Equal opportunity for all

Julie Chaumeil and Jane A Skok*

Department of Pathology, New York University School of Medicine, New York, NY, USA

*Correspondence to: jane.skok@nyumc.org

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By sequencing tens of millions of TCR α transcripts from naive mouse CD8⁺ T cells, Genolet *et al* (2012) show that the TCR α repertoire is at least as diverse as the TCR β repertoire. This overturns a long held view in the field that recombination of the *Tcr* locus occurs in a co-ordinate sequential bidirectional manner that relies on proximity of V α and J α gene segments. The observation that rearrangement of all possible V α -J α combinations can occur is consistent with an alternative model in which intralocus loop formation/locus contraction enables an opportunity for all V α gene segments to recombine with J α gene segments.

For an effective immune response, the spectrum of antigen-receptor specificity must be roughly equivalent to the spectrum of antigen diversity. To fulfill this requirement, developing B and T cells of the adaptive immune system make use of limited genomic resources to generate diversity within three *Immunoglobulin* (*Igh*, *Igk* and *Igl*) and four *T-cell receptor* (*Tcr γ* , *Tcr δ* , *Tcr β* and *Tcr α*), loci respectively. This involves co-ordinated lineage and stage-specific recombination of variable (V) diversity (D) and joining (J) genes within the different loci. To some extent, this process is like reshuffling the pieces of a puzzle, with the limitations of the design being determined by the number of available pieces that can fit together. V(D)J recombination has inbuilt restraints on joining that enables different V, D or J gene segments to recombine with each other but not with members of the same family. These restrictions are determined by the type of recombination signal sequence (RSS) that is located upstream or downstream of each of the individual V, D or J gene segments. The recombinase proteins, RAG 1 and 2 recognize and bind to RSSs to join together two separated gene segments through the introduction of double-strand breaks, deletion of intervening sequences and subsequent repair by the non-homologous end-joining pathway (Figure 1A). Some antigen-receptor loci such as *Tcr β* and *Igh* contain D as well as V and J gene segments and recombination occurs as a two-step process, which involves recombining D to J prior to V-DJ regions. For others (*Tcr α* , *Igk* and *Igl*) recombination is a one step process that involves recombination of V to J gene segments. Rearrangement involving V and J gene segments alone is less restrictive and the loci can go through successive rounds of rearrangement until all available V or J gene segments are used up.

The first step of the recombination process is synapsis: RAG proteins bind to and bring together the two recombining gene segments prior to the introduction of breaks. However, since the V, D and J gene segments that make up the individual germline antigen-receptor loci can occupy as much as 2.8 Mb of DNA, individual gene segments that are potential joining partners can be separated by a large linear chromosomal expanse. Locus contraction provides a mechanism for gene segments, separated by a small or a large linear distance to recombine with an equivalent potential. This process brings widely dispersed gene segments together in nuclear space through the formation of intralocus chromatin loops (Fuxa *et al*, 2004; Roldan *et al*, 2005; Skok *et al*, 2007; Jhunjhunwala *et al*, 2009). Thus, locus contraction can impact on the ability of the immune system to mount an effective response through generating a diverse repertoire of receptors that maximizes the recombinatorial potential of each individual locus. The substructure of looping at each of the antigen-receptor loci is complex and is still being worked out (Chaumeil and Skok, 2012). Clearly, the looping conformation of the individual loci will determine what gene segments can make contacts and be joined together through the action of the RAG proteins.

The *Tcr α* /d locus is particularly complex because in its germline form it encompasses the *Tcr δ* locus, which recombines in CD4[−]CD8[−] double-negative (DN) T cells while *Tcr α* rearrangement takes place at the subsequent CD4⁺CD8⁺ double-positive (DP) stage of development. Productive rearrangement of *Tcr δ* and *Tcr γ* gives rise to $\gamma\delta$ T cells while a failure of their rearrangement relies on productive rearrangement of the *Tcr β* locus to drive development forward to the DP stage where *Tcr α* rearrangement takes place. Functional rearrangement of *Tcr β* and *Tcr α* will generate single positive $\alpha\beta$ CD8⁺ cytotoxic and CD4⁺ helper T cells.

Most of the *Tcr δ* locus (with the exception of a few V δ genes) is embedded between the V α and J α gene segments and thus will be deleted during *Tcr α* recombination (Figure 1B). Initiation of *Tcr α* rearrangement relies on activation of the *Tcr α* enhancer (*E α*), which in turn acts on the T early activation (TEA) and J gene promoters to open up proximal J α and V α gene segments for primary rearrangement events (Krangel, 2003). Activation of this region of the locus depends on cohesin-mediated interaction between the TEA promoter and the *E α* (Seitan *et al*, 2011) (Figure 1B, black box on the right). Primary rearrangements between

Figure 1 Recombination of the *Tcrα* locus. **(A)** Outline of V(D)J recombination of antigen-receptor loci: (i) RAG1/2 recognizes, binds and cleaves DNA at specific RSSs flanking the V, D and J segments; (ii) double-stranded breaks are then repaired by the non-homologous end-joining (NHEJ) pathway. **(B)** In DP cells, activation of the *Tcrα* enhancer (Eα) and the T early activation (TEA) promoter open up proximal Jα and Vα segments for primary rearrangement. Activation of this region depends on the formation of an Eα-TEA loop mediated by cohesin/CTCF (grey box on the right). Subsequently, *Tcrα* can go through multiple rounds of secondary rearrangement. The prevailing view is that secondary recombination occurs in a non-random bidirectional manner where 3' Jα segments join with proximal 5' Vα segments, giving rise to a limited TCRα repertoire (i). Now sequencing of tens of millions of TCRα transcripts from naive mouse CD8⁺ T cells shows that all Vα-Jα combinations are possible. This is consistent with an alternative model in which intralocus loop formation/locus contraction enables an equal opportunity for all Vα segments to recombine with Jα segments (ii).

proximal Vα-Jα genes will result in the deletion of the TEA and proximal J gene promoters (Figure 1B). But this is not the end point of the process: in the absence of Dα gene segments, the *Tcrα* locus can go through multiple rounds of secondary rearrangement deleting previously recombined Vα and Jα gene segments.

The prevailing view is that secondary *Tcrα* recombination also occurs in a non-random manner and is governed by the position of both the Vα and Jα gene segments so that 3' Jα gene segments join with proximal 5' Vα gene segments in an ordered, coordinated bidirectional manner that is conserved in mouse and humans (Roth *et al*, 1991; Huang and Kanagawa, 2001; Pasqual *et al*, 2002; Fuschiotti *et al*, 2007) (Figure 1B (i)). Since rearrangement involves gene segments in close proximity, recombination does not have to rely on the formation of chromatin loops, to bring other Vα gene segments into equivalent proximity with Jα gene segments (Skok *et al*, 2007). Rather, ordered, position-dependent recombina-

tion is compatible with a 5' decontracted, 3' contracted model of locus conformation that has been postulated by the Krangel laboratory (Shih and Krangel, 2010). Position biased recombination shapes a considerably more limited TCRα repertoire than one predicted by random recombination in which all gene segments have an equal opportunity to rearrange.

Although previous studies that support a model for a restricted TCRα repertoire were as far as possible designed to examine combinatorial diversity by eliminating bias, they could not cover the entire locus as comprehensively as current sequencing techniques. It is difficult to determine the actual diversity of the TCRα repertoire from analysing a limited fraction of VJα recombination events by PCR assays, which make use of a restricted range of primers. Indeed, using high throughput sequencing to examine TCR distribution in different developing and effector human T cell subsets, Wang *et al* (2010) arrive at an increased estimate for TCRα diversity.

Now, by sequencing tens of millions of TCR α transcripts from naive mouse CD8⁺ T cells, Genolet *et al* (2012) show that the TCR α repertoire is even greater than these estimates, and is in fact comprised of 94% of possible VJ α combinations.

Consistent with coordinated, ordered, position biased primary rearrangement, there is infrequent representation of the most 5' V α gene segments. These are likely deleted during secondary rearrangement and preferential rearrangement of proximal J α and V α is limited to three V α gene segments. The latter constitute only a minor fraction of recombinants, while the frequency of distribution of the majority of recombinants depends more on V α gene versus J α gene usage: V α gene segments can recombine with nearly all J α gene segments independent of V α gene position, but the frequency of recombination varies in a manner that is dependent on V α location. Furthermore, VJ α recombination frequencies determine the generation of diversity of CDR3 α (a region of the receptor that contacts antigen and is thus

important in the immune response). These data are not consistent with a model in which secondary rearrangement occurs in a coordinated sequential position-dependent manner. Rather, the data suggest that in secondary rearrangements all V α gene segments have equal access to the J α region, which is compatible with a model in which contraction can occur over the entire Tcr α locus so that all V α gene segments have an equivalent potential to recombine (Figure 1B (ii)). How the frequency of VJ α recombination, which is dependent on V α location, is linked to a substructure of intralocus loops remains to be determined. Clearly, maximized combinatorial diversity will provide better immune coverage and influence the outcome of diseases that result from infection by foreign antigen.

Conflict of interest

The authors declare that they have no conflict of interest.

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