Outline

- Antibody
  - Structure
  - Antigen recognition
  - Primary Ab repertoire
  - Antigen-mediated Ig Diversification

Plasma Cell Secreted Ab Specificity is Derived from Its B Cell Precursor Surface Ab

Surface coated antibody is the B cell receptor

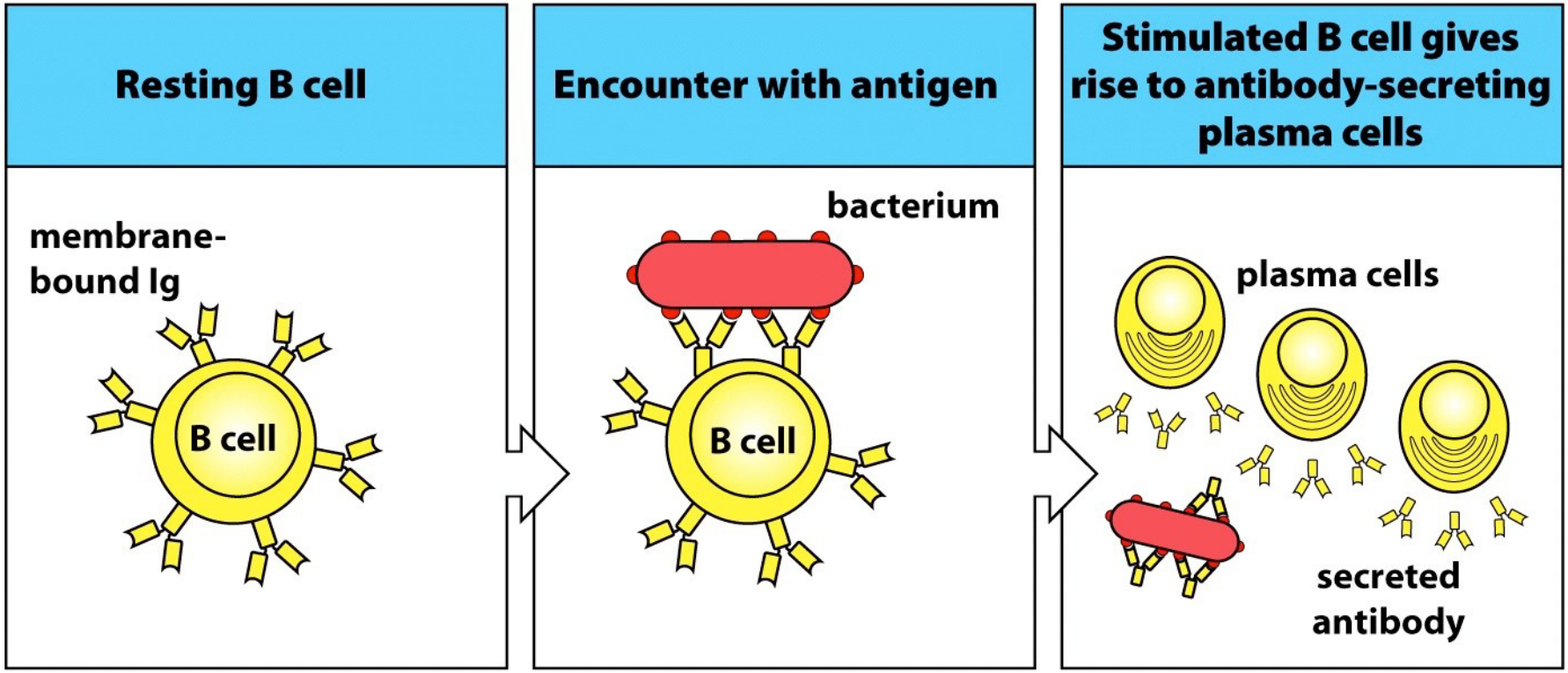
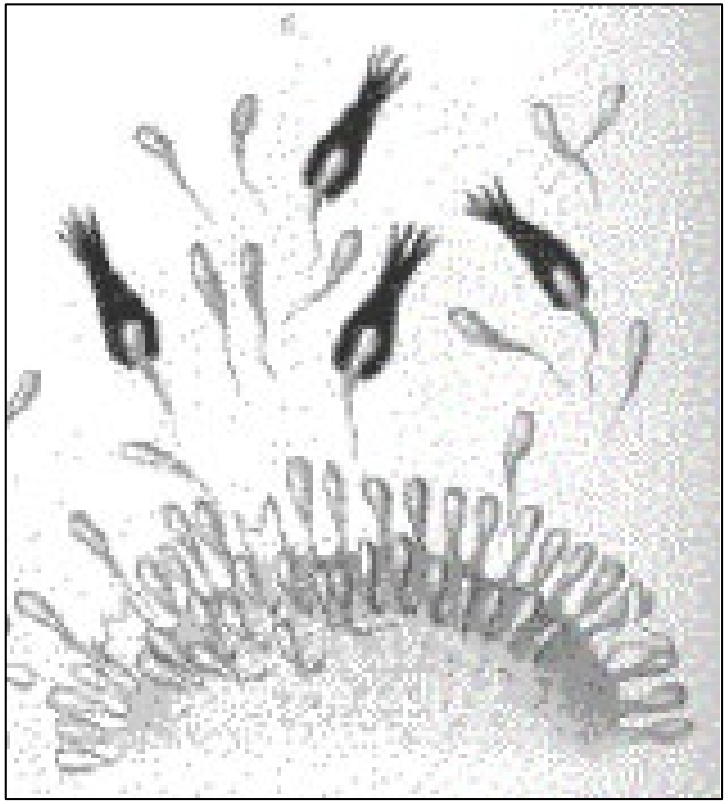


Figure 4.1 The Immune System, 3ed. (© Garland Science 2009)

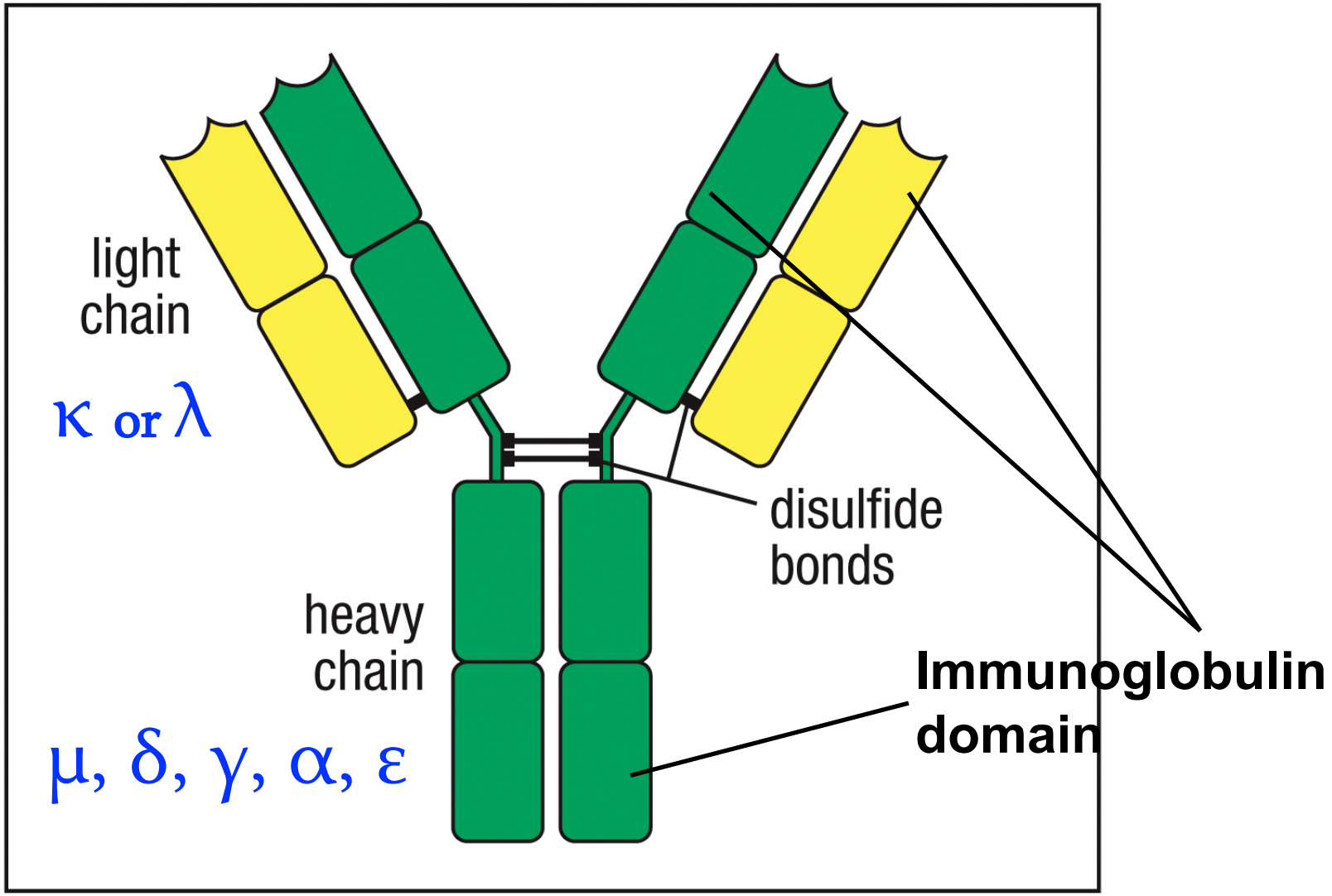
Antibodies Are Responsible for Immunity

In **1891**, Paul Ehrlich proposed that antibodies are involved in an immune response.

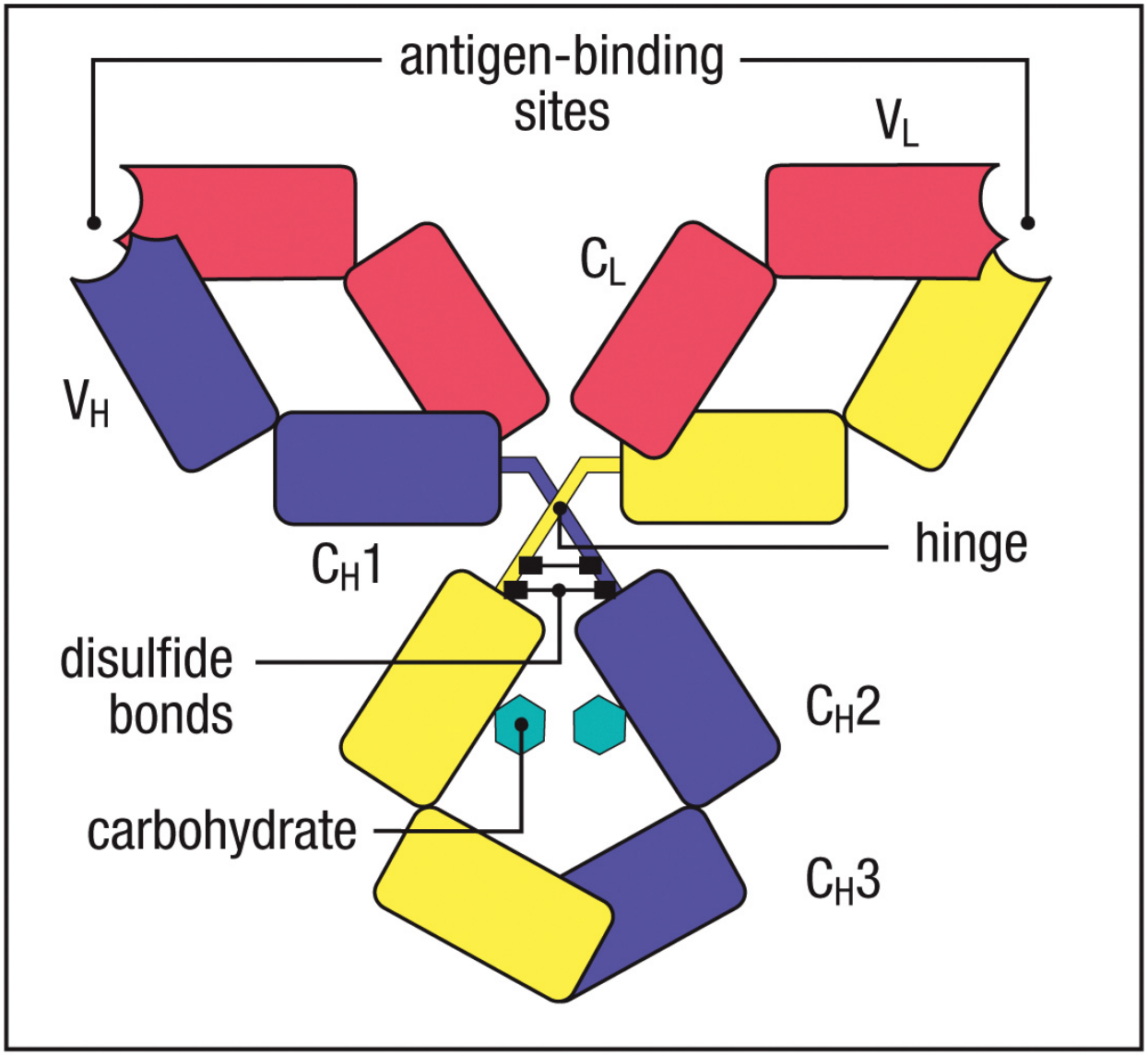


Ehrlich's drawing of a "haemotopoietic" cell bearing "side chains" and releasing "immune bodies".

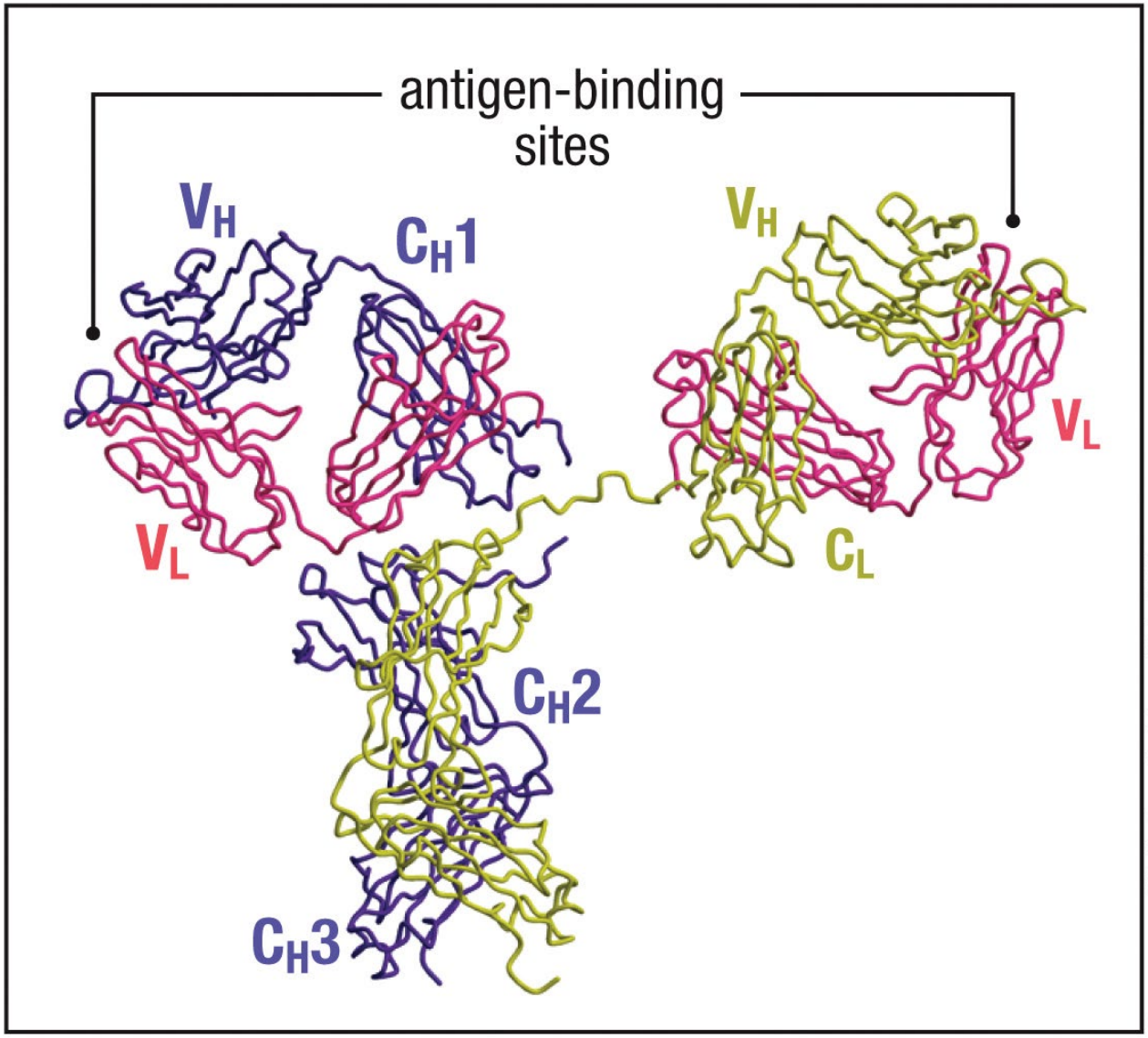
Antibody Structure



Antibody Structure

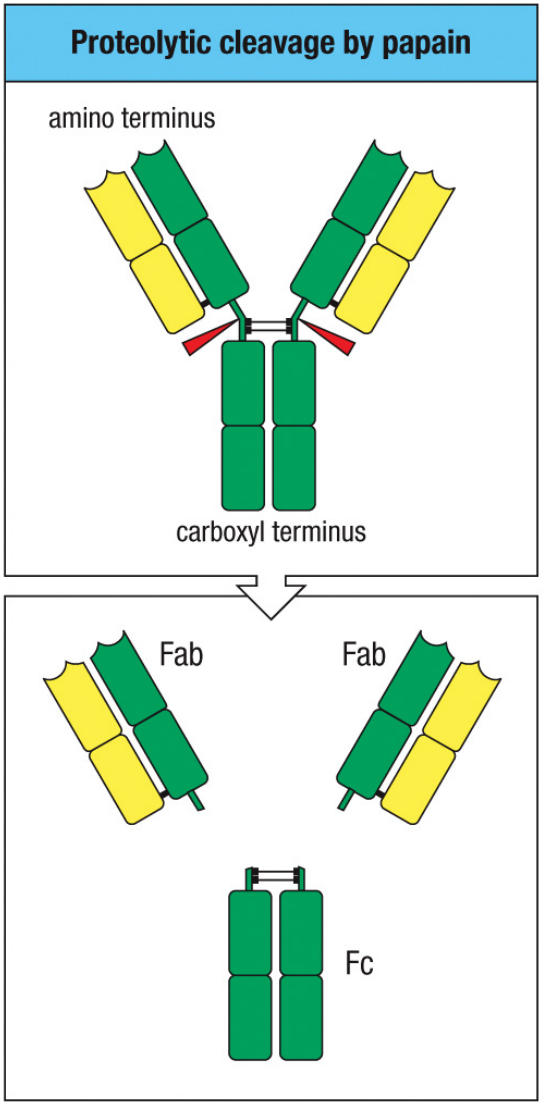


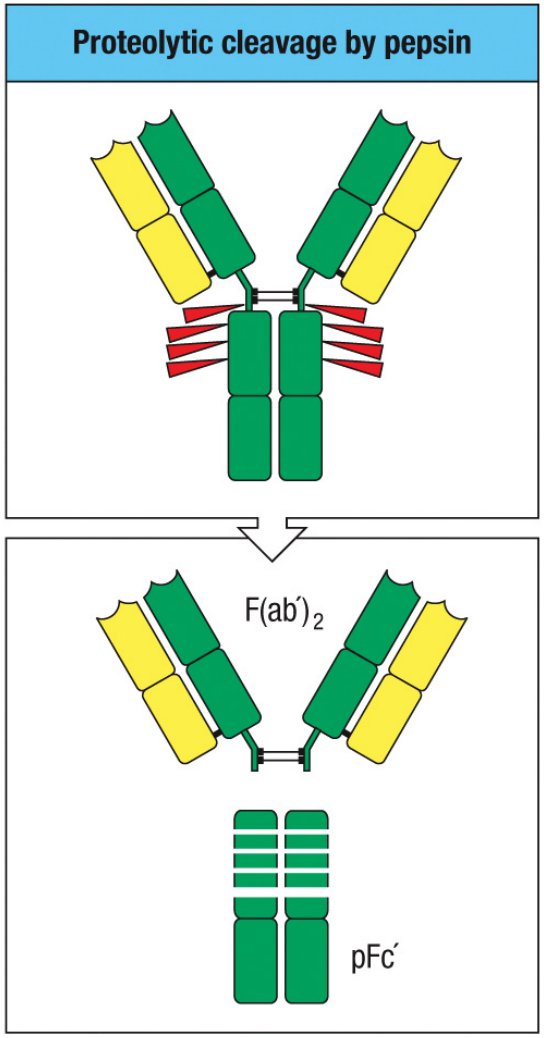
Globular Regions of Antibodies

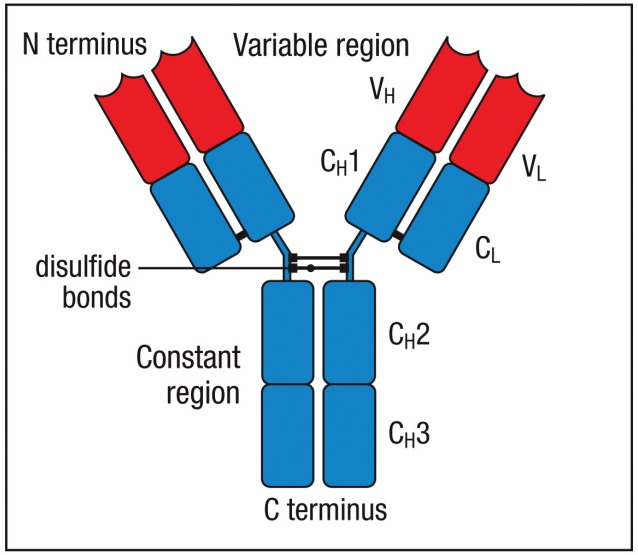


Courtesy of R.L. Stanfield and I.A. Wilson

Antibody Fragments Have Distinct Functions







Antigen binding fragments

Crystalizable fragment

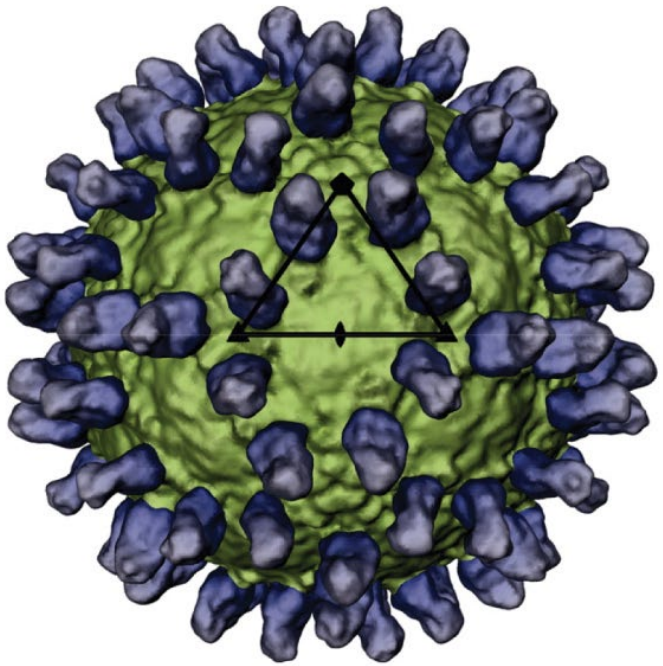
Outline

- Antibody
  - Structure
  - Antigen recognition
  - Primary Ab repertoire
  - Antigen-mediated Ig Diversification

Definition

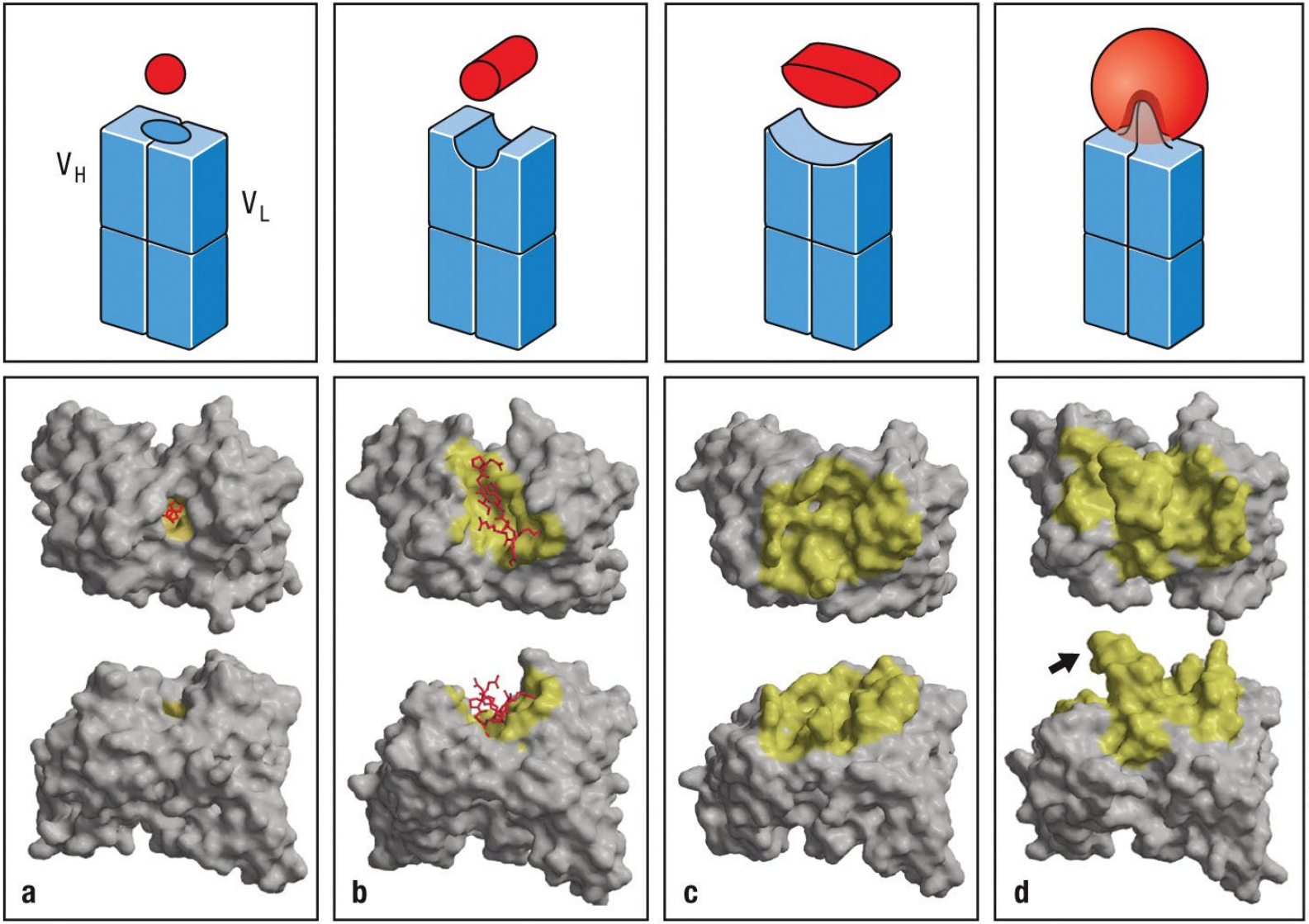
- Antigen:
  - any substance that causes an immune system to produce antibodies against it
- Epitope:
  - also known as **antigenic determinant**, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells.

Cryo-EM reconstruction of E16 Fab bound to mature WNV particle



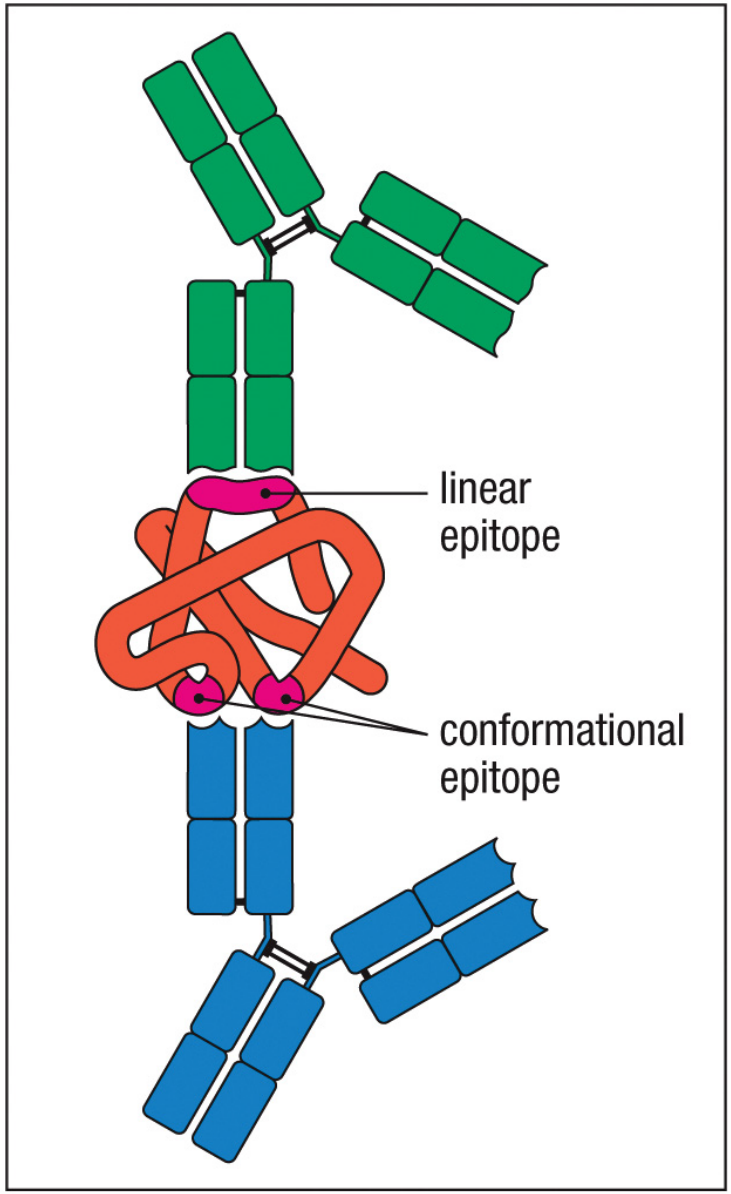
Kaufman et al. (2006). West Nile virus in complex with the Fab fragment of a neutralizing monoclonal antibody. *PNAS* August 15, 2006 103 (33) 12400–12404. Copyright (2006) National Academy of Sciences, USA.

Spatial Ab:Antigen Complementarity

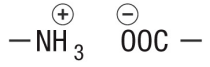
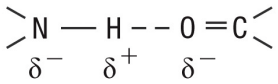
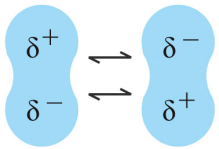
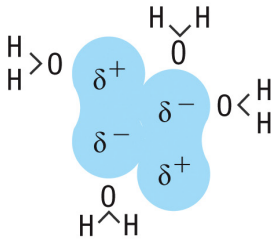
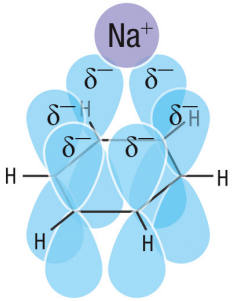


Courtesy of R.L. Stanfield and I.A. Wilson

Linear and Discontinuous Epitopes



Antibody-Antigen Interactions Disrupted by High Salt or Detergent

Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	
Hydrogen bonds	Hydrogen shared between electronegative atoms (N, O)	
Van der Waals forces	Fluctuations in electron clouds around molecules polarize neighboring atoms oppositely	
Hydrophobic forces	Hydrophobic groups interact unfavorably with water and tend to pack together to exclude water molecules. The attraction also involves van der Waals forces	
Cation-pi interaction	Noncovalent interaction between a cation and an electron cloud of a nearby aromatic group	

Antibodies Recognize Different Antigens on the Same Pathogen

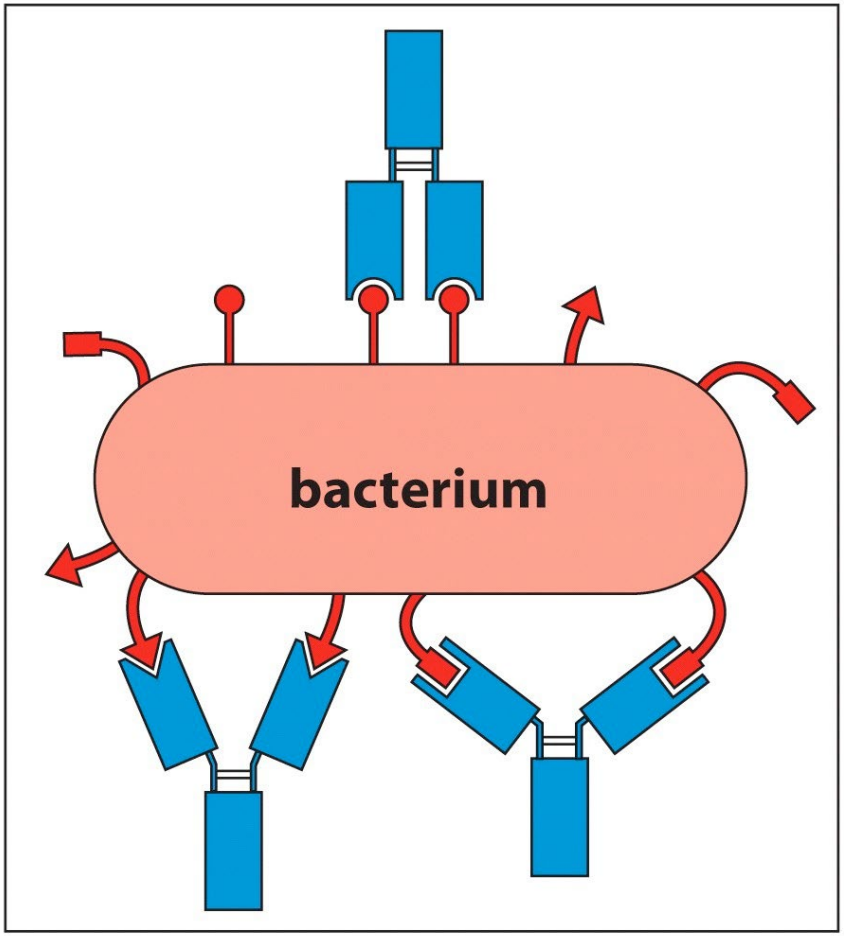
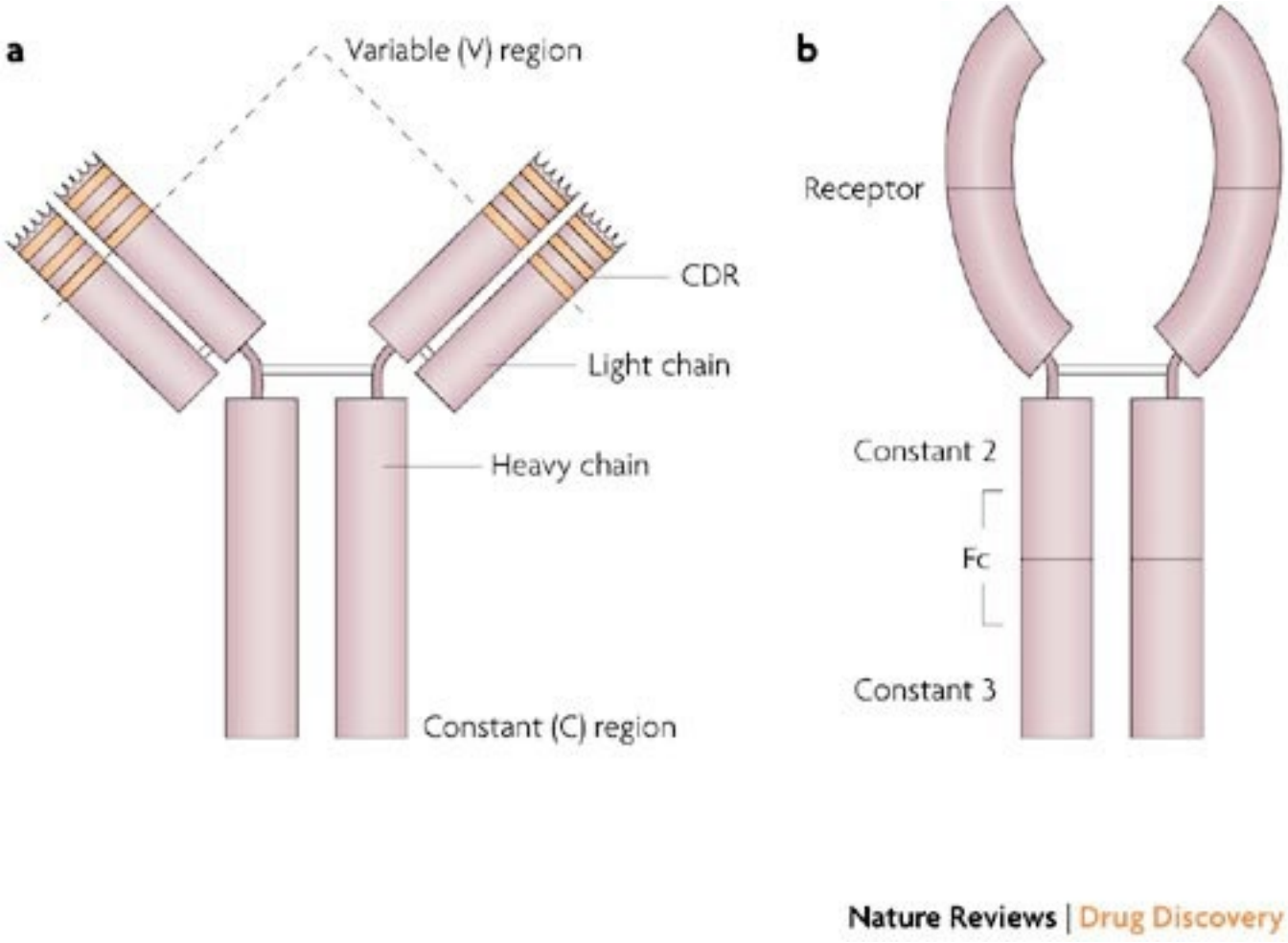


Figure 4.4 The Immune System, 3ed. (© Garland Science 2009)

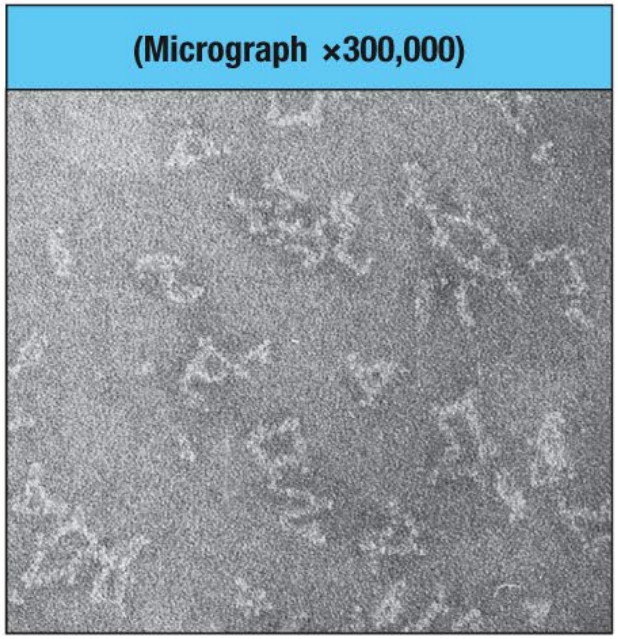
A pathogen needs to heavily coated with antibody

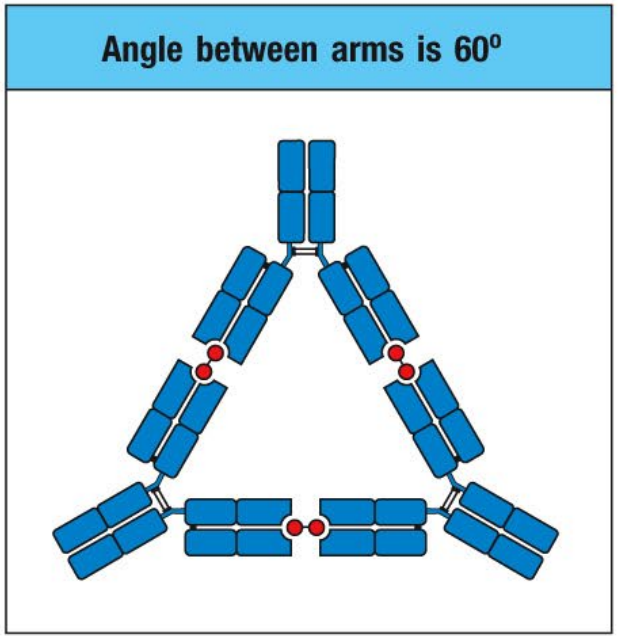
Hinge Adds Flexibility to Ab Molecule



Nature Reviews Drug Discovery 6, 75-92 (January 2007)

Ab Hinge Region Allows Formation of Antigen:Ab Complexes





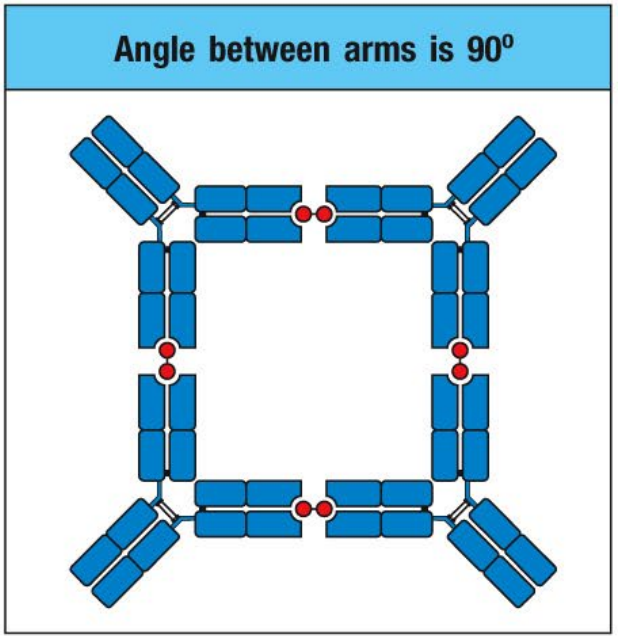
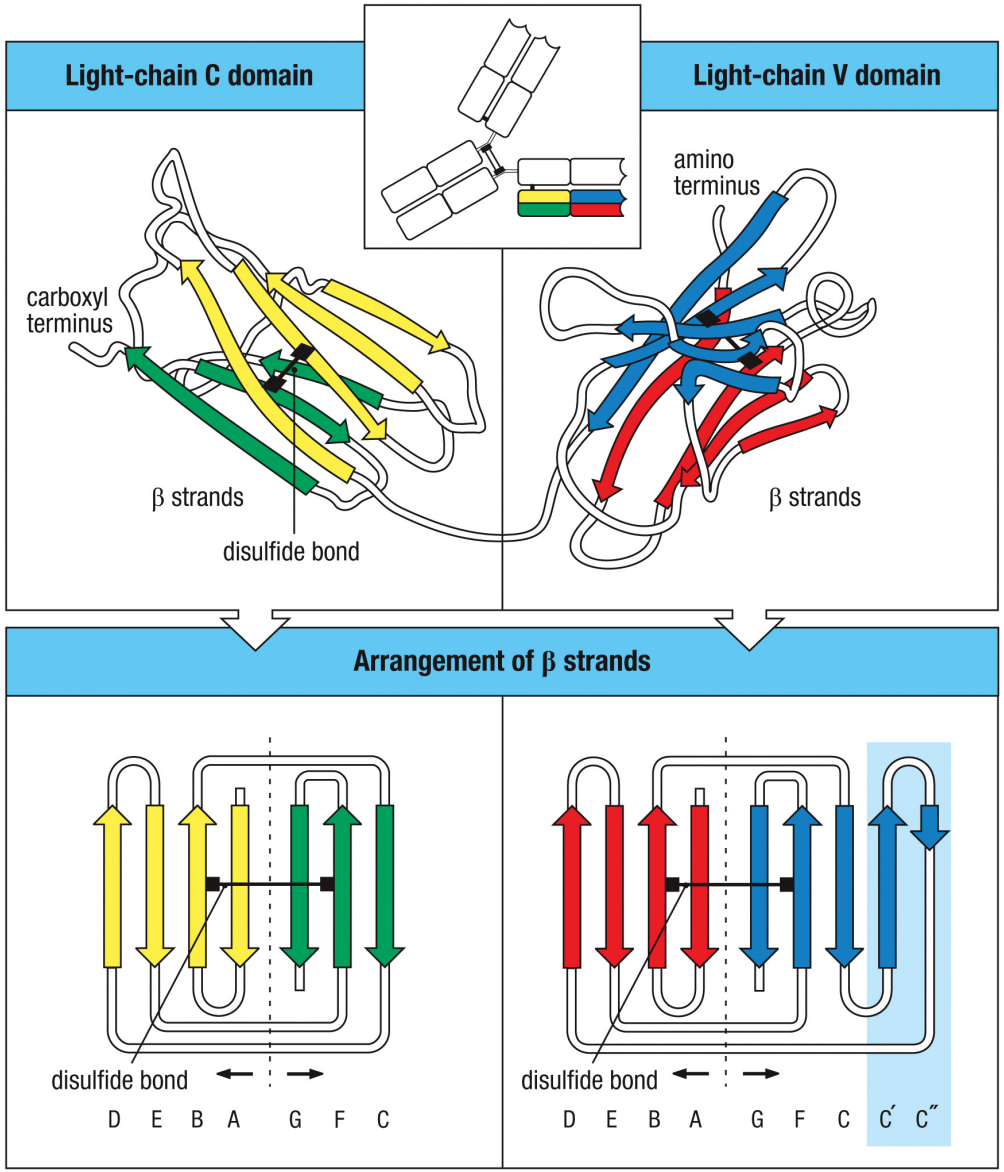


Figure 4.5 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Structure of Ig Constant and Variable Domains

immunoglobulin fold (β barrel)

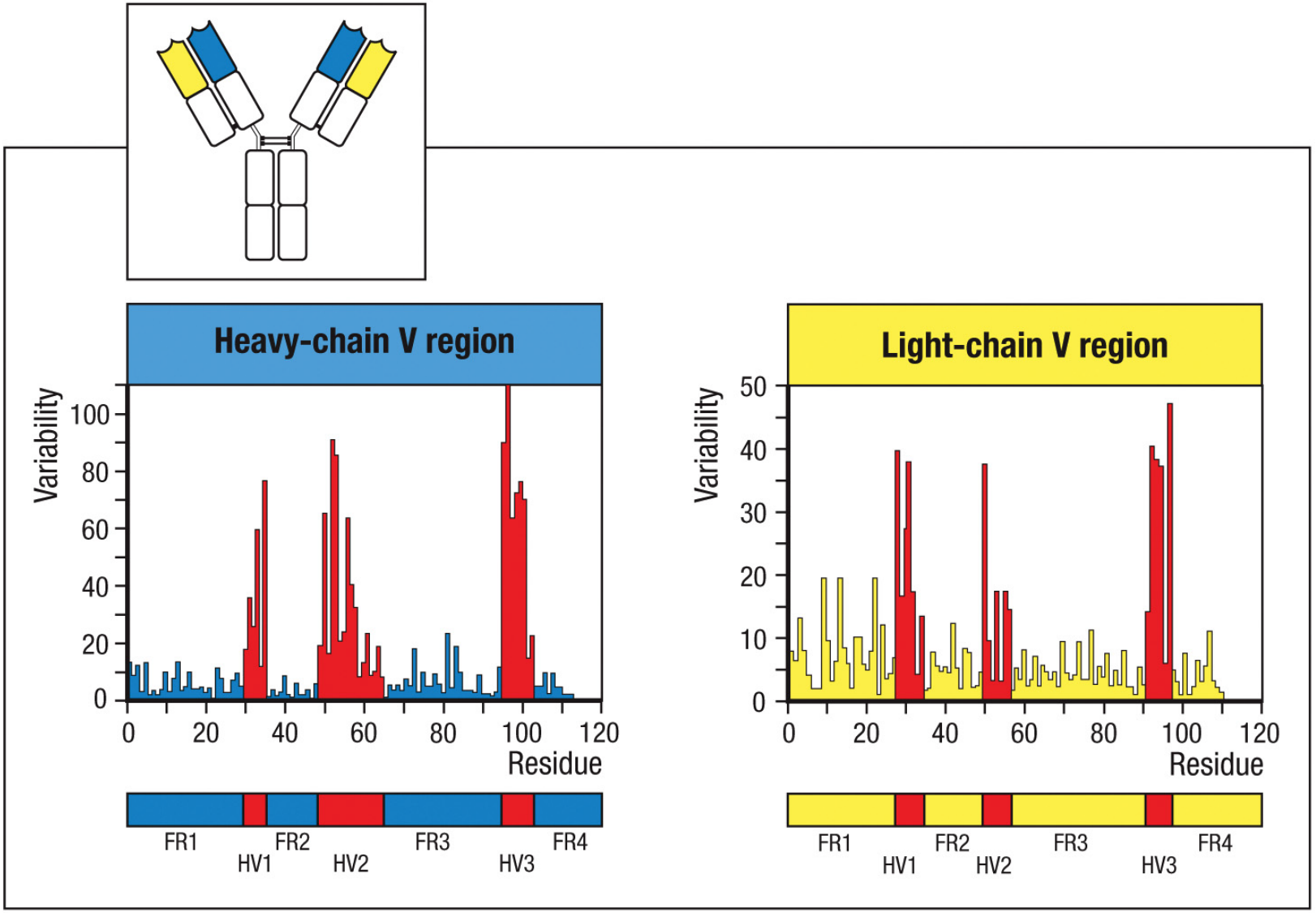


β-sheets w/ anti-parallel β-strands

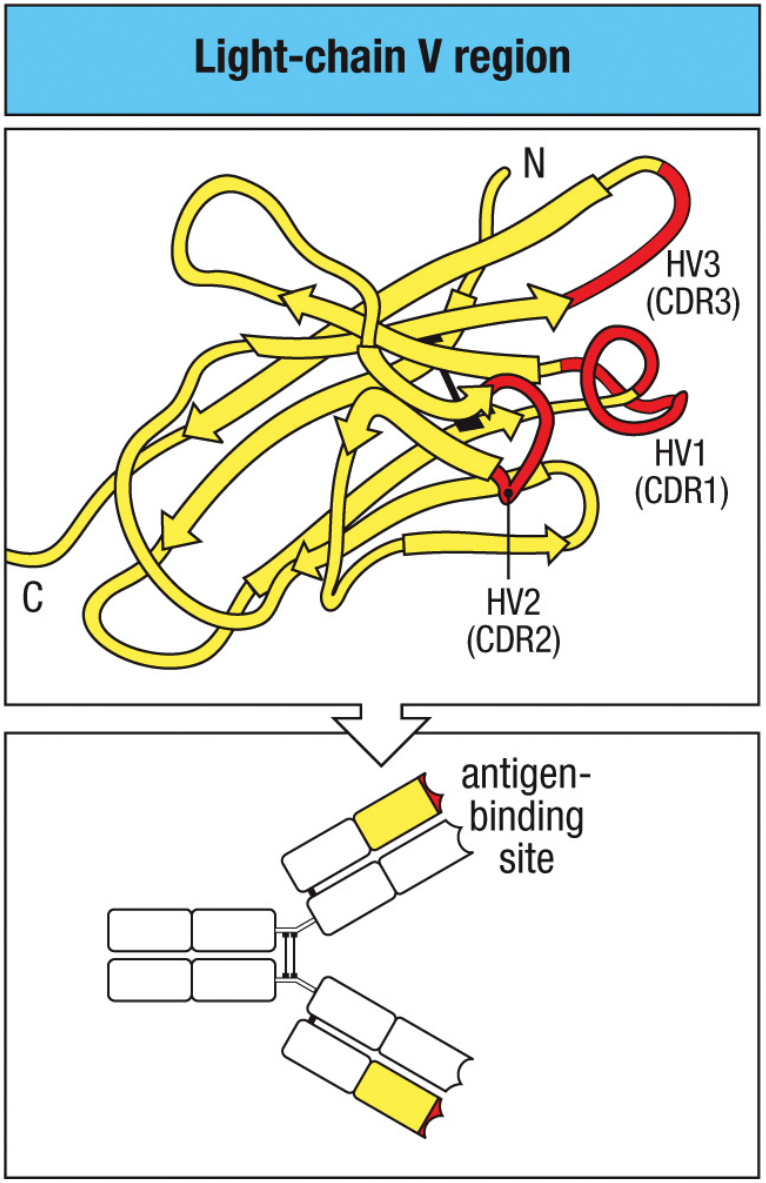
Sheets: structural

Loops: recognition

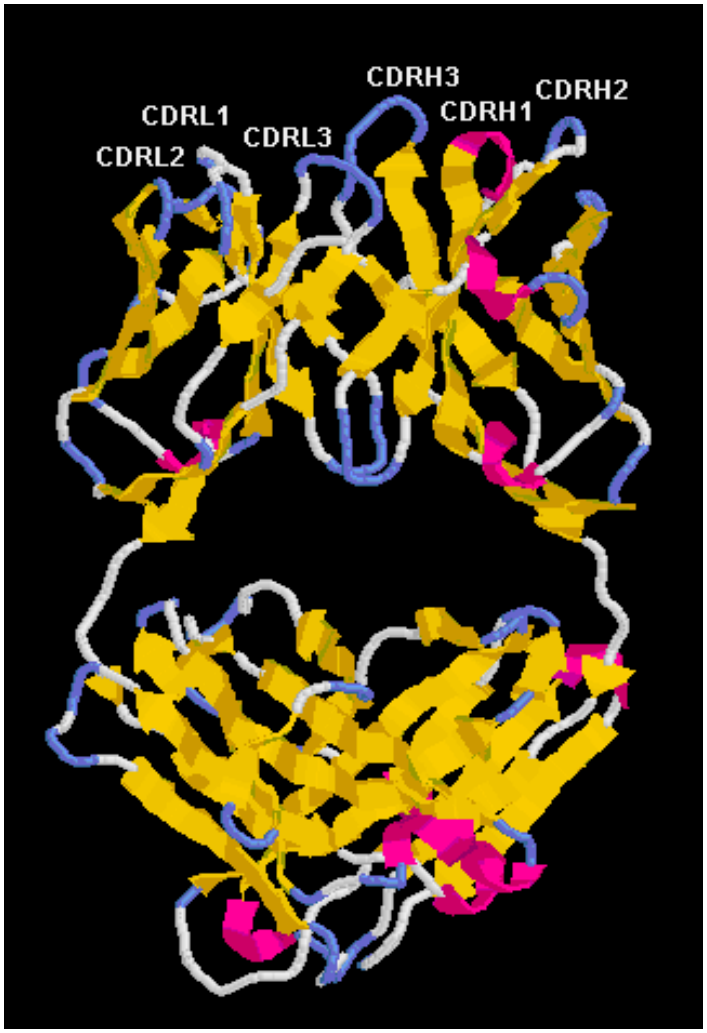
V Regions of Ab Molecules Are Unique



Hypervariable Loops of V_L and V_H Regions Fold to Form Antigen Binding Site



Complementarity Determining Regions Form Antigen Binding Site



Question

- What does an antibody look like?
- Which part determines antigen specificity?

Outline

- Antibody
  - Structure
  - Antigen recognition
  - Primary Ab repertoire
  - Antigen-mediated Ig Diversification

Immunoglobulin Repertoire

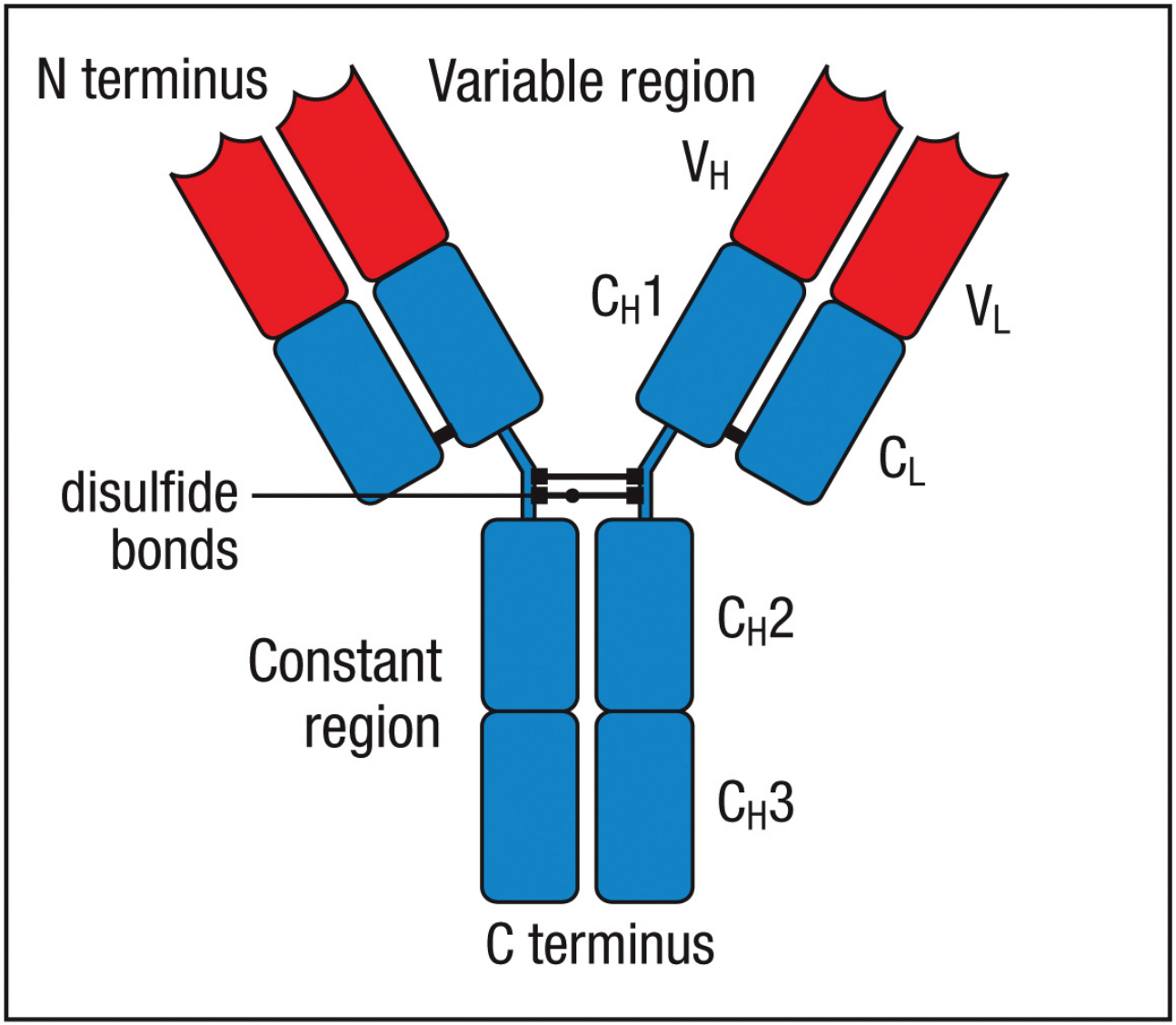
- Total human antibody repertoire is about **10^{11}** specific molecules.

- Antibody repertoire at any give time $t_{(x)}$ is based on:
  - total number of B cells
  - previous encounters with antigen

Sources of Antibody Diversity

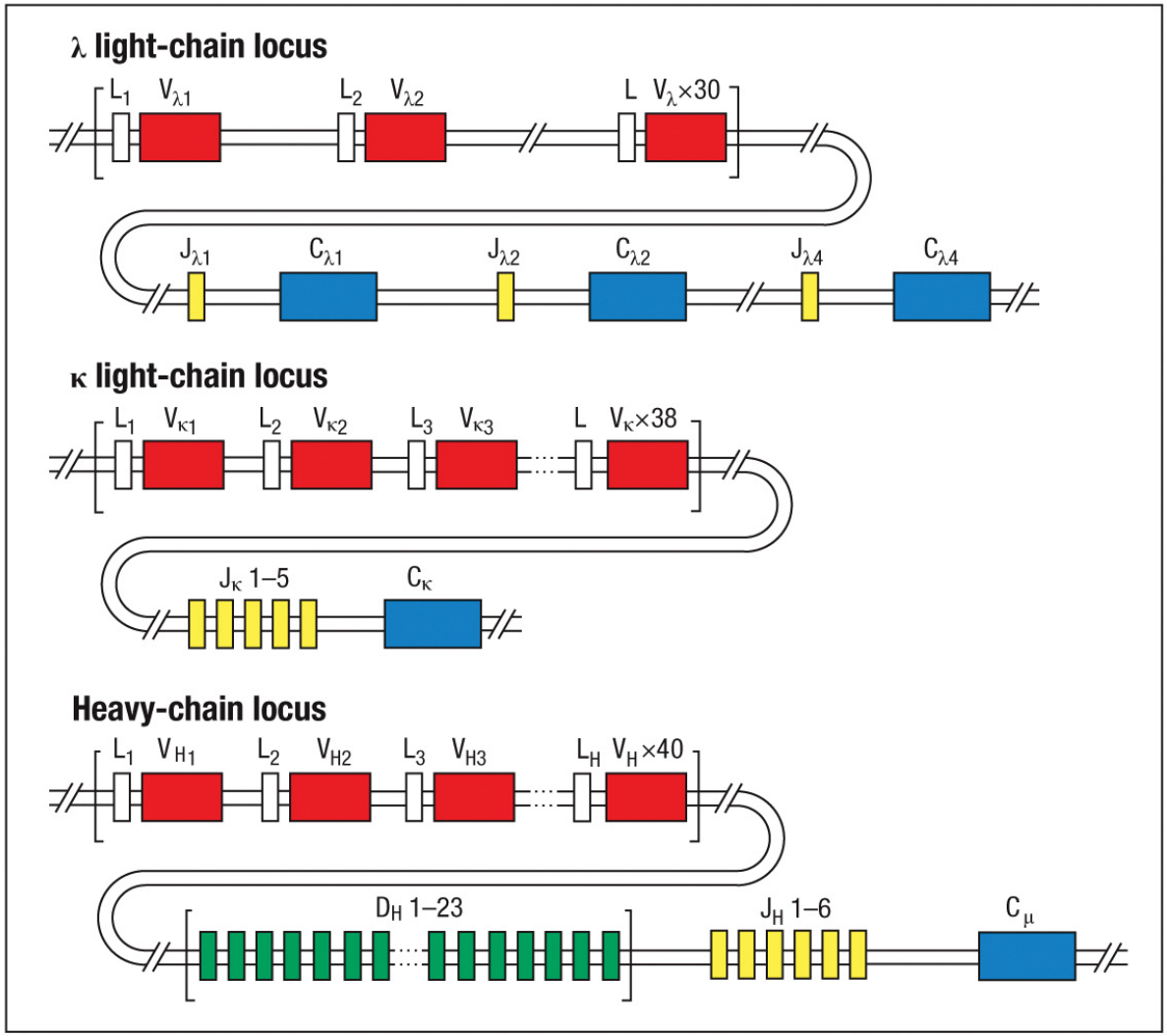
- Combinatorial diversity
  - -- Multiple segments of the variable region
    - Allelic exclusion (Once recombined, no other VDJ combination can be expressed by the same B cell)
  - – Combination of multiple heavy and light chains
- Junctional diversity
  - – VDJ recombination
- Somatic hypermutation
  - – Affinity maturation
    - Generation of antibodies with increased binding affinities for the specific antigen
- Class switch (isotype switch)

Antibody Structure

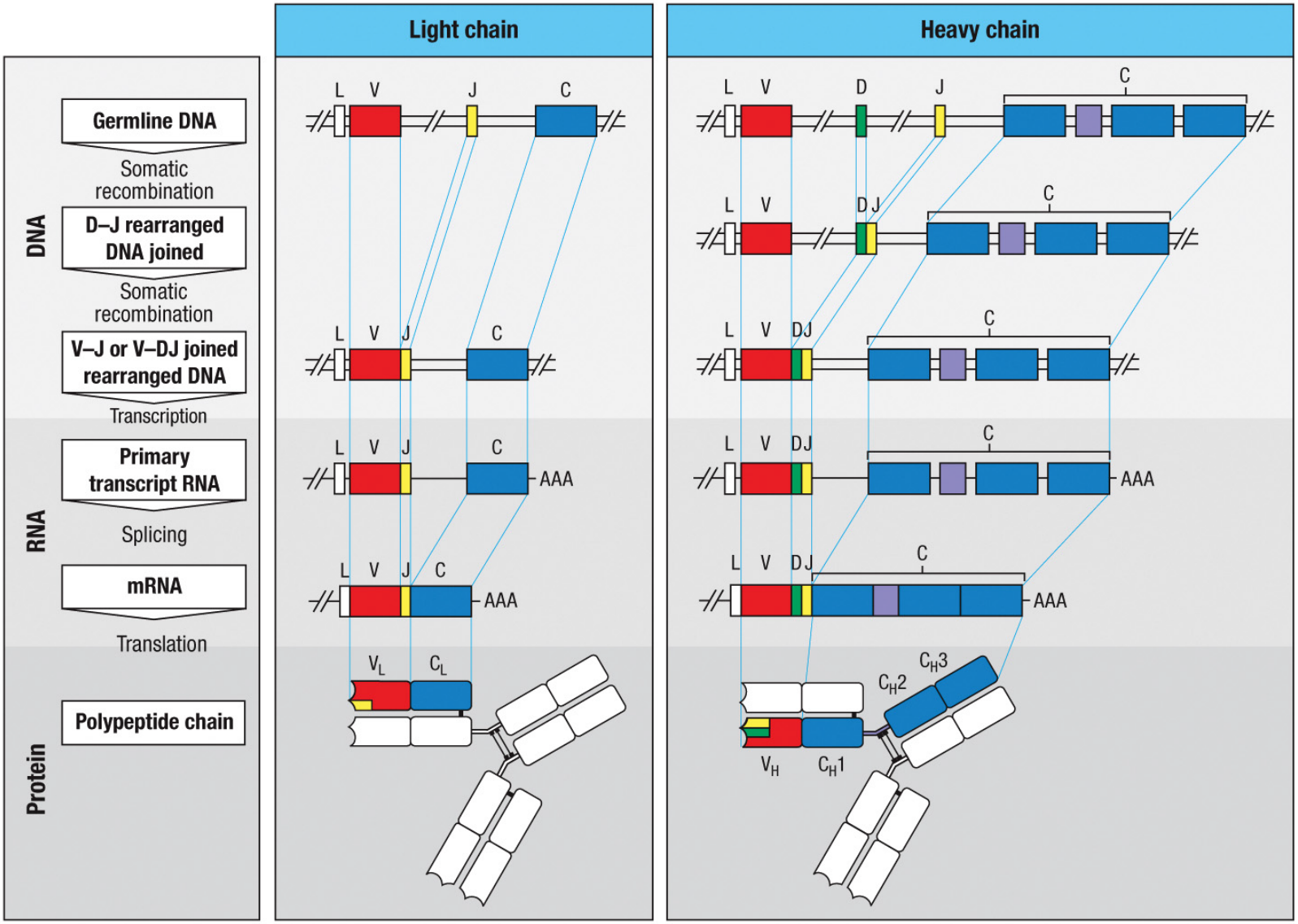


Germline Organization of Ig Loci

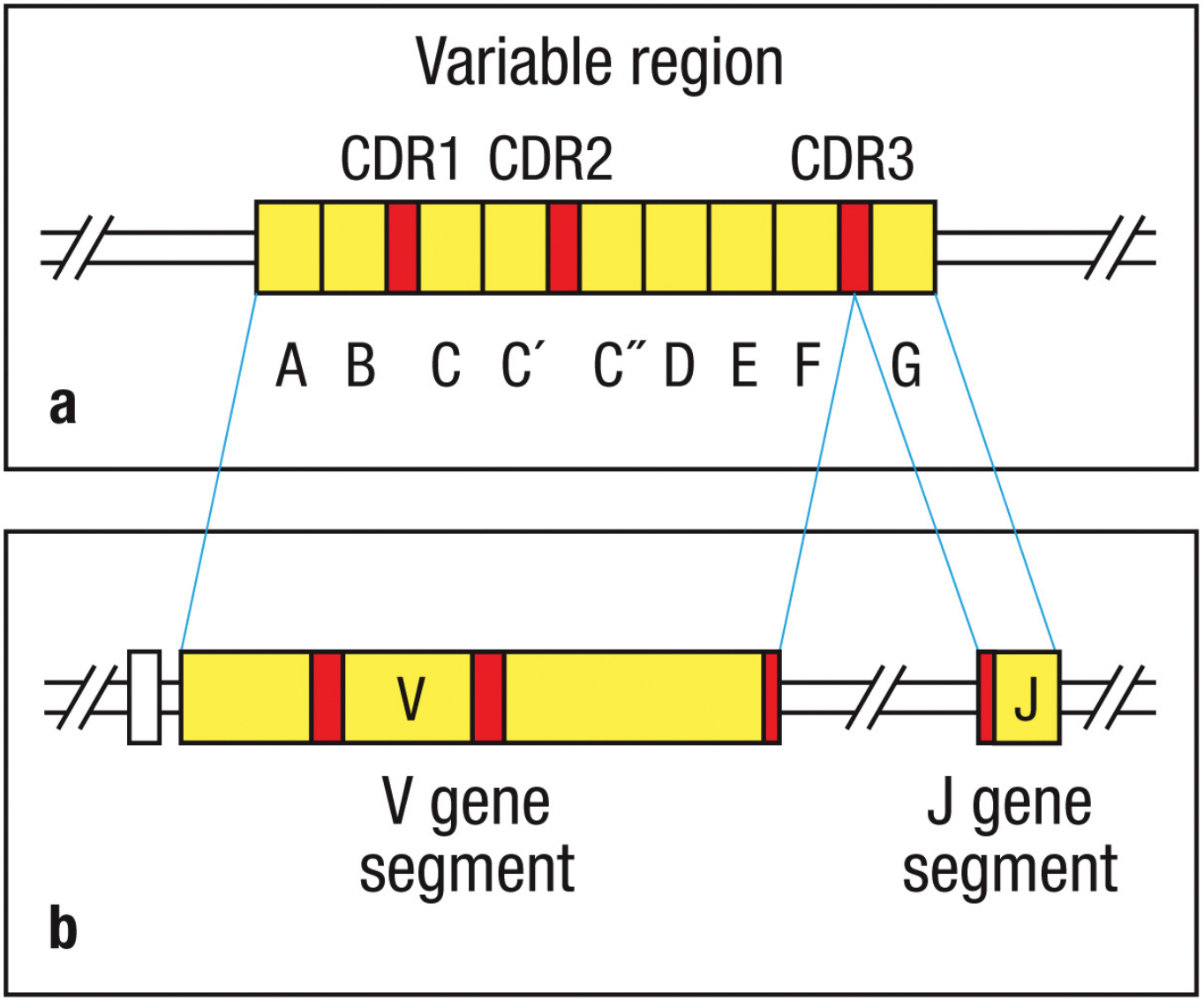
Two light chain loci in human
Human κ:λ ratio is 2:1



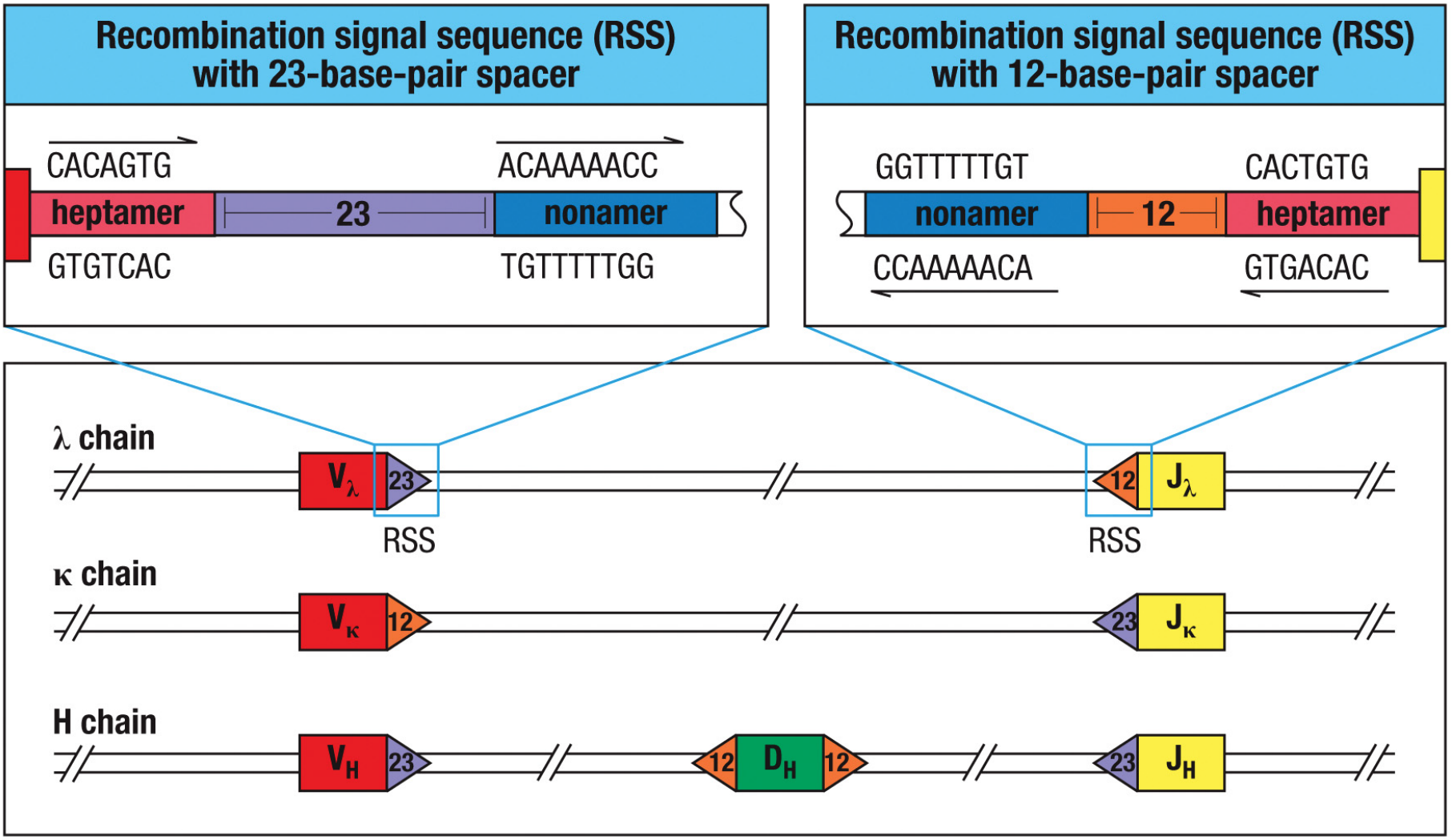
Construction of the Variable Region



VDJ Recombination



12/23 Rule


Recombination signal sequence (RSS) with 23-base-pair spacer
CACAGTG
ACAAAAACC
heptamer
23
nonamer
GTGTCAC
TGTTTTTGG
Recombination signal sequence (RSS) with 12-base-pair spacer
GGTTTTTGT
CACTGTG
nonamer
12
heptamer
CCAAAAACA
GTGACAC
λ chain
Vλ
23
RSS
12
Jλ
RSS
κ chain
Vκ
12
23
Jκ
H chain
VH
23
12
DH
12
23
JH

Number of Functional Ig Gene Segments

Number of functional gene segments in human immunoglobulin loci			
Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	31–35	29–32	38–46
Diversity (D)	0	0	23
Joining (J)	5	4–5	6
Constant (C)	1	4–5	9

Somatic Recombination Generated Diversity

Segment	κ	λ	*H*
Variable (V)	40	30	65
Diversity (D)	0	0	27
Joining (J)	5	4	6

Light chain diversity:

κ chain: 40 V_κ x 5 J_κ = 200 chains

λ chain: 30 V_λ x 4J_λ = 120 chains

Heavy chain diversity:

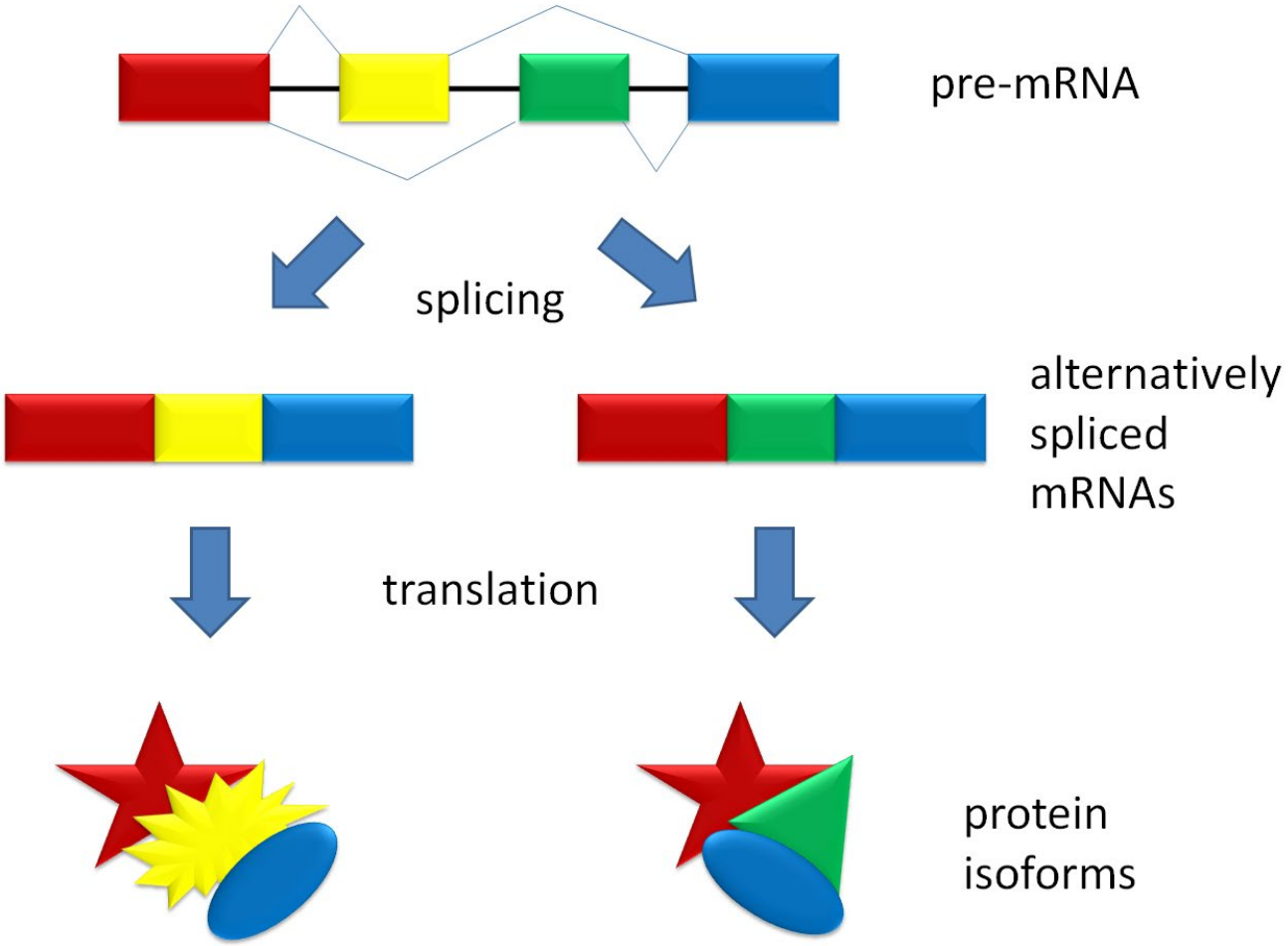
65 V_H x 27 D_H x 6J_H = 10,530 chains

Combinatorial diversity:

200 IgL_κ x 10,530 IgH = 2,106,000 IgM_κ

120 IgL_λ x 10,530 IgH = 1,263,600 IgM_λ

Alternative Splicing



Mature naïve B Cells Express Surface IgM and IgD

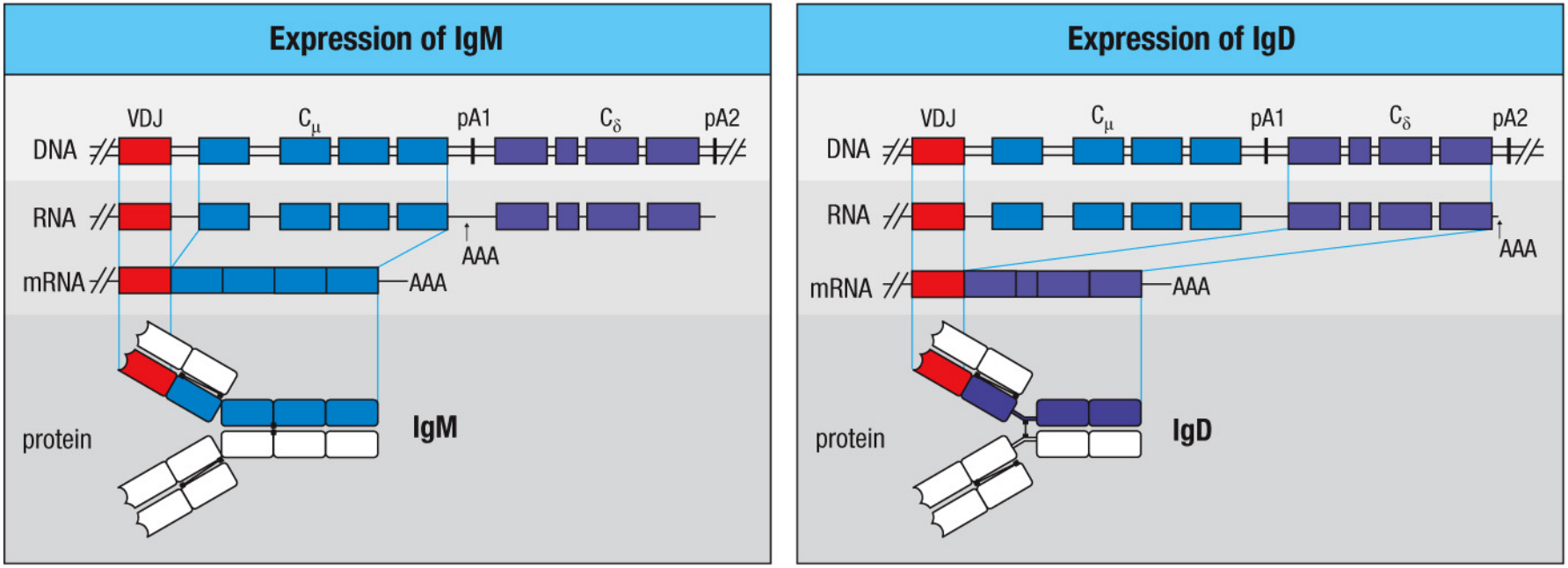
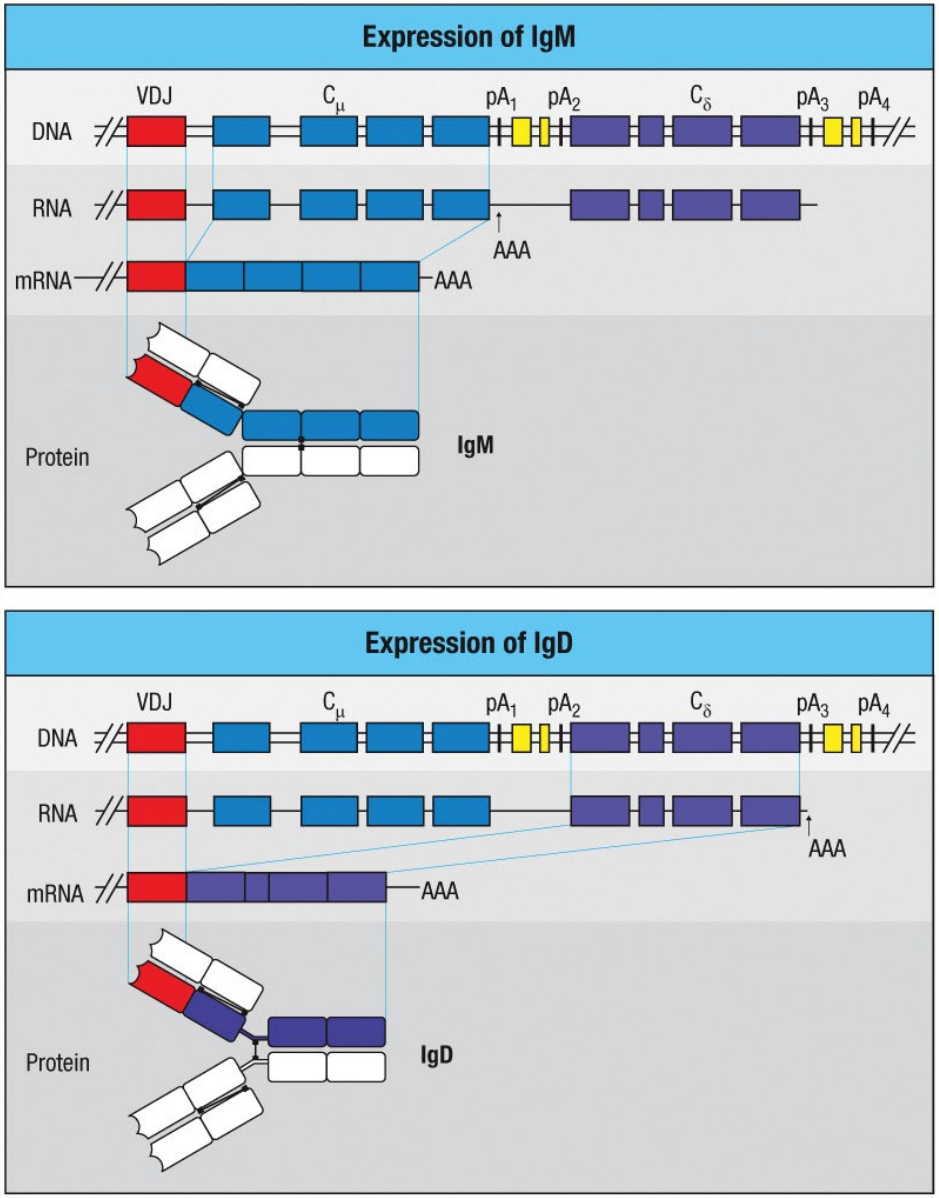


Figure 5.21 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Mature naïve B Cells Express Surface IgM and IgD



IgM and IgD C_H Are Created by Splicing

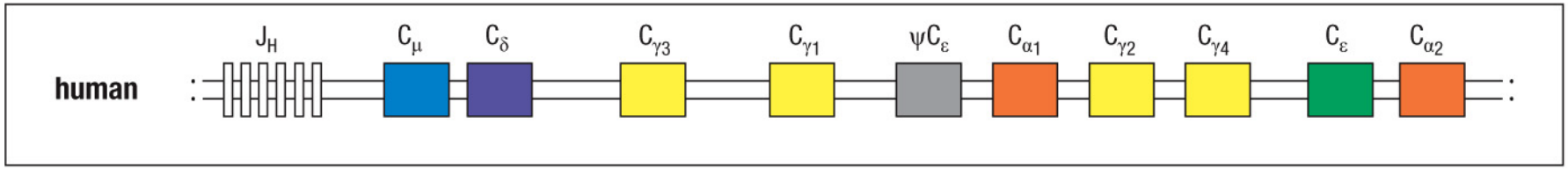
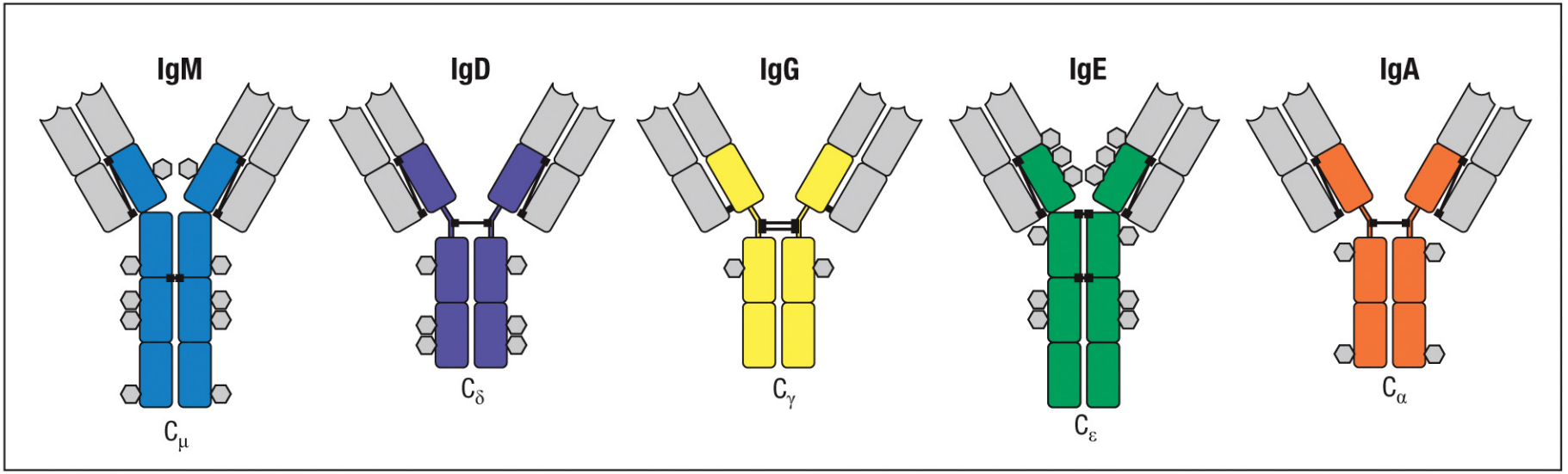
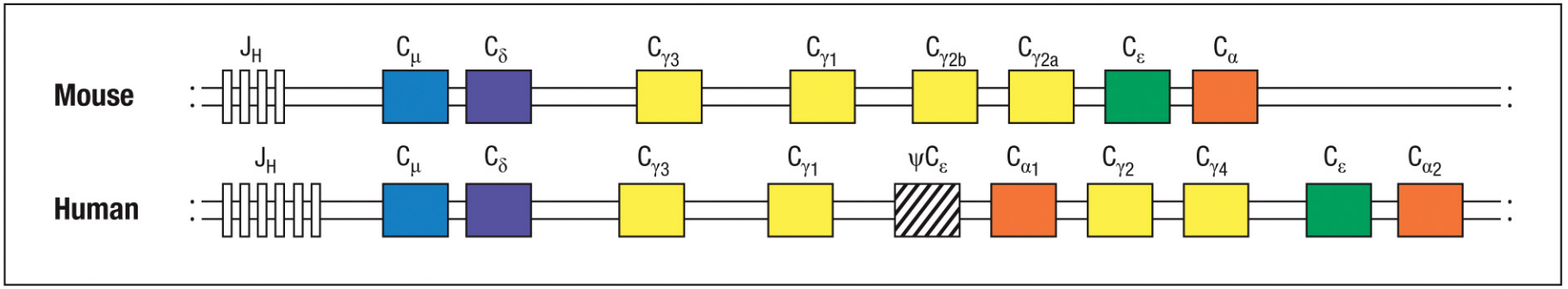


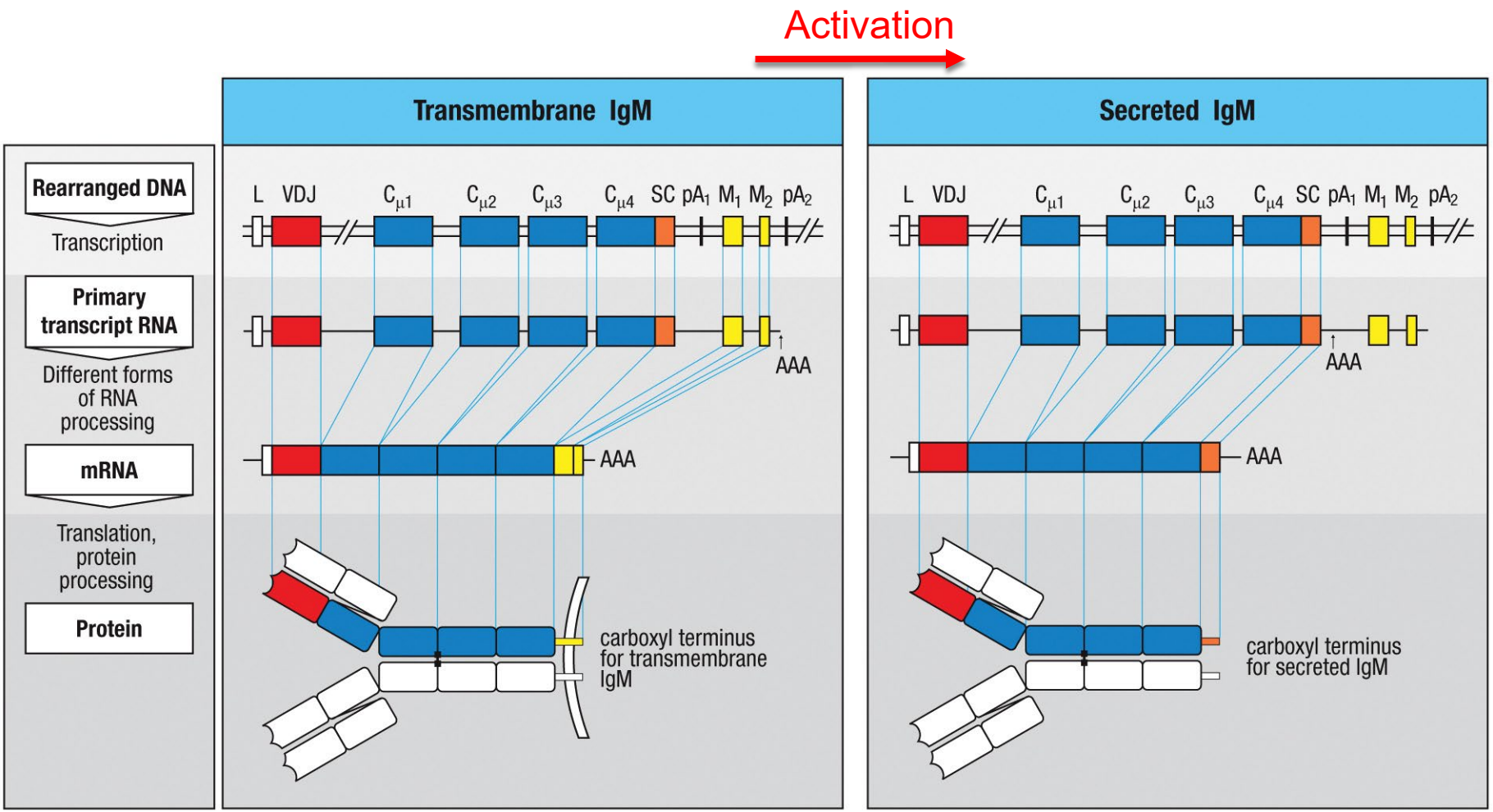
Figure 5.19 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

C-regions Are Encoded by Different Ig C_H genes





B-cells Initially Express Transmembrane IgM



Outline

- Antibody
  - Structure
  - Antigen recognition
  - Primary Ab repertoire
  - Antigen-mediated Ig Diversification

Acquisition of Ig Specificity

- Acquisition of primary Ab repertoire
  - V(D)J recombination (genomic)
  - C_H splicing
    - expression of surface IgM and IgD
    - expression of secreted pentameric IgM
- Antigen-mediated Ig Diversification
  - Somatic hypermutation (genomic)
  - Class switch (genomic)
    - expression of secreted IgG, IgA, IgE,

Question

- Which steps in antibody generation is controlled by alternative splicing?

- A) VDJ recombination
- B) Changing from membrane bound to secreted IgM
- C) Class switching
- D) Affinity maturation

Question

- What is the B cell receptor?