Published in final edited form as:

Biochim Biophys Acta. 2012 July ; 1819(7): 733–738. doi:10.1016/j.bbagr.2012.01.019.

Roles for histone H3K4 methyltransferase activities during immunoglobulin class switch recombination

Jeremy A. Daniel^{1,2} and André Nussenzweig¹

¹Laboratory of Genome Integrity, National Cancer Institute, National Institutes of Health, Bethesda, MD USA

Abstract

Germ-line transcription of an antigen receptor gene segment is an essential feature of the targeting mechanism for DNA double-strand break formation during physiological DNA rearrangements in lymphocytes. Alterations in chromatin structure have long been postulated to regulate accessibility of recombinase activities for lymphocytes to generate antibody diversity; however, whether or not germ-line transcripts are the cause or the effect of chromatin changes at antigen receptor loci is still not clear. Methylation of histone H3 at lysine 4 is one of the most well-studied histone post-translational modifications yet we have only recently begun to understand the significance of the MLL-like H3K4 methyltransferase activities in lymphocyte function. While it is clear during lymphocyte development that H3K4me3 plays a critical role in targeting and stimulating RAG1/2 recombinase activity for V(D)J recombination, recent work suggests roles for this histone mark and different MLL-like complexes in mature B cells during immunoglobulin class-switch recombination. In this review, we focus our discussion to advances on how MLL-like complexes and H3K4 methylation may function during the germ-line transcription and recombinase targeting steps of class-switch recombination.

Antigen receptor gene rearrangements

The humoral immune response relies on lymphocytes to produce a diverse repertoire of antibodies with the appropriate isotype and affinity for removal of pathogens from our body. Each lymphocyte expresses a single B cell (BCR) or T cell receptor (TCR), presenting a unique interaction surface for recognition of a foreign epitope. To generate antibody diversity, lymphocytes have evolved to tolerate physiological DNA damage during V(D)J (variable/diversity/joining gene segment) recombination and class-switch recombination (CSR) at antigen receptor gene loci [1]. V(D)J recombination occurs in developing B cells within the bone marrow at *Ig* genes and in developing T cells within the thymus at *Tcr* genes. Developmental signals induce limited expression of recombination activating genes 1 and 2 (RAG1/2) in developing lymphocytes undergoing V(D)J recombination. Recombination signal sequence (RSS) DNA within antigen receptor genes targets RAG1/2-mediated cleavage during V(D)J recombination, guiding RAG1/2 away from generating genomic instability at other chromosomal locations [1-4].

Correspondence to Jeremy A. Daniel: jeremy.daniel@cpr.ku.dk or André Nussenzweig: andre_nussenzweig@nih.gov.

²Present address: The Novo Nordisk Foundation Center for Protein Research, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3b, 2200 Copenhagen N, Denmark

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

In addition to V(D)J recombination, mature naïve IgM-expressing B cells in the periphery have the ability to undergo a second rearrangement event called CSR. This recombination reaction occurs downstream of the V(D)J gene segments at the *Igh* locus and aims to swap constant regions of an antibody to eliminate a particular pathogen through interaction with different cell surface receptors [1, 5]. During an immune response, peripheral B cells stimulated by antigen and the cytokine milieu become activated, begin to undergo *Igh* class-switching, and concentrate in germinal center structures of lymph nodes and the spleen for this process to continue [1, 5]. As activated B cells enter cell cycle, they express AID (activation-induced cytidine deaminase), which gets targeted to the *Igh* locus and initiates DNA lesions leading to DNA double-strand break (DSB) formation [6, 7]. For productive CSR, AID-induced DSBs must occur at two switch (S) repeat regions (i.e. S μ , S γ 3, S γ 1, S γ 2b, S γ 2a, S ϵ , or S α in the mouse) that precede participating constant region gene segments [5]. Through mechanisms similar to those which occur during V(D)J recombination, subsequent synapsis and DNA repair of the two broken DNA ends is mediated by factors functioning in the DNA damage response (DDR) and the non-homologous end-joining (NHEJ) pathways. This process results in a switch from IgM expression to expression of either IgG, IgE, or IgA [8]. Failure to resolve unrepaired DNA DSBs during DNA rearrangements in lymphocytes can lead to the formation of oncogenic chromosomal translocations by fusing a transcriptional control element from the antigen receptor locus to an oncogene. Indeed, B and T cell leukemias and lymphomas have characteristic chromosomal translocations that often involve the antigen receptor genes [8-10].

Chromatin accessibility and histone modifications

To explain how RAG1/2 can target different *Ig* and *Tcr* loci in a lineage-specific and ordered manner, the accessibility hypothesis was put forth by Yancopoulos and Alt in 1985 [11]. Since then, many lines of evidence support the notion that germ-line transcription of an antigen receptor gene segment is an essential feature of the targeting mechanism for RAG1/2-mediated DNA cleavage during V(D)J recombination *in vivo* [1, 12]. A similar accessibility model underlies the mechanism of CSR in mature B cells [13, 14], with activation signals directing promoter-driven germ-line transcription and AID targeting to particular switch regions at the *Igh* locus [1, 5, 15]. Germ-line transcripts coinciding with recombination at a particular gene segment have been observed at all antigen receptor loci and deletion of promoter or enhancer *cis*-acting elements at endogenous loci impairs both transcription and receptor recombination [16, 17]. However, the precise role of germ-line transcripts is still not understood. Accessibility may also be regulated by spatial organization and nuclear positioning of antigen receptor loci and this is an active area of investigation [18-20].

Local changes in chromatin structure have long been postulated to influence accessibility at antigen receptor loci for regulating recombination [21]. For example, the SWI/SNF ATP-dependent chromatin remodeling complex can promote RAG1/2-mediated cleavage of nucleosomal V(D)J recombination substrates *in vitro* in the absence of transcription [22, 23]. Post-translational modifications of the histone H3, H2B, H2A, and H4 components of chromatin also regulate DNA-mediated processes by altering chromatin structure and generating recognition sites for mediating effector protein stabilization [24-26]. Two histone modification interactions important for DNA repair are with the histone H2AX variant phosphorylated at S139 (γ -H2AX) and the tandem BRCT domains of MDC1 as well as recognition of histone H4K20 mono- and dimethylation by the 53BP1 tandem Tudor domains [27-30]. With respect to chromatin accessibility prior to DSB formation, multiple studies have correlated localized changes in other histone modifications with antigen receptor gene recombination. For example, antigen receptor loci poised to undergo V(D)J

recombination are marked by peaks of H3K4me2 and H3K4me3 [31, 32], two histone post-translational modifications that strongly correlate with active gene expression [33].

H3K4me3 is found at sites of transcription initiation and many of the known H3K4me3-binding proteins associate with large transcription regulatory complexes [33-36]. Evidence from yeast and human systems indicate that the PAF transcription elongation complex and the H2B K120 ubiquitination machinery are required for H3K4me3 and that H2B ubiquitination can directly stimulate H3K4me2 and me3 [33, 34, 37, 38], suggesting that transcription elongation may be a prerequisite for catalysis of H3K4me3. However, some MLL-like methyltransferase complexes have been shown to directly interact with site-specific DNA-binding transcription factors, leading to the notion that recruitment of these complexes likely involves a combination of gene-specific and general mechanisms [33].

Recently, a paradigm shift in the mechanism of RAG1/2 targeting has directly linked H3K4me3 to the chromatin accessibility important for V(D)J recombination. The PHD domain of RAG2 can directly interact with H3K4me3 and V(D)J recombination is severely impaired in vivo by mutation of the PHD H3K4me3-binding interface or by suppressing global H3K4me3 [39-41]. Surprisingly, RAG2 binding was found to be widely spread across the genome and mirror sites of H3K4me3 in thymocytes with a remarkable 99% overlap [42]. Moreover, an H3K4me3 histone tail peptide alone was shown to enhance RAG1/2 catalytic activity in vitro in a manner dependent on an intact RAG2 PHD domain [43, 44]. In sum, the RAG2-H3K4me3 interaction has been proposed to be important both for RAG2 association with chromatin as well as for stimulation of DNA cleavage [4] and these findings help explain mutations identified in the PHD domain of RAG2 from patients with SCID and Omenn syndrome [45]. The current model for initiation of V(D)J recombination is that actively transcribed antigen receptor gene elements expose RSS DNA recognized by RAG1 and are marked by H3K4me3 recognized by RAG2 and these activities together impart specificity for promoting restricted DNA cleavage [4].

With respect to CSR, targeting of AID to the *Igh* locus occurs, at least in part, by RNA Polymerase II, Spt5, and the RNA exosome [46-48], and additional studies have suggested roles for histone modifications in targeting AID. For example, there is evidence that acetylation of histones H3 and H4 occurs at switch regions actively undergoing CSR [46, 49-53]. Analogous to the surprising RAG2 localization at many sites in the genome of developing lymphocytes undergoing V(D)J recombination [42], AID recruitment in activated mature B cells also occurs at many sites in the genome [54]. Interestingly, histone acetylation and H3K4me marks were significantly enriched at sites of AID localization, as was RNA Pol II [54]. Amidst these correlative studies, only recently has evidence provided some direct support for the role of histone modifications in CSR. Nevertheless, whether or not germ-line transcripts are the cause or the effect of chromatin changes at antigen receptor loci is still not clear.

Recent studies have suggested that the H3K9me3 mark, normally associated with gene repression [55], may function in targeting of AID to the *Igh* locus. H3K9me3 was surprisingly enriched at switch regions in both human and murine B cells undergoing CSR [51, 53, 56] and H3K9me3 histone peptide pull-down experiments precipitated AID and KAP1 from activated B cells [56]. In support of this idea, B cells deficient in the SUV39H1 H3K9 methyltransferase or KAP1 show defects in CSR [56, 57]. However, AID localization is most significantly associated with paused RNA Pol II and active chromatin marks including H3K4me [54]. Therefore, further work is needed to determine how H3K9me3 plays a role in AID targeting.

In this review, we focus our discussion on recent genetic and biochemical evidence that support roles for histone H3K4 methylation and methyltransferase activities of MLL-like complexes during the germ-line transcription and recombinase targeting steps of CSR.

MLL-like histone H3K4 methyltransferase complexes

The MLL-like family of methyltransferases have activity for histone H3 at lysine 4 [33]. This family includes SET1A, SET1B, MLL1, MLL2, MLL3, and MLL4. MLL1 is the founding member of this family and was identified as a partner in chromosomal translocations in some leukemias [58]. Recently, MLL1 has been shown to be phosphorylated by the ATR kinase and suggested to function in the S-phase DNA damage checkpoint by inhibiting CDC45 loading and thereby delaying DNA replication [59]. Further work is needed to determine whether this function is also mediated in part through histone H3K4 methylation. The other members of the family are expressed from different genes, but all contain a conserved SET domain containing the methyltransferase activity [33, 60]. As part of multi-protein complexes, the MLL-like enzymes catalyze mono-, di-, and trimethylation of histone H3 at lysine 4 [33, 55]. However, null mutations of many components of MLL-like complex members result in murine embryonic lethality [33], which has greatly limited our understanding of the roles and specificities of these complexes in normal tissues including during lymphocyte maturation. Recent studies have suggested that MLL-like complexes directly function in CSR.

Roles for H3K4 methyltransferases during class-switch recombination

Most genes display H3K4me3 and RNA Pol II at their promoters and exhibit transcription initiation; however, genes lacking both RNA Pol II and H3K4me3 tend to be localized within clusters of highly homologous genes [35]. H3K4me3 and the transcription elongation-associated H3K36me3 mark [55] are induced at downstream *Igh* switch regions when stimulated to undergo CSR and profiles of these marks at S μ do not change with stimulation [52, 61]. These data are consistent with IgM expression in resting B cells and support a mechanism for controlled silencing of the remaining *Igh* constant region locus until B cell activation. Thus, *Igh* switch regions are normally refractory to H3K4me3 and RNA Pol II recruitment, and only upon B cell stimulation do they acquire H3K4me3 and RNA Pol II association [52, 61]. One particular feature of H3K4me3 at S μ and activated downstream switch regions is that the peak is localized from the promoter-proximal initiator exon to the end of the switch region [52, 61], which can extend between 1-12kb in length. This is in contrast to the H3K4me3 localization pattern within 2kb of the transcription start site (TSS), as genome-wide studies have shown [55]. Indeed, the peak of H3K4me3 at *Igh*- γ 3 is among the most broad of all H3K4me3 peaks in LPS-stimulated B cells demarcating near 7kb of DNA [61]. Importantly, H3K4me3 at the *Igh* locus in activated B cells is independent of AID [52, 61], consistent with H3K4me3 associating with transcription rather than with DNA DSBs [37].

We have recently shown that PTIP (Pax interaction with transcription-activation domain protein-1, also known as Paxip1) recruits an MLL-like complex to the *Igh* locus and plays a critical role in promoting H3K4me3 and germ-line transcription at switch regions, leading to CSR of multiple isotypes [61]. As PTIP stably associates with a subset of MLL-like complexes containing the MLL3/KMT2C and MLL4/KMT2B methyltransferases [62-64], our study was the first demonstration of a histone modifying complex that directly controls expression of *Igh* germ-line transcripts, establishing that specific chromatin changes may in fact control the accessibility of the *Igh* locus to CSR [61]. Specifically, activated B cells from B lymphocyte-specific PTIP conditional deletion mice displayed defects in IgG3, IgG2b, and IgG1 class-switching concomitant with loss of RNA Pol II association and

initiating transcripts at the $\gamma 3$, $\gamma 2b$, and $\gamma 1$ downstream switch regions, respectively [61]. This study demonstrated that PTIP promotes H3K4me2/3 and histone acetylation and suggested that a hierarchy of histone modifications may regulate chromatin accessibility at *Igh* switch regions [61]. However, with the available evidence at this time, we cannot distinguish whether H3K4me3 precedes transcription or vice versa. A recent study using the same B lymphocyte-specific PTIP deletion mouse model confirmed many of our observations and provided evidence that PTIP may regulate long-range chromatin interactions between the 3'E α enhancer and switch promoter regions [65]. Given that the *Igh* locus contains clustered and highly repetitive switch regions controlled by the 3'E α enhancer [5], we propose that *Igh* switch regions are an example of a tightly repressed gene class which requires PTIP to alleviate regulatory mechanisms that ensure proper targeting and stability of the locus.

In addition to its transcriptional role, PTIP contains six BRCT domains and plays a role in the cellular response to DNA DSBs [66]. For example, upon treatment of cells with ionizing radiation, PTIP forms nuclear foci dependent on the γ -H2AX/MDC1 damage response pathway [66]. In stimulated B cells, we showed that PTIP accumulation at DSBs contributes to CSR and genome stability independently of *Igh* switch transcription [61]. Therefore, PTIP is a rare example of a protein that displays dual functions during a physiological DNA rearrangement in which chromatin accessibility and recombination need to be precisely coordinated.

Since disruption of PTIP does not appear to alter the integrity of the MLL3/4 complex [62], our data suggest that PTIP confers specificity for recruitment of MLL3/4 activity to the *Igh* locus. Based on observations that PTIP can directly interact with PAX and SMAD transcription factors [67, 68] and mediate recruitment of H3K4me activity to a PAX-dependent promoter [63], we speculate that PTIP recruitment to *Igh* results from direct interaction with a DNA-binding transcription factor. PAX5 is the only member of the PAX family expressed in the B lymphoid lineage and has putative binding sites near every *Igh* switch region [17]. PAX5 also strongly associates with the 3'E α enhancer [69], and promotes production of serum IgG [70]. However, further work is required to determine the extent of direct PAX5 association with germ-line promoter regions. Different from PTIP-deficient B cells, PAX5-deficient mice exhibit a complete block in early B cell development and show severely impaired B cell proliferation [70, 71], indicating that PAX5 functions are clearly not mediated solely by PTIP. Other components of the MLL-like PTIP complex may also function in the recruitment or stabilization of PTIP to *Igh*. For example, the PA1 (PTIP-associated 1) subunit of the methyltransferase complex directly interacts with PTIP and there is evidence that another pool of PTIP/PA1 may exist as a lower molecular weight complex independent from the MLL-like methyltransferase complex [62, 72]. In any case, further investigation is needed to determine the interacting proteins of PTIP in B lymphocytes that control PTIP targeting during CSR.

A different study has provided evidence that MLL-like methyltransferase activities are dispensable for *Igh* germ-line transcription at switch regions, but are required for H3K4me3, DSB formation, and CSR [73]. Specifically, siRNA knock-down of the common MLL-like complex components WDR5 or ASH2L, or the SET1A/B complex-specific subunits CXXC1 or WDR82, led to deficient IgA class-switching and decreased H3K4me3 at both μ and α switch regions, without affecting μ or α germ-line transcript levels. Double siRNA knock-down of WDR5 and ASH2L led to decreased DSB formation at both S μ and S α as assessed by γ -H2AX ChIP and biotin-dUTP incorporation assays [73]. The authors suggest that H3K4me3 may serve as a mark for recruiting the recombinase machinery for CSR [73]. It remains to be determined whether decreased H3K4me3 at the switch regions in mutant

cells simply correlates with impaired DSB formation or whether H3K4me3 directly functions in AID (and AID cofactor) targeting.

While the experiments investigating the role of PTIP in CSR were performed using primary splenic B cells, the aforementioned study used the CH12F3-2A murine B lymphoma cell line that is predisposed to switch to IgA and will not switch to other isotypes. Thus, the cell line differs from normal splenic B cells, which could in theory lead to germ-line transcription being resistant to knock-down of MLL-like complex components. A surprising aspect of the Stanlie et al study is that there were no differences observed in H3K4me3 at the downstream S α region between unstimulated and CD40/IL4/TGF β stimulated conditions. Other studies, including ours by ChIP-Seq, have indicated that H3K4me3 is induced upon stimulation across S γ 3, S γ 2b, and S γ 3 in primary murine B cells ex vivo [52, 61]. It is possible that methyltransferase activities regulate IgA switching differently than IgG switching. For example, Suv39h1 does not stimulate switching to any isotype but IgA [57]. Thus, further work is needed to better clarify the requirements for methyltransferase activities during CSR.

Taken together, we propose a model to explain how MLL-like complexes may function to promote accessibility during CSR (Figure 1). Upon B cell activation during an immune response, PTIP recruitment and stabilization by a transcription factor– DNA complex at the *Igh* locus leads to more efficient transcription initiation by RNA Pol II and promotes H3K4me3 across the switch region. PTIP functions to recruit and/or stabilize an MLL-like complex, likely containing MLL3/4, to switch promoter regions, which further promotes H3K4me3 at these sites. The SET1A/B methyltransferase complexes promote H3K4me3 and DSB formation at switch regions, either directly or indirectly. The H3K4 methyltransferase activity mobilizes in the direction of germ-line transcription and heavily methylates H3K4 across switch regions because of RNA Pol II stalling [47, 74]. This model takes into account data indicating that the CH12F3-2A B cell line disrupted in the common WDR5 subunit of MLL-like complexes displays CSR defects but has relatively normal *Igh* germ-line transcription [73]. Further investigation is required to understand which methyltransferases are required for H3K4me3 at the *Igh* switch regions and whether H3K4me3 directly functions in CSR.

Recently, exome sequencing has identified mutations in human *Mll2* (homologue of murine MLL4/KMT2B) to be a cause of Kabuki syndrome, a rare multiple malformation disorder characterized by developmental defects and immunodeficiency showing decreased immunoglobulin production [75]. It is intriguing to speculate that our study of PTIP-deficient mice may provide a mechanistic explanation for the immunodeficiency observed in this disease [76]. Two other studies characterizing the genome, exome, and transcriptome of B cell lymphoma cases have demonstrated that human *Mll2* is the most significantly mutated gene in this disease [77, 78]. Specifically, 32% of diffuse large B cell lymphoma and 89% of follicular lymphoma cases had somatic mutations in *Mll2* [78]. Further investigation is needed to understand the mechanistic basis for MLL2 in B cell lymphoma, but it is clear that disrupting the function of this methyltransferase complex causes significant problems in mature B cells.

Concluding Remarks

The H3K4me marks are arguably the most well-studied histone modifications, in terms of enzymatic machinery and effector protein recognition. Only recently have we begun to understand the significance of methylation at this particular lysine in lymphocyte biology. While it is clear that H3K4me3 plays a critical role in targeting and stimulating the RAG1/2 recombinase activity during V(D)J recombination, further investigation is required to clarify

its role and those of the different MLL-like complexes in CSR. Novel mechanistic insights on how chromatin accessibility regulates the targeting and chromosomal stability of the *Igh* locus may be useful for understanding and developing treatments for patients with immunodeficiencies or with the diffuse large B cell and follicular types of B cell lymphoma.

Acknowledgments

Work in the Nussenzweig laboratory is supported by the Intramural Research program of the NIH, National Cancer Institute, and Center for Cancer Research. J.A.D is the recipient of a K99/R00 NIH Pathway to Independence Award.

References

1. Dudley DD, Chaudhuri J, Bassing CH, Alt FW. Mechanism and control of V(D)J recombination versus class switch recombination: similarities and differences. *Advances in immunology*. 2005; 86:43–112. [PubMed: 15705419]
2. Matthews AG, Oettinger MA. RAG: a recombinase diversified. *Nature immunology*. 2009; 10:817–821. [PubMed: 19621044]
3. Brandt VL, Roth DB. Recent insights into the formation of RAG-induced chromosomal translocations. *Advances in experimental medicine and biology*. 2009; 650:32–45. [PubMed: 19731799]
4. Schatz DG, Swanson PC. V(D)J recombination: mechanisms of initiation. *Annual review of genetics*. 2011; 45:167–202.
5. Stavnezer J, Guikema JE, Schrader CE. Mechanism and Regulation of Class Switch Recombination. *Annu Rev Immunol*. 2008; 26:261–292. [PubMed: 18370922]
6. Ramiro AR, Stavropoulos P, Jankovic M, Nussenzweig MC. Transcription enhances AID-mediated cytidine deamination by exposing single-stranded DNA on the nontemplate strand. *Nature immunology*. 2003; 4:452–456. [PubMed: 12692548]
7. Chaudhuri J, Tian M, Khuong C, Chua K, Pinaud E, Alt FW. Transcription-targeted DNA deamination by the AID antibody diversification enzyme. *Nature*. 2003; 422:726–730. [PubMed: 12692563]
8. Nussenzweig A, Nussenzweig MC. Origin of chromosomal translocations in lymphoid cancer. *Cell*. 2010; 141:27–38. [PubMed: 20371343]
9. Gostissa M, Alt FW, Chiarle R. Mechanisms that promote and suppress chromosomal translocations in lymphocytes. *Annu Rev Immunol*. 2011; 29:319–350. [PubMed: 21219174]
10. Kuppers R. Mechanisms of B-cell lymphoma pathogenesis. *Nature reviews. Cancer*. 2005; 5:251–262.
11. Yancopoulos GD, Alt FW. Developmentally controlled and tissue-specific expression of unrearranged VH gene segments. *Cell*. 1985; 40:271–281. [PubMed: 2578321]
12. Krangel MS. Gene segment selection in V(D)J recombination: accessibility and beyond. *Nature immunology*. 2003; 4:624–630. [PubMed: 12830137]
13. Stavnezer-Nordgren J, Sirlin S. Specificity of immunoglobulin heavy chain switch correlates with activity of germline heavy chain genes prior to switching. *The EMBO journal*. 1986; 5:95–102. [PubMed: 3007121]
14. Yancopoulos GD, DePinho RA, Zimmerman KA, Lutzker SG, Rosenberg N, Alt FW. Secondary genomic rearrangement events in pre-B cells: VHDJH replacement by a LINE-1 sequence and directed class switching. *The EMBO journal*. 1986; 5:3259–3266. [PubMed: 3028778]
15. Ramiro A, Reina San-Martin B, McBride K, Jankovic M, Barreto V, Nussenzweig A, Nussenzweig MC. The role of activation-induced deaminase in antibody diversification and chromosome translocations. *Advances in immunology*. 2007; 94:75–107. [PubMed: 17560272]
16. Cobb RM, Oestreich KJ, Osipovich OA, Oltz EM. Accessibility control of V(D)J recombination. *Advances in immunology*. 2006; 91:45–109. [PubMed: 16938538]
17. Stavnezer J. Molecular processes that regulate class switching. *Curr Top Microbiol Immunol*. 2000; 245:127–168. [PubMed: 10533321]

18. Jhunjhunwala S, van Zelm MC, Peak MM, Murre C. Chromatin architecture and the generation of antigen receptor diversity. *Cell*. 2009; 138:435–448. [PubMed: 19665968]
19. Hewitt SL, Chaumeil J, Skok JA. Chromosome dynamics and the regulation of V(D)J recombination. *Immunological reviews*. 2010; 237:43–54. [PubMed: 20727028]
20. Johnson K, Reddy KL, Singh H. Molecular pathways and mechanisms regulating the recombination of immunoglobulin genes during B-lymphocyte development. *Advances in experimental medicine and biology*. 2009; 650:133–147. [PubMed: 19731807]
21. Mostoslavsky R, Alt FW, Bassing CH. Chromatin dynamics and locus accessibility in the immune system. *Nature immunology*. 2003; 4:603–606. [PubMed: 12830134]
22. Kwon J, Morshead KB, Guyon JR, Kingston RE, Oettinger MA. Histone acetylation and hSWI/SNF remodeling act in concert to stimulate V(D)J cleavage of nucleosomal DNA. *Mol Cell*. 2000; 6:1037–1048. [PubMed: 11106743]
23. Du H, Ishii H, Pazin MJ, Sen R. Activation of 12/23-RSS-dependent RAG cleavage by hSWI/SNF complex in the absence of transcription. *Mol Cell*. 2008; 31:641–649. [PubMed: 18775324]
24. Felsenfeld G, Groudine M. Controlling the double helix. *Nature*. 2003; 421:448–453. [PubMed: 12540921]
25. Ruthenburg AJ, Li H, Patel DJ, Allis CD. Multivalent engagement of chromatin modifications by linked binding modules. *Nat Rev Mol Cell Biol*. 2007; 8:983–994. [PubMed: 18037899]
26. Jenuwein T, Allis CD. Translating the histone code. *Science*. 2001; 293:1074–1080. [PubMed: 11498575]
27. Sanders SL, Portoso M, Mata J, Bahler J, Allshire RC, Kouzarides T. Methylation of histone H4 lysine 20 controls recruitment of Crb2 to sites of DNA damage. *Cell*. 2004; 119:603–614. [PubMed: 15550243]
28. Lou Z, Minter-Dykhouse K, Franco S, Gostissa M, Rivera MA, Celeste A, Manis JP, van Deursen J, Nussenzweig A, Paull TT, Alt FW, Chen J. MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. *Mol Cell*. 2006; 21:187–200. [PubMed: 16427009]
29. Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. *Cell*. 2005; 123:1213–1226. [PubMed: 16377563]
30. Botuyan MV, Lee J, Ward IM, Kim JE, Thompson JR, Chen J, Mer G. Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. *Cell*. 2006; 127:1361–1373. [PubMed: 17190600]
31. Morshead KB, Ciccone DN, Taverna SD, Allis CD, Oettinger MA. Antigen receptor loci poised for V(D)J rearrangement are broadly associated with BRG1 and flanked by peaks of histone H3 dimethylated at lysine 4. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:11577–11582. [PubMed: 14500909]
32. Perkins EJ, Kee BL, Ramsden DA. Histone 3 lysine 4 methylation during the pre-B to immature B-cell transition. *Nucleic acids research*. 2004; 32:1942–1947. [PubMed: 15051812]
33. Ruthenburg AJ, Allis CD, Wysocka J. Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. *Mol Cell*. 2007; 25:15–30. [PubMed: 17218268]
34. Ng HH, Robert F, Young RA, Struhl K. Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol Cell*. 2003; 11:709–719. [PubMed: 12667453]
35. 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]
36. Eberl HC, Mann M, Vermeulen M. Quantitative proteomics for epigenetics. *Chembiochem : a European journal of chemical biology*. 2011; 12:224–234. [PubMed: 21243711]
37. Shilatifard A. Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. *Annu Rev Biochem*. 2006; 75:243–269. [PubMed: 16756492]
38. Kim J, Guermah M, McGinty RK, Lee JS, Tang Z, Milne TA, Shilatifard A, Muir TW, Roeder RG. RAD6-Mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. *Cell*. 2009; 137:459–471. [PubMed: 19410543]

39. Matthews AG, Kuo AJ, Ramon-Maiques S, Han S, Champagne KS, Ivanov D, Gallardo M, Carney D, Cheung P, Ciccone DN, Walter KL, Utz PJ, Shi Y, Kutateladze TG, Yang W, Gozani O, Oettinger MA. RAG2 PHD finger couples histone H3 lysine 4 trimethylation with V(D)J recombination. *Nature*. 2007; 450:1106–1110. [PubMed: 18033247]
40. Liu Y, Subrahmanyam R, Chakraborty T, Sen R, Desiderio S. A plant homeodomain in RAG-2 that binds Hypermethylated lysine 4 of histone H3 is necessary for efficient antigen-receptor-gene rearrangement. *Immunity*. 2007; 27:561–571. [PubMed: 17936034]
41. Ramon-Maiques S, Kuo AJ, Carney D, Matthews AG, Oettinger MA, Gozani O, Yang W. The plant homeodomain finger of RAG2 recognizes histone H3 methylated at both lysine-4 and arginine-2. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:18993–18998. [PubMed: 18025461]
42. Ji Y, Resch W, Corbett E, Yamane A, Casellas R, Schatz DG. The in vivo pattern of binding of RAG1 and RAG2 to antigen receptor loci. *Cell*. 2010; 141:419–431. [PubMed: 20398922]
43. Shimazaki N, Tsai AG, Lieber MR. H3K4me3 stimulates the V(D)J RAG complex for both nicking and hairpinning in trans in addition to tethering in cis: implications for translocations. *Mol Cell*. 2009; 34:535–544. [PubMed: 19524534]
44. Grundy GJ, Yang W, Gellert M. Autoinhibition of DNA cleavage mediated by RAG1 and RAG2 is overcome by an epigenetic signal in V(D)J recombination. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:22487–22492. [PubMed: 21149691]
45. Couedel C, Roman C, Jones A, Vezzoni P, Villa A, Cortes P. Analysis of mutations from SCID and Omenn syndrome patients reveals the central role of the Rag2 PHD domain in regulating V(D)J recombination. *The Journal of clinical investigation*. 2010; 120:1337–1344. [PubMed: 20234091]
46. 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]
47. Pavri R, Gazumyan A, Jankovic M, Di Virgilio M, Klein I, Ansarah-Sobrinho C, Resch W, Yamane A, Reina San-Martin B, Barreto V, Nieland TJ, Root DE, Casellas R, Nussenzweig MC. Activation-induced cytidine deaminase targets DNA at sites of RNA polymerase II stalling by interaction with Spt5. *Cell*. 2010; 143:122–133. [PubMed: 20887897]
48. Basu U, Meng FL, Keim C, Grinstein V, Pefanis E, Eccleston J, Zhang T, Myers D, Wasserman CR, Wesemann DR, Januszyk K, Gregory RI, Deng H, Lima CD, Alt FW. The RNA exosome targets the AID cytidine deaminase to both strands of transcribed duplex DNA substrates. *Cell*. 2011; 144:353–363. [PubMed: 21255825]
49. 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]
50. Li Z, Luo Z, Scharff MD. Differential regulation of histone acetylation and generation of mutations in switch regions is associated with Ig class switching. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:15428–15433. [PubMed: 15486086]
51. Chowdhury M, Forouhi O, Dayal S, McCloskey N, Gould HJ, Felsenfeld G, Fear DJ. Analysis of intergenic transcription and histone modification across the human immunoglobulin heavy-chain locus. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:15872–15877. [PubMed: 18836073]
52. 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]
53. Kuang FL, Luo Z, Scharff MD. H3 trimethyl K9 and H3 acetyl K9 chromatin modifications are associated with class switch recombination. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:5288–5293. [PubMed: 19276123]
54. Yamane A, Resch W, Kuo N, Kuchen S, Li Z, Sun HW, Robbiani DF, McBride K, Nussenzweig MC, Casellas R. Deep-sequencing identification of the genomic targets of the cytidine deaminase AID and its cofactor RPA in B lymphocytes. *Nature immunology*. 2011; 12:62–69. [PubMed: 21113164]

55. Wang Z, Schones DE, Zhao K. Characterization of human epigenomes. *Curr Opin Genet Dev*. 2009; 19:127–134. [PubMed: 19299119]
56. Jeevan-Raj BP, Robert I, Heyer V, Page A, Wang JH, Cammas F, Alt FW, Losson R, Reina-San-Martin B. Epigenetic tethering of AID to the donor switch region during immunoglobulin class switch recombination. *J Exp Med*. 2011; 208:1649–1660. [PubMed: 21746811]
57. Bradley SP, Kaminski DA, Peters AH, Jenuwein T, Stavnezer J. The histone methyltransferase Suv39h1 increases class switch recombination specifically to IgA. *J Immunol*. 2006; 177:1179–1188. [PubMed: 16818776]
58. Thirman MJ, Gill HJ, Burnett RC, Mbangkollo D, McCabe NR, Kobayashi H, Ziemer-van der Poel S, Kaneko Y, Morgan R, Sandberg AA, et al. Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. *The New England journal of medicine*. 1993; 329:909–914. [PubMed: 8361504]
59. Wang JK, Tsai MC, Poulin G, Adler AS, Chen S, Liu H, Shi Y, Chang HY. The histone demethylase UTX enables RB-dependent cell fate control. *Genes Dev*. 2010; 24:327–332. [PubMed: 20123895]
60. Lachner M, O'Sullivan RJ, Jenuwein T. An epigenetic road map for histone lysine methylation. *Journal of cell science*. 2003; 116:2117–2124. [PubMed: 12730288]
61. 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
62. Cho YW, Hong T, Hong S, Guo H, Yu H, Kim D, Guszczynski T, Dressler GR, Copeland TD, Kalkum M, Ge K. PTIP associates with MLL3- and MLL4-containing histone H3 lysine 4 methyltransferase complex. *The Journal of biological chemistry*. 2007; 282:20395–20406. [PubMed: 17500065]
63. Patel SR, Kim D, Levitan I, Dressler GR. The BRCT-domain containing protein PTIP links PAX2 to a histone H3, lysine 4 methyltransferase complex. *Dev Cell*. 2007; 13:580–592. [PubMed: 17925232]
64. Issaeva I, Zonis Y, Rozovskaia T, Orlovsky K, Croce CM, Nakamura T, Mazo A, Eisenbach L, Canaani E. Knockdown of ALR (MLL2) reveals ALR target genes and leads to alterations in cell adhesion and growth. *Molecular and cellular biology*. 2007; 27:1889–1903. [PubMed: 17178841]
65. Schwab KR, Patel SR, Dressler GR. Role of PTIP in class switch recombination and long-range chromatin interactions at the immunoglobulin heavy chain locus. *Molecular and cellular biology*. 2011; 31:1503–1511. [PubMed: 21282469]
66. Munoz IM, Rouse J. Control of histone methylation and genome stability by PTIP. *EMBO Rep*. 2009; 10:239–245. [PubMed: 19229280]
67. Lechner MS, Levitan I, Dressler GR. PTIP, a novel BRCT domain-containing protein interacts with Pax2 and is associated with active chromatin. *Nucleic acids research*. 2000; 28:2741–2751. [PubMed: 10908331]
68. Shimizu K, Bourillot PY, Nielsen SJ, Zorn AM, Gurdon JB. Swift is a novel BRCT domain coactivator of Smad2 in transforming growth factor beta signaling. *Molecular and cellular biology*. 2001; 21:3901–3912. [PubMed: 11359898]
69. Michaelson JS, Singh M, Birshtein BK. B cell lineage-specific activator protein (BSAP). A player at multiple stages of B cell development. *J Immunol*. 1996; 156:2349–2351. [PubMed: 8786288]
70. Cobaleda C, Schebesta A, Delogu A, Busslinger M. Pax5: the guardian of B cell identity and function. *Nature immunology*. 2007; 8:463–470. [PubMed: 17440452]
71. Wakatsuki Y, Neurath MF, Max EE, Strober W. The B cell-specific transcription factor BSAP regulates B cell proliferation. *J Exp Med*. 1994; 179:1099–1108. [PubMed: 7511679]
72. Gong Z, Cho YW, Kim JE, Ge K, Chen J. Accumulation of Pax2 transactivation domain interaction protein (PTIP) at sites of DNA breaks via RNF8-dependent pathway is required for cell survival after DNA damage. *The Journal of biological chemistry*. 2009; 284:7284–7293. [PubMed: 19124460]
73. Stanlie A, Aida M, Muramatsu M, Honjo T, Begum NA. Histone3 lysine4 trimethylation regulated by the facilitates chromatin transcription complex is critical for DNA cleavage in class switch

- recombination. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:22190–22195. [PubMed: 21139053]
74. 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]
75. Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, Beck AE, Tabor HK, Cooper GM, Mefford HC, Lee C, Turner EH, Smith JD, Rieder MJ, Yoshiura K, Matsumoto N, Ohta T, Niikawa N, Nickerson DA, Bamshad MJ, Shendure J. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nature genetics*. 2010; 42:790–793. [PubMed: 20711175]
76. Hoffman JD, Cipraro KL, Sullivan KE, Kaplan PB, McDonald-McGinn DM, Zackai EH, Ming JE. Immune abnormalities are a frequent manifestation of Kabuki syndrome. *Am J Med Genet A*. 2005; 135:278–281. [PubMed: 15887282]
77. Pasqualucci L, Trifonov V, Fabbri G, Ma J, Rossi D, Chiarenza A, Wells VA, Grunn A, Messina M, Elliot O, Chan J, Bhagat G, Chadburn A, Gaidano G, Mullighan CG, Rabadan R, Dalla-Favera R. Analysis of the coding genome of diffuse large B-cell lymphoma. *Nature genetics*. 2011; 43:830–837. [PubMed: 21804550]
78. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, Johnson NA, Severson TM, Chiu R, Field M, Jackman S, Krzywinski M, Scott DW, Trinh DL, Tamura-Wells J, Li S, Firme MR, Rogic S, Griffith M, Chan S, Yakovenko O, Meyer IM, Zhao EY, Smailus D, Moksa M, Chittaranjan S, Rimsza L, Brooks-Wilson A, Spinelli JJ, Ben-Neriah S, Meissner B, Woolcock B, Boyle M, McDonald H, Tam A, Zhao Y, Delaney A, Zeng T, Tse K, Butterfield Y, Birol I, Holt R, Schein J, Horsman DE, Moore R, Jones SJ, Connors JM, Hirst M, Gascoyne RD, Marra MA. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature*. 2011; 476:298–303. [PubMed: 21796119]

Highlights for Daniel and Nussenzweig

- Germ-line transcription of an antigen receptor gene segment is required for DNA recombination
- Not clear whether germ-line transcripts are the cause or effect of chromatin changes
- H3K4me3 plays a critical role in targeting and stimulating RAG1/2 for V(D)J recombination
- PTIP subunit of MLL-like complex required for H3K4me3 and germ-line transcription during CSR
- MLL-like complex required for H3K4me3 and DSB formation during CSR

Figure 1. Model for how MLL-like complexes function to promote accessibility during the transcription and DNA damage steps of CSR. The model assumes that regulation of *Igh*- γ and α switch regions are similar and that the SET1A/B complex is directly recruited to *Igh*; further work is needed to test these assumptions. See text for details. Green rectangles indicate constant (C) region exon segments, blue circles indicate switch (S) regions, orange ovals indicate enhancers (E), and the black rectangle indicates the antigen recognition V(D)J gene segment. The μ , δ , and γ/α isotypes correspond to immunoglobulins M, D, G, and A. TF, transcription factor.