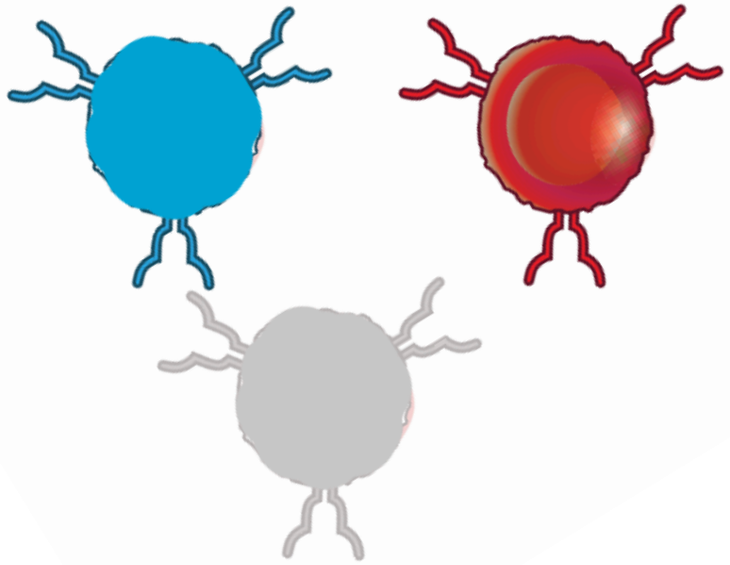
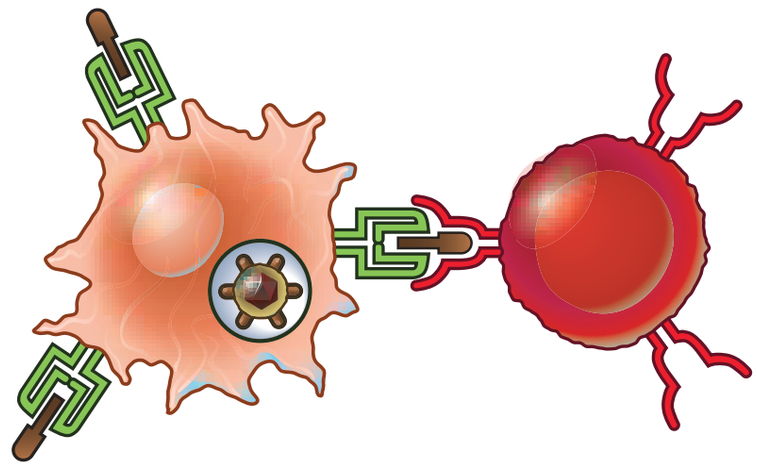
DIVERSITY

multiple clones with different TCRs exit the thymus

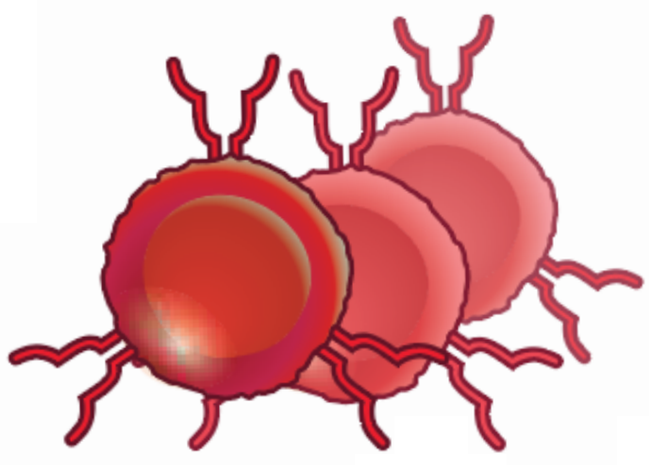


ACTIVATION

antigen-specific clones interact with antigen-presenting DCs



CLONAL EXPANSION



MHC-I and MHC-II

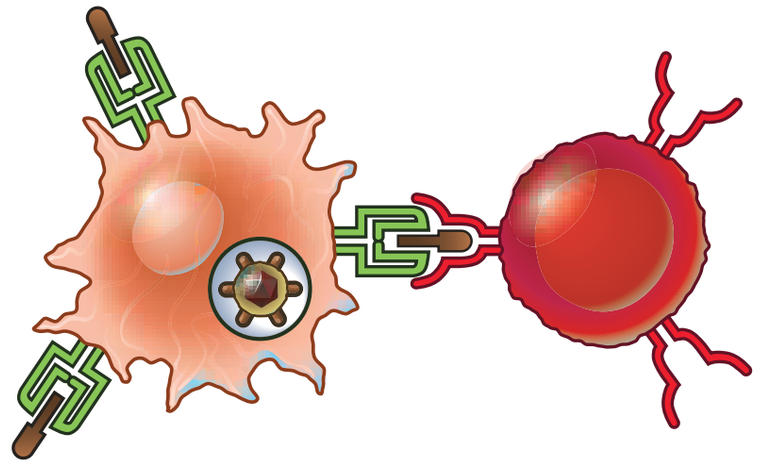
MHC-I presents “intracellular” antigens to CD8+ T cells, while MHC-II presents “extracellular” antigens to CD4+ T cells

HLA genes and their polymorphism

The concept of MHC restriction

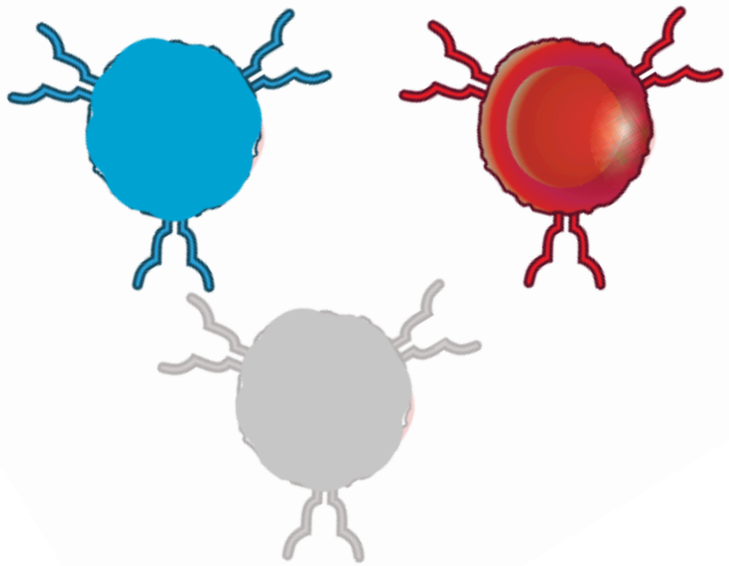
ACTIVATION

antigen-specific clones interact with antigen-presenting DCs



DIVERSITY

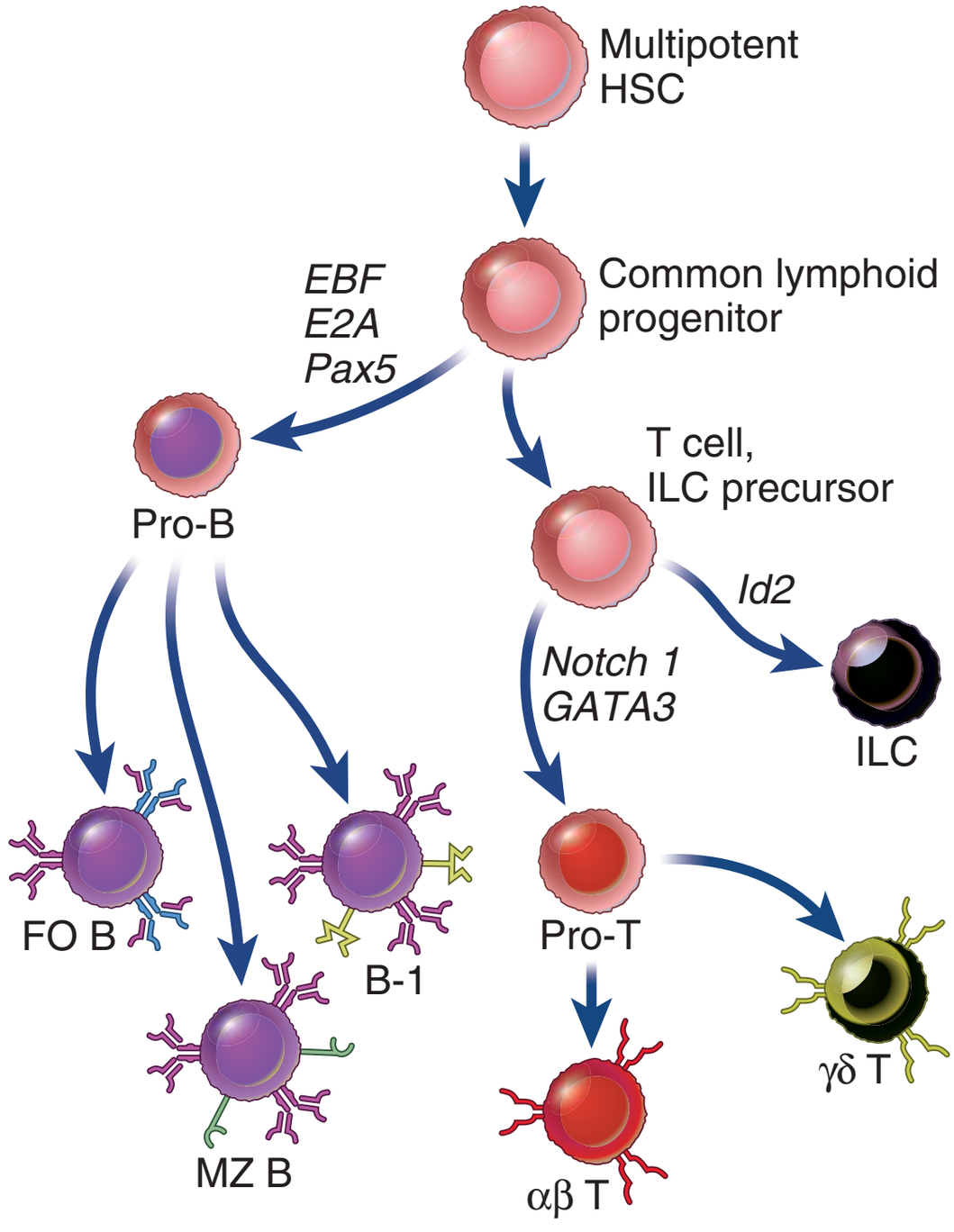
multiple clones with different TCRs exit the thymus



How the immune system generate diversity?

TCR recombination, testing and selection
(similar process for the BCR)

Multipotent stem cells give rise to distinct B and T lineages



The steps

TCR recombination

TCR tested for functionality and
self reactivity
(positive and negative selection)

Proliferation

Pre-B/T antigen receptor expression

Proliferation

Antigen receptor expression

Positive and negative selection

Pro-B/T cell

Pre-B/T cell: expresses one chain of antigen receptor

Failure to express pre-antigen receptor; cell death

Immature B/T cell: expresses complete antigen receptor

Failure to express antigen receptor; cell death

Weak antigen recognition

Mature T/B cell

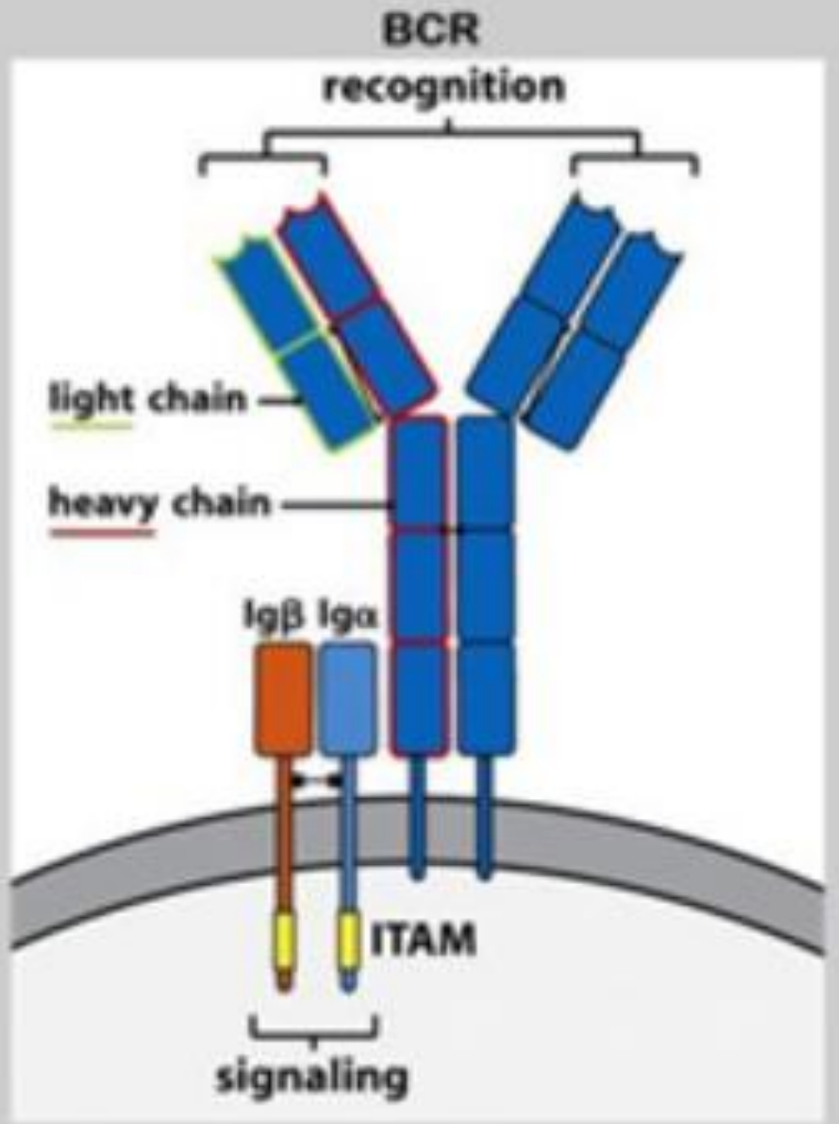
Positive selection

Strong antigen recognition

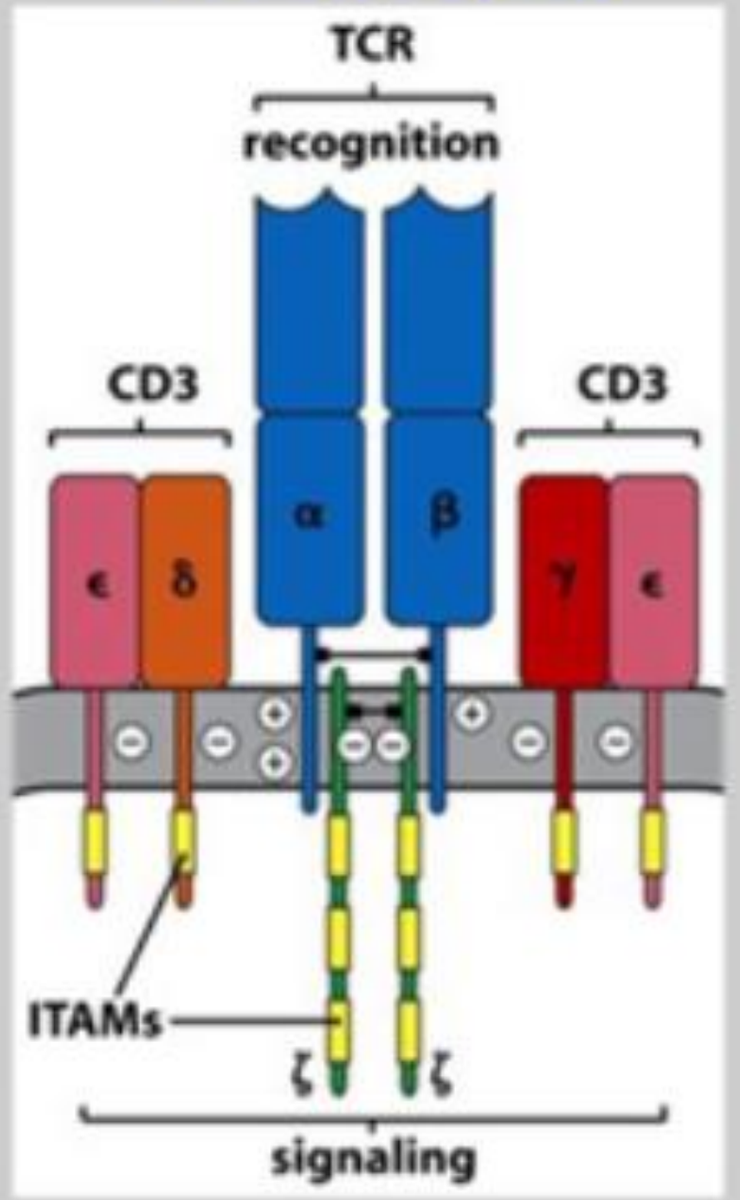
Negative selection

T Cell and B Cell Antigen Receptors
(TCR and BCR)

B cell Receptor



T cell Receptor



TCRs and BCRs are generated by recombination of V, D, J gene segments

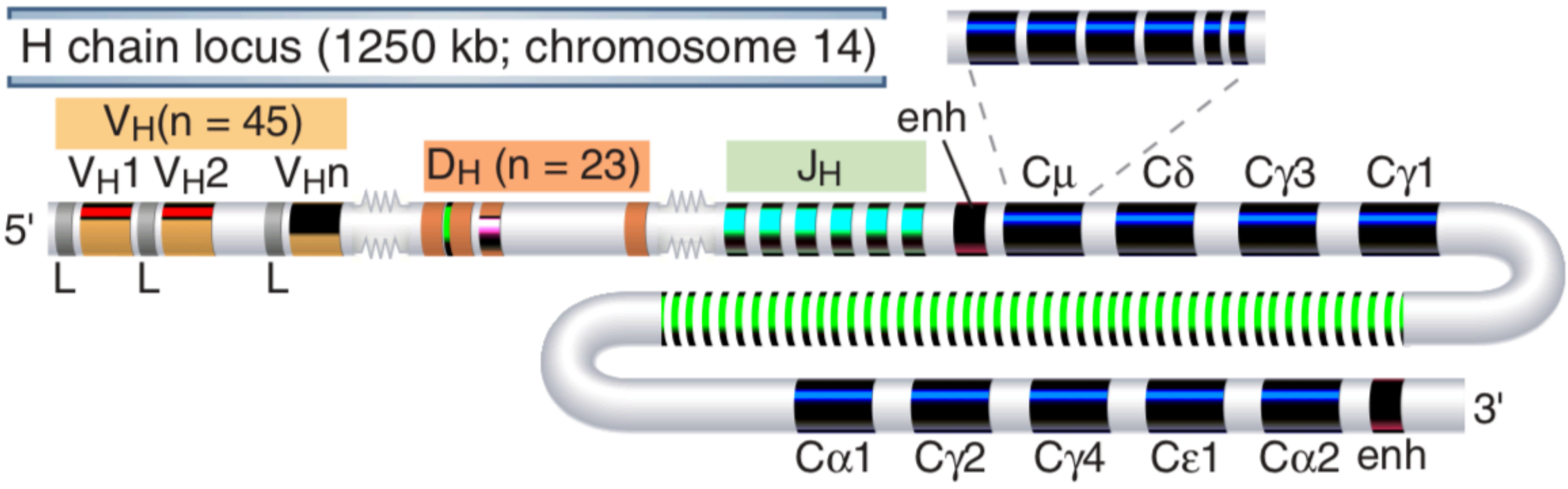
Both TCR and BCR have a variable region and a constant region

The variable region is composed by the assembly of a V, a D and a J gene fragment

There are many V, D and J gene fragments to choose from

Organization of the Ig loci

Three separate loci: Ig heavy chain; Ig κ light chain k; Ig λ light chain



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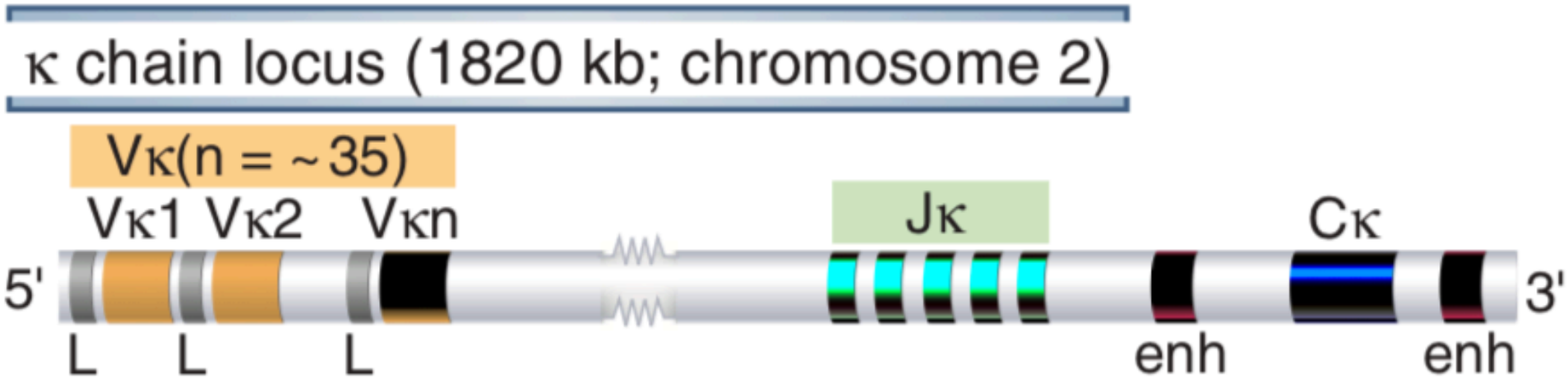

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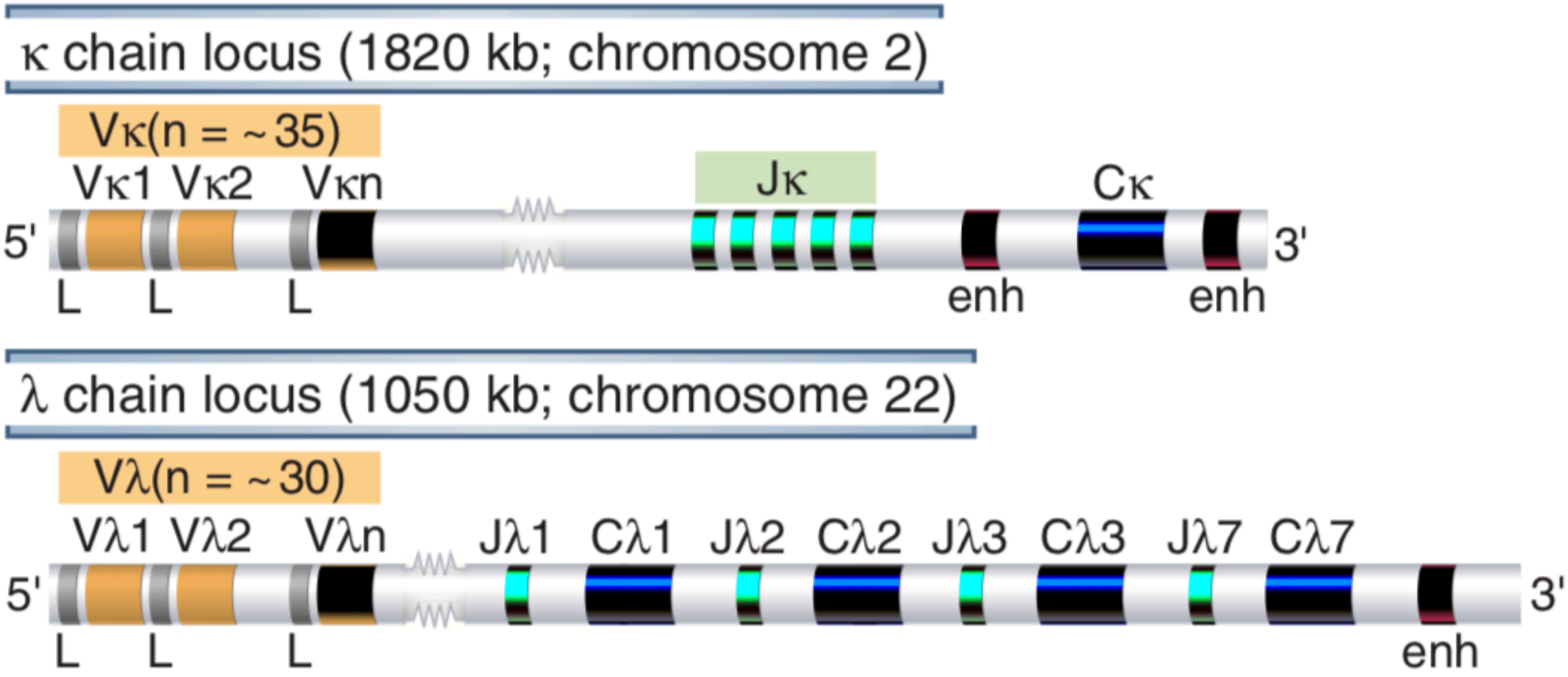
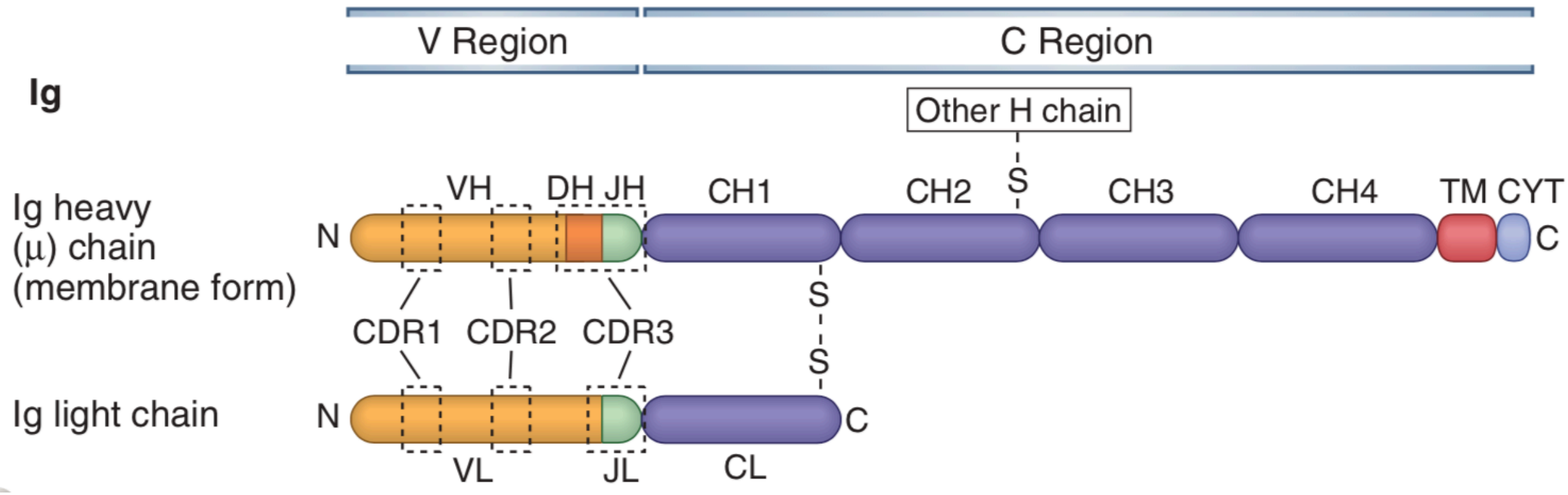
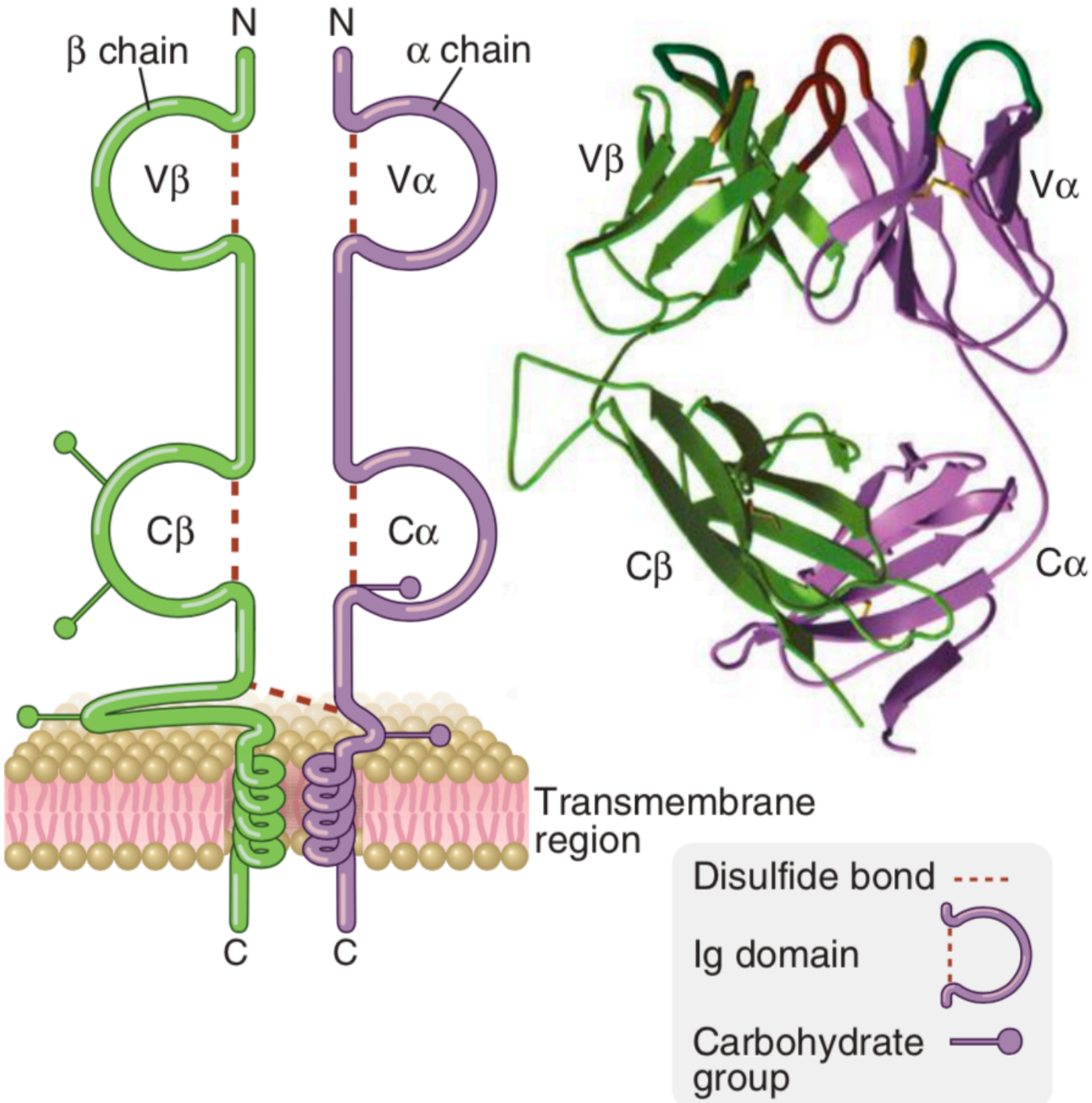


In light chain loci, D segments are not present

V, (D) and J gene product correspond to the variable regions



Structure of TCR

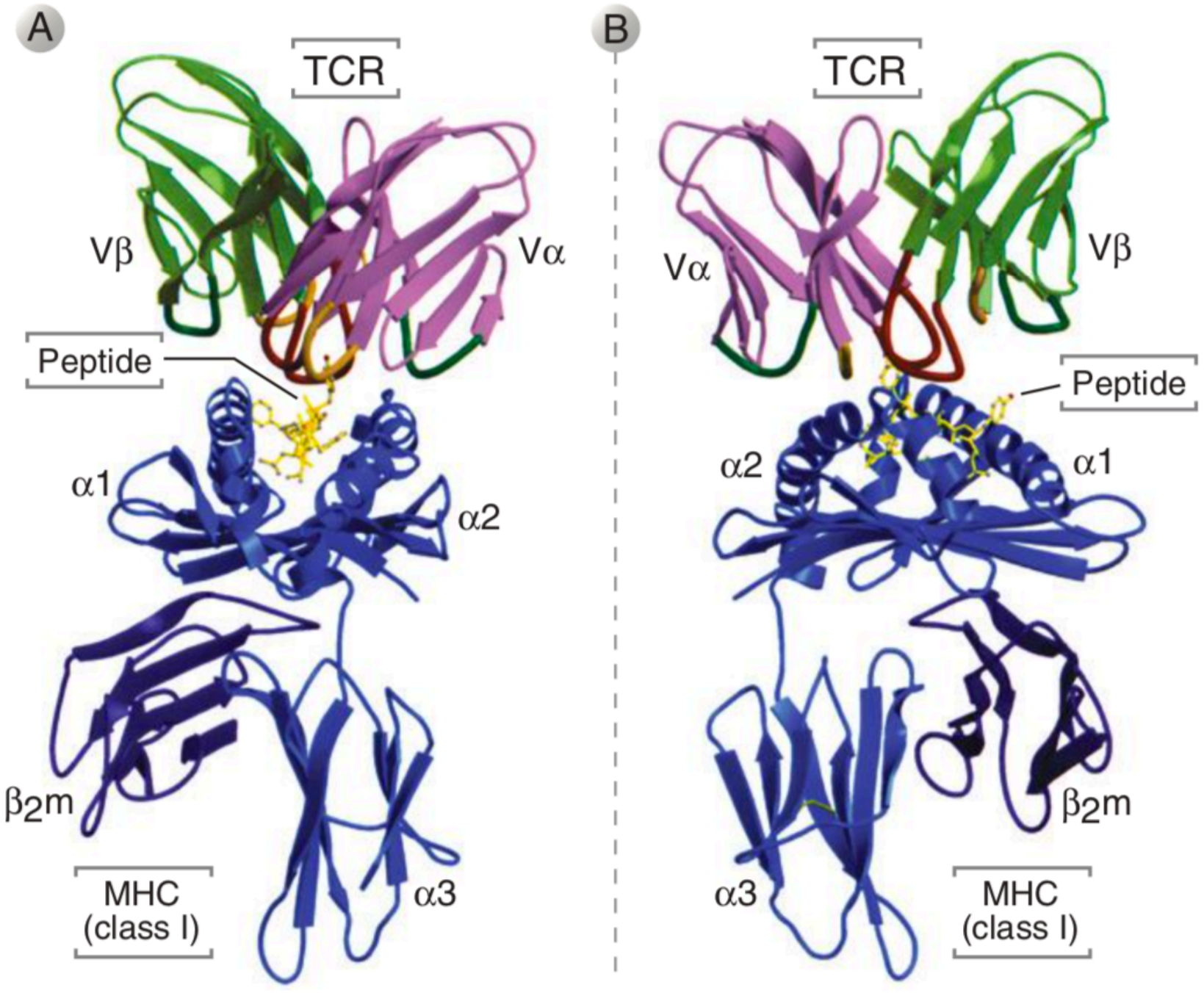


Alpha chain

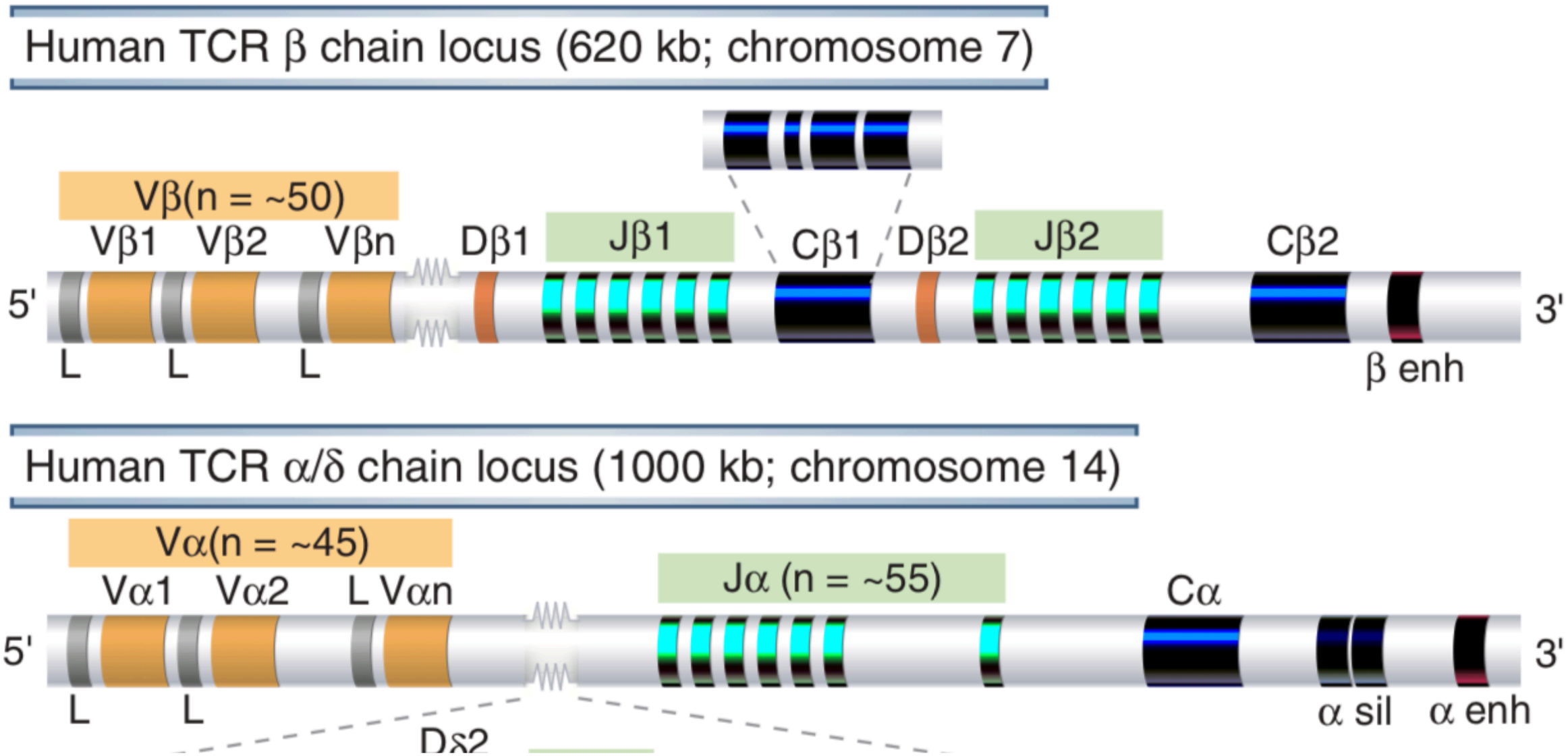
Beta chain

Gamma chain

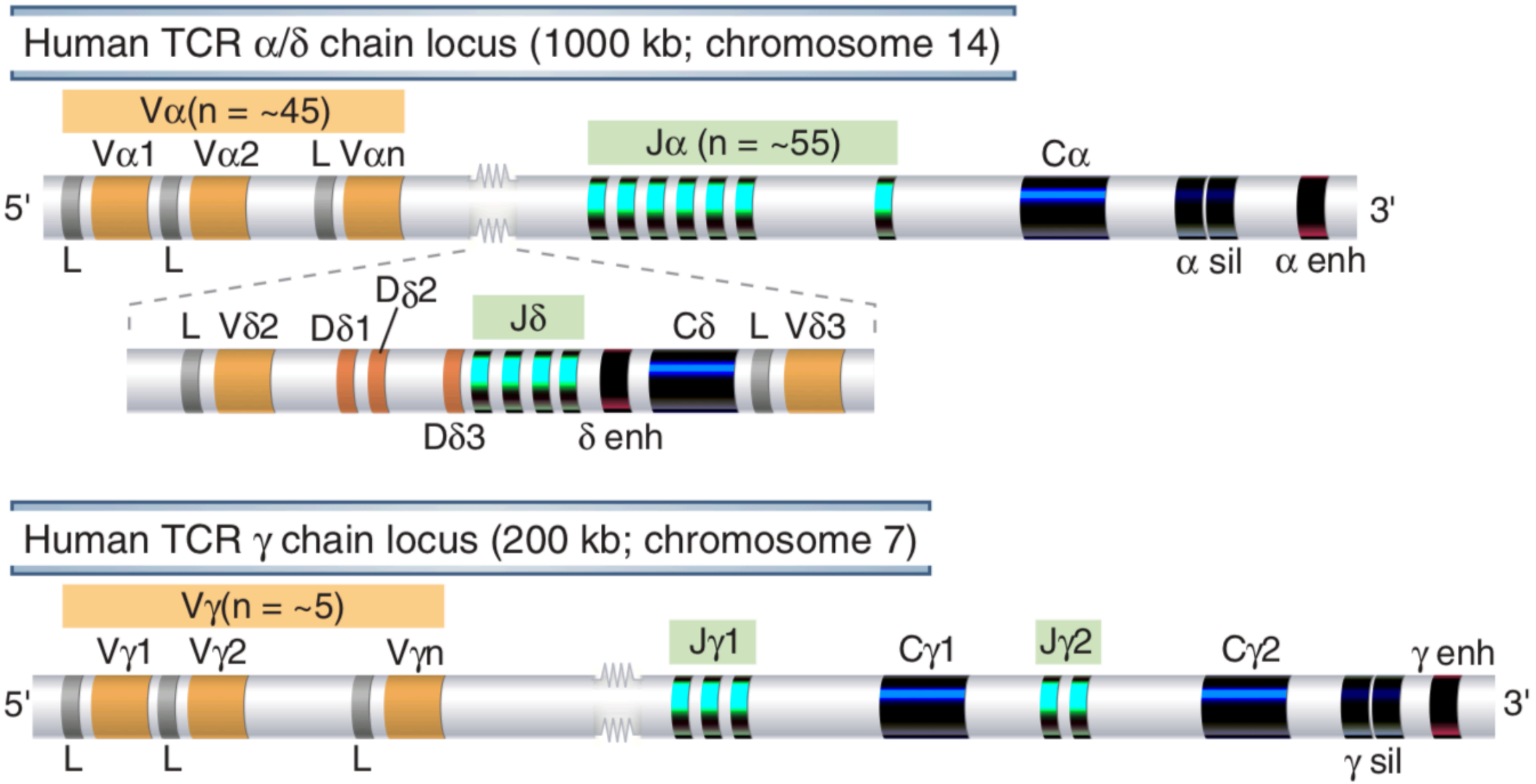
Delta chain


A
TCR
Vβ
Vα
Peptide
α1
α2
β2m
MHC
(class I)
α3
B
TCR
Vα
Vβ
Peptide
α2
α1
β2m
α3
MHC
(class I)

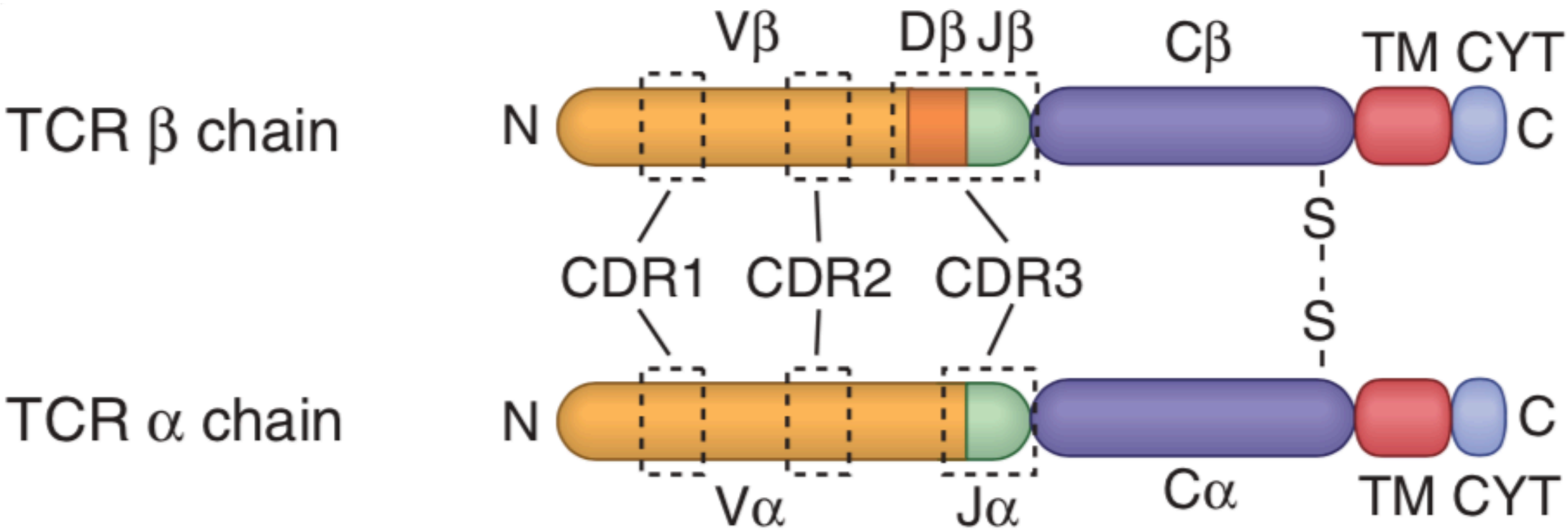
Organization of the TCR loci



Organization of the TCR loci



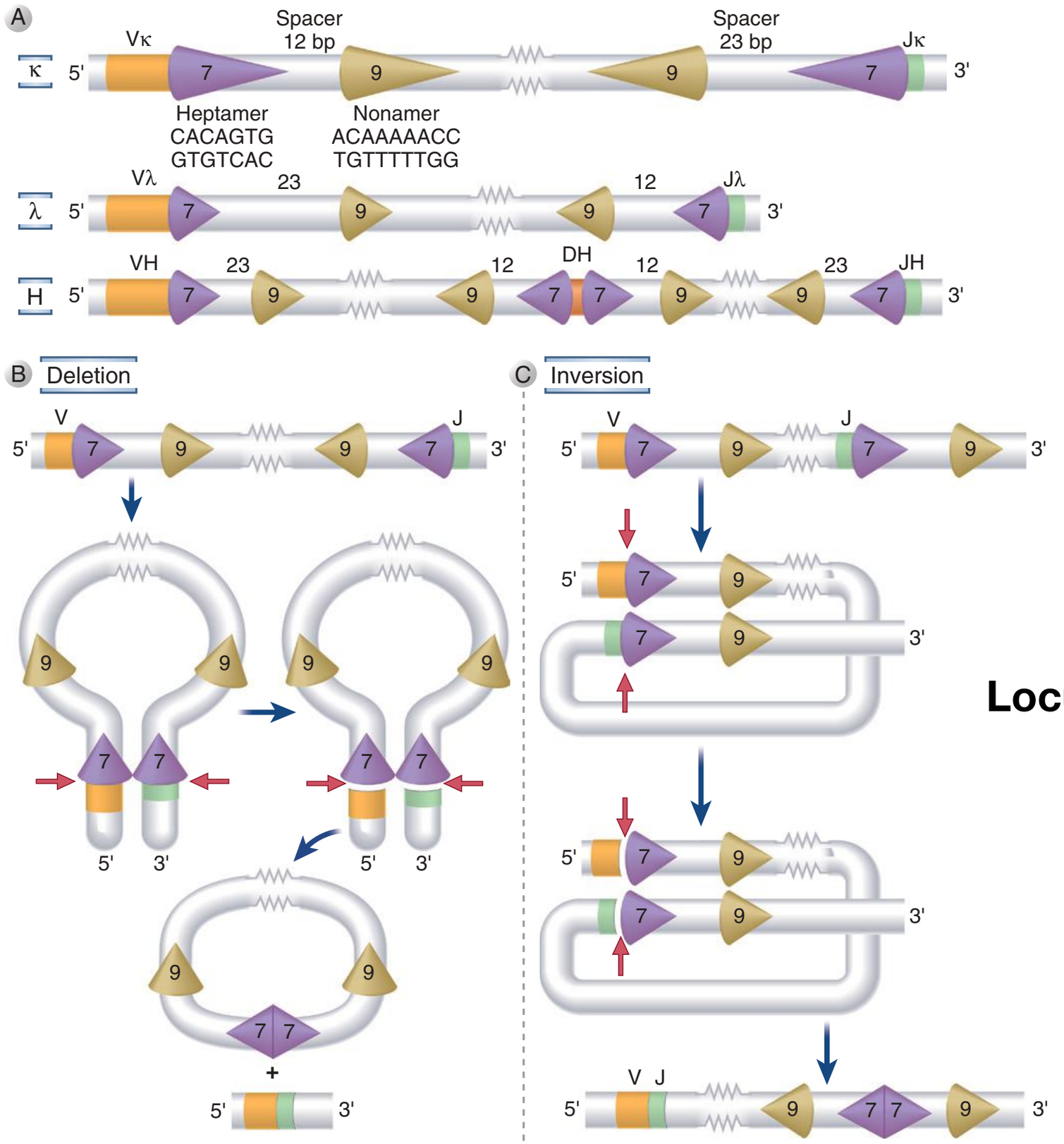
V, (D) and J gene product correspond to the variable regions



The germline organization of the Ig and TCR loci is present as described in all cells, but there is no transcription of a functional mRNA

During the development of T and B cells, DNA recombines bring in proximity randomly chosen V-D-J fragments

V(D)J recombination



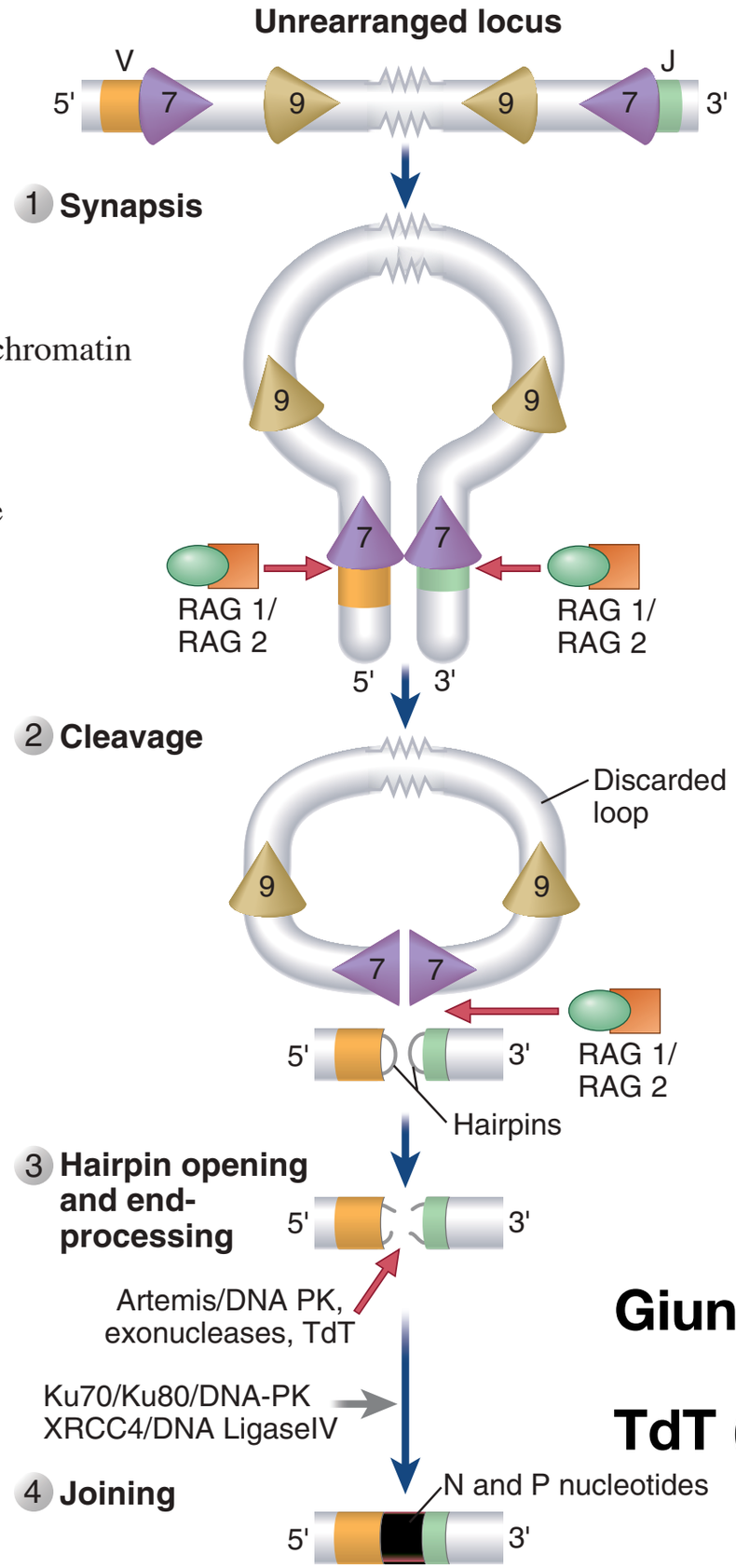
Locus K per Ig

RSS (Recombination Signal sequences)

Sequential events during V(D)J recombination

1 RSSs located in open euchromatin exposed

2 gene segments undergoing recombination acquire histone hypermethylation



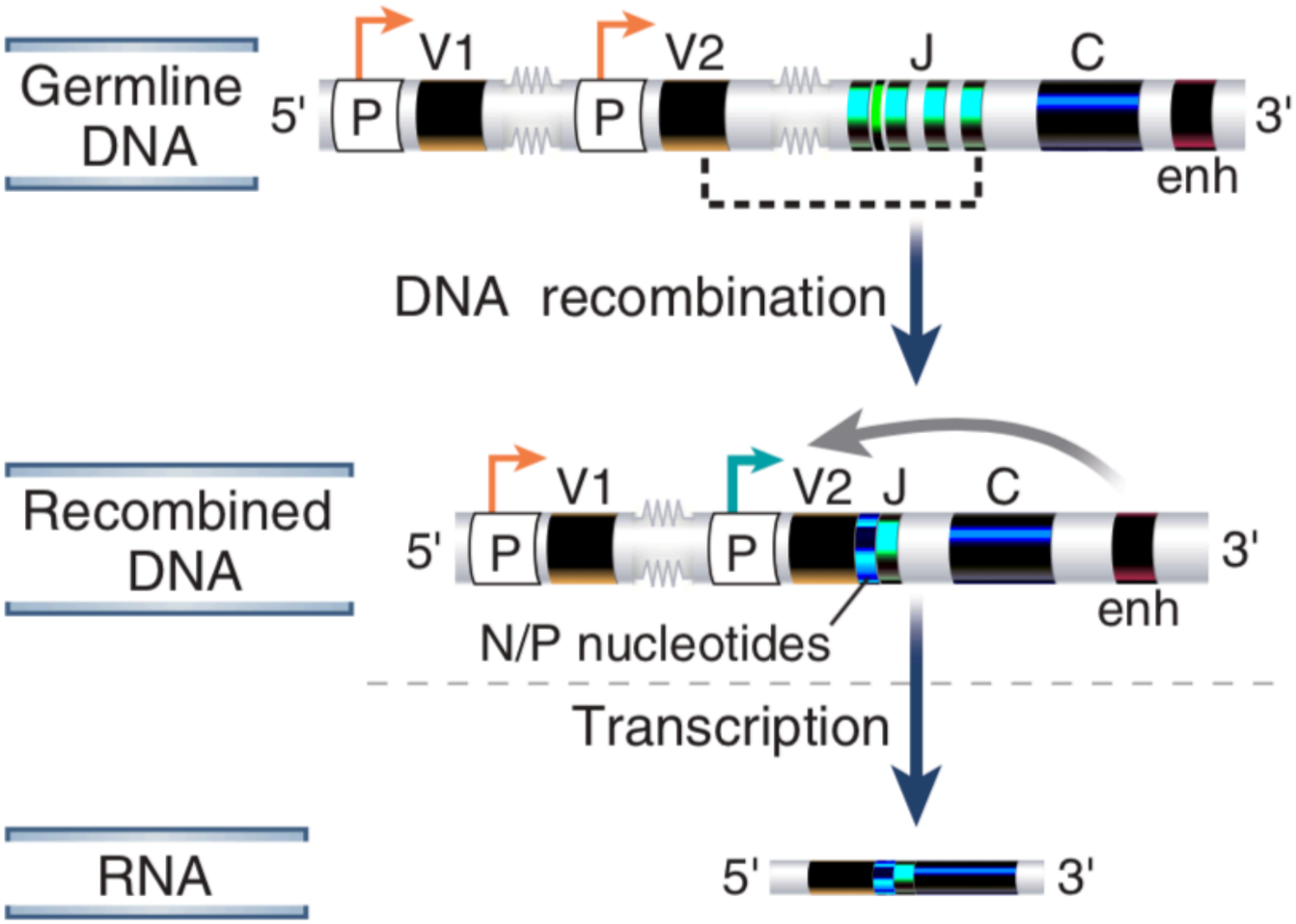
RAG (Recombination Activating Gene)

RAG1 is endonuclease
RAG2 bind Histones modified

Giunzione terminale non omologa

TdT (Terminal deoxynucleotidyl Transferase)

Recombination of V, D and J segments



Combinatorial diversity (RAG enzymes)

Junctional diversity (TdT Terminal deoxynucleotidyl transferase)

Junctional Diversity

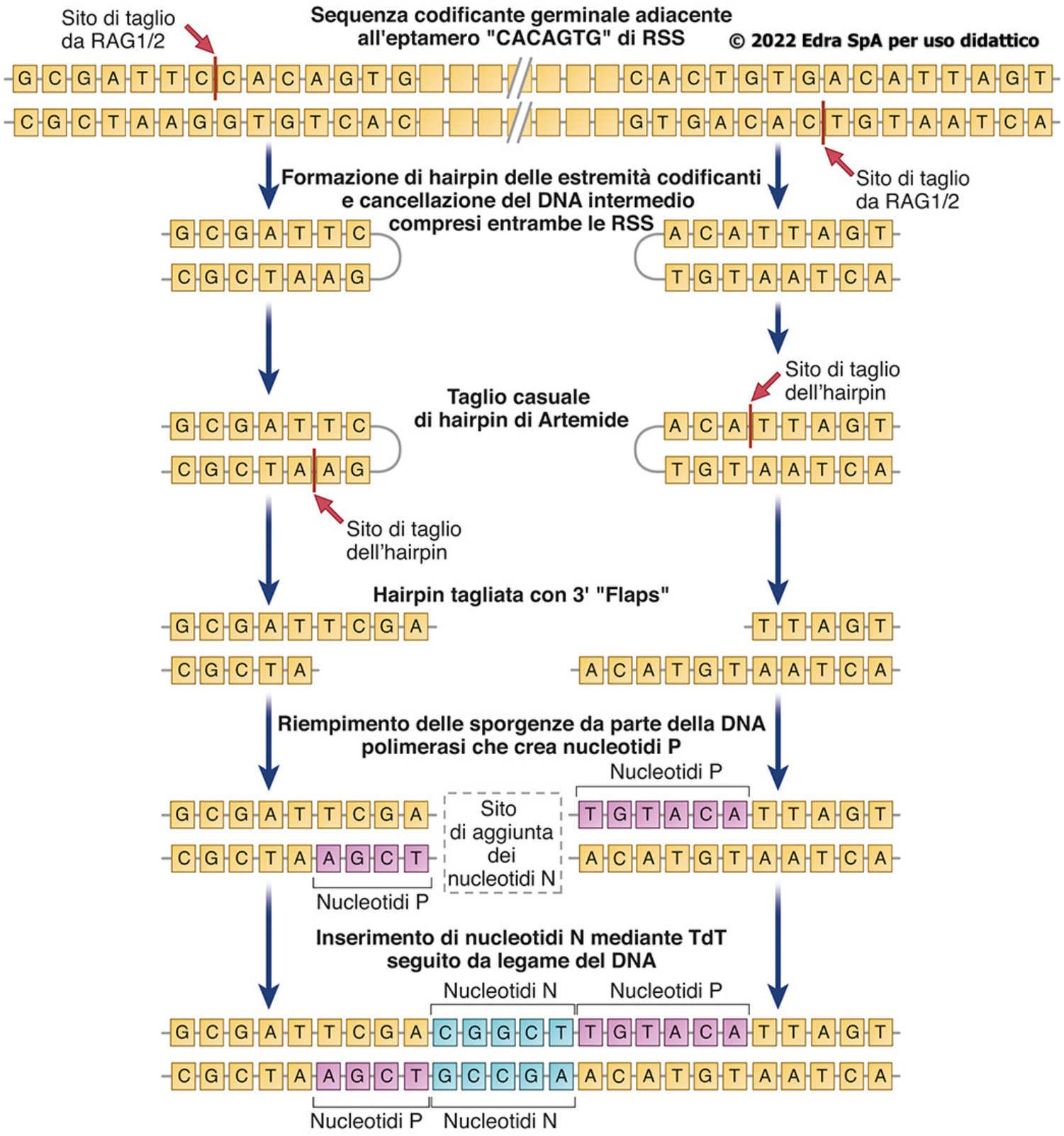


TABLE 8.1 Contributions of Different Mechanisms to the Generation of Diversity in Immunoglobulin and T Cell Receptor Genes

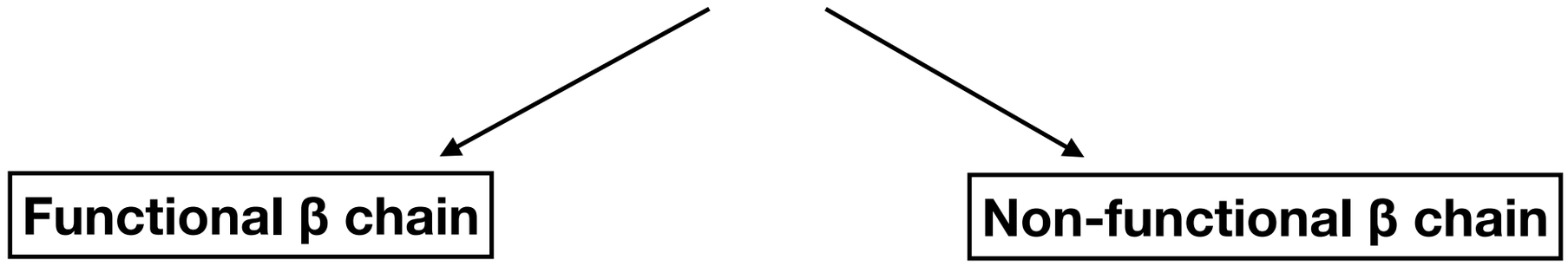
	Immunoglobulin			T Cell Receptor αβ		T Cell Receptor γδ	
Mechanism	**Heavy Chain**	**κ**	**λ**	**α**	**β**	**γ**	**δ**
Variable (V) segments	45	35	30	45	50	5	2
Diversity (D) segments	23	0	0	0	2	0	3
D segments read in all three reading frames	Rare	—		—	Often	—	Often
N region diversification	V-D, D-J	None		V-J	V-D, D-J	V-J	V-D1, D1-D2, D1-J
Joining (J) segments	6	5	4	55	12	5	4
Total potential repertoire with junctional diversity	$\sim 10^{11}$		—	$\sim 10^{16}$		$\sim 10^{18}$	

The potential number of antigen receptors with junctional diversity is much greater than the number that can be generated only by combinations of V, D, and J gene segments. The calculated figures for lymphocyte repertoire magnitudes should be considered very gross approximations. The calculations for the Ig repertoire do not account for the phenomenon of somatic hypermutation, which will be discussed in Chapter 12.

Maturation of T cells

T cell precursors enter the thymus (in the cortex); they don’t express any TCR chain at this moment. They also don’t express CD4 or CD8 (double negatives)

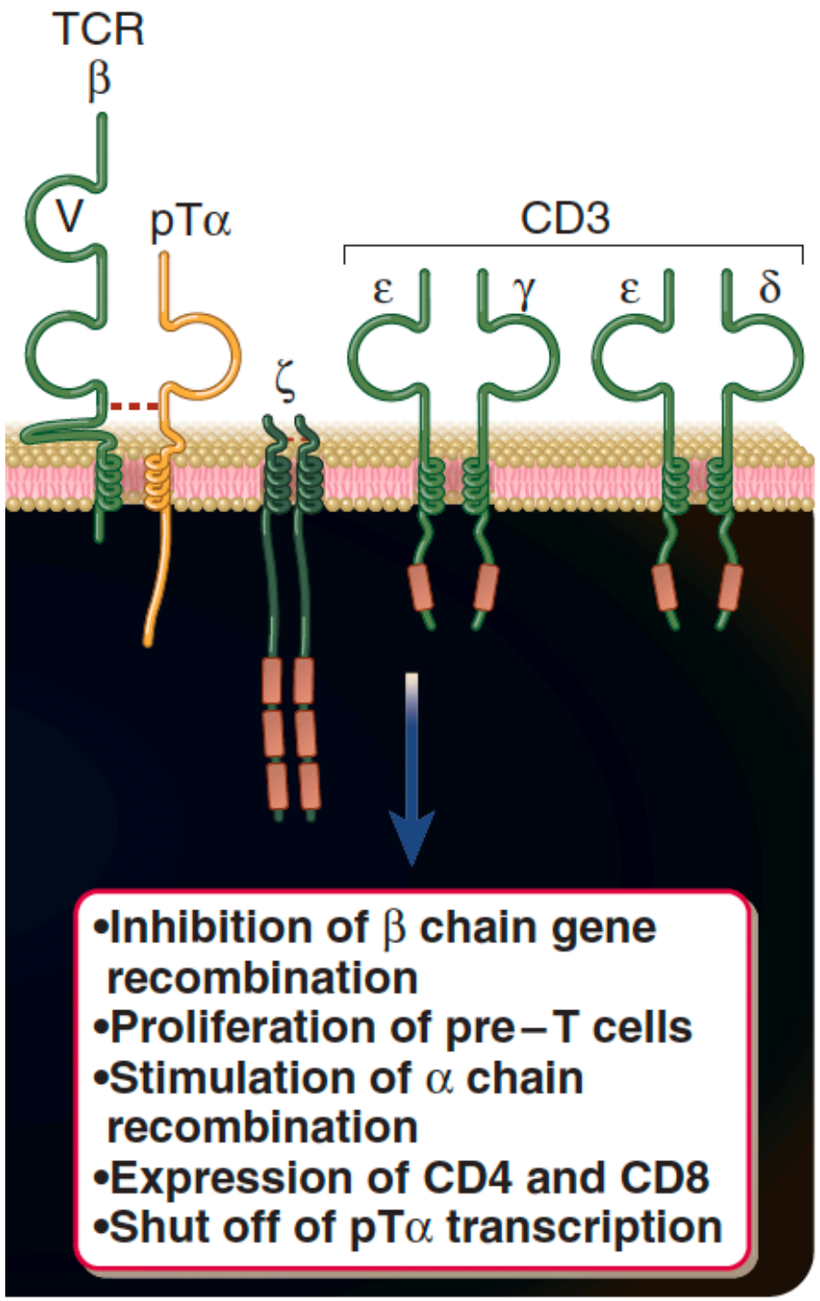
Rag1 and Rag2 expression > rearrangement of the β chain



Expression of pre-TCR and survival

No expression of pre-TCR and death

Pre-TCR is made by rearranged β chain and invariant pre-Tα



Pre-TCR assembly lead to signaling in thymocytes
(in the cortex)

Expression of CD4 or CD8 (double positives)
(before expression of alpha chain)
Allelic exclusion (rearrangment only in one allele)

Rag1 and Rag2 expression > rearrangement of the α chain

Functional α chain

Expression of αβTCR,
Go through positive and negative selection

Non-functional α chain

Death

Thymic epithelial cells express MHC-I and MHC-II loaded with self peptides

Too week recognition of self MHC complexes> death by apoptosis (*death by neglect*)

Recognition of self MHC complexes > survival

Too strong recognition > death by apoptosis, or Treg fate

During the selection process, double positive thymocytes become single positive

They enter in the medulla area

Positive selection

Goal: SELECT thymocytes that recognize self MHC complexes

Thymic epithelial cells express MHC-I and MHC-II loaded with self peptides

Too week recognition of self MHC complexes> **death** by apoptosis (*death by neglect*)

Moderate recognition of self MHC complexes > **survival** and proliferation

Negative selection

Goal: REMOVE thymocytes that recognize with high affinity self peptide-MHC complexes

Strong recognition of self peptide MHC complexes > **death by apoptosis**
Treg fate

The transcription factor AIRE expressed in thymic epithelial cells induce the ectopic expression of tissue-restricted antigens

⇨ **Removal of self reactive T cell clones**

Thanks to negative selection, central tolerance is established

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED)

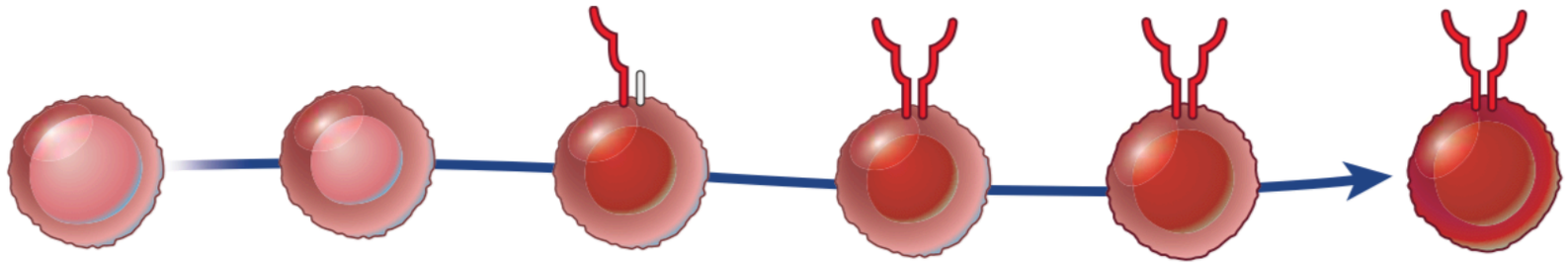
Caused by *AIRE* deficiency, 1:14,500 in Sardinians

Highly variable in its presentation, diagnosis is made when:

1. Mucocutaneous candidiasis
2. Hypoparathyroidism
3. Adrenal insufficiency

Or by sequencing of the *AIRE* gene

T cell maturation summary



Stage of maturation	Stem cell	Pro-T	Pre-T	Double positive	Single positive (immature T cell)	Mature T cell
Proliferation						
RAG expression						
TdT expression						
TCR DNA, RNA	Unrecombined (germline) DNA		Recombined β chain gene [V(D)J-C]; β chain mRNA	Recombined β and α chain genes [V(D)J-C]; β and α chain mRNA		
TCR expression	None	None	Pre-T receptor (β chain/pre-Tα)	Membrane αβ TCR		
Surface markers	c-*kit*$^+$ CD44$^+$ CD25$^-$	c-*kit*$^+$ CD44$^+$ CD25$^+$	c-*kit*$^+$ CD44$^-$ CD25$^+$	CD4$^+$CD8$^+$ TCR/CD3^{lo}	CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$ TCR/CD3^{hi}	
Anatomic site	Bone marrow	Thymus				Periphery
Response to antigen	None	None	None	Positive and negative selection		Activation (proliferation and differentiation)

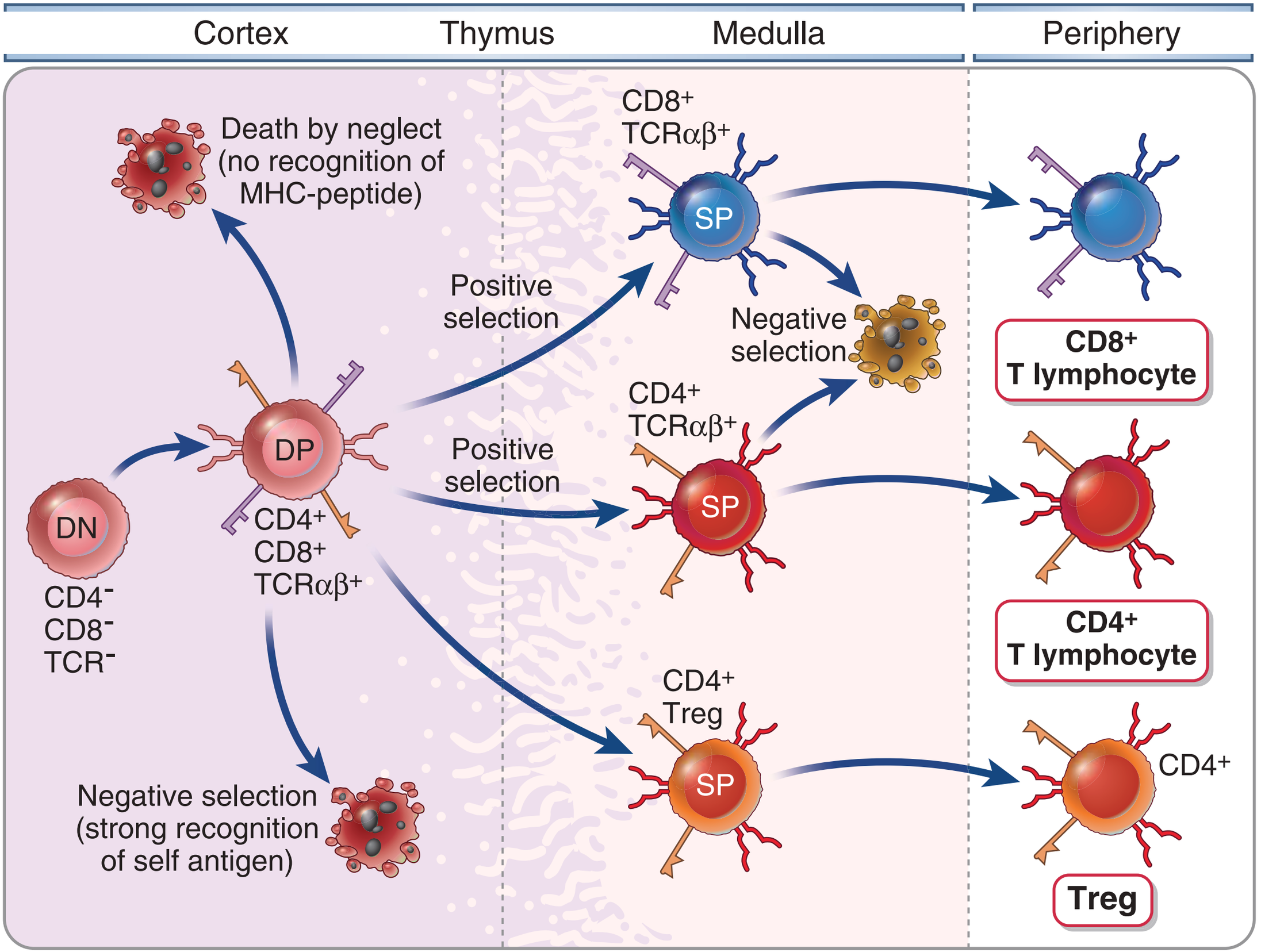
But how to choose the co-receptor?

Stochastic model

CD4 or CD8 are stochastically expressed, then selection occurs

Instructive model

High CD4 low CD8 phase, based on signals downstream of the TCR a decision is taken


Cortex
Thymus
Medulla
Periphery
Death by neglect
(no recognition of
MHC-peptide)
CD8+
TCRαβ+
SP
Positive
selection
Negative
selection
CD8+
T lymphocyte
DP
CD4+
TCRαβ+
Positive
selection
DN
CD4-
CD8-
TCR-
CD4+
CD8+
TCRαβ+
SP
CD4+
T lymphocyte
CD4+
Treg
SP
CD4+
Negative selection
(strong recognition
of self antigen)
Treg

Non conventional T cells: γδ T cells and NKT cells

Are considered T cells since they **express a TCR**

Differently from conventional T cells, γδ T cells and NKT cells are not MHC-I or MHC-II restricted, i.e. their TCR **don't recognize peptides loaded on MHC-I or MHC-II**

Once activated, these cells can secrete different **cytokines**

γδ T cells express γδTCR

About 10% of T cells

Mostly present at epithelial and mucosal barriers

Characterized by a limited TCR diversity (even if the loci contain many V-(D)-J)

NKT cells express αβTCR and are CD1 restricted

Called NKT because they express some markers typical of NK cells

Characterized by a limited TCR diversity

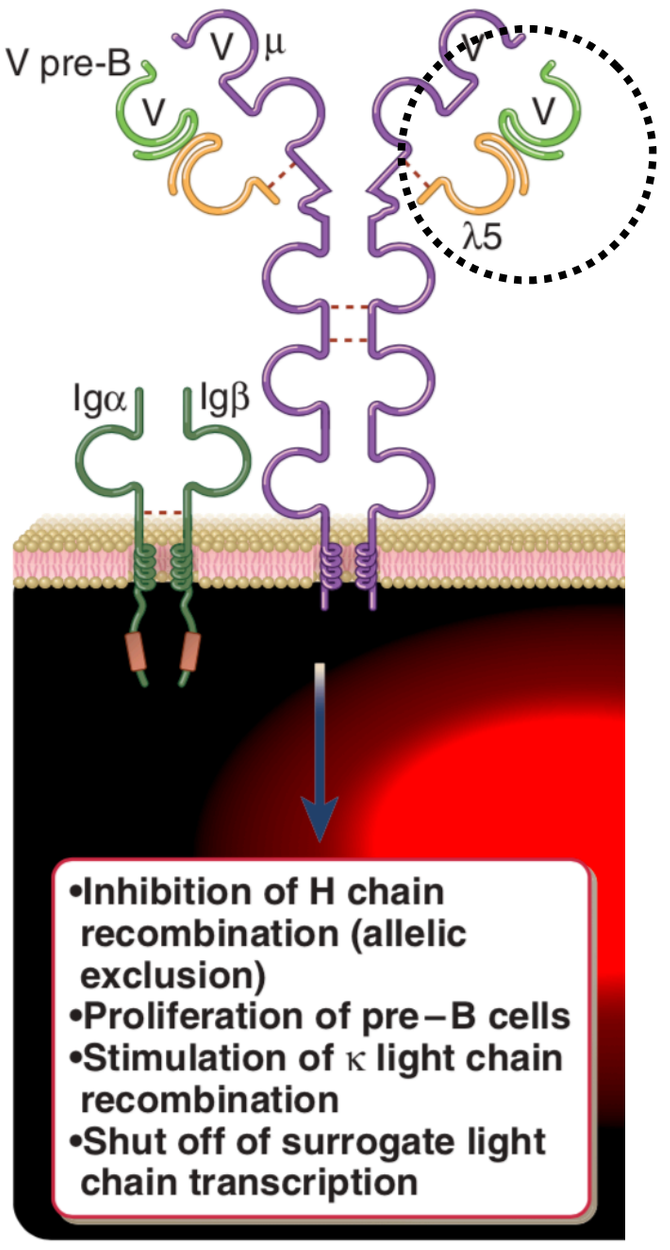
Recognize lipids and glycolipids presented on CD1, a membrane complex similar to MHC

B cell maturation (BM)

Pro-B cells — no BCR, but expression of B cell markers as CD19 and CD10

Pre-B cells — rearrangement of $Ig_{H\ (\mu\ chain)}$; expression of pre-BCR, constituted by two heavy chains, surrogate light chains, Igα and Igβ

Pre-BCR expression lead to light chain rearrangement



Surrogate light chains

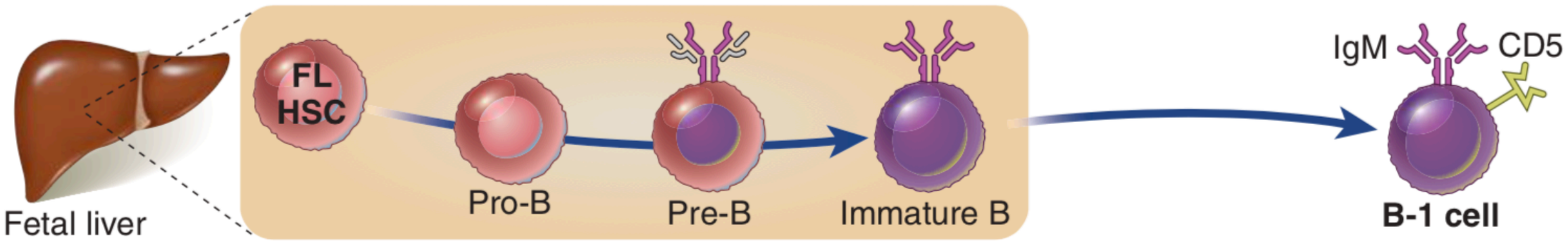
Allelic exclusion (otherwise recombination of 2nd allele)

Kappa locus is recombined first. If recombination in the kappa locus is not functional, recombination occurs at the lambda locus.

Isotype exclusion

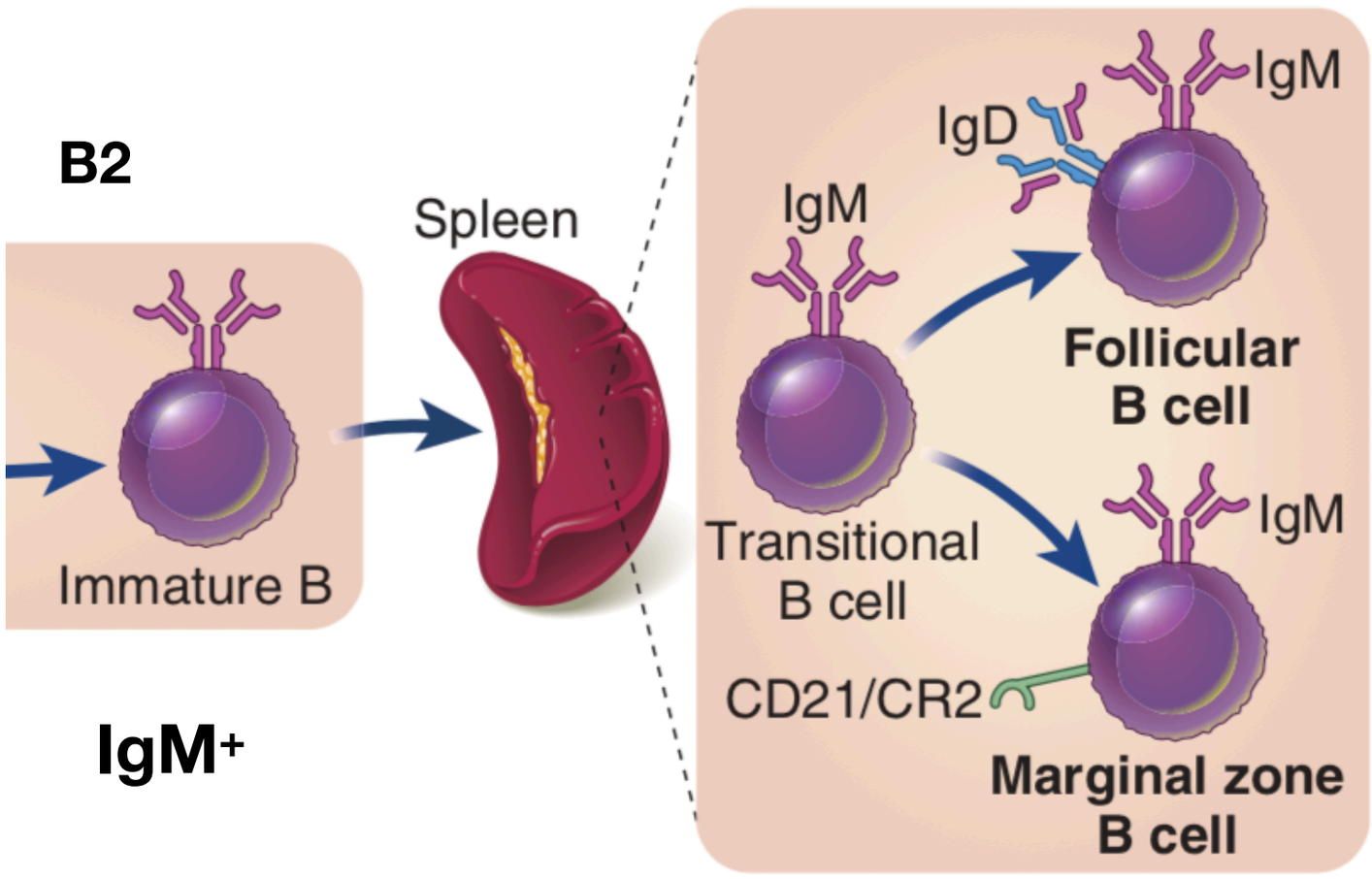
> **IgM** surface expression (immature B cell)

Immature B cells further commit to three possible programs



Immature cells deriving from fetal liver hematopoietic stem cells become B-1 cells (IgM^+ $CD5^+$). B-1 cells are abundant in mucosal tissues, have limited diversity and spontaneously secrete **natural antibodies**. Respond to lipids and polysaccharides

Immature B cells further commit to three possible programs



Follicular B cell (IgM^+IgD^+) functional active and they can recirculate

Same specificity same V domain generated by alternative splicing

Marginal zone B cell (IgM^+)

Quality control in the B cell repertoire

Positive selection

Correct BCR lead to signaling important for survival (**tonic signal**)

Negative selection

Antigen recognition by immature B cells (so in the bone marrow) lead to **receptor editing** (deletion of recombined light chain, new recombination of light chain)

If receptor editing fails, B cell undergoes apoptosis