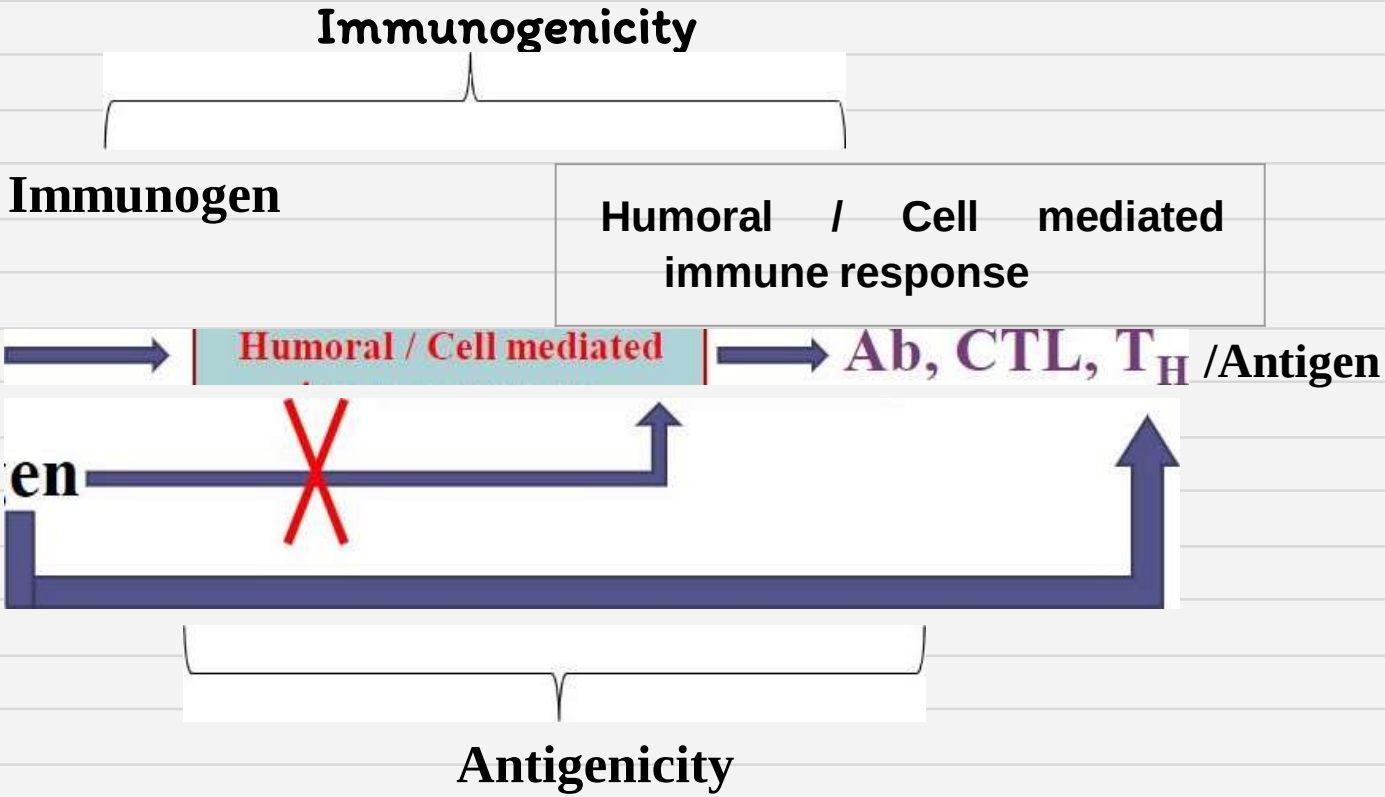
IMMUNOLOGY

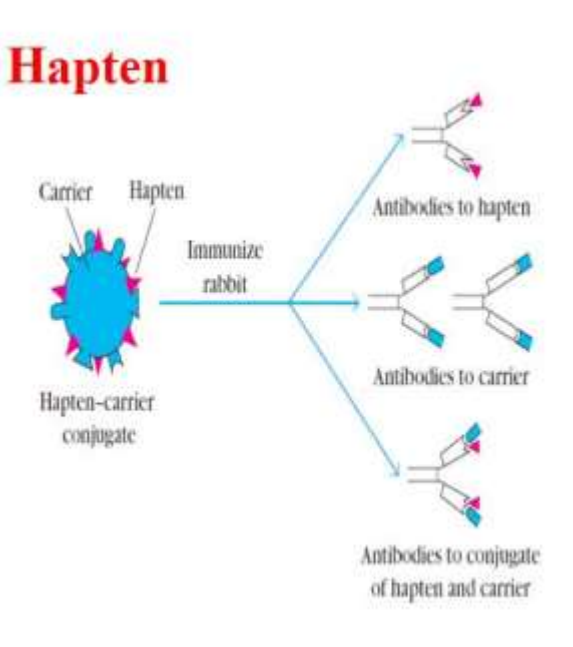
Immunogenicity vs Antigenecity



Immunogen

- Properties of Immunogen
- Foreignness
- Molecular size
- Chemical and structural complexity/ heterogencity
- Degradability
- Genetic make up of host
- Dosage & route of administration
- All immunogens are antigen but all antigen are not immunogens.

Hapten

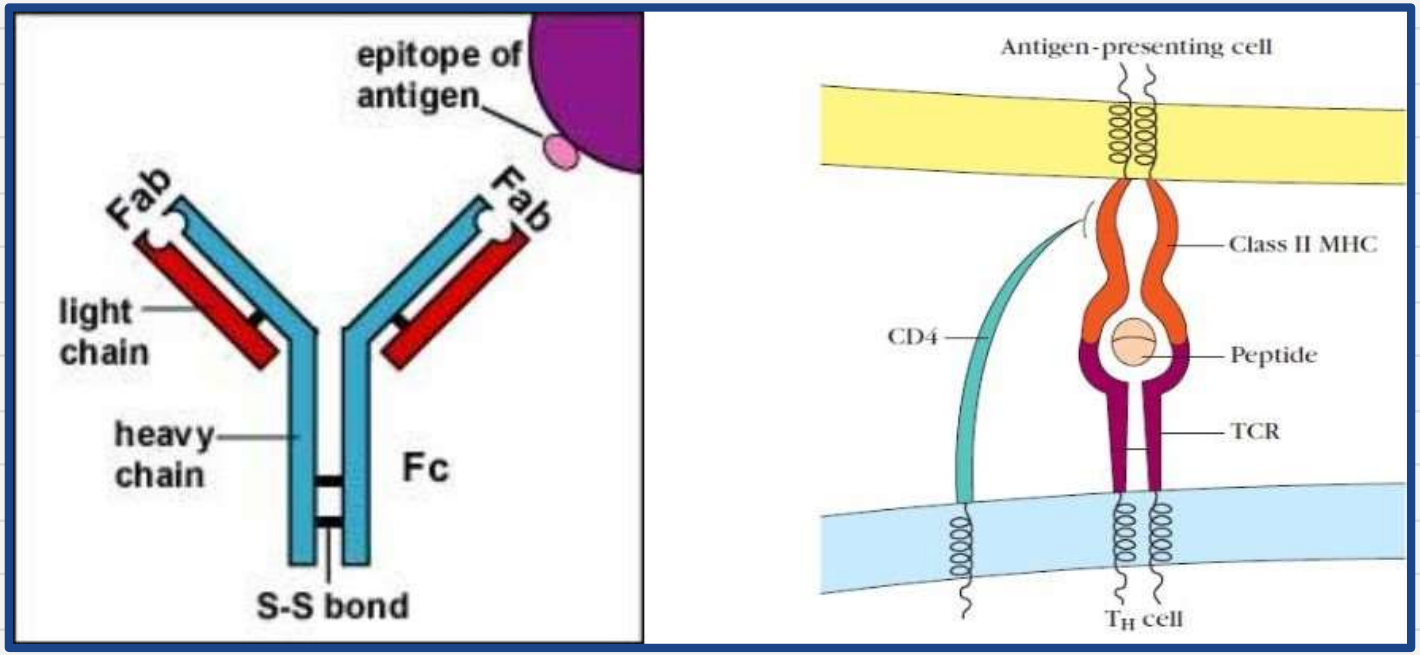


Adjuvent

- Prolonged persistence
- Enhanced co-stimulatory signals
- Increased local inflammation
- Stimulated non-specific

Epitope

- Part of imm
- unogen
- Immunogenically active
- Antigenic Determinant Region
- Bind to specific membrane receptors/Antibody



Receptors

Pattern Recognition Receptor

•Innate immunity — lectin like and bind multivalently Present on phagocytic cells recognise pathogen associated molecular patterns (PAMPs) • PAMPs

- Recognize self and non-self
- Broad structural motif absent in host, conserved, shared among pathogens
- Gram-negative lipopolysaccharide (LPS), Gram-positive lipoteichoic acid, yeast cell wall mannans and mycobacterial glycolipids
- Un Methylated CpG (guanosine-cytosine) sequences in bacterial DNA and the double-stranded RNA from RNA viruses

Types

- Present in the bloodstream and tissue fluids as soluble circulating proteins :

- Mannose- binding lectin (MBL)
- C-reactive protein (CRP)
- Lipopolysaccharide-binding protein

- Membrane bound in cells such as macrophages, neutrophils, and dendritic cells :

- Scavenger receptors (SRS)
- T011-1ike receptors (TLRs)

Membrane bound Receptor

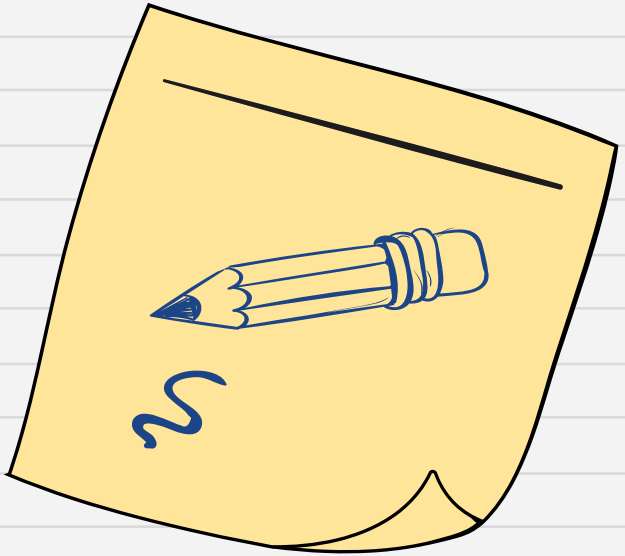
TLR2 (cell membrane)	Cell-wall components of gram-positive bacteria, LPS*. Yeast cell-wall component (zymosan)	Attracts phagocytes, activates macrophages, dendritic cells. Induces secretion of several cytokines
TLR3 (cell membrane)	Double-stranded RNA (dsRNA) (replication of many RNA viruses)	Induces production of interferon, an antiviral cytokine
TLR4 (cell membrane)	LPS*	Attracts phagocytes, activates macrophages, dendritic cells. Induces secretion of several cytokines
TLR5 (cell membrane)	Flagellin (flagella of gram-positive and gram-negative bacteria)	Attracts phagocytes, activates macrophages, dendritic cells. Induces secretion of several cytokines
TLR9 (cell membrane)	CpG	Attracts phagocytes, macrophages, dendritic cells. Induces secretion of several cytokines
Scavenger receptors (many) (cell membrane)	Many targets; gram-positive and gram-negative bacteria, apoptotic host cells	Induces phagocytosis or endocytosis

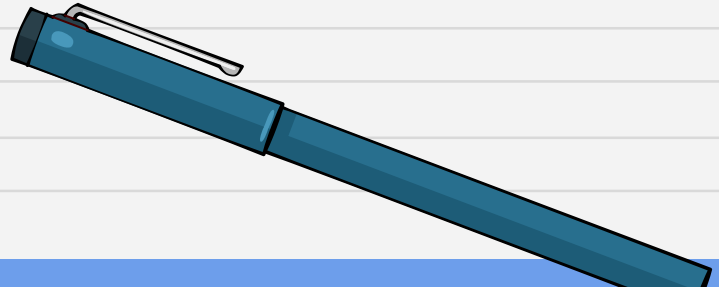
Receptor of Adaptive Immune System

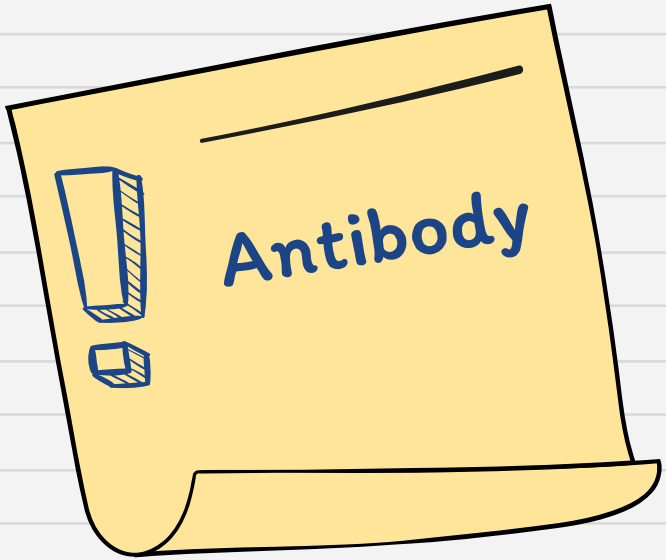
Receptor (location)	Target (source)	Effect of recognition
Antibody (B-cell membrane, blood, tissue fluids)	Specific components of pathogen	Labeling of pathogen for destruction and removal
T-cell receptor (T-cell membrane)	Proteins or certain lipids of pathogen	Induction of pathogen-specific humoral and cell-mediated immunity

Characteristic	B cells	T cells
Interaction with antigen	Involves binary complex of membrane Ig and Ag	Involves ternary complex of T-cell receptor, Ag, and MHC molecule
Binding of soluble antigen	Yes	No
Involvement of MHC molecules	None required	Required to display processed antigen
Chemical nature of antigens	Protein, polysaccharide, lipid	Mostly proteins, but some lipids and glycolipids presented on MHC-like molecules
Epitope properties	Accessible, hydrophilic, mobile peptides containing sequential or nonsequential amino acids	Internal linear peptides produced by processing of antigen and bound to MHC molecules

Antibody!







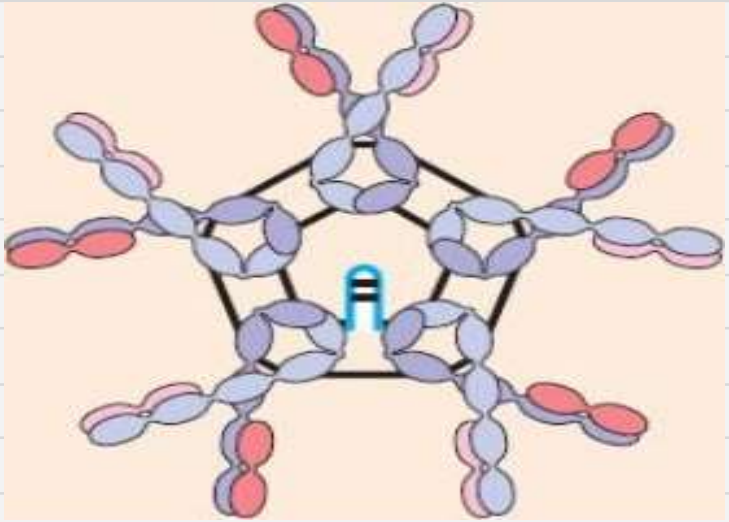
- Antigen binding glycoprotein
- Immunoglobulin
- B cell membrane and secreted by plasma cells
- Specific binding sites

Structure

- Antibody molecules have a common structure of four peptide chains- heterodimer
- 2 identical Light (L) (approx 25 000 Da) — K & lambda
- 2 identical heavy (H) chains (approx 50 000 Da)- y, u, g, e & ö
- L-H chain joined together — by disulphide linkage and noncovalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L)
- Similar forces link the two identical heavy and light (H-L) chain combinations to each other to form the basic four-chain (H-L)2 antibody structure, dimer of dimers
- proline-rich hinge region — y, u & ö - flexible

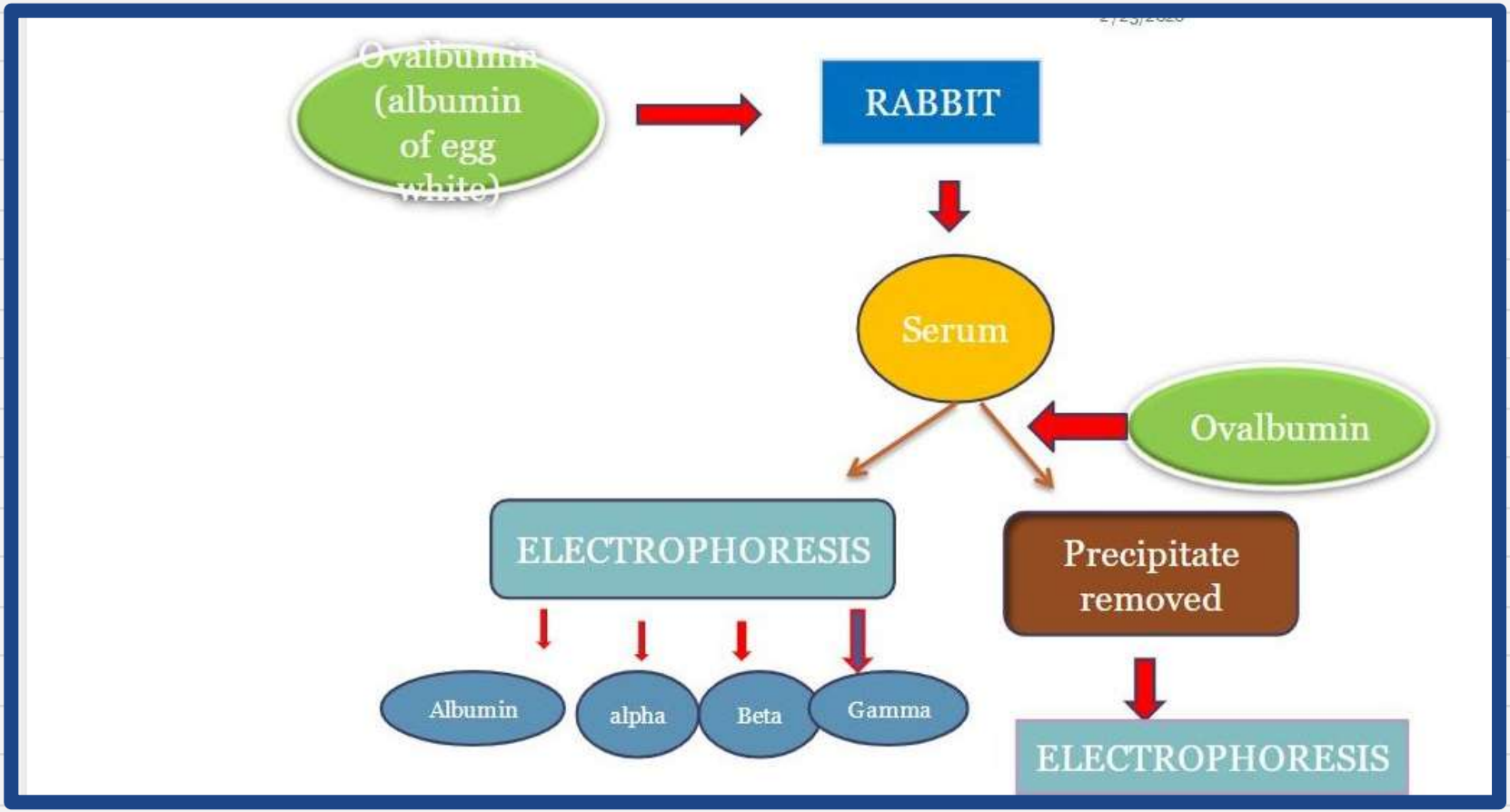
Antibody

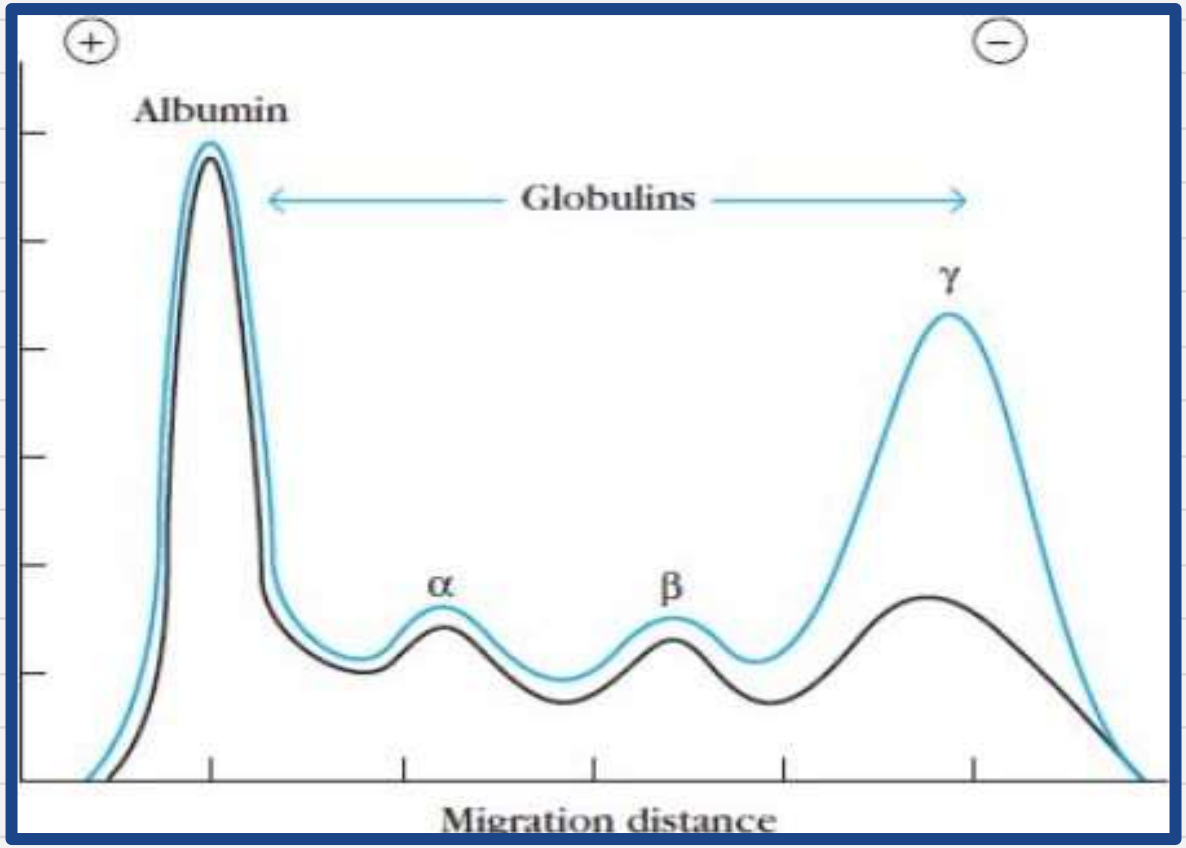
- Antibodies are the antigen-binding protein present on the B-cell membrane and secreted by plasma cells



- The first evidence that antibodies were contained in particular serum protein fractions came from a classic experiment by A. Tiselius and E. A.Kabat, in 1939

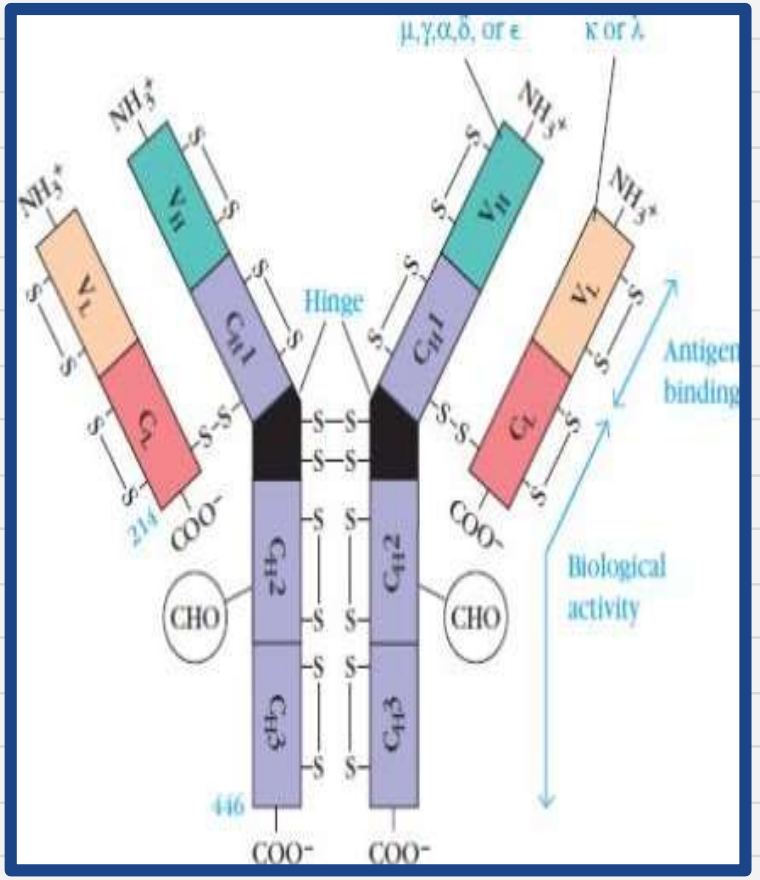
- Gamma-globulin fraction was identified as containing serum antibodies, which were called Immunoglobulins, to distinguish them from any other proteins that might be contained in the gamma globulin fraction


Ovalbumin (albumin of egg white)
RABBIT
Serum
Ovalbumin
ELECTROPHORESIS
Precipitate removed
Albumin
alpha
Beta
Gamma
ELECTROPHORESIS


+
−
Albumin
Globulins
γ
α
β
Migration distance

Basic Structure of antibody

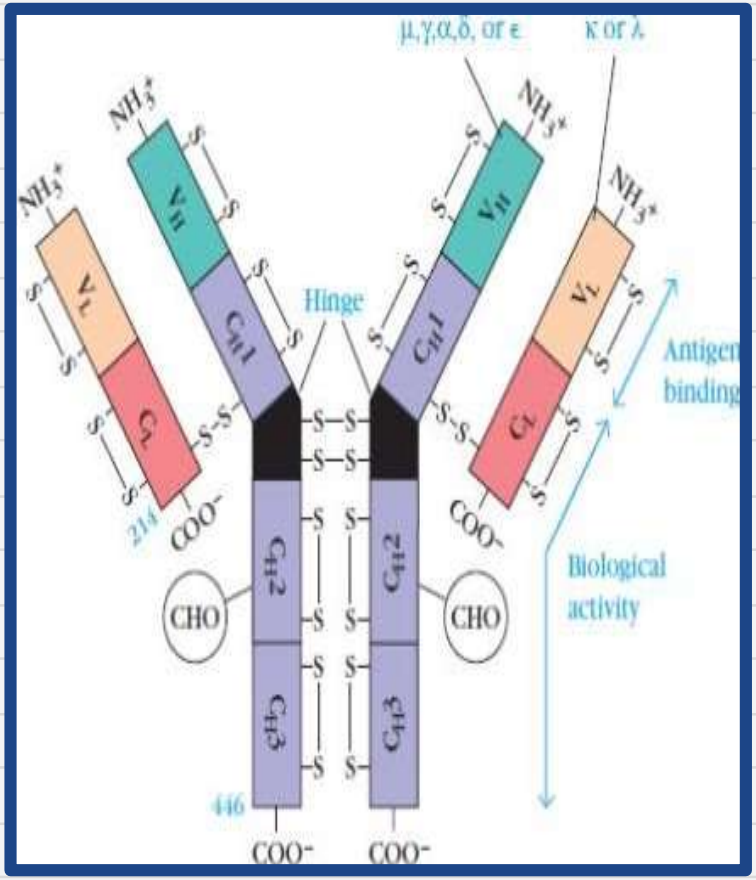
- Antibody molecules have a common structure of four peptide chains
- This structure consists of two identical light (L) chains, polypeptides of about 25,000 molecular weight, and two identical heavy (H) chains, larger polypeptides of molecular weight 50,000 or more Like the antibody molecules they constitute, H and L chains are also called immunoglobulins.
- Each light chain is bound to a heavy chain by a di sulfide bond, and by such non covalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L).
- Similar non covalent interactions and di sulfide bridges link the two identical heavy and light (H-L) chain combinations to each other to form the basic four-chain (H-L)2 antibody structure, a dimer of dimers



- Most of the differences among antibodies fall within areas of the V regions called complementarity determining regions (CDRs), and it is these CDRs, on both light and heavy chains, that constitute the antigen binding site of the antibody molecule.

- Within the same antibody class, far fewer differences are seen when one compares sequences throughout the rest of the molecule. The regions of relatively constant sequence beyond the variable regions have been dubbed C regions, CL on the light chain and CH on the heavy chain

- Antibodies are glycoproteins; with few exceptions, the sites of attachment for Antigen carbohydrates are binding restricted to the constant region



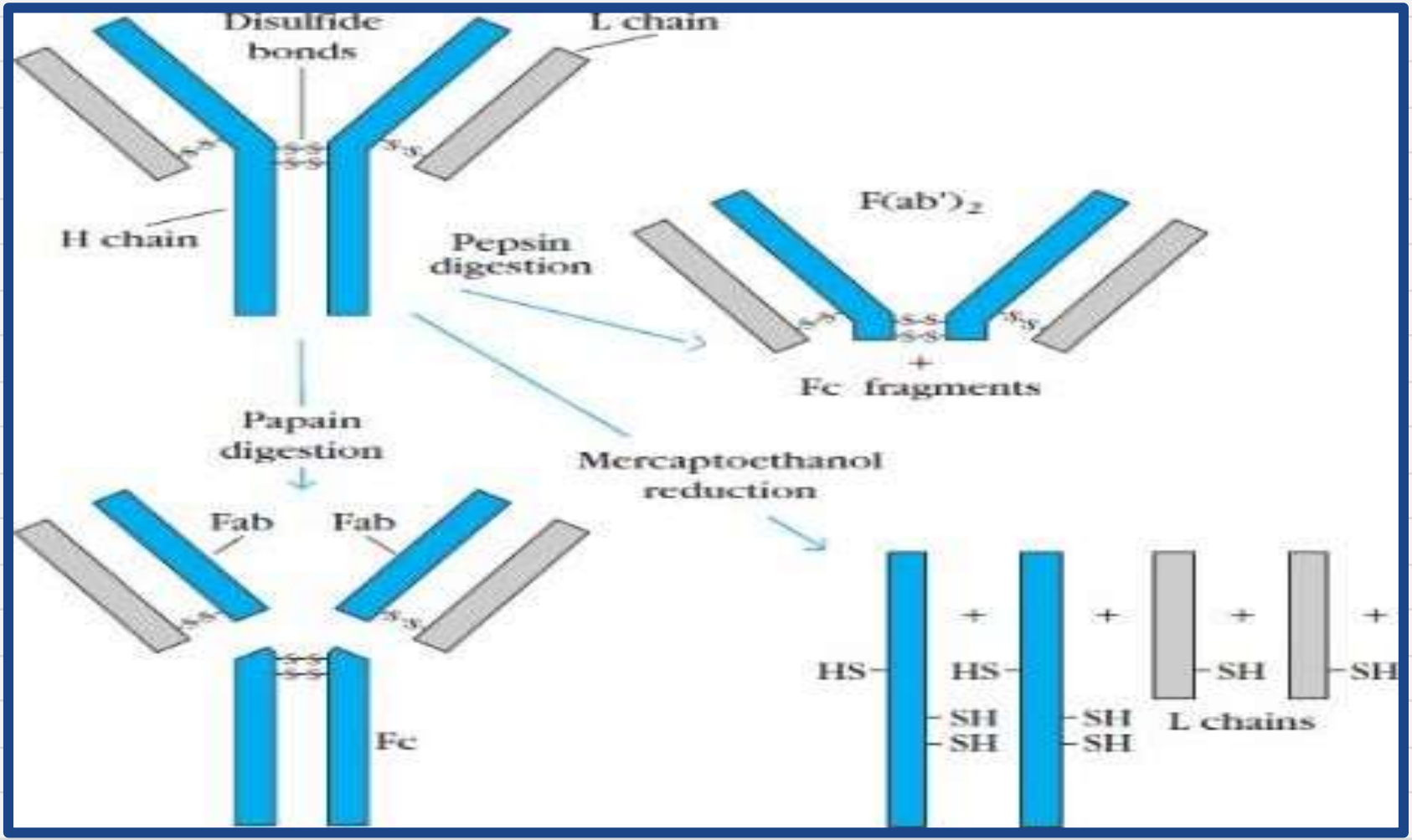
What is the role of this glycosylation?

- It probably increases the solubility of the molecules. Inappropriate glycosylation, or its absence, affects the rate at which antibodies are cleared from the serum, and decreases the efficiency of interaction between antibody and the complement system and between antibodies and FC receptors

How Chemical and Enzymatic Methods Revealed Basic Antibody Structure

- When the —globulin fraction of serum is separated into high- and low molecular weight fractions, antibodies of around 150,000MW, designated as immunoglobulin G (IgG) are found in the low molecular-weight fraction Brief digestion of IgG with the enzyme papain produced three fragments, two of which were identical fragments and a third that was quite different.

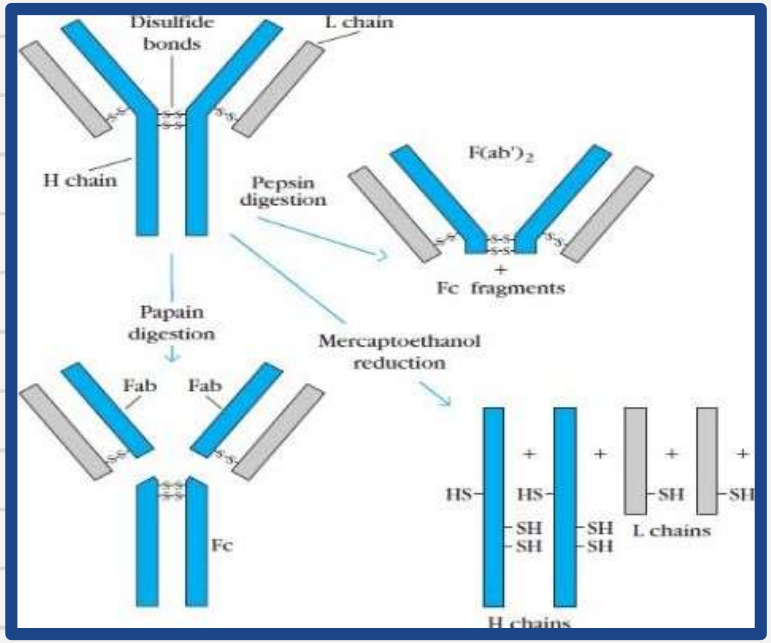
- The two identical fragments (each with a MW 45,000), had antigen-binding activity and were called Fab fragments ("fragment, antigen binding"). The other fragment (MW of 50,000) had no antigen binding activity at all. Because it was found to crystallize during cold storage, it was called the FC fragment ("fragment, crystallisable").


Disulfide bonds
L chain
H chain
Pepsin digestion
F(ab')2
+
Fc fragments
Papain digestion
Mercaptoethanol reduction
Fab
Fab
Fc
HS
HS
SH
SH
SH
SH
+
+
+
+
SH
SH
L chains

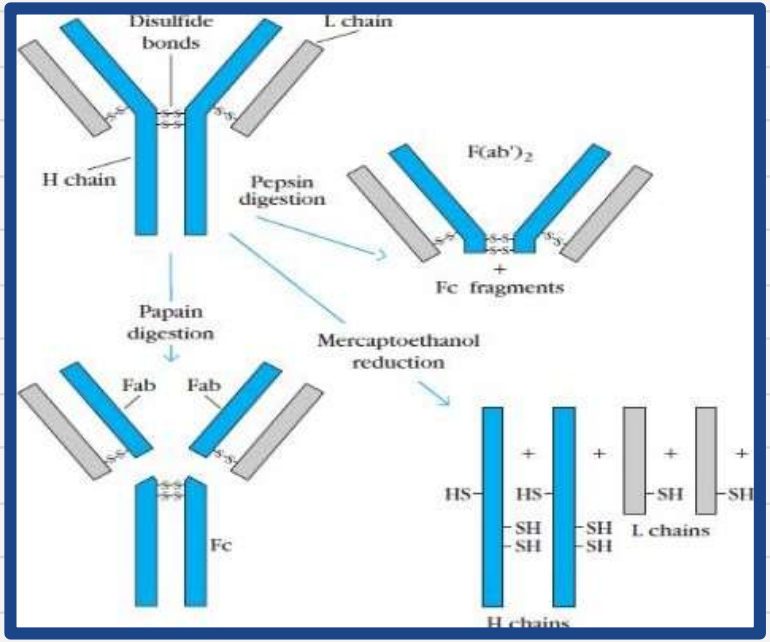
- Pepsin digestion generated a single 100,000- MW fragment composed of two Fab-like fragments designated the F(ab)2 fragment, which binds antigen. The FC fragment was not recovered from pepsin digestion because it had been digested into multiple fragments

- Mercaptoethanol reduction and alkylation, a chemical treatment that irreversibly cleaves di sulphide bonds. Sample is chromatographed on a column that separates molecules by size following cleavage of di sulphide bonds, it is clear that the intact 150,000-MW IgG molecule is, in fact, composed of subunits.

- Each IgG molecule contains two 50,000-MWpolypeptide chains, designated as heavy (H) chains, and two 25,000-MW chains, designated as light (L) chains



- According to this model, the IgG molecule consists of two identical H chains and two identical L chains, which are linked by disulfide bridges. The enzyme papain cleaves just above the inter chain disulfide bonds linking the heavy chains, whereas the enzyme pepsin cleaves just below these bonds, so that the two proteolytic enzymes generate different digestion products. Mercaptoethanol reduction and alkylation allow separation of the individual heavy and light chains.



TO FURTHER UNDERSTAND

Antisera from goats were taken that had been immunized with either the Fab fragments or the Fc fragments of rabbit IgG

The antibody to the Fab fragment could react with both the H and the L chains, whereas antibody to the FC fragment reacted only with the H chain. These observations led to the conclusion that the Fab fragment consists of portions of a heavy and a light chain and that FC contains only heavy-chain components.

A cancerous plasma cell, called a myeloma cell, has been transformed, BUT its protein-synthesizing machinery and secretory functions are not altered; thus, the cell continues to secrete molecularly homogeneous antibody.

This antibody is indistinguishable from normal antibody molecules but is called myeloma protein to denote its source.

In most patients, the myeloma cells also secrete excessive amounts of light chains These excess light chains were first discovered in the urine of myeloma patients and were named Bence Jones proteins , for their discoverer

The clones of malignant plasma cells tha develop are called , and many of these ar designated MOPCs, denoting the mineral oil induction of plasmacytoma cells

Light-Chain

- **The amino-terminal half of the chain, consisting of 100—110 amino acids, was found to vary among different Bence-Jones proteins. This region was called the variable (V) region**

The carboxyl-terminal half of the molecule, called the **constant (C) region, had two**basic amino acid sequences. This led to the recognition that there were two light chain types, **kappa (k) and lambda**

The amino acid sequences of light chains show minor differences that are used to classify light chains into subtypes. In mice, there are three subtypes (1, 2, and 3); in humans,there are four subtypes

Heavy Chain

- The amino-acid end terminal part of the chain, consisting of 100—110 amino acids, showed great sequence variation among myeloma heavy chains and was therefore called the variable region

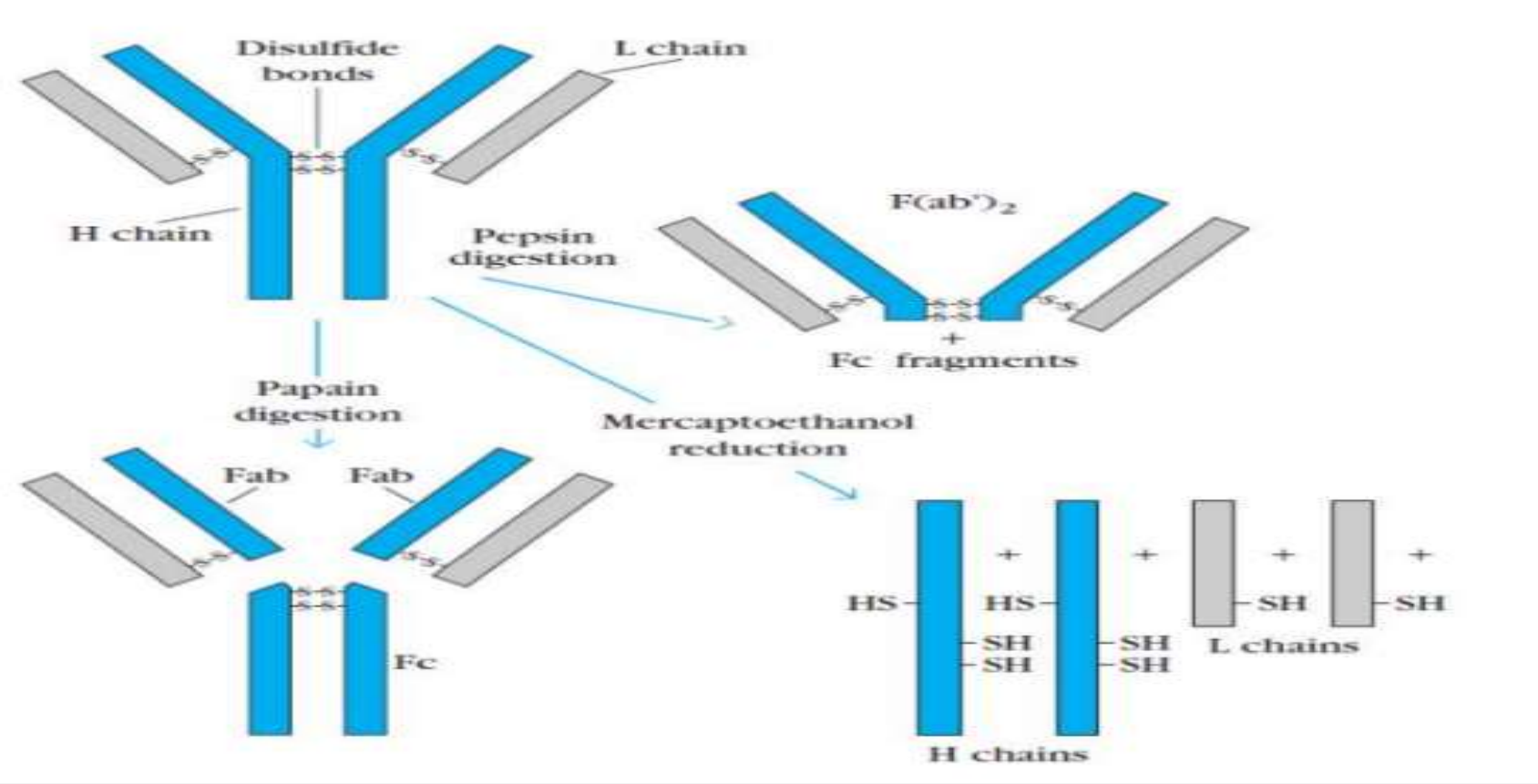
- The remaining part of the protein revealed have basic sequence patterns, corresponding to five different heavy-chain constant (C) regions Each of these five different heavy chains is called an iso type.

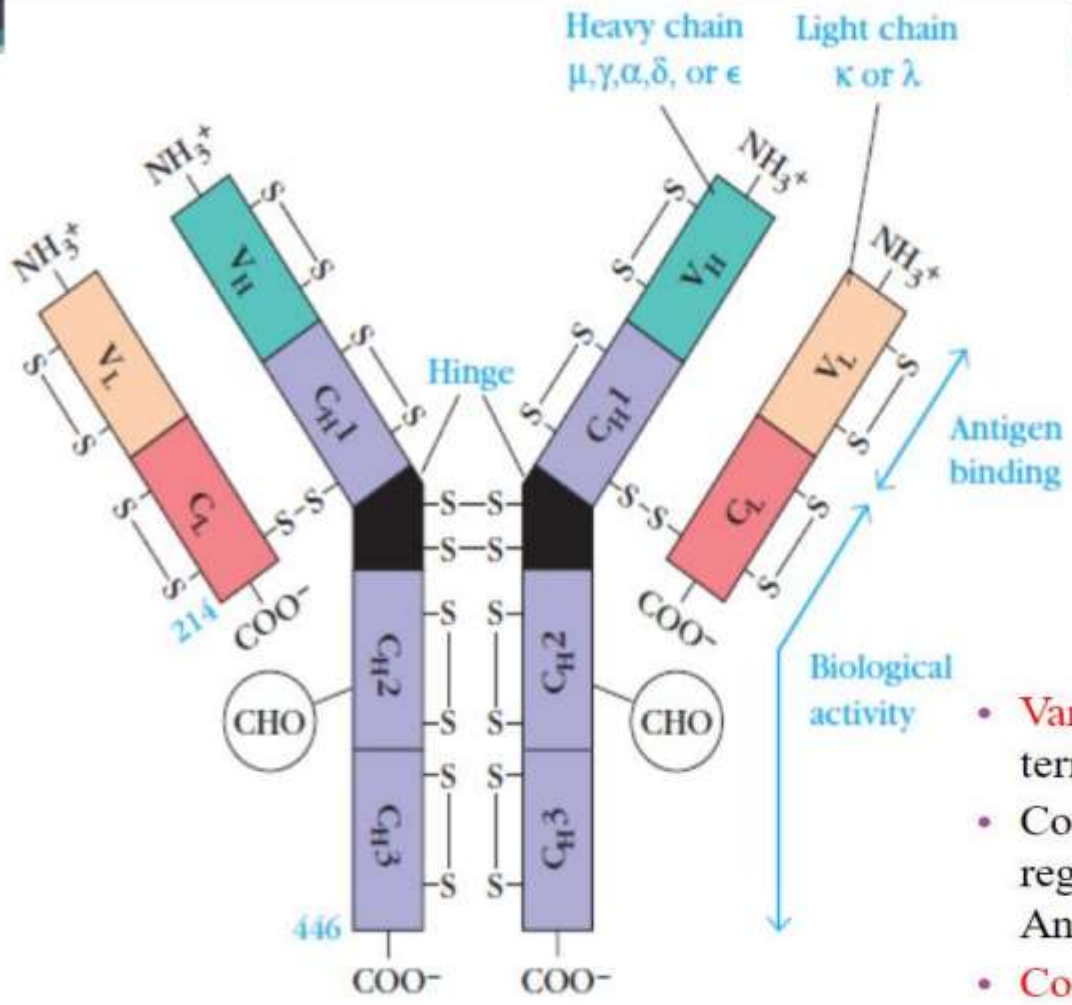
- The length of the constant regions is approximately 330 amino acids for delta, lambda, and alpha , and 440 amino acids for μ and Ɛ . The heavy chains of a given antibody molecule determine the class of that antibody: IgM(μ), IgA(alpha), IgD(delta), or IgE(Ɛ).

TABLE 4-1 Chain composition of the five immunoglobulin classes in humans

Class	Heavy chain	Subclasses	Light chain	Molecular formula
IgG	γ	$\gamma1$, $\gamma2$, $\gamma3$, $\gamma4$	κ or λ	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_n$ $(\mu_2\lambda_2)_n$ $n = 1$ or 5
IgA	α	$\alpha1$, $\alpha2$	κ or λ	$(\alpha_2\kappa_2)_n$ $(\alpha_2\lambda_2)_n$ $n = 1, 2, 3$, or 4
IgE	ϵ	None	κ or λ	$\epsilon_2\kappa_2$ $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

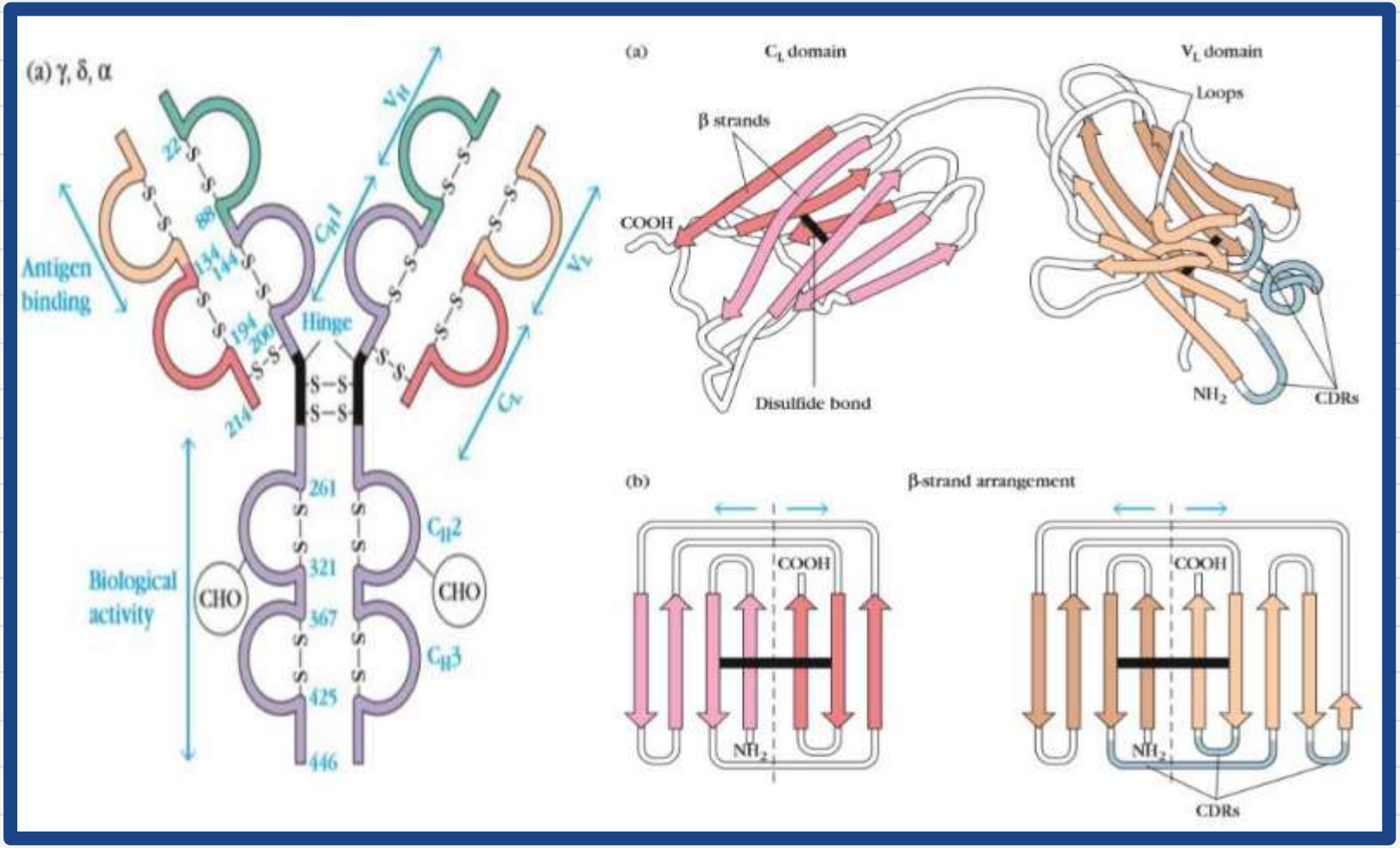
Enzymatic Digestion Of Ab

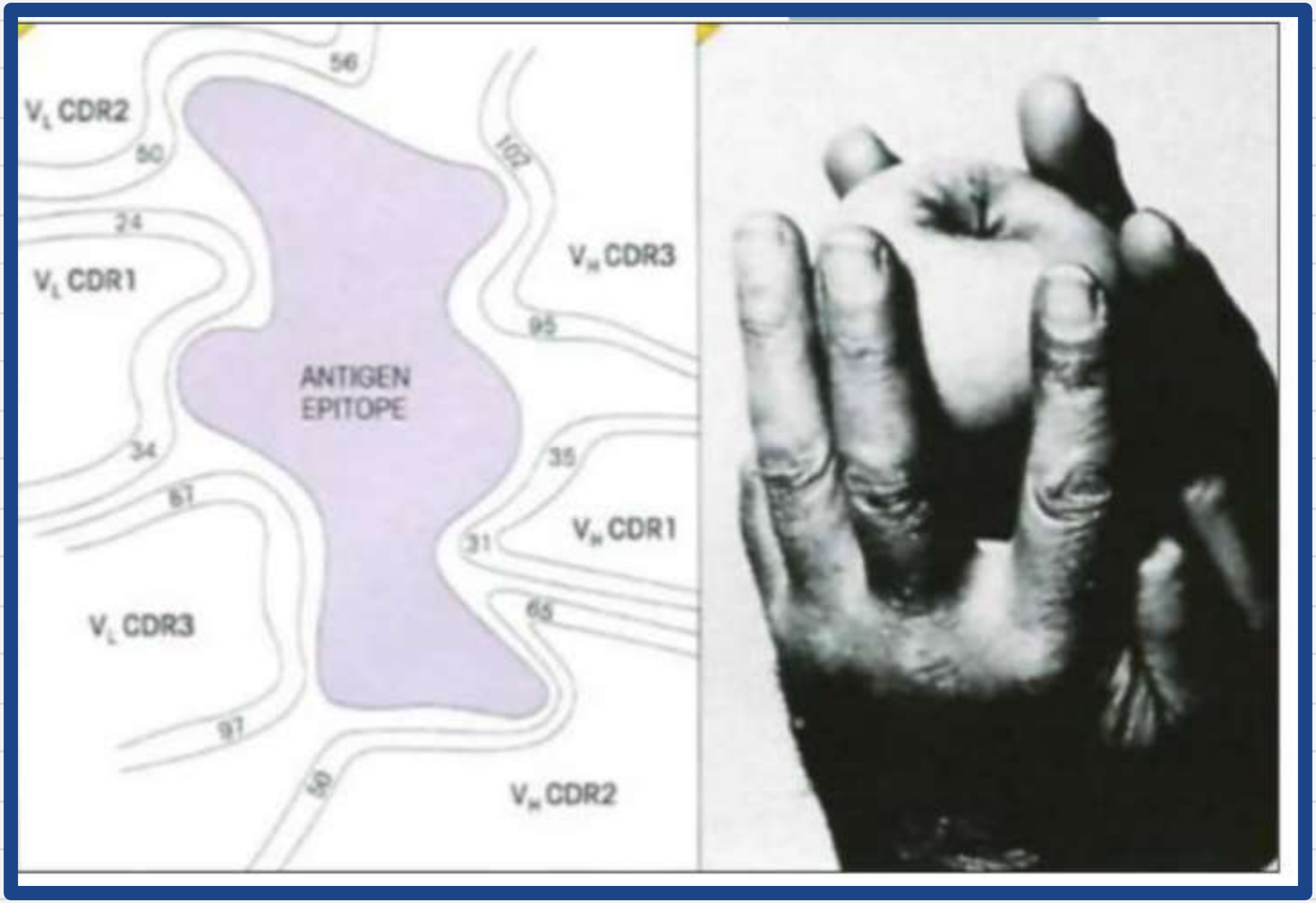




- Variable region – V_L & V_H , N terminal first 110 amino acids
- Complementarity-determining regions (CDRs) in V region – Antigen binding site
- Constant region – C_L & C_H

Immunoglobulin fold




56
V_L CDR2
50
24
V_L CDR1
34
97
V_L CDR3
97
89
V_H CDR2
ANTIGEN
EPITOPE
102
V_H CDR3
95
35
V_H CDR1
31
65

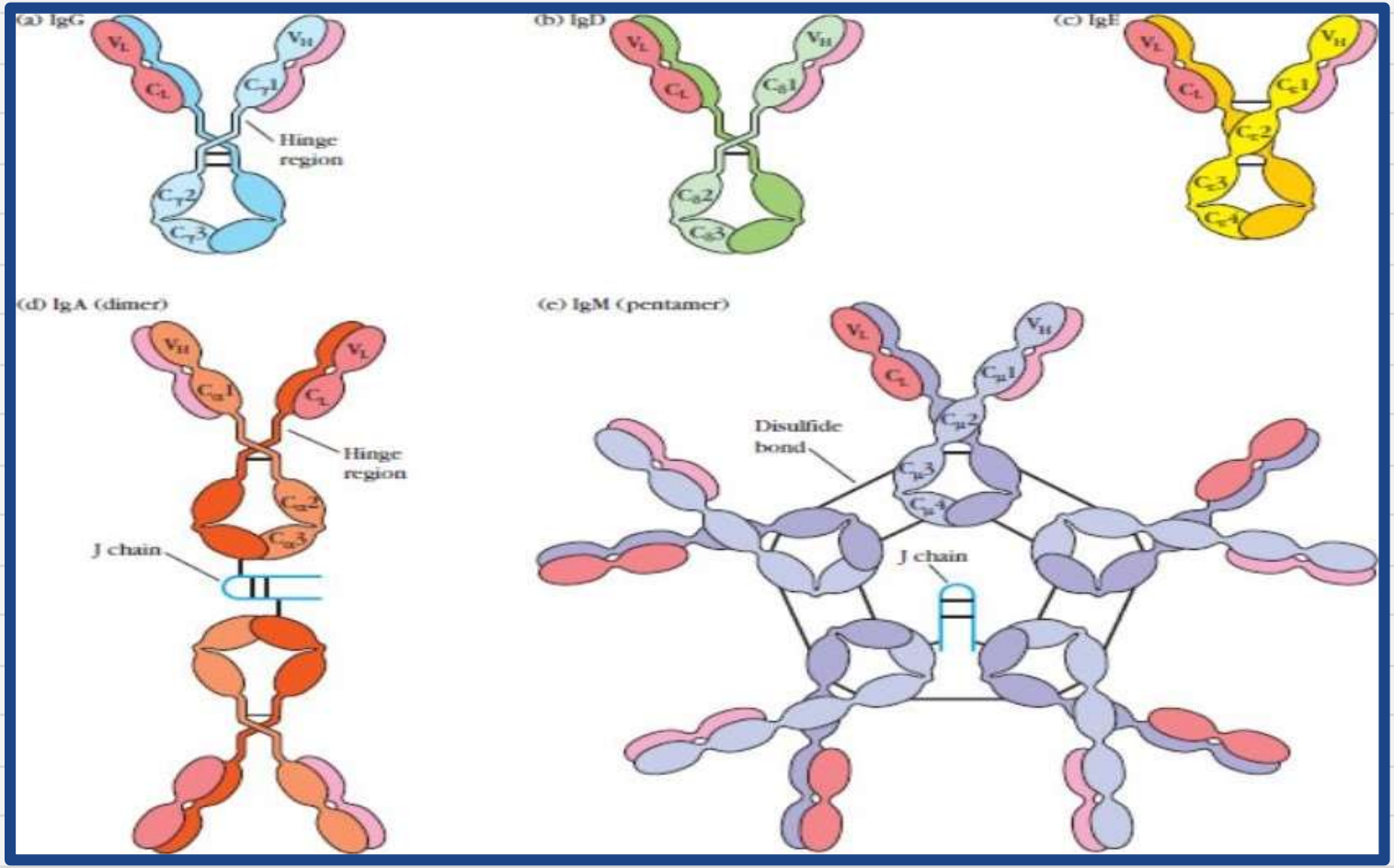
Components Of antibody

Class	Heavy chain	Subclasses	Light chain	Molecular formula
IgG	γ	$\gamma1$, $\gamma2$, $\gamma3$, $\gamma4$	κ or λ	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_n$ $(\mu_2\lambda_2)_n$ $n = 1$ or 5
IgA	α	$\alpha1$, $\alpha2$	κ or λ	$(\alpha_2\kappa_2)_n$ $(\alpha_2\lambda_2)_n$ $n = 1, 2, 3$, or 4
IgE	ϵ	None	κ or λ	$\epsilon_2\kappa_2$ $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

Functions Of Antibody

- Opsonisation
- Neutralization
- Antibody activated complement
- Antibody Directed Cell mediated Cytotoxicity

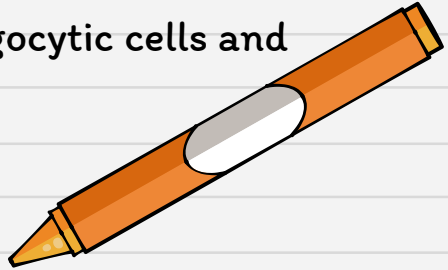
Classes of Ab

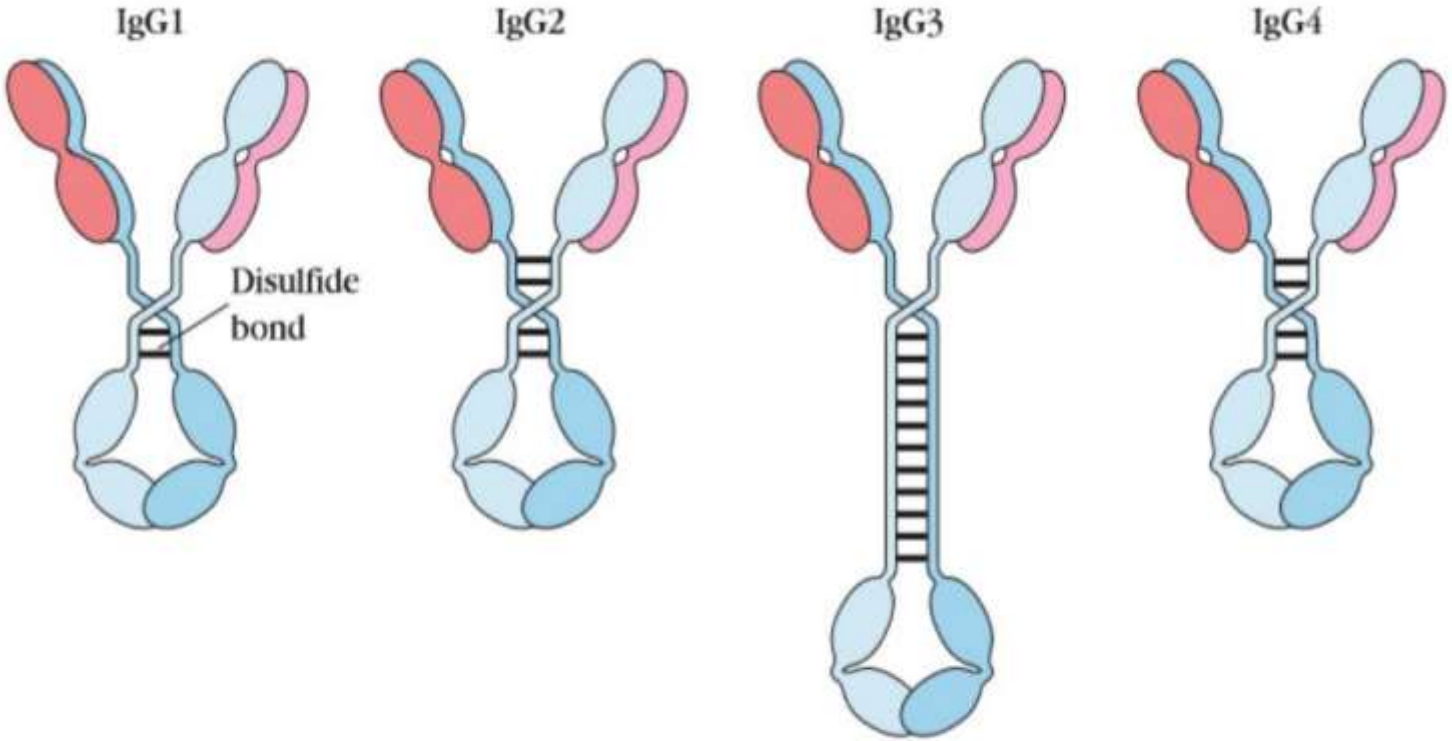


IgG	IgA	IgM	IgD	IgE
Cross the placenta – immunity to foetus Major Ig in secondary immune response Protection to most of blood borne infections	Major Ig in external secretions First line defense at mucosal membrane	Major Ig in primary response First Ig produced by and expressed on B cell surface	Act as antigen receptor on B lymphocytes	Acute inflammation Allergic reaction Protection against helminthic infections

Immunoglobulin G (IgG)

- Most abundant class in serum, constitutes about 80% of the total serum immunoglobulin
- Two y heavy chains and two K or two light chains
- Differences in y -chain sequence - IgGl,IgG2,IgG3, and IgG4
- sub iso types - the size of the hinge region and the number and position of the interchain di sulfide bonds between the heavy chains
- IgG1, IgG3, and IgG4 - cross the placenta
- IgG3 is the most effective complement activator, followed by lgG1 and lgG2
- IgG1 and IgG3 bind with high affinity to FC receptors on phagocytic cells and thus mediate opsonisation





Vidarsson G, Dekkers G, Rispens T. IgG Subclasses and Allotypes: From Structure to Effector Functions. *Frontiers in Immunology*. 2014;5:520. doi:10.3389/fimmu.2014.00520.

Immunoglobulin M (IgM)

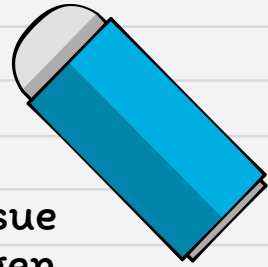
- Constitutes only 10% 15% of the total immunoglobulin in serum, predominant immunoglobulin class in external secretions

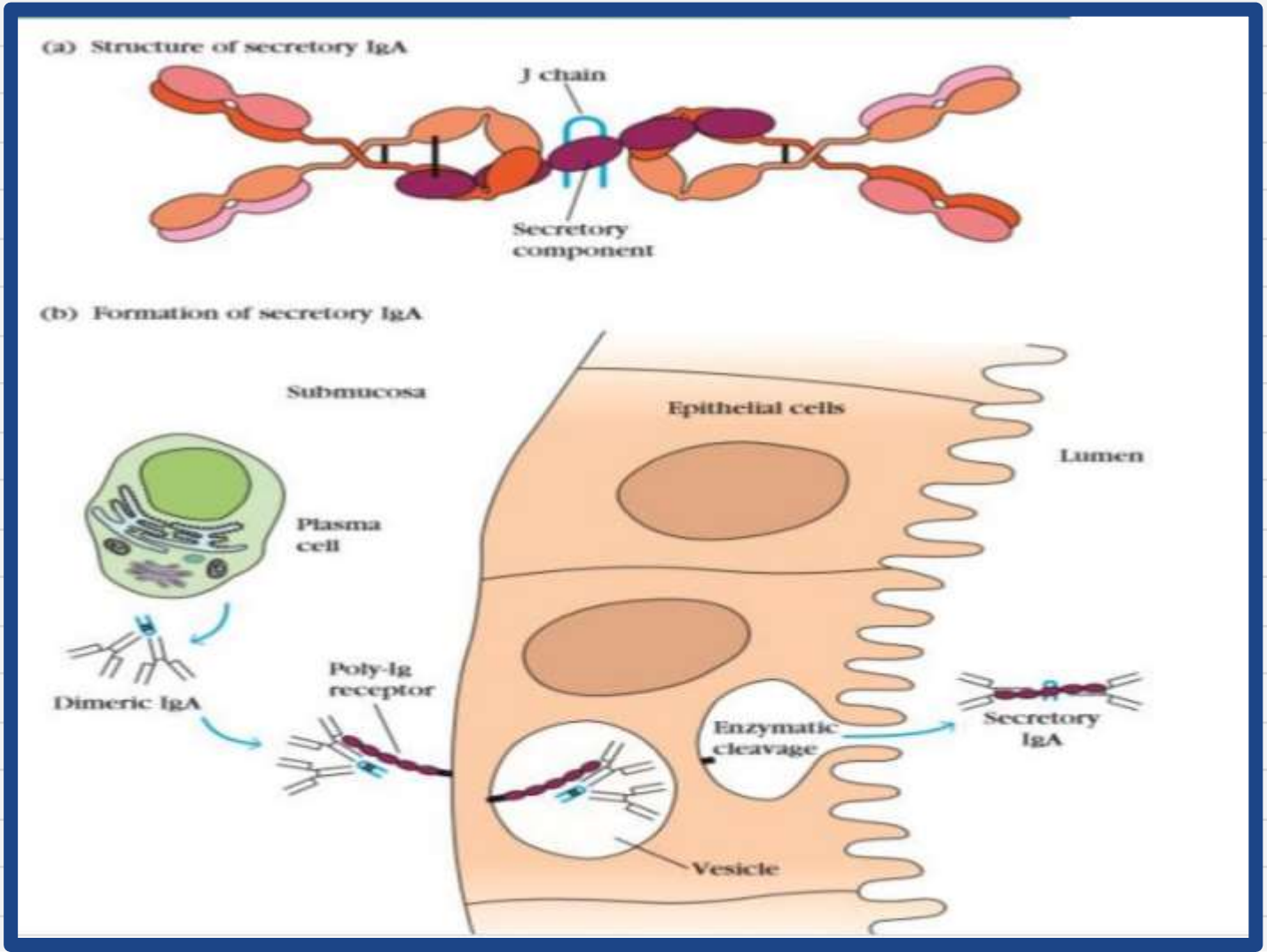
- Polymeric forms (dimers, trimers, and some tetramers) are sometimes seen, all containing a J-chain

- IgA of external secretions, called secretory IgA, consists of a dimer or tetramer, a J-chain polypeptide, and a polypeptide chain (70,000 Da) called secretory component

Immunoglobulin A (IgA)

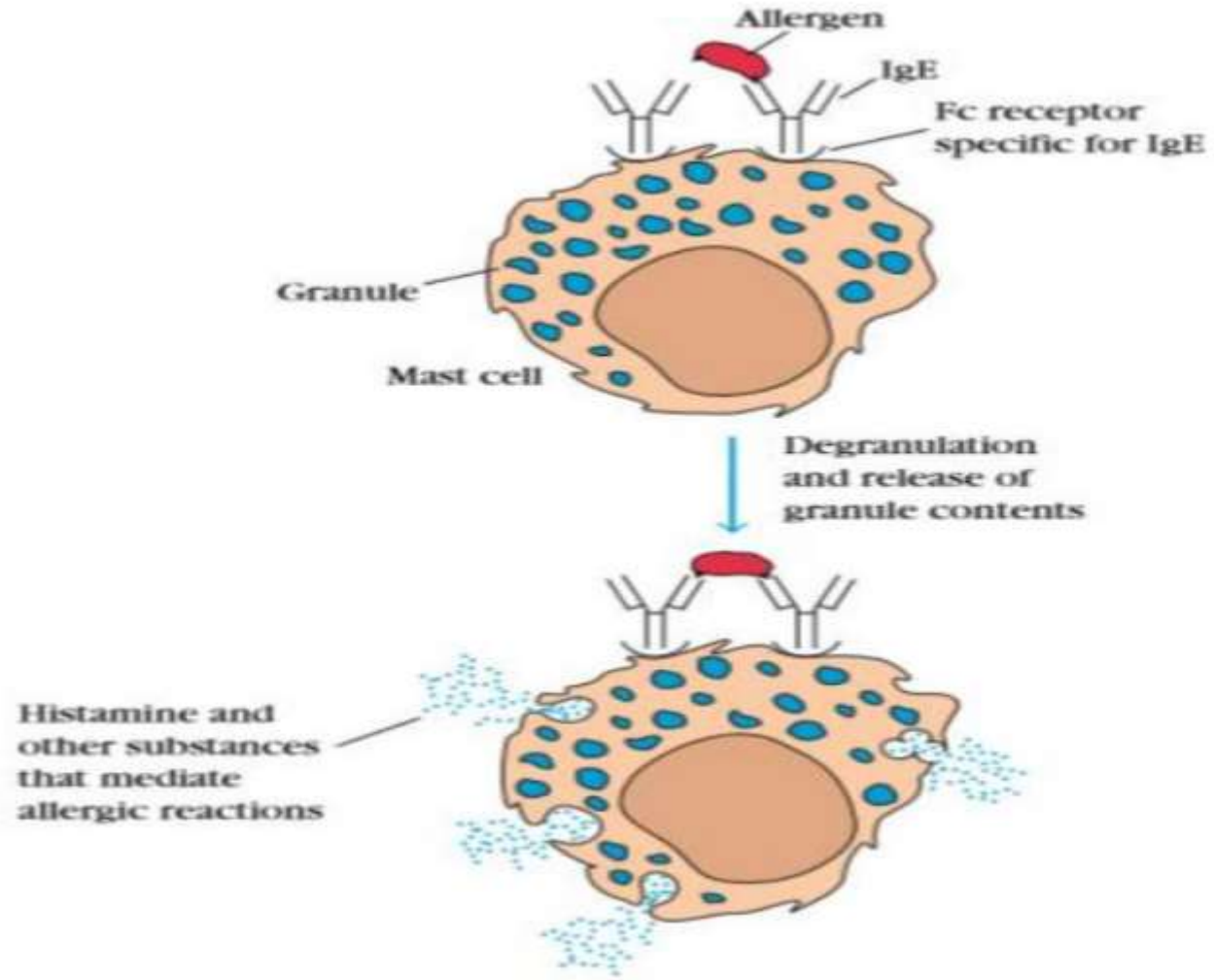
- Extremely low average serum concentration (0.3 gg/ml)
- Mediate the immediate hypersensitivity
- Binds to FC receptors on the membranes of blood basophils and tissue mast cells. Cross-linkage of receptor- bound IgE molecules by antigen (allergen) induces basophils and mast cells to translocate their granules to the plasma membrane and release their contents to the extracellular environment, a process known as degranulation




(a) Structure of secretory IgA
J chain
Secretory component
(b) Formation of secretory IgA
Submucosa
Epithelial cells
Lumen
Plasma cell
Dimeric IgA
Poly-Ig receptor
Enzymatic cleavage
Secretory IgA
Vesicle

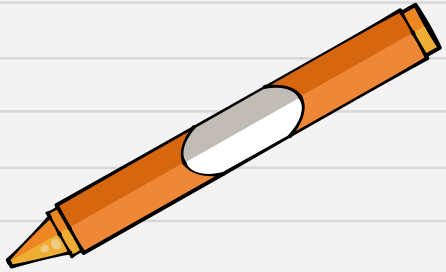
Immunoglobulin E (IgE)

- Extremely low average serum concentration (0.3 gg/ml)
- Mediate the immediate hypersensitivity
- Binds to FC receptors on the membranes of blood basophils and tissue mast cells. Cross-linkage of receptor- bound IgE molecules by antigen (allergen) induces basophils and mast cells to translocate their granules to the plasma membrane and release their contents to the extracellular environment, a process known as degranulation

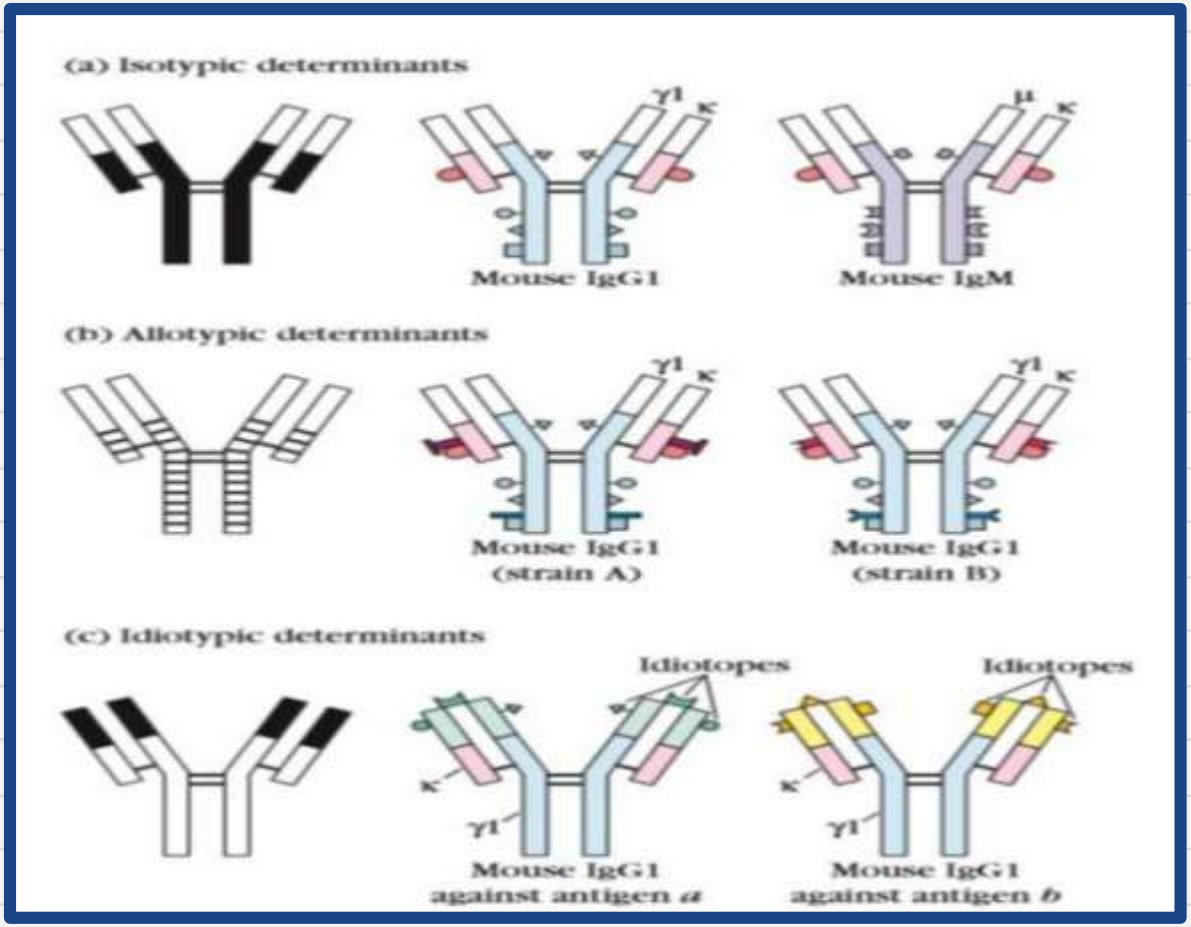

Allergen
IgE
Fc receptor
specific for IgE
Granule
Mast cell
Degranulation
and release of
granule contents
Histamine and
other substances
that mediate
allergic reactions

Immunoglobulin D (IgD)

- It constitutes about 0.2% of the total immunoglobulin
- IgD, together with IgM, is the major membrane- bound immunoglobulin expressed by mature B cells



Antigenic determination on immunoglobulins



B cell Receptor

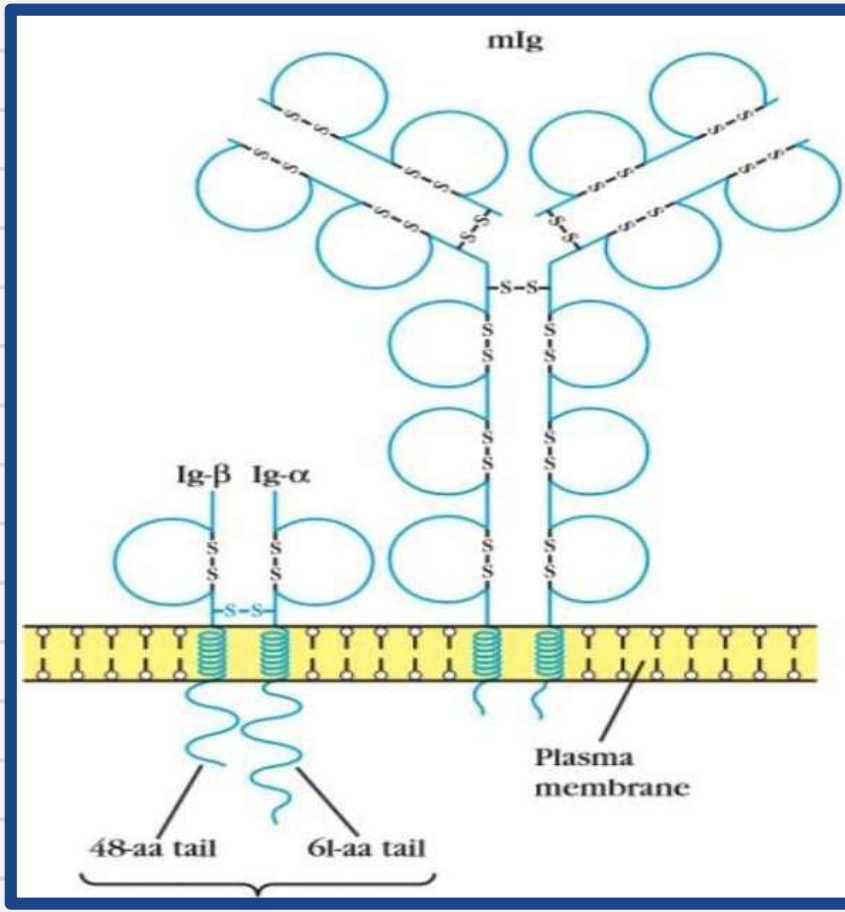
Dilemma is that all iso types of mIg have very short cytoplasmic tails how the signal will be transmitted

A transmembrane protein complex composed (BCR) of mIg and disulfide-linked heterodimers called Ig-u/lg-ß

Ig-u chain has a long cytoplasmic tail containing 61 amino acids; the tail of the Ig-ß chain contains 48 amino acids

Interact with intracellular signalling molecules by immuno receptor tyrosine-based activation motif (ITAM)

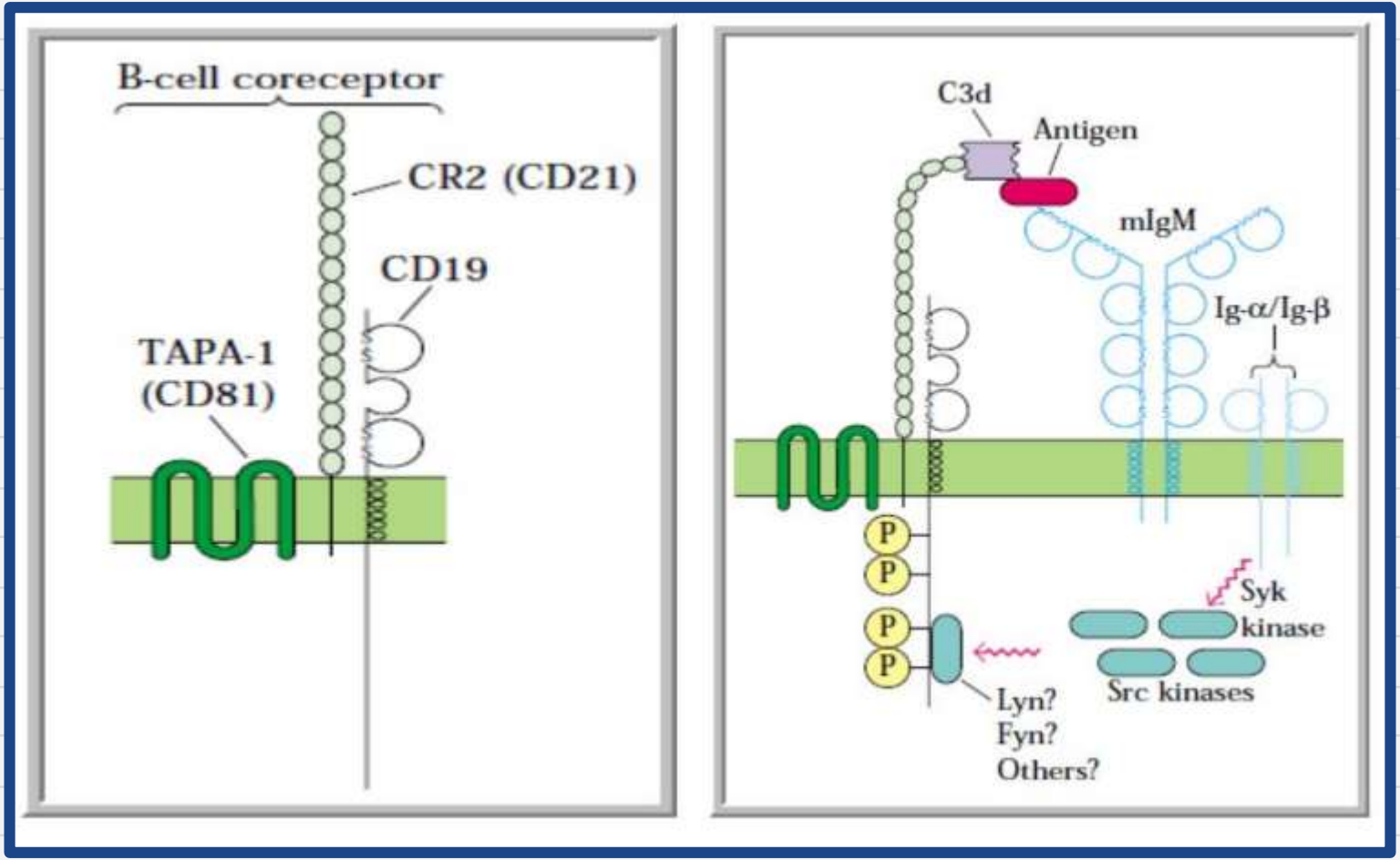
ITAM sites have been shown to interact with tyrosine kinases and to play an important role in signal transduction



Cyloplasmic tails

B-cell co receptor

- The B-cell co receptor is a complex of three proteins:CD19,
- CR2 (CD21), and TAPA-I (CD81)
- CD19, a member of the immunoglobulin superfamily, has a long cytoplasmic tail and three extracellular domains
- CR2 component is a receptor ofC3d, a breakdown product of the complement system
- TAPA-I (Target of the Anti proliferative Antibody l)
- Another molecule,CD22,which is constitutively associated with the B-cell receptor in resting B cells, delivers a negative signal that makes B-cells more difficult to activate


B-cell coreceptor
CR2 (CD21)
CD19
TAPA-1
(CD81)
C3d
Antigen
mIgM
Ig-α/Ig-β
P
P
P
P
Syk
kinase
Src kinases
Lyn?
Fyn?
Others?

Fc Receptor

Membrane glycoproteins called FC receptors (FcR) that have an affinity for the FC portion of the antibody molecule

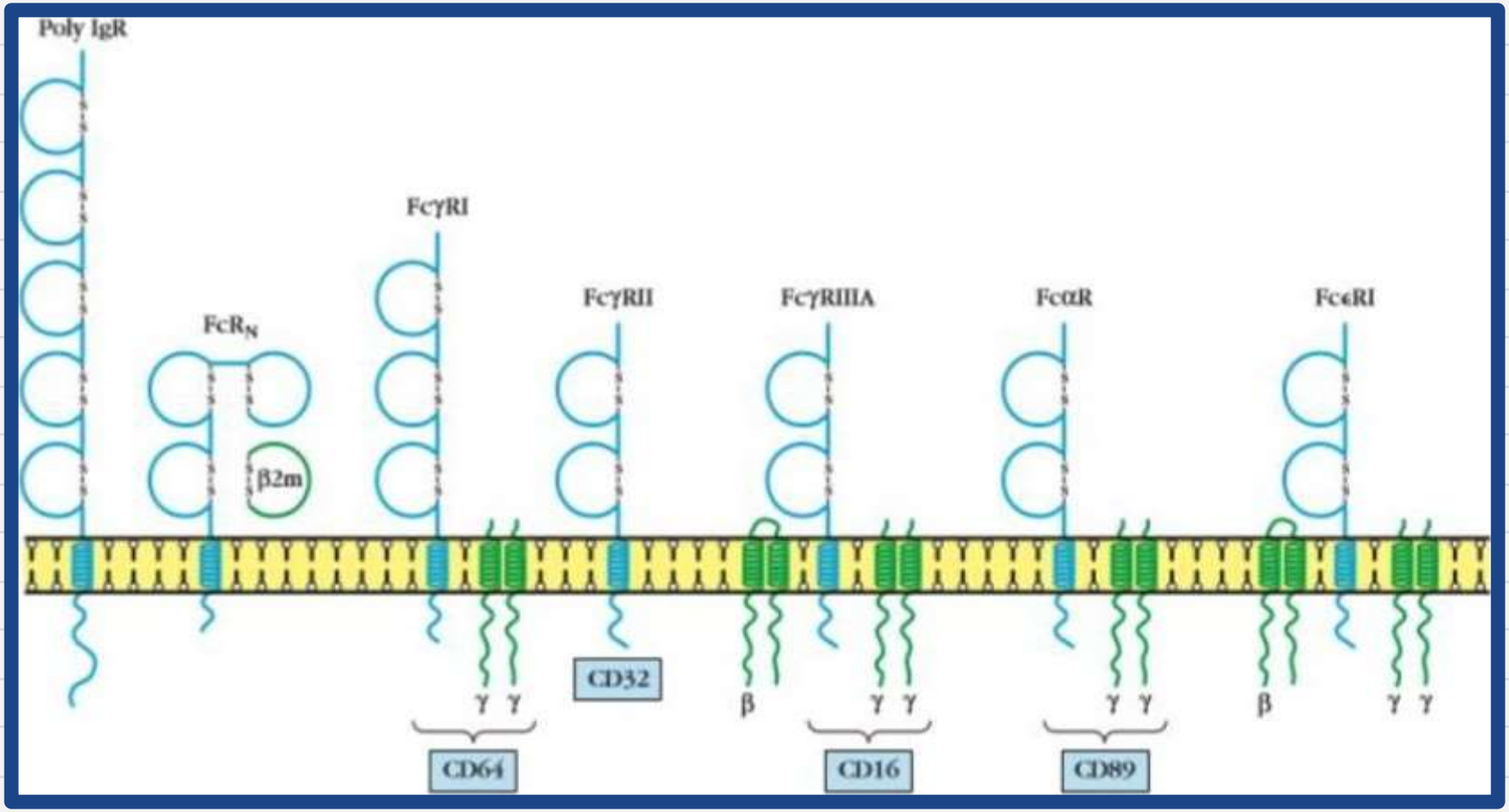
Essential for many of the biological functions of antibodies

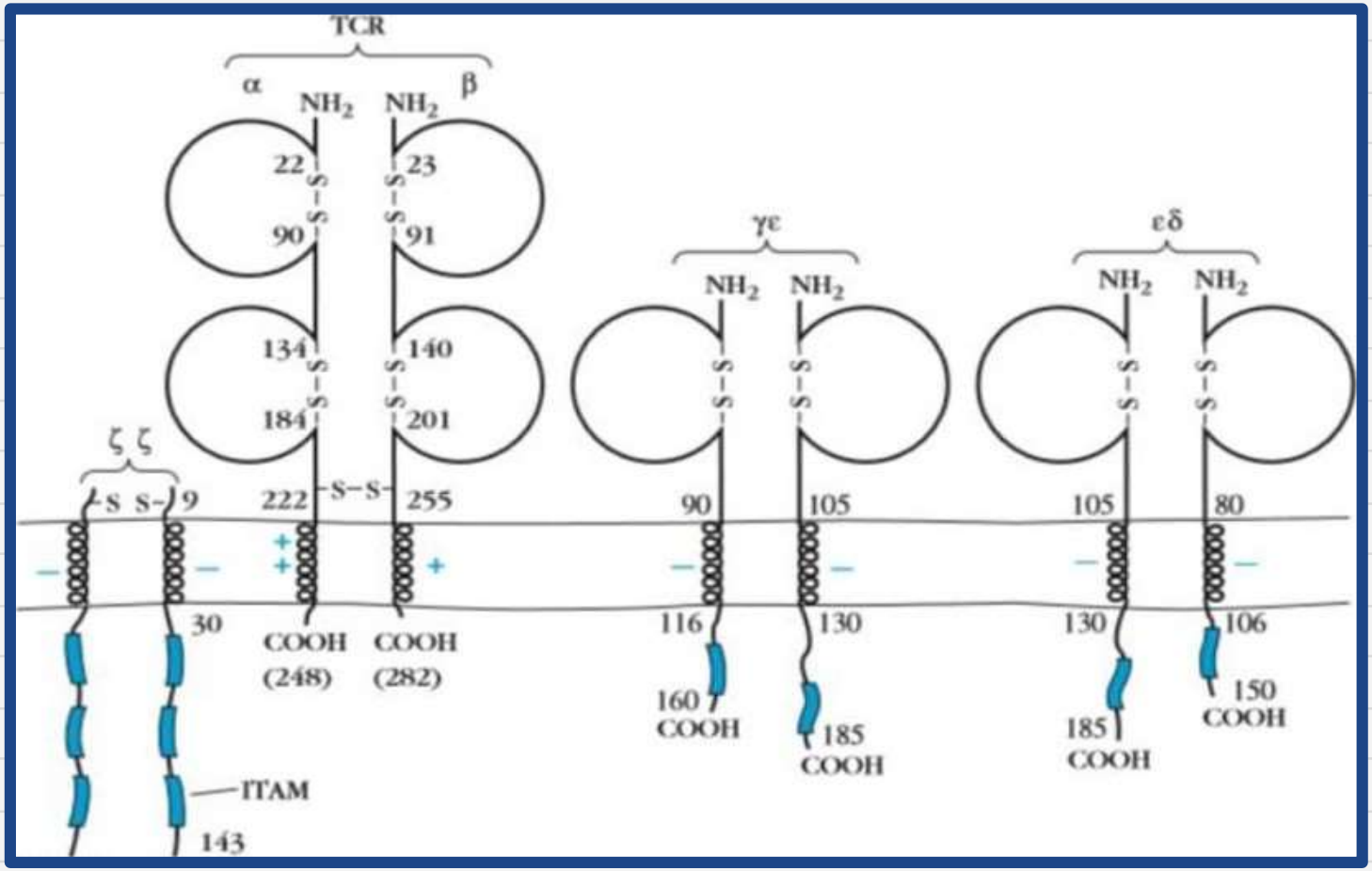
Allow passive acquisition of antibody by many cell types, including T lymphocytes, neutrophils, mast cells, eosinophils, macrophages, and natural killer cells

Triggers such effector functions as opsonisation or ADCC

Crosslinking of FC receptors generates immuno regulatory signals that affect cell activation, induce differentiation

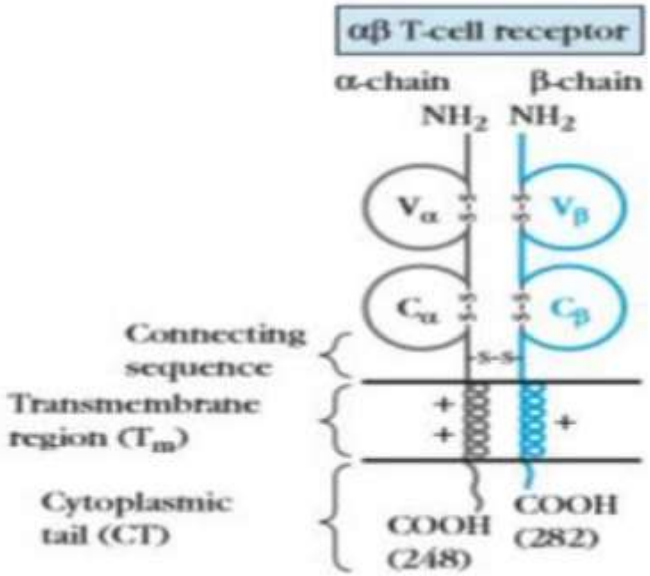
- Poly Ig receptor is essential for the transport of polymeric immunoglobulins (polymeric IgA and to some extent, pentameric IgM) across epithelial surfaces
- Neonatal FC receptor (FcRN) transfers IgGs from mother to feotus during gestation
- FcƐR that binds lgE >FcöR that binds lgD
- FcuR receptor that binds IgA
- FcgR that binds IgM
- FcyR that binds IgG


Poly IgR
FcγRI
FcRN
β2m
FcγRII
FcγRIIIA
FcαR
FcεRI
γ γ
CD32
β
γ γ
γ γ
β
γ γ
CD64
CD16
CD89


TCR
α
β
NH2
NH2
22
23
90
91
134
140
184
201
ζ ζ
S S
9
222
S–S
255
γε
NH2
NH2
90
105
εδ
NH2
NH2
105
80
30
COOH
(248)
COOH
(282)
116
130
160
COOH
185
COOH
130
106
150
COOH
185
COOH
ITAM
143

T cell Receptor

- Domain structures of uß and yö TCR heterodimers — Ig
- Each chain in a TCR has two domains containing an intrachain disulfide bond that spans 60—75 amino acids.
- Amino-terminal domain in both chains exhibits marked sequence variation, but the sequences of the remainder of each chain are conserved - one variable (V) and one constant (C)
- TCR variable domains have three hyper variable regions, which appear to be equivalent to the complementarity determining regions (CDRs)
- TCR chain contains a short connecting sequence, in which a cysteine residue forms a disulphide link with the other chain of the heterodimer
- A transmembrane region of 21 or 22 amino acids, which anchors each chain in the plasma membrane - positively charged amino acid
- A short cytoplasmic tail of 5 12 amino acids at the carboxyl terminal end



Comparison of αβ and γδ T cells

Feature	αβ T cells	γδ T cells
Proportion of $CD3^+$ cells	90–99%	1–10%
TCR V gene germ-line repertoire	Large	Small
CD4/CD8 phenotype		
$CD4^+$	~60%	<1%
$CD8^+$	~30%	~30%
$CD4^+CD8^+$	<1%	<1%
$CD4^-CD8^-$	<1%	~60%
MHC restriction	$CD4^+$: MHC class II	No MHC restriction
	$CD8^+$: MHC class I	
Ligands	Peptide + MHC	Phospholipid antigen

T-Cell Receptor Complex: TCR-CD3

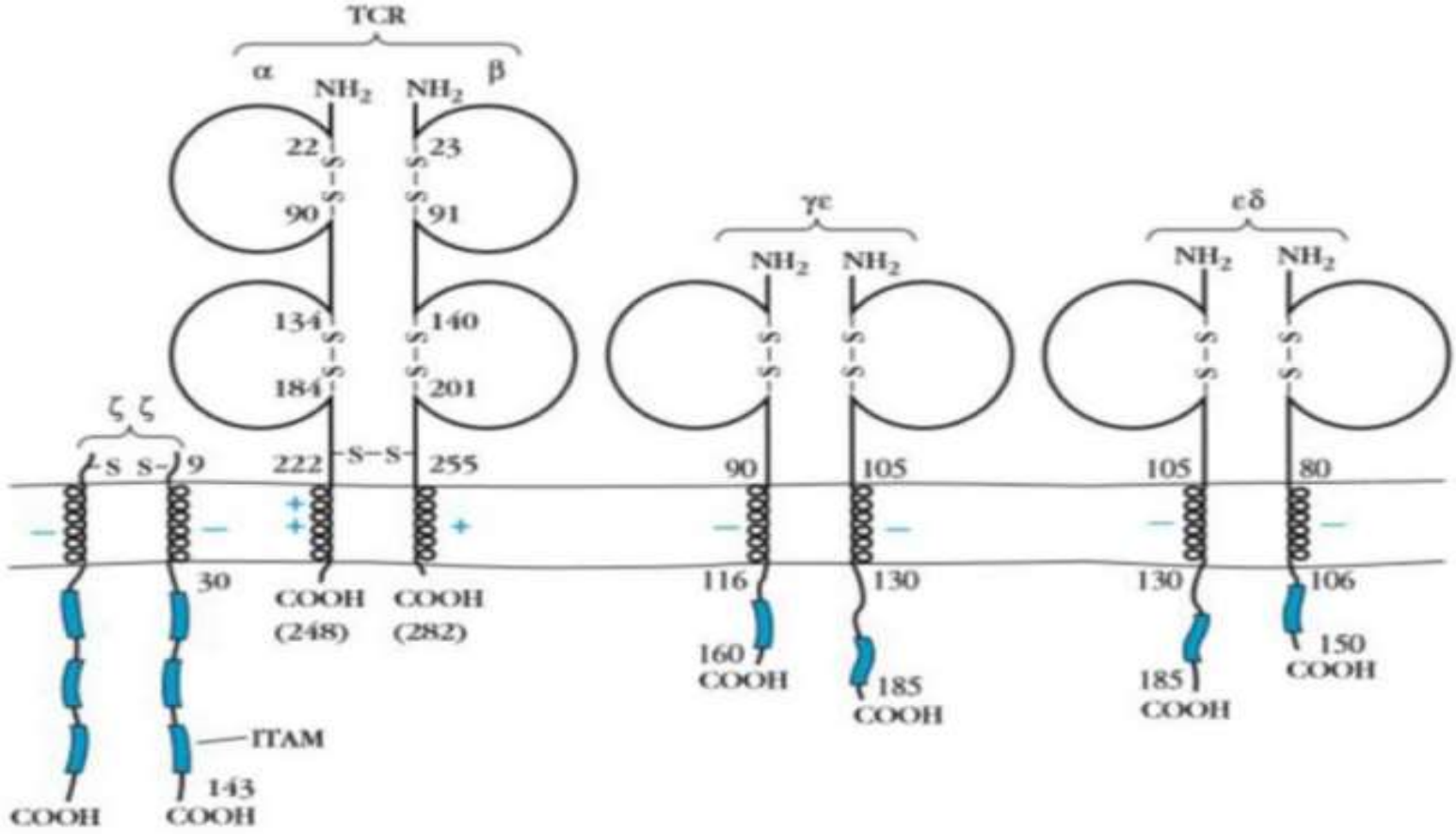
- T-cell receptor associates with CD3, forming the TCR-CD3 membrane complex.
- A complex of five invariant polypeptide chains that associate to form three dimers:

➢ heterodimer of γε

➢ heterodimer of δε

➢ homodimer of ζζ or heterodimer of ζη

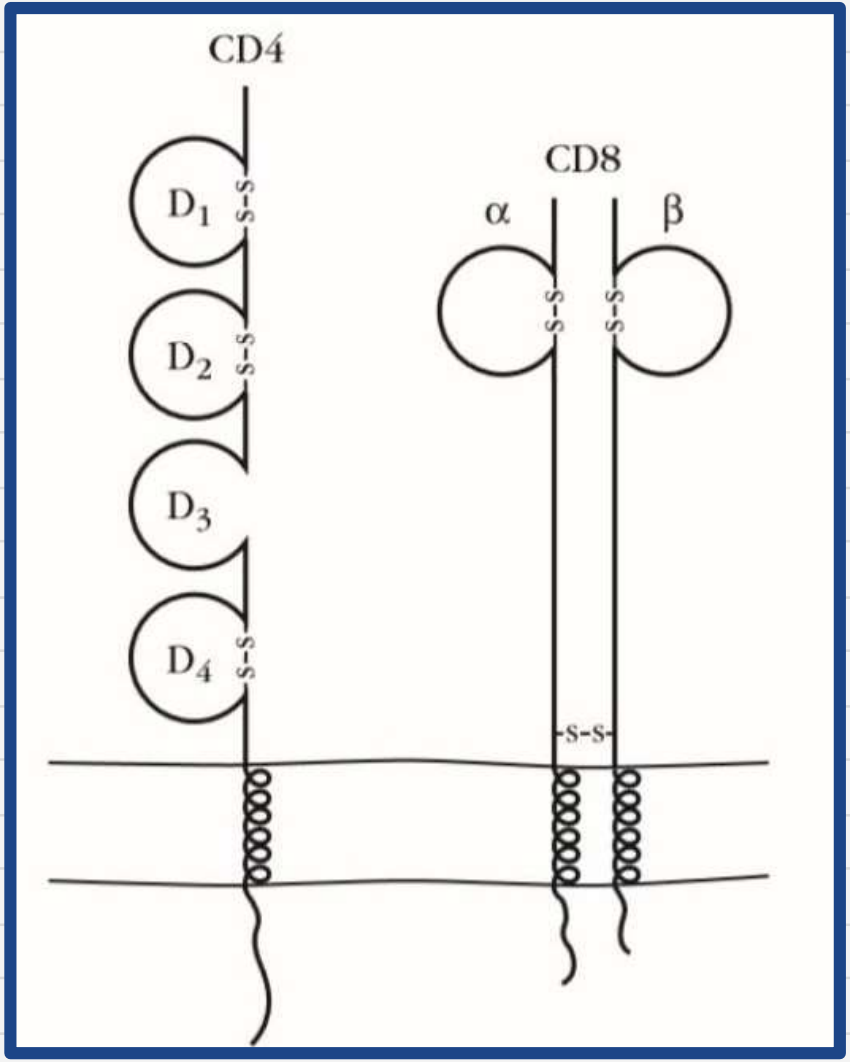
- γ, δ ,and ε chains of CD3 contain an immunoglobulin- like extracellular domain, a transmembrane region and a cytoplasmic domain of more than 40 amino acids
- ζ chain has a very short external region of only 9 amino acids, and a long cytoplasmic tail containing 113 amino acids
- Transmembrane region of all the CD3 polypeptide chains contains a negatively charged aspartic acid residue
- Cytoplasmic tails of the CD3 chains contain a motif called the immunoreceptor tyrosine-based activation motif (ITAM)
- γ, δ ,and ε chains of CD3 contain single copy of ITAM, ζ chain has 3 copies.


TCR
α
β
NH2
NH2
22
23
90
91
134
140
184
201
ζ ζ
S S
9
222
S–S
255
30
COOH
(248)
COOH
(282)
ITAM
143
COOH
COOH
γε
NH2
NH2
90
105
116
130
160
COOH
185
COOH
εδ
NH2
NH2
105
80
130
106
150
COOH
185
COOH

CD4 and CD8 co receptors

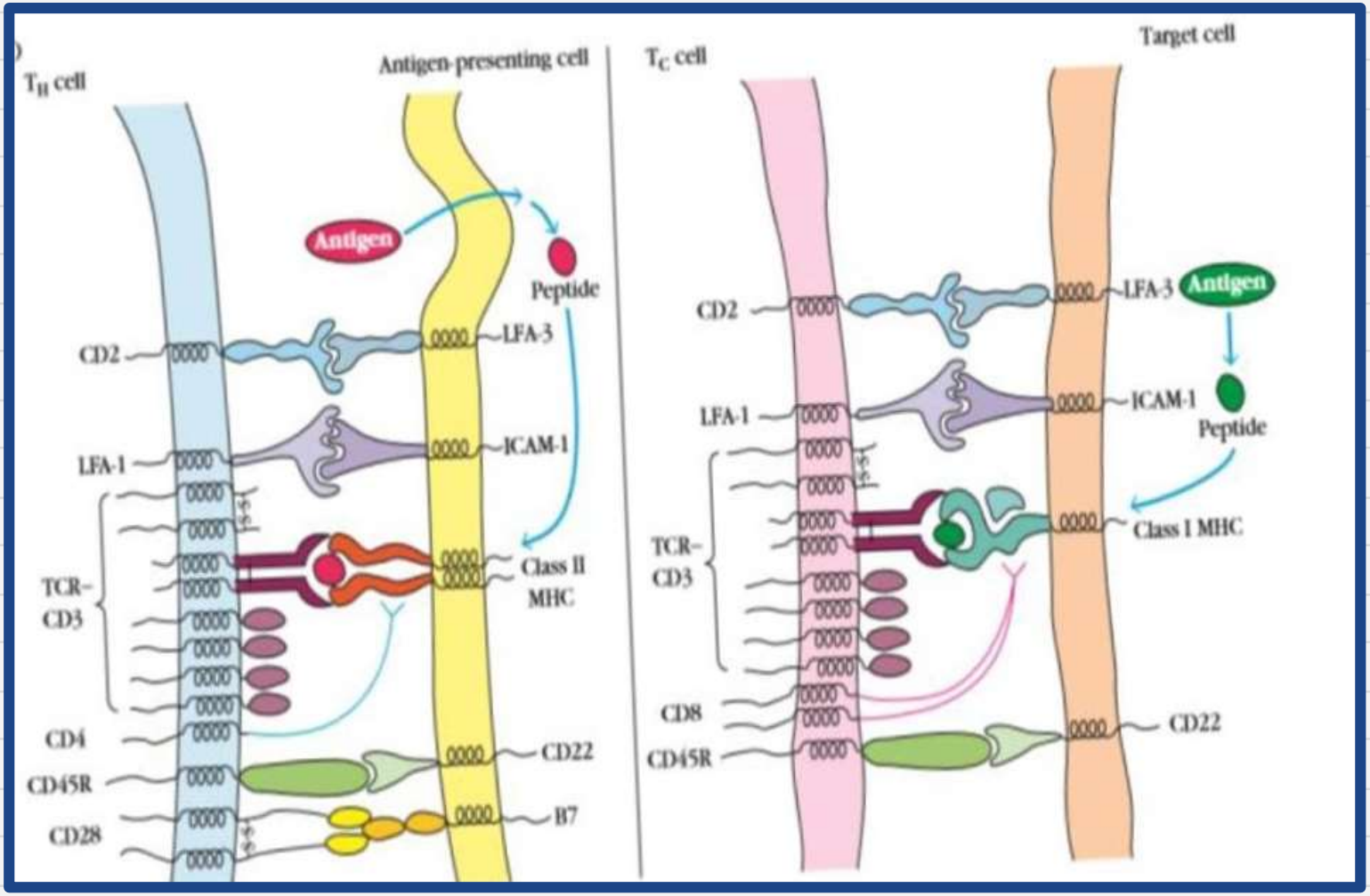
- CD4 and CD8 are classified as co receptors based on their abilities to recognize the peptide-MHC complex and their roles in signal transduction
- CD4+ T cells recognize antigen that is combined with class Il MHC molecules
- A monomeric membrane glycoprotein (55-kDa) that contains four extracellular immunoglobulin-like domains (DI—D4)
- A hydrophobic transmembrane region, and a long cytoplasmic tail containing three serine residues that can be phosphorylated

- CD8+ T cells recognize antigen that is combined with class I MHC molecules
- A di sulfide-linked u13 heterodimer or of an alpha alpha homo dimer
- u and ß chains of CD8 are small glycoproteins of approx 30—38 kDa
- Each chain consists of a single extracellular immuno globulin like domain, a hydrophobic transmembrane region, and a cytoplasmic tail containing 25—27 residues, several of which can be phosphorylated
- The extracellular domains of CD4 and CD8 bind to the conserved regions of MI--IC molecules on antigen-presenting cells (APCs) or target cells



TCR- Cell Adhesion Molecule

- The affinity of T-cell receptors for peptide-MHC complexes is low to moderate
- T-cell interactions do not depend solely on binding by the TCR
- Cell-adhesion molecules strengthen the bond between a T cell and an antigen-presenting cell or a target cell
- CD2, LFA-I, CD28, and CD45R bind independently to other ligands on antigen-presenting cells or target cells
- Once cell-to-cell contact has been made by the adhesion molecules, the T-cell receptor may scan the membrane for peptide-MHC complexes.


T_H cell
Antigen-presenting cell
Antigen
Peptide
CD2
LFA-3
LFA-1
ICAM-1
TCR–CD3
Class II MHC
CD4
CD45R
CD22
CD28
B7
T_C cell
Target cell
LFA-3
Antigen
ICAM-1
Peptide
Class I MHC
CD8
CD45R
CD22

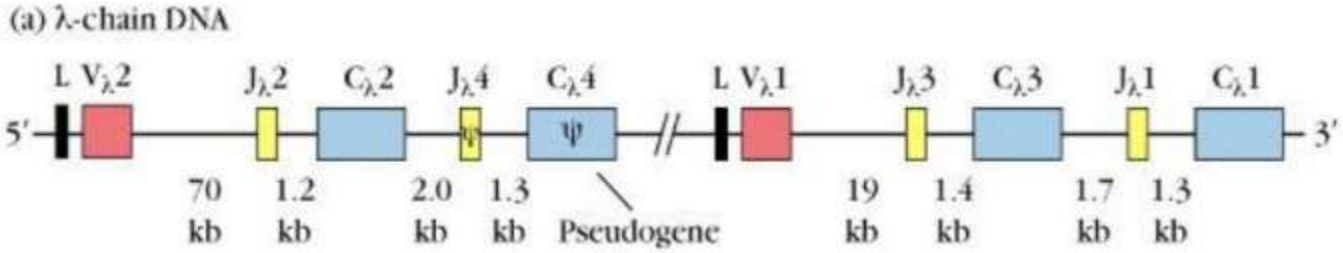
ORGANIZATION OF IMMUNOGLOBULIN GENES

- Germ-line DNA contains multiple gene segments encode portions of a single Ig H or L chain
- V region= Unique amino acids sequence
- C region= limited number of variable sequences
- During B-cell maturation in the bone marrow, Ig gene segments are rearranged and generated into more than 10^{10} combinations of V region
- Each B cell has a unique combination and is antigenically committed to a specific epitope
- After antigenic stimulation, further rearrangement of C-region gene segments can generate changes in isotypes without changing the specificity of Ig

Multigene organization

	CHROMOSOME	
Gene	Human	Mouse
λ Light chain	22	16
κ Light chain	2	6
Heavy chain	14	12

Lambda Chain MultiGene Family

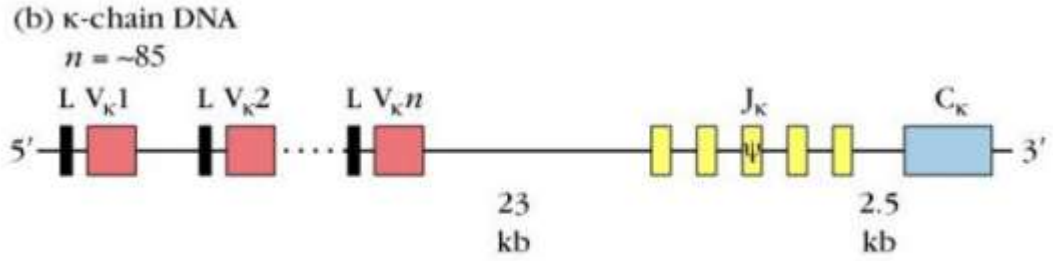


V region: 2 **Vλ gene segments**
4 Jλ gene segments(13 aa)

C region: 4 **Cλ gene segments – λ1, λ2, λ3 subtypes (mouse)**

In humans: 31 **Vλ, 4 Jλ and 7 Cλ segments**

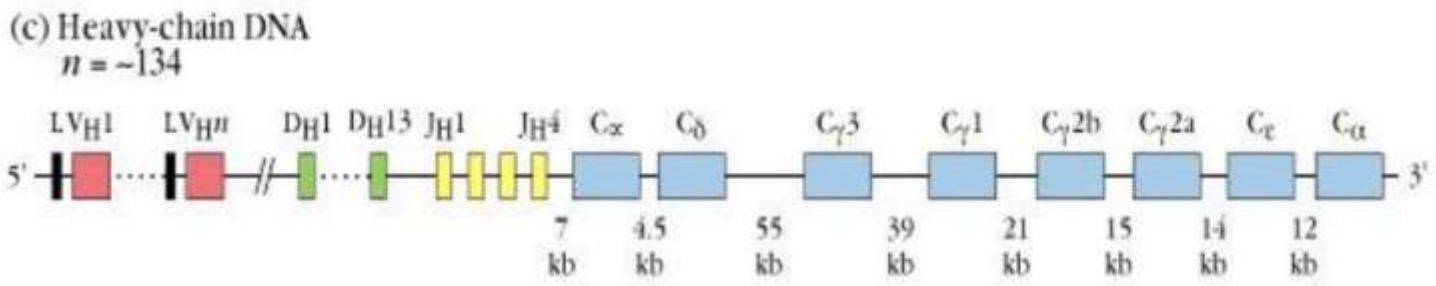
Kappa Chain MultiGene Family



V region: 85 **Vκ gene segments**
5 **Jκ gene segments**
C region: 1 **Cκ gene segments (mouse)**

In humans: 40 **Vκ, 5 Jκ and 1 Cκ segments**

H Chain MultiGene Family

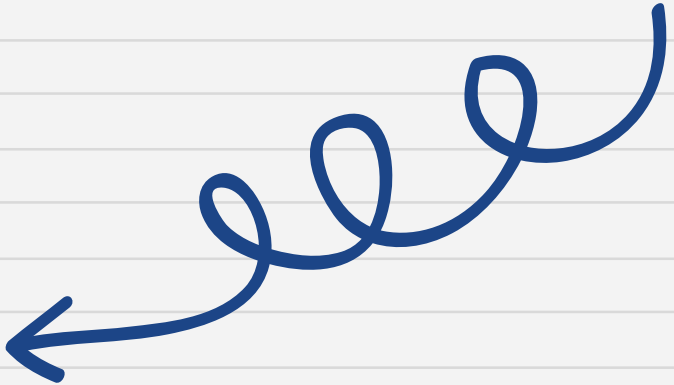


V region: 134 **VH gene segments**
13 **DH gene segments**
4 **JH gene segments**
C region: 8 **CH gene segments(mouse)**

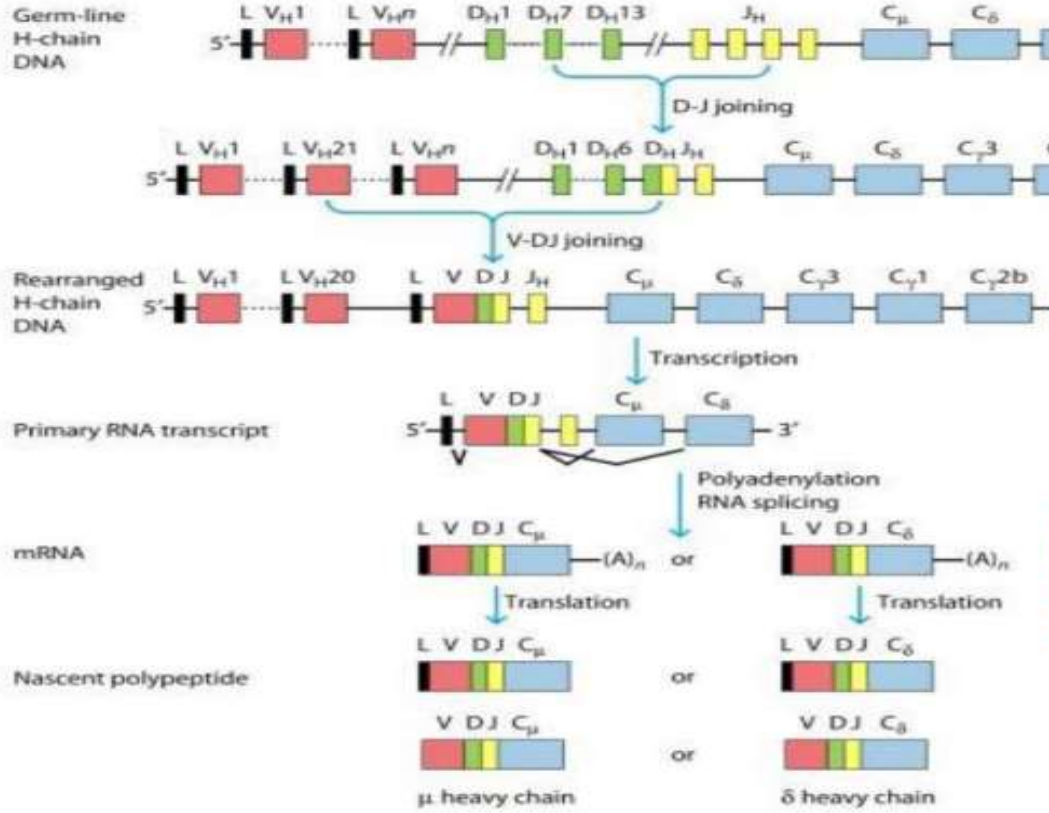
In humans: 51 **VH, 27 DH, 6 JH and 5 CH segments**

V Region Gene Rearrangement

- The H-chain V-region genes rearrange first, then the L-chain V-region genes
- The rearrangements are random events

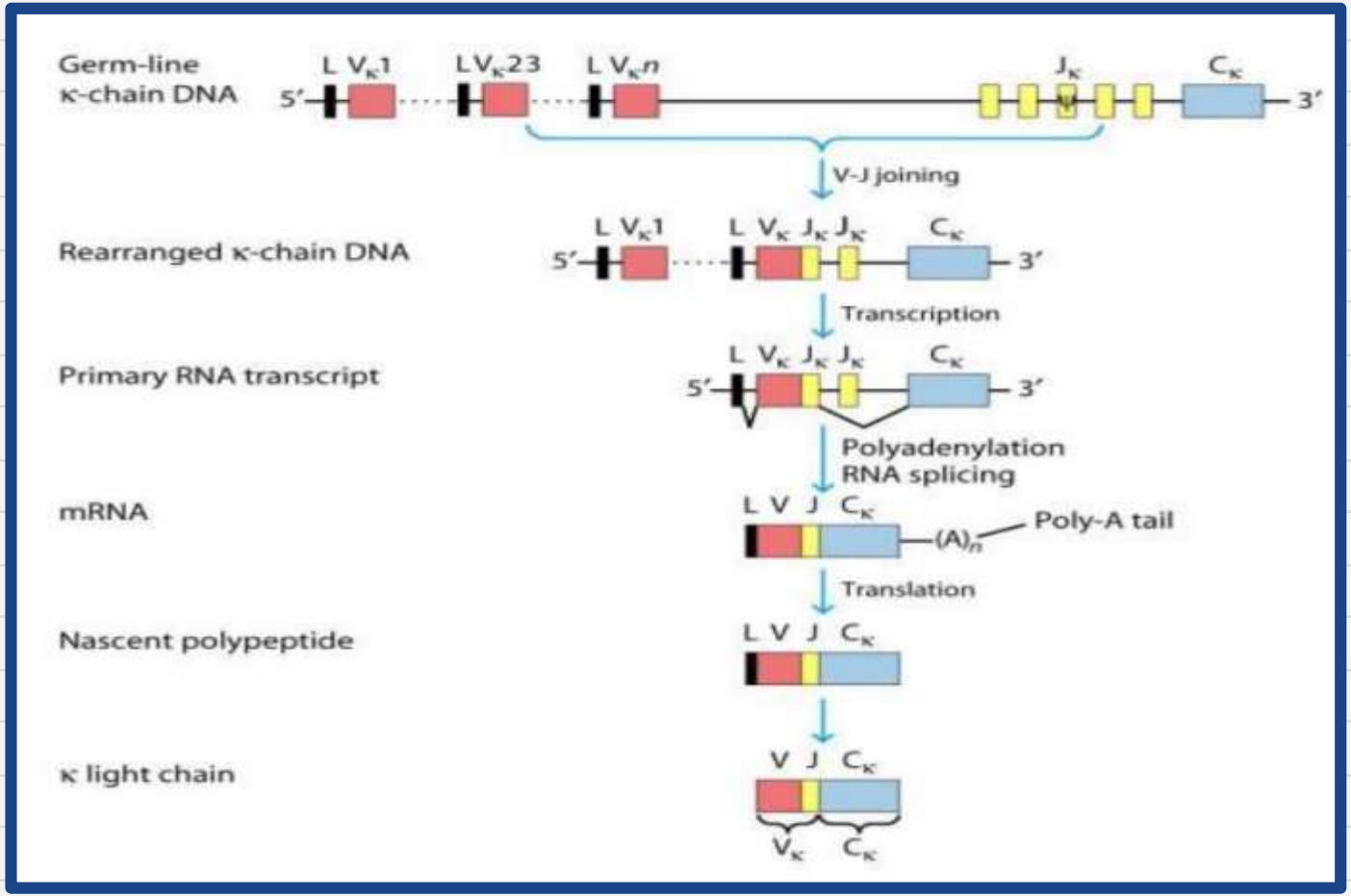


Heavy Chain gene Rearrangement



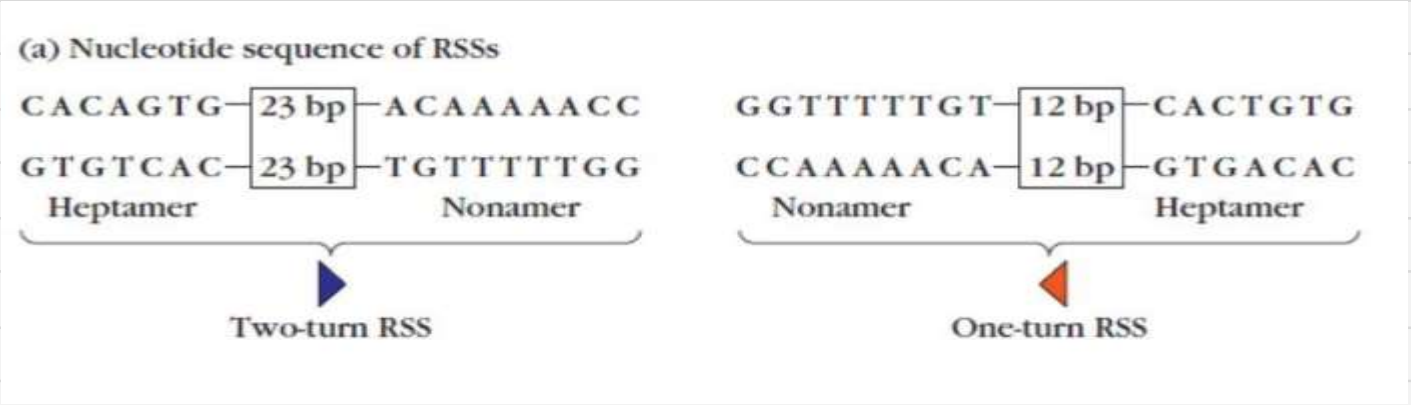
A mature , immunocompetent B cell expresses both IgM & IgD with identical antigenic specificity on its surface.

Kappa Light Chain Recognition

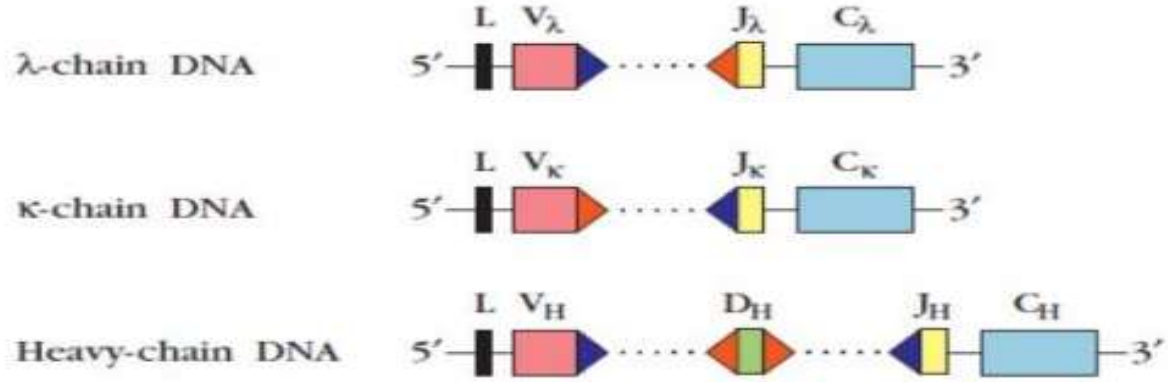


Mechanism

- Two unique recombination signal sequences (RSSs) flanking each germ-line V, D, and J gene segment
- One-turn RSS: located at 3' to each VIC, 5' to each JR, and both sides of each DH gene segment
- Two-turn RSS: located at 3' to each V), & VH and 5' to each
- JR & JH gene segment



(b) Location of RSSs in germ-line immunoglobulin DNA



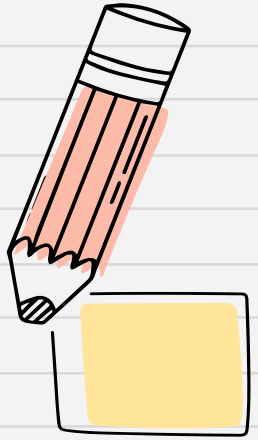
Signal sequences having a one-turn spacer (12 bp) can join only with sequences having a two-turn spacer (23 bp) (one-turn/two turn joining rule)

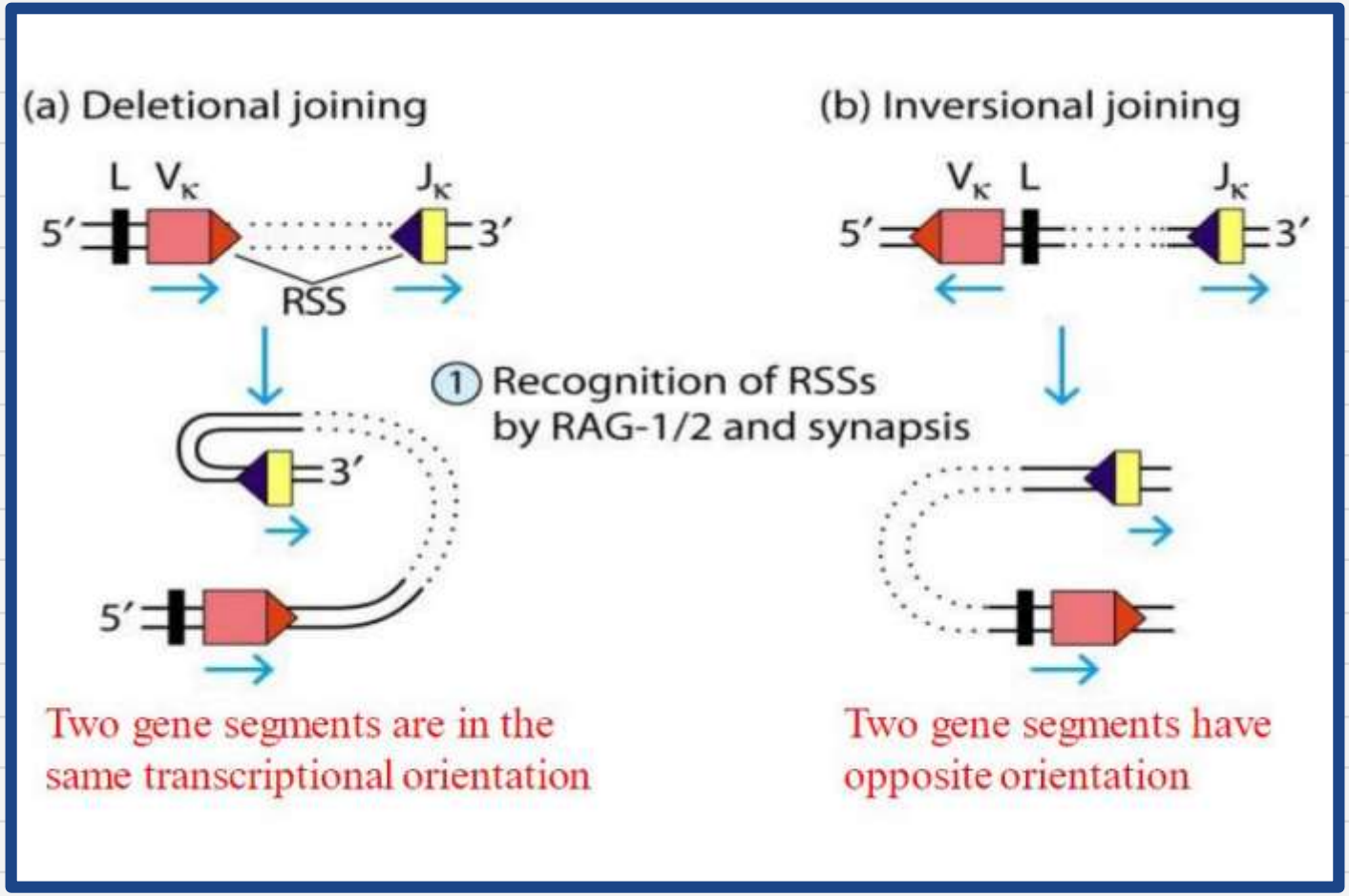
This joining rule ensures that a VL segment joins only to a JL segment and not to another VL segment

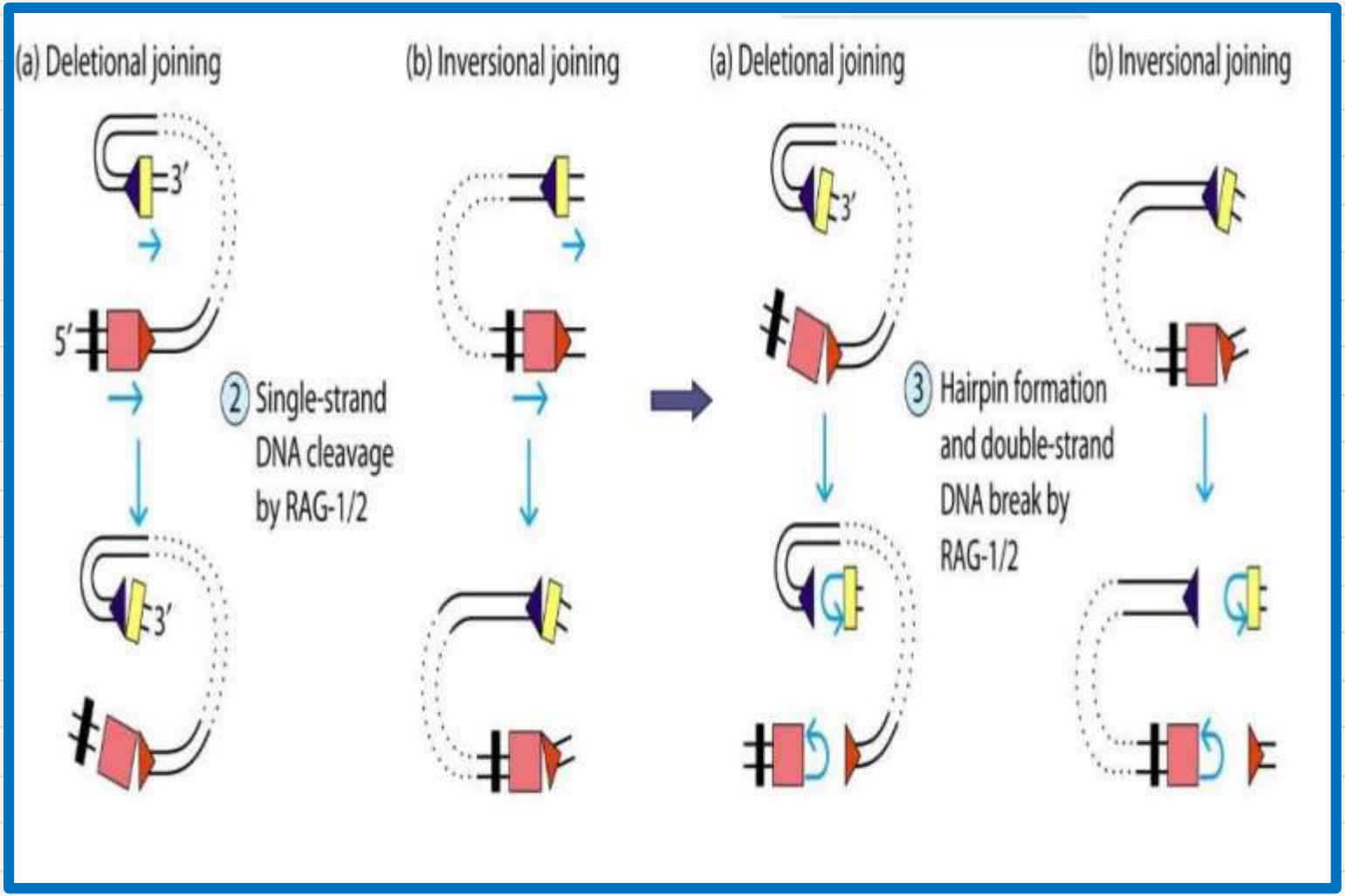
The rule likewise ensures that VH, DH, and JH segments join in proper order and that segments of the same type do not join each other

Enzymes

- Recombination-Activating Genes: RAG-I, RAG-2 - mediate V-(D)-J joining
- TdT: terminal deoxy nucleotidyl transferase
- DSBR: double strand break repair enzymes

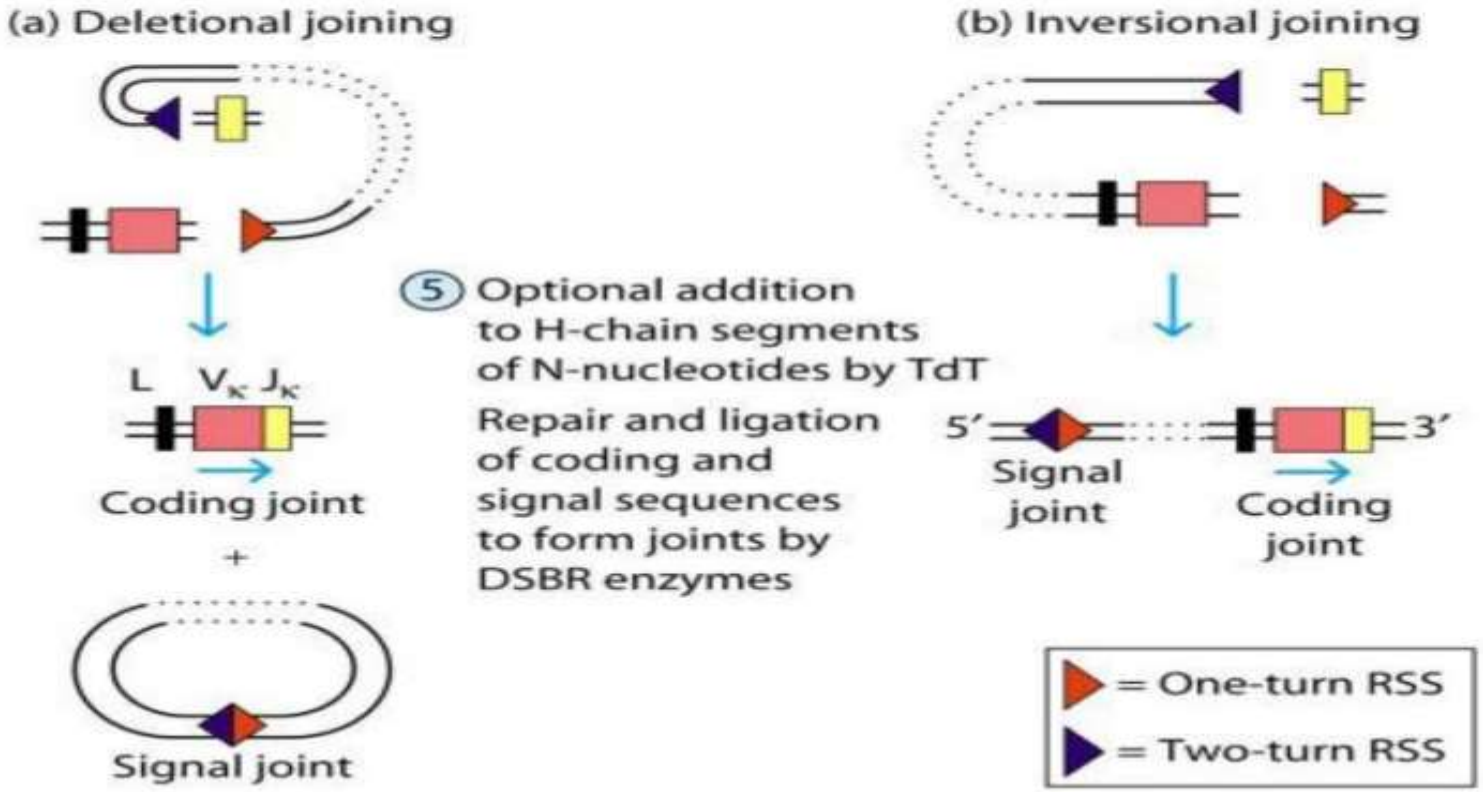



(a) Deletional joining
(b) Inversional joining
L
V_κ
J_κ
5′
3′
RSS
① Recognition of RSSs by RAG-1/2 and synapsis
Two gene segments are in the same transcriptional orientation
Two gene segments have opposite orientation


(a) Deletional joining
(b) Inversional joining
(a) Deletional joining
(b) Inversional joining
3′
5′
3′
3′
2 Single-strand DNA cleavage by RAG-1/2
3 Hairpin formation and double-strand DNA break by RAG-1/2

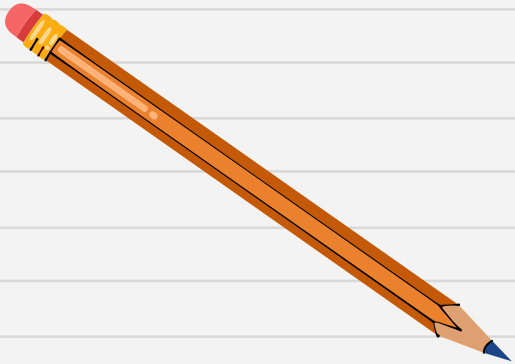
Deletion of the signal joint and intervening DNA as a circular excision product

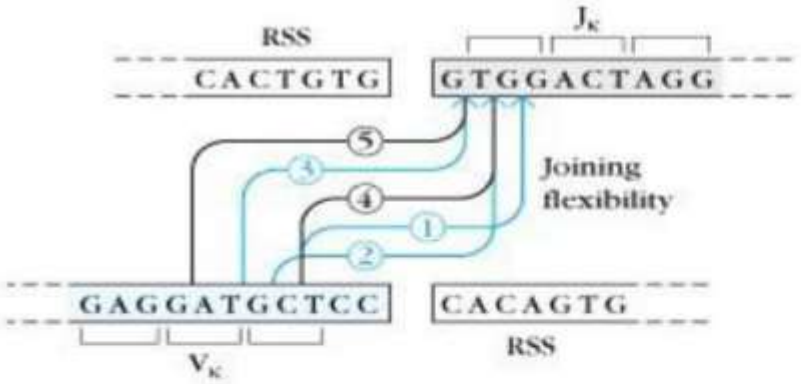
Retention of both the coding joint and the signal joint (and intervening DNA) on the chromosome



Ig-Gene Rearrangements May-B Productive or Non productive

- Non-productive rearrangement — gene segments may be joined out of phase, so that the triplet reading frame for translation is not preserved
- Numerous stop codons — interrupts translation
- Productive rearrangement - When gene segments are joined in phase, the reading frame is maintained - resulting VJ or VDJ unit can be translated in its entirety, yielding a complete antibody.





Productive rearrangements

1. Glu Asp Ala Thr Arg — GAGGATGCGACTAGG
2. Glu Asp Gly Thr Arg — GAGGATGGGACTAGG
3. Glu Asp Trp Thr Arg — GAGGATTGGACTAGG

Nonproductive rearrangements

4. Glu Asp Ala Asp Stop — GAGGATGCGGACTAGG
5. Glu Val Asp Stop — GAGGTGGACTAGG

Imprecise Joining

- Productive and nonproductive rearrangements

- Productive rearrangement in one allele is enough

- If rearrangement is not produced, the B cell dies by apoptosis

Generation of Antibody Diversity

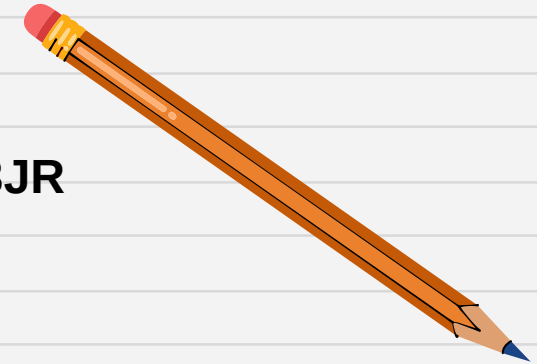
- Seven means of generation of Ab diversity:

1. Multiple germ-line V, D, and J gene segments
2. Combinatorial V-(D)-J joining
3. Junctional flexibility
4. P-region nucleotide addition (P-addition)
5. N-region nucleotide addition (N-addition)
6. Somatic hyper mutation
7. Combinatorial association of light and heavy chains

Germ Line Gene Segments

- In Humans
  a) Heavy Chain – 51 VH, 25 DH, 6JH
  b) Light Chain—40 VR and 5 JR & 31 VR and 4 JR

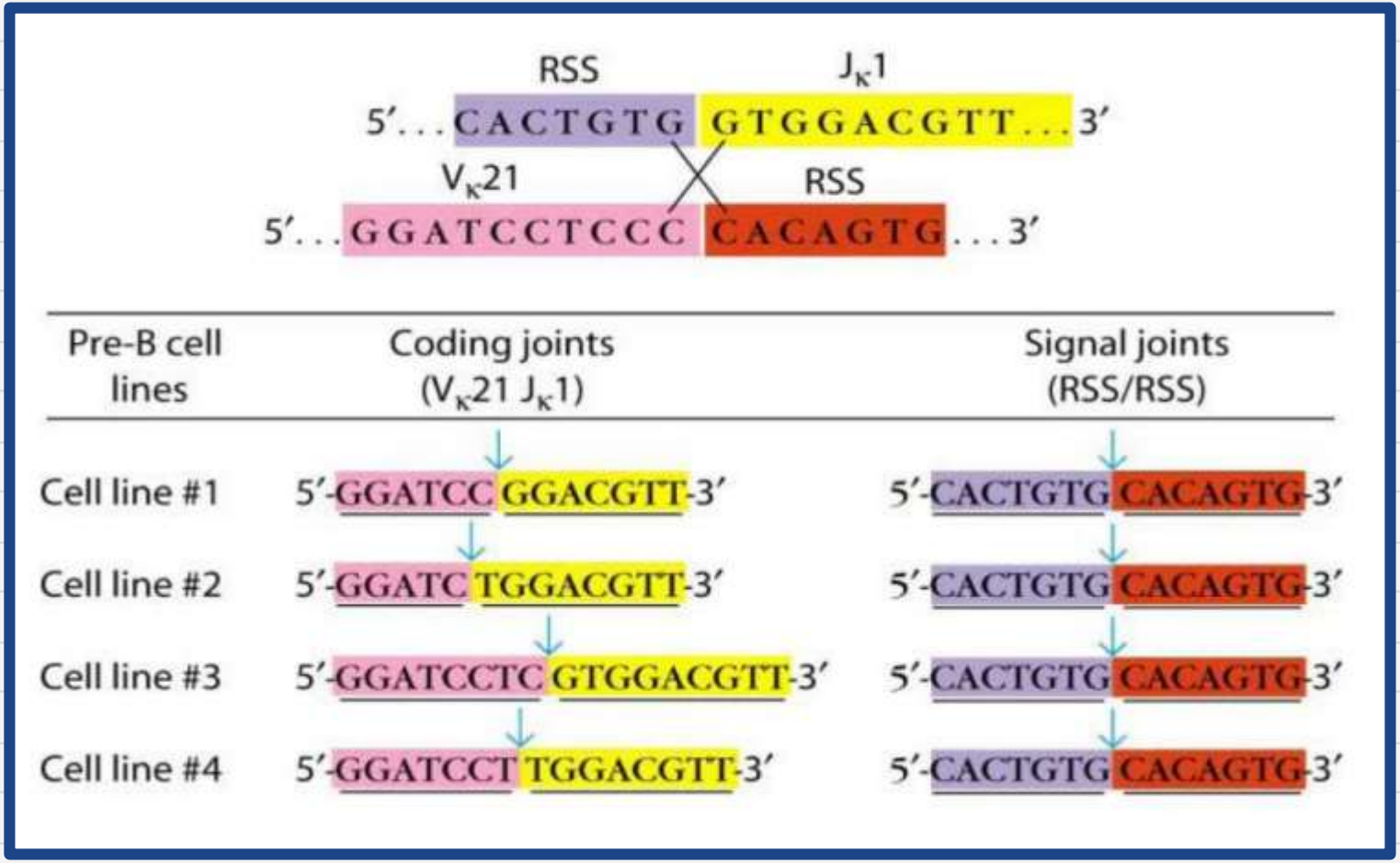
- In mice
  a) Heavy Chain – 134 VH, 13 DH, 4JH
  b) Light Chain—85Vk and 4Jk & 3 V lambda and 3JR



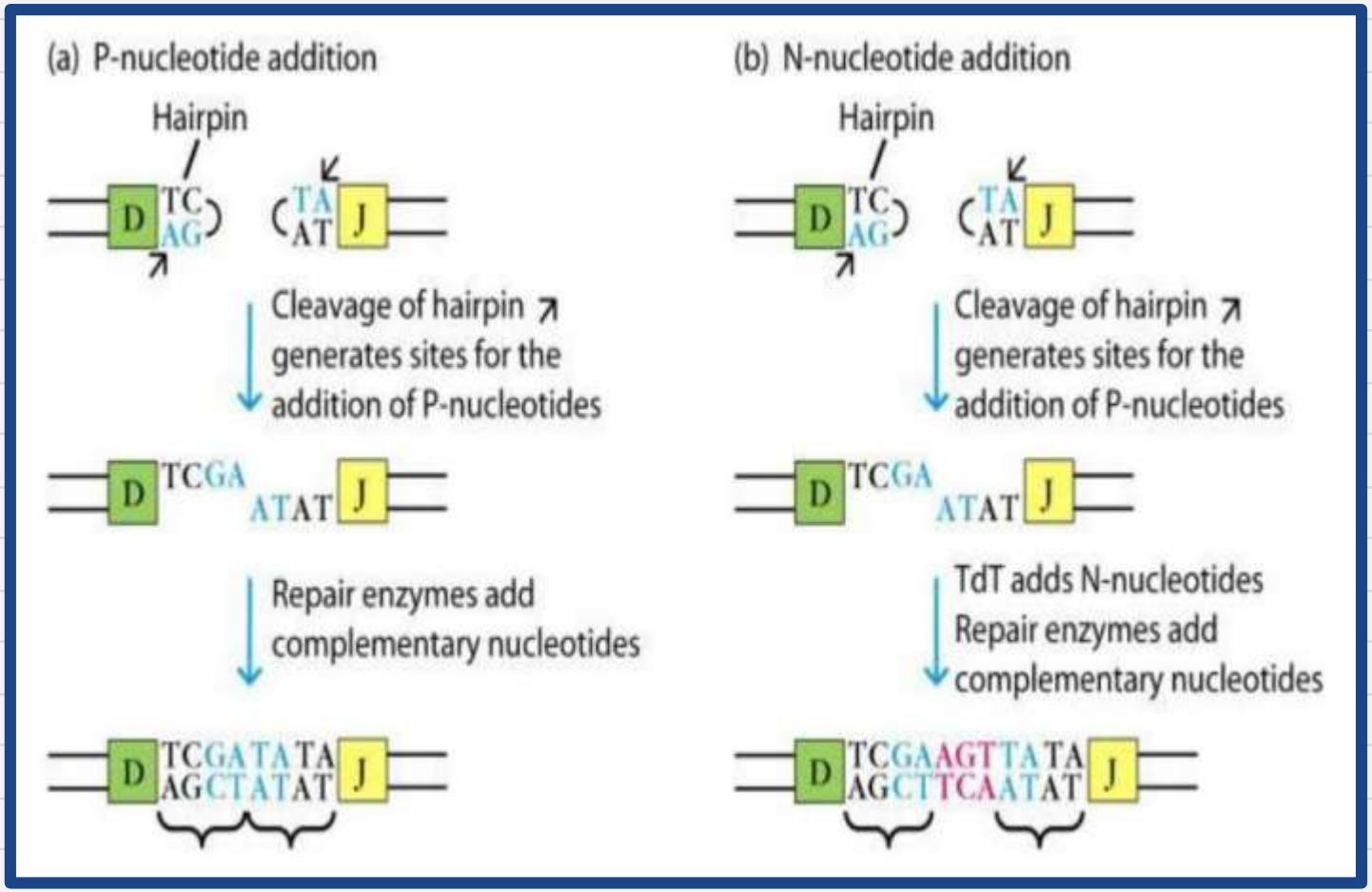
Combinatorial V-J and V-D-J Joining

Multiple germ-line segments	Heavy chain	LIGHT CHAINS κ	LIGHT CHAINS λ
	ESTIMATED NUMBER OF SEGMENTS IN HUMANS*		
V	51	40	30
D	27	0	0
J	6	5	4
Combinatorial V-D-J and V-J joining (possible number of combinations)	51 × 27 × 6 = 8262	40 × 5 = 200	30 × 4 = 120
Possible combinatorial associations of heavy and light chains†		8262 × (200 × 120) = 2.64×10^6	

Junctional flexibility



P and N nucleotide Addition

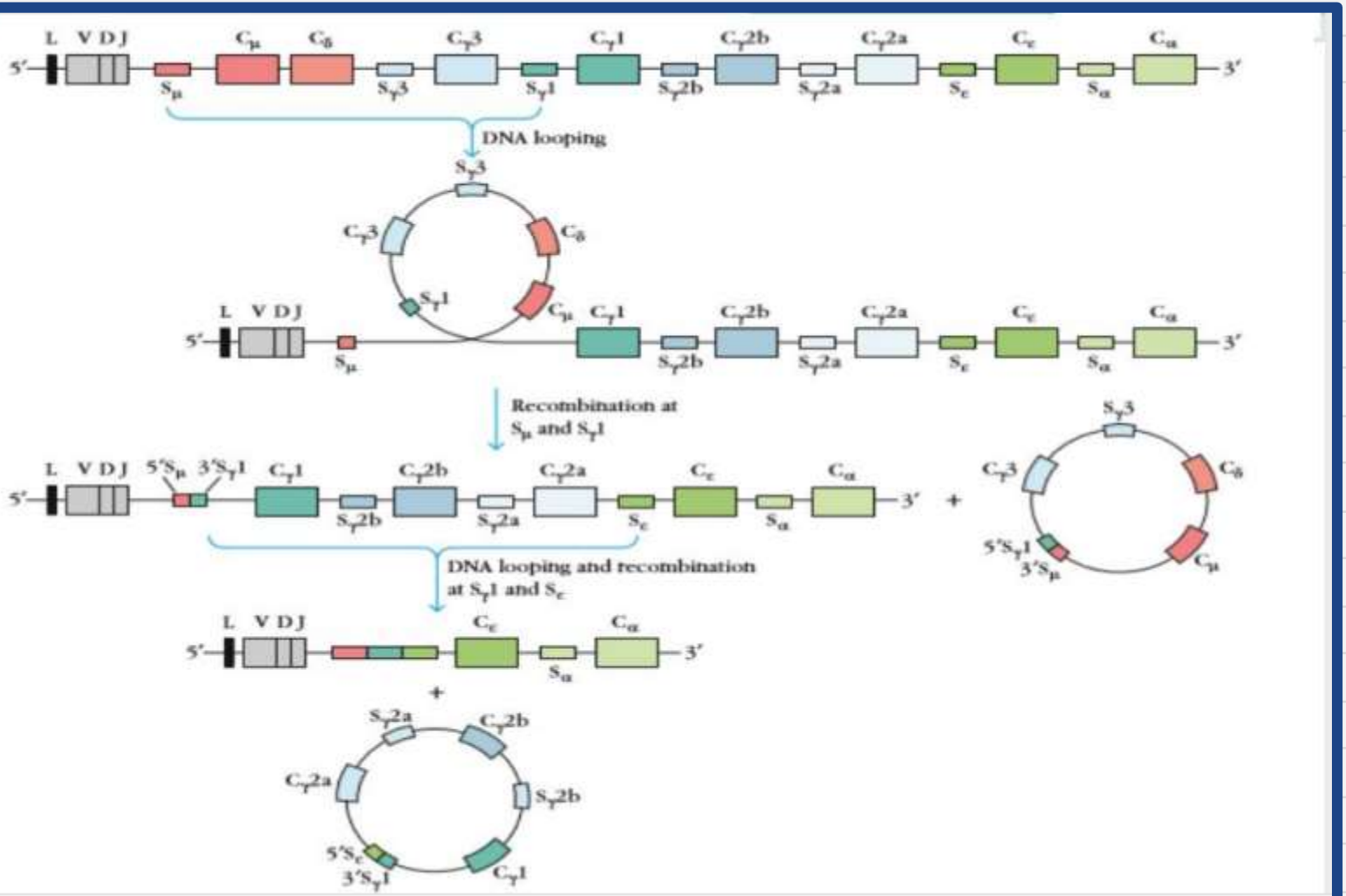


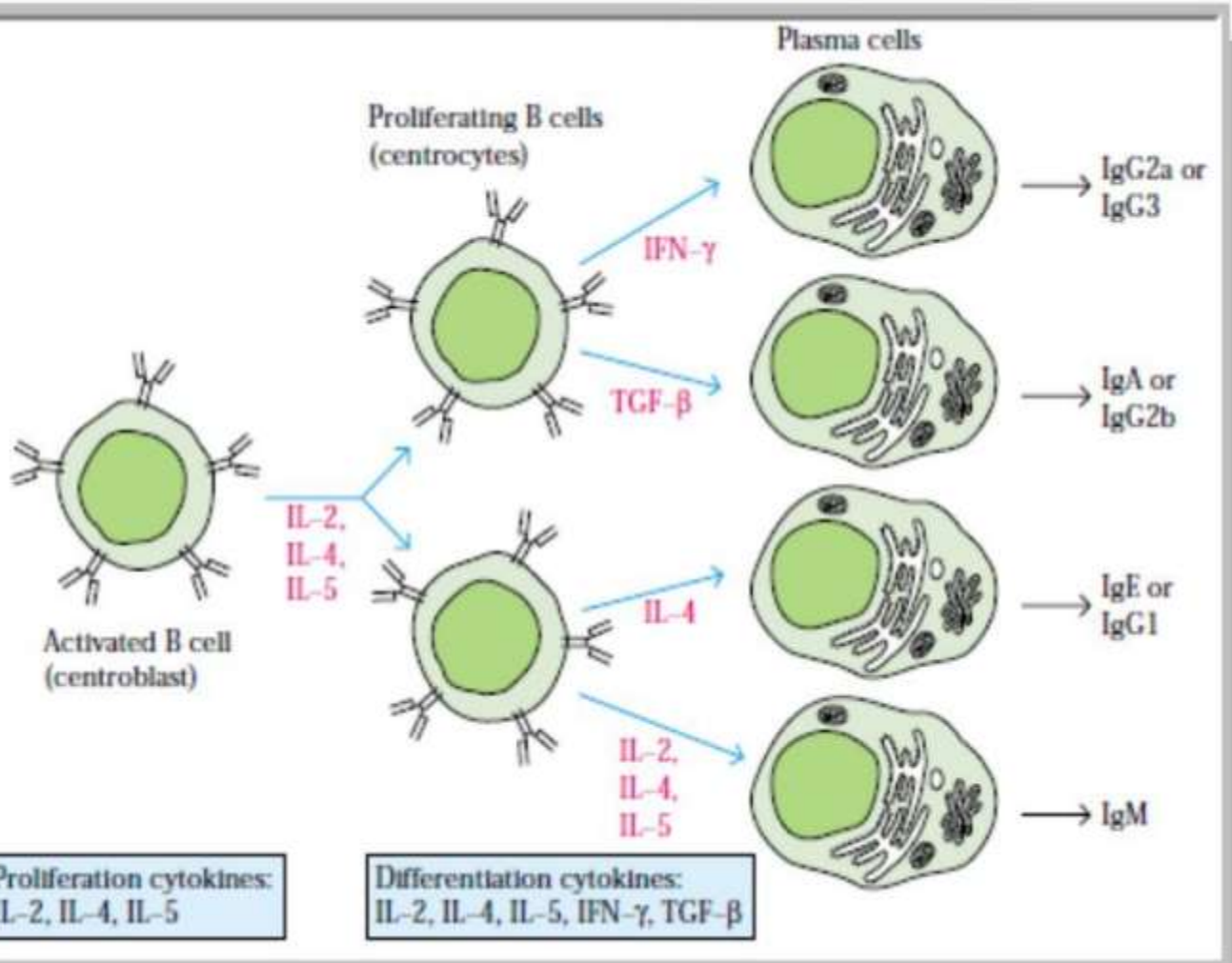
Somatic Hyper mutation

- Additional antibody diversity is generated in rearranged variable region gene units by a process called somatic hyper mutation
- Occurs within the germinal centres
- Somatic hyper mutation is targeted to rearranged V regions located within a DNA sequence — IO^{A} -3 per base pair per generation
- Nucleotides in VJ or VDJ units are replaced with alternatives, thus potentially altering the specificity of the encoded immunoglobulins
- Somatic hyper mutation is targeted to rearranged V regions located within a DNA sequence — IO^{A} -3 per base pair per generation
- Mutations are nucleotide substitutions rather than deletions or insertions
- B cells with higher-affinity Ig receptors will be preferentially selected for survival because of their greater ability to bind to the Ag Affinity Maturation

Class Switching among Constant-Region Genes

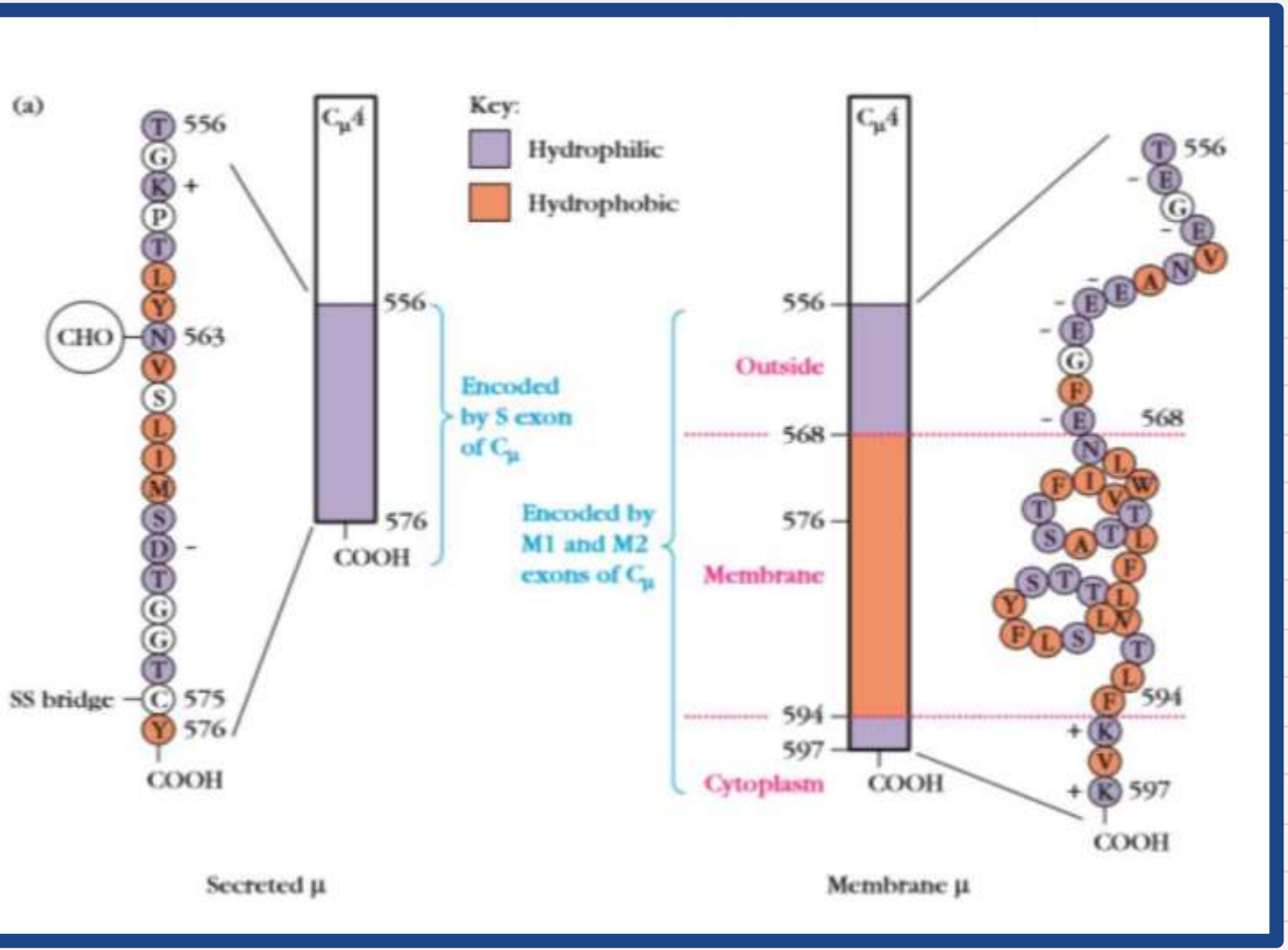
- ✓ The heavy-chain DNA can undergo a further rearrangement in which the V-D-J unit can combine with any CH gene segment.
- ✓ Class switching or iso - type switching requires
- ✓ DNA flanking region (switch regions) located 2—3 kb upstream from each CH segment, length of 2 to 10 kb as copies of short repeats (GAGCT and TGGGG)
- ✓ Switch recombinases
- ✓ Intercellular regulatory proteins - cytokines

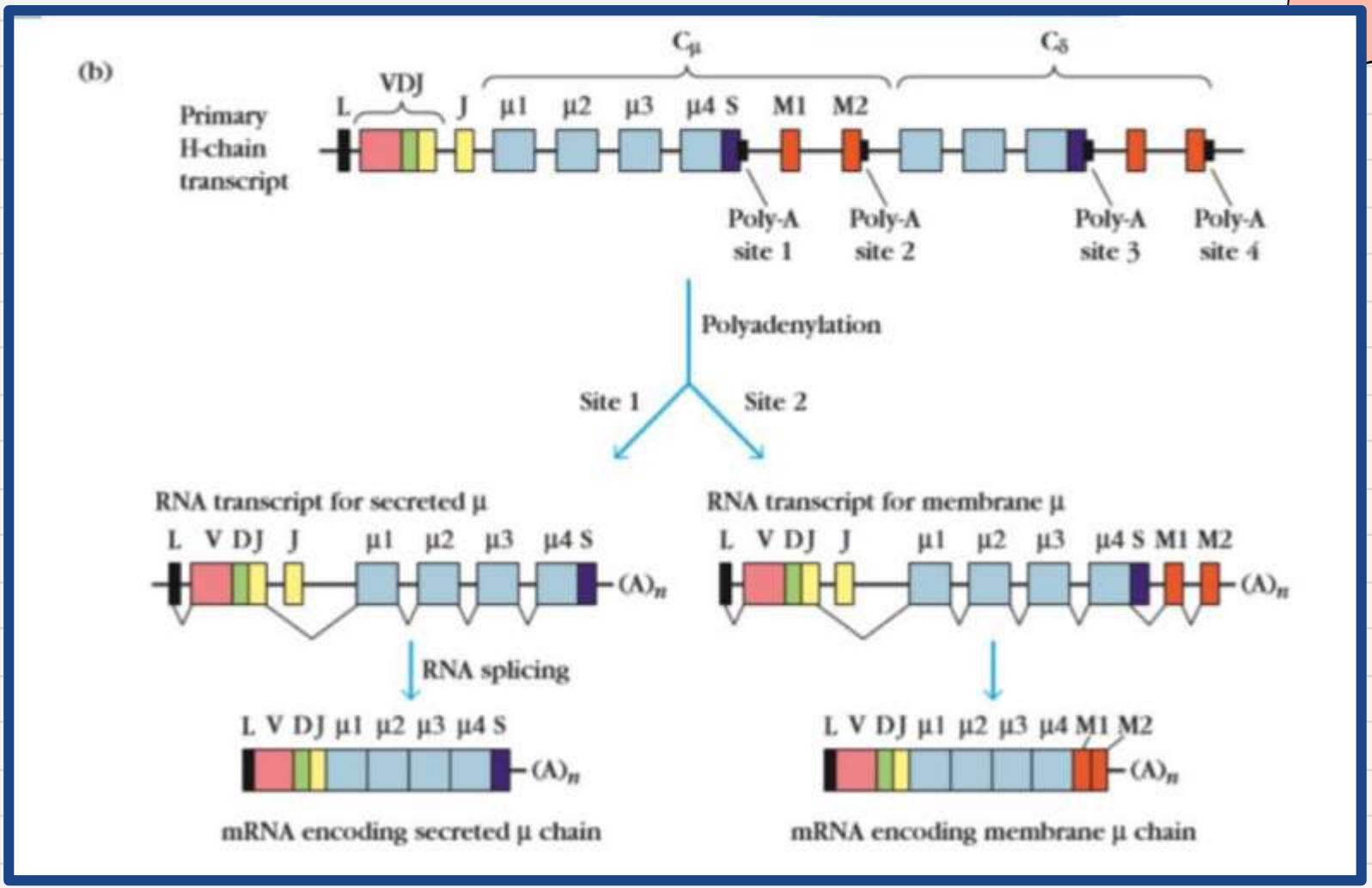

L V D J
5′
3′
DNA looping
Recombination at
Sμ and Sγ1
DNA looping and recombination
at Sγ1 and Sε
+


Plasma cells
Proliferating B cells
(centrocytes)
IFN-γ
TGF-β
IgG2a or
IgG3
IgA or
IgG2b
IL-2,
IL-4,
IL-5
Activated B cell
(centroblast)
IL-4
IgE or
IgG1
IL-2,
IL-4,
IL-5
IgM
Proliferation cytokines:
IL-2, IL-4, IL-5
Differentiation cytokines:
IL-2, IL-4, IL-5, IFN-γ, TGF-β

EXPRESSION OF MEMBRANE OR SECRETED IMMUNOGLOBULIN

- A particular immunoglobulin can exist in either membrane bound or secreted form
- The secreted form has a hydrophilic sequence of about 20 bound or secreted form amino acids in the carboxyl- terminal domain
- This is replaced in the membrane-bound form with a sequence of about 40 amino acids containing a hydrophilic segment that extends outside the cell, a hydrophobic transmembrane segment, and a short hydrophilic segment at the carboxyl terminus that extends into the cytoplasm


(a)
T 556
G
K +
P
T
L
Y
CHO N 563
V
S
L
I
M
S
D -
T
G
G
T
SS bridge C 575
Y 576
COOH
$C_\mu 4$
556
576
COOH
Key:
Hydrophilic
Hydrophobic
Encoded by S exon of C_μ
Encoded by M1 and M2 exons of C_μ
$C_\mu 4$
556
Outside
568
576
Membrane
594
597
Cytoplasm
COOH
T 556
E -
G
E -
V
N
A
E -
E
E -
G
F
E - 568
N
L
F
I
W
V
T
T
S
A
T
L
F
S
T
T
L
Y
L
V
F
L
S
T
L
F 594
K +
V
K + 597
COOH
Secreted μ
Membrane μ


(b)
Cμ
Cδ
VDJ
L
J
μ1
μ2
μ3
μ4
S
M1
M2
Primary H-chain transcript
Poly-A site 1
Poly-A site 2
Poly-A site 3
Poly-A site 4
Polyadenylation
Site 1
Site 2
RNA transcript for secreted μ
L V DJ J μ1 μ2 μ3 μ4 S
(A)n
RNA splicing
L V DJ μ1 μ2 μ3 μ4 S
(A)n
mRNA encoding secreted μ chain
RNA transcript for membrane μ
L V DJ J μ1 μ2 μ3 μ4 S M1 M2
(A)n
L V DJ μ1 μ2 μ3 μ4 M1 M2
(A)n
mRNA encoding membrane μ chain