1. Give brief definitions or unique descriptions of the following terms:
   a. Antisense strand
   b. Open RNA-Polymerase-promoter complex
   c. Codon
   d. Unsaturated fatty acid
   e. Ganglioside
   f. Micelle
   g. Paracrine hormone
   h. Pyranose
   i. Mutarotation

2. Below you can find the sequencing of the DNA coding strand.
   CAGGCTTGACACTTTATGCTTCCGGCTCGTATAATTTCTCCATATTGTGA

   GCCGTTTG<u>TAAGGAGGT</u>GATCATGGTTCGCTATACCTAAGTATGATAGCG
   <u>Shine-Dalgarno sequence</u>

   CGCATGCTGCGCGCTTTTTTTTTGCATAGACT

   a. Identify in this sequence the promoter and termination signal for transcription as well as the start and stop codon for translation.
   b. What is the amino acid sequence of the encoded protein? (Use the table provided at the end of the questions)

3. What is the function of the σ factor for RNA-Polymerase?

4. Write the reaction occuring during transcription initiation when the first phosphodiesterbond is formed between adenosine and uridine. Draw the structures as a stick diagram.

5. Provide four reasons why errors during transcription are not necessarily harmful.

6. Translation
   a. What are the four key features of the genetic code. Explain each feature in one sentence.
   b. What is the functional difference between mRNA and tRNA?
   c. Give the sequence of one codon matching the following anticodons and state which amino acid is attached to the tRNA. (Use the table of the genetic code provided in the lecture notes.)
   anticodon 3' – C G U – 5' 3' – A A G – 5'

   d. The mRNA specifying the a chain of human hemoglobin contains the base sequence ...UCCAAAUACCGUUAAGCUGGA..... The C-terminal tetrapeptide of the normal 'A' chain, which is specified by part of this sequence, is ...-Ser-Lys-Tyr-Arg. In a mutant hemoglobin called hemoglobin “Constant Spring”, the corresponding region of the 'A'-chain has the sequence ...-Ser-Lys-Tyr-Arg-Gln-Ala-Gly-...

Specify the mutation on the mRNA level which causes hemoglobin “Constant Spring”. (Again use the genetic code table.)

7. Aminoacyl-tRNA
   a. Write down the first reaction during charging of a tRNA with an amino acid by aminoacyl-tRNA Synthetases (no structures required).
   b. Draw the structure of the last A of the tRNA and the attached amino acid (use R as a symbol for the side chain).

8. Translation
   a. What are the names of the three tRNA binding sites on the ribosome? Which tRNAs typically bind to each of these sites?
   b. Describe with one sentence each phase of the translation elongation cycle.
   c. Fill the following table which indicates which type of tRNA occupies the given tRNA-binding site on the ribosome or wether the site is empty. Note that we ask for the occupancy BEFORE the indicated phase of the elongation cycle.

	A-site	P-site	E-site
Before A-site binding			
Before peptide bond formation			
Before translocation			

9. Draw the structures of the following lipids:
   a. a triacylglycerol containing the following fatty acids: stearic acid (18:0), palmitoleic acid (16:1Δ^{9}), and linoleic acid (18:2$\Delta^{9,12}$).
   b. Beewax consisting of palmitic acid (16:0) and a 30-carbon saturated alcohol.
   c. Phosphatidylethanolamine (a glycerophospholipid) with your choice of fatty acids.
   d. A ceramide, the simplest shingolipid.

10. Identify each as a fatty acid, steroid, triacylglycerol, glycerophospholipid or sphingolipid:
    1. cholesterol

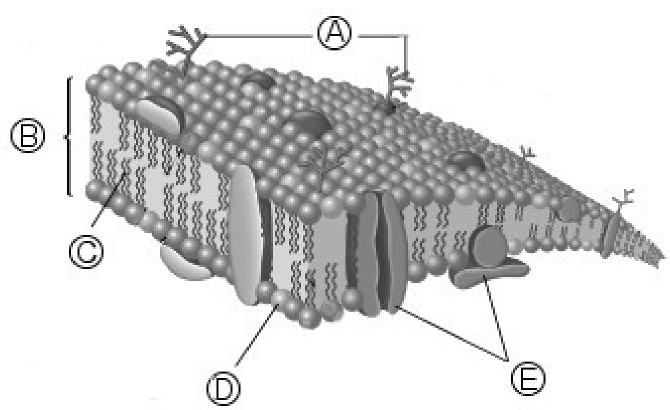
2. glycerol, 2 fatty acids, phosphate, and choline
3. glyceryl tristearate
4. sphingosine, fatty acid, phosphate, and choline

11. Which of the following biomolecules are found in cell membranes?
    a. cholesterol
    b. triacylglycerol
    c. carbohydrates
    d. proteins
    e. waxes
    f. glycerophospholipids
    g. sphingolipids

12. Imagine the planet Gibo in a far-off galaxy where the life forms have a similar appearance to those on earth. However, there is one huge difference: heptane on Gibo has the role played by water on earth.
    If the molecules that form cell membranes are the same as those on earth, what is the major difference in the construction of cell membranes on Gibo compared with earth?

13. Membrane compositions of fish and other cold-blooded animals change when their environmental temperature is lowered. The unsaturated fatty acid content of the lipids in the cell membranes increases when the organism becomes adapted to the lower temperature.
    What is the purpose of this increase?

14. Identify in the diagram the following components of the cell membrane: Integral and peripheral membrane protein, carbohydrates, lipid bilayer, polar heads of lipids, and nonpolar tails of fatty acids.



15. The biochemists Eugene Kennedy and James Rothman have investigated the biosynthesis of membrane lipids. They gave growing bacteria a short pulse of radioactive phosphate which enters the cell so as to label the newly synthesized phospholipids. Immediately afterwards, they added a membrane-

impermeable TNBS label to the bacteria that combines with phopholipids. Analysis of the resulting membrane showed that no TNBS-labeled phospholipid was radioactively labeled.

a. Based on this experiment, where do you conclude are the phospholipids synthesized?
b. Next, they repeated the experiment but allowed 3 min to elapse between the pulse of radioactive phosphate and the TNBS addition. In this case, about half of the TNBS-labeled phospholipid is also radioactive. How can you explain this result?

16. Identify the general type of transport which is described in the following examples.

a. Chloride ions pass through protein channels into cells under conditions where the extracellular chloride concentration exceeds the intracellular concentration.
b. Lactose is transported into cells through integral membrane proteins under conditions in which the intracellular concentration of lactose exceeds the extracellular concentration.

17. Identify the type of active transport which is describe in the following examples:

a. The Ca^{2+}-ATPase pumps Ca^{2+} out of the cytosol as ATP is hydrolysed.
b. The (H^+-K^+)-ATPase of the gastric pumps protons out of the cell. Each proton is accompanied by the transport of a K^+ into the cell.
c. Subsequently to the action of the describe (H^+-K^+)-ATPase, the K^+ is again transported out of the cell in parallel to the export of Cl^- by a different transporter.

18. Draw the structures of the following carbohydrates:

a. As many D-aldopentoses as possible in the linear form
b. A ketohexose in the linear and cyclic, α-anomeric form
c. A disaccharide of two riboses conected by a (β1-α1) glycosidic bond.
d. A disaccharide building block of the polysaccharide amylose.

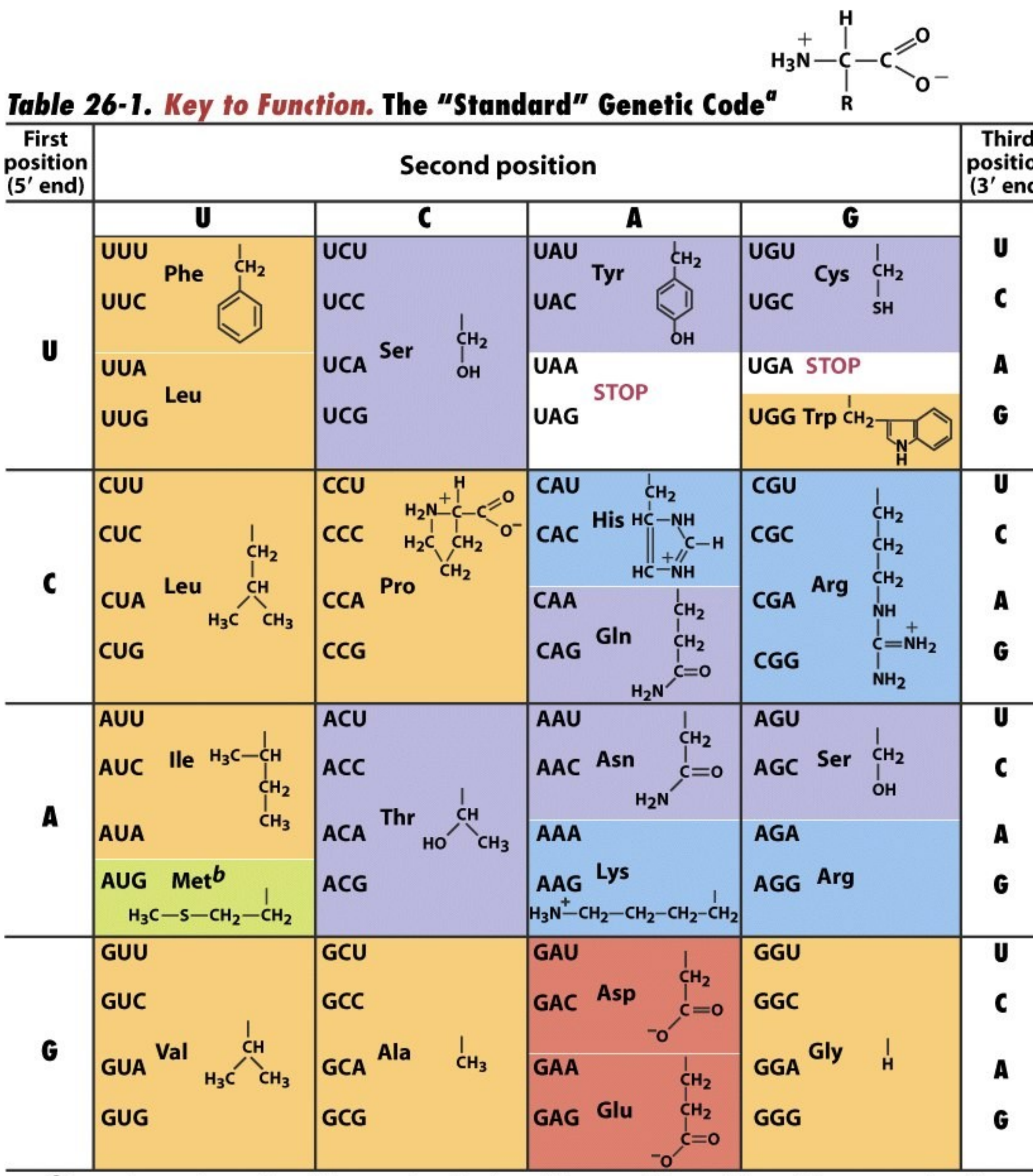


Table 26-1. Key to Function. The "Standard" Genetic Code[a]

First position (5′ end)	Second position: U	Second position: C	Second position: A	Second position: G	Third position (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
U	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
U	UUA Leu	UCA Ser	UAA STOP	UGA STOP	A
U	UUG Leu	UCG Ser	UAG STOP	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
C	CUC Leu	CCC Pro	CAC His	CGC Arg	C
C	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
C	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
A	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
A	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
A	AUG Met[b]	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
G	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
G	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
G	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

[a] Nonpolar amino acid residues are tan, basic residues are blue, acidic residues are red, and polar uncharged residues are purple

[b] AUG forms part of the initiation signal as well as coding for internal Met residues.

Table 26-1 Fundamentals of Biochemistry, 2/e

Answers:

1. Definitions
   a. Antisense strand – the antisense strand of DNA is complementary to the RNA generated from this DNA (because it is the template strand during transcription).
   b. In the open RNA-Polymerase-promoter complex, the DNA template is unwound and is single-stranded thus forming the transcription bubble.
   c. A codon is a base triplet in an mRNA that encodes one amino acid as specified in the genetic code.
   d. An unsaturated fatty acid is a long chain carboxylic acid with one or more double bonds in the hydrocarbon tail.
   e. A ganglioside is a complex shpingolipid consisting of a ceramide and 3 or more sugars.
   f. A micelle is a globular aggregate of single-tail lipids in water where the hydrocarbon tails are out of contact with water.
   g. A paracrine hormone affects cells that are in the immediate environment of the site of secretion.
   h. A pyranose is a cyclic monosaccharide with a 6-membered ring.
   i. Mutarotation is the interconversion of the α- and β-anomeric forms of a cyclic monosaccharide.

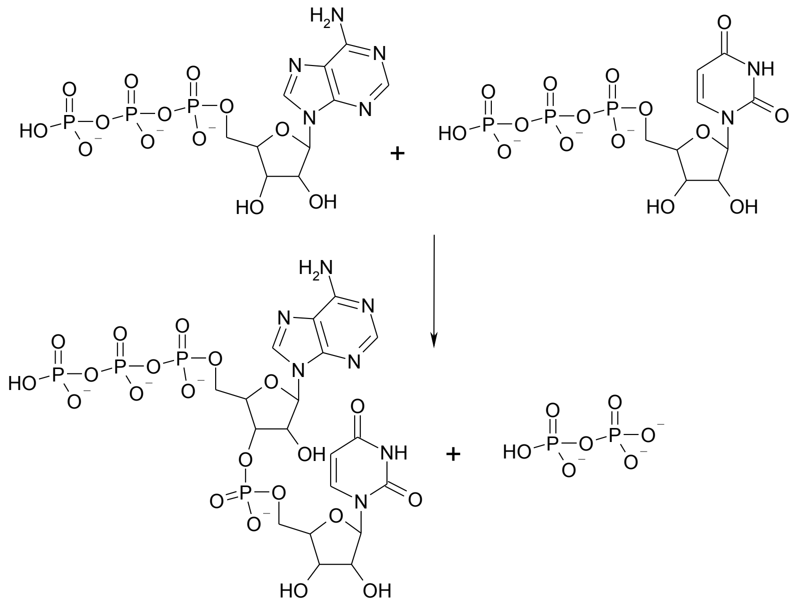
2. a. Promoter, Termination signal, start and stop codon:

CAGGC**TTGACA**CTTTATGCTTCCGGCTCG**TATAAT**TTCTC**CAT**ATTGTGA
----------------------Promoter--
GCCGTTTGTAAGGAGGTGATC**ATG**GTTCGCTATACC**TAA**GTATGATA**GCG**
Shine-Dalgarno sequence **Start codon** **Stop codon**
CGCATGCTGCGCGCTTTTTTTTTGCATAGACT
-------------Termination signal------------

b. Encoded amino acid sequence: Met-Val-Arg-Tyr-Thr-(Stop)

3. The σ factor helps the RNA-Polymerase to find the promoter. Holoenzyme (RNA-Polymerase including σ factor) binds weakly to non-promoter DNA such that it can move along DNA in search of the promoter. Holoenzyme binds tightly only to the promoter. After transcription initiation, the σ factor dissociates and the resulting core enzyme (RNA-Polymerase without σ factor) binds tightly and continues with transcription elongation.

4. pppA + pppU → pppApU + PPi



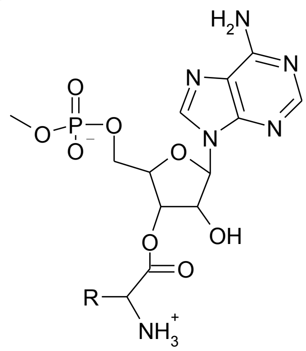
5. (1) Multiple transcripts arise from the same gene so a correct copy is typically produced. (2) Because of the redundancy of the "genetic code", many mutations do not cause an amino acid change in the resulting proteins. (3) Many mutations in proteins are functionally innocuous (unless they occur at invariant positions). (4) In eucaryotes, excision of introns means errors may not appear in mature transcript.

6. a. (1) The genetic code has a certain <u>frame</u> which determines the starting point for each codon encoding one amino acid. (2) The genetic is <u>degenerate</u> since one amino acid can be encoded by up to six different codons. (3) It is <u>nonrandom</u> – the codons encoding the same amino acid are similar to each other and often differ only in the third position. (4) The genetic code is <u>widespread, but not universal</u>: it is the same in almost all organisms, and only a few organisms have a slightly different genetic code.
   b. The function of the <u>mRNA</u> is to <u>encode the sequence for a protein</u> by being a copy of the genetic information (DNA), while the <u>tRNA</u> has the function to <u>decode the mRNA</u> by functioning as an <u>adapter</u>, i.e. the anticodon of the tRNA base-pair with the codon of the mRNA and carries at its other end the amino acid encoded by this codon.
   c.

anticodon	3' – C G U – 5'	3' – A A G – 5'
codon	5' – G C A – 3'	5' – U U C – 3'
amino acid	Ala	Phe

   d. In the sequence CCAAAUACCGUUAAGCUGGA the U of the stop codon following the Arg codon is changed to a C which generates a Gln codon. The C is indicated in bold in the mutant sequence:
   CCAAAUACCGU**C**AAGCUGGA

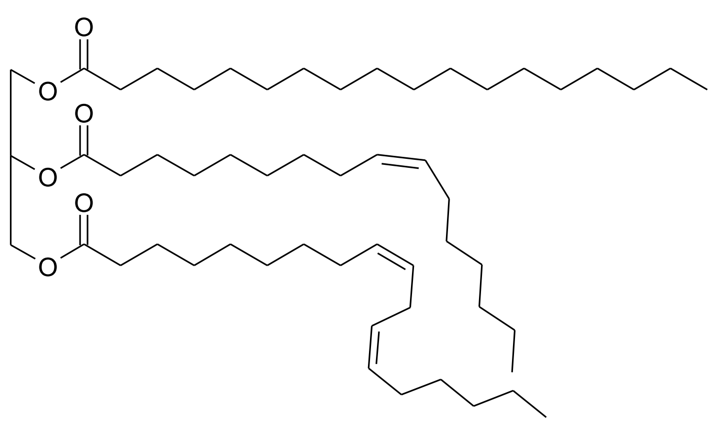
7. a. Amino acid + ATP → Aminoacyl-adenylate (aminoacyl-AMP) + PPi
   b.



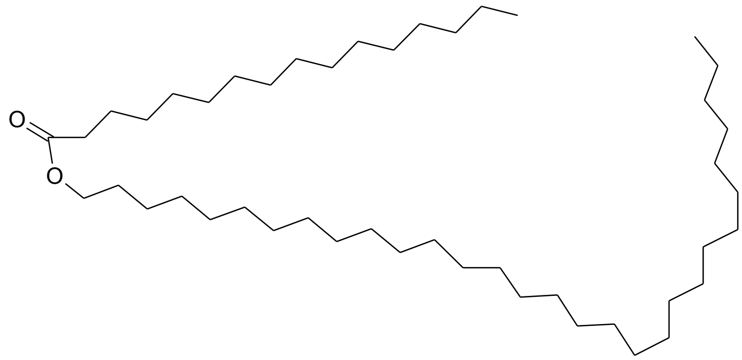
8. Translation
   a. The three tRNA binding sites are called A site, P site and E site. Typically, aminoacyl-tRNA binds to the A site, peptidyl-tRNA to the P site and deacylated tRNA to the E site.
   b. During aminoacyl-tRNA binding, the next aminoacyl-tRNA is delivered by elongation factor Tu to the ribosomal A site. Next, the peptide bond is formed resulting in an peptidyl-tRNA in the A-site. During translocation, the tRNA-mRNA complex is moved within the ribosome by elongation factor G such that the A-site becomes emptly, the peptidyl-tRNA resides in the P-site and the deacylated tRNA can exit from the E-site.
   c.

	A-site	P-site	E-site
Before A-site binding	empty	peptidyl-tRNA	empty (deacylated tRNA)
Before peptide bond formation	aminoacyl-tRNA	peptidyl-tRNA	empty
Before translocation	peptidyl-tRNA	deacylated tRNA	empty

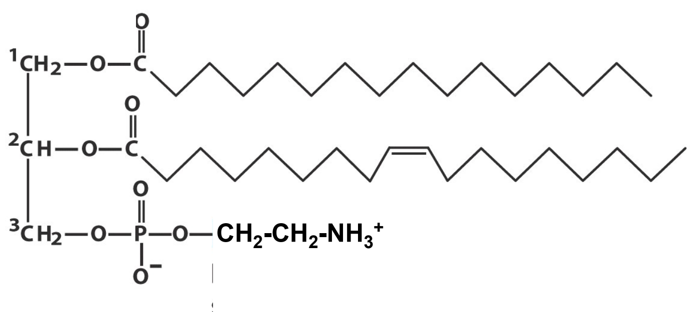
9. a.



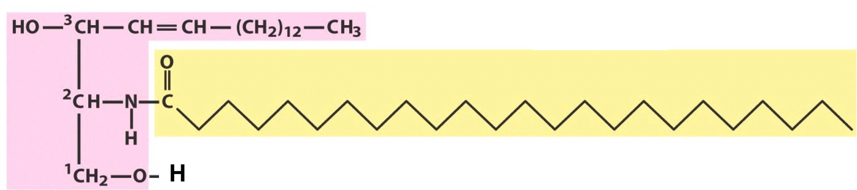
b.



c.



d.



10. a. cholesterol = steroid
    b. glycerol, 2 fatty acids, phosphate, and choline = glycerophospholipid
    c. glyceryl tristearate = triacylglycerol
    d. sphingosine, fatty acid, phosphate, and choline = Sphingolipid

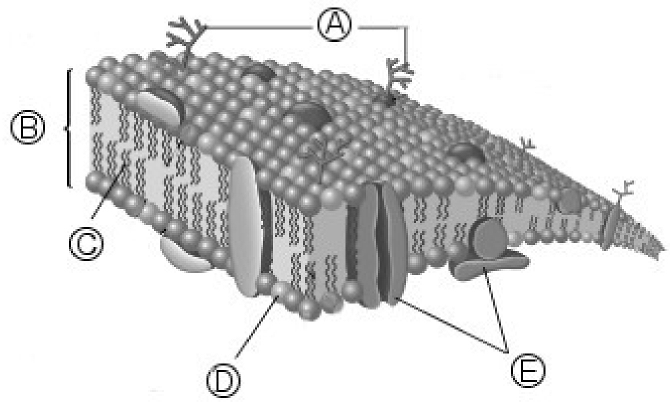
11. cholesterol, carbohydrates, proteins, glycerophospholipids and sphingolipids are found in biological membranes.

12. If the same molecules form cell membranes, then these are lipids (phosphoglycerolipids, sphingolipids) with a polar head and a nonpolar tail. The polar heads (hydrophilic) are not soluble in heptane, but the nonpolar tails are. Therefore the tails will point outwards, and the polar heads will form

the inner core of the lipid bilayer. That is the opposite orientation compared to the lipid bilayers on earth.

13. Increasing the unsaturated fatty acid contents will create chains that cannot pack as well within the membrane due to the kink within the chains. Therefore, the unsaturated fatty acids have lower melting points than saturated fatty acids. The loose packing means that the cell membranes remain fluid and do not become too rigid even when the temperature is decreasing.

14.
A) Carbohydrates (attached to proteins or as glycosphingolipids)
B) Lipid bilayer
C) Nonpolar tails of fatty acids
D) Polar heads of lipids
E) Integral and peripheral membrane protein



15. a. The added TNBS remains outside the cells and labels only the outer lipid layer of the bilayer in the cell membrane since it is membrane impermeable. Consequently, the newly synthesized phospholipids can not be in this outer lipid layer, but must be in the inner lipid layer of the membrane. This indicates that newly made phospholipids are synthesized on the cytoplasmic face of the membrane.
b. The newly synthesized, radioactive phospholipids must reach the outer layer of the membrane to become also labeled with TNBS. Obviously, this can occur in only 3 min and requires a flip-flop (transverse diffusion) of the lipids. Usually, a flip-flop is extremely slow (in contrast to lateral diffusion). The only possibility to explain the fast flip flop observed here is to assume active transport of the phospholipids. (Indeed, this flip-flop is catalyzed by enzymes known as flipases which are passive transporters.)

16. Transport
   a. Mediated, passive transport
   b. Mediated, active transport

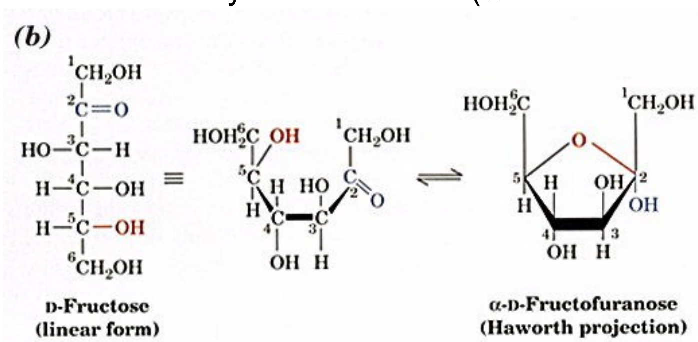
17. Active Transport

a. electrogenic uniport
b. electroneutral antiport
c. electroneutral symport

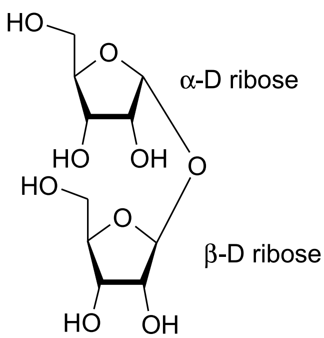
18. Carbohydrates

a. D-Aldopentoses

b. Linear and cyclic ketohexose (α-anomeric form), e.g. fructose



c. two riboses conected by a (β1-α1) glycosidic bond



d. Amylose