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Epigenetic aspects of lymphocyte antigen receptor gene rearrangement or 'when stochasticity completes randomness'

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Summary

To perform their specific functional role, B and T lymphocytes, cells of the adaptive immune system of jawed vertebrates, need to express one (and, preferably, only one) form of antigen receptor, i.e. the immunoglobulin or T-cell receptor (TCR), respectively. This end goal depends initially on a series of DNA *cis*-rearrangement events between randomly chosen units from separate clusters of V, D (at some immunoglobulin and TCR loci) and J gene segments, a biomolecular process collectively referred to as V(D)J recombination. V(D)J recombination takes place in immature T and B cells and relies on the so-called RAG nuclease, a site-specific DNA cleavage apparatus that corresponds to the lymphoid-specific moiety of the VDJ recombinase. At the genome level, this recombinase's mission presents substantial biochemical challenges. These relate to the huge distance between (some of) the gene segments that it eventually rearranges and the need to achieve cell-lineage-restricted and developmentally ordered routines with at times, mono-allelic versus bi-allelic discrimination. The entire process must be completed without any recombination errors, instigators of chromosome instability, translocation and, potentially, tumorigenesis. As expected, such a precisely choreographed and yet potentially risky process demands sophisticated controls; epigenetics demonstrates what is possible when calling upon its many facets. In this vignette, we will recall the evidence that almost from the start appeared to link the two topics, V(D)J recombination and epigenetics, before reviewing the latest advances in our knowledge of this joint venture.

Keywords: allelic exclusion; chromatin; epigenetics; immunoglobulin; T-cell receptor; V(D)J recombination.

Introduction; a short trip back in (adaptive) immunological time

Antigen receptor gene rearrangement and epigenetics are two indisputably complementary phenomena. Discovered in the mid-1970s[1] and later known as V(D)J recombination,[2,3] the former is the somatic process that reshuffles and joins together – generally in a random and imprecise manner – two or three pieces of DNA (V and J segments, or V, D and J segments) from immunoglobulin genes (in developing B lymphocytes) or T-cell receptor (TCR) genes (in developing T lymphocytes), to achieve vast repertoires of custom-made immunoglobulin and TCRs and thereby afford adaptive immunity. Over the years, systematic studies to untangle the many mysteries of V(D)J recombination and its sophisticated controls (see below)

have led to numerous exciting new findings, including in the early 1990s the discovery of the Recombination Activating Genes 1 and 2 (RAG1 and RAG2), two adjacent lymphoid-specific genes that code for the core components of the long-sought VDJ recombinase (reviewed in ref. 4). Extensively covered in this *Focus Issue*, epigenetics conversely keeps out of all things involving the revision of germline DNA, at least so it would first seem. In short, this idiom refers to the functionally relevant modifications of, and heritable traits conveyed by the genome – in particular by the chromatin and nucleosome building block – which orchestrate regulated changes in gene expression and in cellular phenotype, yet do not involve the alteration of the underlying primary DNA sequence (e.g. ref. 5). Though coined relatively long ago,[6] this field truly exploded during the past decade with a maintained exponential growth thanks to technical breakthroughs allowing depiction and interrogation at the genome scale of the so-called epigenetic code, i.e. the dynamic chromosomal marks and structural features that carry the epigenetic information. Indeed, it has now become clear that a bulk of molecular procedures (and associated enzymatic factors thereof) have an epigenetic impact that is cell-type specific and developmentally regulated. These include DNA methylation and demethylation,[7,8] the active (energy-consuming) disruption or displacement of positioned nucleosomes, the exchange of nucleosomal histones and a variety of biochemical modifications of histone N-terminal tails,[9–13] as well as diverse forms of non-coding transcription[14,15] and three-dimensional (3D) organization of the genomic material through chromatin folding and long-range interaction via DNA looping.[16,17] Over the years, many of these procedures were shown to also have an impact on V(D)J recombination.

It did not take long before V(D)J recombination and epigenetics met. A few years after the discovery of immunoglobulin gene recombination in B lymphocytes,[1,18] and the subsequent description of sequential rearrangements of B-cell antigen receptor genes [i.e. immunoglobulin heavy (H) chain gene rearrangement preceding immunoglobulin light (L) chain gene rearrangement; and, within the IgH locus, D_H-to-J_H rearrangement preceding V_H-to-DJ_H rearrangement[19,20] (and see below)] two types of seminal observations were reported by Alt's group. First, unrearranged endogenous V_H gene segments are expressed in a developmentally controlled and tissue-specific manner, with unrearranged V_H expression being limited to the very early stages of B-lymphocyte differentiation and most prominent in cells undergoing V_H-to-DJ_H rearrangement.[21] Second, exogenous immunoglobulin and TCR gene segments rearrange equally well when introduced as plasmid substrates into an immature, pre-B-cell line in which endogenous immunoglobulin genes, but not TCR genes, rearrange with substrate recombination enhanced by transcription of a flanking selectable gene.[22,23] Based on these observations, these authors formulated the so-called accessibility hypothesis whereby the accessibility of a locus to a unique VDJ recombinase determines the choice of the antigen receptor gene that will recombine, with chromatin structure anticipated as a key determinant of accessibility.[24] Since then, evidence has accumulated in support of the accessibility hypothesis, to a 'no doubt about it' general agreement. Modern epigenetics has enlightened molecular features that could confidently distinguish between accessible and inaccessible immunoglobulin/TCR loci. What is more, epigenetics appears to play an even broader role in V(D)J recombination than originally anticipated, impinging not only on the direct recruitment of the RAG nuclease to discrete rearranging loci but also on its catalytic activity; and probably, through some of the molecular procedures mentioned above, also on the 3D organization of the actively recombining chromosome with important implications for the generation of antigen receptor immune repertoires.

V(D)J recombination, basic features

A succinct description of the molecular and mechanistic aspects of V(D)J recombination is provided below. Detailed reviews on these various aspects can be found elsewhere.[25–27]

Overall, seven distinct gene loci are normally subjected to V(D)J recombination, including three immunoglobulin genes (IgH, IgLκ and IgLλ), and four TCR genes (TCRα, TCRβ, TCRδ and TCRγ) two of which (TCRα and TCRδ) are intermingled on the same chromosome. The assembly of IgH, TCRβ and TCRδ genes is achieved from separate sets of V, D and J gene segments, whereas that of IgLκ, Iglλ, TCRα and TCRγ genes uses V and J gene segments only. Unrearranged segments are flanked on their rearranging end(s) by conserved recombination signal sequences (RSSs), which represent direct targets for the RAG nuclease. Within a given locus, V(D)J recombination is initiated following RAG binding (assisted by high mobility group proteins) to and synapsis of pairs of compatible gene segments, i.e. segments that are flanked by dissymmetric RSSs in which evolutionarily conserved 7-base-pair (bp) (heptamer) and 9-bp (nonamer) sequences are separated by either 12 or 23 bp of less-conserved sequences. [Note: Subsequent biochemical studies on RAG-mediated RSS cleavage *in vitro* in fact provided evidence of a two-step orchestrated 'capture' model of RSS synapsis involving RAG binding to a first RSS before capturing a fitting partner.[28,29] Moreover, based on the detection of initial cleavage mostly occurring at 12RSSs *in vivo*, a '12RSS binding first' model was proposed.[30] However, further investigations have questioned such a universal scenario because – depending on the locus considered – prefer-

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ential RAG binding to either 12RSS-associated or 23RSS-associated clusters is equally observed.[31]].

Of note, though theoretically 12/23 compatible, the direct joining of some gene segments such as for instance the Vβ (23RSS) and Jβ (12RSS) segments, is in fact prohibited. Termed beyond (B)12/23, this additional constraint is enforced during the V(D)J recombination reaction itself, with implications on the ordered assembly of TCRβ genes in T-cell development.[32]

Once brought closer together, RSSs of the paired segments are precisely cleaved by the RAG nuclease at their boundary with the coding sequences to make double-strand breaks. The neighbouring coding DNA is converted to a hairpin during breakage. Broken ends are then processed and joined with the help of ubiquitously expressed DNA repair factors, including members of the non-homologous end joining pathway (the DNA-dependent protein kinase and the Ku, Artemis, DNA ligase IV, Cernunnos/XLF and Xrcc4 proteins) and, possibly, histone H2AX and the Mre11/Rad50/Nbs1 complex.[33,34] The resulting signal joint (SJ) and coding joint (CJ) products present different structures, with the former corresponding to the back-to-back fusion of RSS heptamers and the latter possibly displaying sequence variability at DNA junctions because of nucleotide deletion and non-templated nucleotide addition. V(D)J rearrangement maintains a correct reading frame in roughly one-third of cases – 'out-of-frame' types of CJ accounting for the remaining two-thirds – yielding one productive rearrangement (one that enables production of potentially functional immunoglobulin or TCR chains) for every three attempts on average. Importantly, concurrent analysis of the biochemistry of the RAG proteins provided evidence that these factors are also capable of transposing RSS-ended fragments into new DNA sites. Such a parallel activity helps to explain the mechanism of RAG action and supports proposals that, unexpectedly, V(D)J recombination has evolved from an ancient mobile DNA element.[35,36] The importance of the role of VDJ recombinase was further highlighted when misrepair of DNA double-strand breaks produced at immunoglobulin and TCR loci was implicated in the pathogenesis of lymphoid malignancies in humans and in mice (reviewed in ref. 37; also see ref. 38).

Regulated controls of V(D)J recombination

Schematically, three levels of control appear to constrain the activity of the VDJ recombinase. As mentioned above, V(D)J recombination is accurately coordinated to the cell-lineage and developmental stage of differentiating lymphocytes. Hence, immunoglobulin gene complete rearrangements occur in early B lymphocytes whereas TCR gene rearrangements occur in early T lymphocytes. Within the B-cell lineage, IgH genes rearrange first, followed by IgL genes; likewise, within the T lineage, TCRβ genes rearrange first followed by TCRα genes. Moreover, IgH and TCRβ genes also achieve ordered recombination each beginning with a D-to-J rearrangement before being completed by the appendage of a V gene segment to the pre-formed DJ complex. Finally, at some antigen receptor gene loci including the IgH, IgLκ and TCRβ loci, V(D)J recombination appears also to be regulated in the context of allelic exclusion. This phenomenon – whereby antigen receptor chains are eventually encoded on only one of two opposite alleles – ensures the specificity of the immune response depending on antigenic selection of discrete clones of B or T cells, each restricted to expression of a homogeneous set of immunoglobulin receptors or TCR, respectively (the clonal selection theory[39]). In these cases, the prevailing, so-called regulated model of allelic exclusion proposes that antigen receptor V gene assembly proceeds one allele at a time and that protein products from a functionally relevant rearrangement (one that encodes IgH, IgLκ or TCRβ chains that can contribute to, respectively, the pre-B or B-cell receptors, or the pre-TCRs[40,41]) mediate allelic exclusion through feedback inhibition of further rearrangement at the corresponding locus. The accessibility hypothesis originally formulated to interpret cell-lineage and developmental stage specificities of V(D)J recombination was extended to the regulation of allelic exclusion assuming in particular that feedback inhibition down-modulates the accessibility of the remaining allele. Because allelically excluded B and T cells generally display DJ rearrangement on, respectively, the two IgH or the two TCRβ alleles and a functional VDJ rearrangement on only one of these, it is thought that down-modulation of the accessibility in the context of allelic exclusion would essentially impinge on V gene segments, at least at the IgH and TCRβ loci.[42,43]

Accessibility hypothesis: first principles and consolidation

Despite its elegance, the accessibility hypothesis – with a lack of an identified recombinase – for a while remained purely hypothetical, although it was endorsed by strong and evocative correlations (i.e. DNA and chromatin features that mimicked those accompanying gene expression or silencing). Indeed, V(D)J rearranging substrates or endogenous clusters of gene segments were found to generally display hypomethylation of scattered CpG dinucleotides and hypersensitivity to DNase and restriction endonucleases in contrast to the hypermethylation and hyposensitivity exhibited by their non-rearranging control counterparts. Furthermore, immunoglobulin and TCR loci undergoing V(D)J recombination commonly overlapped, in a lineage-specific and developmentally regulated way, with transcriptionally active domains comprised of unrearranged gene segments (hence the

term germ-line transcription) – though it remained uncertain whether transcription induces or is merely a by-product of locus accessibility.[24] More recently, germ-line transcription was updated, especially where heading in the opposite direction is concerned: within the IgH locus, B-cell developmentally regulated D_H-J_H antisense intergenic transcripts and V_H antisense intergenic transcripts, each appeared to be sequentially produced before D_H-to-J_H recombination and during the transition from DJ_H-to-V_HDJ_H recombination, respectively; and were likewise hypothesized to remodel the corresponding domains for V(D)J recombination.[44,45]

The accessibility hypothesis predicts the existence of accessibility control elements (ACE) at the distinct antigen receptor loci, that would be required to promote V(D)J recombination. Because of the widespread association of V(D)J recombination with germline transcription, it was anticipated that ACE would be connected to the control of the latter process. This prediction was first verified using mouse transgenesis, which demonstrated that transcriptional enhancer elements act to make a linked antigen receptor gene minilocus accessible to VDJ recombinase,[46–49] a role that subsequent studies confirmed for most endogenous immunoglobulin and TCR locus enhancer elements through gene-targeted mutation in mouse embryonic stem cells.[50] In addition to enhancers, such mouse knockout studies also identified endogenous immunoglobulin and TCR transcriptional promoters as potential ACEs. Generally speaking, enhancer mutation led to inhibition of V(D)J recombination on a whole-locus scale, whereas promoter mutation had a more limited regional impact;[51–55] however, both resulted in chromatin remodelling as evidenced by changes in epigenetic marking.[56,57] In line with these results, targeted gene mutation of transcription factors or chromatin-modifying factors, known to bind immunoglobulin and TCR ACEs, generally affected, though to varying degrees, V(D)J recombination at the corresponding loci.[58–62]

Finally, that cell type-specific chromatin structure indeed determines the targeting of VDJ recombinase was elegantly demonstrated by Schlissel's group.[63] Using an *in vitro* system, these authors analysed RAG nuclease-mediated cleavage of RSSs flanking immunoglobulin and TCR gene segments in cell nuclei. They found that both the lineage-specificity and temporal ordering of gene rearrangement is reflected in the accessibility of RSSs within chromatin to *in vitro* cleavage, so definitively quietening any scepticism that remained about the accessibility hypothesis.

Accessibility hypothesis: the modern age

With chromatin officially holding centre stage, the time came to ascertain the molecular features that distinguish RAG accessible and inaccessible immunoglobulin and TCR loci. This was made possible by the opportunely and newly developed technical approach chromatin immunoprecipitation (ChIP), which uses antibodies raised against, in particular, specific activating or suppressing histone modifications followed by PCR amplification to accurately locate the given mark within the genome (ChIP-PCR). [Note: Later, interrogation of genomic location also used microarray hybridization or deep-sequencing of amplified ChIP DNA: referred to as (ChIP-chip) and (ChIP-seq), respectively.]

Available immunoglobulin/TCR enhancer or promoter mouse mutants also provided invaluable sources of lymphoid cell nuclei for these studies. Overall, none too surprisingly, the immunoglobulin and TCR accessible loci were found to be enriched in epigenetic marks commonly associated with gene activation [including histone H3/H4 acetylation (H3ac; H4ac); di-/tri-methylation of Lys 4 of histone H3 (H3K4me2/me3)]; whereas the inaccessible loci were mostly decorated with epigenetic marks associated with gene silencing [including di-/tri-methylation of Lys 9 or Lys 27 of histone H3 (H3K9me2 and H3K27me3, respectively)].[64–69] Interestingly, hotspots of specific activating marks (H3K4me2/me3) or of marks differentially affected by enhancer mutations, for example, were identified, which may represent discrete domains perhaps important in, respectively, primary recruitment of the RAG nuclease,[65,66] and the hierarchical establishment of locus-specific, chromosomal accessibility.[65,69]

At this point, epigenetic ChIP-based analyses were also combined with further genetic manipulations of the mouse genome (gene knockin) to intentionally modulate accessibility and V(D)J recombination at discrete antigen receptor loci, with the aim of challenging the molecular connection between and improving our mechanistic understanding of the two processes. Hence, remarkably, targeting the histone methyl transferase enzyme G9a (mediating H3K9 methylation) to chromosomal recombination substrates containing functional ACEs induced revisions in the local chromatin environment, over-rode the ACEs' function, and crippled V(D)J recombination of linked chromosomal gene segments.[70] Moreover, introducing a transcription terminator into the 5′ end of the mouse TCR-Jα locus to block transcriptional elongation effectively suppressed chromatin remodelling and Vα-to-Jα recombination of 3′ adjacent Jα segments.[71] Altogether, these and other studies yielded results compatible with a scenario in which epigenetic chromatin modifications introduced during transcriptional elongation of antigen receptor genes might recruit chromatin remodelling complexes that displace or remodel nucleosomes positioned over RSSs (and thereby increase RSS accessibility to RAG proteins) or might even recruit the RAG nuclease itself, as further discussed below.

RAG2 contains a non-canonical plant homeodomain finger in a part of the protein that is dispensable for

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RAG-mediated DNA cleavage *in vitro* (RAG2 non-core region).[72,73] In a number of proteins associated with epigenetic regulation, a similar domain specifically binds H3K4me2 or H3K4me3. Two groups, led respectively by M.A. Oettinger and S. Desiderio, have shown that this non-canonical plant homeodomain finger mediates direct binding of RAG2 to H3K4me2 and (preferentially) H3K4me3.[74,75] The functional significance of the latter interaction was demonstrated by showing that, *in vivo*, (i) mutations in the plant homeodomain finger that abolished H3K4me3 recognition severely impaired V(D)J recombination, and (ii) DNA binding and recombination depended on the amount of H3K4me3 deposition. Therefore, recognition of the post-translational H3K4me3 epigenetic mark by RAG2 appears critical to V(D)J recombination. Strikingly, this may not be confined to a function in recombinase recruitment only. Indeed, building on these previous findings, biochemical investigations further indicated that recognition of H3K4me3 by RAG2 also stimulates the catalytic activity of the RAG nuclease;[76,77] and, possibly, stabilizes the newly excised recombination ends within the RAG post-cleavage complex and their transfer to the non-homologous end joining repair machinery.[78]

In this context, what about *in vivo* binding of the RAG nuclease to RSS-containing domains of endogenous antigen receptor loci? This issue was beautifully addressed by Schatz's group who used transgenic mice expressing an active site mutant RAG1 protein that binds DNA normally (and interacts with RAG2) but lacks catalytic activity, so avoiding the formation of recombination products that could complicate the interpretation of ChIP data at immunoglobulin and TCR loci. They demonstrated that RAG protein binding occurs in a focal manner to small regions rich in activating histone modifications (H3Ac, H3K4me3), which they referred to as 'recombination centres'.[31] Notably, these comprised regions encompassing Igκ and TCRα J gene segments and IgH and TCRβ J and J-proximal D gene segments. Interestingly, while RAG1 binds mostly to RSS-containing regions, RAG2 binds broadly to H3K4me-rich sequences throughout the mouse genome. A later study using mutant TCRα and TCRβ alleles found that enhancers control RAG1 binding globally at Jα or Dβ-Jβ gene segments, and that promoters direct RAG1 binding locally, a profile that recapitulates the V(D)J recombinational function of these ACEs defined in previous knockout studies.[79] Overall, 'recombination centres' were interpreted as specialized sites of high local RAG concentration that facilitate RSS binding and synapsis and help to regulate recombination order and fidelity.[80] Indeed, recent analysis of DJ_H-recombined alleles provided evidence that DJ_H junctions are selectively epigenetically marked and bind RAG proteins, thereby probably permitting DJ_H-5′ intact RSSs to initiate the second step of IgH gene assembly.[81]

Accessibility in 3D

At several immunoglobulin and TCR loci, RSS synapsis poses a real challenge as V, D (or DJ), and J gene segments may be located far apart [> 1–2 Megabases (Mb)] on the chromosome. A variety of studies have implicated conformational changes of such loci as important determinants of long-distance V(D)J recombination events. 3D fluorescence *in situ* hybridization analyses have revealed large-scale compaction of immunoglobulin and TCR loci, which is developmentally regulated and therefore assumed to punctually facilitate the synapsis of distant RSSs.[82–85] For example, the IgH locus looks compacted in pro-B cells undergoing V_H-to-DJ_H rearrangement; whereas such compaction is released in the subsequent pre-B stage, forcing the physical separation of the distal V_H genes from the proximal IgH domain at a stage where further IgH rearrangement is prevented.[83] A detailed analysis of the topography of the IgH locus in pro-B cells predicted that the entire locus is organized into dynamic compartments containing clusters of loops with V_H regions juxtaposed to the D_H elements, mechanistically permitting long-range genomic interactions to occur.[86]

The molecular features that may be responsible for the developmental regulation of immunoglobulin/TCR locus compaction/de-compaction are on the verge of disclosure. We know that IgH compaction depends on the transcription factors Pax5, Ikaros and YY1.[87–89] Moreover, putative regulatory sequences of conserved repeat elements with a potential role in these processes were recently discovered in the distal V gene cluster of the IgH locus.[90] These 'PAIR' elements are bound by Pax-5 specifically in pro-B cells and subjected to Pax-5-dependent antisense transcription. They also recruit the transcription factors E2A and CTCF throughout B-cell development. Lately, the CTCF zinc finger protein and its partner, the protein complex cohesin, have attracted attention as potentially important players in the regulation of V(D)J recombination at several immunoglobulin and TCR loci, through the shaping of DNA looping interactions and their contribution to lineage-specific and developmentally ordered accessibility and germline non-coding transcription.[91–98] Last but not least, detailed analysis (using RNA deep-sequencing) of germline non-coding transcription throughout the IgH locus has indicated that the majority of antisense transcripts localize around a limited number of PAIR elements; and provided evidence that this particular activity might affect the 3D chromosomal structure, bringing the distal part of the V_H locus close to the domain comprising the rearranged DJ_H and adjacent enhancer Eμ.[99] The overall emerging picture would be that of chromosomal 'rosettes' folding the dispersed V_H gene segments around a core domain bound by the recombinase, so creating opportunities for V_H-to-DJ_H rearrangement; with nascent non-coding transcription

possibly facilitating locus compaction and the formation of macromolecular structures that serve as transcription and recombination factories[99–101] (Fig. 1a).

The control of allelic exclusion: deterministic versus stochastic

One overarching aim of 3D conformation analyses of immunoglobulin and TCR loci is a better understanding of the molecular rules that enforce allelic exclusion, a still puzzling phenomenon. It appears that allelic exclusion impinges on the assembly of V gene segments, and comprises an initiation phase to dissociate V-to-(D)J rearrangements on the two opposite alleles, followed by a maintenance phase to prohibit these events once a functional V(D)J joint has been made.[42,43] In this respect, it is ostensibly appealing to consider the reversible contraction and subnuclear compartmentalization processes affecting the IgH/IgLκ and TCRβ loci in, respectively, developing pro-/pre-B and pro/pre-T cells as key regulatory mainsprings that enforce allelic exclusion (at least and most obviously during the maintenance phase).[82–84] However, rather than mere locus compaction, the primary importance in enforcing allelic exclusion, at least at the IgH locus, may be that the V and D domains remain functionally separate, perhaps achieved through the insulator/DNA looping activity of CTCF–cohesin complexes.[93,102] Separate studies have suggested additional mechanisms that could possibly also contribute to inducing or maintaining allelic dissociation, including (i) monoallelic epigenetic changes that may occur even before rearrangement (as reflected for example by asynchronous immunoglobulin and TCR locus replication[103,104]), and would eventually dispatch the two differentially packaged alleles

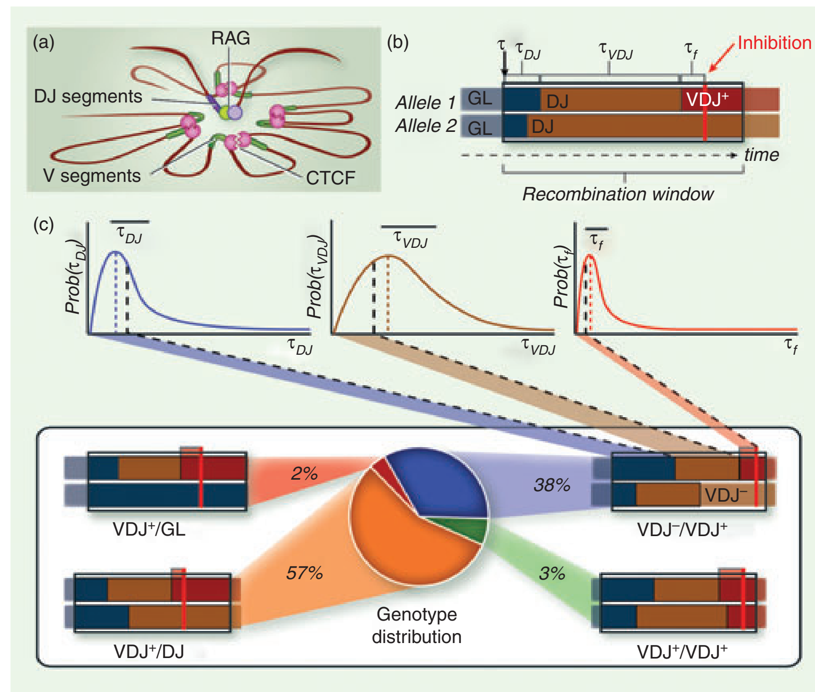

Figure 1. Dynamic combination of deterministic and stochastic features enforces allelic exclusion at V-D-J containing gene loci. (a) Illustration of a V-DJ recombination factory as proposed by Lucas *et al.* (*Curr Opin Cell Biol* 2011;23:318–24). The diagram illustrates rosette-like structures at an antigen receptor allele that would bring remote V gene segments in proximity to a *cis*-linked, RAG-loaded DJ-recombined intermediate product. At homologous alleles, stochastic proceeding of V-to-DJ recombination is assumed to mostly contribute to the V-D-J assembly time of the given locus [illustrated by the dark brown sections in (b)]. (b) Schematic depiction of stochastic modelling of DNA rearrangement at V-D-J containing antigen receptor alleles based on the Markov process formalism, as described by Farcot *et al.* (*J Immunol* 2010;185:1622–32). The recombination window defines the time period along developmental maturation of a single lymphoid cell during which all requirements for sequential rearrangements (including epigenetics) could be met (hence during which recombination of the given locus could occur). The blue, brown and red sections represent the time course of mono-allelic behaviours in a collection of individual single cells from, respectively, (i) the transition from germline (GL) to DJ-rearranged (DJ) allelic statuses, (ii) the transition from DJ-rearranged to VDJ-rearranged (VDJ) allelic statuses, and (iii) the residence of productively rearranged (VDJ^+) alleles [or of non-productively rearranged (VDJ^-) alleles; depicted in light brown in the upper right diagram of part (c)] till the recombination window comes to an end. Transition times τ_{DJ} and τ_{VDJ} correspond to the time lapses required in the particular case to achieve steps (i) and (ii), respectively; τ_f corresponds to the time lapse required to achieve feedback inhibition (red bar). (c) Statistical compilation of biallelic behaviours and their contribution to the VDJ^+/DJ, VDJ^-/VDJ^+, VDJ^+/VDJ^+ and VDJ^+/GL final genomic distribution. The probabilistic curves (*Prob*) for the τ_{DJ}, τ_{VDJ}, and τ_f parameters are shown on the top, with the locations of the mean values indicated ($\overline{\tau_{DJ}}, \overline{\tau_{VDJ}}, \overline{\tau_f}$).

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towards either the centre of the nucleus (active allele) or heterochromatin compartments (silenced allele);[105,106] (ii) pairing of homologous alleles and, following a RAG-induced DNA break on one of them, ATM-dependent repositioning of the other to pericentromeric heterochromatin;[107] and (iii) discrimination between productive and non-productive alleles through differential stability of their mRNA products with a suppressive effect of the remaining stable mRNA on V(D)J recombination.[108] Contrary to such deterministic procedures, however, allelic dissociation in V-to-(D)J joining was also proposed to simply rely on the stochastic accessibility of only a small fraction of alleles, as the result of either a high frequency interaction with repressive nuclear compartments including the nuclear lamina and pericentromeric heterochromatin,[109] or, in a non-mutually exclusive manner, variegated transcriptional activation.[110]

The control of allelic exclusion: deterministic, stochastic and dynamic

Neither a purely deterministic nor a purely stochastic representation alone sufficiently accounts for allelic exclusion. Distinct from strictly mono-allelic, deterministic gene expression systems such as X chromosome inactivation or gene imprinting, allelic exclusion is a 'faulty' developmental process with a low but sizeable fraction of the emerging lymphoid cells being allelically included that display productive rearrangements on, e.g. the two IgH or TCRβ alleles.[43] [Note: It is now clear that multiple mechanisms function in a successive manner to limit the frequency of cells with surface expression of immunoglobulin or TCR chains from productive rearrangement at both allelic copies of the corresponding loci, which depending on the locus considered may vary from 1 to 10% (ref. 43 and references therein)].

On the other hand, highly stochastic scenarios are hard to reconcile with the relatively high proportion (40–45%) of cells displaying fully rearranged, productive and non-productive, IgH or TCRβ alleles. Using TCRβ allelic exclusion as a reference system, we proposed that a dynamic combination of the two concepts, determinism and stochasticity – as modelled using the Markov chain formalism, better accounts for the distribution of TCRβ genotypes emerging from early T-cell development;[111] (Fig. 1b,c). The deterministic features would simply include the basic attribute of randomness in V(D)J recombination (one-third productive / two-thirds non-productive rearrangement outcomes) and, with regards to TCRβ gene recombination, the sequential Dβ-Jβ and Vβ-DJβ rearrangements with no direct Vβ-Jβ joining (B12/23 constraint), followed by and ending with feedback inhibition. Molecular changeovers are characterized by transition rates that are expressed in terms of the mean duration of a given rearrangement step and the average time lapse to achieve inhibition, respectively. At the single cell level, however, stochasticity relies on temporal variations in the execution of these changes (in other words, noise), both between cells (extrinsic noise) and alleles (intrinsic noise), with epigenetics likely occupying the front stage. It is increasingly recognized that cell fate decisions and underlying genetic circuits are subjected to such stochastic fluctuations to generate non-genetic cellular diversity; with, in mammals, intrinsic noise in gene expression mainly depending on epigenetic-regulated chromatin changes.[112,113] When integrated at the level of a whole cell-population, this model makes it possible to readily predict the distinct TCRβ cell genotypes and allelic exclusion/inclusion profiles from wild-type and mutant mice.[111] In keeping with the same concepts, we anticipate that corresponding features at other gene loci could likewise be interpreted. Indeed, within the transcription and recombination factories evoked above, V_H-to-DJ_H recombination might putatively proceed by way of dynamic and stochastic interactions involving on the one hand the V_H domain-folded 'rosettes' and on the other hand the inner RAG-loaded loci comprising the DJ_H intermediate CJ products, respectively.[99–101]

Conclusions

The long-standing joint venture between V(D)J recombination and epigenetics tells a win–win story. Epigenetics has already contributed to ascertaining and clarifying the regulated recruitment of a common recombinase to RSS substrates that vary according to modulated changes in gene accessibility. Deeper examination of these controls has also offered a unique insight into the epigenetic multifaceted potential to promoting accurate interconnection between widely separated gene partners within an intricate chromosomal landscape. The application of increasingly sophisticated genomic approaches to investigate these issues should continue to disclose as yet unforeseen biological resources that enforce the V(D)J recombination–epigenetics partnership. Mathematical modelling based on the dynamics of epigenetic-driven changes allied to (by nature) randomness in V(D)J recombination outcomes may concur and uncover the true intricacy of regulatory controls on such as allelic exclusion. Advances could also arise from the investigation of apparently conflicting results such as those regarding the generation of the TCRα repertoire as analysed by fluorescence *in situ* hybridization in single cells or deep-sequencing from whole $\alpha\beta$ T-cell populations, which either suggested a co-ordinate bidirectional trimming mechanism that relies on the proximity of Vα and Jα gene segments or the formation of intralocus loops whereby all Vα gene segments have equal opportunity to recombine.[85,114] More generally, the development of elaborate tools to improve our understanding of V(D)J recombination events,[115] or the

structural organization of immunoglobulin/TCR chromosomal DNA,[111,116,117] may shed more light on individual differences[118] in immune repertoire development.

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Disclosures

The authors declare no conflict of interest.

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