TD) or lipopolysaccharide (LPS) stimulation (T cell-independent signal, TI) (Arulampalam et al., 1994; Grant et al., 1996). Similar to HS1/2, the activities of HS3b and HS4 are restricted to the late stage of B cell development (Pinaud et al., 2001; Shi and Eckhardt, 2001). Although the function of 3′E in CSR remains elusive (Manis et al., 2003; Pinaud et al., 2001), spontaneous deletion of the entire 3′E causes a severe reduction in IgH expression and CSR in plasmacytoma cell lines (Gregor and Morrison, 1986; Michaelson et al., 1995). In fact, gene-targeting experiments indicated that, in concert with the intronic enhancer (Eμ), the 3′ IgH enhancer plays an essential role in CSR during B cell terminal differentiation (Chaudhuri and Alt, 2004). Transfection experiments also indicate that HS1/2–HS3–HS4 functions as a locus control region active only in mature B cells (Madisen and Groudine, 1994) and can alter the upstream c-*myc* promoter usage by modification of its histone acetylation (Madisen et al., 1998).

The nucleosome is the fundamental unit of chromatin structure in eukaryotic cells and plays crucial roles in virtually all aspects of genomic function (Kornberg and Lorch, 1999; Wolffe and Hayes, 1999). The nucleosome core particle comprises a histone octamer, two copies each of H2A, H2B, H3 and H4, around which are wrapped 146 base pairs of DNA (Felsenfeld and Groudine, 2003; Luger and Richmond, 1998). Therefore, the physical barrier by dense chromatin in a cell becomes the rate-limiting step of transcription initiation and elongation (Adams and Workman, 1993; Felsenfeld, 1992; Studitsky et al., 2004) and it must undergo topological changes from "heterochromatin" (off) to "euchromatin" (on) by means of chromatin remodeling to ensure gene expression. Transcription co-activator complexes comprise factors that either alter nucleosome conformation (e.g., SWI/SNF complex) in an ATP-dependent manner (Lusser and Kadonaga, 2003; Martens and Winston, 2003; Sudarsanam and Winston, 2000) or covalently modify the exposed tails of the core histones (acetylation, phosphorylation, methylation, ubiquitination and ADP-ribosylation) in an ATP-independent fashion. Both ATP-dependent and ATP-independent chromatin remodeling function in concert to regulate transcription. Multi-layer configuration of chromatin structure and crosstalk among various histone modification marks thereby contribute to the heterogeneity and specificity in regulation of gene expression (Jenuwein and Allis, 2001; Narlikar et al., 2002; Peterson and Laniel, 2004; Strahl and Allis, 2000; Turner, 2002). Of these histone modifications, acetylation of the ε-amino group of defined lysine residues has been extensively studied.

The mammalian SWI/SNF complex contains 9–12 BRG1-associated factors (BAF) (Peterson and Logie, 2000; Wang et al., 1996). Genetic and biochemical studies suggested that the mammalian SWI/SNF complex contributes to T cell lineage bifurcation by direct repression of CD4 and activation of CD8 (Chi et al., 2002), reminiscent of the role of the yeast SWI/SNF complex in mediating mating-type switching. The SWI/SNF complex has also been found to regulate V(D)J rearrangement of the variable region genes by selective association with the recombination active regions of both the T cell receptor (TCR) and immunoglobulin loci (Chi, 2004). However, whether the SWI/SNF complex plays a role in CSR still remains unclear. On the other hand, there is a growing body of evidence indicating that histone modification may play a critical role in CSR. For example, although insufficient to guarantee CSR, there is a good correlation between germline transcription activation and histone H3 and/or H4 hyperacetylation in S regions (Li et al., 2004; Nambu et al., 2003). CSR seems to require activation-induced cytidine deaminase (AID), which has recently been proved to involve in the accessibility control of S region chromatin in a germline transcription-coupled fashion (Chaudhuri and Alt, 2004). However, whether chromatin remodeling of 3′E plays any role in CSR is still elusive.

The existence of DNase I hypersensitive regions in 3′E during B cell terminal differentiation strongly suggests that the chromatin configuration of this locus might be temporally regulated and may play a role in CSR. Here, we show that SWI/SNF complex differentially associates with the HS1/2 enhancer and the upstream IgG2b germline promoter in response to LPS (TI) and CD40 (TD) signaling. Concerted with changes of chromatin configuration, a unique histone acetylation pattern might exist to determine the specificity of differential B cell activation signals for CSR.

2. Materials and methods

2.1. Cell culture and reagents

WEHI 231 cells were maintained in RPMI 1640 (HyClone, Utah) supplemented with 10% fetal bovine serum (HyClone), 50 μM β-mercaptoethanol (Serva, Germany), 100 unit/mL penicillin and 100 μg/mL streptomycin and 2 mM L-glutamine (HyClone) at 37 °C and 5% CO_2. Where indicated, WEHI 231 cells were treated with either 50 μg/mL LPS for 8 h or 1 μg/mL anti-CD40 (BD Pharmingen, HM40-3) for 2 h.

All fine chemicals, acetyl coenzyme (Ac-CoA) and histone (Type II-A) were purchased from Sigma. Herring sperm DNA and Superscript II reverse transcriptase were from Invitrogen. Oligo-dT primer was from Promega. Glycogen and sequencing grade trypsin was from Roche (Switzerland), proteinase K from Merck (Germany) and MagnaBind streptavidin beads and ECL SuperSignal was from Pierce (IL, USA). Antibodies used in the work: anti-CD40 (HM40-3, BD Pharmingen); anti-BAF57 (Drs. R. Crabtree, W. Wang and Z Wen; ActiveMotif, Carlsbad, CA); anti-BRG1, anti-NF-κB p65 (F-6), anti-CBP (C-1) and anti-HDAC1 (Santa Cruz, CA); anti-AcH3, anti-AcH4 and antibodies specific to each

individual acetyl-Lys: -H3AcK9, -H3AcK14, -H4AcK5, -H4AcK8, -H4AcK12 and -H4AcK16 (Upstate Biotechnology).

2.2. Affinity purification of HS1/2 binding proteins

Biotinylated DNA probe encompassing nt 791–1105 of murine HS1/2 enhancer (NCBI Accession no.: X62778) was PCR amplified using pGL3-VE (Tang and Sharp, 1999) as the template and PAGE purified as described previously (Tang et al., 1994). The sequences of primers were: forward (5′-biotin-GGCCTATGCTGGGAGTC-3′) and reverse primer (5′-TAGACCCCTAAGGGTAG-3′). A 5 pmol of biotinylated HS1/2 probe was affinity-conjugated to 100 μL MagnaBind streptavidin beads after blocked with 300 ng sonicated Herring sperm DNA according to manufacturer's manual (Pierce). After wash with PBS for three times, beads were mixed with 250 μg nuclear extracts and the final volume was brought up to 500 μL with nuclear buffer D (Dignam et al., 1983). After being rolled for 1 h at 37 °C, beads were magnetically concentrated and washed for three times with wash buffer (10 mM HEPES, pH 7.9, 300 mM NaCl, 1 mM EDTA). The bound nuclear proteins were resolved in 8% SDS-PAGE and visualized by Coomassie Blue staining. Indicated protein bands were sliced out and digested with sequencing grade trypsin. The peptide mixture was solublized in 2 μL matrix solution (10 mg/mL of L-cyano-carboxy-cinnamic acid in 50% acetonitrile/0.1% TFA) and 0.5 μL was spotted onto the plate for MALDI-TOF mass spectrometric analysis (Bruker Reflex III, Germany). Protein identities were scored by Mascot search engine (www.matrixscience.com) using monoisotopic mass values (data not shown) with parameter settings at taxonomy *musculus*, missed cleavage at 2, variable oxidation HW, peptide tolerance ± 1. BAF57 was identified for its top score at 56 ($P < 0.05$).

2.3. On beads HAT assay

Histone acetyltransferase (HAT) activities associated with biotinylated HS1/2 probe were assessed as described previously with minor modifications (He et al., 2003). In brief, the protein–HS1/2 DNA complex on magnetic beads was resuspended in 20 μL HAT buffer [20 mM MOPS (pH 6.5), 5 mM $MgCl_2$, 0.25 mM acetyl-CoA and 200 mM histones] and incubated at 37 °C for 1 h. The reaction was stopped by magnetic separation and the supernatant was boiled in 30 μL SDS-PAGE loading buffer. Acetylated histones were detected by Western blotting with Ac-Lys polyclonal antibody (1:1000 in PBST plus 1% milk powder, overnight at 4 °C) and visualized with ECL (Pierce, SuperSignal).

To assess the HAT activity co-precipitated with BAF complex, 300 μg nuclear extracts from WEHI 231 cells primed or not primed with LPS were first immunodepleted with 3 μL BAF57 polyclonal antibody or 5 μL BRG1 polyclonal antibody. The immunodepletion efficiency was assessed by Western blotting after electrophoretic separation of immunodepleted supernatants in 10 or 6% SDS-PAGE for BAF57 or BRG1, respectively. The remaining fraction was subjected to HAT assays as above.

2.4. Chromatin immunoprecipitation assays

Chromatin immunoprecipitation (ChIP) was performed as previously described (Alberts et al., 1998; Braunstein et al., 1993) with minor modifications. In brief, 2×10^6 WEHI 231 cells in 2 mL phosphate-buffered saline (PBS) were fixed with 1% formaldehyde at room temperature for 10 min and extensively washed with ice-cold PBS. Crosslinked nuclei were sonicated in a ice–water cup horn (Sonifier 450, Branson) to shear DNA length in between 200 and 1000 bp. Nuclear lysates were pre-cleared with 50 μL protein A-sepharose beads and immunoprecipitated with the anti-sera, where indicated at 4 °C for 16 h: anti-BAF57 (5 μL), anti-BRG1 (10 μL), anti-NF-κB (10 μL), anti-CBP (10 μL) and anti-HDAC1 (10 μL), anti-AcH3 (3 μL), anti-AcH4 (3 μL), anti-H3AcK9, anti-H3AcK14, anti-H4AcK5, anti-H4AcK8, anti-H4AcK12 and anti-H4AcK16 (5 μL) antibodies. DNA fragments in the immune complex were precipitated with 50 μL protein A-sepharose slurry at 4 °C for 2 h, after proteinase K digestion, DNA was phenol extracted and ethanol precipitated using glycogen as the carrier. Semi-quantitative PCR was performed to assess transcription activation at HS1/2 enhancer (nt 791–1105) and IgG2b promoter (nt from −283 to −133) forward primer 5′-tctatgggtctcaagaggacc-3′ and reverse primer 5′-tcgaaactaggtgacacgggac-3′) (Zhang et al., 1993).

2.5. Reverse transcription

RNA was isolated for reverse transcription PCR (RT-PCR) using RNeasy mini kit (Qiagen). The first cDNA strand was synthesized using 2.5 μg total RNA and 200 U Superscript II reverse transcriptase and 0.5 μg oligo-dT primer. PCR amplification was conducted as previously described (Strom et al., 1999) using 1 μL first-strand product and 20 pmol specific primers for IgG2b germline transcript (forward primer 5′-AGAGAAAGGTGCCTACCTGC-3′, reverse primer, 5′-TGTGACACTCCTTGCATGGAG-3′). RT-PCR of β-actin was performed in parallel as an internal control (forward primer 5′-GTCGACAACGGCTCCGGC-3′, reverse primer 5′-GGTGTGGTGCCAGATCTTCT-3′).

2.6. Nuclear Western blotting

WEHI 231 cells in a six-well plate (2×10^6) were treated with 50 μg/mL LPS or 1 μg/mL anti-CD40 antibody for the time indicated. Nuclear extracts were prepared as previously described (Tang and Sharp, 1999) and nuclear translocation of NF-κB p65 was determined by Western blotting (1:1000 in PBST with 1% milk) and visualized with ECL.

3. Results

3.1. LPS-stimulated SWI/SNF complex to dissociate from HS1/2 enhancer

CSR is driven by cytokines that modulate both promoter and enhancer activities (Chaudhuri and Alt, 2004; Stavnezer, 2000). By activation of CSR, both LPS and CD40 signaling trigger NF-κB to bind to the HS1/2 enhancer (Laurencikiene et al., 2001; Linderson et al., 1997; Michaelson et al., 1996; Zelazowski et al., 2000). We first determined the optimal conditions for LPS and anti-CD40 crosslinking antibody to activate immature B cells as evidenced by nuclear translocation of NF-κB p65 in WEHI 231 cells (Fig. 1A). To determine the composition of the nuclear proteins assembled at the HS1/2 enhancer, we then used streptavidin beads for affinity pulldown of the nuclear protein complex bound to the biotinylated HS1/2 fragment. The one-dimensional SDS-PAGE resolved several polypeptide bands differentially associated with the HS1/2 enhancer before and after LPS treatment of WEHI 231 cells (Fig. 1B). We observed a decreased binding of the protein with molecular weight ∼55 kDa at HS1/2 in response to LPS activation (black arrow, Fig. 1B). Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) analysis of the protein fingerprints scored it as BAF57. BAF57 is a subunit of murine SWI/SNF complex containing the high-mobility-group box (HMG) with DNA binding activity (Wang et al., 1998). To further confirm the finding, we went on to perform the analogous affinity pulldown experiments and probed the protein complex associated with the HS1/2 fragment using antibodies against BAF57 and BRG1. BRG1 is the ATPase subunit of SWI/SNF complex (Wang et al., 1996) and recombinant BRG1 can hydrolyze ATP and generate superhelical torsion in naked DNA or nucleosome

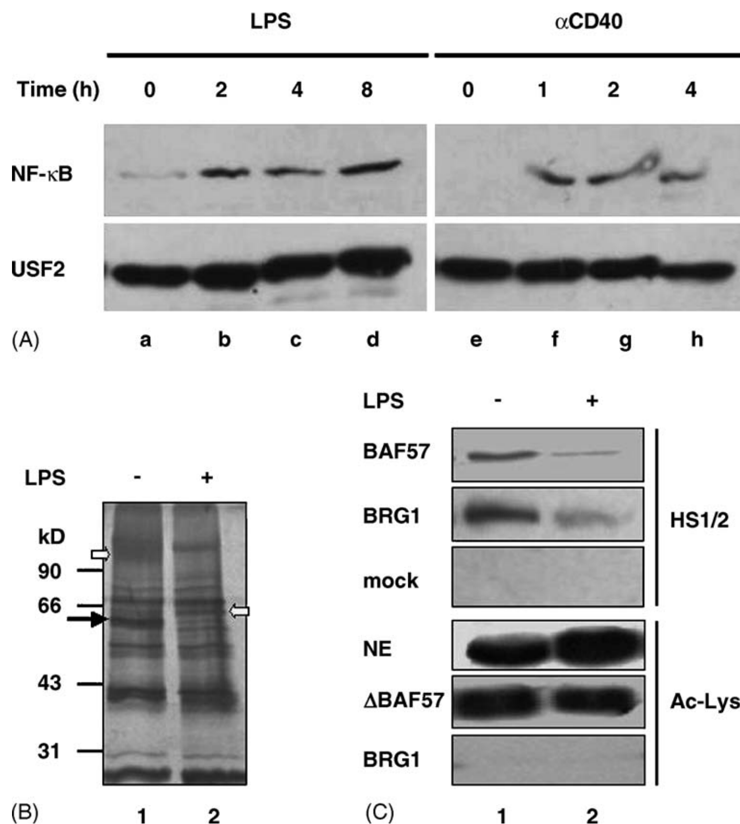

Fig. 1. BAF57 and BRG1 associated with HS1/2 region of Ig H 3′αE. (A) Nuclear translocation of NF-κB p65. Nuclear extracts were prepared from WEHI 231 cells (2×10^6/lane) pre-treated with 50 μg/mL LPS (lanes a–d) or 1 μg/mL anti-CD40 (lanes e–h) in 1 mL serum-free RPMI 1640 for the indicated time. Nuclear translocation of NF-κB p65 was determined by Western blotting and the optimal activation conditions for LPS (d, 8 h) or anti-CD40 treatment (g, 2 h) were used throughout assays. Nuclear transcription factor USF2 was used as a loading control. (B) Mass fingerprinting of BAF57. Nuclear proteins (250 μg) from WEHI 231 not treated (lane 1) or treated (lane 2) with 50 μg/mL LPS were incubated with biotinylated HS1/2 probe (5 pmol absorbed on streptavidin beads) and the bound fractions were eluted and resolved on 8% SDS-PAGE. Differentially bound protein bands (arrows) were identified after Coomassie Blue staining and the most pronounced band (black arrow, BAF57) was excised for MALDI-TOF mass fingerprinting assessment (see Section 2). (C) Association of BRG1 and BAF57 with HS1/2. Affinity pulldown of LPS untreated (lane 1) or treated (lane 2) nuclear extracts assays analogous to (B) was performed and instead of Coomassie staining, immunoblotting using antibodies specific to BAF 57 (panel 1) or BRG1 (panel 2) was performed. The control experiment (mock) was performed similarly except for addition of biotinylated HS1/2 probe. For on-bead HAT assay, nuclear proteins depleted of BAF57 (panel ΔBAF57), BRG1 (panel ΔBRG1) or control depletion using mouse IgG1 (panel NE) were subjected to affinity pulldown by the HS1/2 probe as in (B). Protein–HS1/2 DNA complex on-beads was resuspended in 20 μL HAT buffer containing 200 mM histones was incubated with 0.25 mM acetyl-CoA at 37 °C for 1 h. Acetylated histones were assessed by immunoblotting with Ac-Lys polyclonal antibody (1:1000 in PBST plus 1% milk powder, overnight at 4 °C) and visualized with ECL.

(Havas et al., 2000). HS1/2 could pulldown less BAF57 in LPS-primed WEHI 231 nuclear extracts as compared to the untreated fraction (Fig. 1C, panel 1), which was in good agreement with the observation of Fig. 1B. We could also detect BRG1 binding at the HS1/2 enhancer in the same immunoblot assay (Fig. 1C, panel 2). Similar to that of BAF57, association of BRG1 with HS1/2 decreased after cells were treated with LPS. We could simultaneously detect other SWI/SNF subunits, BAF47, BAF155 and BAF170, differentially associated with HS1/2 in a similar fashion (data not shown). These results, therefore, strongly suggest that chromatin remodeling by SWI/SNF complex might involve in LPS activation of the HS1/2 enhancer that concomitantly remodels and increases the accessibility of transcription activators and co-activators to the HS1/2 enhancer.

3.2. Dissociation of SWI/SNF complex from HS1/2 was coupled with increased HAT activities

Histone acetyltransferase is often coupled with SWI/SNF complex in chromatin remodeling and may regulate S region accessibility to germline transcription activation (Li et al., 2004; Nambu et al., 2003). To assess whether association of SWI/SNF complex with HS1/2 has any correlation with histone acetylation of the locus, we first performed HAT assays as shown in Fig. 1B. Nuclear proteins extracted from WEHI 231 cells treated or untreated with LPS were incubated with the biotinylated HS1/2 probe conjugated to magnetic streptavidin beads. HAT reaction was then carried out on substrate histones after addition of acetyl-CoA and the overall histone acetylation was measured with acetyl-lysine antibody immunoblotting. The HAT activity assembled at the HS1/2 probe slightly increased after LPS stimulation of WEHI 231 cells (Fig. 1C, panel 4). To assess whether HAT activities associated with HS1/2 enhancer were dependent on BAFs, we then immunodepleted BAF57 or BRG1 from the nuclear extracts before HAT activities were measured. The results showed that, after eliminating most BAF57 or BRG1 (data not shown), HS1/2-associated HAT activity was either slightly decreased (Fig. 1C, panel 5, for BAF57 depletion) or completely eliminated (Fig. 1C, panel 6, for BRG1 depletion). Therefore, this correlation between SWI/SNF complex and HAT activity associated with the HS1/2 enhancer in response to LPS suggested a concerted remodeling mode in activation of the enhancer region. The fact that depletion of BRG1 abolished HAT activity assembled at the HS1/2 enhancer further suggested that histone acetylation at HS1/2 enhancer might require a favored chromatin conformation. The difference in abolishment of HAT activity by BRG1 or BAF57 depletion suggests that HAT complex might directly contact BRG1.

3.3. Mutual chromatin remodeling of HS1/2 enhancer in vivo

The above assays utilized a synthetic HS1/2 probe to recruit the chromatin remodelers analogous to the studies of the histone modification marks at the IFN-β promoter (Agalioti et al., 2002) and might not necessarily reflect how the HS1/2 chromatin structure was remodeled intracellularly. We then performed chromatin immunoprecipitation (ChIP) analysis of the HS1/2 enhancer in WEHI 231 cells treated or not treated with LPS. PCR reactions were optimized to ensure the linearity for semi-quantitative analysis (data not shown). Similar to Fig. 1C, the ChIP assay showed the decreased association of BAF57 and BRG1 with HS1/2 after LPS treatment (Fig. 2A, panels 1 and 2).

The overall levels of histone acetylation at HS1/2 enhancer as revealed by ChIP showed slight or no increase after LPS stimulation (Fig. 2A, panel 3). However, detailed ChIP analysis also indicated the existence of differential acetylation patterns for histone H3 and H4, with acetylation of H3 increased and H4 decreased after LPS treatment (Fig. 2A). The different tendencies of H3 and H4 acetylation held the same along the time course of LPS treatment (data not shown). It, therefore, potentially explains why there was no obvious change of overall acetylation at HS1/2 after LPS stimulation since H3 and H4 acetylation might offset each other. Interestingly enough, H3 hyperacetylation was well correlated with the increased binding of CBP/p300, a transcription co-activator possessing HAT activity that might bind HS1/2 (Podojil et al., 2004) and decreased binding of histone deacetylase 1 (HDAC1) to the enhancer (Fig. 2A). ChIP analysis also showed the increased binding of NF-κB with HS1/2 in response to LPS, reminiscent of recent results, where NF-κB was involved in activation of HS1/2 enhancer in transient transfection reporter assays (Laurencikiene et al., 2001; Zelazowski et al., 2000). Therefore, our results were in good agreement with the interdependent feature of SWI/SNF complex and HAT in activation of promoter proximal regions (Narlikar et al., 2002). It is reasonable to speculate that alteration of the chromatin structure of the HS1/2 enhancer by the SWI/SNF complex might make it a better substrate for HAT after B cell activation by LPS.

CD40 is a TD activation signal for B cell terminal differentiation and can activate the HS1/2 enhancer (Arulampalam et al., 1994; Grant et al., 1996). To assess whether there is any difference between TD and TI stimuli in their ability to remodel the chromatin structure at HS1/2, we performed analogous ChIP analyses of the HS1/2 enhancer after anti-CD40 antibody crosslinking of WEHI 231 cells. CD40 engagement could obviously up-regulate histone acetylation at HS1/2 enhancer (Fig. 2A, panel 3). Compared to LPS treatment, CD40 signaling yielded the qualitatively similar binding patterns of ATP-dependent remodelers (BAF57 and BRG1), histone covalent modifiers (CBP/p300 and HDAC1) and transcription activator or co-activator (NF-κB or CBP/p300) to the HS1/2 enhancer. Alteration of H3 and H4 acetylation was also qualitatively comparable (Fig. 2A, right column). Whether the unique acetylation patterns of H3 and H4 are the consequence of SWI/SNF remodeling or the prerequisite for SWI/SNF dissociation from HS1/2 enhancer remains to be investigated.

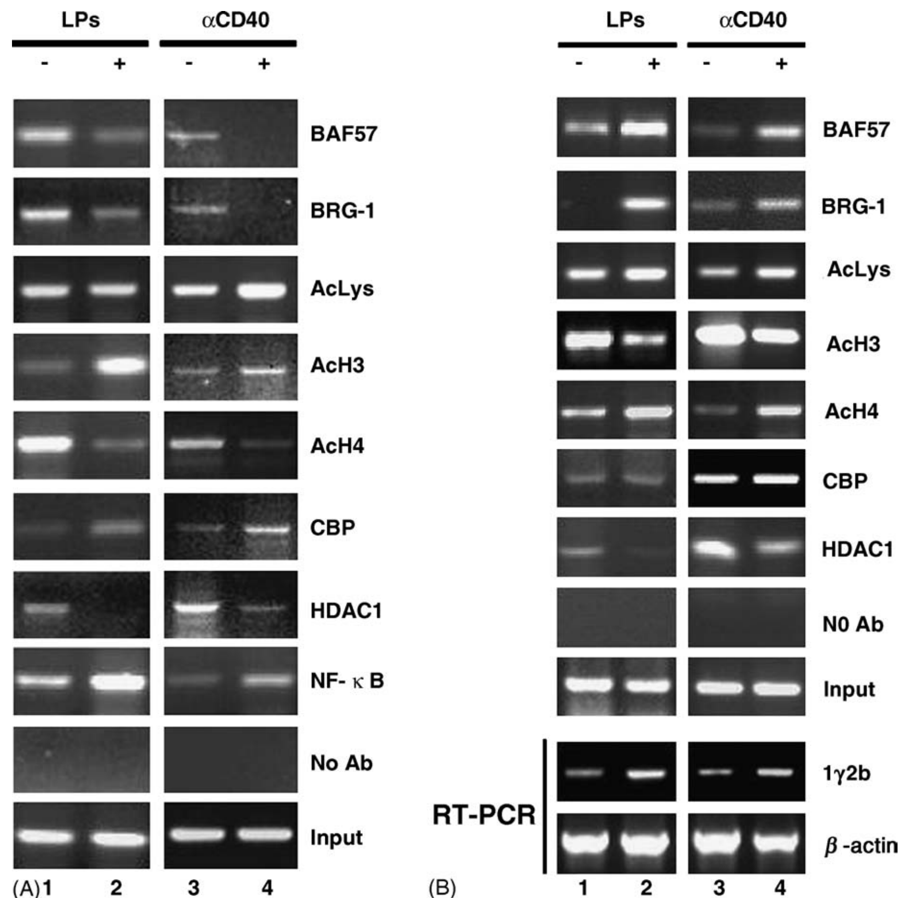

Fig. 2. Chromatin remodeling of HS1/2 enhancer and IgG2b germline promoter. (A) ChIP assays of HS1/2 enhancer. Chromatin from cells treated (+) or not treated (−) with LPS (lanes 1 and 2) or anti-CD40 antibody (lanes 3 and 4) were immunoprecipitated with antibodies against BAF57 (5 μL), BRG1 (10 μL), Ac-Lys (5 μL), Ac-H3 (3 μL), Ac-H4 (3 μL), anti-CBP (10 μL), HDAC1 (10 μL) and NF-κB (10 μL) as indicated. PCR reactions were then performed to determine the amount of HS1/2 fragment precipitated by each antibody. Control ChIP was carried without addition of any antibody (no Ab). An equivalent of 0.5% chromatin lysates used in each ChIP reaction was taken out as internal control of PCR reactions (input). The panels were the representation of at least three independent ChIP analyses. (B) ChIP assays of IgG2b germline promoter. Chromatin precipitated in (A) was subjected to PCR amplification using primers specific to IgG2b germline promoter (Section 2). The panels were the representation of at least three independent ChIP analyses. The amount of γ2b germline transcript (Iγ2b) before and after LPS (lanes 1 and 2) or anti-CD40 (lanes 3 and 4) treatment of WEHI 231 cells were assessed by RT-PCR and that of β-actin in parallel as an internal control.

3.4. Mutual chromatin remodeling of IgG2b germline promoter and HS1/2 enhancer in vivo

Mitogens and cytokines determine the specificity of CSR (Chaudhuri and Alt, 2004; Stavnezer, 2000), which requires a coordinated activation of S region promoters and their accessibility controlled by the 3′ IgH enhancer (Andersson et al., 1999, 2000; Grant et al., 1996; Laurencikiene et al., 2001; Michaelson et al., 1996; Pinaud et al., 2001; Saleque et al., 1999; Shi and Eckhardt, 2001; Zelazowski et al., 2000). It is, therefore, critical to investigate how germline promoters of S regions are regulated by chromatin remodelers in response to different activation signals. ChIP assays showed that, in contrast to what we observed with HS1/2 enhancer, LPS caused increased binding of BAF57 and BRG1 to the γ2b germline promoter (Fig. 2B). It is, therefore, very likely that SWI/SNF complex disengaged from the HS1/2 enhancer and bound at the γ2b germline promoter after immature B WEHI 231 cells were activated by LPS. Early studies suggest that activation of γ2b germline transcription is prerequisite for LPS-induced IgG2b class switching recombination (Lutzker et al., 1988). Although WEHI 231 cells are sterile for CSR, they express normal amounts of AID and suboptimal germline transcription of IgG2b (Spillmann and Wabl, 2004). LPS led to increased γ2b germline transcription as revealed by RT-PCR analysis of the γ2b germline transcript (Fig. 2B, bottom panel), reminiscent of the observation in primary B cells (Lutzker et al., 1988; Strom et al., 1999). In the meantime, LPS could slightly increase (∼two-fold) histone acetylation of the promoter region (Fig. 2B, panel 3). As indicated in previous studies with WEHI 231 (Spillmann and Wabl, 2004), whether such a minor increase in histone acetylation and γ2b germline transcription by LPS could lead to productive CSR remains unknown. Moreover, H3 and H4

acetylation at the γ2b germline promoter was opposite to that at the HS1/2 enhancer, with H3 becoming hypoacetylated and H4 hyperacetylated after LPS stimulation (Fig. 2B). Correlating well with the increase in acetylation, HDAC1 exhibited reduced binding to the promoter in response to LPS. CBP/p300, unlike that at the HS1/2 enhancer, showed no obvious change before or after LPS activation, suggesting that other HAT might involve in histone acetylation at the promoter.

Previous studies suggest that, instead of the required combinatorial effect of different cytokines, activation of CD40 signaling alone can turn on germline transcription, CSR and secretion of IgG2b in vitro (Laurencikiene et al., 2001; Strom et al., 1999). This, therefore, simplified our assay of chromatin remodeling by TD signals in specific regulation of CSR. Like that of LPS, CD40 could induce γ2b germline transcription in WEHI 231 cells (Fig. 2B, bottom panel) and ChIP analysis showed that the chromatin modeling profile was qualitatively similar to LPS at the same germline promoter (Fig. 2B).

These results, therefore, implied that both TD and TI signals could alter the chromatin conformation of HS1/2 and γ2b germline promoter in WEHI 231 cells. For the same *cis*-acting element, there existed a complementary association of SWI/SNF complex and HAT in response to either LPS or CD40 crosslinking, suggesting diverse functions for these chromatin remodelers for CSR. Moreover, H3 and H4 acetylation exhibited asynchronic patterns, with H3 hyperacetylation and H4 hypoacetylation coinciding with dissociation of SWI/SNF complex from HS1/2 and H3 hypoacetylation and H4 hyperacetylation concurring with increased SWI/SNF binding to the γ2b germline promoter. These results strongly imply that differential yet specific chromatin remodeling might involve in regulation of CSR.

3.5. *Site-specific acetylation at HS1/2 and γ2b germline promoter*

A growing body of evidence indicates that site-specific covalent modification of the histone N-terminal tails underlies distinct regulation of gene expression (Jenuwein and Allis, 2001; Peterson and Laniel, 2004; Strahl and Allis, 2000; Turner, 2002). Either LPS or CD40 can induce CSR of IgG2b in resting B cells and in Ablson virus transformed cell lines and our data showed that these stimuli yielded the similar patterns of SWI/SNF remodeling and histone acetylation at HS1/2 or γ2b germline promoter in WEHI 231 cells. To determine how these seemingly non-specific chromatin remodelings responded to different CSR signals, we went further to assess whether the signaling specificity was embedded in the H3 and H4 acetylation marks of each lysine residue critical to transcription activation. ChIP analysis using antibodies to site-specific acetylated Lys in H3 and H4 was performed similarly to previous studies at the IFN-β promoter (Agalioti et al., 2002). The results showed that H3 K14 became hyperacetylated in response to LPS and CD40 crosslinking at HS1/2 (Fig. 3A), while acetylation of H3 K9 was upregulated upon LPS stimulation but remained at the basal level upon CD40 engagement (Fig. 3A). This result suggested that H3 K9 acetylation might be specific to LPS signaling while H3 K14 hyperacetylation could be a shared mechanism for LPS and CD40 signaling at HS1/2. On the other hand, acetylation of H4 K5 and H4 K8 remained constant at the basal level, while H4 K12 and K16 become hypoacetylated in response to LPS (Fig. 3A). Although CD40 crosslinking caused overall down-regulation of H4 acetylation at HS1/2 (Figs. 2A and 3A), a slight increase in acetylation of H4 K5 and H4 K16 was observed, while those of K8 and K12 remained unchanged (Fig. 3A). This apparent conflict between site-specific acetylation increase and overall H4 acetylation decrease suggested that residue(s) other than K5, K8, K12 or K16 might exist and contribute to down-regulation of H4 acetylation for CD40 signaling. Alternatively, since we only detected HDAC1 at the HS1/2 upon CD40 engagement (Fig. 2A), other HDAC(s) might exist that increase the acetylation turnover rate of K5 and K16 to yield overall reduction of H4 acetylation.

Analogous ChIP assays of site-specific acetylation were also performed at the γ2b germline promoter. Marginal reduction of acetylation at H3 K14 was observed for both LPS and CD40 signaling, while decreased or increased H3 K9 acetylation was shown for either LPS or CD40 activation (Fig. 3B). This result suggested that status of H3 K9 acetylation might determine the specificity of LPS or CD40 signaling, while H3 K14 hypoacetylation could be a shared mechanism for both LPS and CD40 at the γ2b germline promoter. On the other hand, while H4 K5 and K12 remained at basal acetylation levels, both LPS and anti-CD40 caused hyperacetylation of H4 K8 and K16 (Fig. 3B). Therefore, hyperacetylation of H4 K8 and K16 might be a shared mechanism for both LPS- and CD40-induced CSR at the γ2b germline promoter.

4. Discussion

Chromatin remodeling controls the extent and dynamics of chromatin higher order folding and is believed to play a critical role in CSR. Our data suggested that BAF57 and BRG1, two essential subunits of the SWI/SNF complex, are differentially associated with essential *cis*-acting elements of CSR, HS1/2 enhancer and IgG2b germline promoter in response to TD and TI signals. LPS stimulation and CD40 engagement caused the SWI/SNF complex to dissociate from the HS1/2 enhancer and associate with the IgG2b germline promoter, suggesting the potential fluidity of chromatin structure and a specific regulatory mode for the ATP-dependent chromatin remodeler during CSR. On the other hand, increase of overall histone acetylation was either inverse or parallel to the action of SWI/SNF complex at HS1/2 or the IgG2b germline promoter, respectively. The alteration of histone H3 and H4 acetylation in response to TD and TI signals shared overall

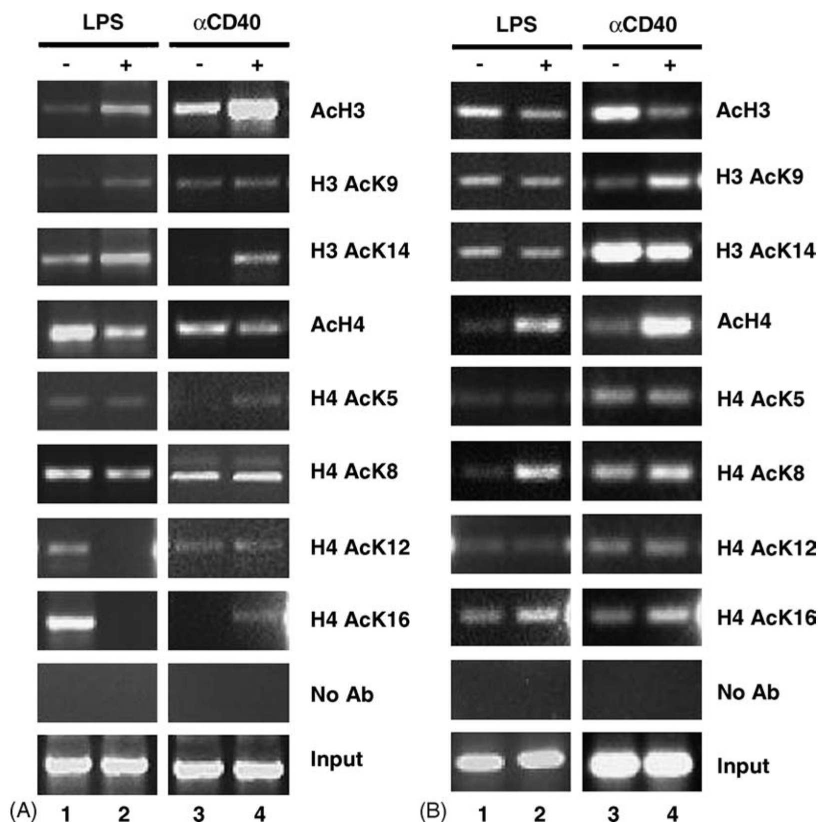

Fig. 3. Site-specific acetylation of H3 and H4 at HS1/2 and IgG2b germline promoter. (A) ChIP assays of HS1/2 enhancer. Analogous ChIP analyses were performed as in Fig. 2A except that additional antibodies as indicated, anti-H3Ac9, anti-H3Ac14, anti-H4Ac5, anti-H4Ac8, anti-H4Ac12, anti-H4 Ac16 (5 μL) were used. Each panel represented one of at least three independent reactions. (B) ChIP assays of IgG2b germline promoter. Analogous ChIP analyses were performed as in Fig. 2B except that additional antibodies as indicated, anti-H3Ac9, anti-H3Ac14, anti-H4Ac5, anti-H4Ac8, anti-H4Ac12, anti-H4 Ac16 (5 μL) were used. Each panel represented one of at least three independent reactions.

similarity, with H3 hyperacetylated and H4 hypoacetylated at the HS1/2 enhancer and reversed modification patterns at the IgG2b germline promoter after WEHI 231 cells encountered activation signals. Finally, the specificity of TD and TI signaling in control of CSR could be partially revealed by the subtle yet specific acetylation marks of N-terminal tails of H3 and H4. In the meantime, it is interesting to investigate the correlation between differential acetylation patterns of H3 and H4 and association of the SWI/SNF complex with the HS1/2 enhancer or the IgG2b germline promoter during B cell activation.

ATP-dependent and ATP-independent chromatin remodeling function in concert to regulate transcription. The order of recruitment of these remodeling complexes is specific to the temporal and spatial regulation of gene expression (Felsenfeld and Groudine, 2003; Peterson and Laniel, 2004). In sharp contrast to those at interferon-β promoter and yeast PHO8 promoter in which acetylated histones may provide specific docking sites for the SWI/SNF complex (Agalioti et al., 2002; Reinke et al., 2001), our results indicated that SWI/SNF complex would increase the accessibility of HATs to the HS1/2 enhancer during B cell activation. This is reminiscent of the notion that ATP-dependent chromatin remodeling is required for the subsequent recruitment of HATs for mitotic gene expression in yeast (Fry and Peterson, 2001). Indeed, kinetic analysis of the recruitment of SWI/SNF and HAT complex to a yeast Gal 4 reporter promoter in mammalian cells indicates that, except for TRRAP, there is a delay of HAT (GCN5, CBP/p300 and PCAF) binding to the promoter (Memedula and Belmont, 2003). Recruitment of chromatin remodeling complexes activates transcription initiation synergistically. However, binding of the SWI/SNF complex and increased acetylation at the HS1/2 enhancer seemed to be mutually exclusive at HS1/2. It is likely that alteration of DNA topology by the SWI/SNF complex might provide a sufficient time window for recruitment of HAT and other transcription factors (Fry and Peterson, 2001) and increased acetylation at HS1/2 appeared to facilitate the loading of the SWI/SNF complex from HS1/2 to the IgG2b germline promoter for specific CSR upon CD40 or LPS stimulation. Alternatively, pre-occupation of HS1/2 by the SWI/SNF complex might provide a repressive checkpoint for CSR in WEHI 231 cells. It is well established that BRG1 can involve in transcriptional repression (Hendricks et al., 2004; Martens and Winston, 2002; Murphy et al., 1999), so, the inactive state of HS1/2 in immature WEHI 231 cells (Arulampalam et al.,

1994; Grant et al., 1996) could be maintained even though the γ2b germline promoter seems to be partially activated suboptimal for CSR (Spillmann and Wabl, 2004). LPS or CD40 crosslinking would now provide anti-repression signal that lead to histone modification of HS1/2 and fully activate γ2b germline promoter.

Previous studies established that coordinated H3 and H4 hyperacetylation and progressive activation of germline transcription of the S regions are required for successive CSR (Bergman and Cedar, 2004). In this work, however, we observed the asynchronous and opposite acetylation pattern of H3 and H4 at the HS1/2 enhancer and IgG2b germline promoter during B cell activation. We propose that H3 hyperacetylation and H4 hypoacetylation provide the specific histone marks for HS1/2 activation and H3 hypoacetylation and H4 hyperacetylation provide the specific marks for IgG2b germline promoter activation. This model does not rule out the possibility that either a different set of HAT with different enzymatic kinetics or different deacetylases or both are involved in modification of H3 or H4 at these *cis*-acting elements (Narlikar et al., 2002; Peterson and Laniel, 2004).

Recent studies have also shown that site-specific combinations of histone modifications correlate with particular biological functions (Jenuwein and Allis, 2001; Peterson and Laniel, 2004; Strahl and Allis, 2000; Turner, 2002). Epigenetic marks and interaction among these markings determine the specificity and complexity of transcription activation or repression. Our observation indicated that, although not all types of histone modification were examined, specific H3 and H4 acetylation marks existed either at the different *cis*-acting elements in response to the same stimuli or at the same *cis*-acting element in response to different signals. The acetylation patterns observed for H3 and H4 differed between HS1/2 and the γ2b germline promoter and deviated from those at the interferon-β promoter (Agalioti et al., 2002), further supporting the notion that patterns of histone modification are specific to genes and cell types and can be differentially interpreted by transcription factors that ultimately determine the specificity of transcription regulation (Peterson and Laniel, 2004).

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References

Adams, C.C., Workman, J.L., 1993. Nucleosome displacement in transcription. Cell 72, 305–308.

Agalioti, T., Chen, G., Thanos, D., 2002. Deciphering the transcriptional histone acetylation code for a human gene. Cell 111, 381–392.

Alberts, A.S., Geneste, O., Treisman, R., 1998. Activation of SRF-regulated chromosomal templates by Rho-family GTPases requires a signal that also induces H4 hyperacetylation. Cell 92, 475–487.

Andersson, T., Furebring, C., Borrebaeck, C.A., Pettersson, S., 1999. Temporal expression of a V(H) promoter-Cmu transgene linked to the IgH HS1, 2 enhancer. Mol. Immunol. 36, 19–29.

Andersson, T., Samuelsson, A., Matthias, P., Pettersson, S., 2000. The lymphoid-specific cofactor OBF-1 is essential for the expression of a V(H) promoter/HS1, 2 enhancer-linked transgene in late B cell development. Mol. Immunol. 37, 889–899.

Arulampalam, V., Grant, P.A., Samuelsson, A., Lendahl, U., Pettersson, S., 1994. Lipopolysaccharide-dependent transactivation of the temporally regulated immunoglobulin heavy chain 3′ enhancer. Eur. J. Immunol. 24, 1671–1677.

Bergman, Y., Cedar, H., 2004. A stepwise epigenetic process controls immunoglobulin allelic exclusion. Nat. Rev. Immunol. 4, 753–761.

Braunstein, M., Rose, A.B., Holmes, S.G., Allis, C.D., Broach, J.R., 1993. Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. Genes Dev. 7, 592–604.

Chaudhuri, J., Alt, W., 2004. Class-switch recombination: interplay of transcription, DNA deamination and DNA repair. Nat. Rev. Immunol. 4, 541–552.

Chi, T., 2004. A BAF-centered view of the immune system. Nat. Rev. Immunol. 4, 965–977.

Chi, T.H., Wan, M., Zhao, K., Taniuchi, I., Chen, L., Littman, D.R., Crabtree, G.R., 2002. Reciprocal regulation of CD4/CD8 expression by SWI/SNF-like BAF complexes. Nature 418, 195–199.

Dignam, J.D., Lebovitz, R.M., Roeder, R.G., 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. 11, 1475–1489.

Felsenfeld, G., 1992. Chromatin as an essential part of transcriptional mechanism. Nature 355, 219–224.

Felsenfeld, G., Groudine, M., 2003. Controlling the double helix. Nature 421, 448–453.

Fry, C.J., Peterson, C.L., 2001. Chromatin remodeling enzymes: who's on first? Curr. Biol. 11, R185–R197.

Grant, P.A., Andersson, T., Neurath, M.F., Arulampalam, V., Bauch, A., Muller, R., Reth, M., Pettersson, S., 1996. A T cell controlled molecular pathway regulating the IgH locus: CD40-mediated activation of the IgH 3′ enhancer. EMBO J. 15, 6691–6700.

Gregor, P.D., Morrison, S.L., 1986. Myeloma mutant with a novel 3′ flanking region: loss of normal sequence and insertion of repetitive elements leads to decreased transcription but normal processing of the alpha heavy-chain gene products. Mol. Cell Biol. 6, 1903–1916.

Havas, K., Flaus, A., Phelan, M., Kingston, R., Wade, P.A., Lilley, D.M., Owen-Hughes, T., 2000. Generation of superhelical torsion by ATP-dependent chromatin remodeling activities. Cell 103, 1133–1142.

He, H., Ding, Y., Cao, Z., Shao, Y., Bartlam, M., Tang, H., Jiang, F., Liu, Y., Liu, J., Zhao, N., Rao, Z., 2003. Crystal structure of tabtoxin resistance protein complexed with acetyl coenzyme A reveals the mechanism for beta-lactam acetylation. J. Mol. Biol. 325, 1019–1030.

Hendricks, K.B., Shanahan, F., Lees, E., 2004. Role for BRG1 in cell cycle control and tumor suppression. Mol. Cell Biol. 24, 362–376.

Jenuwein, T., Allis, C.D., 2001. Translating the histone code. Science 293, 1074–1080.

Khamlichi, A.A., Pinaud, E., Decourt, C., Chauveau, C., Cogne, M., 2000. The 3′ IgH regulatory region: a complex structure in a search for a function. Adv. Immunol. 75, 317–345.

Kornberg, R.D., Lorch, Y., 1999. Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell 98, 285–294.

Laurencikiene, J., Deveikaite, V., Severinson, E., 2001. HS1,2 enhancer regulation of germline epsilon and gamma2b promoters in murine B lymphocytes: evidence for specific promoter–enhancer interactions. J. Immunol. 167, 3257–3265.

Li, Z., Luo, Z., Scharff, M.D., 2004. Differential regulation of histone acetylation and generation of mutations in switch regions is associated with Ig class switching. Proc. Natl. Acad. Sci. U.S.A. 101, 15428–15433.

Linderson, Y., Cross, D., Neurath, M.F., Pettersson, S., 1997. NFE, a new transcriptional activator that facilitates p50 and c-Rel-dependent IgH 3′ enhancer activity. Eur. J. Immunol. 27, 468–475.

Luger, K., Richmond, T.J., 1998. The histone tails of the nucleosome. Curr. Opin. Genet. Dev. 8, 140–146.

Lusser, A., Kadonaga, J.T., 2003. Chromatin remodeling by ATP-dependent molecular machines. Bioessays 25, 1192–1200.

Lutzker, S., Rothman, P., Pollock, R., Coffman, R., Alt, F.W., 1988. Mitogen- and IL-4-regulated expression of germ-line Ig gamma 2b transcripts: evidence for directed heavy chain class switching. Cell 53, 177–184.

Madisen, L., Groudine, M., 1994. Identification of a locus control region in the immunoglobulin heavy-chain locus that deregulates c-myc expression in plasmacytoma and Burkitt's lymphoma cells. Genes Dev. 8, 2212–2226.

Madisen, L., Krumm, A., Hebbes, T.R., Groudine, M., 1998. The immunoglobulin heavy chain locus control region increases histone acetylation along linked c-myc genes. Mol. Cell Biol. 18, 6281–6292.

Manis, J.P., Michaelson, J.S., Birshtein, B.K., Alt, F.W., 2003. Elucidation of a downstream boundary of the 3′ IgH regulatory region. Mol. Immunol. 39, 753–760.

Martens, J.A., Winston, F., 2002. Evidence that Swi/Snf directly represses transcription in *S. cerevisiae*. Genes Dev. 16, 2231–2236.

Martens, J.A., Winston, F., 2003. Recent advances in understanding chromatin remodeling by Swi/Snf complexes. Curr. Opin. Genet. Dev. 13, 136–142.

Memedula, S., Belmont, A.S., 2003. Sequential recruitment of HAT and SWI/SNF components to condensed chromatin by VP16. Curr. Biol. 13, 241–246.

Michaelson, J.S., Giannini, S.L., Birshtein, B.K., 1995. Identification of 3′ alpha-hs4, a novel Ig heavy chain enhancer element regulated at multiple stages of B cell differentiation. Nucleic Acids Res. 23, 975–981.

Michaelson, J.S., Singh, M., Snapper, C.M., Sha, W.C., Baltimore, D., Birshtein, B.K., 1996. Regulation of 3′ IgH enhancers by a common set of factors, including kappa B-binding proteins. J. Immunol. 156, 2828–2839.

Murphy, D.J., Hardy, S., Engel, D.A., 1999. Human SWI–SNF component BRG1 represses transcription of the c-fos gene. Mol. Cell Biol. 19, 2724–2733.

Nambu, Y., Sugai, M., Gonda, H., Lee, C.G., Katakai, T., Agata, Y., Yokota, Y., Shimizu, A., 2003. Transcription-coupled events associating with immunoglobulin switch region chromatin. Science 302, 2137–2140.

Narlikar, G.J., Fan, H.Y., Kingston, R.E., 2002. Cooperation between complexes that regulate chromatin structure and transcription. Cell 108, 475–487.

Peterson, C.L., Laniel, M.A., 2004. Histones and histone modifications. Curr. Biol. 14, 546–551.

Peterson, C.L., Logie, C., 2000. Recruitment of chromatin remodeling machines. J. Cell Biochem. 78, 179–185.

Pinaud, E., Khamlichi, A.A., Le Morvan, C., Drouet, M., Nalesso, V., Le Bert, M., Cogne, M., 2001. Localization of the 3′ IgH locus elements that effect long-distance regulation of class switch recombination. Immunity 15, 187–199.

Podojil, J.R., Kin, N.W., Sanders, V.M., 2004. CD86 and beta2-adrenergic receptor signaling pathways, respectively, increase Oct-2 and OCA-B expression and binding to the 3′-IgH enhancer in B cells. J. Biol. Chem. 279, 23394–23404.

Reinke, H., Gregory, P.D., Horz, W., 2001. A transient histone hyperacetylation signal marks nucleosomes for remodeling at the PHO8 promoter in vivo. Mol. Cell 7, 529–538.

Saleque, S., Singh, M., Birshtein, B.K., 1999. Ig heavy chain expression and class switching in vitro from an allele lacking the 3′ enhancers DNase I-hypersensitive hs3A and hs1, 2. J. Immunol. 162, 2791–2803.

Shi, X., Eckhardt, L.A., 2001. Deletional analyses reveal an essential role for the hs3b/hs4 IgH 3′ enhancer pair in an Ig-secreting but not an earlier-stage B cell line. Int. Immunol. 13, 1003–1012.

Spillmann, F.J., Wabl, M., 2004. Endogenous expression of activation-induced cytidine deaminase in cell line WEHI-231. J. Immunol. 173, 1858–18567.

Stavnezer, J., 2000. Molecular processes that regulate class switching. Curr. Top. Microbiol. Immunol. 245, 127–168.

Strahl, B.D., Allis, C.D., 2000. The language of covalent histone modifications. Nature 403, 41–45.

Strom, L., Laurencikiene, J., Miskiniene, A., Severinson, E., 1999. Characterization of CD40-dependent immunoglobulin class switching. Scand. J. Immunol. 49, 523–532.

Studitsky, V.M., Walter, W., Kireeva, M., Kashlev, M., Felsenfeld, G., 2004. Chromatin remodeling by RNA polymerases. Trends Biochem. Sci. 29, 127–135.

Sudarsanam, P., Winston, F., 2000. The Swi/Snf family nucleosome-remodeling complexes and transcriptional control. Trends Genet. 16, 345–351.

Tang, H., Sharp, P.A., 1999. Transcriptional regulation of the murine 3′ IgH enhancer by OCT-2. Immunity 11, 517–526.

Tang, H., Severinov, K., Goldfarb, A., Fenyo, D., Chait, B., Ebright, R.H., 1994. Location, structure, and function of the target of a transcriptional activator protein. Genes Dev. 8, 3058–3067.

Turner, B.M., 2002. Cellular memory and the histone code. Cell 111, 285–291.

Wang, W., Xue, Y., Zhou, S., Kuo, A., Cairns, B.R., Crabtree, G.R., 1996. Diversity and specialization of mammalian SWI/SNF complexes. Genes Dev. 10, 2117–2130.

Wang, W., Chi, T., Xue, Y., Zhou, S., Kuo, A., Crabtree, G.R., 1998. Architectural DNA binding by a high-mobility-group/kinesin-like subunit in mammalian SWI/SNF-related complexes. Proc. Natl. Acad. Sci. U.S.A. 95, 492–498.

Wolffe, A.P., Hayes, J.J., 1999. Chromatin disruption and modification. Nucleic Acids Res. 27, 711–720.

Zelazowski, P., Shen, Y., Snapper, C.M., 2000. NF-kappaB/p50 and NF-kappaB/c-Rel differentially regulate the activity of the 3′alphaE-hsl, 2 enhancer in normal murine B cells in an activation-dependent manner. Int. Immunol. 12, 1167–1172.

Zhang, J., Bottaro, A., Li, S., Stewart, V., Alt, F.W., 1993. A selective defect in IgG2b switching as a result of targeted mutation of the I gamma 2b promoter and exon. EMBO J. 12, 3529–3537.