Epigenetic regulation of antigen receptor gene rearrangement

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Abstract

V(D)J recombination assembles antigen-specific immunoglobulin and T-cell receptor variable region genes from germline V, D, and J segments during lymphocyte development. Regulation of this site-specific DNA rearrangement process occurs with respect to the cell type and stage of differentiation, order of locus recombination, and allele usage. Many of these controls are mediated via the modulation of gene accessibility to the V(D)J recombinase. Here, we summarise recent advances regarding the impact of nuclear organisation and epigenetic-based mechanisms on the regulation of V(D)J recombination.

Introduction and context

B and T lymphocytes form the adaptive arm of the immune system in jawed vertebrates and can specifically respond to an astounding number of foreign antigens. This property depends largely on a series of site-specific DNA rearrangement events between separate V, D, and J gene segments at antigen receptor (AR)-encoding loci, a process referred to as V(D)J recombination [1]. There are seven AR loci: the immunoglobulin heavy (IgH) and light (Igκ and Igλ) chain loci expressed in B cells and the T-cell receptor (TCR) α, β, γ, and δ loci expressed in T cells. For V(D)J recombination to occur, the presence of the lymphoid-specific proteins RAG1 and RAG2 and the ubiquitously expressed DNA repair factors from the non-homologous end joining pathway is required. Strict regulation of this process ensures B- or T-cell lineage specificity, dictates the temporal order of Ig or TCR rearrangements, respectively, and allows allelic exclusion at certain AR genes (for a recent review, see [2]). The lineage and temporal specificities of the common V(D)J recombinase are regulated primarily at the level of chromosomal accessibility. During the past decade, AR genes have served as tractable models to study the regulation of gene expression at complex genomic loci,

using gene targeting technologies in particular [3,4]. These studies have notably led to a better appreciation of the hierarchical function of transcriptional *cis*-regulatory elements (enhancers and germline promoters) and their impact on the control of V(D)J recombination via the modulation of chromatin structure (e.g., [4-6]). Within a given AR locus, enhancer- or promoter-dependent chromosomal accessibility to the V(D)J recombinase generally correlate with the presence of several epigenetic marks synonymous with euchromatin (germline transcription [GT], accessibility to endonucleases, CpG demethylation, and enrichment in active histone marks) and the delocalisation from heterochromatic regions of the nucleus (the pericentromeric heterochromatin and nuclear periphery) [7]. How these epigenetic features are achieved at the molecular level is currently the focus of intense investigation.

Major recent advances

While we know that the presence of active epigenetic marks at AR loci generally correlates with tissue- and stage-specific V(D)J recombination events, its potential role in effectively targeting the recombinase to the individual loci remains unclear (reviewed in [7]). A first insight came

from studies demonstrating that the PHD (plant homeodomain) finger of RAG2 binds to histone H3 dimethylated or trimethylated at K4 (H3K4me2/3), with a preference for H3K4me3, and is enhanced by the presence of symmetrically dimethylated H3R2 (H3R2me2s) [8-10]. More recently, Lieber and colleagues [11] reported that H3K4me3 stimulates RAG-mediated *cis*-DNA cleavage *in vitro*, an effect that was also achieved by simply adding H3K4me3 peptide *in trans* to the reaction. This supports the notion of a direct impact of epigenetic environment on the recombinase catalytic activity. The authors also observed that most common cryptic recombination signals (RSs) known to be aberrantly used in acute T-cell leukaemia-transformed cells are located nearby H3K4me3-enriched domains in normal T cells. This could link translocation frequency at cryptic RSs with the epigenetic landscape in cells undergoing V(D)J recombination.

The RAG2-H3K4me3 connection raised the possibility that a specific histone code may restrict V(D)J recombination *in vivo*. However, H3K4me3 is not confined to the AR loci, and to date no particular combination of histone marks has been exclusively associated with these loci [12,13]. Irrespective of these concerns, the question of how active epigenetic marks are established through the recombining gene segments and associated RSs remains. One possibility could be that GT, either sense or antisense, impinges on epigenetic marking before AR assembly [14,15]. (Notice that the functional significance, if any, of antisense transcription at a given Ig/TCR locus or cluster [or both] still requires thorough investigation; also, see [16,17].) Accordingly, suppression of GT throughout the 5' TCR-J α region interferes with epigenetic marking and inhibits V α -J α rearrangements in this region [18]. Likewise, in an inducible pre-B cell line, GT at Ig κ / λ loci appears to precede the establishment of active epigenetic marks [13]. In this context, targeted deletion of AR-associated enhancers generally results in reduced levels of germline transcripts and active histone marks [6], even though the magnitude of these effects may vary depending on the locus and, within a given locus, the particular region (i.e., the V versus D/J region) [19,20]. Ultimately, determining the precise enhancer function in chromatin remodelling may require mutational/deletional analysis of the discrete transcription factor (TF)-binding sites within these regulatory modules.

One arm of current research aims to decipher the gene expression programs that coordinate lymphoid cell differentiation and V(D)J recombination. An outstanding example is the dissection of the signalling pathways orchestrating the transition through the pre-B-cell receptor (pre-BCR) checkpoint during pro-B to pre-B

cell differentiation and induction of Ig κ recombination. On the one hand, pre-BCR signalling induces the expression of the TF interferon regulatory factor 4 (IRF4) and activation of the Ras-MEK-Erk phosphotyrosine kinase cascade, which in turn promotes cell cycle exit (via repression of cyclin Ccdn3) and modulates the expression of antagonist TFs E2A and Id3 – two features known to favour Ig gene assembly (notably, E2A is a direct activator of Ig κ transcription and recombination) [21,22]. On the other hand, IL7R signalling acts, via the STAT5 TF, to inhibit Ig κ recombination, in part by blocking E2A access to the intronic Ig κ enhancer [21-23]. Finally, IRF4 also promotes the progressive loss of IL7R signalling, resulting in a full activation of Ig κ recombination. Thus, intricate pre-BCR and IL7R signalling interplay regulates cell cycle exit, Ig κ recombination, and B-cell differentiation.

Pioneering studies using fluorescence *in situ* hybridisation (FISH) have revealed large-scale locus contraction and chromosomal looping as novel processes that may be involved in the developmental regulation of V(D)J recombination at AR loci [7]. More recently, Jhunjhunwala and colleagues [24] used high-resolution microscopy to generate a statistical, three-dimensional (3D) description of the nuclear topology of the IgH locus within pre-pro- and pro-B cells. This 3D imaging revealed the existence of chromosomal domains displaying conformational changes during early B-cell development. It was suggested that the chromatin fibre, once poised for V(D)J recombination, folds into dynamic loops of variable sizes, which may depend on 'bridging' factors such as CCCTC-binding factor (CTCF). Indeed, the DNA-binding profile of CTCF throughout the IgH locus, together with that of Rad21 (a component of the cohesin complex that physically and functionally interacts with CTCF [25]), tends to support this assumption [26]. Intriguingly, another potential candidate, the TF Pax5, which is known to impinge on IgH locus contraction and rearrangements of distal V_H gene segments ([7] and references therein), may do so, at least in part, by promoting enrichment of the suppressive H3K27me3 mark at the proximal side of the V_H gene cluster [27].

FISH analyses of the subnuclear organisation of AR genes have, in addition, revealed a strong correlation between gene repression and positioning at the nuclear periphery or pericentromeric heterochromatin [7]. Using an inducible system, Singh and colleagues [28] demonstrated that recruitment of a chromosomal reporter to the nuclear lamina indeed results in its physical interaction with proteins associated with the inner nuclear membrane and transcriptional repression. Whether the

subnuclear organisation of Ig/TCR loci impacts on the initiation of allelic exclusion, a process that essentially relies on the dissociation of V-to-(D)J rearrangement between the two alleles, is a matter of debate. Experimental evidence supports both a deterministic (or instructive) and a stochastic (or probabilistic) model of gene activation (or a combination of both) to explain this phenomenon [29]. In the former scenario, AR alleles are believed to randomly display distinct epigenetic marking acquired during development such that, in individual cells, one will be preferentially used for initial rearrangement. Instead, stochastic models argue that allele dissociation results from inter-allelic competition and, usually, a low probability of locus activation. In this context, primary FISH-based studies have revealed a preferential mono-allelic association of Ig loci with pericentromeric heterochromatin, suggesting that a deterministic mechanism may in this way direct the inactivation of the non-functional allele ([30] and references therein). Conversely, a similar approach applied later on to the analysis of the TCR β locus instead demonstrated high levels of biallelic association with the nuclear lamina or pericentromeric compartments (or both), arguing for a stochastic component in allelic primary choice [31]. In the meantime, however, re-examination of a GFP knockin mouse model long considered to provide strong evidence for probabilistic gene expression at Ig κ alleles (hence supporting the stochastic view for initiation of allelic exclusion at this locus) has questioned the validity of this experimental system, thus casting doubt on prior interpretation [32,33]. Two novel observations regarding Ig locus intra-nuclear interaction fuelled the debate. First, the IgH and Ig κ loci appear to frequently associate in pre-B cell nuclei [34]. This association requires the Ig κ 3' enhancer and contributes to the pericentromeric recruitment and decontraction of the IgH locus but appears dispensable for allelic exclusion. Second, the two alleles of the IgH and Ig κ loci may at some point during B-cell differentiation also be paired in a stage-specific way [35]. This pairing would require the RAG1 protein, with the resulting DNA cut at one single allele then inducing the repositioning of the opposite allele toward pericentromeric chromatin, a process mediated by the DNA damage response protein ataxia telangiectasia mutated (ATM), which could thereby prevent biallelic recombination. Whether similar processes also occur at TCR alleles during T-cell development remains to be investigated. This could be relevant considering, for example, all the evidence that germline V β segments adjacent to a V β DJ β -C β domain maintain a chromosomal accessible configuration following TCR β feedback signalling, and yet are not subjected to recombination [36,37].

Little is known about the epigenetic pattern or patterns that correlate with the late, DNA repair joining phase of V(D)J recombination. Recombinase-generated DNA double-strand breaks induce phosphorylation of the histone variant H2AX over long distances [38]. The aforementioned involvement of ATM in the nuclear positioning of Ig loci also suggests a link between the regulation of V(D)J recombination and DNA repair. Indeed, ATM appears to exhibit various effects on AR gene assembly [39,40], and additional DNA repair factors may also be involved [41]. Likewise, the 53BP1 protein that functions in a subset of ATM-dependent responses may also play a more specific role in long-range contraction of AR loci or the maintaining of genomic stability during their recombination (or both) [42,43]. Given all of these connections, it might also be interesting to investigate whether epigenetic marks contribute to the recruitment/spreading and activity of at least some DNA repair factors at V(D)J recombination foci.

Future directions

The sophisticated mechanisms behind the regulation of V(D)J recombination are progressively emerging. New concepts require further investigation, in particular regarding their generalisation to all Ig/TCR loci (and other immune loci). It will be equally important to decipher the precise links between epigenetic modifications, intergenic transcription, chromosomal organisation and connection with DNA-transacting machineries at these loci, and the precise mechanisms behind the establishment and enforcement of allelic exclusion. Significant progress may come from the thorough characterisation of TF and nucleoprotein complexes bound at AR loci, ideally in a developmental order, using high-throughput technologies (chromatin immunoprecipitation [ChIP]-on-chip, ChIP-Seq) [20,23,26,27,44]. Given the complexity of these biological systems in which the molecular/cellular outcome may not at first be obvious, mathematical models and simulations could also help in connecting hypotheses with experimental observations [7,45].

Abbreviations

3D, three-dimensional; AR, antigen receptor; ATM, ataxia telangiectasia mutated; BCR, B-cell receptor; ChIP, chromatin immunoprecipitation; CTCF, CCCTC-binding factor; FISH, fluorescence *in situ* hybridisation; GT, germline transcription; Ig, immunoglobulin; IgH, immunoglobulin heavy; IRF4, interferon regulatory factor 4; RS, recombination signal; TCR, T-cell receptor; TF, transcription factor.

Competing interests

The authors declare that they have no competing interests.

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