Introduction to Carbohydrate and Structure

Part II: Complex Carbohydrates (Functional Implications, i.e., Glycobiology©)

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Glycoconjugates©

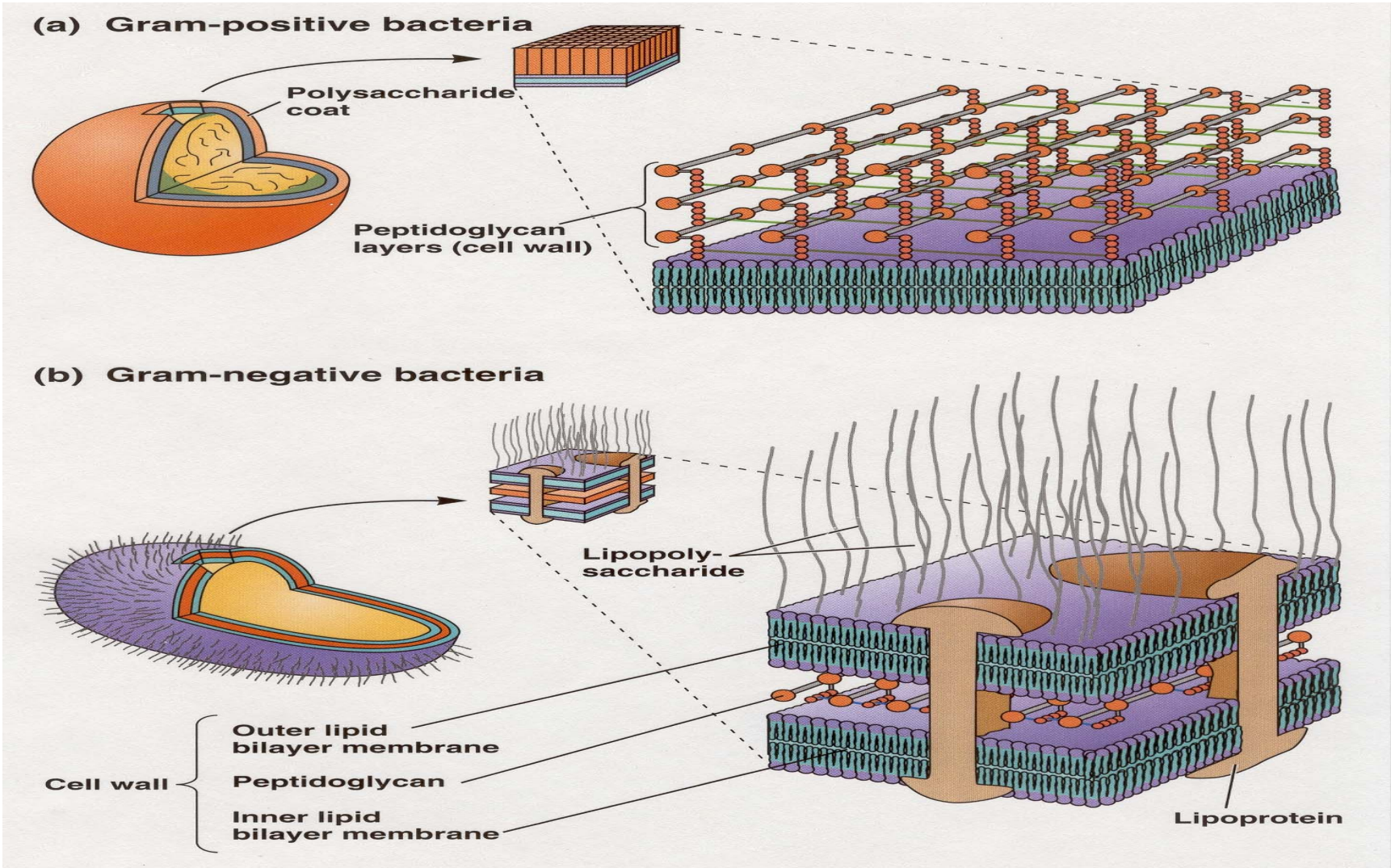
1. Proteoglycans
2. Glycoproteins
3. Glycolipids
4. Peptidoglycan

Peptidoglycan©

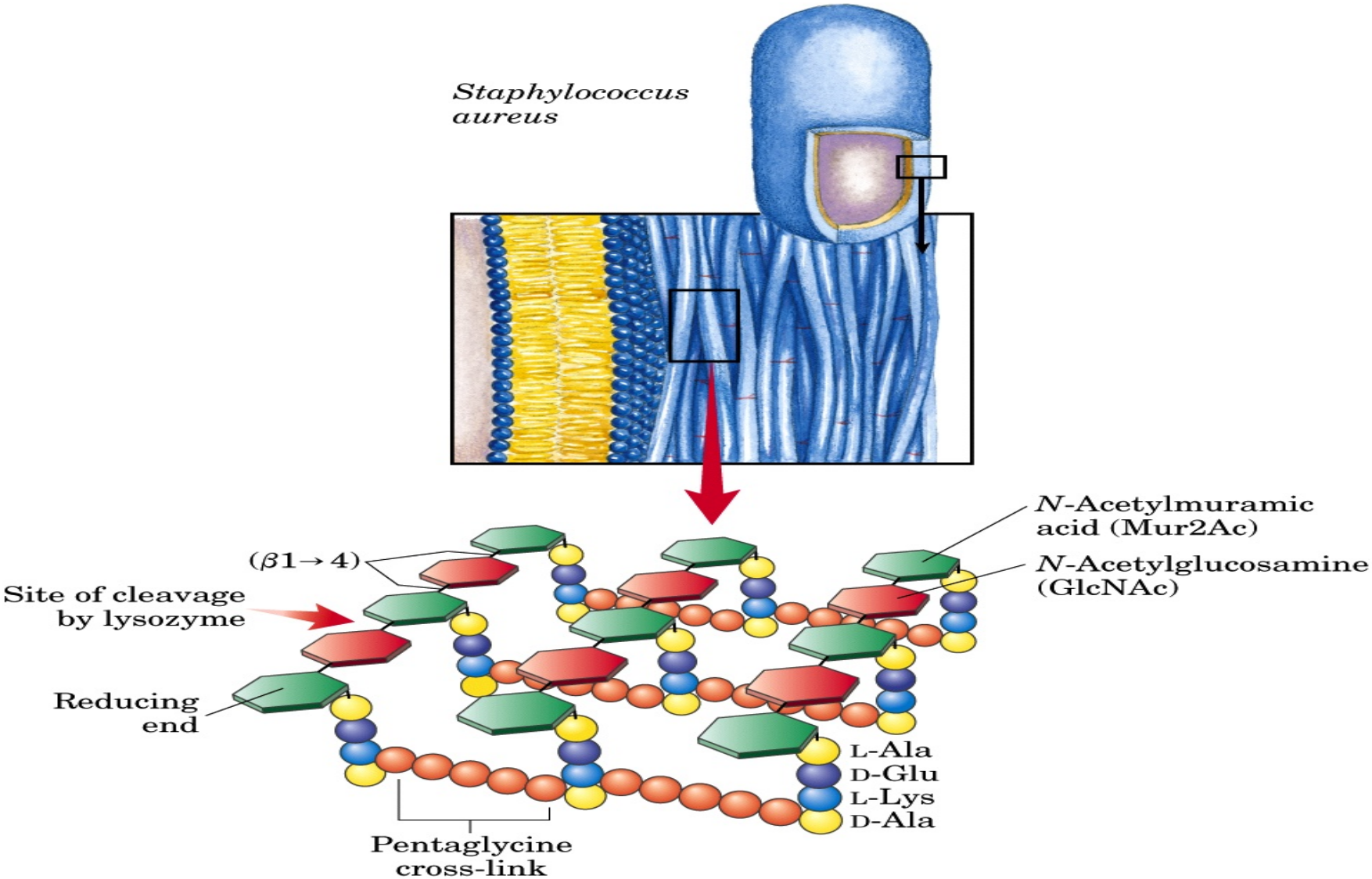
- An unique feature of the bacteria is their **cell wall** that surrounds the plasma membrane and provides the mechanical strength that enables bacteria to resist shear and osmotic shock.

- The cell wall is composed of a network of linear heteropolysaccharides cross-linked by peptide. A structure of this sort is called **peptidoglycan.**

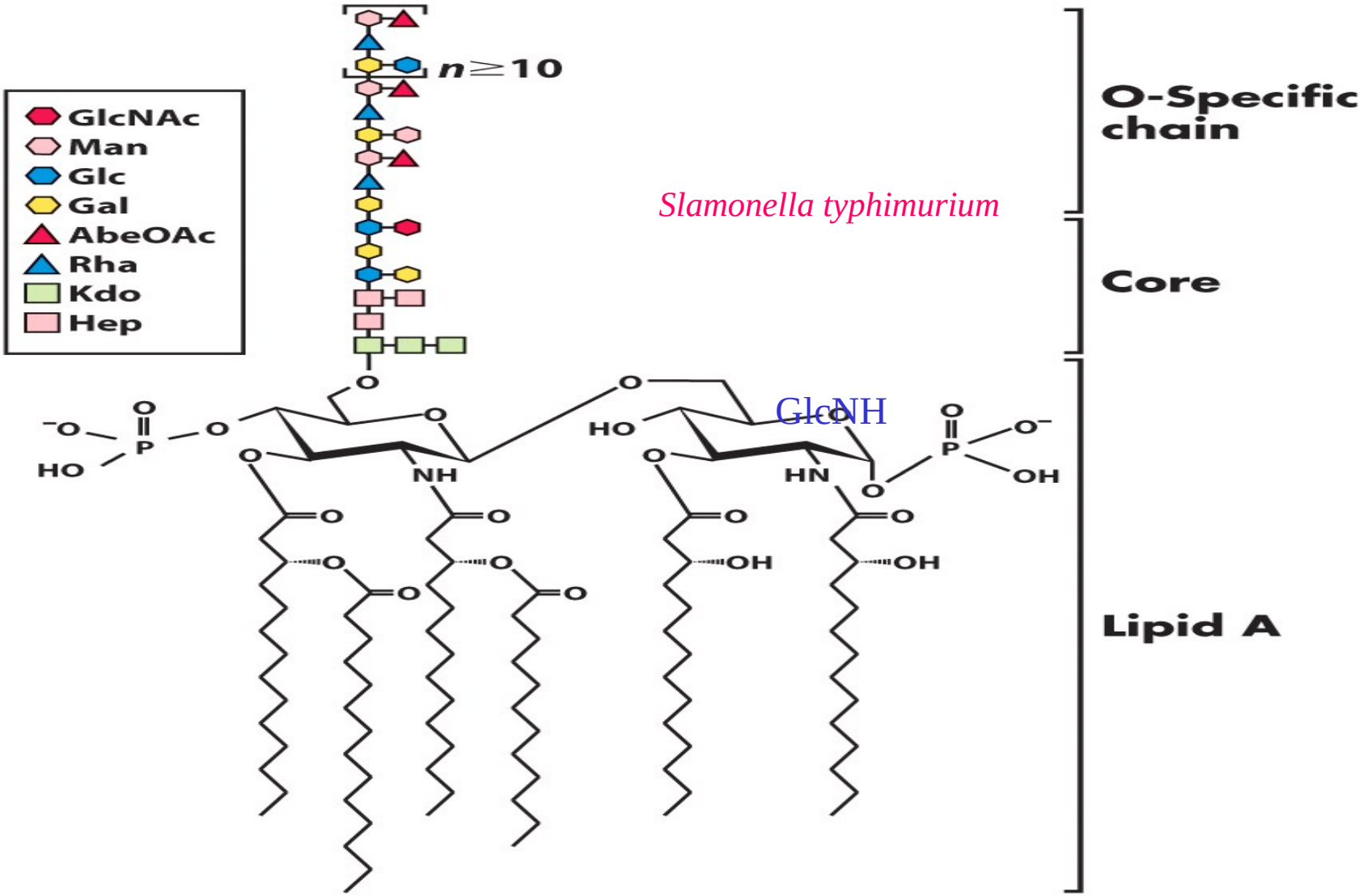
Bacterial Cell Wall: Peptidoglycan©



Peptidoglycan of Bacterial Cell Wall©



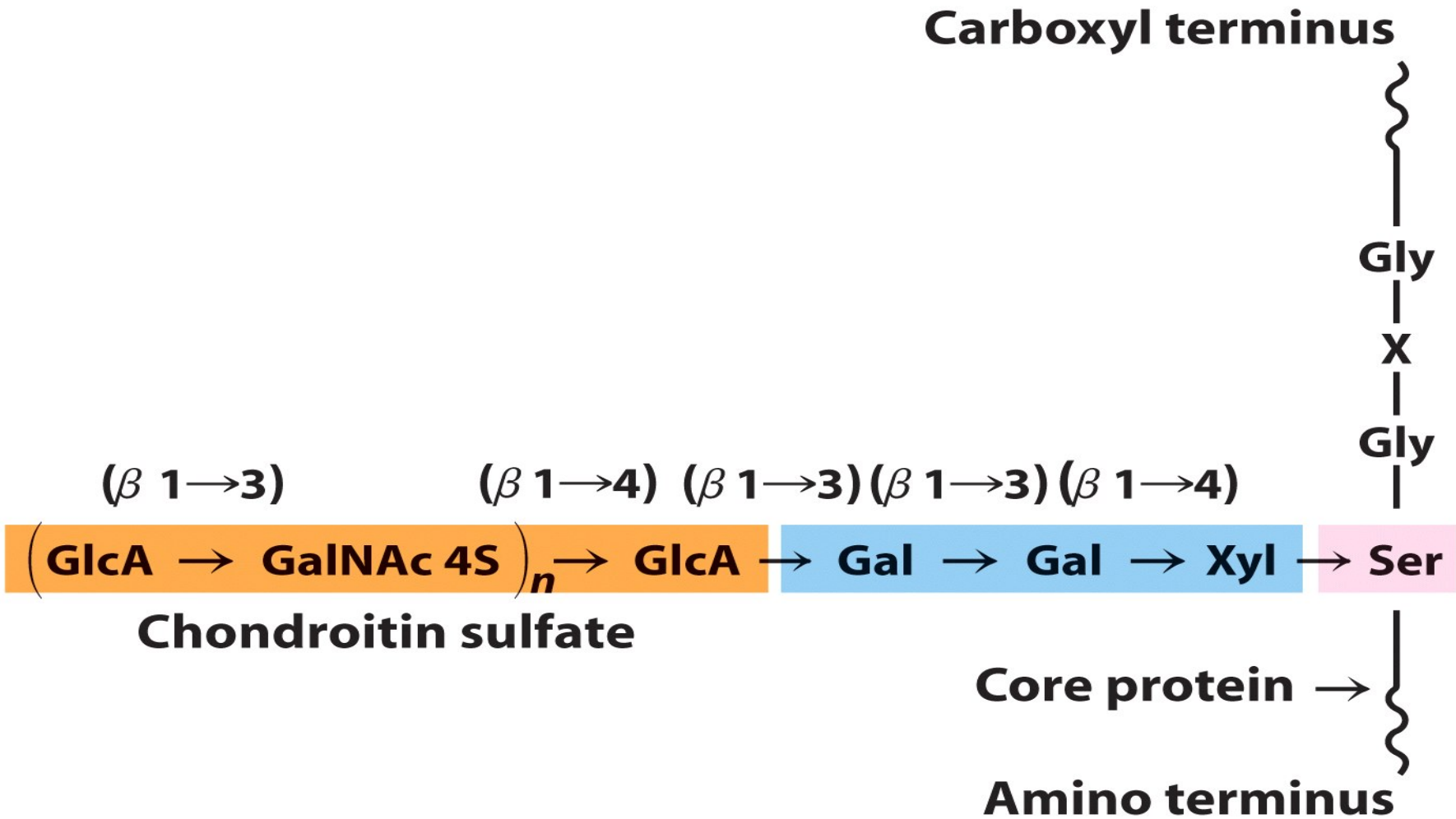
Bacterial Lipopolysaccharide©



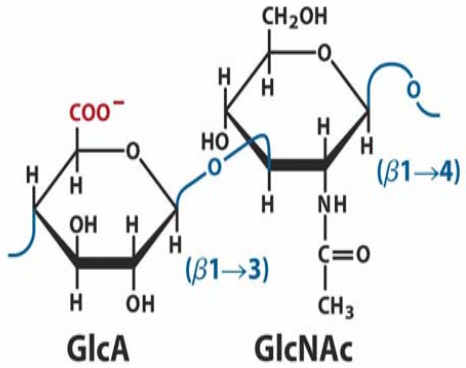
Proteoglycans©

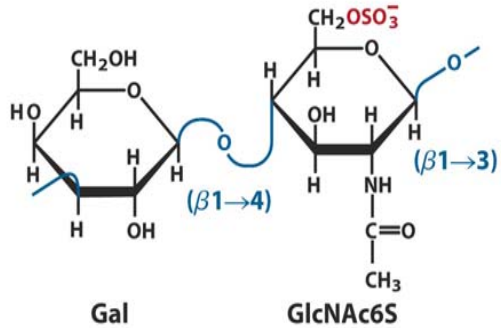
- A class of complex macromolecules contains 95% or more carbohydrate, and are polyanionic.
- The carbohydrate chains are called **glycosaminoglycans (GAGs) (or mucopolysaccharides)**.
- **GAG** chains are linked covalently to a protein core, are predominantly components of the extracellular matrices and cell surfaces. Six distinct classes of GAGs are now recognized, but certain features are common to all classes.
- The long un-branched heteropolysaccharide chains are made up largely of disaccharide repeating units consisting of a hexosamine and a uronic acid. Other common constituents are sulfate groups, linked by ester bonds to certain monosaccharides or by amide bonds to the amino group of glucosamine (exception: Hyaluronate)
- Electrical charge & macromolecular structure aid in their biological role as lubricants and support elements in connective tissue and more dynamic roles in cell adhesion and signaling..

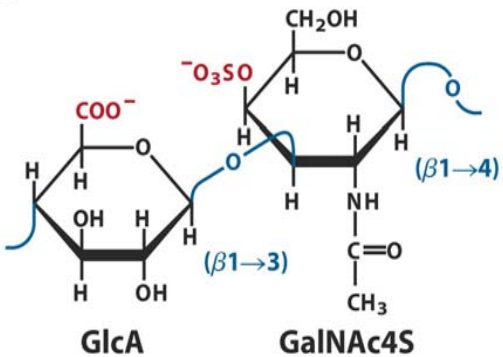
Linkage Region of the Proteoglycan©

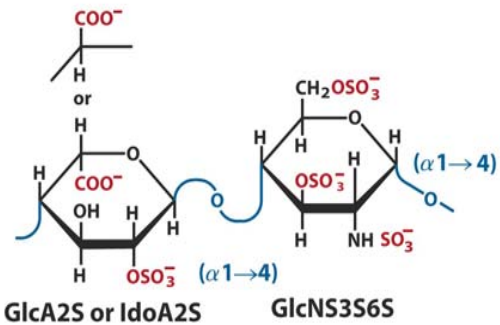


Repeating Units of Some Common Glycosaminoglycans of Extracellular Matrix

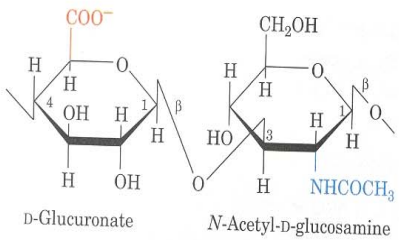
Glycosaminoglycan	Number of disaccharides per chain	Repeating disaccharide
Hyaluronate	~50,000	

Glycosaminoglycan	Number of disaccharides per chain	Repeating disaccharide
Keratan sulfate	~25	

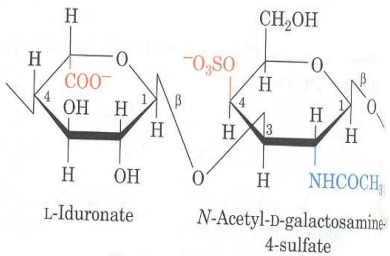
Glycosaminoglycan	Number of disaccharides per chain	Repeating disaccharide
Chondroitin 4-sulfate	20–60	

Glycosaminoglycan	Number of disaccharides per chain	Repeating disaccharide
Heparin	15–90	

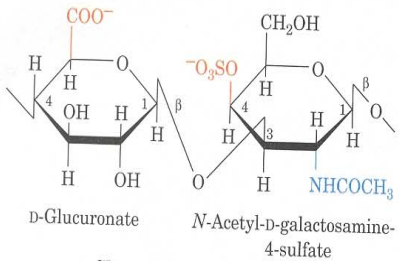
Disaccharide Repeating Units in Glycosaminoglycans©



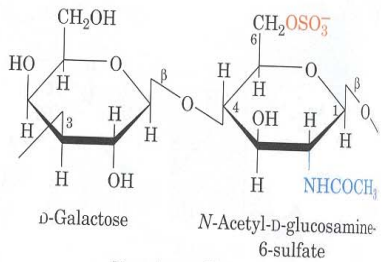
Hyaluronate (MW 10^5 - 10^7)



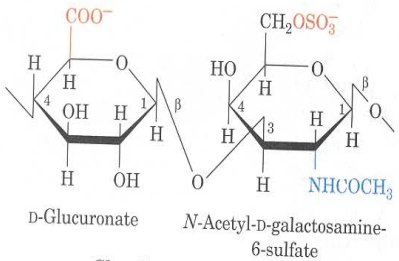
Dermatan sulfate (MW 2-5 x 10^4)



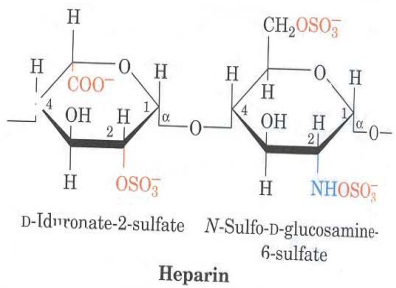
Chondroitin-4-sulfate (MW 1.5 - 2 x 10^6)



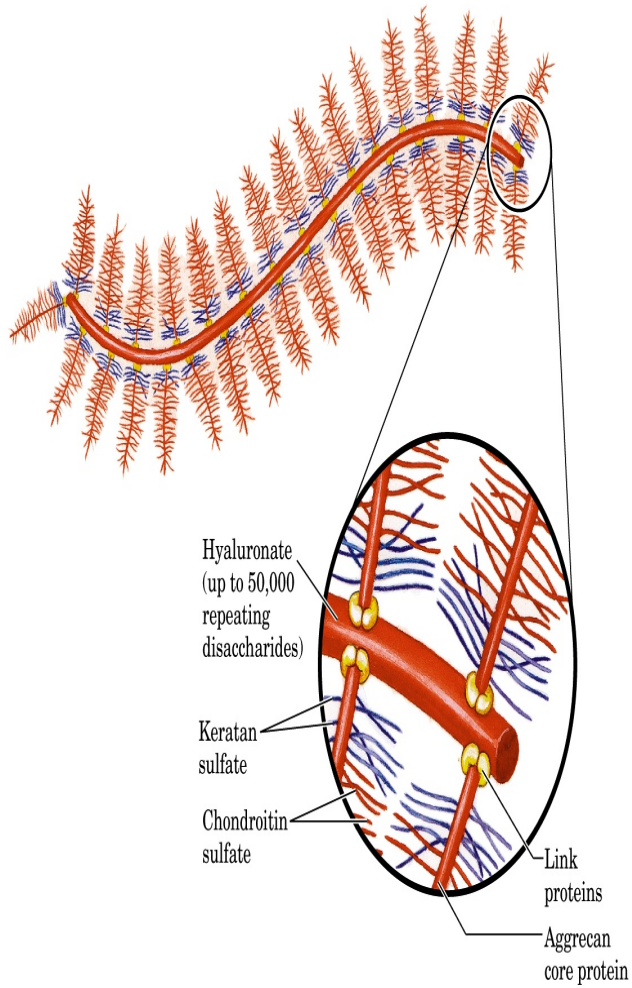
Keratan sulfate



Chondroitin-6-sulfate



Heparin



I. Functions of Proteoglycans©

1. Proteoglycans may be *soluble* and located in the extra-cellular matrix, or they may be *integral transmembrane proteins.*
2. Proteoglycans may modulate cell growth.

   a) Heparin and heparin sulfate are known to inhibit cell proliferation by internalization of glycosaminoglycan (GAG) moiety.

   b) Fibroblast growth factor (FGF) binds tightly to heparin and other GAGs and gets protected.

   c) Transforming growth factor β (TGF β) has been shown to stimulate the synthesis and

II. Functions of Proteoglycans©

secretion of proteoglycans in certain cells.

d) Several proteoglycan core proteins, such as *lymphocyte homing receptor*, have domains similar in sequence to *epidermal growth factor* (EGF) and *complement regulatory factor*.

Functions of Glycosaminoglycans©

- **Hyaluronic Acid (HA):** binds cations and water molecules; present in connective tissue, synovial fluid, and the vitreous of the eye; at low shear stress it forms tangled masses that impedes flow. The viscoelastic nature makes HA an excellent shock absorbers & lubcricants. HA is an essential component of cartilage and tendons. Umbilical cord is rich in HA. Hyaluronidase secreted by some pathogenic bacteria can hydrolyze HA and making tissues more susceptible to bacterial invasion. A similar

Functions of Glycosaminoglycans (*Contd.*)©

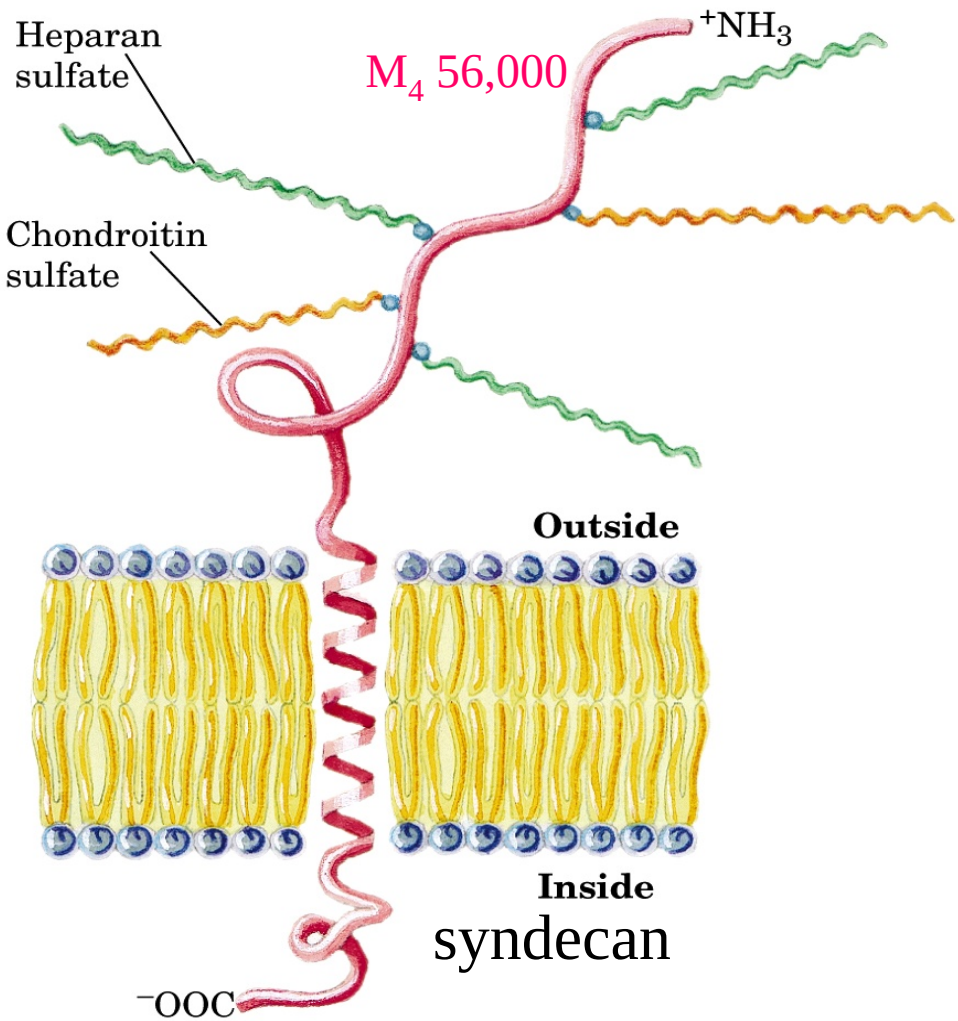
enzyme in sperm hydrolyzes an outer GAG coat around the ovum, allowing sperm penetration.

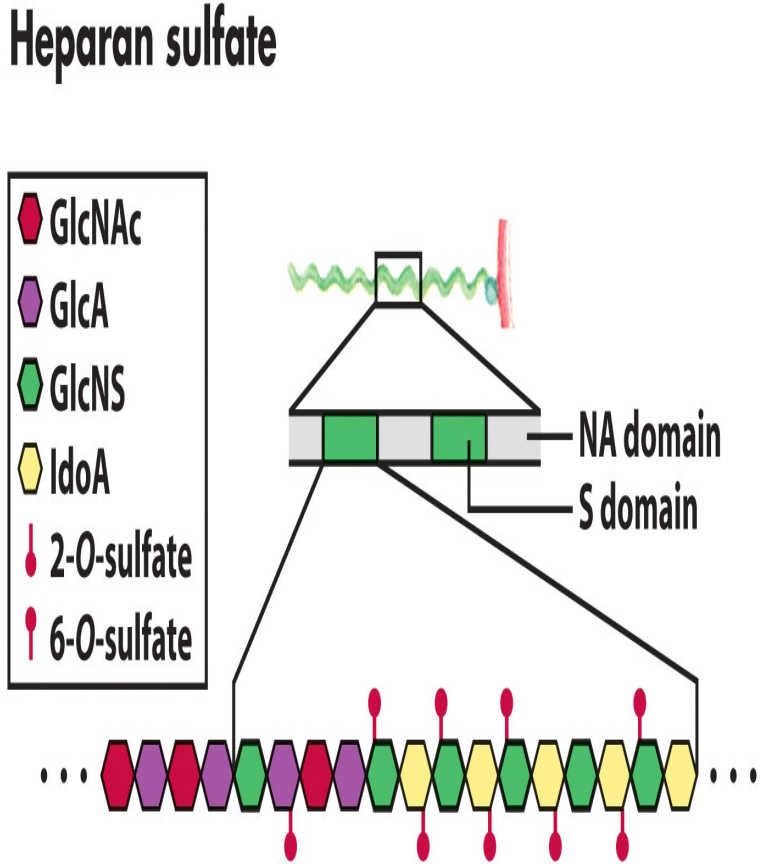
Chondroitin Sulfate: Contributes to the tensile strength of cartilage, tendons, ligaments, and the walls of aorta. **Dermatan Sulfate:** It is present in skin, blood vessels and heart valves.

Keratan Sulfate: A most heterogeneous GAG with no uronic acid, present in cornea, cartilage, bone, as well as in horn, hair, hoofs, nails, and claws.

•**Heparin:** Not a constituent of connective tissue, present exclusively in mast cells, and inhibits blood clotting.

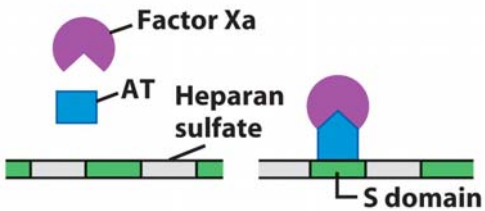
Proteoglycan of an Integral Membrane Protein©





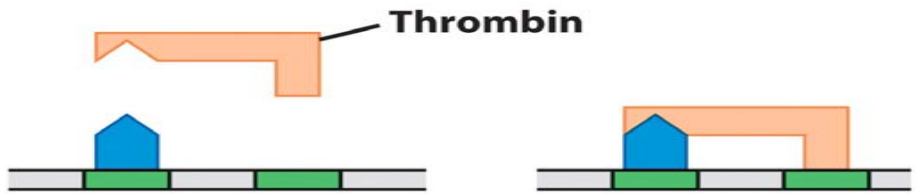
Four Types of Protein Interactions with S Domains of Heparan Sulfate

(a) Conformational activation



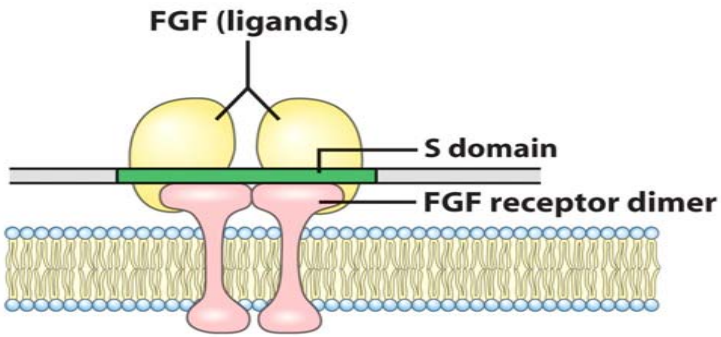
A conformational change induced in the protein antithrombin (AT) on binding a specific pentasaccharide S domain allows its interaction with Factor Xa, a blood clotting factor, preventing clotting.

(b) Enhanced protein-protein interaction



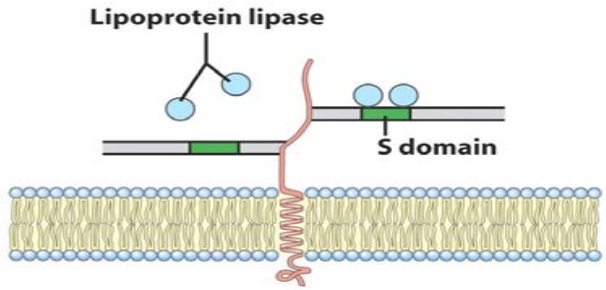
Binding of AT and thrombin to two adjacent S domains brings the two proteins into close proximity, favoring their interaction, which inhibits blood clotting.

(c) Coreceptor for extracellular ligands



S domains interact with both the fibroblast growth factor (FGF) and its receptor, bringing the oligomeric complex together and increasing the effectiveness of a low concentration of FGF.

(d) Cell surface localization/concentration



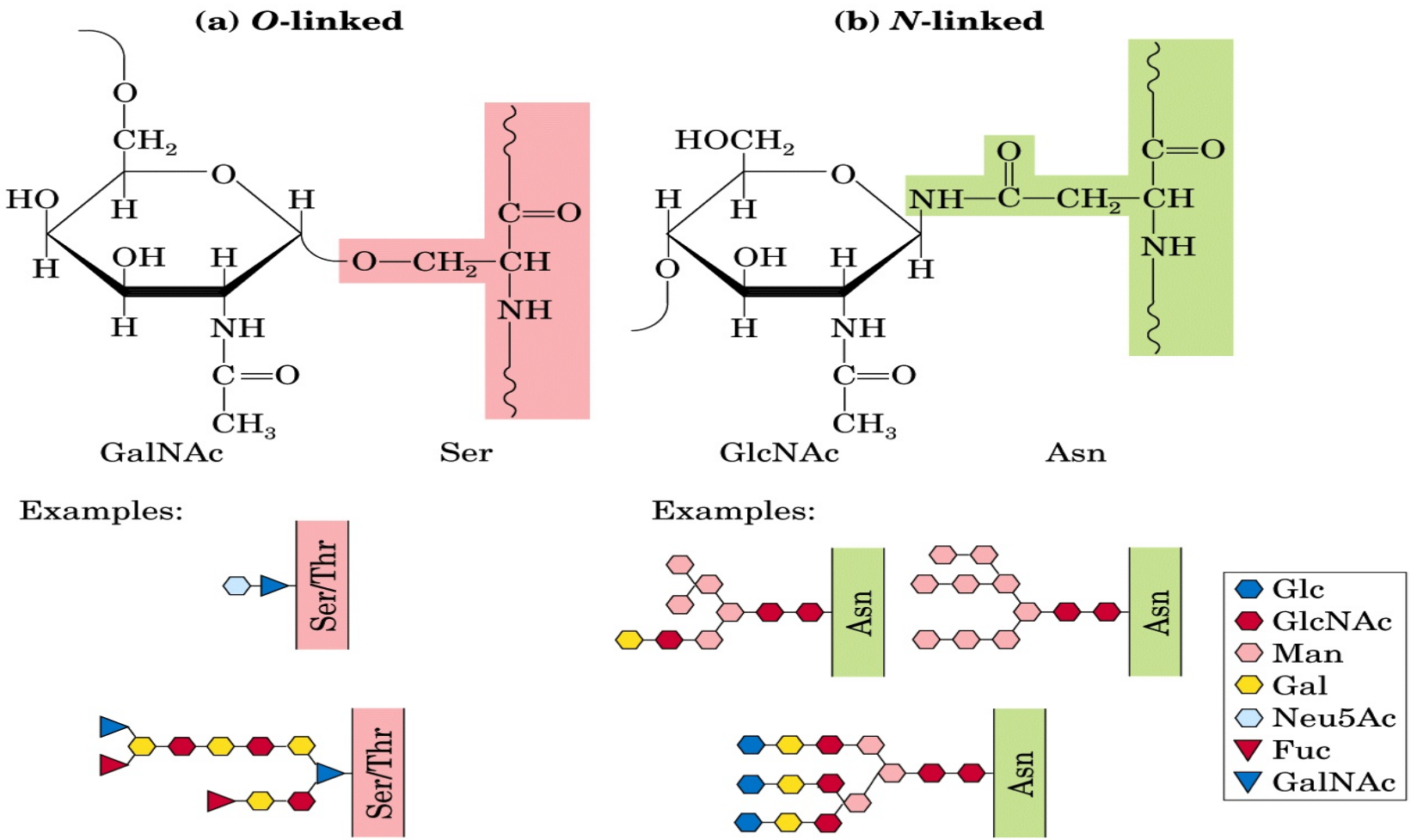
The high density of negative charges in heparan sulfate brings positively charged molecules of lipoprotein lipase into the vicinity and holds them by electrostatic interactions as well as by sequence-specific interactions with S domains. Such interactions are also central in the first step in the entry of certain viruses (such as herpes simplex viruses HSV-1 and HSV-2) into cells.

Glycoproteins©

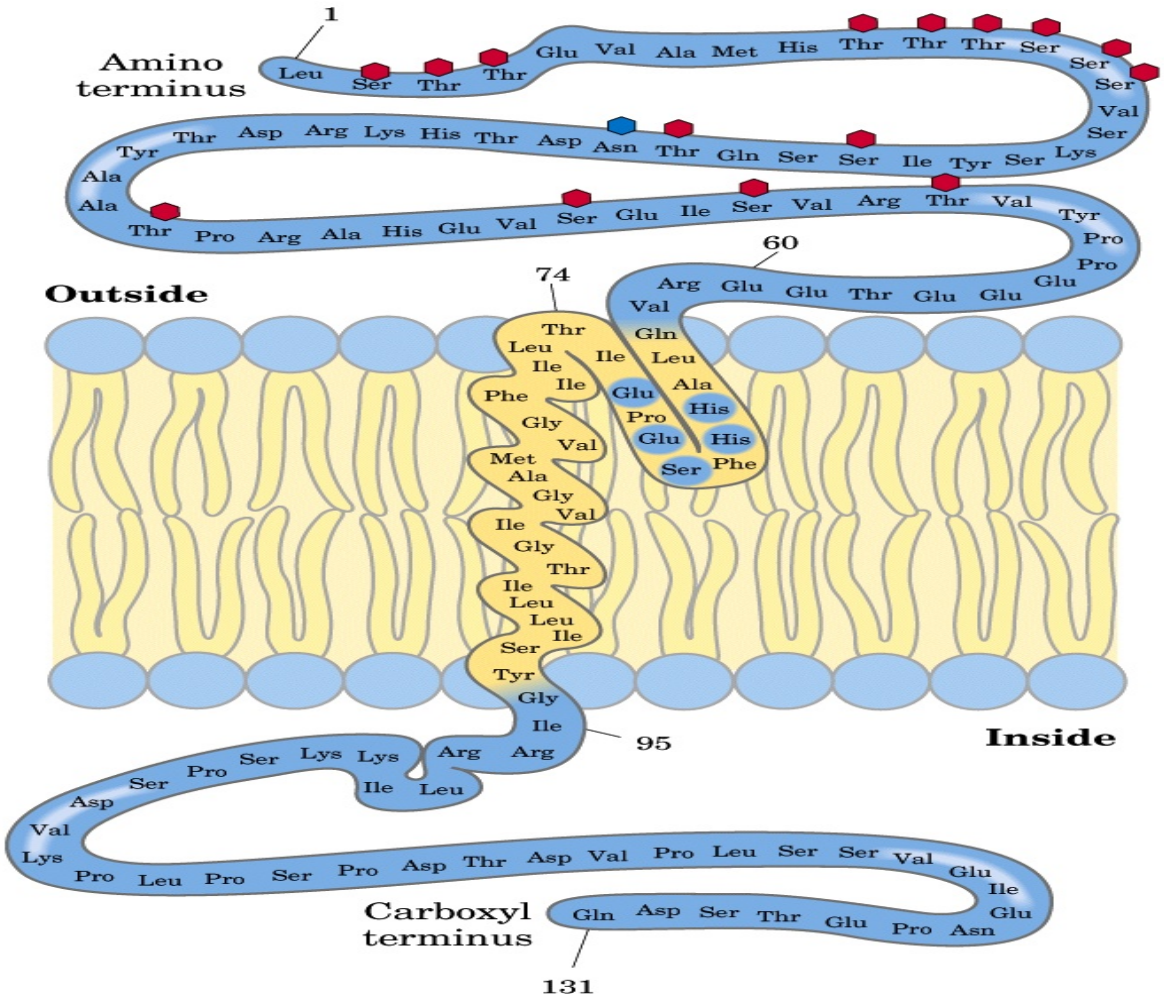
- Almost all secreted and membrane-associated proteins in eukaryotic cells are **glycosylated.**
- Oligosaccharides are covalently attached to proteins by either N-glycosidic or O-glycosidic bonds/linkages.
- A single protein may contain several N- and O-linked oligosaccharide chains, although different molecules of the same glycoproteins may differ in their sequences, locations, and numbers of covalently attached carbohydrates (**glycoforms).**

Glycoproteins Have Covalently Attached Oligosaccharides

O-linked and N-linked Glycans©



Glycophorin of the Erythrocyte Membrane©



Red hexagon:O-linked tetrasaccharide with two Neu5Ac, Gal and GalNAc;

Blue hexagon: N-linked glycan chain.

Some Properties of Mucins©

- Found in secretions of the gastrointestinal, respiratory, and reproductive tracts and also in membranes of various cells.
- Exhibit high content of O-glycan chains, usually containing NeuAc.
- Contain repeating amino acid sequences rich in serine, threonine, and proline.
- Extended structure contributes to their high visco-elasticity
- Form protective physical barrier on epithelial surfaces, are involved in cell-cell interactions, and may contain or mask certain surface antigens.
- Submandibular gland secretes mucin

Carbohydrates As Informational Molecules: The Sugar Code

1. Lectins that read the sugar code and mediate many biological processes
2. Lectin-Carbohydrate interactions are very strong and highly specific

Recognition of Oligosaccharide Structures by Lectins©

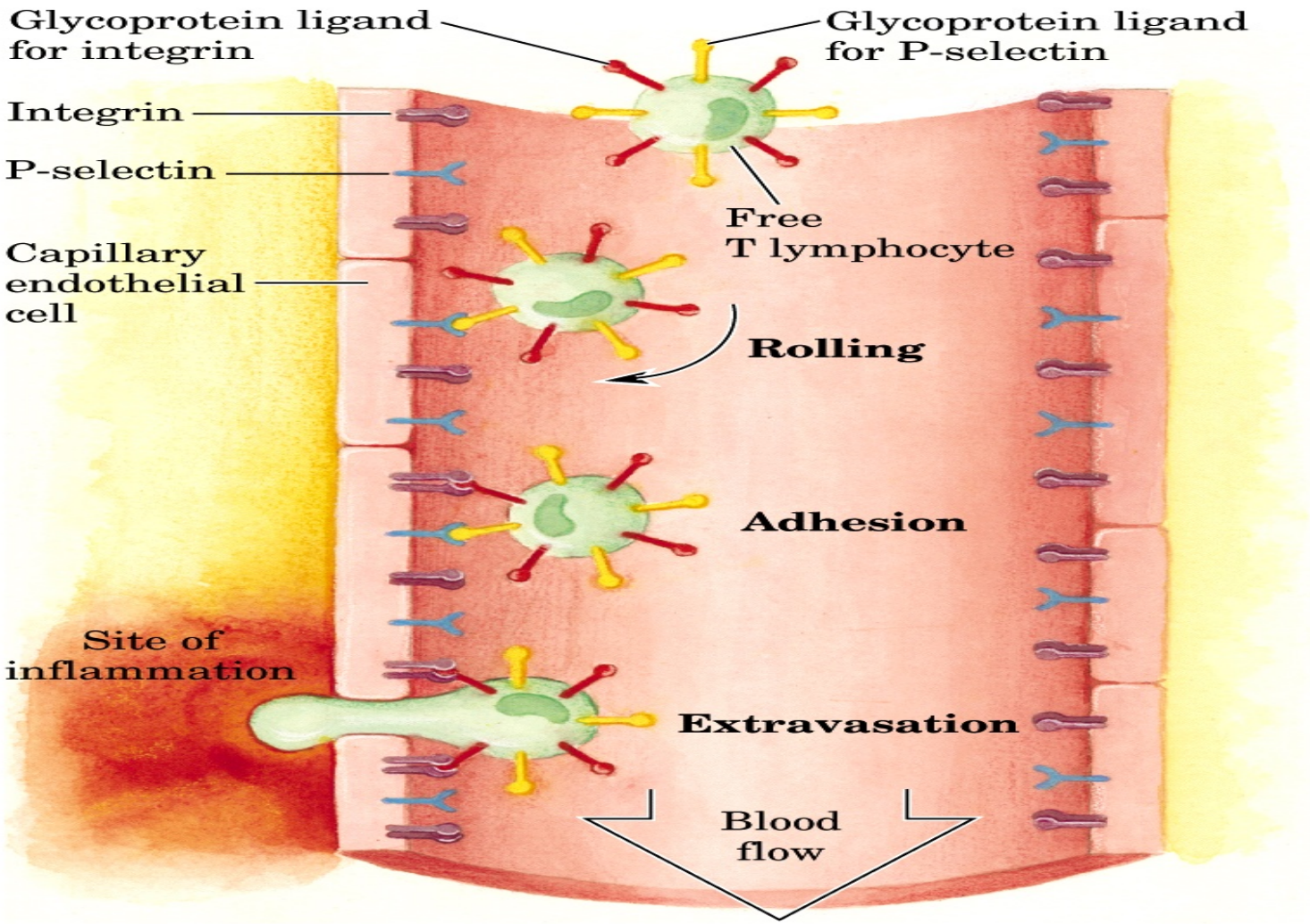
table 9–3

Lectins and the Oligosaccharide Ligands That They Bind

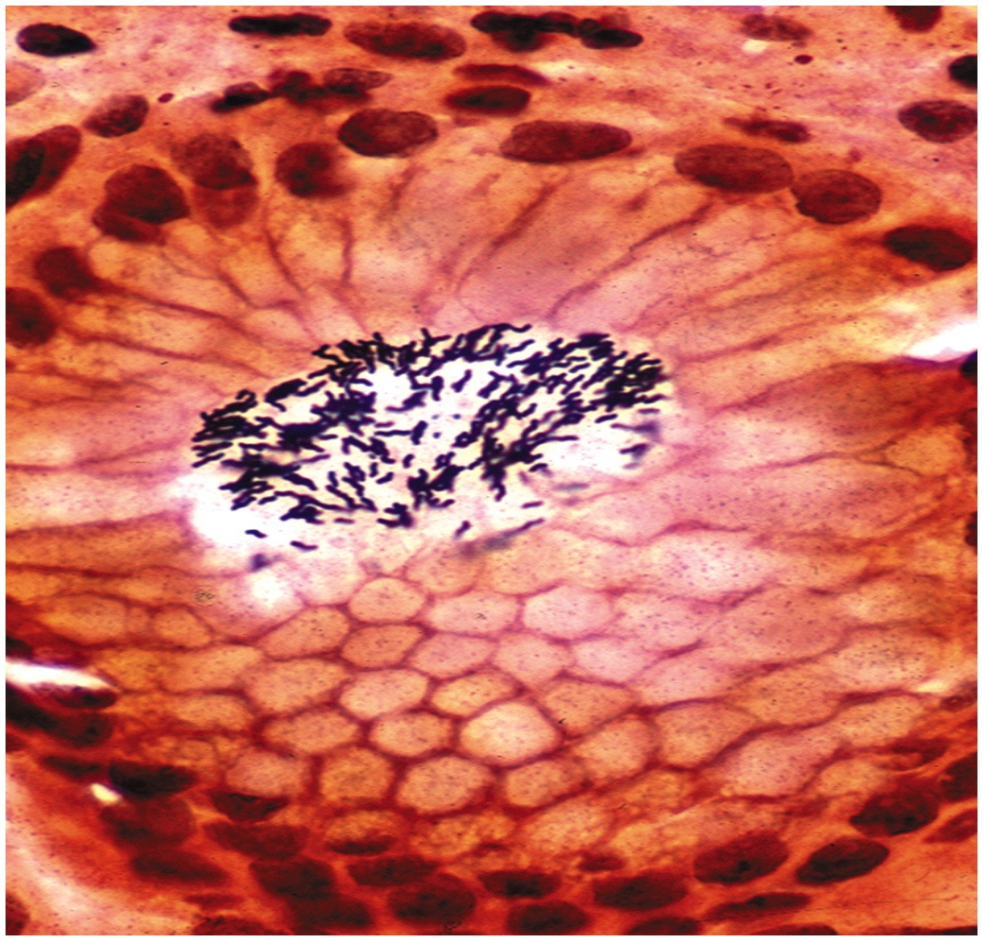
Lectin family and lectin	Abbreviation	Ligand(s)
Plant		
Concanavalin A	ConA	Manα1—OCH_3
Griffonia simplicifolia lectin 4	GS4	Lewis b (Le^b) tetrasaccharide
Wheat germ agglutinin	WGA	Neu5Ac(α2⟶3)Gal(β1⟶4)Glc GlcNAc(β1⟶4)GlcNAc
Ricin		Gal(β1⟶4)Glc
Animal		
Galectin-1		Gal(β1⟶4)Glc
Mannose-binding protein A	MBP-A	High-mannose octasaccharide
Viral		
Influenza virus hemagglutinin	HA	Neu5Ac(α2⟶6)Gal(β1⟶4)Glc
Polyoma virus protein 1	VP1	Neu5Ac(α2⟶3)Gal(β1⟶4)Glc
Bacterial		
Enterotoxin	LT	Gal
Cholera toxin	CT	GM1 pentasaccharide

Source: Weiss, W.I. & Drickamer, K. (1996) Structural basis of lectin-carbohydrate recognition. *Annu. Rev. Biochem.* **65,** 441–473.

Lectin-Ligand Interactions in Lymphocyte Movement to the Site of an Infection or Injury©

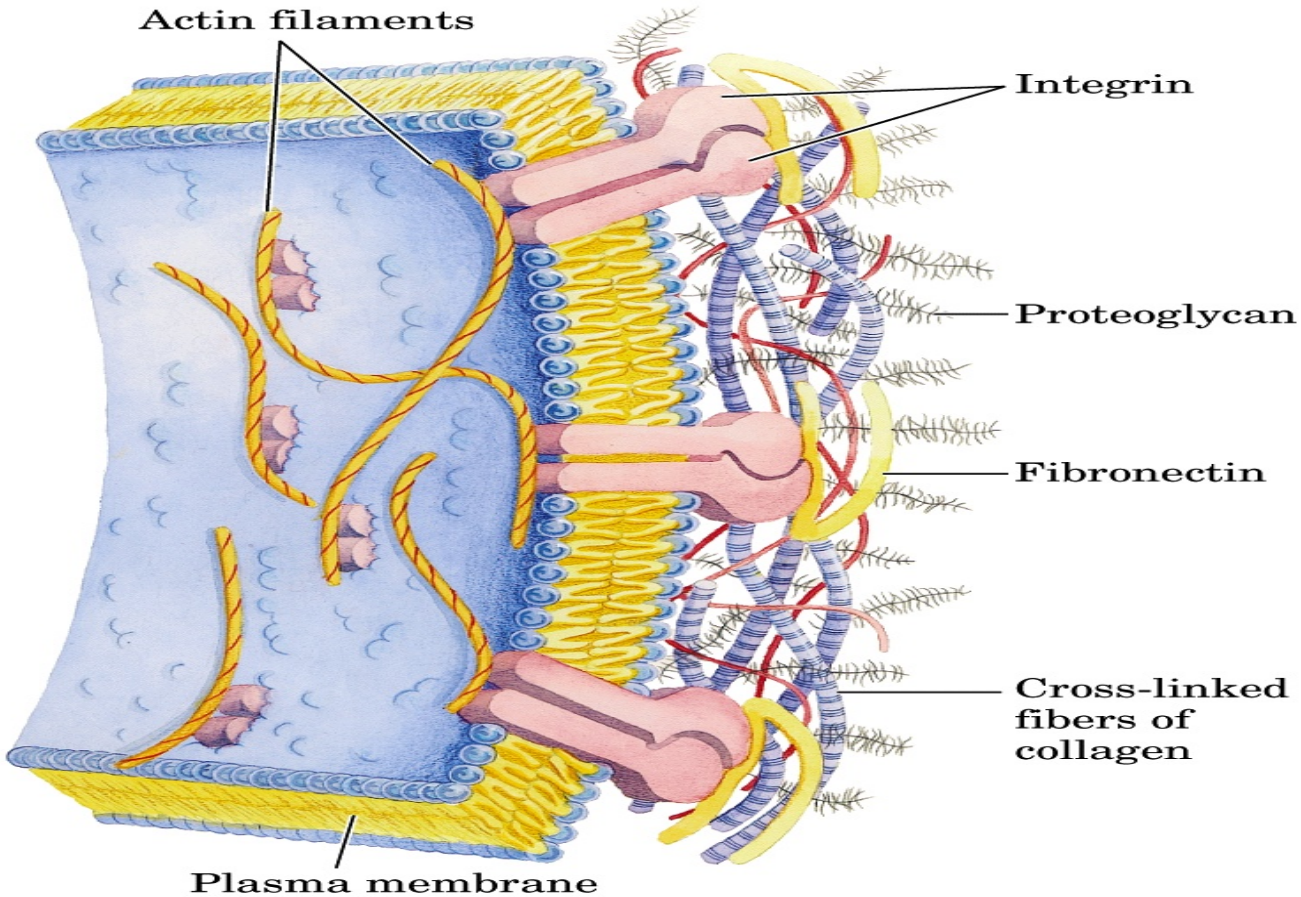


Adherence of *Helicobacter pyroli* to the Gastric Surface©

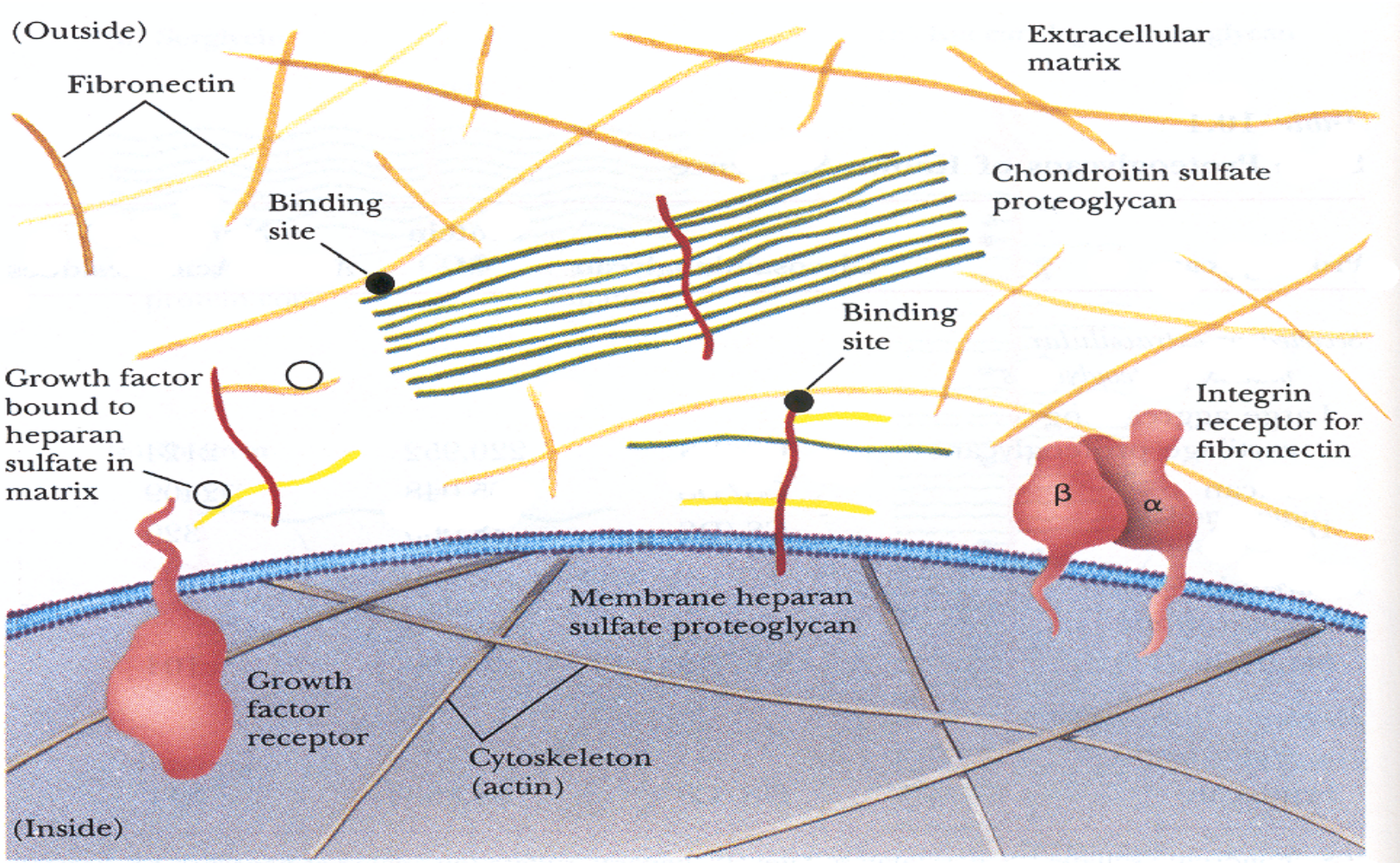


Interaction between a bacterial surface lectin and the Le^b oligosaccahride (a blood group antigen) of the gastric epithelium

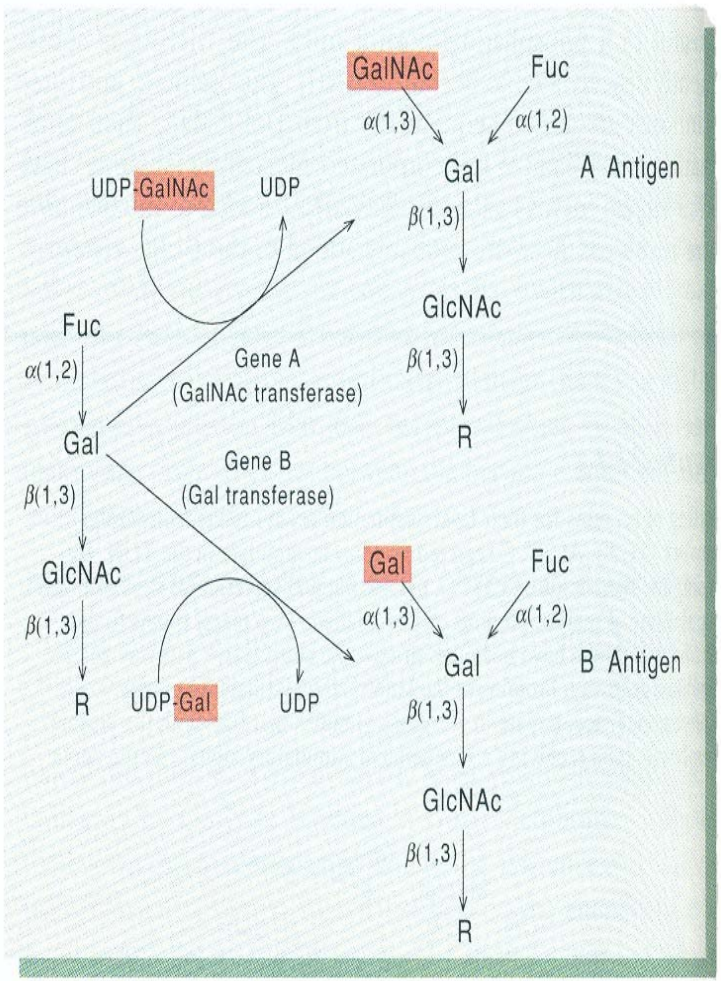
Interactions Between Cells and Extracellular Matrix©

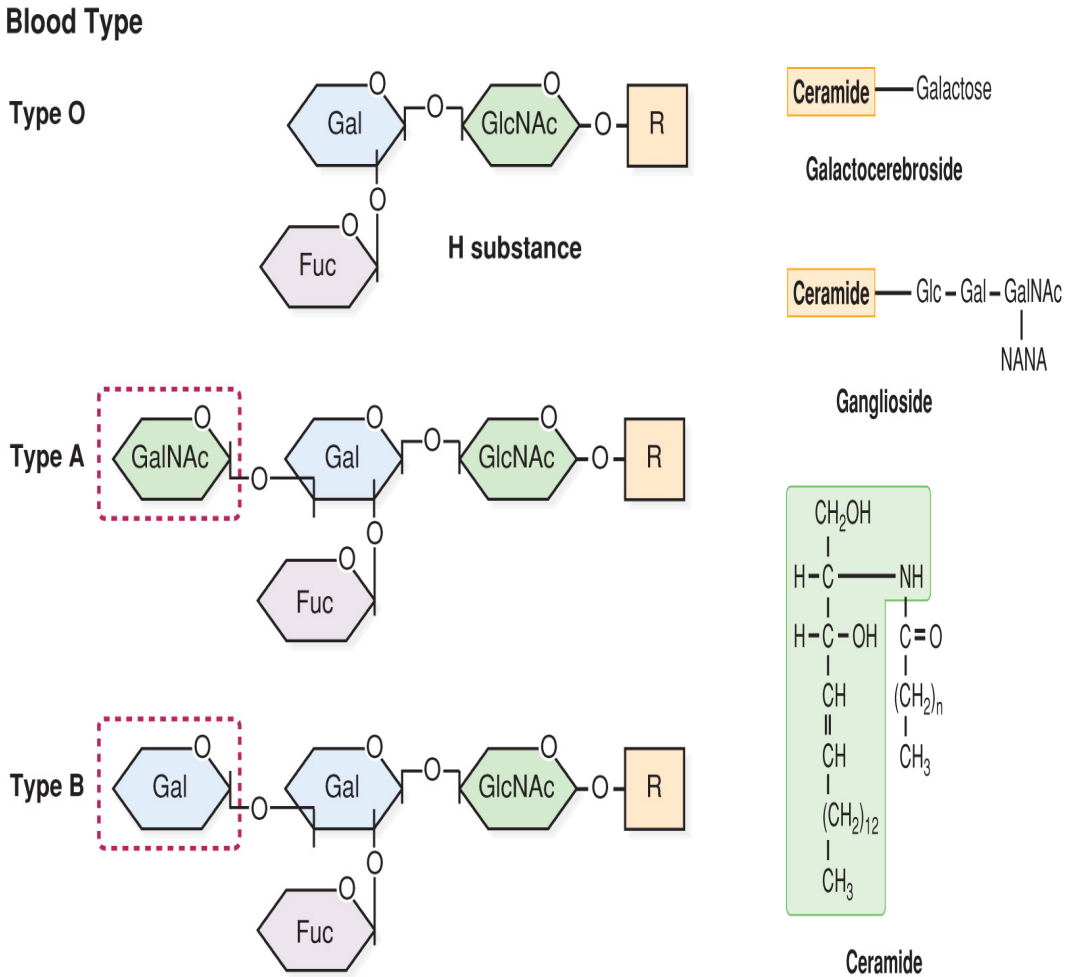


Interactions Between Cells and Extracellular Matrix©



Oligosaccharide Termini of the ABO Human Blood Group Antigens©





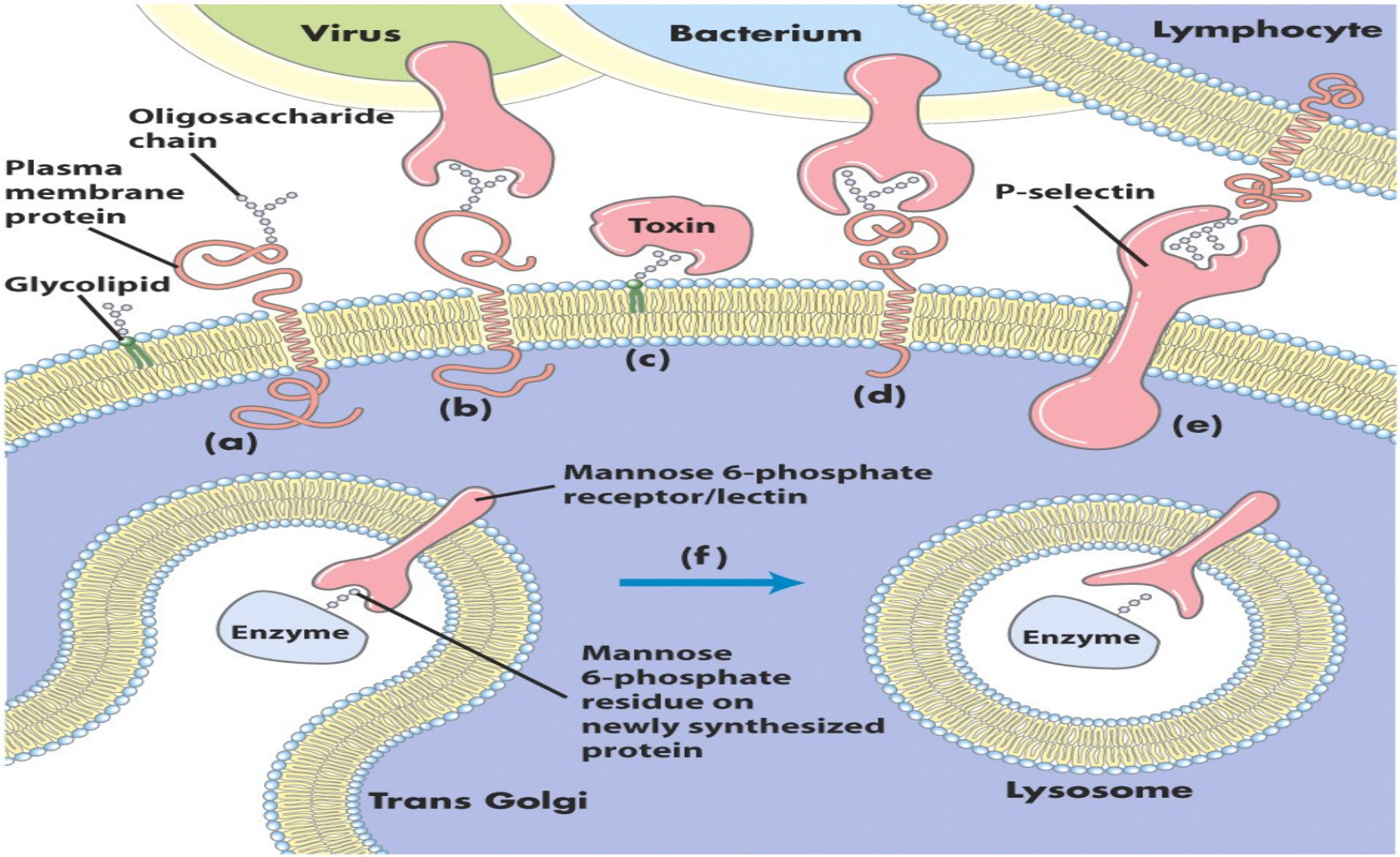
R = Either a protein or the lipid ceramide. **Lieberman and Marks; Figs 30.17 & 30.16**

Characteristics of the ABO Blood Groups

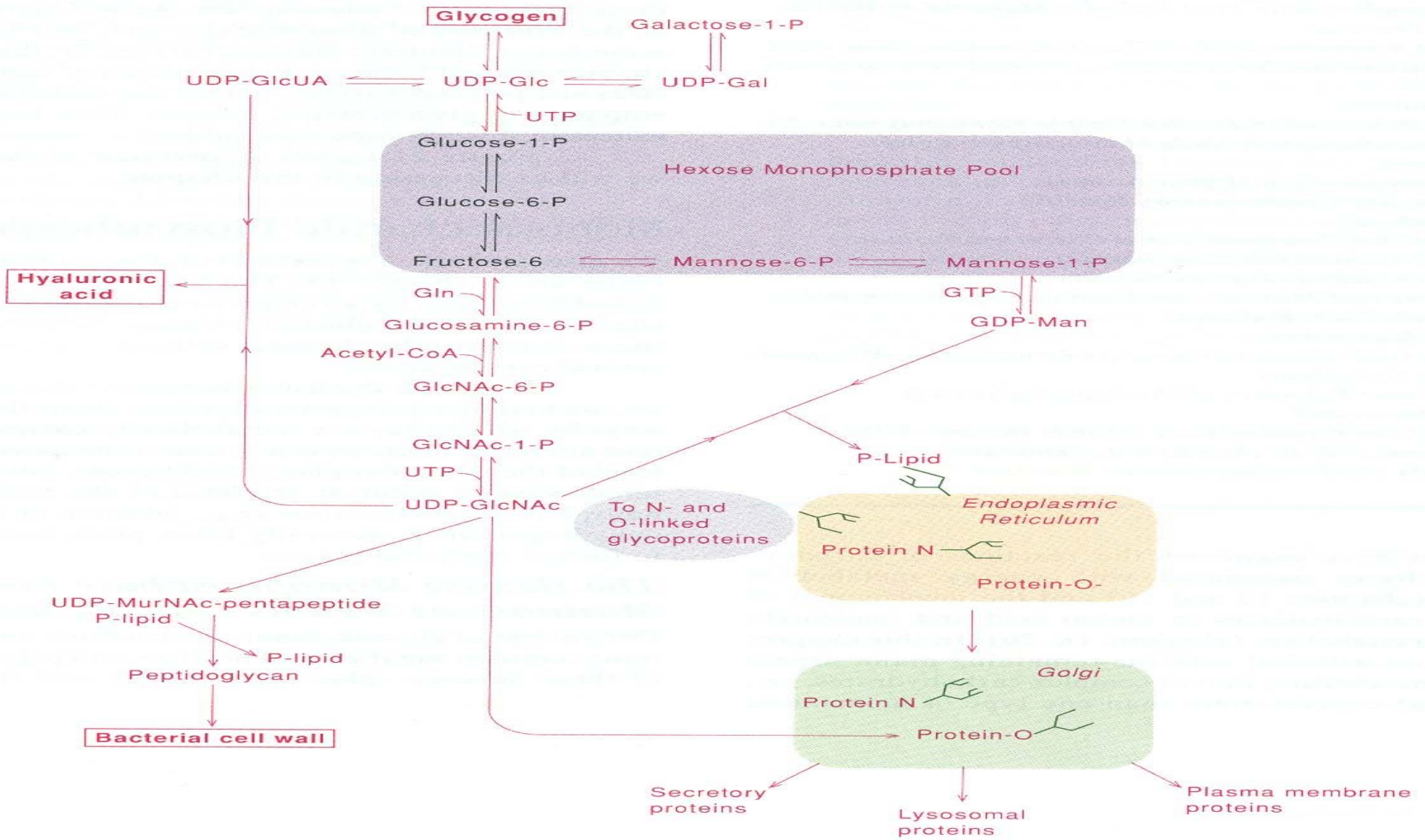
Red cell Type	O	A	B	AB
Possible genotype	OO	AA or AO	BB or BO	AB
Antibodies in serum	Anti-A and B	Anti-B	Anti-A	None
Frequency (in Caucasians)	45%	40%	10%	5
Can accept blood types	O	A, O	B, O	A, B, AB, O

Lieberman and Marks; Table 3.4

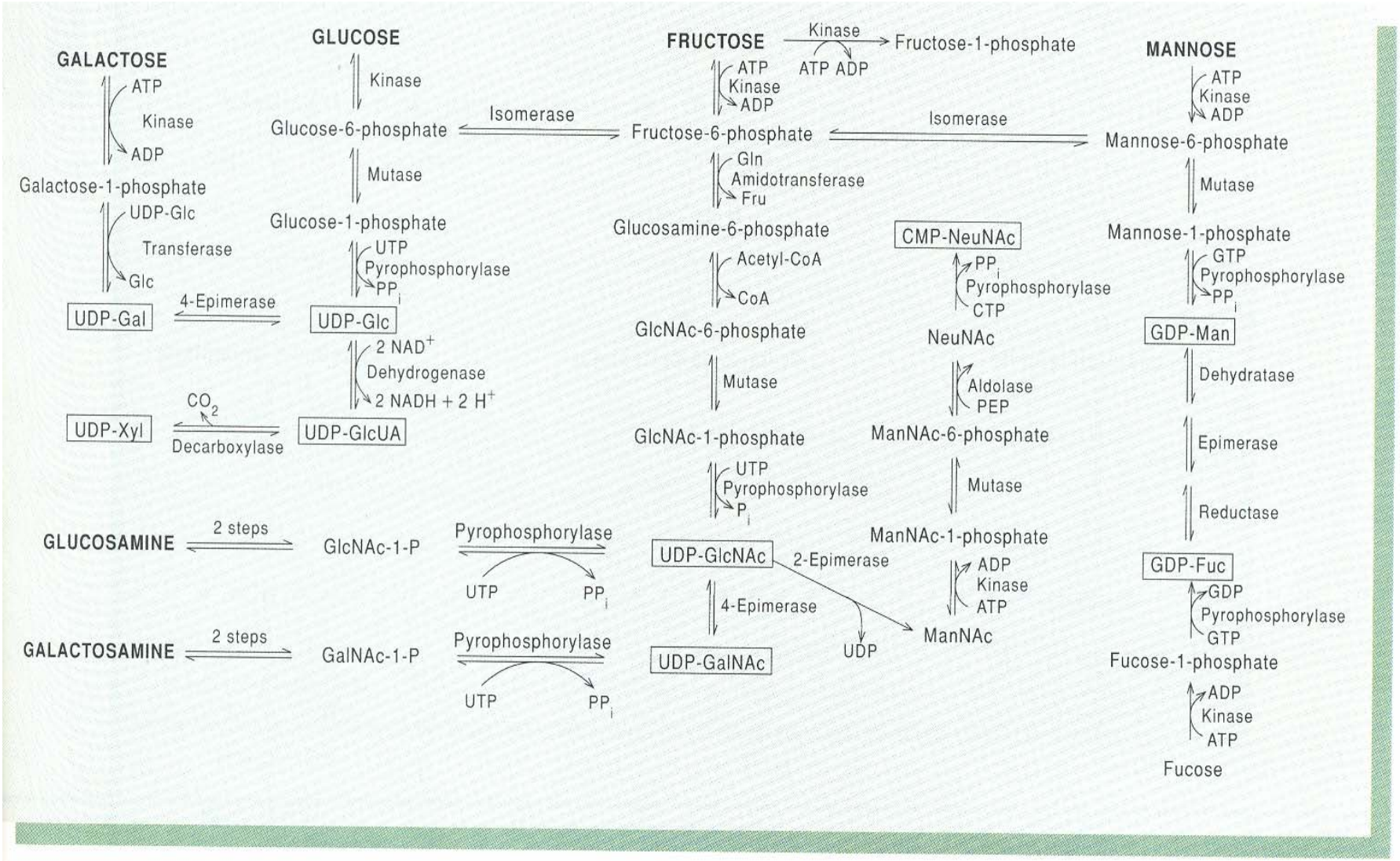
Roles of Oligosaccahrides in Recognition and Adhesion at the Cell Surface©



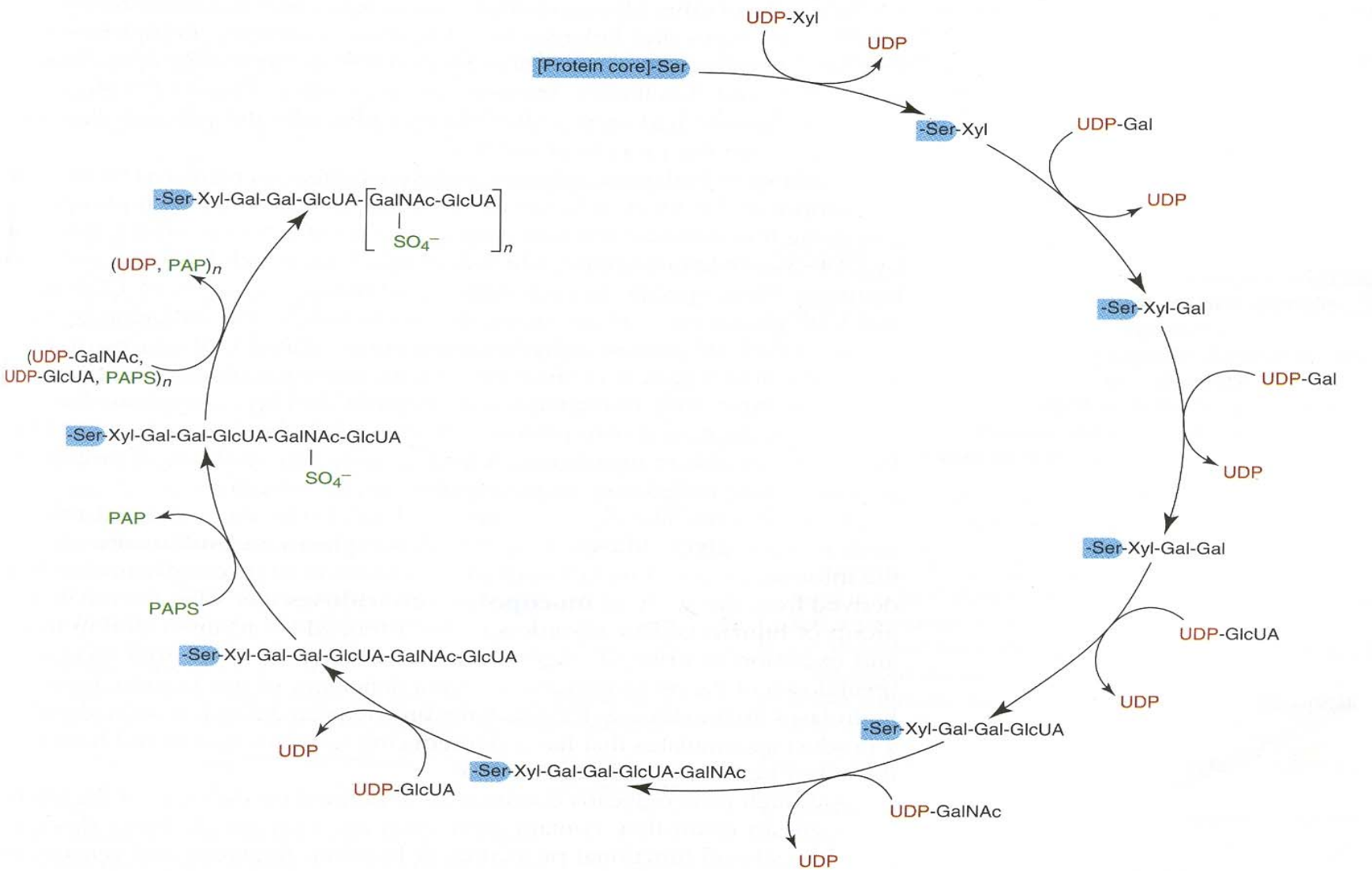
Outlines of the Synthesis of Complex Carbohydrates©



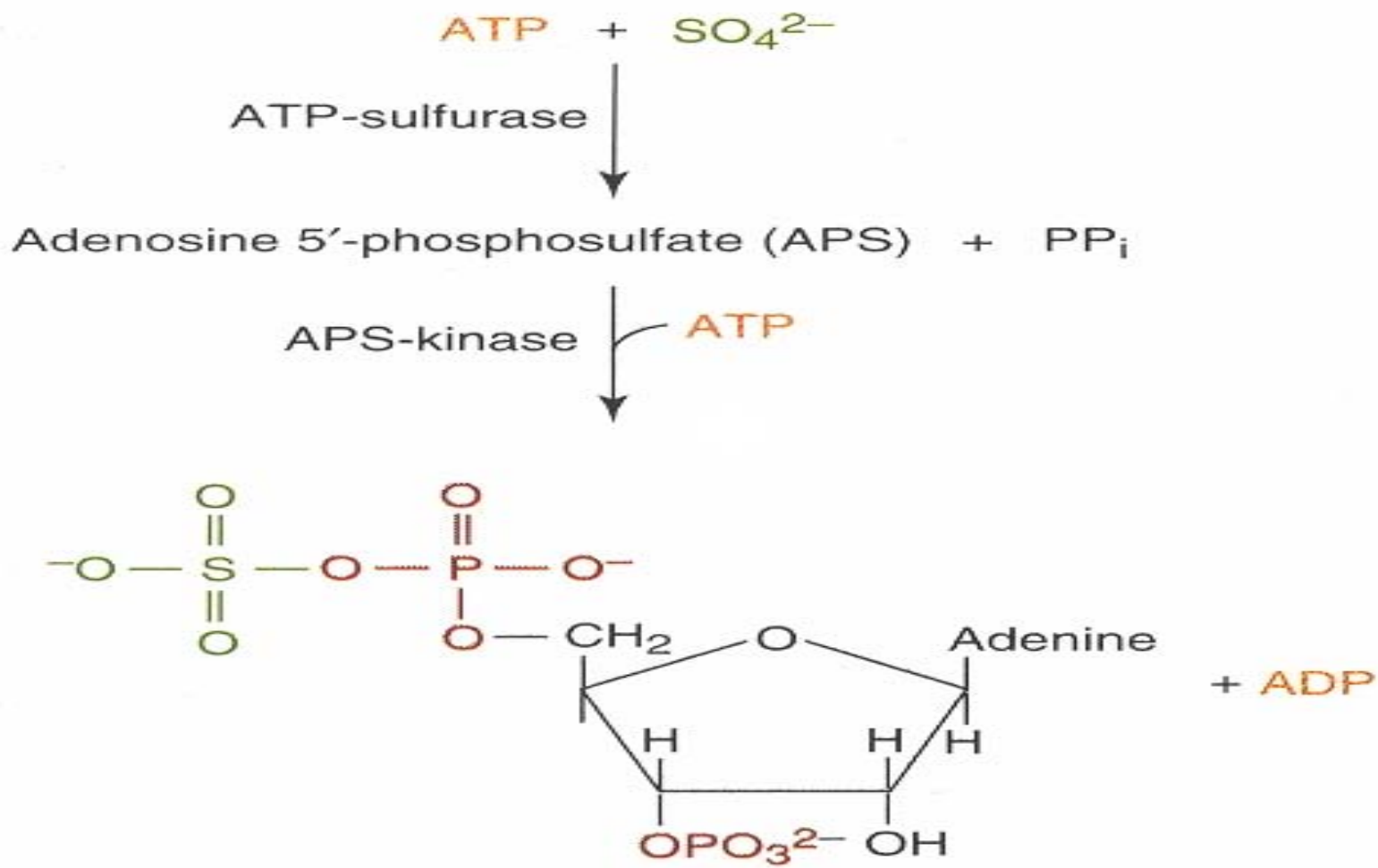
Monosaccharide Interconversions©



Biosynthesis of Chondroitin Sulfate©



Biosynthesis of 3’-phosphoadenosine 5’-phosphosulfate (PAPS)©



Enzymic Defects in the Mucopolysaccharidoses©

Enzymic Defects in the Mucopolysaccharidoses

Disease	*Accumulated Products*[a]	*Deficient Enzyme*[b]
Hunter	Heparan sulfate Dermatan sulfate	Iduronate sulfatase (1)
Hurler + Scheie	Heparan sulfate Dermatan sulfate	α-L-Iduronidase (2)
Maroteaux–Lamy	Dermatan sulfate	*N*-Acetylgalactosamine (3) sulfatase
Mucolipidosis VII	Heparan sulfate Dermatan sulfate	β-Glucuronidase (5)
Sanfilippo A	Heparan sulfate	Heparan sulfamidase (6)
Sanfilippo B	Heparan sulfate	*N*-Acetylglucosaminidase (9)
Sanfilippo D	Heparan sulfate	*N*-Acetylglucosamine 6-sulfatase (8)

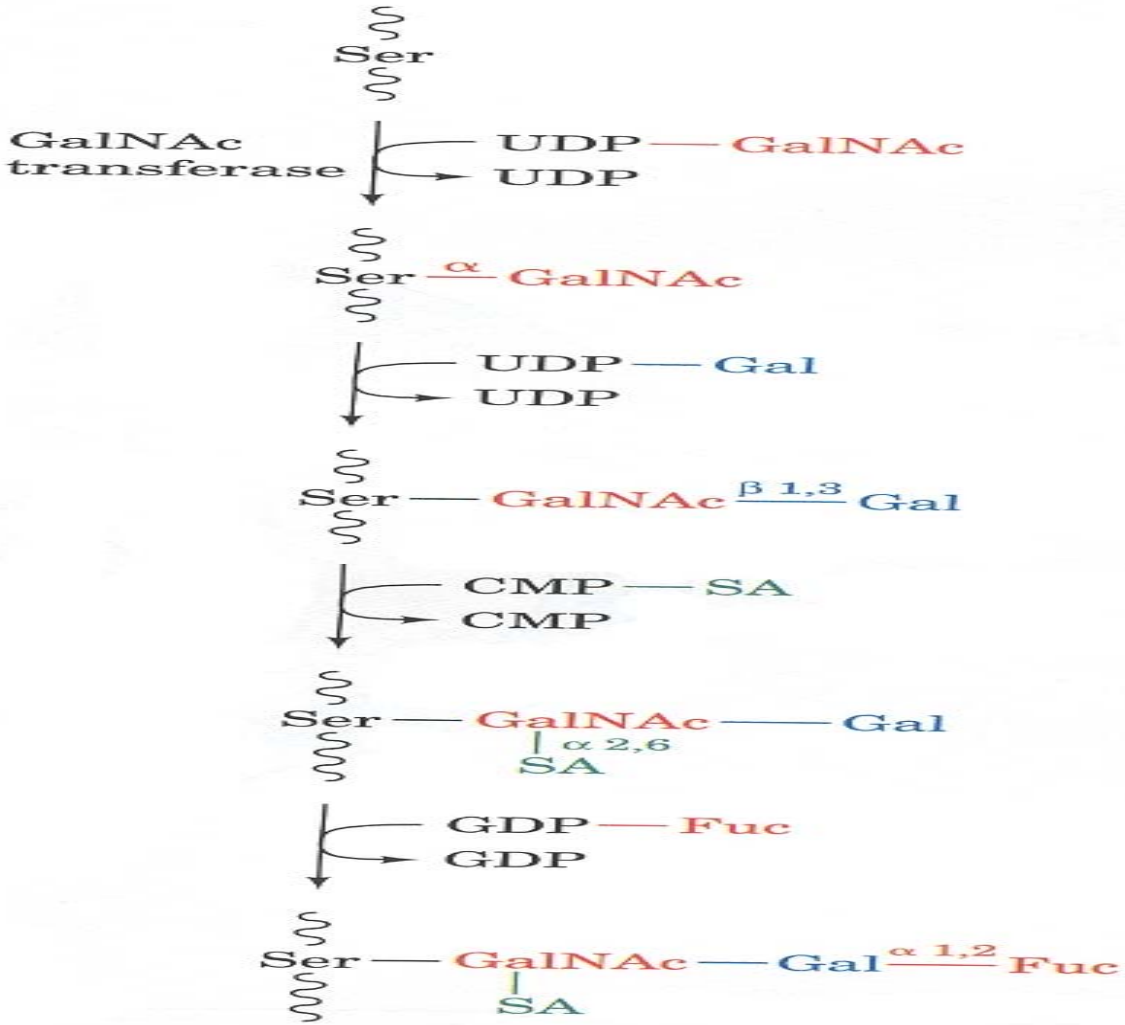
[a] Structures of dermatan sulfate and heparan sulfate.

Glycoprotein Synthesis©

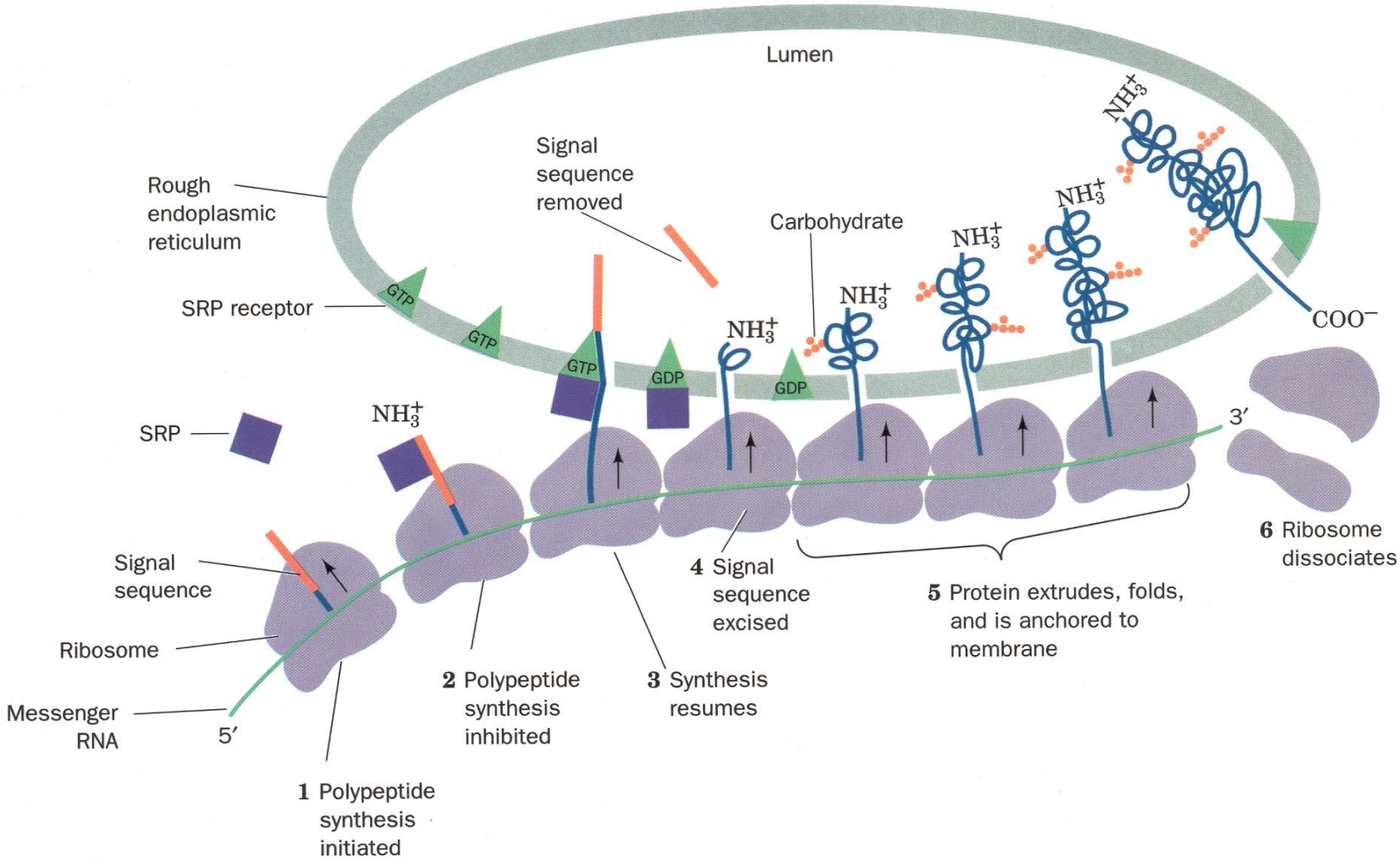
• In the early stages of N-linked oligosaccharide synthesis, sugar residues are sequentially added to a lipid carrier, **dolichol phosphate**. In the final steps, the oligosaccharide is transferred to the Asn residue present in the sequence Asn-X-Ser/Thr (where X is any residue except Pro and Cys) on a growing polypeptide chain

• Glycan chain of O-linked oligosaccharides are synthesized in the Golgi apparatus by the serial addition of monosaccharide units to a completed polypeptide chain. The location of the glycosylation site is thought to be specified by the secondary or tertiary structure of the polypeptide.

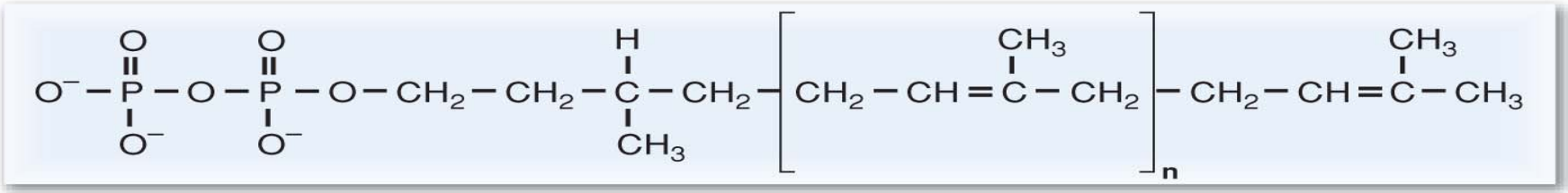
Synthesis of an O-linked Oligosaccharide Chain©



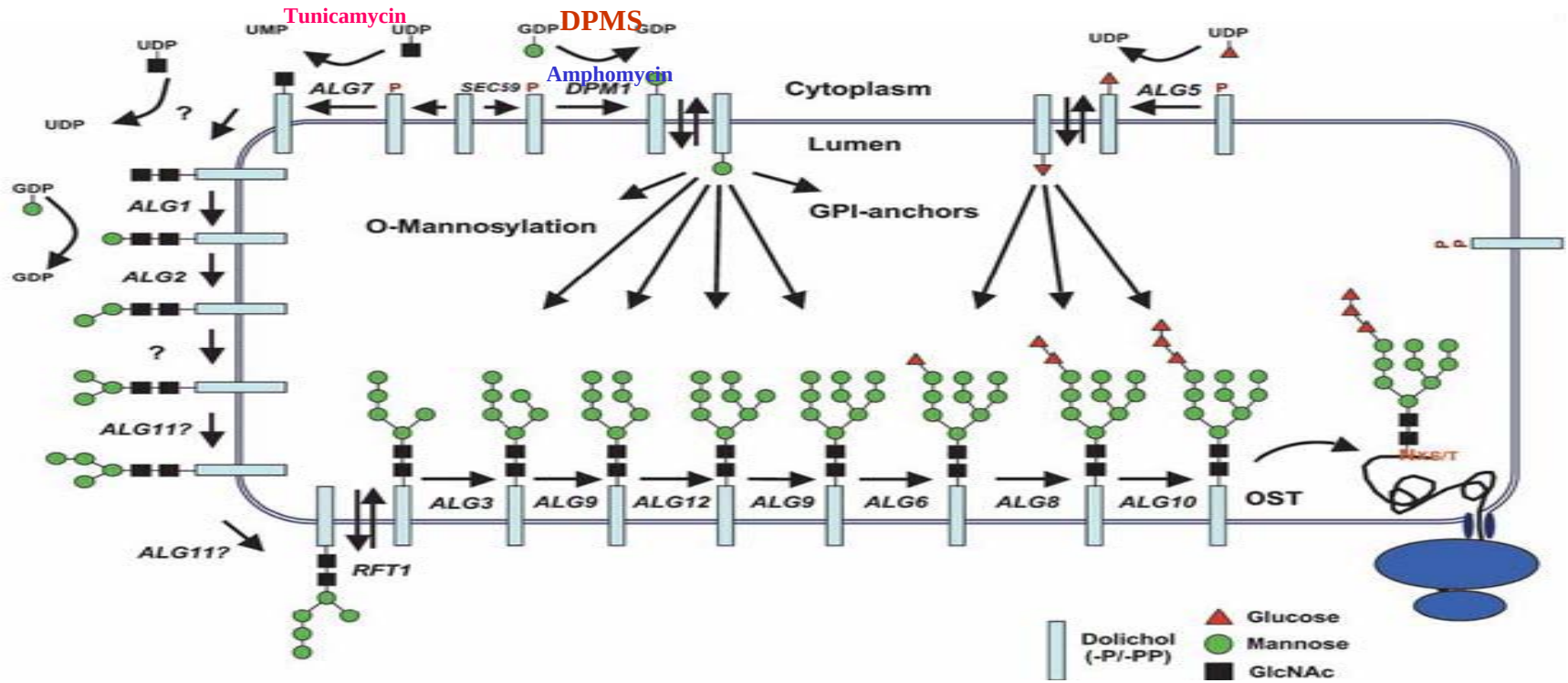
Targeting and Translocation of Proteins©



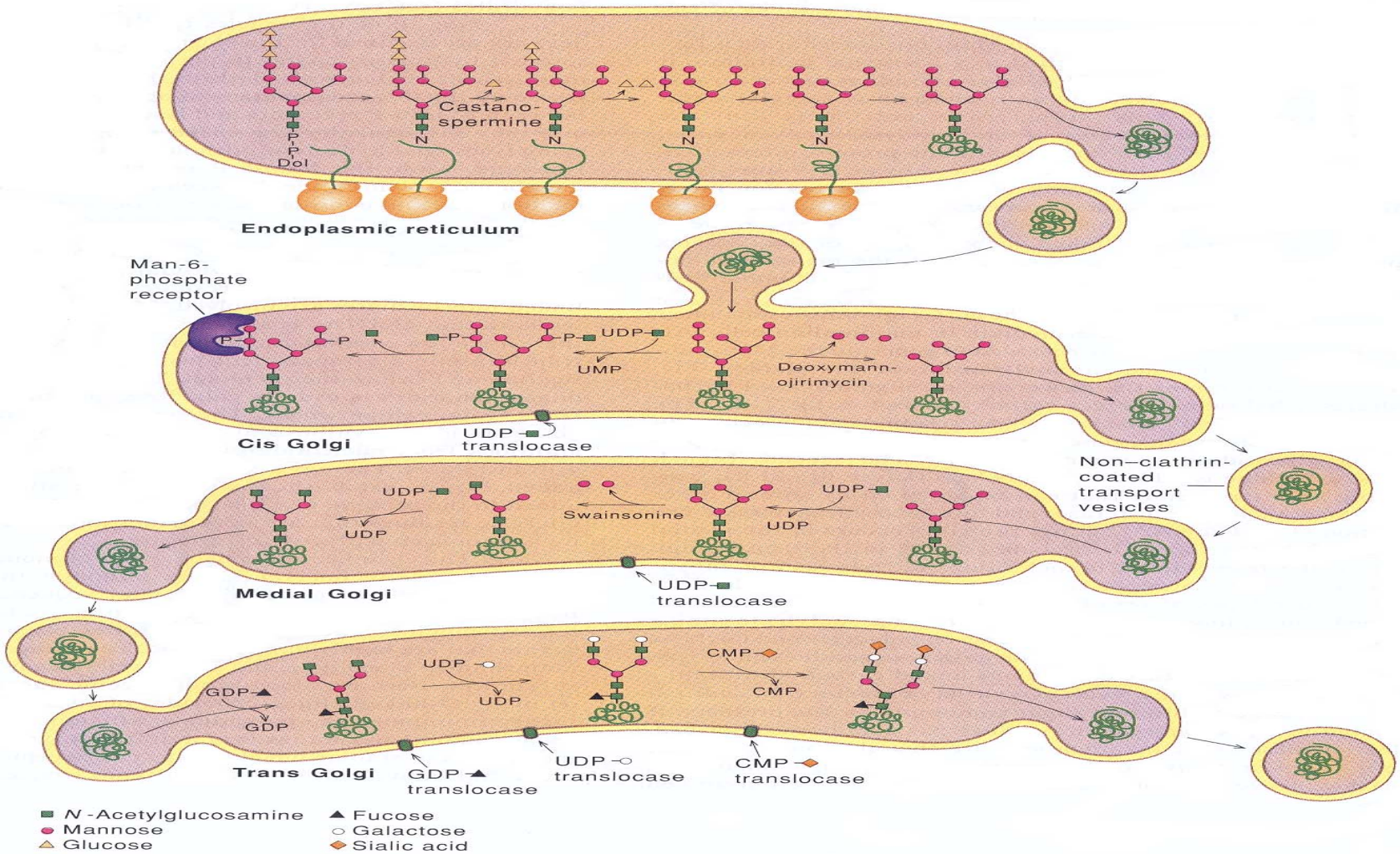
Action of Dolichol Phosphate in Transferring Oligosaccharide to Proteins



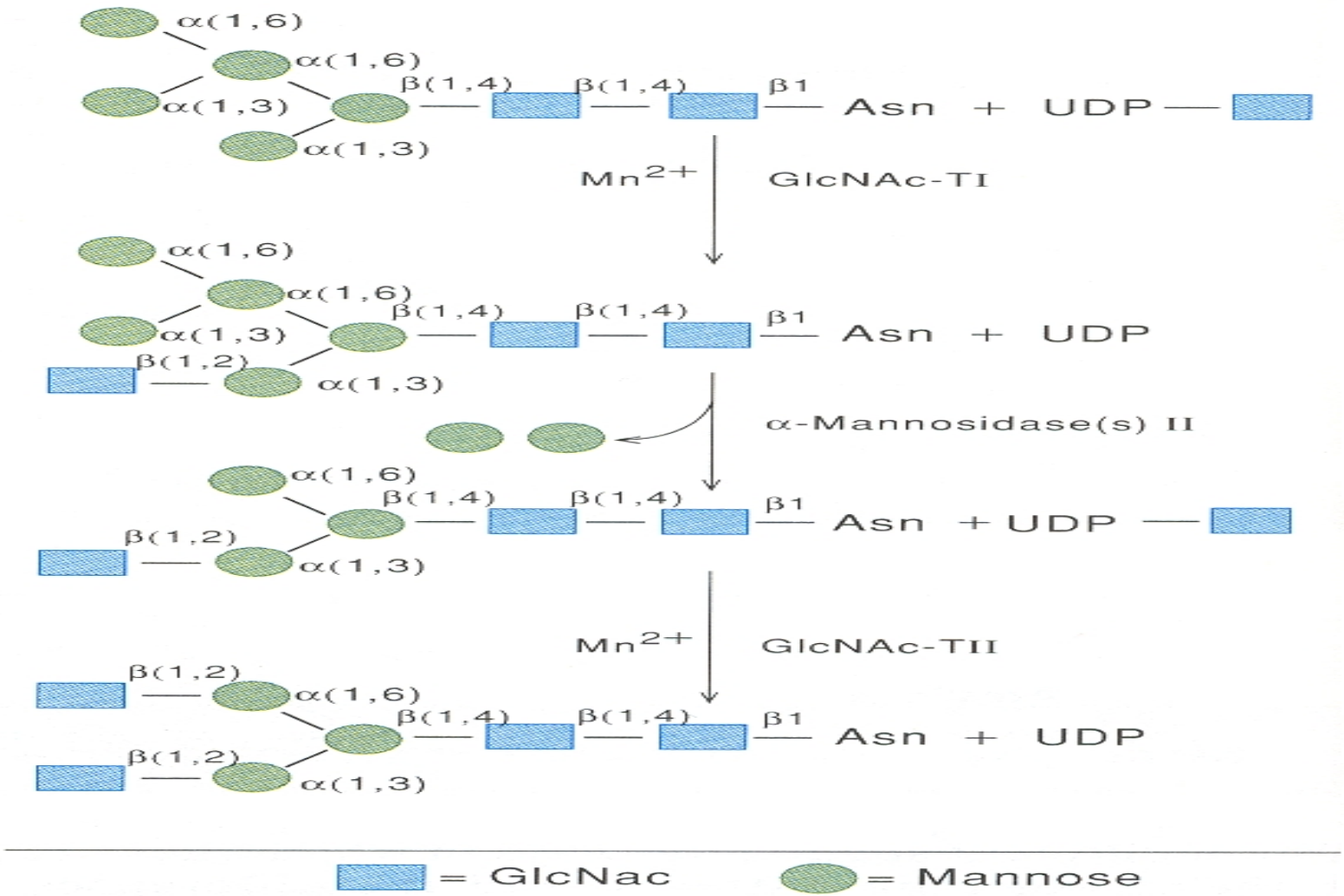
Structure of Dolichol Phosphate. **Lieberman and Marks; Fig. 30.13**



Transfer, Processing and Maturation of Oligosaccharides©



Specificity of Glycosyltransferases©



Clinical Correlations©

1. **Common Carbohydrate Marker of Lysosomal Targeting and I-Cell Disease:**

   Large inclusion bodies observed in cells cultured from patients; Clinical and radiological features include congenital dislocations, thoracic deformities, hernia, restricted joint mobility, and retarded psychomotor development; Lysosomal function is low due to a defect in processing the glycoprotein acid hydrolases, causing failure to generate the Man-6-P residue.

2. **Aspartylglycosylaminuria: Absence of 4-L-Aspartylglycosamine Amidohydrolase:**

   A group of human inborn errors of metabolism; Storage of glycolipids, glycopeptides, mucopolysaccharides, and

Clinical Correlations (*Cont.*)©

oligosaccharides; Defects in lysosomal glycosidase activity; Gradual accumulation in tissues and urine of compounds derived from incomplete degradation of the oligosaccharides accompanied by skeletal abnormalities, hepatosplenomegaly, cataracts, or mental retardation. One disorder allows accumulation of aspartyl-glucosamine-linked structures. Others involve accumulation of oligosaccharides derived from both glycoproteins and glycolipids, which may share common oligosaccharide structures.

3. **Congenital Defect in Glycoprotein Syndrome (CDGS):** A genetic disorder associated with a defect in protein N-glycosylation.

Bacterial Cell Wall Biosynthesis©

Biosynthesis of the cell wall is unusual in two respects:

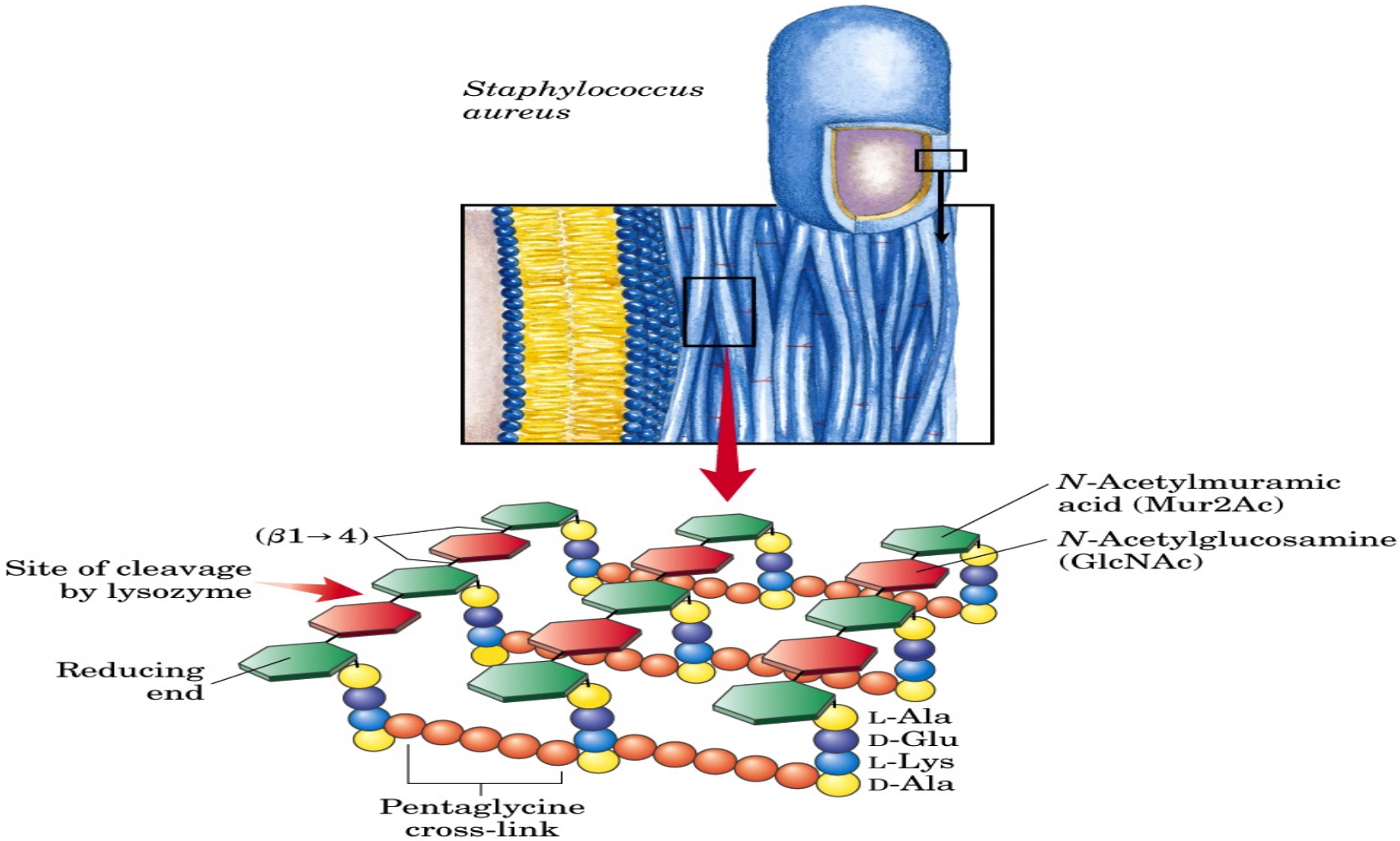
1. It is an example of the synthesis of a regularly cross-linked polymer.
2. Part of the synthesis takes place inside the plasma membrane and part takes place outside the plasma membrane.

Bacterial Cell Wall Biosynthesis©

The synthesis can be conveniently broken into three stages:

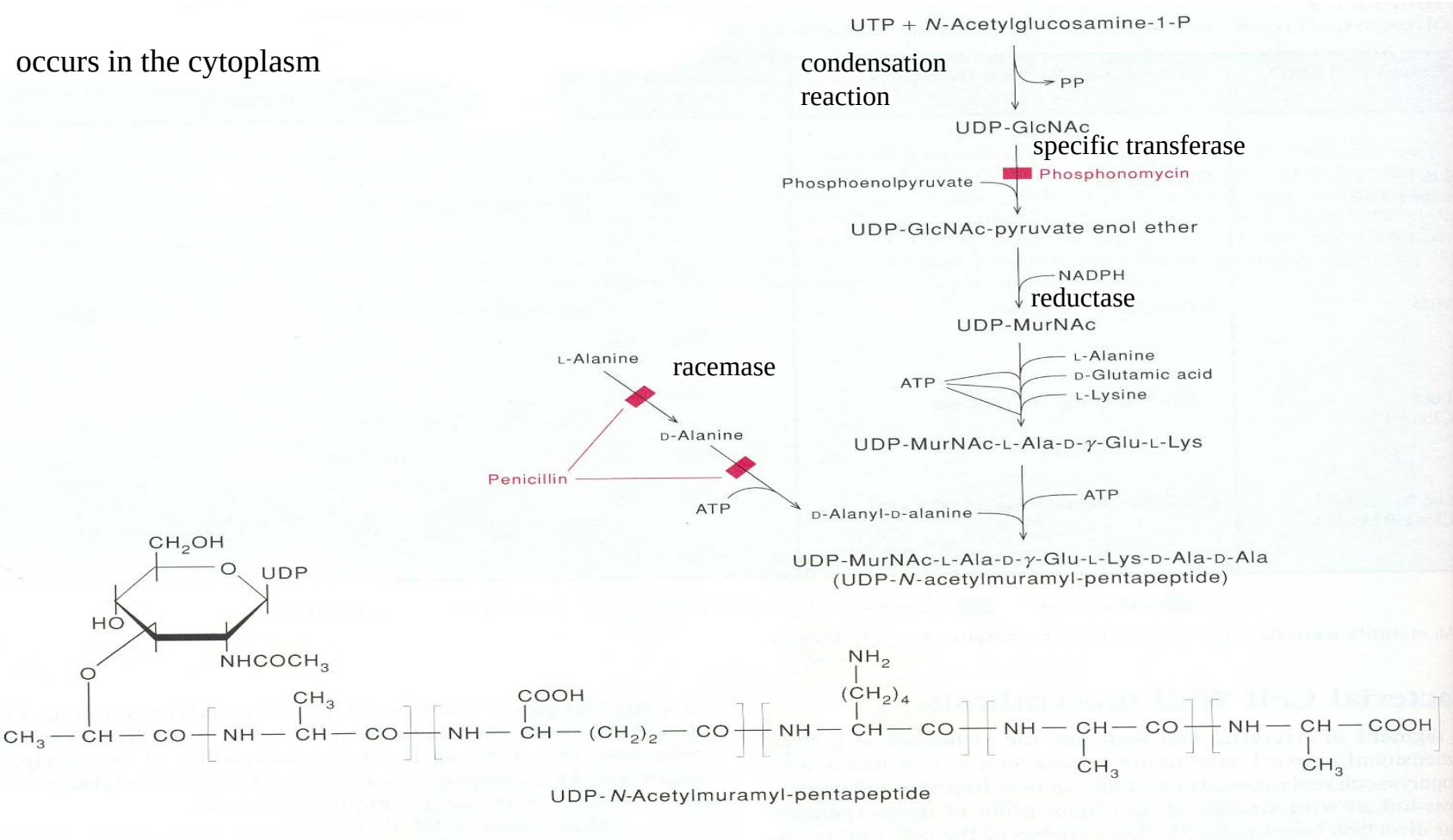
1. Synthesis of UDP-N-acetylmuramyl-penta-peptide
2. Polymerization of N-acetylglucosamine and N-acetylmuramyl-pentapeptide to form the linear pentapeptide strands.
3. Cross-linking of the pentapeptide strands.

Peptidoglycan of Bacterial Cell Wall©

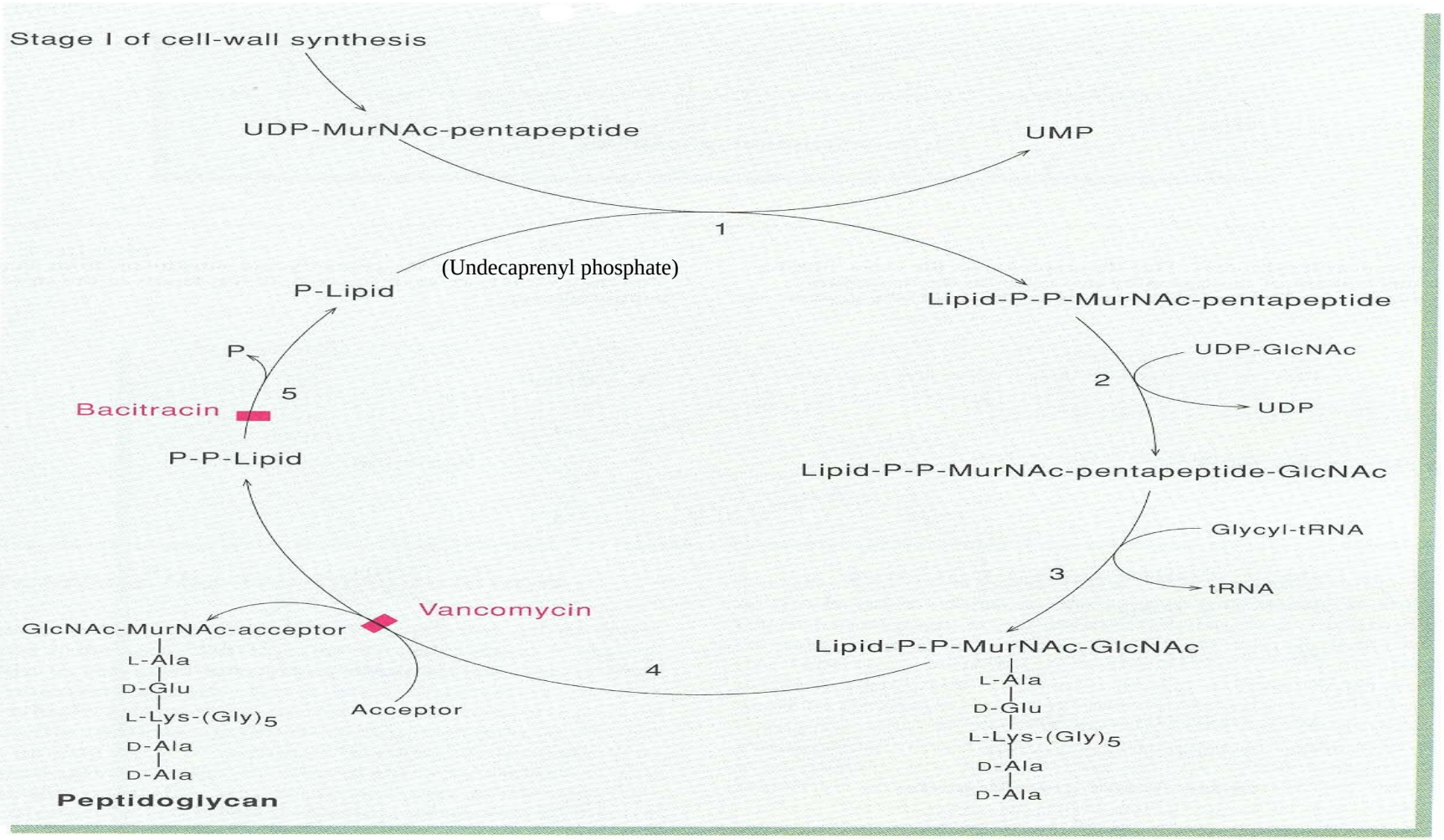


Synthesis of UDP-N-Acetylmuramyl-Pentapeptide Monomer: Stage I©

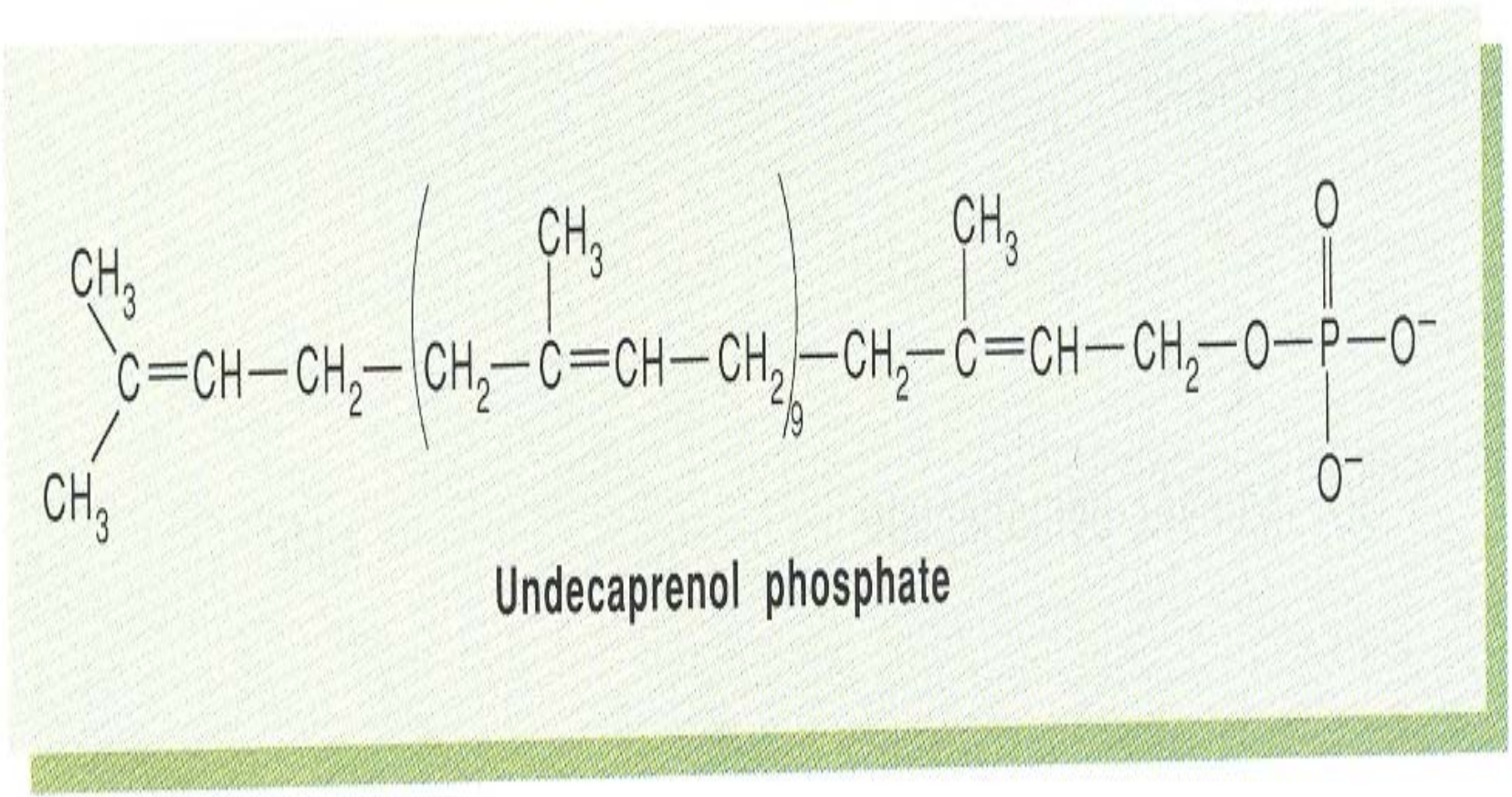
occurs in the cytoplasm



Formation of Linear Polymers of Peptidoglycan: Stage II©



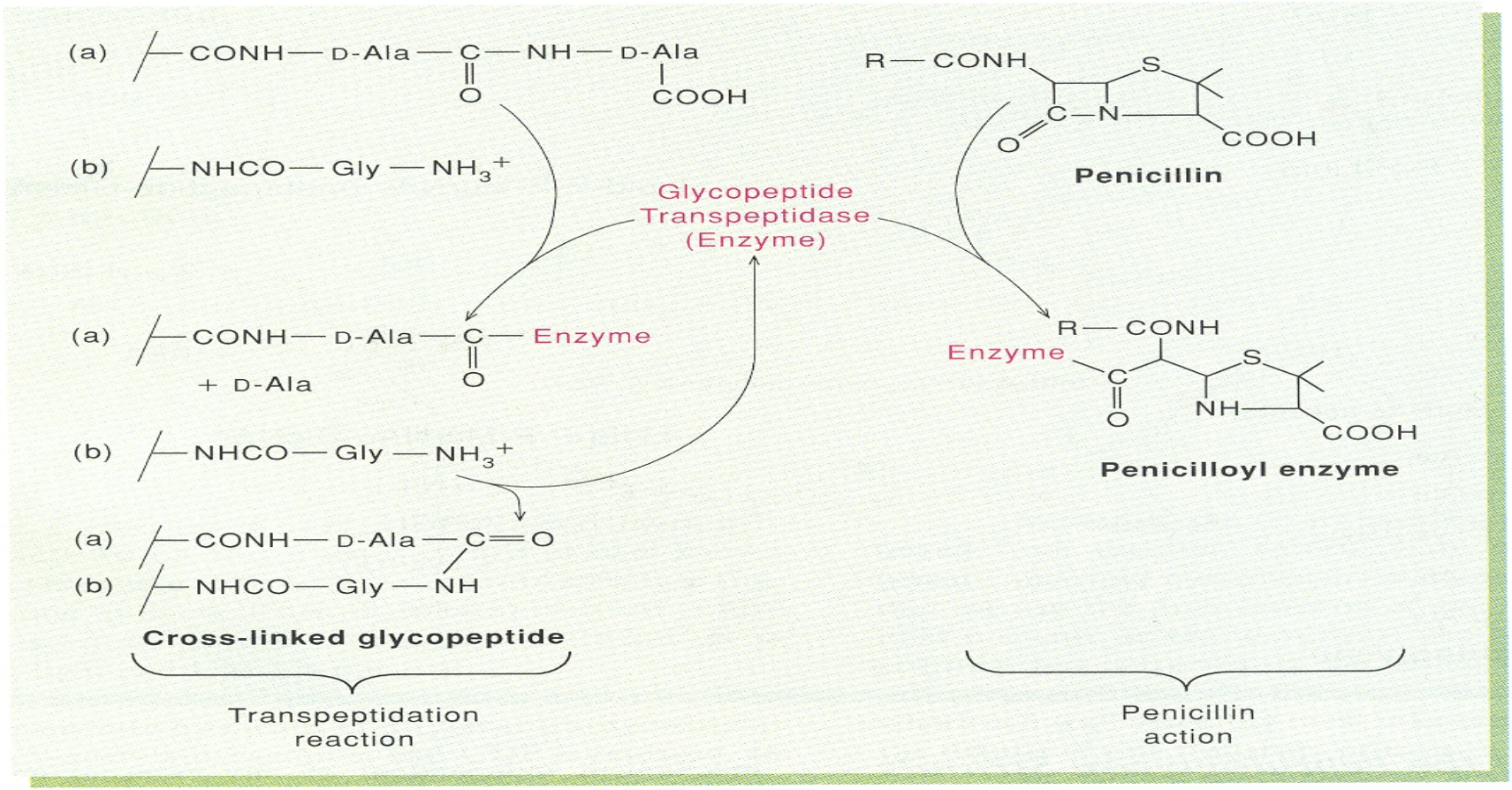
Undecaprenyl Phosphate©



Undecaprenol phosphate

Cross-Linking of the Peptidoglycan Strands: Stage III©

(Occurs on the noncytoplasmic side of the plasma membrane)



Stereomodels of Penicillin and D-alanyl-D-alanine©