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Chromatin architecture, CTCF and V(D)J recombination: managing long-distance relationships at antigen receptor loci[1]

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Abstract

The rearrangement of T and B lymphocyte antigen receptor loci occurs within a highly complex chromosomal environment and is orchestrated through complex mechanisms. Over the past decade, a large body of literature has highlighted the significance of chromatin architecture at antigen receptor loci in supporting the genomic assembly process: in preparation for recombination, these loci tend to contract and form multiple loops that shorten the distances between gene segments and facilitate recombination events. A CCCTC binding factor, CTCF, has received much attention in this regard since it has emerged as an important regulator of chromatin organization and transcription. In this review, we will summarize recent work outlining conformational dynamics at antigen receptor loci during lymphocyte development and we will discuss the role of CTCF in antigen receptor locus conformation and repertoire development.

Introduction

Adaptive immunity in jawed vertebrates is mediated by T and B lymphocytes that express highly diverse and clonally distributed antigen receptors. The diversity of lymphocyte antigen receptors is generated primarily by the assembly of variable (V), diversity (D) and joining (J) gene segments at T cell receptor (TCR) and immunoglobulin (Ig) loci (1). This process, known as V(D)J recombination, is catalyzed by the recombination-activating gene-1 and -2 proteins (RAG-1 and RAG-2, hereafter referred to as RAG). The RAG proteins recognize recombination signal sequences (RSSs)[3] that flank all V, D and J gene segments, and with two RSSs held in a synaptic complex, create double-strand breaks that can be rejoined to assemble V, D and J gene segments with tremendous combinatorial diversity. Antigen receptor loci undergo recombination in a manner that is regulated according to cell lineage and developmental stage in T and B lymphocyte precursors (2, 3). During T lymphocyte development in the thymus, *Tcrb*, *Tcrd* and *Tcrg* genes recombine at the $CD4^{-}CD8^{-}$ double negative (DN) stage, whereas *Tcra* genes recombine at the $CD4^{+}CD8^{+}$ double positive (DP) stage. B lymphocyte development in the bone marrow is similarly characterized by developmentally-staged recombination of Ig heavy (*Igh*) and light (*Igk* and *Igl*) chain genes, with *Igh* recombination in pro-B cells and *Igk* and *Igl* recombination in pre-B cells. Recombination events are also regulated within individual loci; for example, D-to-J recombination precedes V-to-DJ recombination at both *Igh* and

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[3]Abbreviations used in this paper: 3C, chromosome conformation capture; 3′CBE, 3′ CTCF binding element; 3D-FISH, three-dimensional fluorescence *in situ* hybridization; 3′RR, 3′ regulatory region; Cer, contracting element for recombination; CTCF, CCCTC binding factor; DN, double negative; DP, double positive; E_{α}, *Tcra* enhancer; E_{μ}, intronic *Igh* enhancer; iE_{κ}, intronic *Igk* enhancer; IGCR1, intergenic control region 1; PAIR, Pax5 activated intergenic repeat; RSS, recombination signal sequence; Sis, silencer in the intervening sequence; TEA, T early α; YY1, Yin-Yang 1

Tcrb, and proximal V-to-J recombination precedes distal V-to-J recombination at *Tcra* (2, 3).

The V(D)J recombination programs at TCR and Ig loci are controlled at multiple levels (3–5). Most fundamental is the restriction imposed by RAG protein expression, which, except in special circumstances (6), is limited to developing pro- and pre-B cells and DN and DP thymocytes (7). Beyond that, the individual antigen receptor loci carry complex arrays of *cis*-regulatory elements, including enhancers and promoters, that dictate regional changes to transcription and chromatin structure that, in turn, allow RAG proteins to access particular RSSs at the appropriate developmental stage (3–5). In many instances, functional communication between enhancers and promoters must occur over long distances across a landscape that includes many such elements, raising questions as to how these functional interactions are targeted and regulated (8). Most recently, the spatial dynamics of antigen receptor loci within immature lymphocyte nuclei has also emerged as a critical aspect of their regulation (4, 9, 10). The seminal observation relied on three dimensional fluorescence *in situ* hybridization (3D-FISH) to visualize immunoglobulin loci in cell nuclei: *Igh* and *Igk* moved away from the nuclear periphery and the two ends of the *Igh* locus were less separated in nuclei of pro-B cells as compared to other cells (11). Subsequent studies revealed “contraction” to be a general property of antigen receptor loci that occurs during the developmental stage in which V segments undergo recombination, with “decontraction” occurring subsequently (12–16). Because contraction and decontraction can be detected even in recombinase-deficient nuclei, they are thought to set the stage for and to terminate long-distance recombination events, respectively, during lymphocyte development. Since RAG proteins bind preferentially to J or D-J clusters to form “recombination centers”, contraction and decontraction would move distant V gene segments into or out of these recombination centers to allow regulated assembly of a V gene repertoire (4).

Chromatin architecture, CTCF and cohesin

Eukaryotic genomes are packaged at multiple levels to solve a critical “space” problem in the nucleus while simultaneously facilitating transcription and replication of the DNA template (17). Recent technological advances in the analysis of genomic spatial relationships have allowed insights into the principles of higher-order chromatin organization (18). These technologies are all derivative of the chromosome conformation capture technique (3C), in which interacting DNA fragments are initially trapped by chemical crosslinking; following a restriction enzyme digest and addition of DNA ligase under conditions favoring intramolecular ligation, any two interacting DNA fragments can then be identified and quantified by PCR using oligonucleotide primers specific for the two fragments (19). In a version of this technology known as 4C, circular DNA resulting from ligation of both ends of two interacting fragments is amplified by inverse PCR using two primers in a “bait” or “viewpoint” sequence, and the entire universe of sequences interacting with the bait is determined by microarray or deep sequencing (20, 21). In a further adaptation of this technology known as Hi-C, there is no defined “bait”; rather, deep sequencing is used to identify the entire genomic universe of interacting sequences (22). Over the last several years, 4C and Hi-C analysis has revealed segregation of the genome into discrete spatial compartments of up to a few megabases in length that correspond to domains of active or inactive chromatin. Long-distance interactions are more frequent within a domain but also occur between domains, with active chromatin domains interacting more frequently with other active domains, and inactive chromatin domains interacting more frequently with other inactive domains (20, 22–24). The data support a “fractal globule” model of the genome in which locally looped and packaged globular units are then further assembled to package the entire genome.

CTCF (CCCTC-binding factor) is a highly conserved, ubiquitously expressed transcription factor that binds a GC-rich consensus sequence (25). CTCF has been attributed multiple functions, including transcriptional activation, transcriptional repression and transcriptional insulation (the ability to block an enhancer from activating a promoter when CTCF is situated between the two elements). In addition, CTCF has been shown to function as a chromatin organizer that mediates long-distance looping interactions in the genome (25). The ability of CTCF to mediate such loops may underlie some or all of the above activities; CTCF-mediated loops could activate transcription by bringing together enhancer and promoter elements that are associated with nearby CTCF sites, or could insulate or suppress transcription by segregating enhancers and promoters that have intervening CTCF sites. Indeed, there are tens of thousands of CTCF binding sites distributed in a range of contexts across mammalian genomes (26–30). As might be expected for a role in insulation, the boundaries between topologically and functionally defined chromatin domains are highly enriched for CTCF sites. However, only 15% of all CTCF sites are located at these boundaries (24); other CTCF sites are distributed at enhancers, promoters, and other genic and intergenic sites and mediate interactions between these sites (24, 30–32). Recent analysis of the pro-B cell genome indicated that long-distance interactions within chromatin spatial compartments were associated with CTCF binding, whereas those between spatial compartments were associated with the binding of lineage-specific transcription factors (23).

Cohesin is a multi-subunit protein complex that is well-known for its role in mediating sister-chromatid cohesion during cell division; it is thought to function by forming a closed ring around the two newly replicated DNA double strands (33). Cohesin is now also appreciated to play important roles in gene expression and chromatin organization in interphase cells: notably, it binds in a CTCF-dependent fashion to about 70% of CTCF binding sites genome-wide (34–36) and is necessary for CTCF-dependent insulation and looping (37–39). Although in this manner CTCF and cohesin function together at many sites in the genome, they can also interact with other transcription factors and can function independent of each other (40, 41).

ChIP-chip and ChIP-Seq analyses have revealed antigen receptor loci to be particularly enriched in binding sites for CTCF and cohesin (26, 42–47). As a result, both proteins have drawn attention as potential regulators of locus conformation, transcriptional activity and V(D)J recombination. We focus below on recent studies of the *Igh*, *Igk* and *Tcra/Tcrd* loci. Much less is known about *Tcrb* locus architecture and there have been no studies of the *Tcrg* and *Igl* loci.

The studies of locus architecture and transcription discussed below have generally been conducted using immature cell populations isolated from recombinase-deficient mice, because this eliminates the confounding effects of changes in spatial relationships that are a consequence of V(D)J recombination. That said, it is an assumption that the RAG proteins themselves would not substantially impact the parameters being measured; this could be addressed by analyzing cells isolated from mice expressing a catalytically inactive RAG protein complex (48).

Igh locus

The murine *Igh* locus on chromosome 12 contains nearly 200 V_H gene segments spanning 2.7 Mb of DNA, followed by 10–13 D_H, four J_H, and eight C_H gene segments (2) (Fig. 1A). *Igh* locus V(D)J recombination occurs in a strictly ordered fashion, with D_H-to-J_H recombination preceding V_H-to-D_HJ_H recombination in pro-B cells. The V_H-to-D_HJ_H step is further regulated to support allelic exclusion, as it is terminated as a consequence of feedback inhibition in pre-B cells. Notably, each *Igh* allele generally undergoes V_H-to-D_HJ_H

recombination only once, since this rearrangement eliminates all unrearranged D_H gene segments. Therefore, although several D_H-proximal V_H gene segments do rearrange at elevated frequencies, D_H-distal V_H gene segments must be able to compete effectively with these proximal V_H gene segments to allow the assembly of a diverse *Igh* repertoire.

3D-FISH data have shown that the *Igh* locus contracts specifically in pro-B cells (11, 12, 14, 49) and that this conformation brings all V_H gene segments into proximity of the D_HJ_H cluster (13). Spatial-distance measurements and computer modeling suggested that in its extended conformation in pre-pro B cells, the *Igh* locus is organized into at least three rosette-like compartments, each composed of multiple DNA loops, and that these compartments merge in pro-B cells (13). Unrearranged *Igh* loci then appear to decontract in pre-B cells, correlating with the suppression of V_H-to-D_H recombination associated with allelic exclusion (14). Molecular mechanisms of *Igh* contraction and decontraction are only partly understood. Transcription factors Pax5, Yin-Yang 1 (YY1) and Ikaros have been shown to be essential for complete *Igh* locus contraction as well as normal frequencies of distal V_H rearrangement (12, 50, 51). Since distal V_H segments appear to be "accessible" in Pax5- or YY1-deficient pro-B cells based on their germline transcription and histone modifications, it has been inferred that distal V_H-to-D_HJ_H rearrangement relies on *Igh* locus contraction (12, 50). Elimination of the *Igh* intronic enhancer (E_μ), located between J_H and C_μ segments, also causes a loss of *Igh* contraction (52). YY1 may directly regulate *Igh* contraction by binding to E_μ (50, 52) and to other sites in the V_H array (53). However, it may also influence *Igh* contraction indirectly by regulating the expression of Pax5 (50, 53). *Igh* locus contraction was also reduced by CTCF knockdown, but the effect was rather modest (54). The data suggest that multiple factors cooperate to shape the contracted configuration of the *Igh* locus in pro-B cells.

The *Igh* locus contains 85 or more CTCF sites, the majority of which are distributed across the V_H region (43, 54) (Fig. 1A). CTCF sites in the proximal portion of the V_H array are located immediately downstream of V_H RSSs, whereas those in the distal portion are intergenic (42, 44). Included among the latter are CTCF sites at a series of 14 homologous Pax5-activated intergenic repeat (PAIR) elements, some of which express Pax5-dependent and pro-B cell-specific antisense transcripts (43, 53, 54). Only a few CTCF binding sites are found outside of the V_H region. Two are located ~3.2 and 5.6 kb upstream of DFL16.1, the most 5′ D_H gene segment, in a region called 5′DFL16 or intergenic control region 1 (IGCR1); nine others are densely arrayed in an area called the 3′ CTCF-binding element (3′CBE) that flanks the 3′ regulatory region (3′ RR) downstream of the C_H gene segments (42, 54–56). The majority of *Igh* locus CTCF sites also bind cohesin (54). Notably, neither CTCF nor cohesin bind to E_μ (52, 54).

Long-distance DNA interactions across the *Igh* locus have recently been probed at high resolution by 3C and 4C (52–54, 56) (Fig. 1A). These studies have described a 300 kb, multiple-loop structural domain at the 3′ end of the *Igh* locus, defined by interactions among IGCR1, E_μ, 3′RR, and 3′CBE, that are present in both pre-pro-B cells and pro-B cells. The interaction between IGCR1 and 3′CBE is CTCF- and cohesin-dependent (54), whereas E_μ interactions with IGCR1 and 3′CBE are CTCF- and cohesin-independent (52, 54). E_μ interactions with these sites may be mediated by YY1, since this factor binds not only to E_μ, but in one study was found to bind to IGCR1 and 3′CBE as well (52). Further 4C analyses conducted using either a proximal V_H site or a distal V_H site as bait described two additional multiple-loop regions, each spanning several hundred kilobases. Most, but not all, interacting sequences bound CTCF; however, the CTCF-dependency of these interactions requires further study (52). Because the two multiple-loop regions do not interact with each other, they were hypothesized to reside in distinct structural domains (52). Nevertheless,

more comprehensive studies of long-distance interactions will be needed for a better picture of *Igh* domain structure in pre-pro- and pro-B cells.

In addition to the relatively local interactions described above, E_μ was shown to interact with two distant sites, one in the proximal portion of the V_H array (near the 5′ end of the V_H7183 gene segments) and one in the distal portion of the V_H array (near the 3′ end of the V_HJ558 gene segments) (Fig. 1A). These long-distance interactions are E_μ-dependent and may also involve YY1, since YY1 binding was detected at both sites (52). It was also recently shown that E_μ interacts across 1.8–2.0 Mb with two PAIR elements (PAIR4 and PAIR6) that are major sites of intergenic antisense transcription in the distal portion of the V_H array (53). These long-distance interactions are pro-B cell-specific and YY1-dependent; hence, they correlate with, and may reflect, *Igh* locus contraction. Notably, long-distance E_μ-PAIR interactions do not reflect a functional role for E_μ in PAIR activation, since PAIR antisense transcription is E_μ-independent. Nevertheless, PAIR transcription, like *Igh* contraction, is YY1-dependent (53). Therefore, it was suggested that PAIR and E_μ elements might interact because they are independently recruited into the same transcription factory and that this co-recruitment may be the basis for *Igh* locus contraction (44, 53, 57). Consistent with this, knockdown of CTCF in pro-B cells caused increased PAIR transcription and increased PAIR-E_μ interactions (53, 54). However, this observation is inconsistent with the notion that CTCF promotes *Igh* locus contraction; the modest *Igh* conformational change detected by 3D-FISH in CTCF knockdown pro-B cells (54) might reflect more local changes in loop organization within *Igh* domains, rather than decontraction per se.

How do changes in CTCF-mediated loop structures impact *Igh* recombination? Deletion of the 4kb IGCR1 region or mutation of its two CTCF binding sites led to dramatic dysregulation of the V_H repertoire, with substantially elevated transcription and recombination of the most D_H-proximal V_H gene segments (56) (Fig. 1A). Remarkably, proximal V_H gene segments were also found to rearrange to D_H gene segments prior to D_H-J_H joining and to D_H or D_HJ_H segments even in thymocytes, and they were not subject to feedback inhibition from productively assembled *Igh* alleles (56). Deletion of the IGCR1 region also disrupted the IGCR1-3′CBE loop, as well as E_μ interactions with both elements. However, since the functional dysregulation requires only mutation of the IGCR1 CTCF binding sites, it may specifically reflect the loss of the CTCF-dependent IGCR1-3′CBE loop (54, 56). The data suggest that CTCF plays a critical role in insulating the proximal V_H gene segments from the influence of E_μ, perhaps a direct result of CTCF-mediated looping between the IGCR1 and 3′CBE regions. Consistent with this interpretation, deletion of hs5-7, containing 7 of 9 CTCF sites in the 3′CBE region, partially reduced the IGCR1-3′RR/3′CBE interaction and caused a mild increase in proximal V_H usage (58). Moreover, a dysregulation similar to that documented for IGCR1 deletion was observed for a distal V_H gene segment that was repositioned between IGCR1 and the D_H cluster on an otherwise wild-type *Igh* allele (59).

If IGCR1 functions as an insulator, it might be expected to suppress physical interactions between E_μ and upstream sites in the *Igh* locus. However, it is apparent that E_μ can interact with numerous distant sites in the locus in pro-B cells, even in the presence of IGCR1 and an IGCR1-3′CBE loop (52, 53). Therefore, the dynamics of IGCR1-mediated looping and the basis for its impacts on long-distance DNA contacts, transcriptional activation and V(D)J recombination will be important areas for future study.

A note of caution that applies to the interpretation of all studies involving knockout or knockdown of pleiotropic transcription or architectural proteins is that it is difficult to know whether observed effects are direct (as is often assumed) or indirect. Studies involving

binding site mutations, as in the example of IGCR1, can be particularly revealing in this regard.

Igk locus

Studies of the murine *Igk* locus are yielding a picture rather similar to that of the *Igh* locus. The murine *Igk* locus on chromosome 6 contains 96 functional V_κ gene segments that are distributed across 3.2 Mb and are situated upstream of four J_κ gene segments and C_κ (Fig. 1B). Although *Igk* alleles can undergo secondary V_κ-to-J_κ recombination to replace an initial $V_\kappa J_\kappa$ rearrangement that is out-of-frame or that provokes autoreactivity, opportunities for secondary rearrangements are limited by the small number of J_κ segments. Therefore, *Igk* conformational features should be important to foster the development of a broad V_κ repertoire by providing J_κ-proximal and -distal V_κ gene segments similar opportunities for rearrangement. Based on 3D-FISH, the *Igk* locus was reported to undergo contraction in pre-B cells to facilitate V_κ-to-J_κ recombination at this stage (14). However, a recent Hi-C study documented an extensive, locus-wide network of physical interactions involving V_κ segments and the intronic κ enhancer (iE_κ, situated in the J_κ-C_κ intron) that, although absent in pre-pro-B cells, is apparent in pro-B cells well before the initiation of *Igk* recombination (23). The presence of this interaction network in pro-B cells suggests that *Igk* contraction may occur earlier during B cell development than previously thought.

The *Igk* locus contains approximately 60 sites to which CTCF binds in pre-B cells; binding was found to be lower in pro-B cells (42, 45). Strong CTCF binding was detected at the silencer in the intervening sequence (Sis), a recombination silencer in the intergenic region between V_κ and J_κ segments (60, 61), at the newly described contracting element for recombination (Cer) immediately upstream of Sis (62), and at the 5′ and 3′ boundaries of the locus (42, 45) (Fig. 1B). Other CTCF sites are distributed at intergenic locations across the 3.2 Mb V_κ array. A functional role for CTCF in *Igk* locus recombination was evaluated in mb1-cre $Ctcf^{f/f}$ mice supplied with a pre-rearranged Igμ transgene (45). The pre-B cell V_κ repertoire in these mice was strongly biased towards usage of proximal V_κ gene segments. Consistent with this, germline transcription of proximal V_κ gene segments was substantially increased, as were interactions of iE_κ and the 3′ κ enhancer ($3'E_\kappa$) with sites distributed across the proximal 1 Mb of the V_κ array (45). The enhancers also displayed increased interactions with sites outside of the *Igk* locus. The data suggest that CTCF plays a role in insulation, and that dysregulation of the V_κ repertoire is a consequence of hyper-activation of proximal V_κ segments by the *Igk* enhancers in the absence of CTCF.

Sis-deficient mice also displayed a proximally biased V_κ repertoire, indicating that the Sis CTCF site may contribute to insulation (61) (Fig. 1B). Cer deletion imparted an even stronger proximal bias to V_κ rearrangement and also allowed V_κ-J_κ rearrangement in thymocytes (62). Thus, Cer may contribute to insulation as well. However, Cer (unlike Sis) deletion modestly reduced *Igk* locus contraction, suggesting locus conformation as a potential explanation for the proximal V_κ bias (62). Additional experiments will be required to determine whether the conformational change and proximal V_κ bias reflect lost binding of CTCF or of another factor to Cer, and analysis on a recombinase-deficient background may be needed to fully evaluate effects of Sis and Cer on transcription and long-distance E_κ interactions. Although Sis and Cer have both been attributed other functions (60–62), their potential roles as insulators separating V_κ segments from the E_κ-regulated J_κ domain appear similar to that of IGCR1 in the *Igh* locus.

CTCF likely contributes to the network of long-distance interactions at the *Igk* locus detected by Hi-C in pro-B cells (23). However, many of the described iE_κ interactions mapped to sites of E2A occupancy. This suggests the possibility that E2A may drive the

clustering of widely distributed V_κ gene segments with iE_κ and may in this way facilitate contraction and V_κ-to-J_κ rearrangement (23). This possibility warrants further study.

Tcra/Tcrd locus

As compared to either *Igh* or *Igk*, the *Tcra/Tcrd* locus on murine chromosome 14 (Fig. 1C) boasts a more nuanced program of conformational states and distinct regulatory functions for CTCF. These features are thought to facilitate a complex developmental program that transitions from *Tcrd* gene assembly in DN thymocytes to *Tcra* gene assembly in DP thymocytes (3). The locus contains approximately one hundred V genesegments that are distributed across 1.5Mb. A small subset of these V segments rearrange to two D_δ and J_δ gene segments to assemble a *Tcrd* repertoire. In contrast, most V segments can rearrange to a large array of 61 J_α gene segments to assemble a *Tcra* repertoire.

Notably, similar to *Igh* recombination, complete V-to-D_δ-to-J_δ recombination can only occur once per allele due to elimination of D_δ gene segments. In contrast, the large arrays of V_α and J_α gene segments can support multiple rounds of V_α-to-J_α recombination on each allele, allowing thymocytes multiple chances to assemble a *Tcra* gene that can promote positive selection (3). It is well established that initial (primary) rearrangements in early DP thymocytes are targeted to J_α segments at the extreme 5′ end of the J_α array by the activity of the T early α (TEA) and J_α49 promoters, and that as a function of DP thymocyte lifespan, subsequent (secondary) rearrangements are targeted to progressively more 3′ J_α segments by the activities of the introduced V_α gene segment promoters (63–66). This 5′-to-3′ J_α progression is coupled with an inevitable, reciprocal 3′-to-5′ progression V gene segment utilization, but there is controversy over the extent to which this progression is regulated and coordinated with the J_α progression. PCR analysis of thymic V_α-to-J_α rearrangements has shown that the most J_α-proximal V_α gene segments are biased to rearrange to the most 5′ J_α gene segments, that the most J_α-distal V_α gene segments are biased to rearrange to substantially more 3′ J_α gene segments, and that broadly distributed multi-member V_α families tend to rearrange to the entire set of J_α gene segments (67–69). This has been interpreted as a regulated and coordinated progression of V_α gene segment availability. Recent deep-sequencing analysis of *Tcra* transcripts in peripheral $CD8^+$ T cells painted a picture that was generally consistent with the observations outlined above (70). However, features of the observed combinatorial diversity conflicted with the notion of coordinated V_α and J_α progressions, leading the authors to propose that following primary rearrangements between 3′ V_α and 5′ J_α gene segments, the remaining V_α segments simultaneously become available for recombination to J_α gene segments (70). Further analysis may be required to conclusively resolve this issue. For example, the study discussed above analyzed a peripheral CD8 T cell repertoire that had been shaped by thymic selection (70), rather than the complete spectrum of recombination events occurring in DP thymocytes. Moreover, additional factors, including duplicated or triplicated portions of the V array featuring nearly identical V gene segments, make analysis of the *Tcra/Tcrd* locus repertoire particularly challenging.

Analysis of *Tcra/Tcrd* locus conformation by 3D-FISH revealed that, as compared to control B cells, the locus is fully contracted in DN thymocytes but undergoes 5′ end extension to adopt a unique 3′ contracted and 5′ decontracted configuration in DP thymocytes (16) (Fig. 1C) (although an earlier study had reached different conclusions for unknown reasons (15)). What would be the rationale for this behavior? In DN thymocytes the locus is limited to a single round of *Tcrd* gene recombination per allele and both proximal and distal V_δ gene segments serve as recombination substrates (reviewed in (71)); this *Igh*-like behavior is best facilitated by a fully contracted configuration of the locus. In contrast, in DP thymocytes the locus undergoes multiple rounds of recombination with primary recombination biased

towards use of 3′ V_α and 5′ J_α gene segments. A 3′ contracted and 5′ decontracted configuration would favor the initial usage of 3′ V_α gene segments and thereby save more 5′ V_α gene segments for subsequent rounds of recombination (16). It should be noted that the conformations described above are adopted by the unrearranged *Tcra/Tcrd* locus in recombinase-deficient thymocytes. Whether and how locus conformation in DP thymocytes adjusts to prior *Tcrd* gene or primary *Tcra* gene recombination is not known.

Recent 3C studies have documented a network of interactions within the 3′-contracted domain of the *Tcra/Tcrd* locus that forms in DP thymocytes (Fig. 1C). The *Tcra* enhancer (E_α) becomes active in DP thymocytes and is known to activate promoters distributed across 500 kb (71), a region that may correspond to the contracted 3′ domain. Included among these promoters are the TEA promoter associated with 5′ J_α gene segments and the promoters of proximal V gene segments. E_α was shown to contact individual V and J promoters and to bring these promoters in contact with each other to form a “chromatin hub” (47). Because RAG proteins preferentially bind to 5′ J_α gene segments to form a recombination center in DP thymocytes (48), hub formation would bring proximal V gene segments into this recombination center and would facilitate the synapsis of V and J RSSs to support primary *Tcra* recombination. Notably, although E_α is required to establish this network of DNA contacts within the contracted portion of the *Tcra/Tcrd* locus, it is not required for 3′ end contraction *per se* (16). This differs from the reported role for E_μ in *Igh* locus contraction (52). CTCF also plays no role in 3′ end contraction in DP thymocytes (47). Hence, the molecular basis for *Tcra/Tcrd* locus contraction is unknown.

As is the case for *Igh* and *Igk*, there are many binding sites for CTCF and cohesin in the *Tcra/Tcrd* locus (46, 47) (Fig. 1C). However the distribution of binding sites is strikingly different than for *Igh* and *Igk*, since in the *Tcra/Tcrd* locus, CTCF sites generally mark *cis*-regulatory elements, including most V gene segment promoters, the TEA promoter and E_α (47). Conditional knockout of *Ctcf* or *Rad21* (which encodes a cohesin subunit) caused reduced interactions between E_α and the TEA promoter, reduced TEA transcription, and reduced 5′ J_α accessiblity (46, 47). Loss of CTCF also partially disrupted interactions between E_α and proximal V_α gene segments and between V_α and J_α gene segments (47). As a result, CTCF- and cohesin-deficiency were associated with reduced V_α-to-J_α rearrangement (46, 47). Surprisingly, CTCF-deficiency, or deletion of the TEA promoter and its associated CTCF site, caused increased interaction between E_α and the $D_\delta J_\delta C_\delta$ cluster and increased transcription and recombination of D_δ and J_δ gene segments. Because the $D_\delta J_\delta C_\delta$ cluster lacks CTCF sites, it was suggested that CTCF normally synergizes with E_α-bound factors to specify CTCF-marked promoters (eg., TEA) as E_α targets, and that this suppresses E_α interactions with suboptimal, CTCF-free targets (eg. D_δ and J_δ promoters). With CTCF eliminated, this specificity is lost, leading to reduced *Tcra* gene rearrangement and increased *Tcrd* gene rearrangement in DP thymocytes (47). CTCF-dependent long-distance interactions are thought to serve primarily a targeting function at the *Tcra/Tcrd* locus, rather than an insulating function as at *Igh* and *Igk*, because *Tcra/Tcrd* locus CTCF sites are positioned at, rather than between, critical *cis*-regulatory elements.

Conclusions

Antigen receptor locus conformation is manipulated at multiple levels to support gene assembly by V(D)J recombination. The evidence suggests at least two layers of organization: multiple-loop rosette-like structures span hundreds of kilobases and interactions between such structures mediate global spatial relationships. Long-distance interaction networks appear to be orchestrated by ubiquitous and lineage-specific transcription factors (YY1, Pax5 and E2A) as well as chromatin architectural proteins (CTCF and cohesin). However, there appears not to be a single paradigm that fits all antigen

receptor loci. CTCF-mediated looping can suppress or stimulate V(D)J recombination through effects on RSS accessibility, depending on whether those loops insulate enhancers from promoters (eg. *Igh* and *Igk*) or target enhancers to promoters (eg. *Tcra*). CTCF-mediated looping may also regulate V(D)J recombination by influencing RSS synapsis. Synapsis may be facilitated when RSSs are positioned near *cis*-regulatory elements brought into contact by CTCF (eg. *Tcra*). Synapsis was also shown to be inhibited when accessible RSSs are segregated into different chromatin loops by CTCF (72), although this result is difficult to reconcile with the obvious need for RSS synapsis between loops at antigen receptor loci. It seems clear that the development of antigen receptor repertoires must occur as an exceedingly complex function of conformational states and spatial relationships dictated by the distribution of binding sites for CTCF and other factors. It will take additional time and effort to make sense of it all.

References

1. Schatz DG, Swanson PC. V(D)J recombination: mechanisms of initiation. Annu Rev Genet. 2011; 45:167–202. [PubMed: 21854230]
2. Jung D, Giallourakis C, Mostoslavsky R, Alt FW. Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. Annu Rev Immunol. 2006; 24:541–570. [PubMed: 16551259]
3. Krangel MS. Mechanics of T cell receptor gene rearrangement. Curr Opin Immunol. 2009; 21:133–139. [PubMed: 19362456]
4. Schatz DG, Ji Y. Recombination centres and the orchestration of V(D)J recombination. Nat Rev Immunol. 2011; 11:251–263. [PubMed: 21394103]
5. Osipovich O, Oltz EM. Regulation of antigen receptor gene assembly by genetic-epigenetic crosstalk. Sem Immunol. 2010; 22:313–322.
6. Hale JS, Ames KT, Boursalian TE, Fink PJ. Cutting Edge: Rag deletion in peripheral T cells blocks TCR revision. J Immunol. 2010; 184:5964–5968. [PubMed: 20435935]
7. Kuo TC, Schlissel MS. Mechanisms controlling expression of the RAG locus during lymphocyte development. Curr Opin Immunol. 2009; 21:173–178. [PubMed: 19359154]
8. Krivega I, Dean A. Enhancer and promoter interactions-long distance calls. Curr Opin Genet Dev. 2012; 22:79–85. [PubMed: 22169023]
9. 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]
10. Bossen C, Mansson R, Murre C. Chromatin topology and the regulation of antigen receptor assembly. Annu Rev Immunol. 2012; 30:337–356. [PubMed: 22224771]
11. Kosak ST, Skok JA, Medina KL, Riblet R, Le Beau MM, Fisher AG, Singh H. Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. Science. 2002; 296:158–162. [PubMed: 11935030]
12. 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]
13. Jhunjhunwala S, van Zelm MC, Peak MM, Cutchin S, Riblet R, van Dongen JJ, Grosveld FG, Knoch TA, Murre C. The 3D structure of the immunoglobulin heavy-chain locus: implications for long-range genomic interactions. Cell. 2008; 133:265–279. [PubMed: 18423198]
14. Roldan E, Fuxa M, Chong W, Martinez D, Novatchkova M, Busslinger M, Skok JA. Locus 'decontraction' and centromeric recruitment contribute to allelic exclusion of the immunoglobulin heavy-chain gene. Nat Immunol. 2005; 6:31–41. [PubMed: 15580273]
15. Skok JA, Gisler R, Novatchkova M, Farmer D, de Laat W, Busslinger M. Reversible contraction by looping of the *Tcra* and *Tcrb* loci in rearranging thymocytes. Nat Immunol. 2007; 8:378–387. [PubMed: 17334367]
16. Shih HY, Krangel MS. Distinct contracted conformations of the *Tcra/Tcrd* locus during *Tcra* and *Tcrd* recombination. J Exp Med. 2010; 207:1835–1841. [PubMed: 20696701]

17. Hubner, MR.; Eckersley-Maslin, MA.; Spector, DL. Chromatin organization and transcriptional regulation. Curr Opin Genet Dev. 2012. (in press)http://dx.doi.org/10.1016/j.gde.2012.11.00618
18. de Wit E, de Laat W. A decade of 3C technologiesinsights into nuclear organization. Genes Dev. 2012; 26:11–24. [PubMed: 22215806]
19. Dekker J, Rippe K, Dekker M, Kleckner N. Capturing chromosome conformation. Science. 2002; 295:1306–1311. [PubMed: 11847345]
20. Simonis M, Klous P, Splinter E, Moshkin Y, Willemsen R, de Wit E, van Steensel B, de Laat W. Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). Nat Genet. 2006; 38:1348–1354. [PubMed: 17033623]
21. Zhao Z, Tavoosidana G, Sjolinder M, Gondor A, Mariano P, Wang S, Kanduri C, Lezcano M, Sandhu KS, Singh U, Pant V, Tiwari V, Kurukuti S, Ohlsson R. Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. Nat Genet. 2006; 38:1341–1347. [PubMed: 17033624]
22. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, Sandstrom R, Bernstein B, Bender MA, Groudine M, Gnirke A, Stamatoyannopoulos J, Mirny LA, Lander ES, Dekker J. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science. 2009; 326:289–293. [PubMed: 19815776]
23. Lin YC, Benner C, Mansson R, Heinz S, Miyazaki K, Miyazaki M, Chandra V, Bossen C, Glass CK, Murre C. Global changes in the nuclear positioning of genes and intra- and interdomain genomic interactions that orchestrate B cell fate. Nat Immunol. 2012; 13:1196–1204. [PubMed: 23064439]
24. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature. 2012; 485:376–380. [PubMed: 22495300]
25. Phillips JE V, Corces G. CTCF: master weaver of the genome. Cell. 2009; 137:1194–1211. [PubMed: 19563753]
26. Kim TH, Abdullaev ZK, Smith AD, Ching KA, Loukinov DI, Green RD, Zhang MQ, Lobanenkov VV, Ren B. Analysis of the vertebrate insulator protein CTCF-binding sites in the human genome. Cell. 2007; 128:1231–1245. [PubMed: 17382889]
27. Cuddapah S, Jothi R, Schones DE, Roh TY, Cui K, Zhao K. Global analysis of the insulator binding protein CTCF in chromatin barrier regions reveals demarcation of active and repressive domains. Genome Res. 2009; 19:24–32. [PubMed: 19056695]
28. Jothi R, Cuddapah S, Barski A, Cui K, Zhao K. Genome-wide identification of *in vivo* protein-DNA binding sites from ChIP-Seq data. Nucleic acids research. 2008; 36:5221–5231. [PubMed: 18684996]
29. Xie X, Mikkelsen TS, Gnirke A, Lindblad-Toh K, Kellis M, Lander ES. Systematic discovery of regulatory motifs in conserved regions of the human genome, including thousands of CTCF insulator sites. Proc Natl Acad Sci U S A. 2007; 104:7145–7150. [PubMed: 17442748]
30. Handoko L, Xu H, Li G, Ngan CY, Chew E, Schnapp M, Lee CW, Ye C, Ping JL, Mulawadi F, Wong E, Sheng J, Zhang Y, Poh T, Chan CS, Kunarso G, Shahab A, Bourque G, Cacheux-Rataboul V, Sung WK, Ruan Y, Wei CL. CTCF-mediated functional chromatin interactome in pluripotent cells. Nat Genet. 2011; 43:630–638. [PubMed: 21685913]
31. Sanyal A, Lajoie BR, Jain G, Dekker J. The long-range interaction landscape of gene promoters. Nature. 2012; 489:109–113. [PubMed: 22955621]
32. Shen Y, Yue F, McCleary DF, Ye Z, Edsall L, Kuan S, Wagner U, Dixon J, Lee L, Lobanenkov VV, Ren B. A map of the *cis*-regulatory sequences in the mouse genome. Nature. 2012; 488:116–120. [PubMed: 22763441]
33. Wood AJ, Severson AF, Meyer BJ. Condensin and cohesin complexity: the expanding repertoire of functions. Nat Rev Genet. 2010; 11:391–404. [PubMed: 20442714]
34. Parelho V, Hadjur S, Spivakov M, Leleu M, Sauer S, Gregson HC, Jarmuz A, Canzonetta C, Webster Z, Nesterova T, Cobb BS, Yokomori K, Dillon N, Aragon L, Fisher AG, Merkenschlager M. Cohesins functionally associate with CTCF on mammalian chromosome arms. Cell. 2008; 132:422–433. [PubMed: 18237772]

35. Wendt KS, Yoshida K, Itoh T, Bando M, Koch B, Schirghuber E, Tsutsumi S, Nagae G, Ishihara K, Mishiro T, Yahata K, Imamoto F, Aburatani H, Nakao M, Imamoto N, Maeshima K, Shirahige K, Peters JM. Cohesin mediates transcriptional insulation by CCCTC-binding factor. Nature. 2008; 451:796–801. [PubMed: 18235444]

36. Rubio ED, Reiss DJ, Welcsh PL, Disteche CM, Filippova GN, Baliga NS, Aebersold R, Ranish JA, Krumm A. CTCF physically links cohesin to chromatin. Proc Natl Acad Sci U S A. 2008; 105:8309–8314. [PubMed: 18550811]

37. Hou C, Dale R, Dean A. Cell type specificity of chromatin organization mediated by CTCF and cohesin. Proc Natl Acad Sci U S A. 2010; 107:3651–3656. [PubMed: 20133600]

38. Nativio R, Wendt KS, Ito Y, Huddleston JE, Uribe-Lewis S, Woodfine K, Krueger C, Reik W, Peters JM, Murrell A. Cohesin is required for higher-order chromatin conformation at the imprinted IGF2-H19 locus. PLoS Genet. 2009; 5:e1000739. [PubMed: 19956766]

39. Chien R, Zeng W, Kawauchi S, Bender MA, Santos R, Gregson HC, Schmiesing JA, Newkirk DA, Kong X, Ball AR Jr, Calof AL, Lander AD, Groudine MT, Yokomori K. Cohesin mediates chromatin interactions that regulate mammalian beta-globin expression. J Biol Chem. 2011; 286:17870–17878. [PubMed: 21454523]

40. Schmidt D, Schwalie PC, Ross-Innes CS, Hurtado A, Brown GD, Carroll JS, Flicek P, Odom DT. A CTCF-independent role for cohesin in tissue-specific transcription. Genome Res. 2010; 20:578–588. [PubMed: 20219941]

41. Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, Ebmeier CC, Goossens J, Rahl PB, Levine SS, Taatjes DJ, Dekker J, Young RA. Mediator and cohesin connect gene expression and chromatin architecture. Nature. 2010; 467:430–435. [PubMed: 20720539]

42. 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]

43. Ebert A, McManus S, Tagoh H, Medvedovic J, Salvagiotto G, Novatchkova M, Tamir I, Sommer A, Jaritz M, Busslinger M. 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. 2011; 34:175–187. [PubMed: 21349430]

44. Lucas JS, Bossen C, Murre C. Transcription and recombination factories: common features? Curr Opin Cell Biol. 2011; 23:318–324. [PubMed: 21169003]

45. Ribeiro de Almeida C, Stadhouders R, de Bruijn MJ, Bergen IM, Thongjuea S, Lenhard B, van Ijcken W, Grosveld F, Galjart N, Soler E, Hendriks RW. The DNA-binding protein CTCF limits proximal V_{κ} recombination and restricts κ enhancer interactions to the immunoglobulin κ light chain locus. Immunity. 2011; 35:501–513. [PubMed: 22035845]

46. Seitan VC, Hao B, Tachibana-Konwalski K, Lavagnolli T, Mira-Bontenbal H, Brown KE, Teng G, Carroll T, Terry A, Horan K, Marks H, Adams DJ, Schatz DG, Aragon L, Fisher AG, Krangel MS, Nasmyth K, Merkenschlager M. A role for cohesin in T-cell-receptor rearrangement and thymocyte differentiation. Nature. 2011; 476:467–471. [PubMed: 21832993]

47. Shih HY, Verma-Gaur J, Torkamani A, Feeney AJ, Galjart N, Krangel MS. *Tcra* gene recombination is supported by a *Tcra* enhancer- and CTCF-dependent chromatin hub. Proc Natl Acad Sci U S A. 2012; 109:E3493–3502. [PubMed: 23169622]

48. 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]

49. Sayegh CE, 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]

50. Liu H, Schmidt-Supprian M, Shi Y, Hobeika E, Barteneva N, Jumaa H, Pelanda R, Reth M, Skok J, Rajewsky K. Yin Yang 1 is a critical regulator of B-cell development. Genes Dev. 2007; 21:1179–1189. [PubMed: 17504937]

51. Reynaud D I, Demarco A, Reddy KL, Schjerven H, Bertolino E, Chen Z, Smale ST, Winandy S, Singh H. Regulation of B cell fate commitment and immunoglobulin heavy-chain gene rearrangements by Ikaros. Nat Immunol. 2008; 9:927–936. [PubMed: 18568028]

52. Guo C, Gerasimova T, Hao H, Ivanova I, Chakraborty T, Selimyan R, Oltz EM, Sen R. Two forms of loops generate the chromatin conformation of the immunoglobulin heavy-chain gene locus. Cell. 2011; 147:332–343. [PubMed: 21982154]

53. Verma-Gaur J, Torkamani A, Schaffer L, Head SR, Schork NJ, Feeney AJ. 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 U S A. 2012; 109:17004–17009. [PubMed: 23027941]

54. Degner SC, Verma-Gaur J, Wong TP, Bossen C, Iverson GM, Torkamani A, Vettermann C, Lin YC, Ju Z, Schulz D, Murre CS, Birshtein BK, Schork NJ, Schlissel MS, Riblet R, Murre C, Feeney AJ. 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 U S A. 2011; 108:9566–9571. [PubMed: 21606361]

55. Garrett FE, Emelyanov AV, Sepulveda MA, Flanagan P, Volpi S, Li F, Loukinov D, Eckhardt LA, Lobanenkov VV, Birshtein BK. Chromatin architecture near a potential 3′ end of the *Igh* locus involves modular regulation of histone modifications during B-Cell development and in vivo occupancy at CTCF sites. Mol Cell Biol. 2005; 25:1511–1525. [PubMed: 15684400]

56. Guo C, Yoon HS, Franklin A, Jain S, Ebert A, Cheng HL, Hansen E, Despo O, Bossen C, Vettermann C, Bates JG, Richards N, Myers D, Patel H, Gallagher M, Schlissel MS, Murre C, Busslinger M, Giallourakis CC, Alt FW. CTCF-binding elements mediate control of V(D)J recombination. Nature. 2011; 477:424–430. [PubMed: 21909113]

57. Marenduzzo D, Faro-Trindade I, Cook PR. What are the molecular ties that maintain genomic loops? Trends Genet. 2007; 23:126–133. [PubMed: 17280735]

58. Volpi SA, Verma-Gaur J, Hassan R, Ju Z, Roa S, Chatterjee S, Werling U, Hou H Jr, Will B, Steidl U, Scharff M, Edelman W, Feeney AJ, Birshtein BK. Germline deletion of *Igh* 3′ regulatory region elements hs 5, 6, 7 (hs5-7) affects B cell-specific regulation, rearrangement, and insulation of the *Igh* locus. J Immunol. 2012; 188:2556–2566. [PubMed: 22345664]

59. Bates JG, Cado D, Nolla H, Schlissel MS. Chromosomal position of a V_H gene segment determines its activation and inactivation as a substrate for V(D)J recombination. J Exp Med. 2007; 204:3247–3256. [PubMed: 18056289]

60. Liu Z, Widlak P, Zou Y, Xiao F, Oh M, Li S, Chang MY, Shay JW, Garrard WT. A recombination silencer that specifies heterochromatin positioning and Ikaros association in the immunoglobulin κ locus. Immunity. 2006; 24:405–415. [PubMed: 16618599]

61. Xiang Y, Zhou X, Hewitt SL, Skok JA, Garrard WT. A multifunctional element in the mouse *Igk* locus that specifies repertoire and Ig loci subnuclear location. J Immunol. 2011; 186:5356–5366. [PubMed: 21441452]

62. Xiang Y, Park SK, Garrard WT. $V_κ$ gene repertoire and locus contraction are specified by critical DNase I hypersensitive sites within the $V_κ$-$J_κ$ intervening region. J Immunol. 2013; 190:1819–1826. [PubMed: 23296705]

63. Guo J, Hawwari A, Li H, Sun Z, Mahanta SK, Littman DR, Krangel MS, He YW. Regulation of the TCRα repertoire by the survival window of $CD4^+CD8^+$ thymocytes. Nat Immunol. 2002; 3:469–476. [PubMed: 11967541]

64. Hawwari A, Krangel MS. Role for rearranged variable gene segments in directing T cell receptor α recombination. Proc Natl Acad Sci U S A. 2007; 104:903–907. [PubMed: 17210914]

65. Mauvieux L, Villey I, de Villartay JP. T early alpha (TEA) regulates initial TCRVAJA rearrangements and leads to TCRJA coincidence. Eur J Immunol. 2001; 31:2080–2086. [PubMed: 11449361]

66. Villey I, Caillol D, Selz F, Ferrier P, de Villartay JP. Defect in rearrangement of the most 5′ TCR-Jα following targeted deletion of T early α (TEA): implications for TCR α locus accessibility. Immunity. 1996; 5:331–342. [PubMed: 8885866]

67. Pasqual N, Gallagher M, Aude-Garcia C, Loiodice M, Thuderoz F, Demongeot J, Ceredig R, Marche PN, Jouvin-Marche E. Quantitative and qualitative changes in V-J alpha rearrangements during mouse thymocytes differentiation: implication for a limited T cell receptor alpha chain repertoire. J Exp Med. 2002; 196:1163–1173. [PubMed: 12417627]

68. Thuderoz F, Simonet MA, Hansen O, Pasqual N, Dariz A, Baum TP, Hierle V, Demongeot J, Marche PN, Jouvin-Marche E. Numerical modelling of the V-J combinations of the T cell receptor TRA/TRD locus. PLoS Comput Biol. 2010; 6:e1000682. [PubMed: 20174554]

69. Jouvin-Marche E, Fuschiotti P, Marche PN. Dynamic aspects of TCRα gene recombination: qualitative and quantitative assessments of the TCRα chain repertoire in man and mouse. Adv Exp Med Biol. 2009; 650:82–92. [PubMed: 19731803]

70. Genolet R, Stevenson BJ, Farinelli L, Osteras M, Luescher IF. Highly diverse TCRα chain repertoire of pre-immune $CD8^+$ T cells reveals new insights in gene recombination. EMBO J. 2012; 31:1666–1678. [PubMed: 22373576]

71. Hawwari A, Krangel MS. Regulation of TCR δ and α repertoires by local and long-distance control of variable gene segment chromatin structure. J Exp Med. 2005; 202:467–472. [PubMed: 16087716]

72. Shrimali S, Srivastava S, Varma G, Grinberg A, Pfeifer K, Srivastava M. An ectopic CTCF-dependent transcriptional insulator influences the choice of V_β gene segments for VDJ recombination at TCRβ locus. Nucleic Acids Res. 2012; 40:7753–7765. [PubMed: 22718969]

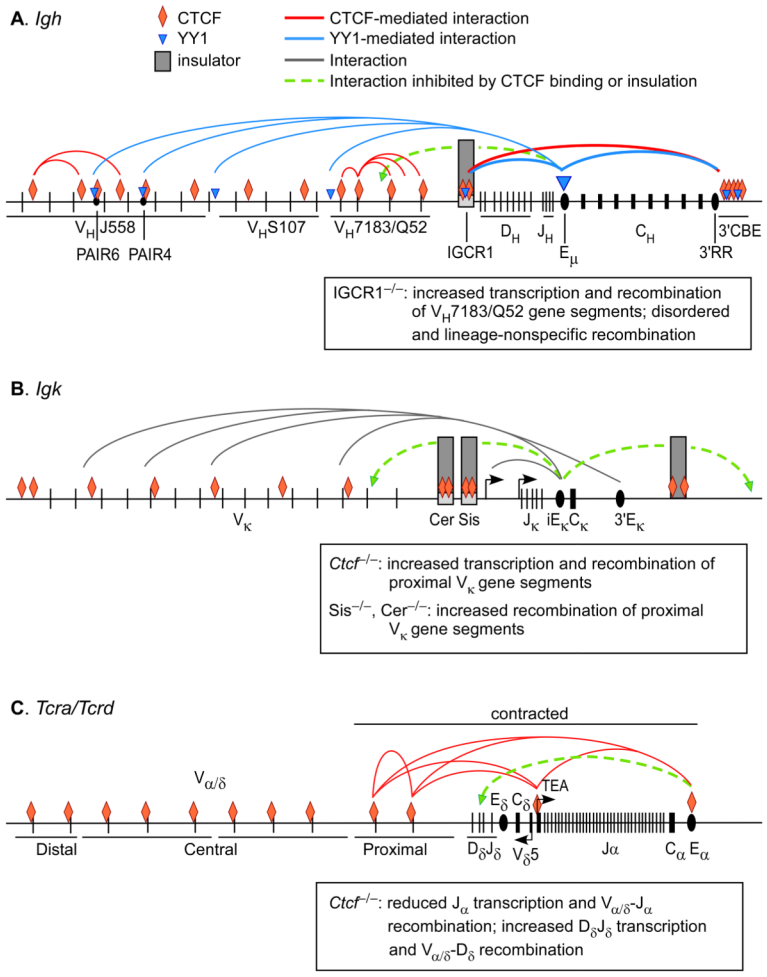

Figure 1.

Long-distance interactions at antigen receptor loci. Long-distance DNA contacts are depicted for the contracted conformation of the *Igh* locus in pro-B cells **(A)**, the contracted conformation of the *Igk* locus in pre-B cells **(B)** and the 3′ contracted but 5′ extended conformation of the *Tcra/Tcrd* locus in DP thymocytes **(C)**. Interactions known or presumed to be mediated by CTCF or YY1 are indicated. Transcription and recombination phenotypes of various genetic models are summarized (boxes). *Igk* interactions involving Sis (45) are not depicted.