CHAPTER 5

Large-Scale Chromatin Remodeling at the Immunoglobulin Heavy Chain Locus:

A Paradigm for Multigene Regulation

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Abstract

V(D)J recombination in lymphocytes is the cutting and pasting together of antigen receptor genes in cis to generate the enormous variety of coding sequences required to produce diverse antigen receptor proteins. It is the key role of the adaptive immune response, which must potentially combat millions of different foreign antigens. Most antigen receptor loci have evolved to be extremely large and contain multiple individual V, D and J genes. The immunoglobulin heavy chain (*Igh*) and immunoglobulin kappa light chain (*Igk*) loci are the largest multigene loci in the mammalian genome and V(D)J recombination is one of the most complicated genetic processes in the nucleus. The challenge for the appropriate lymphocyte is one of macro-management—to make all of the antigen receptor genes in a particular locus available for recombination at the appropriate developmental time-point. Conversely, these large loci must be kept closed in lymphocytes in which they do not normally recombine, to guard against genomic instability generated by the DNA double strand breaks inherent to the V(D)J recombination process. To manage all of these demanding criteria, V(D)J recombination is regulated at numerous levels. It is restricted to lymphocytes since the Rag genes which control the DNA double-strand break step of recombination are only expressed in these cells. Within the lymphocyte lineage, immunoglobulin recombination is restricted to B-lymphocytes and TCR recombination to T-lymphocytes by regulation of locus accessibility, which occurs at multiple levels. Accessibility of recombination signal sequences (RSSs) flanking individual V, D and J genes at the nucleosomal level is the key micro-management mechanism, which is discussed in greater detail in other chapters. This chapter will explore how the antigen receptor loci are regulated as a whole, focussing on the *Igh* locus as a paradigm for the mechanisms involved. Numerous recent studies have begun to unravel the complex and complementary processes involved in this large-scale locus organisation. We will examine the structure of the *Igh* locus and the large-scale and higher-order chromatin remodelling processes associated with V(D)J recombination, at the level of the locus itself, its conformational changes and its dynamic localisation within the nucleus.

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V(D)J Recombination, edited by Pierre Ferrier.

Introduction

B-Cell Development

In order to generate the primary repertoire of immunoglobulins and T-cell receptors, antigen receptor loci undergo variable, diversity and joining (V(D)J) recombination in B- and T-lymphocytes. This involves generation of DNA double strand breaks at recombination signal sequences (RSSs) flanking individual genes, followed by removal of the intervening DNA and juxtapositioning and ligation of the recombining gene segments. This process is regulated at several levels. First, recombination is catalyzed by a recombinase complex containing the protein products of the recombinase activating genes *Rag1* and *Rag2*.[1] RAG expression is restricted to precursor lymphocytes, thereby restricting V(D)J recombination to these cells. Second, within precursor lymphocytes, this process is strictly lineage-specific with heavy (*Igh*) and light (*Igk* and *Igl*) immunoglobulin loci only fully recombining in B-lymphocytes and T-cell receptor loci (*Tcra*, *Tcrb*, *Tcrg* and *Tcrd*) only recombining in T-cells. Third, within lineages, loci are recombined in a precise order. Recombination of the *Igh* locus in pro-B-cells is the earliest step in the generation of the mature antibody repertoire in B-lymphocytes and is followed by *Igk* and then *Igl* recombination in preB-cells. Fourth, the order is also strictly maintained within loci: D_H-to-J_H recombination occurs on both *Igh* alleles before V_H-to-DJ_H recombination takes place.[2] Finally, RAG activity is targeted to RSSs flanking individual V, D and J genes. Apart from restriction to lymphocytes by restricted RAG expression, this ordered regulation is effected by several levels of immunoglobulin locus accessibility.

Description of the Igh Locus

This need for multiple levels of regulation is both necessitated and complicated by the enormous size of the antigen receptor loci. The mouse *Igh* and *Igk* loci are the largest multigene loci known, with sizes of 3Mb and 3.2 Mb respectively.[3,4] The *Igh* locus of the C57BL/6 mouse has recently been completely assembled and annotated. It comprises 195 V_H genes spanning 2.5Mb, 10 D_H genes (~60kb), 4 J_H genes (2kb) and 8 constant (C_H) genes (200kb) (Fig. 1).[3,5] The V genes are organized into 16 families of varying sizes, based on sequence homology. The majority are functional, but a large proportion (85) are classed as pseudogenes, some of which nevertheless recombine, although they do not make functional Ig polypeptides. All of the functional V, D and J genes are used in multiple different combinations and this large choice of V, D and J recombination partners provides the first step in immunoglobulin diversity. However there is a bias in recombination frequency between the 3' and 5' ends of the V region i.e., the 3' end is recombined more frequently in fetal liver and in the earliest bone marrow B-cells. The extent of the bias varies between mouse strains and recombination frequency normalises in later B-cells.[6-8] Large-scale mechanisms which may contribute to the bias will be discussed below.

Each V and D gene has its own promoter and all genes are transcribed in the same orientation (Fig. 1), although this is not the case for all antigen receptor loci. Promoters have several features in common, but also family-specific differences which may be a factor in observed family-specific differences in recombination frequency.[3] The human Igh locus is smaller (1Mb) and contains only 123 V genes, 79 of which are pseudogenes.[9] The V regions of the *Igh* and other antigen receptor loci are believed to have evolved from much smaller V gene clusters that were frequently duplicated, possibly due to ability of the Rag enzymes to act as general transposases.[10,11] Consequently even within species there are significant differences in numbers and family distribution of V genes, particularly in the mouse.[3,12] For example the 7183 gene family at the 3' end of the V region has 21 V genes in the C57BL/6 strain and 49 V genes in the 129 strain. This is an extremely important consideration when comparing *Igh* locus recombination between mouse strains. In the future it is likely that studies on the C57BL/6 strain will predominate as this is the strain in which the mouse genome was sequenced and thus contains all other relevant sequence information.

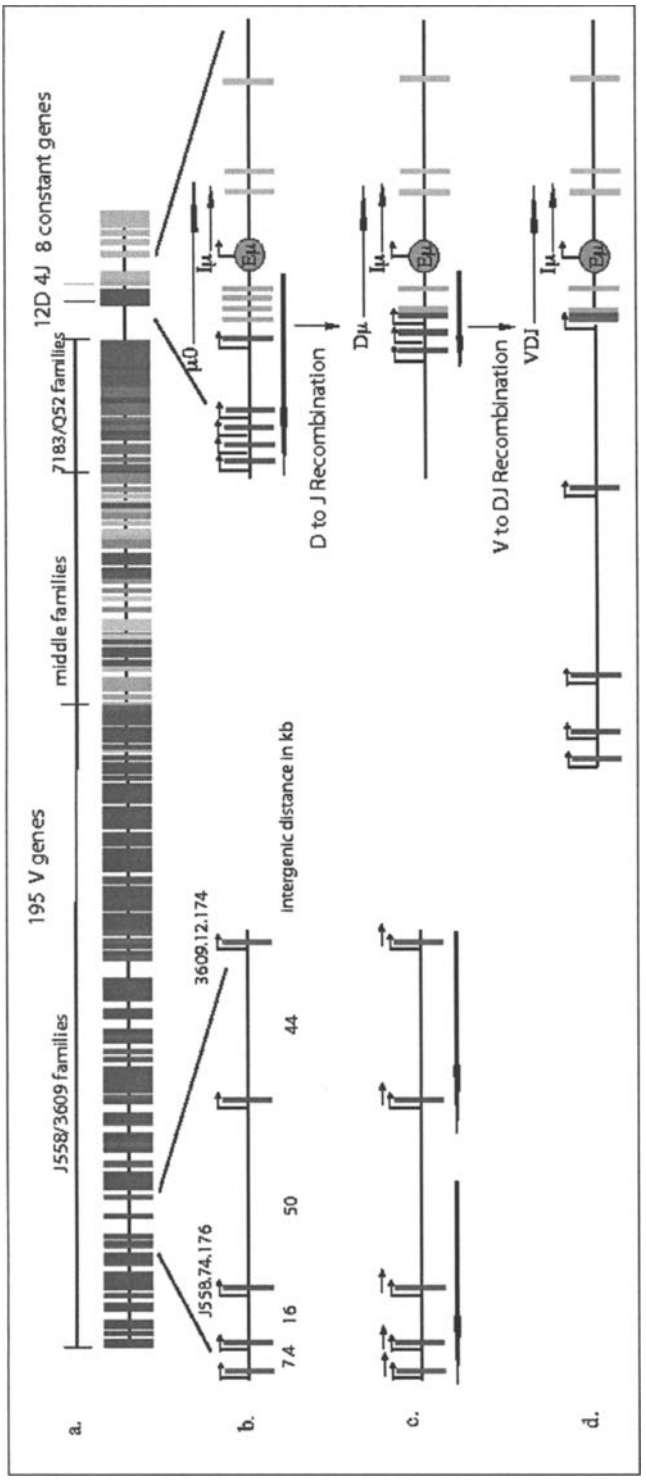

Figure 1. Schematic drawing of the mouse C57BL/6 *Igh* locus and noncoding RNA transcription during V(D)J recombination. a) Approximate scale drawing of the V, D and J gene families, with different colors/shades representing different V gene families. Approximate distances between genes are depicted, albeit for clarity the genes occupy a disproportionately large linear space. For example the J558/3609 region which occupies the 5′ 1.5Mb contains widely spaced J558 genes interspersed with 3609 genes in the 5′ 1Mb (see zoomed out picture), but highly clustered J558 genes with no intervening 3609 genes in the 3′ 500kb. Transcripts (sense above, antisense below the genes) are presented by horizontal arrows. b) Magnified picture of the germline *Igh* locus, showing noncoding RNA transcripts. Numbers above the V genes indicate gene identity (see ref. 3). c) Magnified picture of the DJ recombined locus, as above. d) Magnified picture of the VDJ recombined locus, as above.

Chromatin Remodeling

How is such an enormous piece of DNA manipulated in the nucleus to ensure that its many genes are accessible for V(D)J recombination in pro-B-cells, but not in T-cells or later stage B-cells? While recombination itself is a genetic process i.e., alterations are made in the DNA sequence of the locus, it is regulated by a multitude of epigenetic processes i.e., heritable changes in chromatin structure that do not involve a change to the primary sequence. It is important to bear in mind that structurally this extremely long DNA sequence is not simply a piece of string, but occupies a 3-dimensional space in the nucleus. It is estimated that the linear length of DNA helix contained in a mammalian genome is 3 meters and this must be accommodated in a cell nucleus with a diameter of 5-10μm. This is achieved at the basic level by wrapping the DNA helix around the histone octamer in the nucleosome, followed by several levels of higher order folding of nucleosomes over each other, in ways that are not well understood (Fig. 2). To facilitate general gene transcription, this higher order chromatin must first be unravelled to achieve a more open and ultimately single nucleosomal structure. This kind of multi-tiered regulation also controls V(D)J recombination[13] and recent studies have explored the extent to which these mechanisms are involved in V(D)J recombination. This chapter will explore several aspects in detail—noncoding RNA transcription, nuclear localization and regulatory elements, while placing these in context with other processes including histone modification, which will be explored in detail in other chapters in this volume. We will explore what is currently known, what current studies may predict and what the future directions are likely to be.

Noncoding RNA Transcription

Contrary to the 'central dogma' that 'DNA makes RNA makes protein', numerous genome-wide transcriptional analyses have estimated that over half of all transcribed mammalian genomic sequences are nonprotein-coding[14] and some of this transcription is predicted to play key roles in gene regulation. Notably, over 20 years ago, the *Igh* locus was the one of the first loci shown to express noncoding RNAs. This transcription was originally termed 'sterile' or 'germline' to distinguish it from coding transcription from V(D)J recombined genes. In the *Igh* locus, the first germline transcripts occur before D_H-to-J_H recombination and initiate from two regions; the intronic enhancer E_μ (I_μ transcript)[15] and from a promoter, PDQ52, immediately upstream of the most 3' D_H gene segment, DQ52 ($_\mu$0 transcript)[16] (Fig. 1). Following D_H-to-J_H recombination, the DJ_H gene segment produces D_μ transcripts[17] and sense germline transcription initiates over the V_H genes (Fig. 1).[18,19] Subsequently, noncoding RNA transcripts have been identified in all antigen receptor loci across gene segments competent for recombination.[20] The discovery of V_H gene germline transcription formed the basis of the accessibility hypothesis, which proposed that

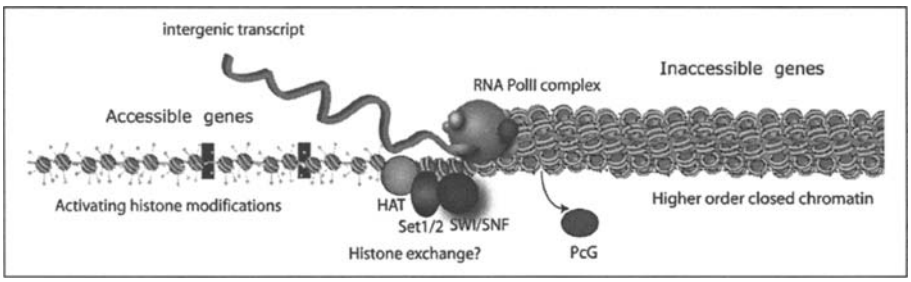

Figure 2. Model of intergenic transcription. The RNA Pol II complex, depicted as a sphere, with associated smaller spheres denoting transcription factors of the basal complex, is pictured processing through closed chromatin, recruiting activating chromatin remodelling factors and promoting egress of PcG (Polycomb) proteins. HAT: histone acetyltransferase; Set1/2: members of Trithorax family of histone H3 HMTs; SWI/SNF: SWItch/sucrose nonfermentable.

lineage- and stage-specificity of recombination are regulated by differential chromatin accessibility of antigen receptor gene segments to the recombinase machinery, with germline transcription associated with open chromatin.[18,21] However, a function for V_H germline transcription has not been formally demonstrated and it has been argued that it may be a secondary effect of the V_H gene promoters becoming accessible for V_H-to-DJ_H recombination. Neither have functions yet been assigned to the $_\mu 0$ and I_μ transcripts. However, quantitative RNA-FISH visualization of I_μ transcription[22,23] have classed this transcript as a 'supergene' i.e., a gene that is transcribed almost continuously from both alleles in an individual nucleus.[24] This property applies to surprisingly few genes, β-globin among them. I_μ is the first noncoding 'supergene' to be identified and would more correctly be termed a 'super-transcription unit', since it is a noncoding, intergenic transcript. The possible implications of this high level transcription for the role of noncoding RNA transcription in the *Igh* locus will be discussed below.

Intergenic Transcription

Recent studies suggest that intergenic transcription may play a role in opening up the *Igh* locus. In the large V region, the relatively small V genes (500bp) are separated by enormous intergenic distances (10-20kb).[3] The chromatin remodeling processes previously discovered are largely confined to V genes (germline transcription above, histone modifications, discussed in detail in other chapters). Such focused alterations are unlikely to be sufficient to open the closed chromatin conformation of the locus, the default state in nonB-cells[25] and additional large-scale processes were investigated. In numerous loci, including β-globin and the MHC complex, intergenic transcription delineates domains of modified chromatin that surround active genes and their regulatory elements.[26-29] RNA polymerase II (PolII) recruits a wide range of chromatin remodeling and histone-modifying factors, including histone acetyltransferases (HATs) and Set1 and Set2 histone methyltransferases (HMTs), required for histone modifications associated with gene activation.[30-34] Furthermore, transcription triggers histone turnover and the deposition of variant histone H3.3, enriched with active modifications.[35] Collectively these activities suggest several mechanisms by which the processing activity of elongating PolII complex can achieve chromatin accessibility.[36,37] Accordingly, intergenic transcription has been proposed to drive through repressive chromatin in several multigene loci, recruiting remodeling factors and opening up large chromatin domains into a poised state, thus facilitating further focused chromatin opening over genes to regulate gene expression (Fig. 2).[38] In several large developmentally regulated loci,[28] this is believed to occur by transcription-dependent[39,40] higher order chromatin remodeling and looping out of their chromosome territories.[41,42]

In many cases, intergenic transcription may only need to drive through once or twice to open up the chromatin. However, in other instances, including the Drosophila homeotic bithorax complex, continuous intergenic transcription is required to prevent binding of repressive Polycomb proteins with H3K27 HMT activity and to recruit activating Trithorax H3K4 HMTs.[43] Furthermore, many enhancers and Locus Control Regions undergo transcription, which is essential for activation of their target genes and thus transcription from an intergenic regulatory region can influence expression of a distal gene.[44]

Intergenic Transcription in the Mouse *Igh* Locus V Region

Analysis of transcription from genes and intergenic regions throughout the *Igh* V_H region, using RT-PCR to measure steady-state levels and RNA-FISH to visualize primary transcripts on individual alleles in single cells revealed that intergenic transcription occurs throughout the Igh V region. It is absent on germline alleles that have not yet recombined D_H to J_H in early B-cells, is expressed on the majority of D_HJ_H recombined alleles and disappears once V to DJ recombination has occurred. This tightly developmentally regulated pattern of expression is characteristic of a large-scale functional process. Furthermore, patterns of transcripts detected by RNA-FISH were extended over large regions, suggesting extensive transcription on individual alleles (Fig. 3).

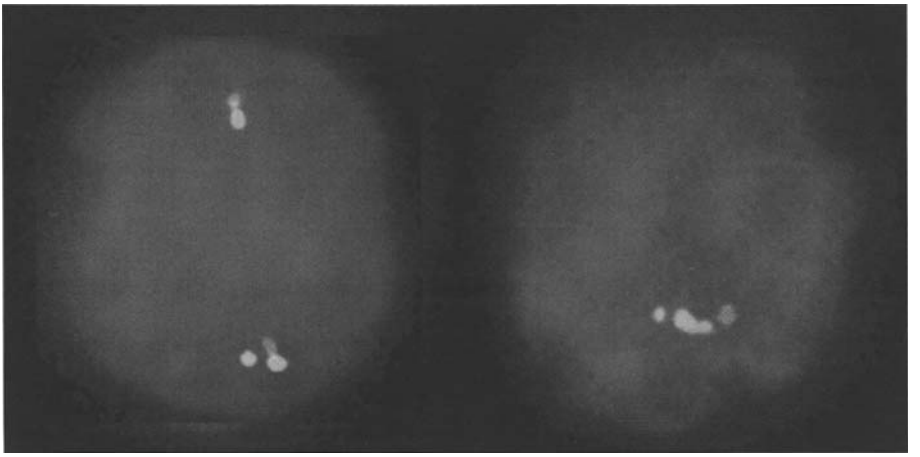

Figure 3. Visualization of antisense transcription in the *Igh* locus by RNA-FISH. Nuclei from ex vivo wild-type bone marrow sorted for Fraction B-cells (the majority of cells are DJ recombined). I_μ sense transcripts, hybridized with a single-stranded antisense probe, are detected by Texas Red (red/light grey punctate signals). J558 gene family antisense transcripts, hybridized with a single-stranded sense probe, are detected by fluoroisothiocyanate (FITC) (green/dark grey extended signals). Nuclei are counterstained with 4′, 6-diamidino-2-phenylindole (blue). (adapted with permission from ref. 22). A color version of this figure is available at www.landesbioscience.com/curie.

Antisense Transcription

Surprisingly intergenic transcription only occurred on the antisense strand, where transcription also occurred over the V_H genes. Antisense transcription has classically been associated with transcription repression in imprinted loci, in which it appears to silence gene expression in *cis* from the allele on which it is expressed. The best studied example is Air (Antisense to Igf2 receptor) transcription (108kb transcript), which silences expression of the Igf2R, with which it partially overlaps, in *cis*.[45] Antisense transcription has also been documented in several lower eukaryotic systems to generate dsRNA and heterochromatin formation.[46,47] However, it is now thought that the majority of mammalian transcription units display overlapping sense and antisense transcription.[48] This high incidence and co-ordinate regulation of many sense-antisense pairs, indicates that antisense transcription is involved in mechanisms other than its classical association with transcriptional repression.[45,49] For example, antisense transcription across the yeast PHO5 gene promoter is required to increase the rate of transcription and is believed to evict histones to enable greater access of RNAPolII to the gene.[50] In the mammalian HOXA cluster, antisense intergenic transcription is required to activate neighboring HOX genes, in part by disrupting interaction with repressive PcG complexes.[51] These examples may be the first of many in which antisense transcription plays an activating role.

Antisense Transcription in the *Igh* Locus V Region

In the *Igh* locus, the absence of V region antisense transcription on germline alleles argues against this transcription keeping the V_H region closed, since it would have to be present before D_H to J_H recombination to do this. Rather, it it is consistent with a role for intergenic transcription in opening up the V_H region and thus it doesn't appear on germline alleles, since the V_H region must be kept closed until D_HJ_H recombination has taken place. Furthermore antisense transcription is biallelic, arguing against a monoallelic mechanism of silencing one allele to prevent recombination. The expression pattern of antisense transcription in the *Igh* locus thus argues in favor of its having an activating rather than a repressive role in V(D)J recombination. Further, this transcription is not controlled by V_H gene promoters and thus cannot be regarded as a by-product of the activation of

these promoters for V(D)J recombination. This is the first evidence in support of a functional role for germline transcription in *Igh* V(D)J recombination. We proposed this large-scale transcription remodels the V_H region to facilitate accessibility for V_H-to-DJ_H recombination, perhaps by directing chromatin remodeling factors to direct other changes in chromatin structure that precede V(D)J recombination (Fig. 4).[22] These occur mostly over the V_H genes and include loss of histone H3K9

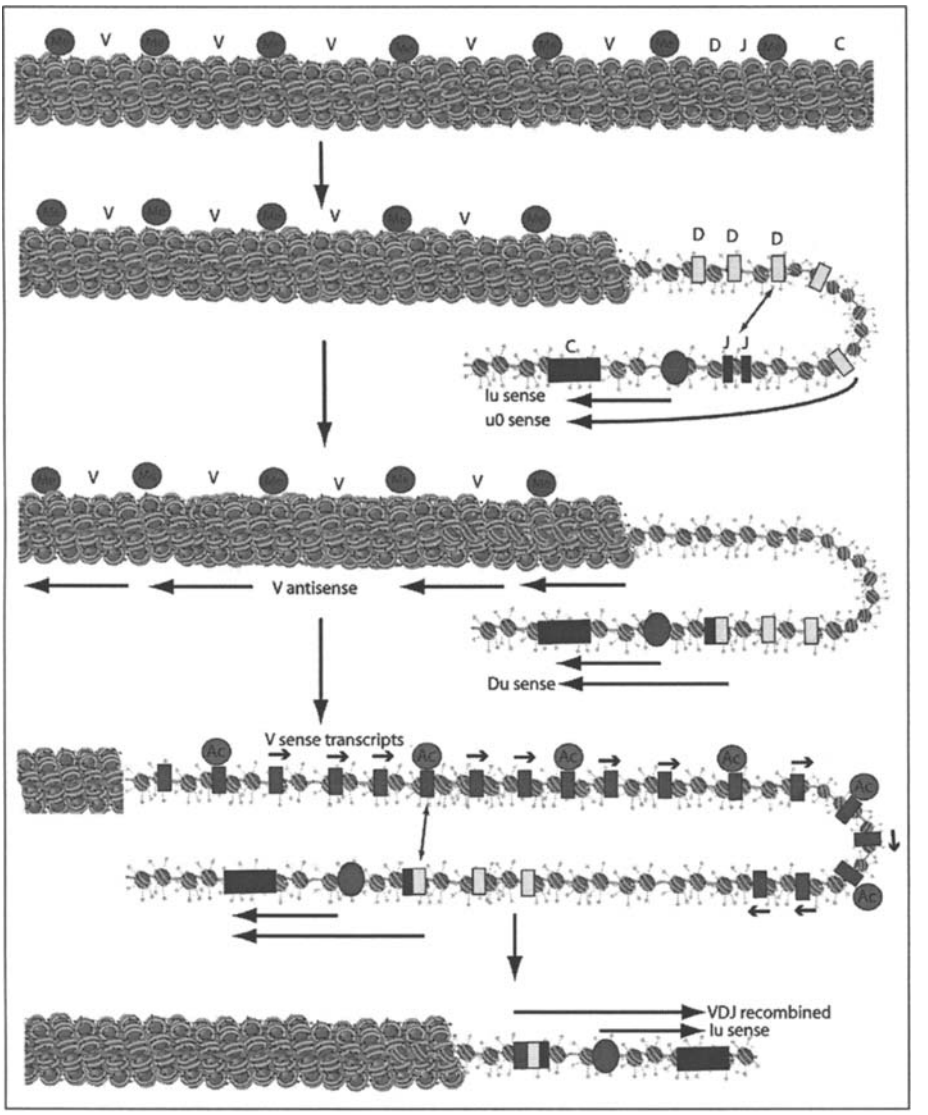

Figure 4. Model of role of antisense intergenic transcription in *Igh* V(D)J recombination. Schematic of order of events, depicting alterations in chromatin structure. Key: Multiple red/light grey boxes: V genes; yellow/light grey boxe: D genes; blue/dark grey boxes: J genes; E_μ: green oval; large rectangular box: constant region; black arrows: sense/antisense transcripts; Me in red circle: repressive histone modifications; Ac in green circle: activating histone modifications. A color version of this figure is available online at www.landesbioscience.com/curie.

methylation, acetylation of histones H3 and H4, markers of accessible chromatin, histone H3.3 exchange and methylation of H3 lysine 27 (H3-27).[25,52-55]

Antisense and Intergenic Transcription in the *Igh* D Region

The discovery of intergenic antisense transcription over the *Igh* V region before V_H-to-D_HJ_H recombination raised the question of whether similar transcriptional processes precede other V(D)J recombination events. Antisense intergenic transcription also occurs throughout the D_H (60kb) and J_H regions of the mouse *Igh* locus in pro-B-cells poised for D_H-to-J_H recombination and is thus a widespread process during V(D)J recombination.[56] It is activated on germline alleles before D_H-to-J_H recombination. Notably, it initiates near to and is regulated by the intronic enhancer E_μ.[56] E_μ was originally proposed to regulate V_H to DJ_H recombination.[57,58] However, recent studies have shown that targeted deletion of E_μ causes a defect in D_H-to-J_H recombination, suggesting that E_μ primarily regulates this process and that defects in V_H to DJ_H recombination may be secondary to this earlier defect.[59,60] It is not yet understood how E_μ regulates D_H to J_H recombination. Transcription of the I_μ 'supergene' initiates immediately downstream. Deletion of E_μ results in loss of both I_μ sense[60] and D region antisense transcription, up to 50 kb away.[56] This suggests that E_μ controls D_H-to-J_H recombination at least in part by activating germline Igh transcription and that in particular, the processivity of the antisense transcription renders the D_H and J_H regions accessible for D_H-to-J_H recombination (Fig. 4).

This model is supported by concomitant increases in DNase I sensitivity, histone H3 and H4 acetylation, H3K4 methylation and nucleosome remodeling enzymes over D_H and J_H genes in pro-B-cells.[52,61,62] Histone acetylation is widespread throughout the D_H region,[52] but is highest over the J_H region and the DQ52 gene,[52,61] which is preferentially used in early D_H-to-J_H recombination.[63] This model is in agreement with a recent suggestion that the region encompassing DQ52, the four J genes and E_μ forms a separate chromatin domain to the rest of the D_H region.[62] Strikingly, DQ52 is the only D_H gene that expresses both sense and antisense germline transcripts and this transcription overlap extends into the J_H region.[56] These data suggest strongly that the transcripts do not produce dsRNAs that lead to heterochromatin. Indeed, they are coordinately up-regulated by E_μ. Additionally, there is no sense germline transcription in the remainder of the D_H region, precluding dsRNA formation.[56,64] Nevertheless, a recent report of active retention of repressive histone marks over the middle D_H genes has led to the opposite hypothesis that antisense transcription may contribute to repression of these genes, by formation of dsRNA and Dicer-mediated heterochromatinization, albeit no dsRNA was detected.[64] Definitive resolution of these opposing models must await clarification of the functional role of antisense transcription by targeted removal of this transcription in vivo. Similar gene targeting studies have shown that intergenic transcription is functionally required for V(D)J recombination at the *Tcra* locus, but in this case it originates from the sense strand.[65] This suggests that the strand origin is not important, which supports the model that the processing activity is the key function of this transcription.

D_H antisense transcripts initiate on germline alleles and V_H transcripts on DJ recombined alleles and D_H and V_H antisense transcripts are rarely associated on individual alleles.[56] Thus there is a stepwise progression of antisense intergenic transcription, in a strikingly similar pattern to the stepwise progression of active histone modifications during *Igh* V(D)J recombination. These occur first over the D_HJ_H region, then sequentially over the 3' end, the middle region and the 5' end of the V_H region.[53,66-68] Thus antisense intergenic transcription may facilitate the exchange of repressive histone marks associated with the locus in nonB-cells with active histone marks, perhaps by histone exchange in favor of active histones e.g., H3.3 (Fig. 4).[25] Notably in the *Tcra* locus, intergenic transcription has been shown to increase active histone marks over genes.[65]

Subnuclear Relocalisation

In addition to these localized and large-scale epigenetic changes over the *Igh* locus, the location of the locus in the nucleus has an enormous impact on its recombination potential. In nonB-lymphoid cells, the *Igh* and *Igk* loci are maintained at the nuclear periphery, generally

regarded as a repressive chromatin environment, although it is not clear whether the *Igh* is specifically associated with repressive chromatin at this location.[69] The D_HJ_H distal J558 V_H genes are oriented towards the nuclear envelope and the locus is effectively 'tethered' at the periphery via the J558 genes, while the D_HJ_H region is oriented towards the centre of the nucleus, which may contribute to D_HJ_H occurring before V_H to D_HJ_H recombination.[70] In early B-cells undergoing V(D)J recombination, both *Igh* and *Igk* alleles are repositioned to the euchromatic interior of the nucleus, a region permissive for transcription.[69] The relocation is dependent on interleukin-7 receptor signalling, but is independent of RAG[69] or Pax5[71] expression. This nuclear repositioning appears to be sufficient for D_HJ_H recombination and V_H to D_HJ_H recombination of D_H-proximal V_H genes in the *Igh* locus.

3-Dimensional Alterations in Chromatin Structure

However, to achieve recombination of middle and D_H-distal V_H genes, central nuclear repositioning is not sufficient, presumably due to the enormous size of the locus. An additional process, termed locus contraction, is required. This juxtaposes the distal V_H genes with the D_HJ_H recombined gene segment in pro-B-cells and is mediated by higher-order chromatin looping of individual IgH subdomains.[72,73] It is regulated by the transcription factor Pax5 (Fig. 5).[71] Pax5 is the pivotal transcription factor that regulates establishment and maintenance of B-lymphocyte identity and its absence prevents recombination of middle and D-distal genes.[74] Looping is also regulated by the multifunctional transcription factor, YY1, which binds E_μ.[75] It is unclear how

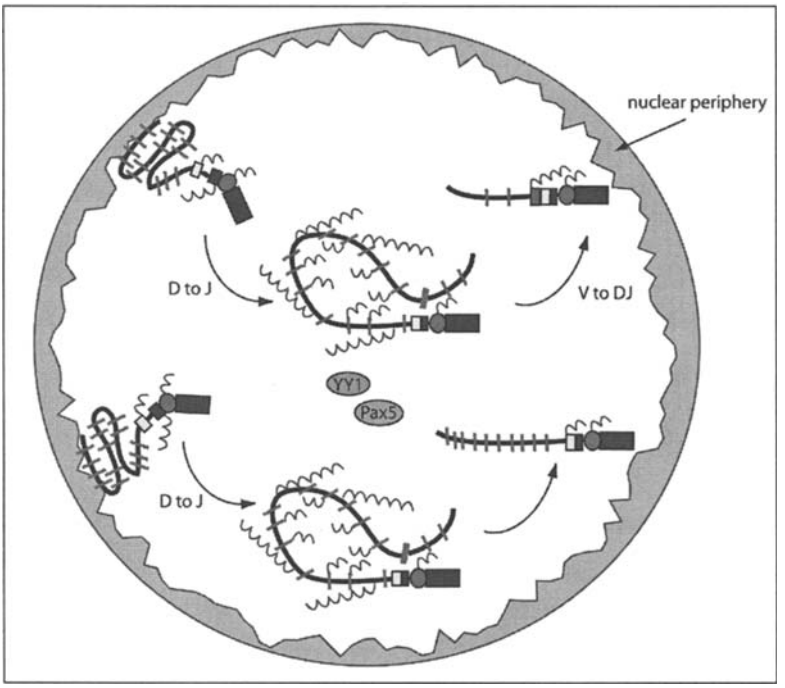

Figure 5. Nuclear organisation of the *Igh* locus. The sequential stages of *Igh* V(D)J recombination are represented in the context of the spatial location of the *Igh* loci in the nucleus and their large-scale conformation changes. The locus is initially tethered at the nuclear periphery via the 5′ end of the V region. Key: Multiple red/light grey lines: V genes; yellow/light grey box: D region; blue/dark grey box: J region; E_μ: green oval; large rectangular box: constant region; short (blue) squiggles: sense transcripts; long (purple) squiggles: antisense transcripts. A color version of this figure is available online at www.landesbioscience.com/curie.

either Pax5 or YY1 enable DNA looping. Neither is required for germline transcription or histone acetylation of these genes,[67] suggesting that these processes are either necessary but not sufficient, or independent of looping. One possibility is that YY1 may recruit other parts of the locus to the enhancer and future studies on the role of E_μ in looping will be informative. YY1 binds Ezh2, a polycomb group protein with H3K27 HMTase activity, although this binding has not yet been shown in B-cells. Ezh2 is required also for recombination of distal V genes.[55] Its mechanism of action is currently unclear, but intriguingly it also appears to be required for DNA looping of the Igh V_H region (A Tarahkovsky, personal communication).

Transcription Factories

A large body of recent evidence has shown that transcription does not occur homogeneously throughout the nucleus, but appears to be concentrated in sub-nuclear foci of active RNA PolII complexes, termed transcription factories.[76,77] Individual transcription factories are believed to contain up to ten RNA PolII complexes and to transcribe several genes simultaneously.[76] These genes can be up to 40 MB apart on the same chromosome and even on separate chromosomes.[23,78] These are dynamic interactions that reflect the frequency of transcription of individual genes.[78] Most genes are not transcribed continuously, but rather switched on and off stochastically.[79] The I_μ 'supergene' is transcribed almost all of the time in both proB and mature B-cells and is thus almost continuously associated with a transcription factory.[22,23] It has recently been shown that enhancers can relocate genes away from the nuclear periphery by recruiting them to a transcription factory.[80] In a similar manner, E_μ may promote nuclear relocation by recruiting the DJ region to a transcription factory in the nuclear interior, where E_μ facilitated transcription may then keep the D_HJ_H region in the transcription factory, providing a relatively stable focal point for DNA looping.

Biased Recombination Frequency Explained by Numerous Mechanisms

The studies above provide several large-scale contributing reasons for preferential recombination of 3' V_H genes in early B-cells. First, the *Igh* is tethered at the nuclear periphery in nonB-cells via the J558 genes at the 5' end, thus the 3' genes are oriented towards and relocated into the central euchromatin first. Following relocation, it appears that proximal V_H genes are less dependent on DNA looping of the V_H region for recombination, presumably due to their proximity to the D_HJ_H region.[71,72] Furthermore, all the factors that regulate looping (Pax5, YY1, Ezh2) are only required for recombination of distal V_H genes. The interleukin 7 receptor is also required for recombination of 5' genes, but not 3' V_H genes in the bone marrow.[19] Since it activates germline transcription over 5' V_H genes, but not 3' V_H genes, it was proposed that it increased V region chromatin accessibility to the recombinase.[19] Subsequent studies have identified other contributory mechanisms regulated by the IL7R. It is required for relocation from the nuclear periphery[69] and histone acetylation of 5' V_H genes.[66,67]

Allelic Choice and Allelic Exclusion

Ultimately the goal of the B-lymphocyte is to express a $V_HD_HJ_H$ recombined *Igh* gene from only one allele at the cell surface. Surface expression of the immunoglobulin polypeptide is believed to lead to a feedback signaling cascade that silences the second allele, a mechanism termed allelic exclusion.[2] This ensures that each lymphocyte produces monoclonal antibodies that recognize a single antigen with high specificity. Several processes contribute to this monoallelic expression. In the *Igh* locus, V_H to D_HJ_H recombination is asynchronous—i.e., one allele undergoes recombination first. This reduces the danger of simultaneously producing two productive recombination events. However, unlike the *Igk* (see below), it is unclear how this allelic choice is achieved in the *Igh* locus. Relocation and antisense intergenic transcription appear to be biallelic. It is currently unclear whether locus contraction is mono or biallellic and further studies are required to reveal whether it plays a role in allelic choice.[72,73] However, it is clear that

the opening mechanisms required for V(D)J recombination are mirrored by a set of opposing processes designed to stop further V(D)J recombination once a productive recombination event has yielded a protein product. Histone acetylation is reduced over V_H genes,[54,81] sense and antisense germline transcription is lost,[22] locus de-contraction occurs.[72] These processes occur on both alleles. An additional mechanism occurs specifically on the second allele that has either yielded a nonproductive $V_HD_HJ_H$ rearrangement or has not yet managed to rearrange the V_H gene (D_HJ_H rearranged allele). In either case, the allele is believed to be recruited to repressive pericentromeric heterochromatin, which may preclude further V to DJ recombination.[72,82] It is recruited via the 5' end of the V region and silencing of the locus is not complete. The I_μ,[22] DJ rearranged[83] and sense germline transcripts from 3' V genes[84] continues to be transcribed. This is presumably because D to J recombination has already occurred on both alleles and thus only the V region needs to be prevented from further recombination.

Other Antigen Receptor Loci

We have focused on the *Igh* locus, which has proved to be a useful paradigm for other antigen receptor loci, since, with some exceptions, processes discovered in the *Igh* locus, also occur in other antigen receptor loci. For example, noncoding sense RNA transcription over V genes has been observed in most other antigen receptor loci.[20] Similarly relocation from the nuclear periphery and locus contraction by DNA looping has been reported in the *Igk*, *Tcra* and *Tcrb* loci.[69,72,85] However, it is not yet known how widespread the process of antisense and/or intergenic transcription is. The biggest difference between recombination of *Igh* and *Igk* is the order and nature of the events that ensure monoallelic expression. In contrast to the *Igh*, in which this appears to be controlled after V(D)J recombination, the *Igk* loci undergo several monoallelic processes before V to J recombination, which render one allele preferentially more available for the initial recombination event. One allele is preferentially DNA demethylated[86] and acquires active histone marks before V to J recombination, while the second allele remains DNA methylated and is recruited to heterochromatin before V to J recombination.[87]

Future Directions

Further studies are required to unequivocally determine the function of antisense intergenic transcription in the *Igh* locus in vivo. Furthermore is it the processivity of the transcription that is important, its strand-specificity, or indeed the transcripts themselves? These are also important considerations for other antigen receptor loci.

There is also little known about other chromatin remodeling processes in *Igh* intergenic regions. It is unclear whether noncoding RNA transcription is regulated by the same histone modifications as coding transcription. Since there are now more than 150 known histone modifications,[88] it will be important to explore the possibility that recombination may have a unique histone code which does not correspond to the code for transcription.

There is as yet no regulatory element defined for the *Igh* V region. However, a novel pro-B-cell specific HS site has recently been identified 5' of the V region.[89] It will be interesting to see if this element regulates V to DJ recombination, albeit initial characterization indicates a repressive role. How might this or another regulatory element function? It might activate V region antisense transcription or enable DNA looping by interacting with elements close to the DJ region. Further, the large size of the V region and the differences in recombination timing and dependence on the IL7R, Pax5, Ezh2 and YY1 in different domains, suggest that there may be boundary elements separating different regions. Furthermore there is 90kb of uncharacterized sequence between the last V_H and first D_H gene and it will be interesting to see if it contains any enhancers, or insulator elements to prevent the V region recombining before the DJ region.

Acknowledgements

The authors thank members of the Corcoran group for helpful discussions. This work was supported by the Biotechnology and Biological Sciences Research Council.

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