LECTURE: 22

Title **IMMUNOGLOBULIN DIVERSITIES**

LEARNING OBJECTIVES:

The student should be able to:

- **Identify the chromosome that contains the gene segments that encode the surface immunoglobulin heavy chain proteins.**
- **Enumerate the gene segments that assemble to form a complete gene for determining the immunoglobulin specificities.**
- **Determine if immunoglobulin gene organization is similar or different in heavy or light chains or in the different heavy chain classes.**
- **Explain how the human immune system is capable of producing a vast number of different antibody molecules, each with its own antigenic specificity while B-cell has a limited number of genes.**
- **Define the terms involved in antibody diversity such as:**

- **Isotypic variation.**
- **Allotypic variation.**
- **Idiotypic variation.**

- **Enumerate the mechanisms contributing to antibody diversity.**
- **Define exons and introns**
- **Explain the gene rearrangement, light, and heavy chain organization.**
- **Explain the mechanisms contributing to ab diversity.**
- **Explain the immunoglobulin class switching.**
- **Explain the nonproductive rearrangement.**
- **Explain the allelic exclusion and clonal restriction.**
- **Explain the defect genes in developing of malignancies (Examples).**
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LECTURE REFRENCE:

1. TEXTBOOK: ROITT, BROSTOFF, MALE IMMUNOLOGY. 6th edition. Chapter 6. pg. 78-85.

2. TEXTBOOK: ABUL K. ABBAS. ANDREW H. LICHTMAN. CELLULAR AND MOLECULAR IMMUNOLOGY. 5TH EDITION. Chapter 8. pg 61- 63. 207-210. Chapter 9 .pg 189-214.

3. TEXTBOOK: NATIONAL MEDICAL SERIES FOR INDEPENDENT STUDY. RICHARD M. HYDE. Chapter 2. pg 51-58. pp 27-43.

IMMUNOGLOBULIN DIVERSITY

A. Allotypic and idiotypic variation. In addition to the isotypic (class) variation , immunoglobulins also display allotypic and idiotypic variation.

1. Allotypes are allelic variants of isotypes.

- **a.** Allotypes exist in different individuals within the same species, and are inherited in a Mendelian manner.
- **b.** Allotypes are identified by **allotypic markers** [i.e., structural (antigenic) differences] found in the constant regions of H and L chains.
- **c.** **Nomenclature** for allotypic markers has been established for gamma, alpha, and kappa chains.

    - **(1)** Markers on **gamma H chains** are designated as **Gm** (for gamma, referring to IgG).

        - **(a)** There are over 20 antigenically different Gm markers.
        - **(b)** All are not found on all IgG molecules; they seem to be restricted to certain subclasses.

    - **(2)** Markers on **alpha H chains** are designated **Am** (for alpha, referring to IgA). There are only two alleles at this locus.
    - **(3)** Markers on **kappa H chains** are designated **Km**.

        - **(a)** There are three alleles at this locus, and the markers vary only in amino acid composition at positions 153 and 191.
        - **(b)** Km was originally called **Inv** from the source of the antiserum used to identify one of the allelic gene products (form patient "V").

2. Idiotypes represent the antigen-binding specificities of immunoglobulins.

- **a. Overview.** Idiotypes represent unique structural (antigenic) determinants (i.e., amino acid sequences) in the variable region that are associated with the antigen-binding capability of the antibody molecule.

    - **(1) Idiotypic variability** pertains to the generation of the antigen-binding site in the variable region of the H and L chains.
    - **(2)** Variability in amino acid sequence in these regions is concentrated in three to four CDRs surrounded by relatively invariant residues.
    - **(3)** These CDRs are the areas that make contact with the epitope of the antigen.

- **b. Idiotopes and idiotypes**

(1) Idiotopes are antigenic epitopes that occur in the variable region of the Fab portion of an antibody molecule. They may be a part of the CDRs or they may be associated with framework sequences of the molecule.

(2) The **iditype** of a particular immunoglobulin is the sum of the individual idiotopes.

c. Anti-idiotypic antibodies (Figure-1)

(1) If an antibody is used as an immunogen, it is possible to induce the production of anti-idiotypic antibodies that structurally resemble the original epitope.

(2) These second-generation anti-idiotypic antibodies could be used in artificial vaccines, as an immunizing antigen to induce the original antibodies in a "naïve" recipient.

(3) A second potential significance to this anti-antibody circuit is as a mechanism for regulation of the immune response.

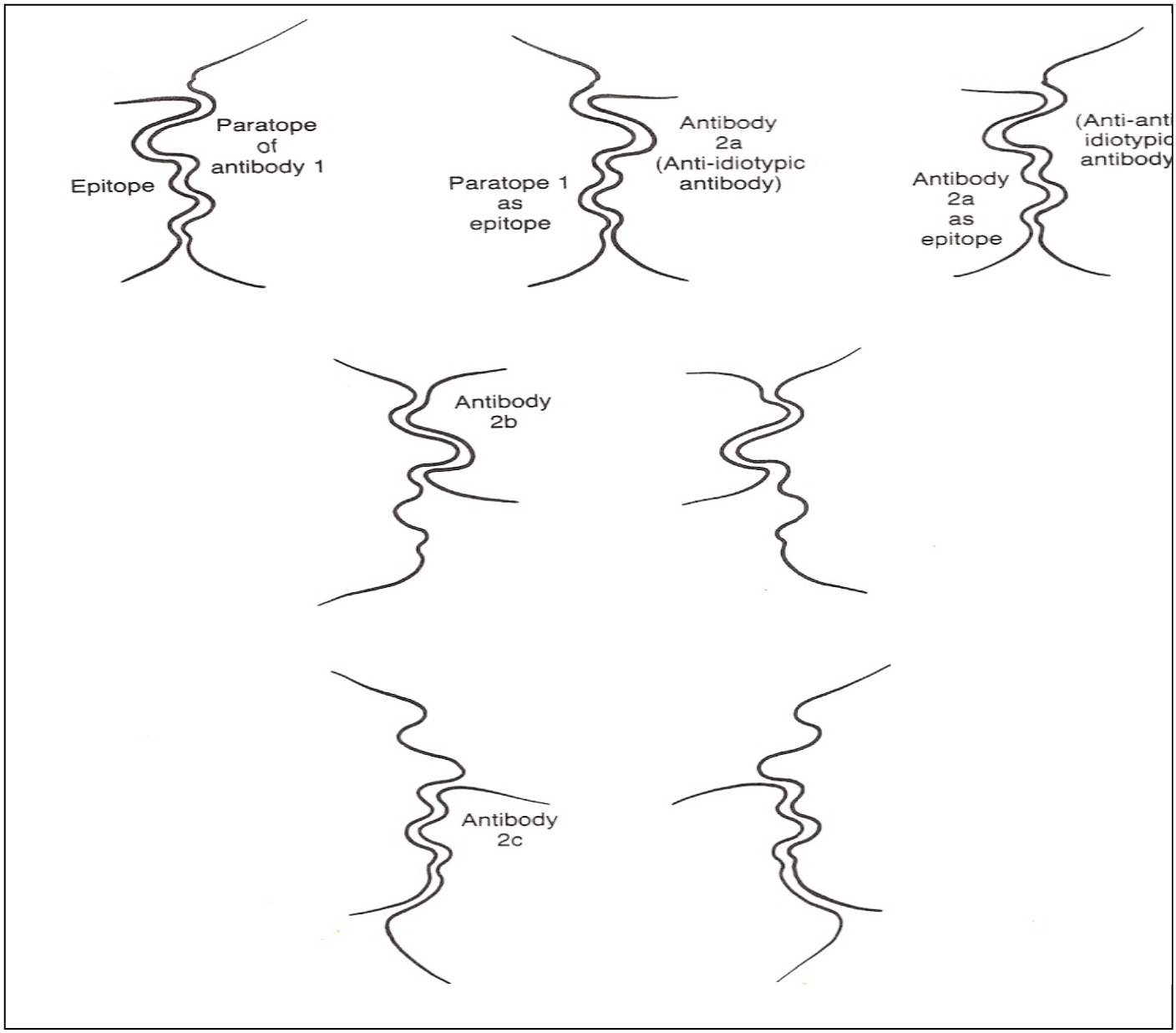

Figure-1 The production of anti-idiotypic antibodies. The paratope of an antibody can become immunogenic in an appropriate host and will constitute one of the idiotypes of that antibody. An antibody homologous with the paratope (an anti-idiotypic antibody- e.g., antibody 2a) will structurally mimic the original epitope. Similarly, anti-idiotypic antibodies can induce anti-anti-idiotypic antibodies that would structurally mimic the original paratope. Different segments of an antibody's paratope can serve as epitopes, raising antibodies with different specificities (antibodies 2a, and 2 c).

Genetics of immunoglobulin diversity

1. Overview

a. Similarity

(1) Immunoglobulin gene organization is remarkably similar, whether dealing with H or L chains, or with the different H chain classes.
(2) In fact, the genetics of the T cell receptor also has a pattern of locus clusters that resembles that of immunoglobulins very closely, although different genes are involved.

b. Diversity

(1) The human immune system is capable of producing a vast number of different antibody molecules, each with its own antigenic specificity.

(a) This vast diversity is possible because immunoglobulin genes undergo an unusual type of interaction.

(i) Embryonic DNA contains a great many genes for the variable regions of the H and L chains.
(ii) A process of **somatic recombination** (DNA rearrangement and deletion), followed by **RNA splicing,** results in a large variety of plasma cell lines that encode different H chains and L chains.
(b) A fairly high rate of **somatic mutation** in κ, λ, and H chains further adds to the diversity.

(2) A similar process of DNA rearrangement gives the T cell receptor its epitope-binding specificity.

2. Chromosomes, exons and introns, and gene rearrangements

a. Chromosomes. H chains and κ, λ, and H chains are each encoded on separate chromosomes. In human these are as follows:

(1) All of the H chain immunoglobulin classes are coded at one site on **chromosome 14.**
(2) The λ chain gene complex is at one site on chromosome 22.
(3) The κ chain gene complex is at one site on chromosome 2.

b. Variable and constant regions

(1) The H and L chains vary markedly in the amino acid composition of their amino terminal potion (constant region).
(2) Analysis of immunoglobulin genes has revealed that the variable and constant regions are separately encoded and located on different fragments of DNA.

c. Exons and introns

(1) As in other genes, **coding sequences (exons)** in the DNA code for the amino acid sequences in immunoglobulin molecules.
(2) The exons are separated by **intervening noncoding nucleotide sequences (introns)**.

(3) Both exons and introns are transcribed into RNA, but RNA splicing then removes the introns, leaving the exons joined together.

d. Gene rearrangement

(1) The exons that code for variable domains are split up into smaller segments of DNA along the chromosome.
(2) Making proper exons from these segments requires rearranging and rejoining the segments to form immunoglobulin gene sequences.

3. L chain gene organization

a. Three genes code for each immunoglobulin L chain; the human κ chain (**Figure-2**) is used here as an example.

(1) Two gene segments encode the variable domain.

(a) The initial gene segment, the variable (**V_κ**) region, encodes the first 95 amino acids of the variable region protein. Over 200 V_κ region genes exist in this region of human chromosome 2.
(b) A second gene, the **joining (J_κ) segment,** encodes the remaining 13 amino acids (96-108) of the V exon (this J is unrelated to the J chain found in IgM and IgA). There are five genes at this locus.

(2) A third gene dictates the amino acid sequence of the constant region. There is only one **C_κ region** on this final segment, which codes for amino acids 109-214.

b. The **λ L chains** arise from a similar gene complex on chromosome 22.

(1) However, there are six to nine slightly different copies of the C_λ region gene, which correspond to the various subtypes of λ protein. Each λ gene I s accompanied by an adjacent J gene segment.

(2) A V gene segment may fuse with any of these alternative gene combinations, resulting in a **V/J exon** that can be transcribed, in the B cell, with the adjacent **C exon.**

4. H chain gene organization. The **H chain of IgM** (**Figure-3**) is used as an example.

a. Although similar to that of L chain genes, H chain gene organization is more complex.

(1) Whereas a L chain gene region encodes only one constant domain, the H chain gene region must code for three or four constant domains.

(2) Also, the H chain C gene region must code for each of the five immunoglobulin classes, and for four IgG and two IgA subclasses as well.

(3) Furthermore, the exon coding for the variable portion of the H chain is composed of three, not two, segments of DNA.

b. Assembly of the V/D/J exon

(1) The additional variable region DNA segment is designated the **diversity (D_H gene region.**

(2) The D_H gene region accounts for the third CDR of the H chain; it comprises only two or three amino acids.

(3) Gene rearrangement links a D_H to a J_H segment, and then joins these to a V_H, to produce the **V/D/J exon.**

c. There are over 200 V_H genes, at least 20 D_H genes, and 6 J_H genes. This enormous gene pool, with its large potential of combinations, underlies the great diversity of the H chain variable region.

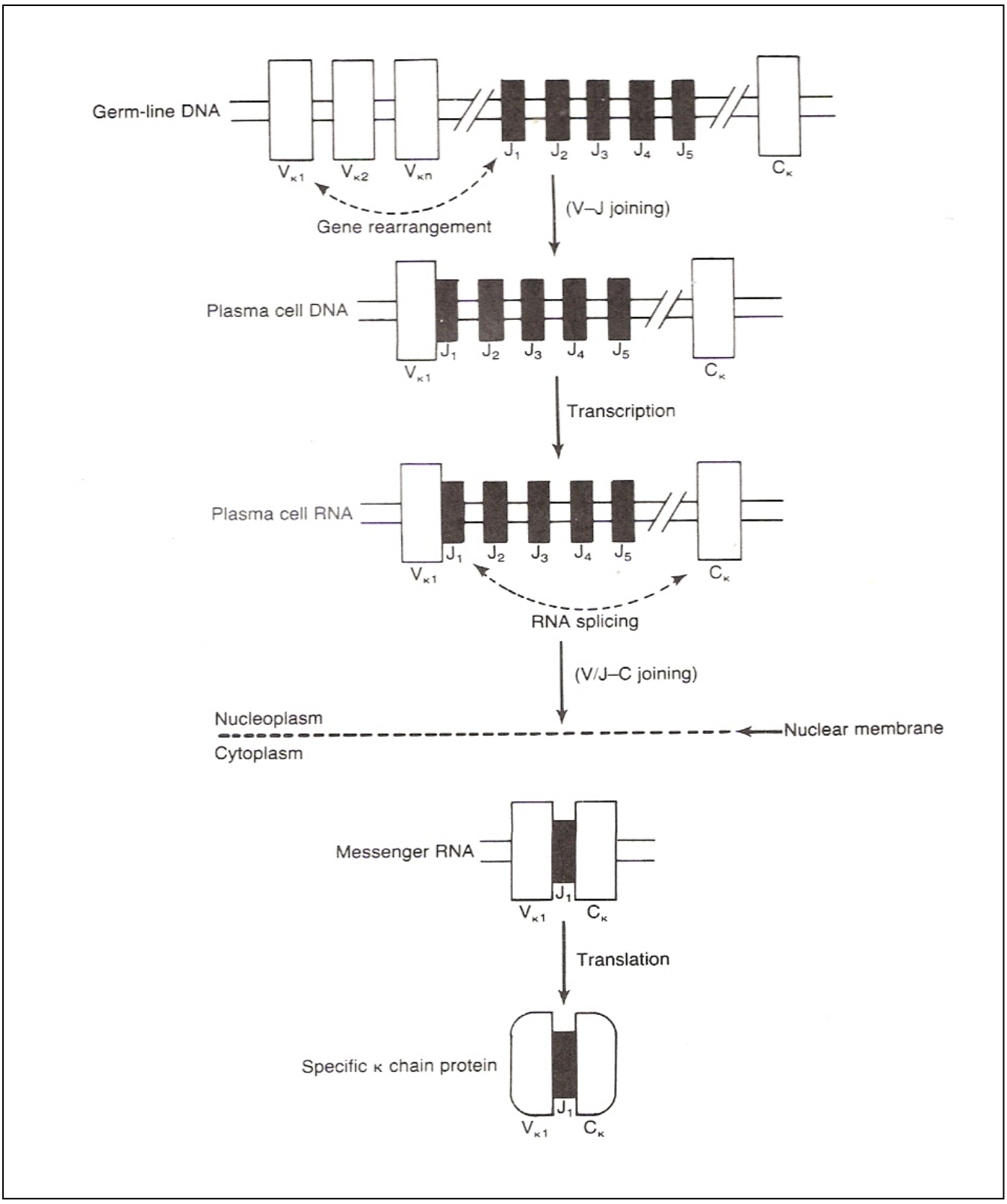

Figure-2 Human **κ** light (L) chain gene organization the potential for a large variety of **κ** chains exists due to somatic recombination in the DNA and RNA splicing. As the B cell precursor differentiates into a mature B cell, DNA deletion brings one of the variable (V **κ**) genes next to one of the joining (J) genes, in this example, $V_{\kappa 1}$ and J_1. This unit and the remaining J genes are separated from the constant (C**κ**) region by an intervening sequence (intron) of DNA. The $V_{\kappa 1}$/ J_1 unit codes for one of the numerous possible κ chain variable exons. The plasma cell DNA is transcribed into nuclear RNA, which is spliced to form messenger RNA (mRNA), with $V_{\kappa 1}$, J_1, and Cκ messages joined and ready for translation into the κ chain.

5. The 12/23 spacer rule-a mechanism for joining immunoglobulin gene segments

a. Each gene segment (H, λ, or κ) is flanked by noncoding **recombination signal sequences (RSSs)** that specify the direction of joining the segments.

(1) Each RSS consists of a highly conserved sequence containing three parts:

(a) A **heptamer** (seven DNA base pairs)

(b) A **spacer** containing either 12 nucleotides (equaling one turn of the DNA helix) or 23 nucleotides (two turns).

(c) A **nonamer** (nine DNA base pairs)

(2) Joining of the gene segments occurs only when the spacer nucleotide units are of different lengths.

b. Joining of L chain genes

(1) For **κ chain** genes, the RSS on the downstream side of each V_κ signets has a 12 nucleotide spacer, while the signal on the upstream side of the J_κ segment is composed of 23 nucleotides, a combination which permits joining.

(2) For **λ chain** genes, the rule is preserved, but the order is reversed.

c. Joining of H chain genes. This is more complex because three segments are involved.

(1) The spacers for V_H and J_H genes all have 23 nucleotides and hence cannot be joined.

(2) However, the spacers that flank the D gene are 13 nucleotides in length, and thus the 12/23 spacer rule is followed once again.

6. VJ and VDJ recombinases

a. The cleavage and rejoining of the DNA strands are presumed to be carried out by endonucleases and ligases, respectively. These enzymes recognize the heptamer and nonammer RSSs when they are separated by one or two turns of the DNA helix.

b. Recently, two genes that function in immunoglobulin gene recombination have been identified in mouse pre-B cells. It is not known whether these **recombination-activation genes 1 and 2 (RAG-1 and RAG-2)** code for enzymes or for other regulatory proteins.

c. These enzymatic processes are active only in the early stages of B and T lymphocyte maturation, during the development of immunologic commitment. The gene products are not functional in mature cells, and thus the immunologic specificity of the B or T cell is maintained.

7. T cell receptor gene organization

a. The cell receptor for antigen is similar to the immunoglobulin molecule in gene organization as well as in molecular structure.

b. During T cell development in the thymus, the genes for the T cell receptor undergo DNA rearrangements much like those for the immunoglobulin molecule.

(1) The variable region of the T cell receptor's α chain involves rearrangements of V and J gene segments.

(2) The variable region of the receptor's β chain involves rearrangements of V, J, and D gene segments.

C. Mechanisms contributing to antibody diversity

1. Chance recombination creates a large amount of antibody diversity.

a. If all events occurred randomly, somatic recombination in the DNA followed by RNA splicing (**Figure-2, and 3**) could produce:

(1) More than 1000 varieties of κ L chains.

(2) A similar number of λ L chains

(3) Perhaps as many as 20,000 varieties of H chains

2. Imprecision in the joining of the V, D, and J genes can also be a source of immunoglobulin diversity. (Wherever genes join, imprecision in joining can occur.)

3. N region additions can produce changes in the specificity and reactivity of the immunoglobulin molecule.

a. N regions are very short peptides of variable sequence, often found near the third CDR of the H chain.

(1) This region, the **most variable region** of the immunoglobulin molecule, contains amino acids encoded by the D_H gene region.

(2) Furthermore, because the DH segment is very small, the N region contains two joining boundaries, where exons V and J join D at opposing ends.

b. Immature lymphoid cells contain an enzyme, **terminal deoxynucleotidyl** transferase, which catalyzes the generation of N regions by the addition of nucleotides at the 3" end of the DNA strands.

4. Extensive mutation involving variable region genes produces even further diversity.

a. Mutations can occur by several mechanisms.

(1) Point mutations. These single-nucleotide substitutions can occur in the variable region of a functional immunoglobulin gene.

(a) Moat mechanisms that generate diversity are active **before** any exposure to antigen.

(b) The **somatic mutation theory** of antibody diversity suggests that a small number of genes diversify during lymphocyte differentiation, that is, in the pre-B cell or B cell stage, either by point mutation or by recombination events.

(i) These mutations occur **after** the cells have encountered antigen, especially during intensive immunization.

(ii) The nucleotide substitutions are found only in variable domains of either H or L chains, predominantly in the CDRs.

(3) Gene conversion. In some immunoglobulin gene families (e.g., chicken λ chain genes), it appears that stretches of nucleotides are translocated form one V gene segment to another in the same V locus. This process may also contribute to the diversity of mammalian variable domain.

c. The antibodies produced by mutations in variable domain genes may confer a **selective advantage** to the lymphocyte if they posses a **higher affinity** for the antigen.

(1) Cells coated with high-affinity antibody are better able to interact with antigen and perpetuate the immune response.

(2) A characteristic of prolonged immunizations the increase in antibody affinity for the antigen as the duration of exposure to that antigen increases, because the longer time increases the chance that a "good" mutation will occur.

D. Immunoglobulin class switching (isotype switching)

1. During the immune response, plasma cells switch from producing IgM to IgG or to another immunoglobulin class.

2. There is no alteration in the L chain or in the variable portion of the H chain; thus, there is **no change** in antigen-binding specificity.

3. The switch involves a change in the **H chain constant domain (C_H).**

a. Chromosome 14 contains C_H gene sequences for each of he nine H chain isotypes; these are designated C_{μ}, C_{δ}, $C\gamma_2$, $C\gamma_1$, and so forth.

b. When the H chain gene is assembled, all but one of the C_H gene sequences are deleted, thereby producing the desired isotype.

c. In the process, DNA rearrangement and RNA splicing place the remaining C_H gene sequence adjacent to the V/D/J exon (**Figure-3**).

4. Repetitive sequences in H chain genes permit immunoglobulin class switching.

a. The switch is accomplished by recombination between special repetitive switching sequences in the introns upstream from the constant region exons.

b. The transcriptional enhancer in the introns between the J_H cluster and C_μ exon is upstream of the switching sequence. Therefore every switch carries the enhancer with it, and active transcription os ensure d regardless of the H chain class expressed.

5. Plasma cells can switch successively from $C_{\mu,}$ to $C\gamma_1$ to $C_{\alpha 2}$, and so forth.

a. Regulatory proteins secreted by **T cells** stimulate plasma cells to undergo particular switch recombinations.

b. For example, **interleukin-4 (IL-4)** promotes switching to the ε gene with the resulting production of IgE.

E. Nonproductive rearrangements

1. The H and L chain are formed during the pre-B cell phase of immunologic maturation.

a. In the formation of a functional gene, DNA segments must be joined properly to ensure that the correct reading frame is maintained.

(1) If the joining is erroneous (off by one or two nucleotides), the downstream sequences are out of frame, preventing translation into a functional polypeptide.

(2) Such erroneous rearrangements occur frequently, and in this case the cell continues to rearrange and join its immunoglobulin gene segments until a functional immunoglobulin is made; then rearrangement ceases.

b. The appearance of an intact H chain in the cytoplasm seems to be the signal for the end of H chain gene shuffling.

c. It is also the signal for commencement of L chain gene rearrangement.

d. The trail and error process is repeated until a functional L chain, either κ or λ, is produced.

2. The pre-B cell then enters the B lymphocyte stage of development, in which the H and L chains are joined by disulfide bonds and are expressed in the B cell membrane.

3. It is likely that many lymphocytes have nonproductive rearrangements and are of no value to the immune system. This appears to be the price that must be paid to preserve the generation of immunologic diversity by so elaborate a system.

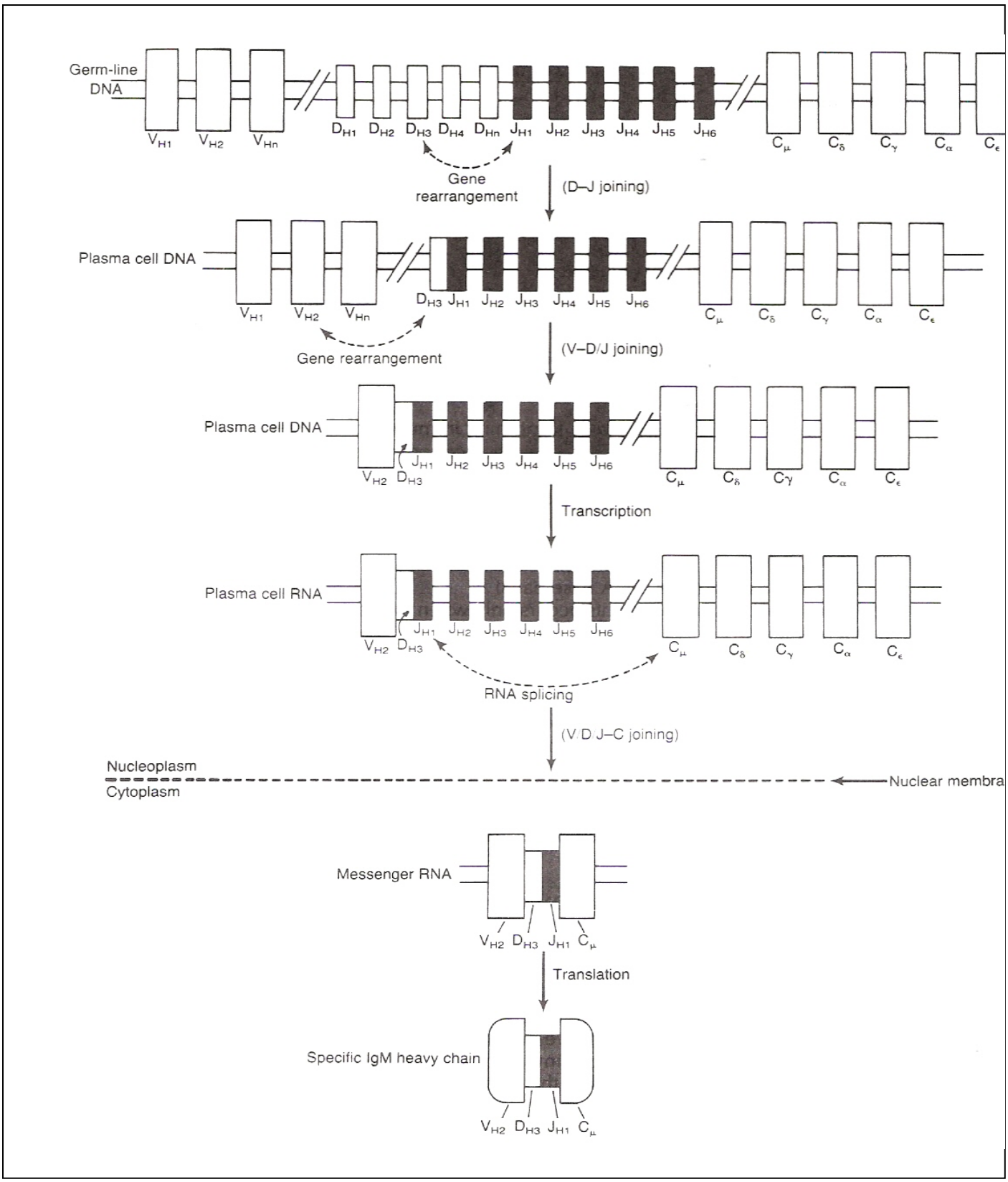

Figure-3 Human μ heavy (H) chain gene organization the potential for variety in H chains, as in κ chains, is due to somatic recombination in the DNA and to RNA splicing. As the B cell precursor differentiates into a mature B cell, DNA deletion brings one of the variable (V_H) genes, one of the diversity (D_H) genes, and one of the joining (J_H) genes together, in this example, V_{H2}, D_{H3}, J_{H1}. This unit and the remaining J genes are separated from the constant (C) region by an intervening sequence of DNA. The plasma cell DNA is transcribed into nuclear RNA, which is spiced to form messenger RNA (mRNA). In this process, the C_μ gene is selected and joined to the $V_{H2}/D_{H3}/J_{H1}$ complex, and the entire unit is ready for translation into a μ chain

F. Allelic exclusion and clonal restriction. These phenomena are peculiar to antibody-producing cells.

1. Allelic exclusion is the expression, in a single cell, of **only one allele** at a particular locus.

a. Two or more alleles frequently exist at the genetic loci for the various components of H and L chains.

b. Because of allelic exclusion, only given B lymphocyte.

(1) In individuals who are heterozygous for allotypic forms of H and L chain, individual B cells will express one or the other allele, but not both.

(2) When a single H or L chain gene is successfully assembled and expressed, this prevents all other genes of that type from undergoing rearrangement in the same cell.

2. Clonal restriction

a. 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.

b. This is why all the immunoglobulin molecules produced by a given clone (a B lymphocyte and its progeny) are identical in epitope specificity and in κ or λ L chain isotype.

G. Development of malignancy. Errors in immunoglobulin gene rearrangement are thought to contribute to the genesis of several B cell malignancies.

1. Follicular lymphoma

a. In many patients with follicular lymphoma, the most common B cell cancer, a putative proto-oncogene (called *bcl-2*) on chromosome 18 is translocated into the H chain gene region on chromosome 14.

b. It is thought that the close proximity of *bcl-2* to the active H chain gene region enhances the expression of the proto-oncogene and thus contributes to malignant transformation.

2. Burkitt's lymphoma

a. Similarly, in the malignant cells of this B cell cancer, a portion of chromosome 8 containing the cellular proto-oncogene c-myc is translocated into chromosome 14 so that C-myc lies right next to the H chain gene region.

b. The c-myc proto-oncogene is thought to be activated as a result of this proximity to the genetically active H gene region.