Histone methylation and V(D)J recombination

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Received: 14 May 2014 / Revised: 8 July 2014 / Accepted: 8 July 2014 / Published online: 25 July 2014
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Abstract V(D)J recombination is the process by which the diversity of antigen receptor genes is generated and is also indispensable for lymphocyte development. This recombination event occurs in a cell lineage- and stage-specific manner, and is carefully controlled by chromatin structure and ordered histone modifications. The recombinationally active V(D)J loci are associated with hypermethylation at lysine4 of histone H3 and hyperacetylation of histones H3/H4. The recombination activating gene 1 (RAG1) and RAG2 complex initiates recombination by introducing double-strand DNA breaks at recombination signal sequences (RSS) adjacent to each coding sequence. To be recognized by the RAG complex, RSS sites must be within an open chromatin context. In addition, the RAG complex specifically recognizes hypermethylated H3K4 through its plant homeodomain (PHD) finger in the RAG2 C terminus, which stimulates RAG catalytic activity via that interaction. In this review, we describe how histone methylation controls V(D)J recombination and discuss its potential role in lymphoid malignancy by mistargeting the RAG complex.

Keywords DNA repair · Chromatin · Lymphoma · RAG · Recombination activating gene · Histone H3K4

Introduction

Early T and B lymphocytes carry out V(D)J recombination to produce a wide variety of antigen receptor coding exons. This somatic cell programmed DNA recombination event is regulated in a lineage- and stage-specific manner. The antigen receptor variable domain exons are assembled from variable (V), diversity (D), and joining (J) gene segments (subexons). V(D)J recombination is initiated by the RAG1 and RAG2 protein complex, by introducing DNA double-strand breaks (DSBs) specifically at the recombination signal sequences (RSS) located adjacent to each V, D, and J coding sequence. The RSS consists of conserved heptamer (CACAGTG) and nonamer (ACAAAAACC) sequences separated by non-conserved 12 or 23 base spacer sequences, so called 12RSS and 23RSS, and recombination occurs only between 12- and 23RSS (12/23 rule) [1–3]. Broken DNA ends created by the RAG complex are repaired by the general DSBs repair machinery, called the nonhomologous end joining (NHEJ) pathway, to join functional antigen receptor genes (Fig. 1) [4].

Three immunoglobulin (Ig) genes (Ig heavy, Ig lambda, and Ig kappa genes) are rearranged completely in B cells, while four T cell receptor (TCR) genes (alpha, beta, gamma, and delta) are assembled only in T cells, indicating that there are mechanisms to control the chromatin accessibility to the V(D)J loci lineage specifically [5–7]. The accessibility of the RAG complex to the RSS sites at the Ig and the TCR genes is controlled by at least two chromatin mechanisms. First, RSSs must be located within open euchromatin to be exposed. Second, recent studies reveal there are dynamic changes in histone modification at recombinationally active V(D)J loci. Only the loci undergoing recombination have hypermethylation of lysine4 of histone H3 and acetylation of H3/H4 [8, 9]. Those modifications

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Fig. 1 Schematic model of V(D)J recombination. V(D)J recombination consists of two biochemical steps: RAG-dependent cleavage phase and NHEJ-dependent joining phase. The RAG complex binds and brings two RSSs together (12/23 synapsis) and introduces double-strand DNA breaks. Both coding ends and signal ends are joined by NHEJ pathway. Functional coding joint encodes variable domain of immunoglobulin or T cell receptor

are often found at transcriptionally active regions as well. Interestingly, the RAG complex has a conserved PHD finger, which specifically recognizes the hypermethylated H3K4 mark and stimulates the RAG catalytic activity upon this interaction [10–13]. Importantly, mutations in the PHD finger cause severe combined immunodeficiency (SCID) or Ommen syndrome (mild form of SCID), indicating this interaction is essential in the normal V(D)J recombination and lymphocyte development [14, 15]. On the other hand, RAG proteins are known to misrecognize suboptimal RSS-like sites at off-target genomic locations, causing DNA double-strand breaks outside of the V(D)J loci, and thereby resulting in chromosomal translocations and lymphoid malignancies [16]. Because the RAG complex selectively binds to H3K4me3 marks and stimulates its activity upon this interaction, the presence of H3K4me3 at translocation hotspots contributes to mistargeting the RAG complex, leading to lymphoid malignancies [13]. Here, we discuss recent progress regarding the histone methylation around V(D)J loci and how epigenetic marks control normal and neoplastic V(D)J recombination.

Biochemistry of the RAG complex

The RAG complex, sometimes called the V(D)J recombinase, consists of two polypeptides, RAG1 and RAG2 [17]. The expression of RAG1 and RAG2 proteins are limited in

developing B and T cells, thereby restricting the V(D)J recombination in a lineage and timing specific manner. Since the discovery of the RAG genes, extensive biochemistry of the protein complex has been conducted. RAG1 contains a conserved sequence-specific DNA-binding motif (nonamer binding motif, NBD) and three acidic amino acids that coordinate divalent cations (DDE motif) necessary for the nucleolytic reaction (Fig. 2a) [18]. RAG2 does not have detectable DNA binding or catalytic activity by itself, yet the nucleolytic activity becomes active only when RAG1 and RAG2 form a complex [19]. High-mobility group protein B1 (HMGB1) and HMGB2 stimulate RAG cleavage activity in vitro, yet its function within in vivo V(D)J recombination is less clear. DNA cleavage by the RAG complex is achieved by a series of molecular events (Fig. 2b). First, the RAG complex recognizes RSS and then introduces nicking at the 5' end of each 12- or 23RSS. The RAG complex then catalyzes transesterification to produce DNA hairpins at the coding ends, thereby resulting in DSBs. DSBs occur efficiently between 12/23RSS (12/23 synapsis). An active unit of the RAG complex consists of heterotetramer including 2 RAG1 and 2 RAG2; however, the kinetic order of the RAG reaction is not clear yet [20–22]. One RAG tetramer may carry out the nicking step at each RSS and then two RAG tetramers may work together to create DSBs. Or one RAG tetramer may first synapse the two RSS sites and then catalyze both nicking and DSB formation. Because of the limitations of

Fig. 2 Biochemistry of the RAG complex. **a** Model of the DNA cleavage by the RAG complex. The RAG complex binds and catalyzes nicking by hydrolysis at each RSS in 12/23 synapsis complex. The 3'-OH of the top strand attacks the bottom strand in the presence of the RAG complex, thereby resulting in double strand DNA breaks. Coding ends form DNA hairpins, while signal ends are blunt ends with 3'-OH. **b** Domain organization of the murine RAG proteins. RAG1 core region (a.a. 384–1,008) contains, nonamer binding domain (NBD; a.a. 389–446), zinc finger B (ZFB; a.a. 727–750) and an active site, DDE motif (a.a. 600, 708, 962). RAG1 non-core region contains ring finger (RING) a.a. 299–330 and zinc finger A (ZFA) a.a. 353–374. RAG2 core region (a.a. 1–387); PHD plant homeodomain finger a.a. 414–481; T490 phosphorylation site by cyclin-dependent kinase; (Ub)_n, ubiquitylation site

biochemical assays, we employed mathematical simulations to find kinetic models that fit experimentally determined kinetic data of *in vitro* cleavage assay [23]. Based on this approach, it is predicted that one RAG tetramer brings 12RSS and 23RSS into synapsis, followed by nicking and hairpinning (Fig. 2b).

Within the RAG proteins, the regions necessary for *in vitro* biochemical cleavage assays and cellular extra-chromosomal V(D)J recombination assays are called core RAG1 and core RAG2 (Fig. 2a) [17]. On the other hand, non-core regions of RAG proteins are necessary for efficient recombination of the genomic V(D)J loci. Importantly, mutations in the non-core regions cause SCID, but their functions are not well understood [24]. The RAG1 N terminus in the non-core region contains a RING finger

domain which has ubiquitin ligase (E3) activity itself or when in complex with VprBP/DBP1/Cul4A/Roc1 proteins [25–27]. It was reported that this region binds and ubiquitinates histone H3, possibly linking the RAG complex to the DSB repair process. The RAG2 non-core region in the C terminus plays a role in genome instability by contributing to the discrimination of sequence specificity [28–30]. This region also contains a phosphorylation site for cyclin A-Cdk2 at Thr490 and a PHD finger [31]. Thr490 and the interval spanning amino acids 499–508 are linked to the periodic destruction of RAG2 in G1-S transition by Skp1-Cul1-Skp2 (Skp2-SCF) dependent polyubiquitylation [32, 33]. Since V(D)J recombination occurs in G1 phase, the destruction of RAG protein may function to prevent an aberrant RAG activity outside of G1. The RAG2 PHD finger specifically binds hypermethylated histone H3 lysine4, thereby contributing to efficient DSB formation, which we will discuss later in this review [10–13].

Histone methylation associated with V(D)J loci

TCR genes are assembled only in T cells, while Ig genes are completely rearranged only in B cells (partial D to J at Ig heavy chain gene [D_H to J_H] rearrangement occurs in T cells). The Ig genes undergoing V(D)J recombination are sensitive to nucleases in accessibility assays, and this accessibility coincides with transcription from the unrearranged V genes (germline transcriptions) [9, 34, 35]. From this, it is clear that the recombinationally active V(D)J loci are associated with open chromatin conformation, while inactive loci are associated with closed chromatin in non-lymphoid cells or recombinationally inactive lymphoid cells. In agreement with this hypothesis, biochemical assays show that an RSS packaged into reconstituted chromatin inhibits RAG accessibility [36–39]. Recent study documented the requirement of the SWI/SNF ATP-dependent chromatin remodeling complex to alter the nucleosome structure at the IgH locus [40]. Another factor that is lineage specific at the V(D)J loci is histone modifications. One unit of chromatin fiber is the nucleosome, which consists of approximately 147 bp of dsDNA wrapping around histone octamer, H2A, H2B, H3, and H4 dimers. Histones can be chemically modified on their amino terminus tail and associate with regulatory elements of the genome [41]. Correlating with the presence of transcription, recombinationally active V(D)J loci is associated with transcriptionally active modifications, such as hypermethylation of lysine4 of histone H3 (H3K4me2/3), H3K79me2, and histone H3/H4 heparacetylation [8, 42–44].

The process of recombination is highly ordered at all V(D)J loci and the D to J recombination precedes V to DJ recombination. Among all V(D)J loci, histone modification

Fig. 3 Histone modifications at the IgH locus undergoing V(D)J recombination. V, D, J, and C (constant region exon) gene segments are indicated at the top. Subexons are depicted as boxes. Among D cluster, the distal D, DFL16.1 and the proximal D, DQ52 genes from intronic enhancer E are indicated. Histone modifications associated with each location are marked with flags; blue flag indicates H3K4me2, red indicates H3K4me3, green indicates H3K9ac and yellow indicates H3K9me2

associated with IgH gene was extensively investigated (Fig. 3) [9, 44, 45]. In the recombinationally inactive state, the IgH locus is associated with repressive epigenetic marks, including DNA methylation at CpG sites and H3K9me2 methylation [46]. As B cells develop, CpG methylation and H3K9me2 are removed, and the level of H3K4me2 and H3/H4Ac at D_H to J_H gene segments increase. At the D_H region, the association of active histone modifications is restricted to the most 5'- and 3'-D_H gene segment, DFL16.1 and DQ52, whereas the other intervening 7 D_H genes are still associated with suppressive modifications, reflecting the frequent usage of DFL16.1 and DQ52 during D_H to J_H rearrangement [9, 44, 45]. This differential status of activation of D_H gene segment is dependent on the presence of the intronic enhancer of Ig gene (E) at the downstream of the J_H gene segments. The J_H gene segments are associated with more distinct features of histone modification, H3K4me3, which is the typical epigenetic mark associated with 5' of the transcribed genes and their promoter regions [35, 47]. These striking features of association with active histone modifications centering on the J gene cluster applies for other V(D)J loci because almost all J clusters are located near their intronic enhancers. Hence, there is a tight correlation between transcription and V(D)J recombination to create chromatin remodeling and changes in histone modifications at V(D)J loci [48].

The V_H segments spans over 2.5 Mbp with more than 100 functional genes, whereas the D_H-C_μ occupies approximately 75 kbp. The V_H segments are divided into distal, intermediate, and proximal gene families. It was found that the V_H segments are associated with different histone modifications at distal and proximal gene families in developing B cells. Active modifications, such as hyperacetylated H3/H4, H3K4me2, and H3K36me3, are only found at distal V_H segments, whereas the suppressive modification, H3K27me3, is associated with proximal V_H

segments, suggesting differential regulation of the accessibility of V_H genes [49–51].

Correlation between the type of histone modifications and regulation of V(D)J recombination is reproducible with a replicating minichromosome system [52]. CpG methylated minichromosomes become chromatinized with repressive histone modifications such as H3K9me3 upon replication after transfection into cells, and these miniloci have severely decreased V(D)J recombination efficiency, whereas unmethylated miniloci have active recombination because replicating plasmids are generally associated with H3K4me3 [10, 52–54].

RAG recognition of H3K4me3

Whereas the RAG complex initiates V(D)J recombination by selectively recognizing the conserved RSS sequence in open chromatin, the RAG complex is targeted to H3K4me3 sites through the direct interaction via the PHD finger in RAG2 [10, 11]. A PHD finger, which consists of two interleaved atypical zinc fingers, is one of the binding motifs of either methylated or unmodified lysine 4 of histone H3, along with a double chromodomain and a double tudor domain [15].

The PHD finger is located in the C terminus of RAG2, which is outside of the core region of the RAG complex (Fig. 2a). Biochemical assays determined that the RAG2 PHD finger specifically binds to the histone H3 tail peptide with K4me3 with $\sim 30 \mu\text{M}$ K_D [10, 12]. This number is ~ 10 -fold higher than other PHD fingers (ING2 PHD or BPTF-PHD), most likely because the RAG2 PHD finger lacks the Arginine 2 of histone H3 (H3R2) engaging pocket which is conserved in other PHD fingers. On the other hand, methylation of H3R2 does not affect RAG2 PHD binding to H3K4me3 unlike other PHD fingers [12, 55]. While the H3K4me3 mark appears to recruit the RAG complex to the recombinationally active sites, its K_D is much higher (weaker) than that of the RAG complex to the RSSs (5–24 nM) [30, 56]. Note that the K_D of the RAG2 PHD for H3K4me3 was determined in free solution, but not with H3K4me3 associated with an RSS. However, mutations in the RAG2 PHD show a marked reduction in the efficiency of V(D)J recombination in mice [10, 11]. Moreover, human severe combined immune deficiency (SCID) or Ommen syndrome (less severe form of immunodeficiency) patients have mutations in the PHD finger, indicating the interaction between the RAG complex and H3K4me3 in the context of chromatin is essential [15]. Importantly, the interaction between H3K4me3 and the RAG complex not only functions to target the RAG complex to RSS with the epigenetic mark, but also modifies its enzymatic activity [13]. In vitro RAG cleavage assays with either *cis* (coupled with

Fig. 4 H3K4me3 stimulates RAG cleavage activity. RAG complex binds histone H3 tail with trimethylation at lysine 4 (H3K4me3) peptide via the PHD finger of RAG2. Heptamer and nonamer are depicted as “7” and “9”. Cleavage activity is stimulated when the

H3K4me3 peptide is both **a** free in solution (in trans) and **b** coupled to the RSS substrate (in trans). Improvement of the catalytic activity is depicted as the change in the shape of the catalytic center of the RAG complex

substrate) or *trans* (free in solution) form of H3K4me3 peptide to the RSS substrate demonstrated that this interaction also functions to stimulate catalytic activity of the RAG complex by accelerating its catalytic rate constant (Fig. 4). The RAG complex catalyzes nicking and hairpinning to create double-strand DNA breaks at RSS sites, and the interaction with H3K4me3 stimulates both steps, especially the hairpinning step [13, 57]. Therefore, the presence of H3K4me3 positively regulates all the kinetic reactions of the RAG proteins, which are targeting (binding), nicking, and hairpinning at the RSS.

The *in vivo* pattern of RAG binding to RSS sites was examined by ChIP using developing mouse B and T cells, and the association of the RAG complex has been found only at J gene segments where the H3K4me3 is also highly distributed, but not at D or V gene segments [58]. This indicates loading of the RAG complex at the RSS with H3K4me3 mark is seen on only at one RSS site, although the recombination occurs between two RSS signals. The J cluster is located close to the enhancer and is associated with many active histone modifications. However, the partner RSS either at the V or D cluster is located at a distal position from the enhancer, and no significant level of RAG binding or H3K4me3 association was observed there [58].

H3K4me3 mark around suboptimal RSS sites and its role in chromosomal translocation

The RAG complex specifically recognizes highly conserved RSS at V(D)J loci. In genomic DNA, however, there are thousands of RSS-like sequence (suboptimal RSS or cryptic RSS) distributed and these cryptic RSSs are likely mistargeted by the RAG complex. The T cell acute lymphoblastic leukemia (T-ALL) displays all the

Fig. 5 Presence of H3K4me3 possibly facilitates mistargeting of the RAG complex and improves miscleavage. **a** Diagram of the SIL-SCL deletion at chr.1. Adjacent to the breakpoints, heptamer/nonamer-like sequences (indicated as triangles) are present. ~100 kb indicates the approximate length of the interstitial deletion; the triangles are intermediately shaded between the 12- and 23-signals because of the uncertainty as to which signal they are mimicking (12- versus 23 RSS). The thin black upward pointing arrows indicate the translocation breakpoint. The *SCL* gene (also known as *Tal-1* gene) is encoded downstream of the SCL breakpoint. Red flag indicates the position of H3K4me3 mark. **b** Model of the role of the H3K4me3 at cryptic RSS sites. The heptamer/nonamer-like sequences in cRSS are depicted with hatched boxes. Presence of H3K4me3 at cRSS sites stimulate its cleavage by the RAG complex, whereas no cleavage occurs at cRSS without H3K4me3 marks

characteristics of V(D)J recombination; the chromosomal translocations found in T-ALL patient have breakpoints at RSS-like sequence and the joining junctions show the patterns of NHEJ [16, 59]. Some pre-B ALL also show evidence of translocations between cryptic RSS sites [60].

Previous studies using replicating extrachromosomal substrate including cryptic RSSs reproduced the events found in neoplastic cells [61]. Since the active histone modifications are found at recombinationally active loci and stimulate V(D)J recombination, it is conceivable that H3K4me3 positioning contributes to mistargeting of the RAG complex at the cryptic RSS. To support this idea, H3K4me3 marks were found at exact or relatively close positions (up to ~1.5 kbp) to the major breakpoint regions (e.g., SIL, SCL, Hox11, LMO2 and Ttg-1 sites) in human T cells [13, 62]. The deletion of SIL-SCL is a common gene alteration and accounts for 25–30 % of all T-ALL, yet their ‘strength’ as an RSS is very poor (Fig. 5a) [59]. However, biochemical assays showed that the H3K4me3 stimulates RAG cleavage activity at the cRSS of the SIL and SCL as well. Hence, the presence of H3K4me3 at poor signals threatens the genome integrity by increasing the chance to mistarget the RAG complex (Fig. 5b).

Future direction

Almost all the biochemical studies for the RAG complex have been examined using naked oligonucleotide substrates with RSS sequence. In order to examine the biochemistry of the RAG activity in the context of chromatin, it will be necessary to create a chromatinized recombination assay system. Several biochemical studies have attempted to understand how the nucleosome structure affects RAG accessibility to RSS substrate [36–39, 63]. The common view of these studies is nucleosome structure inhibits cleavage of RSS by the RAG complex, especially preventing it from binding to the RSS. To relieve the repression, chromatin remodeling by the SWI/SNF complex is necessary for the chromatinized RSS substrate with acetylated histones [38, 39, 63]. These chromatinized substrates are assembled with either bulk histones prepared from cultured cells or *Drosophila* embryo, or recombinant histones expressed in bacteria. Bulk histones contain mixture of active and inactive histone modifications and recombinant histones have no modifications. Therefore, these systems have this limitation for reconstitution of active or suppressive chromatin. To address RAG accessibility for the active chromatin, it is necessary to create a chromatinized recombination system containing H3K4me3 as well as other histone modifications found at *in vivo* recombinationally active V(D)J loci.

Another interesting issue is RAG binding and the high level of H3K4me3 association at the J cluster, but not the D or V cluster. It was proposed that the J cluster with high level of H3K4me3 distribution and RAG binding functions as the “recombination center,” and it captures the partner RSS through large-scale chromatin organization [35, 58].

Yet the most recent paper describes limited association of H3K4me3 at distal V_H genes also [50]. Further experiments will be needed to elucidate this model to uncover the mechanism to activate distal V and D gene segments to be rearranged.

Conclusion

There has been much progress in understanding the chromatin structure and histone modifications at the V(D)J loci undergoing the recombination, especially in the past 10 years. Finding of a direct association between the RAG complex and the specific epigenetic mark, H3K4me3, through the RAG2 PHD finger is also a great advantage to understand the regulation of V(D)J recombination. Mutations in the RAG2 PHD finger cause SCID or Ommen syndrome, indicating that the binding of the RAG complex and H3K4me3 has an essential role in normal V(D)J recombination and normal lymphocyte development. On the other hand, there is a possible role of this interaction in generation of lymphoid malignancy by mistargeting the RAG complex to the suboptimal sites with H3K4me3 marks. Hence, it is important to understand the mechanism of the recruitment and activation of the RAG complex by H3K4me3 further to elucidate how the genome integrity is maintained during V(D)J recombination.

Acknowledgments This work was supported by grants from NIH.

Conflict of interest The authors declare no conflicts of interest.

References

1. Gellert M. V(D)J recombination: RAG proteins, repair factors, and regulation. *Annu Rev Biochem.* 2002;71:101–32.
2. Bacolla A, Wojciechowska M, Kosmider B, et al. The involvement of non-B DNA structures in gross chromosomal rearrangements. *DNA Repair (Amst).* 2006;5:1161–70.
3. Jung D, Giallourakis C, Mostoslavsky R, Alt FW. Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. *Annu Rev Immunol.* 2006;24:541–70.
4. Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu Rev Biochem.* 2010;79:181–211.
5. Yancopoulos GD, Alt FW. Developmentally controlled and tissue-specific expression of unrearranged VH gene segments. *Cell.* 1985;40:271–81.
6. Stanhope-Baker P, Hudson KM, Shaffer AL, et al. Cell type-specific chromatin structure determines the targeting of V(D)J recombinase activity *in vitro*. *Cell.* 1996;85:887–97.
7. Roth DB, Roth SY. Unequal access: regulating V(D)J recombination through chromatin remodeling. *Cell.* 2000;103:699–702.
8. Xu CR, Feeney AJ. The epigenetic profile of Ig genes is dynamically regulated during B cell differentiation and is modulated by pre-B cell receptor signaling. *J Immunol.* 2009;182:1362–9.

9. Subrahmanyam R, Sen R. RAGs' eye view of the immunoglobulin heavy chain gene locus. *Semin Immunol.* 2010;22:337–45.
10. Liu Y, Subrahmanyam R, Chakraborty T, et al. A plant homeo-domain in RAG-2 that binds Hypermethylated lysine 4 of histone H3 is necessary for efficient antigen-receptor-gene rearrangement. *Immunity.* 2007;27:561–71.
11. Matthews AG, Kuo AJ, Ramon-Maiques S, et al. RAG2 PHD finger couples histone H3 lysine 4 trimethylation with V(D)J recombination. *Nature.* 2007;450:1106–10.
12. Ramón-Maiques S, Kuo AJ, Carney D, et al. The plant homeo-domain finger of RAG2 recognizes histone H3 methylated at both lysine-4 and arginine-2. *Proc Natl Acad Sci USA.* 2007;104:18993–8.
13. Shimazaki N, Tsai AG, Lieber MR. H3K4me3 stimulates V(D)J RAG complex for both nicking and hairpinning in trans in addition to tethering in cis: implications for translocations. *Mol Cell.* 2009;34:535–44.
14. Elkin SK, Ivanov D, Ewalt M, et al. A PHD finger motif in the C terminus of RAG2 modulates recombination activity. *J Biol Chem.* 2005;280:28701–10.
15. Baker LA, Allis CD, Wang GG. PHD fingers in human diseases: disorders arising from misinterpreting epigenetic marks. *Mutat Res.* 2008;647:3–12.
16. Tsai AG, Lu H, Raghavan SC, et al. Human chromosomal translocations at CpG sites and a theoretical basis for their lineage and stage specificity. *Cell.* 2008;135:1130–42.
17. Swanson PC. The bounty of RAGs: recombination signal complexes and reaction outcomes. *Immunol Rev.* 2004;200:90–114.
18. Swanson PC. The DDE motif in RAG-1 is contributed in trans to a single active site that catalyzes the nicking and transesterification steps of V(D)J recombination. *Mol Cell Biol.* 2001;21:449–58.
19. Schatz DG, Swanson PC. V(D)J Recombination: mechanisms of Initiation. *Annu Rev Genet.* 2011;45:167–202.
20. Bailin T, Mo X, Sadofsky MJ. A RAG1 and RAG2 tetramer complex is active in cleavage in V(D)J recombination. *Mol Cell Biol.* 1999;19:4664–71.
21. Grundy GJ, Ramon-Maiques S, Dimitriadis EK, et al. Initial stages of V(D)J recombination: the organization of RAG1/2 and RSS DNA in the postcleavage complex. *Mol Cell.* 2009;35:217–27.
22. Shlyakhtenko LS, Gilmore J, Kriatchko AN, et al. Molecular mechanism underlying RAG1/RAG2 synaptic complex formation. *J Biol Chem.* 2009;284:20956–65.
23. Askary A, Shimazaki N, Bayat N, Lieber MR. Modeling of the RAG reaction mechanism. *Cell Rep.* 2014;7:307–15.
24. Jones JM, Simkus C. The roles of the RAG1 and RAG2 “non-core” regions in V(D)J recombination and lymphocyte development. *Arch Immunol Ther Exp (Warsz).* 2009;57:105–16.
25. Grazini U, Zanardi F, Citterio E, et al. The RING domain of RAG1 ubiquitylates histone H3: a novel activity in chromatin-mediated regulation of V(D)J joining. *Mol Cell.* 2010;37:282–93.
26. Jones JM, Bhattacharyya A, Simkus C, et al. The RAG1 V(D)J recombinase/ubiquitin ligase promotes ubiquitylation of acetylated, phosphorylated histone 3.3. *Immunol Lett.* 2011;136:156–62.
27. Kassmeier MD, Mondal K, Palmer VL, et al. VprBP binds full-length RAG1 and is required for B cell development and V(D)J recombination fidelity. *EMBO J.* 2012;31:945–58.
28. Curry JD, Schlissel MS. RAG2's non-core domain contributes to the ordered regulation of V(D)J recombination. *Nucleic Acids Res.* 2008;36:5750–62.
29. Deriano L, Chaumeil J, Coussens M, et al. The RAG2 C terminus suppresses genomic instability and lymphomagenesis. *Nature.* 2011;471:119–23.
30. Shimazaki N, Askary A, Swanson PC, Lieber MR. Mechanistic basis for RAG discrimination between recombination sites and the off-target sites of human lymphomas. *Mol Cell Biol.* 2012;32:365–75.
31. Desiderio S. Temporal and spatial regulatory functions of the V(D)J recombinase. *Semin Immunol.* 2010;22:362–9.
32. Jiang H, Chang F-C, Ross AE, et al. Ubiquitylation of RAG-2 by Skp2-SCF links destruction of the V(D)J recombinase to the cell cycle. *Mol Cell.* 2005;18:699–709.
33. Zhang L, Reynolds TL, Shan X, Desiderio S. Coupling of V(D)J recombination to the cell cycle suppresses genomic instability and lymphoid tumorigenesis. *Immunity.* 2011;34:163–74.
34. Bowen AJ, Corcoran AE. How chromatin remodelling allows shuffling of immunoglobulin heavy chain genes. *Mol Biosyst.* 2008;4:790–8.
35. Schatz DG, Ji Y. Recombination centres and the orchestration of V(D)J recombination. *Nat Rev Immunol.* 2011;11:251–63.
36. Kwon J, Imbalzano AN, Matthews A, Oettinger MA. Accessibility of nucleosomal DNA to V(D)J cleavage is modulated by RSS positioning and HMG1. *Mol Cell.* 1998;2:829–39.
37. Golding A, Chandler S, Ballestar E, et al. Nucleosome structure completely inhibits in vitro cleavage by the V(D)J recombinase. *EMBO J.* 1999;18:3712–23.
38. Kwon J, Morshead KB, Guyon JR, et al. Histone acetylation and hSWI/SNF remodeling act in concert to stimulate V(D)J cleavage of nucleosomal DNA. *Mol Cell.* 2000;6:1037–48.
39. McBlane F, Boyes J. Stimulation of V(D)J recombination by histone acetylation. *Curr Biol.* 2000;10:483–6.
40. Osipovich OA, Subrahmanyam R, Pierce S, et al. Cutting edge: SWI/SNF mediates antisense Igh transcription and locus-wide accessibility in B cell precursors. *J Immunol.* 2009;183:1509–13.
41. Zhou VW, Goren A, Bernstein BE. Charting histone modifications and the functional organization of mammalian genomes. *Nat Rev Genet.* 2011;12:7–18.
42. Morshead KB, Ciccone DN, Taverna SD, et al. 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. *Proc Natl Acad Sci USA.* 2003;100:11577–82.
43. Goldmit M, Ji Y, Skok J, et al. Epigenetic ontogeny of the Igh locus during B cell development. *Nat Immunol.* 2005;6:198–203.
44. Chakraborty T, Perlot T, Subrahmanyam R, et al. A 220-nucleotide deletion of the intronic enhancer reveals an epigenetic hierarchy in immunoglobulin heavy chain locus activation. *J Exp Med.* 2009;206:1019–27.
45. Chakraborty T, Chowdhury D, Keyes A, et al. Repeat organization and epigenetic regulation of the DH-Cmu domain of the immunoglobulin heavy-chain gene locus. *Mol Cell.* 2007;27:842–50.
46. Selimyan R, Gerstein RM, Ivanova I, et al. Localized DNA demethylation at recombination intermediates during immunoglobulin heavy chain gene assembly. *PLoS Biol.* 2013;11:e1001475.
47. Feeney AJ. Epigenetic regulation of antigen receptor gene rearrangement. *Curr Opin Immunol.* 2011;23:171–7.
48. Yokota T, Sudo T, Ishibashi T, et al. Complementary regulation of early B lymphoid differentiation by genetic and epigenetic mechanisms. *Int J Hematol.* 2013;98:382–9.
49. Xu CR, Schaffer L, Head SR, Feeney AJ. Reciprocal patterns of methylation of H3K36 and H3K27 on proximal vs. distal IgVH genes are modulated by IL-7 and Pax5. *Proc Natl Acad Sci USA.* 2008;105:8685–90.
50. Choi NM, Loguercio S, Verma-Gaur J, et al. Deep sequencing of the murine IgH repertoire reveals complex regulation of nonrandom V gene rearrangement frequencies. *J Immunol.* 2013;191:2393–402.
51. Chowdhury D, Sen R. Stepwise activation of the immunoglobulin mu heavy chain gene locus. *EMBO J.* 2001;20:6394–403.
52. Hsieh C-L, Lieber MR. CpG methylated minichromosomes become inaccessible for V(D)J recombination after undergoing replication. *EMBO J.* 1992;11:315–25.

53. Okitsu CY, Hsieh CL. DNA methylation dictates histone H3K4 methylation. *Mol Cell Biol.* 2007;27:2746–57.
54. Okitsu CY, Hsieh JC, Hsieh CL. Transcriptional activity affects the H3K4me3 level and distribution in the coding region. *Mol Cell Biol.* 2010;30:2933–46.
55. Yuan CC, Matthews AG, Jin Y, et al. Histone H3R2 symmetric dimethylation and histone H3K4 trimethylation are tightly correlated in eukaryotic genomes. *Cell Rep.* 2012;1:83–90.
56. Zhao S, Gwyn LM, De P, Rodgers KK. A non-sequence-specific DNA binding mode of RAG1 is inhibited by RAG2. *J Mol Biol.* 2009;387:744–58.
57. 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. *Proc Natl Acad Sci USA.* 2010;107:22487–92.
58. Ji Y, Resch W, Corbett E, et al. The in vivo pattern of binding of RAG1 and RAG2 to antigen receptor loci. *Cell.* 2010;141:419–31.
59. Swerlow SH, Campo E, Harris NL, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: WHO Press; 2008.
60. Papaemmanuil E, Rapado I, Li Y et al. RAG-mediated recombination is the predominant driver of oncogenic rearrangement in ETV6-RUNX1 acute lymphoblastic leukemia. 2014; 46: 116–25.
61. Raghavan SC, Kirsch IR, Lieber MR. Analysis of the V(D)J recombination efficiency at lymphoid chromosomal translocation breakpoints. *J Biol Chem.* 2001;276:29126–33.
62. Barski A, Cuddapah S, Cui K, et al. High-resolution profiling of histone methylations in the human genome. *Cell.* 2007;129:823–37.
63. 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;31:641–9.