Seminars in Immunology 22 (2010) 330–336

Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

Review

Epigenetic control of *Tcrb* gene rearrangement

Salvatore Spicuglia[a,b,c], Aleksandra Pekowska[a,b,c], Joaquin Zacarias-Cabeza[a,b,c], Pierre Ferrier[a,b,c,*]

[a] Centre d'Immunologie de Marseille-Luminy, Inserm, UMR-S 631, F-13009 Marseille, France
[b] CNRS, UMR 6102, F-13009 Marseille, France
[c] Université de la Méditerranée, UM 631, F-13009 Marseille, France

ARTICLE INFO

Keywords:
V(D)J recombination
Allelic exclusion
Tcrb
Epigenetics

ABSTRACT

V(D)J recombination assembles antigen receptor genes from germline V, D and J segments during lymphocyte development. In αβT-cells, this leads to the subsequent expression of T-cell receptor (TCR) β and α chains. Generally, V(D)J recombination is closely controlled at various levels, including cell-type and cell-stage specificities, order of locus/gene segment recombination, and allele usage to mediate allelic exclusion. Many of these controls rely on the modulation of gene accessibility to the recombination machinery, involving not only biochemical changes in chromatin arrangement and structural modifications of chromosomal organization and positioning, but also the refined composition of the recombinase targets, the so-called recombination signal sequences. Here, we summarize current knowledge regarding the regulation of V(D)J recombination at the *Tcrb* gene locus, certainly one for which these various levels of control and regulatory components have been most extensively investigated.

1. Introduction

B and T lymphocytes form the adaptive arm of the immune system in jawed vertebrates, which can specifically respond to an astounding number of foreign antigens. This property largely depends on V(D)J recombination events at antigen receptor (AR)-encoding loci [1,2]. There are seven AR loci, comprising the immunoglobulin heavy (*IgH*) and light (*Igk* and *Igl*) chain loci expressed in B-cells and the T-cell receptor (*Tcra*, *Tcrb*, *Tcrd* and *Tcrg*) 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 (NHEJ) pathway are required. Regulation of these factors' recruitment to and function at their genomic targets – primarily effected at the level of chromosomal accessibility – ensures B- or T-cell lineage-specificity of V(D)J recombination, dictates the temporal order of Ig or TCR rearrangements, and allows allelic exclusion at certain AR genes ([3–5]; for recent reviews, see Ref. [6]). In particular, the *Tcrb* locus is subjected to distinct levels of controls of gene expression, which collectively contribute to determining the developmental order of gene rearrangement events and allelically excluded expression of a productively (in-frame) rearranged *Tcrb* gene. During the past 15 years, this locus has served as a tractable model to study these controls, in particular via the utilization of gene targeting technologies for the generation and analysis of >23 genomic deletions ('knockout') or replacements ('knockin') of different scales [7]. Notably, these studies led to a better appreciation of the hierarchical organization of transcriptional *cis*-regulatory elements (enhancers and germline promoters) and their impact on the control of V(D)J recombination at the *Tcrb* locus via the modulation of the chromatin and chromosomal structures [8,9]. How this is achieved at the molecular level is at the focus of continuing and intense investigations. Here, we review relevant aspects of the control of V(D)J recombination at the *Tcrb* locus, including the structural characteristics and organization of *Tcrb*-RSs, as well as the dynamics of discrete epigenetic marking along the locus and changes in chromosomal conformation and spatial localization within the nucleus. We discuss how these aspects could influence *Tcrb* gene rearrangement; and eventually, be integrated into a dynamical model of allelic exclusion at this locus.

2. *Tcrb* gene rearrangement: overview

T-cell development is characterized by temporally regulated expression and rearrangement of the various *Tcr* genes; with *Tcrd*/*g*/*b* and *Tcra* gene rearrangements being carried out at two distinct stages of thymic-cell development along the γδ and αβ T-cell differentiation pathways, respectively. In the case of the αβ T-cell pathway, VDJ assembly at the *Tcrb* locus is initiated and

Abbreviations: AR, antigen receptor; DN, double-negative; DP, double-positive; Eβ, Tcrb enhancer; FISH, fluorescence in situ hybridization; GT, germline transcription; Ig, immunoglobulin; kb, kilobases; Pol, RNA polymerase; RS, recombination signal sequence; Tcr, T-cell receptor; TF, transcription factor.

* Corresponding author at: Dept. of Immunology, Marseille Luminy (CIML), Parc Scientifique & Technologique de Luminy, Case 906, 13288 Marseille Cedex 9, France. Tel.: +33 491 26 9435; fax: +33 491 26 9430.
E-mail address: ferrier@ciml.univ-mrs.fr (P. Ferrier).

1044-5323/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.smim.2010.07.002

achieved in $CD4^-CD8^-$ double-negative (DN) thymocytes (more precisely the so-called DN2 and DN3 sub-compartments; see Ref. [10]); and proceeds stepwise with Dβ-to-Jβ occurring first prior to Vβ-to-DJβ joining (NB: a description of *Tcrd* and *Tcrg* gene rearrangements and impact on γδ and αβ T-cell development is beyond the scope of this article; dedicated reviews can be found in Refs. [11,12]). Expression of a functionally (in-frame VDJ-C) rearranged *Tcrb* gene leads thymocytes to successfully pass β-selection and differentiate into $CD4^+CD8^+$ double-positive (DP) cells, in which *Tcra* gene expression and recombination in turn take place. Afterwards, TCRαβ-expressing cells might be further selected into mature $CD4^+$ or $CD8^+$ single-positive (SP) T-cells (reviewed in Ref. [13]).

In the mouse germline, the approximately 700-kilobases (kb) *Tcrb* locus lies on the long arm of chromosome 6 and consists of a large (~425-kb) 5′ region containing 22 functional Vβ gene segments as well as 13 additional Vβ pseudogenes, and a shorter (~25-kb) 3′ region comprised of a duplicated cluster of Dβ–Jβ–Cβ gene segments (Fig. 1 and Ref. [14]). A separate Vβ gene segment (Vβ14), lying in the opposite transcriptional direction, is located at the 3′ end of the locus. As determined by the type and orientation of their flanking RSs, recombination of all 5′Vβ, Dβ and Jβ gene segments is deletional, except that of Vβ14 which occurs by inversion. Non-rearranged Vβ and Dβ segments are linked with upstream promoters that are thought to dictate the regional initiation of germline transcription (GT), and thus V(D)J recombination, in a developmentally regulated fashion (reviewed in Ref. [8]). Besides each of the long-known Vβ-associated promoter and the Dβ1-flanking promoter (the latter also referred to as pDβ1), two promoters located on either sides of the Dβ2 gene segment have been lately characterized [15,16]. A single transcriptional enhancer (Eβ) lies between the Cβ2 coding region and Vβ14, which is critical for this locus's functional activation including GT and V(D)J recombination (detailed further below). The formation of a complete VDJβ variable region places the promoter of the rearranged Vβ segment within the Eβ activation area, thus significantly enhancing the transcriptional activity of the newly assembled VβDJβ unit. Recently, putative additional *cis*-regulatory regions were identified within the Vβ–DJβ intervening region [17] and across the Jβ gene segments [18], although their function and hierarchical relation (*i.e.*, relative to the above mentioned *cis*-regulatory elements) with respect to *Tcrb* gene expression and recombination are not clear yet. The partitioned organization of the *Tcrb* locus and structure of the particular RSs impose a number of constraints to the regulation of V(D)J recombination at these particular sites.

3. Structural attributes impinging on *Tcrb* gene rearrangement

3.1. Recombination signal sequences (RSs)

Despite their overall conservation, RSs in general, and *Tcrb*-RSs in particular, exhibit marked sequence variations compared to the canonical RSs [14,19–22]. As the RS itself, together with a few nucleotides of immediately flanking sequences within the adjacent gene coding segment, represents the loading platform for the RAG1/RAG2 recombinase [23], such structural variability naturally impacts the effectiveness of rearrangement of the given gene segment. Actually, the *Tcrb* genomic repertoire indeed reflects this subtle interplay between the RSs and the flanking coding sequences [24–26]. Moreover, nucleotide sequences of some V_H and Vβ RSs cause them to recombine less efficiently relative to RSs (such as the Dβ-RSs) the sequences of which better match the RS consensus [25,27]; and Jβ-RSs appear highly heterogeneous within each cluster, displaying only a few conserved nucleotides, which may account for their apparent low efficacy in focusing RAG-mediated cleavage [27]. Besides, at the *Tcrb* locus, a spectacular example on how RSs can influence, and may in fact dictate, V(D)J recombination profiles was illustrated by the discovery of a novel paradigm referred to as 'beyond the 12/23', or B12/23 [28]. Bypassing the usual 12/23 rule, B12/23 restriction allows Dβ-12RSs, but not Jβ-12RSs, to efficiently target Vβ-23RSs for rearrangement, making direct Vβ-to-Jβ joining practically inexistent within the endogenous locus—a phenomenon that (i) depends uniquely on the RAG1/2 apparatus (*i.e.*, with no participation of any extra lymphoid-specific factor); (ii) relies entirely on the RSs' nucleotide sequences; and (iii) occurs at or prior to RAG1/2-mediated DNA coupled cleavage of V(D)J recombination (reviewed in Ref. [29]).

No matter how remarkable the B12/23 restriction, its actual impact on the regulation of V(D)J recombination at the *Tcrb* locus and basic timing of ordered Dβ-to-Jβ and Vβ-to-DJβ rearrangements is not clear yet. In this respect, additional insight into this phenomenon came from the unexpected findings that the transcription factor (TF) c-Fos, a component of the AP1 transcriptional complex, not only binds the 3′Dβ1-23RS but also directs the recruitment of the RAG1/2 core component of the VDJ recombinase at this site, thereby enabling Dβ-to-Jβ joining [30]. Further, c-Fos deficiency results in decreased *Tcrb* gene recombination and the detection of Vβ–Dβ1 rearrangements, suggesting a disruption of ordered recombination [30]. Independently, we also demonstrated that single-strand DNA nicks (the very initial step of RAG1/2-mediated DNA cleavage at a RS) are primarily observed *in vivo* at both the 3′- and 5′Dβ-RSs, and in a Eβ-dependent way, implying a primary role for these sites during the course of *Tcrb* gene recombination, possibly via recombinase anchoring and the nucleated capture of their respective Vβ and Jβ RS partners (provided the Dβ RSs were first made accessible via Eβ function(s) on chromatin structure, Ref. [27]). In the latter study, we further demonstrated that the presence of the 3′Dβ1-23RS impedes DNA cleavage at the adjacent 5′Dβ1-12RS in some way, hence providing potential molecular hints as to how and why Dβ1–Jβ assembly occurs first, followed by the joining of a Vβ gene segment whose capture by an activated (*i.e.*, RAG1/2 loaded) 5′Dβ1-12RSS would thus require prior excision of the 3′Dβ1-23RS via deletional D–J recombination. Accumulating evidence that Dβ-to-Jβ rearrangement initially involves the Dβ1–Jβ1 cluster during T-cell development [31–33], adds further consistency to this scenario in terms of the regulation of *Tcrb* ordered rearrangement. However, following analysis of the rearrangements involving Dβ1–Jβ1.1 genomic sequences *outside of* their native *Tcrb* domain, it has been argued that RAG deposition on 3′Dβ RSSs may not be sufficient to direct ordered *Tcrb* rearrangements; and that the location of the Dβ–Jβ segments relative to their germline promoters and/or the Eβ enhancer may instead be critical for directing the assembly of endogenous Vβs through DJβ intermediates [34]. In any case, these results collectively indicate that RSs, especially Dβ-RSs, impose significant constraints on *Tcrb* gene assembly, the effects of which are felt beyond those restricting chromosomal accessibility to the VDJ recombinase. At a minimum, their asset in driving the action of the recombination apparatus at this locus guarantees the utilization of an intermediate Dβ gene segment, hence the expression of a fully diversified TCRβ repertoire [35].

3.2. Chromatin accessibility and epigenetics

Pioneering studies by Alt and collaborators [36–37] led to the concept that locus-specific control and temporal-ordering of V(D)J recombination primary involve the modulation of locus and/or gene segment accessibility to a common VDJ recombinase (accessibility model). Since then, results from countless experiments have confirmed this model (reviewed in Refs. [1,9,38]), including the