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4C-ing the *Igh*landscape

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Abstract

The assembly of antigen receptors in developing B-lymphocytes is determined by the spatio-temporal organization of the immunoglobulin heavy chain locus (*Igh*). In this issue of *Immunity*, Medvedovic*et al.* (2013) provide a comprehensive dynamic view of the *Igh* locus architecture.

The ability of B cellsto recognize an almost infinite array of antigens relies on V(D)J recombination, a process that assembles the variable region genes of antigen receptors (or antibodies) from variable (V), diversity (D) and joining (J) gene segments. V(D)J recombination requires RAG1 and RAG2 endonucleases, which cleave DNA at recognition sites flanking the V, D and J gene segments, and the non-homologous end-joining machinery that ligates the broken DNA ends. The mouse*IgH* locus, which encodes the heavy chain of antibody molecules, spans 3Mbp in length and is comprised of approximately 200 V_H genes dispersed over a 2.5Mbp region divided into distal, central and proximal segments. Downstream of the V_H regions are 13-16 D_H and 4 J_H gene segments, as well as 8 C_H regions that encode the *Igh*constant regions of the various antibody isotypes(Figure 1) (Perlot and Alt, 2008).

In developing pro-B cells, a D_H gene segment first recombines with a J_H segment to form a DJ_H junction; subsequent V_H to DJ_H recombination assembles a complete $V_H(D)J_H$ allele.A salient feature of V(D)J recombination is the unbiased representation of the distal and proximal V_H segments in the overall antigen receptor repertoire (Perlot and Alt, 2008). This leads to the central question: what is the mechanism by which each of the ∼200 V_H gene segments scattered over a 2.5Mb region have an equal opportunity to establish contact and recombine with the DJ_H element? Elegant studies employing fluorescence *in situ* hybridization (FISH) (Fuxa et al., 2004; Kosak et al., 2002; Roldan et al., 2005), combined with painstaking measurement of different contact points throughout the *Igh*locus using 3D-FISH and trilateration (Jhunjhunwala et al., 2008)demonstrated that V_H to DJ_H recombination proceeds via large-scale locus contraction occurring through chromatin looping, thus juxtaposing distal V_H gene segments close to the DJ_H element. Several transcription factors, including Pax5, Ezh2, YY1, Ikaros and the CCCTC-binding factor

CTCF, interact with various *cis*- regulatory elements in the *Igh*locus to mediate locus contraction (Perlot and Alt, 2008).

The *Igh*locus contains four well-characterized regulatory elements (Figure 1). (i) 3′RR: Positioned downstream of the Cα gene, the 3′ *r*egulatory *r*egionis comprised of two distinct modules of DNaseI hypersensitivity (hs) sites- hs3A-hs1.2-hs3B-hs4, and hs5-8 containing a high density of CTCF binding elements (CBEs) interspersed with Pax5-binding sites. (ii) Eμ: Located in the J_H-C_H intron, Em is required for efficient V_H to DJ_H joining. (iii) PAIR elements: The *P*AX5-*a*ctivated *i*ntergenic *r*epeat elements are interspersed in the distal V_H cluster, and contain binding sites for Pax5 and CTCF, and promote Pax5-dependent anti-sense transcription through the*Igh*locus in pro-B cells (Ebert et al., 2011). (iv) IGCR1: The *i*nter*g*enic *c*ontrol region is located between the V_H and D clusters containing two CBEs. The CBEs in IGCR1 act as insulator elements to V(D)J recombination at the *Igh*locus (Guo et al., 2011b).

The DNA-FISH methodology employed thus far has provided an overall idea of the topological architecture of the *Igh*locus. However, these studies are of low resolution and do not provide a detailed map of the precise contact points for the myriad long- and short-range interactions that effect locus contraction. To establish a comprehensive map of the interaction domains in the *Igh*locus poised to undergo recombination, Medvedovic et al., (2013)used chromosome conformation capture sequencing methodology (4C-seq) that allows unbiased identification of genome-wide interaction partners using defined bait elements or “viewpoints”. In aremarkable*tour de force*effort, Medvedovic *et al* used 16 distinct viewpoints spanning the entire *Igh*locus to compare 3D-chromatin topology between *Rag2*$^{-/-}$ and *Pax5*$^{-/-}$ *Rag2*$^{-/-}$ pro-B cells. Rag2 deficiency “freezes” B cell development at the pro-B stage and thus provides a snapshot of the Pax5-dependent interactions at the *IgH* locus poised to undergo V(D)J recombination.

The 4C-seq data generated by Medvedovic*et al*revealed several novel and interesting aspects of *Igh*topology that could not be gleaned from the 3D-FISH analysis. First, in pro B-cells, the majority of long-range *IgH* chromosomal interactions occurred specifically within the *Igh*locus, with the region around the 5′ V_HJ558 gene acting as the 5′ boundary and the 3′CBE in the hs5-8 module serving as the 3′ limit of the *Igh*locus interactions.

Second, even though the regulatory elements 3′RR, Eμ and IGCBR1 form multiple loops across the entire *Igh*locus, individual mutations of each of these elements had no effect on locus contraction of the V_H genes, leaving open the possibilities that there are either redundant interaction domains and/or the interactions in pro-B cells reflect those that can occur in later stages of B cell differentiation, for example during *Igh*class switch recombination (CSR). It is to be noted that the results here differ from a recent report demonstrating the requirement of the Eμ enhancer in *Igh*locus contraction using 3D-FISH analyses(Guo et al., 2011a); the reasons behind this discrepancy is not clear at present. Third, local chromatin loops ranging in length from 0.5 Mbp to 1.3 Mbp were observed not only in the absence of Pax5 but also in thymocytes, indicating that these interactions represent the default folding state of the *Igh*locus that is largely invariant between different cell types, reminiscent of the topological architecture of the entire mouse chromosome.

Fourth, a region between Cγ1 and Cγ2b was found to interact with IGCR1, Eμ, and the 3′RR elements. This novel Cγ1- Cγ2b interaction domain contains two Pax5-dependent DNaseI hypersensitivity sites (DHS) in pro-B cells. Surprisingly, these loops occur even in the absence of both DHS sites in Pax5-deficient pro-B cells. Gene targeting to assess the potential role of this region in regulating loop formation during V(D)J recombination or class switch recombination will be of significant interest. Finally, 4C-seq data obtained with multiple viewpoints across the entire V_H cluster in pro-B cells showed that long-range interaction with the V_H segments occur in a Pax5-dependent fashion. Significantly, all the viewpoints revealed a continuum of flexible long-range interactions across the entire V_H cluster, suggesting that each of the V_H genes has a similar probability of being juxtaposed proximal to the rearranged D_HJ_H element during V_H-DJ_H recombination.

In addition to generating a detailed topological map of the *Igh*interaction domains, Medvedovic*et al*elucidated mechanisms by which transcription factors potentially regulate long-range chromatin interactions. Biochemical experiments demonstrated interaction between CTCF and Pax5, suggesting that this association could be relevant for the dynamics of the *Igh*locus. This is consistent with the 4C-seq data demonstrating that targeted mutation of CBE elements in IGCR1 abrogated Pax5-dependent interaction between IGCR1 and distal V_H gene segments. Medvedovic*et al* also investigated the mechanism by which the transcription factor YY1 promotes Igh locus contraction. While YY1 could potentially mediate chromosomal interactions through binding sites scattered throughout the *IgH*locus, antisense transcripts originating from PAIRelements were found to be severely reduced in YY1-deleted pro-B cells, suggesting that YY1 might control long-range interactions by promoting anti-sense transcription.

In summary, the study by Medvedovic*et al* not only confirmed genomic interactions predicted from low-resolution 3D-FISH but also identified novel *Igh*elements that potentially regulate V(D)J recombination. Furthermore, they provided a mechanistic explanation of how the entire complement of V_H gene segments could be presented to the DJ_H element for recombination *en route* to the generation of an unbiased B cell repertoire. The study also establishes 4C-seq as a *bona fide* and convenient method to probe local and long-range chromosomal interactions in other processes such as CSR.

Results presented by Medvedovic*et al*lead to additional questions. First, in pro-B cells, the *Igh*alleles relocate from repressive heterochromatic regions at the nuclear periphery to the center of the nucleus and this movement has been linked to recombination of distal V_H genes (Fuxa et al., 2004; Kosak et al., 2002; Roldan et al., 2005). Do locus contraction and nuclear repositioning influence each other or are they independent processes? Second, in pro-B cells, antisense non-coding RNAs traverse across the entire D_H-J_H region prior to their rearrangement, following which biallelic antisense transcription is initiated across the entire V_H gene cluster (Perlot and Alt, 2008). How does anti-sense transcription modulate locus contraction? Does it alter the three dimensional structure of distinct chromatin territories to promote localized accessibility, or do anti-sense transcripts act as molecular scaffolds to recruit factors required for chromatin looping?Third, both *Igh*alleles undergo homologous pairing in pro-B cells to ensure that V_H to DJ_H recombination occurs on only one *Igh*allele at a time to effect allelic exclusion (Hewitt et al., 2009). Is there cross-talk

between chromosomal loops on individual *Igh*alleles to "read" the recombination status of the complement? Finally, the study by Medvedovic*et al* was performed in RAG-deficient B cells. However, RAG1and RAG2bind to active chromatin and localize to the J_H segments at the 3′ end of each antigen receptor loci (Perlot and Alt, 2008). How does the topology of the *Igh*locus change in the presence of the RAG proteins and following introduction of DNA breaks? These unanswered questions will constitute the next phase of investigation in this complex recombination reaction.

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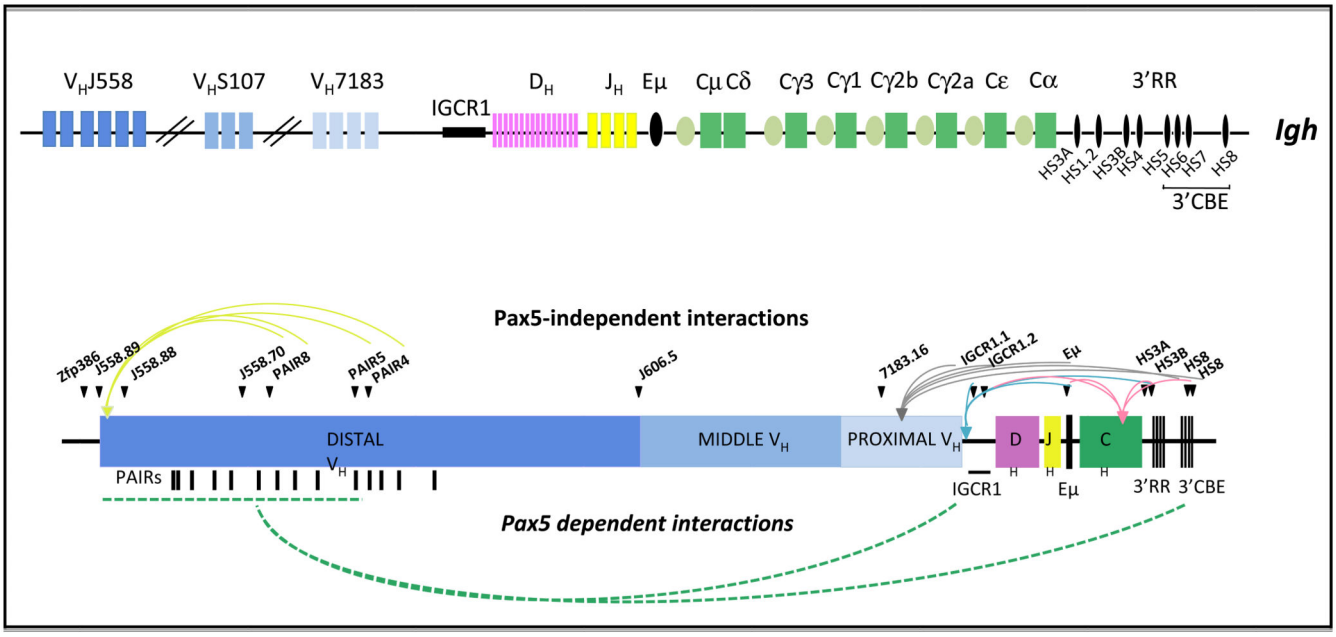

Fig 1. Dynamic architecture of the IgH locus

Top. Schematic representation of the *Igh*locus showing Variable (V), Diversity (D), Joining gene segments (J), Constant regions (C) and the regulatory elements 3′RR, Eμ, IGCR1 and PAIR elements. The CTCF-binding sequence (3′CBE) in the 3′RR region are shown.

Bottom.Interactions in the absence or presence of Pax5. Green, grey, pink and blue arrows represent local interactions between the pointed sequences and the corresponding viewpoints. Broken green arrows represent dynamic long-range interactions. Distal V_H genes interact mainly with CTCF-binding sites in IGCR1 and 3′CBE. Black triangles indicate the relative position of the 16 viewpoints that were employed for 4C-seq analyses.