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Chromatin Architecture, CCCTC-Binding Factor, and V(D)J Recombination: Managing Long-Distance Relationships at Antigen Receptor Loci

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daptive immunity in jawed vertebrates is mediated by T and B lymphocytes that express highly diverse and clonally distributed Ag receptors. The diversity of lymphocyte Ag receptors is generated primarily by the assembly of V, D, and J region gene segments at TCR and Ig loci (1). This process, known as V(D)J recombination, is catalyzed by the RAG-1 and RAG-2 proteins. The RAG proteins recognize recombination signal sequences (RSSs) 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. Ag receptor loci undergo recombination in a manner that is regulated according to cell lineage and developmental stage (2, 3). During T lymphocyte development in the thymus, Tcrb 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 H (Igh) and L (Igk and Igλ) chain genes, with Igh recombination in pro-B cells and Igk and Igλ 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 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 Ag 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 Ag receptor loci within immature lymphocyte nuclei have 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 Ig 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 with other cells (11). Subsequent studies revealed “contraction” to be a general property of Ag 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. Because 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, CCCTC-binding factor, 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; instead, deep sequencing is used to identify the entire genomic universe of interacting sequences (22). During 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.

CCCTC-binding factor, CTCF, 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). Additionally, 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 they 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 multisubunit 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 ~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 independently of one another (40, 41).

Chromatin immunoprecipitation microarray and chromatin immunoprecipitation sequencing analyses have revealed Ag 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(DJ) recombination. We focus below on recent studies of the Igh, Igk, and Teral/Terd loci. Much less is known about Tcrb locus architecture and there have been no studies of the Terg 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(DJ) 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 V11 gene segments spanning 2.7 Mb of DNA, followed by 10–13 D11, 4 J11, and 8 C11 gene segments (2) (Fig. 1A). Igh locus V(D)J recombination occurs in a strictly ordered fashion, with D11-to-J11 recombination preceding V11-to-D11 J11 recombination in pro-B cells. The V11-D11 J11 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 V11-D11 J11 recombination only once, because this rearrangement eliminates all unrearranged D11 gene segments. Therefore, although several D11-proximal V11 gene segments do rearrange at elevated frequencies, D11-distal V11 gene segments must be able to compete effectively with these proximal V11 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 V11 gene segments into proximity of the D11 J11 cluster (13). Spatial distance measurements and computer modeling suggested that in its extended conformation in prepro-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 deconstruct in pre-B cells,
correlating with the suppression of V_{H1}-to-D_{H1} 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 VH rearrangement (12, 50, 51). Because distal VH 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 VH-to-DHJH rearrangement relies on Igh locus contraction (12, 50). Elimination of the Igh intronic enhancer (E\textsubscript{m}), located between J\textsubscript{H} and C\textsubscript{\textmu} segments, also causes a loss of Igh contraction (52). YY1 may directly regulate Igh contraction by binding to E\textsubscript{m} (50, 52) and to other sites in the VH 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, most of which are distributed across the V_{H1} region (43, 54) (Fig. 1A). CTCF sites in the proximal portion of the V_{H1} array are located immediately downstream of V_{H1} 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_{H1} region. Two are located ~3.2 and 5.6 kb upstream of DFL16.1, the most 5’ D_{H1} 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_{\textmu} gene segments (42, 54–56). Most Igh locus CTCF sites also bind cohesin (54). Notably, neither CTCF nor cohesin bind to E\textsubscript{m} (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\textsubscript{m}, 3’RR, and 3’CBE, that are present in both prepro-B cells and pro-B cells. The interaction between IGCR1 and 3’CBE is CTCF- and cohesin-dependent (54), whereas E\textsubscript{m} interactions with these sites may be mediated by YY1, as this factor binds not only to E\textsubscript{m}, 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_{H1} site or a distal V_{H1} site as bait described two additional multiple-loop regions, each spanning several hundred kb. 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 prepro- and pro-B cells.

In addition to the relatively local interactions described above, Eμ was shown to interact with two distant sites, one in the proximal portion of the VH array (near the 5′ end of the V1J7183 gene segments) and one in the distal portion of the VH array (near the 3′ end of the V1J5385 gene segments) (Fig. 1A). These long-distance interactions are Eμ-dependent and may also involve Y1Y1 in YY1 binding was detected at both sites (52). It was also recently shown that Eμ 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 VH 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 Eμ–PAIR interactions do not reflect a functional role for Eμ in PAIR activation, because PAIR antisense transcription is Eμ-independent. Nevertheless, PAIR transcription, similar to Igh contraction, is YY1-dependent (53). Therefore, it was suggested that PAIR and Eμ elements might interact because they are independently recruited into the same transcription factory and that this corecruitment 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–PAIR 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 4-kb IGCR1 region or mutation of its two CTCF binding sites led to dramatic dysregulation of the VH repertoire, with substantially elevated recombination and recombination of the most D1H-proximal VH gene segments (56) (Fig. 1A). Remarkably, proximal VH gene segments were also found to rearrange to D1H gene segments prior to D1H-JH joining and to D1H or D1HJH 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 Eμ interactions with both elements. However, because 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 VH gene segments from the influence of Eμ, perhaps a direct result of CTCF-mediated looping between the IGCR1 and 3′CBE regions. Consistent with this interpretation, deletion of hs5-7, containing seven of nine CTCF sites in the 3′CBE region, partially reduced the IGCR1–3′RR/3′CBE interaction and caused a mild increase in proximal VH usage (58). Moreover, a dysregulation similar to that documented for IGCR1 deletion was observed for a distal VH gene segment that was repositioned between IGCR1 and the D1H cluster on an otherwise wild-type Igh allele (59).

If IGCR1 functions as an insulator, it might be expected to suppress physical interactions between Eμ and upstream sites in the Igh locus. However, it is apparent that Eμ 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 Vk gene segments that are distributed across 3.2 Mb and are situated upstream of 4 Jk gene segments and Cκ (Fig. 1B). Although Igk alleles can undergo secondary Vκ-to-Jk recombination to replace an initial Vκ-Jk rearrangement that is out-of-frame or that provokes autoreactivity, opportunities for secondary rearrangements are limited by the small number of Jk segments. Therefore, Igk conformational features should be important to foster the development of a broad Vκ repertoire by providing Jk-proximal and -distal Vκ gene segments similar opportunities for rearrangement. Based on 3D-FISH, the Igk locus was reported to undergo contraction in pre-B cells to facilitate Vκ-to-Jk recombination at this stage (14). However, a recent Hi-C study documented an extensive, locus-wide network of physical interactions involving Vκ segments and the intronic κ enhancer (iEκ, situated in the Jκ-Cκ intron) that, although absent in prepro-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 Igκ contraction may occur earlier during B cell development than previously thought.

The Igk locus contains ~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 Vκ and Jκ segments (60, 61), at the newly described contracting element for recombination (Cer) immediately upstream of Sis (62) and at the 5′ boundaries of the locus (42, 45) (Fig. 1B). Other CTCF sites are distributed at intergenic locations across the 3.2-Mb Vκ array. A functional role for CTCF in Igk locus recombination was evaluated in mb1-cre CtcfΔ mice supplied with a pre-rearranged Igκ transgene (45). The pre-B cell Vκ repertoire in these mice was strongly biased toward usage of proximal Vκ gene segments. Consistent with this, germline transcription of proximal Vκ gene segments was substantially increased, as were interactions of iEκ and the 3′ κ enhancer (3′Eκ) with sites distributed across the proximal 1 Mb of the Vκ 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 Vκ repertoire is a consequence of hyper-activation of proximal Vκ segments by the Igκ enhancers in the absence of CTCF.
Sis-deficient mice also displayed a proximally biased V<sub>k</sub> repertoire, indicating that the Sis CTCF site may contribute to insolation (61) (Fig. 1B). Cer deletion imparted an even stronger proximal bias to V<sub>k</sub> rearrangement and also allowed V<sub>c</sub>-J<sub>a</sub> rearrangement in thymocytes (62). Thus, Cer may contribute to insolation as well. However, Cer (unlike Sis) deletion modestly reduced Igk locus contraction, suggesting locus conformation as a potential explanation for the proximal V<sub>k</sub> bias (62). Additional experiments will be required to determine whether the conformational change and proximal V<sub>k</sub> 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<sub>a</sub> interactions. Although Sis and Cer have both been attributed other functions (60–62), their potential roles as insulators separating V<sub>k</sub> segments from the E<sub>c</sub>-regulated J<sub>a</sub> domain appear similar to that of IGCR1 in the Igk 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<sub>a</sub> interactions mapped to sites of E2A occupancy. This suggests the possibility that E2A may drive the clustering of widely distributed V<sub>k</sub> gene segments with iE<sub>a</sub> and may be involved in this way to facilitate contraction and V<sub>a</sub>–to–J<sub>a</sub> rearrangement (23). This possibility warrants further study.

**Tcra/Tcrd locus**

As compared with either Igk 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 ~100 V gene segments that are distributed across 1.5 Mb. A small subset of these V segments rearrange to two D<sub>B</sub> and J<sub>a</sub> gene segments to assemble a Tcrd repertoire. In contrast, most V segments can rearrange to a large array of 61 J<sub>a</sub> gene segments to assemble a Tcra repertoire.

Notably, similar to Igk recombination, complete V-to-D<sub>B</sub>-to-J<sub>a</sub> recombination can only occur once per allele due to elimination of D<sub>B</sub> gene segments. In contrast, the large arrays of V<sub>a</sub> and J<sub>a</sub> gene segments can support multiple rounds of V<sub>a</sub>–to–J<sub>a</sub> 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<sub>a</sub> segments at the extreme 5′ end of the J<sub>a</sub> array by the activity of the T early α (TEA) and J<sub>a</sub>49 promoters, and that as a function of DP thymocyte lifespan, subsequent (secondary) rearrangements are targeted to progressively more 3′ J<sub>a</sub> segments by the activities of the introduced V<sub>a</sub> gene segment promoters (63–66). This 5′-to-3′ J<sub>a</sub> 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<sub>a</sub> progression. PCR analysis of thymic V<sub>a</sub>-to-J<sub>a</sub> rearrangements has shown that the most J<sub>a</sub>-proximal V<sub>a</sub> gene segments are biased to rearrange to the most 5′ J<sub>a</sub> gene segments, that the most J<sub>a</sub>-distal V<sub>a</sub> gene segments are biased to rearrange to substantially more 3′ J<sub>a</sub> gene segments, and that broadly distributed multimember V<sub>a</sub> families tend to rearrange to the entire set of J<sub>a</sub> gene segments (67–69). This has been interpreted as a regulated and coordinated progression of V<sub>a</sub> gene segment availability. Recent deep-sequencing analysis of Tcra transcripts in peripheral CD8<sup>+</sup> 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<sub>a</sub> and J<sub>a</sub> progressions, leading the authors to propose that following primary rearrangements between 3′ V<sub>a</sub> and 5′ J<sub>a</sub> gene segments, the remaining V<sub>a</sub> segments simultaneously become available for recombination to J<sub>a</sub> 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 with 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<sub>B</sub> gene segments serve as recombination substrates (reviewed in Ref. 71); this Igk-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 toward use of 3′ V<sub>a</sub> and 5′ J<sub>a</sub> gene segments. A 3′ contracted and 5′ decontracted configuration would favor the initial usage of 3′ V<sub>a</sub> gene segments and thereby save more 5′ V<sub>a</sub> gene segments for subsequent rounds of recombination (16). Note that the conformations described above are adopted by the unarranged Tcrd/Tcra locus in recombinase-deficient thymocytes. Whether and how locus conformation in DP thymocytes adjusts to prior Tcrd gene or primary Tcra gene recombination are 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<sub>a</sub>) 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<sub>a</sub> gene segments and the promoters of proximal V gene segments. E<sub>a</sub> 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<sub>a</sub> 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 synopsis of V and J RSSs to support primary Tcra recombination. Notably, although E<sub>a</sub> 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<sub>a</sub> in Igk locus contraction (52). CTCF also plays no role in 3′ end...
Conclusions

Ag 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 kb, 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 Ag 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 (e.g., Igh and Igk) or target enhancers to promoters (e.g., Tera). CTCF-mediated looping may also regulate V(D)J recombination by influencing RSS synopsis. Synapsis may be facilitated when RSSs are positioned near cis-regulatory elements brought into contact by CTCF (e.g., Tera). 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 synopsis between loops at Ag receptor loci. It seems clear that the development of Ag 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.

Disclosures

The authors have no financial conflicts of interest.

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The long-range interaction
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