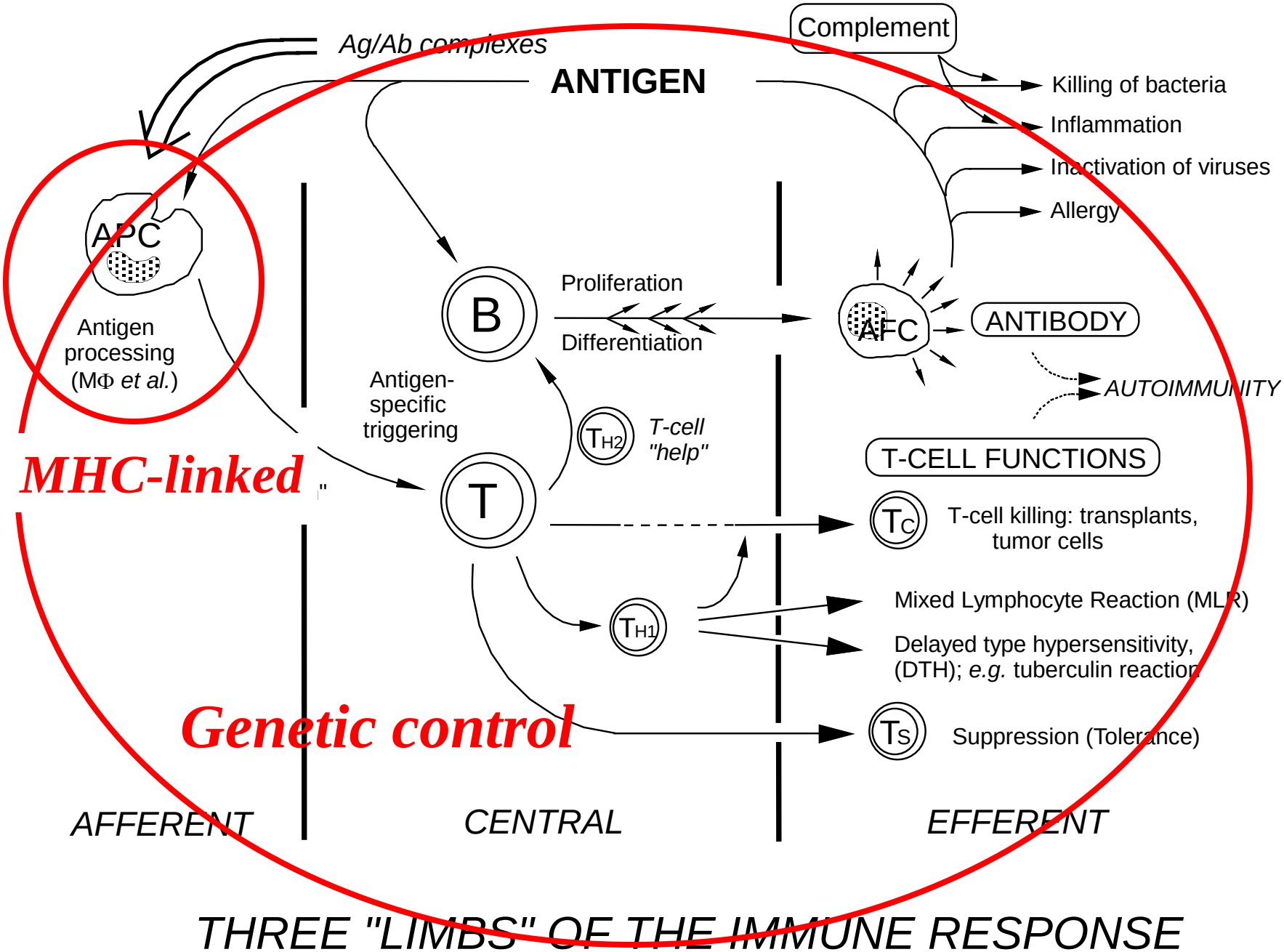
Genetic Control of Immune Responses



THREE "LIMBS" OF THE IMMUNE RESPONSE

Natural killer cells

- Granular lymphocytes, distinct from T- and B-lymphocytes
- **Cytotoxicity** to tumor cells and virally infected autologous cells (perforins)
- Play a role in defense against some bacterial, fungal and helminthic diseases
- Participate in reactions of antibody-dependent cell-mediated cytotoxicity (ADCC)
- They are not subject to MHC restriction (= NK-cells do not need to recognize MHC molecules in the target cells)

Interferons

- Proteins that induce antiviral activity in cells
- We can distinguish two types:

a/ type I: IFN-α (macrophages and other cells)

IFN-β (fibroblasts)

b/ type II: IFN-γ (T-lymphocytes)

Function of interferons

- Induce cells to produce antiviral proteins (protein kinase, oligonucleotide polymerase – interference with the translation of viral mRNA)
- Enhance T-cell activity
- Activate macrophages
- Increase the cytotoxic action of NK-cells

Basophils and mast cells

- Very similar type of cells, however, basophils circulate in blood circulation, whereas mast cells reside in tissues (connective tissue, mucosa)
- IgE antibodies are bound on the surface of basophils and mast cells by FcεRI
- Abundant granules containing biogenic amines (histamine), proteases (tryptase) and proteoglycans (heparin) in cytoplasm

Basophils and mast cells

- If IgE molecules bound on the surface of the cells are cross-linked by an antigen, then occurs:

a/ degranulation – release of content of granules to the cell's surroundings

b/ activation of arachidonic acid's metabolism – production of prostaglandins a leukotriens which are released from cells

- The release of these substances leads to vasodilation, increased vascular permeability, bronchoconstriction, increased mucus secretion etc.

Basophils and mast cells - function

- Defense against helminthic parasites
- Allergic reactions (I.type)
- Mast cells contribute to the normal function of mucosa and connective tissue

Major Histocompatibility Complex

- System of glycoproteins bound on cell membrane which can be recognized by immune system
- Genes coding MHC are localized on chromosome 6, some of these genes are extremely polymorphic (signs of Mendelian heredity, codominancy, en bloc transfer)
- **MHC haplotype** = unique combination of alleles encoding MHC molecules which are localized on one chromosome

Major histocompatibility complex

- **Class I** – HLA A,B,C (E,F,G)
  – expressed on the surface of all nucleated human cells
  – antigen presentation to Tc-lymphocytes
- **Class II** – HLA DR, DP, DQ
  – expressed on the surface of APC (macrophages, B lymphocytes)
  – antigen presentation to Th-lymphocytes

Major histocompatibility complex

- **Class III** – HLA C2, C4, FB etc.
  – numerous genes located in MHC chromosomal region (e.g.gen of two C4-isotypes, C2, factor B, TNF-*alfa* and *beta*)
  – function – processing and transport of
    T-lymphocyte epitopes
    – heat-shock proteins
    – inflammation mediators

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HLA

Human Leukocyte Antigen

human MHC

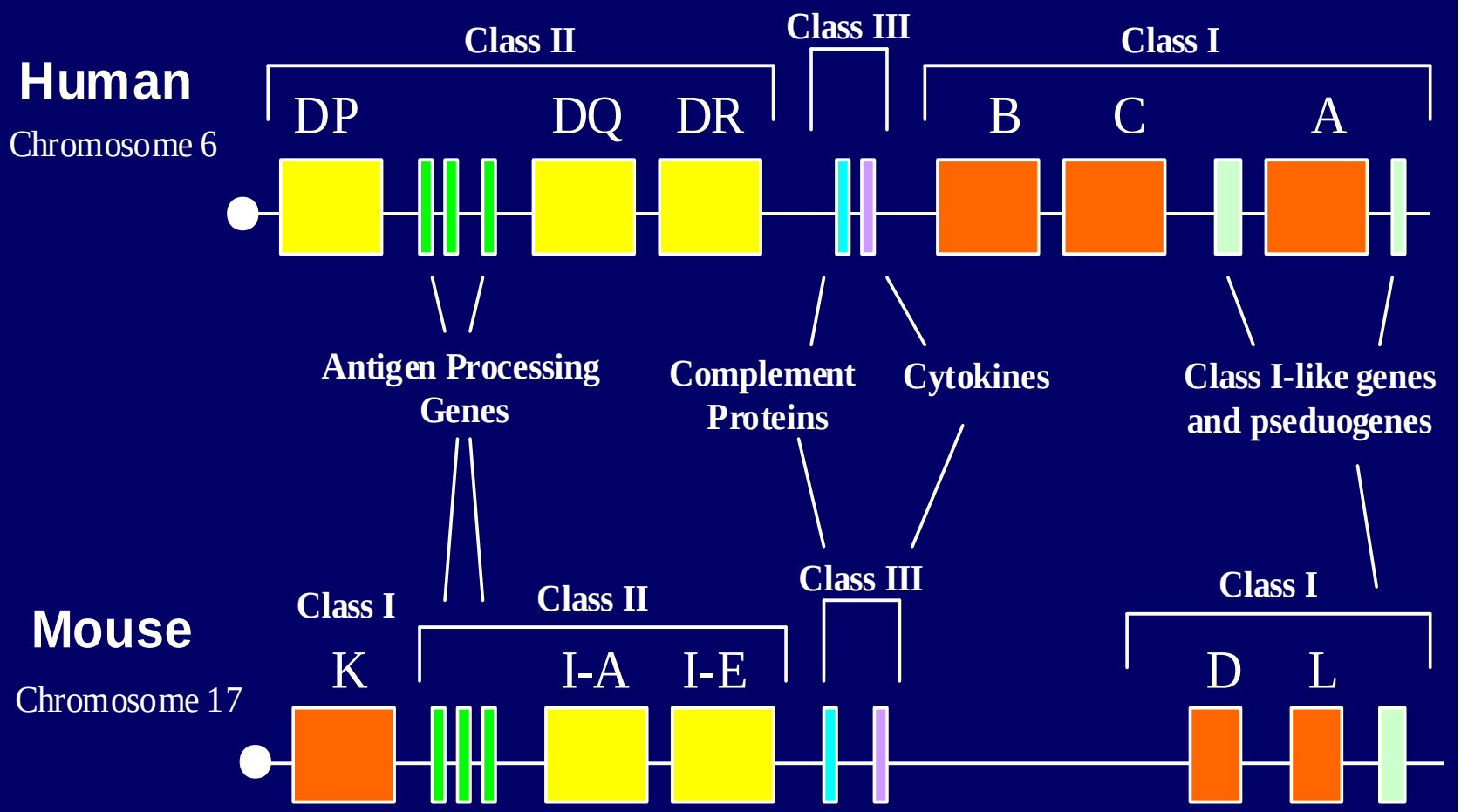
cell-surface proteins

important in self vs. nonself distinction

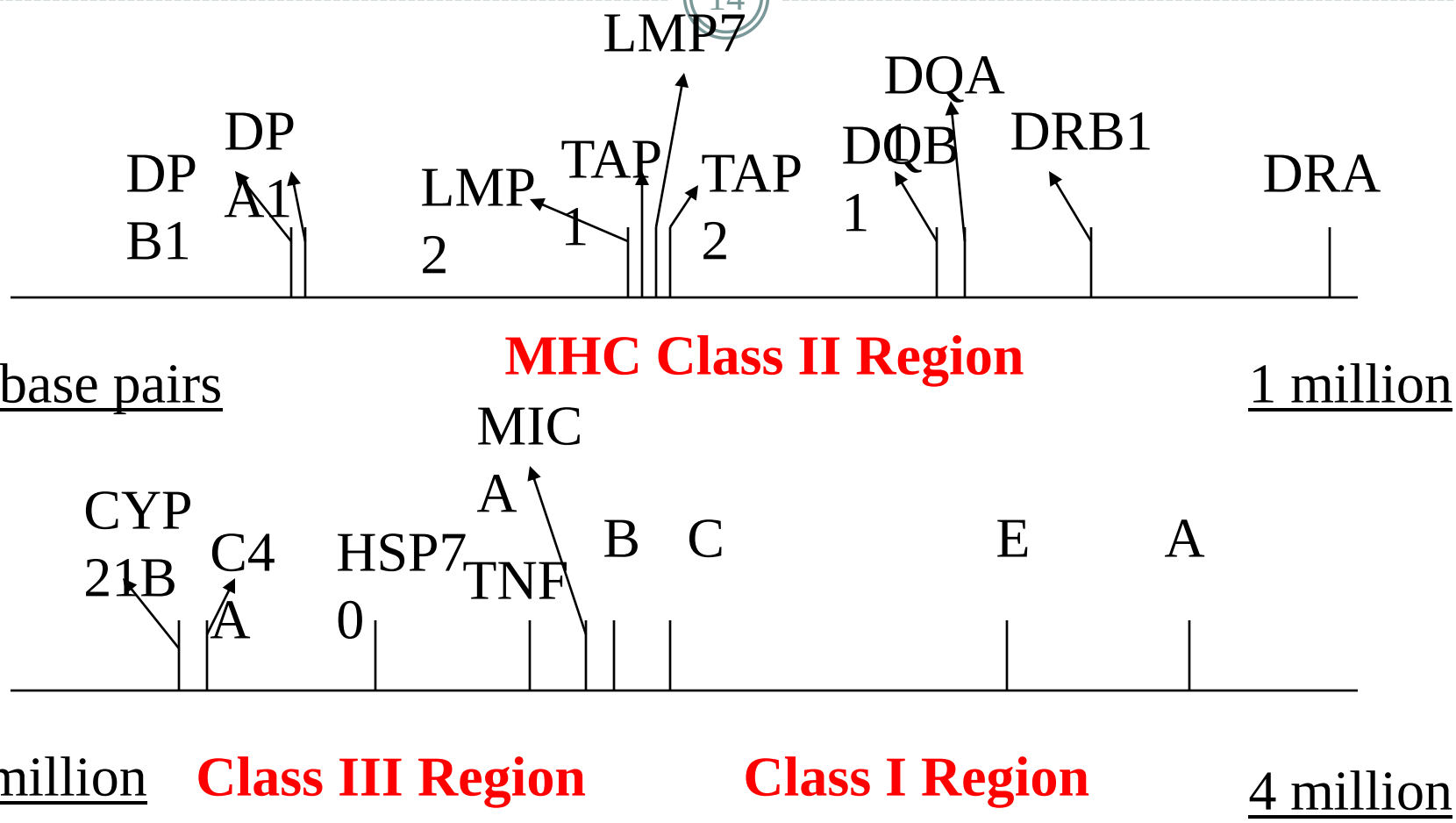
present peptide antigens to T cells

CLASS I: A,B,C **CLASS II: DR,DQ,DP**

The Major Histocompatibility Complex

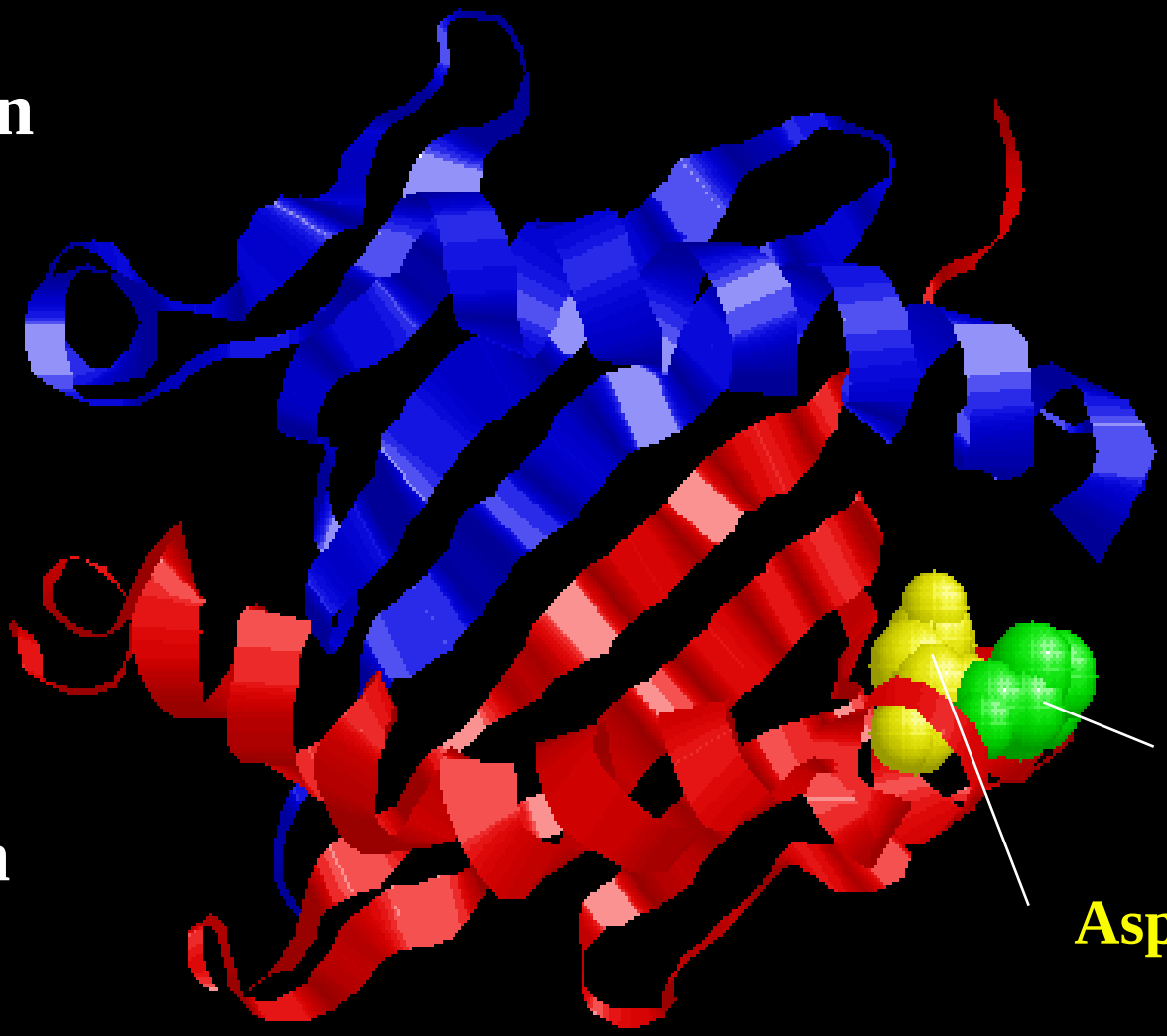


The Major Histocompatibility Complex



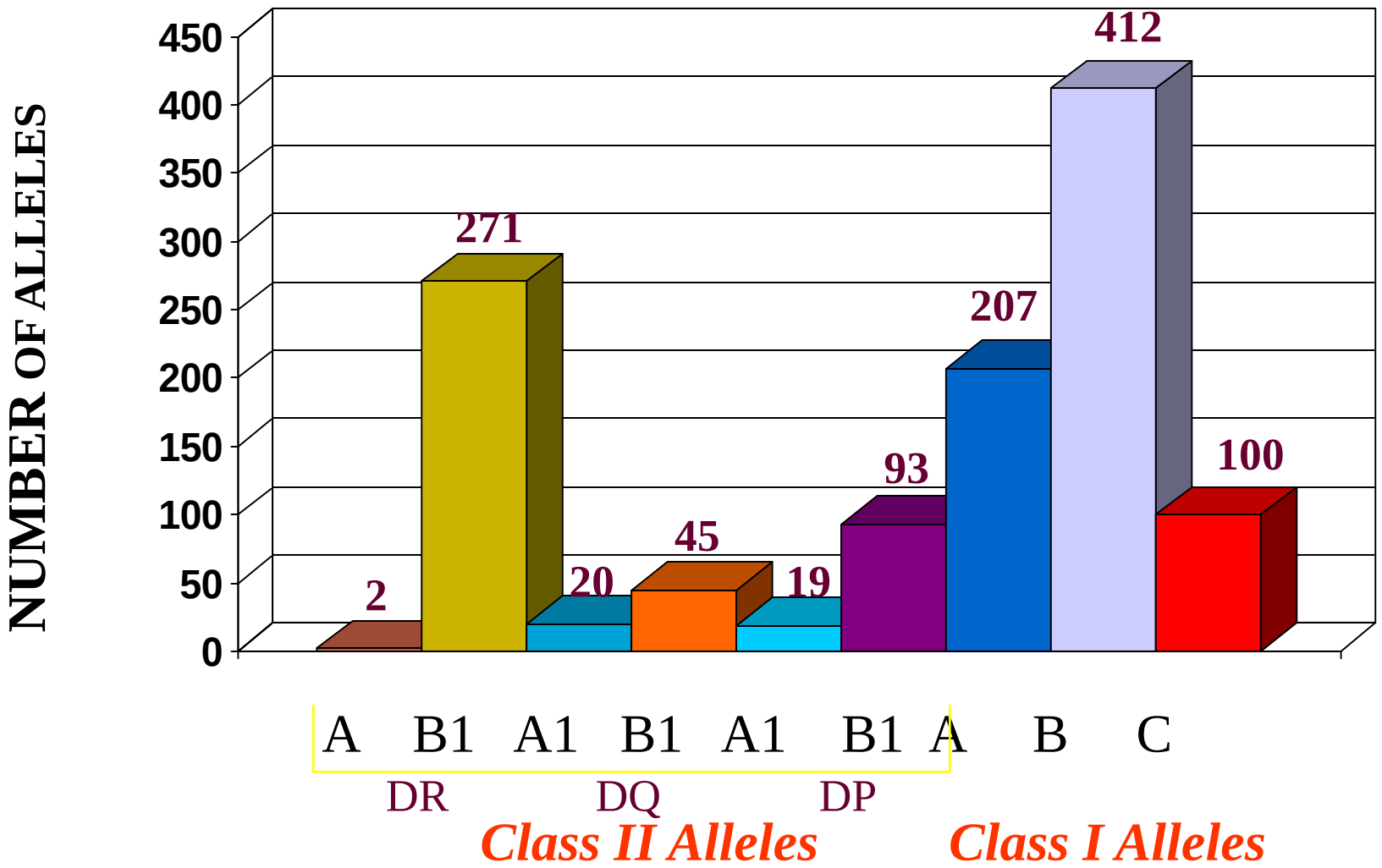
DQB1*0402

α -chain



β-chain

HLA Class I and II Alleles (January 2001)



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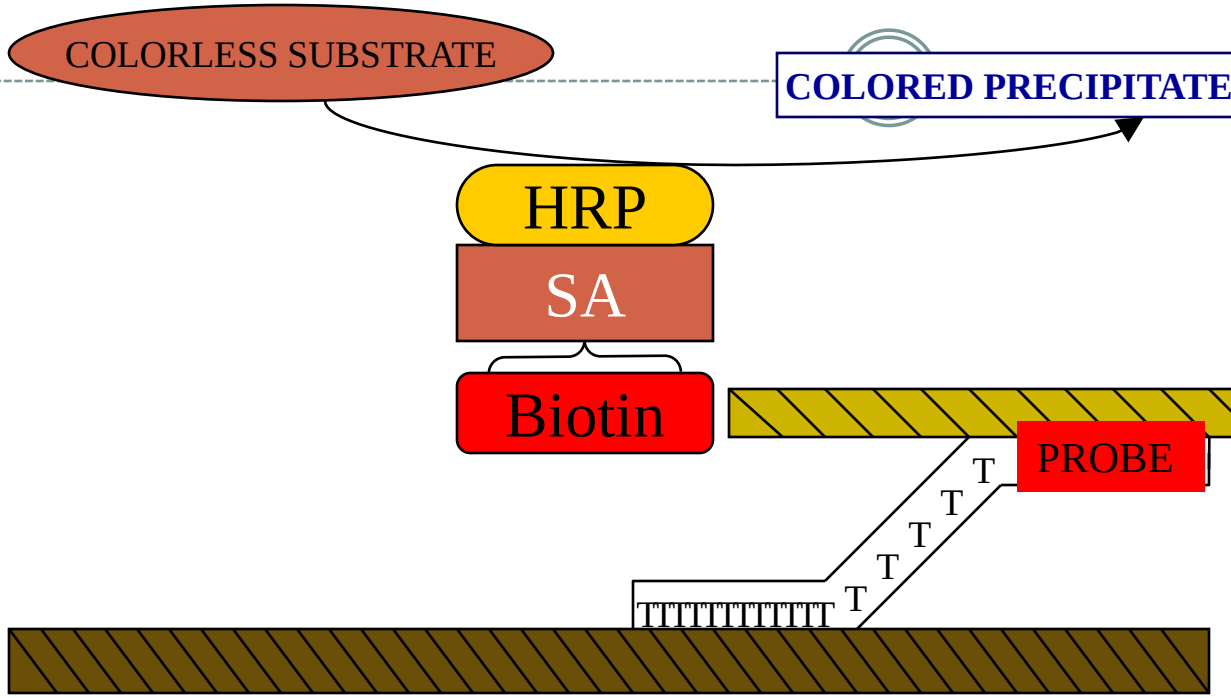
Alleles and Haplotypes in HBDI Type 1 Diabetes Families

Locus/Loci	Unique Alleles/Haplotypes
DRB1	34
DQB1	16
DPB1	23
A	33
B	52
DRB1-DQB1	57
DRB1-DQB1-DPB1	232
DRB1-DQB1-B	313
DPB1-DRB1-DQB1-B	558
DPB1-DRB1-DQB1-B-A	779

MHC testing

- **1/ Sera typing** – identification of specific class I and class II MHC molecules using sera typing
- Less time-consuming method, however, also less accurate
- **2/ DNA typing** – human DNA testing by PCR
- low resolution (groups of alleles), high resolution (single alleles)
- More time-consuming method, however, also highly accurate

The Generation of a Dot Blot for Typing Using Immobilized ASO* Probes ("Erlich" System)



DYE

STREPTAVIDIN-HRP_ ENZYME CONJUGATE

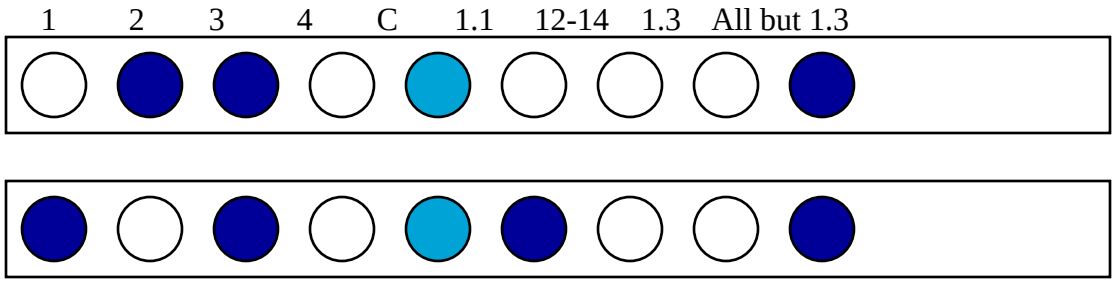
AMPLIFIED DNA

IMMOBOLIZED ASO PROBE

NYLON MEMBRANE

Examples:

Probe Specifications:



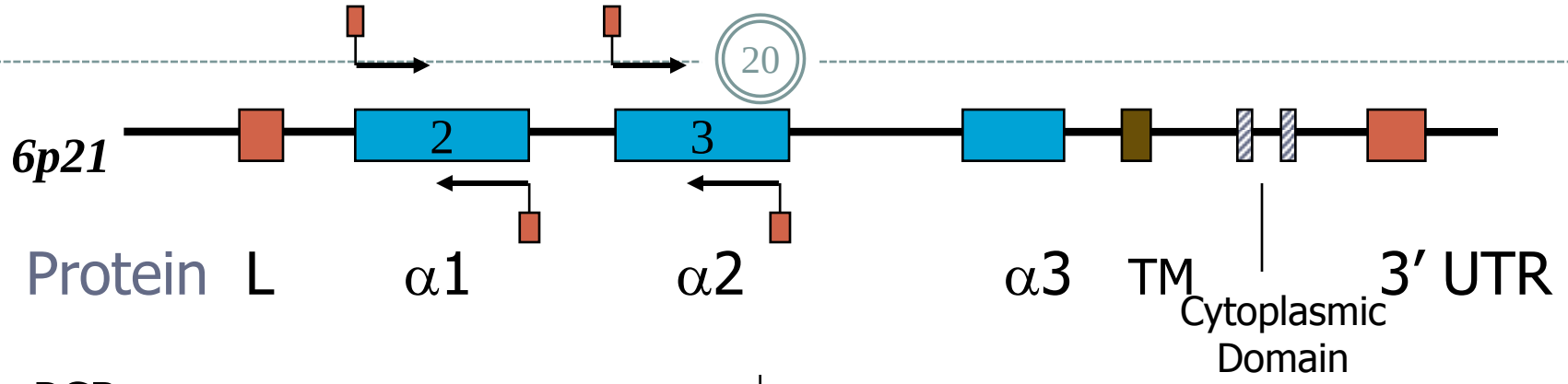
DQA type

2, 3

1.1,3

*Allele-Specific Oligonucleotide

Sequence-Specific Oligonucleotide- SSO HLA-A Tes

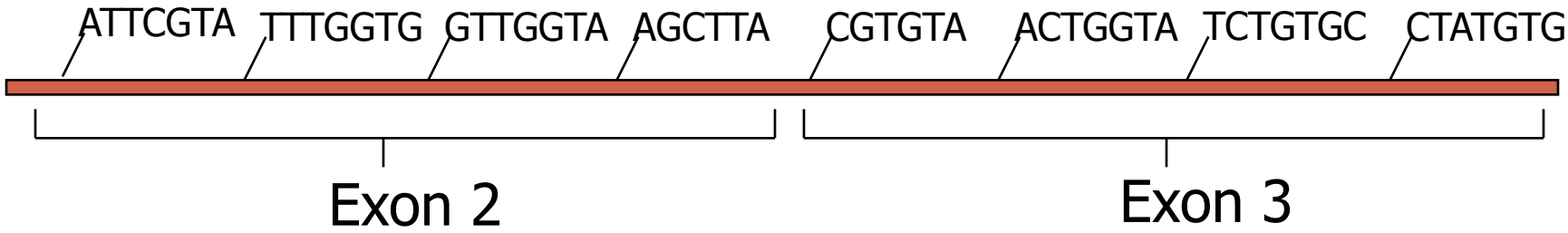


1. PCR
2. Hybridization
3. Detection

PCR 35 Cycles

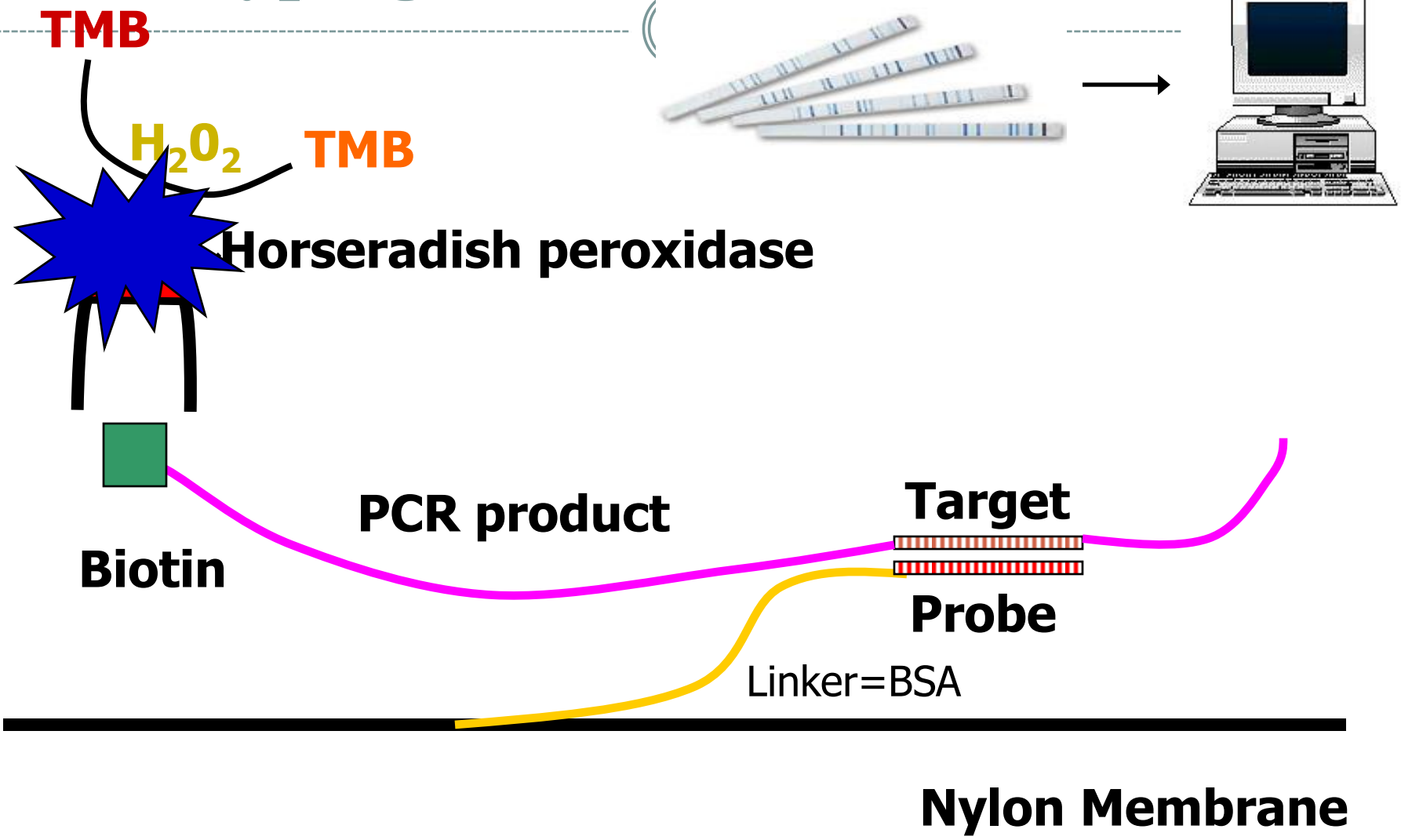
480 bp

314 bp



Nylon Membrane "HLA-A Stips"

HLA Typing: Immobilized Probe Arrays



Antigen presentation

- An antigen is a substance recognized by immune system that reacts to its presence.
- For induction of specific immune response to antigen, first of all antigen processing and its presentation to APC is necessary.
- The professional **antigen presenting cells (APC)** are cells expriming MHC class II molecules (macrophages, dendritic cells, B-lymphocytes).

Processing and presentation of protein antigens

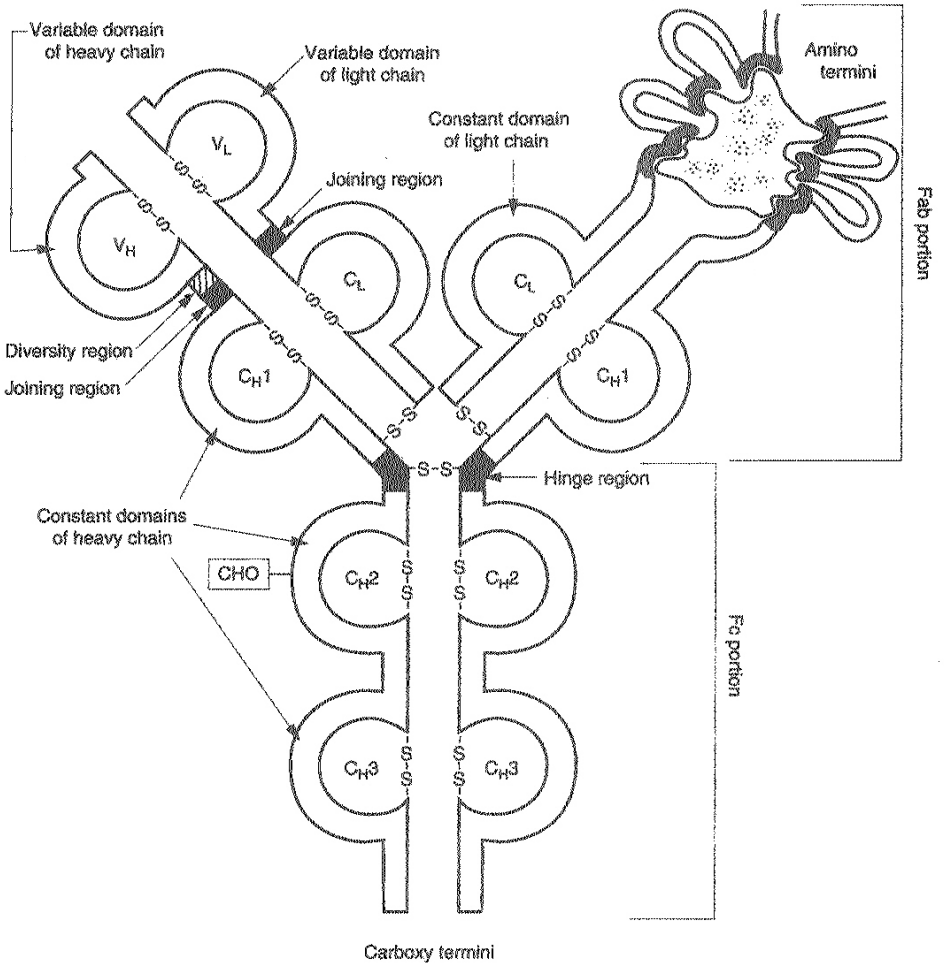
- **1/ Exogenous antigens**
- Bacterial, helminthic or viral antigens (either if they form immune complexes swallowed by APC, or if they are processed together with infected cells)
- They are presented in a complex with MHC class II to T helper (CD4+) cells

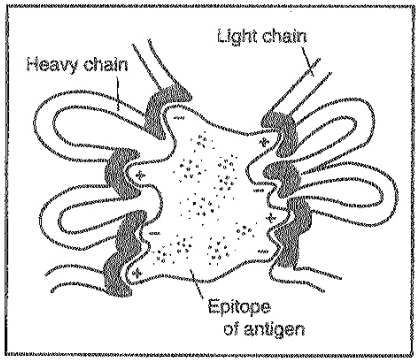
Processing and presentation of protein antigens

- **2/ Endogenous antigens**
- Intracellular auto-antigens, antigens of viruses or other intracellular parasites (infecting APC) or tumorous antigens
- Present in complex with MHC class I molecules to cytotoxic (CD8+) T cells

Immunoglobulins

1. THE STRUCTURE OF IMMUNOGLOBULINS


Variable domain of heavy chain
Variable domain of light chain
Amino termini
Constant domain of light chain
Joining region
Fab portion
Diversity region
Joining region
Hinge region
Constant domains of heavy chain
CHO
Fc portion
Carboxy termini


Light chain
Heavy chain
Epitope of antigen

2. Isotypes

- (in principle) classes of antibodies distinguished on the basis of H chain structure differences
- 5 types: μ (IgM), δ (IgD), γ (IgG), α (IgA) and ε (IgE)
- in addition, we can distinguish subtypes of antibodies within some classes (IgG, IgA) based on their H chain differences ($\gamma_{1\text{-}4}$, $\alpha_{1\text{-}2}$)

3. Domains and their biological function

- In principle: domains of **V regions** form a recognizing unit and domains of **C regions** determine secondary biological functions of antibody (i.e. biological half life, distribution in the body, binding complement, binding to cells through Fc-receptor)

4. Variable region of Ig molecule

- Hypervariable loops are concentrated at the spikes of variable regions where antigen binding sites are localized

- The binding site specificity is determined by aminoacid sequentions and both by morphology and shape of the loop

5. The biological features of distinct Ig classes

IgG

- the most abundant serum Ig
- the most important Ig of secondary immune response
- the only Ig which passes through the placenta
- the main opsonizing Ig
- activates complement via classical pathway
- biological half life 21 day

IgA

- present both in serum and seromucinous secretions
- defence of mucosa
- opsonization
- does not activate complement

IgM

- in pentamer form is present in serum; in monomer form is bounded on membrane of B cells
- prevailing antibody of primary immune response
- high-effective agglutinant and cytolytic agent
- usually isohaemagglutinins and natural antibodies

- the best classical way complement activator
- does not bind phagocytes Fc receptor, but substantially enhances phagocytosis through complement activation

IgD

- free form in serum, bound on B cells membrane
- antigen receptor on B cells

IgE

- in normal conditions low amounts in serum
- mainly bound on mast cells (binds through FcεR)
- anti-helminth defense
- immediate type allergic reactions

6. Allotypic and idiotypic variations

- **Allotypes** = allelic variants of isotypes
- **Idiotypes** = structural determinants localized in variable region having connection with the ability of antigen binding
- **Idiotopes** = epitopes in variable region (idiotype is the sum of idiotopes)
- **Anti-idiotypic antibodies** = in principle reflect the antigen

7. Genetic basis of Ig production

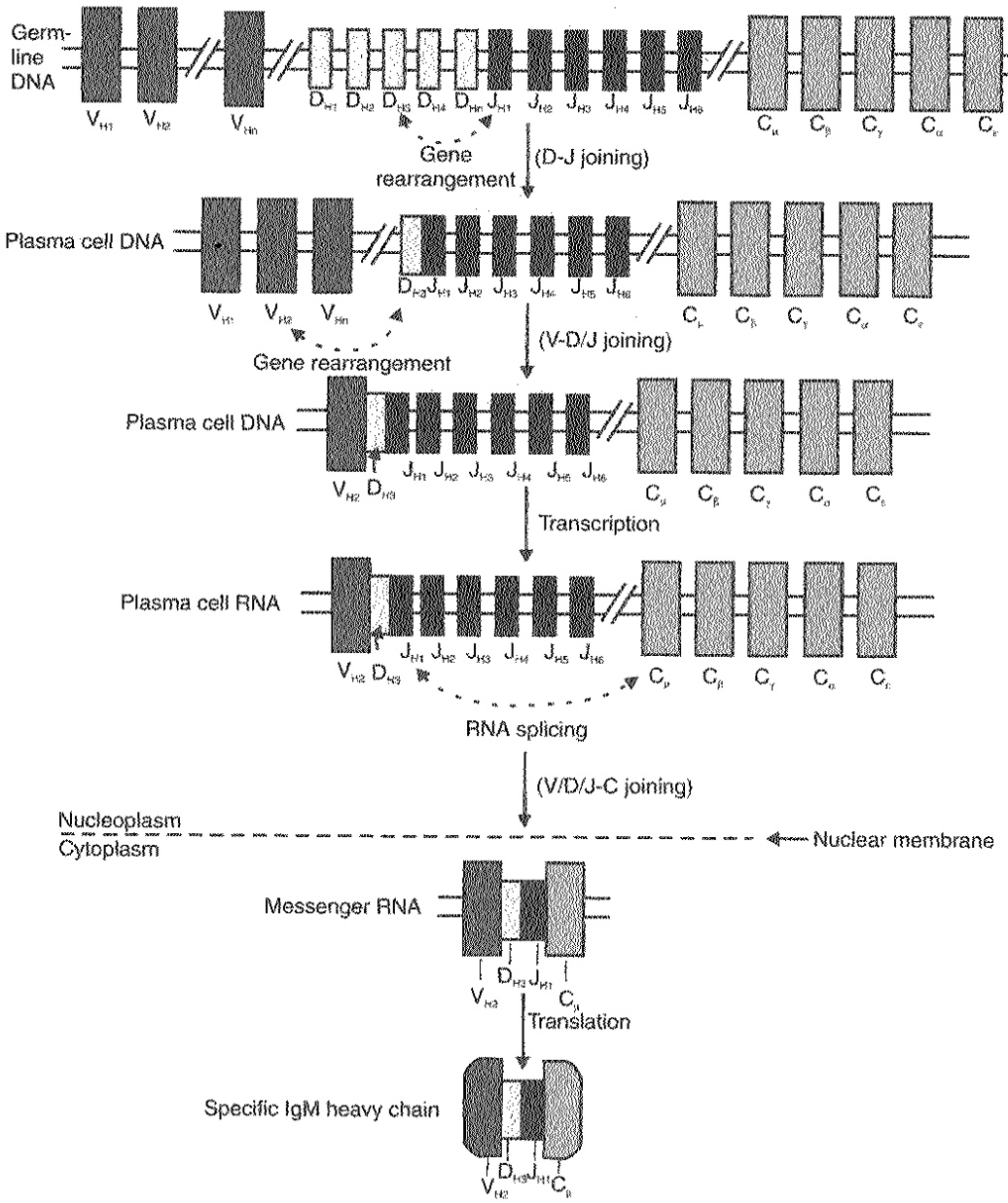
a/ L chains genes

K chain – genes located on chromosome 2
- V, J and C segments

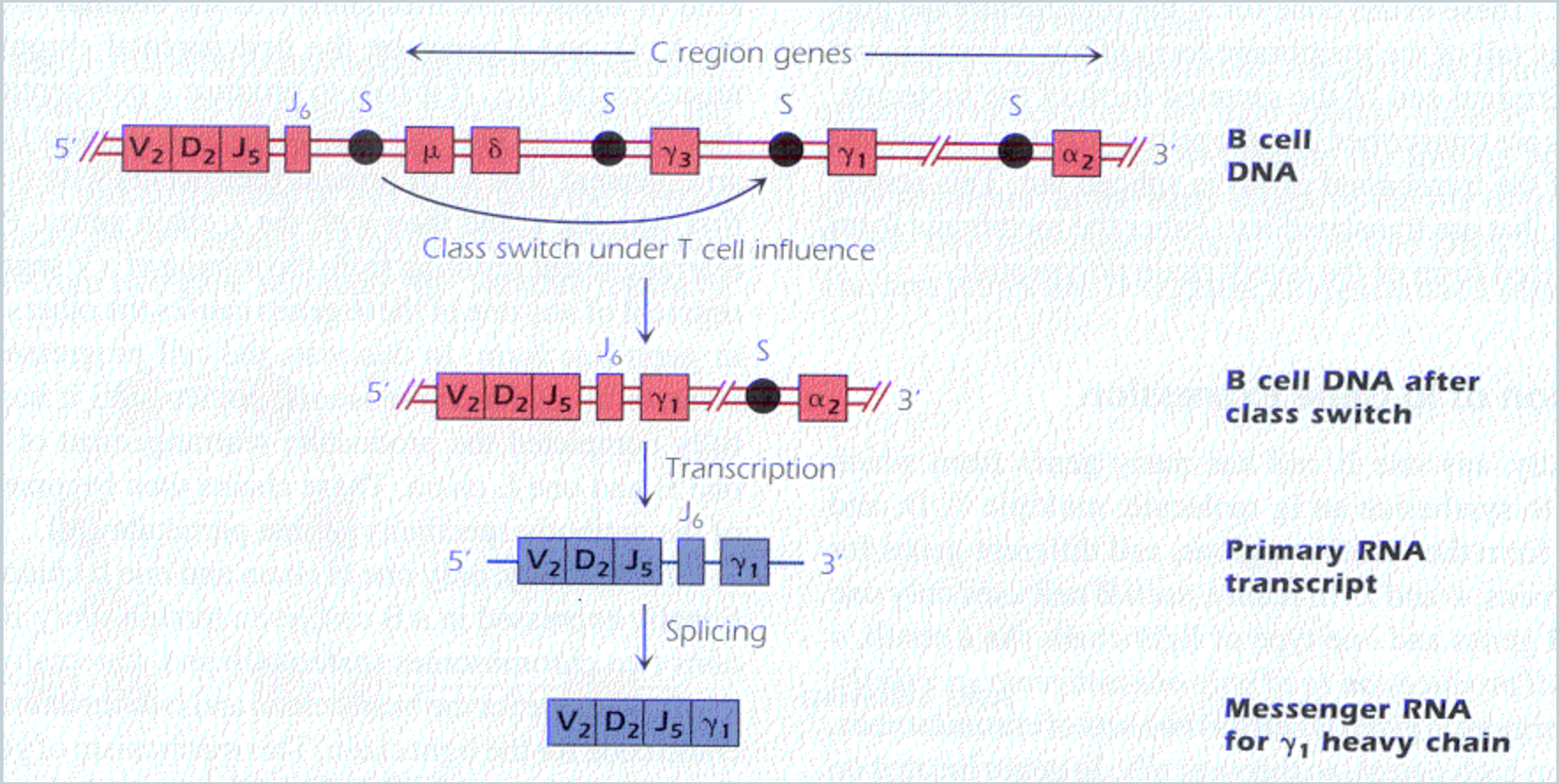
λ chain – encoded in similar complex of genes on chromosome 22

b/ genes encoding H chain

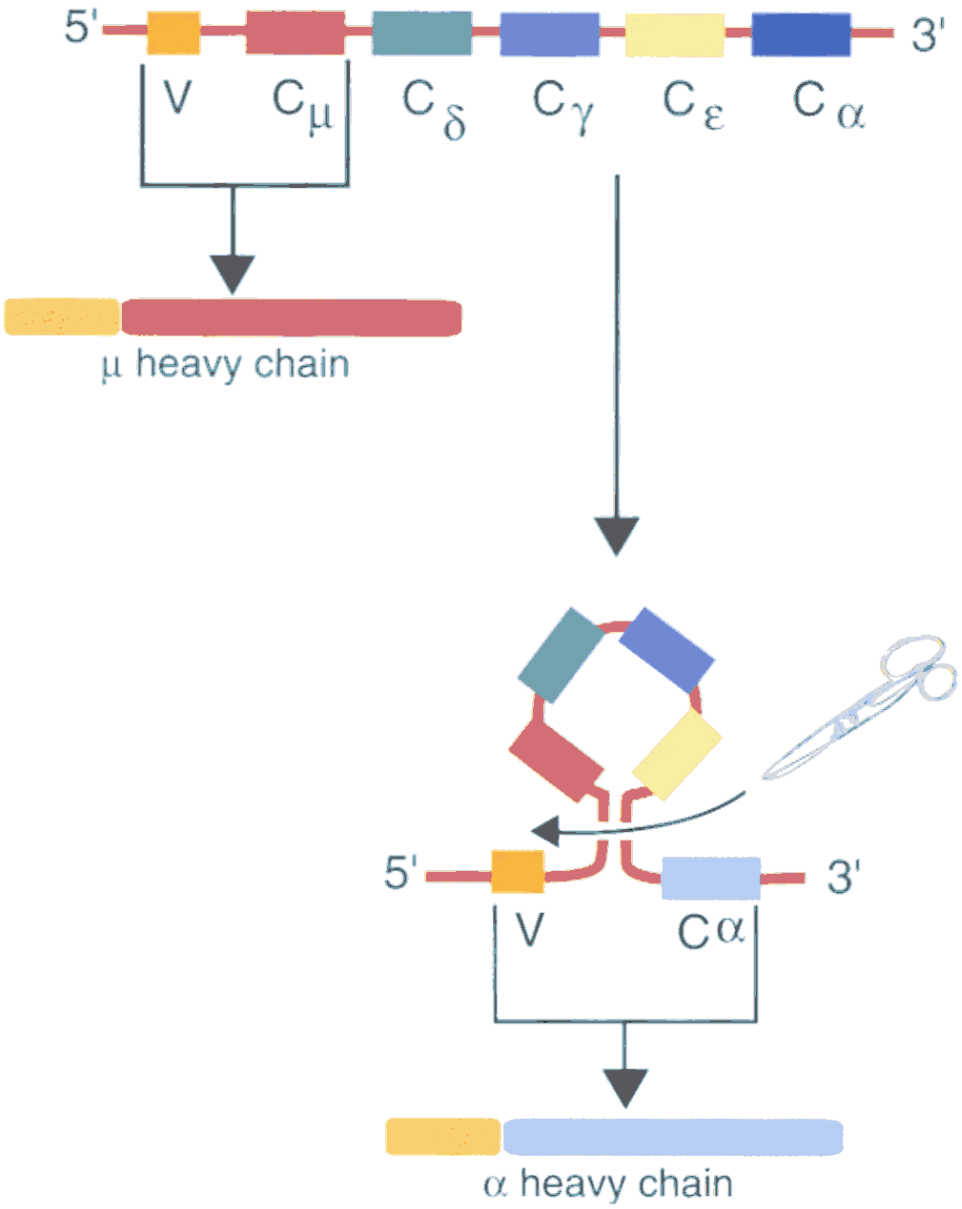
- more complicated
- located on chromosome 14
- V, D, J, C segments (genes encoding individual segments contain more regions compared with L chains)
- during completion of V/D/J exon, gene rearrangement occurs

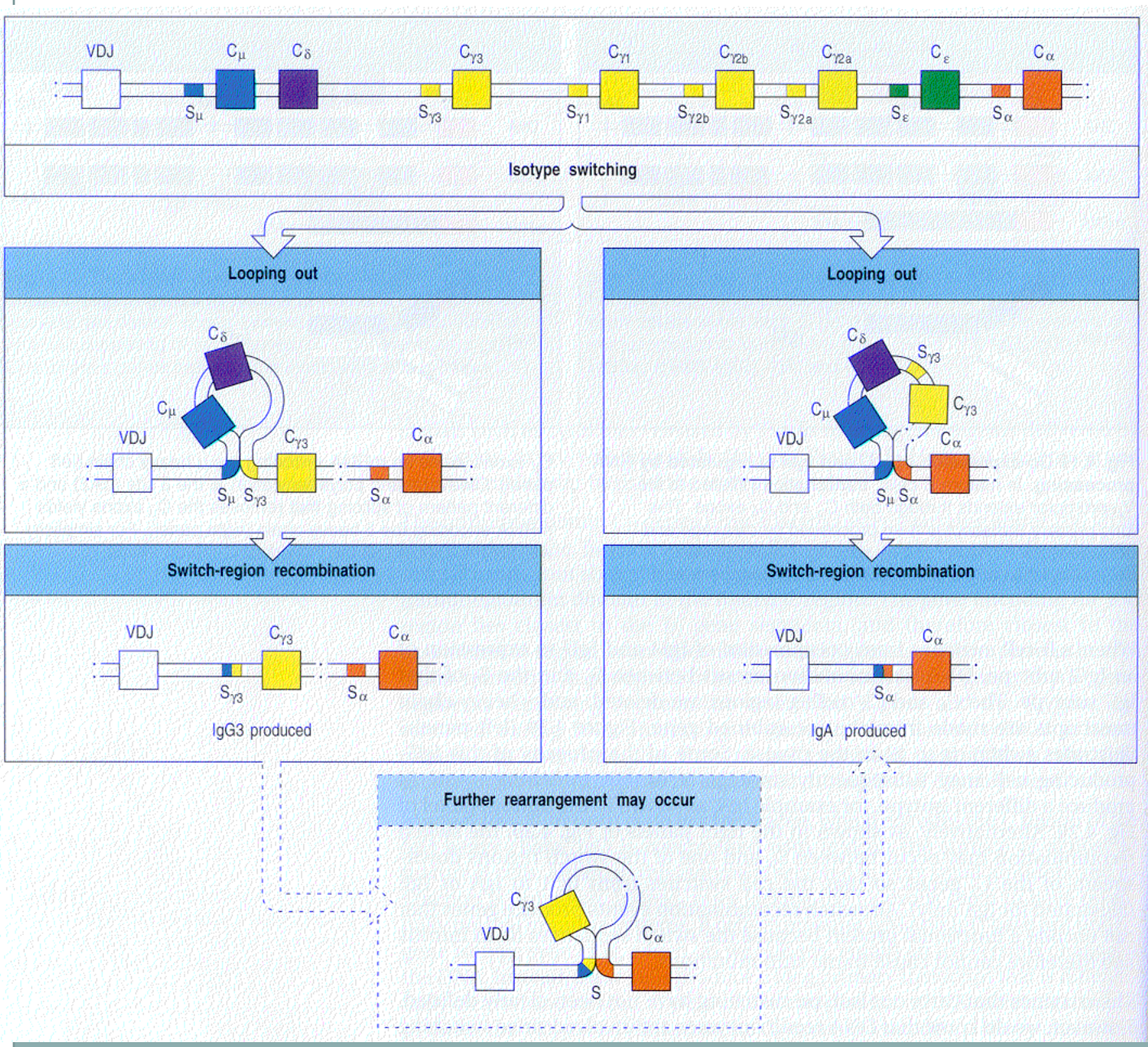

Germ-line DNA
Gene rearrangement
(D-J joining)
Plasma cell DNA
Gene rearrangement
(V-D/J joining)
Plasma cell DNA
Transcription
Plasma cell RNA
RNA splicing
(V/D/J-C joining)
Nucleoplasm
Cytoplasm
Nuclear membrane
Messenger RNA
Translation
Specific IgM heavy chain

Class switching in Ig synthesis



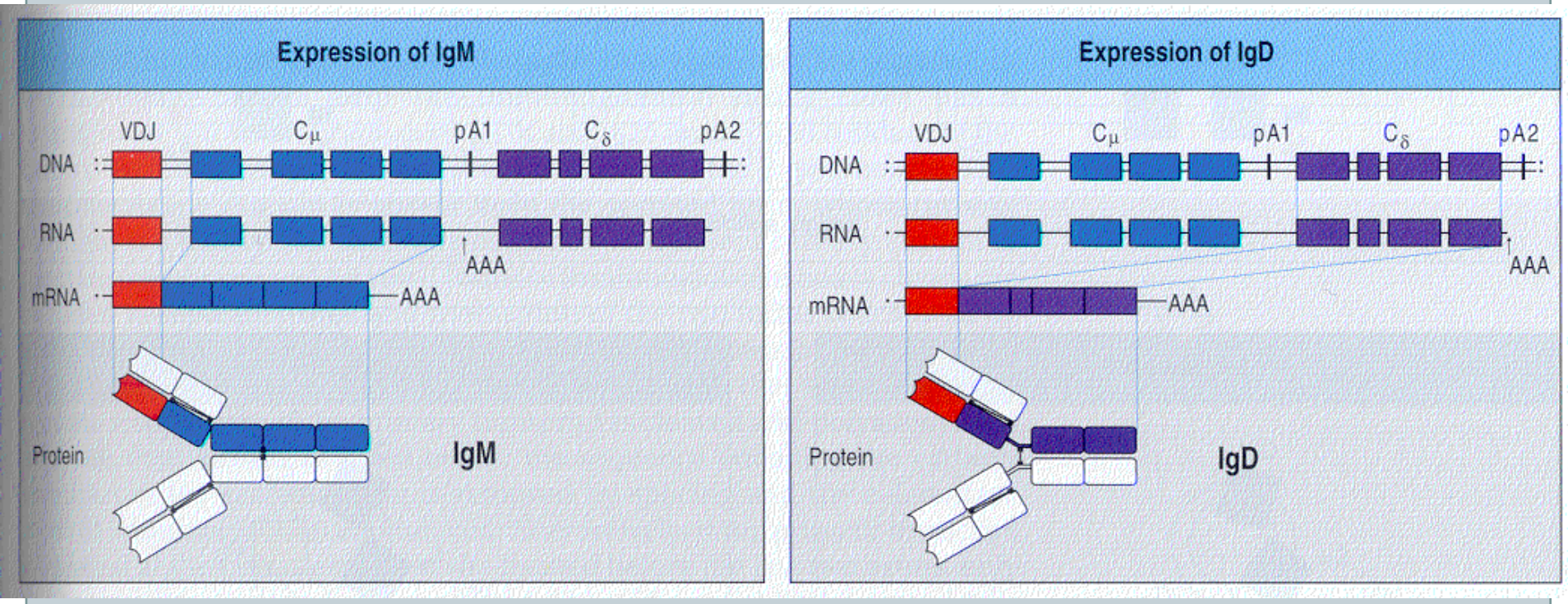
Mechanism of class switching

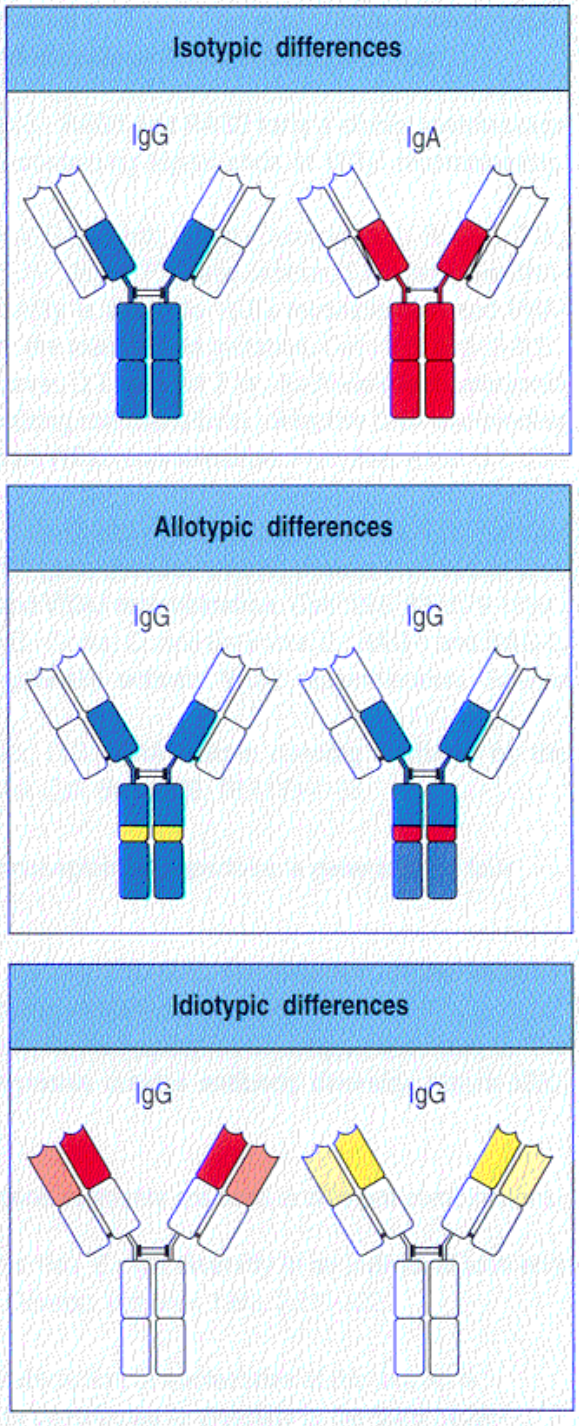




Isotype switching involves recombination between specific switch signals

Co-expression of IgD and IgM regulated by RNA processing





Different types of variation between immunoglobulins

Mechanisms contributing to antibody diversity:

- chance recombinations
- imprecise joining of V, D, J genes
- N-region additions
- extensive mutations involving variable-region genes after antigen exposure

Isotype switching

- during the immune response, plasma cells switch from producing IgM to IgG or to another Ig class (IgA, IgE)
- the switch involves a change in the H-chain constant domains (C_H)
- **no change in antigen-binding specificity !**

  (no alteration in the L chain or in the variable portion of H chain)

Allelic exclusion

- once the process of rearrangement on one of chromosomes is successful, then all attempts on second chromosome are stopped
- the same rule governs both for H- and L-chains
- every single B cell produces only one type of H- and one type of L-chain

Clonal restriction

- each B cell expresses identical copies of an antibody that is specific for single epitope
- when a B cell divides, the chromosomes in its progeny cells bear the selected allelic genes, and these genes do not undergo any further V/J or V/D/J rearrangements
- immunoglobulins produced by given B cell and its progeny are identical in epitope specificity and in κ– or λ–chain isotype

The development of B-lymphocytes

- B-lymphocytes originates from stem-cell
- **Bone marrow:** pre-B-lymphocytes (synthesis of H chains, Ig genes rearrangement → antigen specificity, IgM expression on the surface of the cell)
- **Blood, peripheral lymphoid organs:** mature B-lymphocytes (IgD expression), ready to react with an antigen → contact with an antigen → division of cells and differentiation to plasma cells (secretion of huge amounts of Ig) + generation of memory B-lymphocytes

B-lymphocytes – surface markers

- CD19, CD35 – complement receptors
- IgM, IgD = BCR
- B7 protein – adhesin, contact with T-lymphocyte
- MHC class II – antigen-presenting molecules

B-lymphocytes - function

- B-cells activation:
- **1/ thymus independent** – polysacharide antigens, a cooperation with T cells is not necessary for B cells activation
- **2/ thymus dependent** - first of all, the development of antigen-specific Th cells is necessary, then, thanks to cooperation between B cells and Th cells the antibody production could be sufficient and appropriate

B-lymphocytes - function

- Antibody production
- Antigen presentation

Ontogenesis of the antibody production

- Although the production of specific antibodies already begins about week 20-24 of gestation, IgA+M concentrations are very low until the birth
- IgG production begins only after the birth, but IgG level is at this time sufficient thanks to maternal IgG
- About 4 to 6 months of age maternal IgG is eliminated from the child's organism (possible onset of humoral deficiency symptoms)

Phases of humoral response

- **Primary response** – typical delay of the antibody production (antigen presentation to Th cells is necessary)
- **Secondary response** – thanks to memory antibodies and memory lymphocytes, the response is stronger and faster