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Epigenetic control of recombination in the immune system

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Abstract

Immune receptor gene expression is regulated by a series of developmental events that modify their accessibility in a locus, cell type, stage and allele-specific manner. This is carried out by a programmed combination of many different molecular mechanisms, including region-wide replication timing, changes in nuclear localization, chromatin contraction, histone modification, nucleosome positioning and DNA methylation. These modalities ultimately work by controlling steric interactions between receptor loci and the recombination machinery.

Keywords

DNA methylation; asynchronous DNA replication; chromatin accessibility; V(D)J rearrangement; locus contraction and decontraction

1. Introduction

Immune receptor loci in the genome are organized into multiple variable subunits that can be rearranged to generate a productive gene synthesizing a single unique receptor protein. This process is universally mediated by the trans-acting RAG1/RAG2 enzyme machinery, but the choice of which locus undergoes recombination is precisely regulated in a lineage- and stage-specific manner (reviewed in [1–3]). This clearly indicates that rearrangement must be controlled by chromatin structural features that influence locus accessibility within the nucleus [4–6].

The immune system offers a wonderful opportunity for studying epigenetic structures and their influence on gene function, since it is often possible to isolate cells at different stages of lineage development (Fig. 1), and in this way, follow the precise sequence of chromatin changes. Through the extensive information that has been gathered over the past few years, it is now possible to draw a fairly good picture of how locus accessibility is orchestrated. During lineage development, each immune locus goes through a series of well-programmed events that include the initial opening prior to recombination, long range chromatin maneuvers that control accessibility within the region itself and the subsequent closing of each site by selective placement to more condensed chromatin domains within the nucleus. All of these individual steps appear to be mediated by different molecular mechanisms from long-range regional interactions such as replication timing or nuclear positioning to more local changes at the level

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of histone modification, nucleosome remodeling and DNA methylation [2,3,7,8]. In this review we will attempt to characterize these mechanisms and understand their role in the overall picture of immune system selection.

2. Early developmental events

Even though immune receptor recombination takes place exclusively in the lymphoid lineage, some of the epigenetic features that play a role in this process are actually established very early in development and can already be observed in embryonic stem cells. One of the major marks that ultimately take part in the control of recombination is DNA methylation (Fig. 2). Almost all methyl groups are removed from the genome in the blastula, with a new methylation pattern being reformed at the time of implantation, and this is done according to clear-cut molecular rules with only CpG island-like sequences being protected from this process [9–13]. As a result, CpG islands adopt an accessible structure [14,15], while many tissue-specific genes are automatically packaged into a closed chromatin conformation in a global manner, and this pattern is then maintained following every cell division throughout development. Since rearrangement is strongly inhibited by local methylation, this early mechanism may provide a simple way to prevent cryptic recombination or DNA damage [16].

In addition to CpG islands located at the promoters of many genes, other CpG-island like sequences are also protected from de novo methylation at the time of implantation (Fig. 2) [17]. It was recently demonstrated that some of the protected windows actually represent tissue-specific enhancers. This early embryonic protection keeps these sequences in an open accessible chromatin conformation throughout development and this may explain how pioneer factors ultimately bind to these regions in their cell-type of expression. This has been shown clearly for several lineage-specific enhancers, including an “accessible window” in the P τ ra enhancer domain which could play a significant role in generating α/β expressing thymocytes. The absence of this pre-established undermethylated window resulted in resistance to transcriptional activation in differentiated cells [18,19].

All of the immune receptor loci undergo asynchronous replication in a variety of different cell types throughout the organism, and rearrangement in B cells has been shown to be associated with the early replicating allele in all cases tested [20]. In order to understand how this pattern is established, replication timing was studied in early embryos. Although replication timing at these loci is synchronous in the pre-implantation embryo, it then becomes asynchronous at about the time of implantation, together with many other monoallelically-designated gene regions. Interestingly, while the choice of which parental allele replicates early appears to be switchable in dividing embryonic stem cells [21,22], it ultimately becomes fixed in somatic cells where individual clones all maintain either the maternal allele in an early replication state or the paternal allele [20,23,24]. Since this occurs so early in development and precedes other epigenetic events that are programmed to occur in B and T lineage cells, it is very likely that this inherent asymmetry actually plays a role in directing which allele is chosen for rearrangement at each immune receptor locus.

3. Chromatin accessibility precedes rearrangement

Like other regulatory processes in the nucleus, rearrangement is controlled both in trans and in cis, with the levels of mediating protein factors representing the rate-limiting component of the reaction [25–27], while specificity is determined by local cis-acting chromatin structure [8]. Thus, at all of the immune receptor loci, recombination is always preceded by alterations in protein structure associated with increased accessibility including the appearance of local RNA transcripts, nucleosome remodeling and histone acetylation (reviewed in [2,3]). These epigenetic events are precisely coordinated in keeping with the predetermined program of gene rearrangement. This is particularly evident at the IgH locus, where increased accessibility is

first associated with the DJ region, and only after this domain has undergone rearrangement does the V region become accessible for V(D)J recombination [28–32]. Even within the D region itself, subunits marked with activating histone modifications become the preferred targets for DJ rearrangement while the others are maintained in a heterochromatin conformation by means of ongoing histone deacetylation [33], in a dicer-dependent non-coding RNA mechanism [34].

In the case of the IgL, chromatin changes have been shown to precede rearrangement by several distinct stages of differentiation (Fig. 3). Thus, for example, the J κ region appears to be already packaged with acetylated histones and associated with germ-line transcription in pro- and large pre-B cells [35,36] and remains stably in this open state even though rearrangement with the V region component does not actually occur until the small pre-B cell stage [36,37]. The lambda locus, on the other hand, shows delayed onset of germ line transcription and chromatin activating modifications apparently because they require a higher dosage of key transcription factors [26]. This occurs at about the time when receptor editing is initiated [36].

It is not yet completely understood how these closely related events are connected to each other. The formation of an accessible locus is probably initiated by interactions between stage-specific trans-acting factors and local enhancer sequences, which then activate the nucleosome remodeling machinery at specific sites, and this leads to the local generation of both sense [4,38–42] and antisense [33,43,44] RNA transcripts. This idea was nicely demonstrated at the TCR β locus by showing that SWI/SNF chromatin remodeling complexes are actually recruited in an enhancer-dependent manner, and this is essential for recombinase accessibility throughout this region [45]. Furthermore, it has been shown in vitro that SWI/SNF recruitment to the IgH locus serves to increase cleavage by RAG1 and RAG2, and this can take place even in the absence of transcription or histone acetylation [46].

The actual role of pre-recombination transcription characteristic of all receptor loci has not yet been completely elucidated (Reviewed in [47]). On the one hand, it may just represent an automatic result of open chromatin, but there is also some evidence suggesting that the transcripts themselves could have their own effect on accessibility (Fig. 4). At the IgH locus, for example, DJ rearrangement is preceded by the appearance of an antisense RNA transcript bounded by a stop signal that harbors CTCF binding sites and this apparently has the effect of creating a separate chromatin compartment that actually serves to define the DJ recombination domain. Following this first step of rearrangement, a new antisense transcript extends into the V region, thereby outlining V to DJ recombination boundaries [48]. A similar phenomenon has been observed in the TCR α locus where transcripts initiating 5' of the J region define a preference for the selection of upstream J subunits for rearrangement. In the absence of these transcripts, recombination at the nearby J segment is dramatically reduced, and RNA synthesis is initiated at a secondary downstream location, resulting in the concomitant activation of 3' J segments [49,50].

A recent study has raised the interesting idea that many enhancer regions in the genome may themselves serve as transcription start sites [51,52]. If this is indeed the case in the immune system, as well, it may help explain how enhancers can directly influence transcription and recombination [43,53–57]. RNA transcription itself has been shown to induce local histone acetylation and methylation [58,59], and this might provide a mechanism for increasing chromatin accessibility at immune receptor loci. According to this model, RNA transcripts might serve as a means for “spreading” open chromatin structure.

All the immune receptor loci probably become de novo methylated at the time of implantation and remain in this form throughout the developing organism, only undergoing demethylation prior to rearrangement in their lineage-specific cell types [1,60]. Although little is known about

the ontogeny of DNA methylation at the IgH locus, studies concentrating on the IgL locus clearly indicate that demethylation is the penultimate event prior to recombination [35]. Thus, the κ locus is already primed for rearrangement in pre-B cells having undergone histone acetylation and being associated with germ-line transcripts, but this is not sufficient for the actual rearrangement to occur. Demethylation of the J region occurs on only one allele in each cell [61] (Fig. 3), and is initiated through interaction between RelB and the intronic κ enhancer. Once this is completed, rearrangement occurs almost immediately [62]. This is in keeping with observations in vitro and in transgenic mice demonstrating that the presence DNA methylation strongly inhibits rearrangement [63–65]. Interestingly, although the biochemical mechanism for locus- and tissue-specific demethylation has not yet been worked out, several experts have shown that this occurs as an active process, and studies in other systems have suggested that this may involve a DNA repair-type pathway [66–69].

4. Intra-locus control

As a general feature of receptor gene sites, many V regions are located a great distance from the D or J subunits, making them a relatively poor target for recombination. In order to overcome this problem and ensure equal use of a wide range of different variable regions, epigenetic mechanisms are employed to contract the overlying chromatin structure and thereby generate loops that bring distal V's within effective operational range of the recombination machinery [70–75].

In both the IgH and Igk loci, contraction appears to occur prior to rearrangement and possibly is mediated through interactions between CTCF and cohesin. In keeping with this model, many CTCF binding sites have been observed scattered throughout the V regions of both these B-cell loci [76,77]. In the case of the IgH (but not IgL) chain locus, CTCF binding is largely unchanged throughout development, suggesting that the binding of this factor itself is not responsible for stage-specific looping. This is in contrast to the cohesin subunit RAD21 whose recruitment to CTCF has been found to be lineage and stage specific at all Ig loci. In general, cohesin binding is developmentally regulated so that it only appears following DJ recombination but prior to V rearrangement [76]. Presumably, cohesin works through long-range looping interactions to operate as a type of spring that can contract distal sequences to more proximal positions within the locus [78]. Although little is known about the trans acting factors that regulate this process, it appears that YY1, Ikaros and Pax5 are somehow involved [71,74,79].

Histone modification may represent another mechanism for controlling equal use of distal and proximal V regions. In the IgH locus, for example, IL-7 signaling brings about activation of H3K36 methylation in distal V(H) segments, while Pax5 is responsible for causing trimethylation of H3K27, a repressive modification in the proximal V(H) segments [80]. Interestingly, fetal pro-B cells appear to lack H3K27me3 and, as a result, may predominately rearrange the proximal V's [81,82]. The balance between distal and proximal rearrangement is thus dependent on levels of multiple histone modifications which are, in turn, regulated by IL-7 and Pax5 signaling [29,83].

5. Choosing alleles

One of the most important aspects of immune system biology is that rearrangements occur in a stochastic manner and that this initially takes place on a single allele [84,85]. Early attempts to understand this process suggested that the rearrangement machinery randomly interacts with one allele and within a very short time generates a productive receptor protein which then serves as a signal for inhibiting further recombination [86]. This insures that the second allele will not undergo rearrangement in the same cell that already has a productive set of receptor molecules. Clearly, in order for this type of stochastic mechanism to work, rearrangement must

take place with low efficiency in order to allow enough time before a second recombination event occurs. While reasonable, this hypothesis appears to be inconsistent with other experiments showing that the rearrangement reaction is actually quite efficient and capable of working on multiple targets in the same cell [87,88].

An alternate model is that one allele in each cell is already preselected for recombination. In support of this idea, a number of studies have demonstrated clear-cut monoallelic changes that occur prior to the rearrangement event itself that may be involved in marking one of the two alleles as a preferred substrate [35,37,61,72,89]. In the case of the TCR β locus, for example, one allele becomes associated with either the nuclear lamina or heterochromatin in a subset of early thymocyte cells prior to rearrangement, while the other allele remains available [73]. The best evidence for monoallelic epigenetic selection, however, is at the Igk locus where, despite the presence of an RNA transcript on both copies, only one allele in each cell becomes packaged with acetylated histones in pre-B cells at a very early stage of lymphoid development. The other allele becomes associated with heterochromatin components in the nucleus through a defined cis-acting element that interacts with Ikaros [35,37,90,91]. Furthermore, it has been shown that only a single allele undergoes demethylation in the J region prior to the first rearrangement event [61].

The obvious question is whether these prodromal epigenetic events occur stochastically, on one allele or the other, or alternately, that one of the alleles is already marked from an even earlier stage in development. Studies on the TCR β locus support the idea that this choice is completely stochastic in T cells where it appears that both alleles have an equal propensity to encounter the repressive pericentromeric or nuclear lamina compartments. This in turn, leads to a decreased rate of rearrangement, thereby diminishing the probability that recombination will occur simultaneously on both alleles [92]. In contrast, experiments on the Igk locus provide clear-cut evidence that the two alleles in B cells can be distinguished from each other by their predetermined asynchronous replication timing profile. According to this model, asynchronous replication provides a read-out for setting up downstream monoallelic epigenetic differences that always direct rearrangement to occur on the early allele [20]. This same underlying mechanism is used for allelic selection of V(D)J rearrangement at the μ locus and is probably employed for TCR β rearrangement, which is also known to undergo asynchronous replication [20]. It thus appears that the lymphoid lineage has a built-in sophisticated system for ensuring the use of each parental allele in an almost equivalent manner.

6. Rearrangement

Once all of the initial chromatin components have been put into place, the final rearrangement occurs through interactions with two key proteins, RAG1 and RAG2, and here too, epigenetic marks play a role in dictating the specificity of this reaction (Fig. 3). An example of this has recently been revealed in new studies that detail how RAG2 actually binds to its target sequences and catalyzes recombination [93,94]. As demonstrated by crystallography, RAG2 contains a plant homeodomain (PHD) finger that specifically recognizes histone H3K4me3, and mutations that abrogate this recognition severely impair V(D)J recombination in vivo. In keeping with this, reducing the level of H3K4me3 leads to a decrease in rearrangement. Interestingly, RAG2 actually recognizes two histone modifications simultaneously, with its binding enhanced when the nearby arginine is dimethylated (R2me2), thereby adding an additional dimension to the specificity of this interaction [95]. These observations help explain the defect in Omenn's syndrome which is caused by a mutation (W453R) in the PHD domain of RAG2. Even though this alteration does not affect the core catalytic activity of this enzyme, it evidently brings about severe immunodeficiency by disrupting proper recruitment to genomic recombination sites [94].

These results imply that RAG2 is epigenetically programmed to operate in a site-specific manner. Since many cryptic RSS sites are scattered throughout the genome, there is a general danger that the recombination machinery may cause inappropriate DNA breakage. The additional prerequisite that H3K4me3 must also be present in order to activate recombination serves to restrict this reaction by at least 10–100 fold. This histone modification works in two independent ways. It not only stabilizes interactions between DNA and RAG2, but also serves to stimulate the catalytic reaction itself. Genetic experiments using a core RAG2 construct indicate that this mechanism is particularly important in the regulation of V(D)J recombination [96], and this is in keeping with the idea that V-DJ rearrangement represents the most regulated step in the entire process of generating antibody diversity.

A second player in the recombination machinery is RAG1. Unlike RAG2, this protein has specificity to RSS sequences exclusively. Recent experiments have demonstrated that RAG1 has a Ring domain which brings about histone mono-ubiquitination, and this activity appears to be necessary for V(D)J recombination, probably by affecting the joining phase of V(D)J [97]. A recent study has examined the binding patterns of RAG1 and RAG2 in vivo, and these observations have lead to a general outline of how recombination is managed in these complex loci. Firstly, it should be noted that both RAG1 and RAG2 are recruited to their target sites independently, and this form of binding obviously contributes to the high specificity of this reaction, thereby protecting most of the genome from needless DNA breakage events. It also appears that the RAG complex is initially generated at J or DJ regions, forming “recombination centers,” which then “capture” individual variable region targets in a process that is not yet fully understood [98].

7. Turning off rearrangement in cis

Once rearrangement has taken place, a process for inhibiting further recombination at the same locus is then initiated. In pro-B cells, this is put into motion by the successful V to DJ rearrangement on one allele which is then followed by the generation of a pre-B cell receptor. This key regulatory link immediately causes a chain of epigenetic events that include downregulation of RAG1, RAG2 and TdT factors required for recombination, histone deacetylation, repression of local transcription, repositioning of the unrearranged allele to heterochromatic regions of the nucleus and locus-internal decontraction that removes the distal V genes from the operational range of DJ recombination (Reviewed in [2]). In essence, these events are part of a major change in nuclear organization that takes place in pre-B cells, with one allele of the κ locus pairing with the non-productive μ locus, presumably in association with heterochromatin (Fig. 5) [99]. In the case of κ , this maneuver represents a pre-rearrangement event that may serve as part of the primary allelic exclusion mechanism [35]. This entire process appears to be under central control of the 3' κ enhancer, since none of these events take place if this sequence element is deleted [99]. These molecular biological and genetic observations strongly suggest that down regulation of the heavy chain locus is physically coordinated with the process of choosing a preferred light chain allele.

Overall, inhibition of rearrangement on one allele appears to play an important role in insuring that only one heavy and one light chain are produced in each cell. For the μ locus, this repression must be quite stable, since it is initiated at the pro-B cell stage and remains intact until the κ locus undergoes rearrangement. In the next step, inhibition of recombination on the second κ allele probably plays an additional role by generating a time window that allows rearranged alleles to undergo a number of editing events on the way to selecting a proper antibody molecule. It should be noted that in the event of a non-productive rearrangement, the second allele can be activated for recombination, and this presumably takes place by the same process of chromatin opening together with demethylation.

Once B cells have stably chosen a combination of rearranged heavy and light chain loci, they can then undergo further modification within germinal centers through the process of somatic hypermutation (SHM). Here too, epigenetics appears to play a role in directing this editing mechanism. At both the IgH and IgL chain loci, SHM takes place exclusively on rearranged alleles that have undergone chromatin opening and this could explain why SHM only occurs on transcribed V regions [100–103]. Using an artificial system where both κ alleles are pre-rearranged it was demonstrated that SHM still takes place on a single allele in a process that is probably directed by undermethylation [104]. Epigenetic landmarks may also be involved in insuring that SHM only occurs within the region of immune receptor loci. It has been suggested, for example, that the presence of sense and antisense transcripts over the V region makes it a preferred target over the constant region at the heavy chain locus [105]. In a similar manner, preliminary studies indicate that DNA methylation of the construct helps to distinguish this segment from the unmethylated V region at the κ locus (Y.B. and H.C., unpublished data).

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References

1. Bergman Y, Cedar H. A step-wise epigenetic process controls immunoglobulin allelic exclusion. *Nature Rev Immunol* 2004;4:753–761. [PubMed: 15459667]
2. Cedar H, Bergman Y. Choreography of Ig allelic exclusion. *Curr Opin Immunol* 2008;20:308–317. [PubMed: 18400481]
3. 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]
4. Yancopoulos GD, Alt FW. Developmentally controlled and tissue-specific expression of unrearranged VH gene segments. *Cell* 1985;40:271–281. [PubMed: 2578321]
5. Stanhope-Baker P, Hudson KM, Shaffer AL, Constantinescu A, Schlissel MS. Cell type-specific chromatin structure determines the targeting of V(D)J recombinase activity in vitro. *Cell* 1996;85:887–897. [PubMed: 8681383]
6. Constantinescu A, Schlissel MS. Changes in locus-specific V(D)J recombinase activity induced by immunoglobulin gene products during B cell development. *J Exp Med* 1997;185:609–620. [PubMed: 9034140]
7. Corcoran AE. Immunoglobulin locus silencing and allelic exclusion. *Semin Immunol* 2005;17:141–154. [PubMed: 15737575]
8. Jung D, Giallourakis C, Mostoslavsky R, Alt FW. Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. *Ann Rev Immunol* 2006;24:541–570. [PubMed: 16551259]
9. Kafri T, Ariel M, Brandeis M, Shemer R, Urven L, McCarrey J, et al. Developmental pattern of gene-specific DNA methylation in the mouse embryo and germline. *Genes Dev* 1992;6:705–714. [PubMed: 1577268]
10. Monk M, Boubelik M, Lehnert S. Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. *Development* 1987;99:371–382. [PubMed: 3653008]
11. Brandeis M, Frank D, Keshet I, Siegfried Z, Mendelsohn M, Nemes A, et al. Sp1 elements protect a CpG island from de novo methylation. *Nature* 1994;371:435–438. [PubMed: 8090226]
12. Frank D, Keshet I, Shani M, Levine A, Razin A, Cedar H. Demethylation of CpG islands in embryonic cells. *Nature* 1991;351:239–241. [PubMed: 2041571]
13. Macleod D, Lovell-Badge R, Jones S, Jackson I. A promoter trap in embryonic stem (ES) cells selects for integration of DNA into CpG islands. *Nucleic Acids Res* 1991;19:17–23. [PubMed: 1849255]
14. Thomson JP, Skene PJ, Selfridge J, Clouaire T, Guy J, Webb S, et al. CpG islands influence chromatin structure via the CpG-binding protein Cfp1. *Nature* 2004;429:101–106. [PubMed: 20393567]

15. Blackledge NP, Zhou JC, Tolstorukov MY, Farcas AM, Park PJ, Klose RJ. CpG islands recruit a histone H3 lysine 36 demethylase. *Mol Cell* 38:179–190. [PubMed: 20417597]
16. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nature Genet* 2003;33 Suppl:245–254. [PubMed: 12610534]
17. Straussman R, Nejman D, Roberts D, Steinfeld I, Blum B, Benvenisty N, et al. Developmental programming of CpG island methylation profiles in the human genome. *Nature Struct Mol Biol* 2009;16:564–571. [PubMed: 19377480]
18. Xu J, Pope SD, Jazirehi AR, Attema JL, Papathanasiou P, Watts JA, et al. Pioneer factor interactions and unmethylated CpG dinucleotides mark silent tissue-specific enhancers in embryonic stem cells. *Proc Natl Acad Sci USA* 2007;104:12377–12382. [PubMed: 17640912]
19. Xu J, Watts JA, Pope SD, Gadue P, Kamps M, Plath K, et al. Transcriptional competence and the active marking of tissue-specific enhancers by defined transcription factors in embryonic and induced pluripotent stem cells. *Genes Dev* 2009;23:2824–2838. [PubMed: 20008934]
20. Mostoslavsky R, Singh N, Tenzen T, Goldmit M, Gabay C, Elizur S, et al. Asynchronous replication and allelic exclusion in the immune system. *Nature* 2001;414:221–225. [PubMed: 11700561]
21. Dutta D, Ensminger AW, Zucker JP, Chess A. Asynchronous replication and autosome-pair non-equivalence in human embryonic stem cells. *PLoS ONE* 2009;4:e4970. [PubMed: 19325893]
22. Alexander MK, Mlynarczyk-Evans S, Royce-Tolland M, Plocik A, Kalantry S, Magnuson T, et al. Differences between homologous alleles of olfactory receptor genes require the Polycomb Group protein Eed. *J Cell Biol* 2007;179:269–276. [PubMed: 17954609]
23. Goren A, Cedar H. Replicating by the clock. *Nature Rev Mol Cell Biol* 2003;4:25–32. [PubMed: 12511866]
24. Singh N, Ebrahimi FA, Gimelbrant AA, Ensminger AW, Tackett MR, Qi P, et al. Coordination of the random asynchronous replication of autosomal loci. *Nature Genet* 2003;33:339–341. [PubMed: 12577058]
25. Agata Y, Tamaki N, Sakamoto S, Ikawa T, Masuda K, Kawamoto H, et al. Regulation of T cell receptor Beta gene rearrangements and allelic exclusion by the helix-loop-helix protein, E47. *Immunity* 2007;27:871–884. [PubMed: 18093539]
26. Beck K, Peak MM, Ota T, Nemazee D, Murre C. Distinct roles for E12 and E47 in B cell specification and the sequential rearrangement of immunoglobulin light chain loci. *J Exp Med* 2009;206:2271–2284. [PubMed: 19752184]
27. Lukin K, Fields S, Lopez D, Cherrier M, Ternyak K, Ramirez J, et al. Compound haploinsufficiencies of Ebf1 and Runx1 genes impede B cell lineage progression. *Proc Natl Acad Sci USA* 2010;107:7869–7874.
28. Maës J, O'Neill LP, Cavellier P, Turner BM, Rougeon F, Goodhardt M. Chromatin remodeling at the Ig loci prior to V(D)J recombination. *J Immunol* 2001;167:866–874. [PubMed: 11441093]
29. Chowdhury D, Sen R. Stepwise activation of the immunoglobulin μ heavy chain gene locus. *EMBO J* 2001;20:6394–6403. [PubMed: 11707410]
30. Hesslein DG, Pflugh DL, Chowdhury D, Bothwell AL, Sen R, Schatz DG. Pax5 is required for recombination of transcribed, acetylated, 5' IgH V gene segments. *Genes Dev* 2003;17:37–42. [PubMed: 12514097]
31. Maes J, Chappaz S, Cavellier P, O'Neill L, Turner B, Rougeon F, et al. Activation of V(D)J recombination at the IgH chain JH locus occurs within a 6-kilobase chromatin domain and is associated with nucleosomal remodeling. *J Immunol* 2006;176:5409–5417. [PubMed: 16622008]
32. 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. *Proc Natl Acad Sci USA* 2003;100:11577–11582. [PubMed: 14500909]
33. Chakraborty T, Chowdhury D, Keyes A, Jani A, Subrahmanyam R, Ivanova I, et al. Repeat organization and epigenetic regulation of the DH-Cmu domain of the immunoglobulin heavy-chain gene locus. *Mol Cell* 2007;27:842–850. [PubMed: 17803947]
34. Koralov SB, Muljo SA, Galler GR, Krek A, Chakraborty T, Kanellopoulou C, et al. Dicer ablation affects antibody diversity and cell survival in the B lymphocyte lineage. *Cell* 2008;132:860–874. [PubMed: 18329371]

35. Goldmit M, Yanhong J, Skok J, Roldan E, Jung S, Cedar H, et al. Epigenetic ontogeny of the κ locus during B cell development. *Nature Immunol* 2005;6:198–203. [PubMed: 15619624]
36. 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;182:1362–1369. [PubMed: 19155482]
37. Fitzsimmons SP, Bernstein RM, Max EE, Skok JA, Shapiro MA. Dynamic changes in accessibility, nuclear positioning, recombination, and transcription at the Ig kappa locus. *J Immunol* 2007;179:5264–5273. [PubMed: 17911612]
38. Lennon GG, Perry RP. C mu-containing transcripts initiate heterogeneously within the IgH enhancer region and contain a novel 5'- nontranslatable exon. *Nature* 1985;318:475–478. [PubMed: 3934561]
39. Thompson A, Timmers E, Schuurman RK, Hendriks RW. Immunoglobulin heavy chain germ-line JH-C mu transcription in human precursor B lymphocytes initiates in a unique region upstream of DQ52. *Eur J Immunol* 1995;25:257–261. [PubMed: 7843240]
40. Corcoran AE, Riddell A, Krooshoop D, Venkitaraman AR. Impaired immunoglobulin gene rearrangement in mice lacking the IL-7 receptor. *Nature* 1998;391:904–907. [PubMed: 9495344]
41. Martin DJ, van Ness BG. Initiation and processing of two kappa immunoglobulin germ line transcripts in mouse B cells. *Mol Cell Biol* 1990;10:1950–1958. [PubMed: 2109186]
42. Singh N, Bergman Y, Cedar H, Chess A. Biallelic Germline Transcription at the κ Immunoglobulin Locus. *J Exp Med* 2003;197:743–750. [PubMed: 12629064]
43. Bolland DJ, Wood AL, Afshar R, Featherstone K, Oltz EM, Corcoran AE. Antisense intergenic transcription precedes Igh D-to-J recombination and is controlled by the intronic enhancer Emu. *Mol Cell Biol* 2007;27:5523–5533. [PubMed: 17526723]
44. Bolland DJ, Wood AL, Johnston CM, Bunting SF, Morgan G, Chakalova L, et al. Antisense intergenic transcription in V(D)J recombination. *Nature Immunol* 2004;5:630–637. [PubMed: 15107847]
45. Osipovich OA, Subrahmanyam R, Pierce S, Sen R, Oltz EM. Cutting edge: SWI/SNF mediates antisense Igh transcription and locus-wide accessibility in B cell precursors. *J Immunol* 2009;183:1509–1513. [PubMed: 19596997]
46. 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]
47. Abarrategui I, Krangel MS. Germline transcription: a key regulator of accessibility and recombination. *Adv Exp Med Biol* 2009;650:93–102. [PubMed: 19731804]
48. Featherstone K, Wood AL, Bowen AJ, Corcoran AE. The mouse immunoglobulin heavy chain V-D intergenic sequence contains insulators that may regulate ordered V(D)J recombination. *J Biol Chem* 2010;285:9327–9338. [PubMed: 20100833]
49. Abarrategui I, Krangel MS. Regulation of T cell receptor-alpha gene recombination by transcription. *Nature Immunol* 2006;7:1109–1115. [PubMed: 16936730]
50. Abarrategui I, Krangel MS. Noncoding transcription controls downstream promoters to regulate T-cell receptor alpha recombination. *EMBO J* 2007;26:4380–4390. [PubMed: 17882258]
51. Kim TK, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, et al. Widespread transcription at neuronal activity-regulated enhancers. *Nature* 2010;465:182–187. [PubMed: 20393465]
52. De Santa F, Barozzi I, Miettton F, Ghisletti S, Polletti S, Tusi BK, et al. A large fraction of extragenic RNA pol II transcription sites overlap enhancers. *PLoS Biol* 2010;8:e1000384. [PubMed: 20485488]
53. Inlay M, Alt FW, Baltimore D, Xu Y. Essential roles of the kappa light chain intronic enhancer and 3' enhancer in kappa rearrangement and demethylation. *Nature Immunol* 2002;3:463–468. [PubMed: 11967540]
54. Sleckman BP, Bardon CG, Ferrini R, Davidson L, Alt FW. Function of the TCR alpha enhancer in alphabeta and gammadelta T cells. *Immunity* 1997;7:505–515. [PubMed: 9354471]
55. Perlot T, Alt FW, Bassing CH, Suh H, Pinaud E. Elucidation of IgH intronic enhancer functions via germ-line deletion. *Proc Natl Acad Sci USA* 2005;102:14362–14367. [PubMed: 16186486]
56. Afshar R, Pierce S, Bolland DJ, Corcoran A, Oltz EM. Regulation of IgH gene assembly: role of the intronic enhancer and 5'DQ52 region in targeting DHJH recombination. *J Immunol* 2006;176:2439–2447. [PubMed: 16456003]

57. Bories JC, Demengeot J, Davidson L, Alt FW. Gene-targeted deletion and replacement mutations of the T-cell receptor beta-chain enhancer: the role of enhancer elements in controlling V(D)J recombination accessibility. *Proc Natl Acad Sci USA* 1996;93:7871–7876. [PubMed: 8755569]
58. Shahbazian MD, Grunstein M. Functions of site-specific histone acetylation and deacetylation. *Annu Rev Biochem* 2007;76:75–100. [PubMed: 17362198]
59. Buratowski S. Progression through the RNA polymerase II CTD cycle. *Mol Cell* 2009;36:541–546. [PubMed: 19941815]
60. Bergman Y, Fisher AG, Cedar H. Epigenetic mechanisms that regulate antigen receptor gene expression. *Curr Opin Immunol* 2003;15:176–181. [PubMed: 12633667]
61. Mostoslavsky R, Singh N, Kirillov A, Pelanda R, Cedar H, Chess A, et al. κ chain monoallelic demethylation and the establishment of allelic exclusion. *Genes Dev* 1998;12:1801–1811. [PubMed: 9637682]
62. Kirillov A, Kistler B, Mostoslavsky R, Cedar H, Wirth T, Bergman Y. A role for nuclear NF- κ B in B-cell-specific demethylation of the Igk locus. *Nature Genet* 1996;13:435–441. [PubMed: 8696338]
63. Engler P, Haasch, Pinkert CA, Doglio L, Glymour M, Brinster R, et al. A strain-specific modifier on mouse chromosome 4 controls the methylation of independent transgene loci. *Cell* 1991;65:1–20. [PubMed: 2013090]
64. Cherry SR, Baltimore D. Chromatin remodeling directly activates V(D)J recombination. *Proc Natl Acad Sci USA* 1999;96:10788–10793. [PubMed: 10485904]
65. Hsieh CL, Lieber MR. CpG methylated minichromosomes become inaccessible for V(D)J recombination after undergoing replication. *EMBO J* 1992;11:315–325. [PubMed: 1371250]
66. Rai K, Huggins IJ, James SR, Karpf AR, Jones DA, Cairns BR. DNA Demethylation in Zebrafish Involves the Coupling of a Deaminase, a Glycosylase, and Gadd45. *Cell* 2008;135:1201–1212. [PubMed: 19109892]
67. Barreto G, Schafer A, Marhold J, Stach D, Swaminathan SK, Handa V, et al. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature* 2007;445:671–675. [PubMed: 17268471]
68. Ma DK, Jang MH, Guo JU, Kitabatake Y, Chang ML, Pow-Anpongkul N, et al. Neuronal Activity-Induced Gadd45b Promotes Epigenetic DNA Demethylation and Adult Neurogenesis. *Science*. 2009
69. Gehring M, Reik W, Henikoff S. DNA demethylation by DNA repair. *Trends Genet* 2009;25:82–90. [PubMed: 19144439]
70. Roldan E, Fuxa M, Chong W, Martinez D, Novatchkova M, Busslinger M, et al. Locus 'decontraction' and centromeric recruitment contribute to allelic exclusion of the immunoglobulin heavy-chain gene. *Nature Immunol* 2005;6:31–41. [PubMed: 15580273]
71. Fuxa M, Skok J, Souabni A, Salvagiotto G, Roldan E, Busslinger M. Pax5 induces V-to-DJ rearrangements and locus contraction of the immunoglobulin heavy-chain gene. *Genes Dev* 2004;18:411–422. [PubMed: 15004008]
72. Sayegh C, Jhunjhunwala S, Riblet R, Murre C. Visualization of looping involving the immunoglobulin heavy-chain locus in developing B cells. *Genes Dev* 2005;19:322–327. [PubMed: 15687256]
73. Skok JA, Gisler R, Novatchkova M, Farmer D, de Laat W, Busslinger M. Reversible contraction by looping of the Tcr α and Tcr β loci in rearranging thymocytes. *Nature Immunol* 2007;8:378–387. [PubMed: 17334367]
74. Liu H, Schmidt-Supprian M, Shi Y, Hobeika E, Barteneva N, Jumaa H, et al. Yin Yang 1 is a critical regulator of B-cell development. *Genes Dev* 2007;21:1179–1189. [PubMed: 17504937]
75. Jhunjhunwala S, van Zelm MC, Peak MM, Cutchin S, Riblet R, van Dongen JJ, et al. The 3D structure of the immunoglobulin heavy-chain locus: implications for long-range genomic interactions. *Cell* 2008;133:265–279. [PubMed: 18423198]
76. Degner SC, Wong TP, Jankevicius G, Feeney AJ. Cutting edge: developmental stage-specific recruitment of cohesin to CTCF sites throughout immunoglobulin loci during B lymphocyte development. *J Immunol* 2009;182:44–48. [PubMed: 19109133]
77. Parelho V, Hadjur S, Spivakov M, Leleu M, Sauer S, Gregson HC, et al. Cohesins functionally associate with CTCF on mammalian chromosome arms. *Cell* 2008;132:422–433. [PubMed: 18237772]

78. Hadjur S, Williams LM, Ryan NK, Cobb BS, Sexton T, Fraser P, et al. Cohesins form chromosomal cis-interactions at the developmentally regulated IFNG locus. *Nature* 2009;460:410–413. [PubMed: 19458616]
79. Reynaud D, Demarco IA, Reddy KL, Schjerven H, Bertolino E, Chen Z, et al. Regulation of B cell fate commitment and immunoglobulin heavy-chain gene rearrangements by Ikaros. *Nature Immunol* 2008;9:927–936. [PubMed: 18568028]
80. 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;105:8685–8690. [PubMed: 18562282]
81. Yancopoulos GD, Desiderio SV, Paskind M, Kearney JF, Baltimore D, Alt FW. Preferential utilization of the most JH-proximal VH gene segments in pre-B-cell lines. *Nature* 1984;311:727–733. [PubMed: 6092962]
82. Perlmutter RM, Kearney JF, Chang SP, Hood LE. Developmentally controlled expression of immunoglobulin VH genes. *Science* 1985;227:1597–1601. [PubMed: 3975629]
83. Johnson K, Pflugh DL, Yu D, Hesslein DG, Lin KI, Bothwell AL, et al. B cell-specific loss of histone 3 lysine 9 methylation in the V(H) locus depends on Pax5. *Nature Immunol* 2004;5:853–861. [PubMed: 15258579]
84. Pernis B, Chiappino G, Kelus AS, Gell PG. Cellular localization of immunoglobulins with different allotypic specificities in rabbit lymphoid tissues. *J Exp Med* 1965;122:853–876. [PubMed: 4159057]
85. Cebra JJ, Colberg JE, Dray S. Rabbit lymphoid cells differentiated with respect to alpha-, gamma-, and mu- heavy polypeptide chains and to allotypic markers Aa1 and Aa2. *J Exp Med* 1966;123:547–558. [PubMed: 4160817]
86. Coleclough C. Chance, necessity and antibody gene dynamics. *Nature* 1983;303:23–26. [PubMed: 6405281]
87. Ferrier P, Kripl B, Blackwell TK, Furley AJ, Suh H, Winoto A, et al. Separate elements control DJ and VDJ rearrangement in a transgenic recombination substrate. *EMBO J* 1990;9:117–125. [PubMed: 2153073]
88. Alvarez JD, Anderson SJ, Loh DY. V(D)J recombination and allelic exclusion of a TCR beta-chain minilocus occurs in the absence of a functional promoter. *J Immunol* 1995;155:1191–1202. [PubMed: 7636187]
89. Hewitt SL, Yin B, Ji Y, Chaumeil J, Marszalek K, Tenthorey J, et al. RAG-1 and ATM coordinate monoallelic recombination and nuclear positioning of immunoglobulin loci. *Nature Immunol* 2009;10:655–664. [PubMed: 19448632]
90. Goldmit M, Schlissel M, Cedar H, Bergman Y. Differential accessibility at the kappa chain locus plays a role in allelic exclusion. *EMBO J* 2002;21:5255–5261. [PubMed: 12356741]
91. Liu Z, Widlak P, Zou Y, Xiao F, Oh M, Li S, et al. A recombination silencer that specifies heterochromatin positioning and ikaros association in the immunoglobulin kappa locus. *Immunity* 2006;24:405–415. [PubMed: 16618599]
92. Schlimgen RJ, Reddy KL, Singh H, Krangel MS. Initiation of allelic exclusion by stochastic interaction of Tcrb alleles with repressive nuclear compartments. *Nature Immunol* 2008;9:802–809. [PubMed: 18536719]
93. 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]
94. Matthews AG, Kuo AJ, Ramon-Maiques S, Han S, Champagne KS, Ivanov D, et al. RAG2 PHD finger couples histone H3 lysine 4 trimethylation with V(D)J recombination. *Nature* 2007;450:1106–1110. [PubMed: 18033247]
95. Ramon-Maiques S, Kuo AJ, Carney D, Matthews AG, Oettinger MA, Gozani O, et al. The plant homeodomain finger of RAG2 recognizes histone H3 methylated at both lysine-4 and arginine-2. *Proc Natl Acad Sci USA* 2007;104:18993–18998. [PubMed: 18025461]
96. 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]

97. Grazini U, Zanardi F, Citterio E, Casola S, Goding CR, McBlane F. The RING domain of RAG1 ubiquitylates histone H3: a novel activity in chromatin-mediated regulation of V(D)J joining. *Mol Cell* 2010;37:282–293. [PubMed: 20122409]
98. 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]
99. Hewitt SL, Farmer D, Marszalek K, Cadera E, Liang HE, Xu Y, et al. Association between the Igk and Igh immunoglobulin loci mediated by the 3' Igk enhancer induces 'decontraction' of the Igh locus in pre-B cells. *Nature Immunol* 2008;9:396–404. [PubMed: 18297074]
100. Gorski J, Rollini P, Mach B. Somatic mutations of immunoglobulin variable genes are restricted to the rearranged V gene. *Science* 1983;220:1179–1181. [PubMed: 6857243]
101. Pech M, Hochtl J, Schnell H, Zachau HG. Differences between germ-line and rearranged immunoglobulin V kappa coding sequences suggest a localized mutation mechanism. *Nature* 1981;291:668–670. [PubMed: 6264318]
102. Roes J, Huppi K, Rajewsky K, Sablitzky F. V gene rearrangement is required to fully activate the hypermutation mechanism in B cells. *J Immunol* 1989;142:1022–1026. [PubMed: 2492327]
103. Lebecque SG, Gearhart PJ. Boundaries of somatic mutation in rearranged immunoglobulin genes: 5' boundary is near the promoter, and 3' boundary is approximately 1 kb from V(D)J gene. *J Exp Med* 1990;172:1717–1727. [PubMed: 2258702]
104. Fraenkel S, Mostoslavsky R, Novobrantseva TI, Pelanda R, Chaudhuri J, Esposito G, et al. Allelic 'choice' governs somatic hypermutation in vivo at the immunoglobulin kappa-chain locus. *Nature Immunol* 2007;8:715–722. [PubMed: 17546032]
105. Perlot T, Li G, Alt FW. Antisense transcripts from immunoglobulin heavy-chain locus V(D)J and switch regions. *Proc Natl Acad Sci USA* 2008;105:3843–3848. [PubMed: 18292225]

Figure 1. The order of immune receptor gene recombination in the B-cell lineage

B cell development proceeds from early stages in the bone marrow until mature cells that end up in the peripheral lymphoid system. The heavy chain (μ) is the first to undergo rearrangement in pro-B cells, followed by the κ and λ chains. Pre-BCR: pre-B-cell receptor.

Figure 2. DNA methylation during development

In the gametes most genomic DNA is methylated (blue) with CpG islands and some tissue-specific enhancers being unmethylated (brown). During the preimplantation stage, most DNA methylation is erased, but the bimodal pattern is then established anew at the time of implantation through a wave of de novo methylation coupled with CpG island protection, and this basic pattern is maintained during every cell division throughout development. Many gene promoters are included within constitutively unmethylated CpG islands, as are selected tissue-specific enhancer elements. The generation of individual cell types is accompanied by gene-specific demethylation events (red). In the lymphoid system, each lineage is characterized by its specific demethylation pattern, such as the κ locus in B cells or the TCR β locus in T cells.

Figure 3. Steric interactions control RAG specificity at the κ locus

Both κ alleles are methylated (red oval) in pro-B cells, but one (the early replicating) has histone acetylation (*) in the J region. In pre-BI cells, the unacetylated allele is recruited to heterochromatin regions (gray), while the other allele undergoes H3K4 methylation (black). Finally in small pre-B cells (pre-BII), the RAG proteins specifically interact with H3K4me3 present on the free allele which undergoes demethylation, and this brings about rearrangement.

Figure 4. RNA transcription domain

In the heavy chain locus, domains of rearrangement (yellow) appear to be defined by the presence of long non-coding RNA transcripts . At early stages in pro-B cells, a transcript that apparently initiates at the intronic μ enhancer (E) extends throughout the D region, and this evidently creates a chromatin domain that restricts rearrangement to the DJ region. At later stages, this transcript is turned off, and a new transcript is initiated which extends from the V-D spacer to the outer boundary of the V segments. This serves to restrict recombination to include only V-D rearrangements. It thus appears that the presence of the RNA transcripts plays a role in generating some form of long-range chromatin domain.

Figure 5. Monoallelic selection of the κ locus in pre-B cells

In large pre-B cells, the κ and μ loci are poised to allow κ rearrangement while preserving the previous recombination events that occurred at the μ locus. The μ allele that underwent rearrangement is packaged in acetylated histones and is being actively transcribed, while the second allele is recruited to heterochromatin (gray), making it unavailable for additional rearrangement. At the same time, one κ allele has been placed in heterochromatin, thus allowing only one allele to become packaged in acetylated histones so that it can ultimately serve as a substrate for rearrangement. The 3' κ enhancer appears to be required for pairing between the Ig κ and IgH alleles in heterochromatin.