

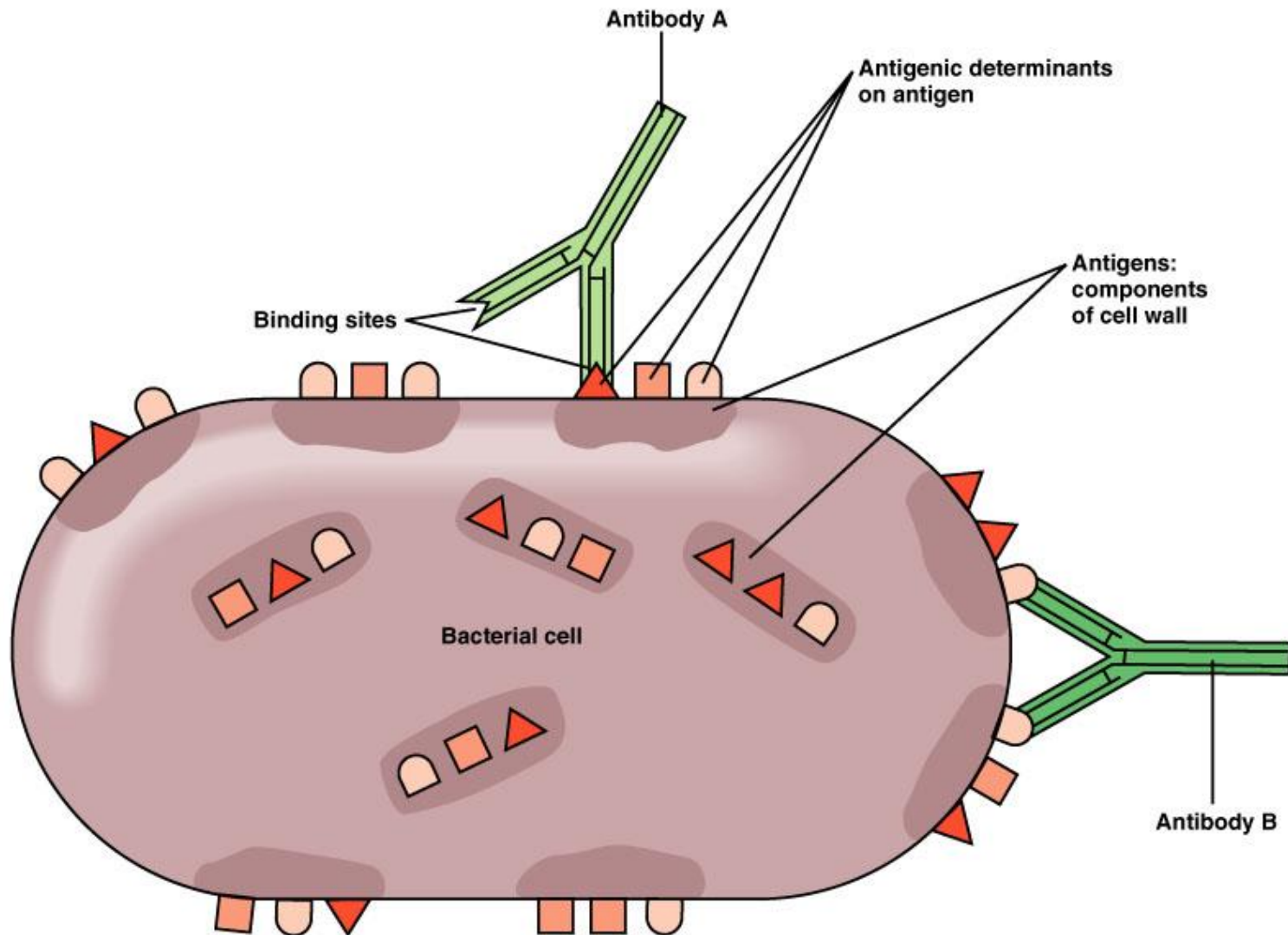
Immunoglobulins – structure and function

Production of immunoglobulins

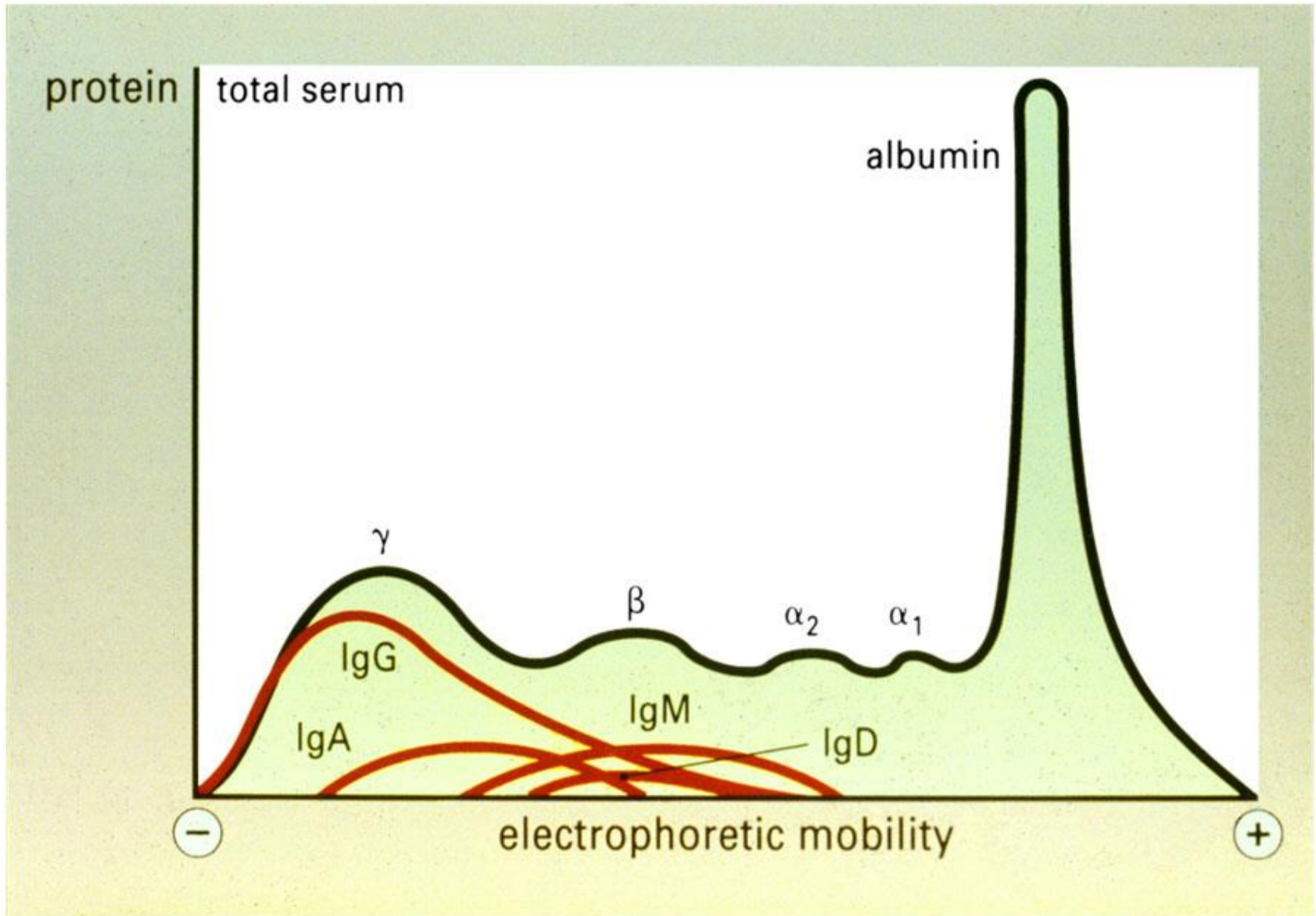
Genetic determination of immunoglobulin production

Clonal selection theory

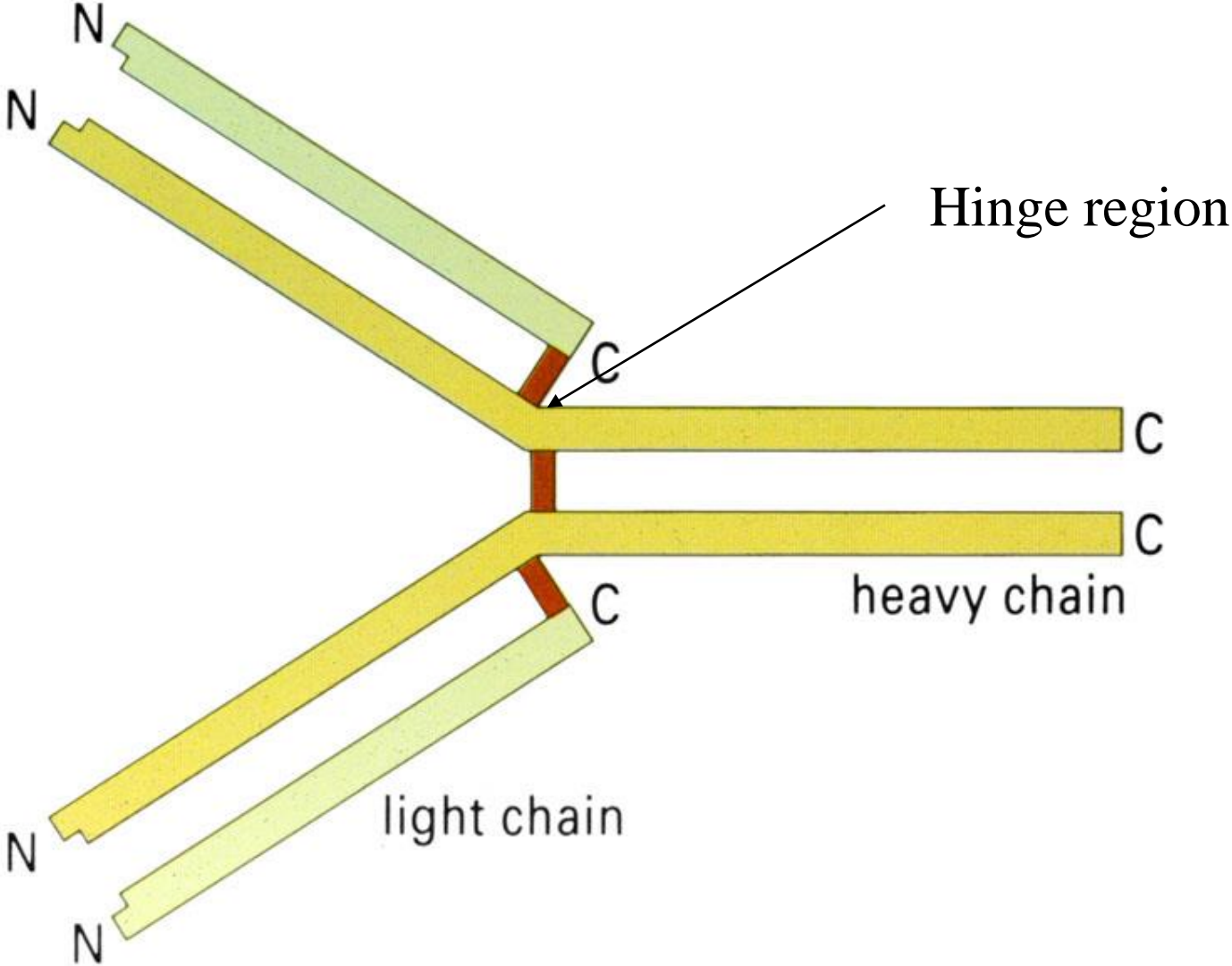
# Antigen and epitope



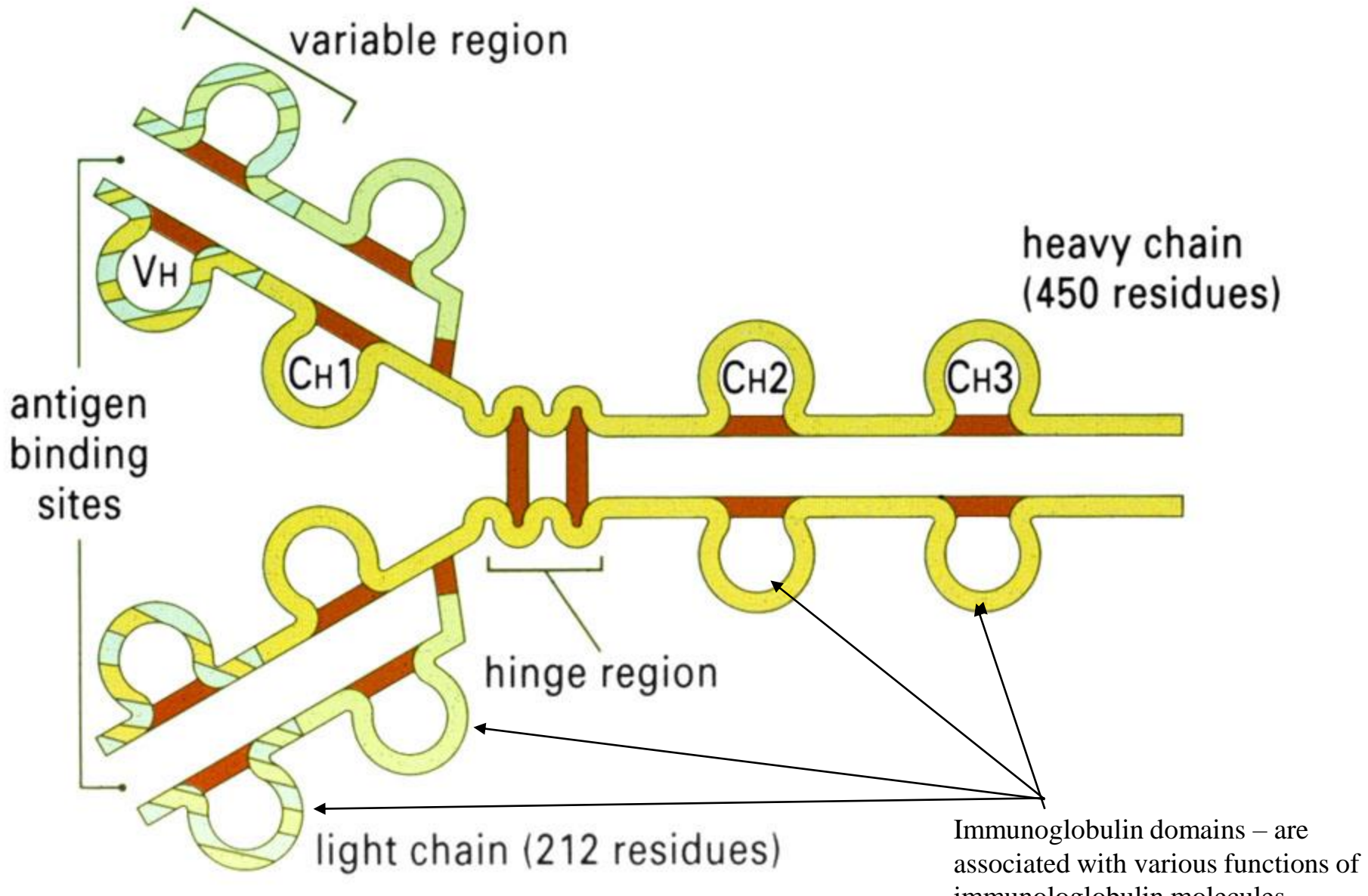
# Distribution of the major human immunoglobulins



# The basic chain structure of immunoglobulins



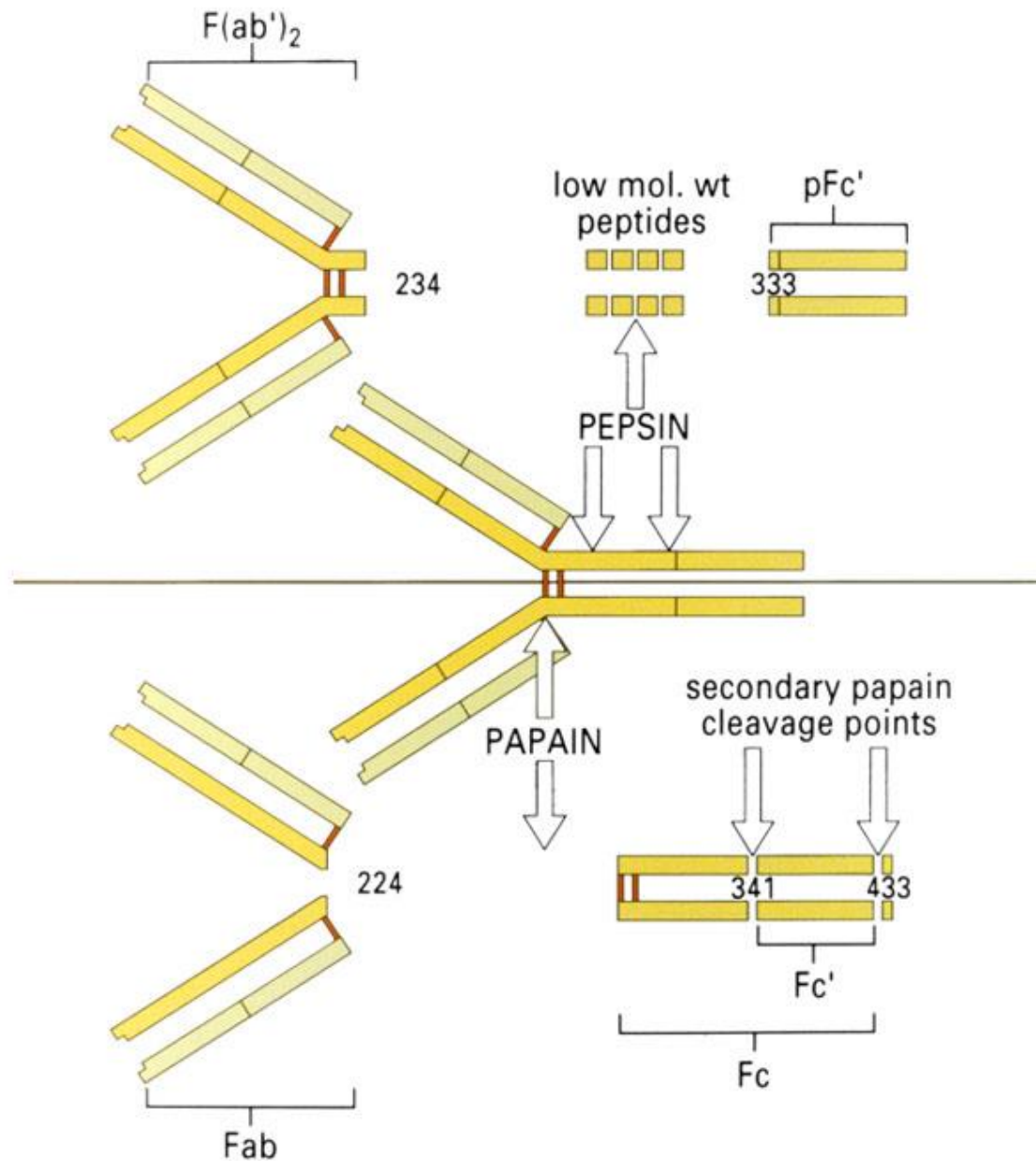
# The basic structure of IgG1



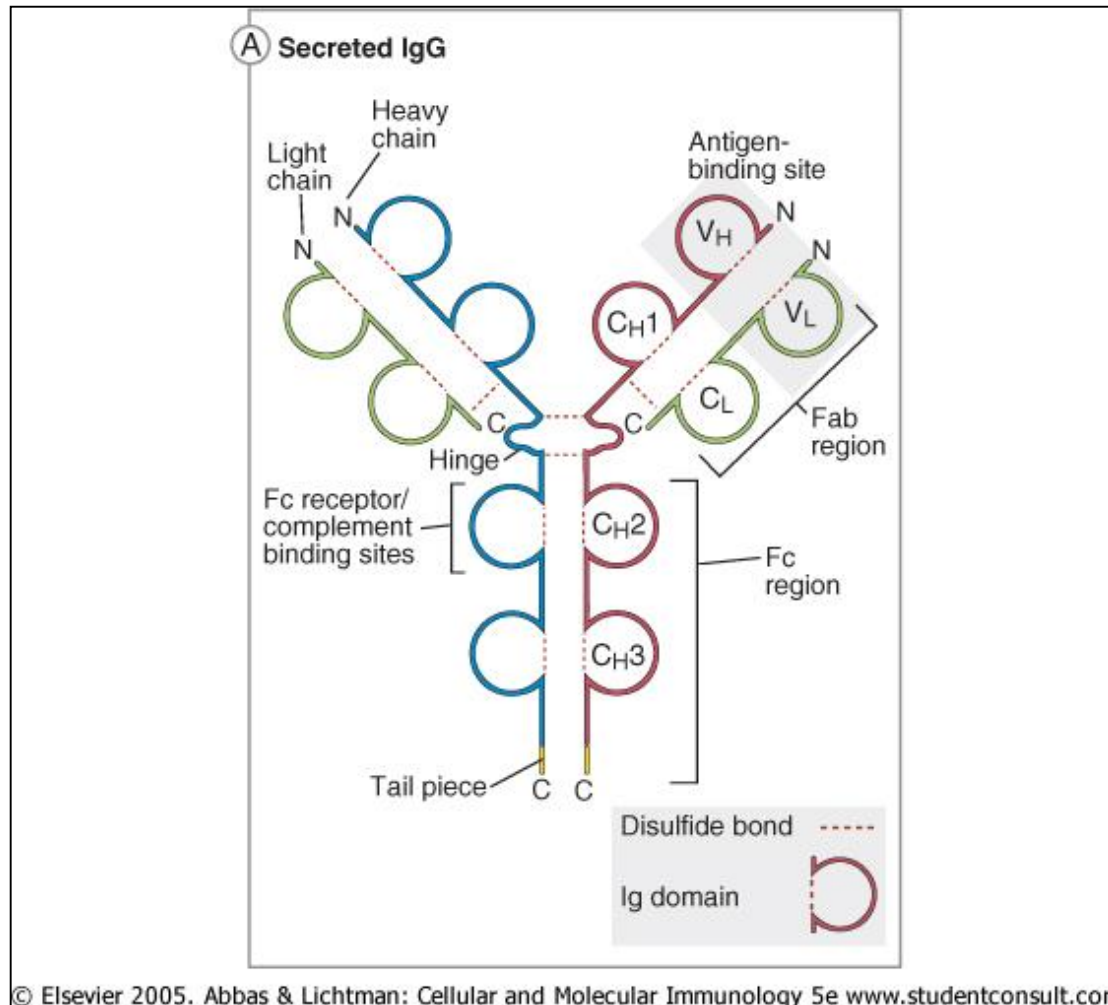
# Protein domain

- is a region of a protein's polypeptide chain that is self-stabilizing and that folds independently from the rest. Each domain forms a compact folded three-dimensional globular structure. Usually held together by a disulfidic bond.

# Enzymic cleavage of human IgG1



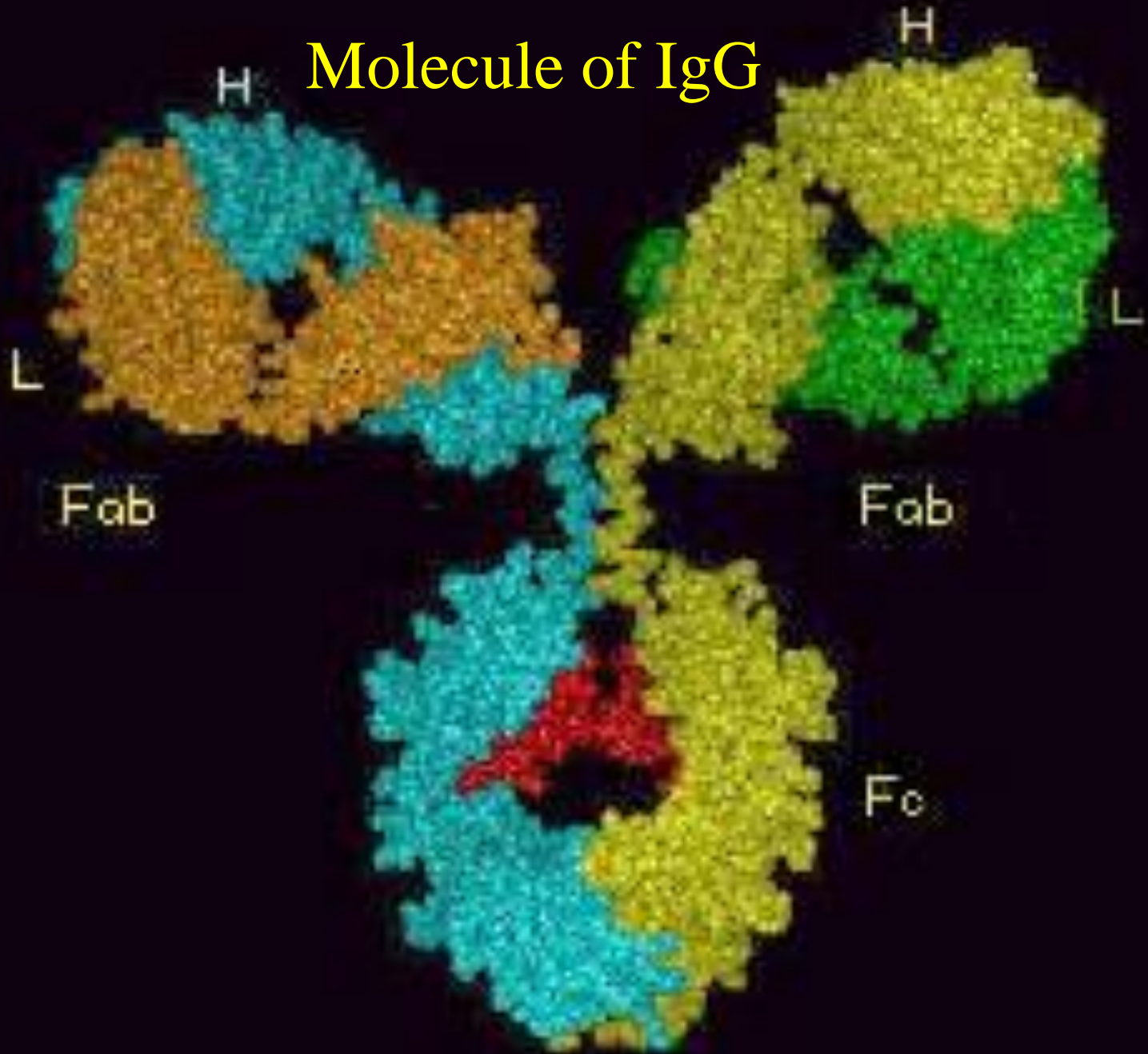
# IgG



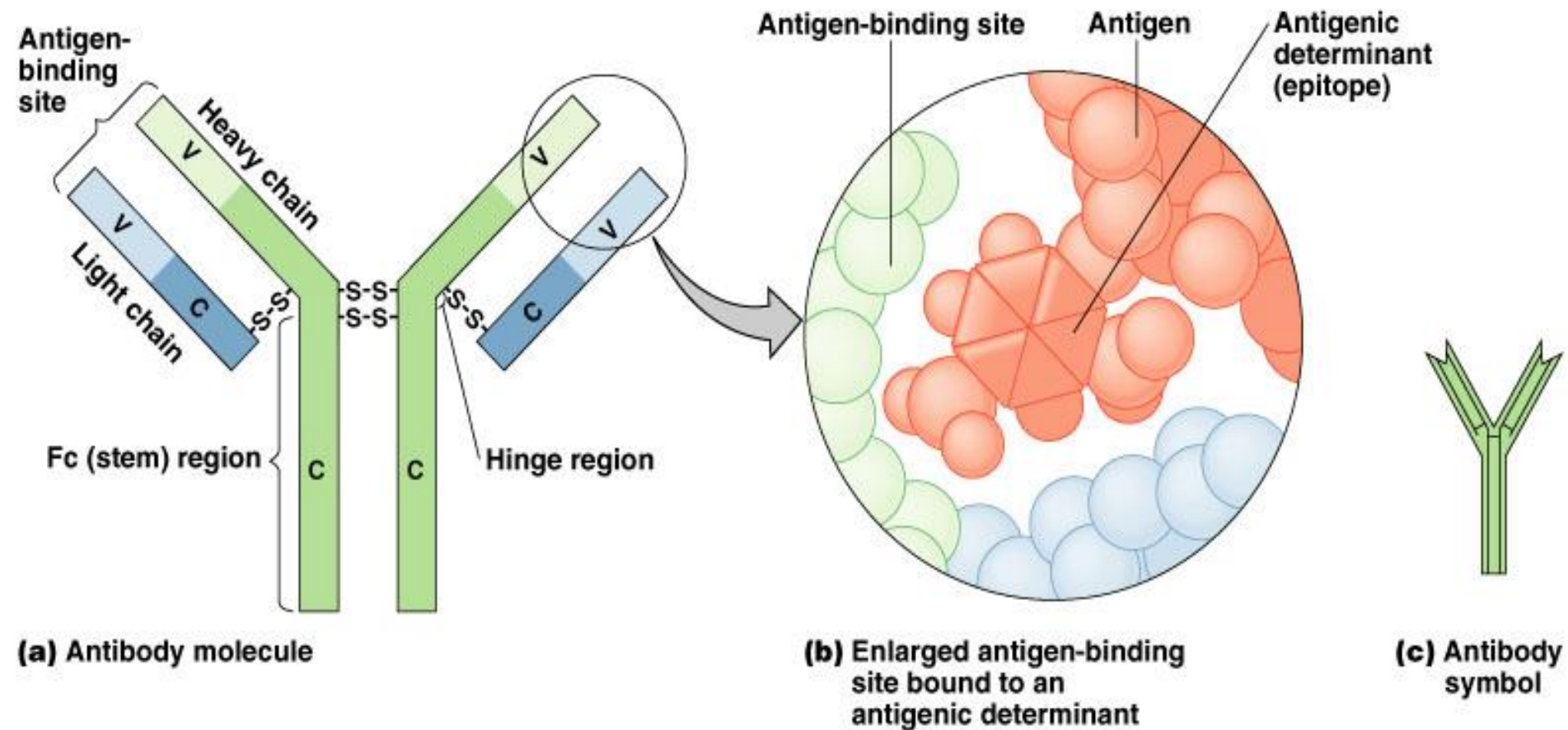


- Proteolytic cleavage (by pepsin or papain) results in formation of two fragments of Ig molecule:
- Fab (antigen binding) associated mainly with antigen specificity
- Fc (crystallizable) – associated with various functions of immunoglobulin molecule

# Molecule of IgG

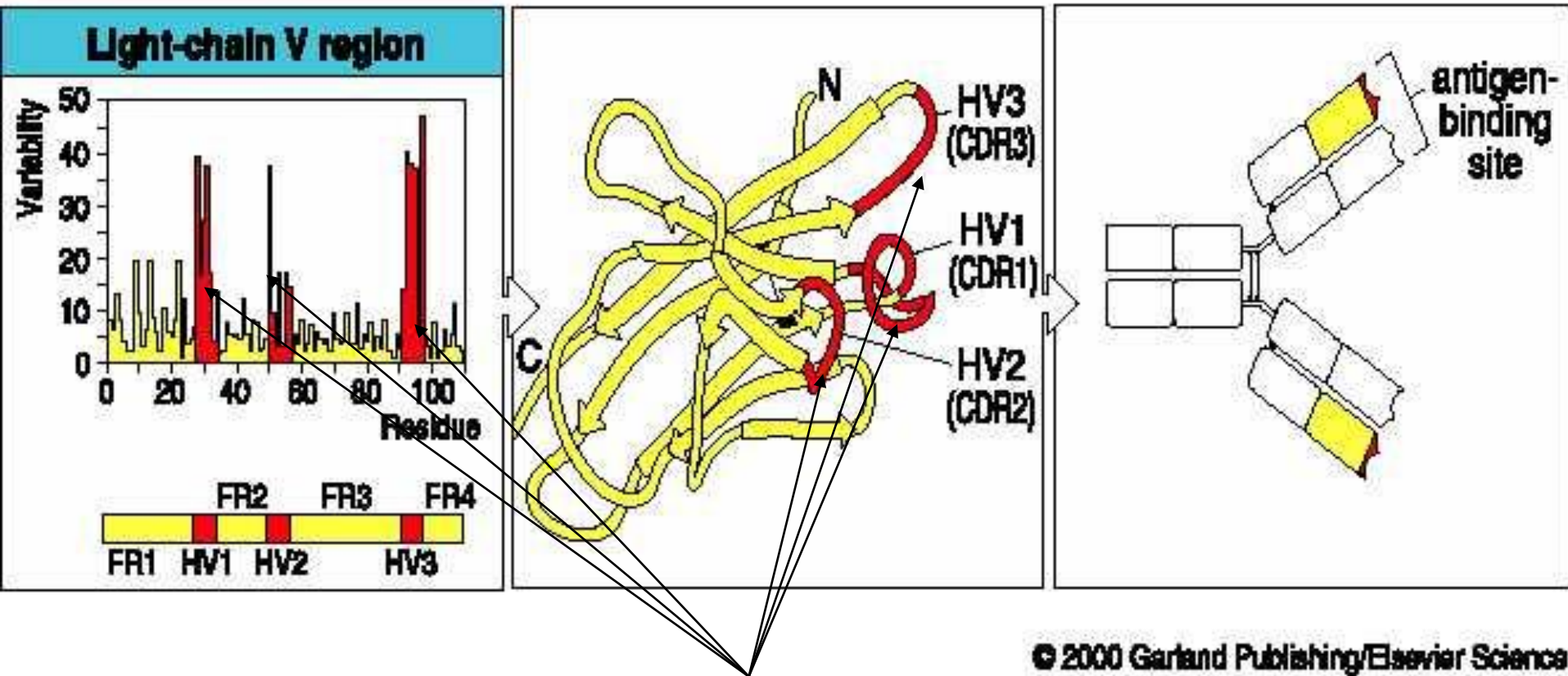


# Hypervariable region of immunoglobulin molecule binds epitope of the antigen



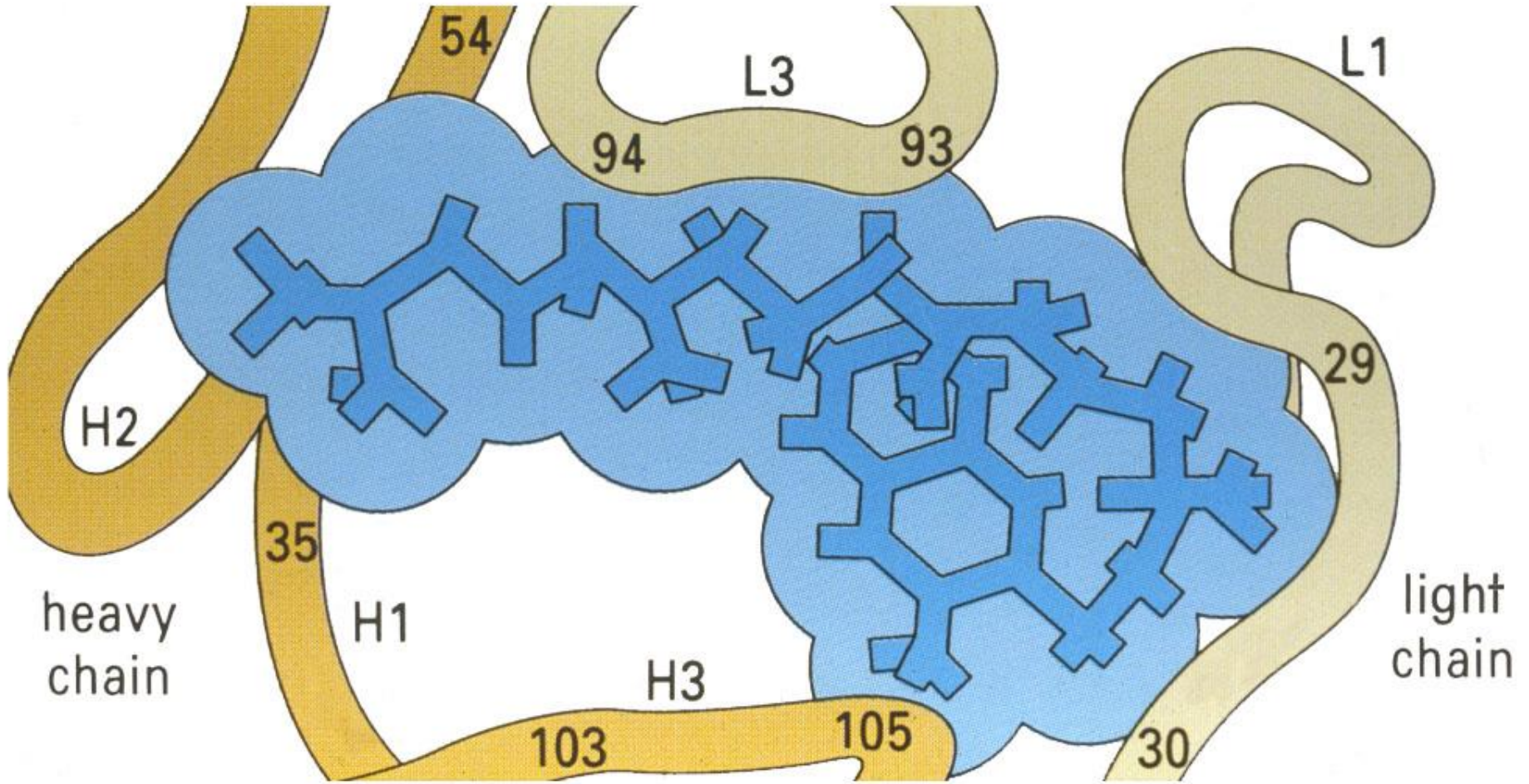
# Variable region of immunoglobulin molecule

Figure 2.7

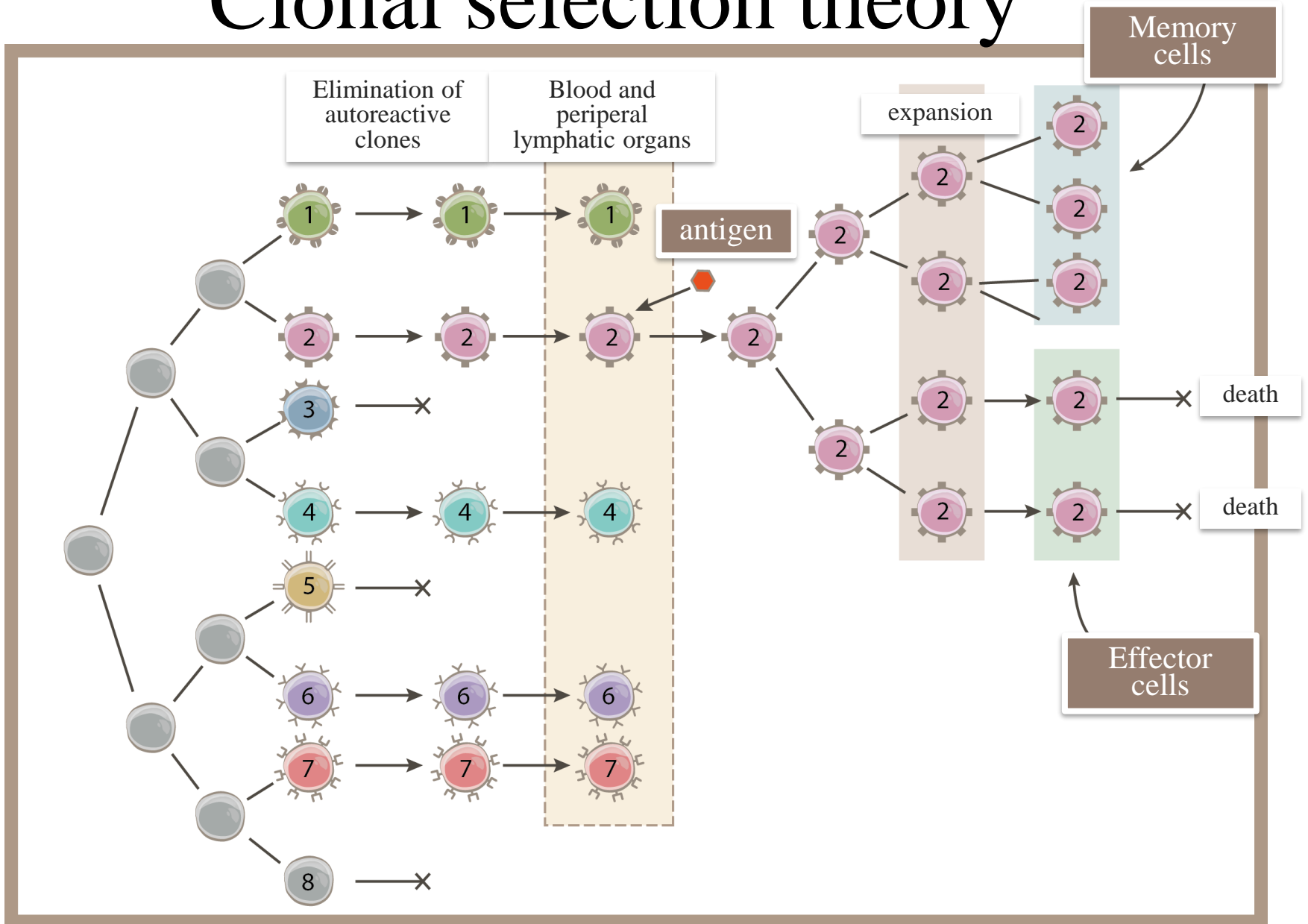


Hypervariable regions of immunoglobulin molecule

# The antibody combining site



# Clonal selection theory



# Clonal selection theory

F.M. Burnet, 1957

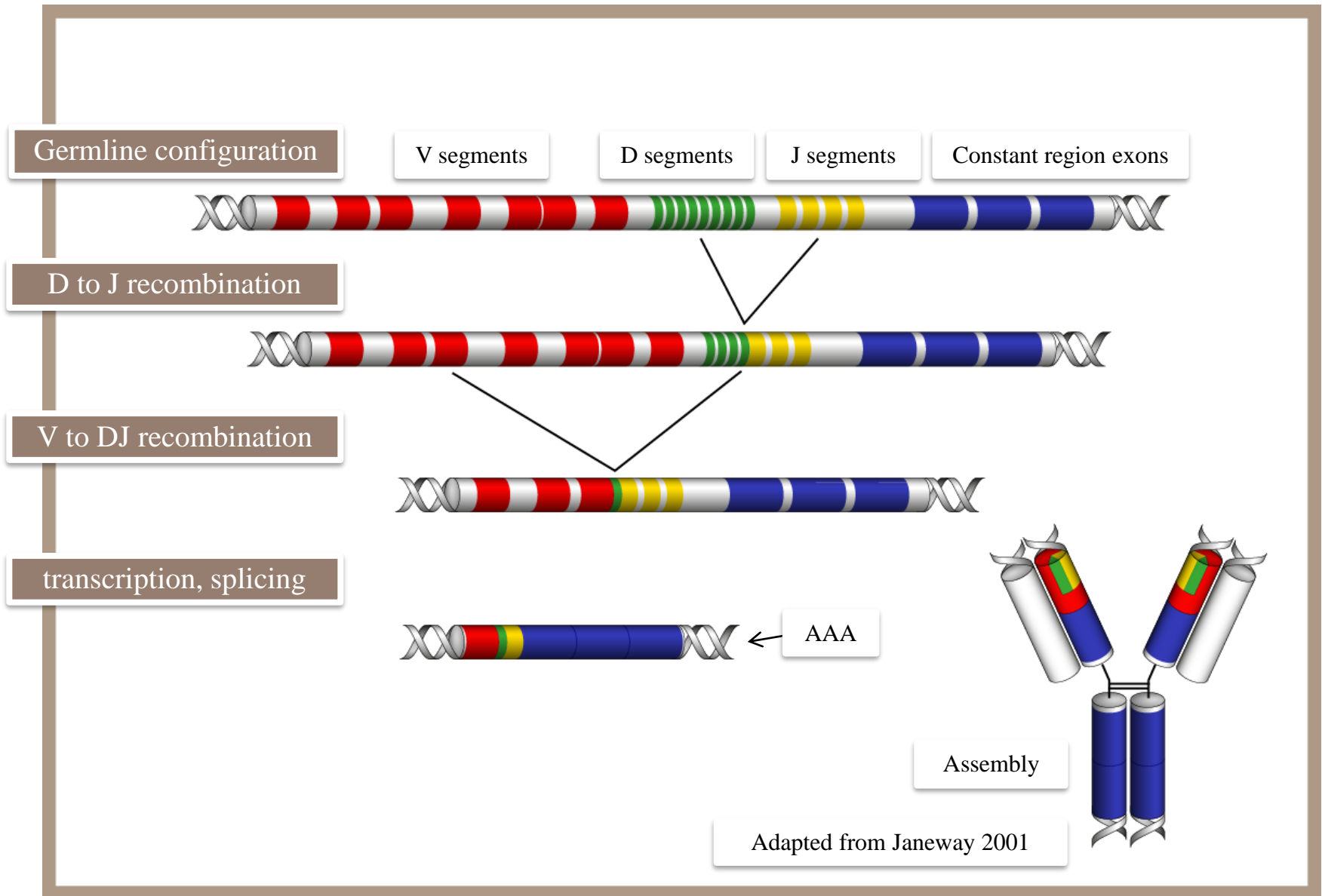
- During (mainly fetal) development immunocompetent cells of the immune system develop. Each cell is characterized by its own antigen specific receptor. Each cell reacts only with one concrete specific antigen.
- After exposure to autoantigen during fetal life autoreactive clones are eliminated („forbidden clones“).
- If a concrete cell recognizes its specific antigen, it is stimulated, proliferates and forms a clone = **clonal selection**.
- After repeated divisions the cells become terminally differentiated cells, that does not proliferate and after some time die.
- The cells of the clone that do not differentiate into the terminal stage become a memory cells which will quickly react after the second exposure to the antigen.

# From the history of immunology

- 1957: Clonal selection theory: 1957
- 1961: Discovery of the thymus as an organ involved in the immune system reaction
- 1965 : T and B- lymphocytes determined
- 1969 discovery of the exact function of the thymus, dichotomy of the immune system
- 1975 Positive and negative selection during the thymocytes' development
- 1978-1980 Organization of the immunoglobulin genes



# VDJ Recombination



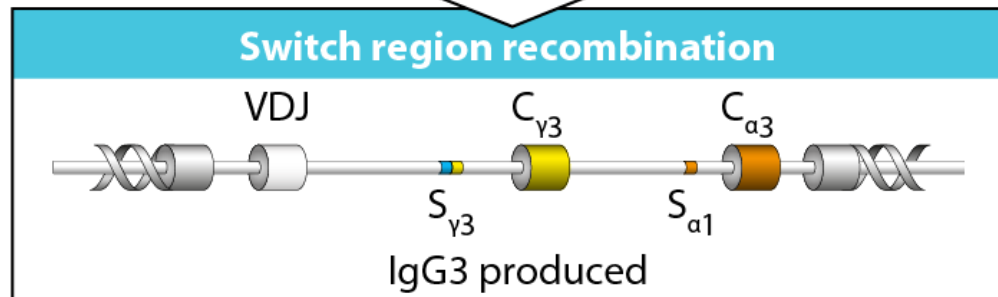
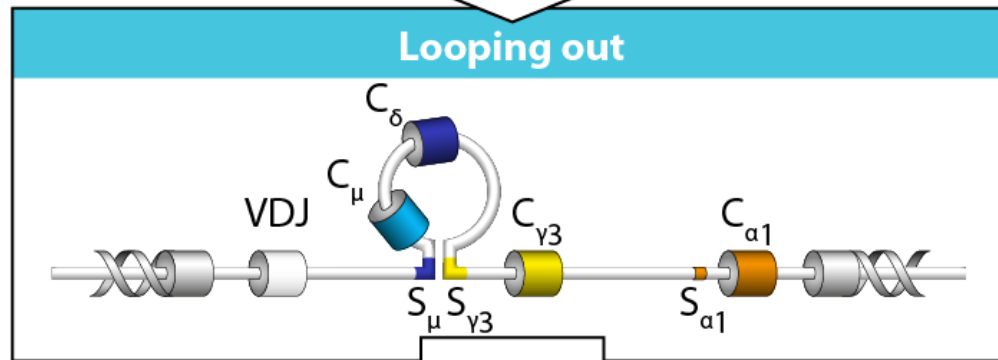
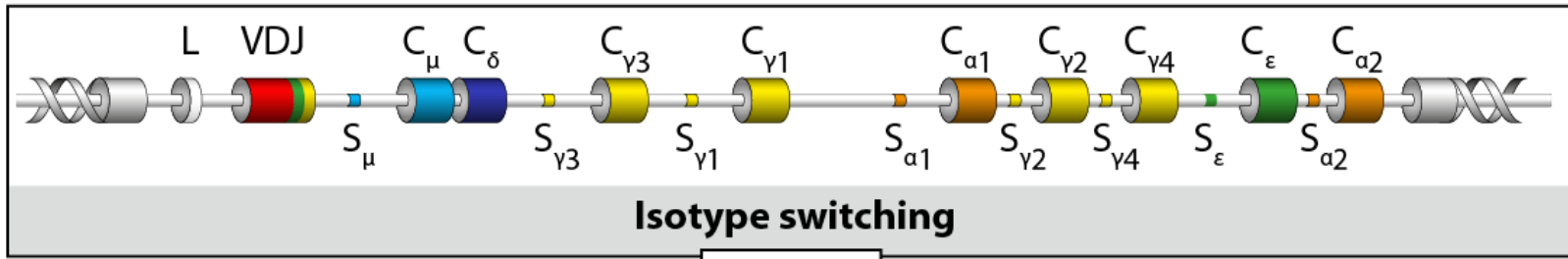
# VDJ genes for BCR, and TCR

	Immunoglobulin		T cell receptor	
	Heavy chain	κ	α	β
Number of V gene segments	45	35	45	50
Number of diversity (D) gene segments	23	0	0	2
Number of joining (J) gene segments	6	5	~50	12
<b>Mechanism</b>				
Combinatorial diversity:				
Number of possible V-(D)-J combinations	Ig: $\sim 10^6$		TCR: $\sim 3 \times 10^6$	
Junctional diversity:				
Total potential repertoire with junctional diversity	Ig: $\sim 10^{11}$		TCR: $\sim 10^{16}$	
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# Somatic hypermutations

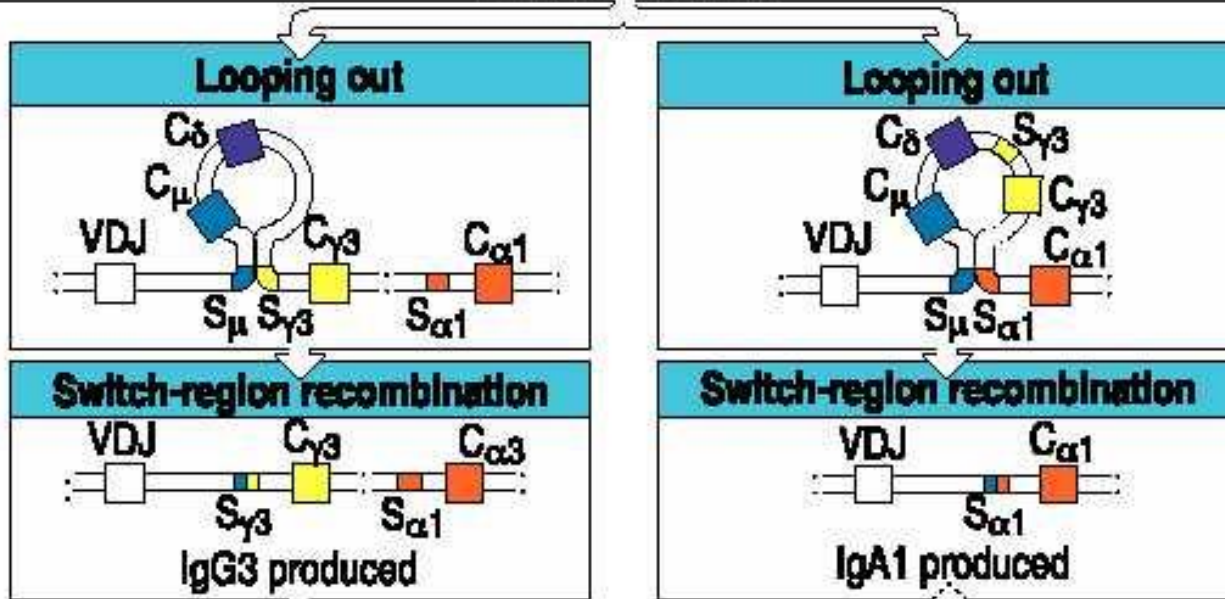
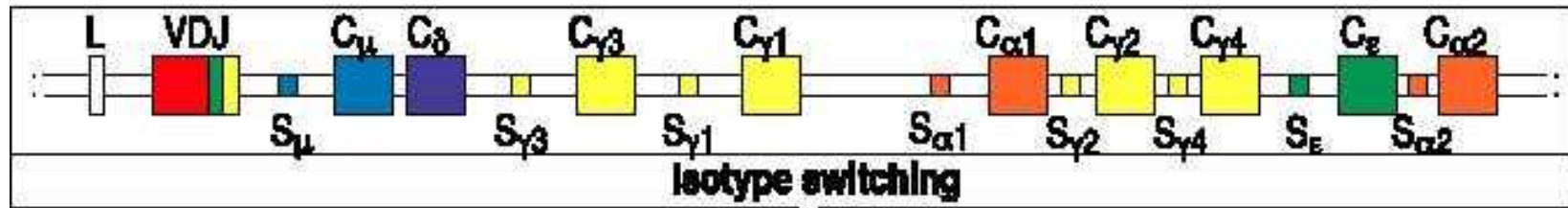
- The process occurs in activated B-lymphocytes, takes place in germinal centers of secondary lymphoid organs.
- Key enzyme is AID (activation-induced deaminase).
- Mutation frequency is approx.  $10^6$  times higher than in other parts of human genome.
- Antigen presentation by lymphoid dendritic cells to B-cells leads to selection of clones with higher affinity – the process is called affinity maturation.

# Isotype switching

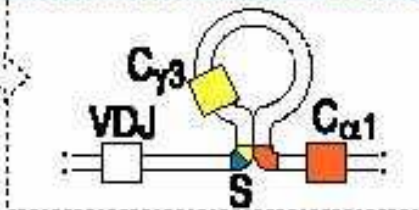


# Isotype switching

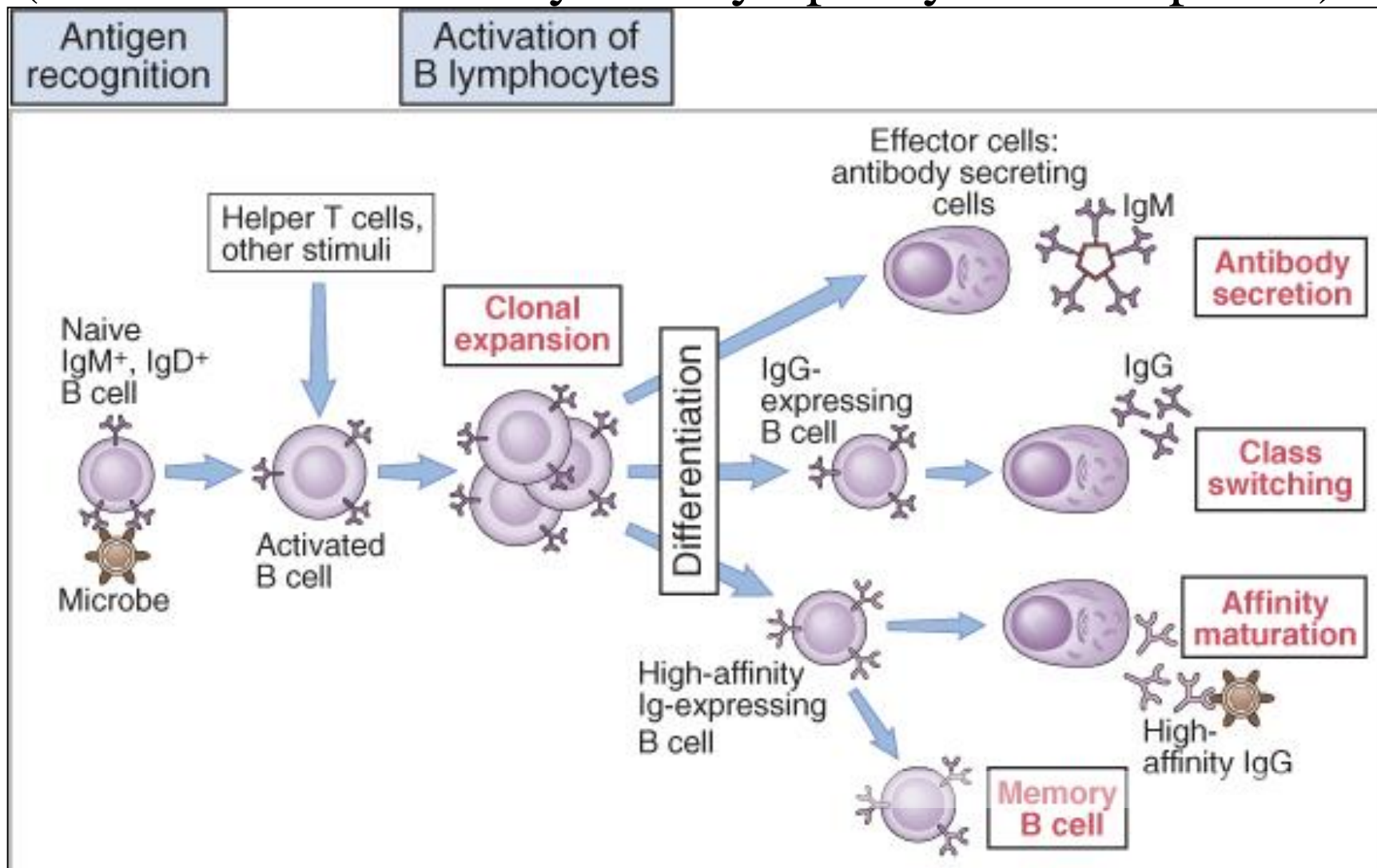
Figure 2.26



A further switch occurs



# Activation and differentiation of B-lymphocytes (clonal selection theory in B-lymphocyte development)



# Primary phase of the antibody response

- Naive or opsonised antigen captured by follicular dendritic cells.
- Primary stimulation of B-cells in lymphoid follicles.
- The antigen also stimulates T cells (after adequate presentation) in T-cell zones. T-cells migrate toward the lymphoid follicles.
- Newly formed plasma cells produce predominantly IgM (mainly in bone marrow).

# Secondary phase of the antibody response

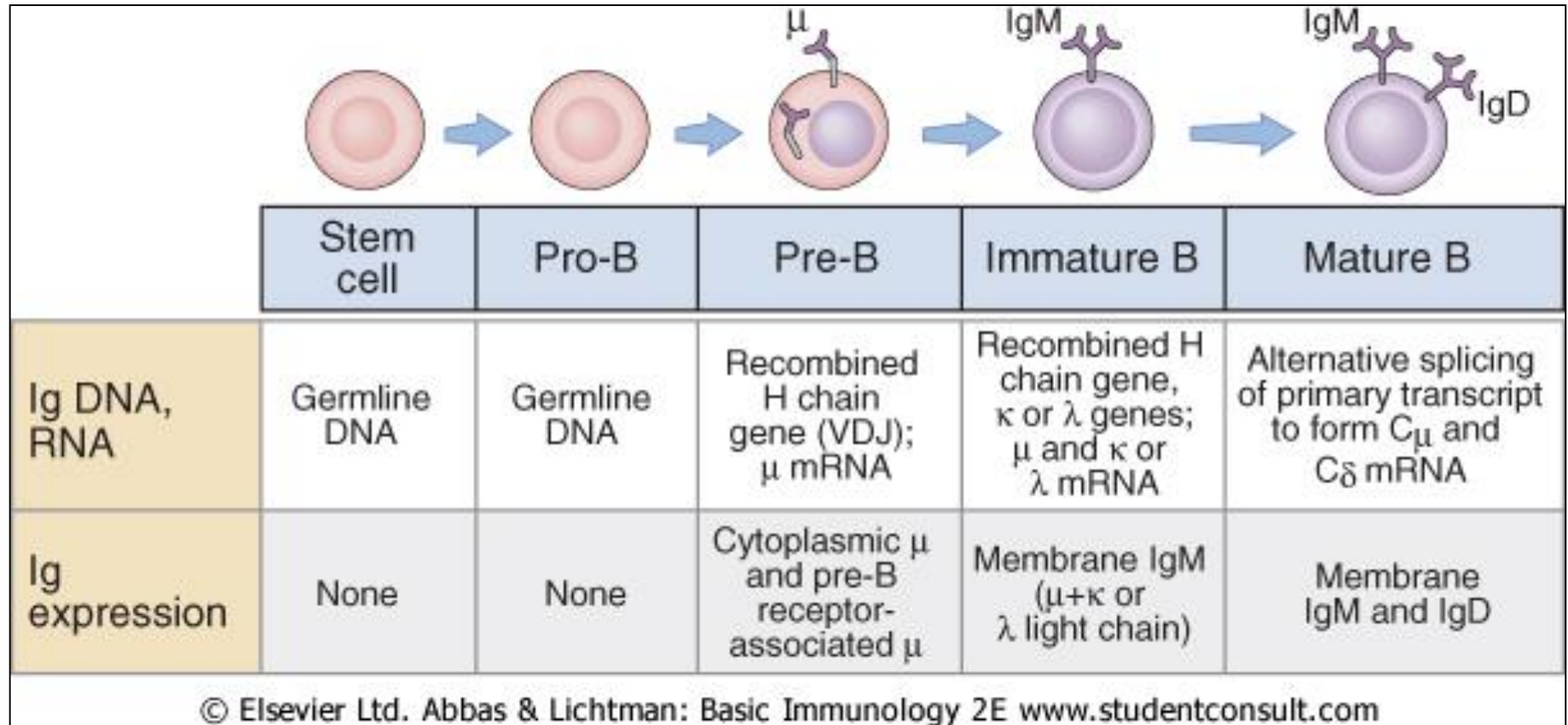
- Occurs in newly formed germinal centers of lymphoid follicles.
- Th lymphocytes stimulate B-lymphocytes to **somatic hypermutations** and **isotype switching**.
- This leads to selection of B- cells producing high-affinity antibodies (**affinity maturation**).
- Majority of B-cells producing low-affinity antibodies die.



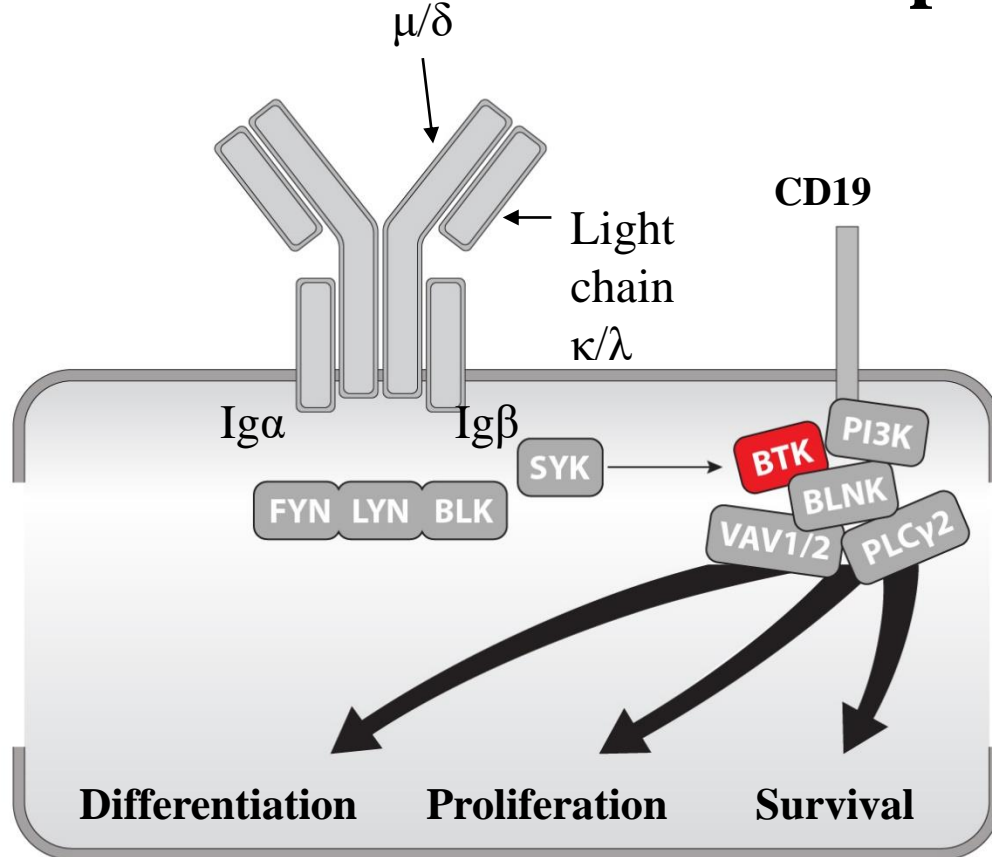
# Development of B-cells in the bone marrow

- **Stem cells:** no B-cell surface markers, no rearrangement of Ig genes.
- **Pro-B lymphocyte** – rearrangement of heavy chain , expression of several B-cell surface markers (e.g. CD19).
- **Pre-B-lymphocytes** VDJ of heavy chain has been completed,  $\mu$  chain can be detected in cytoplasm. Pre-B receptor – composed of  $\mu$  chain and surrogate chains V-preB and  $\lambda 5$  is expressed on the surface of the cell. Signal transduction through this receptor is essential for B- cell development.
- **Immature B-cell** – light chain rearrangement (V-J) completed B-cell receptor is composed of monomeric IgM.
- **Mature B-lymphocyte** has IgM and IgD B-cell receptors.

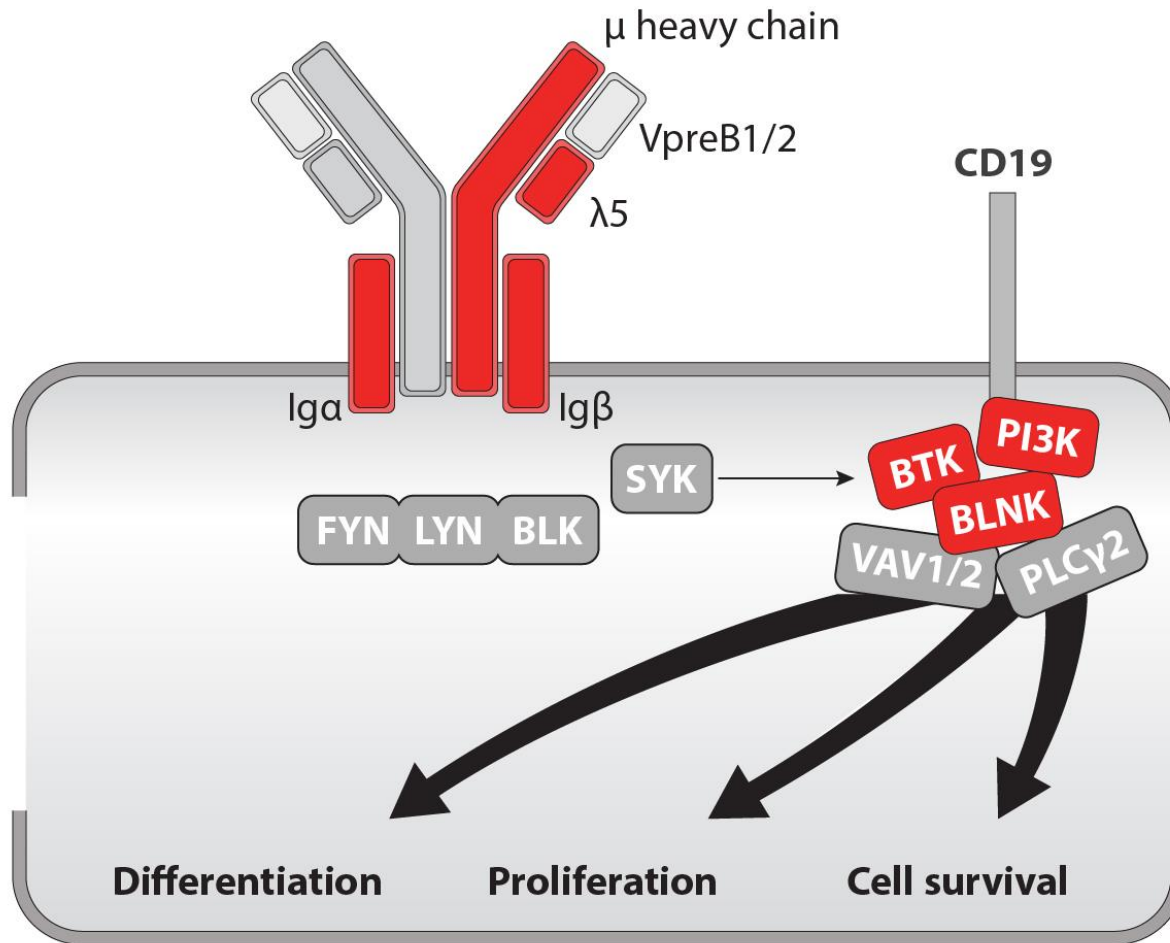
# Development of B-cells in the bone marrow



# Heavy chain **B-cell receptor**



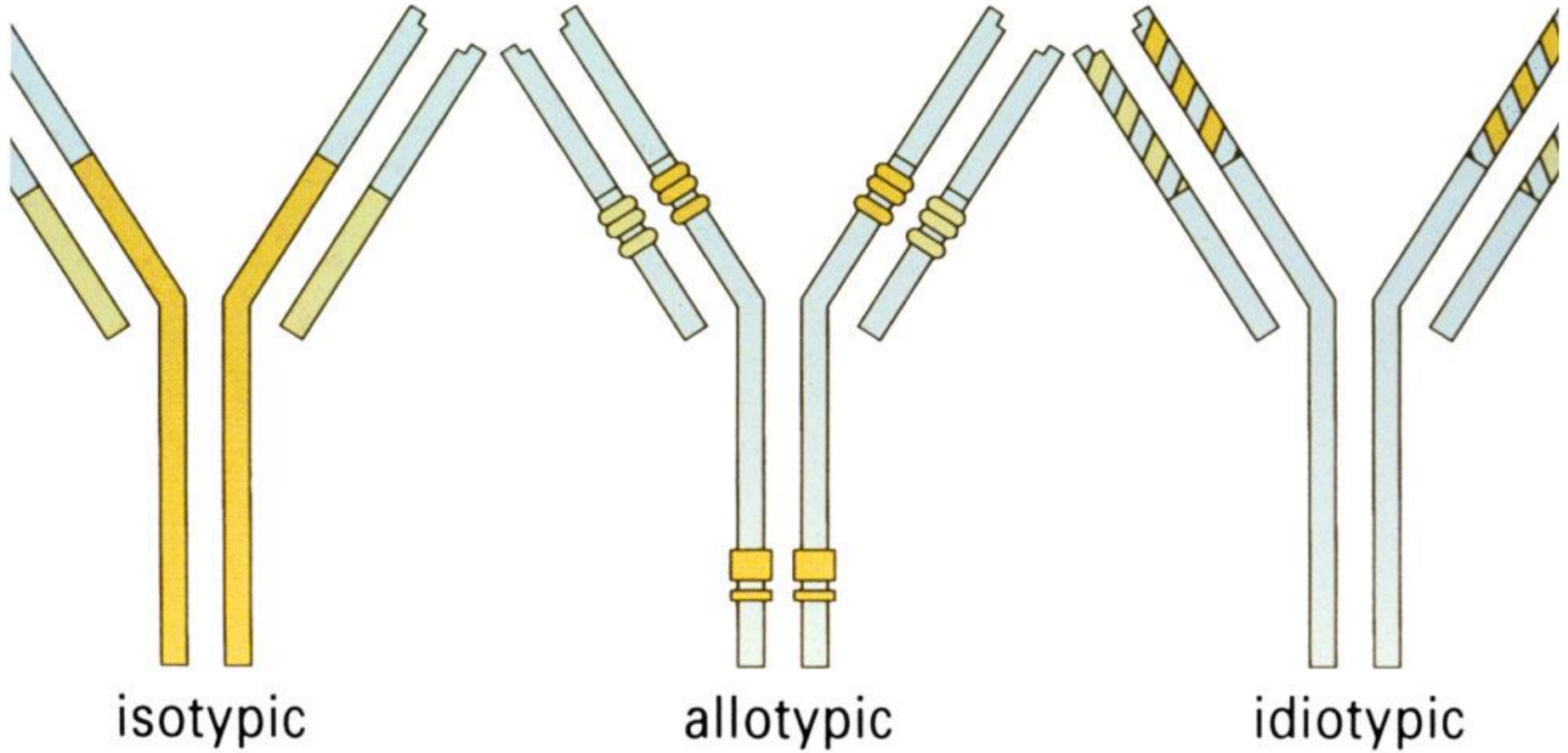
# Pre B-cell receptor



# Bruton's tyrosine kinase (BTK)

- Key tyrosine kinase in activation, differentiation and development of B-cells.
- Mutations of BTK lead to X-linked (Bruton's) agammaglobulinemia.
- BTK blockers (e.g. ibrutinib) are used for the treatment of B-cell malignancies.

# Antibody variants



# Isotype

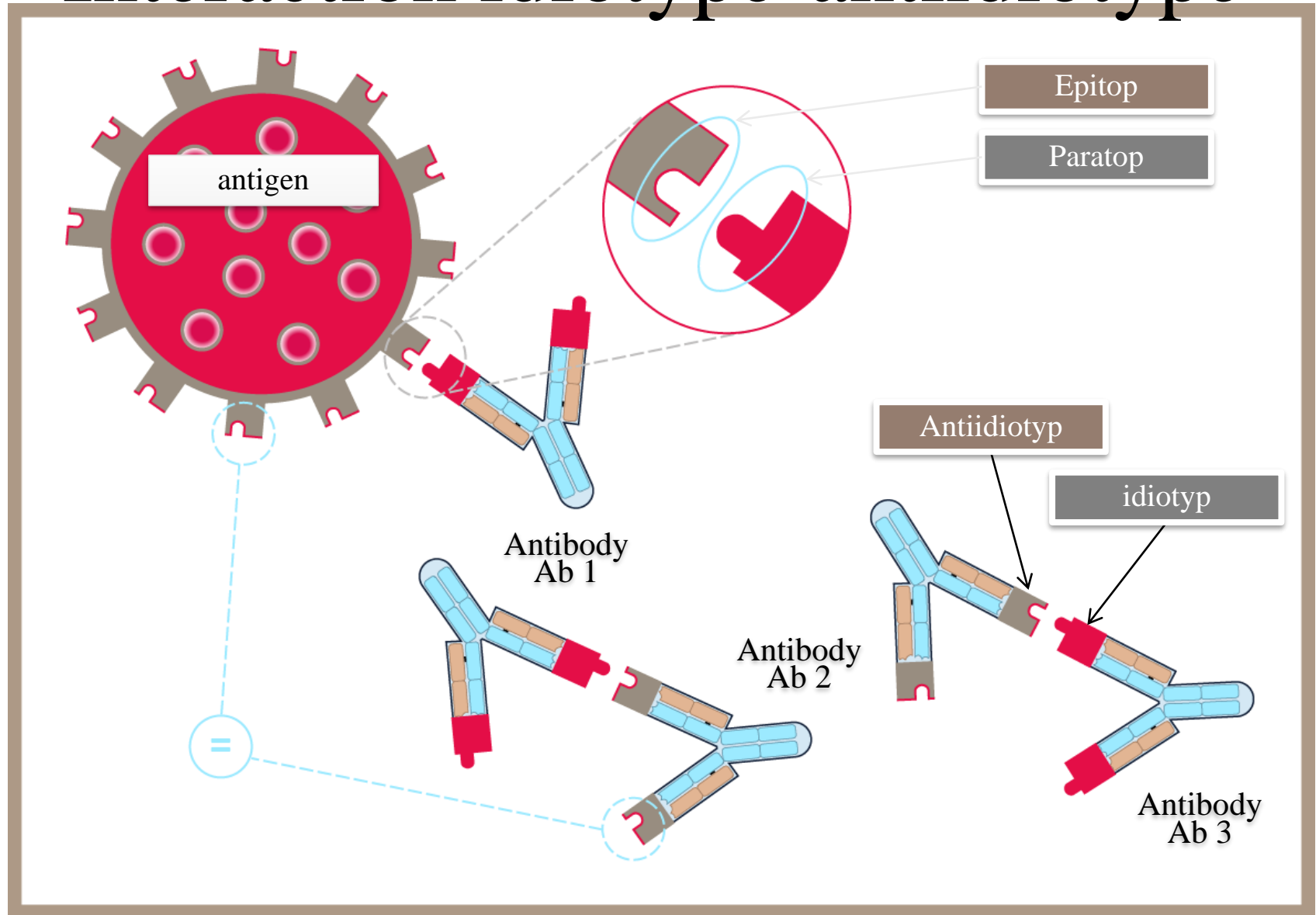
- The class or subclass of an immunoglobulin.
- Antigenic determinants are on constant part of immunoglobulin molecule.

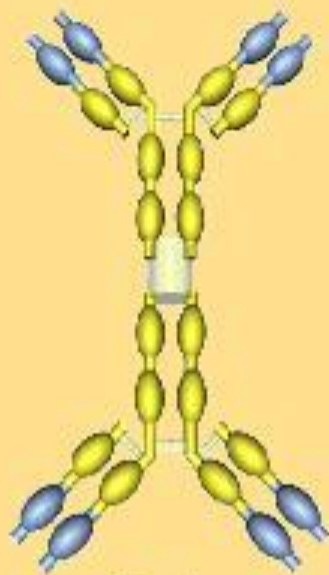
# Idiotypic

- An antigenic determinant on the variable region of immunoglobulin molecule.

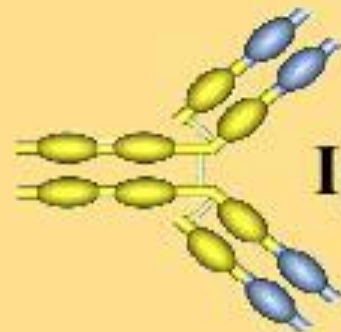


# Interaction idiotypic-antiidiotypic

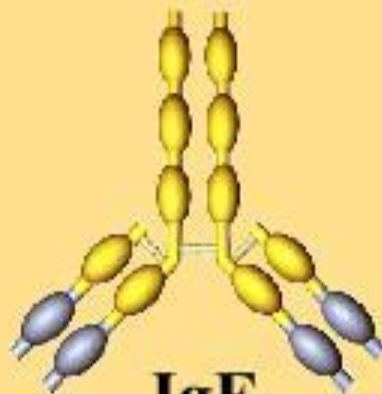




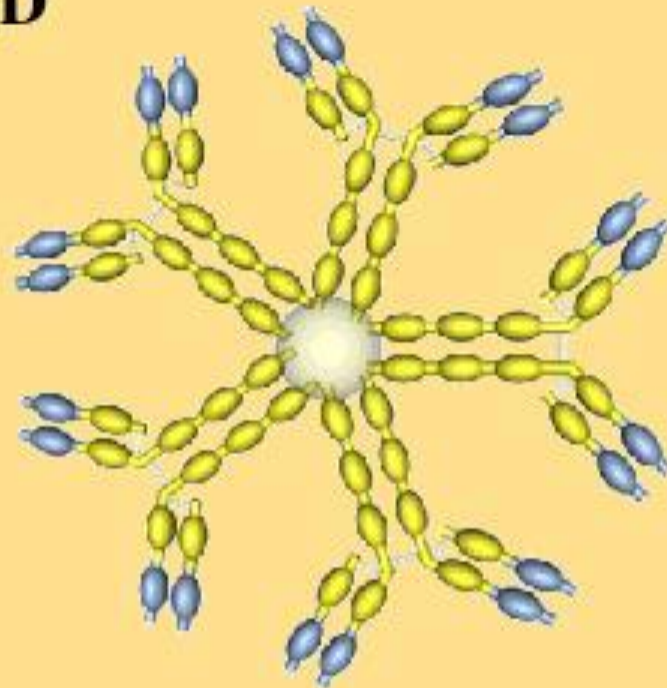
**IgA**



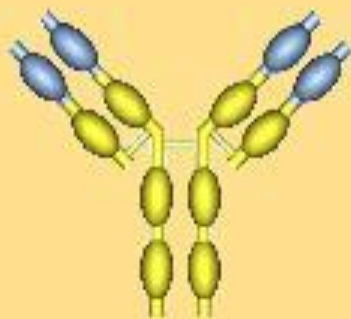
**IgD**



**IgE**

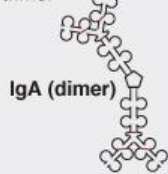





**IgM**



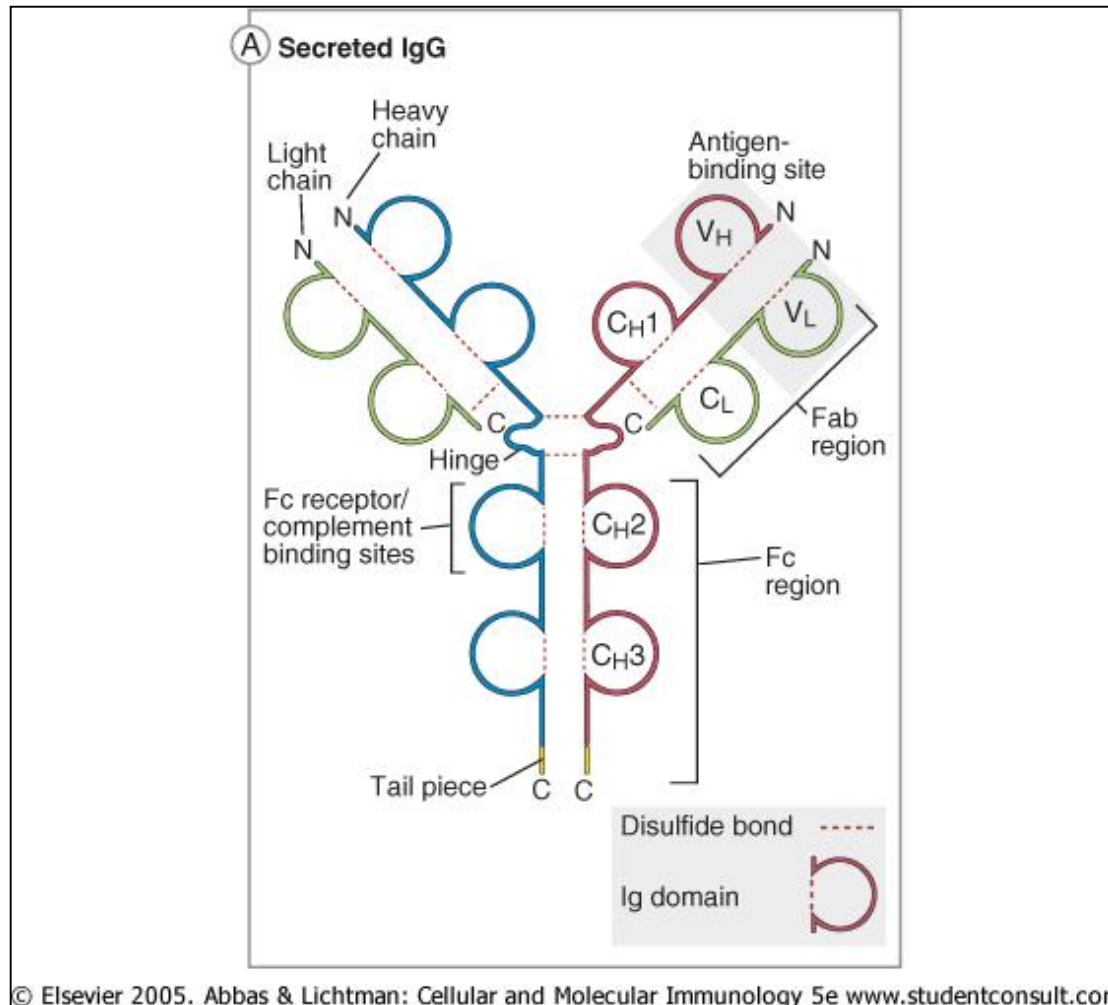
**IgG**

# Characteristics of immunoglobulin classes

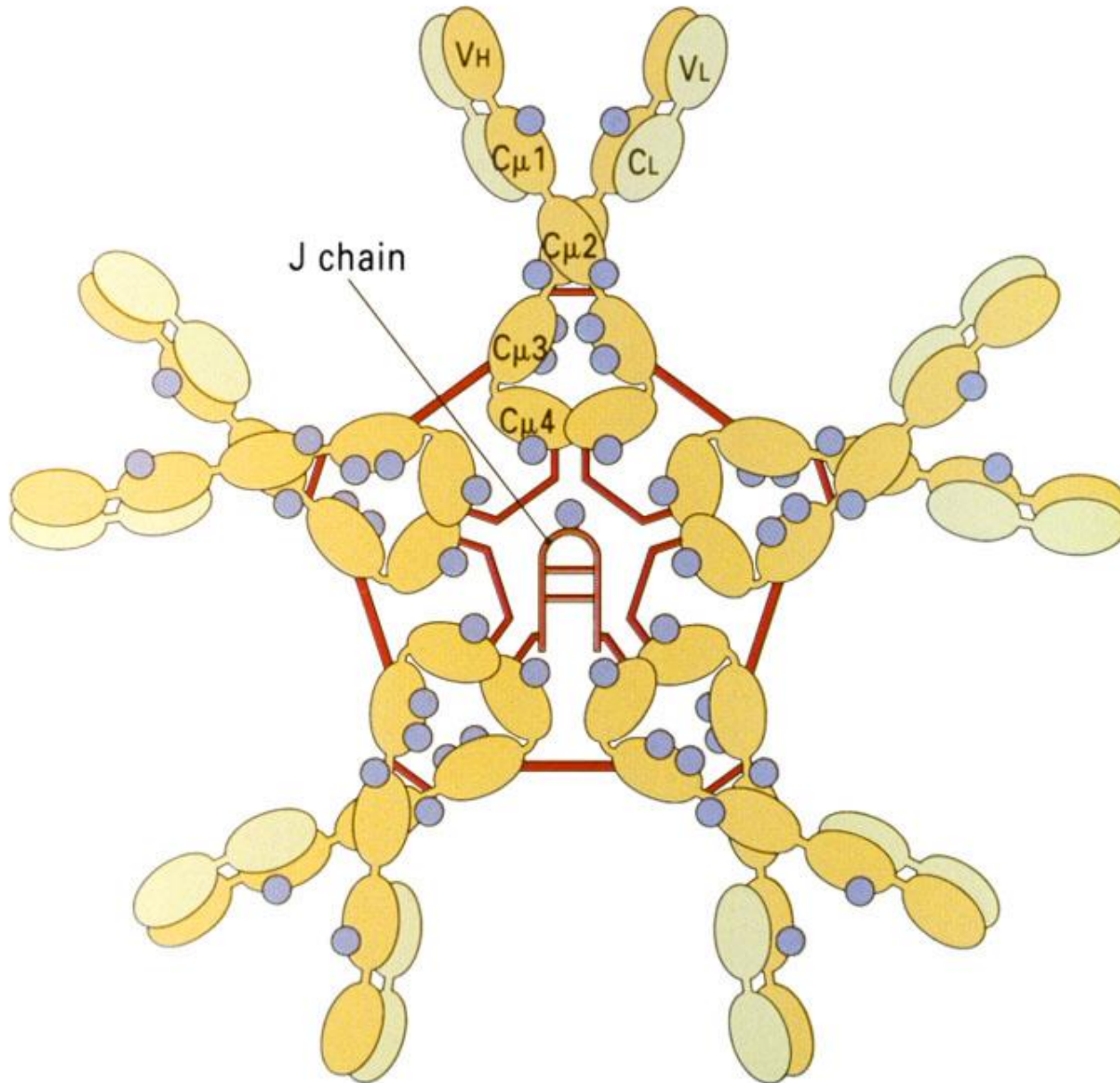
Isotype of antibody	Subtypes	H chain	Serum concentr. (mg/mL)	Serum half-life (days)	Secreted form	Functions
IgA	IgA1,2	$\alpha$ (1 or 2)	3.5	6	Monomer,dimer, trimer  IgA (dimer)	Mucosal immunity, neonatal passive immunity
IgD	None	$\delta$	Trace	3	None	Naive B cell antigen receptor
IgE	None	$\epsilon$	0.05	2	Monomer  IgE	Mast cell activation (immediate hypersensitivity)
IgG	IgG1-4	$\gamma$ (1,2,3 or 4)	13.5	23	Monomer  IgG1	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None	$\mu$	1.5	5	Pentamer  IgM	Naive B cell antigen receptor, complement activation

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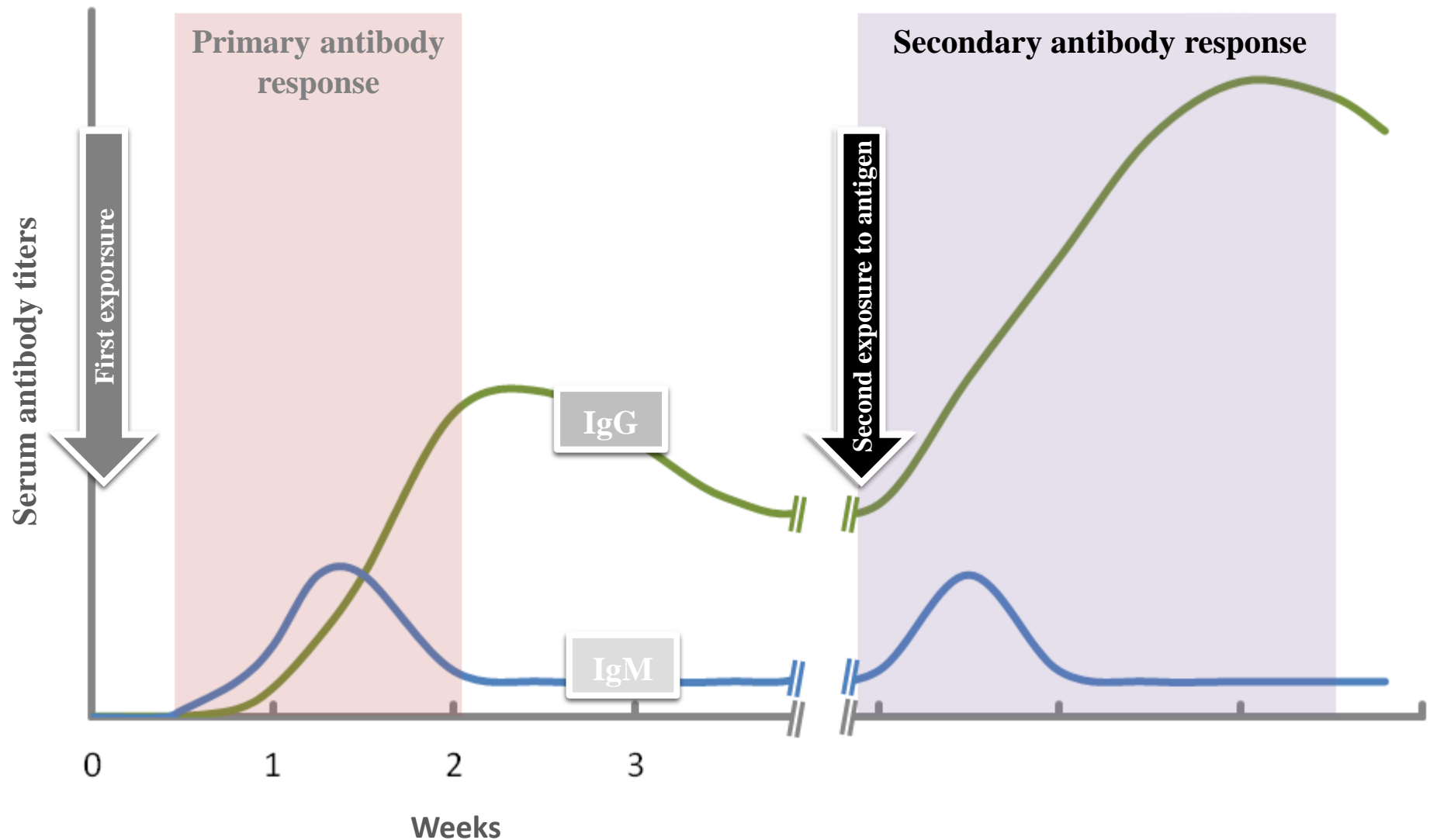
# IgG



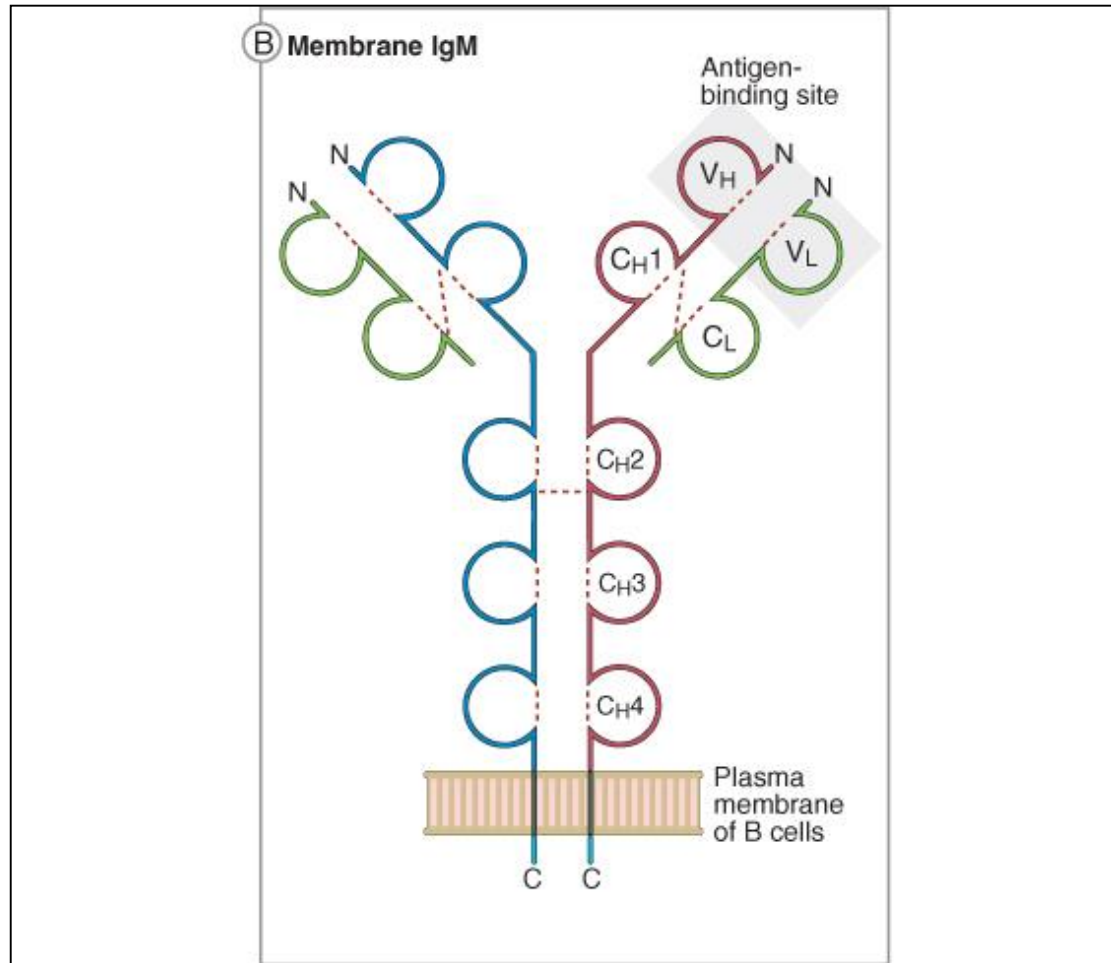
# Structure of human IgM



# Antibody response after primary and secondary antigen exposure



# IgM on B-cell membrane



# Expression of surface immunoglobulins on B-cells

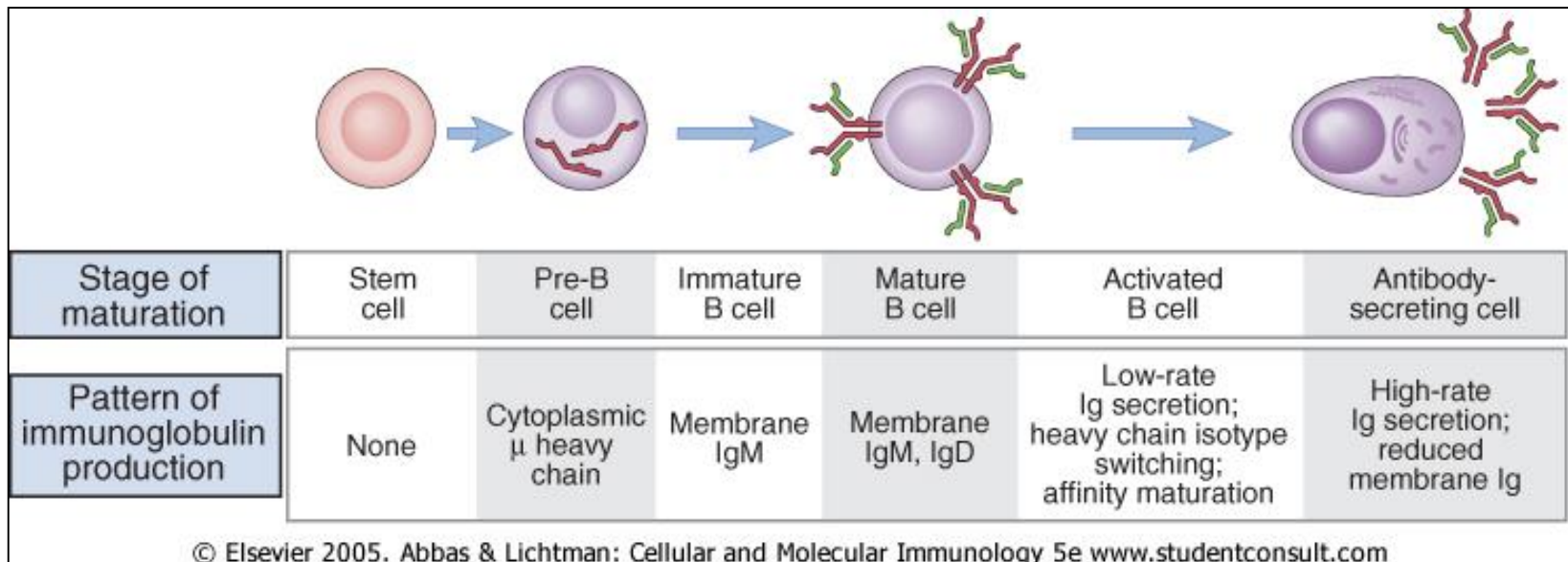
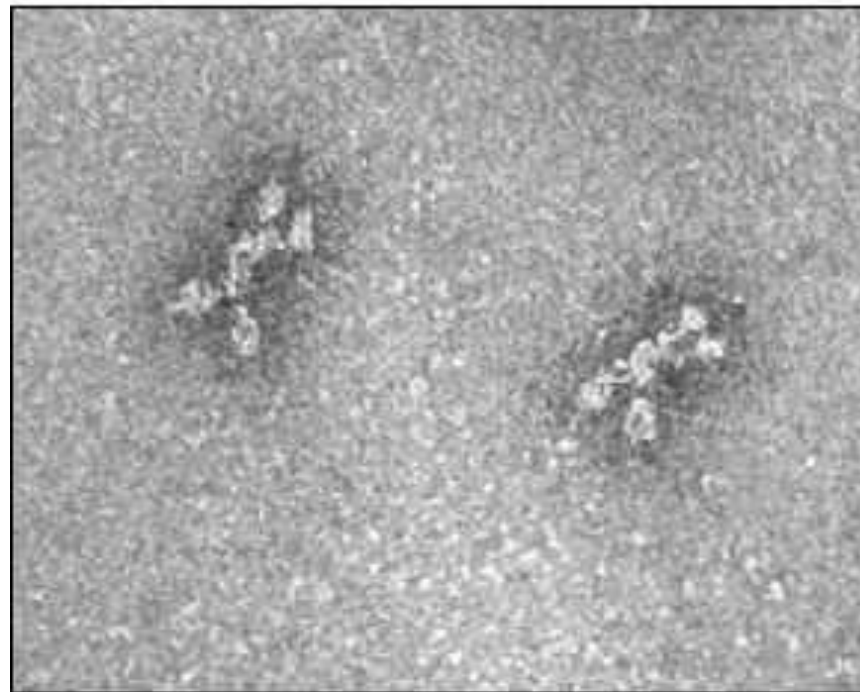
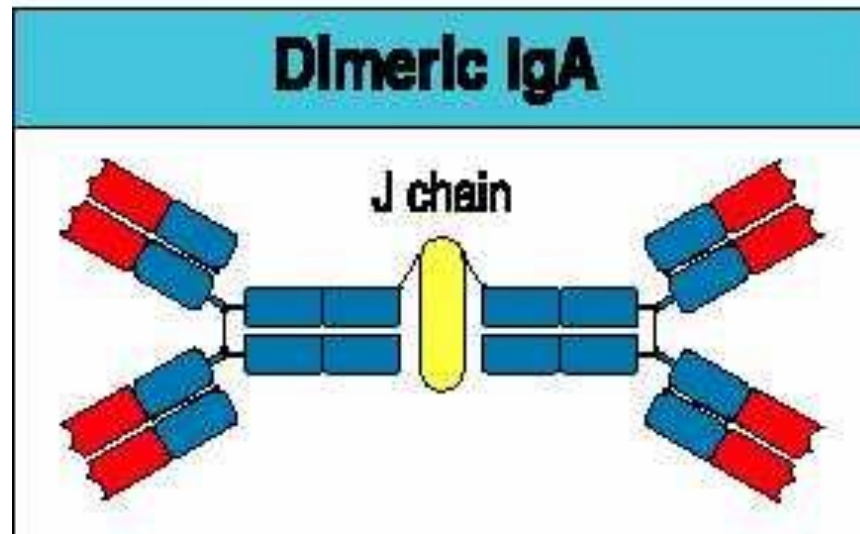
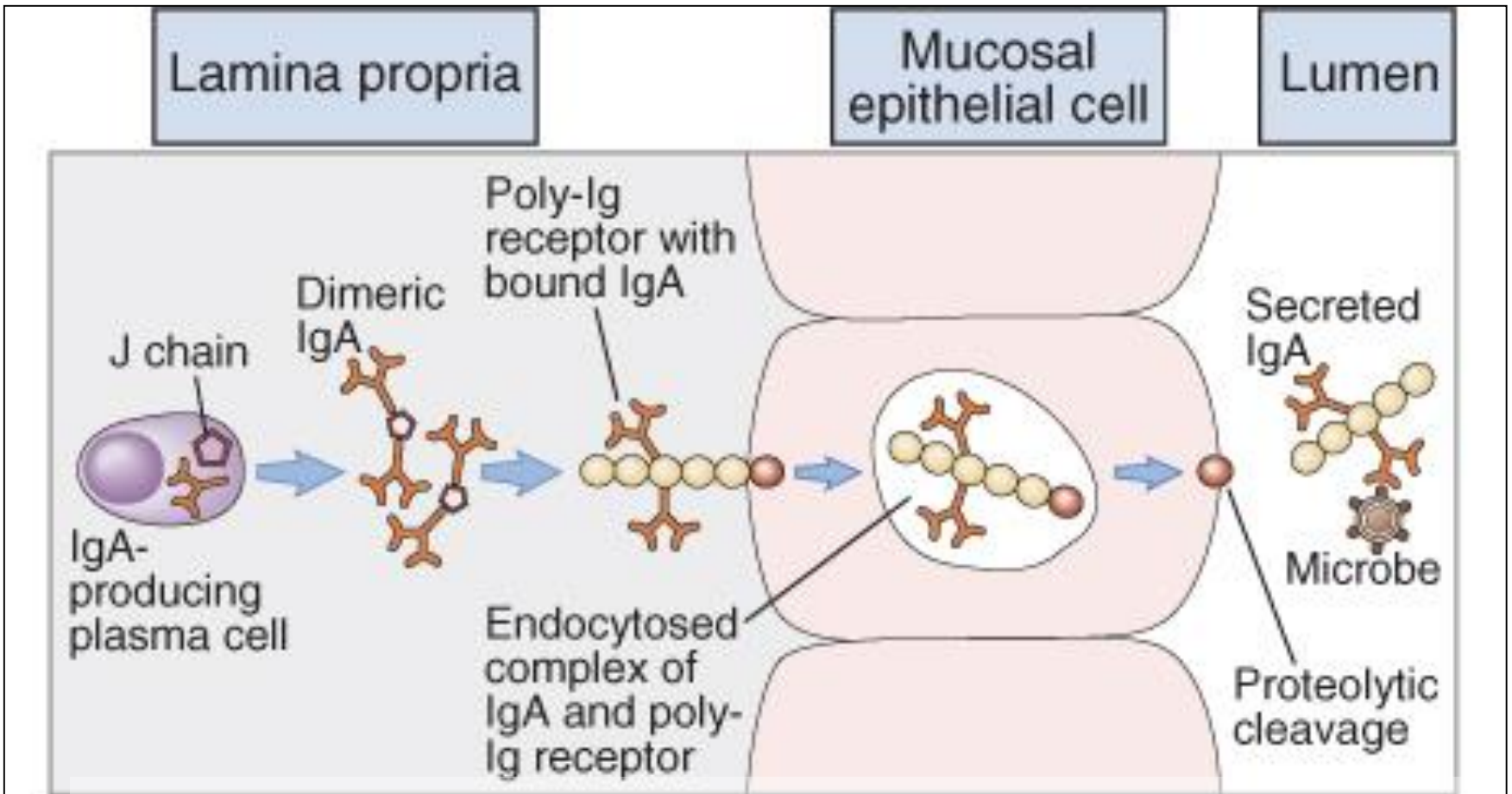




Figure 2.29



# Formation of Secretory IgA



- **Affinity:** The strength of the binding between a single site of an antibody (one variable region) and an epitope.
- **Avidity:** The overall strength of interaction between antibody and antigen. The avidity depends on affinity and the valency of interactions.

## Biological half-life and serum levels of immunoglobulin classes

- IgG: half life approx. 3-4 weeks, serum level approx. 10 g/l.
- IgA, IgM: half life 5-6 days, serum level approx. 1-3 g/l.
- IgE: half life in plasma approx. 1 day (much more on IgE receptors on mast cells), serum levels very variable, several mg/l (IU/ml are used).

B Antibody isotype	Isotype specific effector functions
IgG	Neutralization of microbes and toxins Opsonization of antigens for phagocytosis by macrophages and neutrophils Activation of the classical pathway of complement Antibody-dependent cellular cytotoxicity mediated by NK cells Neonatal immunity: transfer of maternal antibody across placenta and gut Feedback inhibition of B cell activation
IgM	Activation of the classical pathway of complement
IgA	Mucosal immunity: secretion of IgA into lumens of gastrointestinal and respiratory tracts, neutralization of microbes and toxins
IgE	Antibody-dependent cellular cytotoxicity mediated by eosinophils Mast cell degranulation (immediate hypersensitivity reactions)

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# Biological functions of immunoglobulin molecules

- Activation of complement system (IgG, IgM)
- Opsonization (particularly IgG)
- Neutralization of antigens (IgG, IgA, IgM)
- Adherence interference (IgA, IgG)
- Antibody dependent cellular cytotoxicity (ADCC)
- Agglutination, precipitation (IgG, IgM)
- Mast cells degranulation (IgE)
- Transport through placenta (IgG)
- Immunoregulation (mainly IgG)

# Antibody dependent cellular cytotoxicity (ADCC)

