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*J Immunol* 2006; 177:1179-1188; doi: 10.4049/jimmunol.177.2.1179

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The Histone Methyltransferase Suv39h1 Increases Class Switch Recombination Specifically to IgA

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Ab class (isotype) switching allows the humoral immune system to adaptively respond to different infectious organisms. Isotype switching occurs by intrachromosomal DNA recombination between switch (S) region sequences associated with CH region genes. Although isotype-specific transcription of unrearranged (germline) CH genes is required for switching, recent results suggest that isotype specificity is also determined by the sequences of downstream (acceptor) S regions. In the current study, we identify the histone methyltransferase Suv39h1 as a novel Sox-specific factor that specifically increases IgA switching (Sox-Sox recombination) in a transiently transfected plasmid S substrate, and demonstrate that this effect requires the histone methyltransferase activity of Suv39h1. Additionally, B cells from Suv39h1-deficient mice have an isotype-specific reduction in IgA switching with no effect on the level of germline Igα-Cα transcripts. Taken together, our results suggest that Suv39h1 activity inhibits the activity of a sequence-specific DNA-binding protein that represses switch recombination to Igα.

The Journal of Immunology, 2006, 177: 1179–1188.

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1 This work was supported by grants from the National Institutes of Health: R01 AI 23283 and R21 AI 57463 (to J.S.) and F32 AI 56806 (to D.A.K.).

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5 Additional regulation of CSR isotype specificity

A plasmid assay for switch recombination (SR) has been developed to examine the regulation of isotype specificity (13, 14). In this assay, plasmids containing both the Sμ segment and a downstream S region segment are transiently transfected into B cell lines or splenic B cells. Such plasmid substrates undergo AID-dependent Sμ-Sx recombination in B cells capable of undergoing CSR, but not in cell types that do not undergo CSR (14, 15). Importantly, these plasmids show isotype specificity. The transiently transfected plasmid p273 containing Sμ and Sα segments undergoes Sμ-Sα recombination in LPS-activated splenic B cells and in three B cell lines (I.29μ, CH12.LX, and 1B4.B6) capable of undergoing CSR to IgA on their endogenous chromosomes. The pG3.1 plasmid, in which the Sγ3 segment is substituted for the Sα segment, also undergoes Sμ-Sγ3 recombination in LPS-activated splenic B cells, but only recombines in one (1B4.B6) of the three B cell lines, the only one that is capable of switching to IgG3 on its endogenous chromosome. Additional results obtained using other plasmids that differ only in the downstream acceptor S region provided evidence for factors that discriminate between the Sα,
Sy3, Sy1, and Se segments (16). These data have led to the hypothesis that, in addition to GLTs, differentially expressed factors bind different acceptor S regions and regulate isotype-specific CSR by regulating chromatin accessibility. It has also been suggested that such factors might directly bind AID to recruit it to specific downstream S regions (15).

Histone modification and Suv39h1

Enzymatic modification of histones can either increase or decrease chromatin accessibility to transcription, DNA repair, and recombination machinery. Acetylation of the N-terminal tails of histone H3 or H4 generally correlates with an increase in chromatin accessibility. Methylation of H3 or H4 N-terminal tails inhibits or increases gene activity, depending on the specific amino acid residue that is methylated and the level of methylation (17–19). Several R and K residues within the H3 and H4 N-terminal tails can be acetylated or methylated, singly or in combination, determined by the specificity of the enzymes involved. In addition, some K residues can be ubiquitinated, and some S residues can be phosphorylated, but very little is known about the roles of these latter two modifications.

Histone modifications at specific genomic sites can be measured by the chromatin immunoprecipitation assay. Using this assay, it has been found that specific domains within the IgH locus undergo histone acetylation or methylation at defined stages during B cell development, correlating with the rearrangement potential of the genes (20–24). Furthermore, during activation of B cells, histones on specific acceptor S regions undergoing switching are acetylated (25–27). However, H3 acetylation alone does not substitute for GL transcription (25).

One of the best-studied histone-modifying enzymes is the ubiquitously expressed histone methyltransferase (HMT) Suv39h1, the mammalian ortholog of the Drosophila suppressor of position-effect variegation, Su(var)3-9 (28). Suv39h1 trimethylates the K9 residue of H3 (29). The closely related HMT, Suv39h2, also performs the same methylation, but shows much more limited expression in adult mice (17, 30). The HMT activity of these proteins resides in their conserved SET domain, which is also found in mammalian orthologs of the Rb and the E2F transcription factor, resulting in methylation of transcription (25). However, Suv39h1 specifically stimulates IgA CSR.

Suv39h1 is a complex with histone methyltransferase protein-1β (HP-1β/M31), which binds the me3-K9-H3 residue with high affinity (33). HP-1β can self-associate and bind to a series of adjacent nucleosomes to form heterochromatin (17). Suv39h1 is also found in complexes with histone deacetylases 1, 2, 4, and 5 (37). This interaction should allow a concerted process whereby histone deacetylases remove acetyl groups from histone tails, leaving K9 residues available for methylation by the associated Suv39h1.

In addition to initiating the formation of large heterochromatin regions, Suv39h1 is involved in repressing transcription of specific genes. It can specifically target certain promoters due to the ability of the Suv39h1-HP-1β complex to bind Rb, p107, p130, and human T cell leukemia virus-1 Tax (38–41). For example, Suv39h1 can be recruited to the cyclin A and E promoters by the interaction of Rb and the E2F transcription factor, resulting in methylation of H3 and binding of HP-1β to a single nucleosome (42, 43). By this mechanism, Suv39h1 represses transcription of cyclins A and E in HeLa cells, resulting in G1 cell cycle arrest.

Although most data indicate that the me3-K9-H3 modification is associated with heterochromatin and with the promoters of a few inactive genes, it was recently shown that very low levels of me3-K9-H3 and HP-1γ are associated with transcribed segments of active genes in an erythroid cell line and in primary T cells (44). The specific HMT that performs this modification at actively transcribed genes is unknown. Thus, although me3-K9-H3 is generally an inhibitory modification, it is possible that very low levels of this modification, perhaps if combined with certain additional modifications, can be activating.

In this study, we investigate whether Suv39h1 has an effect on CSR. We find that overexpression of Suv39h1 increases SR specifically to the S0 segment in a transiently transfected plasmid, and that this enhancement requires the HMT activity of Suv39h1. Furthermore, B cells from mice deficient in Suv39h1 or both Suv39h1 and Suv39h2 have a reduced ability to switch to IgA, with no effect on the levels of GLTs. We suggest possible models for how Suv39h1 specifically stimulates IgA CSR.

Materials and Methods

S plasmid construction

S plasmids p273, p218, pG3.1, and pE.1 were previously described (13, 16). pG3.1 and pE.1 were gifts from A. Kenter (University of Illinois, Chicago, IL). The Sy2a and Sy2b PCR and S2b PCR sequences obtained by PCR amplification using genomic DNA from the B cell line L294 (clone 22D). PCR primers were designed with incorporated restriction sites (underlined) for cloning into a p218 BamHI-Nol vector backbone. Primers were designed from National Center for Biotechnology Information-Accession D78344, which contains both Sy2b and Sy2a sequences from a BALB/c mouse. Primers for S2a were 5′-CGGATCTTACACGTCGAGATCCGAGGTAACGTAAC-3′ and 5′-ATAAGAATTCCGCGAGGTAACGTCGAGAACCAGAAG-3′. Other cell lines and LPS-stimulated splenic B cells were similarly transfected. L294 cells were cultured in medium described previously (45), but all other cell lines were cultured in medium identical with that used for splenic B cells (see below). T-depleted splenic B cells were purified and activated with LPS (50 μg/ml, Escherichia coli 055:B5, Sigma-Aldrich). On day 3, 1 × 10⁶ cells were transfected and cultured with LPS for another 48 h. Although the frequency of transfection of B cell lines and splenic B cells is very low (<1% of the cells are transfected; our unpublished data), all the S plasmid and the expression plasmids enter the same cells. Due to this very low transfection efficiency, it was not possible to examine the levels of Suv39h1 protein in the transfected cells. Genomic DNA was purified from nuclei prepared by Nonidet P-40 treatment (final concentration, 0.1% (v/v)) and lysed with proteinase K (100 μg/ml). RNAse (100 μg/ml), and proteinase K (100 μg/ml). A total of 2 μg of DNA was used for the S plasmid DC-PCR assay. Samples were digested with SacI and EcoRI, sequentially, purifying with a spin column (QiAQuik; Qiagen) after each digestion. Ligation reactions were performed at 0.4 – 2.0 mg/μl with T4 DNA ligase (5 U; MB Biernettas) in 200 μl overnight at 16°C.

PCR to detect the recircularized vector backbone S10 plasmid was conducted on all S plasmids. The primer set HP-1β primers and EcoRI, as described previously (14). Hotstar Taq (Qiagen) was used for all experiments. The PCR product indicating S-S recombination varies in length specifically to the S segment in the transfected cells. Genomic DNA was purified from nuclei prepared by Nonidet P-40 treatment (final concentration, 0.1% (v/v)) and lysed with proteinase K (100 μg/ml). RNAse (100 μg/ml), and proteinase K (100 μg/ml). A total of 2 μg of DNA was used for the S plasmid DC-PCR assay. Samples were digested with SacI and EcoRI, sequentially, purifying with a spin column (QiAQuik; Qiagen) after each digestion. Ligation reactions were performed at 0.4 – 2.0 mg/μl with T4 DNA ligase (5 U; MB Biernettas) in 200 μl overnight at 16°C.

PCT to detect the recircularized vector backbone S10 plasmid was conducted on all S plasmids. The primer set HP-1β primers and EcoRI, as described previously (14). Hotstar Taq (Qiagen) was used for all experiments. The PCR product indicating S-S recombination varies in length specifically to the S segment in the transfected cells. Genomic DNA was purified from nuclei prepared by Nonidet P-40 treatment (final concentration, 0.1% (v/v)) and lysed with proteinase K (100 μg/ml). RNAse (100 μg/ml), and proteinase K (100 μg/ml). A total of 2 μg of DNA was used for the S plasmid DC-PCR assay. Samples were digested with SacI and EcoRI, sequentially, purifying with a spin column (QiAQuik; Qiagen) after each digestion. Ligation reactions were performed at 0.4 – 2.0 mg/μl with T4 DNA ligase (5 U; MB Biernettas) in 200 μl overnight at 16°C.

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P2/P3; E. coli strains. 5′-CTTCTTCAGGAATGTCCCTGGCCATG TGGAC-3′. Thermocycler conditions for the S-S products were as for the 510-bp band, except 36 cycles were performed. [32P]dCTP was included in the reactions to label the PCR products, and all results shown are from autoradiography or phosphor imaging of dried gels.

Two different template inputs were used for each PCR, and each transfection was performed in duplicate. The intensities of the signals due to SR (generally the 180-bp band) and for the control band (510 bp) were determined by densitometry of autoradiograph films or by phosphor imaging of dried gels. Only data from signals in the linear range were included, and the SR results for each template dose were normalized to the 510-bp band.

Cloning of Suv39h1 cDNA into pcDEF3 and creation of mutants

Mouse Suv39h1 cDNA cloned into pBluescript (46) was excised with BamHI/EcoRI, cloned using BamHI/NotI adaptors into NotI/EcoRI sites of the expression plasmid pcDEF3 (47). The H324L mutation was introduced into this plasmid with the Stratagene Quikchange kit. Internal deletions Δ124–229 and Δ41–81 were created by PCR overlap extension, cloned into pGEM, and then cloned into the KpnI site of pcDEF3. The internal deletion Δ42–229 was a natural variant of Suv39h1 cloned from the I.29 B lymphoma cell line, and inserted into the KpnI site of pcDEF3. The Pax5 binding site was removed as a 83-bp deletion by overlap extension PCR, and the Sα segment was cloned in pGEM and then excised with BamHI and NotI and cloned into these sites in p218.

B cell cultures for induction of CSR and isolation of Peyer’s patch cells

T-depleted splenic B cells were purified and cultured under conditions to induce CSR, and switching was assayed by flow cytometry, as previously described (48, 49). To induce SR to IgG3, LPS (50 μg/ml; Sigma-Aldrich) and anti-α-dextran (0.3 ng/ml) (gift from C. Snapper, Uniformed Services University of the Health Sciences, Bethesda, MD) were added at the initiation of culture. To induce SR to IgG1, LPS and IL-4 (800 U/ml; from W. Paul, National Institutes of Health, Bethesda, MD) were added. LPS and IFN-γ (10 U/ml) were used to induce IgG2a switching, and LPS and dextran sulfate (30 μg/ml; Pharmacia) were used to induce IgG2c switching. LPS, TGF-β1 (2 ng/ml), IL-4, IL-5 (1.5 ng/ml; BD Pharmingen), and anti-α dextran were used to induce IgA CSR. Cells were isolated from excised Peyer’s patches by using forceps to tease apart the isolated patches, followed by straining through a cotton wool column to isolate single cell suspensions. Peyer’s patch cells were analyzed by flow cytometry, as for cultured B cells.

RNA isolation; semiquantitative RT-PCR

Total RNA was isolated from splenic B cell cultures using RNAwiz solution (Ambion). First strand cDNA synthesis used 2 μg of RNA, oligo(dT) primer, and Moloney murine leukemia virus-reverse transcriptase (Promega). PCR primers were previously described (50, 51).

Mice

AID-deficient mice (aid−/−) were obtained from T. Honjo (5). They were backcrossed for several generations with C57BL/6 mice. Wild-type (WT) mice were aid+/− littermates of aid−/− mice. Suv39h1- and Suv39h2-deficient mice were previously described (32). Suv39h1-deficient mice were backcrossed to C57BL/6. Mice were used under protocols approved by the University of Massachusetts Medical School Institutional Animal Care and Use Committee.

Results

Regulation of Sµ-Sα SR by Suv39h1

To determine whether histone modification may regulate CSR, we examined the effect of Suv39h1 on SR in plasmid substrates in transient transfection assays. In this assay, plasmids containing Sµ and one of several different downstream acceptor S regions (Fig. 1A) are transiently transfected into lymphoid cells. SR between the two S regions in the plasmids is assayed after 48 h by DC-PCR (Fig. 1B). The 180-bp PCR product indicates the amount of Sµ-Sα recombination, whereas the 510-bp product, due to circularization of the vector backbone, is an internal control for transfection efficiency, plasmid recovery, restriction enzyme digestion, and ligation efficiency. The plasmids, p273S and pG3.1, contain transcriptional activator elements: the Ig H chain enhancer-VH promoter cassette placed upstream of Sµ, and the promoter for α GLTα promoters; TK, herpes virus thymidine kinase gene; Iκα, GLTα promoter; RI, EcoRI site. p273S is identical with previously described p273 (13), except for the addition of a SacI site at the 3′ end of the Sµ segment to increase the specificity of the assay. pG3.1 also has SacI sites arranged as shown in p273S in B. B. DC-PCR assay for plasmid S-S recombination. The 510-bp PCR product measures the amount of circularized vector backbone. The 180-bp product measures the S-S recombination.
B cells (Fig. 2A). Shown above the graph are the lanes of the corresponding DC-PCR assay for plasmid SR, performed in duplicate with 2-fold doses of input template. The results shown in Fig. 2A demonstrate that whereas the recombined vector band (510 bp) was recovered and amplified from aid−/− cells as well as from WT B cells, the 180-bp band, which indicates Sμ-Sα recombination, could not be amplified from aid−/− cells. This experiment was performed with p218, a plasmid that lacks transcriptional promoters and enhancers upstream of the Sα and Se segments (Fig. 1A). Thus, the effect of Suv39h1 is not due to effects on transcriptional elements. Suv39h1 also stimulates Sμ-Sα recombination in p273S in a variety of B cell lines, even those that rarely undergo endogenous switching and have very low plasmid switch activity (Fig. 2B). However, Suv39h1 was unable to stimulate plasmid switching in two cell lines (J558L plasmacytoma cells and EL-4 T cells) (Fig. 2B and data not shown) that lack AID.

Sα-specific effect of Suv39h1

To test whether Suv39h1 might be isotype specific, we tested the effect of Suv39h1 on SR in I.29μ cells of four additional plasmids in which Sy3, Sy2a, Sy2b, or Se segments replace the Se segment (Fig. 1A). Cotransfected Suv39h1 had no effect on SR of any of these plasmids, except perhaps a small stimulatory effect on the Se plasmid, pE.1 (Fig. 2C). However, because I.29μ cells can only

FIGURE 2. Suv39h1 stimulates plasmid Sμ-Sα recombination. A, SR (Sμ-Sα) of transiently transfected S plasmid p218 in WT, but not aid−/− splenic B cells. The expression plasmid pSuv39h1 and the empty vector control plasmid (pcDEF3) were cotransfected along with p218 into WT or aid−/− splenic B cells. Duplicate transfections were performed 3 days after LPS activation, and cells were harvested 2 days after transfection. Nuclear DNA was isolated and assayed for recombination, as in Fig. 1B. Two-fold doses of each template were assayed, and the data were quantitated by phosphor imaging. The graph shows the average signal (+SEM) of the 180-bp product band normalized to the 510-bp band for each template dose (relative recombination activity) (14). In this experiment only, the relative recombination activity for WT cells transfected with the empty vector were set = 1, and the other data were normalized to this value. Shown above the graph is an autoradiograph of a polyacrylamide gel containing the α-32P-labeled PCR products: 180 bp (S-S recombination) and 510 bp (vector control). B, Suv39h1 stimulates Sμ-Sα recombination in p273S in four different B cell lines, but not in J558L plasmacytoma cells. Relative recombination activity is shown for cells transfected with the control vector (pcDEF3) or the expression plasmid pSuv39h1. Cells were not stimulated. N, Indicates the number of independent transfections/cultures, each of which was analyzed by DC-PCR at least twice. C, Cotransfection of Suv39h1 into I.29μ cells stimulates SR in S plasmids that contain Sα sequences, but not in plasmids containing other S regions (except perhaps Se slightly). Fold stimulation by pSuv39h1 is shown relative to the recombination in cells transfected with pcDEF3 (vector control plasmid). D, Cotransfection of Suv39h1 does not stimulate Sμ-Sy3 SR in pG3.1 in I.29μ, 1B4.B6, I.29μ, or BFO.3 cells.
switch to IgA and IgE (54), and do not support plasmid SR to IgG3 or IgG1 (14, 16), it is possible that the inability of Suv39h1 to stimulate plasmid SR to other isotypes is due to the lack of a required isotype-specific activity in I.29α. Therefore, we tested the ability of Suv39h1 to increase plasmid Sμ-Sγ3 SR (pG3.1) in 1B4.B6, a B cell line with IgG3 switching activity (14). As shown in Fig. 2D, Suv39h1 does not stimulate SR in pG3.1 in 1B4.B6 cells. These data indicate that Suv39h1 specifically stimulates Sμ-Sα recombination.

HMT activity of Suv39h1 is required to stimulate Sα SR

To determine whether Suv39h1 stimulation of Sμ-Sα recombination requires the HMT activity of this enzyme, we created a single amino acid mutation in the SET domain (H324L) of the Suv39h1 gene, which should completely abolish the HMT activity of Suv39h1, according to studies of the human protein (29). This mutation eliminated the stimulatory effect of Suv39h1 on plasmid Sμ-Sα recombination, indicating that the HMT activity is essential for enhancing switching (Fig. 3A). Additionally, a naturally occurring Suv39h1 splice variant in I.29α cells (Δ42–229) (data not shown), which lacks the nuclear localization domain, chromodomain, and cysteine-rich domain, had almost no stimulatory activity (Fig. 3B). Smaller internal deletions were created and resulted in intermediate activity, consistent with the involvement of these domains in nuclear localization, interaction with HP-1 and H3 (46, 55).

To determine whether the effect on plasmid Sμ-Sα recombination is specific to Suv39h1, in similar experiments we overexpressed G9a, another repressive HMT that performs mono- and dimethylation at H3-K9, but which is associated with euchromatin rather than heterochromatin (36, 56). Overexpression of G9a had no effect on plasmid SR (our unpublished data).

Suv39h1 regulates endogenous IgA CSR

To determine whether Suv39h1 regulates SR of endogenous IgH genes, B cells from Suv39h1-deficient mice (32) were examined for their ability to undergo CSR. We used flow cytometry to confirm that the spleen cell populations from Suv39h1-deficient mice did not differ from those of WT mice. In addition to equivalent numbers of CD3−T cells and B220+B cells, there was no difference in the marginal zone (CD23low, CD21high) or follicular (CD23high, CD21low) B cell numbers (our unpublished data). To examine CSR, splenic B cells were isolated and cultured for 4 days under conditions that induce switching to the isotypes shown in Fig. 4A, then stained with Abs to various IgH chain isotypes (48). B cells from Suv39h1-deficient mice showed a 2-fold reduction in switching to IgA compared with the control mice, but unchanged levels of switching to all other isotypes (Fig. 4A). The results from the individual cultures for IgA CSR are shown in Fig. 4B. The difference in percentage of IgA+ cells between WT and Suv39h1-deficient cultures is highly significant (p < 0.003), as determined by a paired t test. We also examined whether mice doubly deficient in Suv39h1 and Suv39h2 (32) would have a further reduction in IgA switching and found they did not. Splenic B cells from two mice deficient in both Suv39h1 and Suv39h2 switched 60% as well to IgA as WT B cells (Fig. 4C and data not shown). These data show that Suv39h1, but probably not Suv39h2, enhances IgA CSR.

CSR is strongly correlated with cell division, and is ongoing as B cells proliferate in LPS cultures (57). Although no differences in

FIGURE 3. Mutations within Suv39h1 reduce its ability to stimulate plasmid SR. A, Mutation of the catalytic domain abolishes Suv39h1 stimulation of plasmid Sμ-Sα recombination. Results of DC-PCR assays of Sμ-Sα recombination in p273S cotransfected into I.29α (22D) cells with either WT or mutant Suv39h1 (H324L) or with empty expression plasmid pcDEF3, as indicated. B, Deletions of the indicated segments of Suv39h1 reduce p273S SR stimulatory activity.
Suv39h1 does not regulate GLα transcripts

The S plasmids p273 and pG3.1 both have a GLα promoter that drives transcription across the Sα segment (13) (Fig. 1A). Thus, the ability of Suv39h1 to stimulate Sμ-Sα recombination cannot be due to stimulation of transcription from this promoter, as both p273 and pG3.1 have the identical GLα promoter, but only p273 switching is stimulated by Suv39h1. Additionally, cotransfection of Suv39h1 increases SR in plasmid p218 as well as it does in p273, although p218 has no transcriptional elements upstream of either the Sμ or Sα segments (Figs. 1A and 2C). In agreement with these results, we found no difference in α GLT levels in Suv39h1-deficient and WT splenic B cells stimulated to switch to IgA (Fig. 6). Therefore, Suv39h1 does not increase CSR to IgA by regulating α GLTs.

The effect of Suv39h1 on plasmid CSR does not require the Sα Pax5 binding site

To reconcile the finding that Suv39h1 specifically increases CSR involving the Sα segment, but not with other acceptor S regions, and the fact that Suv39h1 is a repressor of gene activity, we hypothesize that Suv39h1 activity inhibits a repressor that has specificity for Sα sequences. One known repressor of IgA CSR that binds to Sα is Pax5 (59). In the p273 and p218 plasmids, there are two binding sites for Pax5, one at the 5′ end of Sμ and another at the 5′ end of Sα (13). We have tested the effect of deleting the Pax5 binding site from Sα in p218, the Sμ-Sα plasmid lacking transcriptional regulatory elements (Fig. 1A). Deletion of the known Pax5 binding site from the Sα segment did not alter plasmid recombination or its ability to respond to Suv39h1 (Fig. 7).
Thus, Suv39h1 does not stimulate CSR to IgA by inhibiting the binding of Pax5 to Sα segments.

Discussion

In this study, we show that the HMT Suv39h1 specifically increases Sμ-Sα SR in plasmid S substrates by ~10-fold, and that Suv39h1-deficient B cells have reduced abilities (~2-fold) to undergo CSR to IgA in culture, but switch at normal levels to all the IgG subclasses. Furthermore, we found a 50% reduction in the proportion of freshly isolated Peyer’s patch B cells that express IgA in Suv39h1-deficient mice. The finding that Suv39h1 increases IgA CSR is surprising because Suv39h1 trimethylates the K9 residue of H3, a repressive modification associated with pericentromeric and centromeric heterochromatin (35, 60). Additionally, Suv39h1 binds Rb and is involved in repression of euchromatic genes that are dependent upon E2F transcription factors (39, 40, 42). Because Suv39h1 is known to be a transcriptional repressor, these results suggest that Suv39h1 stimulates CSR specifically to IgA by inhibiting a repressor specific for IgA switching. Interestingly, the regulation of IgA CSR by Suv39h1 does not occur by regulating α GLTs, as their levels are unaffected by Suv39h1 deficiency. As we have found that Suv39h1 stimulates plasmid Sμ-Sα recombination activity, but not SR to other acceptor S regions, it is possible that Suv39h1 HMT activity inhibits binding of a S repressor protein to Sα.

A candidate for a repressor that might be inhibited by Suv39h1 activity is LSF (late SV40 factor/CP2), a protein that binds Sα and Sμ segments in EMSA and has been shown to inhibit IgA CSR (45). There are numerous binding sites in each of the Sα tandem

FIGURE 5. Suv39h1 does not affect B cell cycle or proliferation during CSR. A, DNA content analysis of splenic B cells 48 h after induction to switch to each of the indicated isotypes. The percentage of cells in each phase of the cell cycle is indicated. G1 = G0/G1 and G2 = G2/M. Similar results were obtained in two experiments. B, Proliferation ([^3]H)TdR incorporation) of splenic B cells on day 3 after addition of inducers of switching of the indicated isotypes. Shown are means of two experiments (+SEM), each performed in triplicate.

FIGURE 6. α GLTs are not regulated by Suv39h1. α GLTs were assayed by semiquantitative RT-PCR in splenic B cells from WT and Suv39h1-deficient mice after 4 days of culture with the indicated inducers of CSR. α GLTs are induced in cells during induction of switching to IgA, but not in cells induced to switch with LPS + IL-4. Two-fold doses of input template were amplified. Amplification of GAPDH cDNA served as an internal control for template dose. Similar results were obtained in two experiments.

FIGURE 7. Deletion of the Pax5 binding site from the Sα segment in p218 does not affect the ability of Suv39h1 to stimulate Sμ-Sα recombination. Either p218 or p218-Pax5Δ was cotransfected into L294 cells with the Suv39h1 expression plasmid or the empty vector, and plasmid SR was assayed 2 days later, as described in Fig. 2 legend and Materials and Methods.
repeats and also in the Sμ tandem repeats, but very few LSF binding sites in the other mouse S regions. LSF binds to the SV40 promoter, HIV long-terminal repeat, and α-globin promoter, where it stimulates or inhibits transcription, depending on its binding site and interacting proteins (61). In the I-29μ B cell line, which undergoes CSR from IgM to IgA, stable expression of two different dominant-negative forms of LSF increased IgA CSR 2–fold (45). Therefore, an attractive hypothesis is that by an unknown mechanism, Suv39h1 activity inhibits the binding of LSF to Sμ regions or inhibits its activity when bound to Sμ.

One possible mechanism might be that Suv39h1 inhibits the putative IgA CSR repressor protein by inhibiting its transcription by methylating H3 on a nucleosome(s) at its promoter. Alternatively, Suv39h1 might interact with the putative repressor protein, perhaps methylating it and thereby sequestering it from Sμ regions. Although the only substrate identified to date for Suv39h1 is histone H3, it was shown recently that another HMT, SET9, methylates histone H3 and also TAF10, a component of the general transcription machinery, and that this methylation stimulates transcription in a promoter-specific manner (62). We considered the possibility that Suv39h1 might affect the DNA-binding activity of LSF, but found no difference between LSF-binding activity in WT and Suv39h1-deficient B cells by EMSA (data not shown). Experiments performed by Drouin et al. (45) suggested that LSF is modified in an unknown way upon induction of CSR, and this reduces its ability to bind Sμ regions. It is possible that methylation of the Sμ region by Suv39h1 might inhibit the binding of LSF, but this model would require that transiently transfected nonreplicating plasmids could associate with nucleosomes.

Another possibility is suggested by previous findings that overexpression of Suv39h1 in a variety of cell types alters the intranuclear localization of proteins associated with heterochromatin (the polychrome group protein HPC2 and HP-1β), resulting in concentration of these proteins into large heterochromatin domains away from euchromatin (46, 63). This relocalization depends on the Suv39h1 SET domain, which contains the HMT catalytic site, and also on its chromodomains, and is associated with increased H3-K9 methylation in these heterochromatin domains (63). Both HMT activity and the chromodomains are required for Suv39h1 to stimulate plasmid switching. Perhaps the relocalization of HPC2 and HP-1β to pericentromeric heterochromatin domains results in the sequestration of an isotype-specific inhibitory protein away from the transiently transfected plasmids. In Suv39h1-deficient cells, one could speculate that these same HMTs are more widely distributed in euchromatin regions than in WT cells, and thus associate with the Sμ segment.

It is unclear why the effect of deleting the suv39h1 gene is so small relative to the large stimulation of plasmid SR by overexpression of Suv39h1. Although it could be due to an artifact of overexpression, it is also possible that the endogenous level of Suv39h1 is low and limiting in WT B cells, and thus its loss has a smaller effect than its overexpression. Another possibility is that Eset, another HMT that trimethylates K9-H3 (19) and is inducibly expressed in splenic B cells (64), is partially redundant with Suv39h1.

**Is Suv39h1 regulated during B cell activation?**

Suv39h1 is ubiquitously expressed (55). We have examined whether expression of Suv39h1 mRNA differs among cell lines that can or cannot switch to IgA and found it does not (data not shown). Furthermore, we observed no consistent change in Suv39h1 mRNA levels when splenic B cells are induced to undergo CSR. Suv39h1 associates with centromeric regions during prometaphase and metaphase, but is more broadly distributed over the nucleus during the remainder of the cell cycle (34). Several studies suggest that the activity of Suv39h1 may be regulated during the cell cycle by phosphorylation, rather than at the protein level (34, 40, 65, 66). Suv39h1 undergoes phosphorylation in HeLa cells at the G1/S phase transition, and this phosphorylation appears to be regulated by the anti-phosphatase SET-binding factor 1 (Sbf1) (65), although the kinase(s) involved is unknown. In cotransfection experiments, Sbf1 inhibited the transcriptional repression activity of Suv39h1 (65). The activity of Suv39h1 might be stimulated upon activation of B cells to undergo proliferation and isotype switching, thereby repressing an inhibitor of IgA CSR. One possibility is that Sbf1 is degraded or inhibited in activated B cells, thereby activating Suv39h1.

**Regulation of IgA CSR by S region-specific factors**

Isotype specificity of switching is regulated by GL transcription, which is necessary for CSR. However, the sequences of the acceptor S regions themselves can contribute to regulation of isotype specificity (this study) (14–16). Each S region consists of tandem repeats of sequence elements ranging from 20 to 80 bp in length, up to a total length of 1–10 kb. As each S region is unique, sequence-specific DNA-binding proteins might regulate SR to each isotype, possibly by regulating accessibility and/or AID recruitment (14–16). Kenter et al. (15) have examined SR in plasmids containing minimal-length S regions to study the importance of specific Sy3 and Sy1 sequences. They analyzed a specific sequence motif within the Sy3 consensus repeat that differs between the Sy3 and Sy1 consensus repeats to ask why plasmids with the Sy3 region can switch in LPS-activated cells, whereas plasmids with the Sy1 region switch only if IL-4 is also added. Mutation of the element showed that the Sy1 sequence element prevents plasmid Sμ–Sy1 recombination in splenic B cells treated with LPS alone, but if this motif is replaced by the Sy3 sequence element, plasmid SR occurs in cells treated with LPS alone. This result suggests that a specific factor required for SR binds this Sg3 element. The Kenter studies (16) also suggest that there are Sμ- and Sβ-specific factors, but none of the factors have been identified. In the current study, we identify Suv39h1 as an Sg3-specific factor.

In conclusion, Suv39h1 specifically stimulates IgA CSR, most likely by inhibiting an inhibitor that binds Sμ, as the effect was specific to S plasmid constructs containing the Sg3 segment. Furthermore, mice deficient in Suv39h1 show reduced frequency of IgA + B cells in Peyer’s patches, and splenic B cells from these mice show specific reductions in IgA class switching. Although how Suv39h1 inhibits such an inhibitor is unclear, there are several possibilities that can be investigated.

**Acknowledgments**

We thank Dr. Makoto Tachibana for the mouse cDNA clone for G9a; Dr. Wenyue Bai for assaying whether G9a stimulates plasmid SR; and Dr. Amy Kenter for plasmids pE.1 and pG3.1 and for helpful discussions.

**Disclosures**

The authors have no financial conflict of interest.

**References**


