Control of Antigen Receptor Diversity through Spatial Regulation of V(D)J Recombination

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Lymphocytes recognize a vast variety of pathogens by expressing a diverse repertoire of antigen receptor genes that are assembled by V(D)J recombination in immature B cells (*Igh*, *Igk*) and T cells (*Tcrb*, *Tcra/d*). V(D)J recombination takes place in the 3' proximal domain containing the D, J, and C gene segments, whereas 31 (*Tcrb*) to 200 (*Igh*) V genes are spread over a large region of 0.67 (*Tcrb*) to 3 (*Igk*) Mb pairs. All antigen receptor loci undergo reversible contraction at the developmental stage, where they engage in V-(D)J recombination. This long-range looping promotes the participation of all V genes in V-(D)J recombination by juxtaposing distant V genes next to (D)J segments in the proximal recombination center. The B-cell-specific Pax5, ubiquitous YY1, and architectural CTCF/cohesin proteins promote *Igh* locus contraction in pro-B cells by binding to multiple sites in the V_H gene cluster. These regulators also control the pro-B-cell-specific activity of the distally located PAIR elements, which are likely involved in the regulation of V_H-DJ_H recombination by mediating locus contraction. Notably, the large V_H gene cluster of the *Igh* locus undergoes flexible long-range looping that ensures similar participation of all V_H genes in V_H-DJ_H recombination to generate a diverse antibody repertoire.

The immune system has a sheer unlimited capacity to respond to foreign antigens. Lymphocytes contribute to the enormous adaptive potential of the immune system by expressing a diverse repertoire of antigen receptors that recognize and match the vast diversity of foreign pathogens. The broad antigen receptor repertoire is generated by V(D)J recombination, which assembles the variable regions of immunoglobulin (*Ig*) and T-cell receptor (*Tcr*) genes from discontinuous variable (V), diversity (D), and joining (J) gene segments during B- and T-cell development (Fig. 1) (Perlot and Alt 2008; Bossen et al. 2012). V(D)J recombination is tightly regulated in both lymphocyte lineages. During B-lymphopoiesis, the immunoglobulin heavy-chain (*Igh*) locus rearranges in pro-B cells before recombination of the light-chain genes *Igk* and *Igl* in small pre-B cells. Moreover, D_H-J_H rearrangements precede V_H-DJ_H recombination at the *Igh* locus, whereas the *Igk* locus generally rearranges before the *Igl* locus in pre-B cells (Perlot and Alt 2008). *Tcr* recombination also occurs at two distinct stages in αβ T-cell development. The *Tcrb* locus undergoes D_β-J_β rearrangements before V_β-DJ_β recombination in CD4⁻CD8⁻ double-negative (DN) cells of the thymus, whereas V_α-J_α rearrangements at the *Tcra* locus take place in CD4⁺CD8⁺ double-positive (DP) thymocytes (Bassing et al. 2002).

The V(D)J recombinase consists of lymphoid RAG1 and RAG2 proteins that initiate site-specific recombination by binding to and introducing double-strand breaks at recombination signal sequences (RSSs) flanking all *Ig* and *Tcr* gene segments. Ubiquitous repair factors of the nonhomologous end joining (NHEJ) machinery subsequently complete the recombination reaction by process-

ing and religating the DNA ends (Bassing et al. 2002). The RAG proteins are expressed in all lymphoid progenitors (Igarashi et al. 2002) and immature B and T cells (Janovic et al. 2004). Hence, the temporal and lineage-specific control of V(D)J recombination is largely determined by the restricted access of the RAG1/2 complex to a subset of RSSs of *Ig* or *Tcr* gene segments at the different developmental stages (Perlot and Alt 2008; Bossen et al. 2012). The chromatin accessibility of *Ig* and *Tcr* gene segments is controlled at multiple levels including subnuclear relocation (Kosak et al. 2002), DNA demethylation (Mostoslavsky et al. 1998), chromatin remodeling (Kwon et al. 2000), histone modification (Bossen et al. 2012), intergenic antisense RNA expression (Bolland et al. 2004; Ebert et al. 2011), and germline transcription (Yancopoulos and Alt 1985).

Antigen receptor loci consist of a large V gene cluster ranging from 31 (*Tcrb*) to 200 (*Igh*) V genes and a 3' proximal domain containing the D, J, and constant (C) gene segments together with a potent enhancer (Fig. 1). These two regions are subject to distinct control, because chromatin changes, germline transcription, and D-J recombination in the 3' proximal domain strictly depend on the local enhancer, whereas chromatin accessibility and germline transcription at the V genes are regulated independently of this enhancer (Mathieu et al. 2003; Hawwari and Krangel 2005; Perlot et al. 2005; Afshar et al. 2006; Chakraborty et al. 2009). The size of the two domains is yet another distinctive feature. The D and J segments of the *Igh* and *Tcrb* loci are located within a relatively short 50-kb region that readily facilitates D-J recombination. In contrast, the V genes of all major antigen receptor loci

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Figure 1. Structure of the *Igh* and *Tcrb* loci. (A) Schematic diagram of the *Igh* locus. The distinct V_H gene families (different colors) in the distal, middle, and proximal V_H gene regions (Johnston et al. 2006) are shown together with the D_H (black), J_H (orange), C_H (blue); gene elements; the E_μ enhancer (red) and 3' regulatory region (3'RR; red); and the IGCR1 and 3' CBE insulators (gray) in the 3' proximal *Igh* domain. The mm9 genomic coordinates of mouse chromosome 12 are indicated. HS, DNase I hypersensitive sites; IGCR1, intergenic control region 1; 3'RR, 3' regulatory region; CBE, CTCF-binding element. (B) Schematic diagram of the *Tcrb* locus indicating the location of functional (green) and nonfunctional (black) V_β , D_β (black), J_β (orange), and C_β (blue) gene segments together with the E_β enhancer (red) and 20 trypsinogen genes (grey). The mm9 genomic coordinates of mouse chromosome 6 are indicated.

are spread over a large region of 0.67 (*Tcrb*), 1.5 (*Tcra*), 2.4 (*Igh*), or 3 (*Igk*) Mb pairs, indicating that the V–(D)J recombination step must be spatially restricted (Fig. 1). This is best exemplified by the *Tcrb* locus, where the presence of 13 inactive trypsinogen genes separates the V_β gene cluster from the 3' proximal domain by a distance of 250 kb (Fig. 1B). Participation of the V genes in V–(D)J recombination is therefore only possible if the entire locus undergoes contraction by looping. Here, we discuss the epigenetic and transcriptional mechanisms of this spatial regulation with a particular focus on the *Igh* locus, which has been best studied in this regard.

REVERSIBLE CONTRACTION AND ALLELIC EXCLUSION OF ANTIGEN RECEPTOR LOCI

The nuclear periphery and pericentromeric heterochromatin are two repressive compartments in the nucleus that are important for propagating the inactive state of genes (Deniaud and Bickmore 2009). Depending on their activ-

ity state, genes are repositioned between these repressive compartments and central nuclear positions that facilitate gene transcription. As shown by three-dimensional DNA fluorescence in situ hybridization (3D DNA-FISH), the *Igh* and *Igk* loci are located at the nuclear periphery in all non-B cells including uncommitted lymphoid progenitors (Kosak et al. 2002). The *Igh* locus is thereby anchored via the distal V_H genes at the nuclear periphery and is oriented with the 3' proximal *Igh* domain toward the center of the nucleus, which facilitates D_H – J_H rearrangements in lymphoid progenitors (Fuxa et al. 2004). At the onset of B-cell development, the *Igh* and *Igk* loci are relocated from the nuclear periphery to more central positions within the nucleus (Kosak et al. 2002). This subnuclear repositioning likely promotes chromatin opening and germline transcription of the V_H genes in preparation for V_H – DJ_H recombination.

The 3D DNA-FISH experiments also revealed that the two *Igh* alleles are present in an extended conformation in non-B cells and lymphoid progenitors (Fig. 2) (Kosak et al. 2002; Fuxa et al. 2004). However, in committed pro-B cells, both *Igh* alleles undergo long-range contrac-

Figure 2. Reversible contraction of the *Igh* locus. The *Igh* locus is in an extended configuration in uncommitted progenitors, which allows D_H - J_H recombination to take place in the 3' proximal domain. In committed pro-B cells, all functional V_H genes participate in V_H - DJ_H rearrangements due to contraction of the *Igh* locus by looping. The V_H genes of the incompletely rearranged *Igh* allele are no longer available for V_H - DJ_H recombination due to decontraction of the *Igh* locus in pre-B cells, which results in maintenance of *Igh* allelic exclusion. DNA-FISH data are shown for *Pax5*^{-/-} progenitors, *Rag2*^{-/-} pro-B cells, and wild-type small pre-B cells (Fuxa et al. 2004; Roldán et al. 2005). The contour of the nucleus is indicated by a dashed line.

tion that juxtaposes distal V_H genes next to the rearranged proximal DJ_H domain, thus facilitating V_H - DJ_H rearrangements (Fig. 2) (Kosak et al. 2002; Fuxa et al. 2004). The V_H gene cluster thereby undergoes looping in such a way that the different V_H genes are able to recombine with similar frequency, which is essential for the generation of a diverse immunoglobulin repertoire (Roldán et al. 2005; Sayegh et al. 2005; Jhunjhunwala et al. 2008).

Successful V_H - DJ_H recombination of the *Igh* gene leads to expression of the $Ig\mu$ protein as part of the pre-B-cell receptor (pre-BCR) complex, which acts as an important checkpoint to promote proliferative cell expansion, maintenance of *Igh* allelic exclusion, and differentiation to small pre-B cells undergoing Ig light-chain gene recombination (Melchers 2005). Allelic exclusion refers to the inhibition of V_H - DJ_H recombination at the second, DJ_H -rearranged allele in small pre-B cells and thus ensures that each B lymphocyte expresses only one monospecific B-cell receptor. Importantly, pre-BCR signaling leads to decontraction of the nonfunctional *Igh* allele in pre-B cells (Fig. 2), which physically separates the distal V_H genes from the 3' proximal *Igh* domain, thus preventing further recombination of the second, DJ_H -rearranged *Igh* allele (Roldán et al. 2005). Decontraction is therefore an important mechanism for maintaining *Igh* allelic exclusion in small pre-B cells. Pre-BCR signaling furthermore leads to the relocation of the nonfunctional *Igh* allele from euchromatic regions to pericentromeric heterochromatin (Roldán et al. 2005), which is mediated by interchromosomal pairing with a pericentromerically lo-

cated *Igk* allele (Hewitt et al. 2008). Notably, the decontraction of the *Igh* locus depends on *Igh*-*Igk* pairing in a manner that requires a functional 3' *Igk* enhancer (Hewitt et al. 2008).

Other antigen receptor loci (*Igk*, *Tcrb*, and *Tcra/d*) also show reversible contraction at the developmental stage, where they undergo V(D)J recombination (Roldán et al. 2005; Skok et al. 2007). Hence, reversible locus contraction by looping is a general mechanism that promotes the spatial communication between V genes and the 3' proximal domain and thus allows V genes to participate in V(D)J recombination. On the other hand, decontraction prevents further recombination at the next developmental stage and thereby contributes to the maintenance of allelic exclusion, which ensures expression of a monospecific antigen receptor in each lymphocyte.

TRANSCRIPTION FACTORS AND REGULATORY ELEMENTS INVOLVED IN LOCUS CONTRACTION

To date, only a few *trans*-acting factors have been implicated in the control of locus contraction. 3D DNA-FISH analyses identified the B-cell commitment factor Pax5 as the first transcriptional regulator described to be involved in the pro-B-cell-specific contraction of the *Igh* locus (Fig. 2) (Fuxa et al. 2004). Pax5 was previously shown to control V_H - DJ_H rearrangements, because members of the large V_HJ558 gene family in the distal *Igh* region (black lines in Fig. 1A) undergo recombination

only with a ~ 100 -fold lower efficiency in Pax5-deficient progenitors compared with wild-type pro-B cells, despite normal D_H–J_H recombination in both cell types (Nutt et al. 1997). Subsequent analyses revealed that only the first most proximal of the 200 V_H genes efficiently undergo V_H–DJ_H recombination in the absence of locus contraction, although all V_H genes are accessible and give rise to germline transcripts in Pax5-deficient progenitors (Hesslein et al. 2003; Roldán et al. 2005). A similar V_H–DJ_H recombination phenotype and lack of *Igh* locus contraction were observed in YY1-deficient pro-B cells, indicating that the ubiquitous transcription factor YY1, like Pax5, contributes to long-range looping at the *Igh* locus (Liu et al. 2007). Another potential regulator is the CCCTC-binding factor (CTCF), which has been implicated, through its association with the cohesin complex, in long-range chromatin looping at several complex loci (Merkenschlager and Odom 2013). Indeed, shRNA knockdown experiments have suggested a role for the CTCF/cohesin complex in the regulation of looping at the *Igh* locus in pro-B cells (Degner et al. 2011). Consistent with this finding, CTCF and cohesin bind together to multiple sites along the V_H gene cluster of the *Igh* locus in lymphoid progenitors and committed pro-B cells irrespective of locus contraction (Fig. 3) (Degner et al. 2011; Ebert et al. 2011; Medvedovic et al. 2013). Pax5 also interacts with numerous sites in the *Igh* locus (Fig. 3) (Ebert et al. 2011) as well as with the CTCF protein itself (Medvedovic et al. 2013), suggesting that this protein–protein interaction may contribute to long-range looping by promoting contacts between Pax5-binding sites and CTCF/cohesin-binding elements in committed pro-B cells. In contrast, YY1 binds less frequently to the *Igh* locus, where most of its binding sites are located in the distal V_H gene region (Fig. 3) (Medvedovic et al. 2013). Interestingly however, YY1 also has the potential to interact with the CTCF protein (Donohoe et al. 2007).

The V_H gene region likely contains *cis*-regulatory elements that mediate *Igh* locus contraction. Until recently, however, V_H gene promoters were the only known regulatory elements in the entire V_H gene cluster. By performing a systematic search for intergenic regions with active chromatin, we identified 14 pax5-activated intergenic repeat (PAIR) elements in the distal V_H gene region of the *Igh* locus (Fig. 4) (Ebert et al. 2011). These PAIR elements are bound by Pax5, E2A, YY1, CTCF, and cohesin, carry Pax5-dependent active chromatin, and give rise to Pax5-regulated noncoding antisense RNA exclusively in pro-B cells (Fig. 4) (Ebert et al. 2011; Medvedovic et al. 2013). These antisense transcripts also depend on YY1 (Verma-Gaur et al. 2012; Medvedovic et al. 2013), but not on CTCF, because reduced CTCF expression leads to increased antisense transcription (Degner et al. 2011). Importantly, Pax5 no longer binds to PAIR elements in pre-B and B cells, consistent with the loss of antisense RNA expression in these cells. The pro-B-cell-specific and Pax5-dependent activity therefore implicates the PAIR elements in the regulation of distal V_H–DJ_H recombination possibly by promoting *Igh* locus contraction (Ebert et al. 2011). Because PAIR elements 4, 6, and 11 show the highest transcriptional activity within the V_H gene cluster, they have been postulated to induce long-range looping by relocating the PAIR-containing V_H gene sequences to transcription factories in the nucleus, where they interact with the 3' proximal *Igh* domain containing the highly active E_μ enhancer (Verma-Gaur et al. 2012). As a consequence, these three PAIR elements should constitute interaction hotspots in the distal V_H gene region. However, 4C-seq experiments (described below) failed to confirm this hypothesis, because all V_H gene sequences contact the 3' proximal *Igh* domain with similar probability (Fig. 5C) (Medvedovic et al. 2013).

The E_μ enhancer in the 3' *Igh* domain has recently been implicated in the control of *Igh* locus contraction and thus

Figure 3. Binding patterns of Pax5, YY1, CTCF, and cohesin at the *Igh* locus. The indicated binding sites of the transcription factors Pax5, YY1, and CTCF as well as of the cohesin subunits Smc3 and Rad21, were determined by ChIP analysis of Pax5^{-/-} progenitors (blue) and Rag2^{-/-} pro-B cells (black) combined with paired-end sequencing (Ebert et al. 2011; Revilla-i-Domingo et al. 2012, unpubl.). DNase I hypersensitive (DHS) sites, which were also mapped by using paired-end sequencing, are shown for comparison. All ChIP-seq peaks and DHS sites were called with the MACS program using a stringent *p* value of $< 10^{-10}$. The *Zfp386* gene located upstream of the *Igh* locus is indicated by red lines in the *Igh* annotation.

Figure 4. Pro-B-cell-specific activity of the PAIR elements in the distal *IgH* region. The location of the 14 PAIR elements is indicated together with their chromatin state, transcription factor occupancy, and transcriptional activity in uncommitted progenitors, committed pro-B cells and small pre-B cells (Ebert et al. 2011). Asterisks indicated the three PAIR elements with the highest transcriptional activity (Verma-Gaur et al. 2012). CTCF was shown to interact with cohesin at the PAIR elements (Ebert et al. 2011). The binding of YY1 was so far analyzed only in pro-B cells by ChIP-seq. Binding of YY1 in the progenitors and pre-B cells is currently hypothetical and only inferred due to the ubiquitous expression of this transcription factor.

Figure 5. Flexible long-range loops across the *V_H* gene cluster. (A) Illustration depicting the *IgH* locus in its “extended” conformation with overlapping local interaction domains and in its contracted state with long-range interactions connecting the local interaction domains. (B) Simplified diagram indicating how flexible local interactions in the *V_H* gene region may result in a continuum of chromatin loops across the *V_H* gene cluster in a population of 1 million pro-B cells that were used for 4C sequencing. (C) Comparison of 4C-seq patterns obtained with *Rag2*^{-/-} pro-B cells and random bacterial artificial chromosome (BAC) libraries that were analyzed with an HS3B viewpoint in the 3′ proximal *IgH* domain (Fig. 1A). The 4C-seq data were plotted as reads per million (RPM) mapped sequence reads. The mappability track (gray) indicates to what degree (on a scale from 0 to 2) the end sequences of the analyzed HindIII fragments could be unambiguously mapped to genomic *IgH* sequences. (C, Adapted, with permission, from Medvedovic et al. 2013.)

V_H – DJ_H recombination (Guo et al. 2011a). This conclusion was mainly based on 3D DNA-FISH analyses demonstrating that the *Igh* locus is present in an extended conformation in $E_{\mu}^{-/-}$ *Rag2* $^{-/-}$ pro-B cells in contrast to its contracted state in *Rag2* $^{-/-}$ pro-B cells (Guo et al. 2011a). By performing 3D DNA-FISH and 4C-sequencing experiments with the same pro-B cell types, we could not, however, confirm a role for the E_{μ} enhancer in the regulation of *Igh* locus contraction by these two independent methods (Medvedovic et al. 2013). Previous analyses revealed an essential role of the E_{μ} enhancer in controlling the chromatin accessibility and transcriptional activation of the 3' proximal *Igh* domain, because active histone marks (H3K4me3 and H3K9ac), germline transcripts (μ^0 and I_{μ}), and D_H – J_H recombination are severely reduced in $E_{\mu}^{-/-}$ pro-B cells (Perlot et al. 2005; Afshar et al. 2006; Chakraborty et al. 2009). Hence, the observed block of V_H – DJ_H recombination is likely a secondary consequence of the strongly reduced number of DJ_H -rearranged *Igh* alleles in $E_{\mu}^{-/-}$ pro-B cells (Perlot et al. 2005; Afshar et al. 2006). Notably, the recombination of proximal and distal V_H genes is equally affected by deletion of the E_{μ} enhancer (Perlot et al. 2005), in contrast to the preferential loss of distal V_H – DJ_H recombination in the absence of locus contraction (Fuxa et al. 2004). Moreover, germline V_H gene transcription is unaffected in $E_{\mu}^{-/-}$ pro-B cells, further indicating that the activity of the E_{μ} enhancer is restricted to the 3' proximal *Igh* domain (Perlot et al. 2005; Afshar et al. 2006).

CONTROL OF V(D)J RECOMBINATION BY INSULATORS

CTCF has been implicated not only in chromosome looping but also in chromatin boundary formation, transcriptional insulation, activation, or repression (Phillips and Corces 2009). A large array of regularly spaced CTCF-binding sites characterizes the V gene clusters of all antigen receptor loci, whereas the 3' proximal domains contain CTCF-binding sites only at their 5' and 3' boundaries (Fig. 3) (Degner et al. 2011; Ebert et al. 2011; Ribeiro de Almeida et al. 2011; Seitan et al. 2011; Shih et al. 2012). The CTCF-binding sites at the 5' end of the proximal domain act as insulators, as exemplified by the *Sis* element, which is located between the V_{κ} and J_{κ} segments and functions as a silencer of *Igk* recombination by binding of transcription factors CTCF and Ikaros (Liu et al. 2006; Ribeiro de Almeida et al. 2011). Deletion of the *Sis* element or conditional inactivation of CTCF similarly affect V_{κ} – J_{κ} rearrangements in pre-B cells by increasing germline transcription and recombination of proximal V_{κ} genes at the expense of distal V_{κ} gene recombination (Ribeiro de Almeida et al. 2011; Xiang et al. 2011). The loss of CTCF thereby skews the repertoire of V_{κ} – J_{κ} rearrangements by strongly increasing the interactions of the iE_{κ} and $3'E_{\kappa}$ enhancers with proximal V_{κ} genes (Ribeiro de Almeida et al. 2011). This evidence suggests that the *Sis* element functions as an insulator to negatively regulate proximal V_{κ} gene recombination

by blocking the long-range activity of the E_{κ} enhancers (Ribeiro de Almeida et al. 2011). Long-range looping across the V_{κ} gene cluster is, however, normal in *Sis*-deficient pre-B cells, indicating that the *Sis* element does not contribute to *Igk* locus contraction (Xiang et al. 2011).

The best-characterized insulator of the *Igh* locus is the intergenic control region 1 (IGCR1), containing two CTCF-binding elements (CBEs) that are located 2 kb upstream of the D_H FL16.1 gene segment in the intervening V_H – D_H region (Fig. 1A) (Guo et al. 2011b). Specific mutation of the CBEs showed that the two CTCF-binding sites function as insulator elements to regulate ordered and lineage-specific V(D)J recombination at the *Igh* locus (Guo et al. 2011b). In uncommitted lymphoid progenitors, the IGCR1/CBE insulator restricts the potent E_{μ} enhancer activity as well as all other looping interactions to the 3' proximal domain, which generates a local chromatin structure that promotes D_H – J_H recombination on both *Igh* alleles (Guo et al. 2011b). During normal development, the IGCR1/CBE insulator is only neutralized in committed pro-B cells, possibly by Pax5-dependent contraction of the *Igh* locus, which facilitates V_H – DJ_H recombination. In IGCR1/CBE mutant mice, the activity of the E_{μ} enhancer is, however, no longer restrained and thus induces active chromatin and germline transcription at the first few proximal V_H genes that undergo premature V_H – DJ_H and even V_H – D_H recombination in lymphoid progenitors and developing thymocytes (Guo et al. 2011b). As a consequence of the precocious proximal V_H gene activity in lymphoid progenitors, fewer DJ_H -rearranged *Igh* alleles are available for V_H – DJ_H recombination in committed pro-B cells, which leads to a strong increase of proximal V_H gene rearrangements and a corresponding skewing of the immunoglobulin repertoire. Hence, the CTCF-regulated IGCR1 activity and Pax5-mediated locus contraction fulfill opposing roles by preventing or promoting the participation of V_H genes in V(D)J recombination in lymphoid progenitors or committed pro-B cells, respectively.

THE “EXTENDED” IGH LOCUS CONSISTS OF OVERLAPPING LOCAL INTERACTION DOMAINS IN UNCOMMITTED PROGENITORS

The spatial conformation of antigen receptor loci has so far been studied in single cells at low resolution by DNA-FISH analysis. In contrast, high-resolution analysis of chromatin looping at the cell population is provided by 4C-seq, which is based on the chromosome conformation capture-on-chip technique adapted for deep sequencing (van de Werken et al. 2012). Our recent 4C-seq analyses revealed that the *Igh* locus in its “extended” configuration consists of distinct local interaction domains ranging from 0.5 Mb (3' proximal region) to 1.3 Mb (5' PAIR region) in thymocytes and uncommitted *Pax5* $^{-/-}$ progenitors (Fig. 5A) (Medvedovic et al. 2013). These local interaction domains may consist of rosettes of chromatin loops that have been predicted to be the organizational

units of the *Igh* locus in uncommitted pre-/pro-B cells based on computer simulation of distance measurements among 12 distinct DNA-FISH probes (Jhunjhunwala et al. 2008). Global Hi-C analyses of embryonic stem, pre-/pro-B, and pro-B cells revealed that the entire mouse genome is organized into local chromatin interaction regions referred to as topological domains that range from 0.5 to 3 Mb in size (Dixon et al. 2012; Lin et al. 2012; Nora et al. 2012). The local interaction domains of the *Igh* locus also fit this size range, show a similar 4C-seq pattern in lymphoid progenitors and thymocytes and may thus correspond to topological domains, which are largely invariant between different cell types (Dixon et al. 2012; Lin et al. 2012). Hence, the local interaction domains likely constitute the default folding state of the “extended” *Igh* locus in non-B cells.

Previous 3C analyses of the 3′ proximal *Igh* domain identified loops between the regulatory IGCR1, E_{μ} , 3′RR, and 3′CBE regions (see Fig. 1A) (Degner et al. 2011; Guo et al. 2011a,b). In addition to the known chromatin loops, our 4C-seq analysis detected novel interactions of these regulatory elements with the C γ 1–C γ 2b intervening region as well as with the first proximal V_H genes in thymocytes, lymphoid progenitors, and committed pro-B cells (Medvedovic et al. 2013). The incorporation of these V_H genes into the local 3D architecture of the 3′ proximal *Igh* domain explains why only the first proximal V_H genes undergo V_H –DJ $_H$ recombination in the absence of locus contraction in Pax5-deficient progenitors (Hesslein et al. 2003; Roldán et al. 2005). The same V_H genes also interact with the 3′ proximal *Igh* domain in thymocytes (Medvedovic et al. 2013), although they never undergo V_H –DJ $_H$ recombination during normal T-cell development, unless the IGCR1 insulator is inactivated by mutation of its two CBEs (Guo et al. 2011b). Hence, a functional IGCR1 element prevents V_H –DJ $_H$ recombination even if V_H genes participate in looping with the 3′ proximal *Igh* domain in thymocytes.

FLEXIBLE LONG-RANGE INTERACTIONS ACROSS THE V_H GENE CLUSTER MEDIATE LOCUS CONTRACTION IN PRO-B CELLS

In committed pro-B cells, all local interaction domains engage in long-range looping across the *Igh* locus, which depends on Pax5, YY1, and CTCF as shown by 4C-seq analysis (Fig. 5A) (Medvedovic et al. 2013). 5′ and 3′ boundaries near the first 5′ V_H J558 gene and at the 3′CBE region (Fig. 1A) restrict these long-range interactions to the *Igh* locus, which constitutes one large chromosome-folding unit in pro-B cells. Notably, locus contraction did not affect the interactions within the local domains, but instead provided an additional layer of long-range looping among these domains. As shown with 4C-seq, the 3′ proximal region, which contains the recombination center of the *Igh* locus (Ji et al. 2010), assumes a rigid chromatin structure, where most long-range loops from the V_H gene region contact the IGCR1 and 3′CBE regions containing the only CTCF/cohesin-binding sites

in the 3′ proximal region (Fig. 3) (Medvedovic et al. 2013). Unexpectedly, however, the long-range loops appear to initiate at a multitude of sites in the V_H gene region, which results in a continuum of flexible long-range interactions across the entire V_H gene cluster in a population of pro-B cells (Fig. 5B) (Medvedovic et al. 2013). Given the critical role of the CTCF/cohesin complex in chromatin looping (Merkenschlager and Odom 2013), it is conceivable that the regularly spaced CTCF/cohesin-binding sites (Fig. 3) may anchor the long-range loops in the V_H gene region. Importantly, the 4C-seq patterns of the distal V_H gene region were similar for the local interaction domains in Pax5-deficient progenitors (before locus contraction) and the corresponding long-range loops in committed pro-B cells (undergoing locus contraction) (Medvedovic et al. 2013). This evidence strongly suggests that the observed flexibility may be a characteristic feature of the local interaction domains in the V_H gene region, as depicted in Figure 5B. The 4C-seq data shown in Figure 5C provide the strongest argument for flexible long-range looping, because the chromatin of *Rag2*^{−/−} pro-B cells and randomly ligated DNA fragments of overlapping *Igh* bacterial artificial chromosomes (BACs) gave rise to similar 4C-seq patterns with the HS3B viewpoint in the 3′ proximal region (Medvedovic et al. 2013). We therefore hypothesize that the flexible long-range interactions provide, at the pro-B-cell population level, a similar probability for each V_H gene to be juxtaposed next to the DJ $_H$ -rearranged gene segment in the 3′ proximal *Igh* domain to undergo V_H –DJ $_H$ recombination. By promoting equal participation of all V_H genes in V_H –DJ $_H$ recombination, flexible long-range loops likely contribute to the generation of a diverse antibody repertoire that is essential for an effective immune response to foreign pathogens. It is, however, important to note that individual V_H genes rearrange at different frequencies in pro-B cells (Choi et al. 2013). Hence, in addition to the essential requirement for locus contraction, the recombination rate of a given V_H gene is determined also by other parameters such as the functionality of the V_H RSS elements (Choi et al. 2013).

Our 4C-seq and 3D DNA-FISH analyses furthermore revealed that individual mutation of the IGCR1 element, E_{μ} enhancer, or 3′ regulatory region (3′RR) in the 3′ proximal *Igh* domain does not affect locus contraction. These data further support the notion that the V_H gene region and 3′ proximal domain of the *Igh* locus fulfill distinct roles in V(D)J recombination. The primary function of the V_H gene cluster seems to be the formation of flexible long-range loops to present the different V_H genes to the 3′ proximal domain, which functions as the business end of the *Igh* locus where V(D)J recombination takes place.

ACTIVE CHROMATIN AND THE CONTROL OF SEQUENTIAL REARRANGEMENTS AT THE RECOMBINATION CENTER

The accessibility hypothesis, published already 28 years ago, postulates that the initial step in the control

of V(D)J recombination must be chromatin changes that render the different gene segments of the antigen receptor loci accessible to the V(D)J recombination machinery (Yancopoulos and Alt 1985). In the meantime, it is well established that the 3' proximal domain of antigen receptor loci is activated at the chromatin level by enhancer-mediated recruitment of chromatin-remodeling and histone-modifying enzymes (Mathieu et al. 2000; McMurry and Krangel 2000; Chakraborty et al. 2009). As a consequence, the intronic E_{μ} enhancer and adjacent J_H segments are characterized by an abundance of the three active histone marks H3K4me2, H3K4me3, and H3K9ac in pro-B cells (Fig. 6A) (Chakraborty et al. 2009; Malin et al. 2010), which strongly express the germline transcripts μ^0 and I_{μ} as a proxy for chromatin accessibility (Verma-Gaur et al. 2012). However, it is less clear what defines the accessible state of the V genes before V-(D)J recombination. For instance, the V_H genes of the *Igh* locus are characterized by nucleosomal reorganization and increased DNase I hypersensitivity in *Rag2*^{-/-} pro-B cells (Maes et al. 2001), which may explain the observed low level of germline V_H gene transcription (Yancopoulos and Alt 1985; Verma-Gaur et al. 2012). Initial chromatin precipitation experiments implicated histone acetylation as an important mechanism for activating the chromatin at V genes (Chowdhury and Sen 2001; Tripathi et al. 2002). However, systematic chromatin profiling of the *Igh* locus by ChIP-chip and ChIP-seq analyses subsequently revealed that the active histone marks H3K4me2, H4K4me3, and H3K9ac are either absent or only present at low levels at most V_H genes except for members of the distal V_H3609 gene family that carry relatively high detectable levels of H3K4me2 and H3K9ac (Fig. 6A) (Malin et al. 2010; Choi et al. 2013). Notably, the entire V_H gene cluster contains only three H4K4me3 peaks at the most highly active PAIR4 and PAIR6 elements as well as at another intergenic region located in the middle V_H gene cluster (Fig. 6A) (Malin et al. 2010; Choi et al. 2013). Hence, most V_H genes do not show an active histone modification signature (H3K4me2⁺ H4K4me3⁺ H3K9ac⁺) characteristic of expressed genes, and yet their chromatin must be accessible enough to facilitate V_H -DJ_H recombination in pro-B cells.

A possible explanation for this asymmetric distribution of active chromatin at the *Igh* locus and other antigen receptor loci may be provided by the fact that the RAG2 protein contains at its carboxyl terminus a plant homeodomain (PHD) finger that specifically binds the active histone mark H3K4me3 (Matthews et al. 2007) at active promoters throughout the genome (Ji et al. 2010). Importantly, RAG2 also binds to the H3K4me3 island at the J gene segments of the different antigen receptor loci (*Igh*, *Igk*, *Tcrb*, and *Tcra/d*), once they become accessible during lymphocyte development (Fig. 6B) (Ji et al. 2010). The RAG1 protein interacts in a more restrictive manner only with the RSS elements of the accessible J gene segments and thus further contributes to the focal and stable recruitment of the RAG1/2 complex to the active chromatin region in the 3' proximal region of antigen receptor

loci referred to as the recombination center (Fig. 6B) (Ji et al. 2010). As exemplified for the *Igh* locus, the RAG1/2 complex, once tethered to the J_H segments at the recombination center, can capture a partner RSS element of a D_H segment to undergo synapse formation (Fig. 6B). After D_H - J_H recombination in lymphoid progenitors, the rearranged D_H element is also incorporated into the active chromatin domain and bound by the RAG1/2 complex that can now capture an RSS element of one of the many V_H gene segments to undergo V_H -DJ_H rearrangement in pro-B cells. The H3K4me3-mediated focal targeting of the RAG1/2 endonuclease to the recombination center is a likely reason why active chromatin is asymmetrically distributed at the proximal J_H region and not throughout the D_H and V_H gene clusters in pro-B cells. Importantly, the localized activity of the RAG1/2 complex at the proximal chromatin hub strictly depends on locus contraction to present the V genes to the recombination center for subsequent generation of a functional V(D)J-rearranged antigen receptor gene.

CONCLUDING REMARKS

Locus contraction is essential for the generation of a diverse antigen receptor repertoire, because it promotes V-(D)J recombination at all major antigen receptor loci. Despite its importance for humoral immunity, we still know little about the molecular mechanisms underlying locus contraction. Although Pax5, YY1, and the CTCF/cohesin complex have been identified as important regulators of locus contraction, it is currently unclear how they contribute to this process. For instance, the CTCF/cohesin complex shows a similar binding pattern along the *Igh* locus both in the presence or absence of locus contraction. The question therefore arises as to what signals or factors allow this complex as well as the ubiquitous YY1 protein to participate in long-range interactions only in pro-B cells. Although the B-cell-specific regulator Pax5 is a prime candidate for this job, the molecular mechanism of Pax5 action is also largely unknown except for the observation that CTCF and Pax5 may interact with each other in vivo. Further insight will be provided by identifying a critical subdomain of Pax5 and the respective interacting proteins that mediate the role of Pax5 in locus contraction. Moreover, the function of PAIR elements in V_H -DJ_H recombination needs to be characterized by mutagenesis of these elements in the endogenous *Igh* locus. When compared with the *Igh* locus, even less is known about *trans*-acting factors or *cis*-regulatory elements that control locus contraction at other antigen receptor loci. Hence, elucidating the molecular mechanism of locus contraction will remain an interesting and challenging task for the future.

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Figure 6. Active chromatin and the control of sequential rearrangements at the *Igh* locus. (A) Distribution of active histone modifications at the *Igh* locus. The presence of H3K4me2, H3K4me3, and H3K9ac was determined by ChIP analysis of *Rag2*^{-/-} pro-B cells combined with paired-end Illumina sequencing. The ChIP-seq data are displayed as peak densities that were calculated by extension of each read by 150 bp and subsequent determination of the number of reads at each position (shown as reads per million [RPM] mapped sequence reads). The histone modification peaks detected at V_H3609 genes, PAIR elements, and other intergenic regions in the V_H gene region are indicated. The *Zfp386* gene located upstream of the *Igh* locus is indicated by red lines in the *Igh* annotation. (B) The recombination center model. In lymphoid progenitors, the E_μ enhancer activates the proximal J_H gene region of the *Igh* locus as a recombination center embedded in active chromatin. The RAG1/2 complex (red) is recruited in the recombination center through binding of the RAG2 PHD finger to the active histone modification H3K4me3 (green). The tethered RAG1/2 complex captures one of the D_H gene segments, leading to D_H – J_H recombination in progenitors followed by the subsequent capture of one of the many V_H gene segments upon *Igh* locus contraction in pro-B cells.

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REFERENCES

- Afshar R, Pierce S, Bolland DJ, Corcoran A, Oltz EM. 2006. Regulation of IgH gene assembly: Role of the intronic enhancer and 5'D_{Q52} region in targeting D_HJ_H recombination. *J Immunol* **176**: 2439–2447.
- Bassing CH, Swat W, Alt FW. 2002. The mechanism and regulation of chromosomal V(D)J recombination. *Cell* **109**: S45–S55.
- Bolland DJ, Wood AL, Johnston CM, Bunting SF, Morgan G, Chakalova L, Fraser PJ, Corcoran AE. 2004. Antisense intergenic transcription in V(D)J recombination. *Nat Immunol* **5**: 630–637.
- Bossen C, Mansson R, Murre C. 2012. Chromatin topology and the regulation of antigen receptor assembly. *Annu Rev Immunol* **30**: 337–356.
- Chakraborty T, Perlot T, Subrahmanyam R, Jani A, Goff PH, Zhang Y, Ivanova I, Alt FW, Sen R. 2009. A 220-nucleotide deletion of the intronic enhancer reveals an epigenetic hierarchy in immunoglobulin heavy chain locus activation. *J Exp Med* **206**: 1019–1027.
- Choi NM, Loguercio S, Verma-Gaur J, Degner SC, Torkamani A, Su AI, Oltz EM, Artyomov M, Feeney AJ. 2013. Deep sequencing of the murine *Igh* repertoire reveals complex regulation of nonrandom V gene rearrangement frequencies. *J Immunol* **191**: 2393–2402.
- Chowdhury D, Sen R. 2001. Stepwise activation of the immunoglobulin μ heavy chain gene locus. *EMBO J* **20**: 6394–6403.
- Degner SC, Verma-Gaur J, Wong TP, Bossen C, Iverson GM, Torkamani A, Vettermann C, Lin YC, Ju Z, Schulz D, et al. 2011. CCCTC-binding factor (CTCF) and cohesin influence the genomic architecture of the *Igh* locus and antisense transcription in pro-B cells. *Proc Natl Acad Sci* **108**: 9566–9571.
- Deniaud E, Bickmore WA. 2009. Transcription and the nuclear periphery: Edge of darkness? *Curr Opin Genet Dev* **19**: 187–191.
- Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B. 2012. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* **485**: 376–380.
- Donohoe ME, Zhang LF, Xu N, Shi Y, Lee JT. 2007. Identification of a Ctf cofactor, Yy1, for the X chromosome binary switch. *Mol Cell* **25**: 43–56.
- Ebert A, McManus S, Tagoh H, Medvedovic J, Salvagiotto G, Novatchkova M, Tamir I, Sommer A, Jaritz M, Busslinger M. 2011. The distal V_H gene cluster of the *Igh* locus contains distinct regulatory elements with Pax5 transcription factor-dependent activity in pro-B cells. *Immunity* **34**: 175–187.
- Fuxa M, Skok J, Souabni A, Salvagiotto G, Roldán E, Busslinger M. 2004. Pax5 induces V-to-DJ rearrangements and locus contraction of the immunoglobulin heavy-chain gene. *Genes Dev* **18**: 411–422.
- Guo C, Gerasimova T, Hao H, Ivanova I, Chakraborty T, Selimyan R, Oltz EM, Sen R. 2011a. Two forms of loops generate the chromatin conformation of the immunoglobulin heavy-chain gene locus. *Cell* **147**: 332–343.
- Guo C, Yoon HS, Franklin A, Jain S, Ebert A, Cheng HL, Hansen E, Despo O, Bossen C, Vettermann C, et al. 2011b. CTCF-binding elements mediate control of V(D)J recombination. *Nature* **477**: 424–430.
- Hawwari A, Krangel MS. 2005. Regulation of TCR δ and α repertoires by local and long-distance control of variable gene segment chromatin structure. *J Exp Med* **202**: 467–472.
- Hesslein DGT, Pflugh DL, Chowdhury D, Bothwell ALM, Sen R, Schatz DG. 2003. Pax5 is required for recombination of transcribed, acetylated, 5' IgH V gene segments. *Genes Dev* **17**: 37–42.
- Hewitt SL, Farmer D, Marszałek K, Cadera E, Liang HE, Xu Y, Schlissel MS, Skok JA. 2008. Association between the *Igk* and *Igh* immunoglobulin loci mediated by the 3' *Igk* enhancer induces 'decontraction' of the *Igh* locus in pre-B cells. *Nat Immunol* **9**: 396–404.
- Igarashi H, Gregory SC, Yokota T, Sakaguchi N, Kincade PW. 2002. Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. *Immunity* **17**: 117–130.
- Jankovic M, Casellas R, Yannoutsos N, Wardemann H, Nussenzweig MC. 2004. RAGs and regulation of autoantibodies. *Annu Rev Immunol* **22**: 485–501.
- Jhunjhunwala S, van Zelm MC, Peak MM, Cutchin S, Riblet R, van Dongen JJM, Grosveld FG, Knoch TA, Murre C. 2008. The 3D structure of the immunoglobulin heavy-chain locus: Implications for long-range genomic interactions. *Cell* **133**: 265–279.
- Ji Y, Resch W, Corbett E, Yamane A, Casellas R, Schatz DG. 2010. The in vivo pattern of binding of RAG1 and RAG2 to antigen receptor loci. *Cell* **141**: 419–431.
- Johnston CM, Wood AL, Bolland DJ, Corcoran AE. 2006. Complete sequence assembly and characterization of the C57BL/6 mouse Ig heavy chain V region. *J Immunol* **176**: 4221–4234.
- Kosak ST, Skok JA, Medina KL, Riblet R, Le Beau MM, Fisher AG, Singh H. 2002. Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. *Science* **296**: 158–162.
- Kwon J, Morshead KB, Guyon JR, Kingston RE, Oettinger MA. 2000. Histone acetylation and hSWI/SNF remodeling act in concert to stimulate V(D)J cleavage of nucleosomal DNA. *Mol Cell* **6**: 1037–1048.
- Lin YC, Benner C, Mansson R, Heinz S, Miyazaki K, Miyazaki M, Chandra V, Bossen C, Glass CK, Murre C. 2012. Global changes in the nuclear positioning of genes and intra- and interdomain genomic interactions that orchestrate B cell fate. *Nat Immunol* **13**: 1196–1204.
- Liu Z, Widlak P, Zou Y, Xiao F, Oh M, Li S, Chang MY, Shay JW, Garrard WT. 2006. A recombination silencer that specifies heterochromatin positioning and Ikaros association in the immunoglobulin κ locus. *Immunity* **24**: 405–415.
- Liu H, Schmidt-Suppran M, Shi Y, Hobeika E, Barteneva N, Jumaa H, Pelanda R, Reth M, Skok J, Rajewsky K, et al. 2007. Yin Yang 1 is a critical regulator of B-cell development. *Genes Dev* **21**: 1179–1189.
- Maes J, O'Neill LP, Cavelier P, Turner BM, Rougeon F, Goodhardt M. 2001. Chromatin remodeling at the Ig loci prior to V(D)J recombination. *J Immunol* **167**: 866–874.
- Malin S, McManus S, Cobaleda C, Novatchkova M, Delogu A, Bouillet P, Strasser A, Busslinger M. 2010. Role of STAT5 in controlling cell survival and immunoglobulin gene recombination during pro-B cell development. *Nat Immunol* **11**: 171–179.
- Mathieu N, Hempel WM, Spicuglia S, Verthuy C, Ferrier P. 2000. Chromatin remodeling by the T cell receptor (TCR)- β gene enhancer during early T cell development: Implications for the control of TCR- β locus recombination. *J Exp Med* **192**: 625–636.
- Mathieu N, Spicuglia S, Gorbach S, Cabaud O, Fernex C, Verthuy C, Hempel WM, Hueber A-O, Ferrier P. 2003. Assessing the role of the T cell receptor β gene enhancer in regulating coding joint formation during V(D)J recombination. *J Biol Chem* **278**: 18101–18109.
- Matthews AG, Kuo AJ, Ramón-Maiques S, Han S, Champagne KS, Ivanov D, Gallardo M, Carney D, Cheung P, Ciccone DN, et al. 2007. RAG2 PHD finger couples histone H3 lysine 4 trimethylation with V(D)J recombination. *Nature* **450**: 1106–1110.

- McMurry MT, Krangel MS. 2000. A role for histone acetylation in the developmental regulation of V(D)J recombination. *Science* **287**: 495–498.
- Medvedovic J, Ebert A, Tagoh H, Tamir IM, Schwickert TA, Novatchkova M, Sun Q, Huis in't Veld PJ, Guo C, Yoon HS, et al. 2013. Flexible long-range loops in the V_H gene region of the *Igh* locus facilitate the generation of a diverse antibody repertoire. *Immunity* **39**: 229–244.
- Melchers F. 2005. The pre-B-cell receptor: Selector of fitting immunoglobulin heavy chains for the B-cell repertoire. *Nat Rev Immunol* **5**: 578–584.
- Merkenschlager M, Odom DT. 2013. CTCF and cohesin: Linking gene regulatory elements with their targets. *Cell* **152**: 1285–1297.
- Mostoslavsky R, Singh N, Kirillov A, Pelanda R, Cedar H, Chess A, Bergman Y. 1998. κ chain monoallelic demethylation and the establishment of allelic exclusion. *Genes Dev* **12**: 1801–1811.
- Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, Piolot T, van Berkum NL, Meisig J, Sedat J, et al. 2012. Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* **485**: 381–385.
- Nutt SL, Urbánek P, Rolink A, Busslinger M. 1997. Essential functions of Pax5 (BSAP) in pro-B cell development: Difference between fetal and adult B lymphopoiesis and reduced *V*-to-*DJ* recombination at the *IgH* locus. *Genes Dev* **11**: 476–491.
- Perlot T, Alt FW. 2008. *Cis*-regulatory elements and epigenetic changes control genomic rearrangements of the *IgH* locus. *Adv Immunol* **99**: 1–32.
- Perlot T, Alt FW, Bassing CH, Suh H, Pinaud E. 2005. Elucidation of IgH intronic enhancer functions via germ-line deletion. *Proc Natl Acad Sci* **102**: 14362–14367.
- Phillips JE, Corces VG. 2009. CTCF: Master weaver of the genome. *Cell* **137**: 1194–1211.
- Revilla-i-Domingo R, Bilic I, Vilagos B, Tagoh H, Ebert A, Tamir IM, Smeenk L, Trupke J, Sommer A, Jaritz M, et al. 2012. The B-cell identity factor Pax5 regulates distinct transcriptional programmes in early and late B lymphopoiesis. *EMBO J* **31**: 3130–3146.
- Ribeiro de Almeida C, Stadhouders R, de Bruijn MJ, Bergen IM, Thongjuea S, Lenhard B, van Ijcken W, Grosveld F, Galjart N, Soler E, et al. 2011. The DNA-binding protein CTCF limits proximal V κ recombination and restricts κ enhancer interactions to the immunoglobulin κ light chain locus. *Immunity* **35**: 501–513.
- Roldán E, Fuxa M, Chong W, Martinez D, Novatchkova M, Busslinger M, Skok JA. 2005. Locus ‘decontraction’ and centromeric recruitment contribute to allelic exclusion of the immunoglobulin heavy-chain gene. *Nat Immunol* **6**: 31–41.
- Sayegh C, Jhunjhunwala S, Riblet R, Murre C. 2005. Visualization of looping involving the immunoglobulin heavy-chain locus in developing B cells. *Genes Dev* **19**: 322–327.
- Seitan VC, Hao B, Tachibana-Konwalski K, Lavagnoli T, Mira-Bontenbal H, Brown KE, Teng G, Carroll T, Terry A, Horan K, et al. 2011. A role for cohesin in T-cell-receptor rearrangement and thymocyte differentiation. *Nature* **476**: 467–471.
- Shih H-Y, Verma-Gaur J, Torkamani A, Feeney AJ, Galjart N, Krangel MS. 2012. *Tcr* gene recombination is supported by a *Tcr* enhancer- and CTCF-dependent chromatin hub. *Proc Natl Acad Sci* **109**: E3493–E3502.
- Skok JA, Gisler R, Novatchkova M, Farmer D, de Laat W, Busslinger M. 2007. Reversible contraction by looping of the *Tcrb* and *Tcr* loci in rearranging thymocytes. *Nat Immunol* **8**: 378–387.
- Tripathi R, Jackson A, Krangel MS. 2002. A change in the structure of V β chromatin associated with TCR β allelic exclusion. *J Immunol* **168**: 2316–2324.
- van de Werken HJ, de Vree PJ, Splinter E, Holwerda SJ, Klous P, de Wit E, de Laat W. 2012. 4C technology: Protocols and data analysis. *Methods Enzymol* **513**: 89–112.
- Verma-Gaur J, Torkamani A, Schaffer L, Head SR, Schork NJ, Feeney AJ. 2012. Noncoding transcription within the *Igh* distal V_H region at PAIR elements affects the 3D structure of the *Igh* locus in pro-B cells. *Proc Natl Acad Sci* **109**: 17004–17009.
- Xiang Y, Zhou X, Hewitt SL, Skok JA, Garrard WT. 2011. A multifunctional element in the mouse *Igk* locus that specifies repertoire and Ig loci subnuclear location. *J Immunol* **186**: 5356–5366.
- Yancopoulos GD, Alt FW. 1985. Developmentally controlled and tissue-specific expression of unrearranged V_H gene segments. *Cell* **40**: 271–281.

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