MEDICAL ACADEMY NAMED AFTER S.I.GEORGIEVSKY OF VERNADSKY

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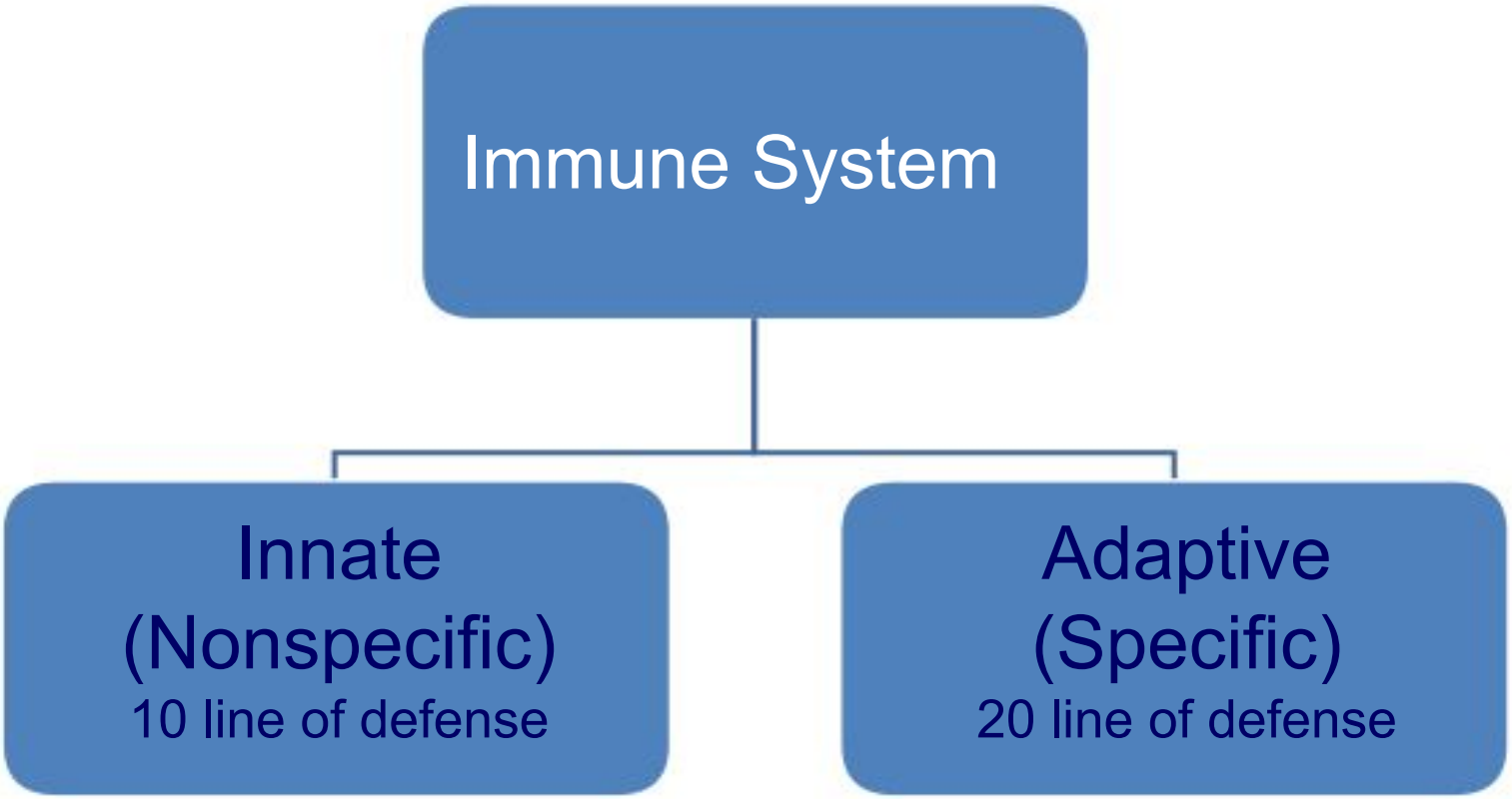
Group: LA1-202(2)
TOPIC: Immuno-genetic method of medical genetics.
Teacher: professor Svetlana smirnova

What is Immuno-Genetics?

- **Immunogenetics** or **immungenetics** is the branch of medical genetics that explores the relationship between the immune system and genetics. The term ‘immunogenetics’ refers to the scientific discipline that studies the molecular and genetic basis of the immune response.
- Genetic conditions that affect either the development or function of components of the immune system lead to an inability to control infectious pathogens or a susceptibility to autoimmunity or cancer.
- These primary immunodeficiency disorders have dramatically increased our understanding that certain components of the immune system are essential for controlling specific pathogens in humans.
- They have also informed our understanding of basic mechanisms involved in immune tolerance (autoimmunity) and immune surveillance (tumor immunity) under normal conditions.

Overview of the Immune System



Innate immunity vs Adaptive Immunity

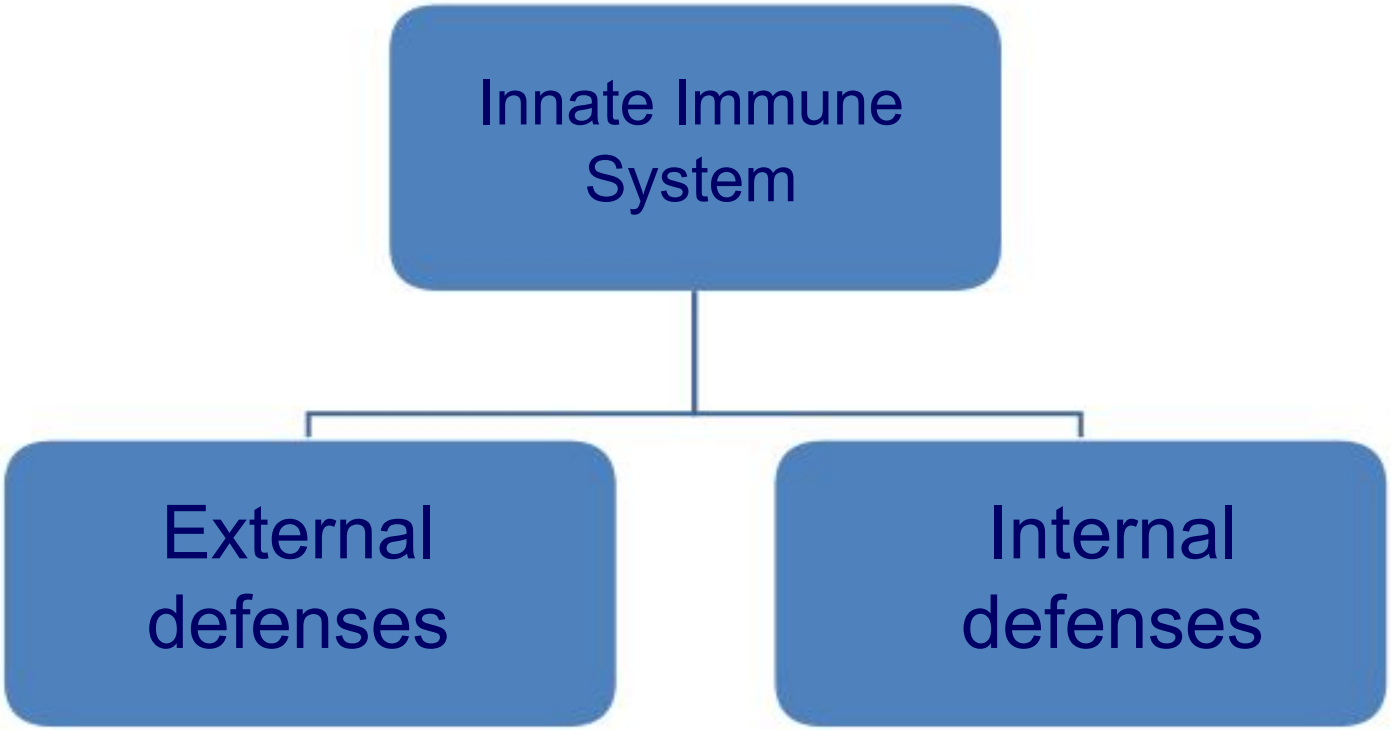
Innate Immunity
(first line of defense)

- No time lag
- Not antigen specific
- No memory

Adaptive Immunity
(second line of defense)

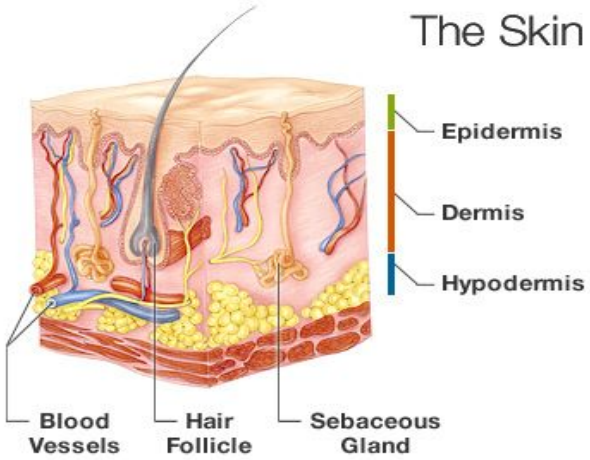
- A lag period
- Antigen specific
- Development of memory

The innate immune System



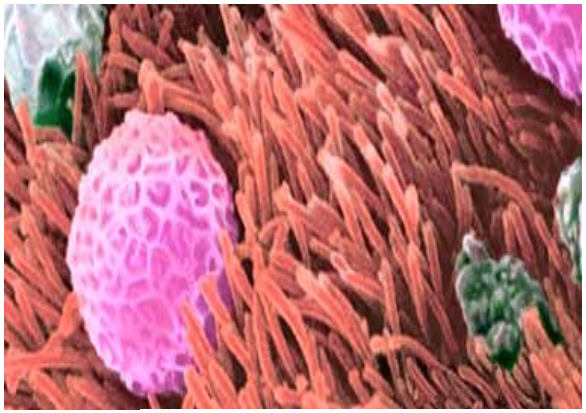
Innate immune system
External defenses

Anatomical Barriers - Mechanical Factors



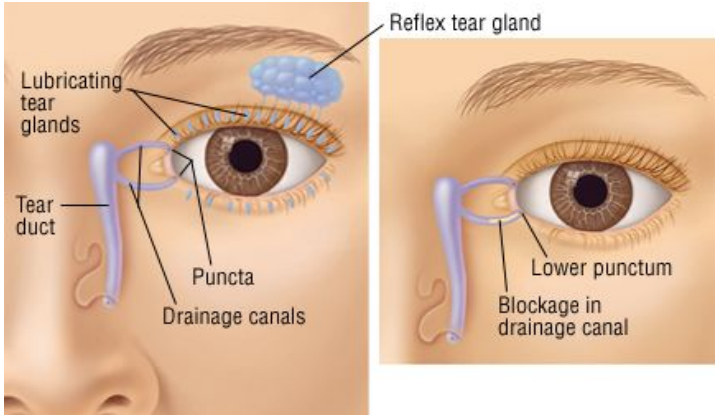
- Skin

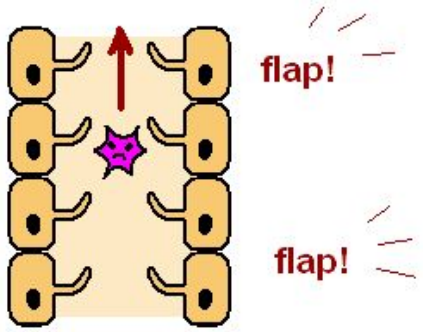
- Mucociliary escalator



The MUCOCILIARY ESCALATOR!

- Flushing action of saliva, tears, urine

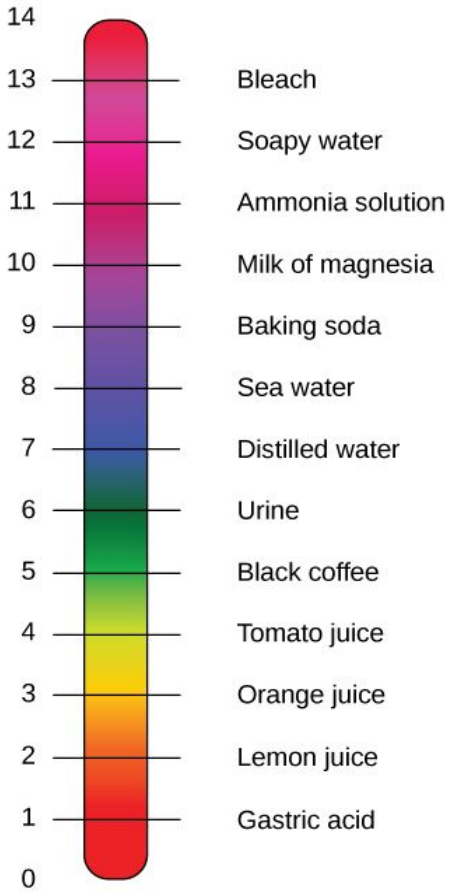




Anatomical Barriers – Chemical factors

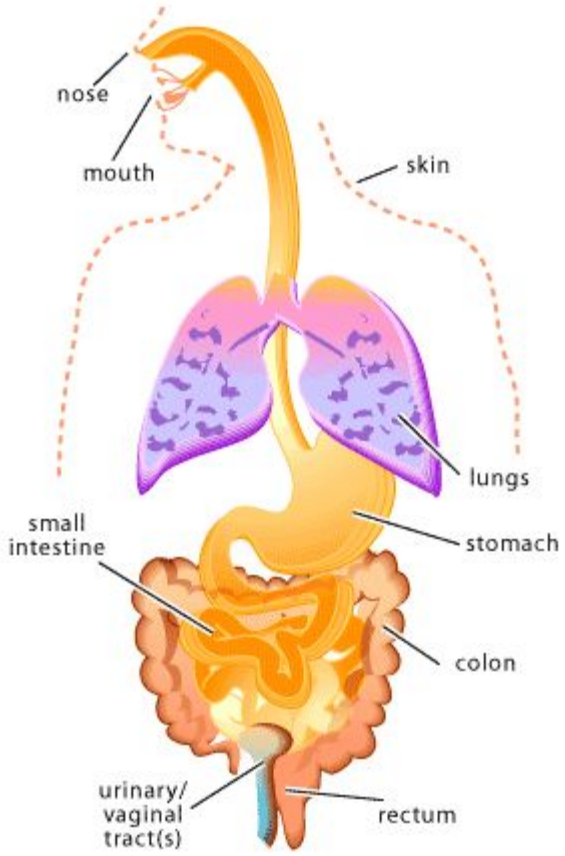
- Antimicrobial Peptides in sweat

- HCl in stomach



- Lysozyme in tears /saliva

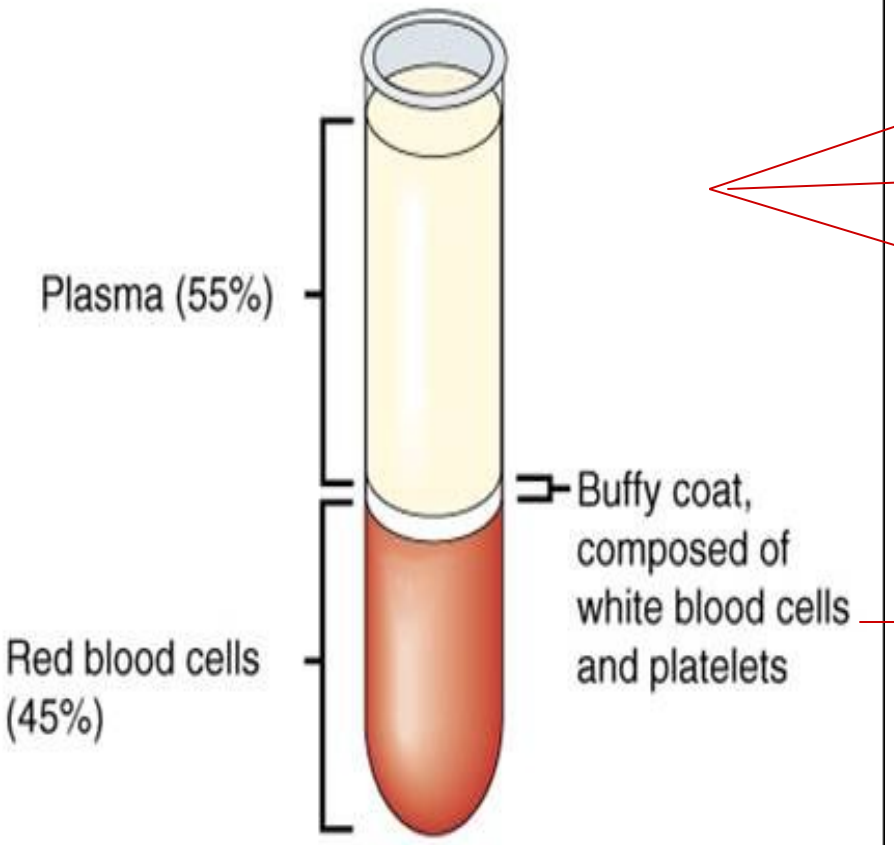
Anatomical Barriers – Biological factors



- Normal flora – microbes in many parts of the body
- Normal flora – > 1000 species of bacteria
- Normal flora – competes with pathogens for nutrients and sp

Innate immune system internal defenses

Innate immune system: components of Blood



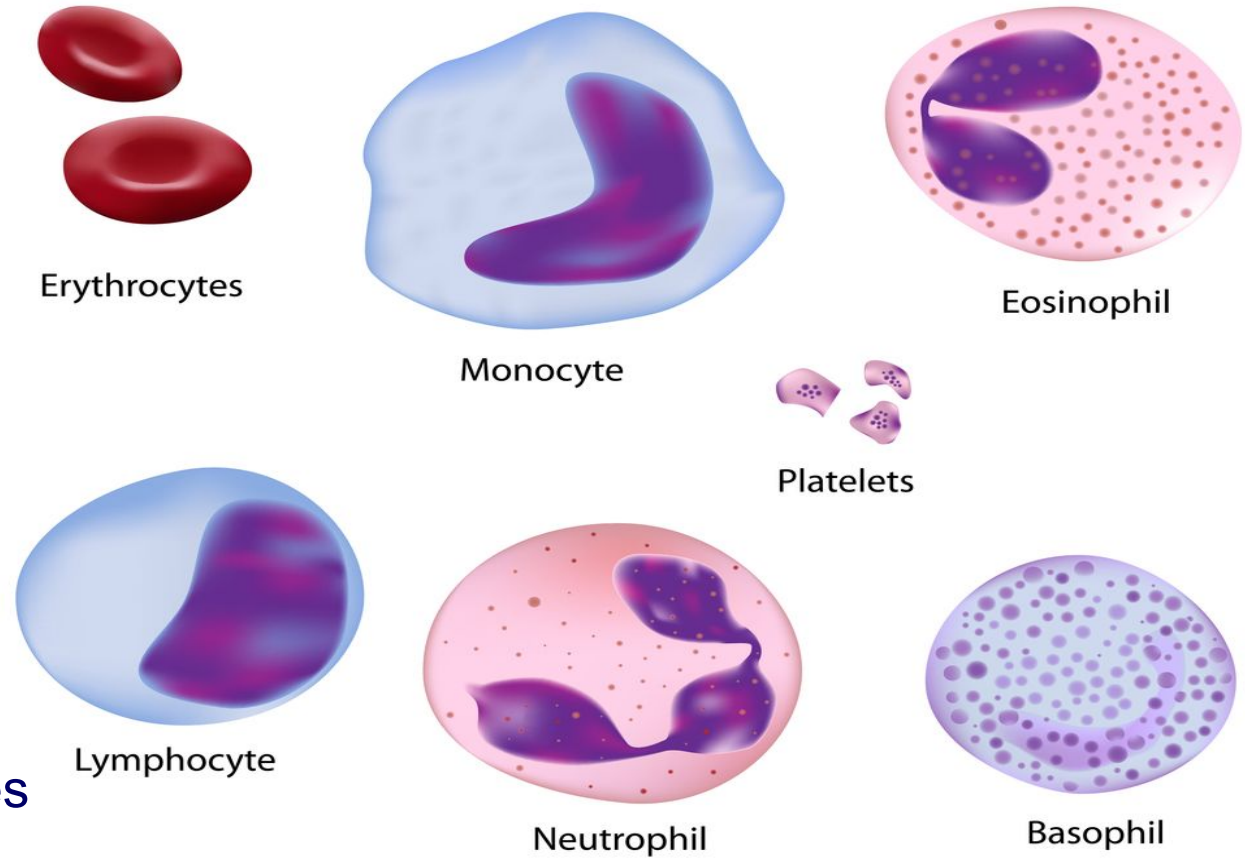
- Complement proteins
- Coagulation proteins
- Cytokines

Extracellular

- WBCs

White blood cells (WBCs)

Macrophages



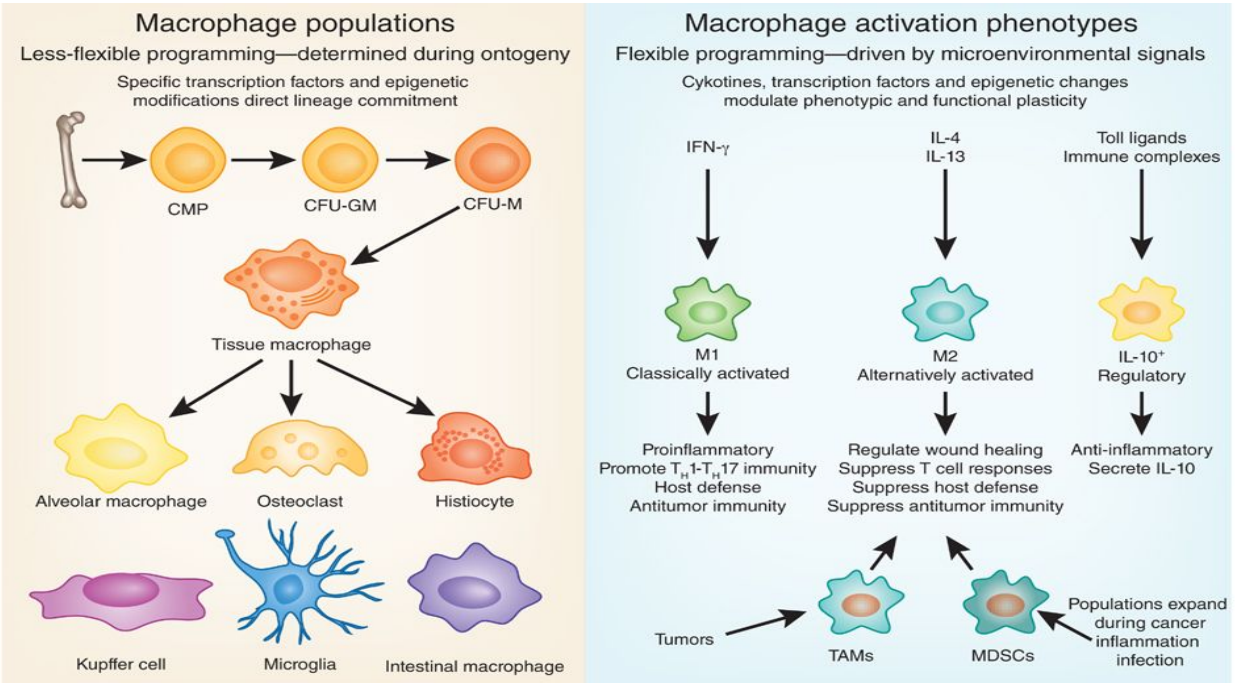
- B- lymphocytes
- T- lymphocytes
- Natural killer(NK) cells

Mast cells

Neutrophils in innate immune response

- Most abundant WBCs (~50-60%)
- Efficient phagocytes
- Most important cells of the innate immune system

Monocytes

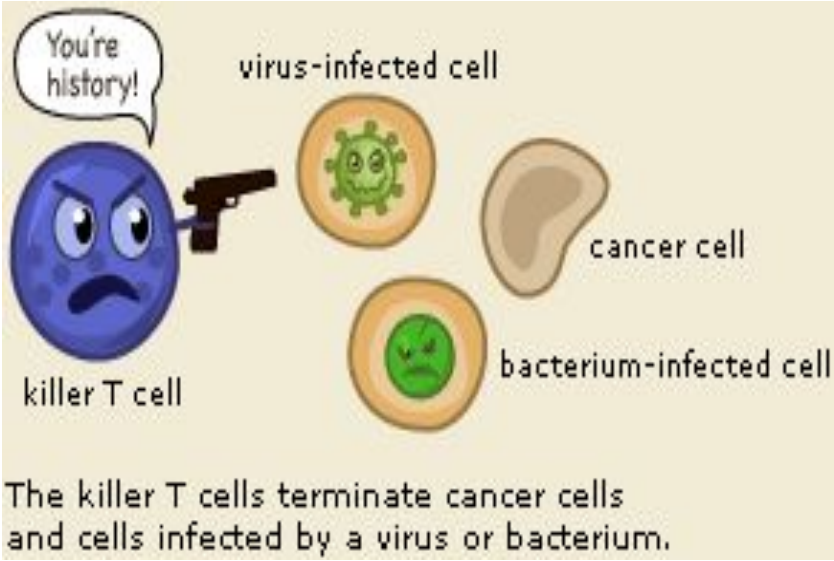


- Monocytes (~5% of WBCs)
- Migrate into the tissues and become Macrophages

Macrophages

- “Big eaters”
- Phagocytosis of microbes in tissue (neutrophils are present only in blood)
- Antigen presentation

Natural killer cells



- Not B-lymphocytes / T lymphocytes
- Important part of the innate immune system
- Kill virus /bacteria infected cells (Intracellular pathogens)

Toll-like receptors (TLRs)

- Transmembrane proteins
- Present on macrophages / few other cells
- Conserved across vertebrates
- Important part of innate immune system

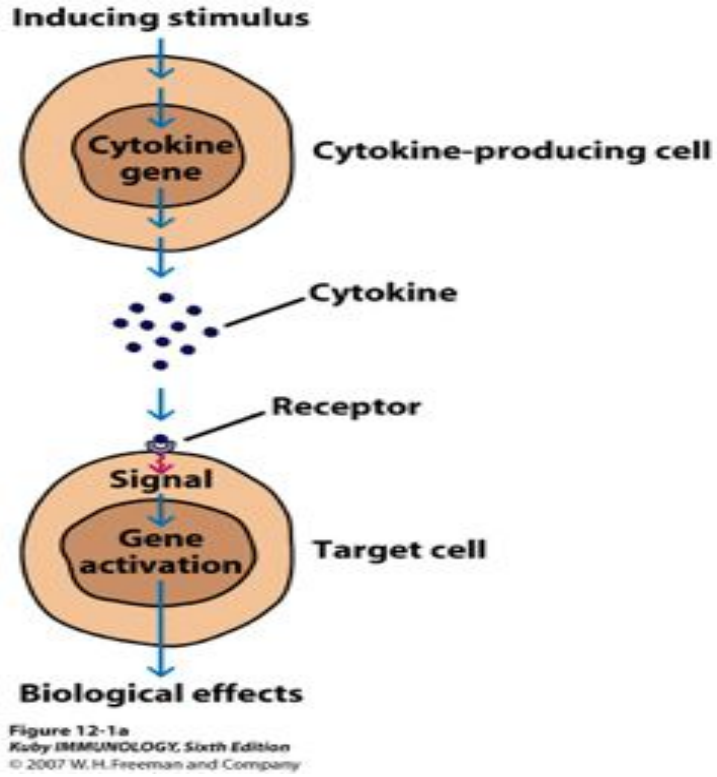
Summary: innate response – internal defenses – Cellular (WBCs)

Come into play when the external defenses are breached

- Neutrophils
- Monocytes /macrophages
- NK cells
- TLRs

Cytokines

- Small proteins – secreted bycells of the immune system
- Affect the behaviour of other cells
- Signalling molecules
- Key players in innate and acquired immunity



Examples of cytokines

- Interferons
- Interleukins
- Tumour necrosis factor (TNF)

Interferons (IFN)

- Signalling proteins produced by by virus infected monocytes and lymphocytes
- Secreted proteins – Key anti-viral proteins
- “Interfere” with virus replication
- Warn the neighbouring cells that a virus is around..

Interleukins

- Interleukins – 1-37
- Not stored inside cells
- Quickly synthesized and secreted in response to infection
- Key modulators of behaviour of immune cells
- Mostly secreted by T-lymphocytes & macrophages

Complement (C`)

- a large number of distinct plasma proteins that react with one another (C1 thro' C9)
- Complement can bind to microbes and coat the microbes
- Essential part of innate immune response
- Enhances adaptive immune resposne (taught later)

Coagulation proteins

- Coagulation: mechanism to stop bleeding after injury to blood vessels

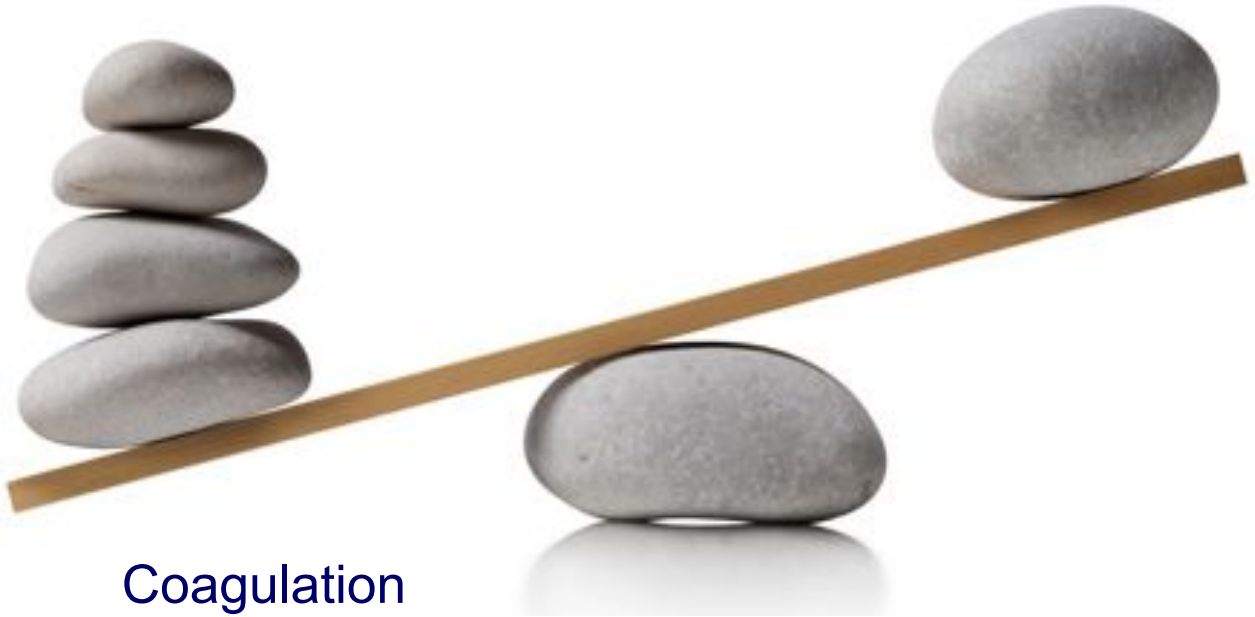
Complex pathway involves

- Platelets
- Coagulation factors
- Vitamin K

Coagulation and innate immunity

Pathogens and cytokines

Anticoagulants



Coagulation proteins

Summary: innate response – internal defenses

Cellular
- Neutrophils
- Monocytes /macrophages
- NK cells
- TLRs

Extracellular
- Cytokines
- Complement
- Coagulation

Inflammation

- Complex biological process by which body responds to pathogens and irritants
- Associated with swelling of tissue
- Key player in innate immune repsone

Summary: role of Inflammation in innate immunity

- Initiation of phagocytosis – killing of pathogen
- Limiting the spread of infection
- Stimulate adaptive immune response
- Initiate tissue repair

Immunogens and antigens

Immunogen / antigen: a substance that elicits an immune response [i.e. a humoral (antibody response) or cell-mediated immune response]

Immune response generator

Though the two terms are used interchangeably – there are differences between the two

Epitope

Epitope: the portion of an antigen that is recognized
and bound by an antibody (Ab) or a T-cell receptor
(TCR)

epitope = antigenic determinant

Isoantigens

- Isoantigens: Antigens present in some but not all members of a species
- Blood group antigens – basis of blood grouping
- MHC (major histocompatibility complex)- cell surface glycoproteins

Autoantigens

- Autoantigens are substances capable of immunizing the host from which they are obtained.
- Self antigens are ordinarily non-antigenic
- Modifications of self-antigens are capable of eliciting an immune response

Haptens

- Haptens are small molecules which are non immunogenic, thus could never induce an immune response by themselves.

What is an antibody?

- Produced by Plasma cell (B-lymphocytes producing Ab) • Essential part of adaptive immunity

- Specifically bind a unique antigenic epitope (also called an
- antigenic determinant)

- Possesses antigen binding sites

The molecular genetics of immunoglobulins

How can the bifunctional nature of antibodies be explained genetically?

Dreyer & Bennett (1965)
For a single isotype of antibody there may be:

- A single C region gene encoded in the GERMLINE and separate from the V region genes
- Multiple choices of V region genes available
- A mechanism to rearrange V and C genes in the genome so that they can fuse to form a complete Immunoglobulin gene.

This was genetic heresy as it violated the then accepted notion that DNA was identical in every cell of an individual

Genetic models of the 1960's were also unable to explain:

- How B cells shut down the Ig genes on just one of their chromosomes.

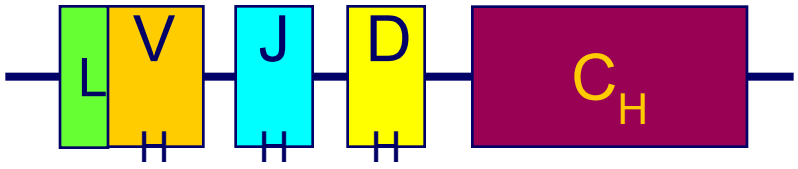
  All other genes known at the time were expressed co-dominantly. B cells expressed a light chain from one parent only and a heavy chain from one parent only (evidence from allotypes).

- A genetic mechanism to account for increased antibody affinity in an immune response

- How a single specificity of antibody sequentially switched isotype.

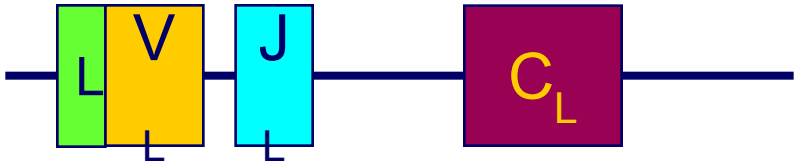
- How the same specificity of antibody was secreted and simultaneously expressed on the cell surface of a B cell.

Further diversity in the Ig heavy chain



Heavy chain: between 0 and 8 additional amino acids between J_H and C_H
The D or DIVERSITY region

Each heavy chain requires three recombination events:
V_H to J_H, V_HJ_H to D_H and $V_HJ_HD_H$ to C_H



Each light chain requires two recombination events:
V_L to J_L and V_LJ_L to C_L

Diversity: Multiple Germline Genes

VH Locus:

- 123 V_H genes on chromosome 14
- 40 functional V_H genes with products identified
- 79 pseudo V_H genes
- 4 functional V_H genes - with no products identified
- 24 non-functional, orphan V_H sequences on chromosomes 15 & 16

JH Locus:

- 9 J_H genes
- 6 functional J_H genes with products identified
- 3 pseudo J_H genes

DH Locus:

- 27 D_H genes
- 23 functional D_H genes with products identified
- 4 pseudo D_H genes
- Additional non-functional D_H sequences on the chromosome 15 orphan locus
- reading D_H regions in 3 frames functionally increases number of D_H regions

Diversity: Multiple germline genes

Vκ & Jκ Loci:

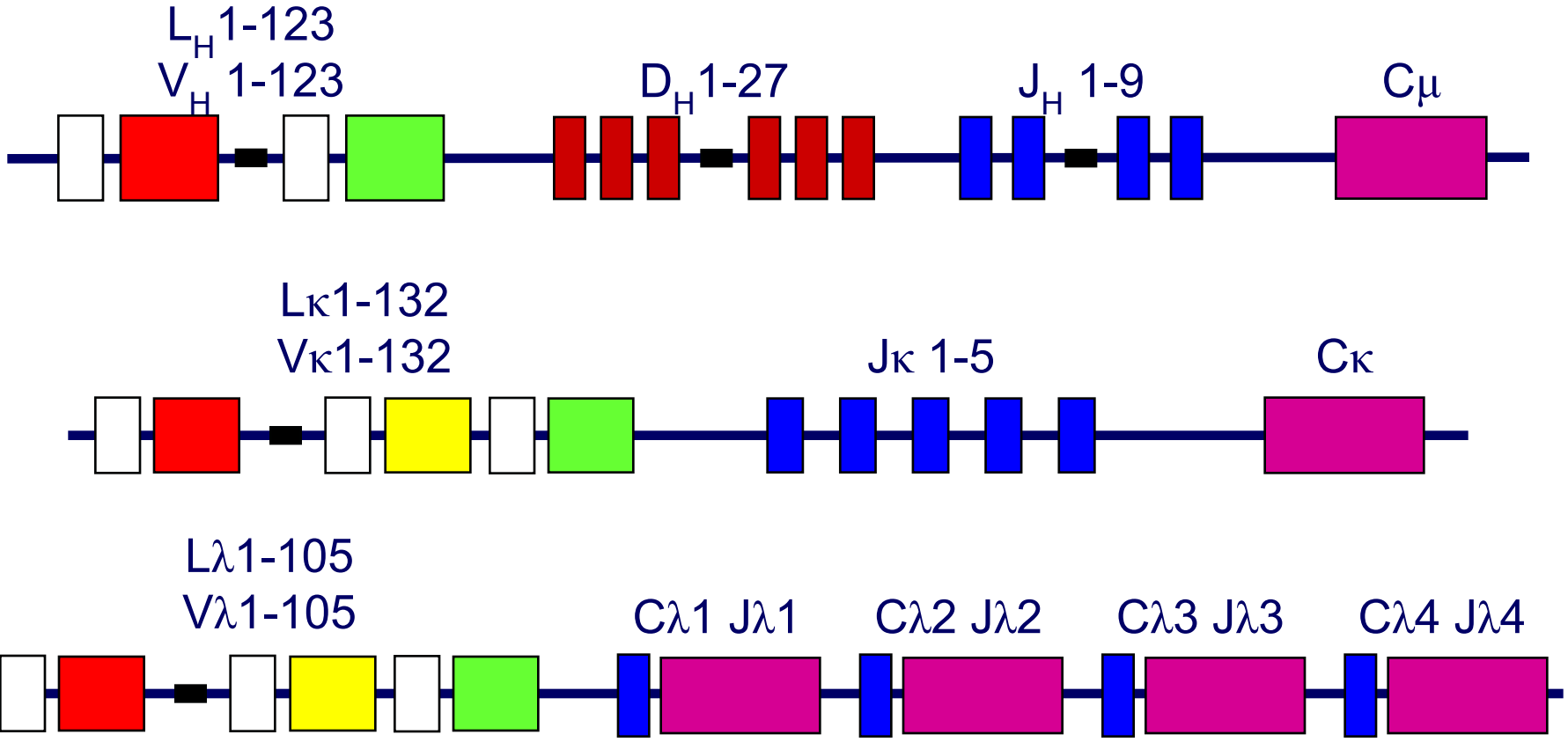
- 132 Vκ genes on the short arm of chromosome 2
- 29 functional Vκ genes with products identified
- 87 pseudo Vκ genes
- 15 functional Vκ genes - with no products identified
- 25 orphans Vκ genes on the long arm of chromosome 2
- 5 Jκ regions

Vλ & Jλ Loci:

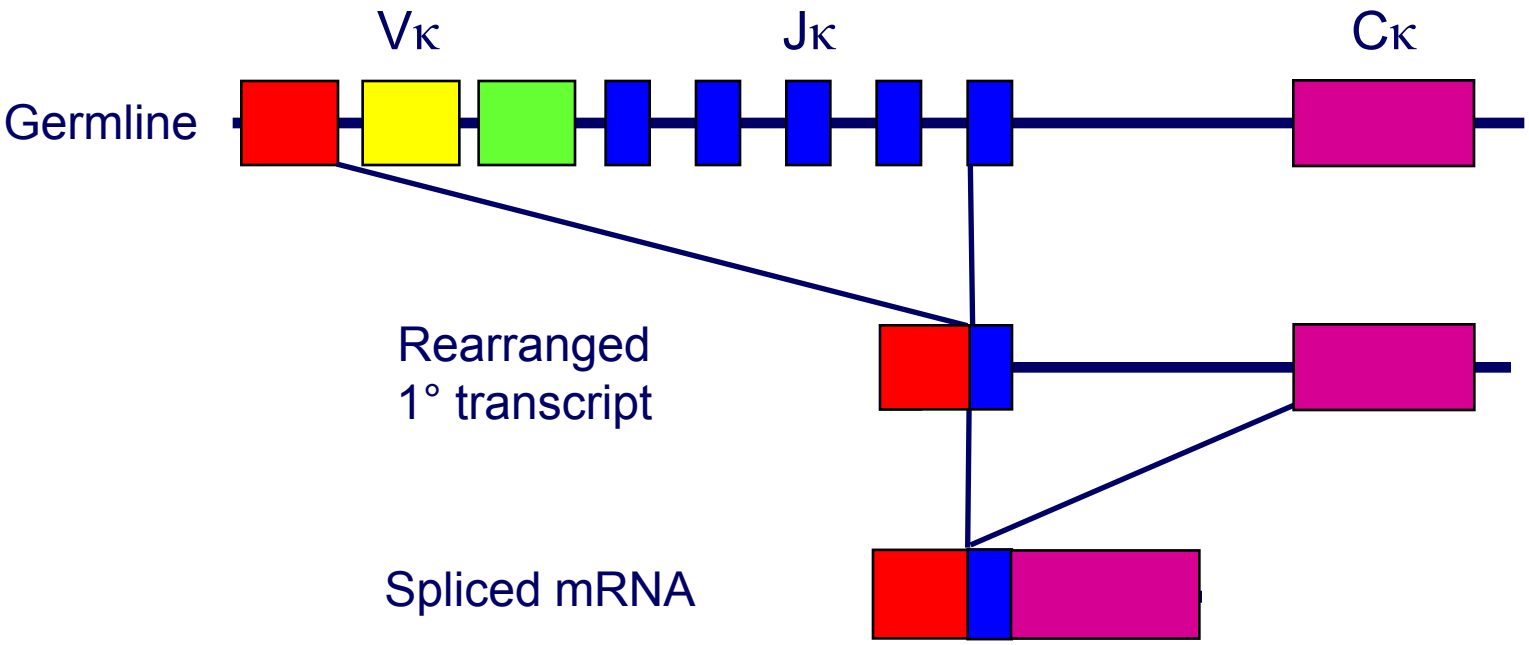
- 105 Vλ genes on the short arm of chromosome 2
- 30 functional genes with products identified
- 56 pseudogenes
- 6 functional genes - with no products identified
- 13 relics (<200bp Vλ of sequence)
- 25 orphans on the long arm of chromosome 2
- 4 Jλ regions

Genomic organisation of Ig genes

(No.s include pseudogenes etc.)

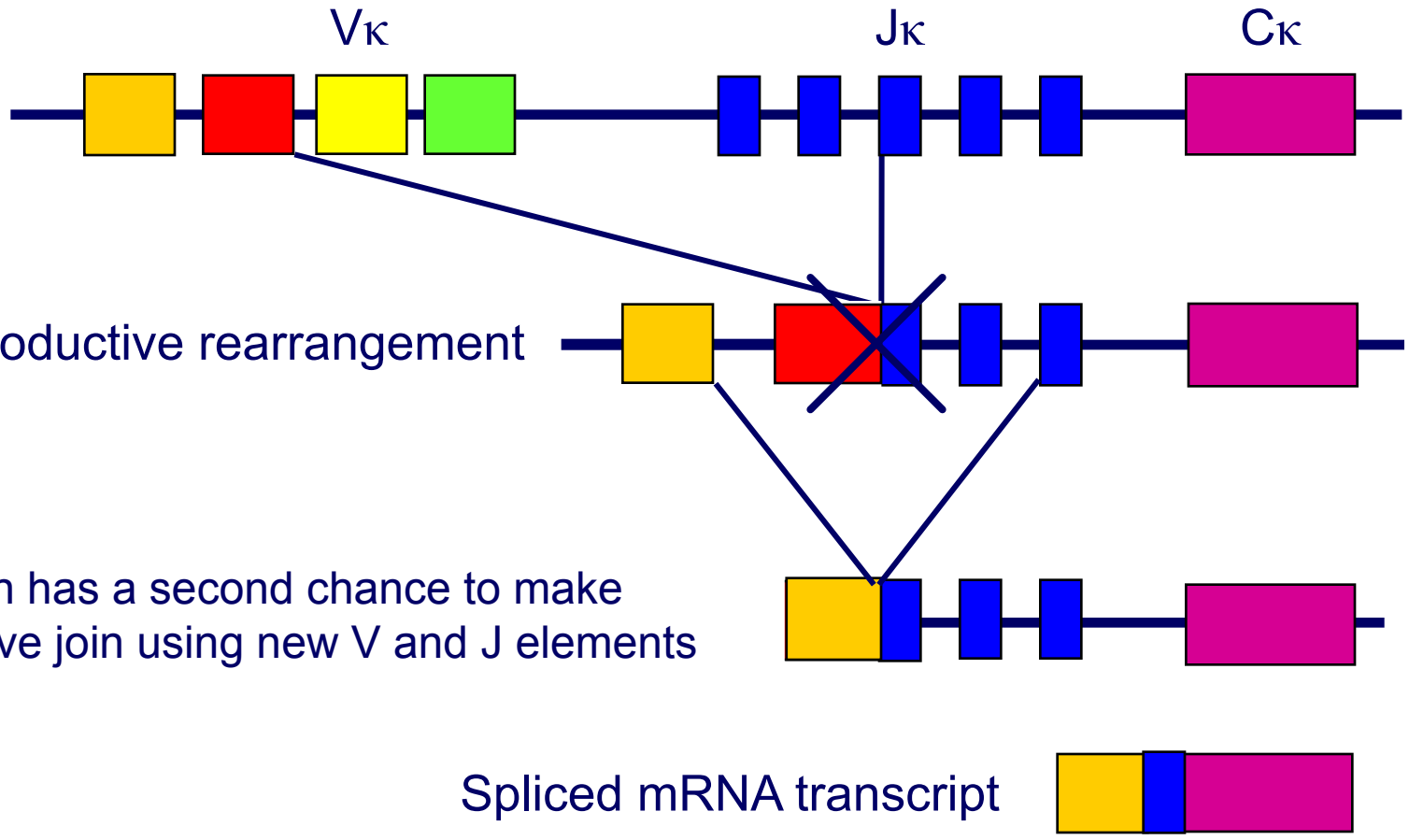


Ig light chain gene rearrangement by somatic recombination

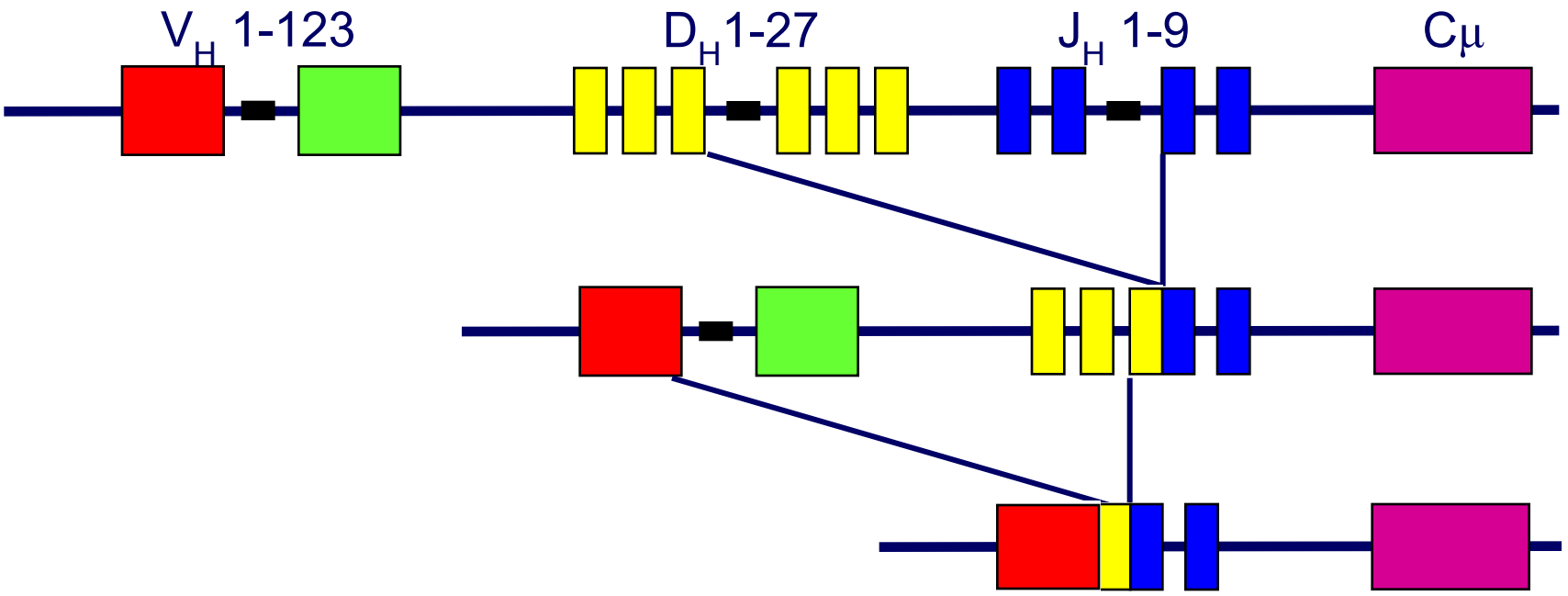


Ig light chain rearrangement: Rescue pathway

There is only a 1:3 chance of the join between the V and J region being in frame



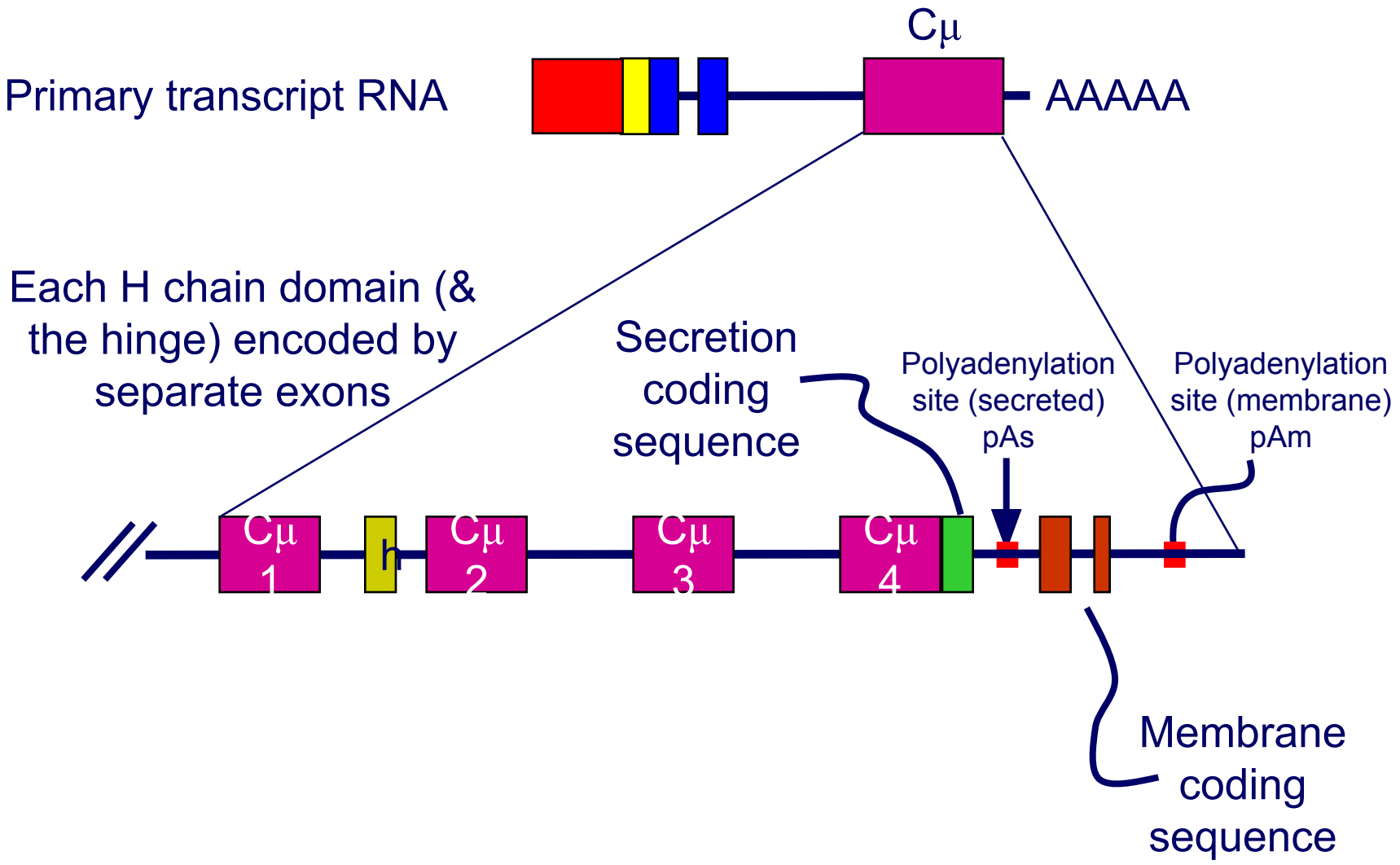
Ig heavy chain gene rearrangement



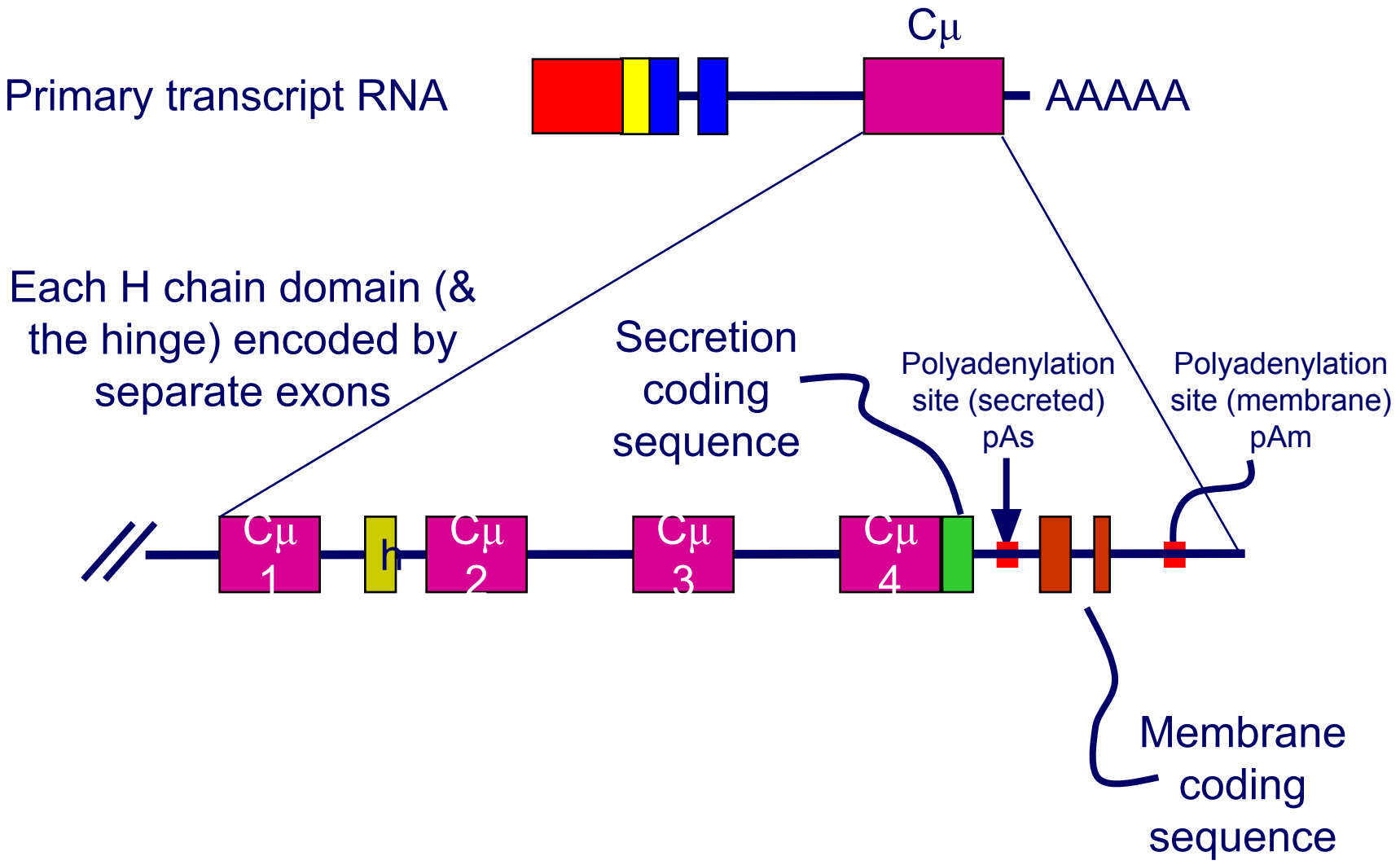
Somatic recombination occurs at the level of DNA which can now be transcribed

BUT:

The constant region has additional, optional exons

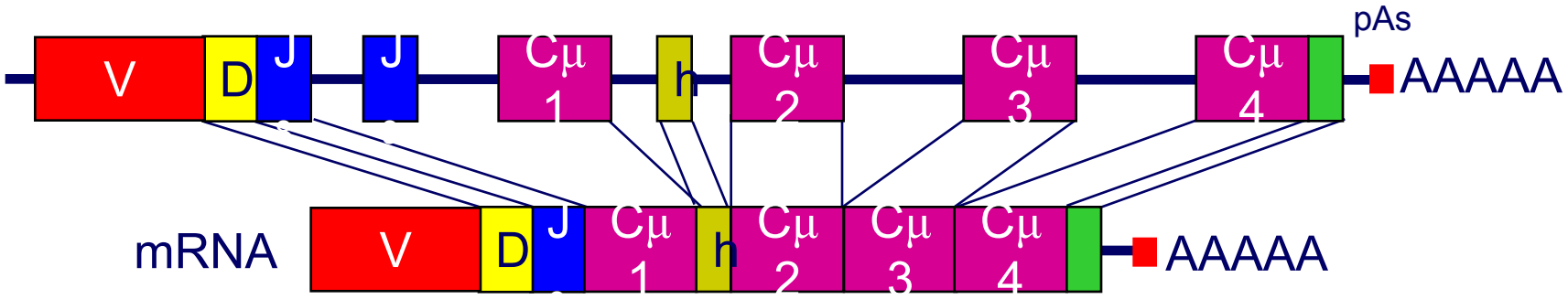


The constant region has additional, optional exons



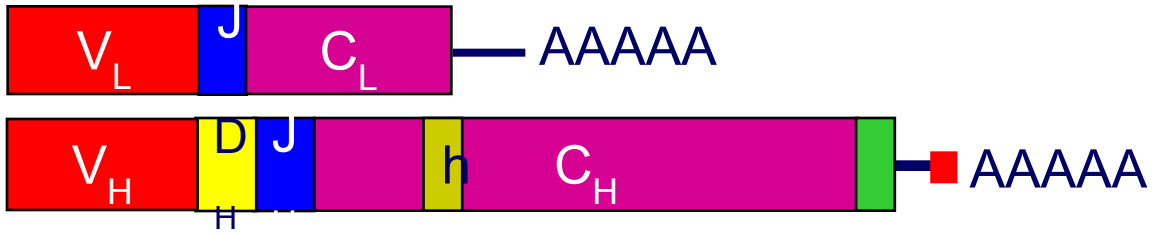
RNA processing

Primary transcript RNA



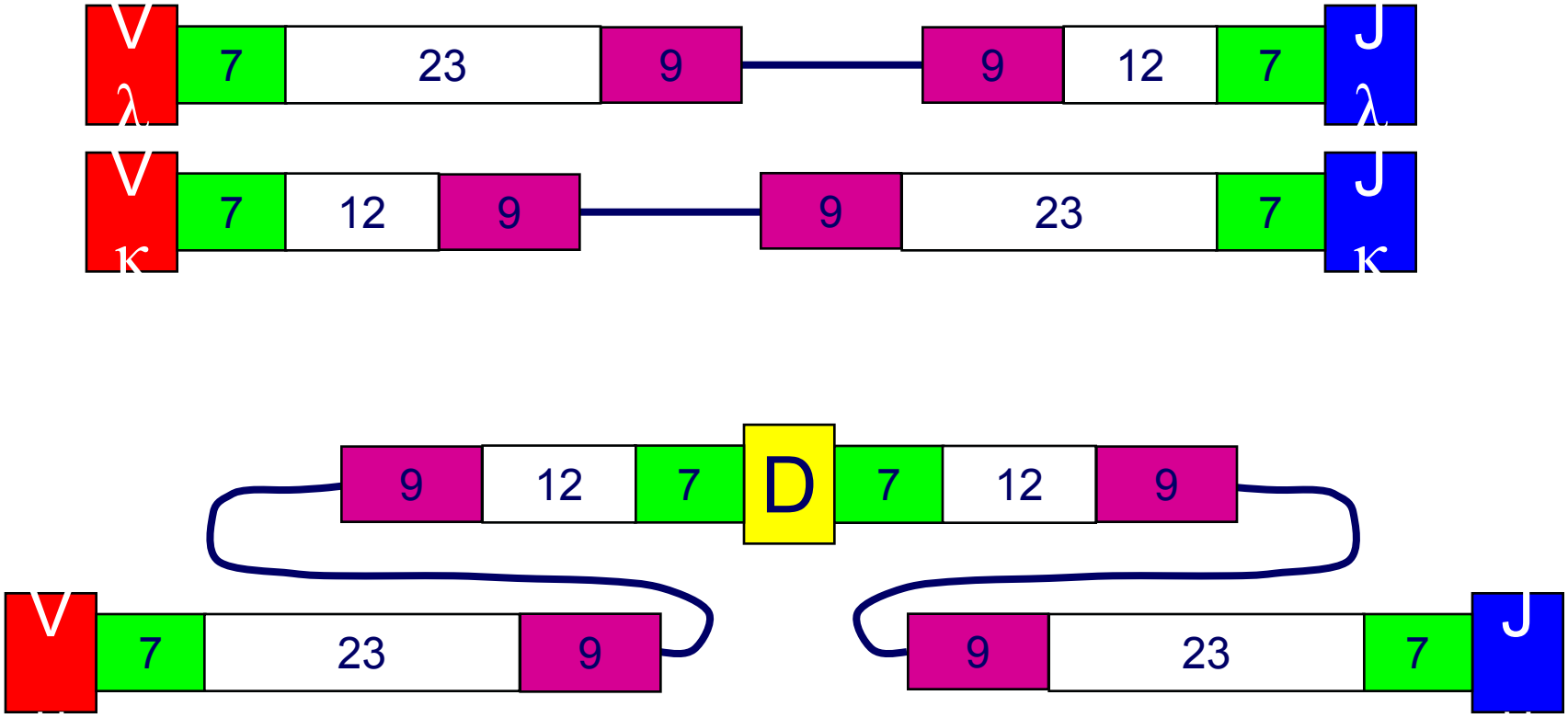
The Heavy chain mRNA is completed by splicing the VDJ region to the C region

The H and L chain mRNA are now ready for translation

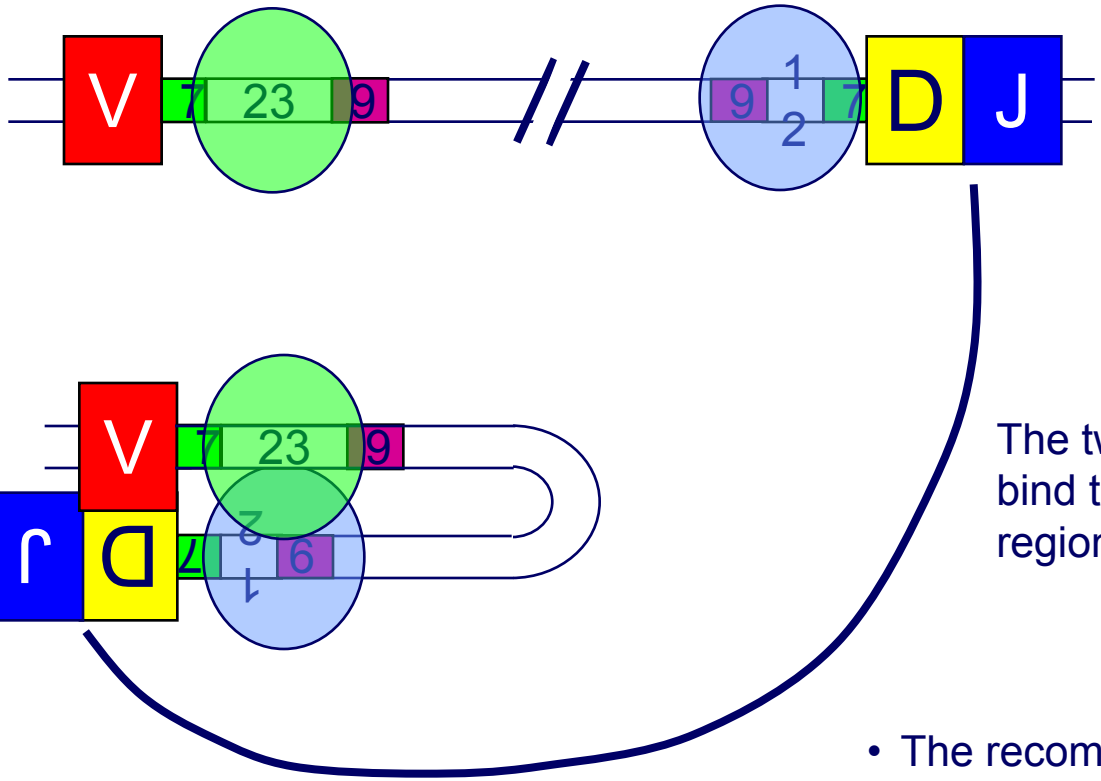


V, D, J flanking sequences

Sequencing up and down stream of V, D and J elements
Conserved sequences of 7, 23, 9 and 12 nucleotides in an arrangement that depended upon the locus

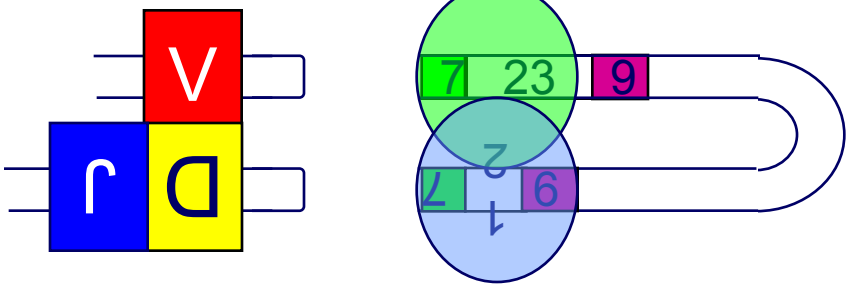


Steps of Ig gene recombination



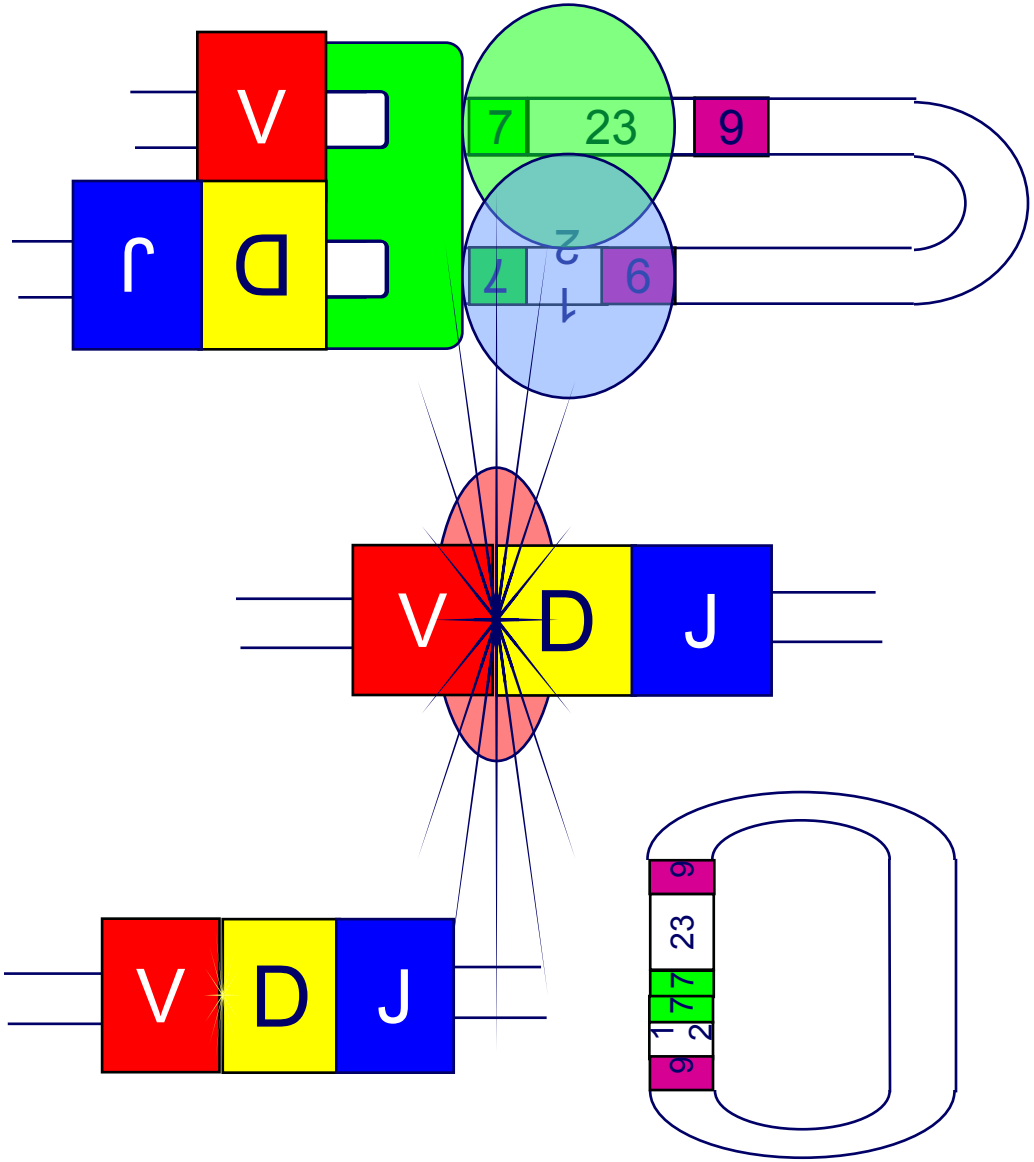
Recombination activating gene products, (RAG1 & RAG 2) and 'high mobility group proteins' bind to the RSS

The two RAG1/RAG 2 complexes bind to each other and bring the V region adjacent to the DJ region



- The recombinase complex makes single stranded nicks in the DNA. The free OH on the 3' end hydrolyses the phosphodiester bond on the other strand.
- This seals the nicks to form a hairpin structure at the end of the V and D regions and a flush double strand break at the ends of the heptamers.
- The recombinase complex remains associated with the break

Steps of Ig gene recombination

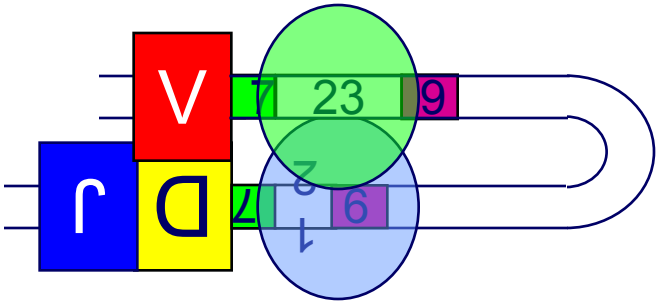


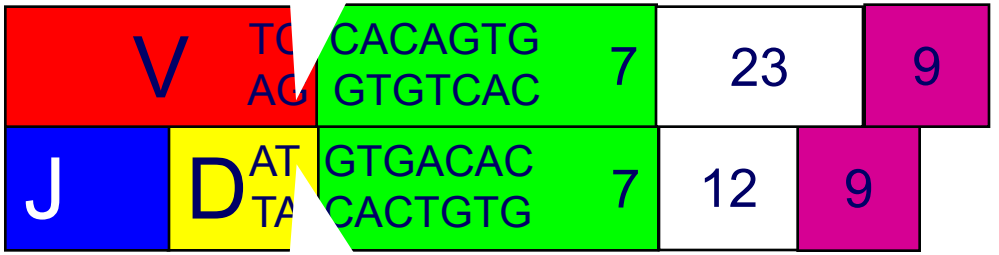
A number of other proteins, (Ku70:Ku80, XRCC4 and DNA dependent protein kinases) bind to the hairpins and the heptamer ends.

The hairpins at the end of the V and D regions are opened, and exonucleases and transferases remove or add random nucleotides to the gap between the V and D region

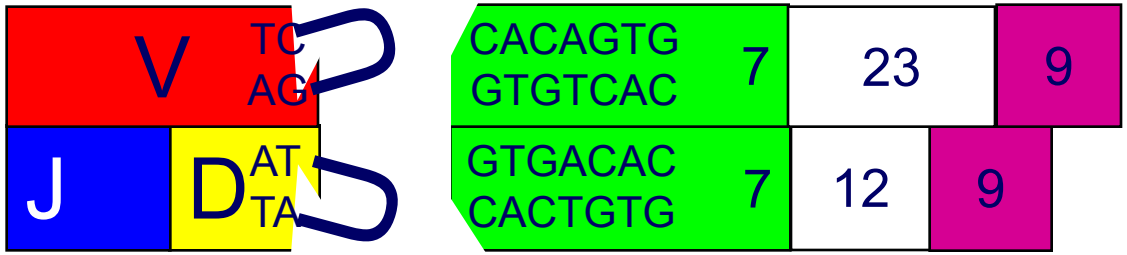
DNA ligase IV joins the ends of the V and D region to form the coding joint and the two heptamers to form the signal joint.

Junctional diversity: P nucleotide additions

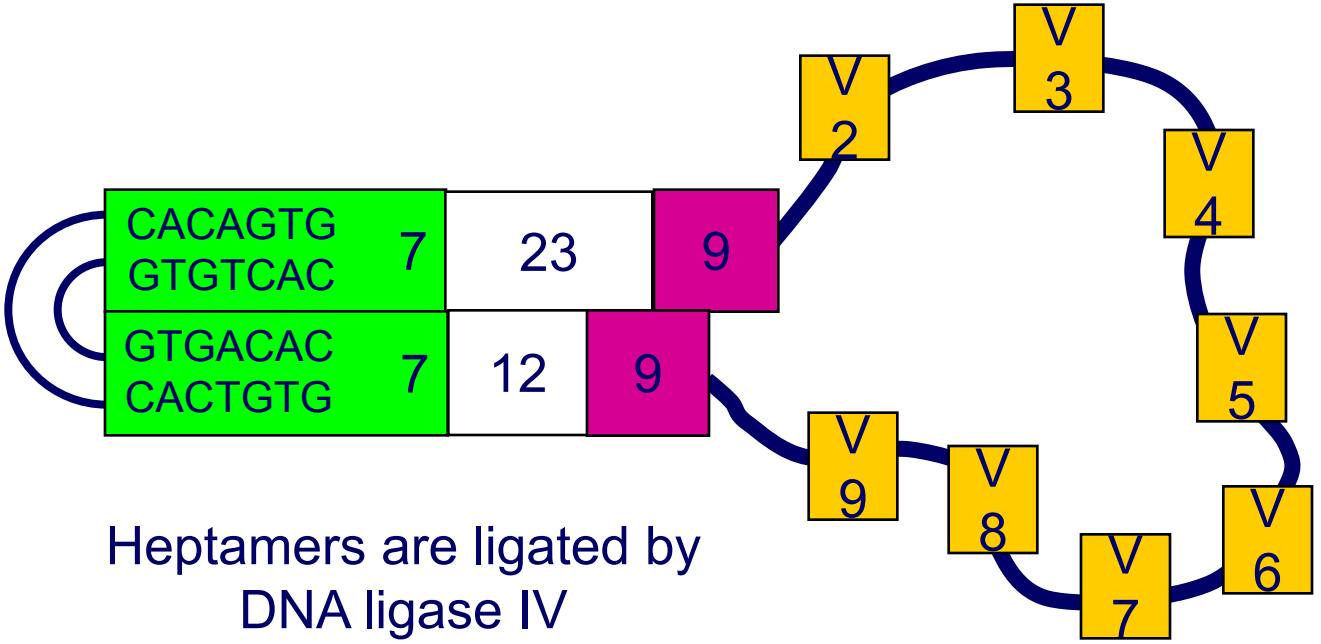




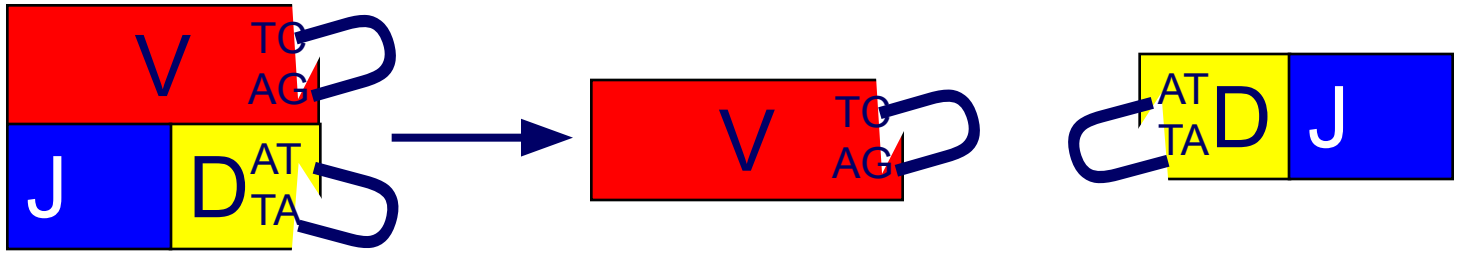
The recombinase complex makes single stranded nicks at random sites close to the ends of the V and D region DNA.



The 2nd strand is cleaved and hairpins form between the complimentary bases at ends of the V and D region.

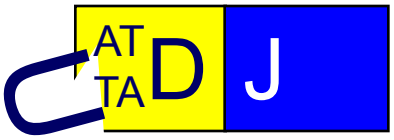


Heptamers are ligated by
DNA ligase IV

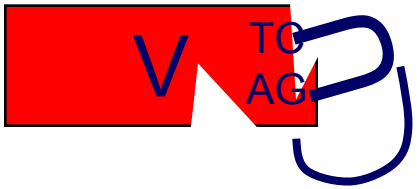


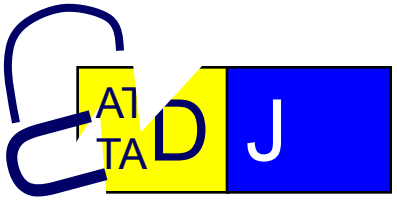
V and D regions juxtaposed

Generation of the palindromic sequence



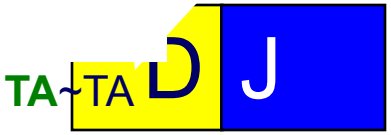
Regions to be joined are juxtaposed





Endonuclease cleaves single strand at random sites in V and D segment

The nicked strand ‘flips’ out



The nucleotides that flip out, become part of the complementary DNA strand

In terms of G to C and T to A pairing, the ‘new’ nucleotides are palindromic. The nucleotides GA and TA were not in the genomic sequence and introduce diversity of sequence at the V to D join.

(Palindrome - A Santa at NASA)

Junctional Diversity – N nucleotide additions

Terminal deoxynucleotidyl transferase (TdT) adds nucleotides randomly to the P nucleotide ends of the single-stranded V and D segment DNA

Complementary bases anneal

Exonucleases nibble back free ends

DNA polymerases fill in the gaps with complementary nucleotides and DNA ligase IV joins the strands

Junctional Diversity

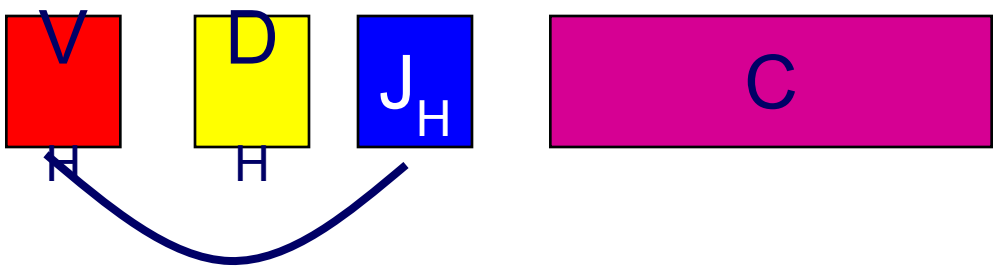
TTTTT Germline-encoded nucleotides

TTTTT Palindromic (P) nucleotides - not in the germline

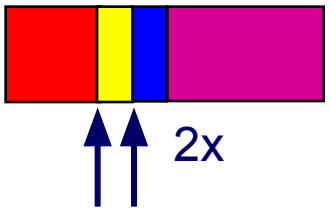
TTTTT Non-template (N) encoded nucleotides - not in the germline

Creates an essentially random sequence between the V region, D region and J region in heavy chains and the V region and J region in light chains.

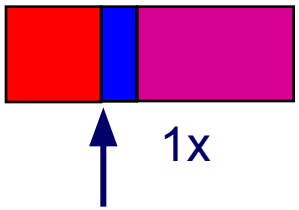
Why do V regions not join to J or C regions?



IF the elements of Ig did not assemble in the correct order, diversity of specificity would be severely compromised



Full potential of the H chain for diversity needs V-D-J-C joining - in the correct order



Were V-J joins allowed in the heavy chain, diversity would be reduced due to loss of the imprecise join between the V and D regions

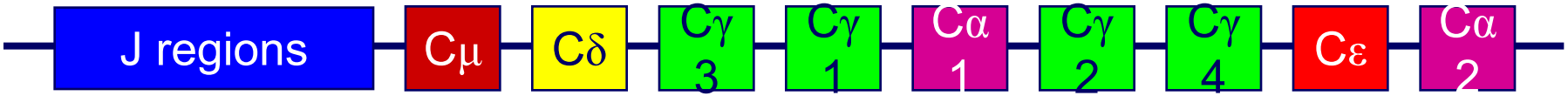
Antibody isotype switching

Throughout an immune response the *specificity* of an antibody will remain the same (notwithstanding affinity maturation)

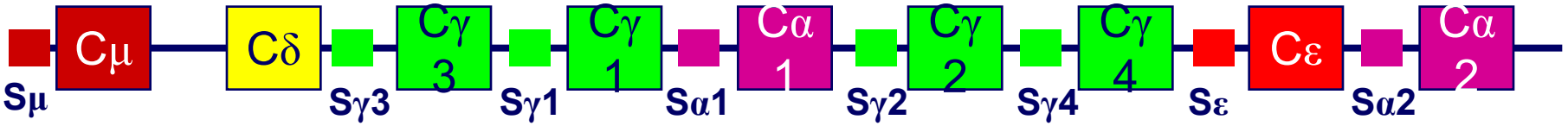
The effector function of antibodies throughout a response needs to change drastically as the response progresses.

Antibodies are able to retain variable regions whilst exchanging constant regions that contain the structures that interact with cells.

Organisation of the functional human heavy chain C region genes

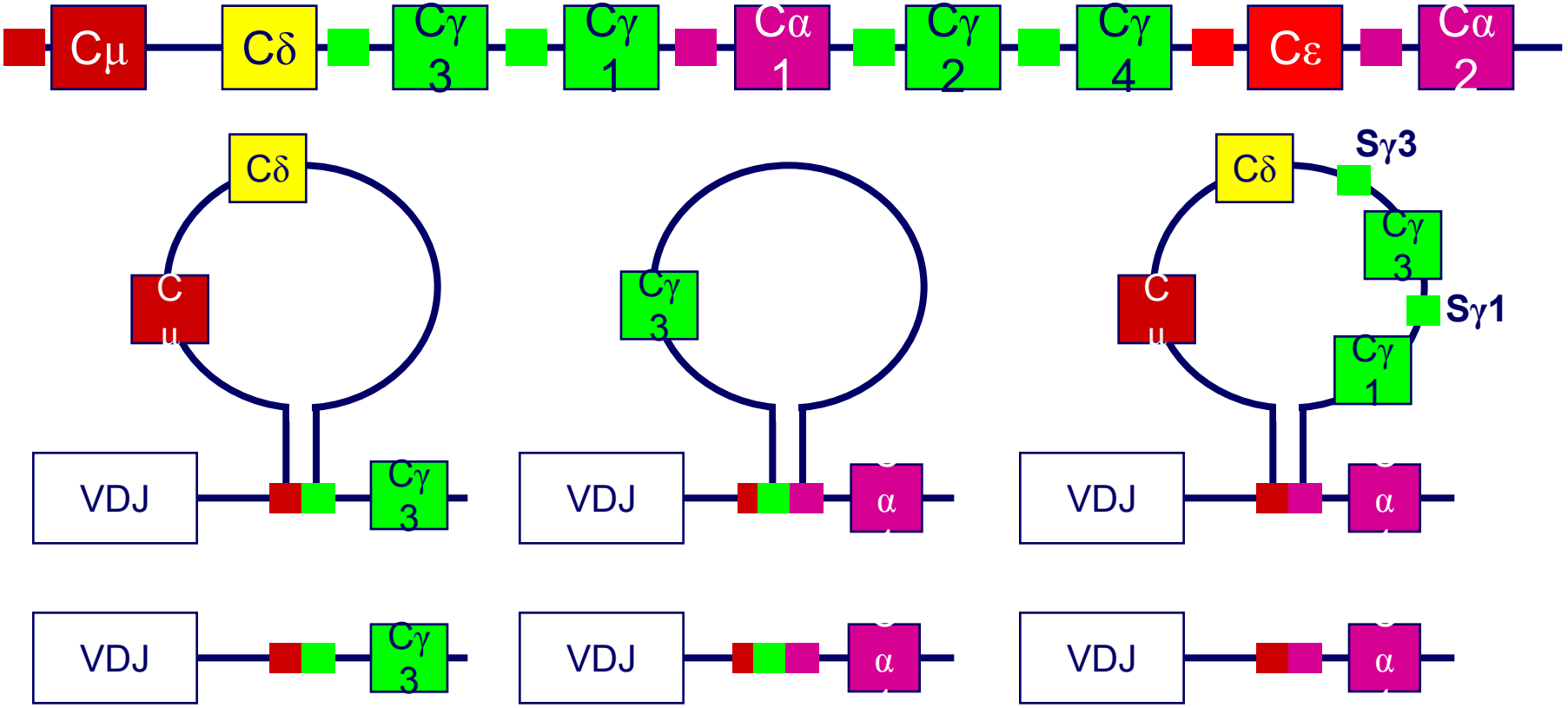


Switch regions



- Upstream of C regions are repetitive regions of DNA called switch regions. (The exception is the Cδ region that has no switch region).
- The Sμ consists of 150 repeats of [(GAGCT)n(GGGGGT)] where n is between 3 and 7.
- Switching is mechanistically similar in may ways to V(D)J recombination.
- Isotype switching does not take place in the bone marrow, however, and it will only occur after B cell activation by antigen and interactions with T cells.

Switch recombination



IgG3 produced.
Switch from IgM

IgA1 produced.
Switch from IgG3

IgA1 produced.
Switch from IgM

At each recombination constant regions are deleted from the genome
An IgE - secreting B cell will never be able to switch to IgM, IgD, IgG1-4 or IgA1

Thank You