Published in final edited form as:

Ann N Y Acad Sci. 2012 September ; 1267(1): 86–94. doi:10.1111/j.1749-6632.2012.06604.x.

Three dimensional architecture of the Igh locus facilitates class switch recombination

Amy L. Kenter¹, Scott Feldman¹, Robert Wuerffel¹, Ikbel Achour, Lili Wang^{1,2}, and Satyendra Kumar¹

¹Department of Microbiology and Immunology, University of Illinois College of Medicine, Chicago, Illinois

Abstract

Immunoglobulin (Ig) class switch recombination (CSR) is responsible for diversification of antibody effector function during an immune response. This region specific recombination event, between repetitive switch (S) DNA elements, is unique to B lymphocytes and is induced by activation induced deaminase (AID). CSR is critically dependent on transcription of noncoding RNAs across S regions. However, mechanistic insight regarding this process remained unclear. New studies indicate that long range intra-chromosomal interactions among Igh transcriptional elements organize the formation of the S/S synaposome, as prerequisite for CSR. This three dimensional chromatin architecture simultaneously brings promoters and enhancers into close proximity to facilitate transcription. Here, we recount how transcription across S DNA promotes accumulation of RNA polymerase II leading to the introduction of activating chromatin modifications and hyperaccessible chromatin that is amenable to AID activity.

Keywords

Chromatin; class switch recombination; transcription; long range interactions

Introduction

B lymphocytes undergo a set of three programmed gene alterations that diversify immunoglobulin (Ig) antigen binding capability or effector function. Antigen binding specificity is encoded by the combinatorial joining of V_H, D_H and J_H or V_L and J_L gene segments on the heavy (H) and light (L) chain, respectively. H and L chain assembly via V-D-J joining occurs in an orderly stepwise process during early B cell development in the bone marrow. Somatic hypermutation (SHM), a mutational process, is focused to rearranged V_HDJ_H and V_LJ_L region genes and leads to increased affinity of antibody binding to antigen, in germinal center B cells. Class switch recombination (CSR) provides for diversification of C_H effector function while maintaining the original V(D)J rearrangement in mature B cells. IgH effector function is encoded by eight constant (C_H) region genes (μ, δ, γ3, γ1, γ2b, γ2a, ε and α) that span a 220 kb genomic region located near the right telomere of chromosome 12. CSR involves an intra-chromosomal deletional rearrangement that focuses on repetitive switch (S) DNA located upstream of each C_H gene (with the exception of Cδ) (reviewed in [1,2]).

Corresponding author: Amy L. Kenter, Department of Microbiology and Immunology, University of Illinois College of Medicine, Chicago, IL 60612-7344, star1@uic.edu.

²Current address: Cancer Vaccine Center, Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts

Conflicts of interest

The authors declare that they have no competing financial interests.

Germline or noncoding transcript (GLT) promoters associated with each of the paired S-C_H regions target CSR to specific S regions by selective transcriptional activation (reviewed in [1,2]). Germline transcripts (GLTs) are so termed because they lack an open reading frame and are not translated [3]. Combinations of cytokines and B cell activators induce transcription from specific GLT promoters [4]. Gene targeting experiments indicate the critical requirement for transcription and transcriptional elements to enable CSR (reviewed in [1,5]). Activation induced deaminase (AID) activity deaminates dC residues in transcribed S regions and is essential for CSR and SHM [6,7]. The mechanism by which AID induced DNA lesions are repaired and culminate in SHM or CSR have been extensively reviewed elsewhere [1,3,8–14].

S regions separated by as much as 150 kb can be targeted for recombination. This distance represents a topological challenge to formation of the S/S synaptosome, a requirement for CSR. However, the mechanism by which two distant S regions targeted for recombination are brought into close proximity has been unclear. Recent studies indicate that regulatory elements act over large genomic distances to bring widely separated elements into close spatial proximity with their target genes or other functional elements [15–21]. The chromatin conformation capture (3C) assay was developed to determine whether distant promoters and enhancers come into contact by long range looping or whether they serve as entry sites or nucleation points for factors that ultimately communicate with the gene [22]. Here, we explore recent findings regarding the influence of GLT transcription on three dimensional chromatin architecture, and its relationship to epigenetic chromatin modifications to suggest a model of how these processes integrate to regulate the CSR reaction.

The looping out and deletion mechanism of CSR

The organization of the C_H region of the *Igh* locus is illustrated in Figure 1A. The E μ intronic enhancer is located at the 5'-end of the C_H subregion. The 3' regulatory region, 3'E α , contains DNase hypersensitive sites (hs) 3a, hs1,2, hs3b and hs4 and functions as a locus control region LCR (reviewed in [23]). Further downstream there are additional hs sites 5–7 [24]. Sequestered between the two enhancers are eight C_H region genes each paired with a S region (with the exception of C δ). S region transcription is a defining feature of CSR. C_H genes are organized in transcription units consisting of a noncoding “intronic” (I) exon, the S region and the C_H coding regions. GLTs emanate from an intronic (I) promoter located upstream of each I exon, run through the S region and then terminate at the 3' end of it's neighboring C_H coding region (Fig. 1A) [4]. Downstream S regions are selectively targeted for recombination with S μ by directed activation of the isotype specific I exon promoters in response to combinations of antigen or mitogen, cytokines and costimulatory signals [2,4]. Targeted deletions of I exon promoters abolish transcription and severely affect CSR frequency [25–27].

Once transcription targets S μ and a downstream S region for recombination, CSR occurs through an intra-chromosomal deletional rearrangement that results in the formation of composite S μ -S_x junctions on the chromosome while the intervening genomic material is looped out and excised (fig. 1B). CSR occurs between two highly repetitive S regions [28]. Concomitant with transcription, AID initiates S region specific double strand breaks (DSBs) [29,30] that are processed through a cascade of events mediated by nonhomologous end joining (NHEJ) [3]. CSR occurs anywhere within the donor S μ and acceptor S region. Each CSR event produces a new composite S/S junction, which as a population are heterogenous with respect to the position of the recombination crossover points (fig. 1C). However, it has been unclear how distantly located S region-specific DSBs, separated by as much as 150 kb are recruited to partner in a CSR reaction that leads to intra-chromosomal deletion.

The mechanism of CSR is tied to the three dimensional structure of the Igh locus

Long range chromatin interactions over large genomic distances bring widely separated elements into close spatial proximity with their target genes. The mouse β -globin genes and their locus control region (LCR) located more than 50 kb apart engage in higher-order loop structures during transcription, and these interactions are tightly correlated with gene-specific expression [16,17,31]. Looped chromatin structures involved in the regulation of gene expression have also been detected in association with imprinted genes [19,32,33], cytokine clusters [18], estrogen receptor [15], MHC [21], α globin [20] loci and between chromatin boundary elements [34]. In the Igh locus, inducible transcription from the downstream GLT promoters requires the 3'E α LCR since targeted deletion of hs3b,4 within 3'E α leads to loss of GLT expression [2,35]. Thus, the direct interaction between GLT promoters and the distant 3'E α may be mediated by chromatin loop formation. Spatial proximity between the various downstream GLT promoters with 3'E α may facilitate transcription but would not support S/S synapsis since the S regions would not be juxtaposed with S μ (Fig. 2A,B). Our chromatin conformation capture (3C) studies indicate that in mature resting B cells, the E μ and 3'E α enhancers are in close spatial proximity and form a chromatin loop [36] (fig. 2C). B cell activation leads to cytokine dependent recruitment of the GLT promoters to the E μ :3'E α complex and enables transcription of S regions targeted for CSR (Fig. 2C) [36]. Our new data indicate that the GLT promoter (and not the S regions) directly interacts with the 3'E α element (unpublished observations). Prior to CSR, this looped structure facilitates S-S synapsis since S μ is proximal to E μ and a downstream S region is co-recruited with the targeted GLT promoter to E μ :3'E α complex. We and others conclude the Igh locus assumes a three dimensional structure that simultaneously supports GLT promoter interactions with the 3'E α regulatory regions to facilitate transcription of the targeted S regions and juxtaposes the S regions targeted for recombination [36–38].

S regions are specialized targets of CSR

S regions are G/C rich and fall into two general groups, sequences incorporating prevalent pentameric repeats into larger tandem repeats such as found in S μ , S ϵ and S α and a 49 bp repeat that is characteristic of S γ 3, S γ 1, S γ 2b, and S γ 2a [28]. S/S junctions are dispersed throughout S regions and occasionally fall in areas flanking the S regions [39,40]. CSR junctions are distinguished by their lack of consensus sequences or significant homology from site-specific or homologous recombination. S region length influences CSR frequency both *in vivo* and *in vitro* [39,41]. Deletion of S regions or their replacement with non-S region sequences by gene targeting methods, reduces CSR indicating that S regions are the specialized targets in this recombination event [41–44].

An intriguing question is how S regions with their divergent sequences are recognized by AID and the CSR machinery. The degeneracy of the S region repeats and the absence of an identifiable recombination motif have led to models in which higher order structures rather than primary sequence provides the recognition code for the CSR machinery [2,45]. S regions are replete with palindromic sequences that have the potential to form stem-loop structures that are proposed to function as recognition substrates [45–47]. Murine and human S regions are G-rich on the nontemplate strand which contributes to R-loop formation [48,49] and G4 tetraplexes ([50] and Chapter Maizels, this volume). Transcribed S regions contain R loops, comprised of RNA:DNA hybrids *in vitro* and *in vivo*, which can provide ssDNA stretches as substrate for AID deamination [48,49]. Targeted inversion of the S γ 1 region, making the G rich strand nontemplate, led to the loss of R-loop formation

and a significant reduction of CSR activity, arguing for the importance of R-loops for CSR efficiency [44].

Transcription and asymmetric AID attack

Early studies suggested that AID may function as an RNA deaminase based on similarities to APOBEC1, the catalytic component of a tissue-specific RNA editing complex ([51,52] and chapter Conticello). Identification of a mutational hotspot for AID in SHM indicated a mechanism by which AID functions directly on DNA [53]. This observation prompted Neuberger and coworkers to propose the DNA deamination model for AID action in which AID initiates SHM and CSR by converting deoxycytidine (dC) to deoxyuracil (dU) that can then be processed by one of several mechanisms (Fig. 3) (reviewed in [1,3,8,14]). The DNA deamination model gained substantial support from the direct demonstration that AID dependent dU residues are detected in the 5'S μ region [54] and that uracil DNA glycosylase (UNG) is required for formation of double strand breaks (DSB) in S regions to enable CSR [29].

During CSR, AID induced S region mutations begin 150 bp downstream of the I exon transcription start site (TSS) [55] (fig.4). The 3' boundary for CSR mutations is located downstream of S regions at a distance of up to 10 kb from the TSS [55] (fig. 4). Studies indicate that ectopically expressed AID binds with RNAP II in co-immunoprecipitation assays [56] and interacts with the transcription apparatus *in vitro* [57] suggesting that AID targeting is linked with transcription. Surprisingly, AID attack is asymmetrically focused within the I-S-C_H region. S regions are substrates for AID activity whereas C_H regions within the same transcription unit are protected [29,30,55] raising the question of how this occurs.

Chromatin accessibility to AID attack

Eukaryotic DNA is wrapped around histone octamers and organized into higher order chromatin fibers which regulate access of trans acting factors to DNA [58–60]. Studies suggest that at least two distinct mechanisms are used to achieve efficient transcription through chromatin. One relies on a pathway based on nucleosome loss and the second involves histone acetylation with little or no loss of histones (reviewed in [61]). Our studies indicate that the second pathway involving histone modification occurs during GLT expression during CSR [62,63], as will be described below. The RNA polymerase II (RNAP II) transcription cycle occurs in distinct steps including RNAP II recruitment to the promoter, formation of the preinitiation complex, promoter clearance, processive elongation, and transcription termination [61]. When RNAP II clears the promoter and transits to the promoter proximal pause site it becomes hyperphosphorylated at Ser5 (p-ser5) on the C-terminal domain (CTD). RNAP II then enters into productive elongation, loses p-ser5 and becomes enriched for p-ser2 CTD [61]. An emerging paradigm links transcription regulation with the phosphorylation status of RNAP II C-terminal domain (CTD) and the recruitment of histone modifying enzymes, which in turn introduce histone marks that alter the status of chromatin accessibility [59,64]. This paradigm is relevant to understanding asymmetric AID targeting away from C_H regions and toward S regions.

Transcription activity is strikingly correlated with significant histone acetylation (Ac) at promoters [65,66]. Genome wide studies reveal that promoter proximal sites in transcriptionally active genes are enriched for tri-methyl histone H3 lysine 4 (H3K4me3), hyper-acetylated (Ac) H3K9, and with RNAP II p-ser5 whereas, downstream coding regions are enriched in H3K36me3 and elongating RNAP II p-ser2 [66–69]. Histone methylation can act as a tag for effector proteins containing methyl-binding domains including chromodomains, tudor domains and PHD finger domains [70]. The NuA3 histone

acetyltransferase (HAT) complex that coordinates transcription activation with histone Ac is directly bound by H3K4me3 (reviewed in [71]). Histone Ac which changes the net charge of nucleosomes and alters chromatin fiber folding properties increases DNA accessibility [72]. Histone Ac may also create binding surfaces for factor-histone contacts that then facilitate recruitment of transcription regulators [59]. Rpd3S, a histone deacetylase (HDAC) complex interacts with H3K36me3, which then functions to reduce histone Ac and thereby repress transcription initiation in downstream coding regions [73,74]. The bifurcation of H3K4me3 and H3K36me3 modifications within a transcription unit serve to recruit HATs and HDACs, respectively, which in turn modulate chromatin accessibility.

Consistent with the observation that S regions and not C_H regions are modified by AID, markers of accessible chromatin are associated with S regions but not with C_H regions. *In vivo*, I-S regions undergoing active transcription are nuclease hypersensitive whereas C_H regions are comparatively inaccessible [62,63]. Accordingly, antisense RNA transcripts are found selectively in S regions and not in C_H regions indicating the reciprocal areas of accessible or repressed chromatin, respectively [75]. Transcriptionally active I-S regions accumulate H3K4me3 and histone Ac modifications while C_H regions become enriched for the repressive countermarks, H3K36me3 and H4K20me1 [56,62,63,76] (Fig. 4). Treatment with trichostatin A, an inhibitor of HDACs, increases H3Ac at S regions concomitant with greater chromatin accessibility and higher frequency CSR demonstrating a direct link between histone Ac and switching frequency [63]. Furthermore, PTIP (PAX interaction with transcription activation domain protein) deficiency displays reduced H3K4me3 and histone Ac marks and impaired transcription initiation at a subset of downstream S regions [77]. PTIP is a component of several histone methyl transferases (HMTases) including MLL3 (mixed lineage leukemia 3) – MLL4 complex that interact with RNAPII p-Ser5 [77,78]. PTIP also indirectly associates with p300/CBP HAT suggesting that PTIP regulates both histone Ac and Me [79]. Interestingly, long range looping interactions between GLT promoters and the 3'E_α enhancer are impaired in PTIP deficient B cells indicative of deficient GLT transcription initiation [80]. Collectively, these observations support the conclusion that histone Ac status and chromatin accessibility in S regions are determined by means of H3K4me3 modifications linked to transcription.

S regions, R-loops and AID targeting

S regions span 1–10 kb beyond the I exons and the TSSs [28], and when transcribed are enriched along their *entire* lengths with H3K4me3, H3Ac, and H4Ac, activating histone modifications [62,63]. This contrasts with genome wide analyses in which these marks are largely relegated to promoter proximal locations [66,67]. What directs the spread of these modifications long distances from the GLT TSSs? This unusual distribution of H3K4me3 in S regions is linked to stalled RNAP II p-ser5 in S DNA. ChIP studies showed accumulation of RNAP II p-ser5 throughout the S_μ and S_{γ3} regions indicating that RNAP II has stalled at multiple sites [63]. In contrast, RNAP II p-ser5 occupancy and H3K4me3 marks remained promoter proximal in S_μ deleted B cells, indicating that it is the S region sequence that stalls RNAP II leading to the unusual H3K4me3 distribution [63,81]. S regions are highly G/C rich and when transcribed develop R-loops over long stretches that enhance CSR efficiency *in vivo* ([48,49] and chapter Maizels). It is likely that R-loops which demonstrably impede transcription elongation [82,83] are responsible for RNAP II accumulation detected in S regions. Further investigation is required to ascribe a causal relationship between R-loop formation and RNAP II accumulation in S regions.

Is there a connection between RNAP II stalling and focused AID activity on S regions? Recent evidence suggests that AID interacts with the transcription elongation factor Spt5. Spt5 was identified in an shRNA screen as a critical regulator of CSR [84]. Upon clearance

from the promoter, RNAP II pausing occurs 25–50 nucleotides downstream of the TSS and is mediated by DSIF (5,6-dichloro-1-b-d-ribofuranosyl-benzimidazole (DRB) sensitivity inducing factor) comprised of Spt4 and Spt5, and NEF (negative elongation factor) [85]. Spt5 interacts with AID and mediates its association with RNAP II [84]. ChIP-seq experiments indicate overlapping positions of Spt5 binding with RNAP II stalling [84,86] and between sites of Spt5 and AID binding genomewide [84]. Spt5 also interacts with several co-transcriptional factors including splicing factors [87], capping enzyme [88] and the RNA exosome [89] that have been functionally associated with CSR [90–92]. Targeted deletion of the GLT splice donor element dramatically impaired CSR [25]. These observations highlight the highly integrated aspect of transcription elongation and RNA processing. Together, these findings suggest that AID is targeted to sites of high occupancy RNAP II by means of its association with Spt5. The unique character of S regions which are prone to forming R-loops, in turn impedes RNAP II elongation. It now appears that Spt5, a cofactor of stalled RNAP II, associates with AID and may function to target it to specific loci. Because C_H regions lack R-loops, stalled RNAP II does not accumulate and Spt5 does not focus AID to these regions.

Summary

Transcription is intrinsic to the mechanism of CSR. The unique structure of the S regions appears to be mechanistically linked to generating open chromatin. These advances highlight the nexus between transcription, long range looping interactions with chromatin remodeling and histone modifications which permit initiation of CSR by AID. Emerging new technologies that allow visualization of higher order chromatin structures are likely to provide new insights into transcription regulation by regulatory elements functioning at great distances from their target genes. Ig CSR is an excellent model to study the intersection of long range chromatin interactions, transcription and recombination.

Acknowledgments

This work was supported in part by the National Institutes of Health (AI052400 to A. L. K.).

References

1. Stavnezer J, Guikema JE, Schrader CE. Mechanism and regulation of class switch recombination. *Annu Rev Immunol.* 2008; 26:261–292. [PubMed: 18370922]
2. Manis JP, Tian M, Alt FW. Mechanism and control of class-switch recombination. *Trends Immunol.* 2002; 23:31–39. [PubMed: 11801452]
3. Chaudhuri J, Basu U, Zarrin A, Yan C, Franco S, Perlot T, Vuong B, Wang J, Phan RT, Datta A, Manis J, Alt FW. Evolution of the immunoglobulin heavy chain class switch recombination mechanism. *Adv Immunol.* 2007; 94:157–214. [PubMed: 17560275]
4. Stavnezer J. Molecular processes that regulate class switching. *Curr Top Microbiol Immunol.* 2000; 245:127–168. [PubMed: 10533321]
5. Perlot T, Alt FW. Cis-regulatory elements and epigenetic changes control genomic rearrangements of the IgH locus. *Adv Immunol.* 2008; 99:1–32. [PubMed: 19117530]
6. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell.* 2000; 102:553–563. [PubMed: 11007474]
7. Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, Catalan N, Forveille M, Dufourcq-Labelouse R, Gennery A, Tezcan I, Ersoy F, Kayserili H, Ugazio AG, Brousse N, Muramatsu M, Notarangelo LD, Kinoshita K, Honjo T, Fischer A, Durandy A. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). *Cell.* 2000; 102:565–575. [PubMed: 11007475]

8. Peled JU, Kuang FL, Iglesias-Ussel MD, Roa S, Kalis SL, Goodman MF, Scharff MD. The biochemistry of somatic hypermutation. *Annu Rev Immunol.* 2008; 26:481–511. [PubMed: 18304001]
9. Neuberger MS, Di Noia JM, Beale RC, Williams GT, Yang Z, Rada C. Somatic hypermutation at A-T pairs: polymerase error versus dUTP incorporation. *Nat Rev Immunol.* 2005; 5:171–178. [PubMed: 15688043]
10. Saribasak H, Rajagopal D, Maul RW, Gearhart PJ. Hijacked DNA repair proteins and unchained DNA polymerases. *Philos Trans R Soc Lond B Biol Sci.* 2009; 364:605–611. [PubMed: 19008198]
11. Teng G, Papavasiliou FN. Immunoglobulin somatic hypermutation. *Annu Rev Genet.* 2007; 41:107–120. [PubMed: 17576170]
12. Kenter AL. Class switch recombination: an emerging mechanism. *Curr Top Microbiol Immunol.* 2005; 290:171–199. [PubMed: 16480043]
13. Stavnezer J. Complex regulation and function of activation-induced cytidine deaminase. *Trends Immunol.* 2011; 32:194–201. [PubMed: 21493144]
14. Di Noia JM, Neuberger MS. Molecular mechanisms of antibody somatic hypermutation. *Annu Rev Biochem.* 2007; 76:1–22. [PubMed: 17328676]
15. Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, Chew EG, Huang PY, Welboren WJ, Han Y, Ooi HS, Ariyaratne PN, Vega VB, Luo Y, Tan PY, Choy PY, Wansa KD, Zhao B, Lim KS, Leow SC, Yow JS, Joseph R, Li H, Desai KV, Thomsen JS, Lee YK, Karuturi RK, Herve T, Bourque G, Stunnenberg HG, Ruan X, Cacheux-Rataboul V, Sung WK, Liu ET, Wei CL, Cheung E, Ruan Y. An oestrogen-receptor-alpha-bound human chromatin interactome. *Nature.* 2009; 462:58–64. [PubMed: 19890323]
16. Vakoc CR, Letting DL, Gheldof N, Sawado T, Bender MA, Groudine M, Weiss MJ, Dekker J, Blobel GA. Proximity among distant regulatory elements at the beta-globin locus requires GATA-1 and FOG-1. *Mol Cell.* 2005; 17:453–462. [PubMed: 15694345]
17. Tolhuis B, Palstra RJ, Splinter E, Grosveld F, de Laat W. Looping and interaction between hypersensitive sites in the active beta-globin locus. *Mol Cell.* 2002; 10:1453–1465. [PubMed: 12504019]
18. Spilianakis CG, Flavell RA. Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. *Nat Immunol.* 2004; 5:1017–1027. [PubMed: 15378057]
19. Kurukuti S, Tiwari VK, Tavoosidana G, Pugacheva E, Murrell A, Zhao Z, Lobanenko V, Reik W, Ohlsson R. CTCF binding at the H19 imprinting control region mediates maternally inherited higher-order chromatin conformation to restrict enhancer access to Igf2. *Proc Natl Acad Sci U S A.* 2006; 103:10684–10689. [PubMed: 16815976]
20. Zhou GL, Xin L, Song W, Di LJ, Liu G, Wu XS, Liu DP, Liang CC. Active chromatin hub of the mouse alpha-globin locus forms in a transcription factory of clustered housekeeping genes. *Mol Cell Biol.* 2006; 26:5096–5105. [PubMed: 16782894]
21. Majumder P, Gomez JA, Chadwick BP, Boss JM. The insulator factor CTCF controls MHC class II gene expression and is required for the formation of long-distance chromatin interactions. *J Exp Med.* 2008; 205:785–798. [PubMed: 18347100]
22. Dekker J, Rippe K, Dekker M, Kleckner N. Capturing chromosome conformation. *Science.* 2002; 295:1306–1311. [PubMed: 11847345]
23. Khamlichi AA, Pinaud E, Decourt C, Chauveau C, Cogne M. The 3' IgH regulatory region: a complex structure in a search for a function. *Adv Immunol.* 2000; 75:317–345. [PubMed: 10879288]
24. Garrett FE, Emelyanov AV, Sepulveda MA, Flanagan P, Volpi S, Li F, Loukinov D, Eckhardt LA, Lobanenko VV, Birshtein BK. Chromatin architecture near a potential 3' end of the igh locus involves modular regulation of histone modifications during B-Cell development and in vivo occupancy at CTCF sites. *Mol Cell Biol.* 2005; 25:1511–1525. [PubMed: 15684400]
25. Jung S, Rajewsky K, Radbruch A. Shutdown of class switch recombination by deletion of a switch region control element. *Science.* 1993; 259:984–987. [PubMed: 8438159]

26. Zhang J, Bottaro A, Li S, Stewart V, Alt FW. A selective defect in IgG2b switching as a result of targeted mutation of the Ig2b promoter and exon. *EMBO J.* 1993; 12:3529–3537. [PubMed: 8253079]
27. Seidl KJ, Manis JP, Bottaro A, Zhang J, Davidson L, Kisselgof A, Oettgen H, Alt FW. Position-dependent inhibition of class-switch recombination by PGK-neor cassettes inserted into the immunoglobulin heavy chain constant region locus. *Proc Natl Acad Sci U S A.* 1999; 96:3000–3005. [PubMed: 10077626]
28. Gritzmacher CA. Molecular aspects of heavy-chain class switching. *Crit Rev Immunol.* 1989; 9:173–200. [PubMed: 2505810]
29. Schrader CE, Linehan EK, Mochegova SN, Woodland RT, Stavnezer J. Inducible DNA breaks in Ig S regions are dependent on AID and UNG. *J Exp Med.* 2005; 202:561–568. [PubMed: 16103411]
30. Wuerffel RA, Du J, Thompson RJ, Kenter AL. Ig Sgamma3 DNA-specific double strand breaks are induced in mitogen-activated B cells and are implicated in switch recombination. *J Immunol.* 1997; 159:4139–4144. [PubMed: 9379005]
31. Carter D, Chakalova L, Osborne CS, Dai YF, Fraser P. Long-range chromatin regulatory interactions in vivo. *Nat Genet.* 2002; 32:623–626. [PubMed: 12426570]
32. Murrell A, Heeson S, Reik W. Interaction between differentially methylated regions partitions the imprinted genes Igf2 and H19 into parent-specific chromatin loops. *Nat Genet.* 2004; 36:889–893. [PubMed: 15273689]
33. Horike S, Cai S, Miyano M, Cheng JF, Kohwi-Shigematsu T. Loss of silent-chromatin looping and impaired imprinting of DLX5 in Rett syndrome. *Nat Genet.* 2005; 37:31–40. [PubMed: 15608638]
34. Blanton J, Gaszner M, Schedl P. Protein:protein interactions and the pairing of boundary elements in vivo. *Genes Dev.* 2003; 17:664–675. [PubMed: 12629048]
35. Pinaud E, Khamlichi AA, Le Morvan C, Drouet M, Nalesso V, Le Bert M, Cogne M. Localization of the 3' IgH locus elements that effect long-distance regulation of class switch recombination. *Immunity.* 2001; 15:187–199. [PubMed: 11520455]
36. Wuerffel R, Wang L, Grigera F, Manis J, Selsing E, Perlot T, Alt FW, Cogne M, Pinaud E, Kenter AL. S-S synapsis during class switch recombination is promoted by distantly located transcriptional elements and activation-induced deaminase. *Immunity.* 2007; 27:711–722. [PubMed: 17980632]
37. Ju Z, Volpi SA, Hassan R, Martinez N, Giannini SL, Gold T, Birshstein BK. Evidence for physical interaction between the immunoglobulin heavy chain variable region and the 3' regulatory region. *J Biol Chem.* 2007; 282:35169–35178. [PubMed: 17921139]
38. Sellars M, Reina-San-Martin B, Kastner P, Chan S. Ikaros controls isotype selection during immunoglobulin class switch recombination. *J Exp Med.* 2009; 206:1073–1087. [PubMed: 19414557]
39. Kenter AL, Wuerffel R, Dominguez C, Shanmugam A, Zhang H. Mapping of a functional recombination motif that defines isotype specificity for mu-->gamma3 switch recombination implicates NF-kappaB p50 as the isotype-specific switching factor. *J Exp Med.* 2004; 199:617–627. [PubMed: 14993249]
40. Schrader CE, Bradley SP, Vardo J, Mochegova SN, Flanagan E, Stavnezer J. Mutations occur in the Ig Smu region but rarely in Sgamma regions prior to class switch recombination. *EMBO J.* 2003; 22:5893–5903. [PubMed: 14592986]
41. Zarrin AA, Tian M, Wang J, Borjeson T, Alt FW. Influence of switch region length on immunoglobulin class switch recombination. *Proc Natl Acad Sci U S A.* 2005; 102:2466–2470. [PubMed: 15684074]
42. Khamlichi AA, Glaudet F, Oruc Z, Denis V, Le Bert M, Cogne M. Immunoglobulin class-switch recombination in mice devoid of any S mu tandem repeat. *Blood.* 2004; 103:3828–3836. [PubMed: 14962903]
43. Luby TM, Schrader CE, Stavnezer J, Selsing E. The mu switch region tandem repeats are important, but not required, for antibody class switch recombination. *J Exp Med.* 2001; 193:159–168. [PubMed: 11148220]

44. Shinkura R, Tian M, Smith M, Chua K, Fujiwara Y, Alt FW. The influence of transcriptional orientation on endogenous switch region function. *Nat Immunol.* 2003; 4:435–441. [PubMed: 12679811]
45. Honjo T, Kinoshita K, Muramatsu M. Molecular mechanism of class switch recombination: linkage with somatic hypermutation. *Annu Rev Immunol.* 2002; 20:165–196. [PubMed: 11861601]
46. Tashiro J, Kinoshita K, Honjo T. Palindromic but not G-rich sequences are targets of class switch recombination. *Int Immunol.* 2001; 13:495–505. [PubMed: 11282989]
47. Musmann R, Courtet M, Schwager J, Du Pasquier L. Microsites for immunoglobulin switch recombination breakpoints from Xenopus to mammals. *Eur J Immunol.* 1997; 27:2610–2619. [PubMed: 9368617]
48. Yu K, Chedin F, Hsieh CL, Wilson TE, Lieber MR. R-loops at immunoglobulin class switch regions in the chromosomes of stimulated B cells. *Nat Immunol.* 2003; 4:442–451. [PubMed: 12679812]
49. Huang FT, Yu K, Balter BB, Selsing E, Oruc Z, Khamlichi AA, Hsieh CL, Lieber MR. Sequence dependence of chromosomal R-loops at the immunoglobulin heavy-chain Smu class switch region. *Mol Cell Biol.* 2007; 27:5921–5932. [PubMed: 17562862]
50. Duquette ML, Handa P, Vincent JA, Taylor AF, Maizels N. Intracellular transcription of G-rich DNAs induces formation of G-loops, novel structures containing G4 DNA. *Genes Dev.* 2004; 18:1618–1629. [PubMed: 15231739]
51. Blanc V, Kennedy S, Davidson NO. A novel nuclear localization signal in the auxiliary domain of apobec-1 complementation factor regulates nucleocytoplasmic import and shuttling. *J Biol Chem.* 2003; 278:41198–41204. [PubMed: 12896982]
52. Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, Davidson NO, Honjo T. Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. *J Biol Chem.* 1999; 274:18470–18476. [PubMed: 10373455]
53. Rada C, Ehrenstein MR, Neuberger MS, Milstein C. Hot spot focusing of somatic hypermutation in MSH2-deficient mice suggests two stages of mutational targeting. *Immunity.* 1998; 9:135–141. [PubMed: 9697843]
54. Maul RW, Gearhart PJ. AID and somatic hypermutation. *Adv Immunol.* 2010; 105:159–191. [PubMed: 20510733]
55. Xue K, Rada C, Neuberger MS. The in vivo pattern of AID targeting to immunoglobulin switch regions deduced from mutation spectra in *msh2*^{-/-}*ung*^{-/-} mice. *J Exp Med.* 2006; 203:2085–2094. [PubMed: 16894013]
56. Nambu Y, Sugai M, Gonda H, Lee CG, Katakai T, Agata Y, Yokota Y, Shimizu A. Transcription-coupled events associating with immunoglobulin switch region chromatin. *Science.* 2003; 302:2137–2140. [PubMed: 14684824]
57. Besmer E, Market E, Papavasiliou FN. The transcription elongation complex directs activation-induced cytidine deaminase-mediated DNA deamination. *Mol Cell Biol.* 2006; 26:4378–4385. [PubMed: 16705187]
58. Woodcock CL, Dimitrov S. Higher-order structure of chromatin and chromosomes. *Curr Opin Genet Dev.* 2001; 11:130–135. [PubMed: 11250134]
59. Li B, Carey M, Workman JL. The role of chromatin during transcription. *Cell.* 2007; 128:707–719. [PubMed: 17320508]
60. Suganuma T, Workman JL. Signals and combinatorial functions of histone modifications. *Annu Rev Biochem.* 2011; 80:473–499. [PubMed: 21529160]
61. Selth LA, Sigurdsson S, Svejstrup JQ. Transcript Elongation by RNA Polymerase II. *Annu Rev Biochem.* 2010; 79:271–293. [PubMed: 20367031]
62. Wang L, Whang N, Wuerffel R, Kenter AL. AID-dependent histone acetylation is detected in immunoglobulin S regions. *J Exp Med.* 2006; 203:215–226. [PubMed: 16418396]
63. Wang L, Wuerffel R, Feldman S, Khamlichi AA, Kenter AL. S region sequence, RNA polymerase II, and histone modifications create chromatin accessibility during class switch recombination. *J Exp Med.* 2009; 206:1817–1830. [PubMed: 19596805]

64. Saunders A, Core LJ, Lis JT. Breaking barriers to transcription elongation. *Nat Rev Mol Cell Biol.* 2006; 7:557–567. [PubMed: 16936696]
65. Workman JL, Kingston RE. Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu Rev Biochem.* 1998; 67:545–579. [PubMed: 9759497]
66. Pokholok DK, Harbison CT, Levine S, Cole M, Hannett NM, Lee TI, Bell GW, Walker K, Rolfe PA, Herbolsheimer E, Zeitlinger J, Lewitter F, Gifford DK, Young RA. Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell.* 2005; 122:517–527. [PubMed: 16122420]
67. Bernstein BE, Kamal M, Lindblad-Toh K, Bekiranov S, Bailey DK, Huebert DJ, McMahon S, Karlsson EK, Kulbokas EJ 3rd, Gingeras TR, Schreiber SL, Lander ES. Genomic maps and comparative analysis of histone modifications in human and mouse. *Cell.* 2005; 120:169–181. [PubMed: 15680324]
68. Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. High-resolution profiling of histone methylations in the human genome. *Cell.* 2007; 129:823–837. [PubMed: 17512414]
69. Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA. A chromatin landmark and transcription initiation at most promoters in human cells. *Cell.* 2007; 130:77–88. [PubMed: 17632057]
70. Daniel JA, Pray-Grant MG, Grant PA. Effector proteins for methylated histones: an expanding family. *Cell Cycle.* 2005; 4:919–926. [PubMed: 15970672]
71. Berger SL. The complex language of chromatin regulation during transcription. *Nature.* 2007; 447:407–412. [PubMed: 17522673]
72. Shahbazian MD, Grunstein M. Functions of site-specific histone acetylation and deacetylation. *Annu Rev Biochem.* 2007; 76:75–100.
73. Carrozza MJ, Li B, Florens L, Suganuma T, Swanson SK, Lee KK, Shia WJ, Anderson S, Yates J, Washburn MP, Workman JL. Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell.* 2005; 123:581–592. [PubMed: 16286007]
74. Lieb JD, Clarke ND. Control of transcription through intragenic patterns of nucleosome composition. *Cell.* 2005; 123:1187–1190. [PubMed: 16377560]
75. Perlot T, Li G, Alt FW. Antisense transcripts from immunoglobulin heavy-chain locus V(D)J and switch regions. *Proc Natl Acad Sci U S A.* 2008; 105:3843–3848. [PubMed: 18292225]
76. Li Z, Luo Z, Scharff MD. 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.* 2004; 101:15428–15433. [PubMed: 15486086]
77. Daniel JA, Santos MA, Wang Z, Zang C, Schwab KR, Jankovic M, Filsuf D, Chen HT, Gazumyan A, Yamane A, Cho YW, Sun HW, Ge K, Peng W, Nussenzweig MC, Casellas R, Dressler GR, Zhao K, Nussenzweig A. PTIP promotes chromatin changes critical for immunoglobulin class switch recombination. *Science.* 2010; 329:917–923. [PubMed: 20671152]
78. Munoz IM, Rouse J. Control of histone methylation and genome stability by PTIP. *EMBO Rep.* 2009; 10:239–245. [PubMed: 19229280]
79. Hoffmeister A, Ropolo A, Vasseur S, Mallo GV, Bodeker H, Ritz-Laser B, Dressler GR, Vaccaro MI, Dagorn JC, Moreno S, Iovanna JL. The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription factors on the glucagon gene promoter. *J Biol Chem.* 2002; 277:22314–22319. [PubMed: 11940591]
80. Schwab KR, Patel SR, Dressler GR. Role of PTIP in class switch recombination and long-range chromatin interactions at the immunoglobulin heavy chain locus. *Mol Cell Biol.* 2011; 31:1503–1511. [PubMed: 21282469]
81. Rajagopal D, Maul RW, Ghosh A, Chakraborty T, Khamlichi AA, Sen R, Gearhart PJ. Immunoglobulin switch mu sequence causes RNA polymerase II accumulation and reduces dA hypermutation. *J Exp Med.* 2009; 206:1237–1244. [PubMed: 19433618]
82. Huertas P, Aguilera A. Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. *Mol Cell.* 2003; 12:711–721. [PubMed: 14527416]

83. Tous C, Aguilera A. Impairment of transcription elongation by R-loops in vitro. *Biochem Biophys Res Commun.* 2007; 360:428–432. [PubMed: 17603014]
84. Pavri R, Nussenzweig MC. AID targeting in antibody diversity. *Adv Immunol.* 2011; 110:1–26. [PubMed: 21762814]
85. Fuda NJ, Ardehali MB, Lis JT. Defining mechanisms that regulate RNA polymerase II transcription in vivo. *Nature.* 2009; 461:186–192. [PubMed: 19741698]
86. Rahl PB, Lin CY, Seila AC, Flynn RA, McCuine S, Burge CB, Sharp PA, Young RA. c-Myc regulates transcriptional pause release. *Cell.* 2010; 141:432–445. [PubMed: 20434984]
87. Pei Y, Shuman S. Interactions between fission yeast mRNA capping enzymes and elongation factor Spt5. *J Biol Chem.* 2002; 277:19639–19648. [PubMed: 11893740]
88. Wen Y, Shatkin AJ. Transcription elongation factor hSPT5 stimulates mRNA capping. *Genes Dev.* 1999; 13:1774–1779. [PubMed: 10421630]
89. Andrulis ED, Werner J, Nazarian A, Erdjument-Bromage H, Tempst P, Lis JT. The RNA processing exosome is linked to elongating RNA polymerase II in *Drosophila*. *Nature.* 2002; 420:837–841. [PubMed: 12490954]
90. Ganesh K, Adam S, Taylor B, Simpson P, Rada C, Neuberger M. CTNNBL1 is a novel nuclear localization sequence-binding protein that recognizes RNA-splicing factors CDC5L and Prp31. *J Biol Chem.* 2011; 286:17091–17102. [PubMed: 21385873]
91. Conticello SG, Ganesh K, Xue K, Lu M, Rada C, Neuberger MS. Interaction between antibody-diversification enzyme AID and spliceosome-associated factor CTNNBL1. *Mol Cell.* 2008; 31:474–484. [PubMed: 18722174]
92. Nowak U, Matthews AJ, Zheng S, Chaudhuri J. The splicing regulator PTBP2 interacts with the cytidine deaminase AID and promotes binding of AID to switch-region DNA. *Nat Immunol.* 2011; 12:160–166. [PubMed: 21186367]

Figure 1. The looping-out and deletion model of Ig switch recombination

(A) A partial schematic map of IgH locus before CSR is shown not to scale. A productive V(D)J rearrangement has occurred allowing expression of the γ and μ IgH chains. Intact S_{μ} and $S_{\gamma 1}$ are separated by approximately 70kb. Stimulation of B cells with antigen or mitogen induces germline transcription through the $I_{\gamma 1}$ - $S_{\gamma 1}$ - $C_{\gamma 1}$ region prior to recombination. (B) The S_{μ} and $S_{\gamma 1}$ regions are aligned causing the intervening genomic DNA to form a loop. (C) A reciprocal crossover between S_{μ} and $S_{\gamma 1}$ results in the formation of a new hybrid transcriptional unit containing the original VDJ exons contiguous with $C_{\gamma 1}$ and the formation of hybrid $S_{\mu}/S_{\gamma 1}$ molecules.

Figure 2. The Igh locus is configured in chromatin loops in B cells

(A) A schematic is shown of the linear Igh locus with the VDJ exons (black box), intronic E μ and 3'E α enhancers (yellow symbols), C $_H$ regions (gray boxes), S regions (ovals), and I exons (rectangles) indicated. This linear configuration is typical for the Igh locus in splenic T cells [36]. (B) Association of the γ 1 locus with the 3'E α will promote γ 1 GLT expression but not S/S synapsis. (C) In resting mature splenic B cells the Igh locus loop is tethered by interactions between E μ and 3'E α . Following treatment of resting B cells with LPS and IL4 for 40 hours, E μ and 3'E α remain in close proximity and the C γ 1 locus has been recruited to the 3'E α regulatory region [36]. S γ 1 is now in close proximity to S μ .

Figure 3. AID deamination model and a role for mismatch repair (MMR) in CSR. AID deaminates dC to dU

(A) The U:G mismatch, can be replicated over to produce T:A, a transition mutation. (B) Using the base excision repair (BER) pathway, dU bases can be excised by a uracil DNA glycosylase (UNG) leaving an abasic site that can then be replicated over by error prone polymerase to produce both transition and transversion mutations. (C) Abasic sites can also be recognized by AP endonucleases (APE) to form ssDNA nicks or double strand breaks (DSBs) when the abasic sites are closely spaced and on complementary strands. (D) Other U:G mismatches could be substrates for mismatch repair (MMR) MSH2/MSH6 binding which in turn recruit PMS2-MLH1 (not shown), and PMS2 nicks the DNA. Exonuclease 1 (Exo I) binds to MSH2 and MLH1, and using the PMS2 induced nick, excises toward the U:G mismatch producing a DSB with a 5' overhang. An error prone DNA polymerase can fill-in the 5' overhang and introduces mutations at A:T bp. 3' overhangs can be excised by Ercc1-XPF. Short overhangs can be used during end joining.

Figure 4. Summary of mutation frequency and differential histone modifications in S and C_H regions of activated B cells

A schematic diagram depicts a generic I-S- C_H region downstream of the GLT promoter (Pr). Above the diagram a summary of mutation frequencies 5' and 3' of the S region for $Ung^{-/-}$ Msh2 $^{-/-}$ B cells is shown [56]. A similar distribution of mutations are found for this region in $Ung^{-/-}$ B cells [82]. Below the I-S- C_H schematic a summary of histone modifications and RNA pol II binding is shown. WT B cells were stimulated with LPS or LPS + IL-4 for 48 hours and then analyzed for mutations of by in chromatin immunoprecipitation (ChIP) assays using antisera against H3K9,14Ac, H3K4me3, H3K36me3, H4K20me1, pol II RNA, and pol II p-ser5. Data was amalgamated from published studies [74,75].