
ORGANIZATION AND EXPRESSION OF
IMMUNOGLOBULIN GENES
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GENETIC MODEL COMPATIBLE WITH IMMUNOGLOBULIN STRUCTURE

Properties of Antibodies

- Vast diversity of antibody specificities.
- Presence in immunoglobulin heavy and light chains of a variable region at the amino terminal end and a constant region at the carboxy terminal end.
- The existence of isotypes with the same antigen specificities, which result from the association of a given variable region with different heavy chain constant region.

GERM LINE AND SOMATIC VARIATION MODELS

Two different sets of theories emerged to explain tremendous diversity of antibody structure.

Germ line

- The genome contributed by the germ cells, eggs and sperm contains a large repertoire of Ig genes.

Somatic variation

- The genome contains a relatively small no: of Ig genes from which a large no: of antibody specificities are generated in the somatic cells by mutation and recombination.

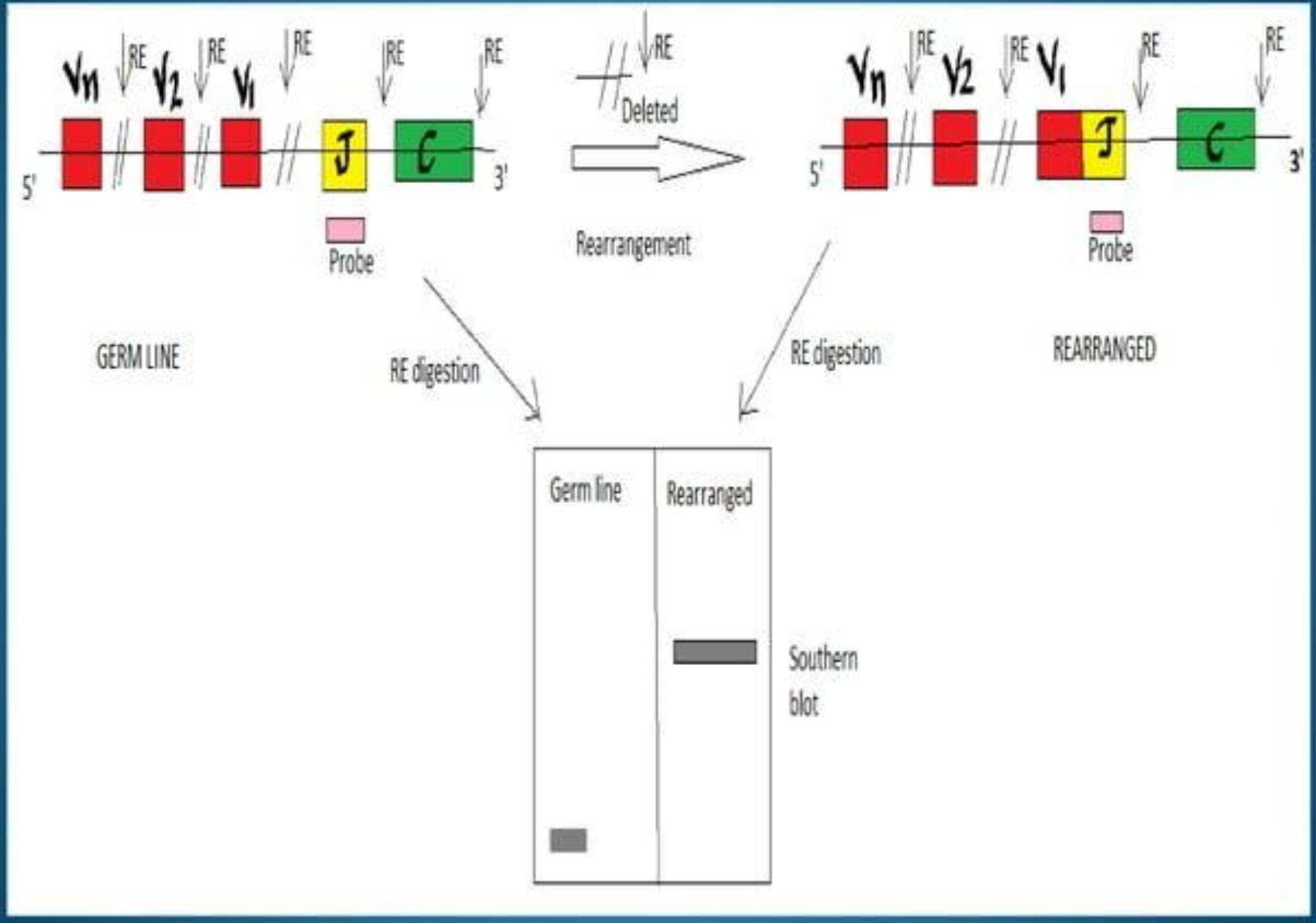
How could stability be maintained in the constant (C) region while some kind of diversifying mechanism generated the variable (V) region?

TWO GENE MODEL OF DREYER AND BENNETT

- In 1965, W. Dreyer and J. Bennett suggested that two separate genes encode a single immunoglobulin heavy or light chain, one gene for the V region (variable region) and the other for the C region (constant region).
- These two genes must come together at the DNA level to form a continuous message that can be transcribed and translated into a single Ig heavy or light chain.

TONEGAWA'S EXPERIMENT

- In 1976, S. Tonegawa and N. Hozumi found that separate genes encode the V and C regions of Immunoglobulins and that the genes are rearranged in the course of B-cell differentiation.
- Tonegawa was awarded Nobel prize for this work in 1987.


Vn
RE
V2
RE
V1
RE
RE
RE
J
C
5'
3'
Probe
GERM LINE
RE
Deleted
Rearrangement
RE digestion
Vn
RE
V2
RE
V1
RE
RE
J
C
5'
3'
Probe
REARRANGED
RE digestion
Germ line
Rearranged
Southern
blot

MULTIGENE ORGANIZATION OF IMMUNOGLOBULIN GENES

- Germ-line DNA contains several coding sequences, called ***gene segments***, separated by non-coding regions.
- Gene segments are rearranged during B cell maturation to form functional Ig genes.
- Each multigene family has distinct features.
- The κ and λ light chain families contain V, J and C segments; the rearranged VJ segments encode the variable region of the light chains and the C gene segments encode the constant region.

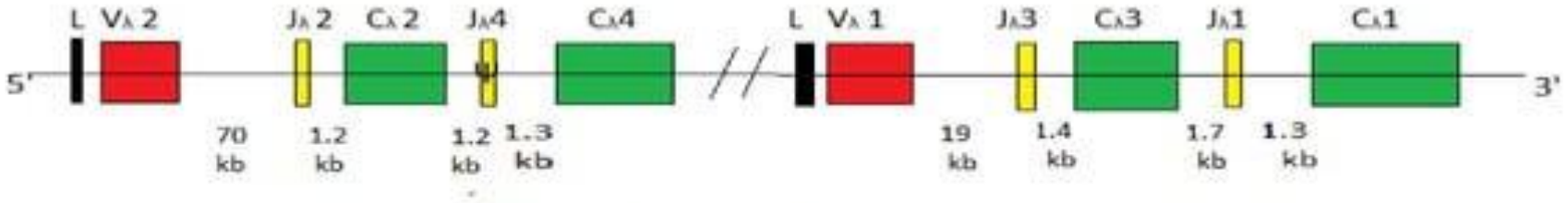
- The heavy-chain family contains V, D, J, and C gene segments; the rearranged VDJ gene segments encode the variable region of the heavy chain.
- Each V gene segment is preceded at its 5' end by a signal or leader (L) peptide that guides the heavy or light chain through the endoplasmic reticulum.
- Signal peptide is cleaved from the nascent light and heavy chains before assembly of the final Ig molecule.

λ CHAIN MULTIGENE FAMILY

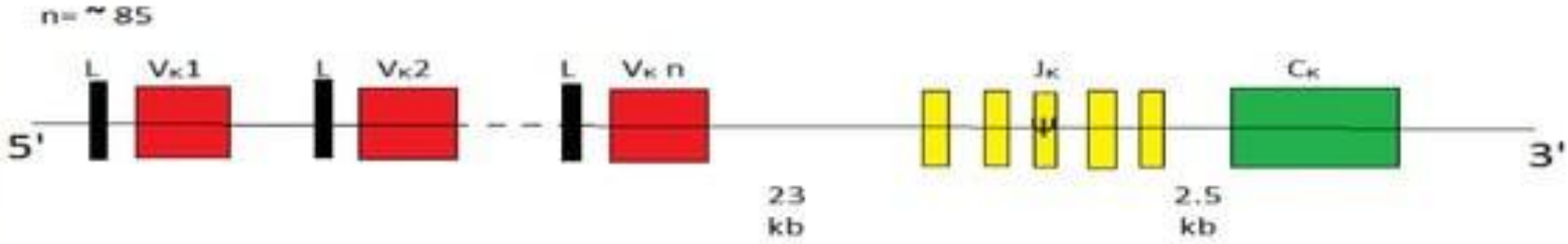
- Functional λ variable-region gene contains two coding segments – a 5' V segment and a 3' J segment – which are separated by a non-coding DNA sequence in unrearranged germ-line DNA.
- J for *joining* (39 bp gene segment).
- In mouse, λ multigene family contains 3 V_λ , 4 J_λ and 4 C_λ gene segments.
- J_λ 4 is a ***pseudogene*** or a defective gene that is incapable of coding proteins, denoted by ψ.
- λ chain have 3 subtypes (λ 1, λ 2 and λ3).
- In humans, there are 31 functional V_λ , 4 J_λ and 7 C_λ gene segments. It also contains many pseudogenes.

K CHAIN MULTIGENE FAMILY

- In mouse, κ chain multigene family contains approximately 85 $V_κ$, 5 $J_κ$ (one pseudogene) and 1 $C_κ$ gene segment.
- $V_κ$ and $C_κ$ encode variable region of the κ light chain and the $J_κ$ gene segment encode the constant region.
- There are no subtypes of κ light chains.
- In humans, approximately 40 $V_κ$, 5 $J_κ$ and 1 $C_κ$ gene segments are present.



λ- Chain DNA

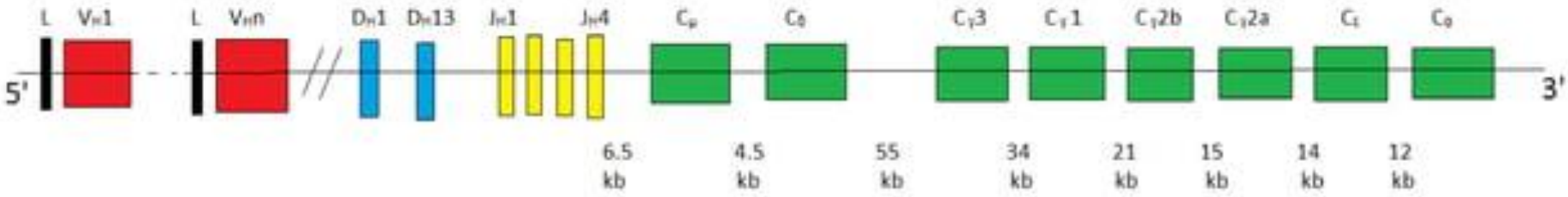


κ-chain DNA

HEAVY CHAIN MULTIGENE FAMILY

- Leroy Hood proposed the existence of an additional gene segment in the heavy chain variable region.
- It was designated D for diversity because of its contribution to the generation of antibody diversity.
- The heavy chain multigene family on human chromosome 14 contain 51 V_H, 27 D_H , 6 J_H and a series of C_H gene segments.
- The C_H gene segments consist of coding exons and non-coding introns.
- In humans and mice, the C_H gene segments are arranged sequentially in the order C_μ , C_δ , C_γ, C_ε, C_α.

n=~ 134



HEAVY CHAIN DNA

GENE	CHROMOSOME	
	HUMAN	MOUSE
λ Light Chain	22	16
κ Light Chain	2	6
Heavy Chain	14	12

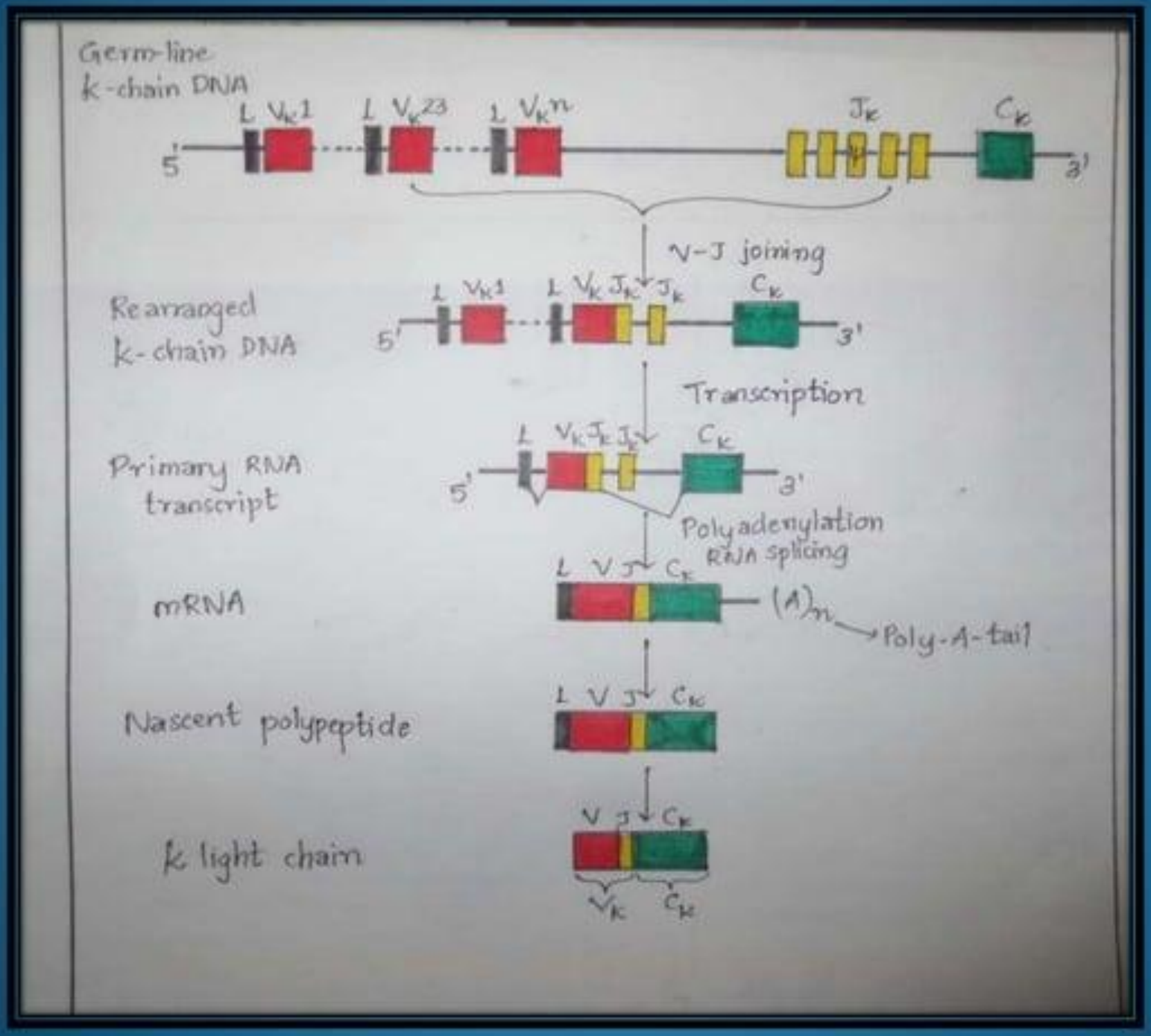
Chromosomal locations of immunoglobulin genes in human and mouse

VARIABLE REGION GENE REARRANGEMENTS

- Variable –region gene rearrangements occur in an ordered sequence during B-cell maturation in the bone marrow.
- The heavy chain variable -region rearrange first, then light chain.
- Process of variable –region gene rearrangement produces mature, immunocompetent B cells which produce antibody with a binding site encoded by the particular sequence of its rearranged V genes.
- Rearrangements of the heavy chain constant region genes will generate further changes in the isotype expressed by a cell.

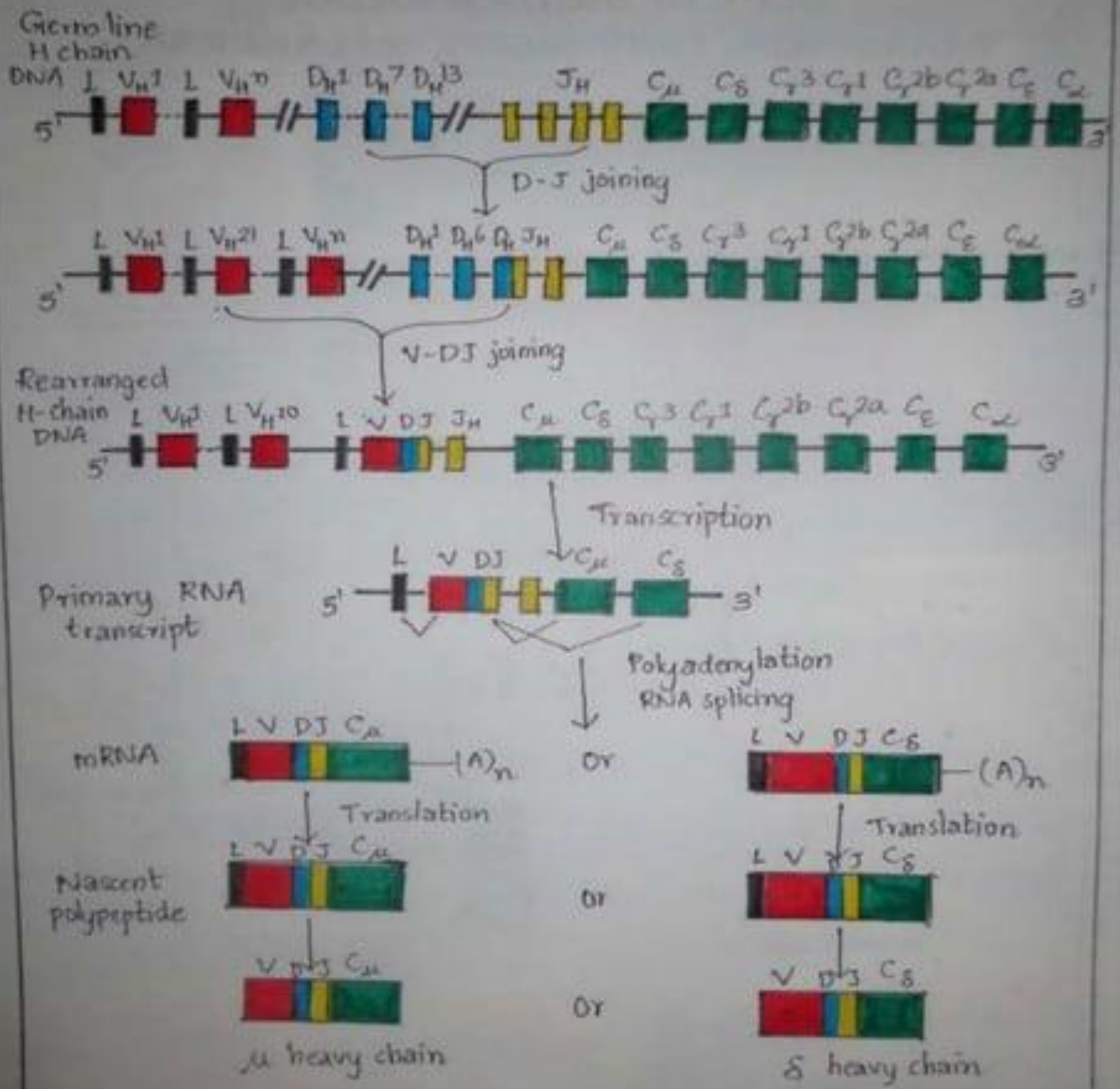
V-J REARRANGEMENTS IN LIGHT CHAIN DNA

- Expression of both κ and λ light chains requires rearrangement of the variable-region V and J gene segments.
- In humans, any of the functional V_λ genes can combine with any of the four functional C_λ- J_λ combinations.
- In mouse, things are slightly more complicated.
- In human or mouse κ light chain DNA, any one of the V_κ gene segments can be joined with any one of the functional J_κ gene segments.
- Rearranged genes have a short leader (L) exon, a non-coding sequence (intron), a joined VJ gene segment, a second intron, and the constant region in order from 5' to 3' end.


Germ-line
k-chain DNA
L
V_K1
L
V_K23
L
V_Kn
J_K
C_K
5'
3'
V-J joining
Rearranged
k-chain DNA
L
V_K1
L
V_K
J_K
J_K
C_K
5'
3'
Transcription
Primary RNA
transcript
L
V_K
J_K
J_K
C_K
5'
3'
Polyadenylation
RNA splicing
mRNA
L V J C_K
$(A)_n$
Poly-A-tail
Nascent polypeptide
L V J C_K
k light chain
V J C_K
V_K
C_K

V-D-J REARRANGEMENTS IN HEAVY CHAIN DNA

- This process requires two separate rearrangement events within the variable region.
- D_H gene segment first joins to a J_H segment; the resulting D_HJ_H segment joins a V_H segment to generate a $V_H D_HJ_H$ unit that encodes the entire variable region.
- Rearrangement produces a short L exon, an intron, a joined VDJ segment, another intron and a series of C gene segments.


Germ line
H chain
DNA
D-J joining
V-DJ joining
Rearranged
H-chain
DNA
Transcription
Primary RNA
transcript
Polyadenylation
RNA splicing
mRNA
Translation
Nascent
polypeptide
or
μ heavy chain
δ heavy chain