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INSIGHTS

Unraveling the *Tcrb* interactome

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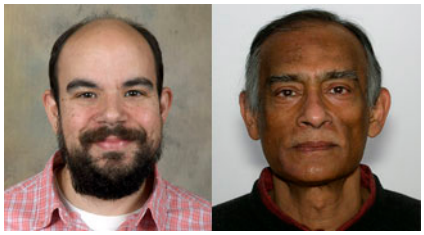
In this issue of *JEM*, Allyn et al. (https://doi.org/10.1084/jem.20230985) provide mechanistic insights into the nuclear organization of the *Tcrb* locus that permits long-range genomic rearrangements.

Diversity of B and T cell antigen receptors underlies the unique specificity of adaptive immune responses. This diversity is generated during lymphocyte development via VDJ recombination, a process that juxtaposes gene segments that are widely separated in the genome. For T cell receptor β chain genes (*Tcrb*) a functional VDJ exon contains one variable (Vβ), one diversity (Dβ), and one joining (Jβ) gene segment assembled from a germline configuration of several Vβ, Dβ, and Jβ gene segments spread over 670 kb (see figure). In this issue of *JEM*, Allyn et al. provide mechanistic insights into the nuclear organization of the *Tcrb* locus that permits long-range genomic rearrangements (Allyn et al., 2024).

The prevailing view of how this occurs is the RAG (recombination activating gene product) scanning model (Zhang et al., 2022). Rag1 and Rag2, proteins that initiate VDJ recombination, are found most prominently near joining gene segments of all antigen receptor genes; such regions are referred to as recombination centers (RCs). The scanning model posits that RAG bound to the RC linearly scans along chromatin, moving the RC into proximity with D or V gene segments. RAG scanning is proposed to be driven by the protein complex cohesin, which brings distal regions of the genome together through a process called loop extrusion (Fudenberg et al., 2016). Formulated based on studies of the immunoglobulin heavy chain gene locus (*Igh*), this model nicely explains deletional orientation of all V_H gene segments that precludes recombination by inversion. The generality of this model was inferred from the observation that most V gene segments at other antigen receptor loci are similarly organized. An essential corollary of the scanning model is that V recombination by inversion would be strongly disfavored. This is, indeed, the case with D_H recombination in *Igh*, where deletional preference exceeds inversion by several orders of magnitude (Zhang et al., 2019).

However, there are aspects of recombination regulation that are not readily explained by the scanning model. One is that Ig κ light chain locus in mice (*Igk*) contains several Vκ gene segments positioned in the inversional orientation relative to the RC. These gene segments are not, as far as is known, under-represented in the *Igk* repertoire. Secondly, unidirectional scanning from the RC predicts that V_H gene segments located closest to the RC would recombine at a higher frequency than those located further away. While the 3′-most functional V_H gene, V_H5-2, recombines most frequently, gene segments further upstream do not follow any discernible pattern. It has been argued that variations from a predicted pattern may result from other factors that affect recombination, such as the strength of gene segment–associated recombination signal sequences (RSSs). Third, V_H recombination to D_H gene segments has been shown to occur by deletion or inversion with comparable efficiency, leading to the proposal that V_H genes may also find target RSSs by diffusion (Qiu et al., 2020).



Insights from Noah Ollikainen and Ranjan Sen.

Allyn et al. (2024) explored mechanistic aspects of recombination control at the *Tcrb* locus by applying genome-wide chromatin structure and epigenetic assays to wild type (WT) and genetically engineered *Tcrb* alleles. They identified three prominent features of WT *Tcrb* alleles (see figure): (a) a central 250-kb portion between *Trbv2-Trbv30* that formed a self-interacting domain, (b) prominent interactions of this domain with the RC (at the 3′ end of the locus), and (c) interactions of the distant *Trbv1* (at the 5′ end) with the central domain and with the RC. Notably, regions between *Trbv1* and the central domain and between the central domain and RC were not involved in these interactions. This organization is reminiscent of the structure of germline *Igh* alleles in pro-B cells that also have V_H gene segments encompassed within self-interacting domains and associations of these domains with the RC-containing 3′ *Igh* domain (Guo et al., 2011; Gerasimova et al., 2015).

The authors then used the unique organization of the *Tcrb* locus in having a remote *Trbv1* gene segment to probe requirements

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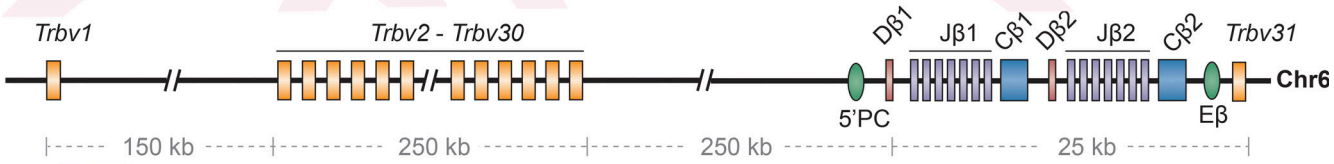

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T cell receptor β chain locus (*Tcrb*) interactome. Schematic representation of the *Tcrb* locus showing multiple variable (*Trbv*), diversity (Dβ1 and 2), and joining (Jβ1 and 2) gene segments. Cβ1 and Cβ2 denote TCRβ constant regions. Eβ identifies a tissue-specific enhancer that controls recombination and transcription, and 5′PC marks a CTCF binding site located 27 kb 5′ of Dβ1. The approximate location of the RC is between Dβ1 and Cβ2. Blue and pink arcs denote cohesin-dependent and homotypic interactions, respectively, identified by Allyn et al. (2024) based on Hi-C analyses of WT, *Trbv1* promoter–deleted, and *Trbv1*-associated CTCF binding site mutated *Tcrb* alleles.

for recombination. *Trbv1* is located ∼2 kb from a CTCF binding element (CBE) that is in a convergent orientation relative to multiple CBEs near the RC. Given that convergently oriented CBEs can form loops via cohesin-dependent loop extrusion (Sanborn et al., 2015), *Trbv1*'s nearby CBE could allow it to interact with the RC. *Trbv1* also has a promoter that is active prior to recombination, which could enable *Trbv1* and the RC to participate in homotypic interactions, a term used to describe interactions between regions of the genome with similar transcriptional and epigenetic states (Misteli, 2020). Interestingly, Allyn et al. (2024) found that mutating *Trbv1*'s CBE and deleting 1.8 kb of the *Trbv1* promoter resulted in virtually identical Hi-C maps. On both mutated alleles, the central Vβ domain and its interactions with the RC were unchanged, whereas interactions of *Trbv1* with both the central domain and the RC were diminished. *Trbv1* rearrangements were reduced fourfold by the CBE mutation and 40-fold by deleting the promoter. These observations lead to the authors' second major conclusion that *Tcrb* structure and rearrangements are regulated by loop extrusion (originating near the *Trbv1* CBE) and by homotypic interactions (via *Trbv1* promoter activity).

Finally, in the most revealing experiment, Allyn et al. (2024) tested the consequences of inverting the *Trbv1*-associated RSS, thereby forcing recombination to proceed only by inversion. They found that inversional recombination occurred almost as efficiently as deletional recombination (the only choice in the WT configuration). Because the scanning model predicts against inversional recombination, the authors conclude that capture of the *Trbv1* RSS by RAG proteins bound to the RC must include a diffusional component. They are cautious in not generalizing their observations to other antigen receptor loci such as *Igh*, where they concur with the current model of V_H RSS search proceeding exclusively by scanning. Nevertheless, this study is the first direct experimental test of scanning versus diffusional mechanisms of locating distal RSSs, and the results support a role for diffusion in this process. It is important to emphasize that the diffusional search for complementary RSSs occurs in the three-dimensional (3D) context established by other means such as cohesin-dependent chromatin extrusion (Qiu et al., 2020).

This study raises several intriguing questions regarding the mechanisms of how genomes fold in 3D, and how 3D genome organization relates to important processes such as VDJ recombination and gene expression. For example, while many proteins that are required for cohesin-mediated loop extrusion are known, much less is known about precisely which proteins are required for the homotypic interactions. Candidates include transcription factors that bind to DNA sequences in promoters and enhancers, proteins that bind to specific histone modifications, or components of the transcriptional machinery itself. We also do not yet know the rules governing homotypic interactions. For instance, if the *Trbv1* promoter were replaced with a stronger or a weaker promoter, how would that impact the frequency of its interactions with the RC and its recombination frequency? Future work targeting specific proteins may lead to insights into which proteins are responsible for these interactions.

While this study demonstrates an instance where loop extrusion and homotypic interactions may work cooperatively, previous work showed that these two mechanisms can be antagonistic. Removal of cohesin has been shown to lead to the loss of chromatin loops but an increase in strength of homotypic interactions (Rao et al., 2017). These results suggest that the process of cohesin loop extrusion facilitates the mixing of chromatin with different histone modifications states. More studies that compare the consequences of perturbing loop extrusion, homotypic interactions, and both at the same time will help us understand in what circumstances are these mechanisms cooperative, antagonistic or independent.

Another intriguing observation in this study is the similarity between chromatin states of CBE-mutated or *Trbv1* promoter–deleted alleles. This indicates that CTCF

binding to *Trbv1* promoter–deleted alleles is insufficient to induce looping via cohesin-dependent loop extrusion with convergently oriented CTCF sites in the RC. It will be interesting to understand how the *Trbv1* promoter contributes to long-distance looping. Allyn et al. (2024) also found that *Trbv1* recombination is 10-fold lower on promoter-less alleles compared to CBE-deleted alleles (though the structure is quite similar). These observations indicate that the promoter does more than enhance looping. One possibility is that transcription-associated H3K4me3 marking at *Trbv1* may stabilize RAG synapsis via the Rag2 PHD domain.

Although this study focused on the *Tcrb* locus, the implications of this work are likely relevant to the folding and function of other antigen receptor loci. Antigen receptor loci can contain many CBEs, and the presence of some CBEs have been shown to be critical to mediate the interactions required for proper VDJ recombination. However, the rules that govern which CBEs matter are unclear. The CBE required for *Trbv1* interactions and recombination is about 2 kb from *Trbv1*. If this CBE were further away from or closer to *Trbv1*, would it have the same function? It remains unclear what the relationship is between the distance a V gene segment is from a CBE and its impact on interactions and recombination.

Future studies that simultaneously measure the effect of these types of perturbations on 3D genome organization and biological processes such as VDJ recombination or gene expression will provide valuable data for building quantitative and predictive models.

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