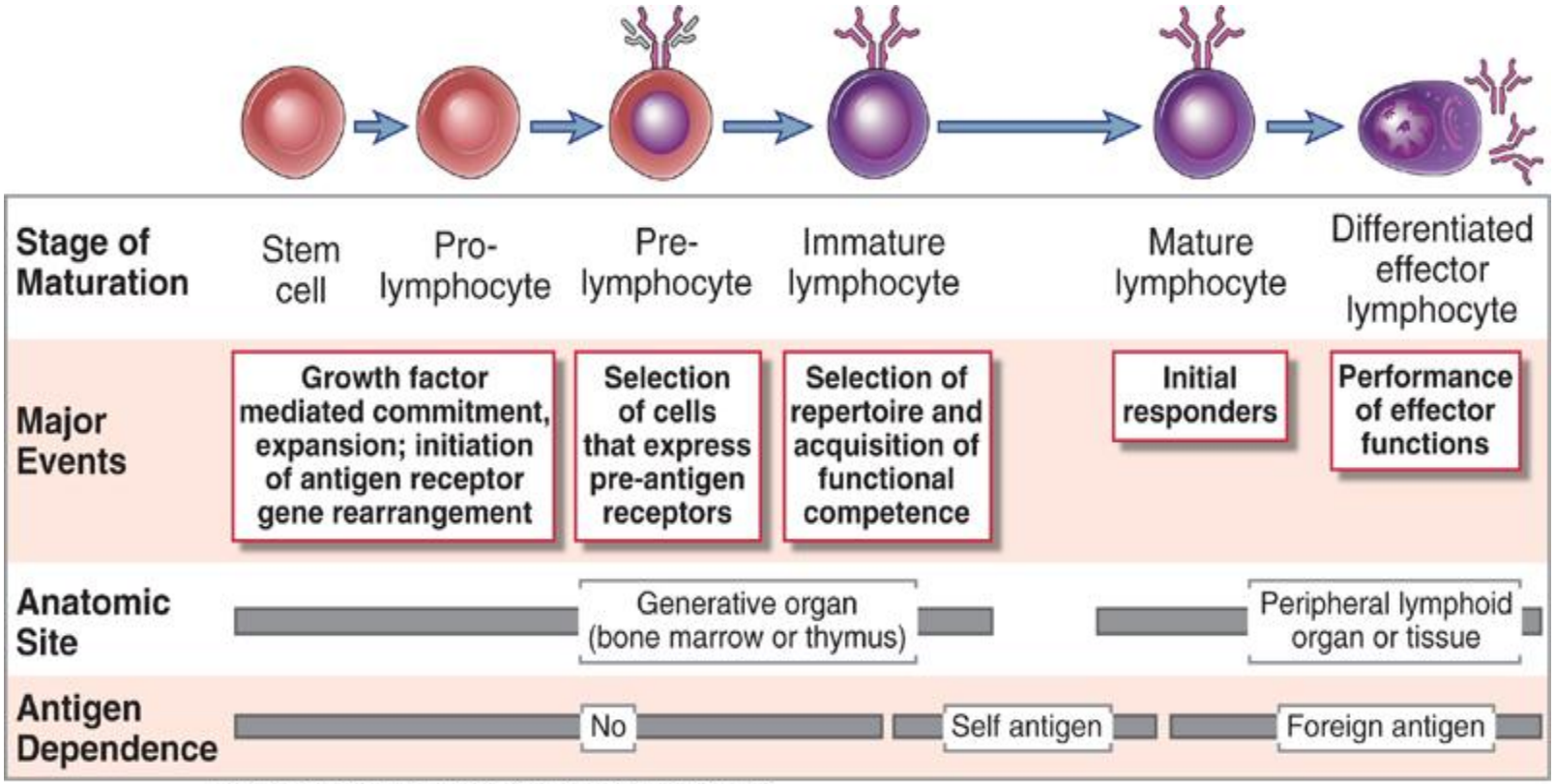
Stages of lymphocyte maturation (Abbas Chapter 8)

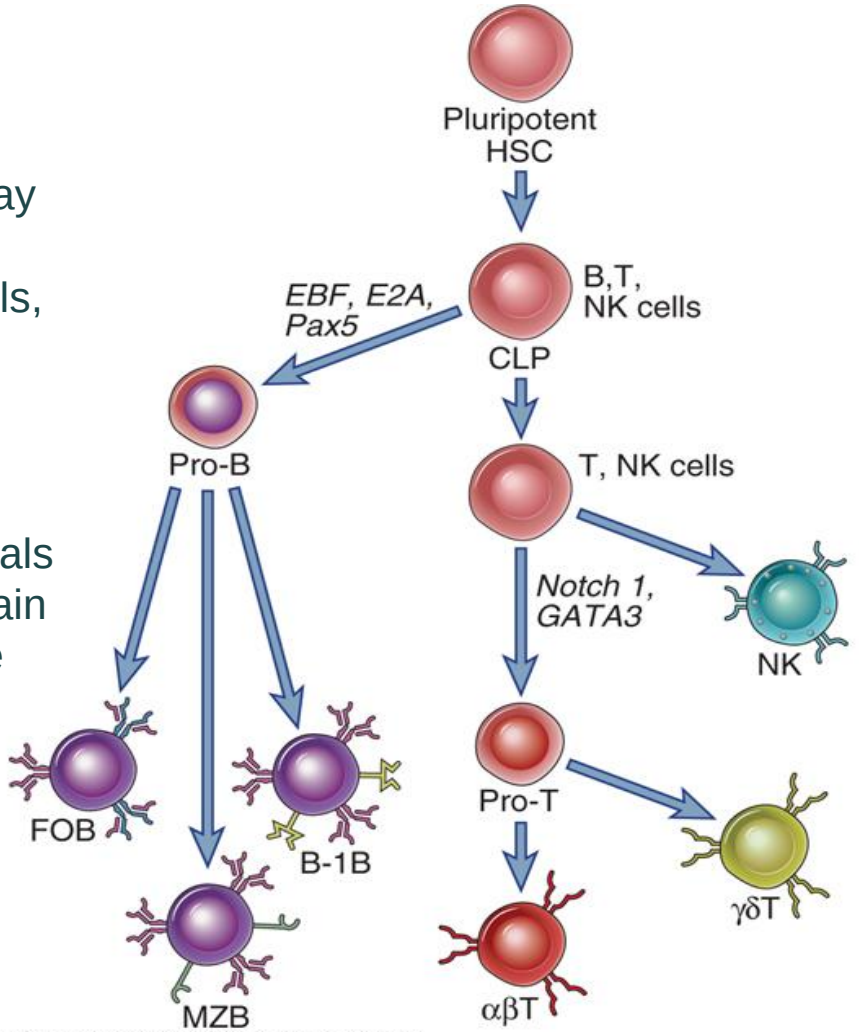


From pluripotent stem cells to B and T cells

Hematopoietic stem cells (**HSCs**) give rise to many distinct progenitors, e.g. a common lymphoid progenitor (CLP).
CLPs give rise mainly to **B** and **T cells**, but may also contribute to **NK** cells and some **DCs**.
Pro-B cells differentiate to follicular (**FO**) **B** cells, marginal zone (**MZ**) **B** cells, and **B-1 B** cells.
Pro-T cells may commit to either the **αβ** or **γδ** T cell lineages.

Commitment to the T lineage depends on signals delivered by Notch-1, whose intracellular domain mediates transcriptional activation of T lineage genes in collaboration with other transcription factors such as GATA-3.
Commitment to the B lineage is mediated by the EBF and E2A transcription factors and subsequently by Pax-5.

Transcription factors are indicated by italics



Early B and T cell development is characterized by the proliferation of committed progenitors induced by cytokine-derived signals

IL-7 = "proliferation" cytokine, produced by stromal cells

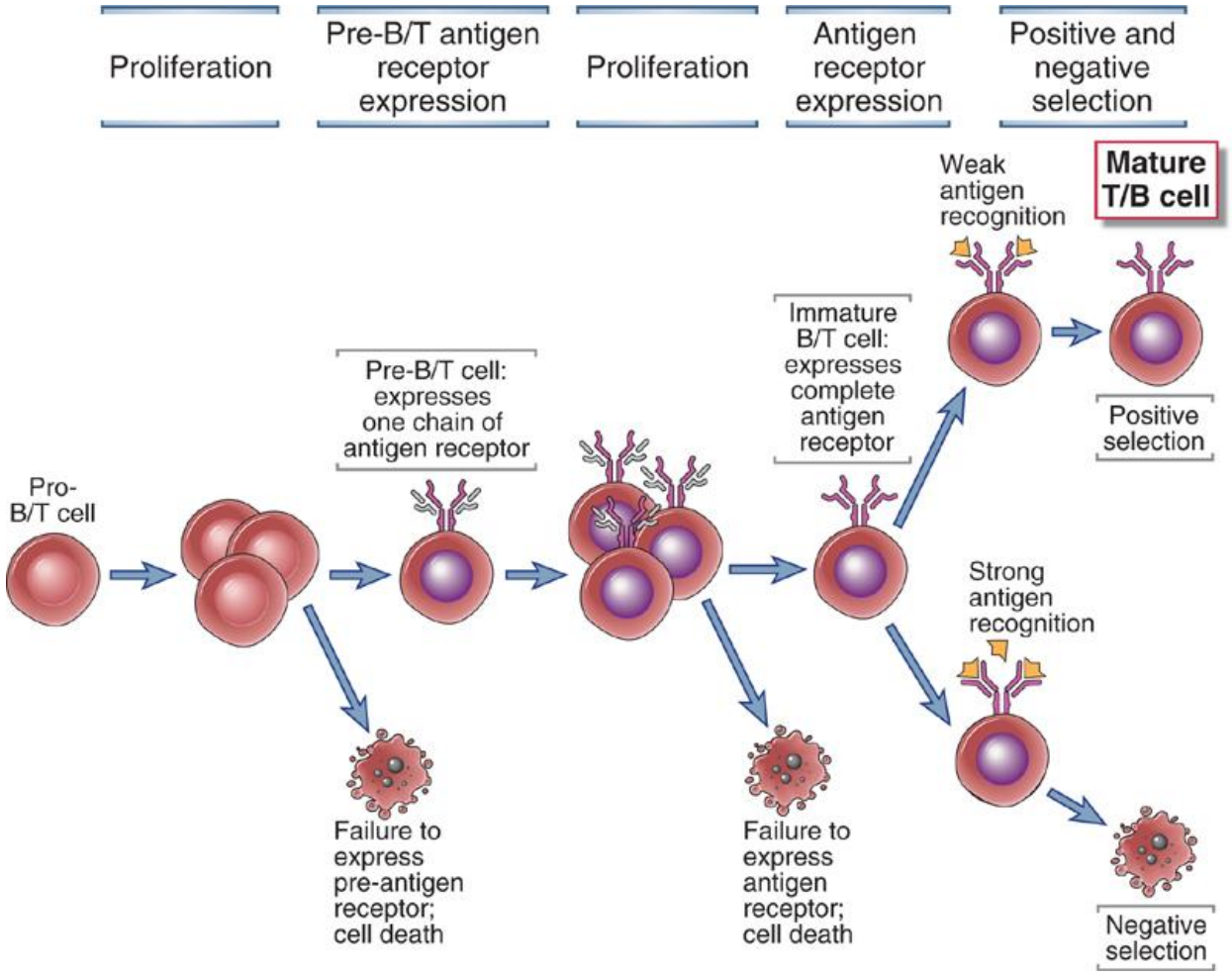
IL-7 for B- and T cells in the mouse
IL-7 for only T cells in humans

IL-7 activity ceases before gene rearrangement

Human X-linked severe combined immunodeficiency disease (X-SCID)

- *Mutation of the common γ-chain of IL-7*
- *Block of T cell and NK cell development*
- *Normal B cell compartment*

Principle checkpoints in lymphocyte maturation



Positive and negative selection during maturation

„Positive“

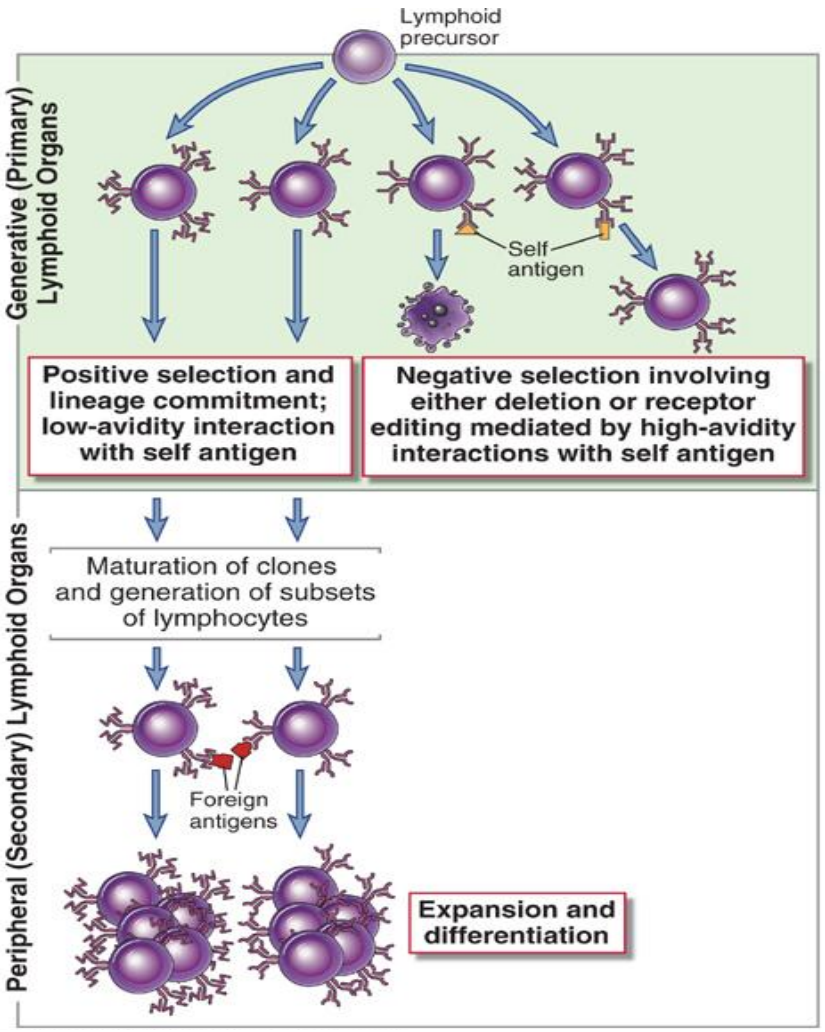
- Building a correct receptor
- Low avidity for self

„Negative“

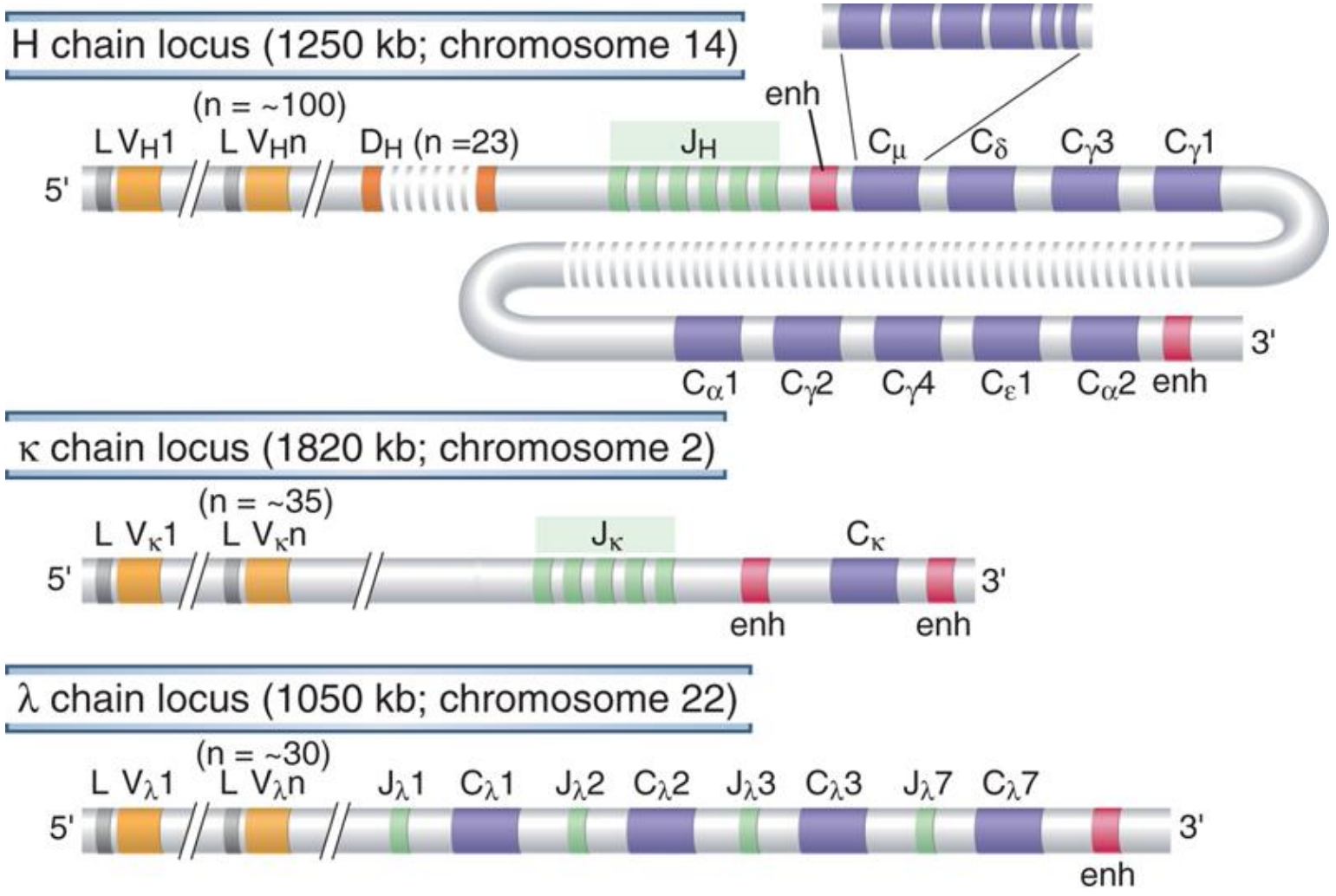
- Non-functional receptor
- High avidity for self

„Rescue mechanisms“

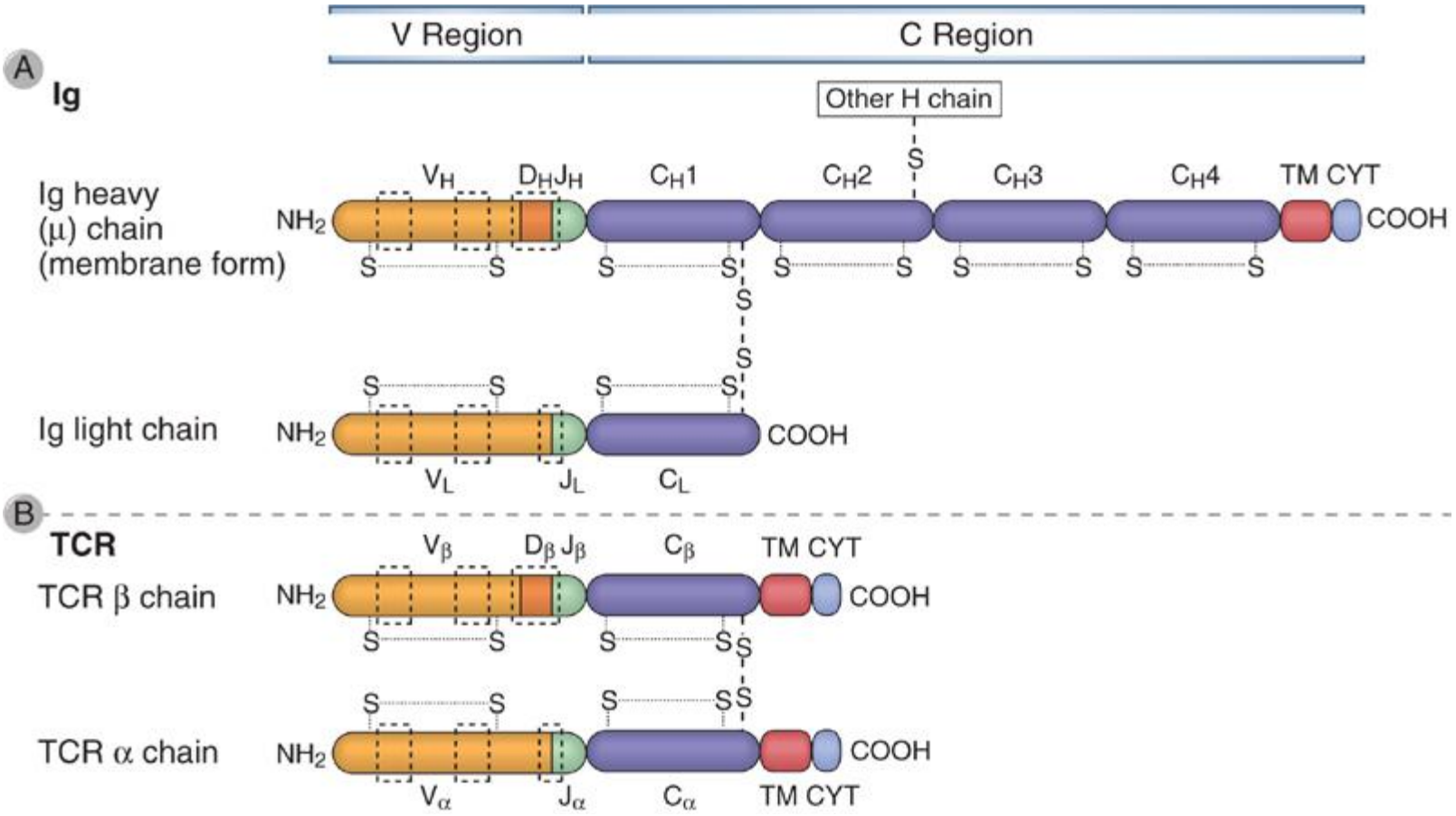
- Second allel
- Receptor editing



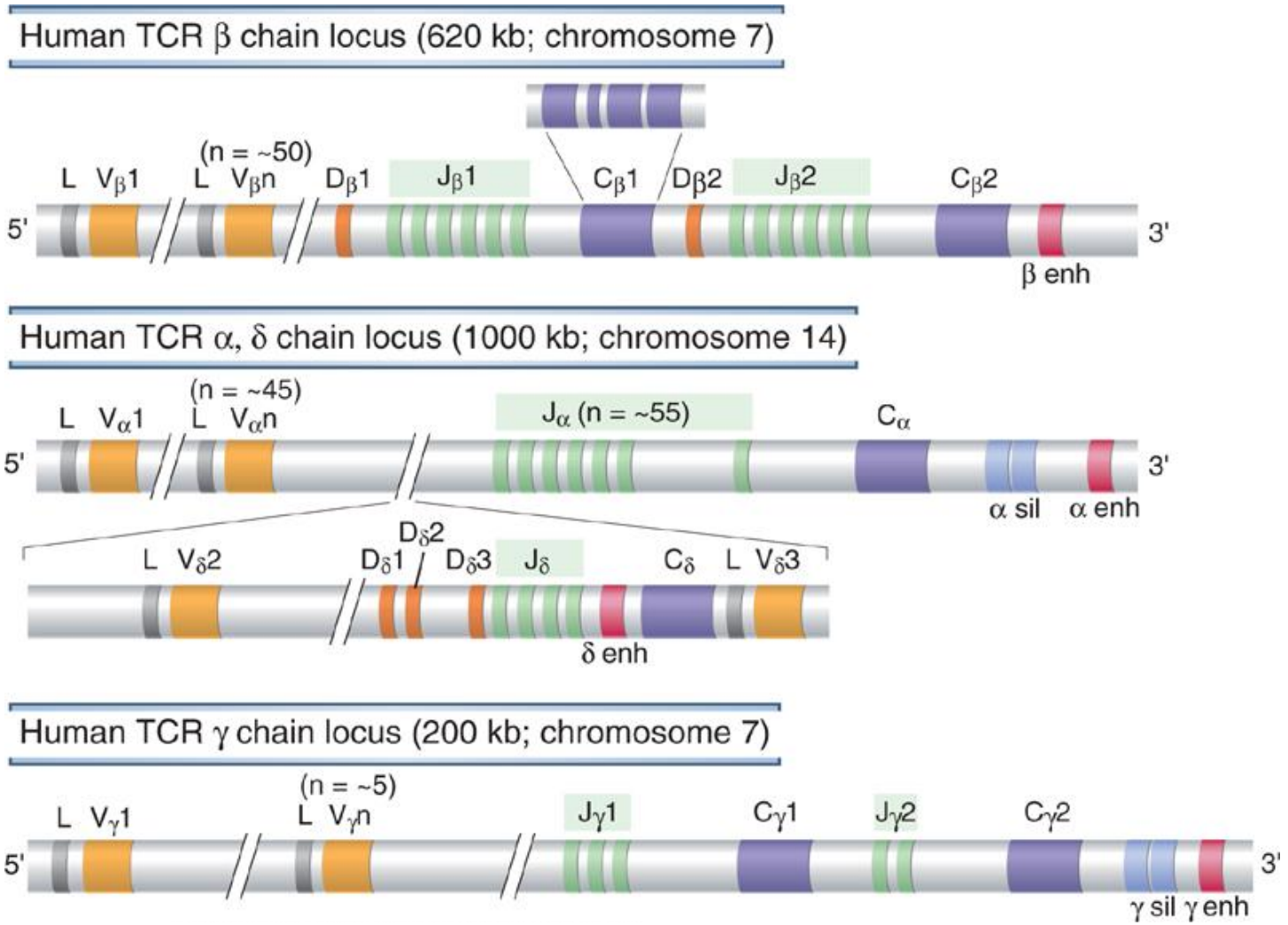
Germline organization of human Ig loci



Domains of Ig and TCR proteins

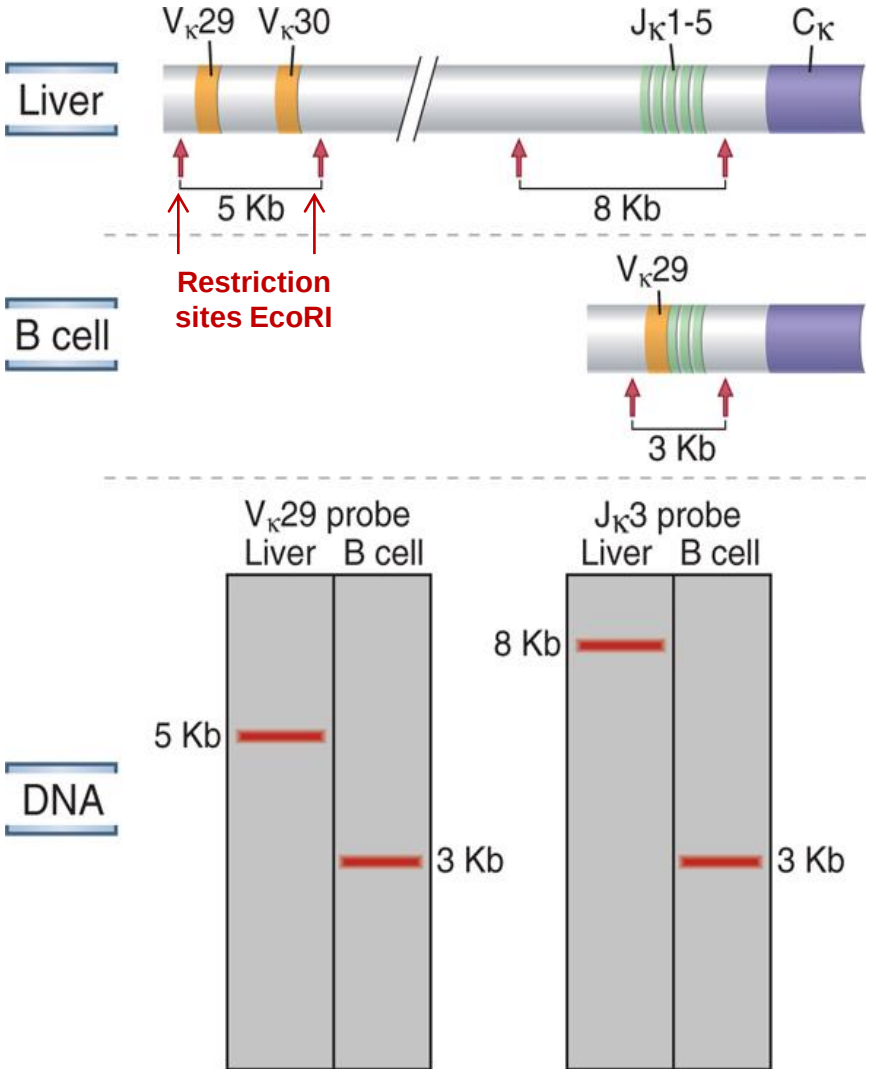


Germline organization of human TCR loci

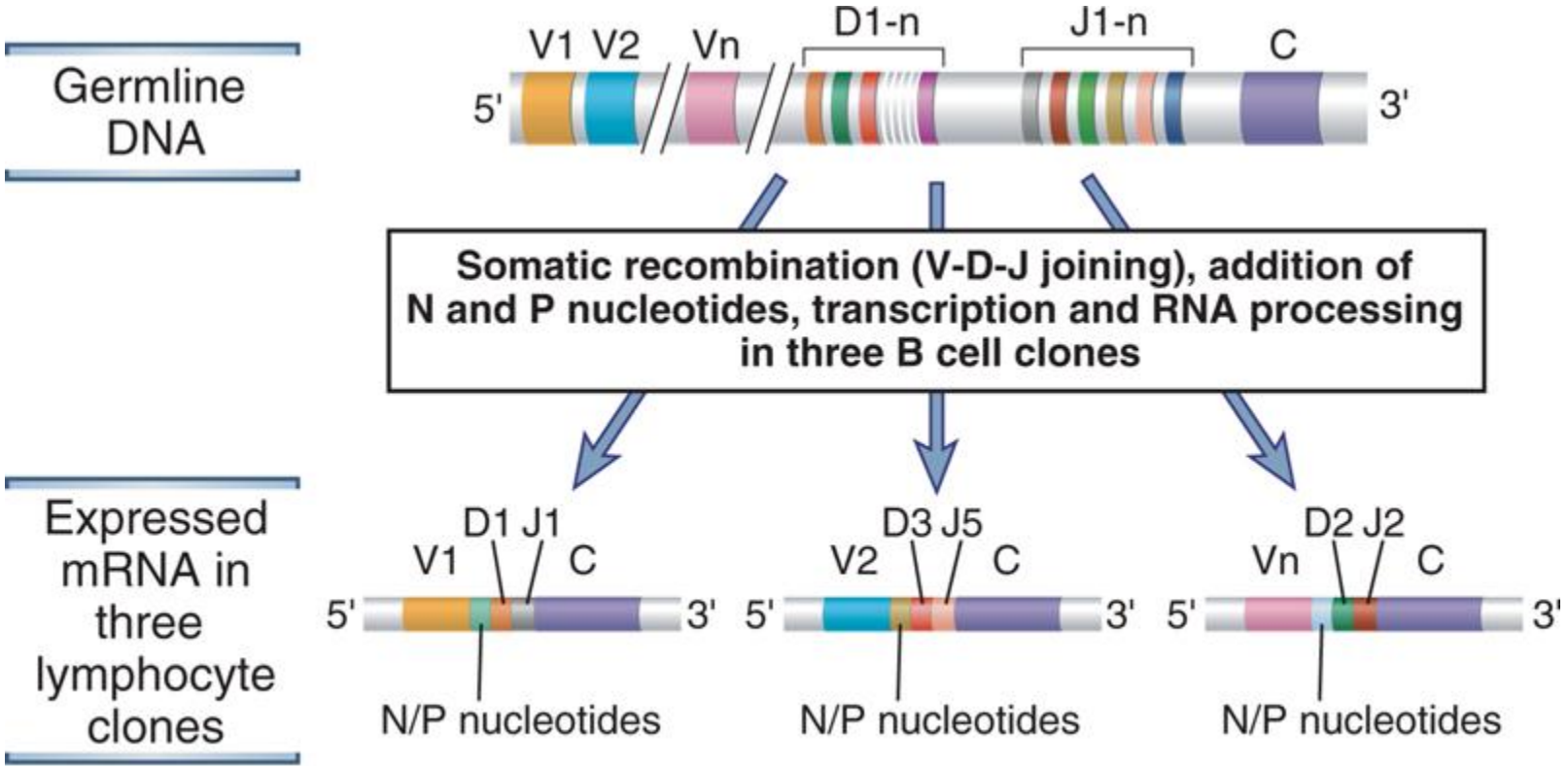


Antigen receptor gene rearrangements

Southern blot of DNA from nonlymphoid (liver) cells and from a monoclonal population of B lymphocyte lineage origin (e.g., a B cell tumor) is shown in schematic fashion. The DNA is digested with a restriction enzyme (*Eco*RI as depicted), different-sized fragments are separated by electrophoresis, and the fragments are transferred onto a filter. The sites at which the *Eco*RI restriction enzyme cleaves the DNA are indicated by arrows. **The size of the fragments containing the Jκ3 segment of the Ig κ light chain gene or the Vκ29 V region gene was determined by use of a radioactive probe that specifically binds to Jκ3 segment DNA or to Vκ29 DNA.** In the hypothetical example shown, Vκ29 is part of a 5-kb *Eco*RI fragment in liver cells but is on a 3-kb fragment in the B cell clone studied. Similarly, the Jκ3 fragment is 8 kb in liver cells but 3 kb in the B cell clone.

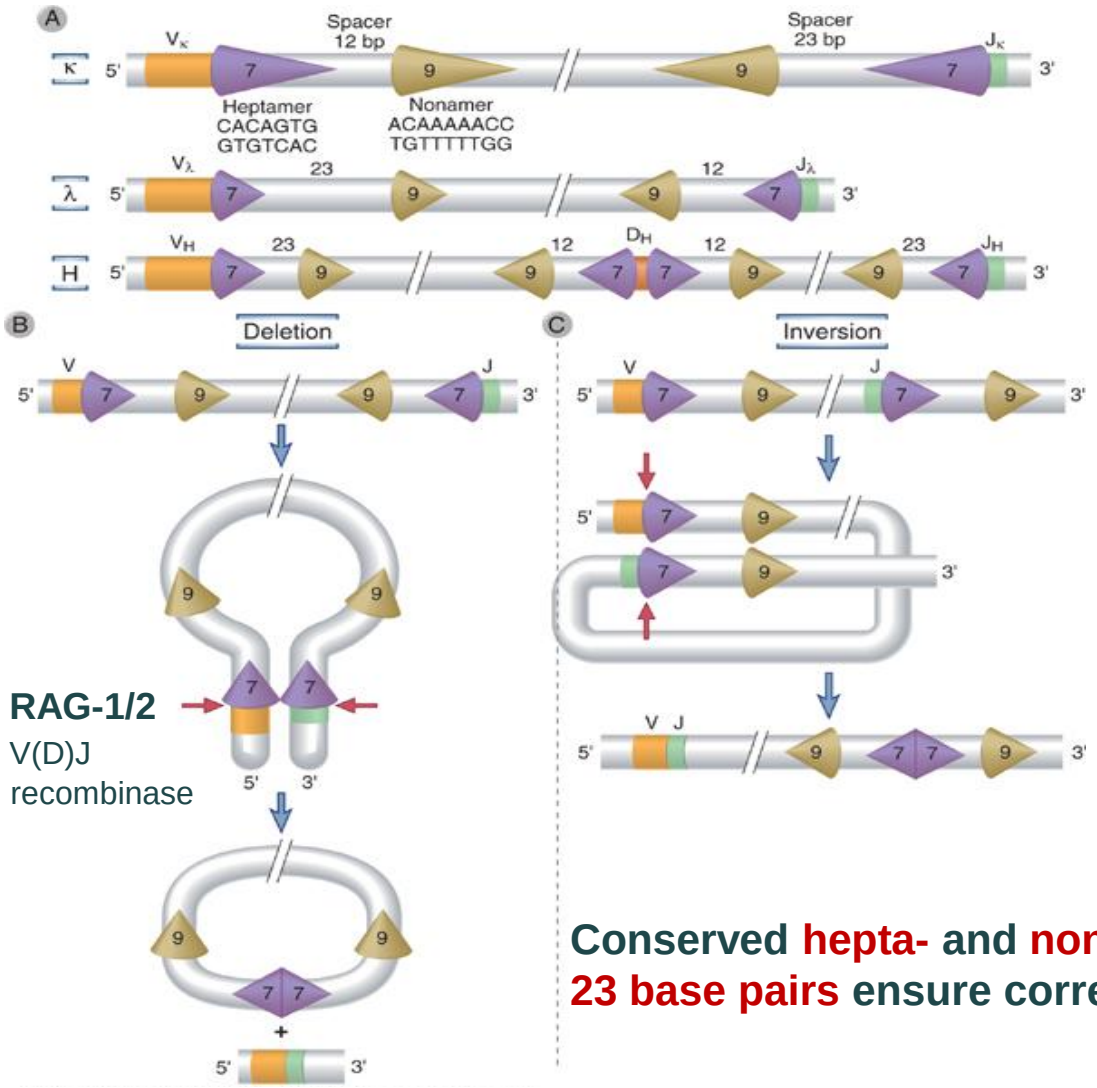


Diversity of antigen receptor genes



From the same germline DNA, it is possible to generate recombined DNA sequences and mRNAs that differ in their V-D-J junctions. In the example shown, **three distinct antigen receptor mRNAs are produced from the same germline DNA** by the use of different gene segments and the addition of nucleotides to the junctions

V(D)J recombination



RSS (recombination signal sequences) **comprise:**

- **a heptamer (CACAGTG)**
- **a spacer (12 or 23 nucleotides)**
- **a nonamer (AT-rich)**

RAG (recombination activating gene)

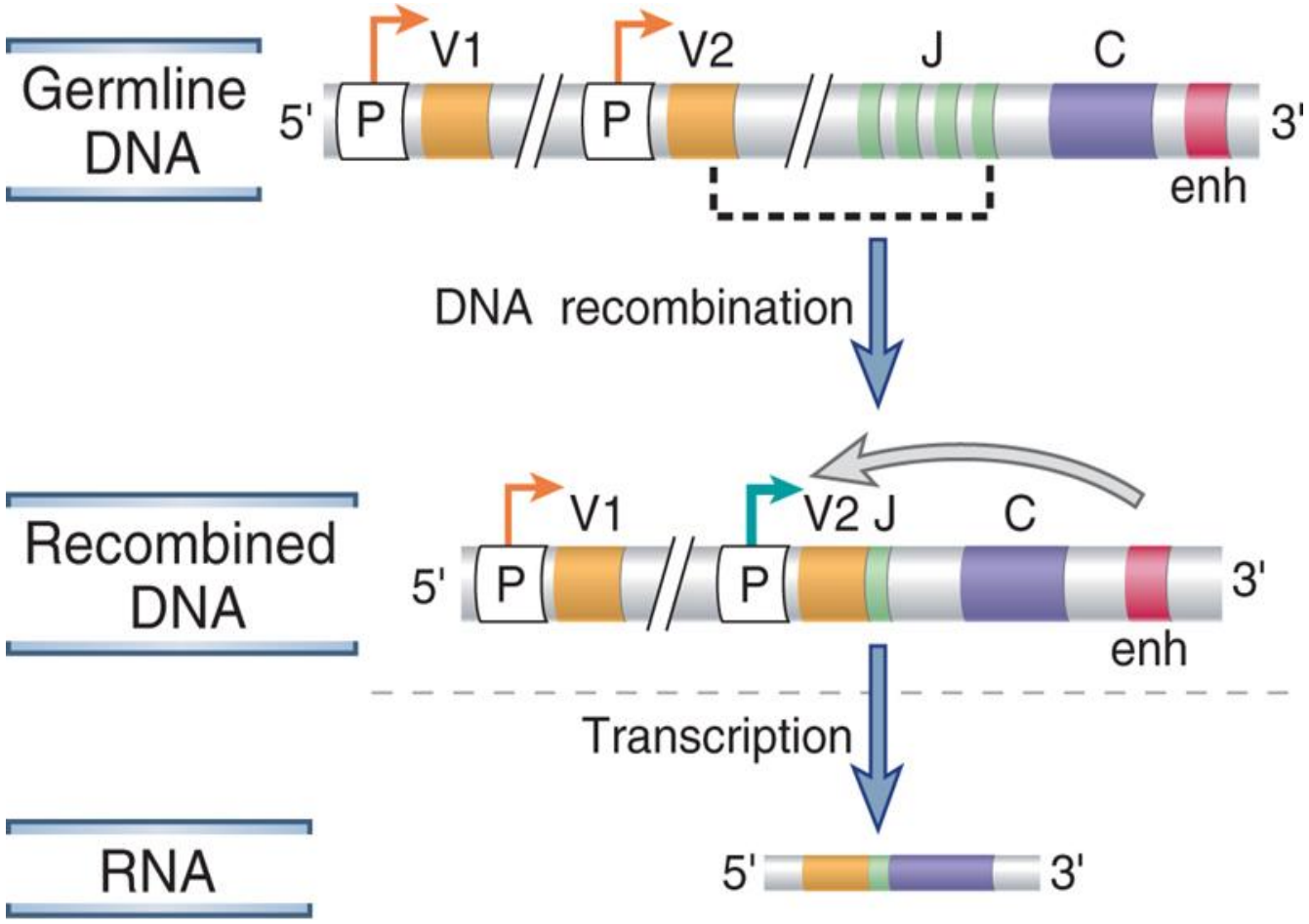
Rag-1/2 = V(D)J recombinase **recognizes:**

a DNA sequence at the junction between a heptamer and the coding sequence

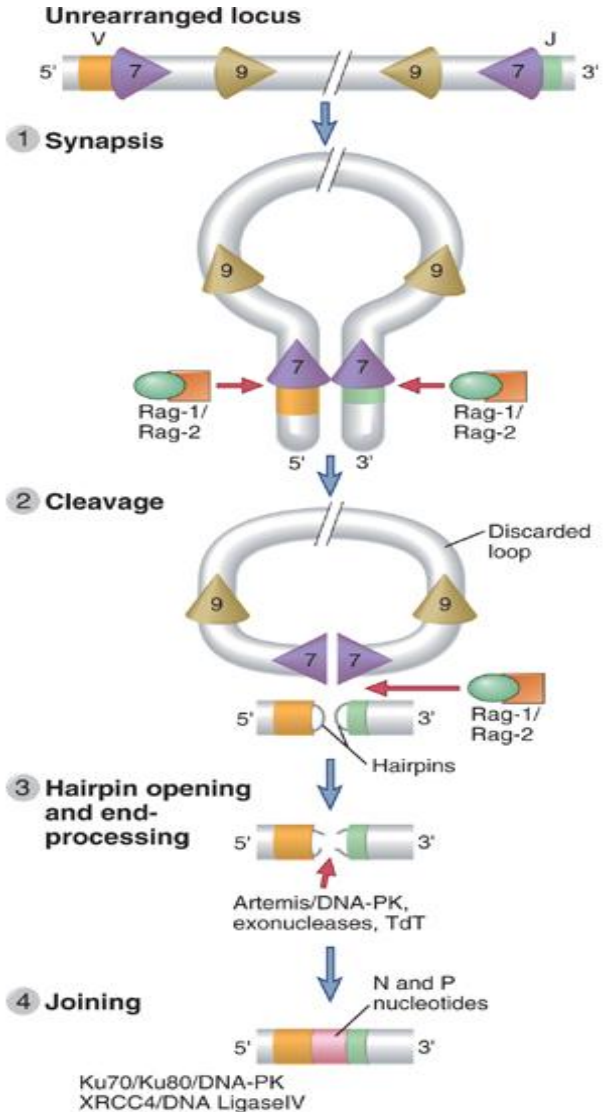
Conserved hepta- and nonamers, and spacers of 12 or 23 base pairs ensure correct recombination

Transcriptional regulation of Ig genes

Recombination brings promotor sequences close to the enhancer



Sequential events during V(D)J recombination



Sequential events during V(D)J recombination

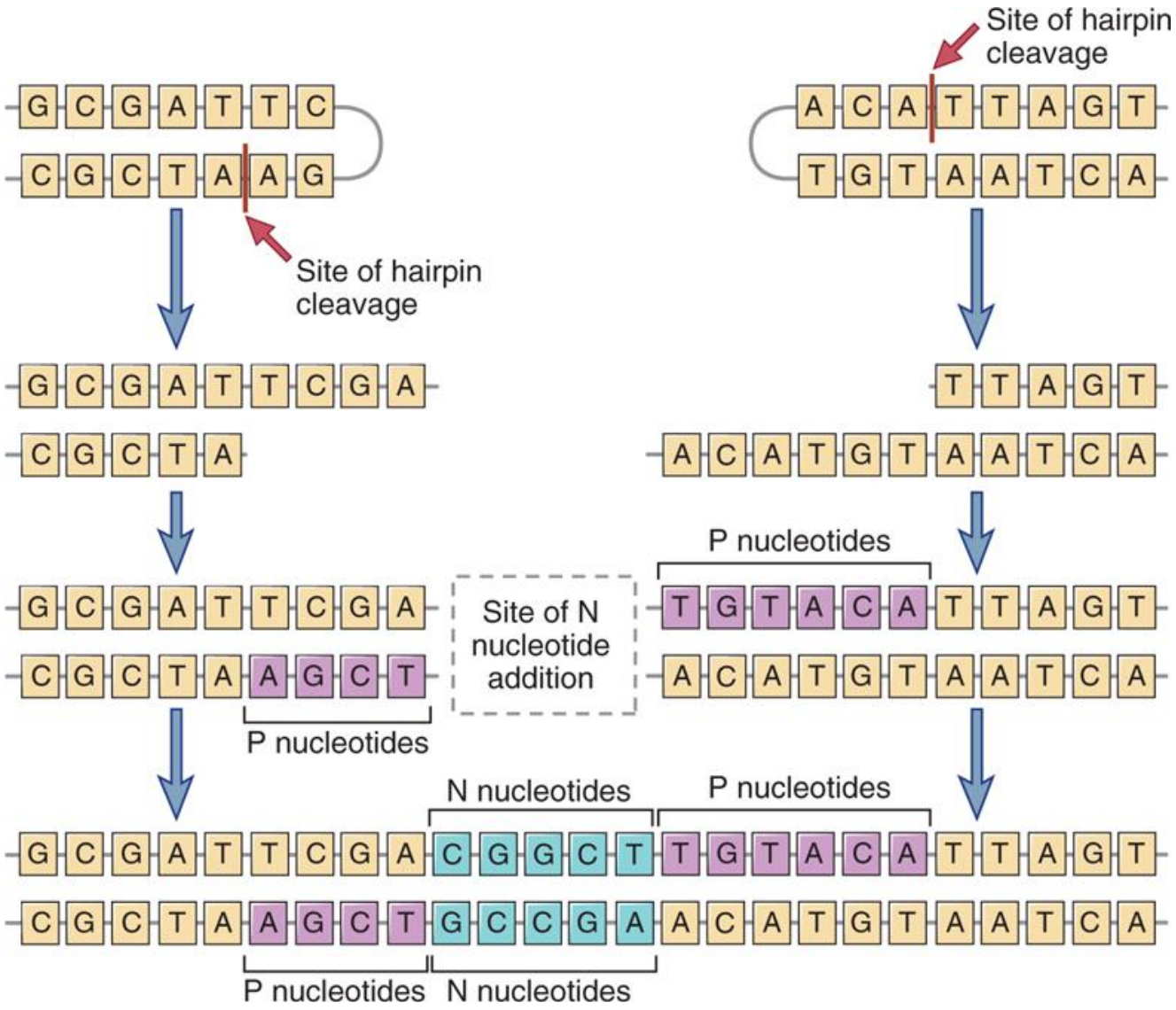
1. ***Synapsis****:* Portions of the chromosome are made accessible to the recombination machinery. Two selected coding segments and their adjacent RSSs are brought together by a chromosomal looping event and held in position for subsequent cleavage, processing, and joining.

2. ***Cleavage****:* Double-stranded breaks are enzymatically generated at RSS-coding sequence junctions by recombination via the Rag-1/Rag-2 complex. *Rag* genes are lymphoid specific and are expressed only in developing B and T cells.

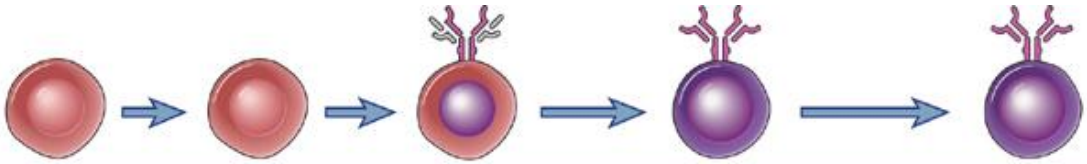
3. ***Hairpin opening and end-processing****:* The broken coding ends (but not the signal/RSS ends) are modified by the addition or removal of bases, and thus greater diversity is generated. *Artemis* is an endonuclease that opens up the hairpins at the coding ends. A lymphoid-specific enzyme, called terminal deoxynucleotidyl transferase (TdT), adds bases to broken DNA ends.

4. ***Joining****:* The broken coding ends as well as the signal ends are brought together and ligated by a doublestranded break repair process found in all cells that is called nonhomologous end joining.

Creation of junctional diversity

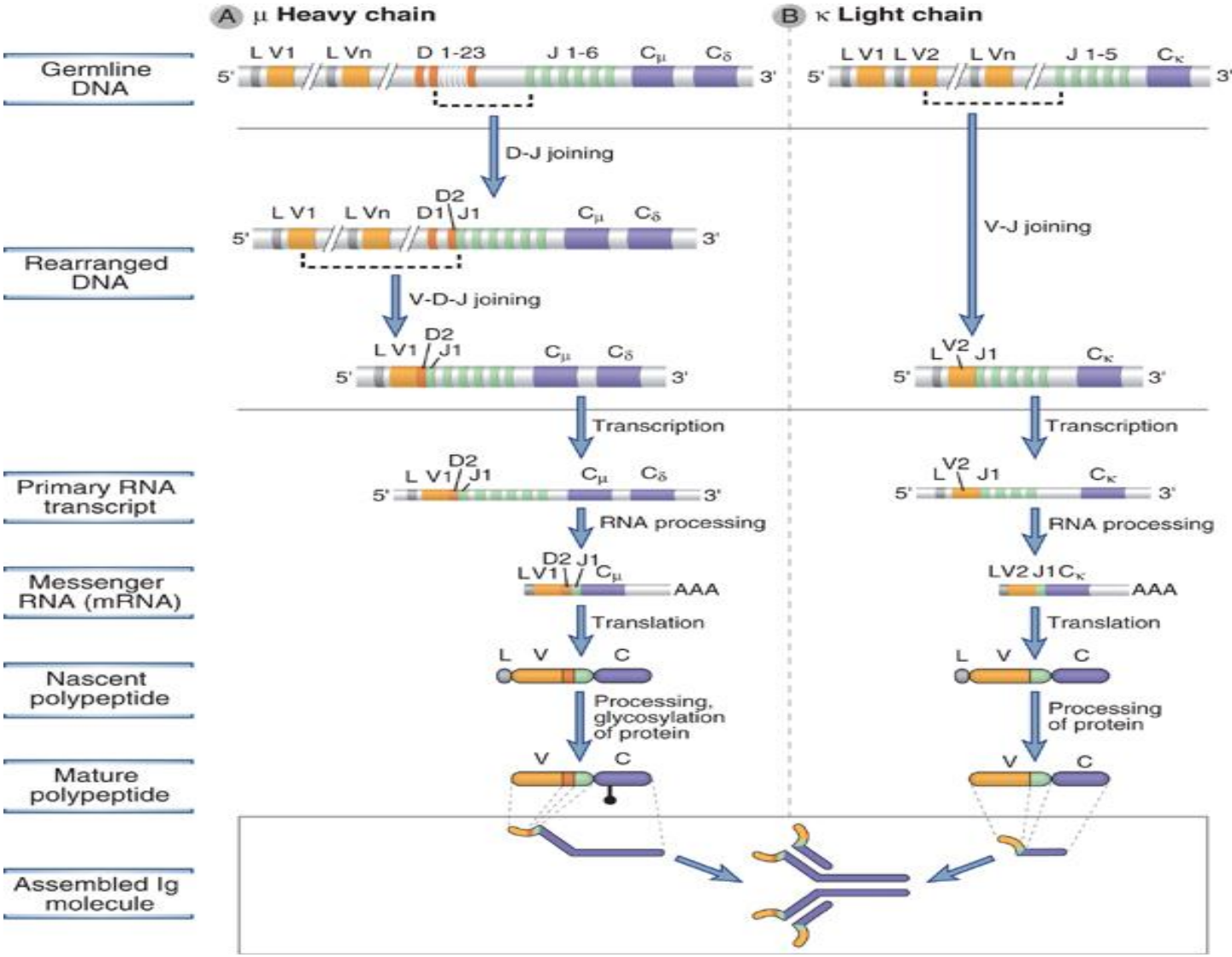


Stages of B cell maturation

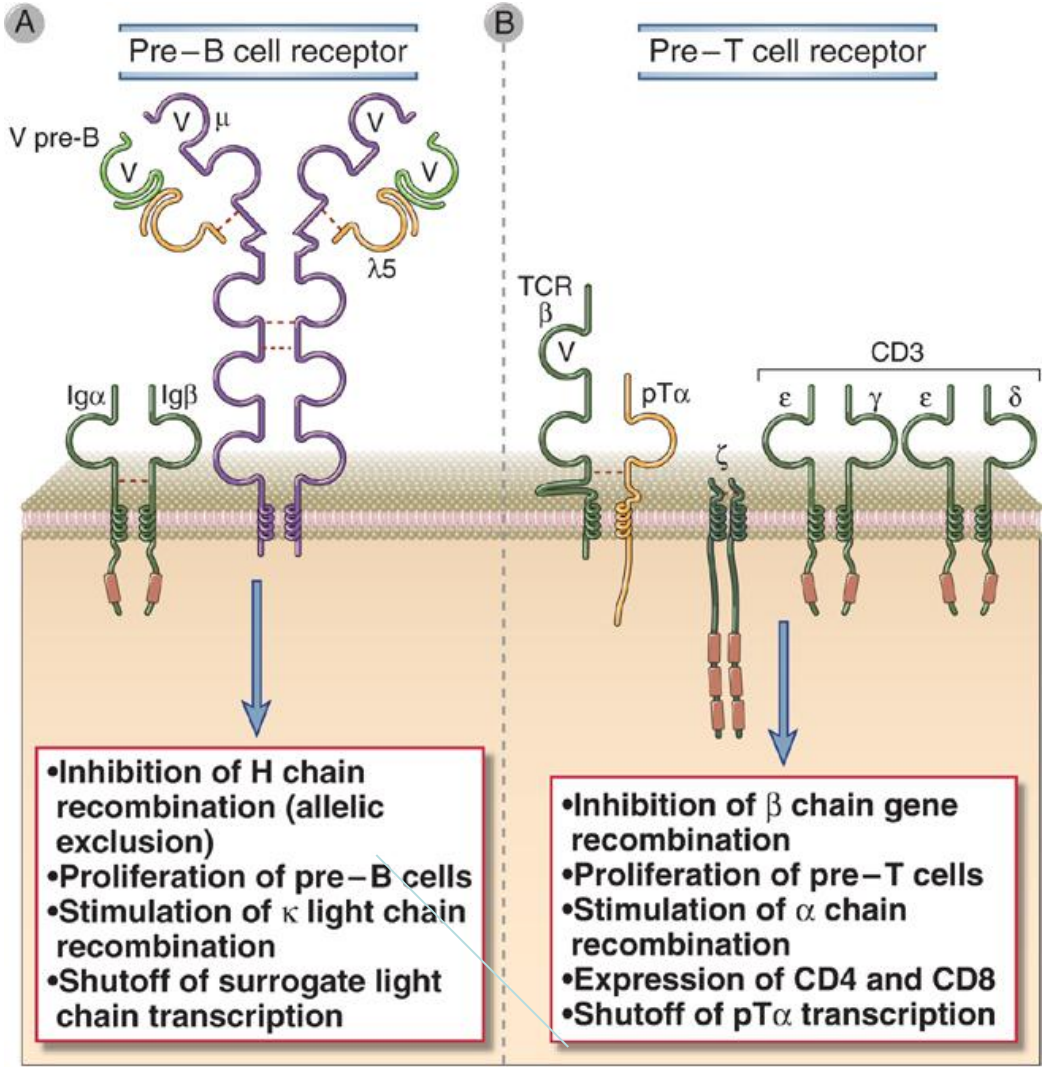


Stage of maturation	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Proliferation					
RAG expression					
TdT expression					
Ig DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene (VDJ), κ or λ genes (VJ); μ or κ or λ mRNA	Alternative splicing of VDJ-C RNA (primary transcript), to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor–associated μ	Membrane IgM (μ+ κ or λ light chain)	Membrane IgM and IgD
Surface markers	$CD43^+$	$CD43^+$ $CD19^+$ $CD10^+$	$B220^{lo}$ $CD43^+$	IgM^{lo} $CD43^-$	IgM^{hi}
Anatomic site	Bone marrow			Periphery	
Response to antigen	None	None	None	Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)

Ig H and L chain recombination and expression



Pre-B cell and pre-T cell receptors



Pre-BcR

μchain+surrogate L chains (VpreB, Vλ5)

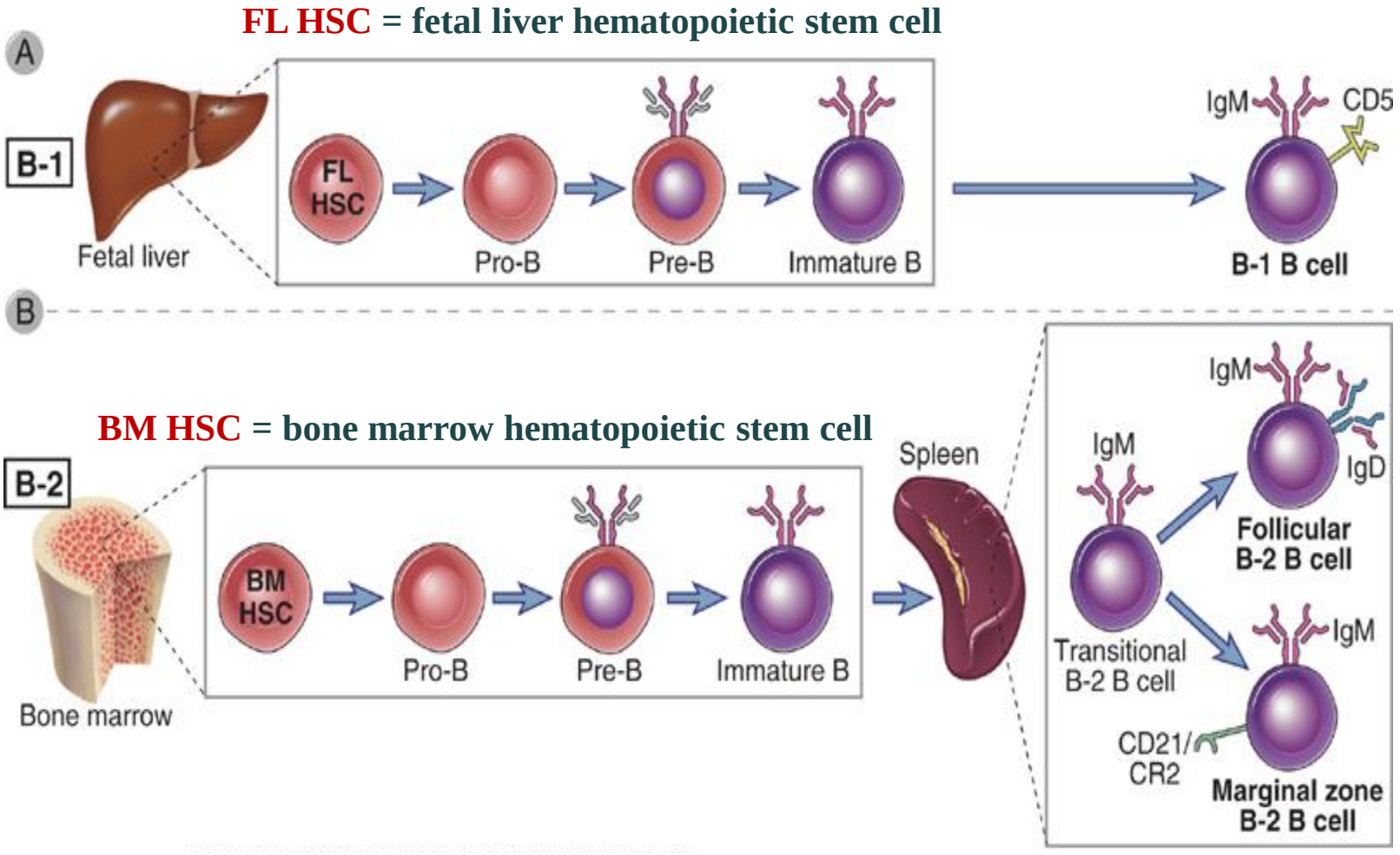
Pre-TcR

ßchain+preTα

First checkpoints for

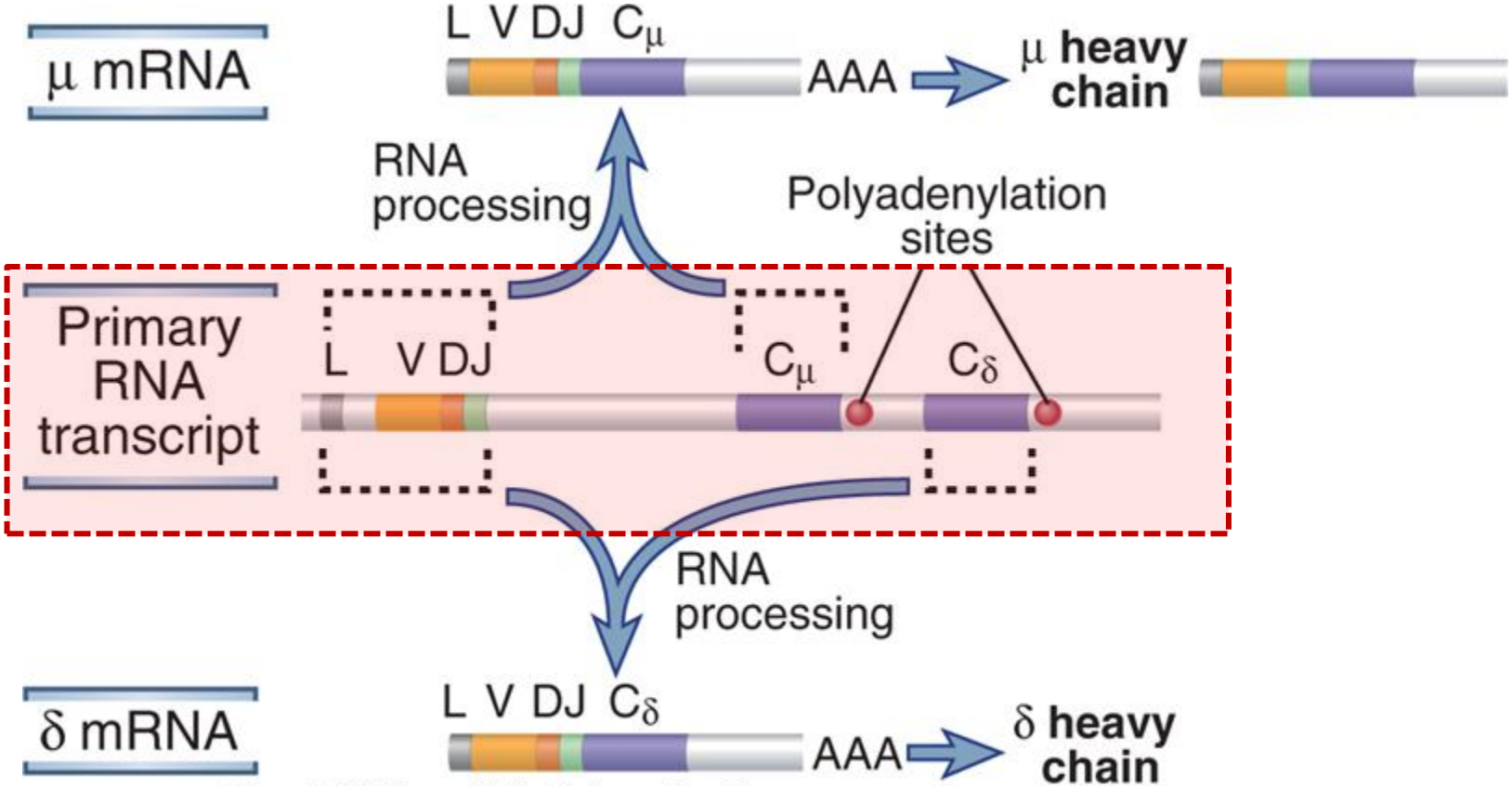
„allelic exclusion“

B lymphocyte subsets (B1 – B2 B cells)

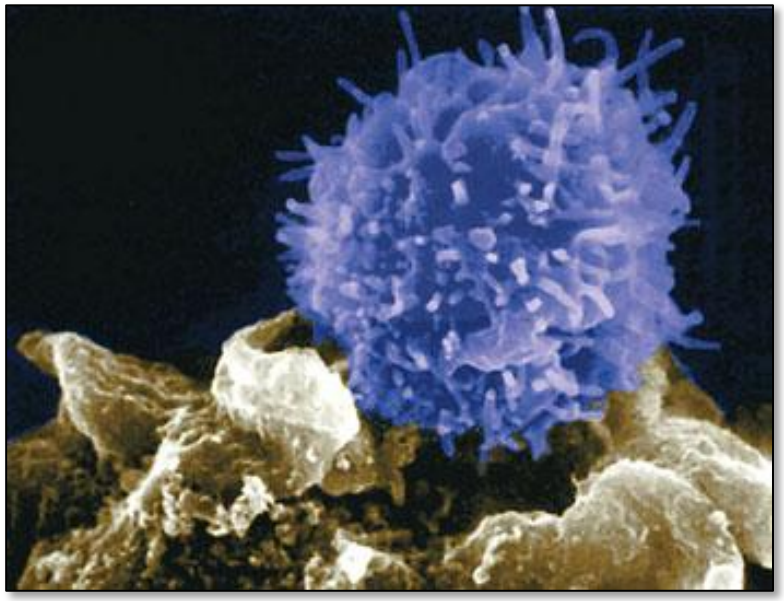


Coexpression of IgM and IgD

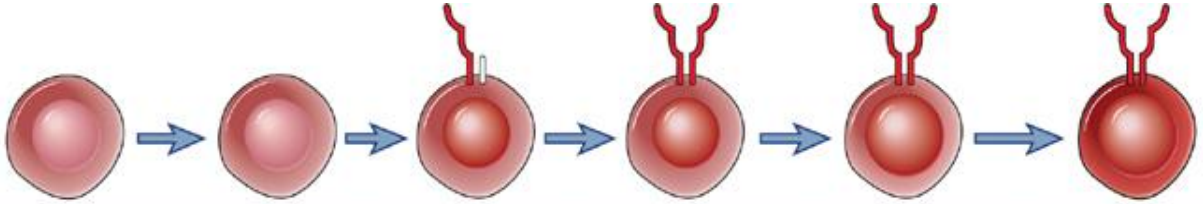
Alternative RNA splicing (of introns)



T cell maturation

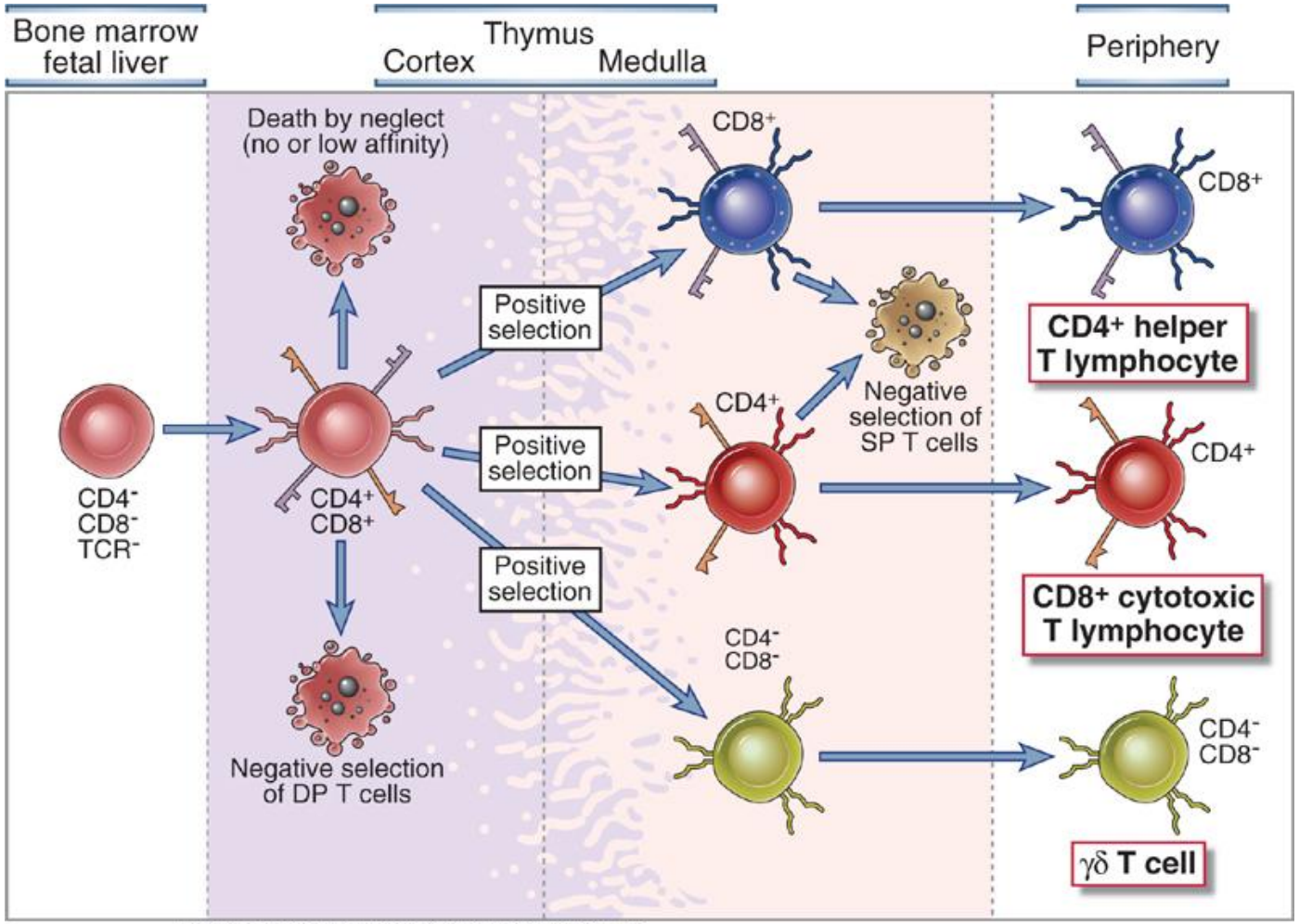


Stages of T cell maturation

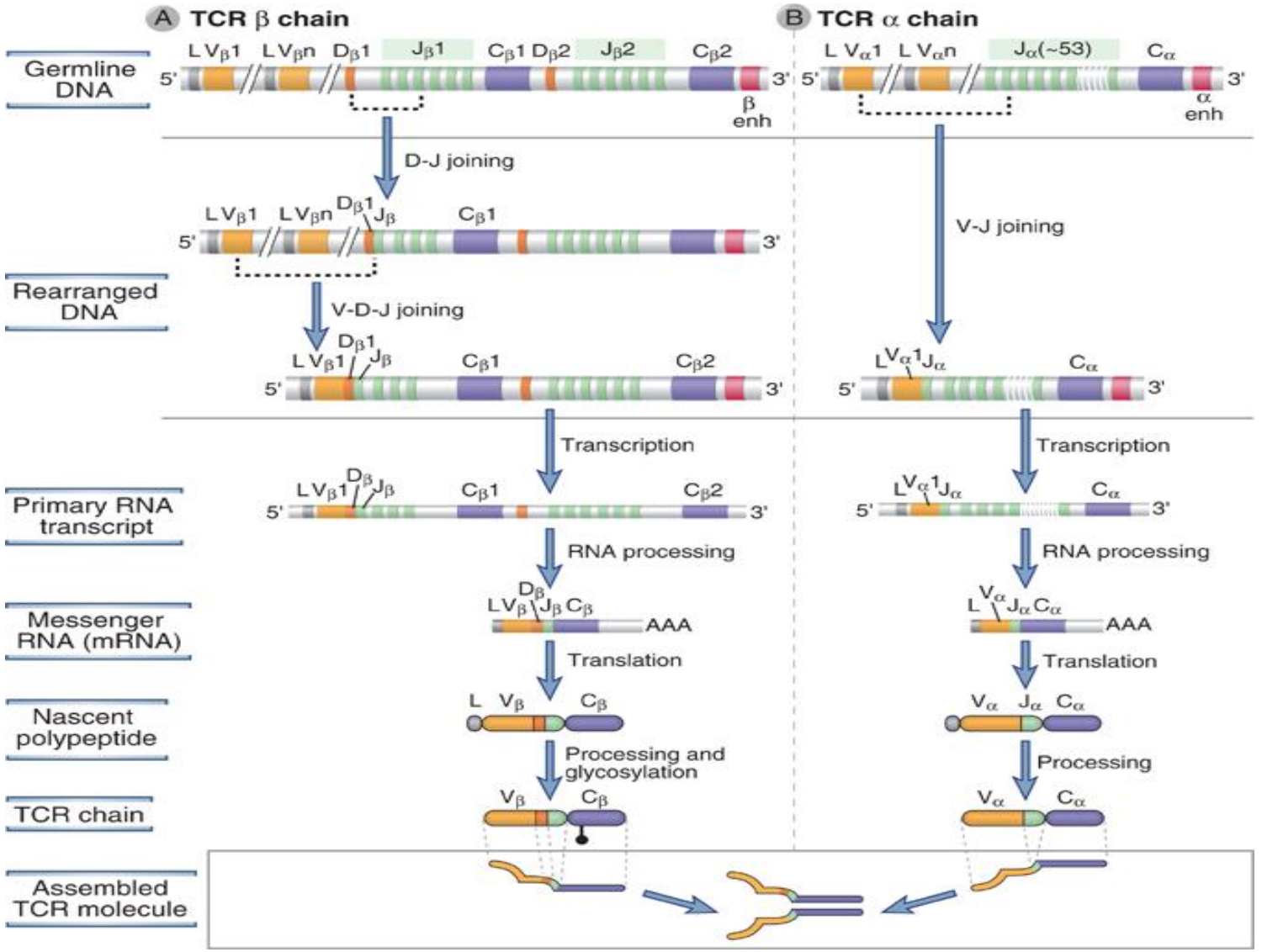


Stage of maturation	Stem cell	Pro-T	Pre-T	Double positive	Single positive (immature T cell)	Naive mature T cell
Proliferation						
RAG expression						
TdT expression						
TCR DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined β chain gene [V(D)J-C]; β chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA
TCR expression	None	None	Pre-T receptor (β chain/pre-T α)	Membrane αβ TCR	Membrane αβ TCR	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}	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)

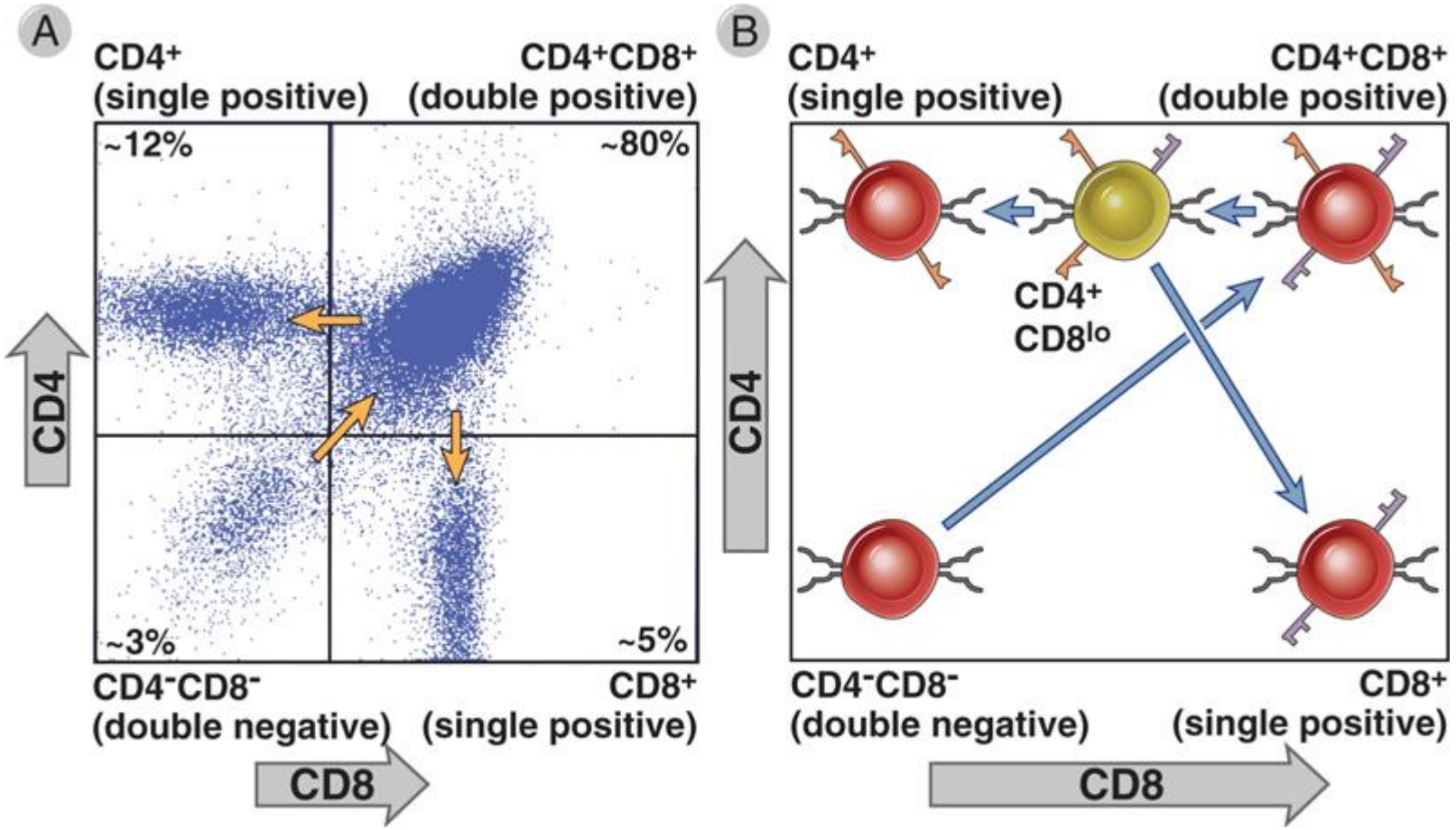
Maturation of T cells in the thymus



TCR α and β chain gene recombination and expression



CD4/CD8 expression and selection



Positive/negative selection in the thymus

Positive selection

the process in which thymocytes with low avidity TCRs to peptide–self MHC complexes are stimulated to survive

Negative selection

Thymocytes whose receptors recognize peptide-MHC complexes in the thymus with high avidity undergo apoptosis or differentiate into regulatory T cells

„Removal“ of self-reactivity in thymus = central tolerance

More aspects of central tolerance induction

AIRE ***(autoimmune regulator)***

induces tissue-specific genes in the thymus

How divers does the TcR repertoir need to be ?

(or: how to balance between non-responder and autoimmunity)

Other cells passing thymic education

γδ T cells

- ***First come – first serve***

  (if rearrangement of γ and δ is successful, the result will be a γδ TcR)

- ***Diversity is theoretically high, practically low***

NKT cells

- ***Are not MHC restricted***
- ***Do not recognize peptide antigens***
- ***Express a NK-like surface marker***
- ***The TcR of NKT cells recognizes lipid antigens on CD1***
- ***Many NKT have an "invariant" TcR***


SCENE
TAKE
2
DAY