Generation of diversity

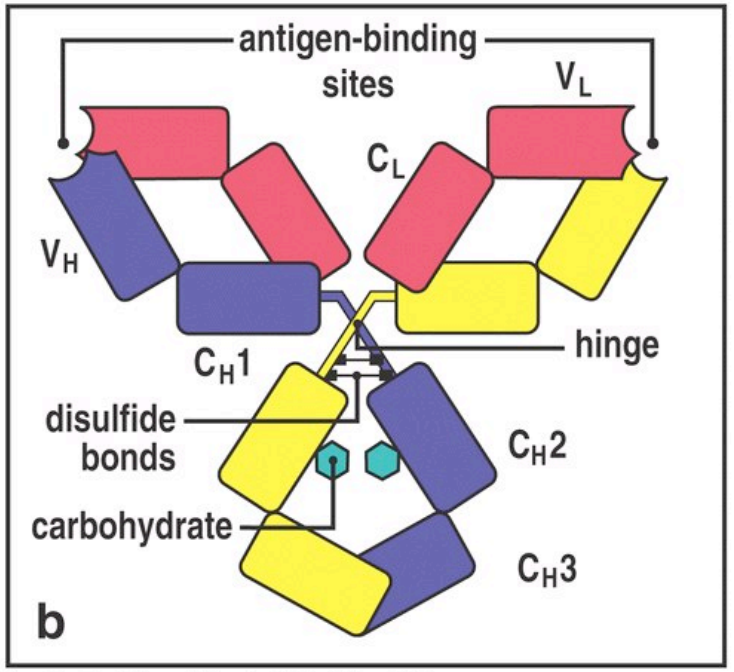

Figure 3-1 part 2 of 3 Immunobiology, 6/e. (© Garland Science 2005)

- Somatic recombination=
  - Several different V regions (V_H, V_L) that can be recombined with constant C regions
  - High number of combinations while not excessive genome costs
  - Occurs in somatic cells, not germline cells
- Occurs in B cells in bone marrow

V(D)J recombination

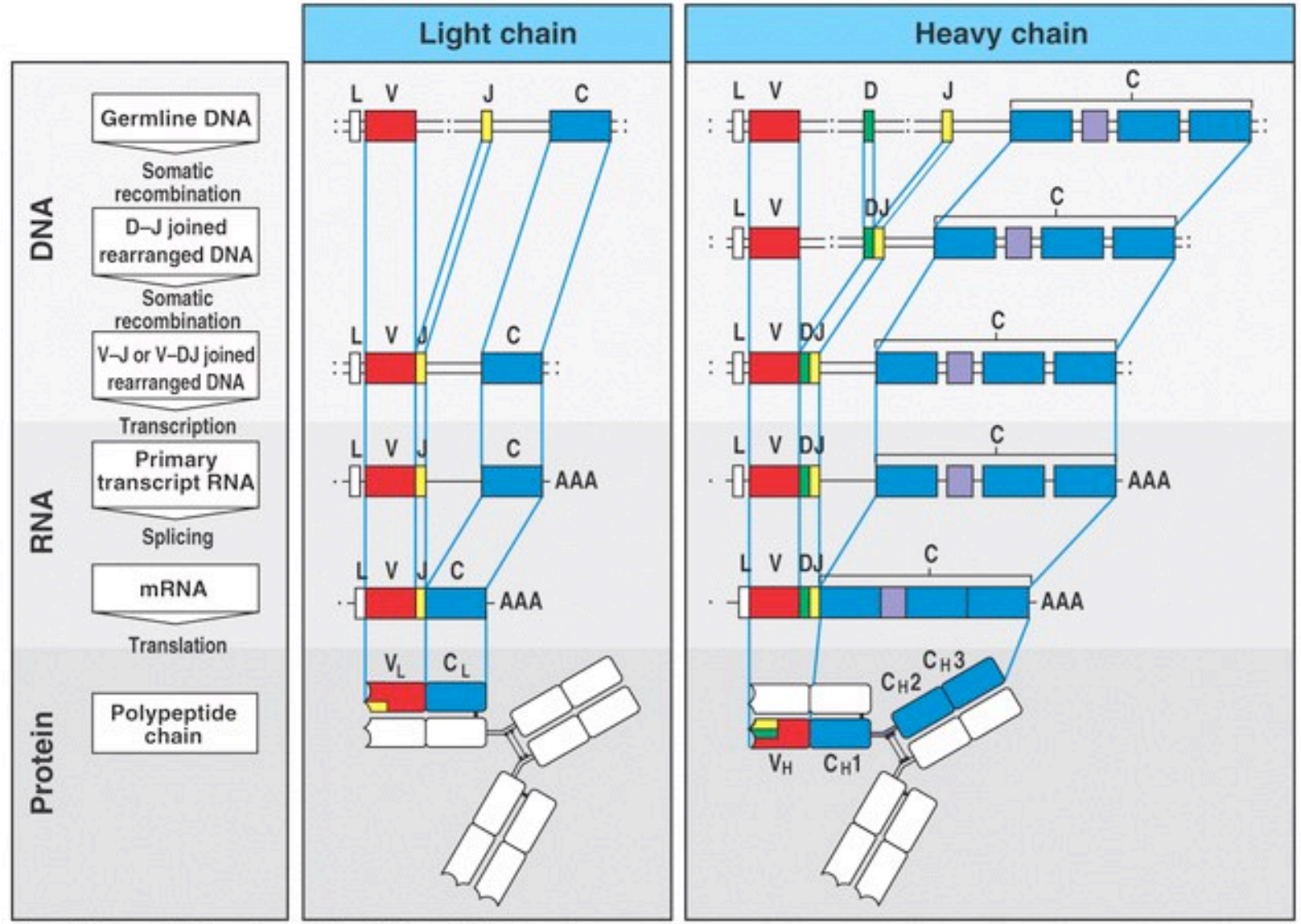

Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)

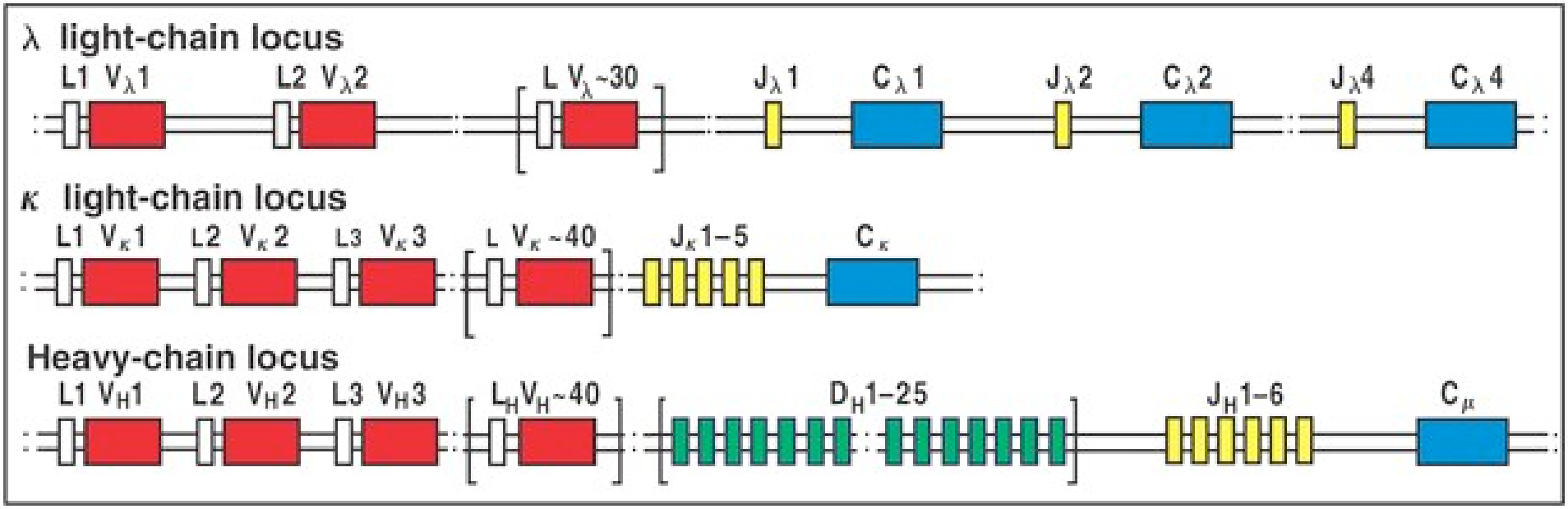

Figure 4-4 Immunobiology, 6/e. (© Garland Science 2005)

CDR1, CDR2 for both chains= contained entirely within V gene segment

Light chain CDR3= crosses V-J joining point

Heavy chain CDR3= crosses D-J joining point

Ig diversity

Number of functional gene segments in human immunoglobulin loci			
Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	40	30	40
Diversity (D)	0	0	25
Joining (J)	5	4	6

Figure 4-3 Immunobiology, 6/e. (© Garland Science 2005)

- VJ recombination for light chains
- VDJ recombination for heavy chains
- Pairing light chains and heavy chains

V(D)J recombination

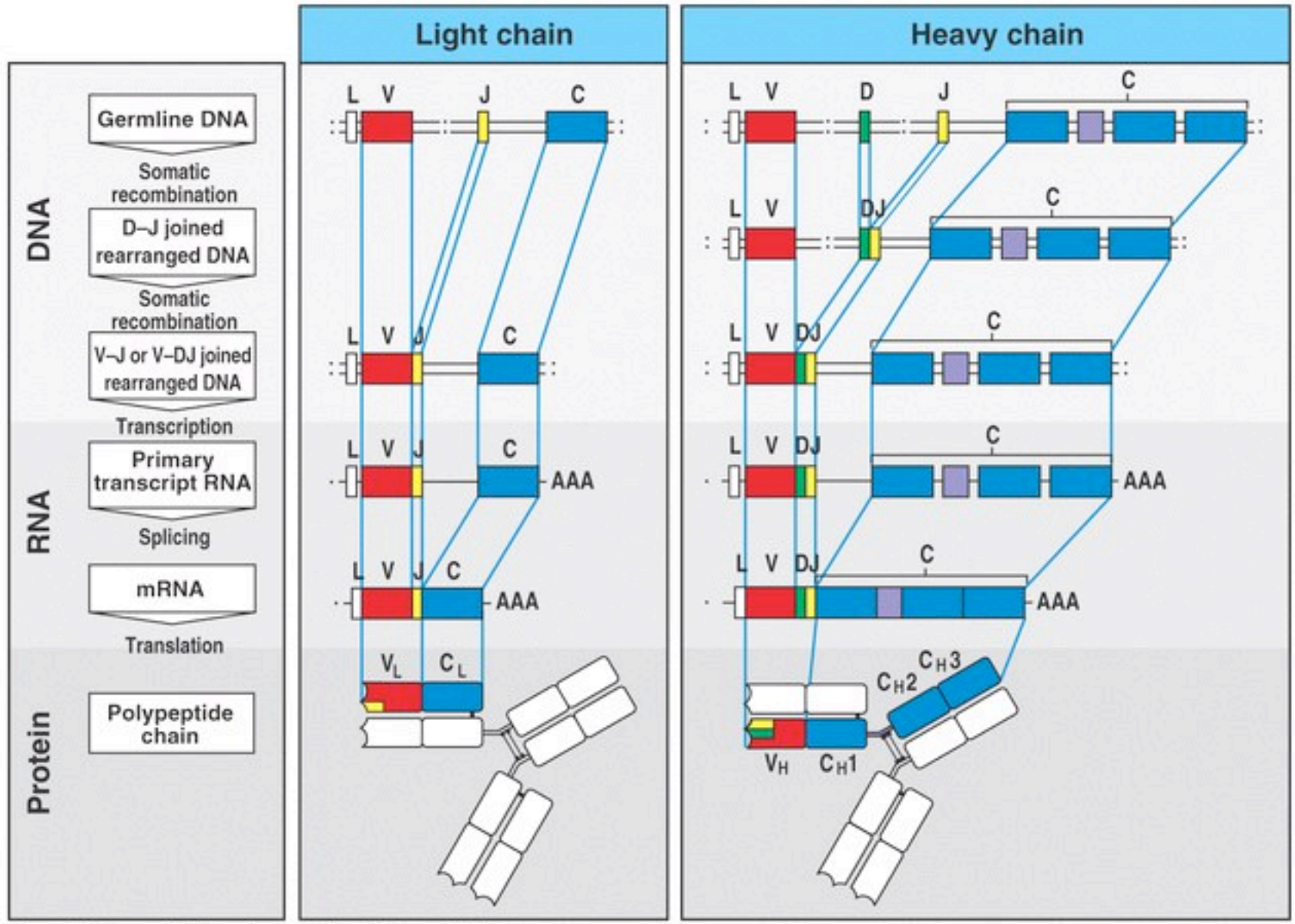

Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)

Recombination sequences

- Hepatamer-nonomer homologous sequences
- 12/23 spacer rule

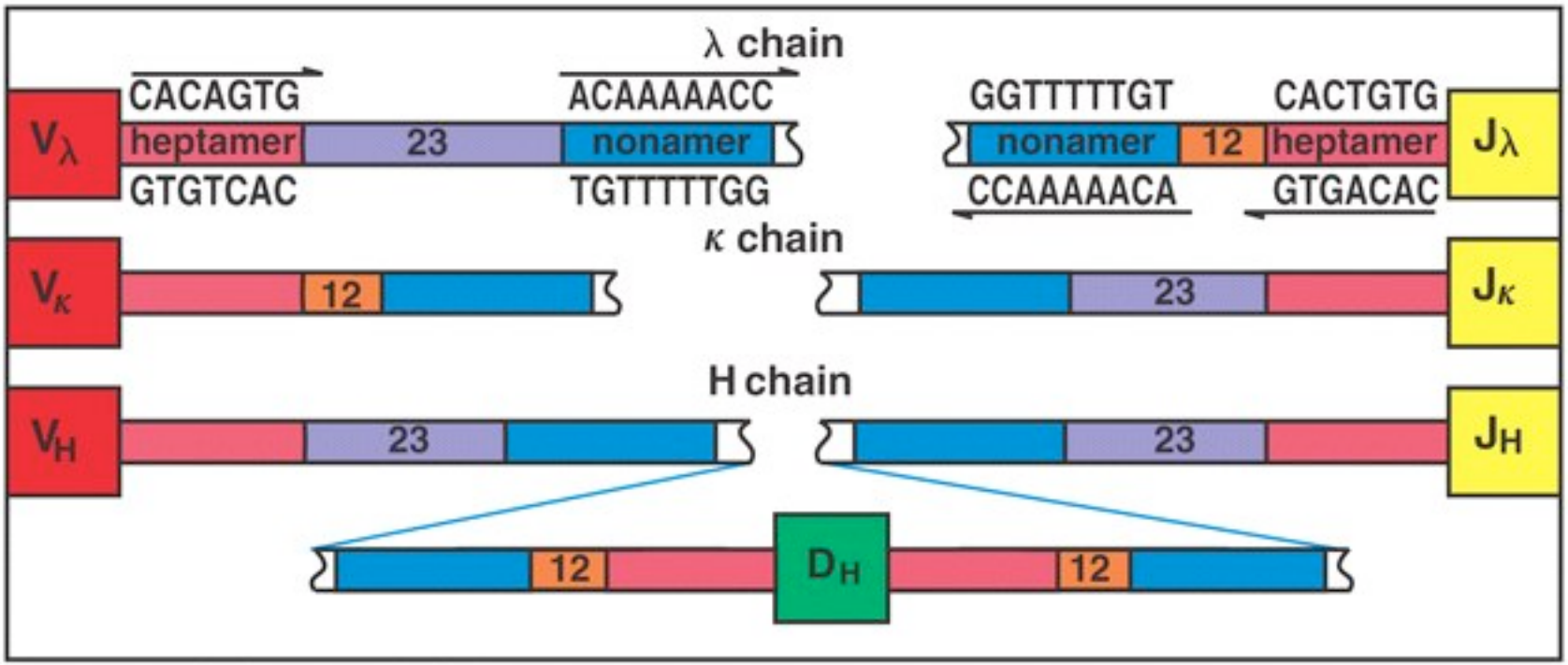

Figure 4-5 Immunobiology, 6/e. (© Garland Science 2005)

Intervening DNA is lost or inverted in recombination

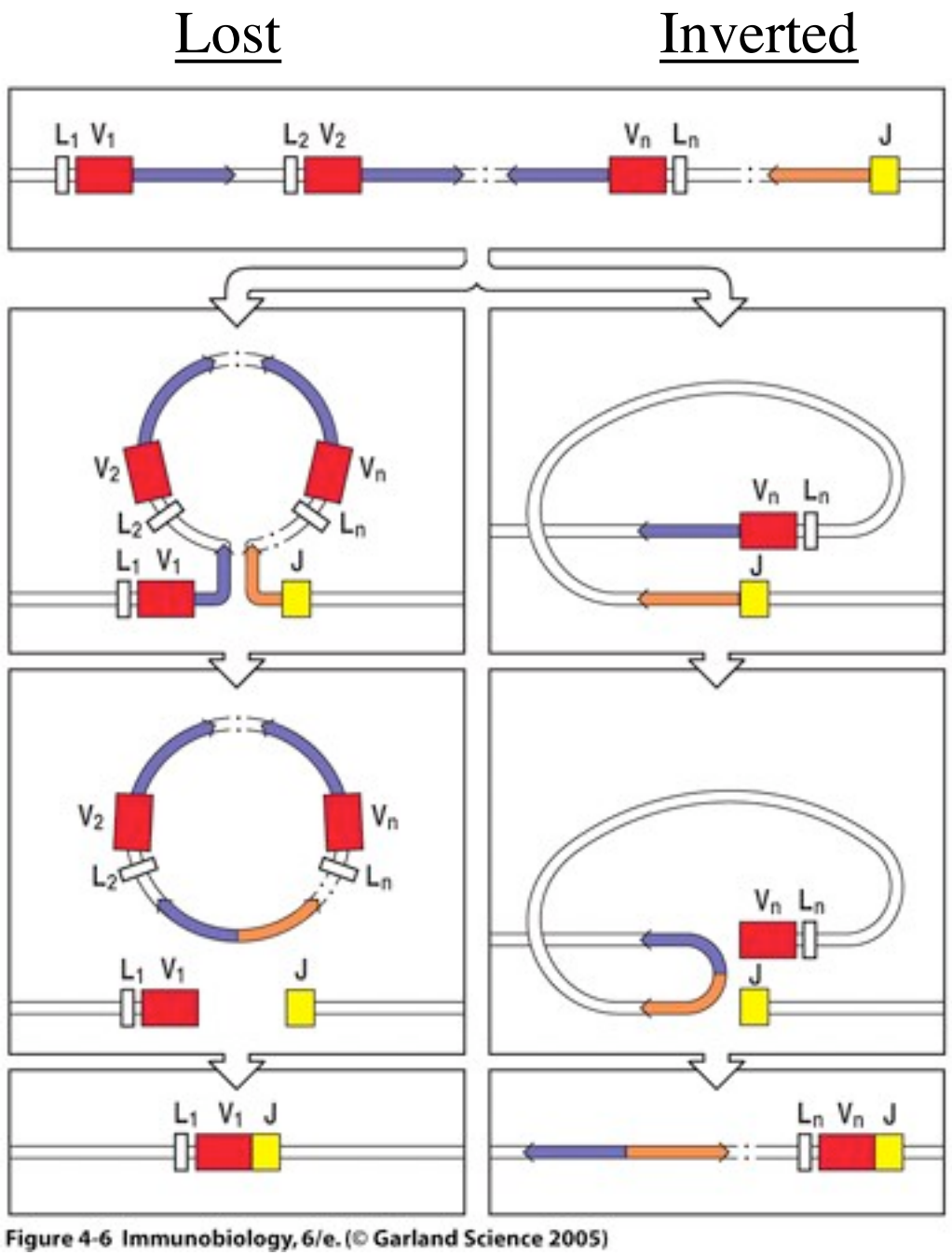

Figure 4-6 Immunobiology, 6/e. (© Garland Science 2005)

Signal joint

Coding joint

Steps in V(D)J joining by the V(D)J recombinase complex

1. Cleavage

-Rag1/Rag-2 endonucleases
-critical to recombination of Ig, TCR

2. Repair/diversity

-Tdt=terminal deoxynucleotidyl transferase

3. Joining

-DNA-PK (defective in SCID mice)
-DNA ligase

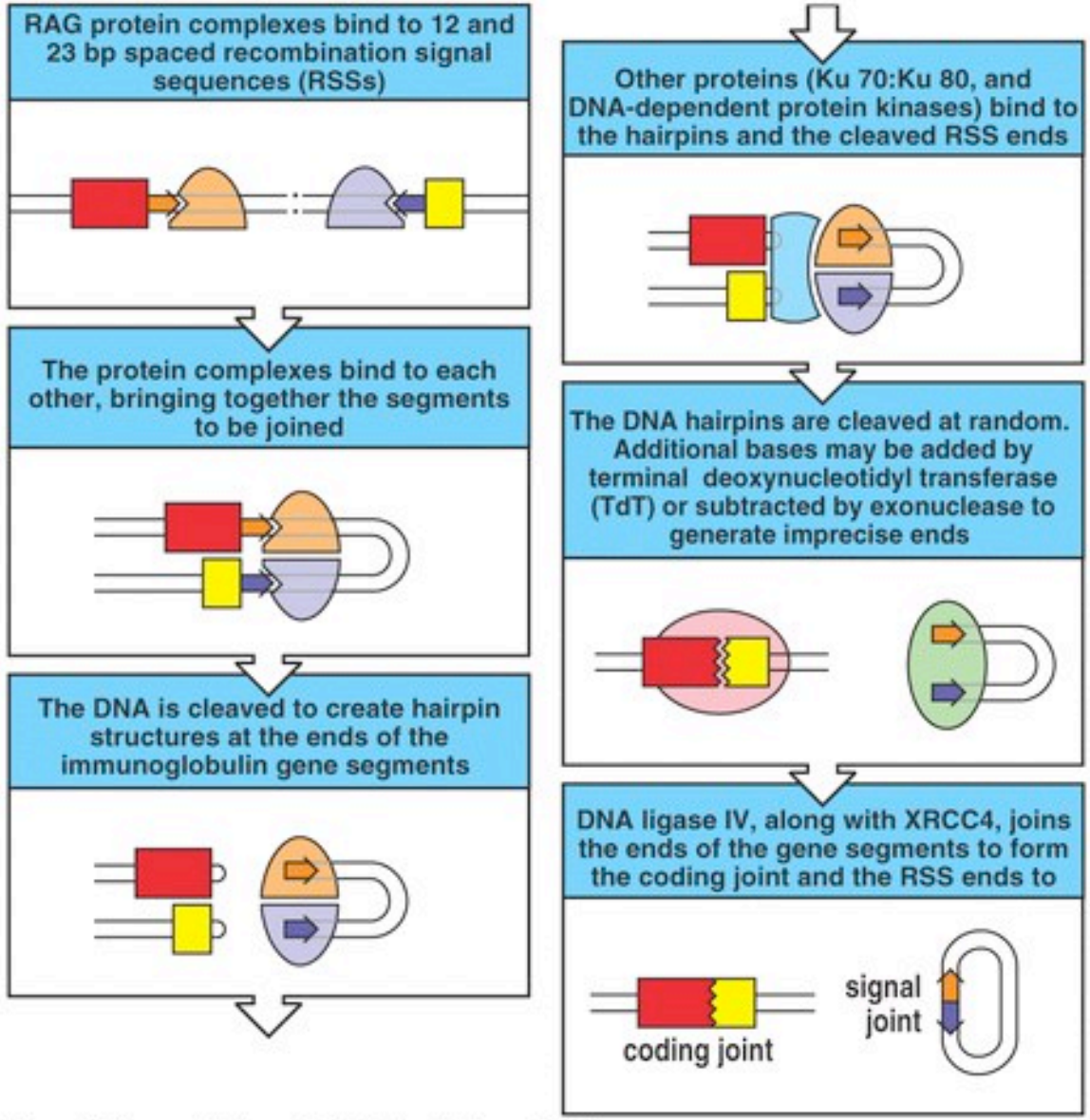

Figure 4-7 Immunobiology, 6/e. (© Garland Science 2005)

TdT yields diversity in CD3

CDR3=
V-J join of light chains
D-J join of heavy chains

CDR1, CDR2=
Within V region

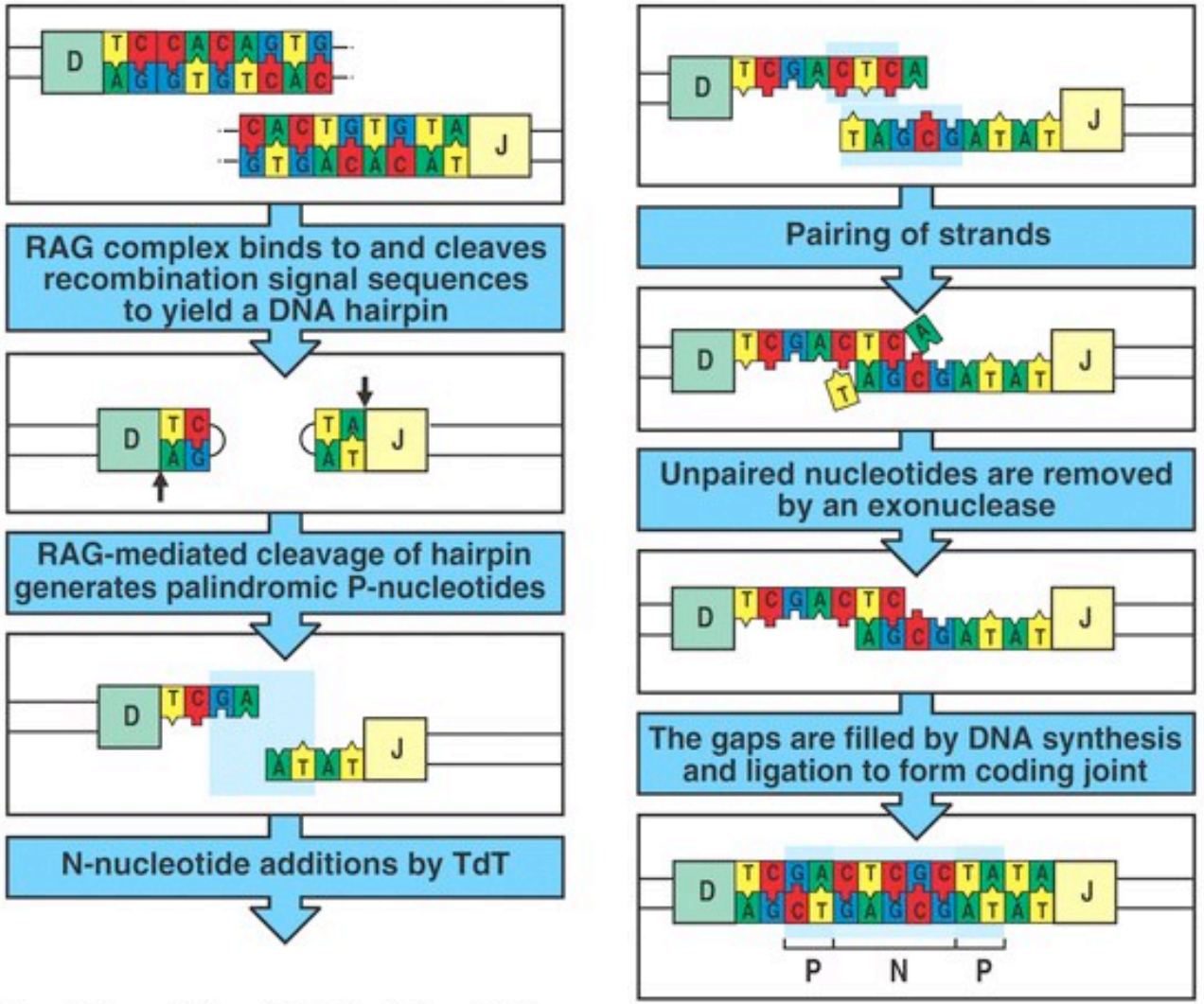

Figure 4-8 Immunobiology, 6/e. (© Garland Science 2005)

Somatic hypermutation

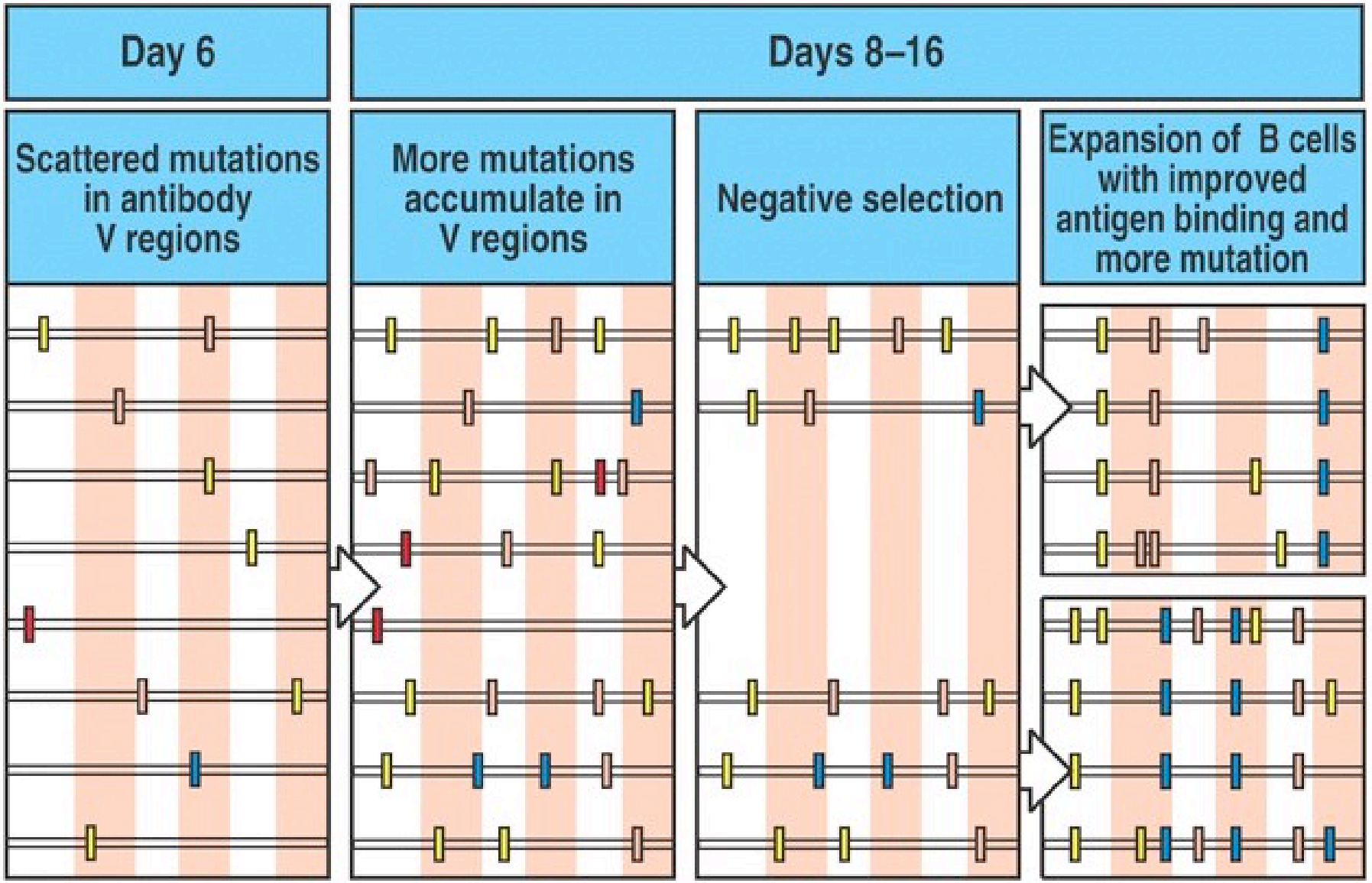

Figure 4-9 Immunobiology, 6/e. (© Garland Science 2005)

Somatic hypermutation

- CDR1, CDR2= hotspots for mutation
- High mutation rate
  - 1/1000 cell divisions vs. normal $1/10^{10}$
- Occurs in 2˚ lymphoid tissue after Ig rearrangement
- Leads to expansion of B cells with Ab of higher binding affinity-”Affinity maturation”

Ab diversity

- 1. V-J, V-D-J recombination
- 2. Pairing of various light chains with heavy chains
- 3. Tdt for junctional diversity
- 4. Somatic hypermutation

Somatic recombination of TCR

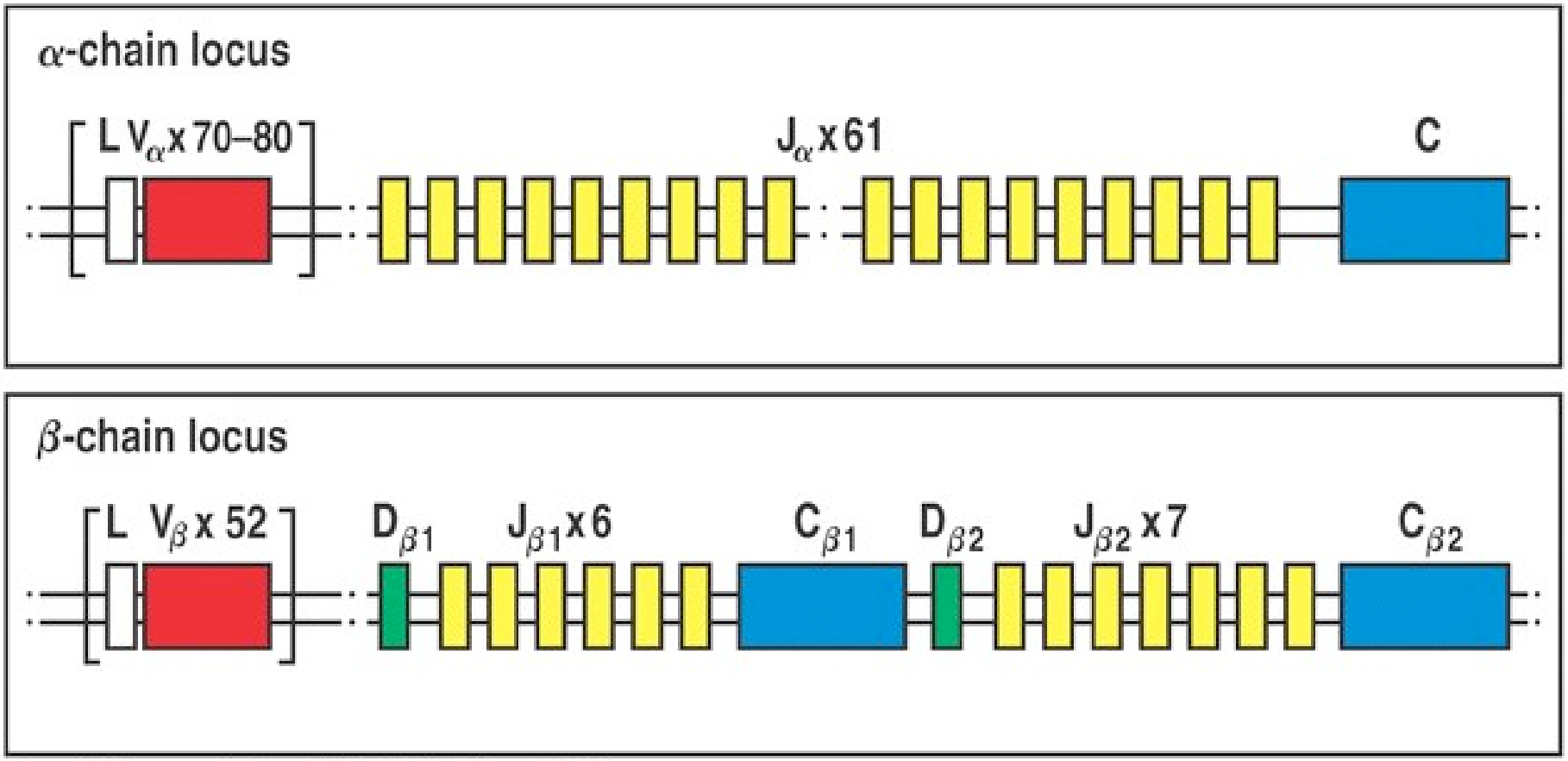

Figure 4-11 Immunobiology, 6/e. (© Garland Science 2005)

TCR somatic recombination

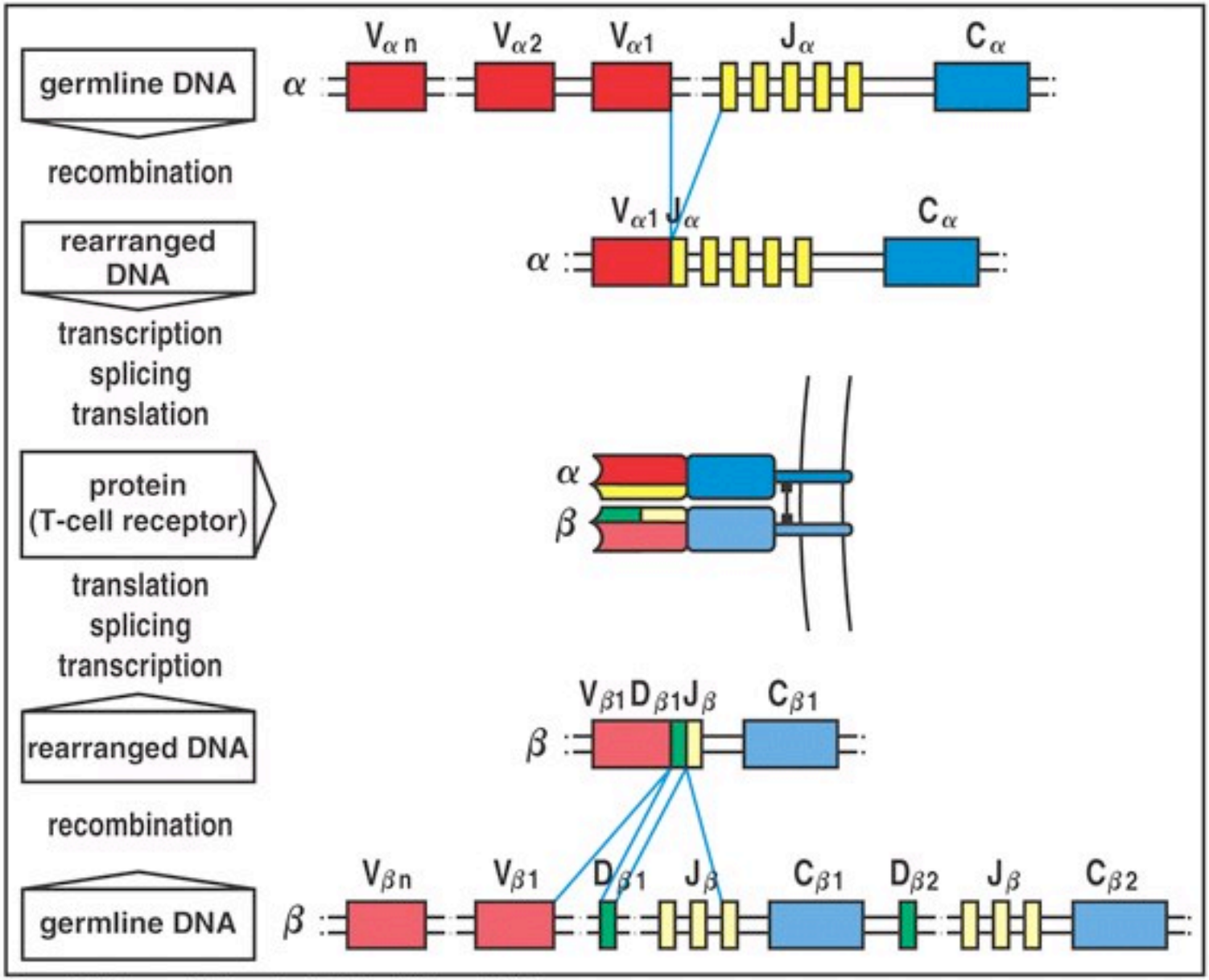

Figure 4-12 Immunobiology, 6/e. (© Garland Science 2005)

TCR diversity

- Joining diversity at CDR3
  - V-J joining in α chain
  - V-D-J joining in β chain
- Relies on same processes/enzymes
  - Recombination signal sequences
  - RAG-1/RAG-2
  - TdT
- No somatic hypermutation!

Incredible diversity!

Element	Immunoglobulin		α:β T-cell receptors	
	H	κ+λ	β	α
Variable segments (V)	40	70	52	~70
Diversity segments (D)	25	0	2	0
D segments read in three frames	rarely	–	often	–
Joining segments (J)	6	5(κ) 4(λ)	13	61
Joints with N- and P-nucleotides	2	50% of joints	2	1
Number of V gene pairs	1.9×10^6		5.8×10^6	
Junctional diversity	$\sim 3 \times 10^7$		$\sim 2 \times 10^{11}$	
Total diversity	$\sim 5 \times 10^{13}$		$\sim 10^{18}$	

Figure 4-13 Immunobiology, 6/e. (© Garland Science 2005)

□□ T cells

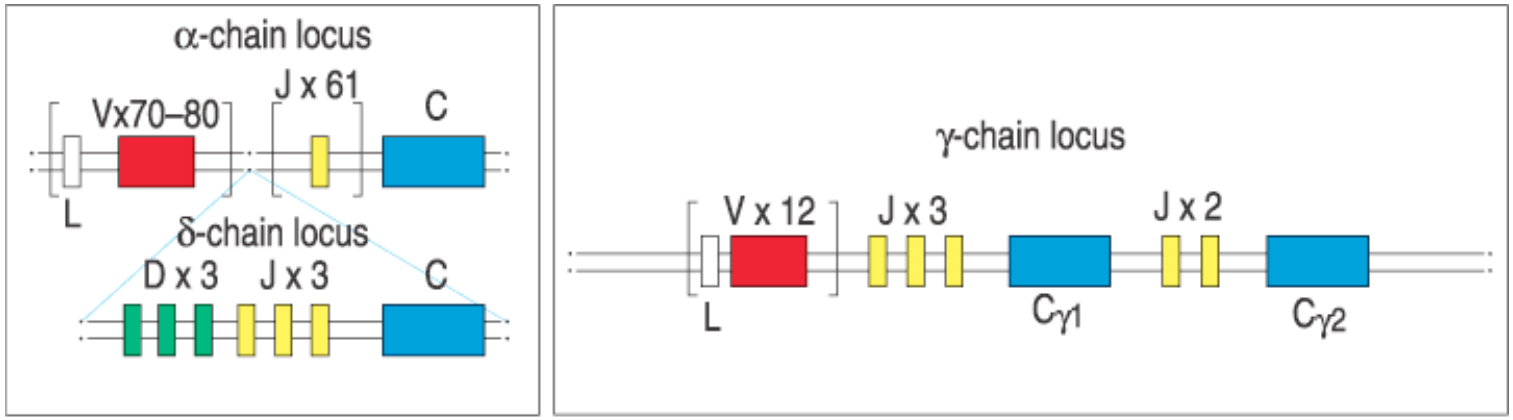

Fig 4.15 © 2001 Garland Science

- Low diversity in TCR
- Do not express CD4 or CD8
- High expression in epithelial tissue, not lymphoid
- Do not circulate through body
- Hypothesis: nonadaptive early line of defense

Ig isotypes

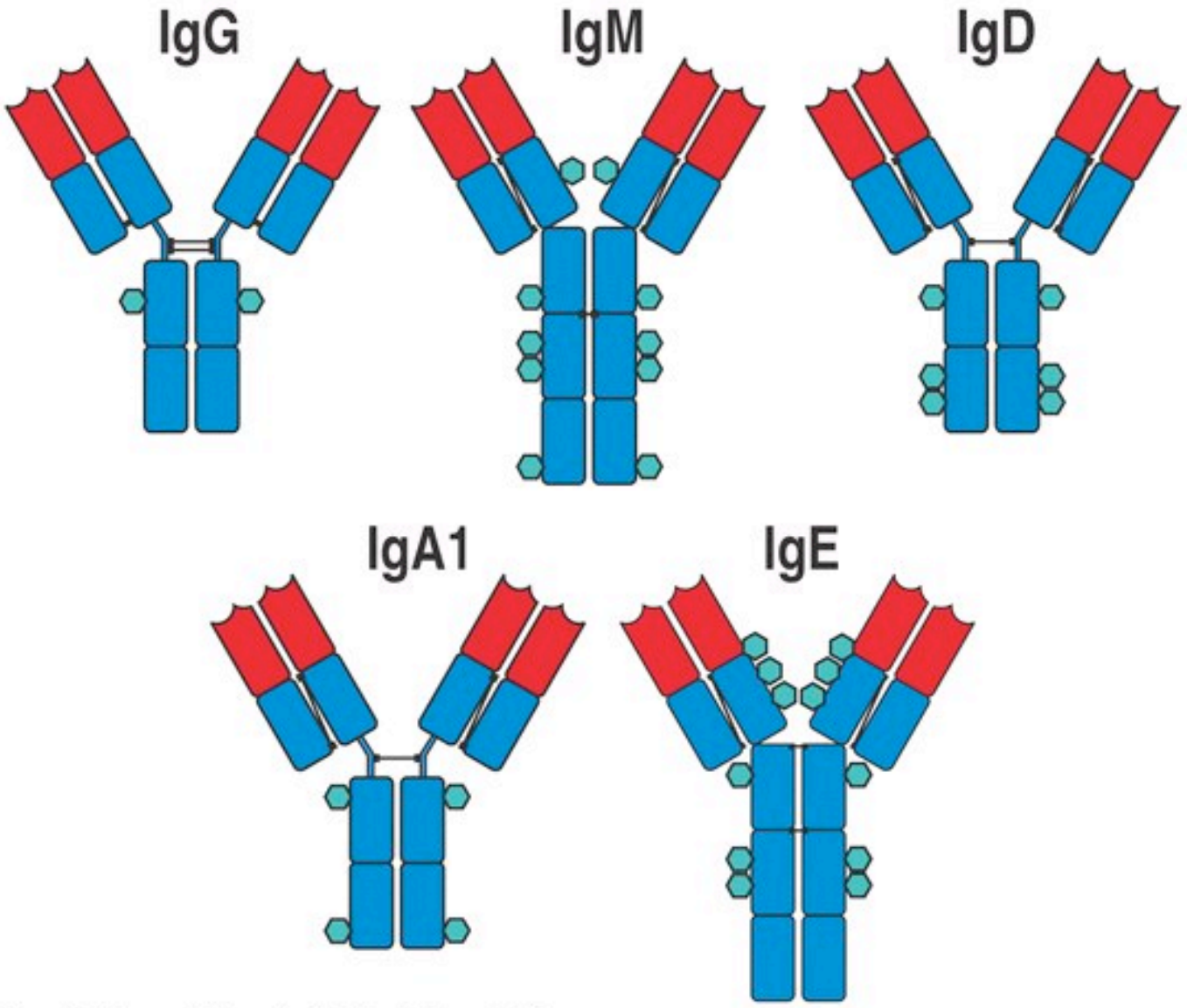

Figure 4-18 Immunobiology, 6/e. (© Garland Science 2005)

Multimeric forms- IgM and IgA

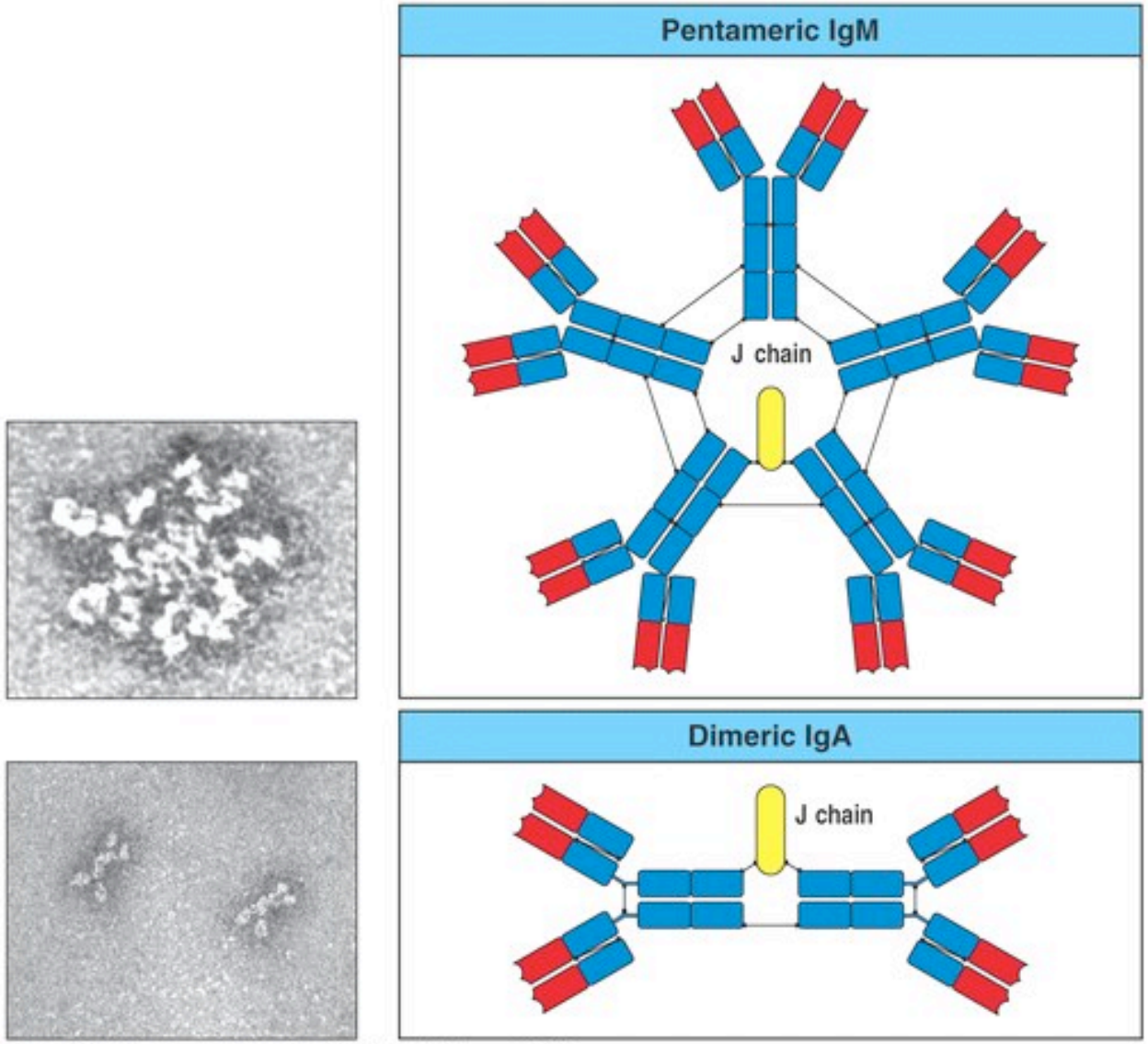

Figure 4-23 Immunobiology, 6/e. (© Garland Science 2005)

Isotype switching

- Constant region can change during immune response
- Stimulated by Ag, T cell cytokines
- Initial expression of IgM, membrane bound

 Can have different B cells with same Ag specificity, different isotypes

Functions of different isotypes

	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	δ	ϵ
Molecular weight (kDa)	146	146	165	146	970	160	160	184	188
Serum level (mean adult mg ml^{-1})	9	3	1	0.5	1.5	3.0	0.5	0.03	5×10^{-5}
Half-life in serum (days)	21	20	7	21	10	6	6	3	2
Classical pathway of complement activation	++	+	+++	–	+++	–	–	–	–
Alternative pathway of complement activation	–	–	–	–	–	+	–	–	–
Placental transfer	+++	+	++	–/+	–	–	–	–	–
Binding to macrophage and phagocyte Fc receptors	+	–	+	–/+	–	+	+	–	+
High-affinity binding to mast cells and basophils	–	–	–	–	–	–	–	–	+++
Reactivity with staphylococcal Protein A	+	+	–/+	+	–	–	–	–	–

Figure 4-17 Immunobiology, 6/e. (© Garland Science 2005)

Genes encode different constant regions

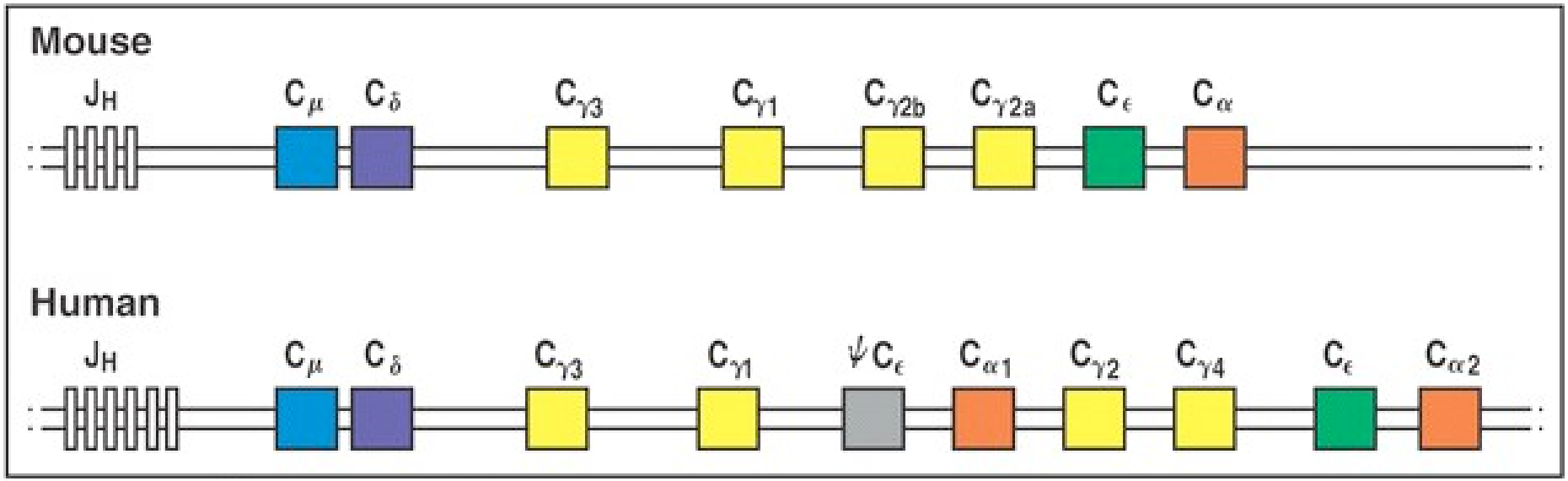

Figure 4-19 Immunobiology, 6/e. (© Garland Science 2005)

IgM vs. IgD expression controlled by alternative splicing

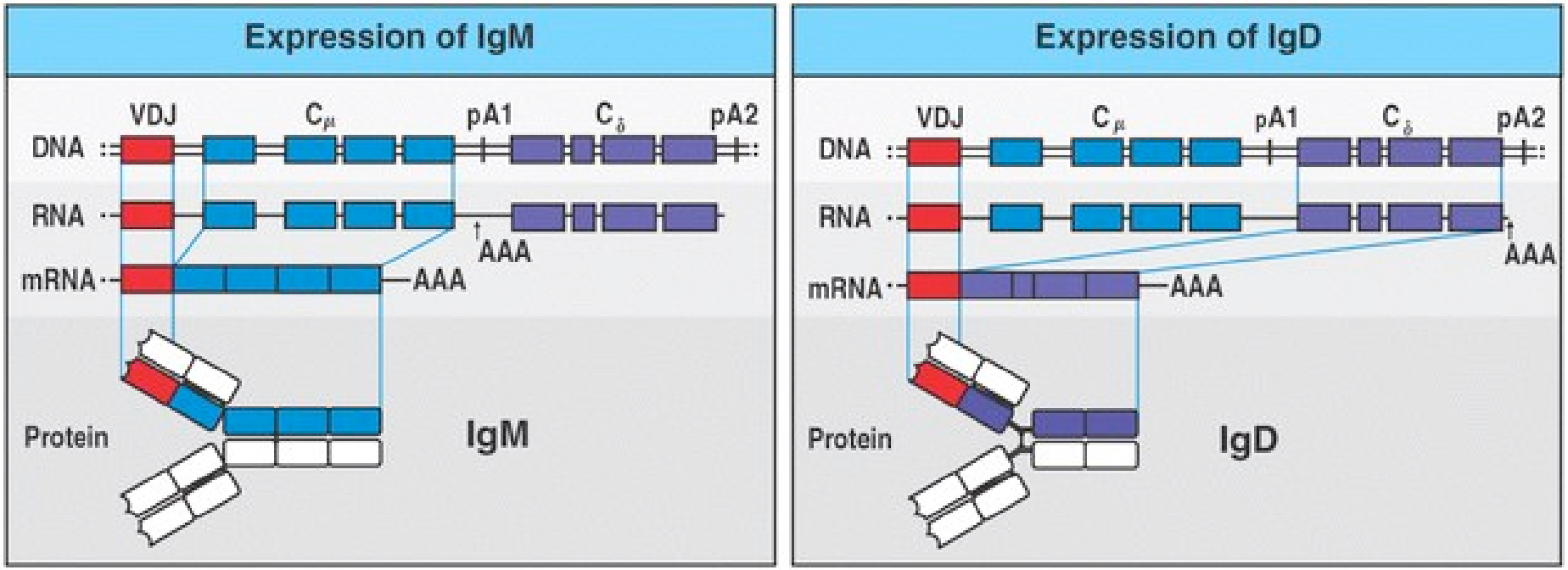

Figure 4-20 Immunobiology, 6/e. (© Garland Science 2005)

IgG/IgE/IgA controlled by recombination

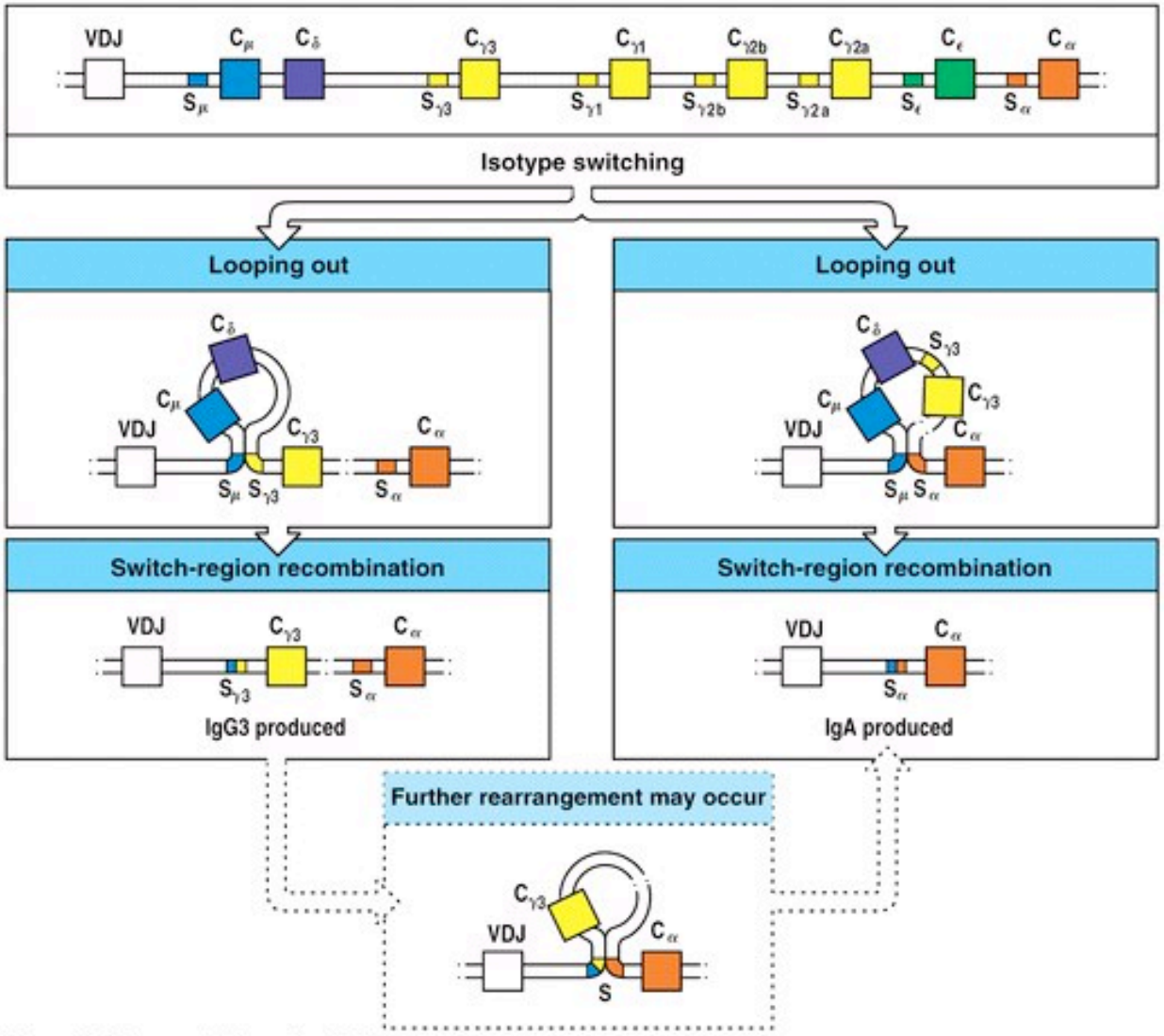

Figure 4-21 Immunobiology, 6/e. (© Garland Science 2005)

Transmembrane Ig

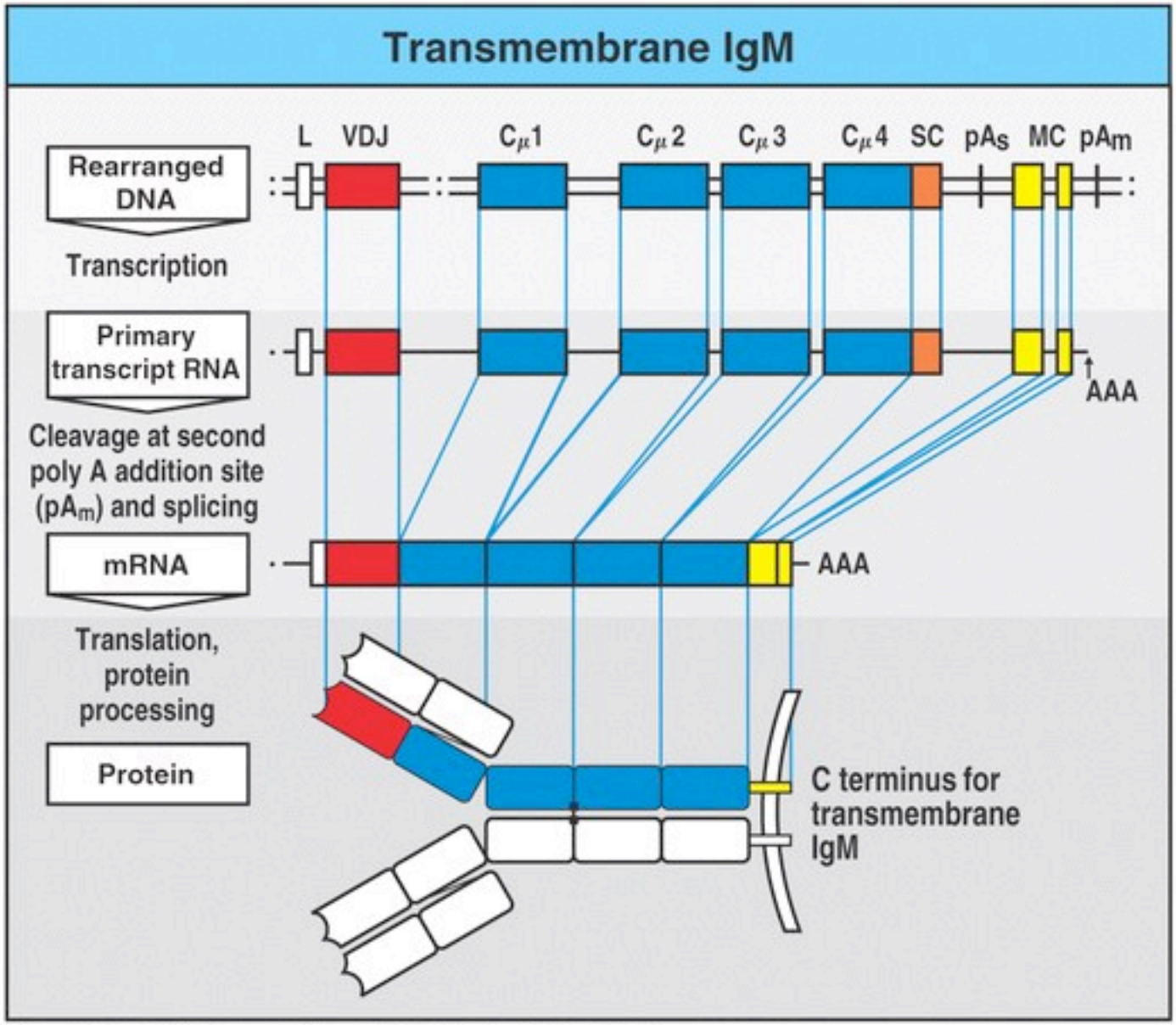

Figure 4-16 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Secreted Ig

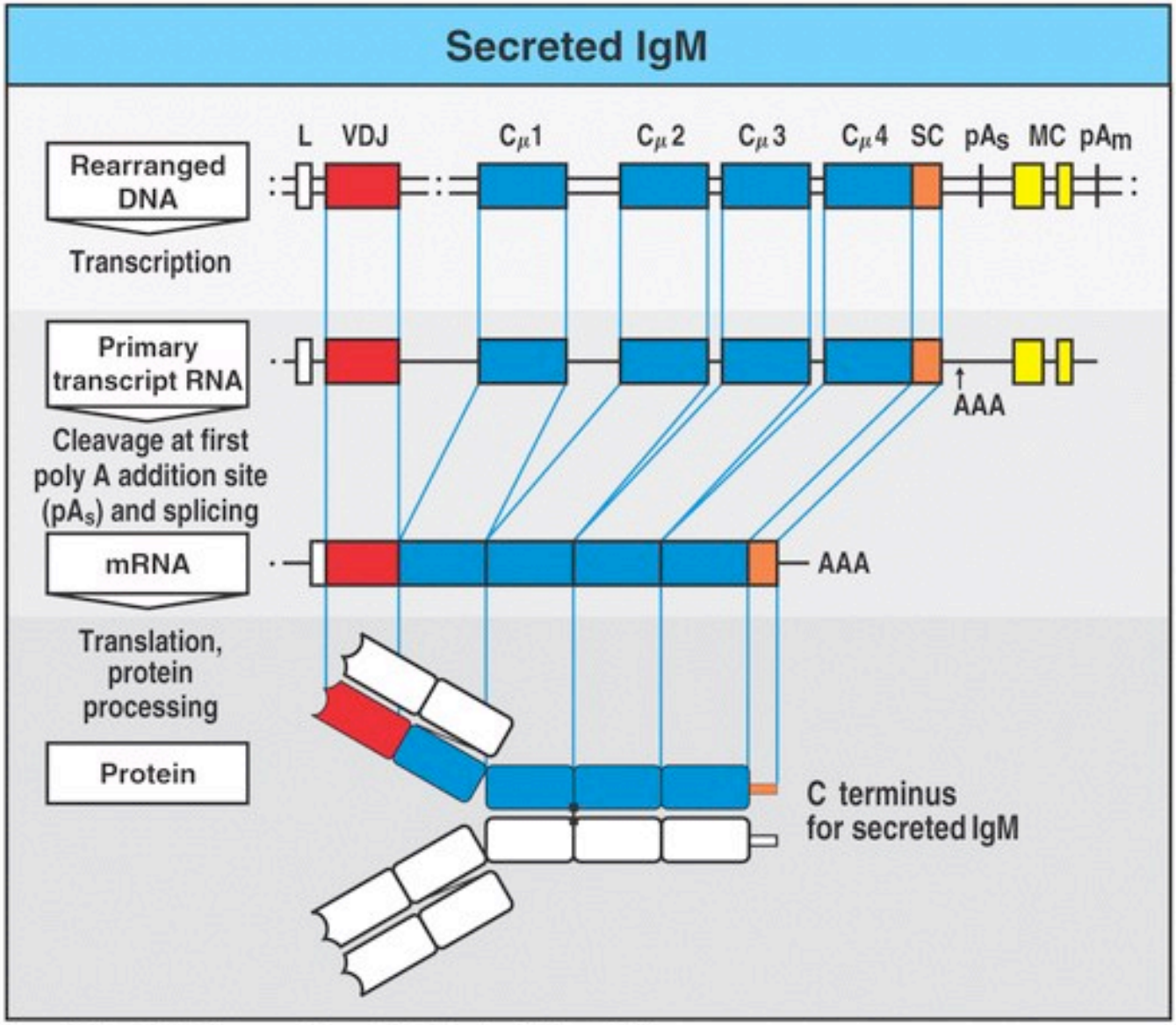

Figure 4-16 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

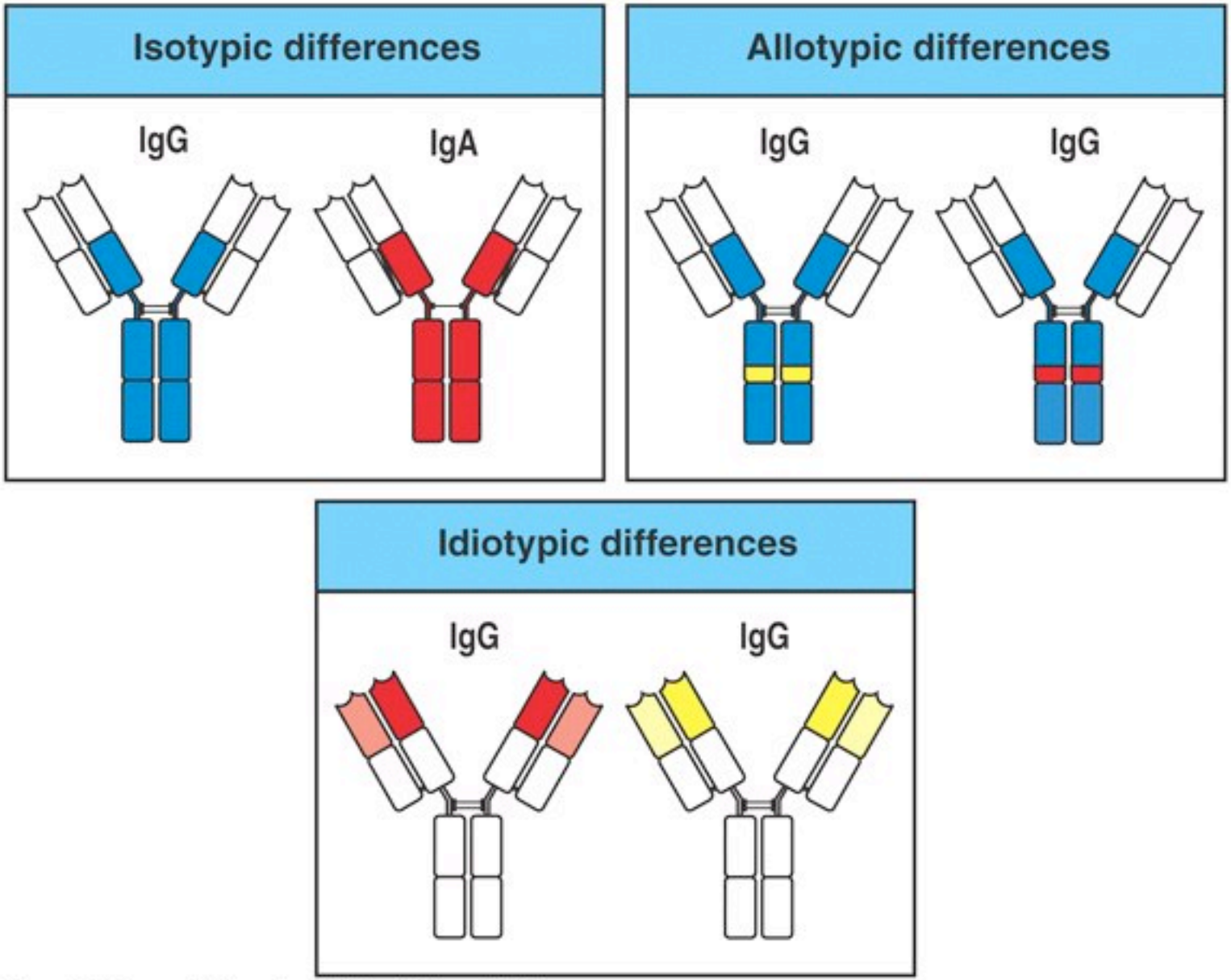

Figure 4-24 Immunobiology, 6/e. (© Garland Science 2005)