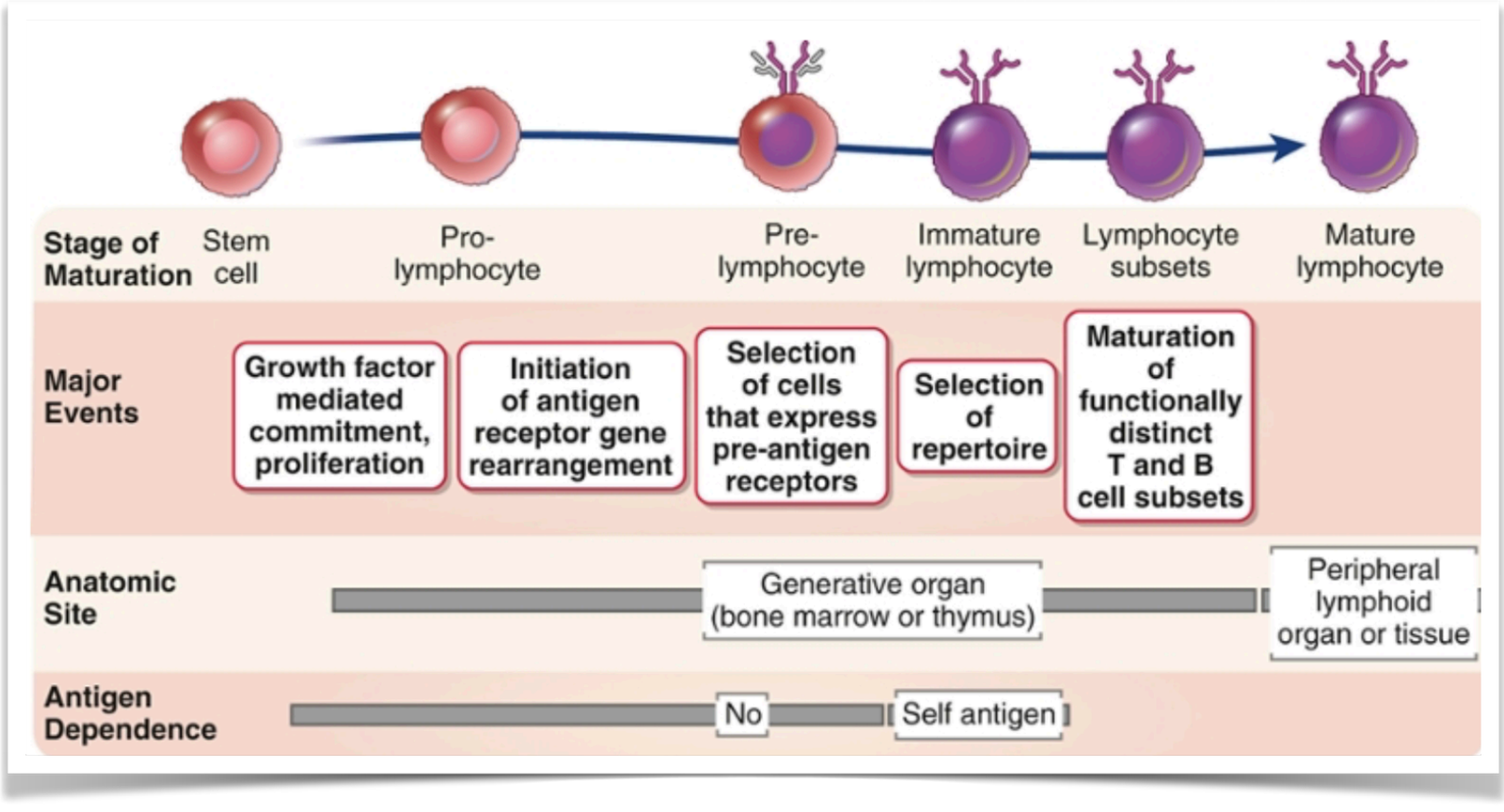
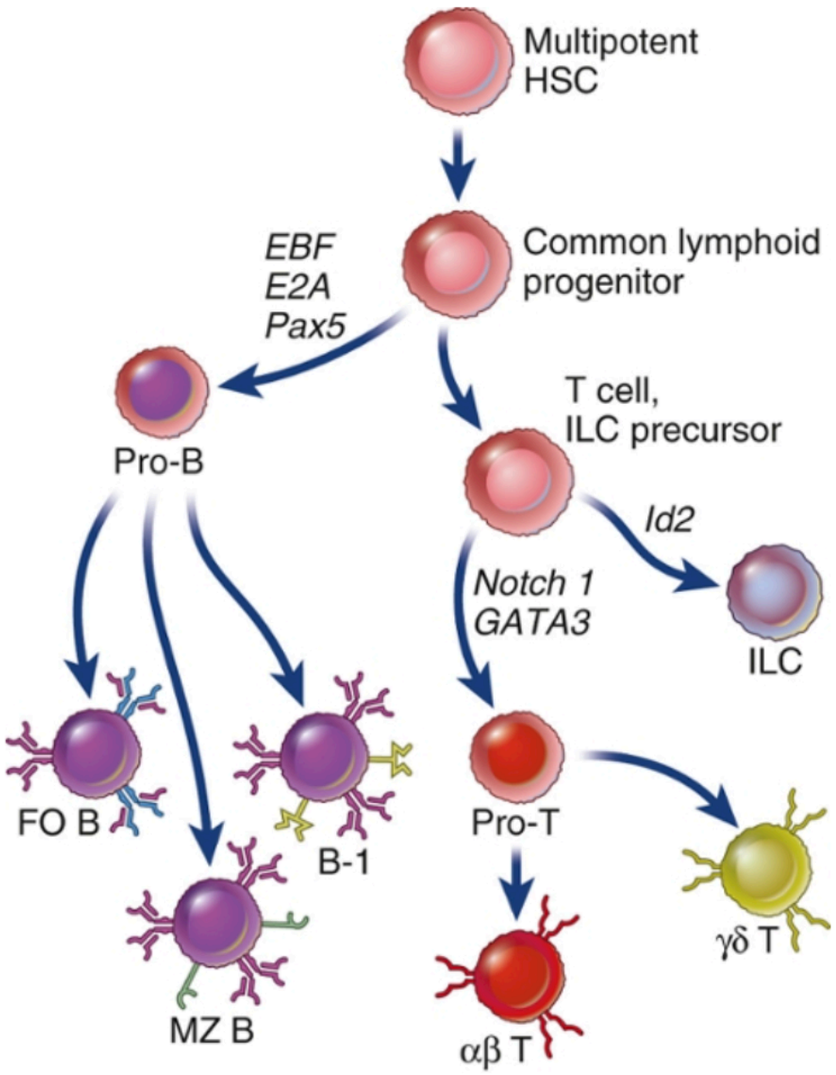
Lymphocyte signalling - diversity and basics of signal transduction

Series of events involved in T and B lymphocyte maturation

1. **Commitment** of progenitor cells to *B or T lineage*
2. **Proliferation** of progenitors and immature committed cells at early stages of development providing a *large pool* of cells that can generate useful lymphocytes
3. **Sequential and ordered rearrangements** (recombination) of antigen receptor genes allowing the expression of *receptor proteins*
4. **Selection** that preserves cells that have produced *functional* antigen receptor proteins and *eliminates potentially dangerous* cells that strongly recognise self antigens (checkpoints ensuring functional receptor with useful specificities)
5. **Differentiation** into functionally and phenotypically distinct *subpopulations*



Multipotent stem cells give rise to distinct B and T lineages



Hematopoietic stem cells (HSC) in fetal liver and bone marrow give rise to all lineages of blood cells, including lymphocytes.

Signals from surface receptors induce *transcriptional regulators* that drive the development of progenitors toward either B or T cells.

NB: **X-linked severe combined immunodeficiency** disease is characterised *impaired T and NK* cell development, caused by a mutation in the gene for common y chain, shared by the receptors for *several cytokines*, including IL-2, IL-7 and IL-15

Antigen receptor gene rearrangement and expression

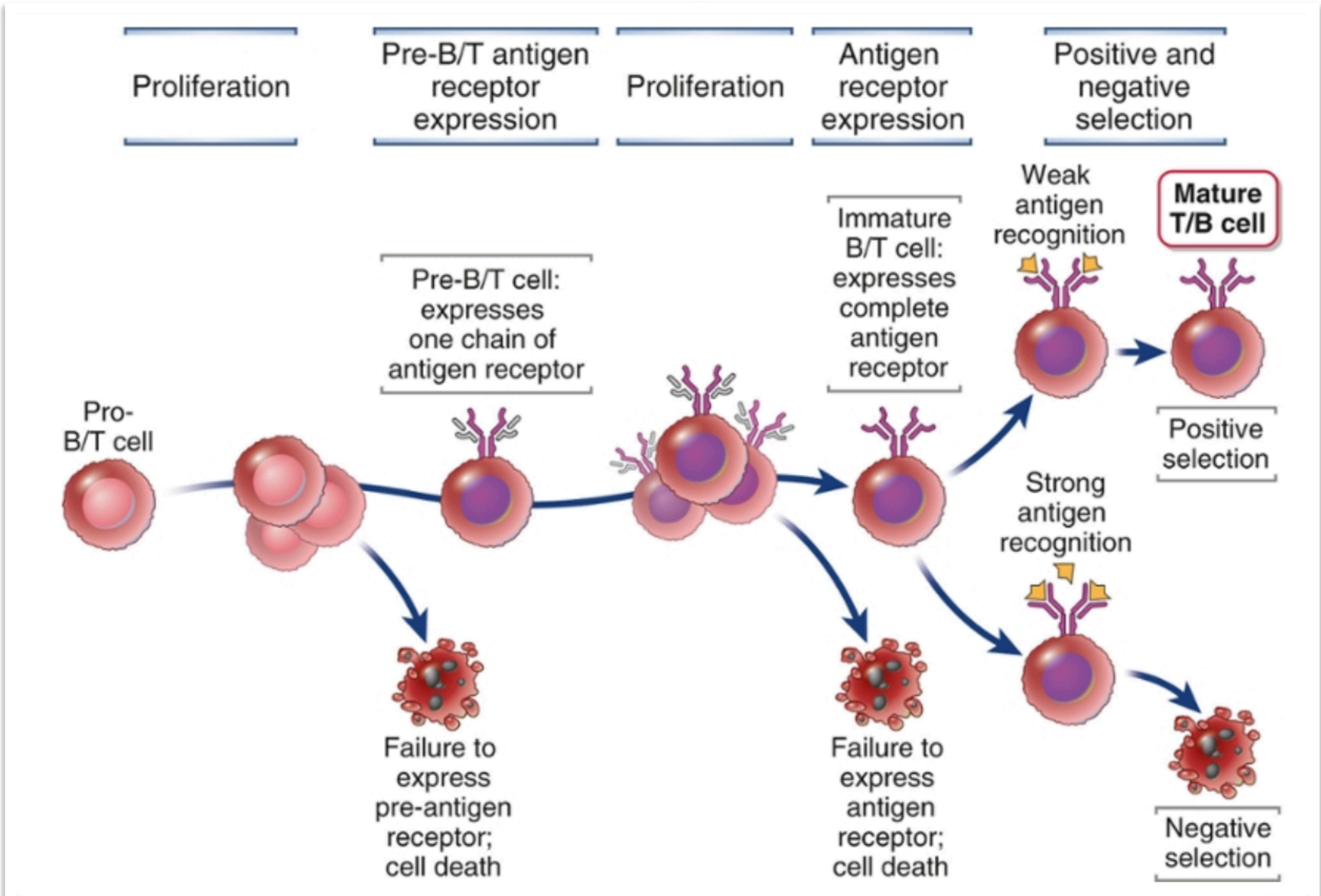
- *Essential* in lymphocyte development
- Responsible for the generation of a *diverse* adaptive immune repertoire: fairly small number of genes can give rise to a vast number of distinct Ig and TCR molecules, each binding to a *different antigen*
- Produced in *immature* B cells (**bone marrow**) and *immature* T cells (**thymus**) via random *recombination* and nucleotide sequences variation introduction
- Independent of the presence of antigens (occurs *before encounter*)

Selection processes shape B and T lymphocyte repertoires

Checkpoints: test developing cells to ensure that only *functional* lymphocytes are selected to mature and that potentially harmful *self-reacting cells are eliminated*

1. **Pre-antigen receptor expression**
   Assembled *pre-BCR* (successfully rearranged Ig heavy chain expressing μ heavy chain protein) and *pre-TCR* (productive b chain gene rearrangement) complexes provide *signals for survival*, proliferation for *allelic exclusion*
2. **Complete antigen receptor expression**
   Rearrangement and expression of the *second chain* of BCR/TCR in immature cells and selection for survival based on *receptor recognition* (cells that strongly recognise self structures are eliminated or induced to change their receptor)
3. **Positive selection:** linked to generation of lymphocyte subsets
   - T cells: maturation of cells recognising *self MHC* molecules and matched coreceptor to MHC type
   - B cells: selection of receptor expressing cells coupled to the generation different subsets
4. **Negative selection:** eliminates/alters lymphocytes whose receptors bind strongly to self antigen ➔ important mechanism maintaining central *tolerance*
   - T cells: elimination by *apoptosis* (clonal deletion)
   - B cells: make further rearrangement (*receptor editing*) otherwise deleted

Selection processes shape B and T lymphocyte repertoires



Rearrangement of antigen receptor genes

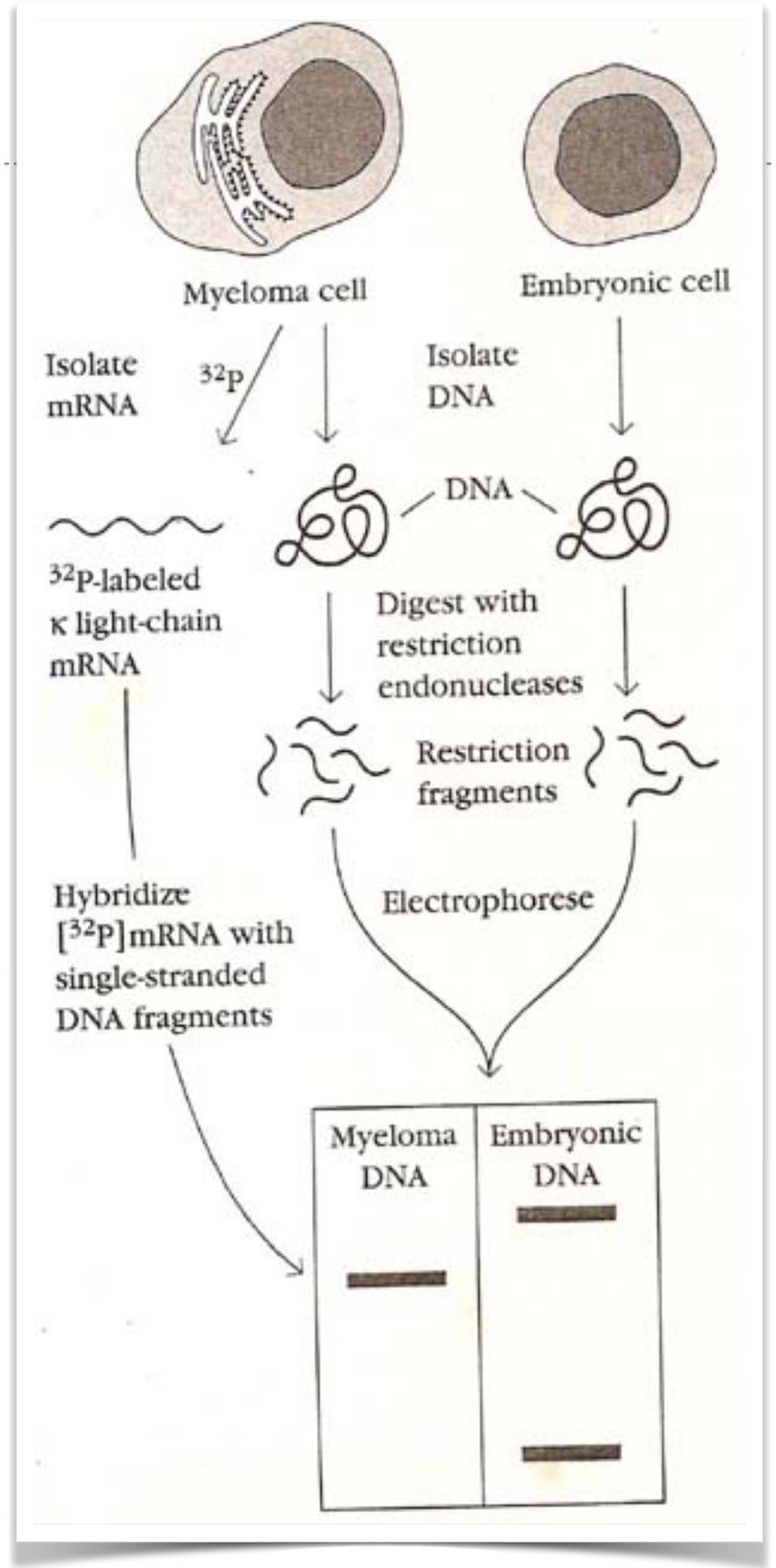
V(D)J recombination: rearrangement of different *variable (V)*, with *diversity (D)* and *joining (J)* gene segments

Germline Ig and TCR genes are separated within inherited loci and brought together and joined by *random combination* only in developing lymphocytes (not in other tissues or cell types)

Rearrangement of Antigen Receptor Genes

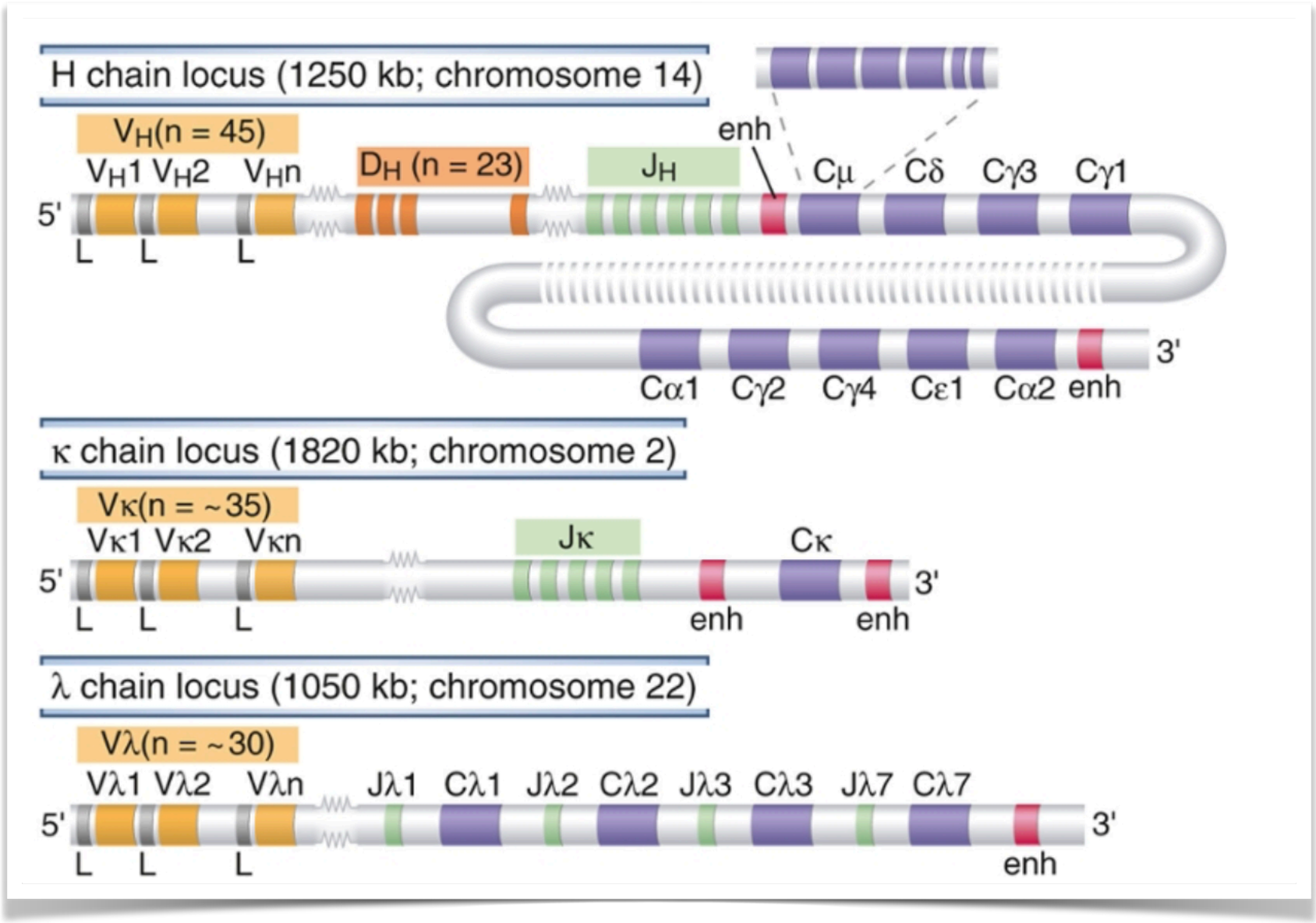
Somatic recombination

The genes that encode the AG receptors on lymphocytes derive from the somatic rearrangement of individual gene segments

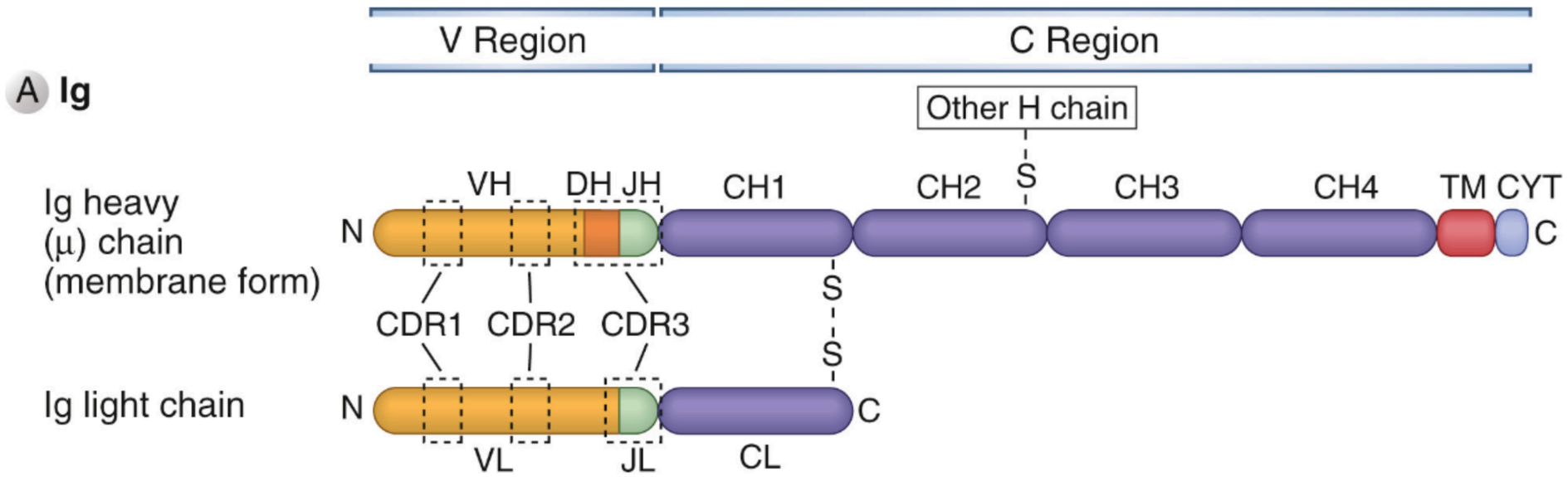


Organization of Immunoglobulin Gene Loci

Three separate loci: heavy chains, κ and λ light chains, each on a different chromosome



A rearranged Ig locus



Organization of Immunoglobulin Gene Loci

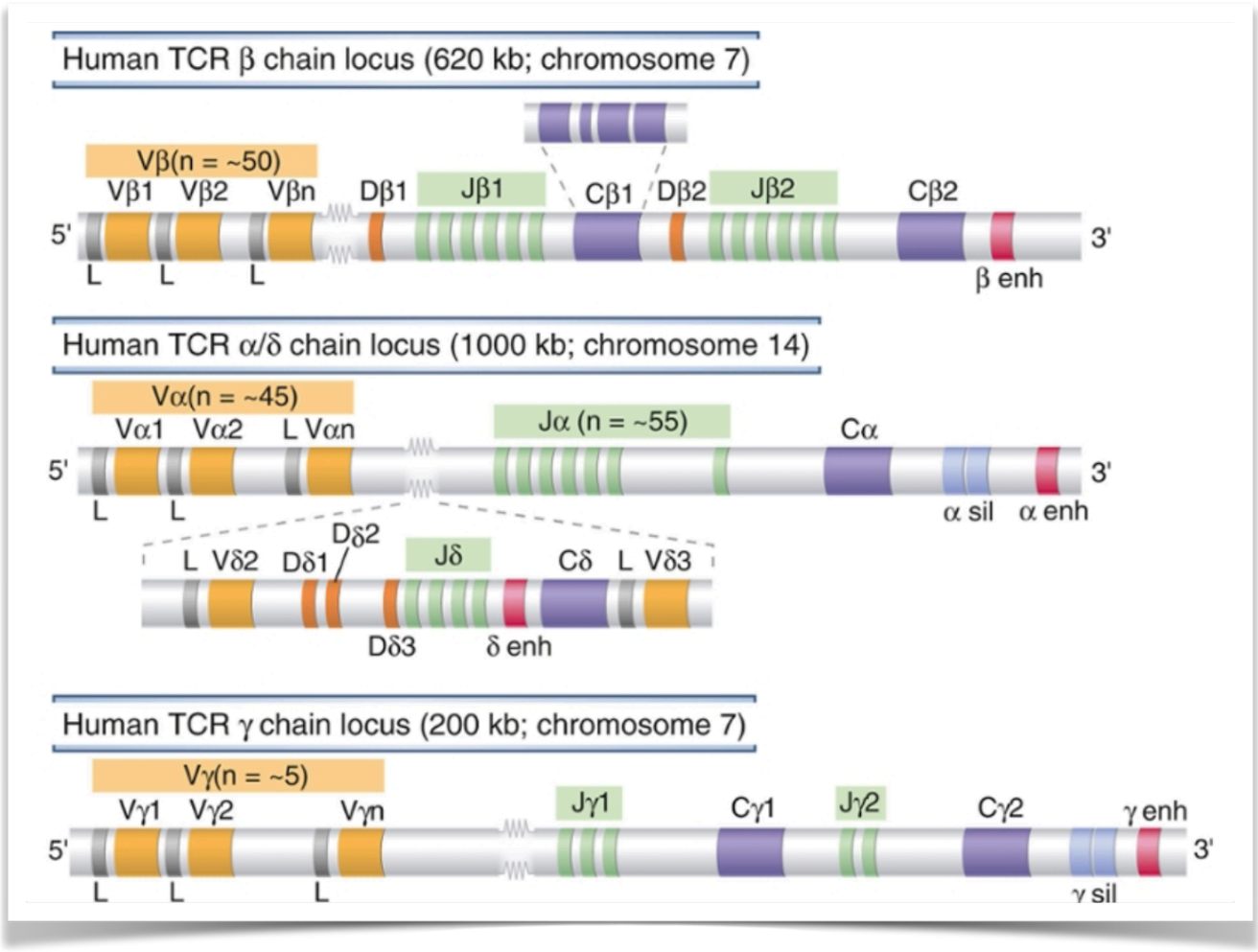
V, J, and D (if present) segments are brought together to create the coding sequence for the **variable domain of antibody chains:**

- V genes segments: spaced over large stretches of DNA, contain a *5' leader exon* important to make the leading peptide signal and guide the nascent polypeptide during translation
- Diversity segments: absent on Ig light chain loci
- Junctional sequences between rearranged V and D, V and J and J itself make up the third *hypervariable regions* (light)

Complete chains contains V domain (rearranged VJ or VDJ) **fused to C domain**:

- Constant (C) regions: different numbers of genes in random array, encode different Ig *isotypes* and subtypes
- Mediated by *RNA-splicing* of the rearranged gene transcript and *noncoding sequences* play an important role in recombination and gene expression (promoters, cis-acting regulatory elements, enhancers etc)

Organization of T cell receptor gene loci

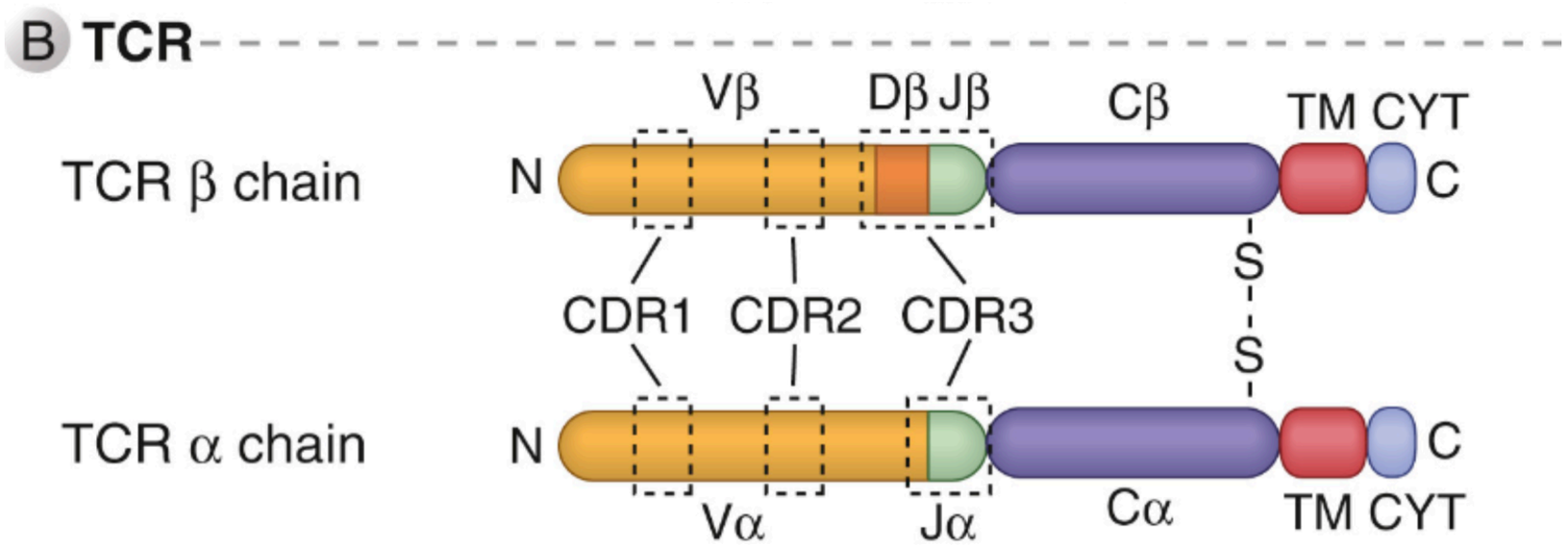


➔ Similar to Ig loci:

Several V segments, D segments and cluster of J segments, upstream of C region genes

γ and δ loci overall have *fewer segments* than classical α and β

A rearranged TCR locus



- V domains: contain the CDR1, CDR2 and CDR3
- C region of each TCR: composed of 4 exons containing the extracellular region Ig domain, short hinge and cytoplasmic tail

V(D)J rearrangement

Functional antigen receptor genes are only generated in developing lymphocytes.

Steps needed to generate a function AR gene:

1) Opening of chromatin

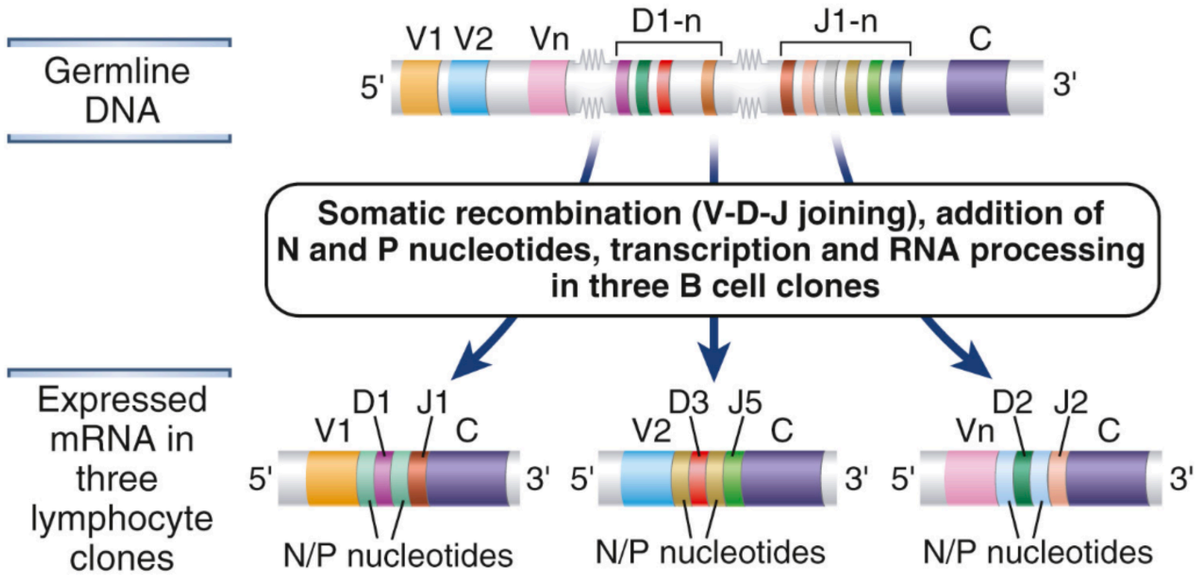
2) Gene segments are brought together

3) DS DNA break

4) Addition/deletion of nucleotides at the break

5) Ligation

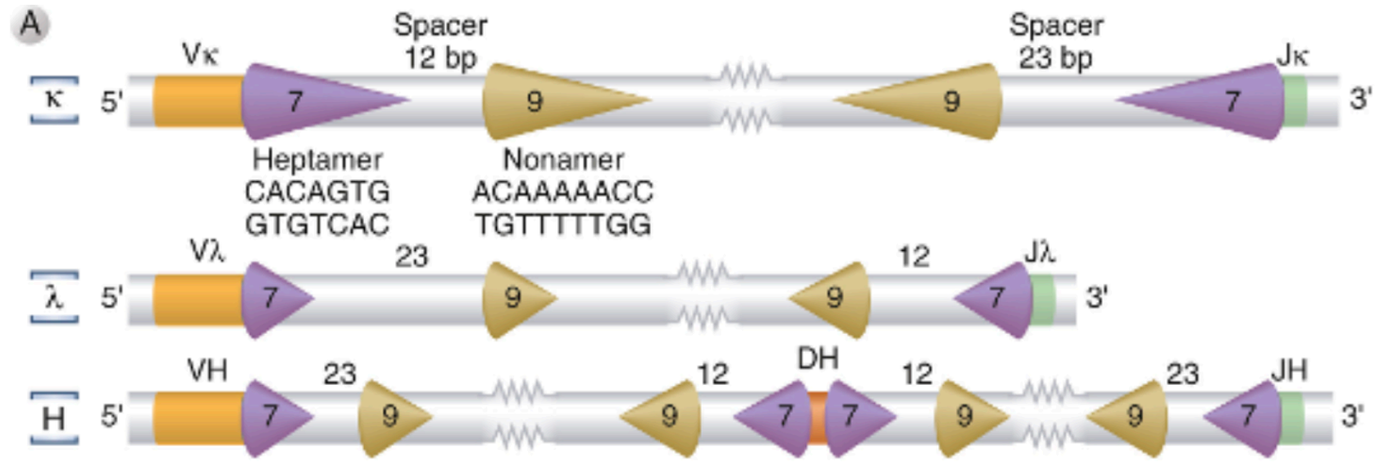
6) RNA splicing



Tremendous diversity arising from:

- Different *combination*
- addition/removal *nucleotides* at junctions

Recognition Signals That Drive V(D)J Recombination



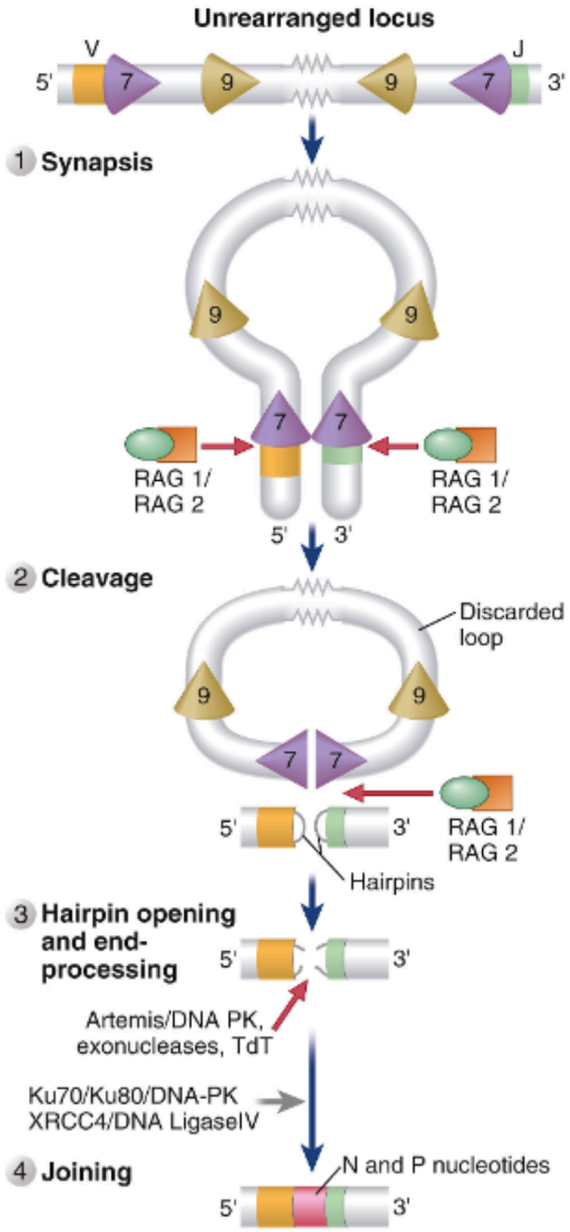
DNA rearrangements are guided by adjacent sequences, called RSS = recombination signal sequences

An RSS consists of

- a conserved block of 7 nucleotides
- a spacer, not conserved - vary in length (12 bp **or** 23 bp)
- a conserved blocked of 9 nucleotides

Recombination occurs in between gene segments located *on the same chromosome*

Mechanism of V(D)J recombination



Enzymes involved in recombination

- **V(D)J recombinase** = RAG1/RAG2 heterodimeric complex that orchestrates the V(D)J recombination
- **RAG-1**, **RAG-2** = *lymphocyte-specific* enzymes (only expressed in **developing** lymphocytes)
- **Ku70/80**, **DNA-PK** (DNA-dependent protein kinase), **Artemis** (nuclease), **DNA ligase IV**, **XRCC4**, **DNA pol μ** = *DNA-modifying* enzymes

V(D)J recombination

1. **Synapsis:** portions of chromosomes made accessible to recombination machinery
   - only RSSs located in *open* euchromatin in *specific cell type* posed to recombination enzyme
   - gene segments undergoing recombination acquire additional *histone marks* to facilitate enzymes recruitment

2. **Cleavage:** *double stranded breaks* generated at RSS coding sequence junctions by lymphoid specific machinery ➔ RAG1 and RAG2 proteins encoded by lymphoid-specific genes form the *recombinase complex*

3. **Hairpin opening and end processing:** nucleotides may be *added or removed* from coding ends to create even greater diversification ➔ Artemis endonuclease opens up hairpins and lymphoid-specific TdT enzyme adds nuclides to broken DNA in the context of *junctional diversity*

4. **Joining:** broken coding ends and signal ends are brought together and ligated by *nonhomologous end joining repair (NHEJ*) ➔ involves Ku70 and Ku80 DNA end-binding proteins, recruitment of DNA-PK repair enzyme and DNA ligase

Generation of diversity in B and T cells

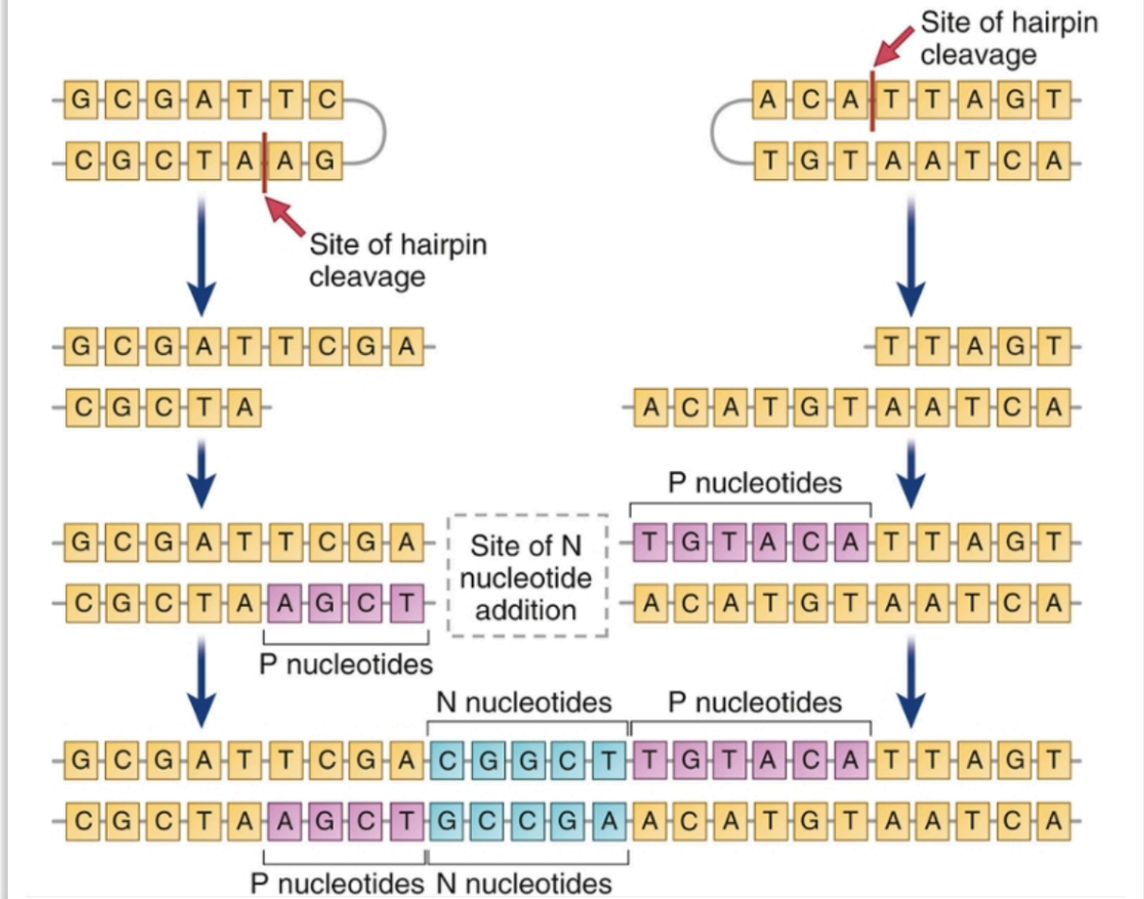
- Combinatorial diversity: union of *different gene segments* produces different receptors
  - Number of possibilities is the product of the number of gene segments at each receptor locus (number of germline gene segments reflects diversity)
  - Enhanced after synthesis by juxtaposition of two different randomly generated V regions, but not all combinations are equally likely to occur and not all pairings form functional receptors
  - Max number of combination = 1-3 million

- Junctional diversity: largest contribution made by *removal/addition of nucleotides* at junctions of V and D, D and J, or V and J segments when joined
  - Segments cleaved by Artemis are often asymmetric and the shorter strand is extended with using the longer strand as a template for the addition of *short P nucleotides*, who introduce new sequences at junctions
  - Random addition of up to 20 non-template-encoded *N nucleotides* is mediated by terminal deoxynucleotidyl transferase (TdT)

- Greatest variability at *junctions of V and C* regions form the CDR3

Generation of diversity in B and T cells

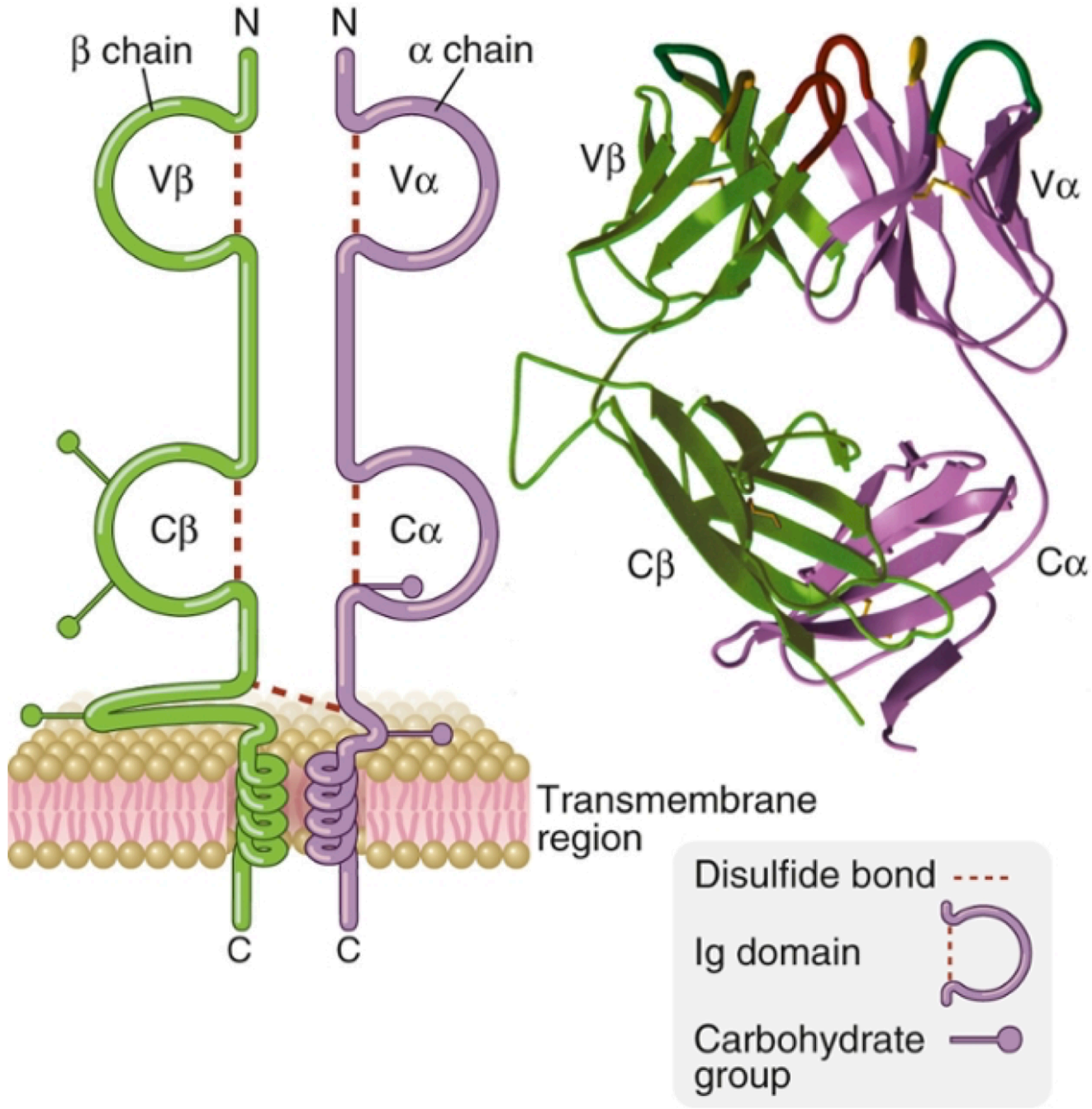
	Immunoglobulin			T Cell Receptor αβ		T Cell Receptor γδ	
Mechanism	**Heavy Chain**	**κ**	**λ**	**α**	**β**	**γ**	**δ**
Variable (V) segments	45	35	30	45	50	5	2
Diversity (D) segments	23	0	0	0	2	0	3
D segments read in all three reading frames	Rare	—		—	Often	—	Often
N region diversification	V-D, D-J	None		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	~10^{11}		—	~10^{16}		~10^{18}	



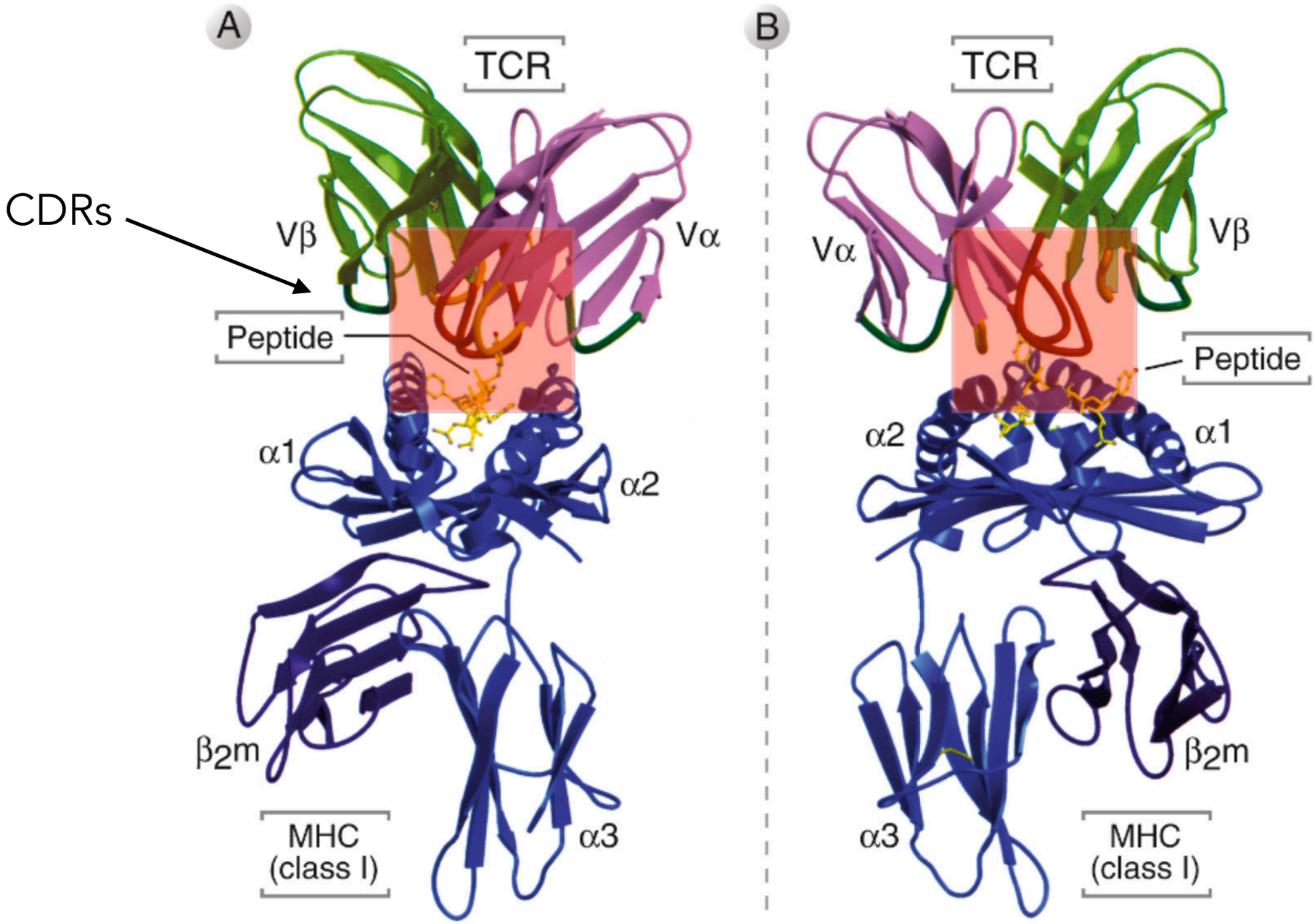
Signal transduction - basics

Structural overview of the TCR

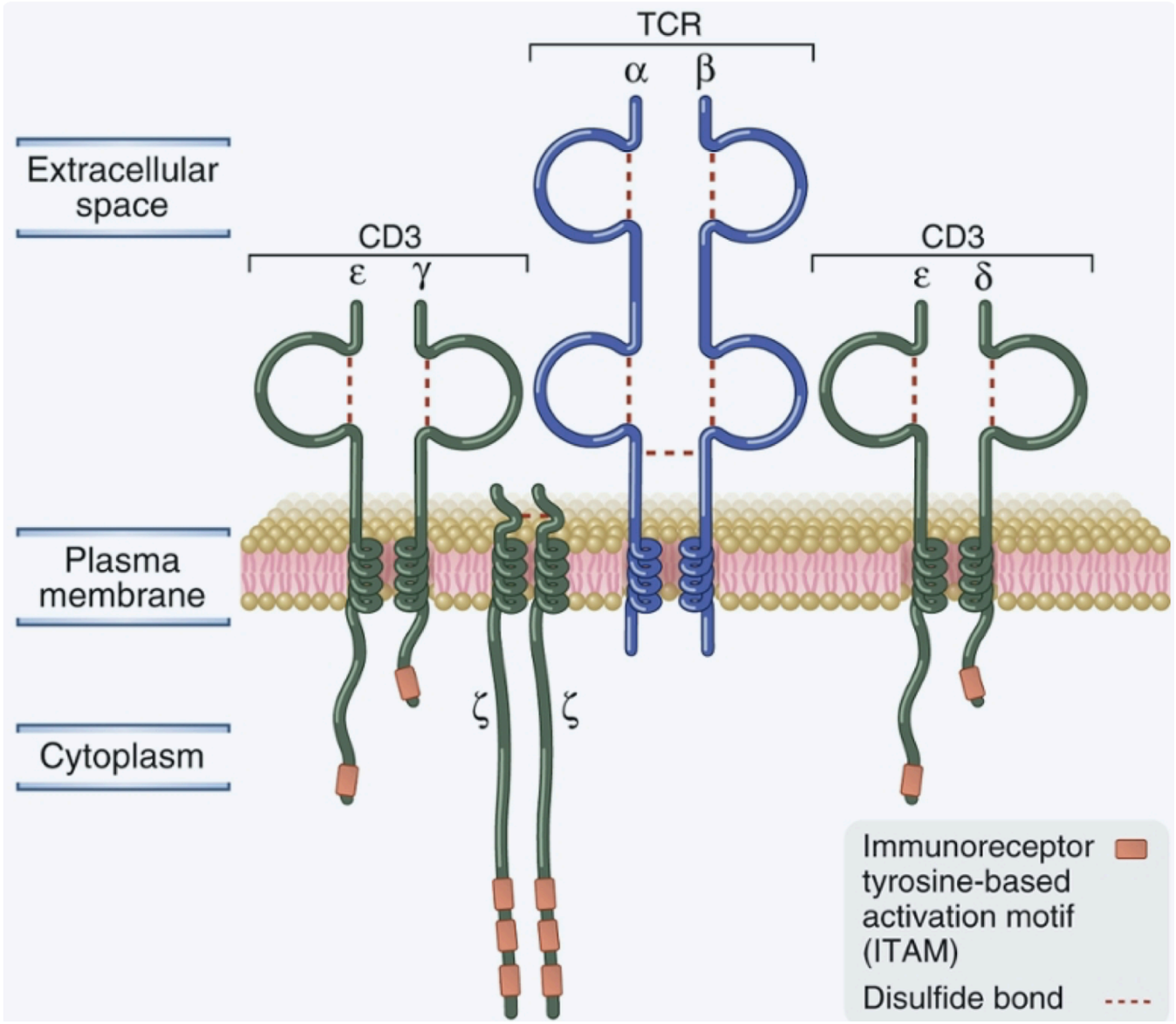
The TCR of αβ T cells is composed of a heterodimer of two transmembrane polypeptide chains (α-chain and β-chain), each of which contains one Ig-like N-terminal variable (V) domain, one Ig-like constant (C) domain, a hydrophobic region, and a short cytoplasmic tail.



Interaction between TCR and pMHC



The TCR complex

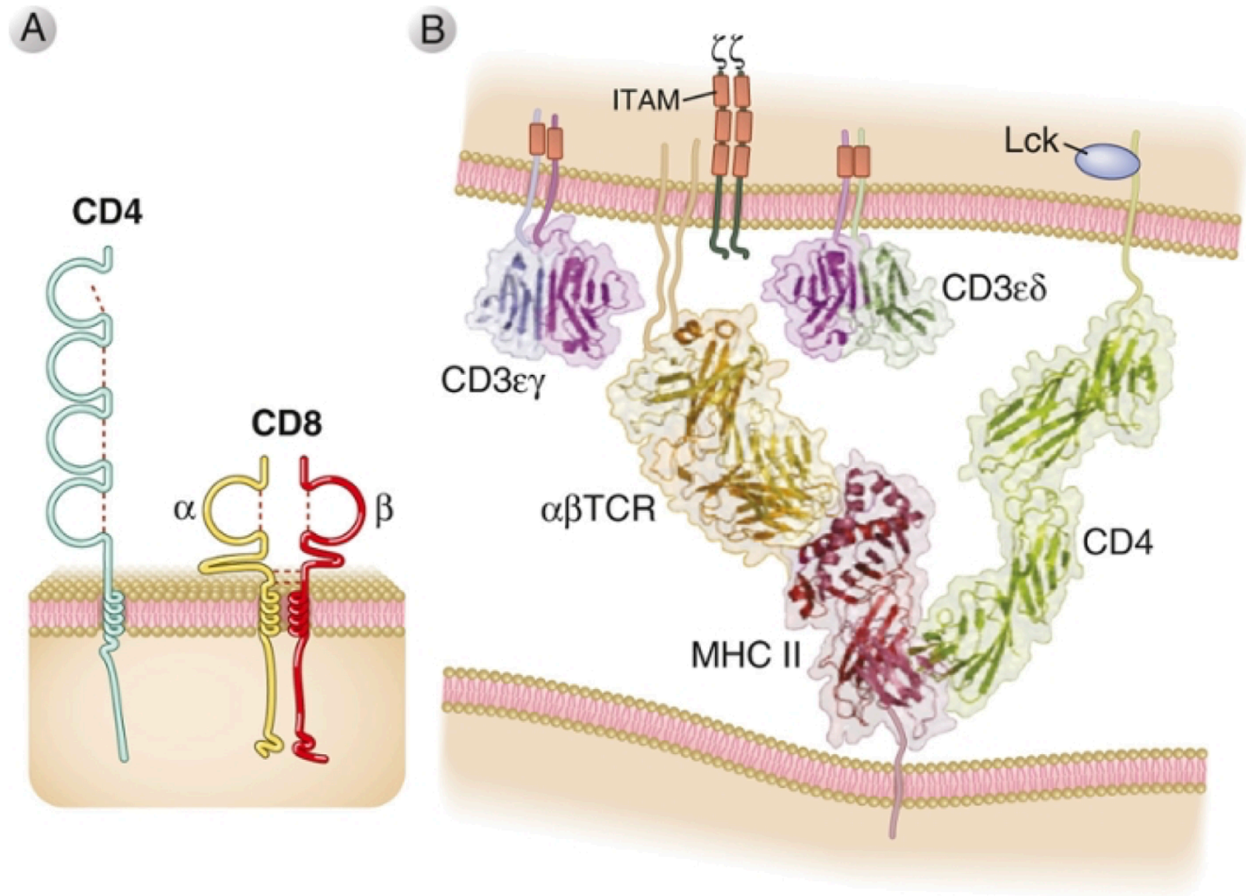


The TCR is non-covalently associated with CD3 proteins and the ζ chain through their transmembrane domains. The CD3 and the ζ chain form the "signalling" component of the TCR complex and is identical for all αβ T cells.

Engagement of the TCR by pMHC triggers clustering of the co-receptors with the TCR and, in turn, signalling through the ITAM motif.

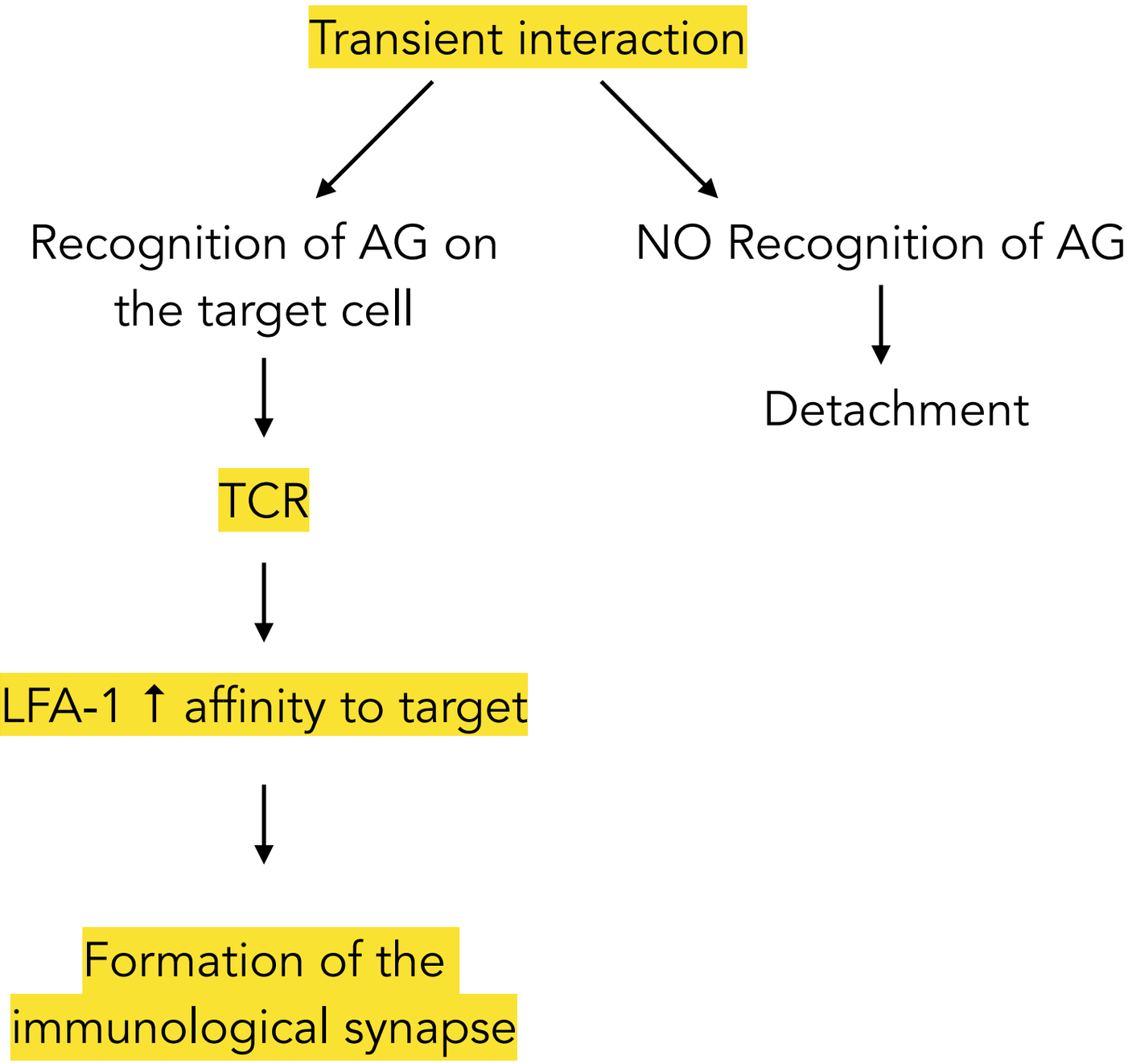
Role of the CD4 and CD8 co-receptors

- CD4 is a transmembrane protein, existing as a monomer with your extracellular Ig domains and recognizes non-polymorphic regions on MHC-II molecules.
- CD8 is a heterodimer, which binds to both constant regions within MHC-I molecules and the β2 microglobulin.
- Mature T cells express only one co-receptor.



Mechanistically, the co-receptors are constitutively associated with a signalling kinase (LCK). Upon binding to MHC molecules, the coreceptors are brought into close proximity of the TCR complex and the associated kinase can phosphorylate the ITAM regions and, thus, initiate downstream signalling.

The immunological synapse (I)



The immunological synapse (II)

Upon binding to peptide:MHC complex, TCR and co-receptor cluster at the site of cell-to-cell contact:

supramolecular activation complex (SMAC) ≈ "*the immunological synapse*"

+ LFA-1/talin cluster ➔ **tight seal**!

Two zones of SMAC:

a) *Central* zone (cSMAC): contains signaling molecules

b) *Outer* zone (pSMAC): contains LFA-1/talin

connects LFA-1 to the cytoskeleton

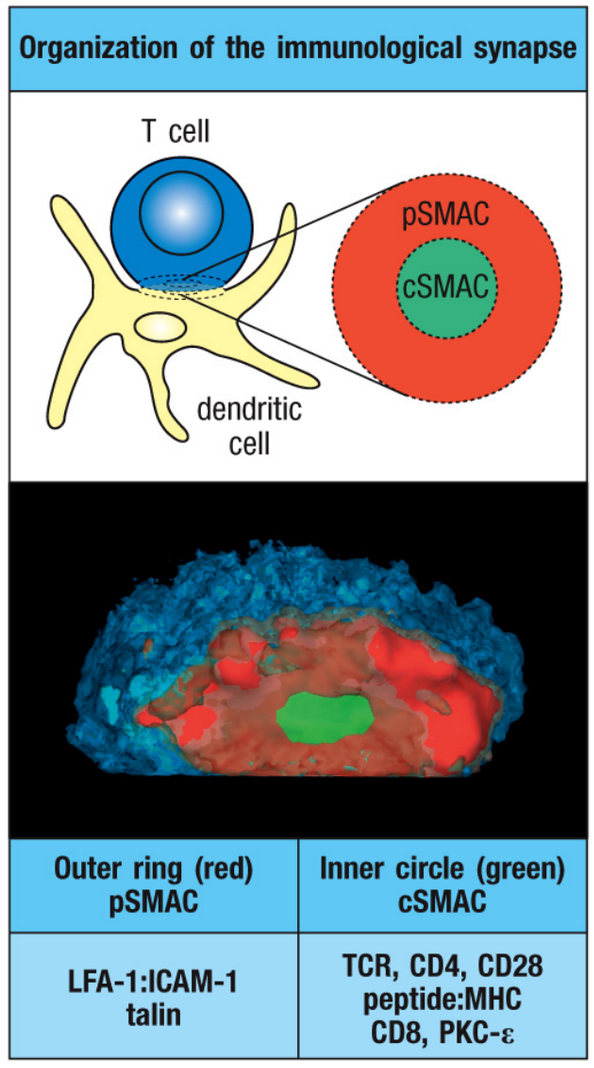


Figure 9.37 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

The immunological synapse (III)

TCR clustering triggers *reorientation* of the **cytoskeleton** + *polarization* of effector cell

➔ release of *effector molecules* at the cell-to-cell contact site!

TCR signaling leads to:

- SMAC + tight contact with target cell
- Polarization of effector cell to guide secretion of cytokines
- Trigger synthesis and release of effector molecules

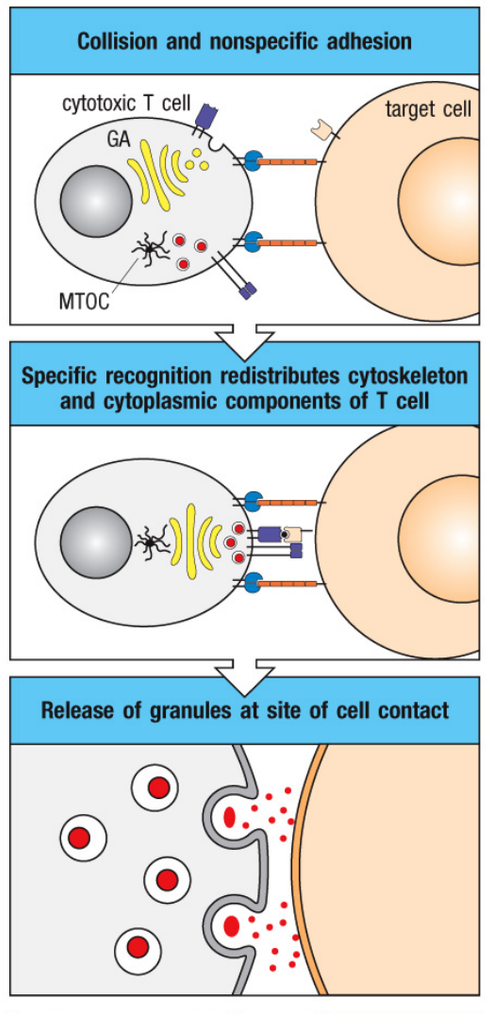
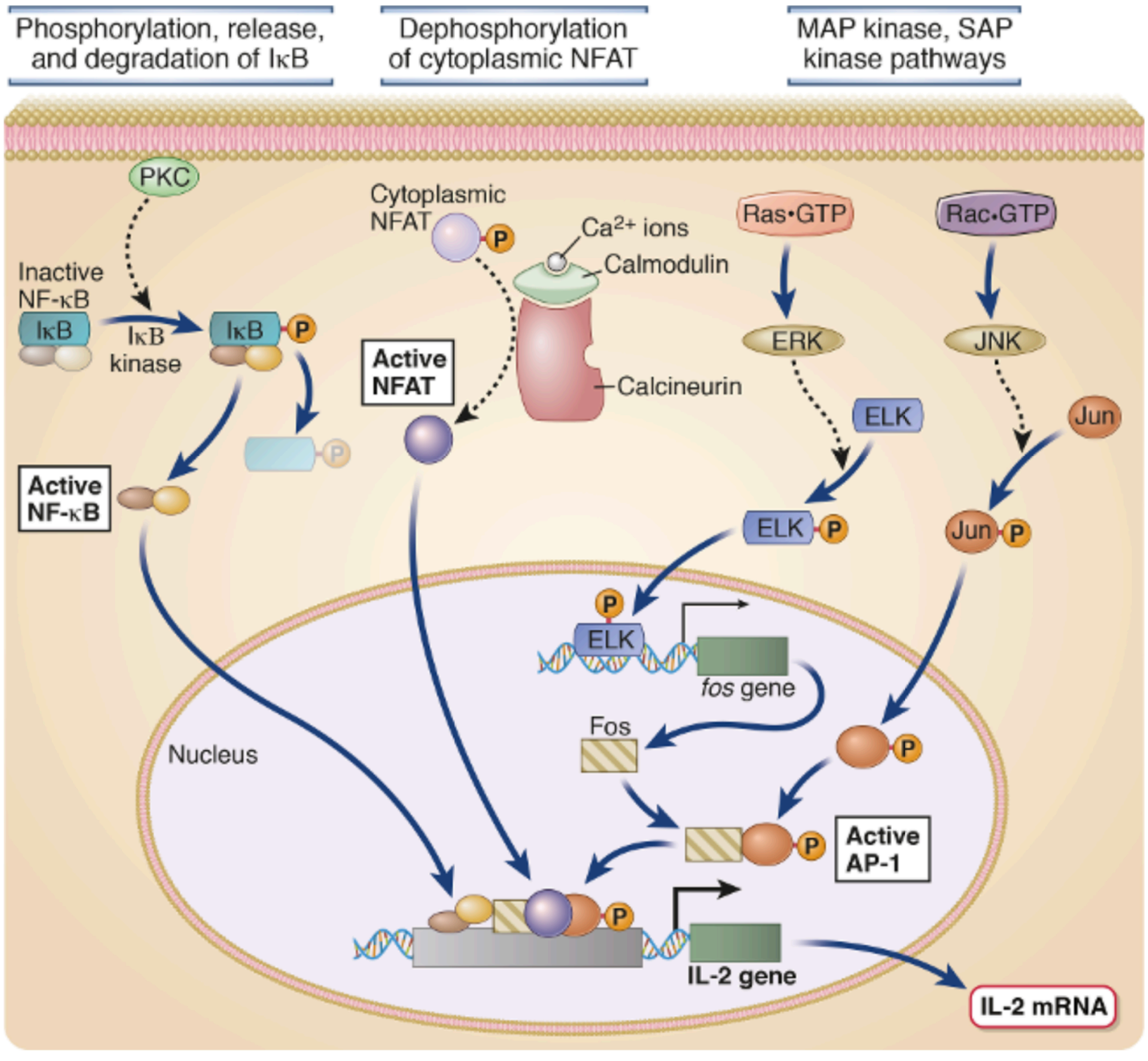


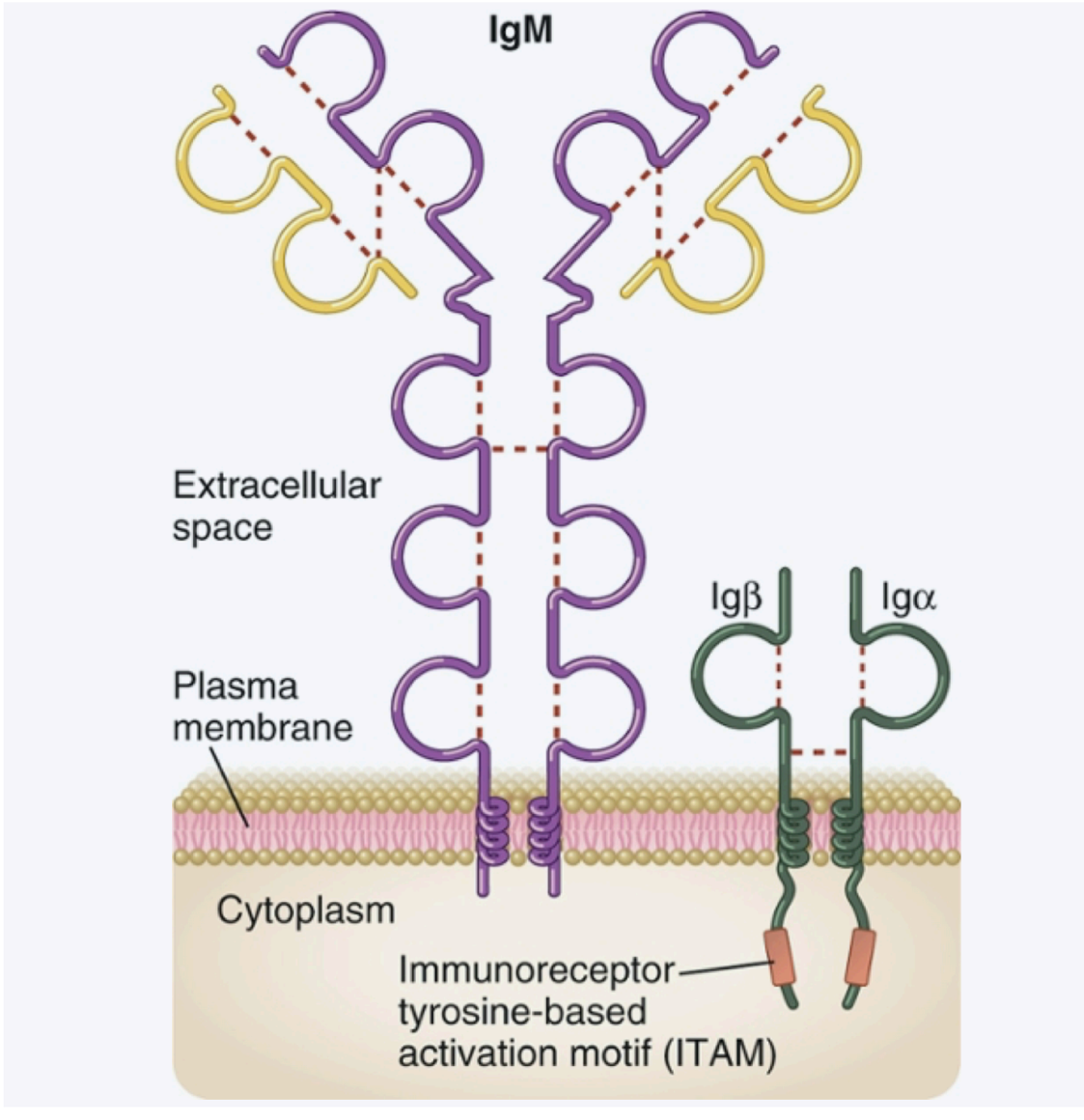
Figure 9.38 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Signalling pathways that regulate T cell functionality



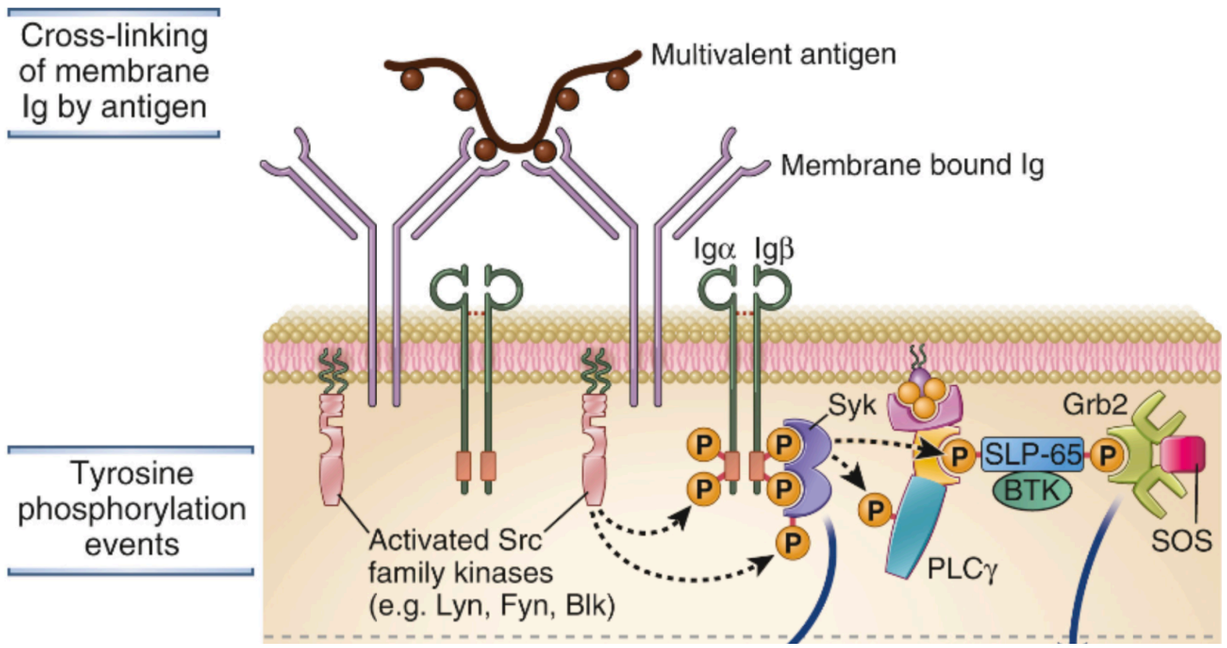
Overview of the BCR complex

- The BCR of naive B cells is a membrane-bound form of IgM (IgD) with a short cytoplasmic domain (3 amino acids).
- Ig-mediated signals are transduced by two non-covalently associated chains, Ig α/β, which contain ITAM motifs.



Initiation of signal transduction

Activation of multivalent antigens



Activation of antigens and co-factors