Inducible CTCF insulator delays the *IgH* 3′ regulatory region-mediated activation of germline promoters and alters class switching

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Class switch recombination (CSR) plays an important role in adaptive immune response by enabling mature B cells to switch from IgM expression to the expression of downstream isotypes. CSR is preceded by inducible germline (GL) transcription of the constant genes and is controlled by the 3′ regulatory region (3′RR) in a stimulus-dependent manner. Why the 3′RR-mediated up-regulation of GL transcription is delayed to the mature B-cell stage is presently unknown. Here we show that mice devoid of an inducible CTCF binding element, located in the α constant gene, display a marked isotype-specific increase of GL transcription in developing and resting splenic B cells and altered CSR in activated B cells. Moreover, insertion of a GL promoter downstream of the CTCF insulator led to premature activation of the ectopic promoter. This study provides functional evidence that the 3′RR has a developmentally controlled potential to constitutively activate GL promoters but that this activity is delayed, at least in part, by the CTCF insulator, which borders a transcriptionally active domain established by the 3′RR in developing B cells.

B lymphocyte | *IgH* locus | insulator | germline transcription | class switch recombination

Expression of complex loci is developmentally programmed or induced by specific stimuli and is often controlled by distant regulatory elements within relatively large chromatin domains. Transcriptional and architectural factors play an important role in the establishment and maintenance of these domains and facilitate long-range interactions between regulatory elements and target promoters (1, 2). The Ig heavy chain (*IgH*) locus is expressed in a lineage- and developmental stage-dependent manner. Various *cis*-acting elements including promoters, enhancers, and insulators control *IgH* locus expression and are engaged in multiple long-range interactions (3, 4).

Factors such as YY1, PAX5, IKAROS, CTCF, and Cohesin play important roles in various aspects of long-range events at the *IgH* locus, including V(D)J recombination, CSR, and promoter/enhancer and enhancer/enhancer interactions (3–6). Multiple CTCF binding elements (CBEs) were reported along the *IgH* locus. The majority of these CBEs lie within the variable domain (7), and two CBEs were identified within the V_H-D intergenic region (7–9). At the 3′ end of the locus, ∼10 CBEs were identified downstream of the 3′RR and are thought to delineate the 3′ border of the *IgH* locus (10). More recently, a discrete CBE was identified within the α constant gene (11), but its role in vivo is presently unknown.

Upon antigen challenge, mature B cells can undergo CSR that allows B cells to change the heavy-chain constant domain of an IgM to IgG, IgE, or IgA. CSR to a particular isotype is induced by specific external stimuli, including antigens, mitogens, cytokines, and intercellular interactions. CSR is mediated by highly repetitive sequences called switch (S) sequences located upstream of the constant exons and is preceded by germline (GL) transcription of the S sequences that originates from GL promoters, named I promoters (12).

The 3′RR is composed of four enhancers—hs3a, hs1.2, hs3b, and hs4—and controls CSR by regulating GL transcription across S sequences. This entails a long-range control of multiple upstream I promoters (6, 13). Gene-targeted deletion of individual enhancers had no effect on GL transcription (14–16). In contrast, deletion of both hs3b and hs4 or of the whole 3′RR dramatically impaired GL transcription (e.g., refs. 17, 18). Thus, the prevailing notion is that the 3′RR-mediated activation of GL transcription preceding CSR is restricted to mature B cells (17–19). This leaves it unknown whether the 3′RR is programmed to activate GL promoters of the *IgH* constant domain only after activation of B cells or whether it can do so in a developmentally regulated, constitutive manner before induction.

Here we show that the 3′RR has the potential to prematurely activate upstream GL promoters in developing and resting B cells, though in an isotype-restricted manner. This activity is delayed by an inducible CTCF insulator that borders an active domain in which the 3′RR displays a bidirectional transcriptional activity.

Results

Specific Increase of Sγ3, Sγ2b, and Sγ2a GL Transcription in 5′hs1RI$^{\Delta/\Delta}$ Resting B Cells. A DNase I hypersensitive site (hs) was detected within the $C\alpha_3$–$C\alpha_{mb}$ intervening sequence of the mouse α constant gene (20) and was recently shown to bind CTCF in resting, but not in activated, splenic B cells (11). The CBE is conserved in

Significance

The spatial and temporal control of complex loci's expression is often effected by distant regulatory elements. At the *IgH* locus, class switch recombination (CSR) is preceded by transcription of the recombining genes and is controlled by the 3′ regulatory region (3′RR) in a stimulus-dependent manner. Why the 3′RR-mediated up-regulation of transcription is delayed to the mature B-cell stage is unknown. We show that an inducible CTCF insulator is involved in this process. Deletion of the insulator led to specific deregulation of transcription and CSR at earlier developmental stages. Insertion of a promoter downstream of the insulator led to its premature activation. Thus, the 3′RR has developmentally controlled potential to constitutively activate target promoters, but its activity is blocked by the insulator.

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the human *α1* and *α2* constant genes (Fig. S1). To elucidate the function of this element in vivo, the Cα3–Cαmb intervening sequence encompassing the hs and the CBE (hereafter called 5′hs1RI) was deleted by gene targeting (Fig. 1*A*). The extent of the deletion was checked by sequencing the relevant region in genomic DNA of 5′hs1RI-deficient splenic B cells and by chromatin immunoprecipitation assays, which confirmed the lack of binding of CTCF and SMC1 and SMC3 subunits of the Cohesin complex in 5′hs1RI$^{\Delta/\Delta}$ B cells (Fig. S2 *A*–*C* and Table S1).

The finding that CTCF bound 5′hs1RI in resting but not in activated B cells (ref. 11 and Fig. S2*C*) led us to hypothesize that the 5′hs1RI may act as a CTCF insulator to the 3′RR in resting B cells, in which case deletion of the 5′hs1RI element would result in premature activation of upstream GL promoters before B-cell activation. To test this hypothesis, we analyzed GL transcription in resting B cells. Total RNAs from CD43^{-} sorted splenic B cells derived from WT and 5′hs1RI$^{\Delta/\Delta}$ littermates were extracted, reverse-transcribed, and analyzed by qPCR. With the exception of the constitutive Sμ GL transcription, derived from Eμ/Iμ promoter, GL transcription across the downstream S regions is barely detectable for most isotypes in unstimulated splenic B cells but is induced upon appropriate stimulation (17, 18, 21).

Strikingly, Sγ3 GL transcripts were markedly up-regulated in unstimulated 5′hs1RI$^{\Delta/\Delta}$ splenic B cells compared with WT controls. There was also a significant increase of Sγ2b and Sγ2a GL transcript levels, though clearly less marked for Sγ2a transcripts. In contrast, no such up-regulation was detected for Sγ1, Sε, and Sα GL transcripts whose levels were extremely low (Fig. 1*B*, *Top*). In 5′hs1RI$^{\Delta/\Delta}$ LPS-activated B cells, Sγ3 transcript levels were comparable to their WT counterparts, and those of Sγ2b were slightly higher than WT levels. The transcript levels of the other isotypes were comparable between mutants and WT controls upon appropriate stimulation (IFNγ stimulation for Sγ2a, IL4 stimulation for Sγ1 and Sε, and TGFβ stimulation for Sα) (Fig. 1*B*, *Bottom*). These findings were obtained regardless of the origin of the WT and 5′hs1RI$^{\Delta/\Delta}$ mice—that is, littermates or from different litters. We conclude that in resting B cells, the 5′hs1RI deletion has a major effect on Sγ3, Sγ2b, and Sγ2a GL transcription specifically.

We then investigated whether up-regulation of Sγ3, Sγ2b, and Sγ2a GL transcription in 5′hs1RI$^{\Delta/\Delta}$ resting B cells correlated with a perturbed expression pattern in marginal zone (MZ) versus follicular (FO) B cells. Although the levels of Sγ1, Sε, and Sα were extremely low and did not vary, Sγ3 transcript levels were higher in both MZ and FO mutant B cells. Sγ2b and Sγ2a transcript levels were also increased in both populations, though the increase was higher in the FO B-cell population (Fig. S3*A*). Thus, 5′hs1RI deletion leads to increased levels of Sγ3, Sγ2b, and Sγ2a transcripts in both MZ and FO B cells.

To investigate whether 5′hs1RI deletion affects GL transcription in *trans*, we quantified GL transcript levels in unstimulated WT, heterozygous, and homozygous splenic B cells from littermates. We focused on Sγ3 and Sγ2b as their levels were higher in mutant B cells. The levels of Sγ3 and Sγ2b GL transcripts in heterozygous mice were roughly half those in homozygous mice (Fig. S3*B*). Upon appropriate stimulation and in agreement with the biallelic nature of GL transcription (22), GL transcript levels were comparable regardless of the genotype (Fig. S3*B*), indicating that in the absence of 5′hs1RI, Sγ3 and Sγ2b GL transcription is likely up-regulated in *cis*. We conclude that 5′hs1RI mainly acts in *cis*.

The 5′hs1RI Deletion Exerts Its Effect Without Altering the 3′RR eRNAs Levels. Although their levels are low, the 3′RR transcripts [3′RR enhancer RNAs (eRNAs)] can readily be detected upon stimulation of splenic B cells and correlate with the 3′RR activity in activated B cells (23, 24). Additionally, recent work involved hs4 eRNA in long-range interactions with a far downstream non-*Ig* sequence (25). Because the deletion of 5′hs1RI may have impacted the long-range interactions of the 3′RR and potentially altered its activity, it was important to check the effect of the mutation on the 3′RR eRNA levels.

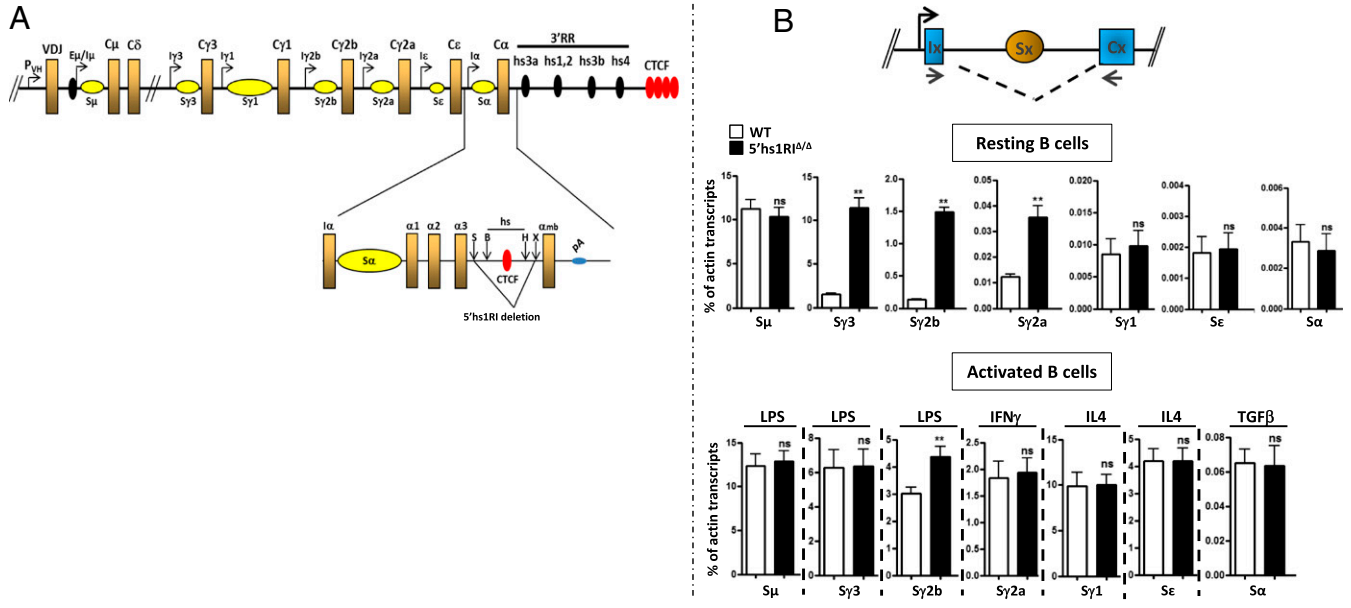

Fig. 1. Specific increase of Sγ3, Sγ2b, and Sγ2a GL transcripts in 5′hs1RI$^{\Delta/\Delta}$ splenic B cells. (*A*) Deletion of 5′hs1RI. (*Top*) Scheme of a rearranged *IgH* locus. The α constant gene is magnified in the scheme below which also highlights the relative position of the hs and the CBE targeted by 5′hs1RI deletion. Only 4 out of ∼10 CTCF sites are shown downstream of the 3′RR. B, B*stE*II; H, H*ind*III; S, S*ph*I; X, X*ba*I. pA, polyadenylation sites of the membrane form of α HC transcript. (*B*) Analysis of GL transcription in resting and activated B cells. (*Top*) A constant gene; x stands for any isotype. The relative position of the primers used to detect spliced GL transcripts is indicated. Total RNAs were prepared from purified CD43^{-} WT and 5′hs1RI$^{\Delta/\Delta}$ splenic B cells or at day 2 poststimulation, reverse-transcribed, and the indicated GL transcript levels quantified by qRT-PCR ($n = 4$). $^{**}P < 0.01$; ns, not significant.

To this end, hs3a, hs1-2, hs3b, and hs4 eRNA levels were quantified by qRT-PCR. The 3′RR eRNA levels were at the background levels in WT and 5′hs1RI$^{\Delta/\Delta}$ pro-B cells. In pre-B cells, only low levels of hs4 eRNAs were detected in both genotypes. Importantly, although still low in unstimulated splenic B cells, the 3′RR eRNA levels did not vary in mutant B cells compared with their WT counterparts. Similarly, we found no difference between the 3′RR eRNA levels in WT and 5′hs1RI$^{\Delta/\Delta}$-activated B cells (Fig. S4).

Thus, the 5′hs1RI deletion did not alter the 3′RR eRNA levels. Therefore, the effect of the mutation on Sγ3, Sγ2b, and Sγ2a GL transcription cannot be ascribed to deregulated expression of the known 3′RR eRNAs.

The 5′hs1RI Deletion Differentially Affects CSR. The specific increase of Sγ3, Sγ2b, and Sγ2a transcript levels in resting B cells and those of Sγ2b in LPS-activated splenic B cells led us to investigate the effect of 5′hs1RI deletion on CSR. To this end, sorted CD43^{-} splenic B cells were induced to switch, and surface expression of IgGs was monitored by FACS. Surprisingly, IgG3 surface expression was reduced, whereas that of IgG2b was increased. In contrast, surface expression of IgG2a and of IgG1 was unaltered (Fig. 2*A*). These findings were confirmed by quantifying the levels of postswitch transcripts by qRT-PCR (Fig. S5*A*) and of CSR events at the genomic level by quantitative digestion/circularization-PCR (qDC-PCR) (Fig. 2*B*).

Because IgG3, IgG2b, and IgG2a production can be induced in a T-independent manner, we quantified their serum levels by ELISA at various time points after i.p. immunization with TNP-Ficoll. At day 28 postimmunization, IgG3 titers were significantly reduced, whereas those of IgG2a were increased. For IgG2b, although the increase was not statistically significant, the trend was consistently toward increased levels in mutant mice (Fig. S5*B*). Thus, at least with regard to TI type II antigens and within the limits of our time course, 5′hs1RI$^{\Delta/\Delta}$ mice display a defective IgG3-mediated and an enhanced IgG2a- and IgG2b-mediated humoral response.

The above data lead us to conclude that 5′hs1RI contributes to the regulation of CSR to IgG3, IgG2b, and IgG2a in vivo and in vitro.

Differential Up-Regulation of GL Transcription and Alteration of CSR in 5′hs1RI-Deleted Mice Start in Developing B Cells. The striking increase of Sγ3, Sγ2b, and Sγ2a transcript levels in unstimulated 5′hs1RI$^{\Delta/\Delta}$ splenic B cells led us to ask at which developmental stage this up-regulation was initiated. To this end, Sγ3, Sγ2b, and Sγ2a GL transcripts were quantified in sorted pro–B-cell and pre–B-cell populations from WT and 5′hs1RI$^{\Delta/\Delta}$ littermates. Sγ3 GL transcripts started to be detected at the pro–B-cell stage onwards, whereas Sγ2b GL transcripts were readily detectable in pre-B cells. In contrast, Sγ2a GL transcripts were at the background level in pro-B and pre-B cells (Fig. 3*A*, *Top*). The mutation targeted Sγ3 and Sγ2b GL transcripts specifically (Fig. 3*A*, *Bottom*).

Accordingly, when purified pro-B cells were grown in vitro and stimulated in the presence of LPS, Sγ3 transcript levels clearly increased. The increase of Sγ2b transcript levels was not statistically significant, although the trend was consistently toward the increase in activated mutant pro-B cells. In contrast, Sγ1 transcript levels were unaltered (Fig. 3*B*). Interestingly, by using postswitch transcript levels as a measure of the efficiency of CSR, we found a defective CSR to Sγ3 and an increased CSR to Sγ2b in LPS-activated mutant pro-B cells. In contrast, no alteration of CSR to Sγ1 was detected upon IL4 stimulation (Fig. 3*C*). Thus, although CSR frequency in activated pro-B cells is manifold lower than in activated splenic B cells, the altered pattern of CSR to Sγ3 and Sγ2b is strikingly similar.

We conclude that the specific effect of 5′hs1RI deletion on Sγ3 and Sγ2b GL transcription starts in developing B cells already.

Premature Activation of a Duplicated Iα GL Promoter Downstream of the 3′RR. Analysis of GL transcription in 5′hs1RI-deleted mice led us to provisionally conclude that the 3′RR had the potential

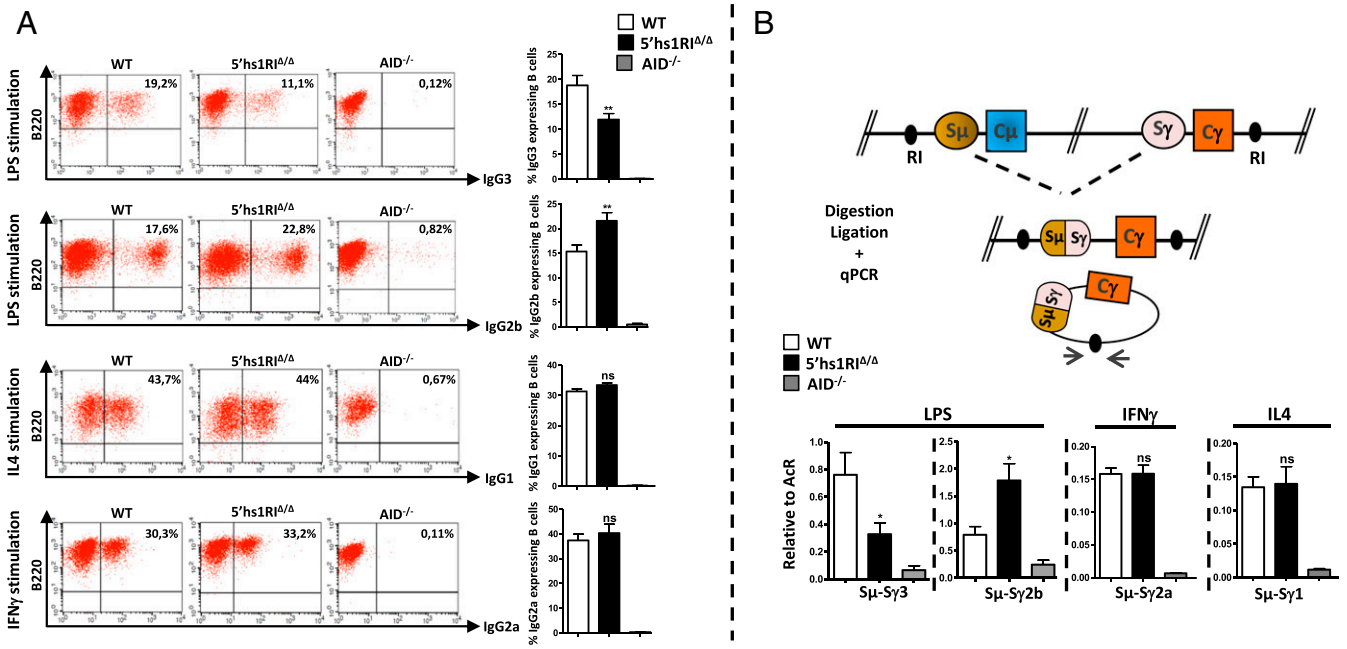

Fig. 2. The 5′hs1RI$^{\Delta/\Delta}$ deletion affects CSR. (*A*) CD43^{-} sorted splenic B cells with the indicated genotypes were induced to switch to IgG3 and IgG2b (LPS stimulation), to IgG1 (IL4 stimulation), or to IgG2a (IFNγ stimulation). At day 4.5 poststimulation, the cells were stained with the indicated antibodies. The statistical data are shown at *Right* ($n = 7$). (*B*) Genomic DNAs were purified from activated splenic B cells (day 4.5) and assayed by qDC-PCR (*Top*; RI, EcoRI). Quantification of CSR events was performed by qPCR ($n = 7$). *$P < 0.05$; **$P < 0.01$; ns, not significant.

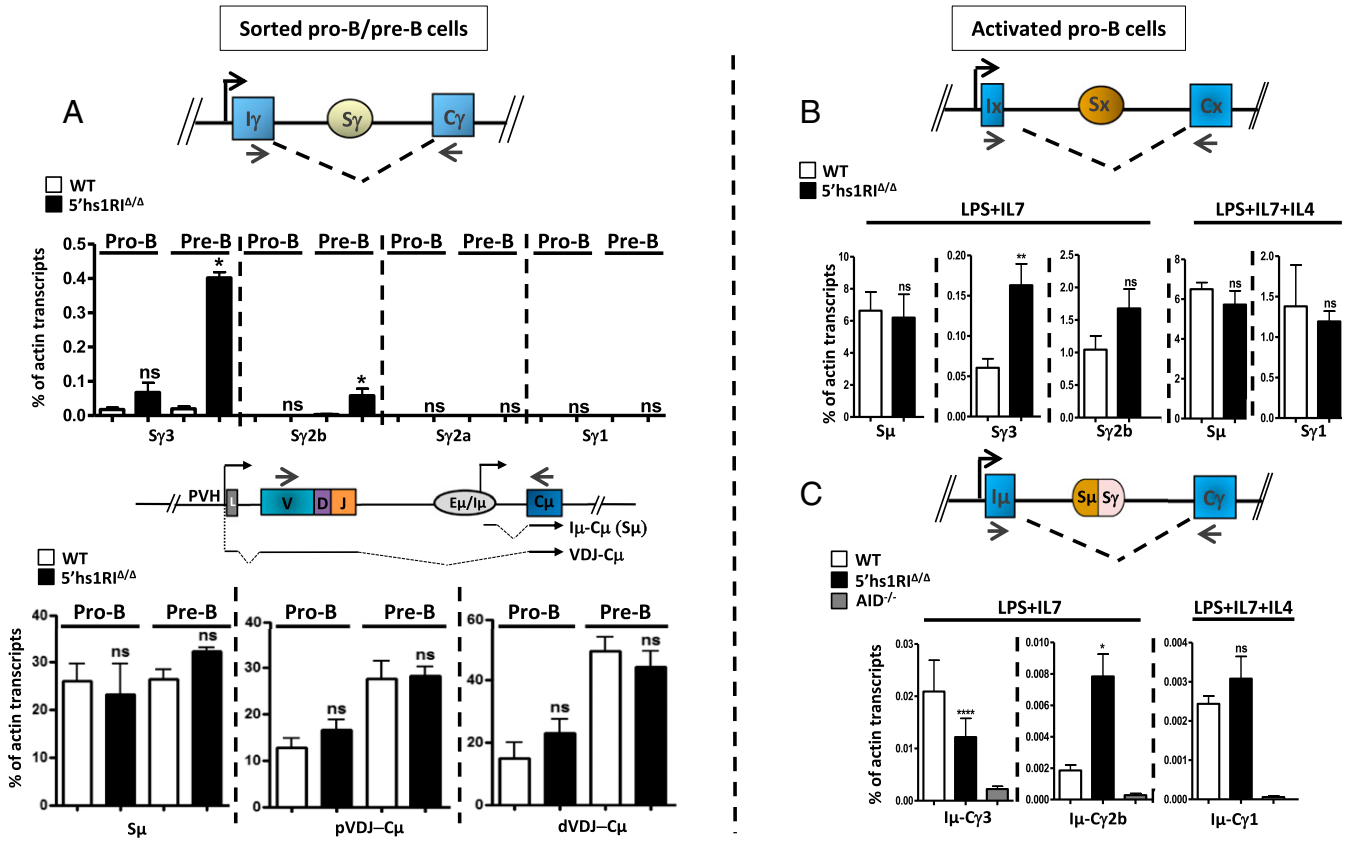

Fig. 3. The effect of 5′hs1RI deletion on GL transcription and CSR starts in developing B cells. (*A*) Total RNAs were prepared from sorted WT and 5′hs1RI$^{\Delta/\Delta}$ pro-B (B220^{+}IgM^{-}CD43^{high}) and pre-B (B220^{+}IgM^{-}CD43^{low}) cells, and GL transcript levels were quantified by qRT-PCR for Sγ GL transcripts (*Top*) and for Sμ GL transcripts (Iμ-Cμ) and μ HC transcripts (pVDJ-Cμ and dVDJ-Cμ) (*Bottom*) ($n = 3$, each experiment starting with a pool of at least three mice per genotype). dVDJ-Cμ, distal V_{HJ558}-containing μ transcripts; pVDJ-Cμ, proximal V_{H7183}-containing μ transcripts. (*B* and *C*) Sorted pro-B cells were grown for 4 d in the presence of IL7 and activated with LPS or LPS+IL4. At day 2 poststimulation, total RNAs were prepared and subjected to qRT-PCR as in *A*. Preswitch (*B*) and postswitch (*C*) GL transcript levels were quantified for the indicated isotypes ($n = 3$ for each set of GL transcripts). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant.

to activate GL promoters in developing B cells already but (*i*) that this activity was insulated from upstream GL promoters by 5′hs1RI and (*ii*) that in the absence of the insulator, the 3′RR activity was prematurely targeted toward Iγ3, Iγ2b, and Iγ2a GL promoters specifically. One implication of these notions is that constitutive activity is an inherent feature of the 3′RR within the chromatin domain downstream of 5′hs1RI, from pro–B-cell stage up to resting splenic B cells. If so, insertion of any GL promoter downstream of 5′hs1RI would lead to premature activation of that promoter. To provide a functional support to this notion, we generated a mouse line in which we inserted a chimeric transcription unit driven by Iα GL promoter, downstream of the 3′RR (hereafter called 2Iα mice; Fig. 4*A* and Fig. S6). All analyses were performed on homozygous 2Iα mice.

To determine at which developmental stage the ectopic Iα GL promoter was activated, we quantified β-globin transcripts in pro-B, pre-B, MZ, and FO B cells and TGFβ-activated splenic B cells. The endogenous Iα-derived GL transcripts were readily detected in TGFβ-activated B cells essentially (Fig. 4 *B* and *C* and Fig. S7). Interestingly, upon TGFβ stimulation, GL transcripts derived from the endogenous Iα GL promoter of 2Iα mice were just as abundant as those derived from the endogenous Iα GL promoter of WT mice (Fig. 4*C*), indicating that the ectopic Iα GL promoter did not interfere with the activity of its endogenous counterpart. Importantly, the transcripts derived from the ectopic Iα GL promoter started to be detected in pro-B cells (Fig. 4*B*) and their levels increased in resting B cells (Fig. 4*C*).

We conclude that insertion of the ectopic Iα GL promoter downstream of the 3′RR results in premature activation of the promoter.

Discussion

The present study reveals an important role for the 5′hs1RI element in *IgH* locus expression along B-cell development. The 5′hs1RI emerges as a *cis*-acting regulatory element that acts, at least in part, as an inducible CTCF insulator. One function of 5′hs1RI is to block premature activation of specific GL promoters—that is, Iγ3, Iγ2b, and Iγ2a GL promoters—before B-cell activation, strongly suggesting that 5′hs1RI is somehow involved in the transcriptional silencing of these promoters. This developmentally controlled, isotype-restricted targeting displayed by 5′hs1RI suggests specific mechanisms and implies that silencing of Iγ1, Iε, and Iα likely involves other mechanisms/elements.

Interestingly, the 5′hs1RI deletion did not alter the 3′RR eRNA levels. Additionally, Sγ3 transcript levels were increased in 5′hs1RI$^{\Delta/\Delta}$ resting B cells in the absence of any obvious increase of 3′RR eRNA levels, suggesting that up-regulation of 3′RR eRNAs was not absolutely required for the 3′RR to exert its constitutive, isotype-specific, activity. It could be that the 3′RR eRNAs are not required for the constitutive activity of the 3′RR and/or for the targeting of GL promoters, before B-cell activation and 3′RR induction. In support of this interpretation, Iγ3 GL promoter in 5′hsRI$^{\Delta/\Delta}$-deleted mice and ectopic Iα GL promoter in 2Iα mice were activated at the pro–B-cell stage at which no 3′RR

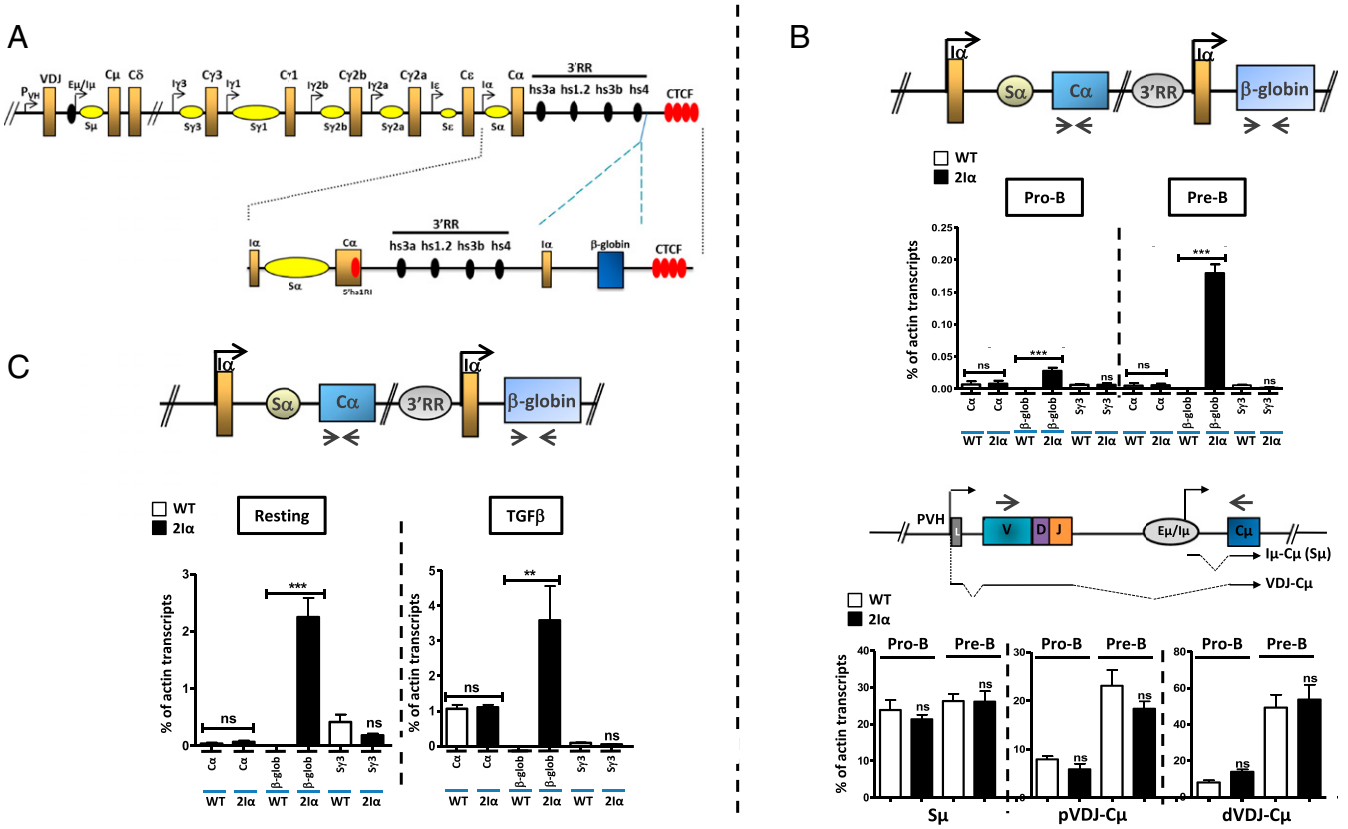

Fig. 4. Duplication of Iα GL promoter downstream of the 3′RR. (*A*) Scheme of a rearranged *IgH* locus. The inserted transcription unit is composed of the mouse Iα GL promoter followed by the terminal intron and exon of the human *β-globin* gene. The localization of 5′hs1RI within the *α* constant gene is indicated. (*B*) For the endogenous *α* constant gene and for the ectopic transcription unit, the primers were designed within Cα1 exon (Cα transcripts) and the β-globin exon (β-glob transcripts), respectively. Total RNAs were prepared from sorted pro-B and pre-B cells, and the indicated transcript levels were quantified by qRT-PCR ($n = 4$). (*C*) Total RNAs were prepared from CD43$^-$ splenic B cells and at day 2 post-TGFβ stimulation, and the endogenous Cα and the β-globin transcript levels were quantified by qPCR ($n = 4$). $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

eRNAs could be detected. Alternatively, low levels of eRNAs may be sufficient for the opening of enhancers' chromatin and the triggering of the 3′RR enhancing activity. In pro-B cells specifically, this would imply that very low levels of hs4 eRNAs would be sufficient to trigger the 3′RR enhancing activity.

Significantly, 5′hs1RI contributes to the regulation of CSR in a relatively complex way. Although increased CSR to IgG2b correlated with increased Sγ2b GL transcription, CSR to IgG3 was defective despite seemingly abundant Sγ3 GL transcription. A likely mechanism could be promoter interference (26, 27) incurred by the downstream, more active Iγ2b GL promoter, which may explain the drop of Sγ3 transcripts to WT levels upon LPS stimulation and the subsequent decrease of CSR to IgG3.

Interestingly, the CBE at the 5′hs1RI element and CBE2 at the IGCR1 share identical sequence (TCCACAAGAGGGCAG) and therefore the same orientation. Building on previous findings on the sequence convergence of interacting CTCF sites (28) and on the interactions engaging IGCR1 CBE2 and the Super Insulator downstream of the *IgH* locus (29, 30), it is plausible that the 5′hs1RI CBE interacts with the CBEs downstream of the *IgH* locus. This may indirectly support the notion that the 5′hs1RI CBE somehow contributes to the insulation of the 3′RR from upstream GL promoters before B-cell activation.

Previous chromosome conformation capture assays on unstimulated splenic B cells (31) or on a pro–B-cell line (32) detected relatively high cross-linking frequencies between the 3′RR and Iγ3 GL promoter and between the 3′RR and Iγ2b GL promoter, suggesting some proximity between the 3′RR and these promoters before B-cell activation. Our study suggests that 5′hs1RI somehow precludes premature 3′RR-mediated activation of Iγ3 and Iγ2b (and to some extent Iγ2a) GL promoters in pro-B cells up to resting splenic B cells. The finding that 5′hs1RI-bound CTCF was evicted upon B-cell activation suggests that CTCF could be involved in this process. Clearly, additional mutational studies are needed to clarify this issue. In immunological terms, it should be noted that MZ B cells mainly switch to IgG3 but can also switch to IgG2b and IgG2a, in response to T-cell–independent antigens (33). We speculate that the developmentally programmed 3′RR/Iγ3 and 3′RR/Iγ2b close-by positioning evolved, at least in part, to enable rapid IgG3 and IgG2b responses by MZ B cells.

Our initial working hypothesis was that deletion of 5′hs1RI would lead to premature activation of all upstream GL promoters with the possible exception of Iγ1 GL promoter (e.g., refs. 17, 18, 21). Strikingly, only Iγ3, Iγ2b, and Iγ2a GL promoters were targeted in the absence of 5′hs1RI. Notwithstanding this isotype restriction, it is clear that the 3′RR displays a constitutive transcriptional enhancer activity and that it already has the potential to activate upstream GL promoters of the *IgH* constant domain before antigenic induction. A reasonable inference is that the 3′RR could activate any GL promoter brought under its control within the transcriptionally active domain established by the 3′RR downstream of the 5′hs1RI element before B-cell activation. The premature activation of the ectopic Iα GL promoter provided a functional support to this notion. The downstream CBEs that mark the 3′ end of the *IgH* locus (10) likely delineate the 3′ border of this active domain. Our data with 2Iα mice suggest, but do not prove, that the 3′RR has a bidirectional activity, just that the effect on further downstream

sequences is likely blocked by the 3′CBEs. However, we do not infer that the mechanisms of action of the 3′RR on upstream and downstream promoters (relatively to the 3′RR) are necessarily the same.

Materials and Methods

Mice, Antibodies, and Cytokines. The generation of the mutant mice, the antibodies, and the cytokines used are described in *SI Materials and Methods*.

Mice and Ethical Guidelines. The WT, heterozygous, and homozygous mutants were of 129Sv background. The experiments on mice have been carried out according to the CNRS ethical guidelines and were approved by the Regional Ethical Committee.

Cell Sorting and Splenic B-Cell Activation. Single cell suspensions from the bone marrows or spleens were obtained by standard techniques. Pro-B cells were sorted as $IgM^{-}B220^{+}CD43^{high}$ and pre-B cells as $IgM^{-}B220^{+}CD43^{low}$. Splenic B cells were negatively sorted by using CD43-magnetic microbeads and LS columns (Miltenyi). Culture conditions used to induce GL transcription and CSR in pro-B cells and splenic B cells and FACS protocol are detailed in *SI Materials and Methods*.

qRT-PCR. Total RNAs were prepared from sorted pro-B cells, pre-B cells, MZ B cells, FO B cells, resting splenic B cells, and B cells at day 2 or 4.5 poststimulation. Total RNAs were reverse-transcribed (Invitrogen) and subjected to qPCR using Sso Fast Eva Green (BioRad). *Actin*, *Gapdh*, and *Ywhaz* transcripts were used for normalization and yielded similar results. Only normalization by *Actin* transcripts is shown. (–RT) controls were included in all of the experiments. The primers used are listed in *SI Materials and Methods*.

qDC-PCR. Genomic DNAs were purified from WT, $5'hs1RI^{\Delta/\Delta}$, and $AID^{-/-}$ splenic B cells at day 4.5 poststimulation. The EcoRI digestion and circularization steps were as described (32). Ligation products were subjected to qPCR. *Acetylcholine receptor* gene was used for normalization.

Statistical Analysis. Results are expressed as mean ± SEM (GraphPad Prism), and overall differences between values from WT and mutant mice were evaluated by an ANOVA parametric test with Newman–Keuls posttest and *t* test with Mann–Whitney posttest. The difference between means is significant if $P < 0.05$ (*), very significant if $P < 0.01$ (**), and extremely significant if $P < 0.001$ (***).

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