Journal of
Clinical & Cellular Immunology

Hernández-Munain et al., J Clin Cell Immunol
2016, 7:2
DOI: 10.4172/2155-9899.1000415

Review Article Open Access

Insights into the Transcriptional Regulation of the Unrearranged and Rearranged *Tcra* and *Tcrd* Genes

Cristina Hernández-Munain*, **Áurea Casal, Belén Juanes, Jennifer López-Ros, and Alonso Rodríguez-Caparrós**

Instituto de Parasitología y Biomedicina "López-Neyra" - Consejo Superior de Investigaciones Científicas (IPBLN-CSIC), Granada, Spain

***Corresponding author:** Cristina Hernández-Munain, Instituto de Parasitología y Biomedicina "López-Neyra" - Consejo Superior de Investigaciones Científicas (IPBLN-CSIC), Avenida del Conocimiento s/n, 18016-Armilla, Granada, Spain, Tel: 34-958-181646; Fax: 34-958-181632; E-mail: chmunain@ipb.csic.es

Received date: March 03, 2016; **Accepted date:** April 27, 2016; **Published date:** April 29, 2016

Abstract

The combined T-cell receptor α and δ locus, *Tcra/Tcrd*, encodes the TCRα and TCRδ chains of the αβ or γδ T-cell receptors (TCRαβ and TCRγδ), respectively, which define the two distinct T-cell lineages, αβ and γδ T lymphocytes. Like other antigen receptor loci, this locus must recombine its variable (V), diversity (D), and joining (J) gene segments to generate a diverse range of TCR that allow vertebrates to respond to an unlimited number of antigens. The *Tcra/Tcrd* germline transcription and subsequent V(D)J gene segment rearrangements are strictly regulated by two distant transcriptional enhancers, Eα and Eδ, respectively, during thymocyte development. Once the *Tcra* locus is productively rearranged, it is assumed Eα remains active for the transcription of the rearranged locus and the expression of the functional TCRα chain in αβ T lymphocytes. However, our recent experiments have shown Eα is significantly inhibited during the final stage of thymocyte development, concomitantly with the expression of the rearranged *Tcra* locus, and remains inhibited in αβ T lymphocytes. These results imply the existence of an Eα-independent mechanism to activate transcription of the rearranged *Tcra* locus in αβ T lymphocytes. Interestingly, Eα is essential for the normal expression of the rearranged *Tcrd* locus in γδ T lymphocytes. In this review, the current knowledge about the regulation of *Tcra/Tcrd* germline transcription and gene segment rearrangement during thymocyte development and the possible mechanisms for transcription of the rearranged *Tcra* locus in mature αβ T lymphocytes are discussed. The knowledge of the detailed mechanisms involved in the regulation of transcription at the *Tcra/Tcrd* locus by distant enhancers is important to understand the cases in which deregulation this process results in disease.

Keywords: Transcription; T-cell receptor; V(D)J recombination; Enhancer

Abbreviations:

A-T: Ataxia-Telangiectasia; ATM: Ataxia-Telangiectasia Mutated Kinase; Cα: *Tcra* Constant Region; Cδ: *Tcrd* Constant Region; Chromosome Conformation Capture Experiments, 3C and 4C; CTCF: CTCCC-Binding Factor; D: Diversity; DN: Double Negative; DP: Double Positive; Eα: *Tcra* Enhancer; Eδ: *Tcrd* Enhancer; eDP: Early DP; ETP: Early T-cell Progenitor; HS: DNaseI Hypersensitivity Site; IL-7R: Interleukin-7 Receptor; ISP: Immature Single Positive; J: Joining; LCR: Locus Control Region; lDP: Late DP; *Rag*: Recombinase Activating Gene; SP: Single Positive; T-ALL: T-cell Acute Lymphoblastic Leukemia; TEAp; T Early α Promoter; TCR: T-cell Receptor; TCRα: T-cell Receptor α; *Tcra*: T-cell Receptor α Gene; TCRαβ: αβ T-cell Receptor; TCRβ: T-cell Receptor β chain; *Tcrb*: T-cell Receptor β Gene; TCRδ: T-cell Receptor δ; *Tcrd*: T-cell Receptor δ gene; TCRγ: T-cell Receptor γ; *Tcrg*: T-cell Receptor γ gene; TCRγδ: γδ T-cell Receptor; TF: Transcription Factor; Traj: T-cell Receptor α J; *Trav*: T-cell Receptor α V; *Trdd*: T-cell Receptor δ D; *Trdj*: T-cell Receptor δ J; *Trdv*: T-cell Receptor δ V; V: Variable.

Temporal Control of TCR Gene Rearrangements

During thymic T-cell development (Figure 1), early T-cell progenitors (ETP) arising from fetal liver or bone marrow enter to the thymus, where they mature progressively through different stages that can be distinguished based on the expression of the CD4 and CD8 surface markers: CD4-CD8- double-negative (DN) thymocytes, immature single-positive (ISP) $CD8^+$ thymocytes, $CD4^+CD8^+$ double-positive (DP) thymocytes, and $CD4^+$ or $CD8^+$ single-positive (SP) thymocytes [1]. Among the DN thymocyte population, four subpopulations can be further distinguished based on the expression of CD25 and CD44 surface markers: DN1 ($CD44^+CD25^-$), DN2 ($CD44^+CD25^+$), DN3 ($CD44^-CD25^+$), and DN4 ($CD44^-CD25^-$) thymocytes. In addition, two DN3 subpopulations can be distinguished based on the expression of CD27: DN3a ($CD27^{low}$) and DN3b ($CD27^{high}$) thymocytes [2]. Furthermore, two DP thymocyte populations can be distinguished based on the expression of CD71: early DP (eDP) ($CD71^+$) and late DP (lDP) ($CD71^-$) thymocytes [3]. For αβ T-cell development, thymocytes transition from DN1 to SP thymocytes by maturing successively through the following populations: DN1, DN2, DN3a, DN3b, DN4, ISP, eDP, lDP, and SP thymocytes; whereas for γδ T-cell development, thymocytes transit only from DN1 to DN2 or DN3a before becoming mature cells [1].

The loci that encode for the TCR chains are composed of dispersed variable (V), diversity (D), and joining (J) gene segments that are rearranged during thymocyte development by a process known as V(D)J recombination to generate a gene configuration capable of expressing the functional receptors, TCRαβ or TCRγδ, on the cell membrane [4,5]. The V(D)J recombination is completed in DN2 and DN3a thymocytes at the *Tcrg* and *Tcrd* loci, in DN3a thymocytes at the *Tcrb* locus, and in DP thymocytes at the *Tcra* locus [4].

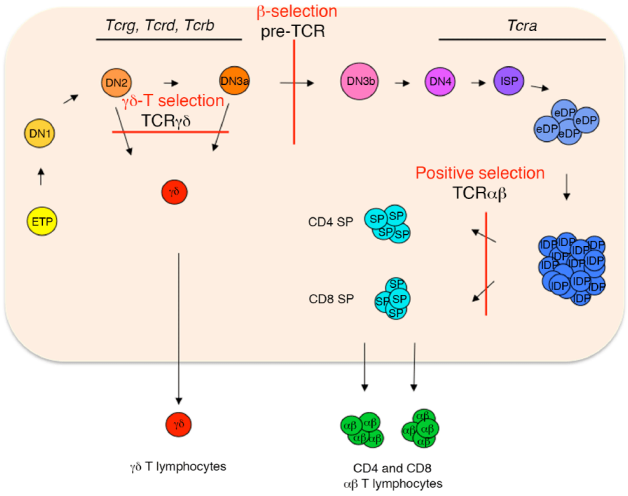

Figure 1: Temporal control of T-cell gene rearrangements and thymocyte development. The thymus is represented as a light pink rectangle. Schematic representation of thymocyte maturation depicting the various developmental stages and the TCR gene rearrangements is shown. β-, γδ-, and positive selection, which depend on the expression of pre-TCR, TCRγδ or TCRαβ, respectively, are indicated in red. T cell maturation is indicated by the transition from yellow to red (maturation to γδ T lymphocytes) or green (maturation to αβ T lymphocytes).

A successful *Tcrg* VJ and *Tcrd* VDJ recombination permits the expression of a TCRγδ, which drives cell differentiation to γδ T lymphocytes in a process known as γδ-selection [4]. A successful *Tcrb* VDJ recombination in DN3a thymocytes permits the expression of a functional TCRβ chain that assembles with the invariant pre-Tα chain to form a pre-TCR, which drives cell differentiation to DP thymocytes in a process known as β-selection [1,4]. A successful *Tcra* VJ recombination in eDP and lDP thymocytes permits the expression of a TCRα chain that associates with the previously expressed TCRβ chain to form a TCRαβ [1,4]. The antigen affinity of the TCRαβ in lDP thymocytes will determine the positive selection of a few DP thymocytes that will survive and differentiate into $CD4^+$ or $CD8^+$ SP thymocytes [1]. SP thymocytes migrate to the periphery as mature αβ T lymphocytes [1].

In addition to the essential roles for pre-TCR- and TCR-mediated signaling on thymocyte development, signals mediated by Notch and interleukin-7 receptor (IL-7R) are required for T-cell commitment, survival, and differentiation [6-10]. Each of these signals has a pivotal role in controlling the process of V(D)J recombination at the different TCR loci [1]. In DN2/3a thymocytes, signaling mediated by IL-7R is essential for the *Tcrg* germline transcription and VJ recombination, as well as for γδ T lymphocyte development [11], whereas signaling mediated by Notch is essential for *Tcrb* gene VDJ recombination and αβ T lymphocyte development [12]. During β-selection, pre-TCR-mediated signaling triggers the *Tcra* germline transcription and VJ recombination and the development of αβ T lymphocytes, and inhibits the transcription of the *Tcrg* and *Tcrd* loci [13-15]. The molecular targets of those signaling pathways are genomic regulatory sequences capable of controlling chromatin structure of the loci, such as the enhancers associated with the *Tcrg, Tcrd,* and *Tcra* loci, and the silencer and promoters associated with the *Tcrg* and *Tcrb* loci [11,13,14,16-25].

Regulatory Cis-elements Present at the *Tcra/Tcrd* Locus

The *Tcra* and *Tcrd* genes are linked in a single genetic locus, *Tcra/Tcrd*, which spans 1.8-Mb with a conserved genomic structure and a location between the olfactory receptor genes and the *Dad1* gene on chromosome 14 in humans and mice [4,5,26]. The 1.7-Mb 5′-locus region includes 132 *Tcra* and *Tcrd* V (*Trav* and *Trdv*) gene segments, while the remaining 0.1-Mb 3′-locus region contains the *Tcrd* D and J (*Trdd* and *Trdj*) gene segments, the *Tcrd* constant region (Cδ), the *Trdv5* gene segment, the *Tcra* J (*Traj*) gene segments, and the *Tcra* constant region (Cα) (Figure 2). Among the *Trav/Trdv* gene segments, some only rearrange with the *Trdd* gene segments, some only with the *Traj* gene segments, and some can rearrange with either *Trdd* or *Traj* gene segments, contributing to both the TCRδ and TCRα chain repertoires [5]. The nested organization of these genes prevents the occurrence of the *Tcrd* and *Tcra* gene segment rearrangements on the same chromosome, because the *Tcra* VJ recombination results in the deletion of the *Tcrd* locus in an extra-chromosomal circle (Figure 2) [4].

Each *Tcra* and *Tcrd* locus is equipped with one transcriptional enhancer, Eα and Eδ, located at the 3′-end of Cα and at the 5′-end of Cδ, respectively, and the numerous promoters that associated with the V, D, and J gene segments along the locus, including the T early α promoter, TEAp, associated with the most 5′-*Traj* gene segment, *Traj61* (Figure 2) [4]. TEAp orchestrates different chromatin loops at the 3′-end of the locus during T-cell development [5,27,28] (see below). Eα is part of a previously described locus control region (LCR) located between Cα and the ubiquitously expressed *Dad1* gene [29]. The *Tcra* LCR spans approximately 7.4-kb, with seven DNase I-hypersensitivity sites (HS): HS1, HS1′, HS2, HS3, HS4, HS5, and HS6 [30]. The most 5′-1.4-kb LCR fragment contains HS1 and HS1′ [30,31]. HS1 contains Eα whereas the 3′-contiguous HS1′ contains two binding sites for the CTCCC-binding factor (CTCF) involved in the *Tcra/Tcrd* locus chromatin organization and Eα function during thymocyte development [27,30-33]. Eα is responsible for activating the endogenous locus germline transcription and the *Tcra* VJ recombination, as well as the generation of αβ T lymphocytes [34]. Eα can also activate transcription and the V(D)J recombination of transgenic reporter constructs in a temporally regulated manner during thymocyte development [29,35-38]. In addition, Eα is required for the normal expression of the rearranged *Tcrd* locus in γδ T lymphocytes [34]. CTCF binding to HS1′, TEAp, and proximal *Trav/Trdv* promoters is important to generate a functional chromatin hub among Eα, TEAp, and proximal *Trav/Trdv* promoters to promote the endogenous *Tcra* VJ recombination in eDP thymocytes [27] (see below). CTCF binding to HS1′ also collaborates with Eα for the expression of transgenic reporter constructs in thymocytes and splenocytes [30]. The 3′-6-kb LCR fragment contains HS2-6 [29]. HS2-6 is transcriptionally active in non T-lineage cells and collaborates with Eα to confer a high-level, position-independent and copy number-dependent transgene expression in T-lineage cells by acting as an insulator that blocks Eα activity to maintain the distinct regulatory programs of the neighboring *Tcra/Tcrd* and *Dad1* genes [29,30,39]. The HS4-HS6 fragment contains the greatest enhancer blocking activity [39], with HS4 and HS6 being the major contributors that confer Eα-dependent high-level, position-independent and copy

number-dependent transgene expression in T-lineage cells in a CTCF-independent manner [32,40,41]. In addition, two other *Tcra/Tcrd* regulatory elements have been recently described (Figure 2): 1) two binding sites for CTCF located upstream of the *Trdv4* gene segment, INT1/2, that creates a functionally relevant chromatin loop with TEAp in DN2/3a thymocytes to increase the *Tcrd* and *Tcra* repertoires (see below), and 2) a new transcriptional enhancer located between the *Traj3* gene segment and Cα, called E3´-Jα, that is active in thymic and peripheral αβ T cells as assessed using transgenic mice [28,42].

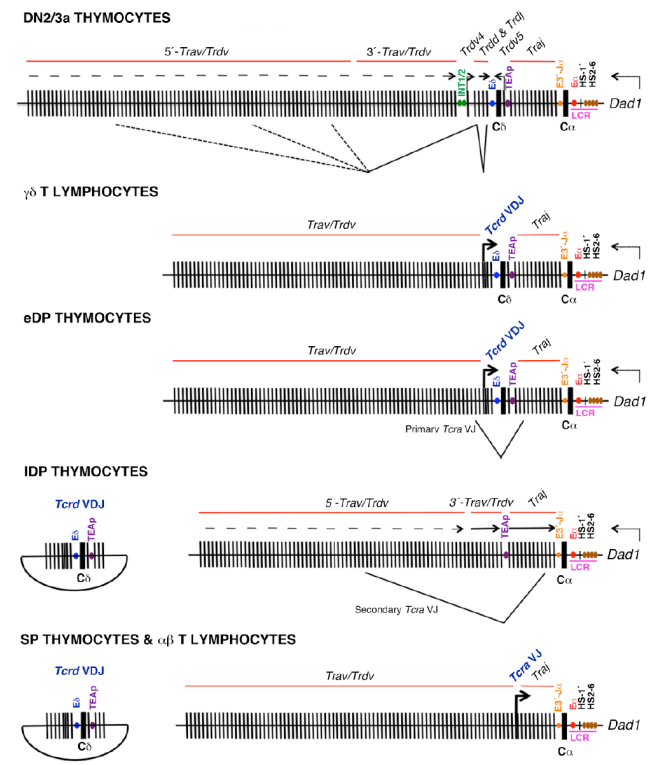

Figure 2: Representation of the different structure of the *Tcra/Tcrd* locus during T-lymphocyte development. The V, D, and J gene segments are represented by black narrow rectangles. The Cδ and Cα regions are represented by black large rectangles. The 1.6-Mb 5´-locus region includes the *Trav/Trdv* gene segments, and the 0.1-Mb 3´-locus region includes the *Trdd* and *Trdj* gene segments, the Cδ region, the *Trdv5* gene segment, the *Traj* gene segments, and the Cα region. Red lines indicate the areas occupied by *Trav/Trdv, Trdd* and *Trdj*, and *Traj* gene segments. The position of the functionally relevant regulatory elements is indicated as follows: INT1/2 as green circles, Eδ as a blue circle, TEAp as a purple circle, E3´-Jα as an orange circle, LCR as a pink line, Eα as a red circle, HS-1´as a black rectangle, and HS2-6 as brown ovals. The dashed lines represent the possible different rearrangements along the *Trav/Trdv* gene segment cluster and the continuous lines represent the *Tcrd* DJ recombination in DN2/3a thymocytes and the primary and secondary *Tcra* VJ recombination in DP thymocytes. The arrows represent regions of active germline transcription. Arrows anchored to a particular V gene segment represent the transcripts originated from specific V promoters. The rearranged *Tcrd* VDJ and *Tcra* VJ transcripts are written in bold blue characters. Low transcription is represented by dashed lines (low transcription level) whereas the width of the continuous lines is proportional to the level of transcription (medium or high). The deleted *Tcrd* locus in DP and SP thymocytes, and in αβ T lymphocytes, is represented as a separate circularized DNA fragment containing the rearranged *Tcrd* and several unrearranged *Trav/Trdv* and *Traj* gene segments produced as a consequence of a *Tcra* VJ recombination.

Developmental Control of the *Tcra/Tcrd* Locus Recombination by Eα and Eδ

Eδ is essential for normal *Tcrd* V(D)J recombination and generation of γδ T lymphocytes [43]. Eδ functions as a local enhancer important to confer the accessibility of the *Trdv5, Trdd*, and *Trdj* gene segments to the recombinase machinery in a 10-20-kb region of adult DN3a thymocytes, while Eα influences a 500-kb region including the proximal 1/3 of the *Trav/Trdv* (3´-*Trav*/*Trdv*) and *Traj* gene segments in DP thymocytes (Figure 2) [44,45]. Eδ and Eα are responsible for the specificity of the *Tcrd* and *Tcra* gene segment rearrangement, respectively; across the developmental stages by regulating the germline transcription and chromatin structure that mediates the accessibility of the recombinase machinery to each specific gene [46]. To permit the generation of functional *Tcrd* VDJ recombination and expression of the TCRδ chain in DN2/3a, Eδ is active whereas Eα remains inactive in these cells [14,46]. *Tcrd* VDJ recombination is accomplished through the activation of the Eδ-dependent promoters associated with the *Trdd* and *Trdj* gene segments, which opens up the chromatin structure to provide accessibility for the recombination machinery in DN2/3a thymocytes (Figure 2) [43,47]. During β-selection, Eα is activated to induce the *Tcra* VJ recombination in DP thymocytes whereas Eδ becomes inactivated [14,34,46]. During γδ-selection, Eα is also activated to contribute to the transcription of the rearranged *Tcrd* locus, being required for normal expression of the TCRδ chain in γδ T lymphocytes (34). Interestingly, Eδ is inactivated during β-selection but presumably not during γδ-selection [14,43]. Therefore, both Eδ and Eα are relevant enhancers to dictate the patterns of the *Tcrd* and *Tcra* gene germline transcription and V(D)J recombination during thymocyte development [14,34,43,46].

The *Tcra* VJ rearrangements are accomplished through activation of germline transcription, which is initiated at Eα-dependent promoters associated with the most 3´-*Trav/Trdv* and 5´-*Traj* gene segments and opens up the chromatin structure to provide accessibility for the recombination machinery in eDP thymocytes (Figure 2) [48-50]. These initial *Tcra* VJ gene segment rearrangements occur in eDP thymocytes and are known as primary *Tcra* VJ recombination (Figure 2) [50]. As a consequence of the nested organization of the *Tcra/Tcrd* locus, the primary *Tcra* VJ recombination results in the deletion of the rearranged *Tcrd* locus in an extra-chromosomal circle (Figure 2) [4]. These extra-chromosomal circles will remain present in all DP and SP thymocytes, as well as in naïve αβ T lymphocytes [4]. If the primary *Tcra* VJ recombination is not productive, then the secondary *Tcra* VJ recombination involving the more 5´-*Trav/Trdv* and 3´-*Traj* gene segments will occur in lDP thymocytes (Figure 2) [51]. This strategy of successive *Tcra* VJ gene segment rearrangements using the further 5´-*Trav/Trdv* and 3´-*Traj* gene segments permits multiple VJ gene segment rearrangements at each *Tcra* allele to assure the expression of a productive TCRα chain in all lDP thymocytes and to provide a greater probability that positive selection and further αβ T lymphocyte maturation can occur.

Eδ is formed by seven protein-bound elements known as δE1, δE2, δE3, δE4, δE5, δE6, and δE7, in a 380-bp DNA fragment [46]. Although this fragment is functional in activating transcription of reporter constructs in transient transfection experiments, it is not able to activate rearrangement of a reporter construct in single-copy transgenic mice requiring the presence of two flanking matrix attachment regions for such function [52]. Eδ activity depends critically on the binding of the transcription factors (TFs) Runx1 and c-Myb to δE3 [53-55]. These TFs are dissociated from Eδ in the transition from DN3a to DP thymocytes, which is concomitant with the inactivation of the enhancer [14].

Eα is formed by four protein-bound elements known as Tα1, Tα2, Tα3, and Tα4, in a 275-bp DNA fragment that constitutes the minimal Eα with the correct temporal regulation during thymocyte development [46]. The 116-bp Tα1-Tα2 fragment constitutes the core enhancer with essential binding sites for the constitutive TFs CREB/ATF, TCF-1/LEF-1, Runx1, and Ets-1 that bind cooperatively in an all-or-none fashion [36,56-58]. These TFs are bound to a primed Eα prior to its activation in DN3a thymocytes as well as when the enhancer is fully active in eDP and lDP thymocytes [14,46,59]. Although the Tα1-Tα2 fragment is efficient in activating transcription and gene segment recombination at short distances in the context of a transgenic recombination reporter construct, is not sufficient to activate endogenous *Tcra* VJ recombination at large distances [36,60]. In addition, it does not display the proper Eα developmental regulation because it is activated prematurely in DN3a thymocytes, being necessary additional Tα3-Tα4-binding TFs including Sp1, GATA-3, E2A, and/or HEB for proper temporal activation of the enhancer [36,37]. Pre-TCR signaling triggers the activation of Eα through the binding of the inducible TFs NFAT, AP-1, and Egr-1 in eDP thymocytes, which are recruited to a pre-assembled Eα enhanceosome formed by the Eα-bound constitutive TFs [18]. In lDP thymocytes, prior to positive selection, Eα remains fully active through the induction of strong binding of constitutive TFs such as E2A [18].

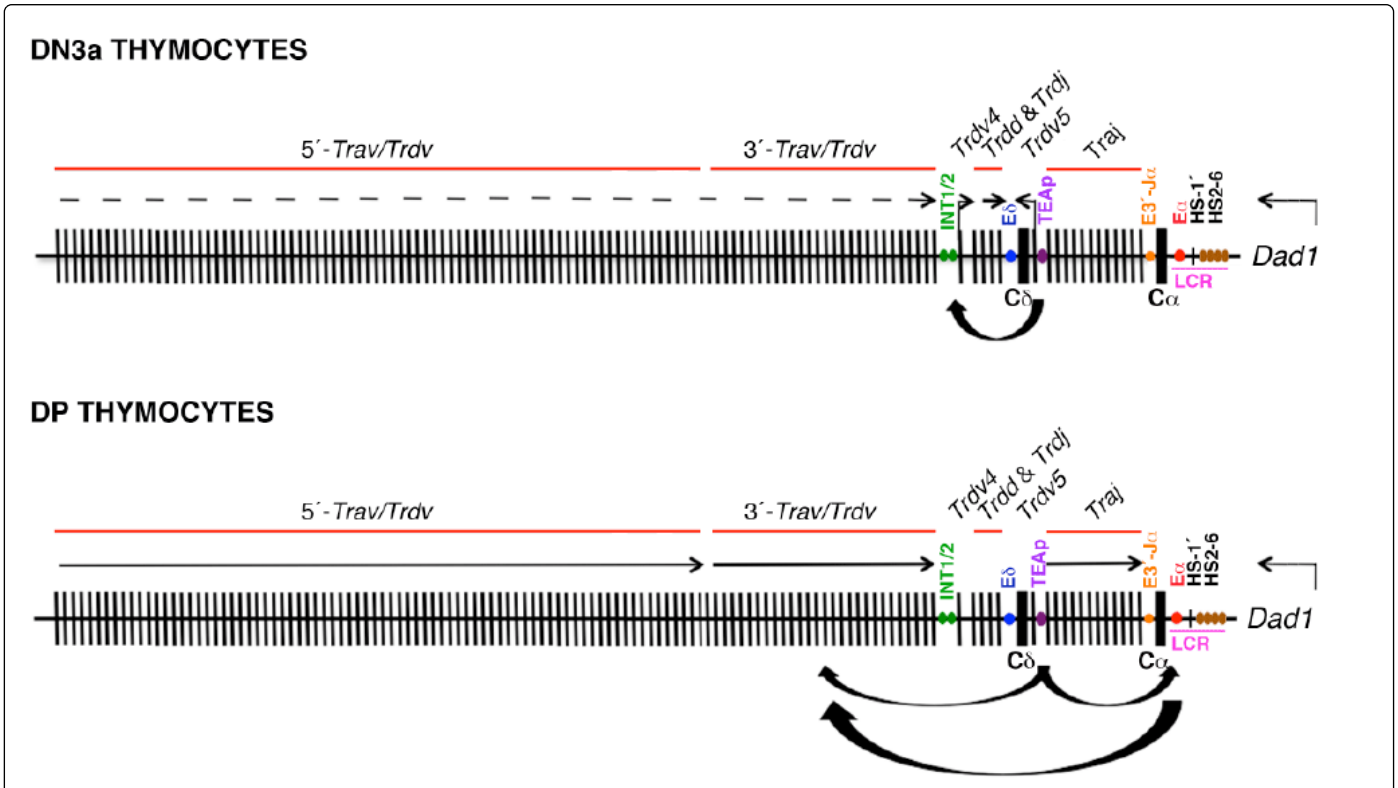

Figure 3: Different chromatin loops are formed at the 3´-*Tcra/Tcrd* locus in DN3a and DP thymocytes. The V, D, and J gene segments are represented by black narrow rectangles. The Cδ and Cα regions are represented by black large rectangles. The diagram indicates the 5´- and the 3´-*Trav/Trdv* gene segments, the *Trdd* and *Trdj* gene segments, the Cδ region, the *Trdv5* gene segment, the *Traj* gene segments, and the Cα region. The red lines indicate the areas occupied by 5´-*Trav/Trdv*, 3´-*Trav/Trdv*, *Trdd* and *Trdj*, and *Traj* gene segments. The position of the functionally relevant described regulatory elements is indicated as follows: INT1/2 as green circles, Eδ as a blue circle, TEAp as a purple circle, E3´-Jα as an orange circle, LCR as a pink line, Eα as a red circle, HS-1´as a black rectangle, and HS2-6 as brown ovals. Curved arrows represent the looping interactions between the regulatory elements demonstrated by 3C and 4C experiments [27,28]. In $Rag^{-/-}$ DN3a thymocytes, high-frequency looping interactions occur between the INT1/2 elements and the TEAp CTCF sites. In $Rag^{-/-}$ DP thymocytes, high frequency looping interactions occur between the Eα-, TEAp-, and the 3´-*Trav/Trdv* promoters-associated CTCF sites and bound TFs. These interactions are thought to promote the nucleation of recombination centers that facilitate both the *Tcrd* VDJ recombination in DN2/3a thymocytes and the *Tcra* VJ recombination in DP thymocytes involving distant gene segments.

Developmental Chromatin Dynamics at *Tcrd/Tcra*

Three-dimensional fluorescence in situ hybridization experiments revealed the *Tcra/Tcrd* locus changes its configuration in DN3a and DP thymocytes [5,61]. In DN3a thymocytes, the *Tcra/Tcrd* locus adopts a fully contracted configuration [61]. In DP thymocytes, the locus adopts a contracted configuration across the most 3´-region of the locus, including the 3´ *Trav/Trdv* and the *Traj* gene segments, as well as the Cα region, in a region of approximately 0.5-Mb; whereas it adopts an extended configuration across the 5´-region of the locus, including the centrally and upstream position *Trav/Trdv* gene segments (5´-*Trav/Trdv*) in a region of over 1-Mb [61]. The fully contracted locus configuration in DN3a thymocytes is thought to facilitate the *Tcrd* VDJ recombination using the disperse *Trdv* gene segments across the entire locus, whereas the *Tcra/Tcrd* configuration in DP thymocytes is believed to facilitate the sequentially ordered primary and secondary *Tcra* VJ recombination (Figure 2) [5,61]. The molecular mechanism involved in the regulation of the different configurations adopted by the *Tcra/Tcrd* locus during thymocyte development is currently unknown.

Although the 3´-end of the locus remains similarly contracted in DN3a and DP thymocytes, chromosome conformation capture experiments (3C and 4C) have distinguished two distinct functional chromatin interactions within this 0.5-Mb region of DN3a and DP thymocytes using recombinase activating gene-deficient (*Rag*$^{-/-}$) mice (Figure 3) [5,27,28]. In *Rag*$^{-/-}$ DN3a thymocytes, a functionally relevant discrete chromatin loop mediated by CTCF-bound INT1/2 and CTCF-bound TEAp has been recently identified [28]. Active Eδ is present within this chromatin loop attached to the *Trdd* and *Trdj* gene segments constituting a recombination center capable of recruiting the distant *Trdv* gene segments in DN2/3a thymocytes [5,28]. In addition to CTCF, other factors are required for loop formation because it is not present in B lymphocytes where occupancy of the relevant CTCF sites remains intact [28]. Interestingly, the formation of this chromatin loop favors the use of the diverse *Trdv* gene segments for *Tcrd* VDJ recombination in DN2/3a thymocytes and indirectly increases the diverse use of the *Trav/Trdv* gene segments for *Tcra* VJ recombination in DP thymocytes [28]. In *Rag*$^{-/-}$ eDP thymocytes, binding of the pre-TCR inducible TFs to Eα triggers the formation of a chromatin hub through the physical interactions of the Eα-bound TFs, TFs bound to the promoters associated with the most 3´-*Trav/Trdv* gene segments and TEAp, and the CTCF bound to HS-1´ and each Eα-dependent promoter (Figure 3) [27,31]. This chromatin hub creates an additional recombination center at the 3´-*Trav/Trdv* and 5´-*Traj* gene segments to activate the primary *Tcra* VJ recombination in DP thymocytes [5].

Eα and Eδ in mature αβ and γδ T lymphocytes

Once the TCRαβ or TCRγδ is assembled on the thymocyte surface, Eα becomes active in γδ T lymphocytes and is essential for normal transcription of the rearranged Tcrd locus in these cells, but surprisingly this enhancer is significantly inhibited in SP thymocytes and αβ T lymphocytes (Figure 4) [34,62]. Although Eδ is accepted to be active in γδ T lymphocytes, its contribution toward the transcription of the rearranged *Tcrd* locus is negligible due to the strong activity of the Eα enhancer in these cells (Figure 4) [43].

In support of inhibition of Eα activity in the transition from DP to SP thymocytes and in αβ T lymphocytes, Eα inhibition is evidenced not only when it is located in its natural location at the unrearranged *Tcra* locus and also when positioned at an ectopic location [62].

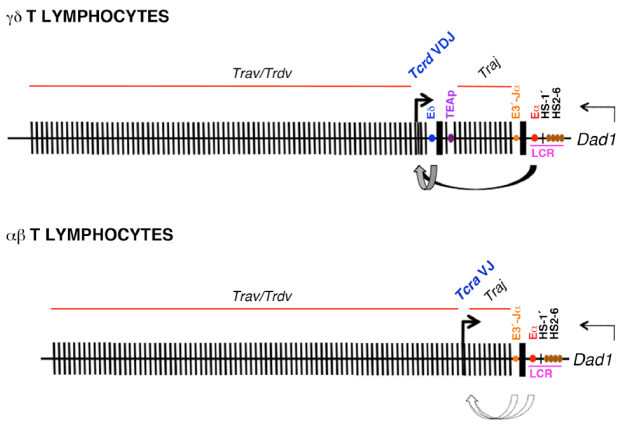

Figure 4: Regulation of transcription of the rearranged *Tcrd* and *Tcra* genes by distant enhancers in γδ and αβ T lymphocytes, respectively. The V, D, and J gene segments are represented by black narrow rectangles. The Cδ and Cα regions are represented by black large rectangles. Red lines indicate the areas occupied by *Trav/Trdv* and *Traj* gene segments. The rearranged *Tcrd* VDJ and *Tcra* VJ transcripts are written in bold blue characters. The position of the functionally relevant described regulatory elements is indicated as follows: Eδ as a blue circle, TEAp as a purple circle, E3´-Jα as an orange circle, LCR as a pink line, Eα as a red circle, HS-1´as a black rectangle, and HS2-6 as brown ovals. Curved arrows represent the predicted enhancer-promoter interactions based on the functional experiments [34,43,62]. In γδ T lymphocytes, Eα, and also presumably Eδ, functionally interact with the rearranged *Trav/Trdv* promoter. The black arrow represents the functionally relevant interaction between Eα and the rearranged *Trav/Trdv* promoter in comparison to the presumed interaction between Eδ and the rearranged *Trav/Trdv* promoter, which is represented in grey. In αβ T lymphocytes, Eα is strongly inhibited and its contribution to the transcription of the rearranged *Tcra* locus is uncertain. The contribution of the recently described E3´-Jα enhancer to the transcription of the rearranged *Tcra* locus is also unknown. The putatively weak or uncertain interactions between these enhancers and the rearranged *Trav/Trdv* promoters are represented as dashed light lines.

Furthermore, expression of reporter transgenes directed by the 7.4-kb LCR containing Eα is significantly inhibited in splenocytes and αβ T lymphocytes compared to thymocytes [30,63]. The transcriptional inhibition of the unrearranged *Tcra* locus by Eα in SP thymocytes and αβ T lymphocytes suggests this enhancer does not contribute to the transcription of the rearranged *Tcra* locus in these cells [62]. In support of this hypothesis, transgenic rearranged *Tcra* constructs containing the 3-kb region from the downstream Cα, including Eα and HS1´, are expressed at very low and variable levels, ranging from 1 to 20% in αβ T lymphocytes [29,64]. Two main questions rise from these findings: How is Eα inactivated and what is the molecular mechanism for transcribing the rearranged *Tcra* locus in SP thymocytes and αβ T cells?

Recent experiments using chromatin immunoprecipitation to compare the active and inactive Eα enhanceosomes assembled in DP thymocytes and αβ T lymphocytes, respectively, have revealed that the

presence of the E2A and HEB TFs is highly diminished in αβ T lymphocytes (Figure 5) [62].

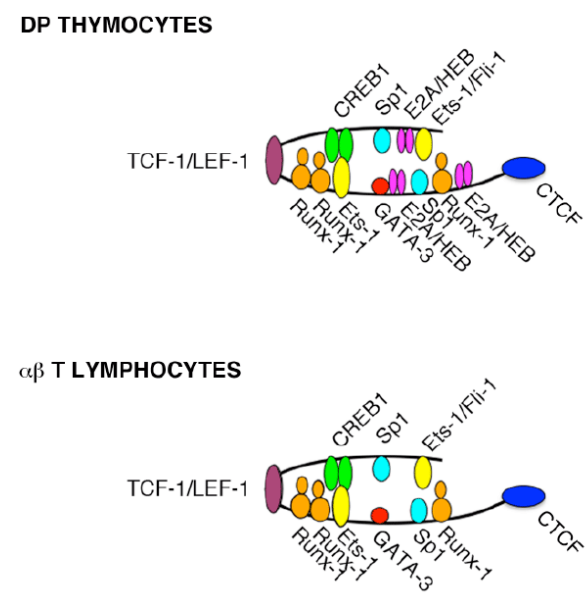

Figure 5: Different Eα enhanceosomes are assembled in lDP thymocytes and αβ T lymphocytes. The diagram depicts the TFs that are bound to Eα in the indicated cell stages based on genomic footprinting and chromatin immunoprecipitation experiments, as well as in vitro experiments [14,57-59,62,76]. The TFs are represented by colored ovals and their identity is indicated. The Eα enhanceosome assembled in αβ T lymphocytes lacks bound E2A and HEB TFs compared to those assembled in lDP thymocytes [62,76].

No differences in the binding of CTCF to HS-1´ were detected between DP thymocytes and αβ T lymphocytes, indicating the binding of this factor is not involved in the inhibition of Eα function in αβ T cells [31,33,62]. These results suggest strong binding of E2A and HEB might be essential for Eα activity. The forced expression of E2A in αβ T lymphocytes through retroviral transduction cannot recover the enhancer activity of Eα, neither alone nor in combination with the upregulation of other TFs in the context of T-cell activation or T helper differentiation [62]. Future experiments are necessary to reveal the molecular mechanism of enhancer inactivation in mature αβ T-cells by evaluating the simultaneous functional effect of E2A and HEB on Eα activity, the analysis of the molecular consequences of different signaling pathways mediated by the pre-TCR and TCRα on Eα function, and a detailed comparison of the Eα enhanceosomes assembled in αβ and γδ T lymphocytes.

The inhibition of Eα in SP thymocytes and αβ T lymphocytes does not preclude the enhanced transcription of the rearranged *Tcra* locus in these cells compared to the unrearranged *Tcra* locus in preselected DP thymocytes in the presence of a fully active Eα [62]. The molecular basis for the transcription of the rearranged *Tcra* locus is currently unknown. Although a possible contribution of Eα to the transcription of the rearranged *Tcra* locus cannot be totally rejected, the inhibition of its activity through the disruption of the functional long-range enhancer-promoter interactions, the loss of activating histone modifications, and the decreased transcription of the unrearranged *Tcra* locus in αβ T lymphocytes compared to lDP thymocytes suggests the existence of an Eα-independent mechanism to activate transcription of the rearranged *Tcra* locus in αβ T cells [62]. In support of this, Eα is not required for copy number-dependent transgenic expression in splenocytes [30]. It is possible that different conformations of the unrearranged and rearranged *Tcra* locus, due to the deletion of intergenic sequences, may reveal a novel enhancer or activate an enhancer-independent activity in the rearranged *Tcra* V promoters. The putative novel enhancer must be located upstream of *Trav1* or downstream of *Traj2* gene segments to ensure its retention upon *Tcra* VJ recombination. Interestingly, transcription of reporter transgenes controlled by the LCR is also significantly inhibited in splenocytes and αβ T lymphocytes compared to thymocytes, suggesting the additional sequences required for proper transcription the rearranged *Tcra* locus in αβ T lymphocytes are not contained within the 7.4-kb LCR fragment [30,31,63]. A new putative enhancer, E3´-Jα, located between the *Traj3* gene segment and Cα region, and is active in both thymocytes and peripheral αβ T lymphocytes has been recently described (Figures 2-4) [42]. However, transgenic constructs containing a rearranged *Tcra* locus with an intact *Traj2* to HS1´ are expressed at very low and variable levels in αβ T lymphocytes, suggesting the genomic region containing E3´-Jα, Eα, and HS1´ is not sufficient to allow for the strong and stable transcription of the endogenous rearranged *Tcra* locus [29,64]. It will be important to test the relevance of Eα and other putative relevant sequences in the transcription the rearranged *Tcra* locus by their conditional deletion in peripheral αβ T lymphocytes and in transgenic mice.

Consequences of Defects in *Tcra/Tcrd* Locus Transcription and Recombination

Although beneficial, V(D)J recombination is a dangerous process. Defects in this process at the TCR loci cause for immunodeficiencies and chromosomal translocations that lead to lethal leukemia [4,65,66]. The most common T-lymphocyte leukemia, T-cell acute lymphoblastic leukemia (T-ALL), is composed by a heterogeneous group of acute leukemias derived from the transformation of thymocytes that are arrested at various developmental stages. 35% of human T-ALLs carry chromosomal translocations involving TCR loci in thymocytes. These aberrant translocations frequently involve the juxtaposition of a strong promoter or enhancer from a TCR gene with a TF gene or a gene involved in cell signaling or differentiation. These illegitimate TCR gene translocations lead to the aberrant expression of their corresponding proteins, resulting in abnormal proliferation and differentiation processes. Among all the aberrant translocations of TCR genes during thymocyte development, those involving the *Tcra/Tcrd* locus have been found in a high percent of human T-ALLs. For example, 5-10% of pediatric and 30% of adult T-ALLs show translocations of the TLX1 and TLX3 genes into the *Tcra/Tcrd* locus. These translocations result in the overexpression of the TFs TLX1 and TLX3 and the arrest of DP thymocyte maturation. This arrest is a direct consequence of the recruitment of these TFs to Eα [67]. Binding of TLX1/TLX3 to Eα interferes with the recruitment of Ets-1 and results in reduced enhancer activity as evidenced by decreased gene chromatin accessibility and a drastic inhibition of *Tcra* gene segment recombination. The expression of a functional TCRα chain is needed for the assembly of the TCRαβ and the maturation of DP to SP thymocytes [34,68]. Other important aberrant translocations involving the *Tcra/Tcrd* locus include those that result in ataxia-telangiectasia (A-T) syndrome, which is rare immunodeficiency disorder due to

mutations in the A-T mutated kinase (ATM) that cause chromosome instability and defects in DNA repair [69]. An important percent of A-T syndrome patients develop the disease due to translocations and inversions involving specific breakpoints at the *Tcra/Tcrd* locus and most of all $ATM^{-/-}$ mice die due to thymic lymphomas derived from and incorrect repair of the breaks that result from V(D)J recombination and aberrant *Tcra/Tcrd* locus translocations [70-75]. The knowledge of the precise mechanisms by which the *Tcra/Tcrd* locus transcription and recombination are regulated is important to understand the defective control of these processes that results in disease.

Acknowledgements

This work was supported by the Spanish government (Grant BFU2013-44660R) and the Andalusian government (Grant CTS-6587), which is financed in part by the European Regional Development Fund (ERDF/FEDER).

References

1. Rothenberg EV, Taghon T (2005) Molecular genetics of T cell development. Annu Rev Immunol 23: 601-649.
2. Taghon T, Yui MA, Pant R, Diamond RA, Rothenberg EV (2006) Developmental and molecular characterization of emerging β- and γδ-selected pre-T cells in the adult mouse thymus. Immunity 24: 53-64.
3. Brekelmans P, van Soest P, Voerman J, Platenburg PP, Leenen PJ, et al. (1994) Transferrin receptor expression as a marker of immature cycling thymocytes in the mouse. Cell Immunol 159: 331-339.
4. del Blanco B, Angulo Ú, Hernández-Munain C (2014) Epigenetic control of T cell receptor locus rearrangements in normal and aberrant conditions. Epigenetic and Human Health. Transcriptional and Epigenetic Mechanisms Regulating Normal and Aberrant Blood Cell Development 12: 295-329.
5. Carico Z, Krangel MS (2015) Chromatin Dynamics and the Development of the TCRα and TCRδ Repertoires. Adv Immunol 128: 307-361.
6. Ciofani M, Schmitt TM, Ciofani A, Michie AM, Cuburu N, et al. (2004) Obligatory role for cooperative signaling by pre-TCR and Notch during thymocyte differentiation. J Immunol 172: 5230-5239.
7. Ciofani M, Zúñiga-Pflücker JC (2005) Notch promotes survival of pre-T cells at the β-selection checkpoint by regulating cellular metabolism. Nat Immunol 6: 881-888.
8. Pui JC, Allman D, Xu L, DeRocco S, Karnell FG, et al. (1999) Notch1 expression in early lymphopoiesis influences B versus T lineage determination. Immunity 11: 299-308.
9. Radtke F, Wilson A, Stark G, Bauer M, van Meerwijk J, et al. (1999) Deficient T cell fate specification in mice with an induced inactivation of Notch1. Immunity 10: 547-558.
10. Boudil A, Matei IR, Shih H-Y, Bogdanoski G, Yuan JS, et al. (2015) IL-7 coordinates proliferation, differentiation and *Tcra* recombination during thymocyte β-selection. Nat Immunol 16: 397-405.
11. Kang J, Coles M, Raulet DH (1999) Defective development of γδ T cells in interleukin 7 receptor- deficient mice is due to impaired expression of T cell receptor γ genes. J Exp Med 190: 973-982.
12. Wolfer A, Wilson A, Nemir M, MacDonald HR, Radtke F (2002) Inactivation of Notch1 impairs VDJβ rearrangement and allows pre-TCR-independent survival of early αβ Lineage Thymocytes. Immunity 16: 869-879.
13. Ferrero I, Mancini SJ, Grosjean F, Wilson A, Otten L, et al. (2006) TCRγ silencing during αβ T cell development depends upon pre-TCR-induced proliferation. J Immunol 177: 6038-6043.
14. Hernández-Munain C, Sleckman BP, Krangel MS (1999) A developmental switch from TCRδ enhancer to TCRα enhancer function during thymocyte maturation. Immunity 10: 723-733.
15. Livák F, Tourigny M, Schatz DG, Petrie HT (1999) Characterization of TCR gene rearrangements during adult murine T cell development. J Immunol 162: 2575-2580.
16. Tani-Ichi S, Satake M, Ikuta K (2011) The pre-TCR signal induces transcriptional silencing of the TCRγ locus by reducing the recruitment of STAT5 and Runx to transcriptional enhancers. Int Immunol 23: 553-563.
17. Tani-ichi S, Satake M, Ikuta K (2009) Activation of the mouse TCRγ enhancers by STAT5. Int Immunol 21: 1079-1088.
18. del Blanco B, García-Mariscal A, Wiest DL, Hernández-Munain C (2012) *Tcra* enhancer activation by inducible transcription factors downstream of pre-TCR signaling. J Immunol 188: 3278-3293.
19. Schlissel MS, Durum SD, Muegge K (2000) The interleukin 7 receptor is required for T cell receptor γ locus accessibility to the V(D)J recombinase. J Exp Med 191: 1045-1050.
20. Maki K, Sunaga S, Ikuta K (1996) The V-J recombination of T cell receptor-γ genes is blocked in interleukin-7 receptor-deficient mice. J Exp Med 184: 2423-2427.
21. Durum SK, Candèias S, Nakajima H, Leonard WJ, Baird AM, et al. (1998) Interleukin 7 receptor control of T cell receptor γ gene rearrangement: role of receptor-associated chains and locus accessibility. J Exp Med 188: 2233-2241.
22. Ye SK, Agata Y, Lee HC, Kurooka H, Kitamura T, et al. (2001) The IL-7 receptor controls the accessibility of the TCRγ locus by Stat5 and histone acetylation. Immunity 15: 813-823.
23. Ye SK, Maki K, Kitamura T, Sunaga S, Akashi K, et al. (1999) Induction of germline transcription in the TCRγ locus by Stat5: implications for accessibility control by the IL-7 receptor. Immunity 11: 213-223.
24. Masui N, Tani-ichi S, Maki K, Ikuta K (2008) Transcriptional activation of mouse TCR Jγ4 germline promoter by STAT5. Mol Immunol 45: 849-855.
25. Jackson AM, Krangel MS (2006) A role for MAPK in feedback inhibition of *Tcrb* recombination. J Immunol 176: 6824-6830.
26. Glusman G, Rowen L, Lee I, Boysen C, Roach JC, et al. (2001) Comparative genomics of the human and mouse T cell receptor loci. Immunity 15: 337-349.
27. Shih HY, Verma-Gaur J, Torkamani A, Feeney AJ, Galjart N, et al. (2012) *Tcra* gene recombination is supported by a *Tcra* enhancer- and CTCF-dependent chromatin hub. Proc Natl Acad Sci U S A 109: E3493-3502.
28. Chen L, Carico Z, Shih HY, Krangel MS (2015) A discrete chromatin loop in the mouse *Tcra-Tcrd* locus shapes the TCR δ and TCRα repertoires. Nat Immunol 16: 1085-1093.
29. Diaz P, Cado D, Winoto A (1994) A locus control region in the T cell receptor α/δ locus. Immunity 1: 207-217.
30. Ortiz BD, Cado D, Winoto A (1999) A new element within the T-cell receptor α locus required for tissue-specific locus control region activity. Mol Cell Biol 19: 1901-1909.
31. Ortiz BD, Cado D, Chen V, Diaz PW, Winoto A (1997) Adjacent DNA elements dominantly restrict the ubiquitous activity of a novel chromatin-opening region to specific tissues. EMBO J 16: 5037-5045.
32. Gomos-Klein J, Harrow F, Alarcon J, Ortiz BD (2007) CTCF-independent, but not CTCF-dependent, elements significantly contribute to TCR-α locus control region activity. J Immunol 179: 1088-1095.
33. Magdinier F, Yusufzai TM, Felsenfeld G (2004) Both CTCF-dependent and -independent insulators are found between the mouse T cell receptor α and *Dad1* genes. J Biol Chem 279: 25381-25389.
34. Sleckman BP, Bardon CG, Ferrini R, Davidson L, Alt FW (1997) Function of the TCR α enhancer in αβ and γδ T cells. Immunity 7: 505-515.
35. Lauzurica P, Krangel MS (1994) Temporal and lineage-specific control of T cell receptor α/δ gene rearrangement by T cell receptor α and δ enhancers. J Exp Med 179: 1913-1921.
36. Roberts JL, Lauzurica P, Krangel MS (1997) Developmental regulation of VDJ recombination by the core fragment of the T cell receptor α enhancer. J Exp Med 185: 131-140.

37. Balmelle N, Zamarreño N, Krangel MS, Hernández-Munain C (2004) Developmental activation of the TCRα enhancer requires functional collaboration among proteins bound inside and outside the core enhancer. J Immunol 173: 5054-5063.
38. Capone M, Watrin F, Fernex C, Horvat B, Krippl B, et al. (1993) TCRβ and TCRα gene enhancers confer tissue- and stage-specificity on V(D)J recombination events. EMBO J 12: 4335-4346.
39. Zhong XP, Krangel MS (1999) Enhancer-blocking activity within the DNase I hypersensitive site 2 to 6 region between the TCRα and *Dad1* genes. J Immunol 163: 295-300.
40. Ortiz BD, Harrow F, Cado D, Santoso B, Winoto A (2001) Function and factor interactions of a locus control region element in the mouse T cell receptor-α/*Dad1* gene locus. J Immunol 167: 3836-3845.
41. Harrow F, Amuta JU, Hutchinson SR, Akwaa F, Ortiz BD (2004) Factors binding a non-classical Cis-element prevent heterochromatin effects on locus control region activity. J Biol Chem 279: 17842-17849.
42. Kučerová-Levisohn M, Knirr S, Mejia RI, Ortiz BD (2015) The 3'-Jα Region of the TCRα Locus Bears Gene Regulatory Activity in Thymic and Peripheral T Cells. PLoS One 10: e0132856.
43. Monroe RJ, Sleckman BP, Monroe BC, Khor B, Claypool S, et al. (1999) Developmental regulation of TCRδ locus accessibility and expression by the TCR δ enhancer. Immunity 10: 503-513.
44. Hawwari A, Krangel MS (2005) Regulation of TCRδ and α repertoires by local and long-distance control of variable gene segment chromatin structure. J Exp Med 202: 467-472.
45. Hao B, Krangel MS (2011) Long-distance regulation of fetal V(δ) gene segment TRDV4 by the *Tcrd* enhancer. J Immunol 187: 2484-2491.
46. Hernández-Munain C (2015) Recent insights into the transcriptional control of the *Tcra/Tcrd* locus by distant enhancers during the development of T-lymphocytes. Transcription 6: 65-73.
47. McMurry MT, Hernandez-Munain C, Lauzurica P, Krangel MS (1997) Enhancer control of local accessibility to V(D)J recombinase. Mol Cell Biol 17: 4553-4561.
48. Abarrategui I, Krangel MS (2006) Regulation of T cell receptor-α gene recombination by transcription. Nat Immunol 7: 1109-1115.
49. Abarrategui I, Krangel MS (2007) Noncoding transcription controls downstream promoters to regulate T-cell receptor α recombination. EMBO J 26: 4380-4390.
50. Hawwari A, Bock C, Krangel MS (2005) Regulation of T cell receptor α gene assembly by a complex hierarchy of germline Jα promoters. Nat Immunol 6: 481-489.
51. Hawwari A, Krangel MS (2007) Role for rearranged variable gene segments in directing secondary T cell receptor α recombination. Proc Natl Acad Sci USA 104: 903-907.
52. Zhong XP, Carabaña J, Krangel MS (1999) Flanking nuclear matrix attachment regions synergize with the T cell receptor δ enhancer to promote V(D)J recombination. Proc Natl Acad Sci U S A 96: 11970-11975.
53. Hernández-Munain C, Krangel MS (1994) Regulation of the T-cell receptor δ enhancer by functional cooperation between c-Myb and core-binding factors. Mol Cell Biol 14: 473-483.
54. Hernández-Munain C, Lauzurica P, Krangel MS (1996) Regulation of T cell receptor δ gene rearrangement by c-Myb. J Exp Med 183: 289-293.
55. Lauzurica P, Zhong XP, Krangel MS, Roberts JL (1997) Regulation of T cell receptor δ gene rearrangement by CBF/PEBP2. J Exp Med 185: 1193-1201.
56. Hernández-Munain C, Roberts JL, Krangel MS (1998) Cooperation among multiple transcription factors is required for access to minimal T-cell receptor α-enhancer chromatin in vivo. Mol Cell Biol 18: 3223-3233.
57. Giese K, Kingsley C, Kirshner JR, Grosschedl R (1995) Assembly and function of a TCRα enhancer complex is dependent on LEF-1-induced DNA bending and multiple protein-protein interactions. Genes Dev 9: 995-1008.
58. Mayall TP, Sheridan PL, Montminy MR, Jones KA (1997) Distinct roles for P-CREB and LEF-1 in TCRα enhancer assembly and activation on chromatin templates in vitro. Genes Dev 11: 887-899.
59. Spicuglia S, Payet D, Tripathi RK, Rameil P, Verthuy C, et al. (2000) TCRα enhancer activation occurs via a conformational change of a pre-assembled nucleo-protein complex. EMBO J 19: 2034-2045.
60. Bassing CH, Tillman RE, Woodman BB, Canty D, Monroe RJ, et al. (2003) T cell receptor (TCR) α/δ locus enhancer identity and position are critical for the assembly of TCR δ and α variable region genes. Proc Natl Acad Sci U S A 100: 2598-2603.
61. Shih HY, Krangel MS (2010) Distinct contracted conformations of the *Tcra/Tcrd* locus during *Tcra* and *Tcrd* recombination. J Exp Med 207: 1835-1841.
62. del Blanco B, Angulo Ú, Krangel MS, Hernández-Munain C (2015) T-cell receptor α enhancer is inactivated in αβ T lymphocytes. Proc Natl Acad Sci U S A 112: E1744-1753.
63. Harrow F, Ortiz BD (2005) The TCRα locus control region specifies thymic, but not peripheral, patterns of TCRα gene expression. J Immunol 175: 6659-6667.
64. von Boehmer H (1990) Developmental biology of T cells in T cell-receptor transgenic mice. Annu Rev Immunol 8: 531-556.
65. Van Vlierberghe P, Ferrando A (2012) The molecular basis of T cell acute lymphoblastic leukemia. J Clin Invest 122: 3398-3406.
66. Aifantis I, Raetz E, Buonamici S (2008) Molecular pathogenesis of T-cell leukaemia and lymphoma. Nat Rev Immunol 8: 380-390.
67. Dadi S, Le Noir S, Payet-Bornet D, Lhermitte L, Zacarias-Cabeza J, et al. (2012) TLX homeodomain oncogenes mediate T cell maturation arrest in T-ALL via interaction with ETS1 and suppression of TCRα gene expression. Cancer Cell 21: 563-576.
68. Shinkai Y, Koyasu S, Nakayama K, Murphy KM, Loh DY, et al. (1993) Restoration of T cell development in RAG-2-deficient mice by functional TCR transgenes. Science 259: 822-825.
69. Bednarski JJ, Sleckman BP (2012) Lymphocyte development: integration of DNA damage response signaling. Adv Immunol 116: 175-204.
70. Liyanage M, Weaver Z, Barlow C, Coleman A, Pankratz DG, et al. (2000) Abnormal rearrangement within the α/δ T-cell receptor locus in lymphomas from Atm-deficient mice. Blood 96: 1940-1946.
71. Zha S, Bassing CH, Sanda T, Brush JW, Patel H, et al. (2010) ATM-deficient thymic lymphoma is associated with aberrant tcrd rearrangement and gene amplification. J Exp Med 207: 1369-1380.
72. Isoda T, Takagi M, Nakagama S, Sato M, Masuda K, et al. (2012) Process for immune defect and chromosomal translocation during early thymocyte development lacking ATM. Blood 120: 789-799.
73. Bredemeyer AL, Sharma GG, Huang CY, Helmink BA, Walker LM, et al. (2006) ATM stabilizes DNA double-strand-break complexes during V(D)J recombination. Nature 442: 466-470.
74. Matei IR, Gladdy RA, Nutter LM, Canty A, Guidos CJ, et al. (2007) ATM deficiency disrupts *Tcra* locus integrity and the maturation of $CD4^+CD8^+$ thymocytes. Blood 109: 1887-1896.
75. Vacchio MS, Olaru A, Livak F, Hodes RJ (2007) ATM deficiency impairs thymocyte maturation because of defective resolution of T cell receptor α locus coding end breaks. Proc Natl Acad Sci U S A 104: 6323-6328.
76. del Blanco B, Roberts JL, Zamarreño N, Balmelle-Devaux N, Hernández-Munain C (2009) Flexible stereospecific interactions and composition within nucleoprotein complexes assembled on the TCR α gene enhancer. J Immunol 183: 1871-1883.

This article was originally published in a special issue, entitled: **"T-cell Immunology"**, Edited by Dr. Kota V Ramana, University of Texas, USA