GENETIC CONTROL OF ANTIBODY PRODUCTION

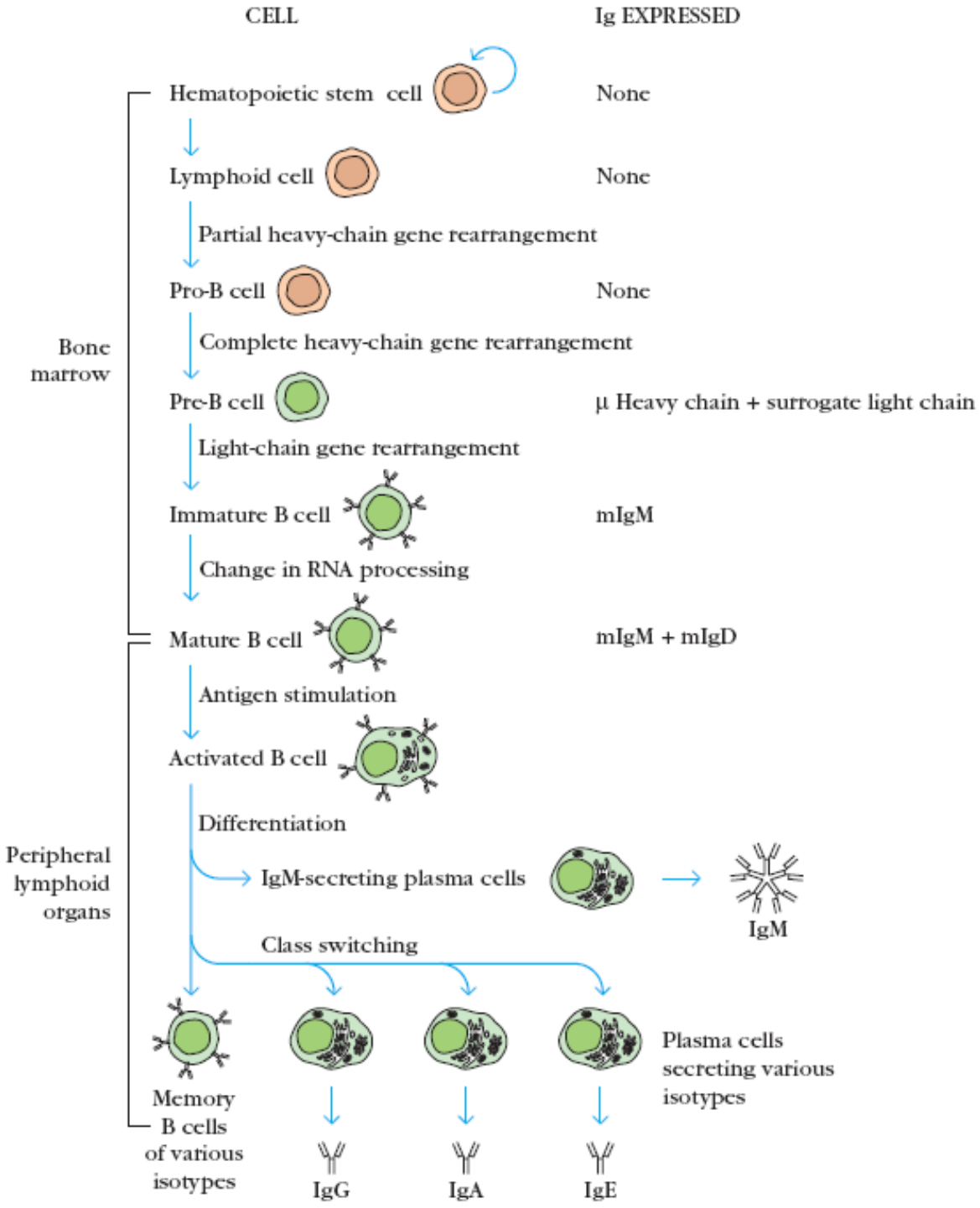
Even in the absence of antigenic stimulus a mouse can make millions of different antibodies. The capacity to generate constancy in the constant regions and generate tremendous variability in the variable regions of both light chains and heavy chains lies in their genetic combinations. Human have around 10^5 genes, and still they are able to produce around 10^6 antibodies, the number which is more than the number of genes. This is explained saying that during the early stages of development, the germ line DNA has multiple gene segments encoding portions of L and H-chains. But these cannot be transcribes and translated until they are rearranged into functional genes.

During B-cell maturation, random shuffling occurs which results in these 10^6 combinations. The B-cell differentiation from a progenitor cell to a mature cell involves an organized progression of Ig gene rearrangements. By the time the B-cell matures it will contain a single functional V-region DNA sequence for its heavy chain and a single functional V-region for its light chain, which will form the specific antigenically committed B-cells Ab's for an epitope. The chromosomal DNA content is no longer identical to the germ line DNA content (figure).

Many models have been proposed to explain the mechanism behind these variations. A viable model of the Ig gene has to take into account the following properties of Ab's.

a) The vast diversity of Ab specificities.
b) The presence of amino terminal at the variable end and carboxy terminal at the constant region end of both heavy chain as well as light chain.
c) The existence of isotopes with the same antigenic specificity which results from the association of a given variable region with different H-chain constant regions.

1) Germ line theory: This theory states that the genome contains large number of Ig genes sufficient to generate 10^8 different Ab's and hence it has failed as the total number of genes is around 10^5.


CELL
Ig EXPRESSED
Hematopoietic stem cell
None
Lymphoid cell
None
Partial heavy-chain gene rearrangement
Pro-B cell
None
Complete heavy-chain gene rearrangement
Bone marrow
Pre-B cell
μ Heavy chain + surrogate light chain
Light-chain gene rearrangement
Immature B cell
mIgM
Change in RNA processing
Mature B cell
mIgM + mIgD
Antigen stimulation
Activated B cell
Differentiation
Peripheral lymphoid organs
IgM-secreting plasma cells
IgM
Class switching
Plasma cells secreting various isotypes
Memory B cells of various isotypes
IgG
IgA
IgE

2) Somatic variation theories: these theories state that genome has very few genes for immunoglobulins but generate large number of Antibodies. But the maintenance of the constancy at c-region and generation of diversity at V-regions could not be explained. Later studies by V.Todd and other workers revealed that allotypic markers in the heavy chain V-regions could be associated with alpha, gama and well as mu heavy chains constant region.

3) Dryer and Bennet two gene Model (1965): this theory suggests that two genes code for a single Heavy chain and Light chain of each Immunoglobulin. That means one for V region and one for C region (V- variable, C- constant). These later come together at DNA level and are then transcribed and translated into a single H or L chain.

But this theory contradicts one gene one polypeptide hypothesis, hence has faced opposition.

Verification of this theory: S.Tonegawa and N.Hozumi provided first direct evidence to this two gene theory. They cleaved DNA form embryonic cells and from adult myeloma cells into fragments using restriction endonucleases. These were subjected to agarose gel electrophoresis and then analysed by their ability to bind to radio labeled k-chain mRNA probe.

The results showed that the mRNA probe hybridized with only one fragment of the myeloma DNA where as it hybridized with two fragments of embryonic DNA. It may

now be concluded that during embryonic stages the V and C genes are separated by large distances and have restriction site inbetween them, while in the adult stage, during the process of differentiation they are brought together and the RE sites are eliminated.

Multi gene Organisation of Immunoglobulin gene: Cloning and sequencing studies of κ and λ light chains and heavy chains revealed that theya re coded by multiple gene families situated on different chromosomes.

Gene	**chromosome**	
	Human	**Mouse**
λ–light chain	22	16
κ –light chan	2	6
Heavy chain	14	12

Each of these families contain coding sequences called gene segments. The κ and λ chain families contain L,V,J and C gene segments. The Heavy chain family contains L,V,D,J and C gene segments. The rearranged V and J gene segments code V-region of L-chain and rearranged VDJ gene segments encode the V-region of H-chain. C-gene segment encodes the constant region of both L or H chain. L-gene segments encode a short signal or leader sequence which guides the chain through the endoplasmic reticulum. But later it is cleaved before Immunoglobulin is assembled.

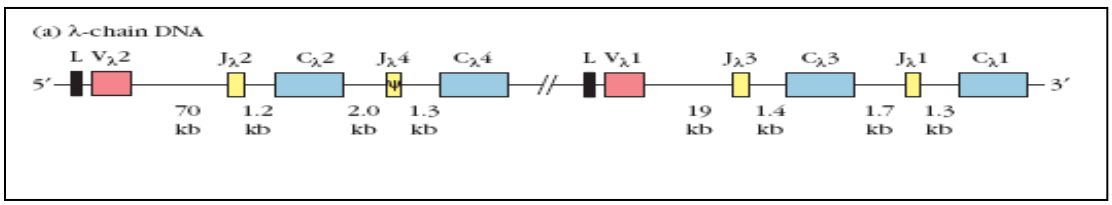
λ chain multigene family: Functional λ V-region gene contains two coding segments (exons). A 5^1 V segment and a 3^1 J segment. These are separated by non-coding DNA sequences (introns).

Mouse λ-multigene family contains 3 V λ gene segments
4 J λ gene segments and
4 C λ gene segments.

Human have
31-Vλgene segments
4-Jλgene segments
7-Cλgene segments
They have many pseudogenes.

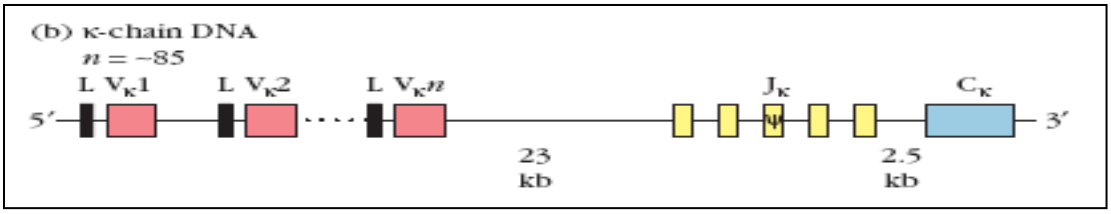
The J λ 4 and Cλ4 are defective genes hence called **pseudogenes.**

The constant region is coded by 3 functional Cλ gene segments which encode 3 λchain subtypes, i.e., λ_1, λ_2, λ_3.

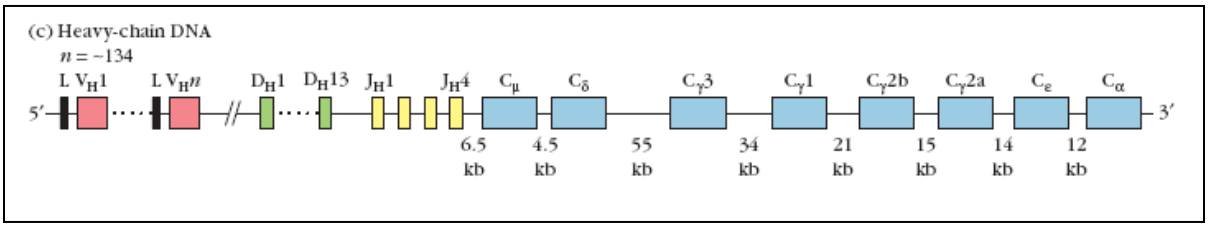


κ-chain multigene family: In mouse this family contains around 85V_K gene segments with an adjacent leader sequence located upstream towards the 5^1 end. There are $5J_k$ gene segments of which one is nonfunctional psuedogene and one is C_k gene segment.

V_k and J_k gene segments code for V-region. There is only one C_k gene segment. Human contain $40V_k$ gene segments, 5 J_k segments and one C_k segments.



Heavy chain multigene family: Along with the V_H and J_H gene segments, a third family of genes D(diversity) are required to generate the Ab diversity. Human chromosome 14, contains 51 V_H gene segments each proceeded by a Leader sequence. 27 D_H gene segments are located down stream the V_H gene segments. Next are located 6 functional J_H gene segments. These gene segments contain coding exons and noncoding introns. The exons encode for the constant region of an Immunoglobulin H-chain isotype. The C_H gene segments are arranged sequentially in the order C_{μ}, C_{δ}, C_{γ}, C_{ε} and C_{α}. This is related to the sequential expression of Ig classes during embryonic development, where IgM is the first to be coded on the B cell surface. The V_H gene segment was found to encode amino acids 1 to 94 and the J_H gene segment encode the amino acids 98 to 113; however, D gene segments carried the information to encode amino acids 95 to 97.



Variable region gene rearrangements:

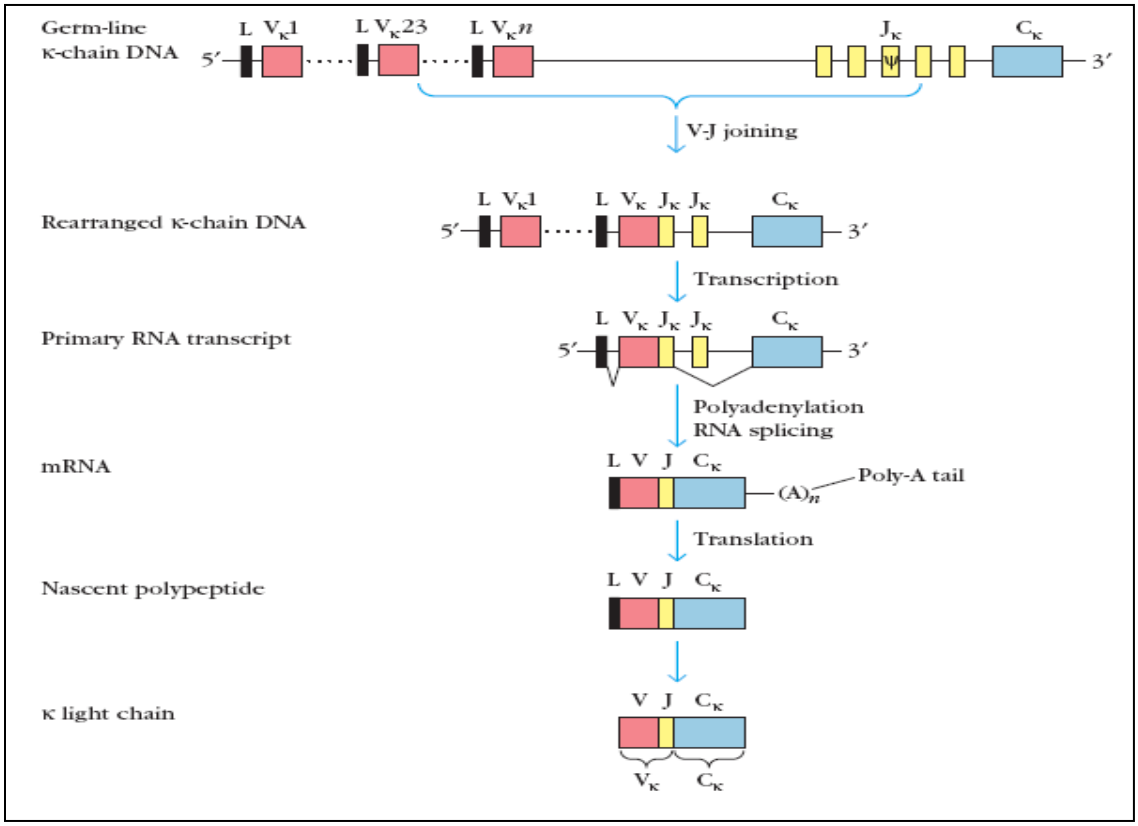
Lchain undergoes V-J Rearrangements: In human, nay of the functional V_{λ} genes can combine with any of the four functional J_{λ} - C_{λ} combinations. Whereas in mice, the V_{λ}^1 gene segment can join either with the J_{λ}^1 or J_{λ}^3 gene segments or V_{λ}^2 can join with J_{λ}^2 gene segment also.

Incase of κ chain rearrangements, any of the V_κ gene segment can be joined with anyone of the J_κ gene segments. A rearranged κ and λ genes contain the following regions inorder from their 5^1 to 3^1 end,

- Short leader (L) exon.
- A noncoding sequence (Intron)
- A joined VJ gene segment
- 2^{nd} intron and
- A constant region.

Upstream the leader gene segment is present a promoter sequence. RNA polymerase transcribes these genes until the stop signal present after the C-segment is reached, resulting in a L chain primary RNA transcript.

The introns present in the primary transcript are removed by RNA-processing enzymes (recombinase) resuting in a Lchain m-RNA with a poly-A tail. This L-chain mRNA is transported out of the nucleus where it is translated into L-chain protein. The leader sequence present at the amino terminus of this protein pulls it into the lumen of Rough Endoplasmic reticulum. The leader sequence is cleaved there finally giving a finished L-chain protein.



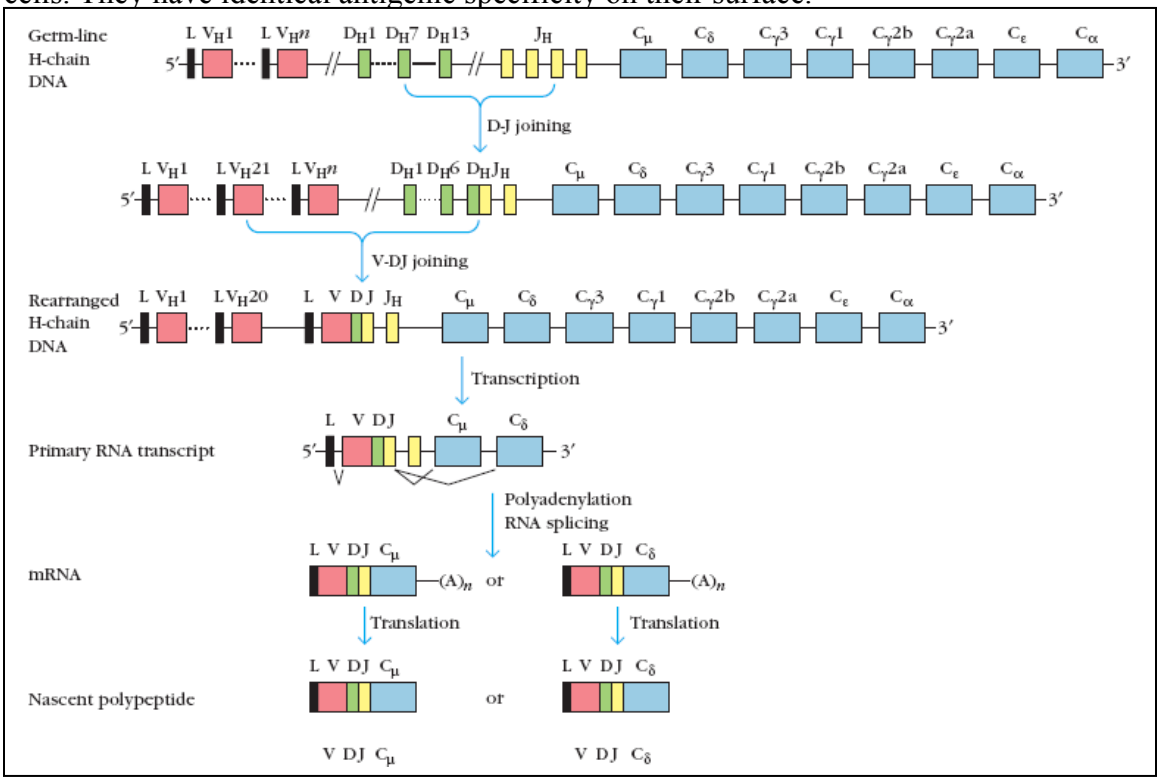
Heavy chain undergoes V-D-J rearrangements:

H-chain genes require two separate rearrangements for the V-region. First a D_H gene segment joins a J_H segment, and the resulting D_HJ_H segment moves and next joins a V_H segment generating $V_HD_HJ_H$ unit, which encodes the entire variable region. The variable region rearrangement results in a gene having the following sequences from 5^1 to 3^1 end,

- a short L exon.
- an intron
- a joined VDJ segment
- another intron
- series of C gene segments.

A promoter sequence is present upstream the leader sequence. To this rearranged H-chain gene, RNA polymerase binds at the promoter sequence and transcribes it. C_μ and C_δ gene segments are both transcribed. Polyadenylation and RNA splicing removes introns and results in a mRNA having one of the C_μ and C_δ coding sequences.

The mRNA are translated in the cytoplasm. The resulting peptides leader sequence is cleaved and finally generates a finished μ H chain or a δ H chain. This explains the explains the expression of both the chains by a mature, immunocompetent B cells. They have identical antigenic specificity on their surface.



Mechanism of V region DNA rearrangements:

Recombination signal sequences: These are located at 3^1 end of each V gene segment, at 5^1 end of each J gene segment and at both the ends of D gene segment.

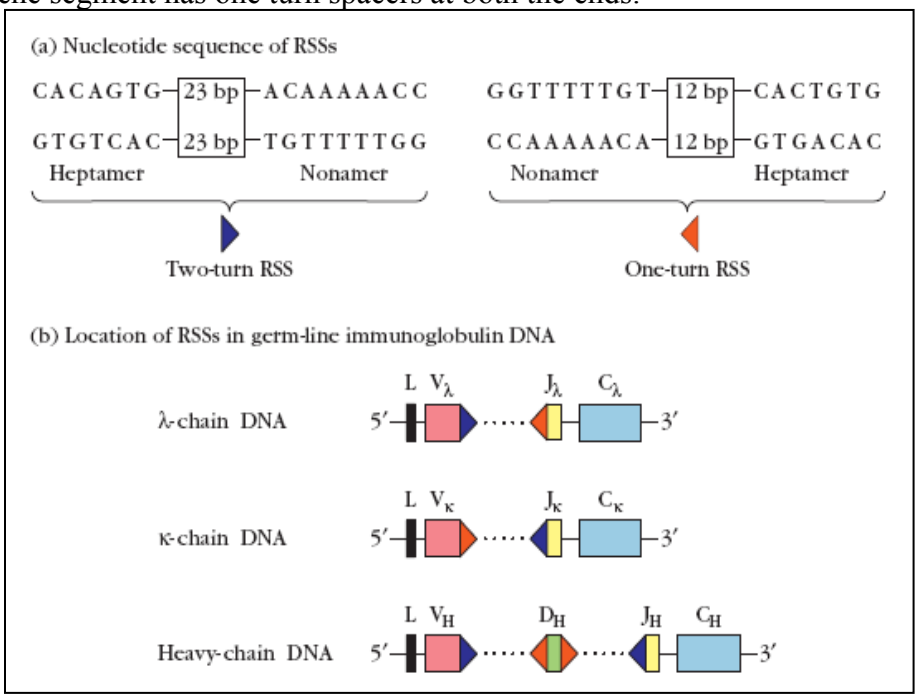
-Each RSS contains, a palindromic heptamer

An intervening 12 or 23 bp sequence and

A nanomer sequence rich in AT.

The 12 or the 23bp sequence correspond to a gap of one or two turns in a DNA double helix. Hence these are also called one-turn recombination signal sequences and two turn signal sequences. This gaps ensure that a V_L segment joins a J segment and so on.

For a L-chain, V_λ signal sequence has a two turn spacer and a J_λ signal sequence has one turn spacer. For a H chain, both V_H and J_H segments have two turn spacers and the D_H gene segment has one turn spacers at both the ends.



Recombinases are the enzymes involved in joining the gene segments. Two proteins RAG-1 and RAG-2 and a terminal deoxynucleotidyl transferees (TdT) enzyme are involved in V-(D)-J rearrangement.

Generation of Antibody diversity: Seven means of Ab diversification have been identified in mice and human:

- Multi germline gene segments.
- Combinatorial V-(D)-J joining.
- Junctional flexibility.
- P-region nucleotide addition (P-addition0
- N-region nucleotide addition (N-addition).
- Somatic hypermutation.
- Combinatorial association of L and H chains.

There are numerous germ line V,D and J gene segments. In human there are 51 V_H, 25 D, $6J_H$, $40V_K$, $5J_k$, $31V_\lambda$ and around 4 J_λ gene segments. In mouse there are about 85 V_k gene segments and 134 V_H gene segments, 4 functional J_H, 4 functional J_H, 3 functional J_λ and around 13 D_H gene segments, but only 3 V_λ gene segments.

Calculation of extimated diversity: In human heavy chian diversity can be attainded by the recombination of $51V_H$ gene segments with any of the 27 D_H gene segments, which inturn can combine with any of the $6J_H$ gene segments. Therefore the number of possible combinations generated are,

51 x 27 x 6 = 8262

Similarly 40 V_k segments can join with any of the 5 J_k segments generating 40 x 5=200 combinations.

In case of λ L chains, 30 V_λ and 4 J_λ gene segments generate 120 combinations.

Junctional flexibility, P and N nucleotide addition and somatic hypermutation also contribute to antigenic diversity.

TABLE 5-2 Combinatorial antibody diversity in humans and mice

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		
ESTIMATED NUMBER OF SEGMENTS IN MICE*			
V	134	85	2
D	13	0	0
J	4	4	3
Combinatorial V-D-J and V-J joining (possible number of combinations)	134 × 13 × 4 = 6968	85 × 4 = 340	2 × 3 = 6
Possible combinatorial associations of heavy and light chains†	6968 × (340 + 6) = 2.41×10^6		

Class switching among constant region genes: After antigenic stimulation of a B cell, the heavy chain DNA can undergo a further rearrangement in which the $V_HD_HJ_H$ unit can combine with any C_H gene segment. The extract mechanism of this process called class switching or isotype switching, is unclear, but it was known to involve DNA flanking sequences called Switch regions, which are laocated 2-3kb upstream form each C_H segment (except C_δ).

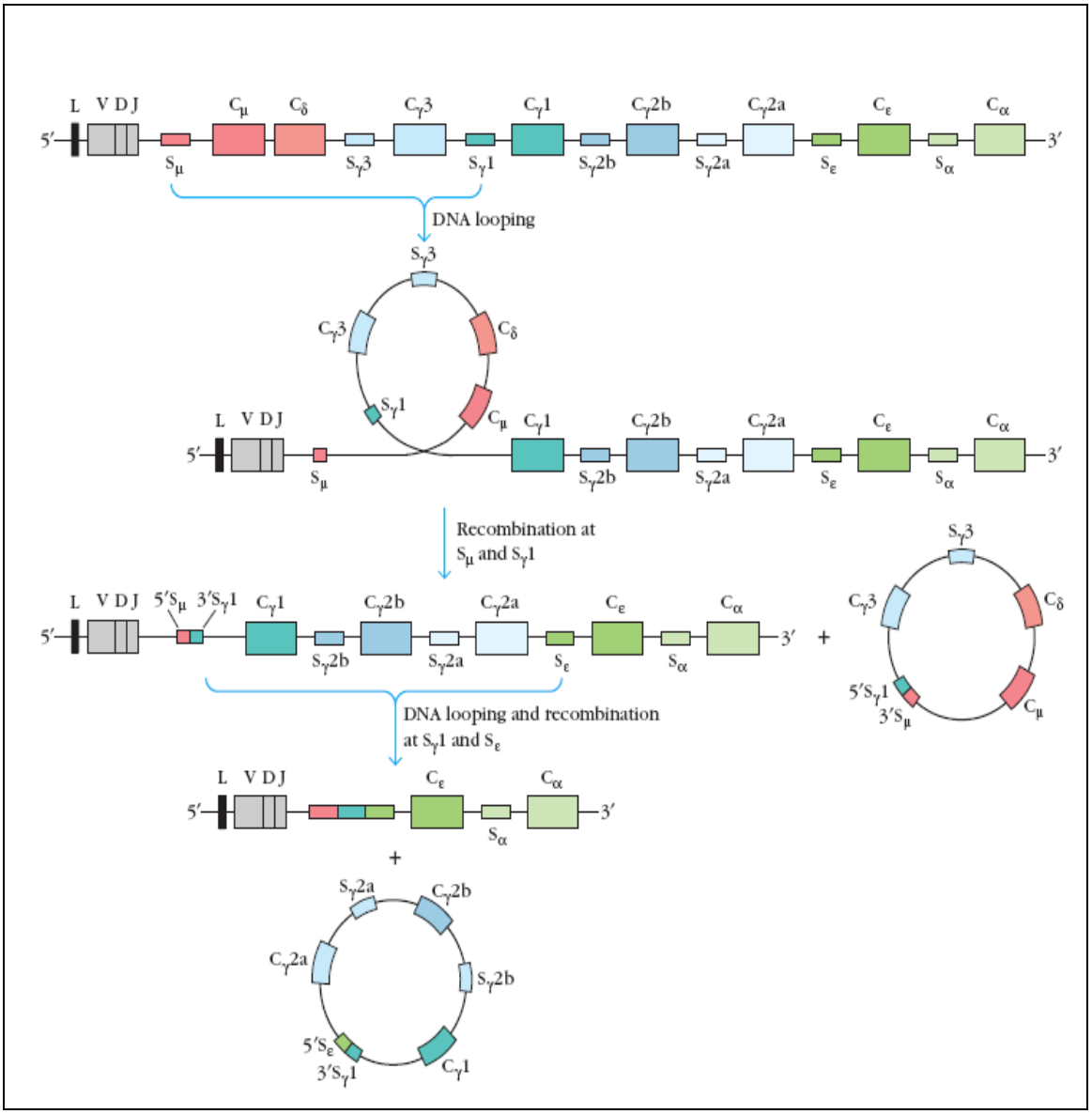
The switch region occupies 2-10kb of segment, which is mostly composed of multiple copies of short repeating sequences. A protein of the switch recombinase recognizes these repeats and upon binding to the switch regions carry out the DNA recombination which finally inclass switching.

Cytokines which are intercellular regulatory proteins act as switch factors and they determine the Ig class to be expressed. Eg: IL-4 induce class switch from Cμ to Cγ1 or $C_{\varepsilon.}$ It is also known to induce class switching in a successive manner.

The excision products produced during class switching from Cμ to Cγ1 showed circular excision product containing switch region of γ1 at its 5^1 end and that of μ at its

3^1 end. Further switch from C_γ to C_ε, leads to a circular excision product containing Cγ1 together with portions of μ, γ & ε switch regions. Therefore class switching depends upon the interplay of 3 elements.

1) Switch regions
2) Switch Recombinase
3) Cytokine signals that dictate the isotype to which the B cell switches.



Synthesis, Assembly and Secretion of Immunoglobulins: The H and L-chains of Ig's are translated on separate polyribosomes of the RER. The newly synthesized chains

contain an amino terminal leader sequences, which guides it into the lumen of RER where this signal sequence is cleaved.

Assembly of these L and H-chains via their disulphide linkages and their glycosylation occurs in the cisternae of the RER, finally yielding a Ig molecule. This is then transported to golgi apparatus which forms a secretory vesicle, that transports the Ig outside. The secretory vesicle fuses with the plasma membrane. The order of chain assembly also varies. Incase of IgM, the H and L-chains assemble first to form half molecules in the RER, and then the two half molecules in the RER, and then the two halfs come together to form a complete IgM.

Incase of IgG, two H chains come together first to form H_2, then it forms H_2L and finally H_2L_2 molecule.