Organization of antibody genes

- ✓ Antibody genes are organized into three main loci: κ, λ, and heavy chain loci.
- ✓ Each locus contains a cluster of variable (V), joining (J), and constant (C) region gene segments.
- ✓ These segments are arranged on different chromosomes and undergo specific rearrangements during B cell development to generate antibody diversity.

Elaboration:

Light Chain Loci (κ and λ):

- The κ locus, found on chromosome 2, and the λ locus, located on chromosome 22, both contain clusters of V genes, followed by J genes, and then a single C gene.
- During B cell development, a single V gene segment is joined with a J gene segment to form the variable region of the light chain.
- The rearranged VJ segment is then spliced with the C region gene to produce the complete light chain.

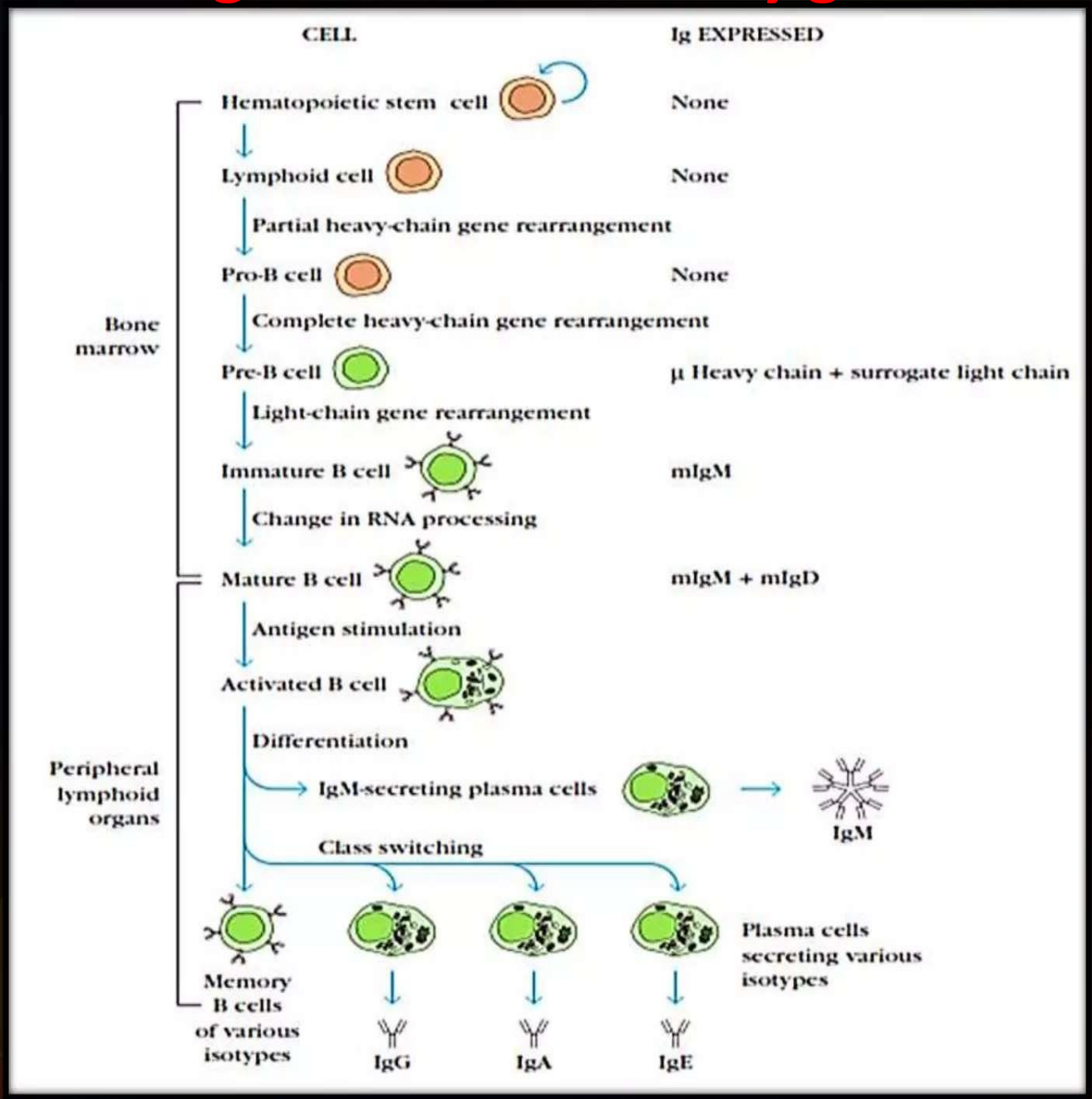
Heavy Chain Locus:

- The heavy chain locus, located on chromosome 14, contains clusters of V, D (diversity), and J gene segments, as well as a series of C region genes.
- Unlike the light chain loci, the heavy chain locus has multiple C region genes, each corresponding to a different antibody isotype (e.g., IgM, IgG).
- During B cell development, a V, D, and J gene segment are joined together to form the variable region of the heavy chain.
- The rearranged VDJ segment is then spliced with a specific C region gene to produce the complete heavy chain.

Gene Rearrangement and Diversity:

- The arrangement of V, D, and J gene segments, and the subsequent joining of these segments during B cell development, allows for a vast range of antibody diversity.
- This process, known as V(D)J recombination, is mediated by the enzyme RAG (Recombination Activation Gene).
- Additional diversity is generated through junctional flexibility (random addition or deletion of nucleotides at the junctions between V, D, and J segments) and somatic hypermutation (point mutations in the variable regions of heavy and light chains).

Rearrangement of antibody genes



Rearrangement of antibody genes

- Antibody gene rearrangement is a crucial process in the development of antibody diversity, where different gene segments are randomly joined to create a vast range of antibody variants. This process, involving the V, D, and J gene segments for heavy chains and V and J segments for light chains, allows for the generation of diverse antigen-binding sites.

Here's a more detailed explanation:

1. Somatic Recombination:

- Antibody-producing cells (B cells) undergo a process called somatic recombination, where DNA segments encoding the antibody variable region (V, D, and J for heavy chains, V and J for light chains) are shuffled and joined.
- This rearrangement occurs at the DNA level, ensuring a unique antibody sequence for each B cell.
- The process is guided by specific DNA sequences called recombination signal sequences (RSS).
- It involves lymphocyte-specific and ubiquitous DNA-modifying enzymes.
- This rearrangement also activates the gene promoter through changes in enhancer and silencer positions.

2. Generation of Diversity:

a. Combinatorial Joining:

- The random joining of different V, D, and J segments creates a vast number of different antibody variable regions.

b. Junctional Diversity:

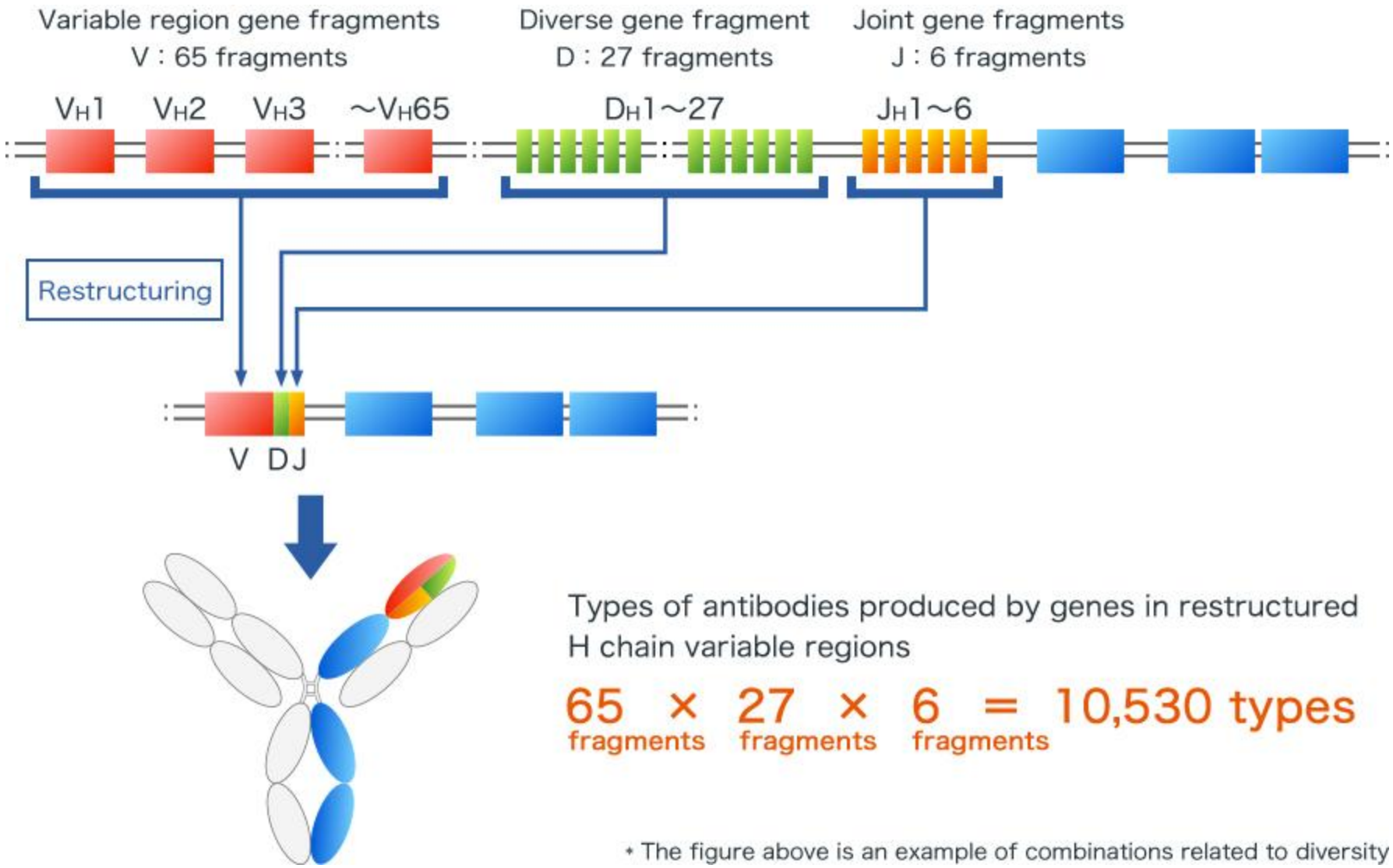
- During the joining process, nucleotides can be added or deleted, further increasing the diversity of the antigen-binding site.

c. Somatic Hypermutation:

- After B cells differentiate into plasma cells, they can undergo somatic hypermutation, where mutations accumulate in the variable region, leading to further diversification.

Somatic recombination-VDJ Recombination

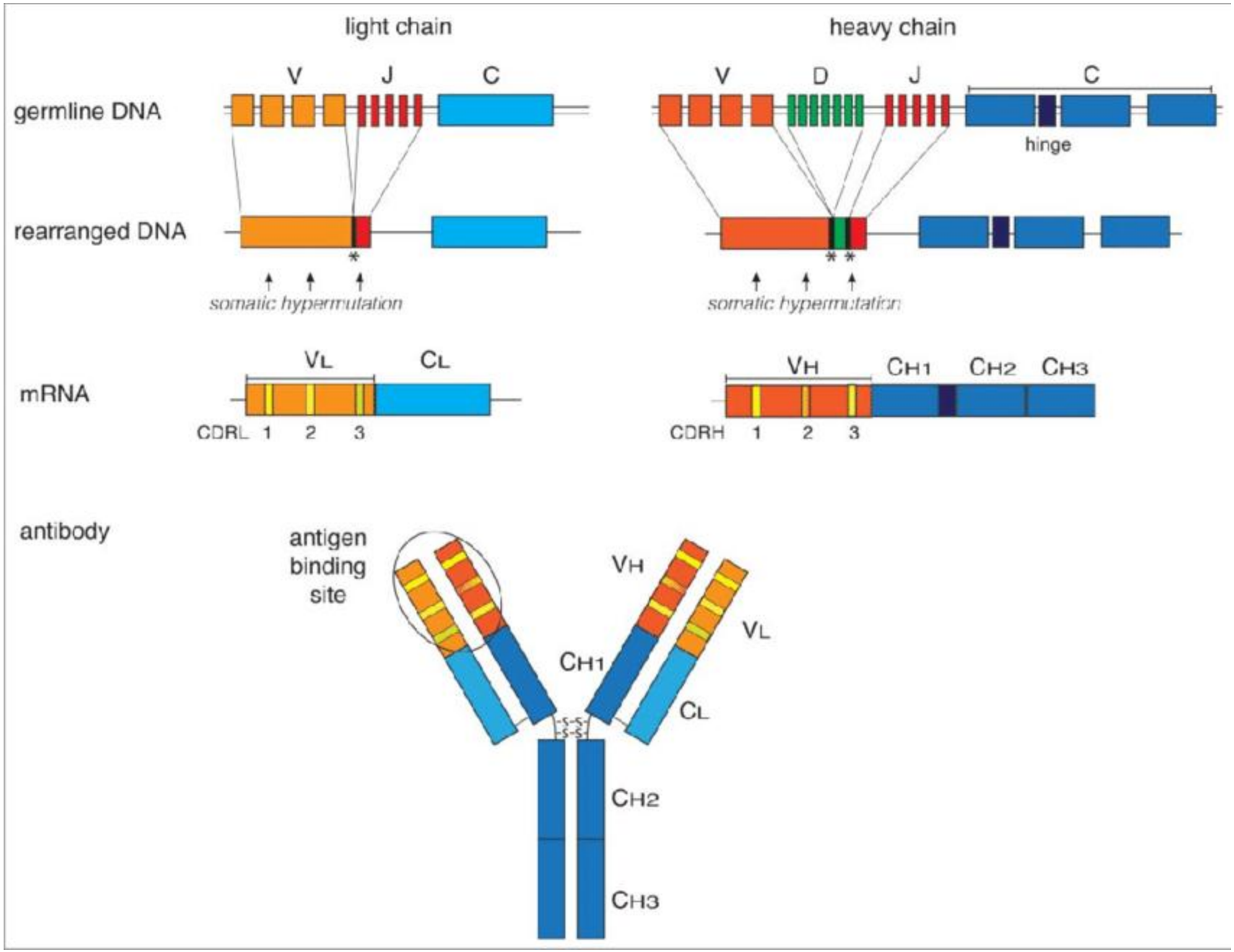
- Somatic recombination is a biological process that occurs in certain cells of the immune system, primarily B cells and T cells, allowing them to generate a vast diversity of receptors that recognize antigens.
- It's a crucial part of the adaptive immune system.
- Somatic recombination refers to the rearrangement of DNA segments within a single somatic (non-reproductive) cell. This is not inherited by offspring but happens during the development of immune cells in each individual.
- **Key Features:** Occurs in: Immature B cells (in bone marrow) and T cells (in thymus).
- **Involves:** V (Variable), D (Diversity), and J (Joining) gene segments (for heavy chains in B cells and β chains in T cells). V and J segments only (for light chains in B cells and α chains in T cells).
- Enzyme involved: RAG-1 and RAG-2 (Recombination Activating Genes) initiate the cutting and rejoining of DNA.
- **How It Works:**
  - ✓ Selection of gene segments: One each from V, (D), and J segments is chosen.
  - ✓ DNA is cut and spliced: Unneeded segments are removed, and the chosen V(D)J segments are joined.
  - ✓ Transcription and translation: The rearranged gene is transcribed into mRNA and then translated into a protein—either a B-cell receptor (antibody) or a T-cell receptor.
- **Purpose and Significance:**
  - ✓ Immune diversity: Allows generation of millions of unique receptors from a limited number of genes.
  - ✓ Antigen recognition: Essential for recognizing and responding to a wide array of pathogens.
  - ✓ Self-tolerance: Cells that bind self-antigens too strongly are usually eliminated (clonal deletion), helping prevent autoimmunity.
- **Errors in Somatic Recombination:**
  - ✓ Can lead to autoimmune diseases or cancers (e.g., lymphomas and leukemias) if recombination is faulty or regulatory mechanisms fail.

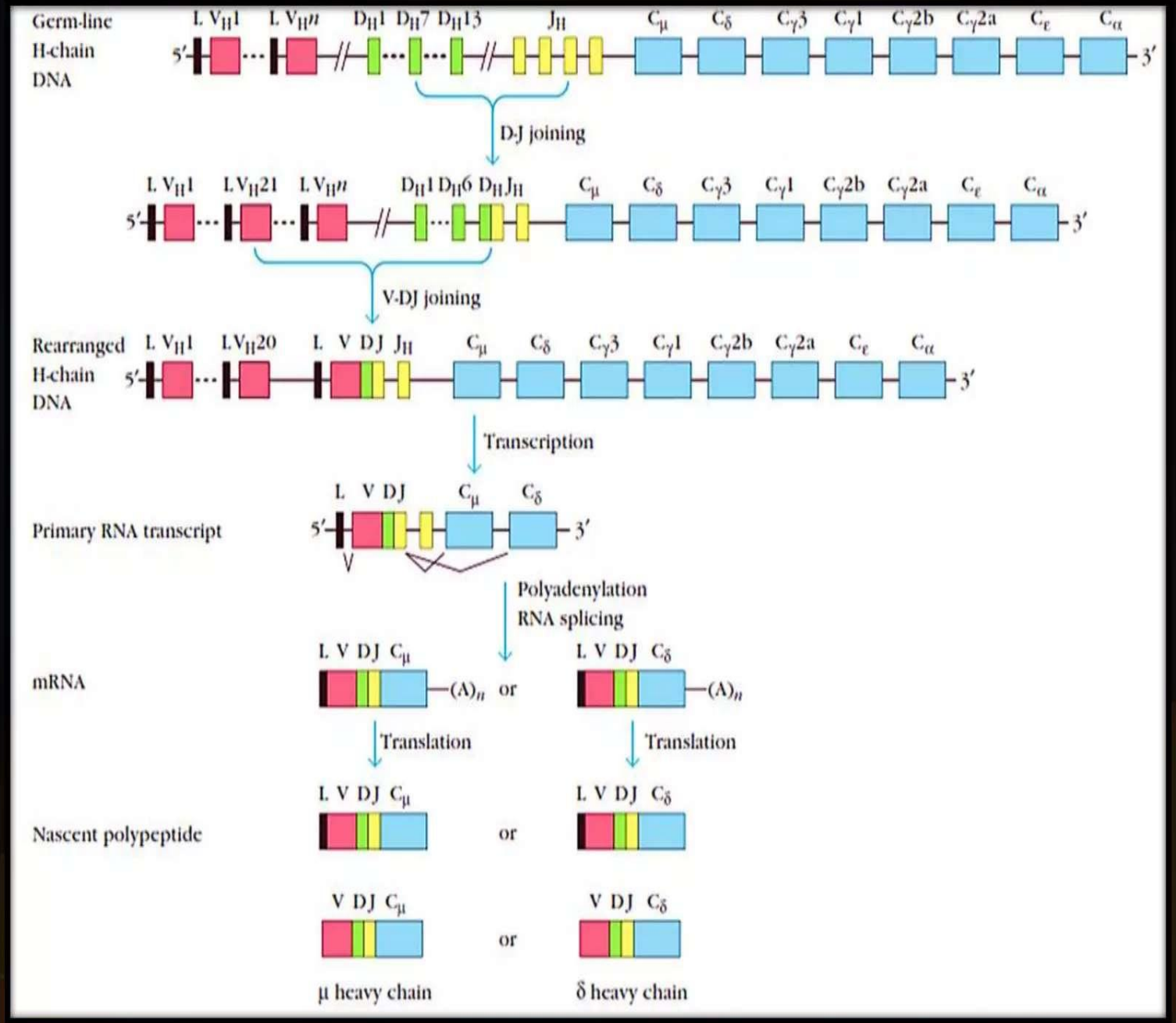


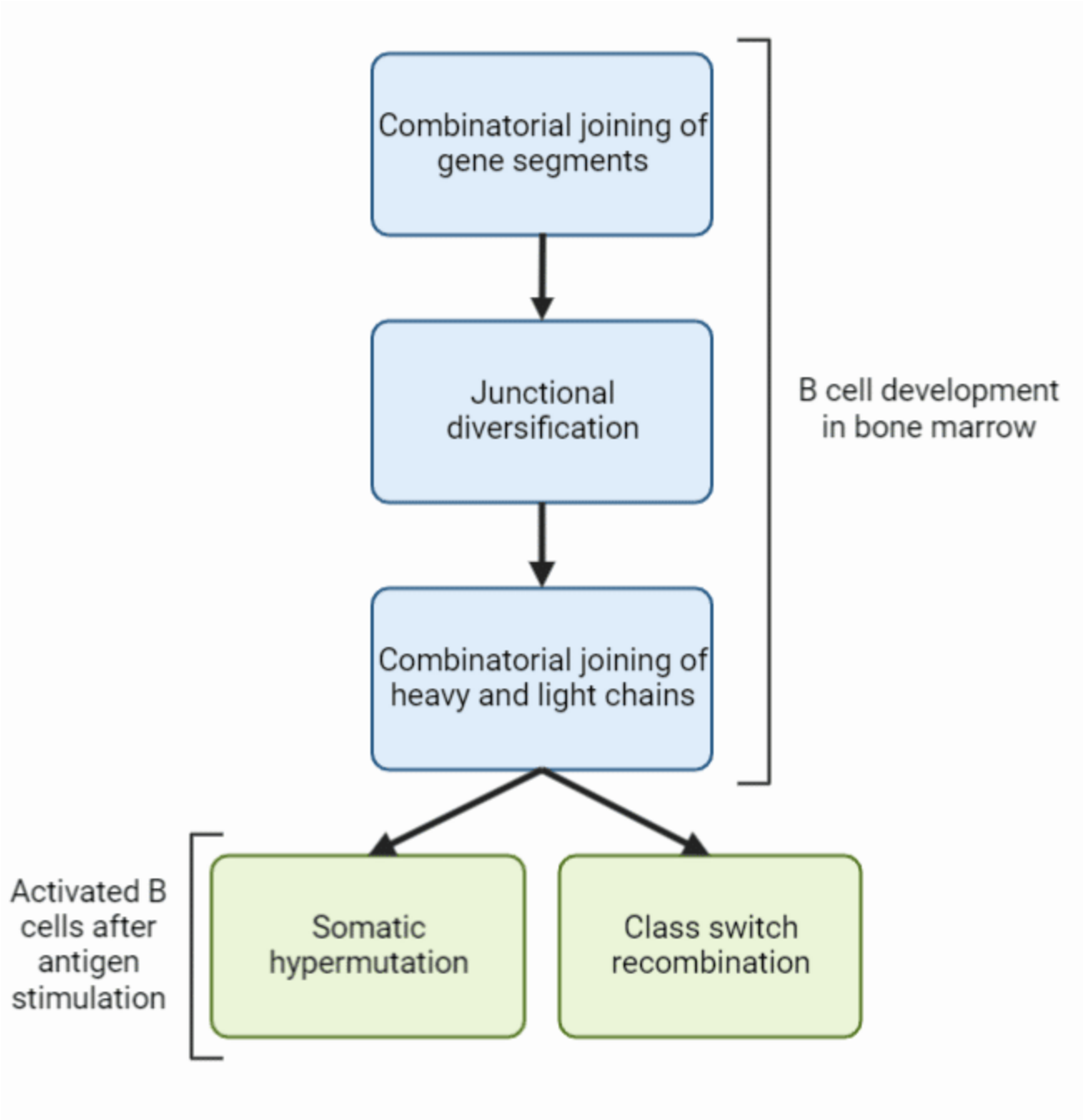
Types of antibodies produced by genes in restructured H chain variable regions

65 fragments × 27 fragments × 6 fragments = 10,530 types

* The figure above is an example of combinations related to diversity


light chain
heavy chain
germline DNA
V
J
C
V
D
J
C
hinge
rearranged DNA
somatic hypermutation
somatic hypermutation
mRNA
VL
CL
CDRL 1 2 3
VH
CH1
CH2
CH3
CDRH 1 2 3
antibody
antigen binding site
VH
VL
CH1
CL
CH2
CH3


Germ-line H-chain DNA
L V_H1
L V_Hn
D_H1
D_H7
D_H13
J_H
C_μ
C_δ
$C_\gamma3$
$C_\gamma1$
$C_\gamma2b$
$C_\gamma2a$
C_ε
C_α
5′
3′
D-J joining
L V_H1
L V_H21
L V_Hn
D_H1
D_H6
D_HJ_H
V-DJ joining
Rearranged H-chain DNA
L V_H1
L V_H20
L V DJ J_H
Transcription
Primary RNA transcript
L V DJ
Polyadenylation
RNA splicing
mRNA
$(A)_n$
or
Translation
Nascent polypeptide
V DJ
μ heavy chain
δ heavy chain


Combinatorial joining of gene segments
Junctional diversification
Combinatorial joining of heavy and light chains
B cell development in bone marrow
Activated B cells after antigen stimulation
Somatic hypermutation
Class switch recombination

Junctional diversity

- Junctional diversity in antibody gene rearrangement arises from imprecise joining of variable (V), diversity (D), and joining (J) gene segments during V(D)J recombination, leading to variations in the amino acid sequence, particularly in the third hypervariable region (CDR3).
- This process involves the loss of nucleotides at the ends of recombining gene segments and the potential insertion of P and N nucleotides, increasing antibody diversity.

Explanation:

✓ **Imprecise Joining:**

- The V, D, and J gene segments are not always joined precisely during recombination.

✓ **Nucleotide Loss and Insertion:**

- Nucleotides can be lost from the ends of the recombining gene segments, and P and N nucleotides can be inserted at the joining sites.

✓ **P Nucleotides:**

- These are palindromic nucleotides that are added at the junction from the opposite strand of the DNA.

✓ **N Nucleotides:**

- These are non-templated nucleotides added by the enzyme TdT (terminal deoxynucleotidyl transferase).

✓ **CDR3 Diversity:**

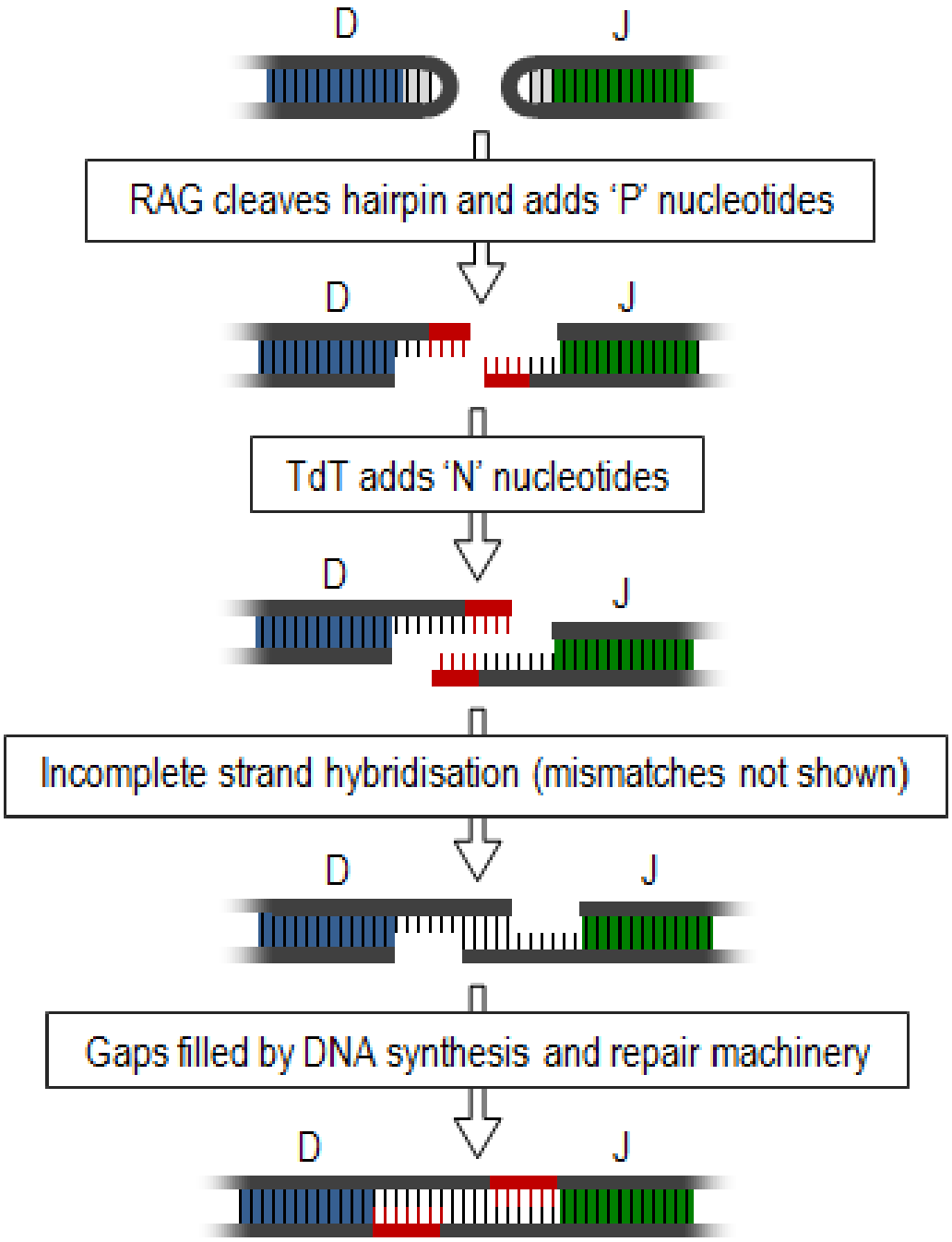
- Junctional diversity significantly increases the diversity of the CDR3 region, which is crucial for antigen recognition.

✓ **Nonproductive Rearrangements:**

- Junctional diversity can also lead to frameshifts, resulting in nonfunctional antibody genes.

✓ **Receptor Editing:**

- B cells that produce nonproductive rearrangements can undergo receptor editing, where they undergo a second round of V(D)J rearrangement to produce a functional antibody.


D
J
RAG cleaves hairpin and adds 'P' nucleotides
D
J
TdT adds 'N' nucleotides
D
J
Incomplete strand hybridisation (mismatches not shown)
D
J
Gaps filled by DNA synthesis and repair machinery
D
J

Somatic Hypermutation

- Somatic hypermutation (SHM) is a biological process where activated B cells undergo a high rate of mutation in the variable regions of their immunoglobulin genes. This process, driven by the enzyme activation-induced deaminase (AID), leads to a significant increase in the diversity of antibody receptors on B cells, ultimately improving the affinity of antibodies for their target antigens.

Here's a more detailed explanation:

1. The Role of AID:

- AID, an enzyme expressed in germinal center B cells, initiates SHM by catalyzing the deamination of cytosine to uracil in the DNA of immunoglobulin genes.
- This deamination creates lesions that are subsequently processed by DNA repair pathways, leading to mutations.

2. Affinity Maturation:

- SHM introduces mutations into the variable regions of antibody genes, which are responsible for binding to specific antigens.
- These mutations can alter the binding affinity of antibodies, with some mutations resulting in higher affinity for the antigen.
- Germinal center B cells that produce high-affinity antibodies are then selected and expand, leading to a more refined antibody response.

3. Germinal Centers:

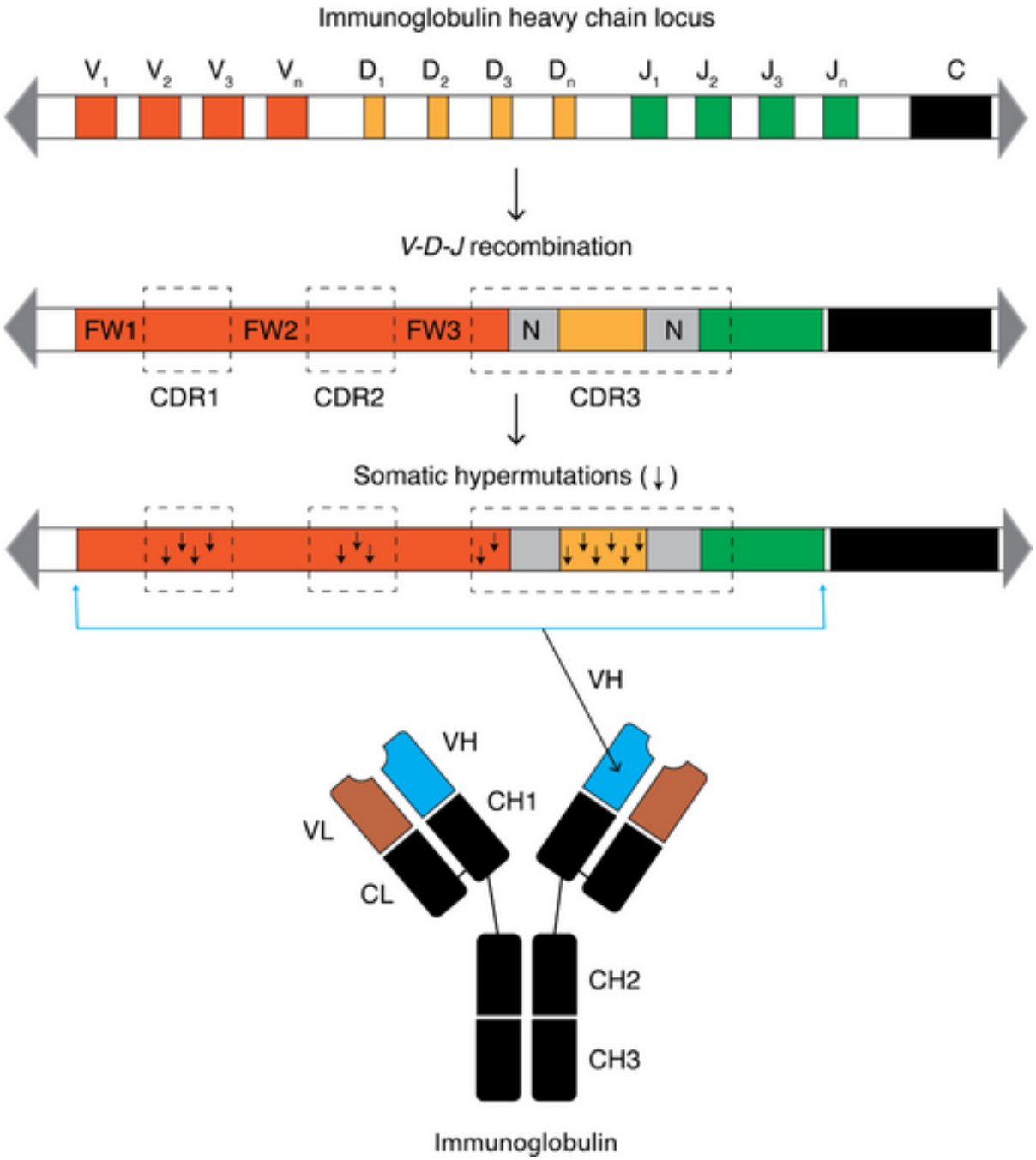
- SHM primarily occurs in germinal centers, which are specialized structures within lymph nodes where B cells are activated and undergo clonal expansion.
- These germinal centers are characterized by a high concentration of B cells and T helper cells, which facilitate the SHM process.

4. Molecular Mechanisms:

- The mutations introduced by SHM are not random but are targeted to specific sites within the variable regions of antibody genes.
- DNA repair pathways, particularly base excision repair (BER), are involved in processing the lesions generated by AID, leading to a range of mutations.
- The specific mutations introduced during SHM can vary depending on the DNA repair pathways used.

5. Importance of SHM:

- SHM is essential for the development of a robust and specific antibody response to foreign antigens.
- By increasing the diversity of antibody receptors, SHM allows for the selection of B cells that produce high-affinity antibodies, which are crucial for effectively neutralizing pathogens.
- SHM also plays a role in the development of memory B cells, which provide long-lasting immunity.


Immunoglobulin heavy chain locus
V1 V2 V3 Vn D1 D2 D3 Dn J1 J2 J3 Jn C
V-D-J recombination
FW1 FW2 FW3 N N
CDR1 CDR2 CDR3
Somatic hypermutations (↓)
VH
VH
CH1
VL
CL
CH2
CH3
Immunoglobulin

Mechanisms

- The mechanism of SHM involves deamination of cytosine to uracil in DNA by the enzyme activation-induced cytidine deaminase, or AID.
- A cytosine: guanine pair is thus directly mutated to a uracil: guanine mismatch.
- Uracil residues are not normally found in DNA, therefore, to maintain the integrity of the genome, most of these mutations must be repaired by high-fidelity base excision repair enzymes.
- The uracil bases are removed by the repair enzyme, uracil-DNA glycosylase, followed by cleavage of the DNA backbone by apurinic endonuclease.
-
- Error-prone DNA polymerases are then recruited to fill in the gap and create mutations.
- The synthesis of this new DNA involves error-prone DNA polymerases, which often introduce mutations at the position of the deaminated cytosine itself or neighboring base pairs.
- The introduction of mutations in the rapidly proliferating population of B cells ultimately culminates in the production of thousands of B cells, possessing slightly different receptors and varying specificity for the antigen, from which the B cell with highest affinities for the antigen can be selected.
- The B cells with the greatest affinity will then be selected to differentiate into plasma cells producing antibody and long-lived memory B cells contributing to enhanced immune responses upon reinfection.
- The hypermutation process also utilizes cells that auto-select against the 'signature' of an organism's own cells. It is hypothesized that failures of this auto-selection process may also lead to the development of an auto-immune response.