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Non-coding transcription and large-scale nuclear organisation of immunoglobulin recombination

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The enormous antigen receptor loci in lymphocytes are a paradigm of dynamic nuclear organisation, which is integral to their need to move extensively in 3D space to achieve distal gene synapse for V(D)J recombination and allelic exclusion. The loci undergo extensive 3D looping to bring distal genes together, controlled by several tissue-specific and ubiquitous factors, but how these factors achieve looping, synapsis and V(D)J recombination has been a mystery. Now several studies provide evidence that non-coding transcription, often proposed to play a role, is indeed an important driver, and furthermore has a specific nuclear destination for recombination. Both local transcription-independent looping and longer range factor-mediated transcription-dependent looping play separate roles in altering AgR architecture to enable V(D)J recombination.

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Introduction

The profound influence of genomic architecture on gene function is nowhere more exemplified than in V(D)J recombination of antigen receptor (AgR) loci in lymphocytes. These are the largest loci in the genome, and include the immunoglobulin heavy chain (Igh), light chain kappa (Igκ) and lambda (Igλ), and the T cell receptors α, β, γ and δ. Before being expressed they must be recombined, requiring large-scale DNA looping in cis. Furthermore monoallelic recombination is a primary goal, and the two alleles ultimately have distinct subnuclear destinations. Taking the Igh as an example, this 3 Mb locus is recombined in progenitor B cells to generate the IgH polypeptide. It includes three sets of genes: 4 J_H (joining) genes, 10 D_H (diversity) genes, and 195 V_H (variable) genes (Figure 1). The goal of V(D)J recombination is to recombine these genes in the correct order in as many combinations as possible to generate antibody diversity. After one D_H and one J_H gene recombine together on both alleles, one of the V_H genes recombines with the DJ_H segment on one allele. The most distal V_H gene is 2.5 Mb away from the DJ_H segment, too far to permit efficient interactions due to random chromatin movement [1]. Thus, to enable synapsis between the D_H genes and distal V_H segments, V_H to DJ_H recombination requires multiple long-range interactions at much greater genomic distance than any enhancer–promoter interactions yet described. Moreover, once V_H to DJ_H recombination is successful on one allele, the second allele is sequestered to pericentric heterochromatin to prevent its V_H to DJ_H recombination. This review will highlight recent progress in understanding the mediators and mechanisms of the architectural changes that underpin V(D)J recombination.

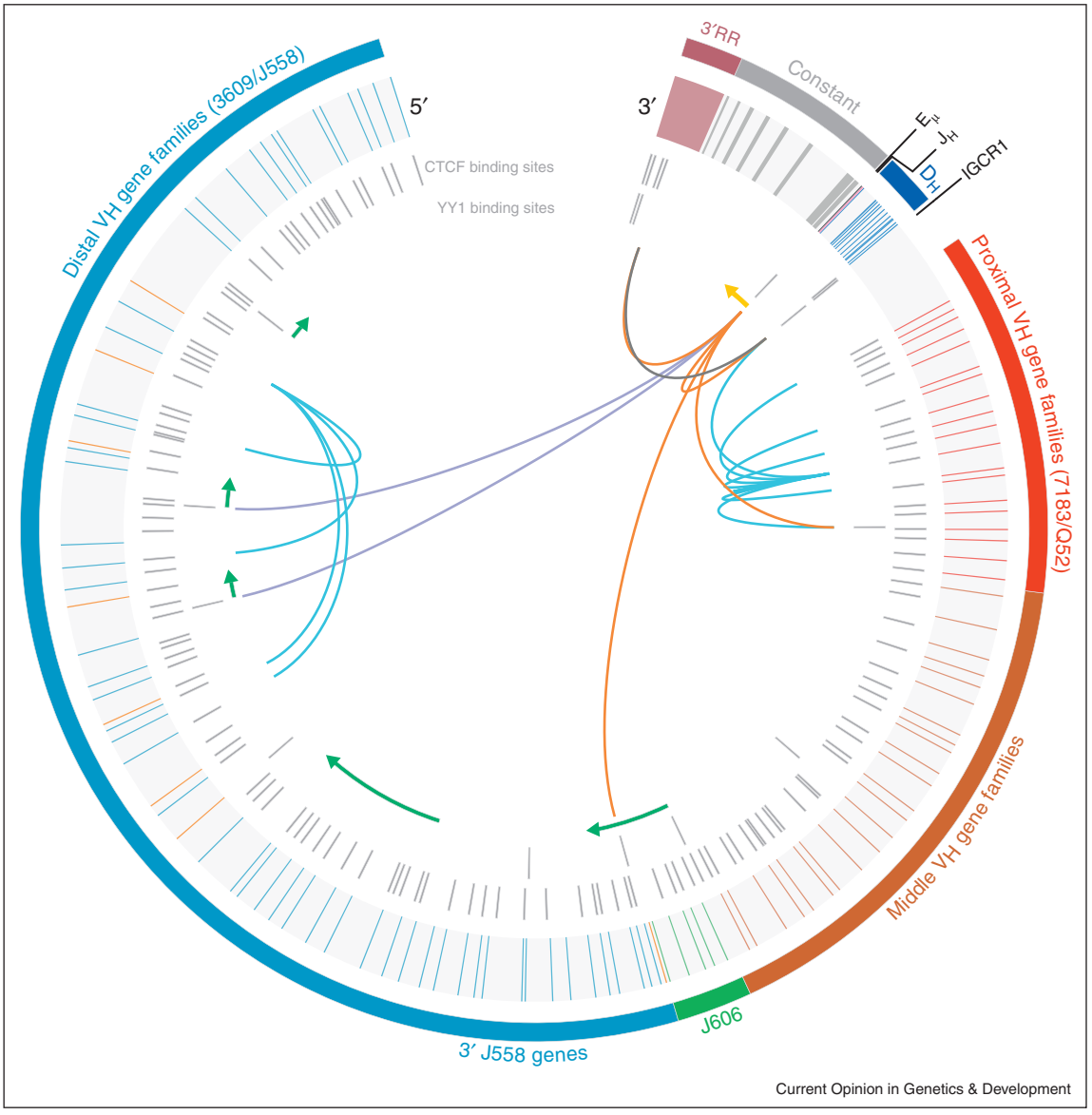
DNA looping of AgR loci

All of the larger AgR deal with the logistic challenge of extensive movement in 3D nuclear space by undergoing developmentally regulated DNA looping and compaction, which brings distal V genes closer to the DJ or J regions of their respective AgR locus [2–8]. Several factors including lineage-specific transcription factors Pax5, ikaros [9,10], and ubiquitous chromatin organisation factors YY1, ezh2 [11,12], regulate Igh V_H region looping, and V_H–DJ_H recombination of D_H-distal V_H genes. However, they all have numerous direct and indirect targets, and it was unclear whether, or how, they acted directly on the Igh locus. So the key question remains: what mechanism drives this essential AgR looping in 3D nuclear space?

Non-coding RNA transcription in Ig loci

Recent insights suggest that part of the answer is non-coding (nc) RNA transcription. The first non-coding transcript to be described was Iμ [13]; it is transcribed from the first eukaryotic enhancer discovered, the mouse Igh intronic enhancer Eμ [14]. Ever since these discoveries, along with those of non-coding transcription from Igh V_H genes [15], it has been clear that non-coding transcription is an unmistakeable sign that preparation is underway for V(D)J recombination of AgR loci. However, its function has remained an elusive enigma. In recent years, there has been a sea-change in our understanding of ncRNA transcription and the enormous importance of ncRNAs in gene regulation and genomic

Figure 1



The architecture of the Igh locus. Circular representation of the Igh locus with the outer coloured bars representing functional V_H, D_H, J_H and constant region genes. Within the V_H genes, blue bars represent members of the V_HJ558 family, orange bars represent the 3609 family and green bars the J606 family. The inner grey bars indicate binding sites for CTCF [32•] and YY1 [23•,34••]. Green arrows indicate major V_H region antisense transcripts seen in pro-B cells [23•] (Matheson *et al.*, in prep.). The yellow arrow represents the Iμ transcript. Curved links across the centre of the plot represent interactions within three-dimensional space observed by chromatin conformation capture and related techniques. The grey link is between IGCR1 and the 3′Regulatory Region [29••], orange links are Eμ-dependent associations whilst blue links are Eμ-independent interactions that involve CTCF [34••]. The purple links are those observed between Eμ and PAIR elements four and six [23•].

architecture [16]. Thousands of long non-coding RNAs play numerous roles as molecular chaperones and sub-nuclear structural components [17], or as enhancer RNAs ('eRNAs') transcribed from developmentally regulated enhancers, with enhancer-like function [18,19]. Furthermore, deep sequencing technologies have enabled interrogation of these processes for the first time in the large AgR loci. These advances have set the stage for several recent studies that have rediscovered the importance of non-coding RNA transcription in AgRs. In this review, we will take a transcription-centric approach, as we explore how multiple factors influence transcription, and transcription influences V(D)J recombination.

Our lab first found that developmentally regulated antisense intergenic transcription occurs extensively in the