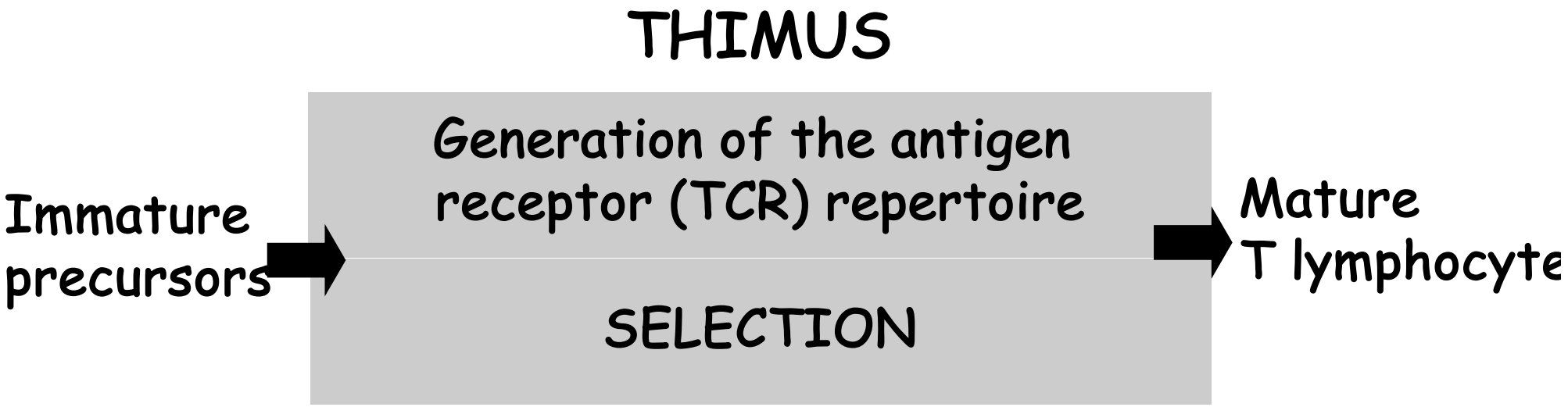
Corso di Immunologia - III anno
Prof. Paolini

Lezione 22/10/2025

«La maturazione dei linfociti T»

THYMIC DIFFERENTIATION OF T LYMPHOCYTES



- **SELF RESTRICTION**

Ability to recognize in the context of self-MHC molecules

- **SELF TOLERANCE**

Inability to react to self-antigens

T cell precursors are born in the bone marrow and "educated" in the thymus

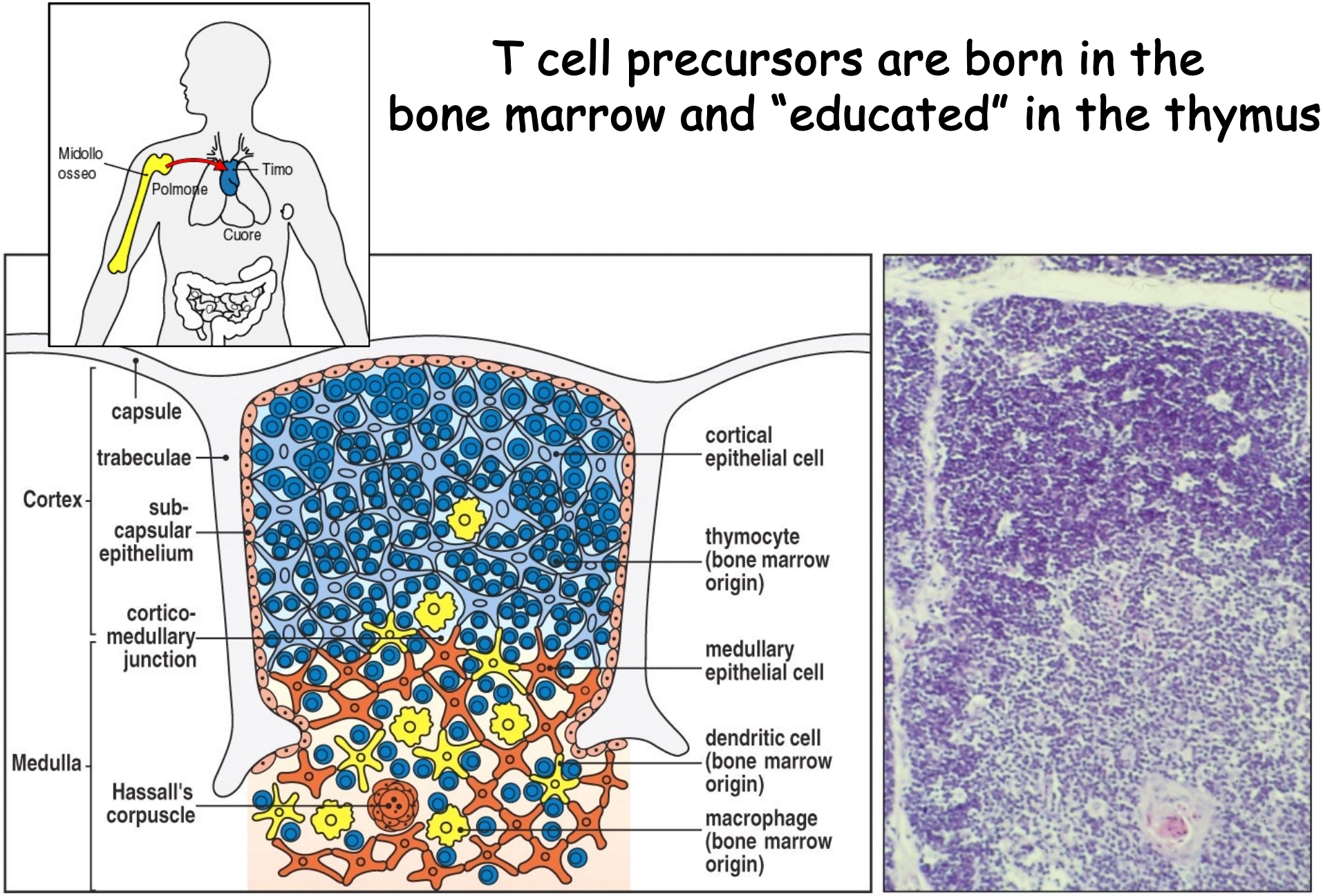


Figure 7-8 Immunobiology, 6/e. (© Garland Science 2005)

The Thymus is required for T cell development

- *Nude* mice

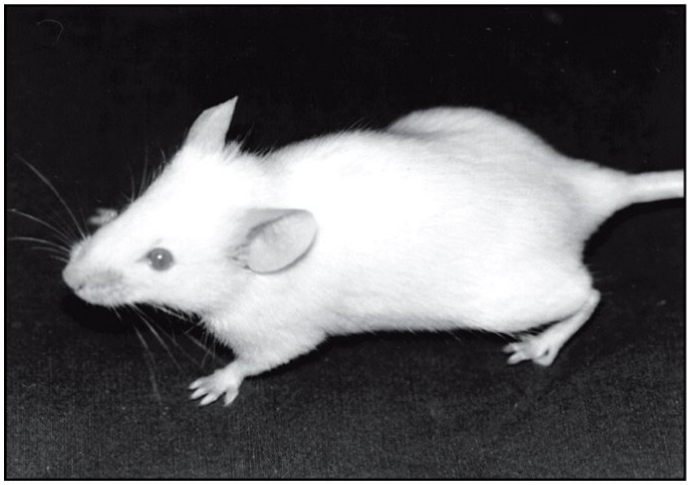
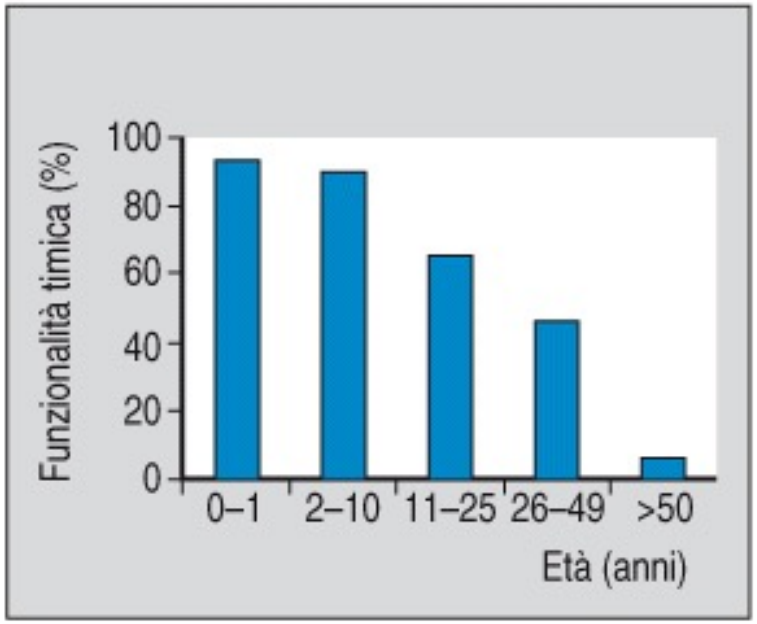
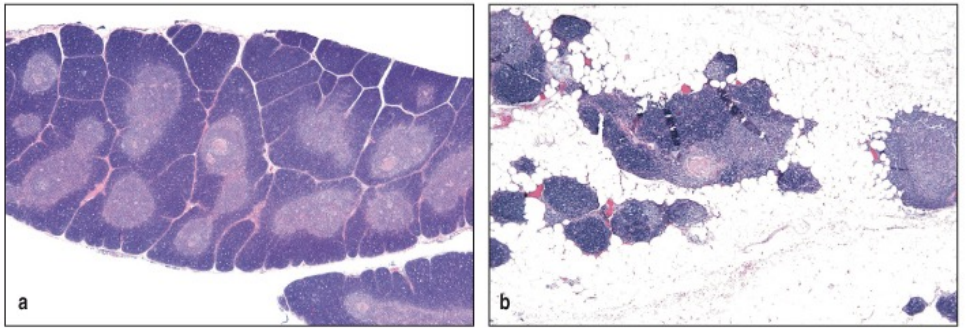


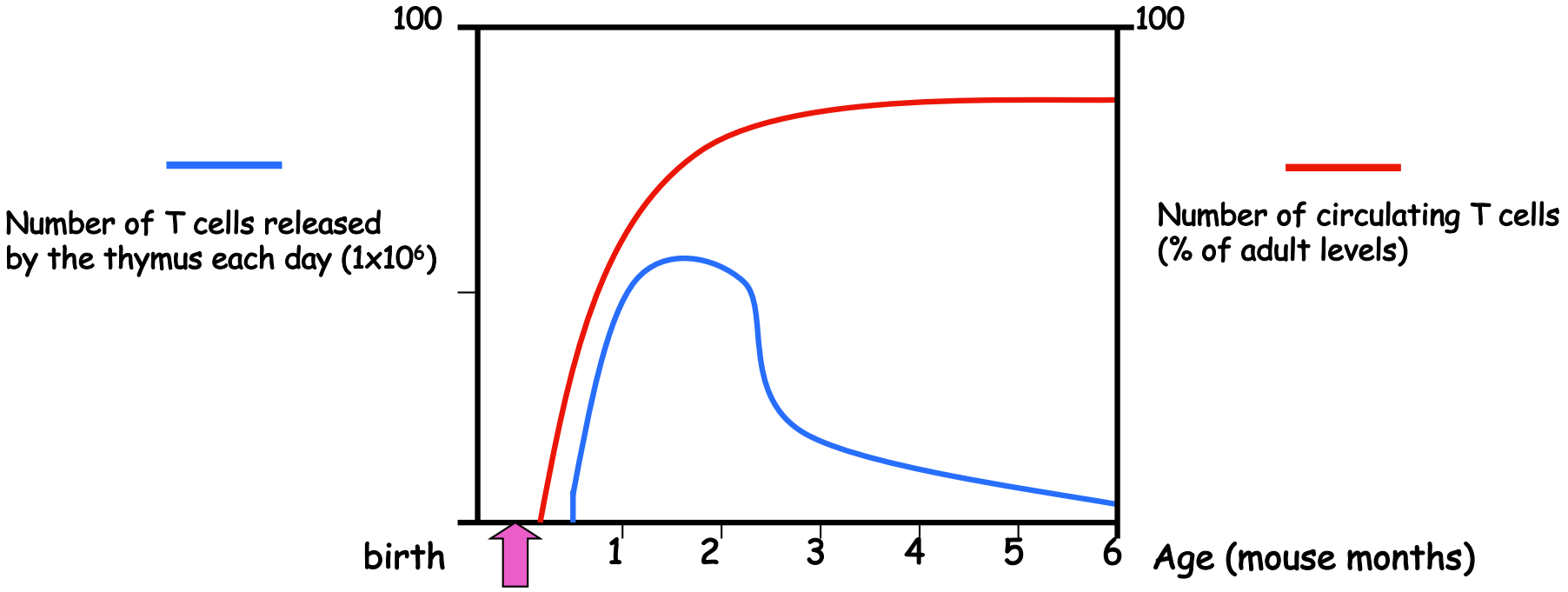
Figure 7-10 part 1 of 3 Immunobiology, 6/e. (© Garland Science 2005)

- Sindrome di DiGeorge

The thymus regresses after birth



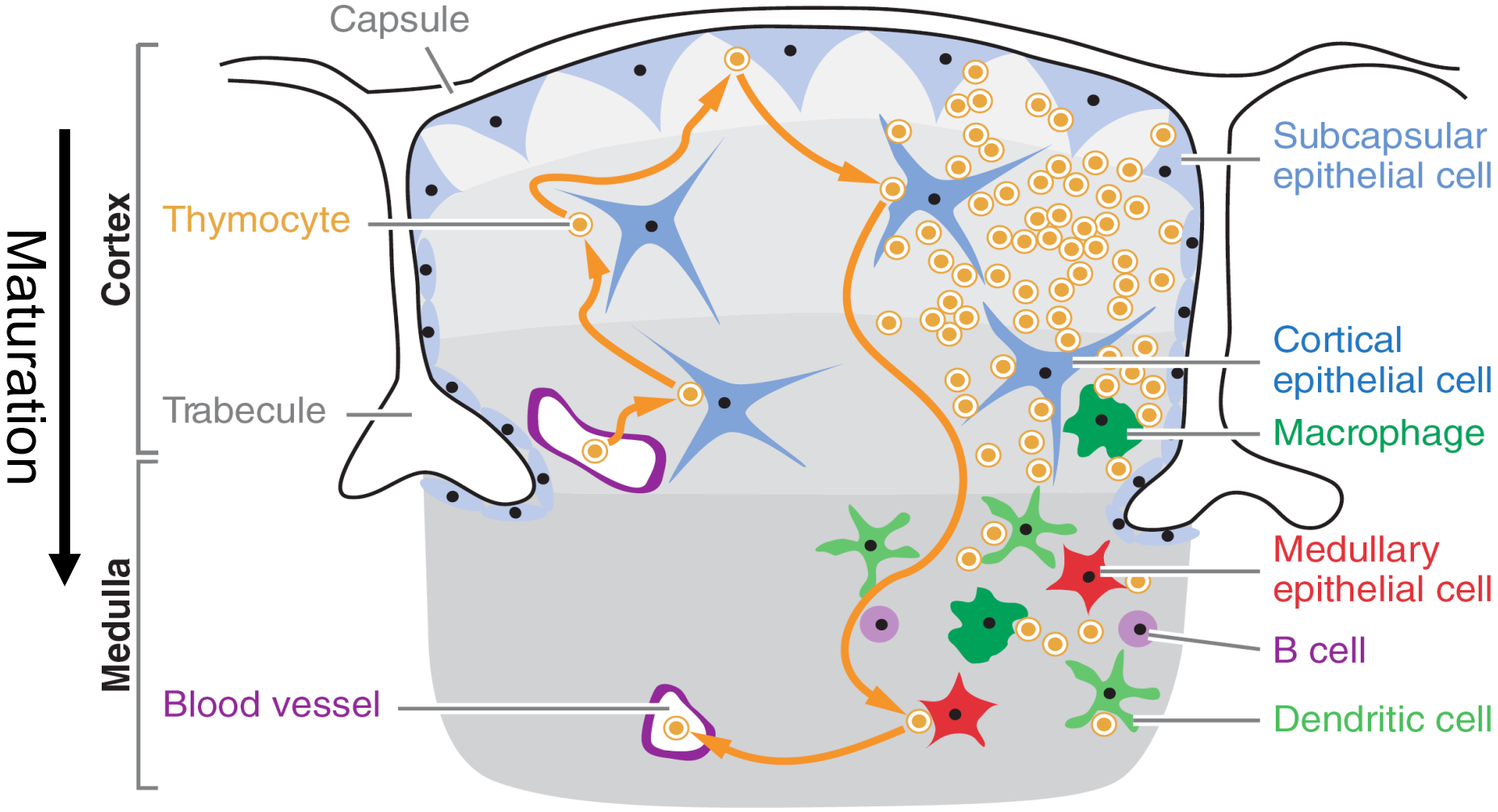




Thymocytes during the differentiation process

- Migrate
- Contact stromal cells
- Change phenotype
- Express an antigen receptor
- Undergo a selection process

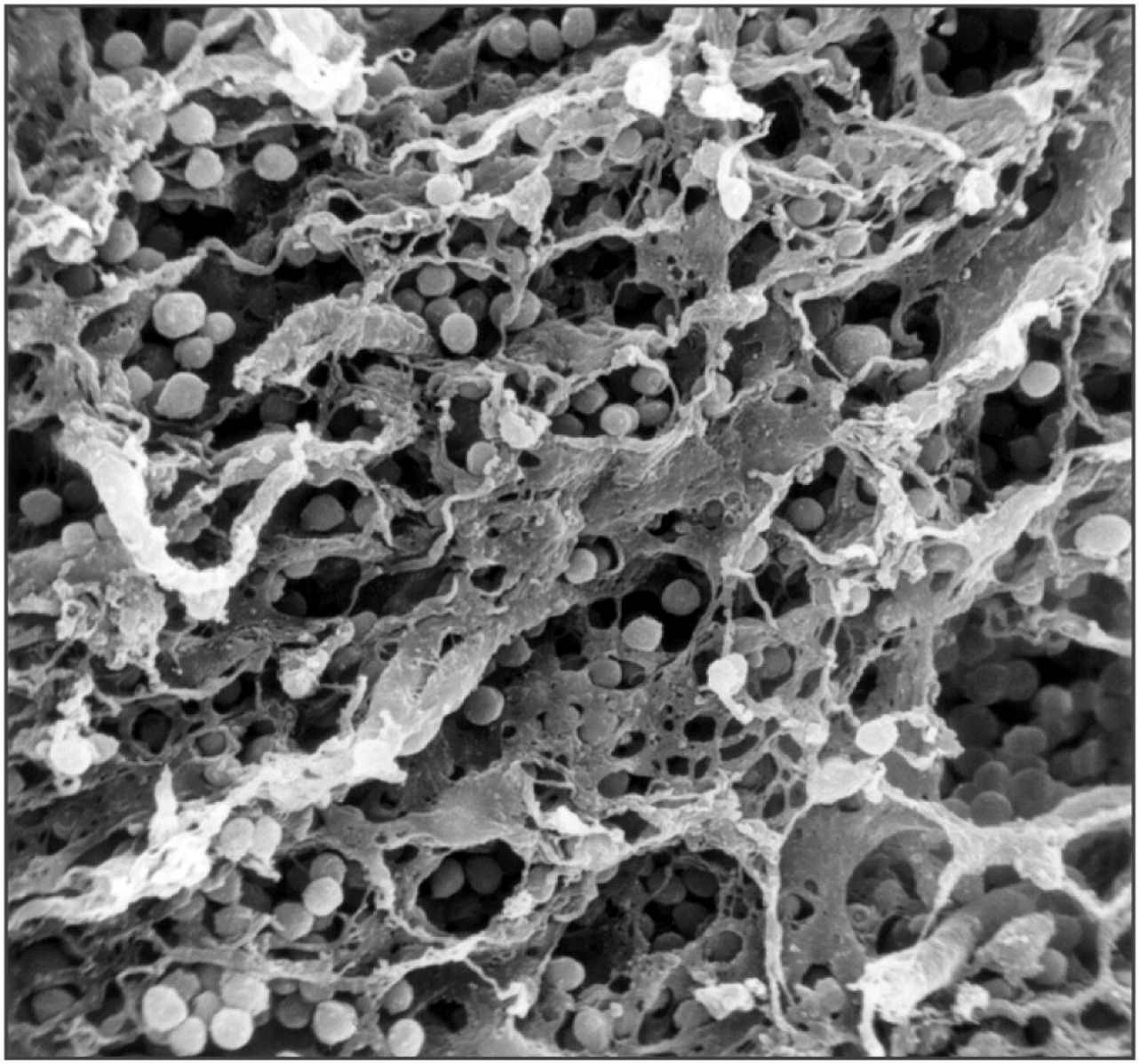
Thymocytes at different developmental stages are found in distinct parts of the thymus



Thymocytes during the differentiation process

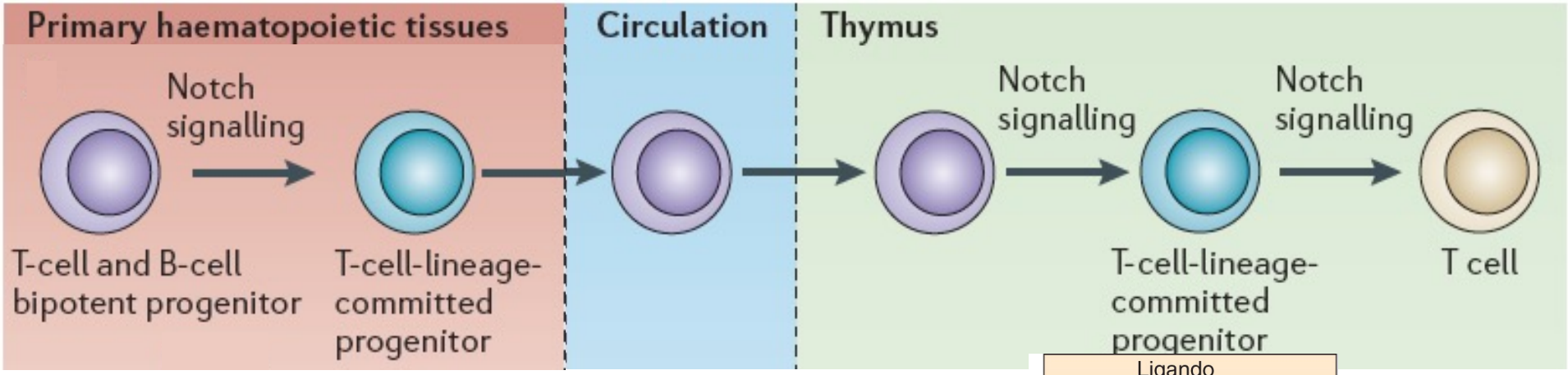
- Migrate
- **Contact stromal cells**
- Change phenotype
- Express an antigen receptor
- Undergo a selection process

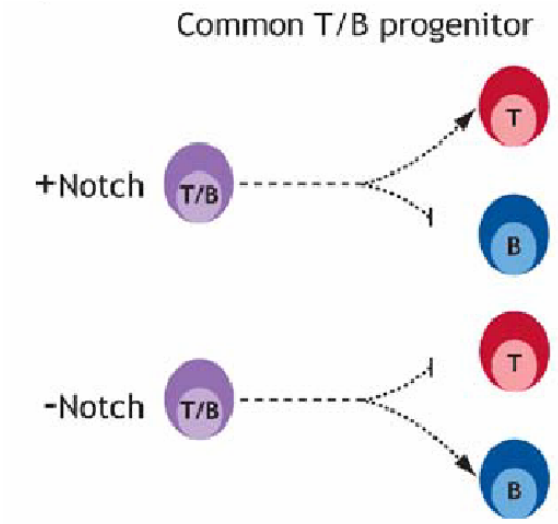
THE EPITHELIAL CELL NETWORK



Stage 1: Commitment

Control of development by transcriptional factors



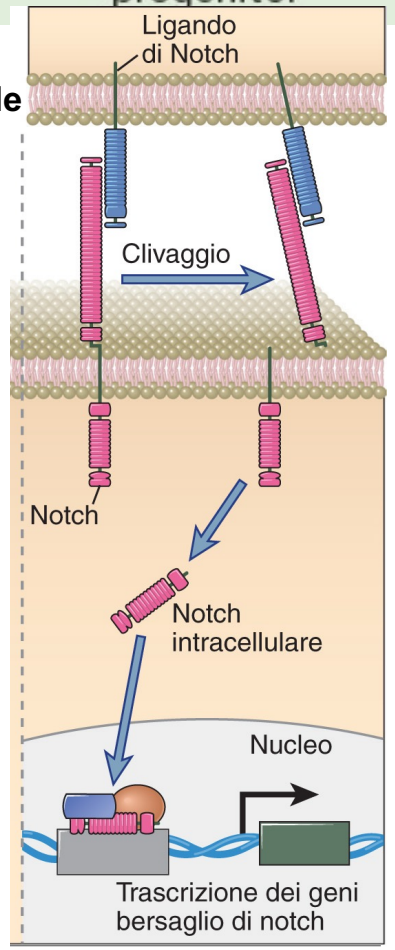


Notch signaling activation results in progenitor commitment to T cell lineage and a block in B cell development. Absence of Notch signaling results in progenitor commitment to B cell lineage and a block in T cell development.

From Maillard, I. et al. Regulation of lymphoid development, differentiation and function by the Notch pathway. *Annu. Rev. Immunol.* 23. 2005. 945-974

Cellula epiteliale timica

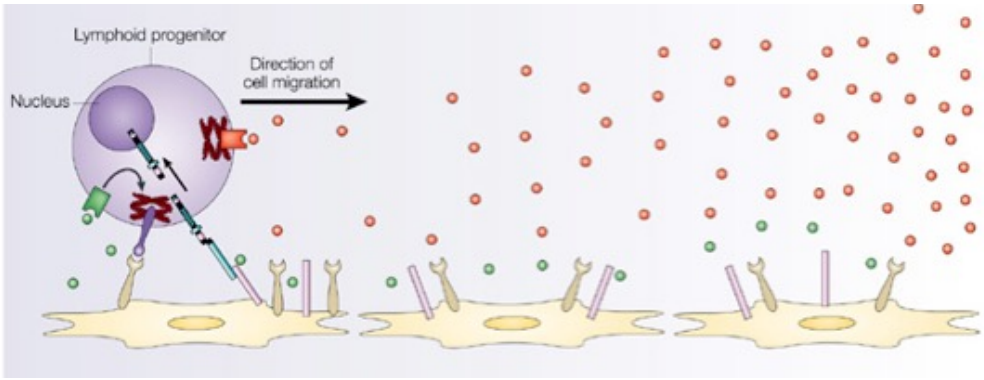
Timocita



Stage 2: Proliferation

Interactions with thymic epithelial cells and CYTOKINES present in the thymus:

- maintain thymocyte survival
- stimulate proliferation
- guide migration through the thymus



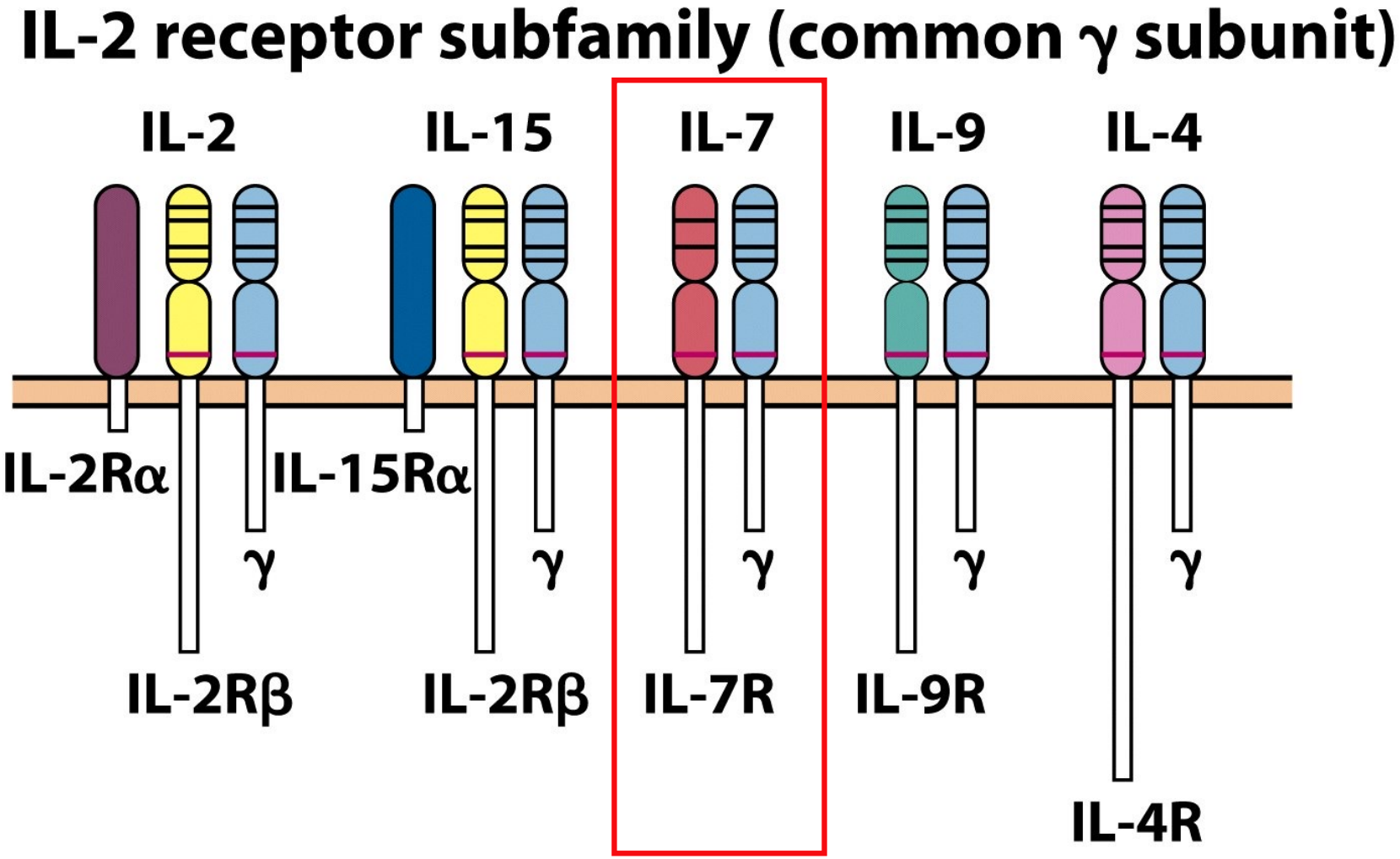
INTERLEUKIN 7

Source: Thymic epithelial cells

Target cells: Immature thymocytes

Main action: Proliferation of lymphoid precursors

The receptor for IL-7 and type I cytokines:



Thymocytes during the differentiation process

- Migrate
- Contact stromal cells
- Change phenotype
- Express an antigen receptor
- Undergo a selection process

T cell development involves the sequential generation, assembly and testing of TCR

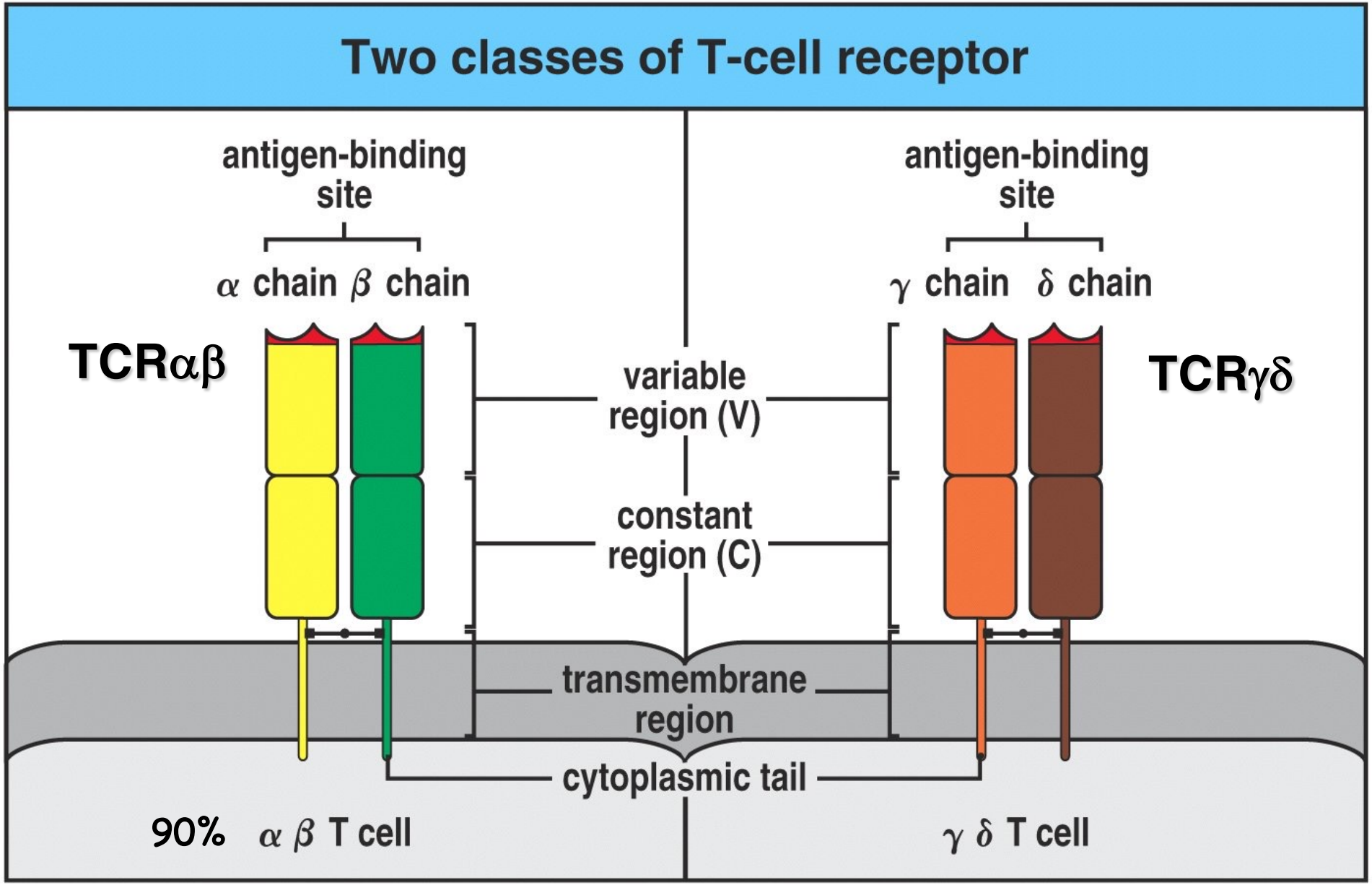
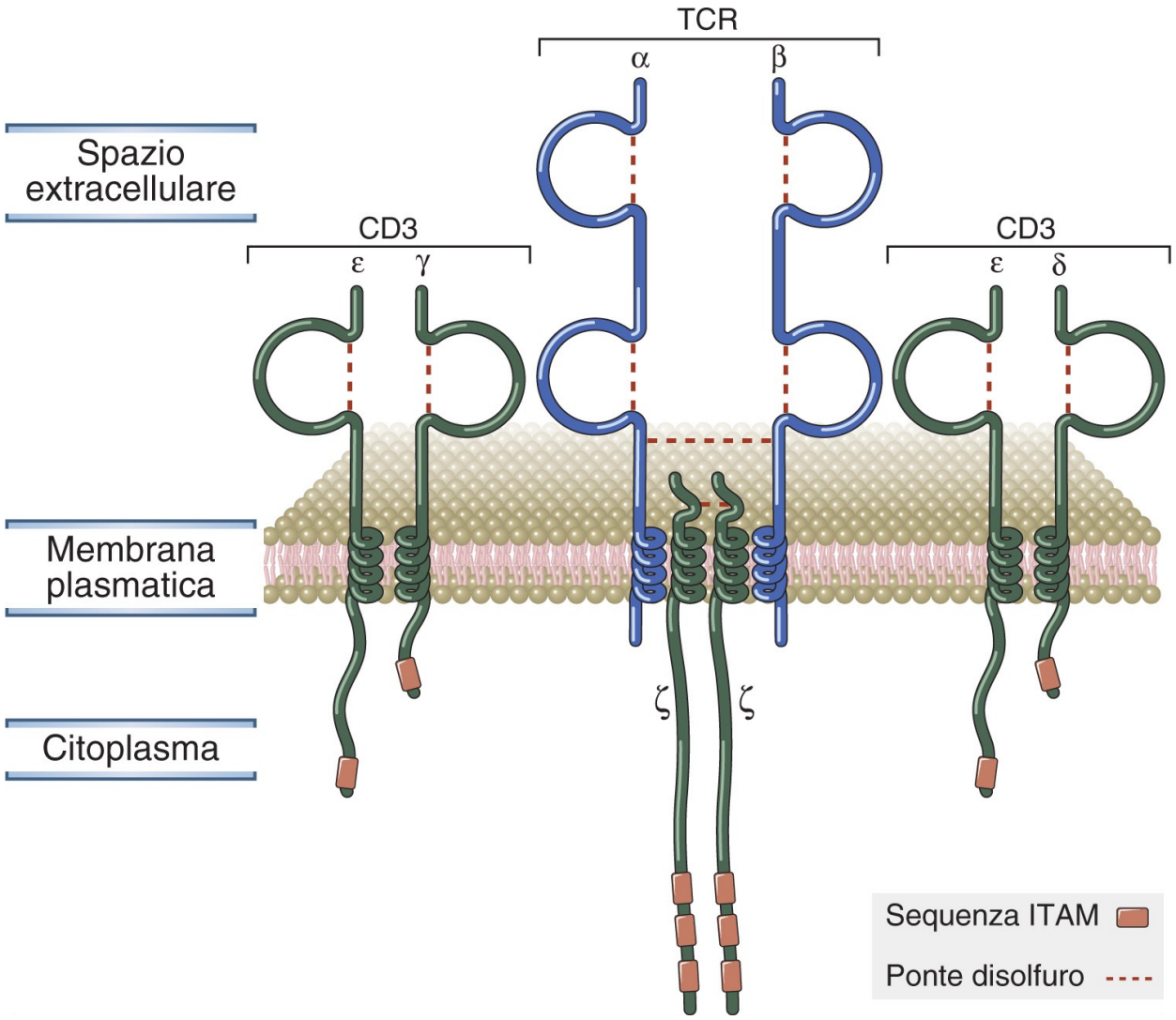


Figure 3-7 The Immune System, 2/e (© Garland Science 2005)

The T-lymphocyte receptor complex



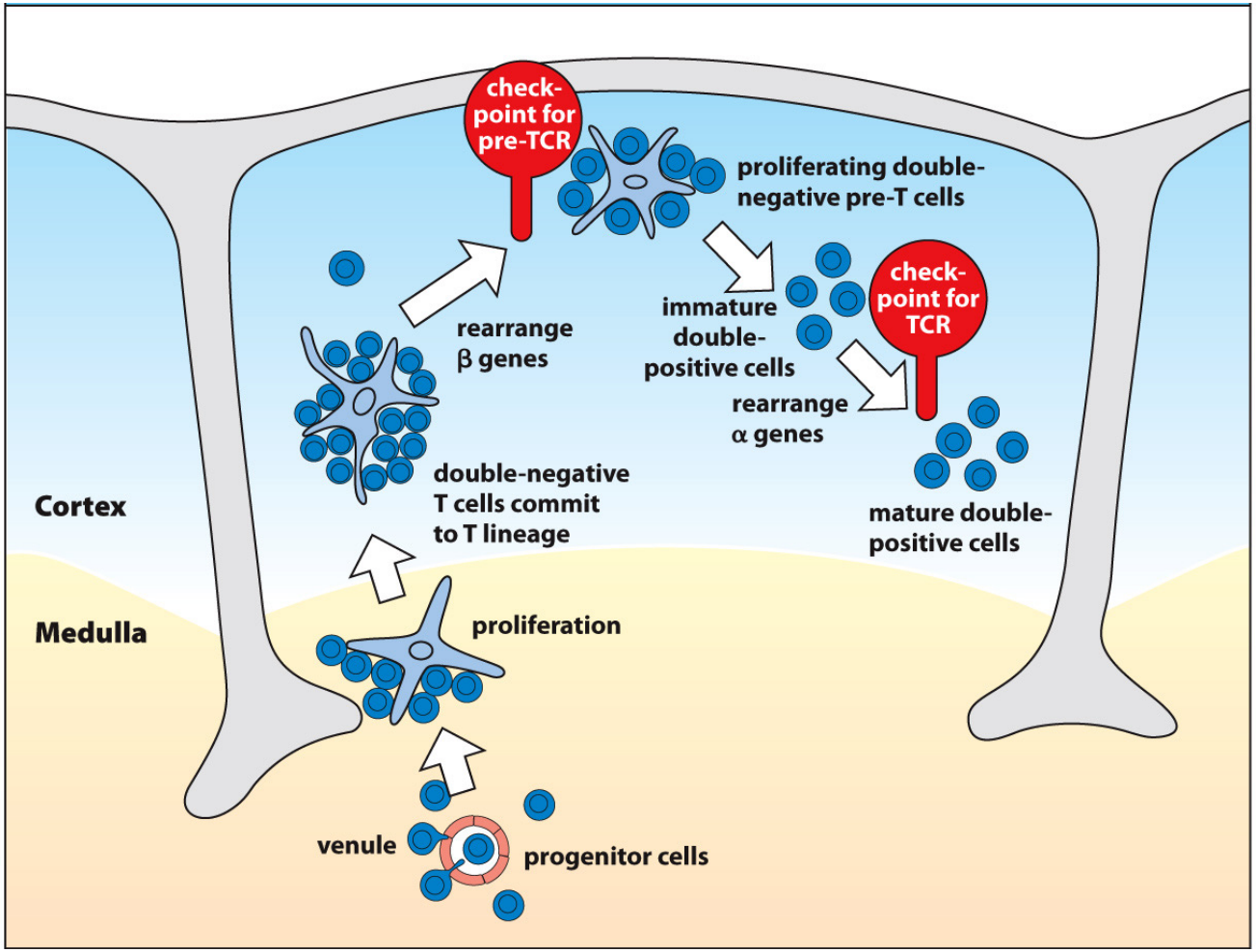
- All TCRs are expressed in association with a CD3 complex
- The alpha/beta TCR recognizes the MHC/peptide complex
- The CD3 complex transduces a signal

The expression of CD4 and CD8 allows to define different maturation stages

Double negative cells (DN)
$TCR^-CD3^-CD4^-CD8^-$

$TCR\alpha\beta^+CD3^+$
$CD4^+CD8^+$
Double positive cells (DP)

$CD4^+8^-$ $CD8^+4^-$
single positive (SP)



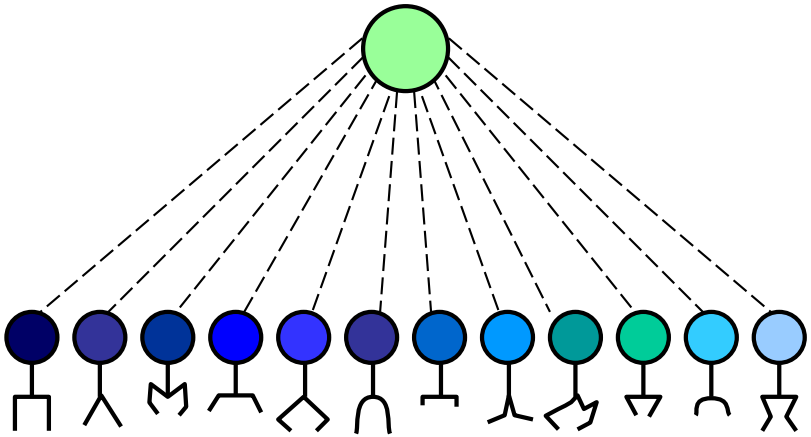
Thymocytes during the differentiation process

- Migrate
- Contact stromal cells
- Change phenotype
- Express an antigen receptor
- Undergo a selection process

Stage 3:

THE GENERATION OF ANTIGEN RECEPTOR DIVERSITY

PRIMARY LYMPHATIC ORGANS: GENERATION OF DIVERSITY



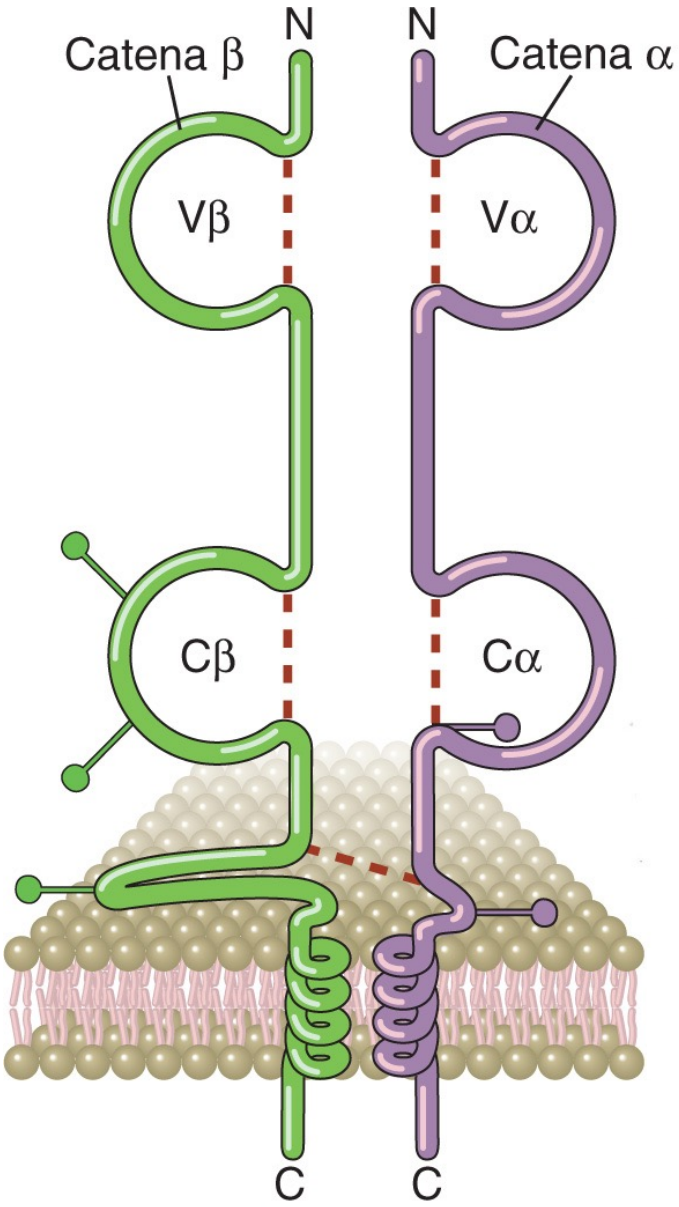
STAMINAL PRECURSORS

"RANDOM" GENERATION OF A LARGE REPERTOIRE OF DIFFERENT MEMBRANE RECEPTORS

The mechanisms by which the TCR repertoire is generated are similar to those that generate the antibody repertoire.

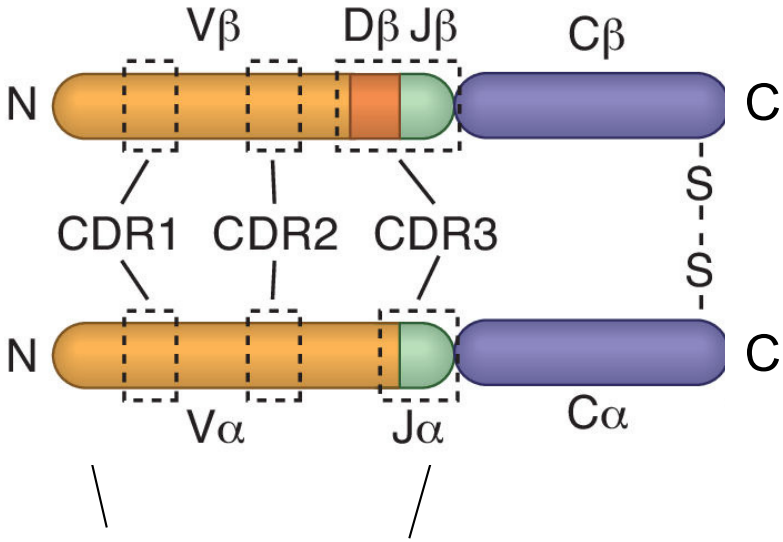
In both cases, the polypeptide chains are encoded by multiple gene segments located on the same chromosome, which must undergo recombination events to become functional genes.

Each TCR polypeptide chain is encoded by multiple gene segments!



Catena β del TCR

Catena α del TCR

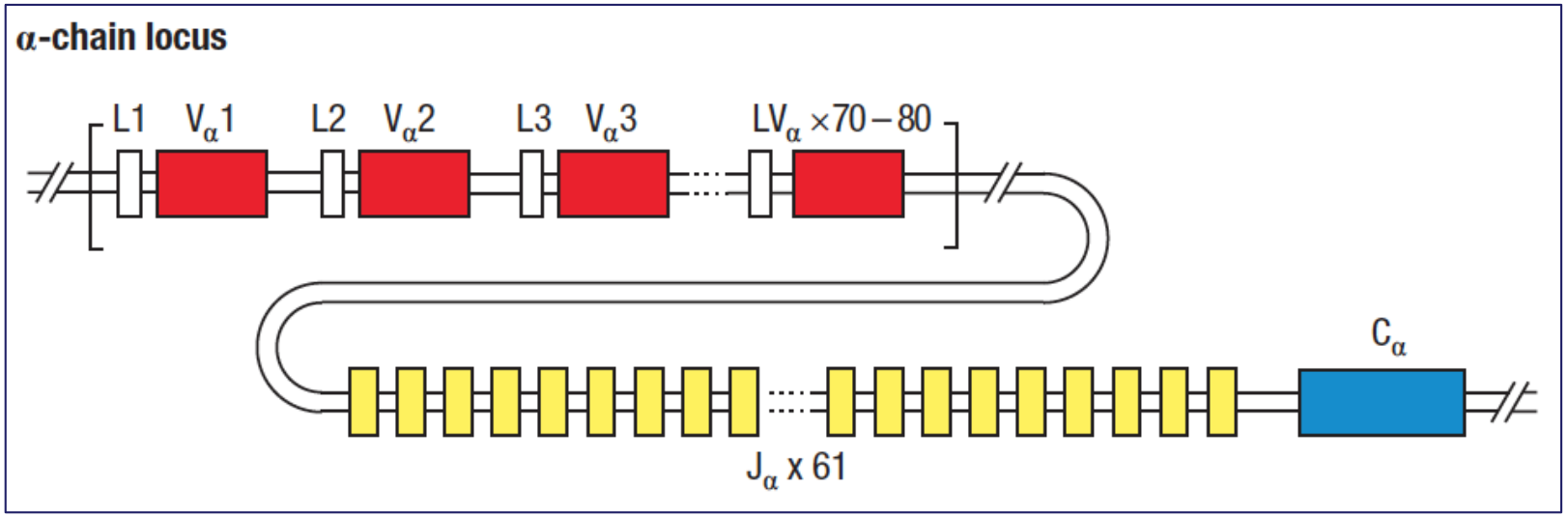


V region

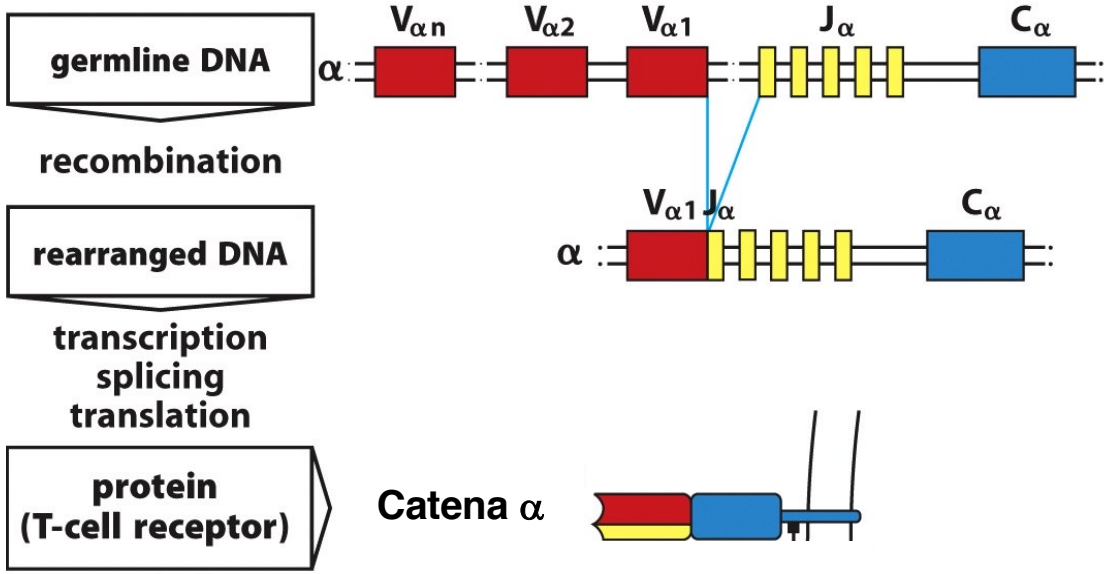
Region V is encoded by three different gene segments:

V = Variability
D = Diversity
J = Junction

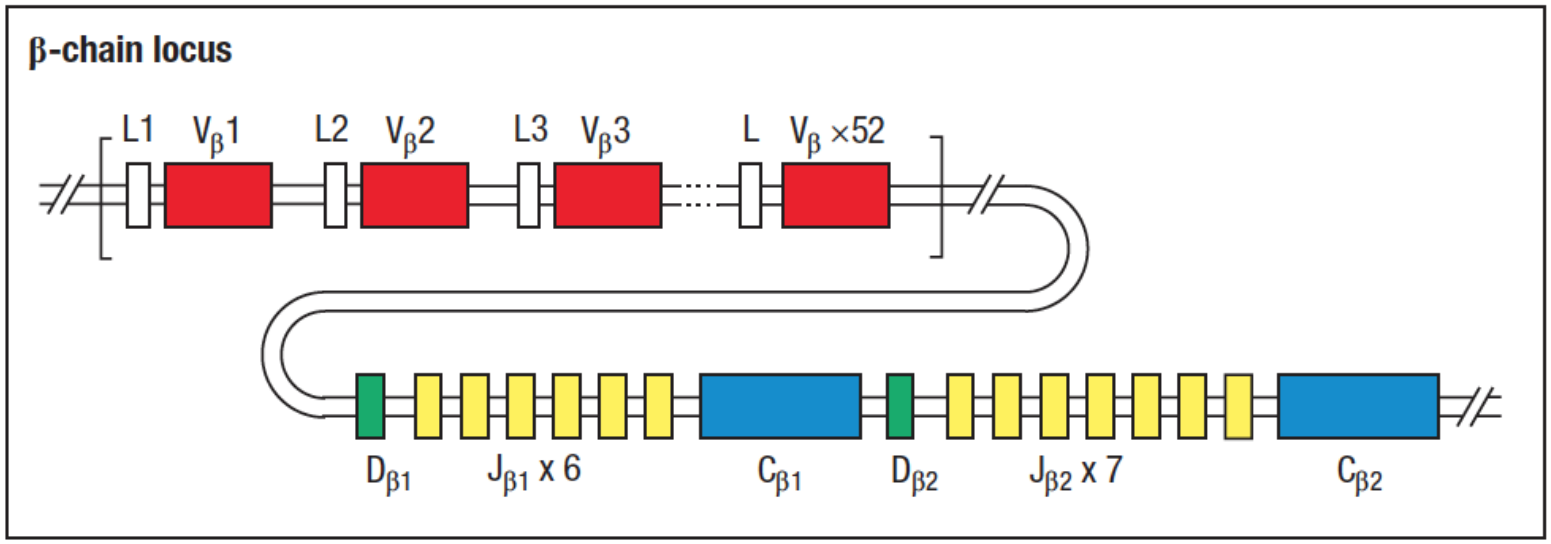
GENOMIC ORGANIZATION of the TCR α LOCUS (1000 kb; chromosome 14)



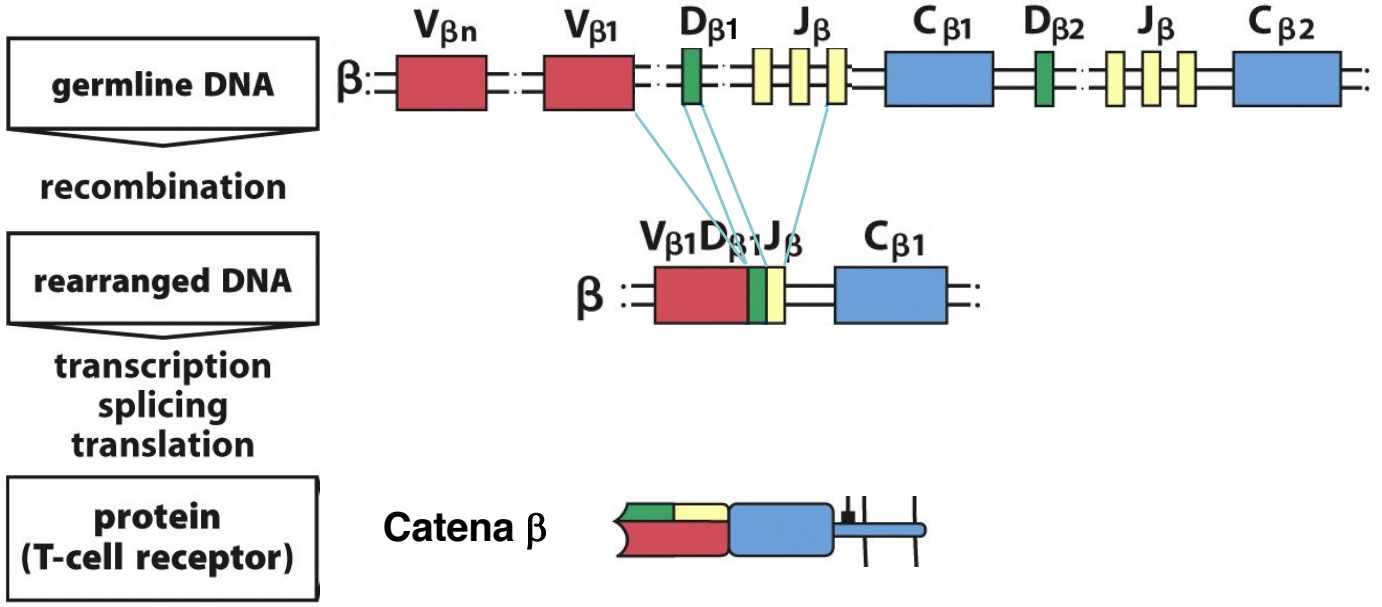
V-D-J recombination events allow the formation of a functional gene for the variable region of the α chain



GENOMIC ORGANIZATION of the TCR β LOCUS (620 Kb; chromosome 7)



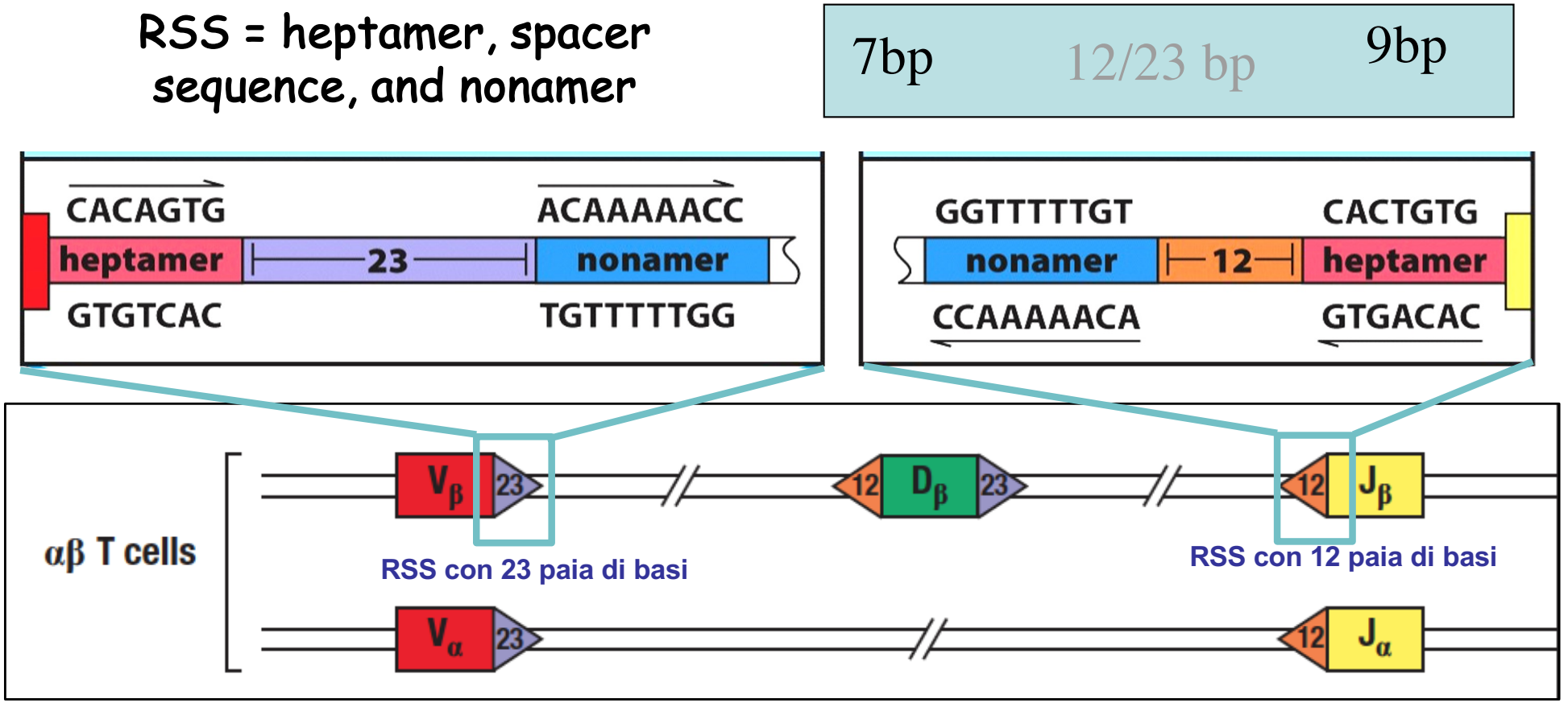
V-D-J recombination events allow the formation of a functional gene for the variable region of the β chain



The individual gene segments V, D and J are flanked by

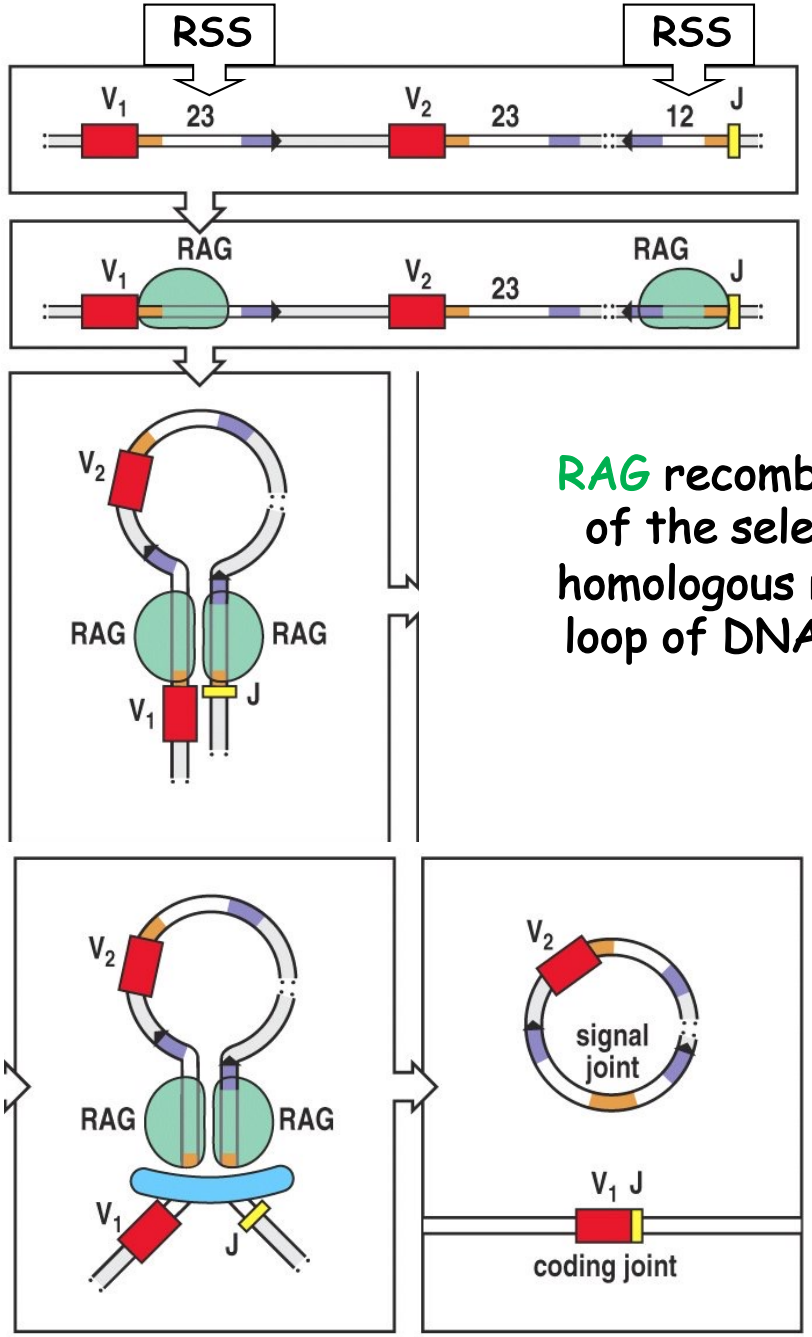
RECOMBINATION SIGNAL SEQUENCES (RSS)

RSS = heptamer, spacer sequence, and nonamer



RSS sequences are recognized by specific recombinases called RAG1 and RAG2

RAG1/RAG2 recombinases recognize RSS sequences and promote rearrangement

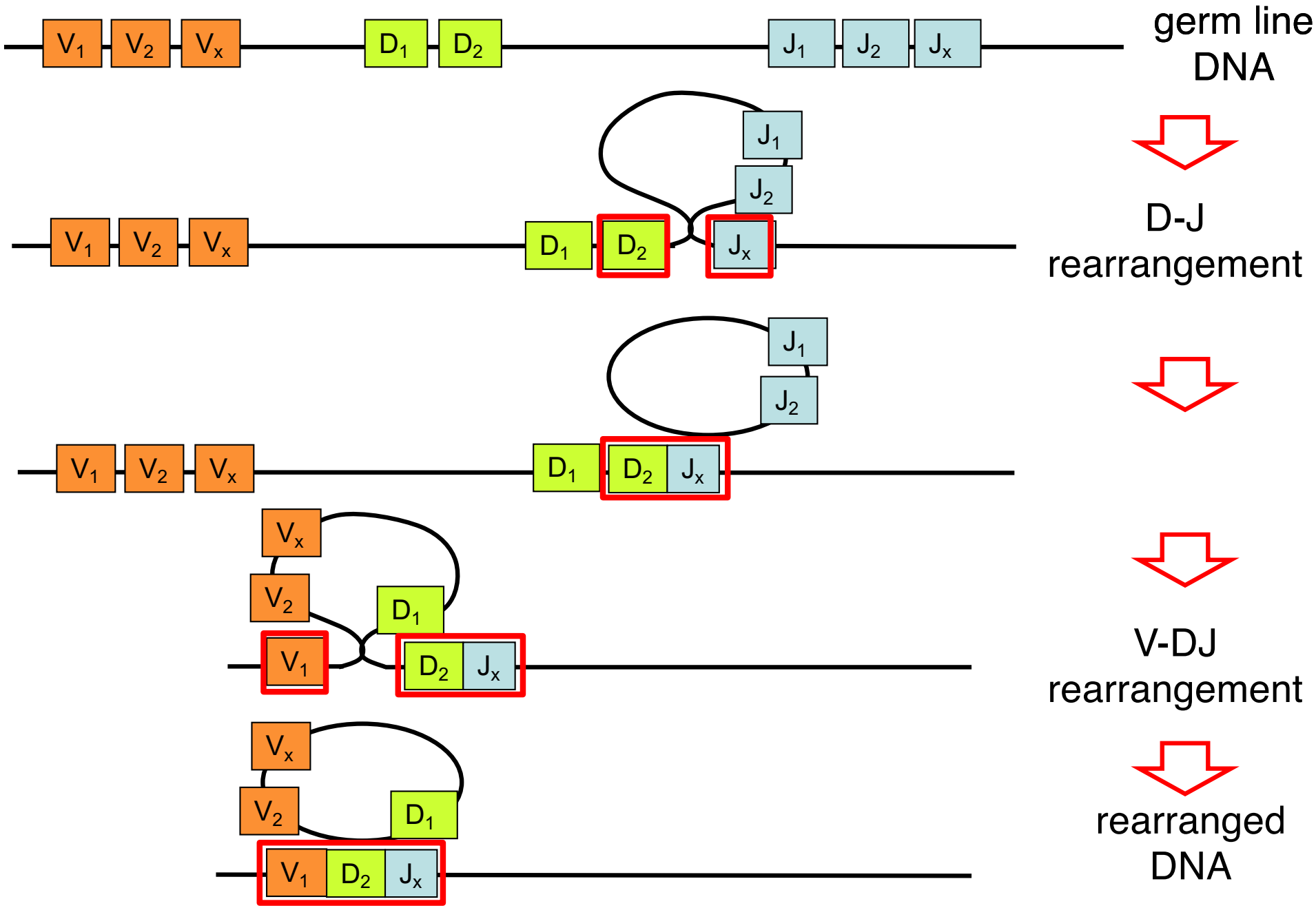


RAG recombinases promote the approach of the selected genes by annealing the homologous regions of the RSS forming a loop of DNA intercalated between them.

This loop is then eliminated by the action of RAG enzymes with endonuclease activity...

...and the ends of the VJ genes are sealed by ligases

The recombinases (RAG-1 and RAG-2) recognize the conserved sequences flanking each gene segment and drive the VDJ rearrangement



Factors contributing to TCR diversity

COMBINATORIAL DIVERSITY

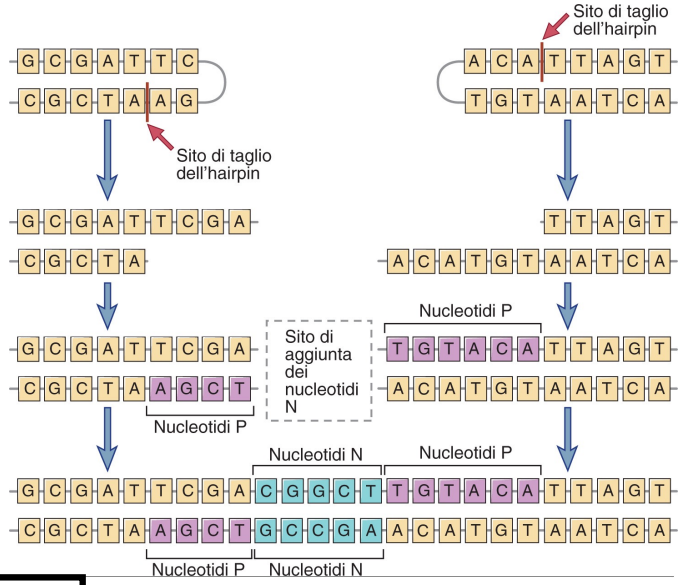
- Multiple genes for the variable region V, D, J (*random selection of only one of the V, D, and J segments*)
- Combinatorial association of α and β chain

Elemento	Recettore α–β	
	β	α
Segmenti varibili (V)	52	~70
Segmenti di diversità (D)	2	0
Segmenti di giunzione (J)	13	61
Numero di coppie di geni V	$5{,}8\times10^6$	

JUNCTIONAL DIVERSITY

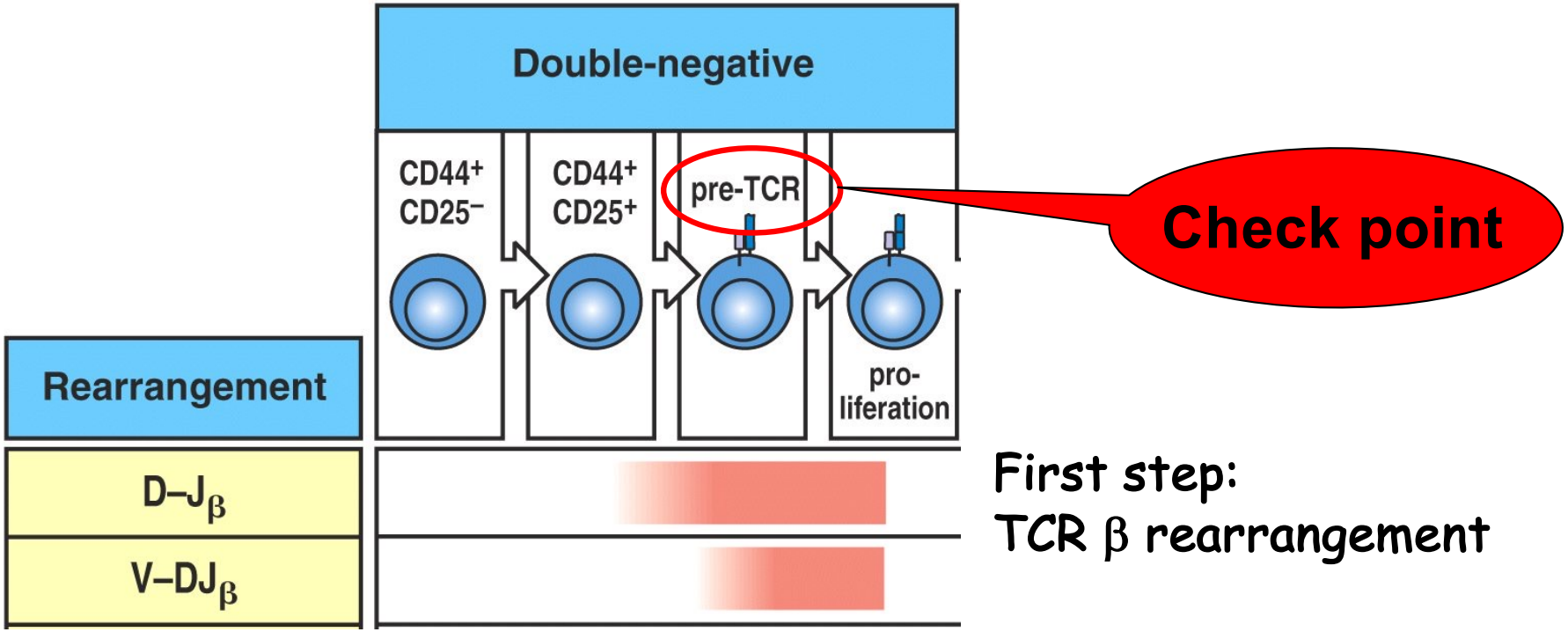
- Removal or addition of nucleotides at the
- junction point

Addition of P-nucleotides and N-nucleotides by terminal deoxynucleotidyl transferase (TdT)



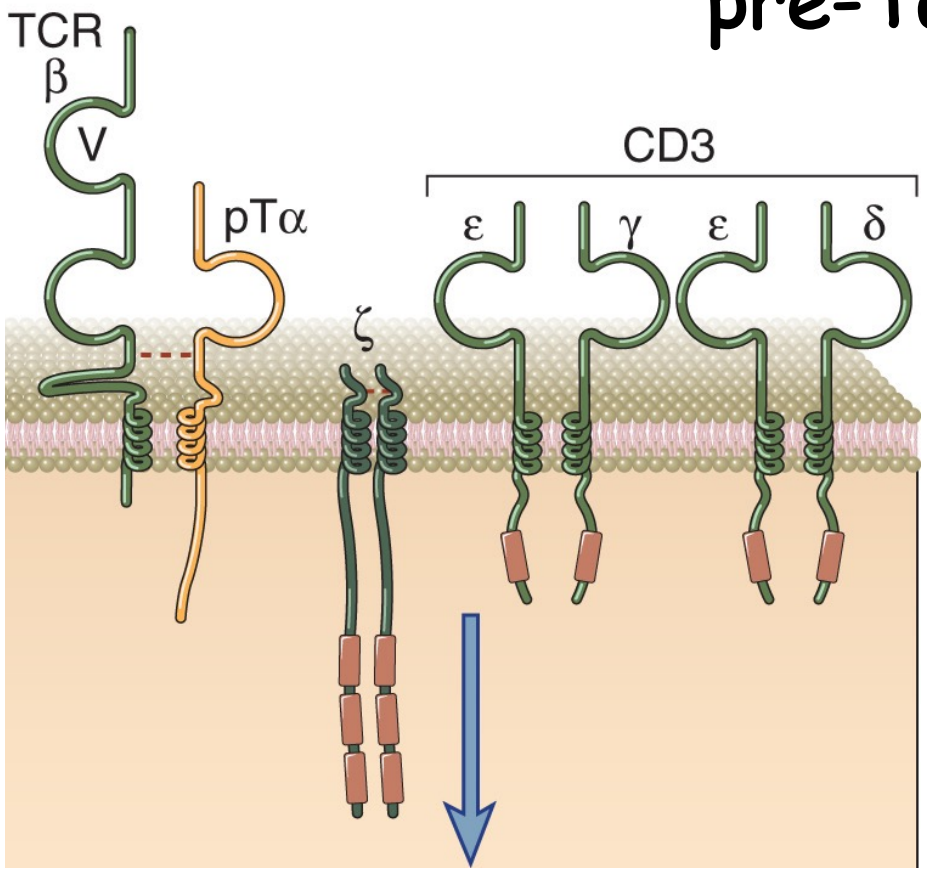
~ 10^{16} different receptors

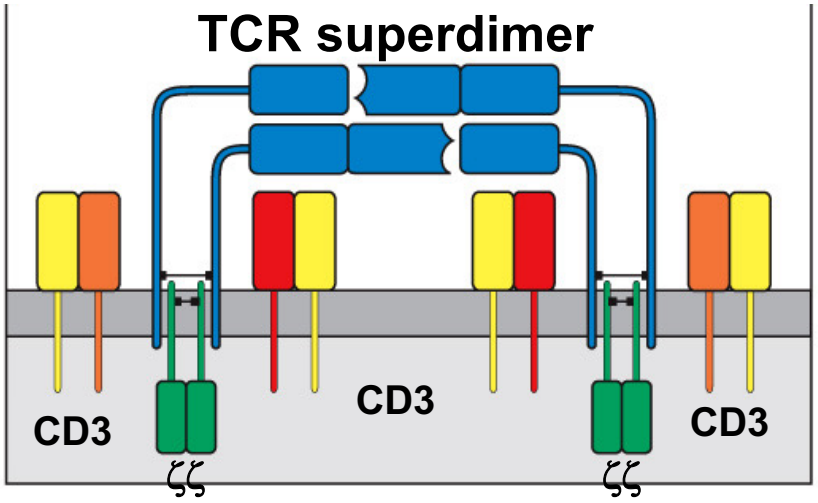
Correlation between T lymphocyte developmental stages and TCR β rearrangement



First step:
TCR β rearrangement

pre-Talpha

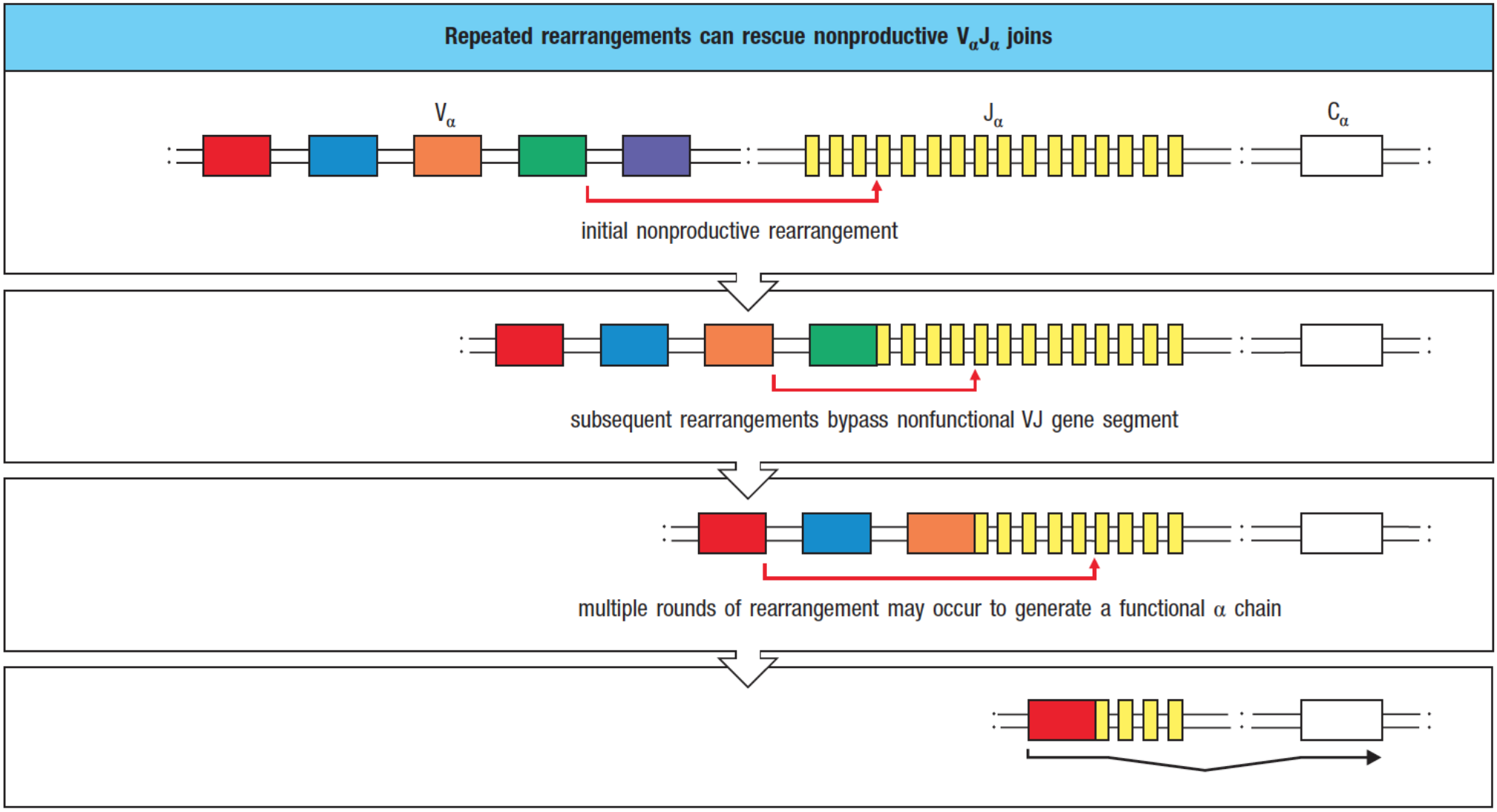




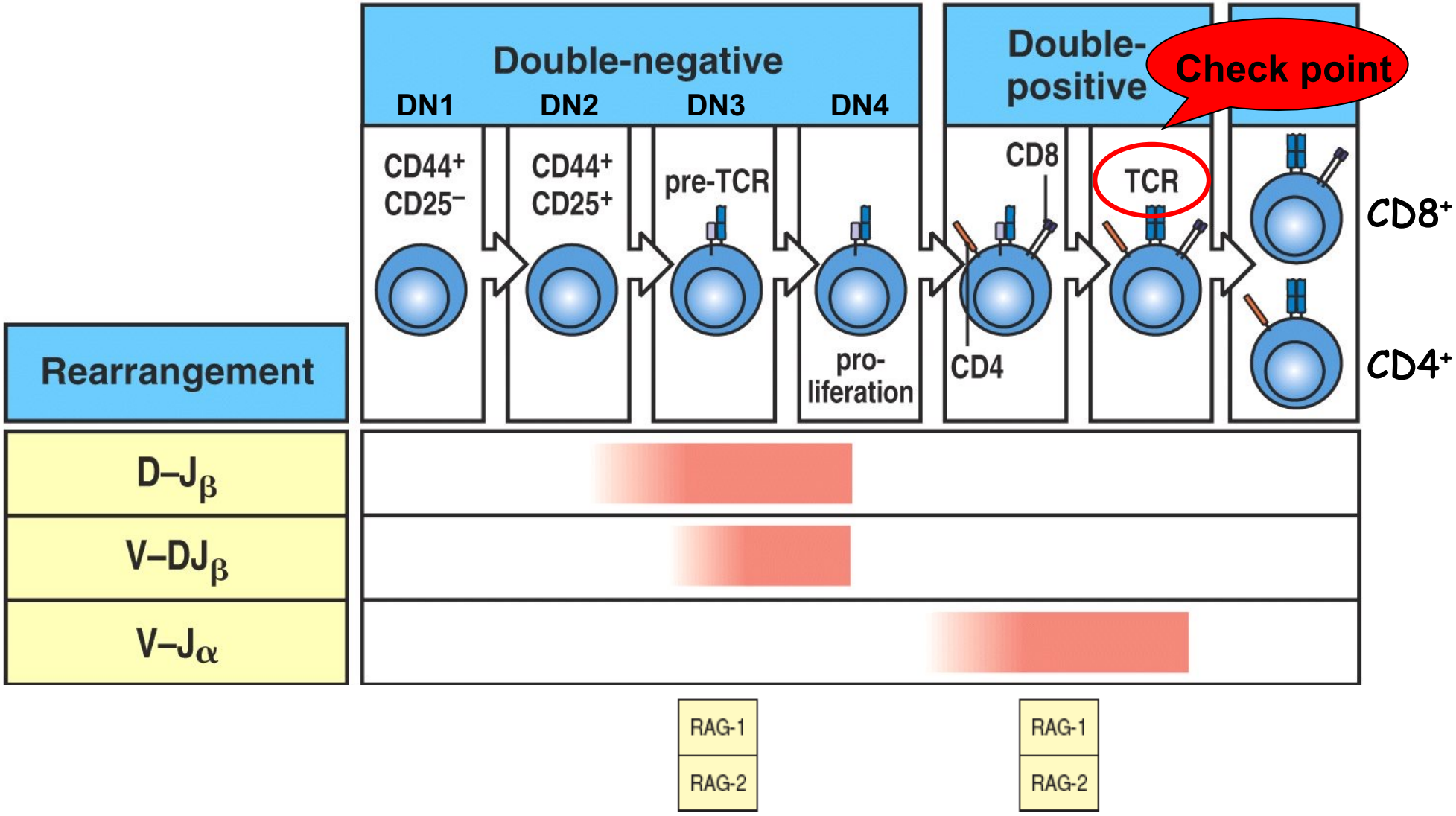
- Inhibition of β chain gene recombination
- Proliferation of pre–T cells
- Stimulation of α chain recombination
- Expression of CD4 and CD8

Allelic exclusion

Va and Ja rearrangement events follow one another until a productive event is achieved



Correlation between T-lymphocyte developmental stages and TCR gene rearrangement



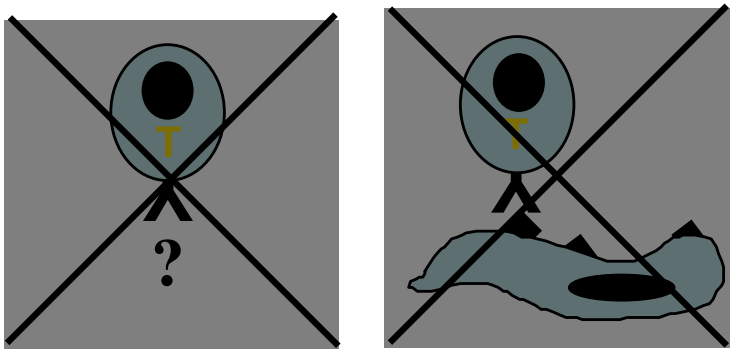
Thymocytes during the differentiation process

- Migrate
- Contact stromal cells
- Change phenotype
- Express an antigen receptor
- Undergo a selection process

Thymic selection

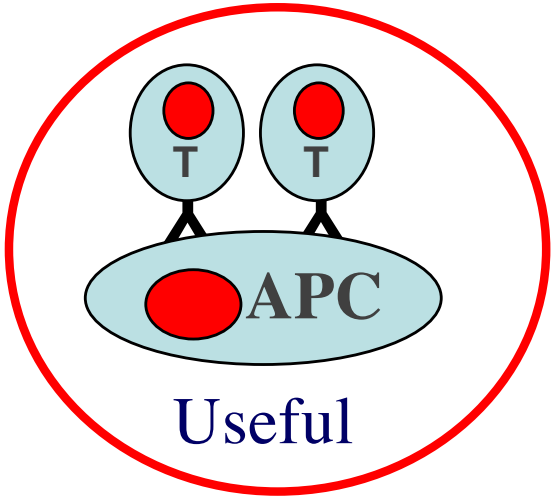
The purpose of thymic selection is:

- Eliminate useless thymocytes (because they are unable to recognize self-MHC molecules) and harmful thymocytes (capable of recognizing self-MHC complexes at high avidity and therefore of inducing autoimmune reactions)



Useless Harmful

- Maintain thymocytes with useful specificities

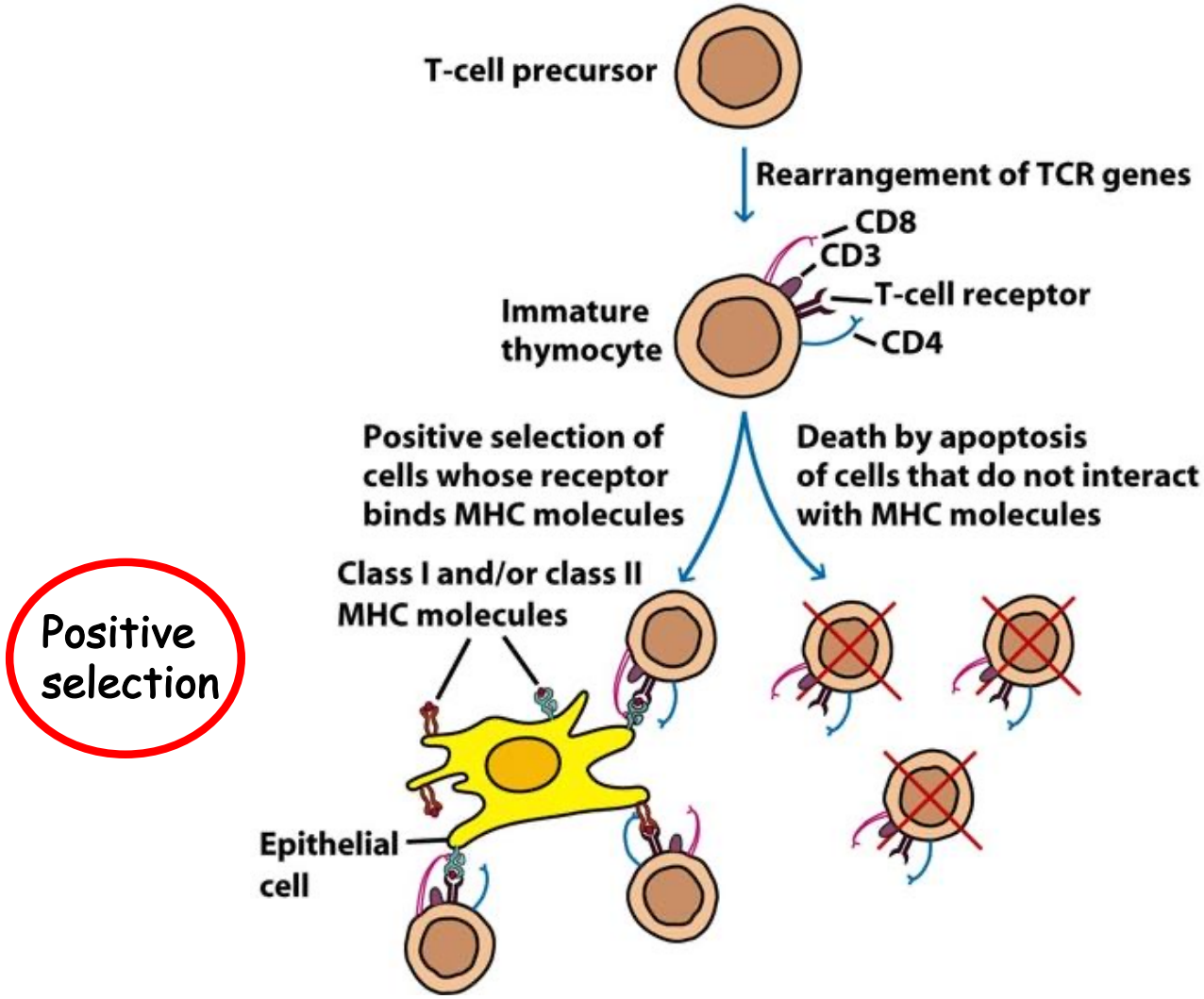


Useful

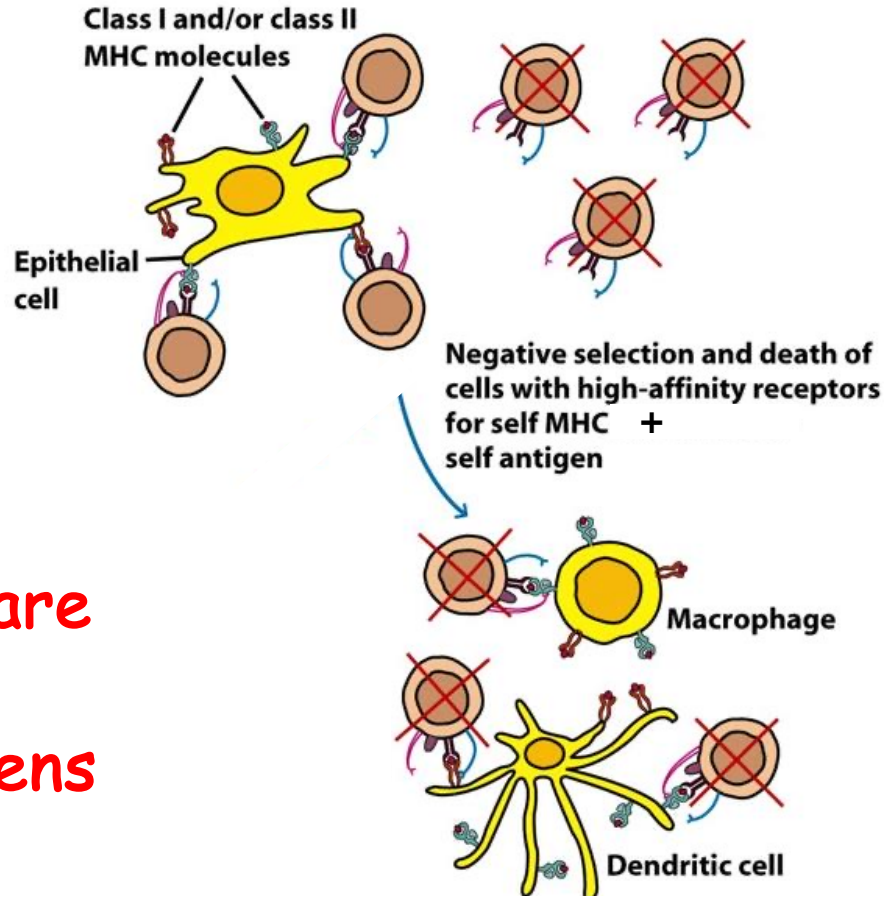
- **Positive selection**

  Thymocytes DP ($CD4^+/CD8^+$)

  Selects for thymocytes bearing receptors capable of binding self-MHC molecules with low affinity, resulting in MHC restriction



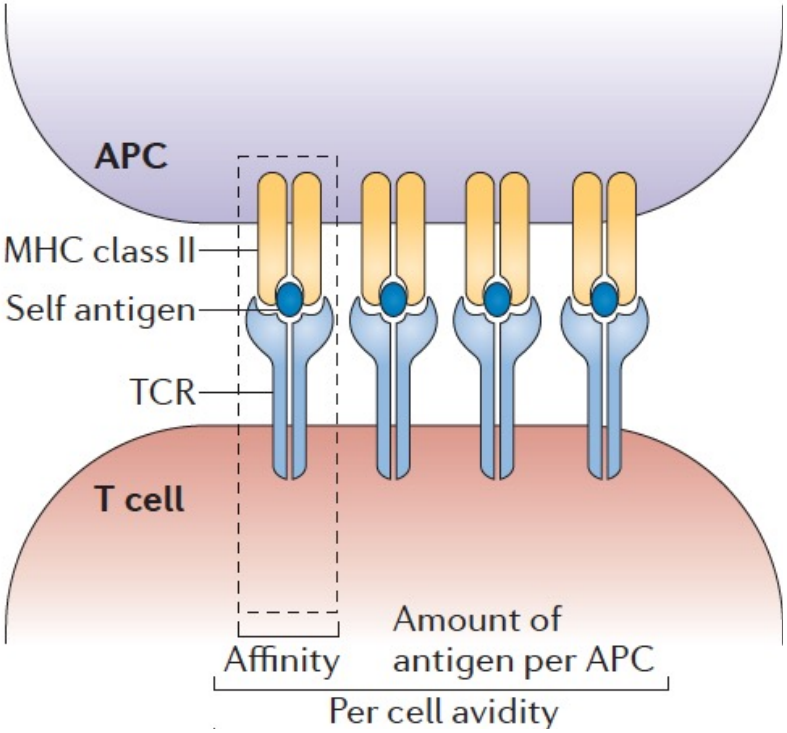
- Negative selection
  - Thymocytes DP (CD4+/CD8+)
  - Selects against thymocytes bearing high-affinity receptors for self-MHC/peptide complexes, resulting in self-tolerance

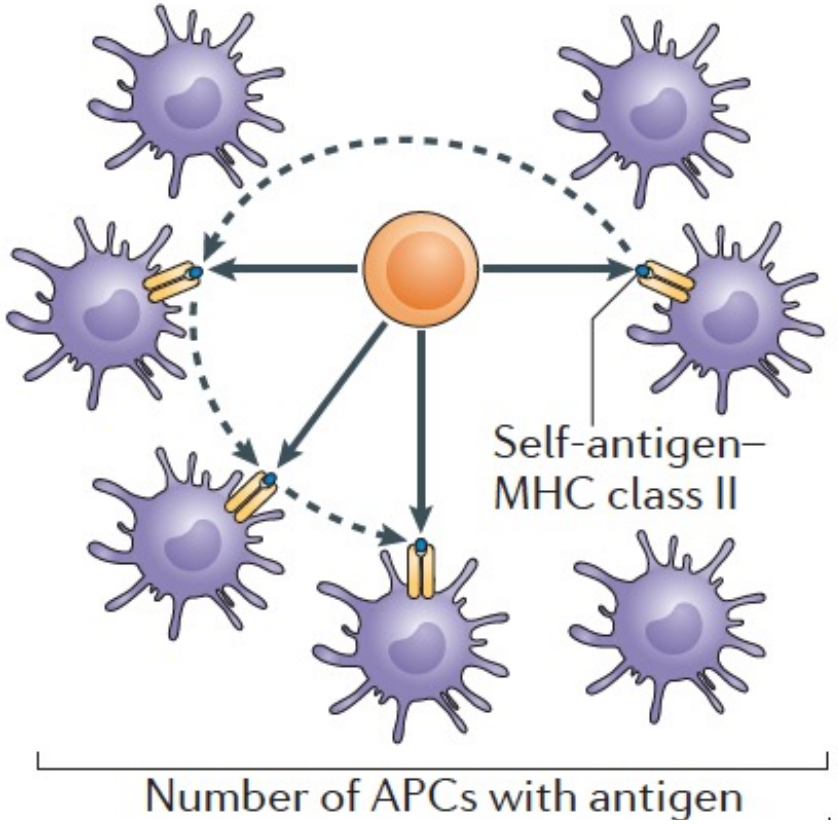


Surviving thymocytes are tolerant to self-antigens

Negative selection

Difference between affinity and avidity….



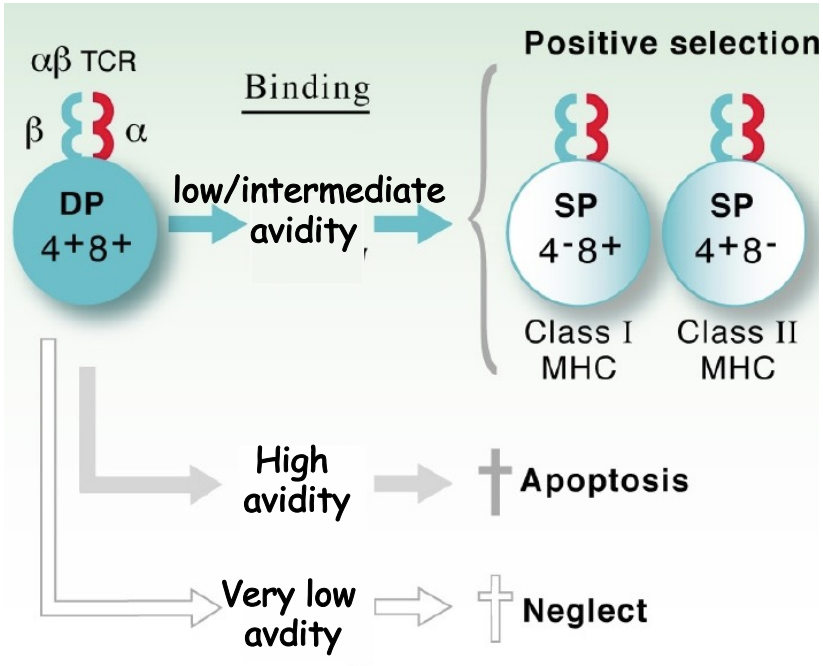


Avidità

- **Affinity** of single interaction TCR/MHC peptide
- **Amount** of different interaction TCR/MHCpeptide/cells

Modified from Hsieh C-S et al, Nature Review, 2012

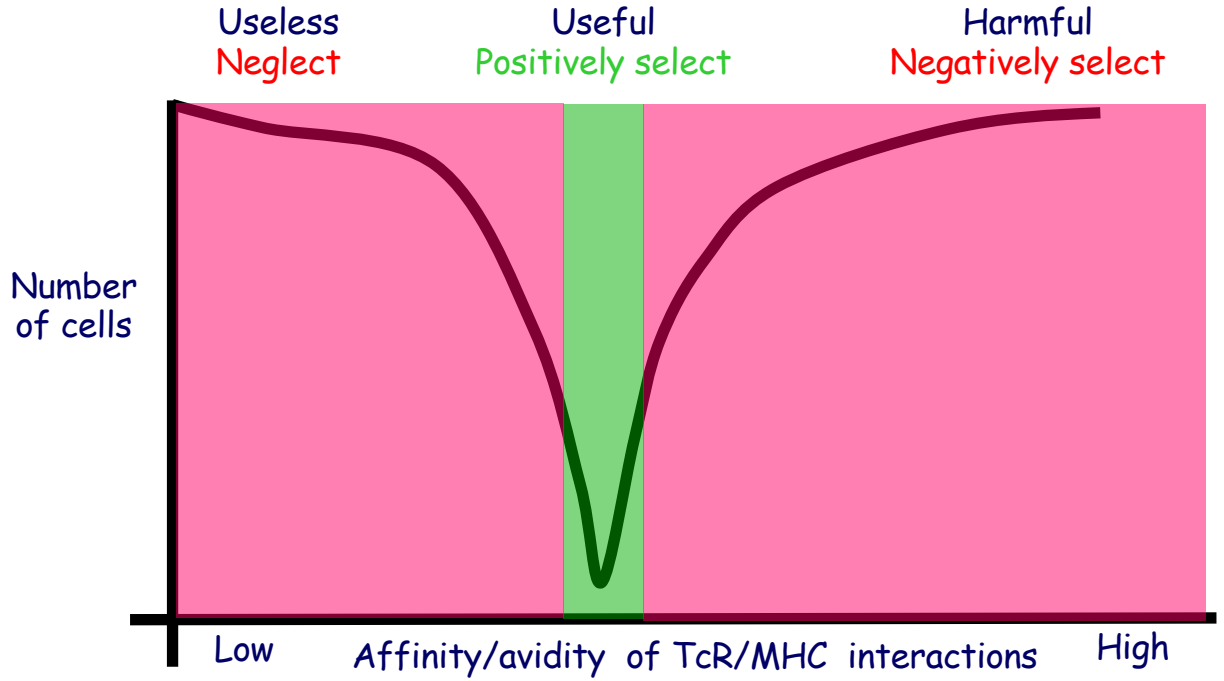
...and selection based on overall signal strength

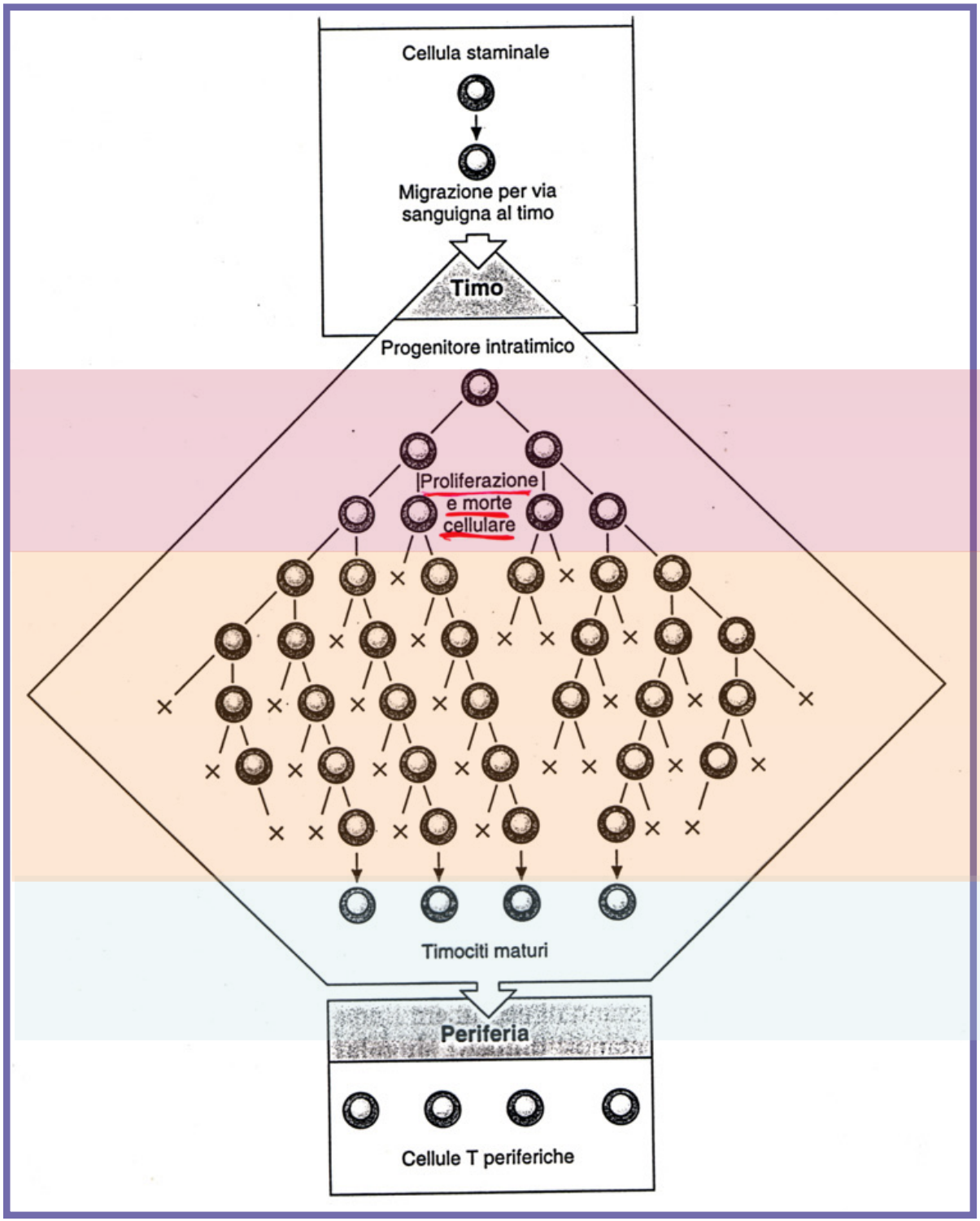


The lymphocyte passes selection and survives

The lymphocyte dies by apoptosis

The neglected lymphocyte dies of loneliness





T cells mature in the thymus but most die there !

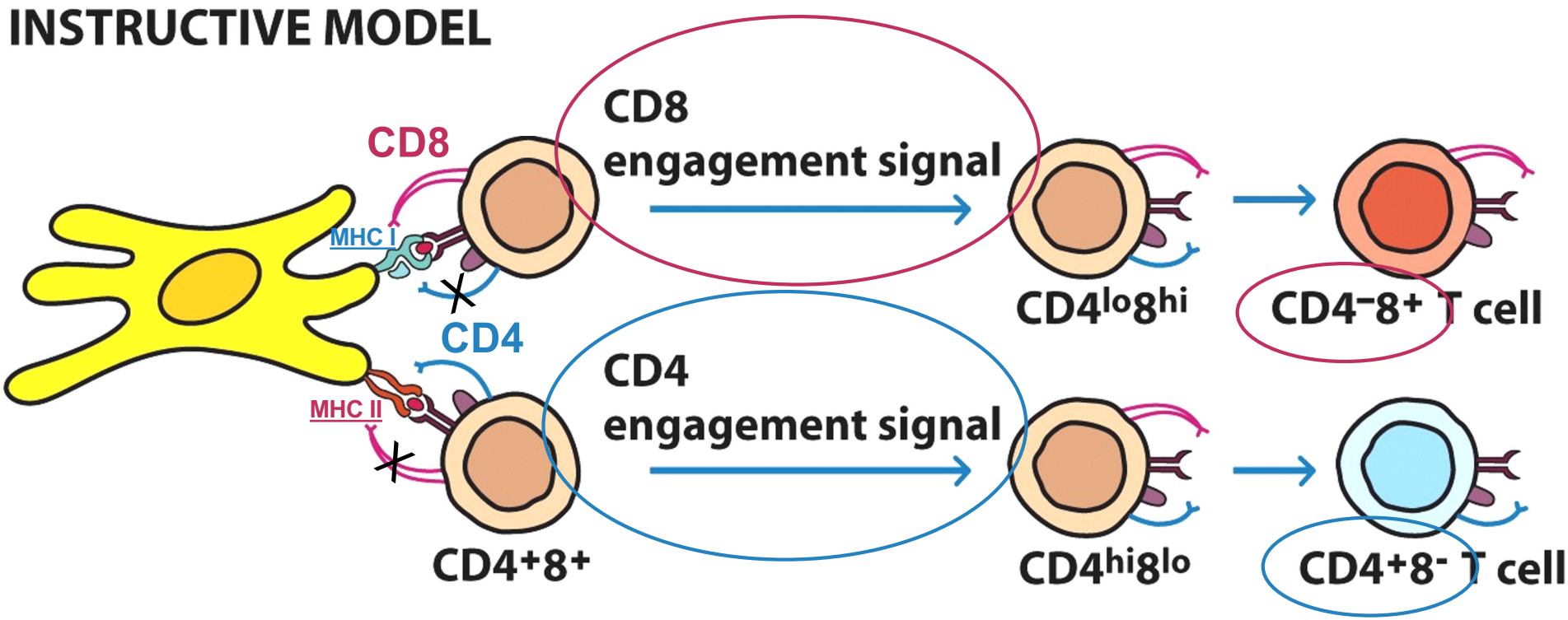
$CD4^-CD8^-$ DN 5%

$CD4^+CD8^+$ DP 80%

$CD4^+$ or $CD8^+$ SP 15%

Young adult:
~$5x10^7$ thymocytes produced/day
~$2x10^6$ mature cells leave/day

CD4 or CD8?
The transition from DP to SP



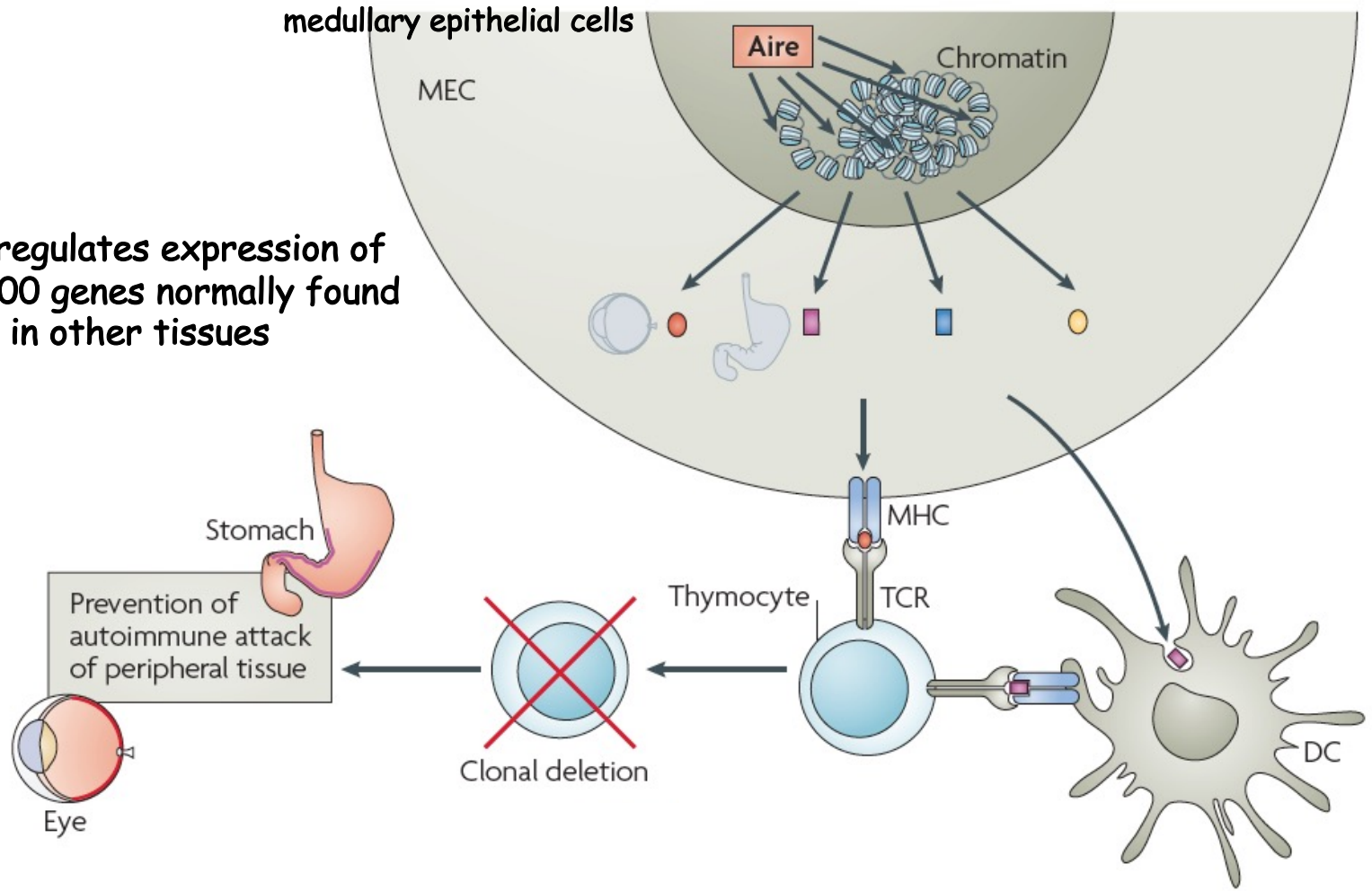
- If the TCR can bind to an MHC class I molecule, it also binds with the CD8 molecule, selecting the cell to the $CD8^+$ subset.
- The opposite happens if the TCR binds to an MHC class II molecule, resulting in selection to the $CD4^+$ subset

How can the thymus express all self antigens – including self antigens only made by specialised tissues?

How do we become self tolerant to these antigens?

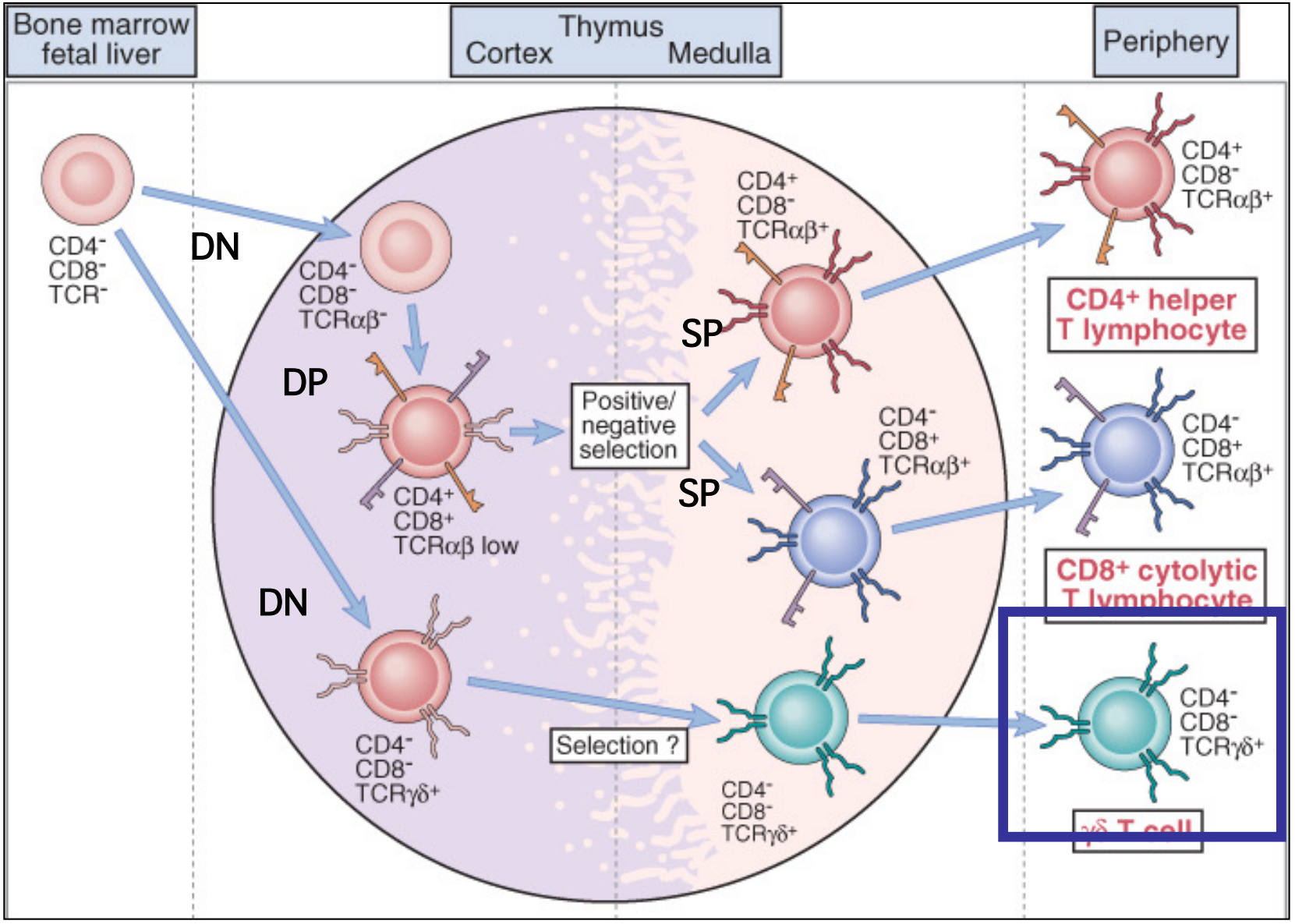
AIRE: from transcriptional regulation to tolerance induction

AIRE regulates expression of 300-1200 genes normally found in other tissues



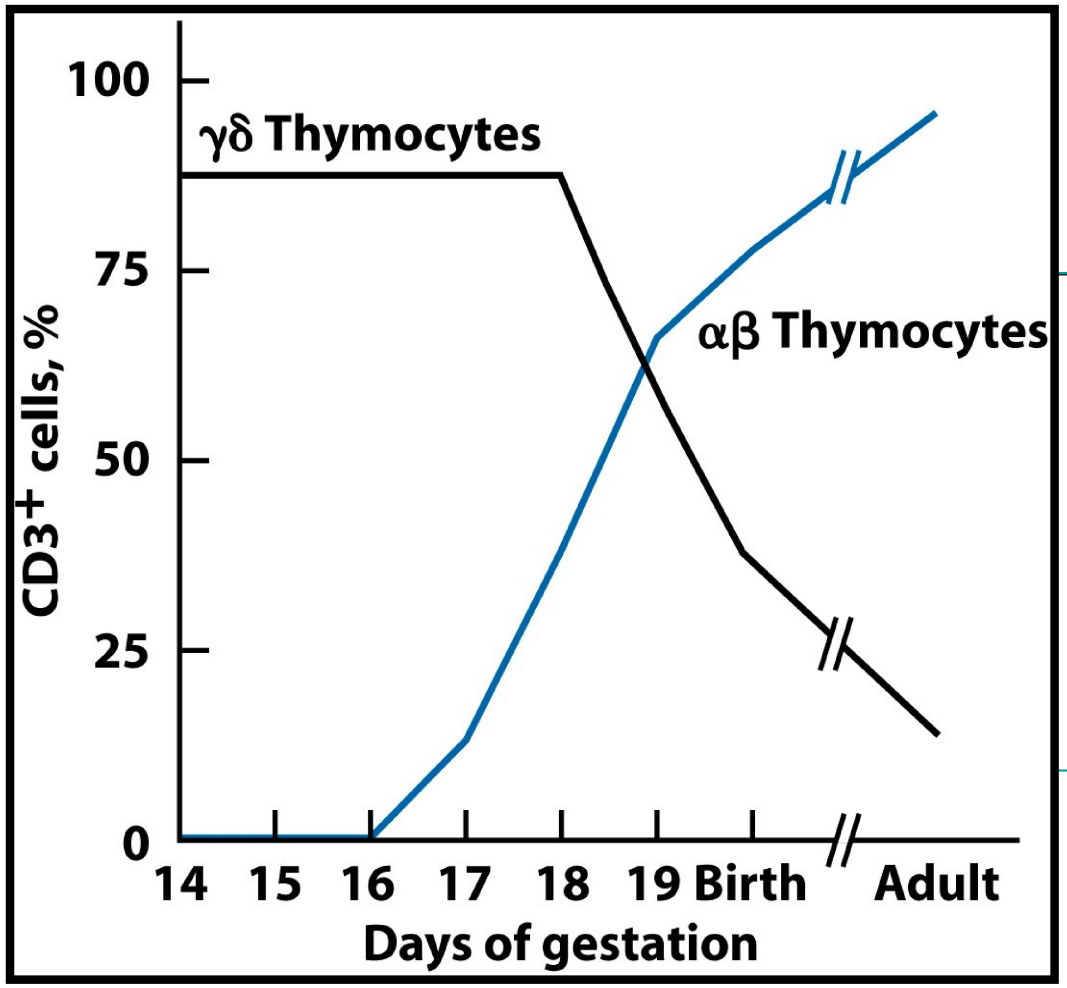
CENTRAL TOLERANCE

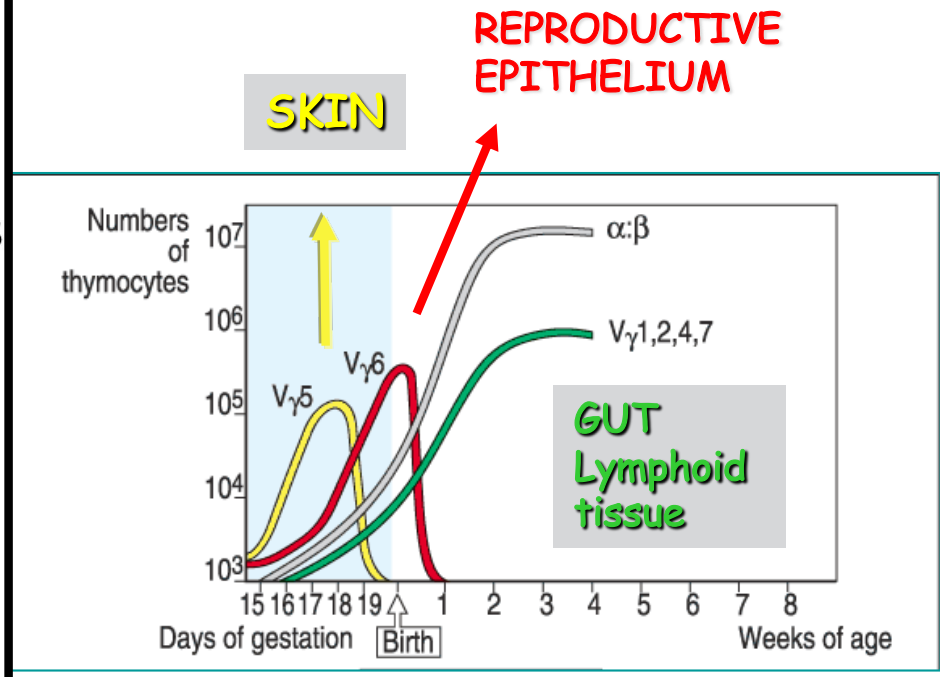
ONTOGENESIS OF T LYMPHOCYTES



10%

γδ T cells are favored during early fetal development





Waves of γδ T cells home to different tissues

CHARACTERISTICS OF THE TWO POPULATIONS OF T LYMPHOCYTES

	$\alpha\beta$	$\gamma\delta$
Frequency in peripheral blood	90% of T cells	1-10% T cells
Localization	peripheral blood	epithelial layer of mucosal and barrier tissues
Recognition	non-self antigen + self MHC	Classical MHC? molecule induced by stress
Phenotype	$CD4^+$ o $CD8^+$	$CD4^-$ $CD8^-$
Function	helper or cytotoxic	cytotossic
Role	adattative response against non-self antigen	first line of defence recognition of altered cells