24.1 Structure and Classification of Lipids

- ***Lipids*** are naturally occurring molecules from plants or animals that are soluble in nonpolar organic solvents.
- Lipid molecules contain large hydrocarbon portion and not many polar functional group, which accounts for their solubility behavior.

Classification of Lipids

Lipids that are ester or amides of fatty acids:

- *Waxes* – are carboxylic acid esters where both R groups are long straight hydrocarbon chain. Performs external protective functions.
- *Triacylglycerol* – are carboxylic acid triesters of glycerols. They are a major source of biochemical energy.
- *Glycerophopholipids* - triesters of glycerols that contain charged phosphate diesters. They help to control the flow of molecules into and out of cells.

- *Sphingomyelins* – amides derived from an amino alcohol, also contain charged phosphate diester groups. They are essential to the structure of cell membranes.
- *Glycolipids* – amides derived from sphingosine, contain polar carbohydrate groups. On the cell surface, they connect with by intracellular messengers.

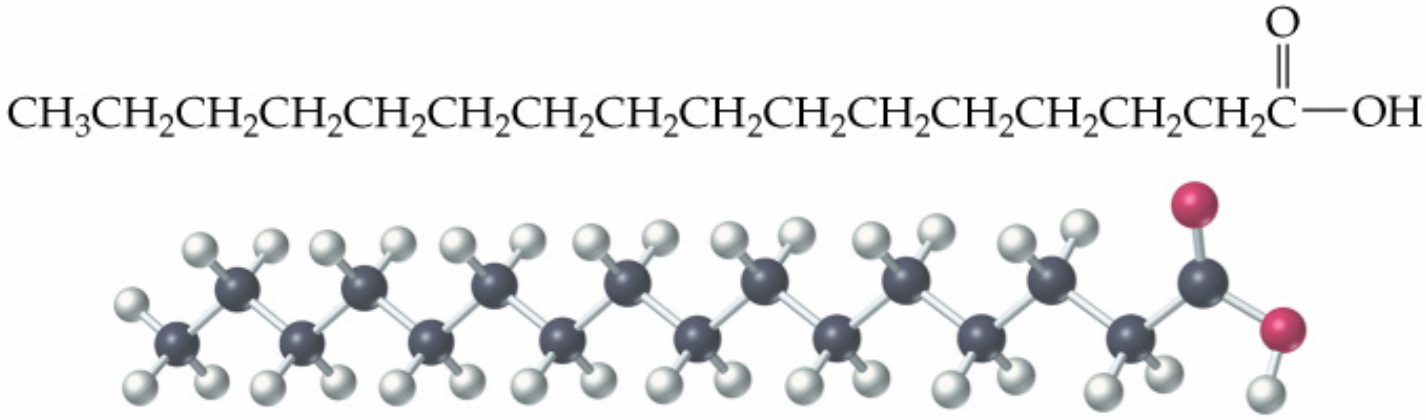
Lipids that are not esters or amides:

- *Steroids* – They performs various functions such as hormones and contributes to the structure of cell membranes.
- *Eicosanoids* – They are carboxylic acids that are a special type of intracellular chemical messengers.

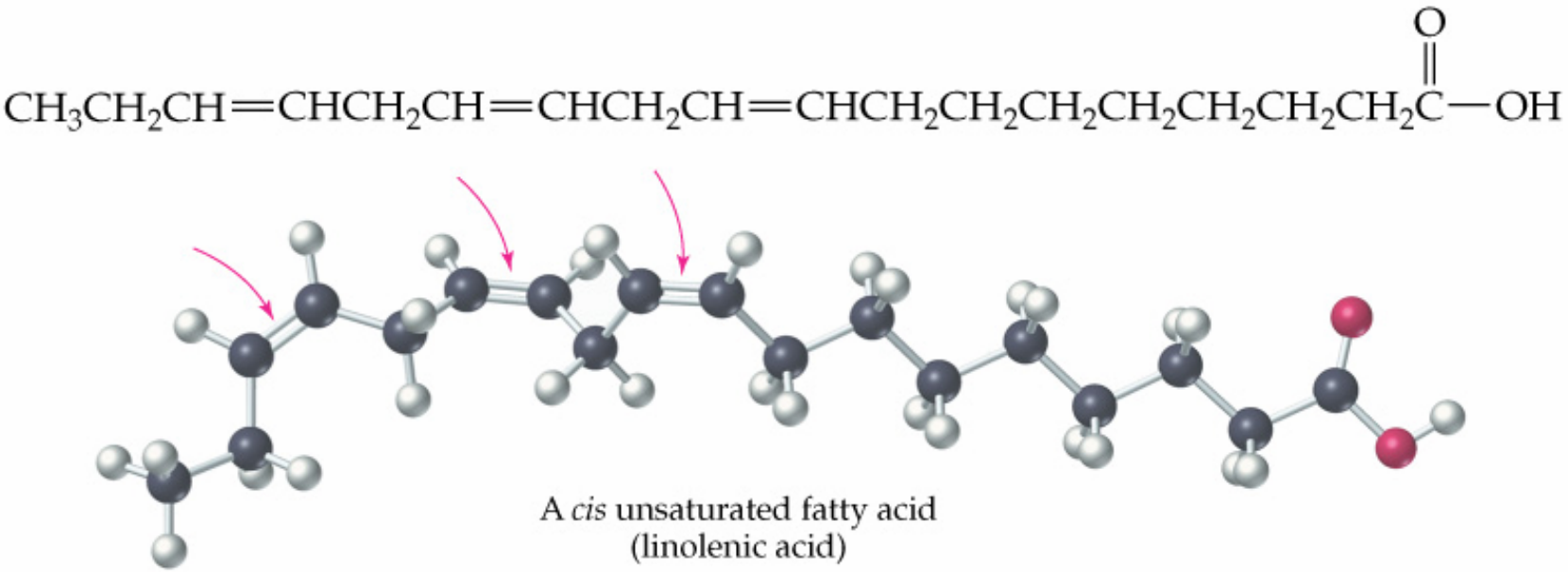
24.3 Properties of Fats and Oils

Oils: A mixture of triglycerols that is liquid because it contains a high proportions of unsaturated fatty acids.

Fats: A mixture of triglycerols that is solid because it contains a high proportions of saturated fatty acids.



A saturated fatty acid
(palmitic acid)



A *cis* unsaturated fatty acid
(linolenic acid)

Properties of triglycerols in natural fats and oils:

- Nonpolar and hydrophobic
- No ionic charges
- Solid triglycerols (Fats) - high proportions of saturated fatty acids.
- Liquid triglycerols (Oils) - high proportions of unsaturated fatty acids.

24.4 Chemical Reactions of Triglycerols

Hydrogenation: The carbon-carbon double bonds in unsaturated fatty acids can be hydrogenated by reacting with hydrogen to produce saturated fatty acids. For example, margarine is produced when two thirds of the double bonds present in vegetable oil is hydrogenated.

Hydrolysis of triglycerols: Triglycerols like any other esters react with water to form their carboxylic acid and alcohol – a process known as hydrolysis.

- In body, this hydrolysis is catalyzed by the enzyme hydrolase and is the first step in the digestion of dietary fats and oils.
- In the laboratory and commercial production of soap, hydrolysis of fats and oils is usually carried out by strong aqueous bases such as NaOH and KOH and is called saponification.

24.5 Cell Membrane Lipids: Phosphilipids and Glycolipids

- Cell membranes establish a hydrophobic barrier between the watery environment in the cell and outside the cell. Lipids are ideal for this function.
- The three major kinds of cell membrane lipids in animals are *phospholipids*, *glycolipids*, and *cholesterol*.

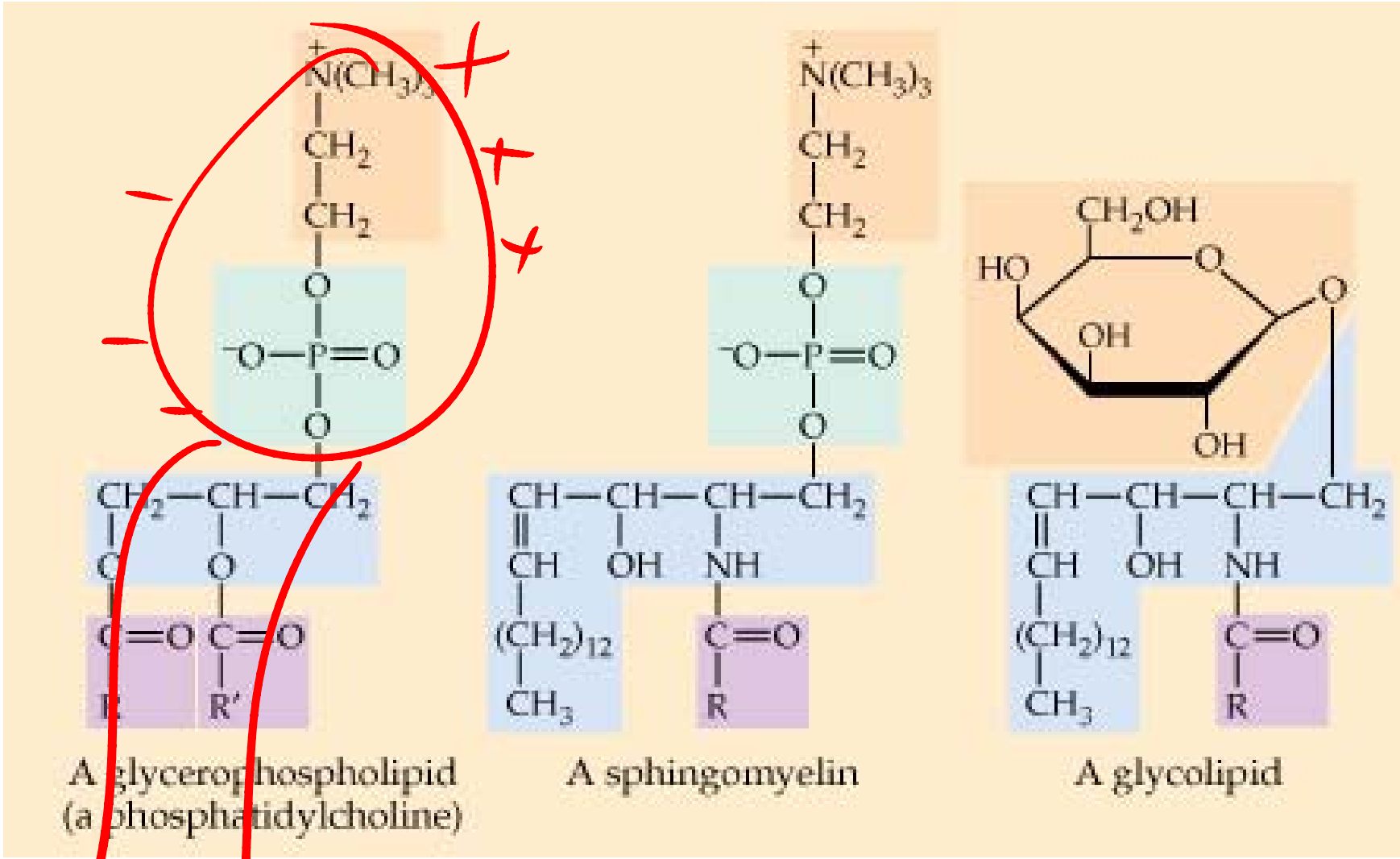
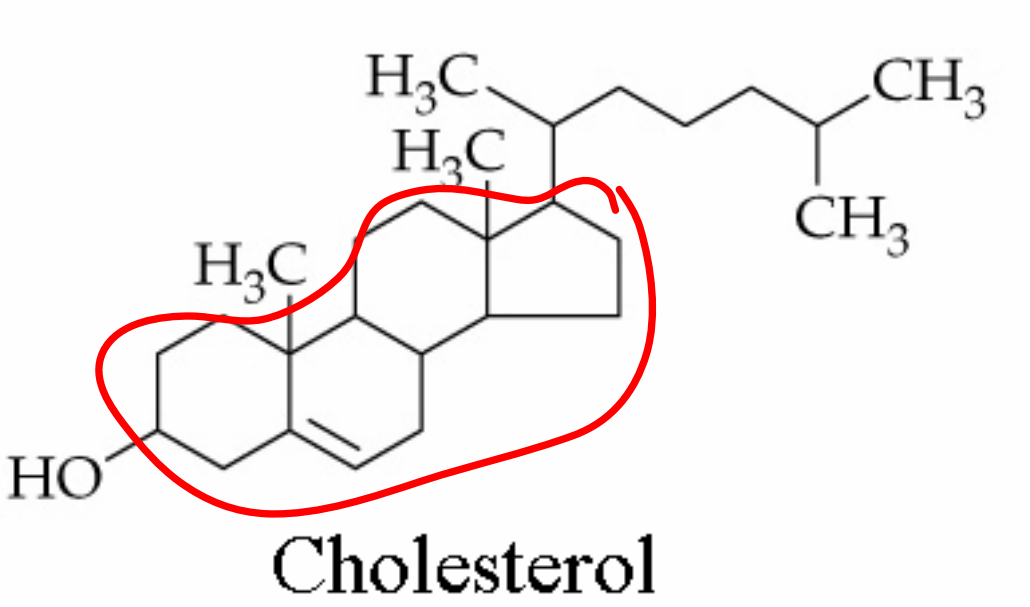


Fig 24.4 Membrane lipids

- Phosphoilipids contain an ester link between a phosphoric acid and an alcohol. The alcohol is either a glycerol to give a glycerophopholipid or a sphingosine to give sphingomyelins.
- Glycolipids: Glycolipids are derived from sphingosine. They differ from sphingomyelins by having a carbohydrate group at C1 instead of a phosphate bonded to a choline.

24.5 Cell Membrane Lipids: Cholesterol

- Animal cell membranes contain significant amount of cholesterol.

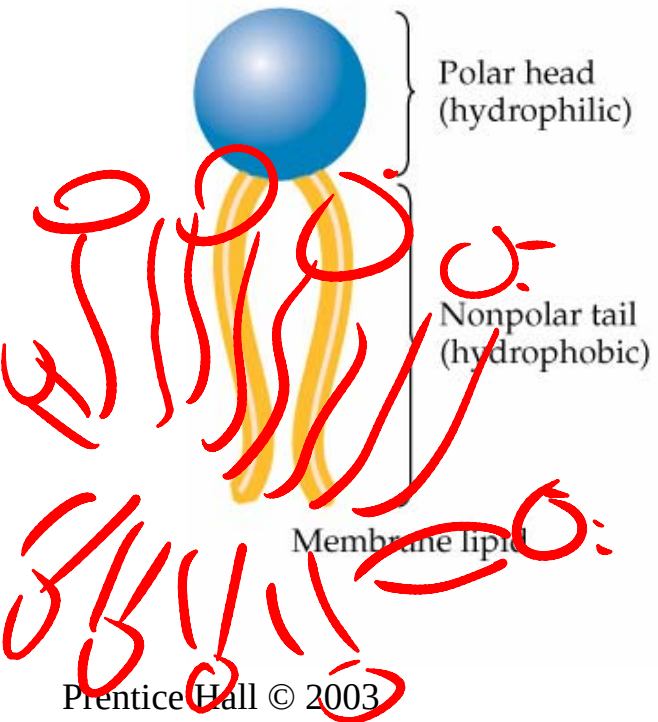


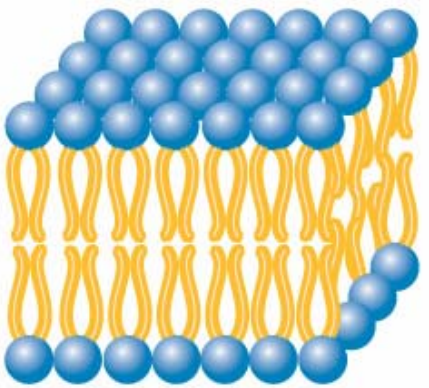
Cholesterol

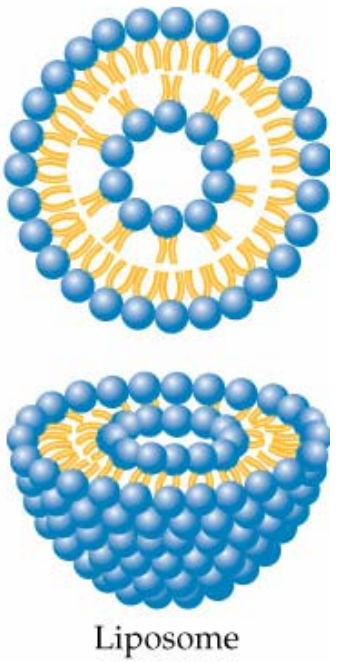
- Cholesterol is a steroid, a member of the class of lipids that all contain the same four ring system.
- Cholesterol serves two important purposes: as a component of cell membranes and as a starting materials for the synthesis of all other steroids.

24.7 Structure of Cell Membranes

The basic structural unit of cell membrane is lipid bilayer which is composed of two parallel sheets of membrane lipid molecules arranged tail to tail. Bilayers are highly ordered and stable, but still flexible.







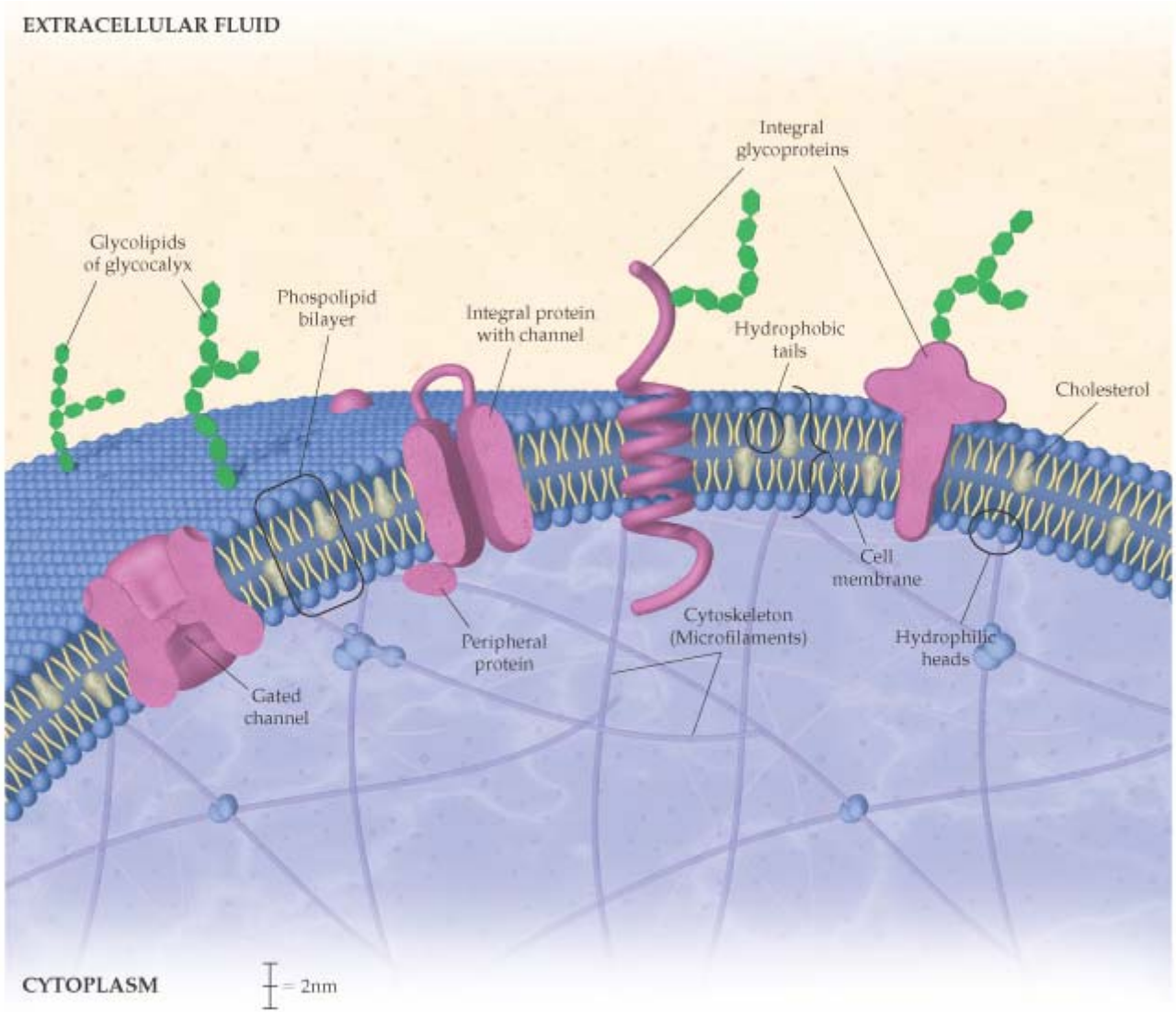


Fig 24.7 The cell membrane

When phospholipids are shaken vigorously with water, they spontaneously form liposome – small spherical vesicle with lipid bilayer surrounding an aqueous center. Water soluble substances can be trapped in the center of the liposome, and lipid-soluble substances can be incorporated into the bilayer.

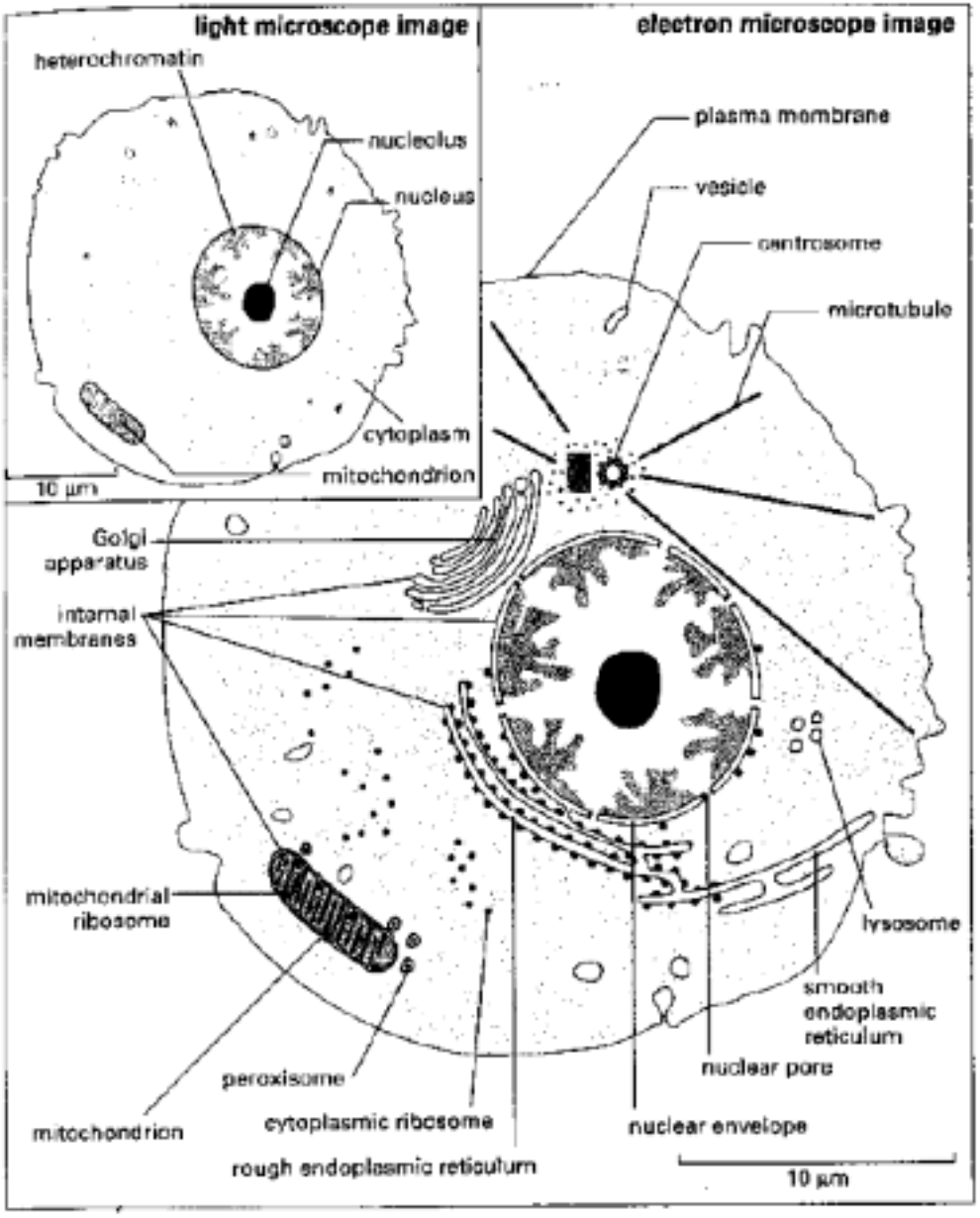
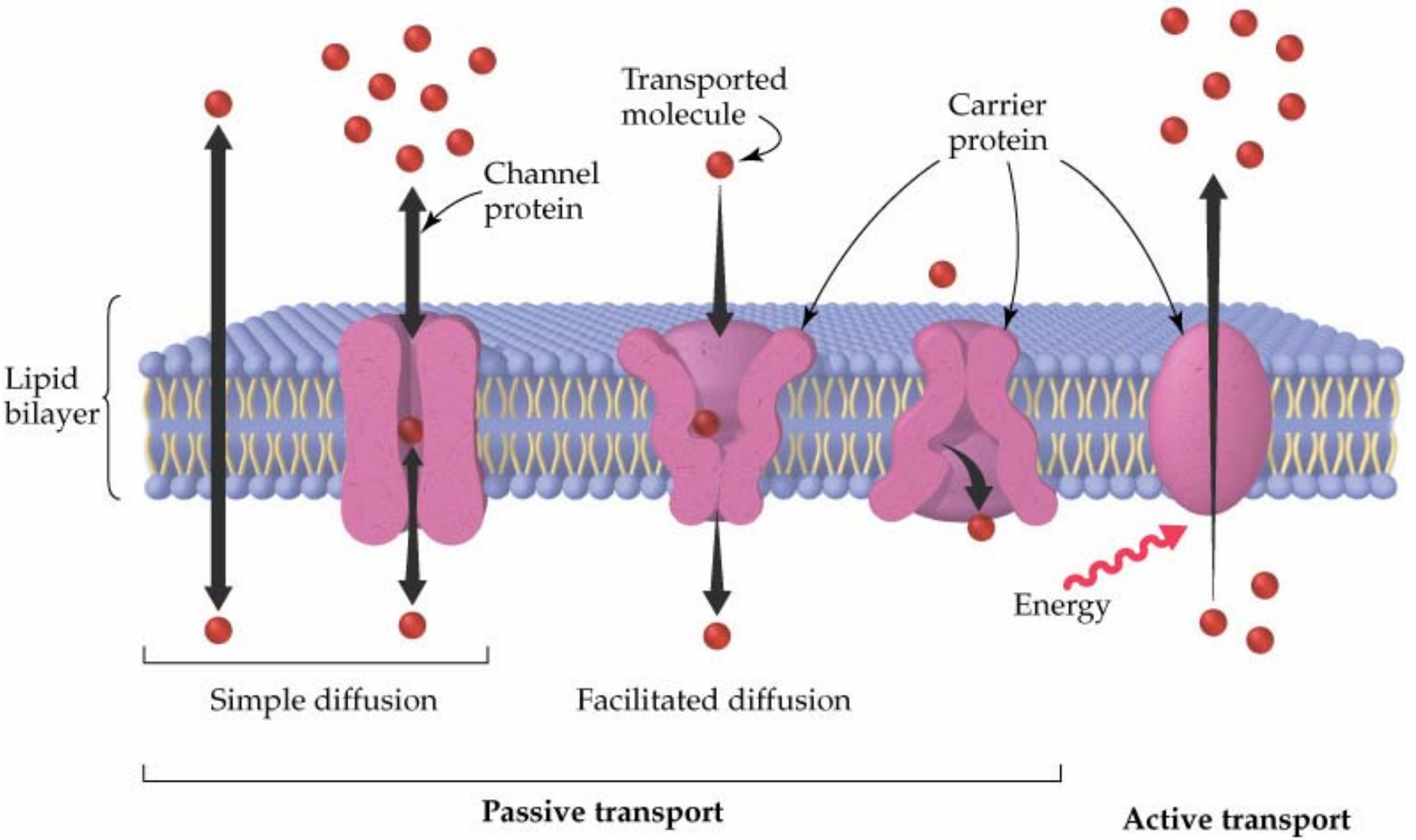


Figure 1.2. Cell structure as seen through the light and transmission electron microscopes.

24.8 Transport Across Cell Membranes

The cell membranes allow the passage of molecules and ions into and out of a cell by two modes; passive transportation and active transportation.

- *Passive transport* – substances move across the cell membrane freely by diffusion from regions of higher concentration to regions of lower concentration. Glucose is transported into many cells in this way.


Transported molecule
Carrier protein
Channel protein
Lipid bilayer
Energy
Simple diffusion
Facilitated diffusion
Passive transport
Active transport

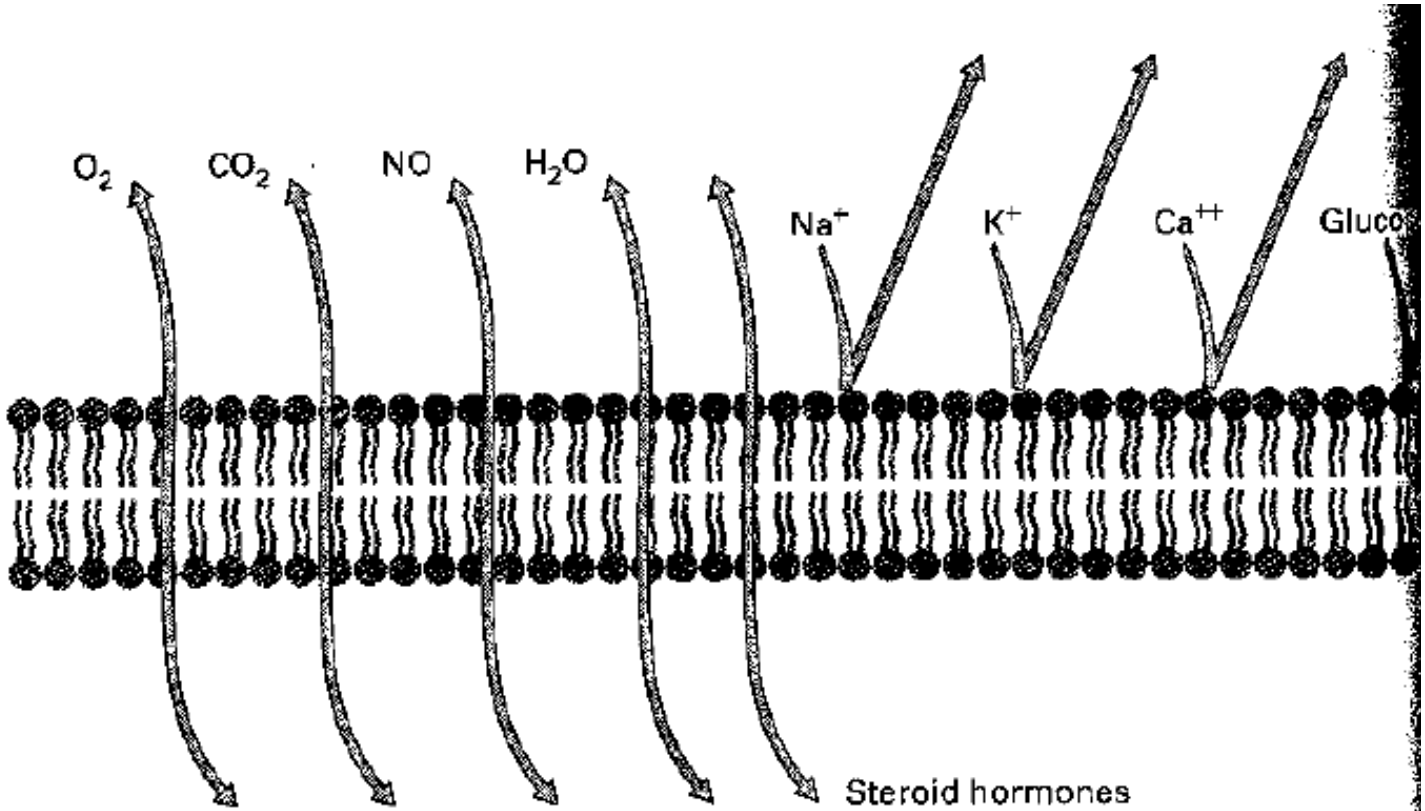


Figure 3.2. Small uncharged molecules can pass through membranes by simple diffusion, but ions can

- *Active transport* - substances move across the cell membrane only when energy is supplied because they must go in the reverse direction from regions of lower to regions of higher concentration. Only by this method, cells maintain lower Na^+ concentration within cells and higher Na^+ concentration in extracellular fluids, with the opposite concentration ratio for K^+.

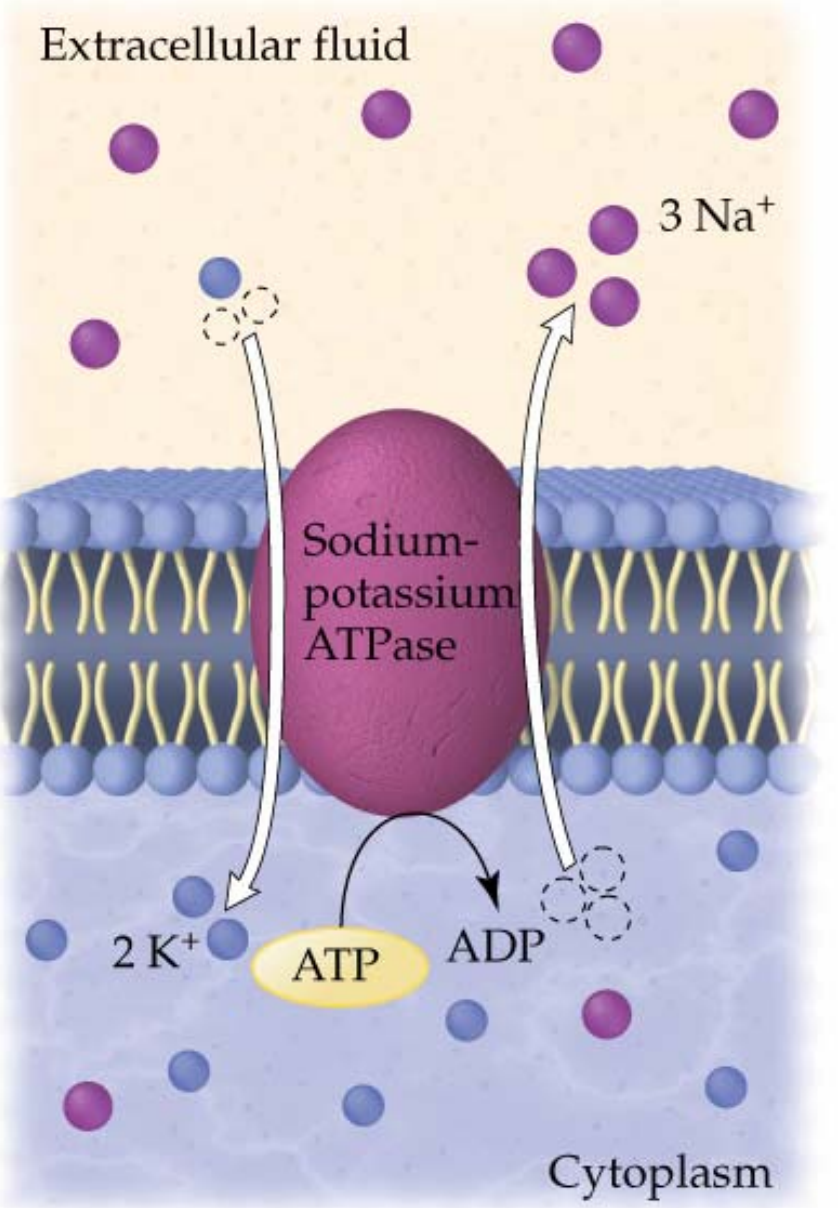


Fig 24.9 An example of active transport

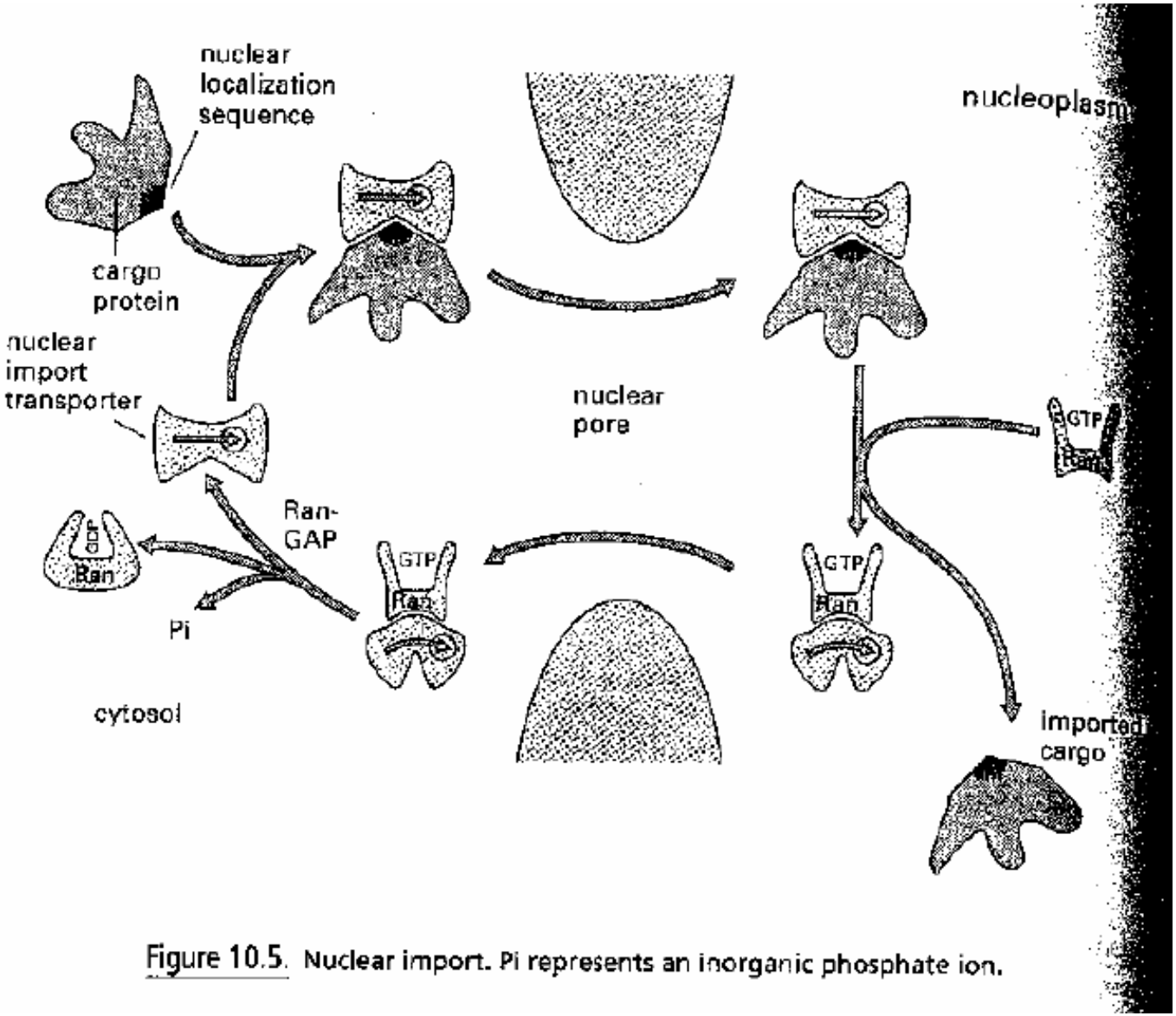


Figure 10.5. Nuclear import. Pi represents an inorganic phosphate ion.

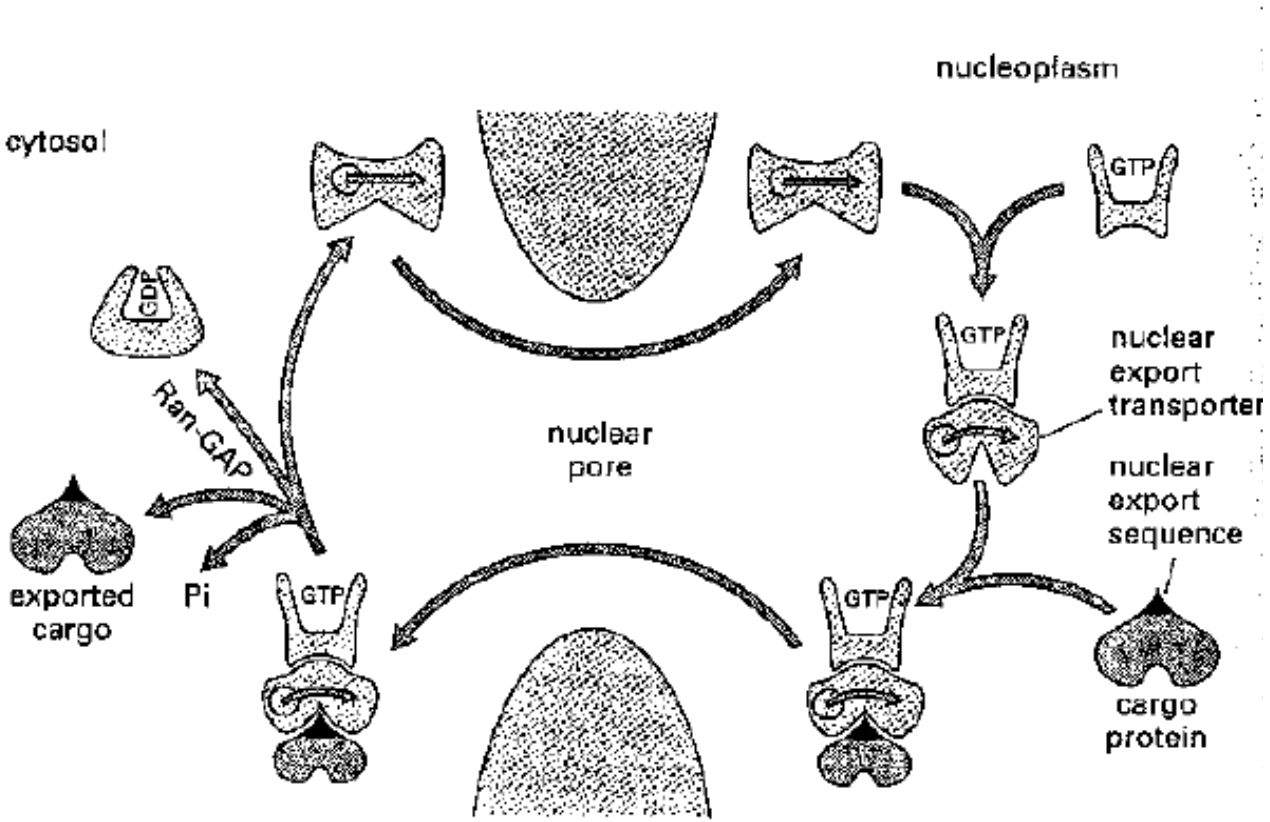


Figure 10.6. Nuclear export. Pi represents an inorganic phosphate ion.

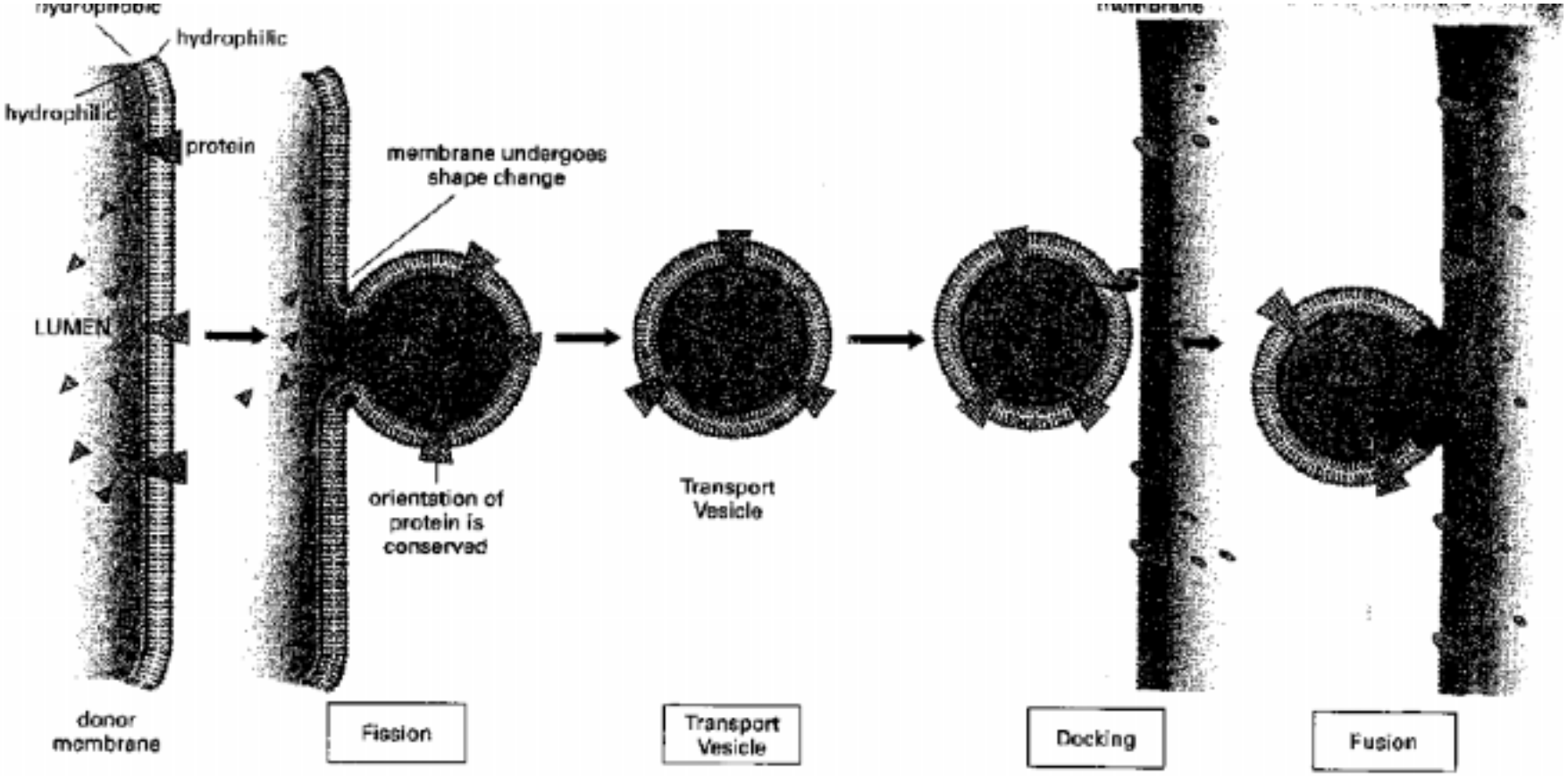


Figure 10.9. Fission and fusion.

Properties of cell membranes:

- Cell membranes are composed of a fluid like phospholipid bilayer.
- The bilayer incorporates cholesterol, proteins, and glycolipids.
- Small nonpolar molecules cross by diffusion through the lipid bilayer.

- Small ions and polar molecules diffuse through the aqueous media in protein pores.
- Glucose and certain other substances cross with the aid of proteins without energy input.
- Na^+, K^+, and other substances that maintain concentration gradients inside and outside the cell cross with expenditure of energy and the aid of proteins.

25.2 Lipoproteins for Lipid Transport

- Lipids enter metabolism from three different sources: (1) the diet, (2) storage in adipose tissue, and (3) synthesis in the liver.
- Whatever their source these lipids must eventually be transported in blood, an aqueous media.
- To become water soluble, fatty acid release from adipose tissue associate with albumin. All other lipids are carried by lipoproteins.

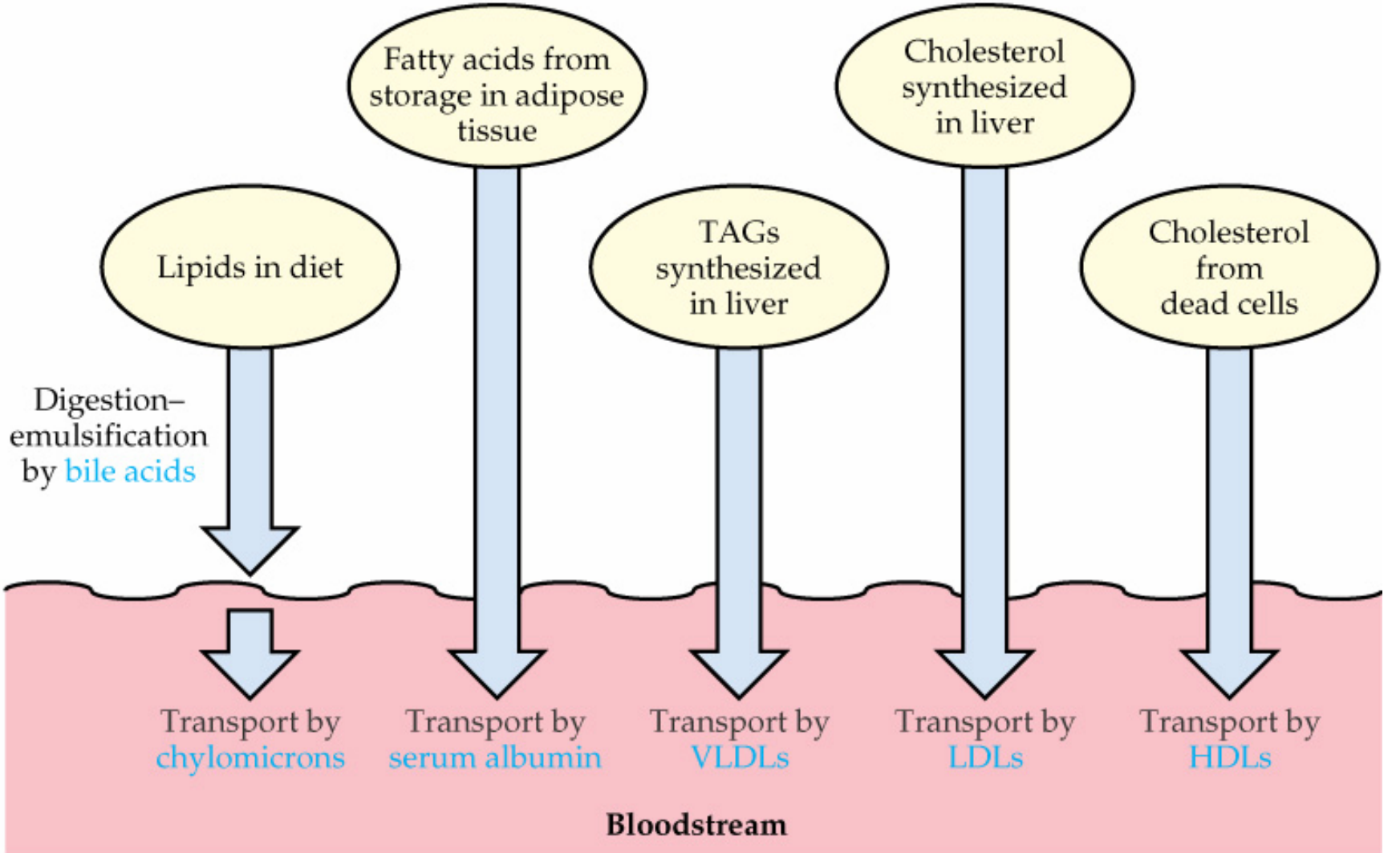
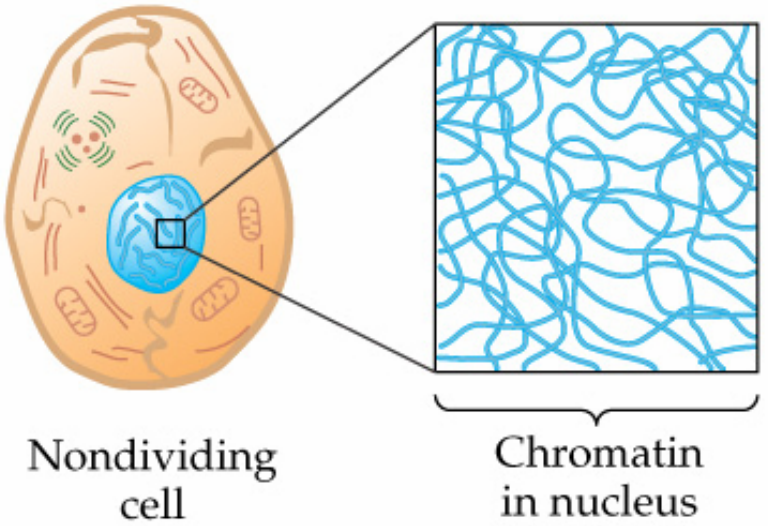
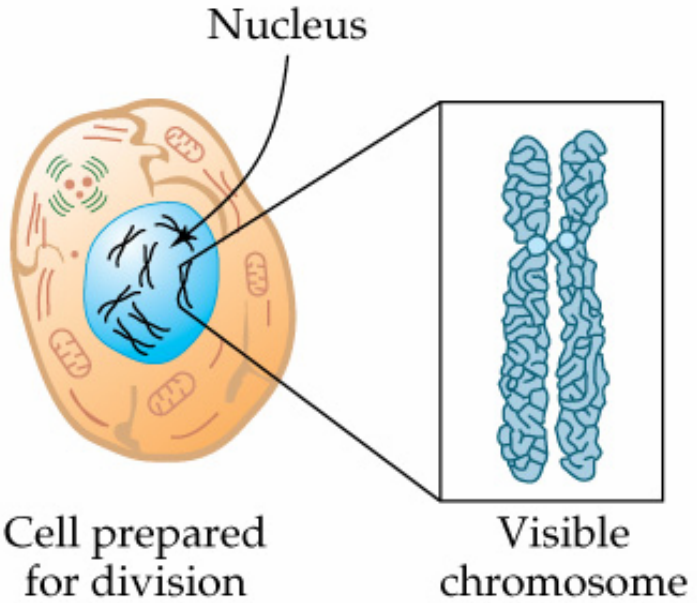


Fig 25.5 Transport of lipids

26.1 DNA, Chromosome, and Genes

- ***DNA*** (deoxyribonucleic acid): The nucleic acid that stores genetic information.
- ***Chromosome***: A complex of proteins and DNA molecule: visible during cell division.
- When a cell is not actively dividing, its nucleus is occupied by chromatin, which is a compact tangle of DNA twisted around proteins known as histones.
- During cell division, chromatin organizes itself into *chromosome*.


Nondividing cell
Chromatin in nucleus


Nucleus
Cell prepared for division
Visible chromosome

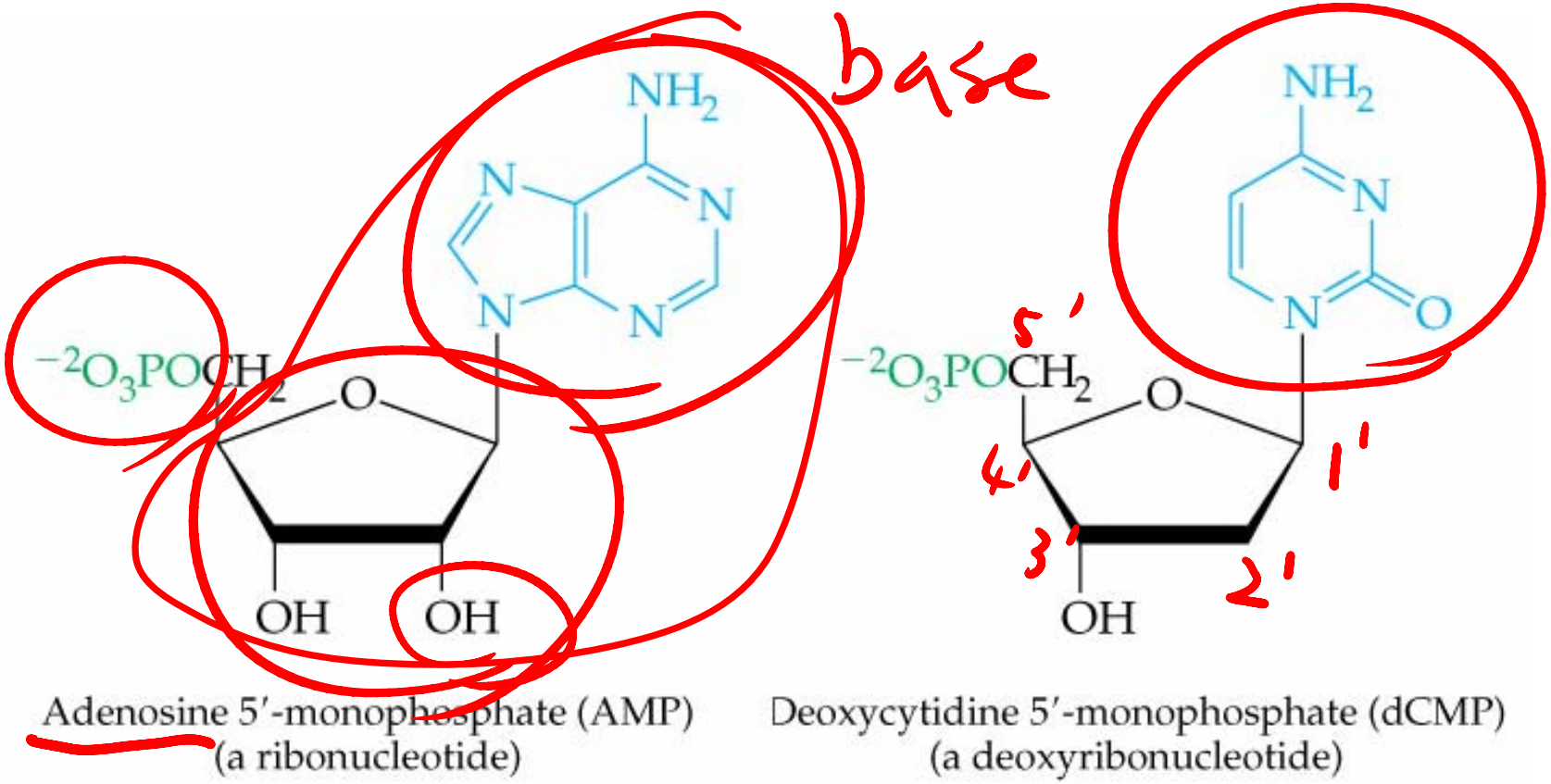
- Each chromosome contains a different DNA molecule, and the DNA is duplicated so that each new cell receives a complete copy.
- Each DNA molecule is made up of many genes – individual segments of DNA that contain the instructions that directs the synthesis of a single polypeptide.
- Number of chromosome varies from organism to organism. For example, a horse has 64 chromosome (32 pairs), a cat has 38 or 19 pairs of chromosomes, a mosquito has 3, a human has 46 or 23 pairs of chromosomes.

26.2 Composition of Nucleic Acids

- Nucleic acids are polymer of nucleotides.
- Each nucleotides has three parts: a five-membered ring monosaccharide, a nitrogen-containing cyclic compound that is a base, and a phosphate group.
- There are two kinds of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The function of RNA is to put the information stored in DNA to use.

In RNA, the sugar is ribose.

In DNA, the sugar is deoxyribose.



Adenosine 5′-monophosphate (AMP)
(a ribonucleotide)

Deoxycytidine 5′-monophosphate (dCMP)
(a deoxyribonucleotide)

Base componenet

- Thymine is present only in DNA molecules. Uracil is present only in RNA. Adenine, guanine, and cytosine bases are present in both DNA and RNA.
- The combination of ribose or deoxyribose and one of the bases listed in Table 261 produces a **nucleoside**.
- Nucleotides are building blocks of nucleic acids. Each nucleotide is a 5'-monophosphate ester of a nucleoside.

26.3 The Structure of Nucleic Acid Chains

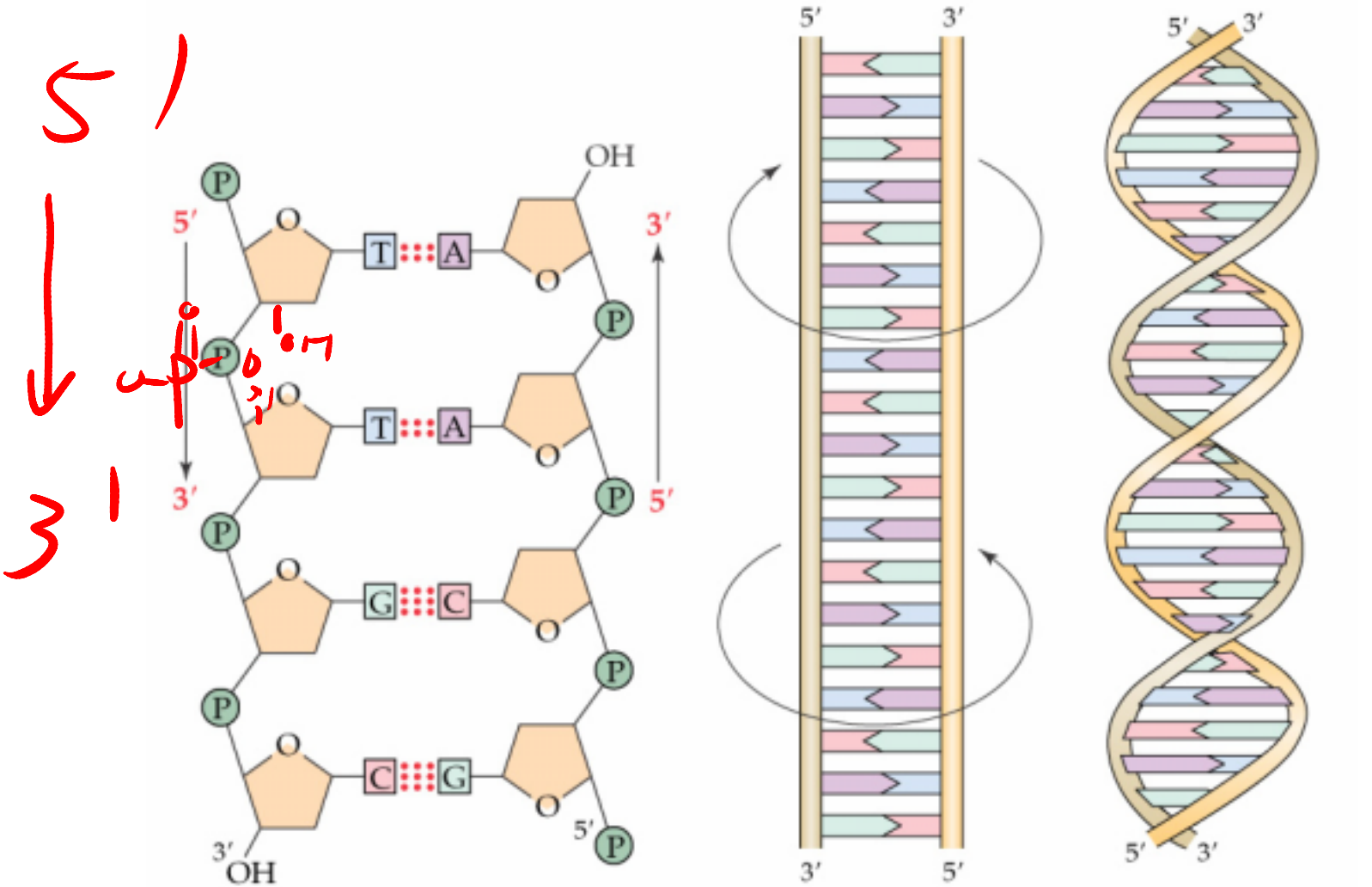
- The nucleotides are connected in DNA and RNA by phosphate diester linkages between the –OH group on C3’ of the sugar ring of one nucleotide and the phosphate group on C5’ of the next nucleotide.
- The structure and function of a nucleic acid depends on the sequence in which its individual nucleotides are connected.
- The differences between different nucleic acids are provided by the order of the groups bonded to the backbone and bases.

26.4 Base Pairing in DNA: The Watson-Crick Model

DNA samples from different cells of the same species have the same proportions of the four heterocyclic bases, but samples from different species have different proportions of bases. For example, human DNA contain 30% each of adenine and thymine, and 20% each of guanine and cytosine.

The bacterium *Escherichia Coli* contains 24% each of adenine and thymine, 26% each of guanine and cytosine. In both cases, A and T are present in equal amounts, are as G and C. The bases occur in pairs.

- In 1953, James Watson and Francis Crick proposed double helix structure for DNA that accounts for base pairing also accounts for the storage and transfer of genetic information.



Double helix The two strands coiled around each other in A screwlike fasion.

- Two strands of DNA coiled around each other in a right-handed screwlike fashion. In most organisms the two polynucleotides of DNA form a double helix.
- The two strands of DNA double helix run in opposite directions-one in the 5’ to 3’ direction, the other in the 3’ to 5’ direction.
- The sugar-phosphate back-bone is on the outside of the right handed double helix, and the heterocyclic bases are on the inside.

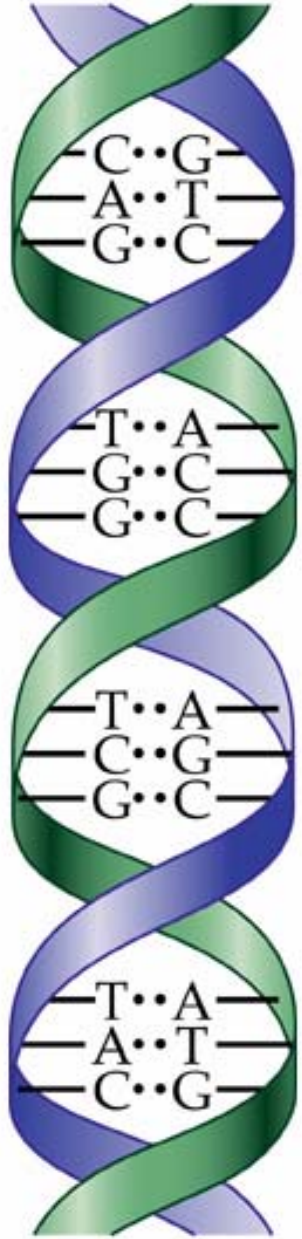
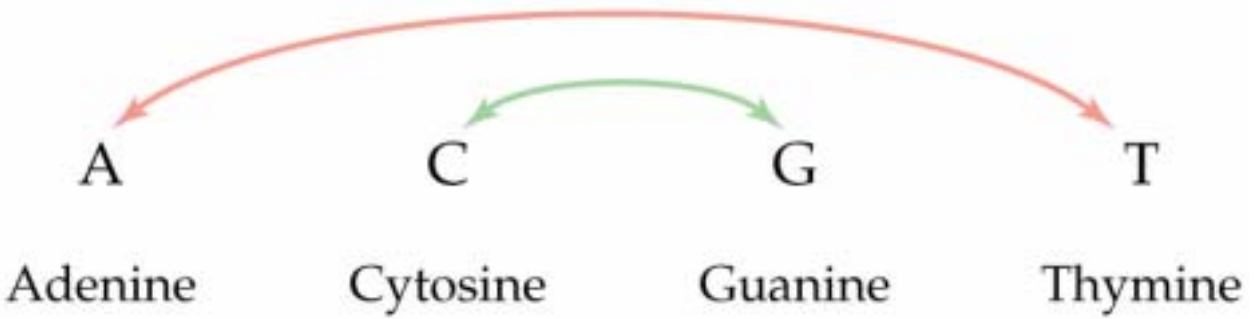


Fig 26.3 A segment of DNA

- A base on one strand points directly toward a base on the second strand.
- The double helix looks like a twisted ladder, with the sugar-phosphate backbone making up the sides and the hydrogen-bonded base pairs, the rungs.
- Wherever a T base occurs in one strand, an A base falls opposite it in the other strand; Wherever a C base occurs in one strand, an G base falls opposite it in the other strand. This ***base pairing*** explains why A and T and why C and G occur in equal amounts in double-stranded DNA.



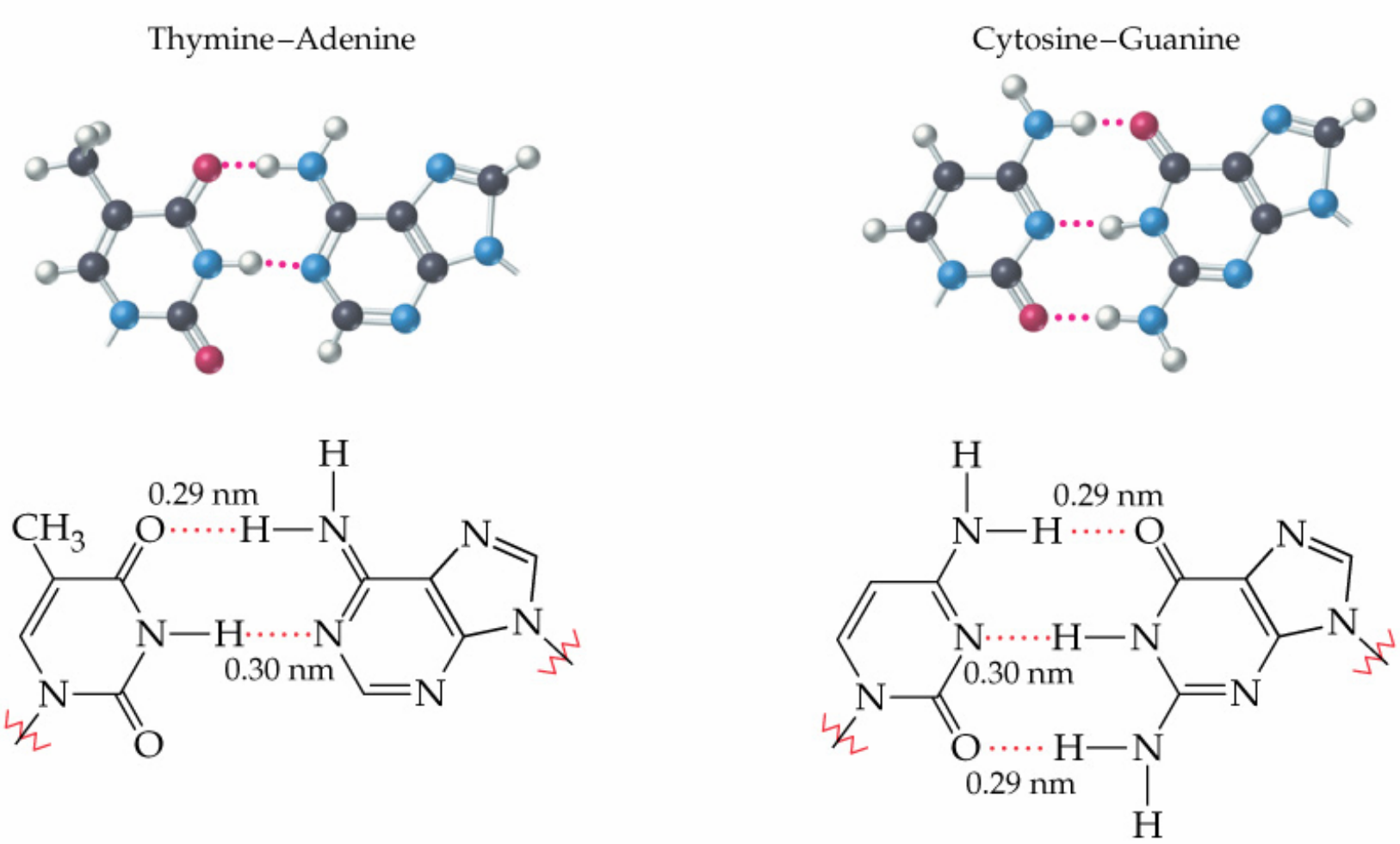


Fig 26.2 Base pairing in DNA

26.5 Nucleic Acids and Heredity

- Each of our 23 pairs of chromosomes contain one DNA copied from that of our father and one DNA copied from that of our mother.
- Most cells in our body contain copies of these originals.
- Our genetic information is carried in the sequence of bases along the DNA strands. Every time a cell divides, the information is passed along to the daughter cells.

- The following three processes are involved in duplication, transfer, and use of genetic information:

  Replication: The process by which a replica, or identical copy, of DNA is made when a cell divides.

  Transcription: The process by which the genetic messages contained in DNA are read and copied.

  Translation: The process by which the genetic messages carried by RNA are decoded and used to build proteins.

26.6 Replication of DNA

- DNA replication involves unwinding of the double helix at several places.
- As the DNA strands separates and the bases are exposed, DNA polymerase enzymes begins their function as a facilitator for replication of DNA.
- Nucleoside triphosphates carrying each of the four bases move into place one by one by forming hydrogen bonds between the base they carry and the base exposed on the DNA strand being copied, the template strand.

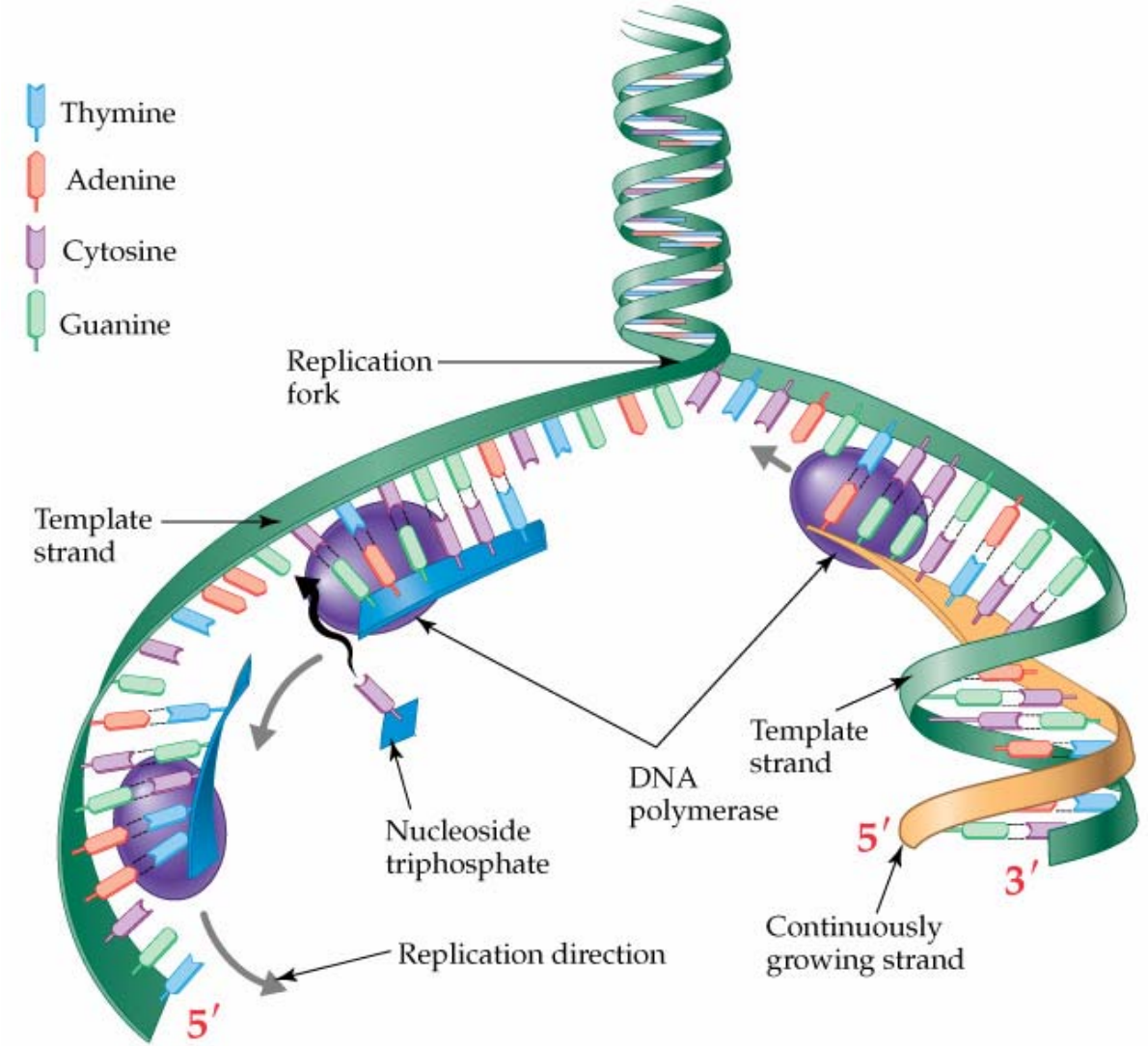
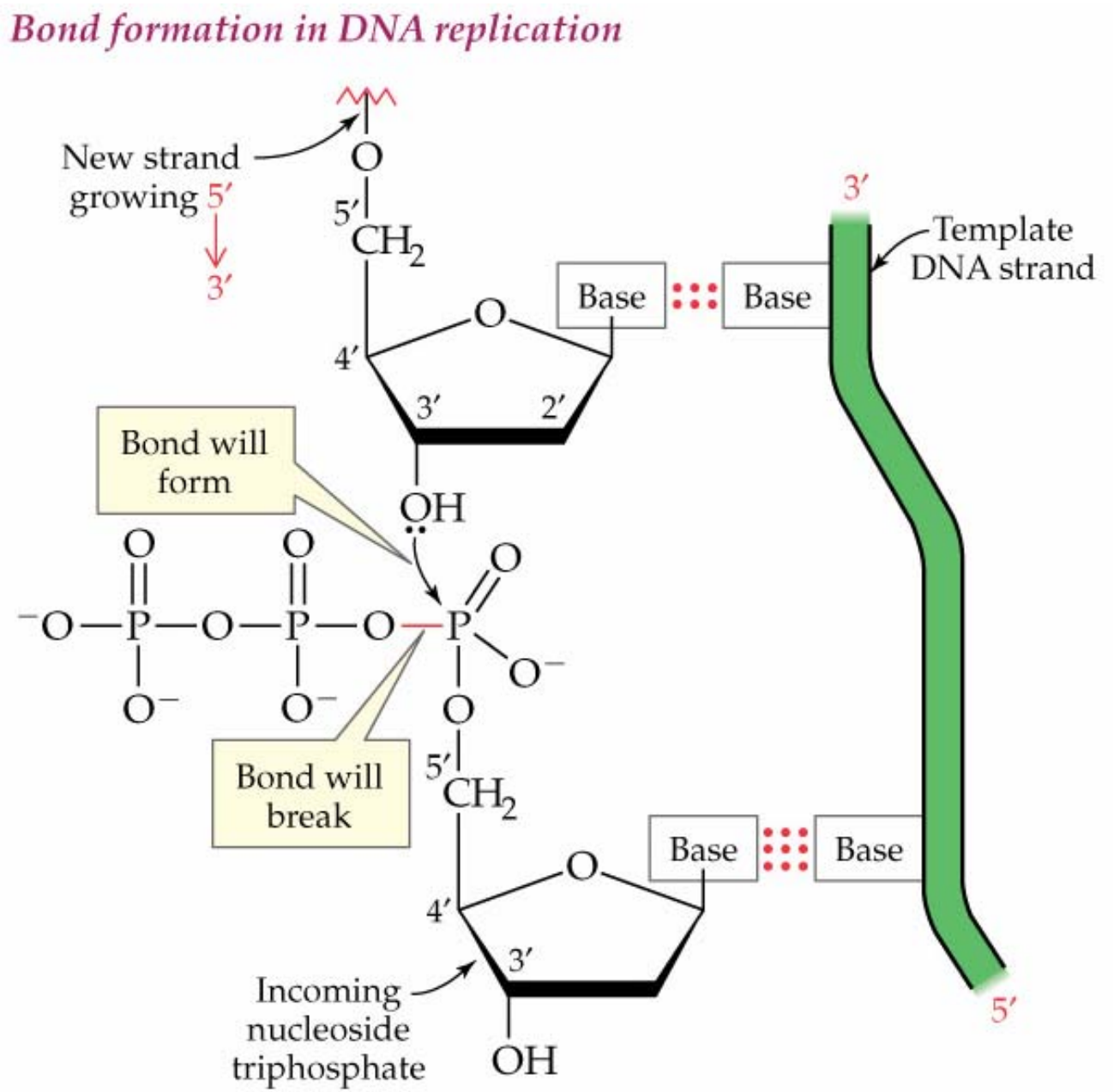


Fig 26.5 DNA replication

- The hydrogen bonding requires that A can pair only with T, and G can pair only with C.
- DNA polymerase then catalyze the bond formation between the 5’ phosphate group of the arriving nucleoside triphosphate and the 3’ –OH group of the growing polynucleotide strand, as the two extra phosphate groups are removed. Therefore, the template strand is copied in the 3’ to 5’ direction.
- Each new strand is complementary to its template strand, two identical copies of the DNA double helix are produced during replication.

ATP, GTP, CTP, TTP


Bond formation in DNA replication
New strand growing 5′
3′
5′
CH2
O
4′
3′
2′
OH
Base
Base
3′
Template DNA strand
Bond will form
Bond will break
O
P
O⁻
5′
CH2
Base
Base
4′
3′
Incoming nucleoside triphosphate
OH
5′

26.7 Structure and Function of RNA

RNA is similar to DNA – both are sugar-phosphate polymers and both have nitrogen-containing bases attached – but there are differences between them.

DNA has only one kind of function-storing genetic information. By contrast, the different kinds of RNA perform different functions.

The following three RNA make it possible for the encoded information carried by the DNA to be put to use in the synthesis of proteins.

Ribosome RNA: The granular organelles in the cell where protein synthesis takes place. These organelles are composed of protein and ribosomal RNA (rRNA).

Messenger RNA (mRNA): The RNA that carries the code transcribed from DNA and directs protein synthesis.

Transfer RNA (tRNA): The smaller RNA that delivers amino acids one by one to protein chains growing at ribosomes. Each tRNA recognizes and carries only one amino acid.

26.8 Transcription: RNA Synthesis

- RNA are synthesized in the cell nucleus. Before leaving the nucleus, all types of RNA are modified in the various ways needed for their various functions.
- In transcription, a small section of the DNA double helix unwinds, the bases on the two strands are exposed, and one by one the complementary nucleotides are attached.

- rRNA, tRNA, and mRNA are all synthesized in essentially the same manner. Only one of the two DNA strands is transcribed during RNA synthesis.
- The DNA strand that is transcribed is the template strand; while the its complementary strand is the informational strand.
- The messenger RNA produced is a duplicate of the DNA informational strand, but with U base wherever the DNA has a T base.

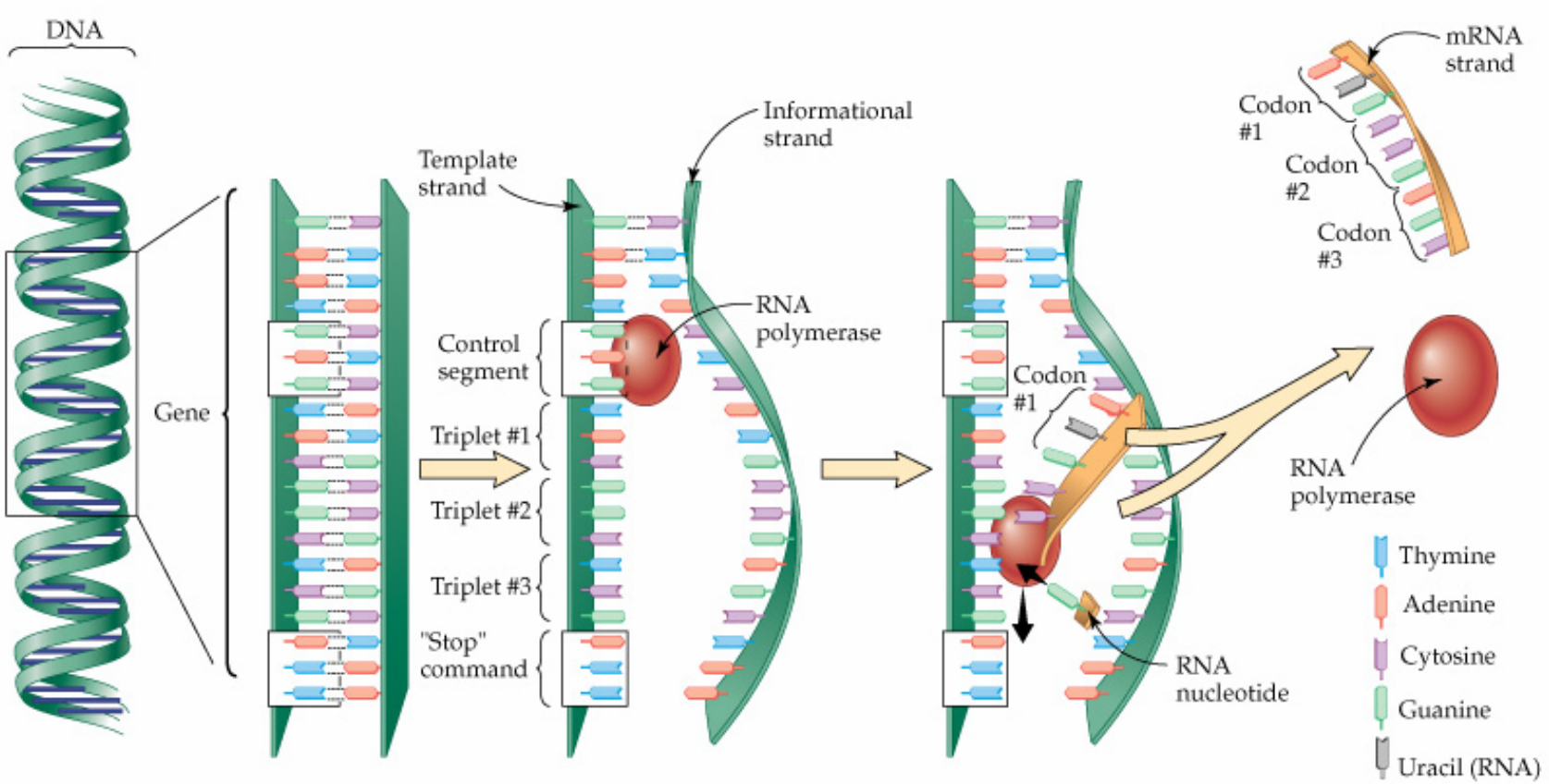


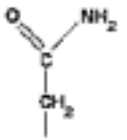
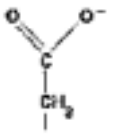
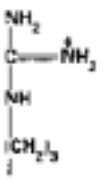
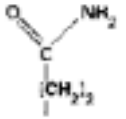
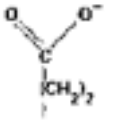
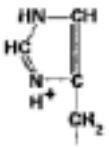
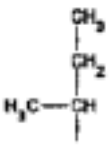
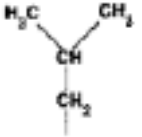
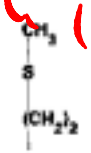
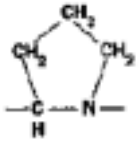
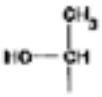
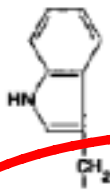
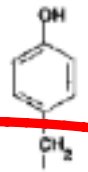
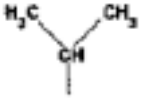
Fig 26.6 Transcription of DNA to produce mRNA

26.9 The Genetic Code

- The ribinucleotide sequence in an mRNA chain is like a coded sentence that species the order in which amino acid residues should be joined to form a protein.
- Each word, or **codon** in the mRNA sentence is a series of three ribonucleotides that code for a specific amino acid.

- For example, the series uracil-uracil-guanine (UUG) on an mRNA chain is a codon directing incorporation of the amino acid leucine into a growing protein chain.
- Of the 64 possible three-base combinations in RNA, 61 code for specific amino acids and 3 code for chain termination.

The genetic code and the corresponding amino acid side chains.

alanine (ala) A	asparagine (asn) N	aspartate (asp) D	arginine (arg) R
 GCU GCC GCA GCG	 AAU AAG	 GAU GAC	 CGU CGC CGA CGG AGA AGG
cysteine (cys) C	**glutamine (gln) Q**	**glutamate (glu) E**	**glycine (gly) G**
 UGU UGC	 CAA CAG	 GAA GAG	 GGU GGC GGA GGG
histidine (his) H	**isoleucine (ile) I**	**leucine (leu) L**	**lysine (lys) K**
 CAU CAC	 AUU AUC AUA	 UUA UUG CUU CUC CUA CUG	 AAA AAG
methionine (met) M	**phenylalanine (phe) F**	**proline (pro) P**	**serine (ser) S**
 AUG	 UUU UUC	 CCU CCC CCA CCG	 AGU AGC UCU UCC UCA UCG
threonine (thr) T	**tryptophan (trp) W**	**tyrosine (tyr) Y**	**valine (val) V**
 ACU ACC ACG ACA	 UGG	 UAU UAC	 GUU GUC GUG GUA
	STOP UGA	STOP UAA UAG	

AAAA

→ AAA

AGAGAG

AGA

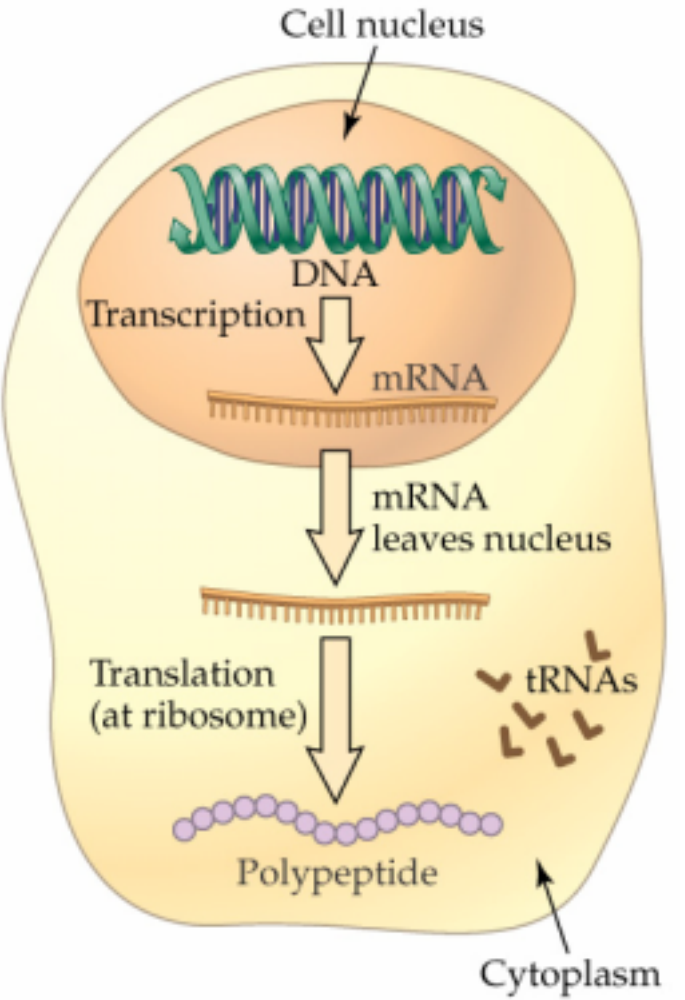
GAG

fMet i

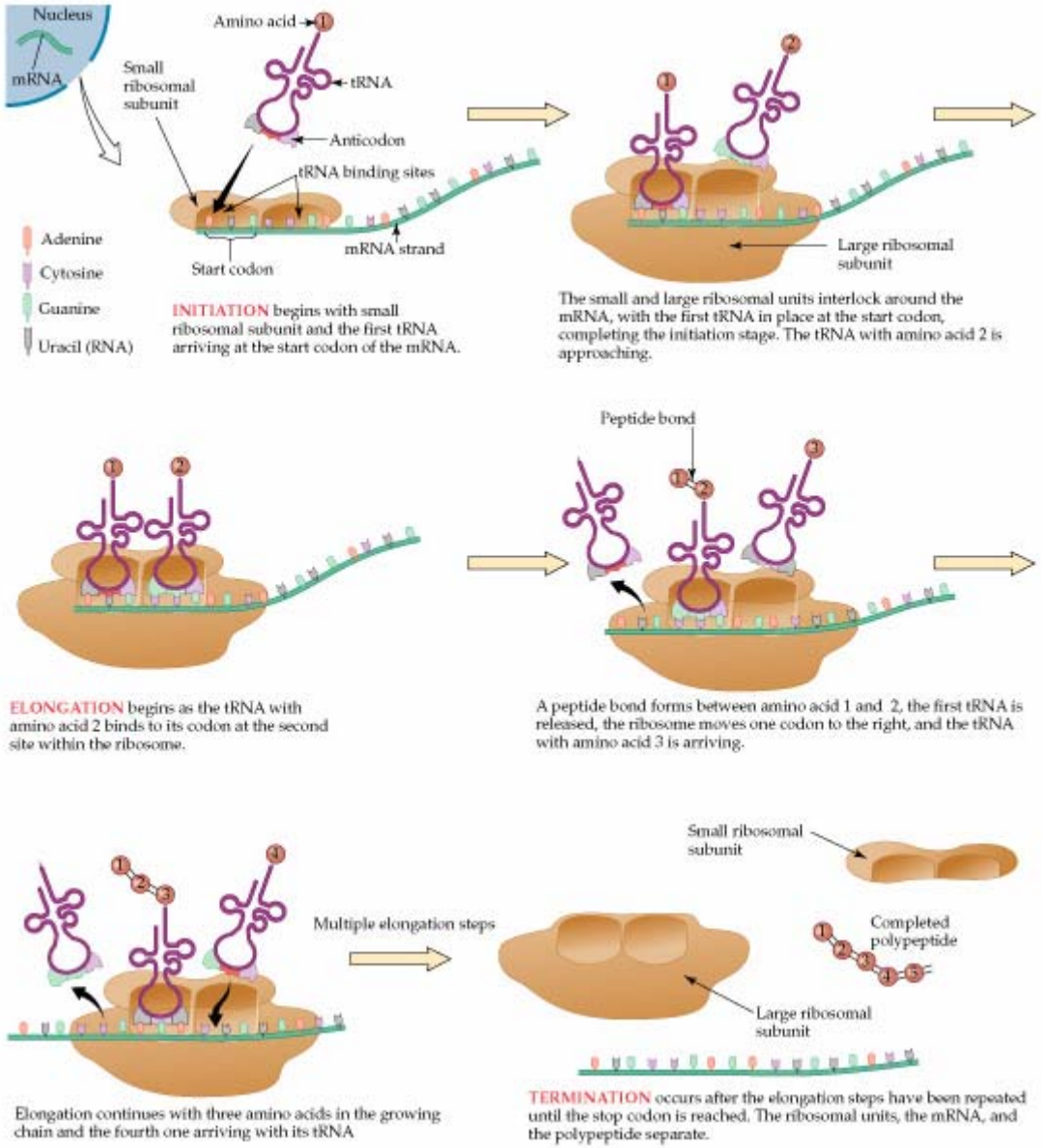
59

26.10 Translation: Transfer RNA and Protein Synthesis

- The synthesis of proteins occur at ribosomes, which are outside the nucleus and within the cytoplasm of cells.
- The mRNA connects with the ribosome, and the amino acids attached to transfer RNA (tRNA) are delivered one by one.



Three stages in protein Synthesis are initiation, elongation, and termination. A diagram of translation is shown in the Fig 26.8.



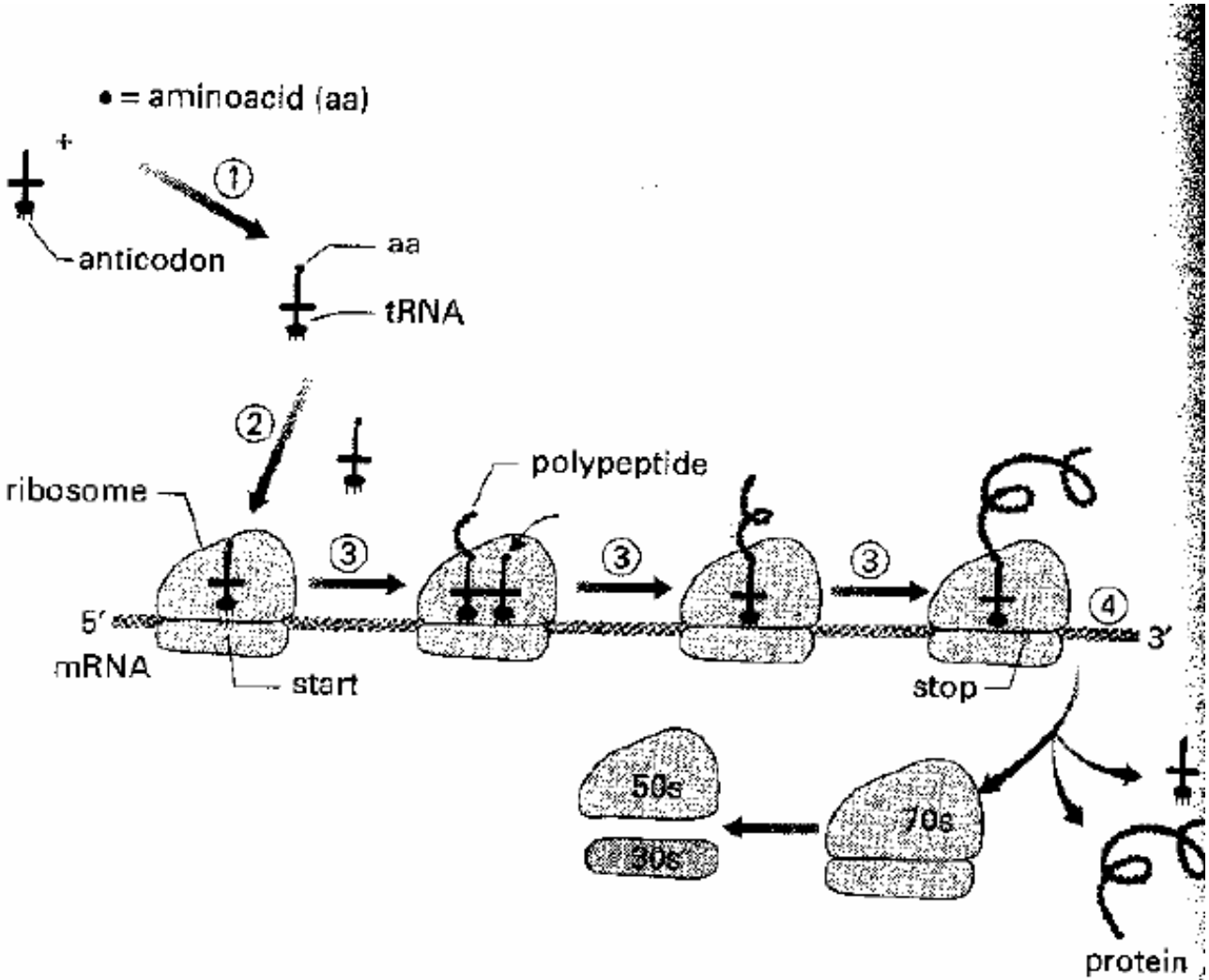


Figure 8.1. Overview of protein synthesis

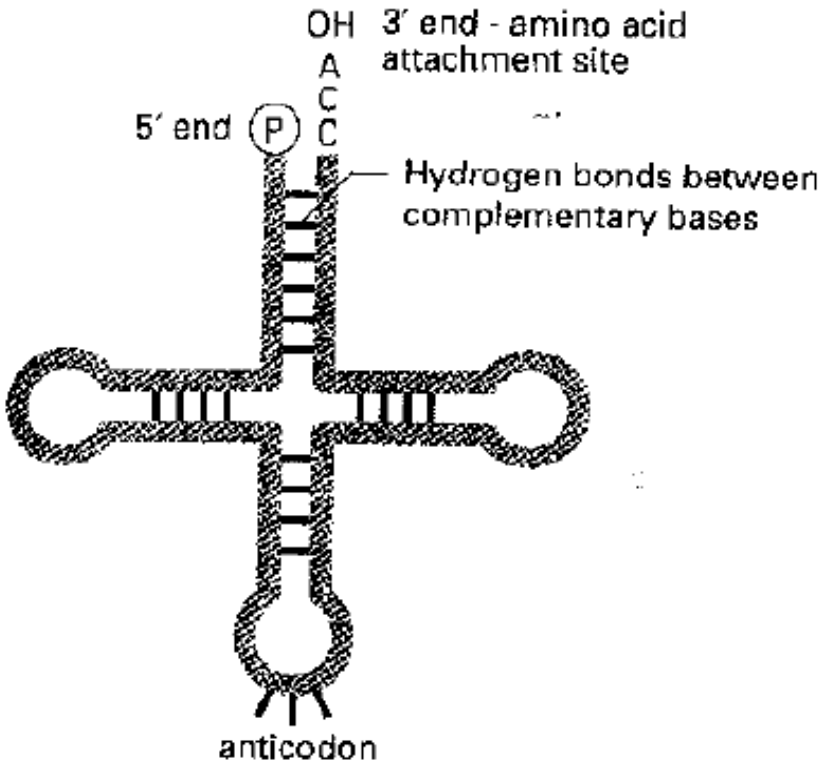


Figure 8.2. Transfer RNA (tRNA).

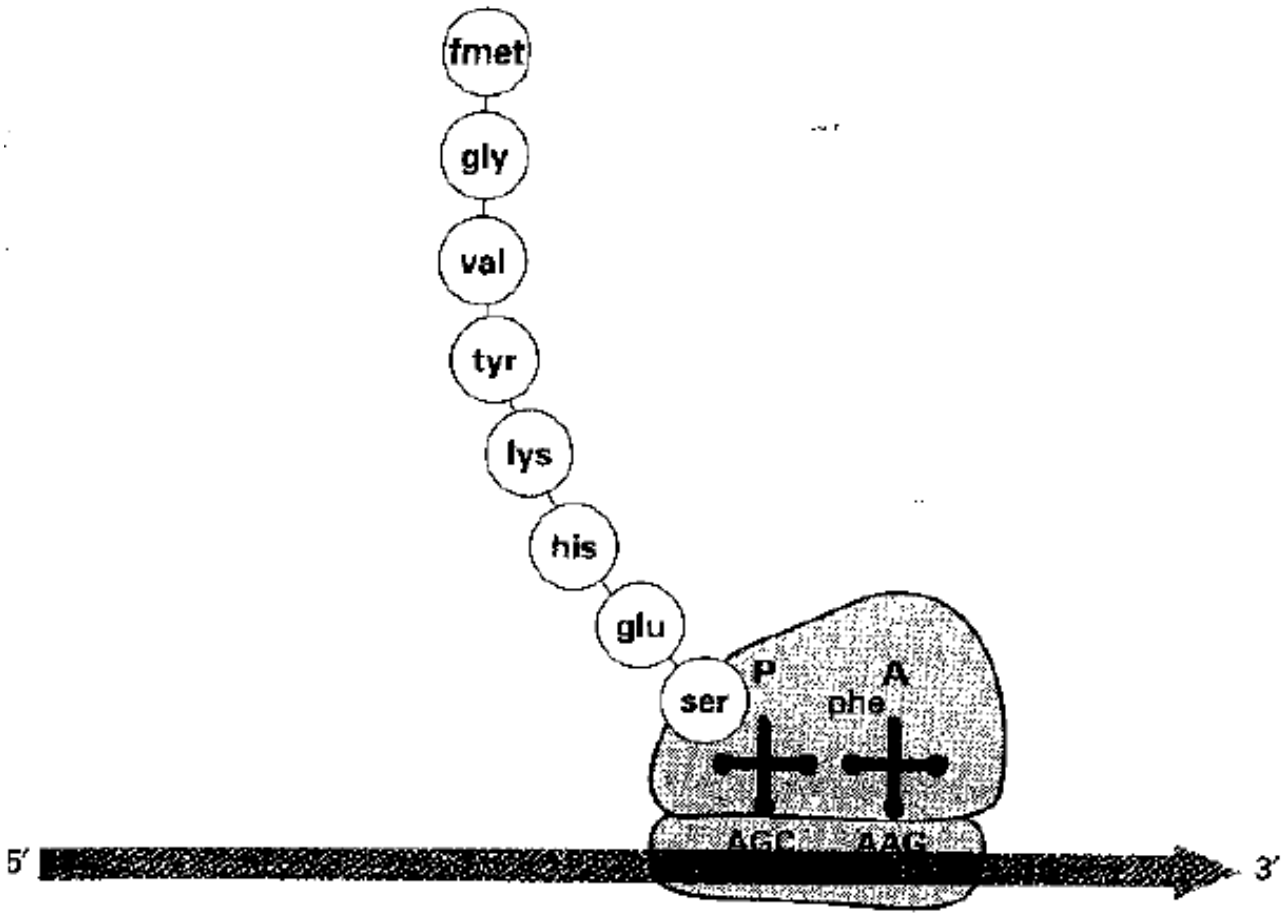


Figure 8.4. The P and A sites on the ribosome.

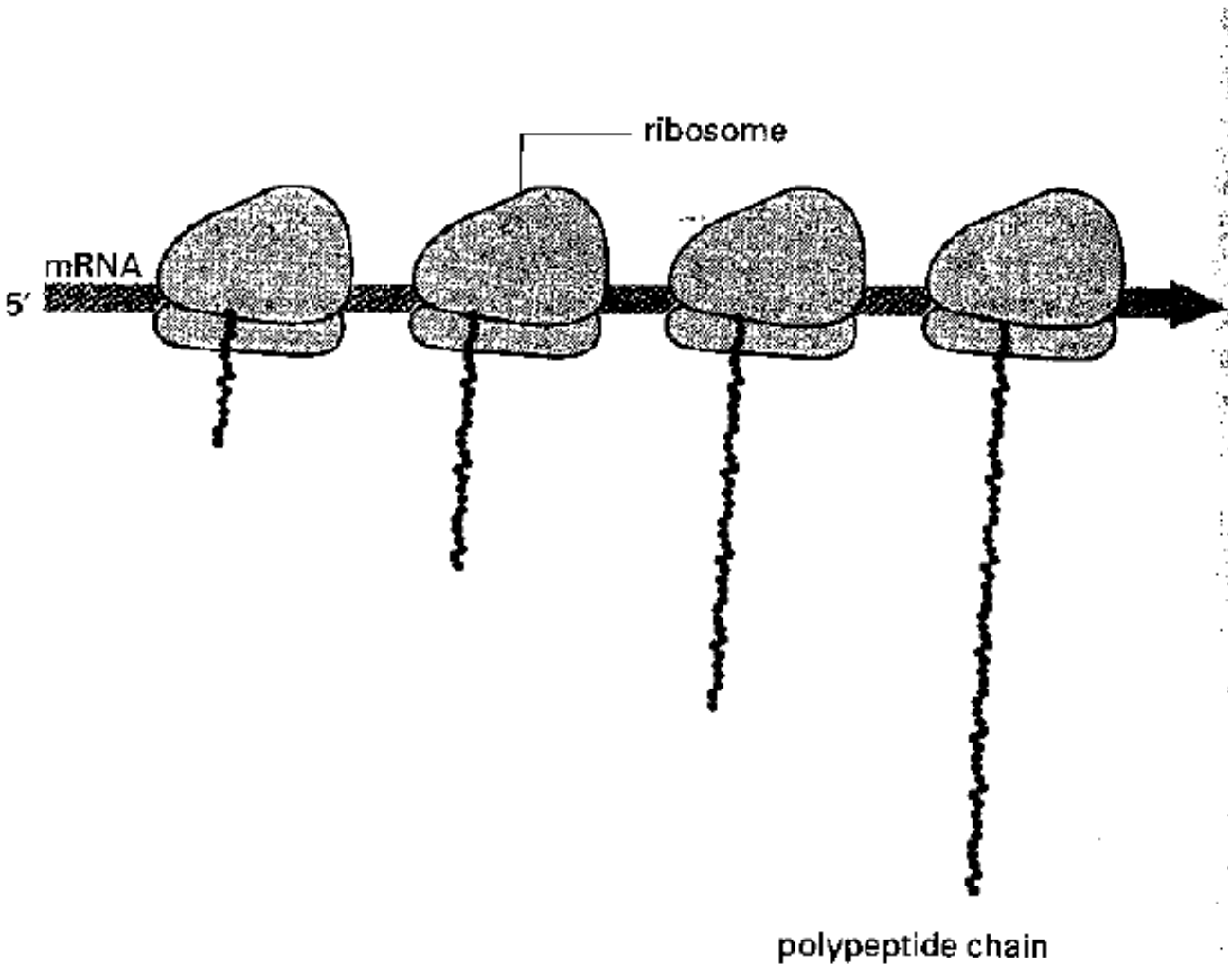
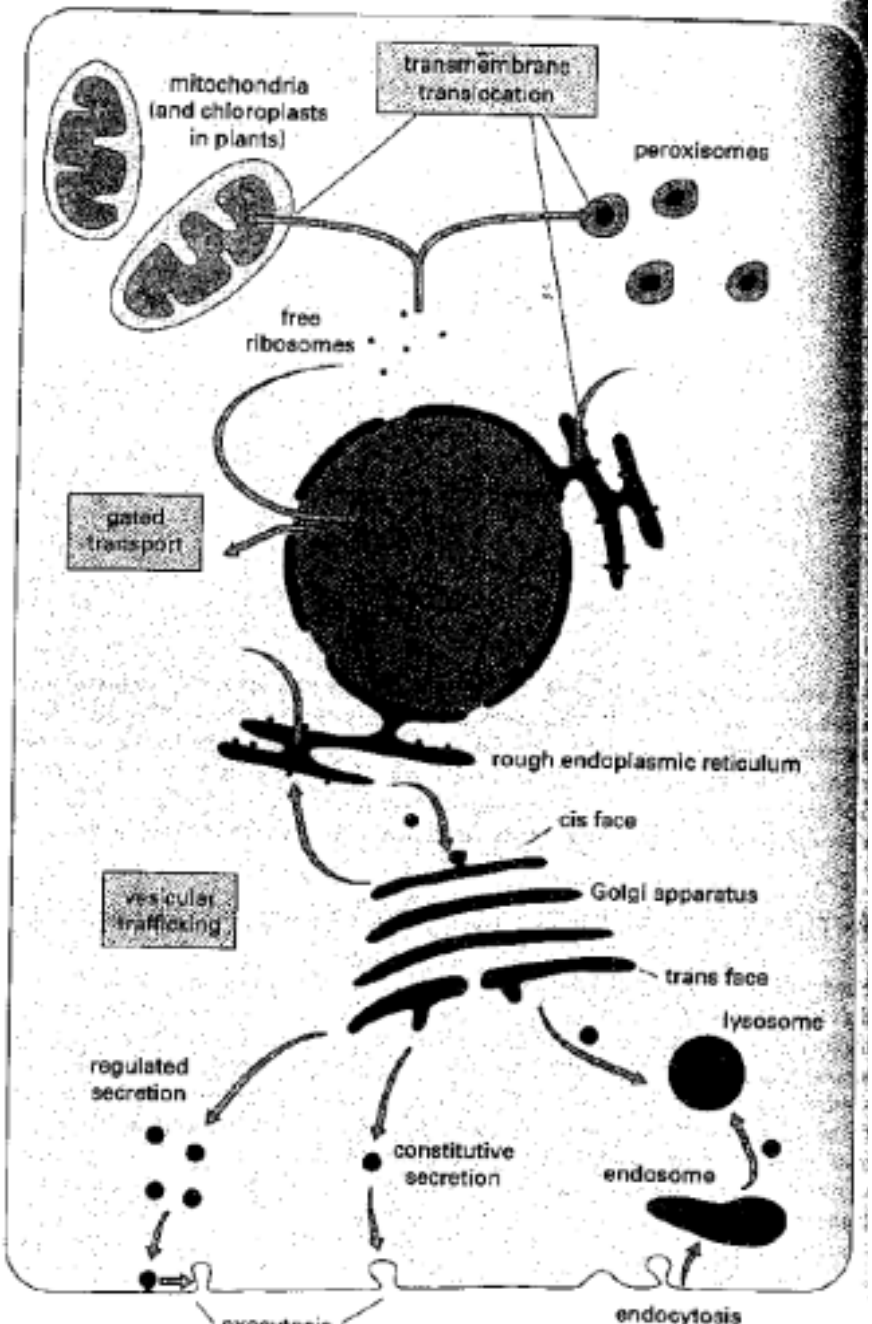


Figure 8.8. Many ribosomes reading one mRNA form a polyribosome.


mitochondria
(and chloroplasts
in plants)
transmembrane
translocation
peroxisomes
free
ribosomes
gated
transport
rough endoplasmic reticulum
cis face
vesicular
trafficking
Golgi apparatus
trans face
lysosome
regulated
secretion
constitutive
secretion
endosome
endocytosis

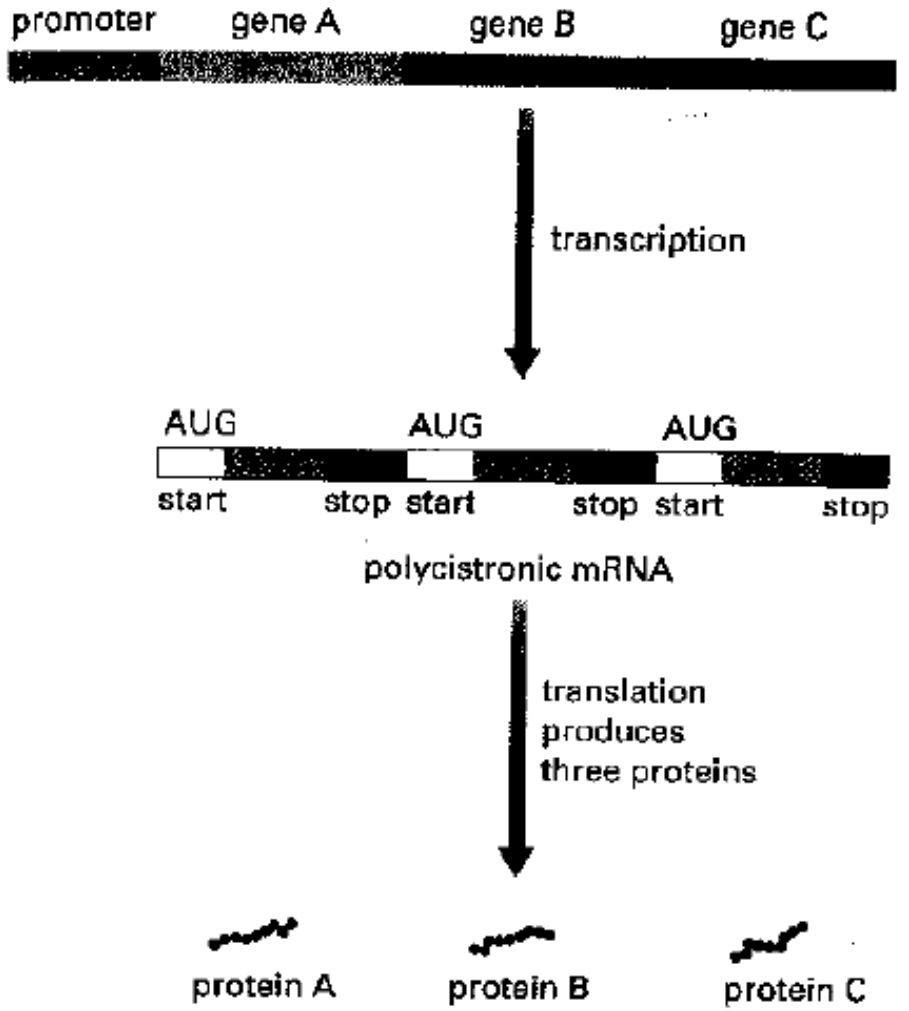


Figure 6.6. A bacterial operon is transcribed into a polycistronic mRNA.

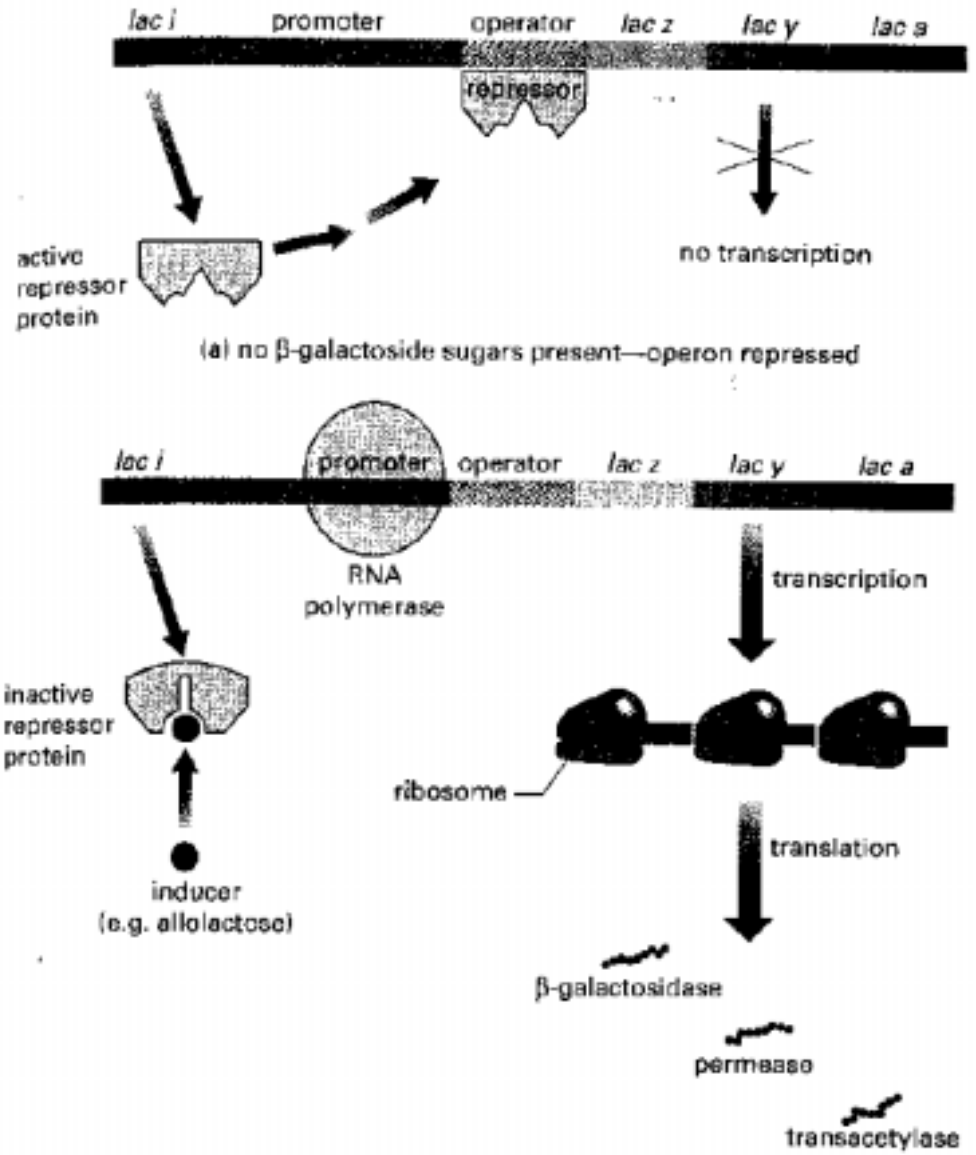


Figure 6.8. Transcription of the *lac* operon requires the presence of an inducer.

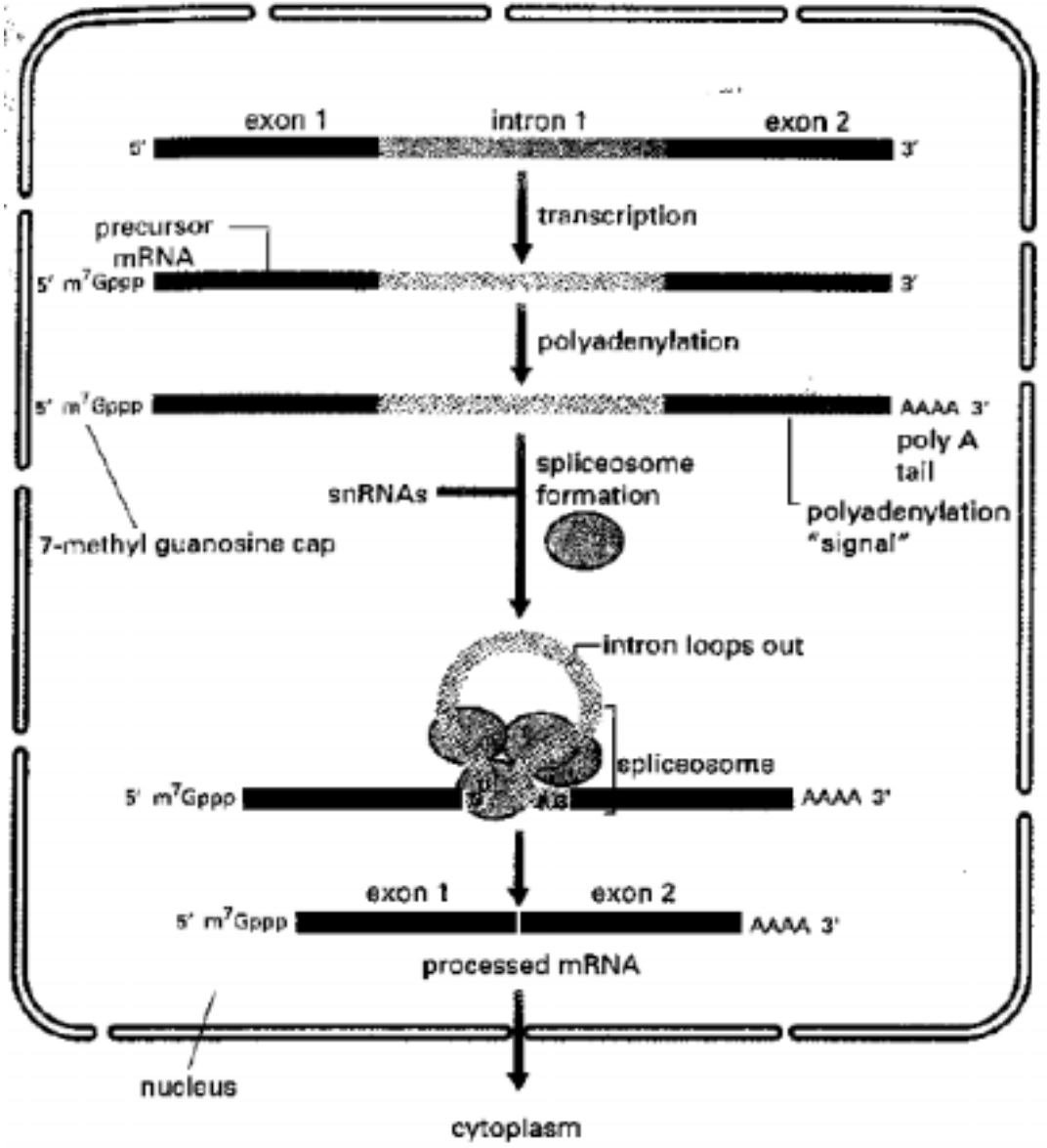


Figure 6.13. mRNA processing in eukaryotes.

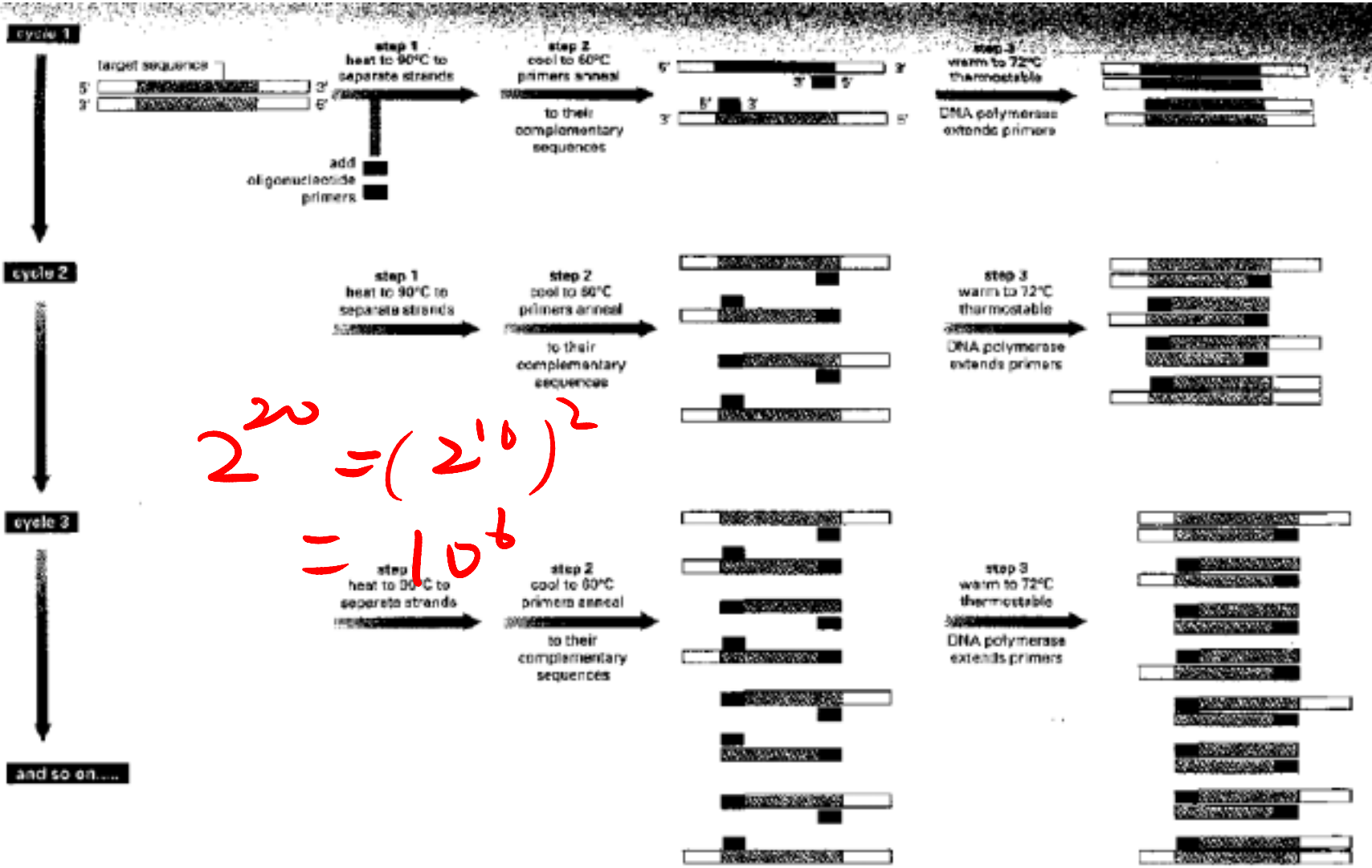
27.4 Recombinant DNA

- Recombinant DNA: DNA that contains two or more DNA segments not found together in nature.
- Recombinant DNA technology made it possible to cut a gene out of one organism and recombine it into the genetic machinery of another organism.

- The two other techniques that play important roles in DNA studies are:

  - Polymerase chain reaction (PCR): PCR allows the synthesis of large quantities of identical piece of DNA.

  - Electrophoresis: A technique that allows separation of proteins or DNA fragments according to their size.

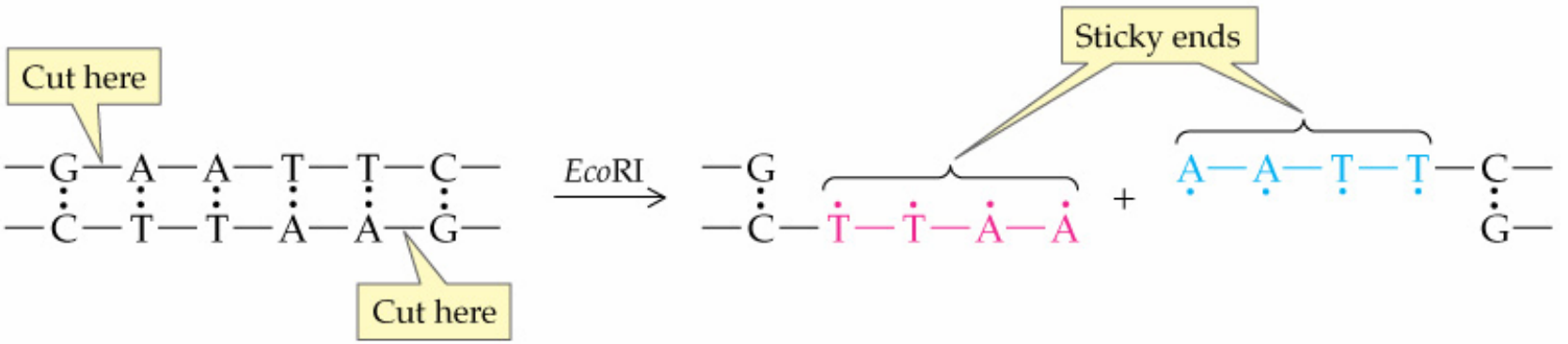


$2^{20} = (2^{10})^2$

$= 10^6$

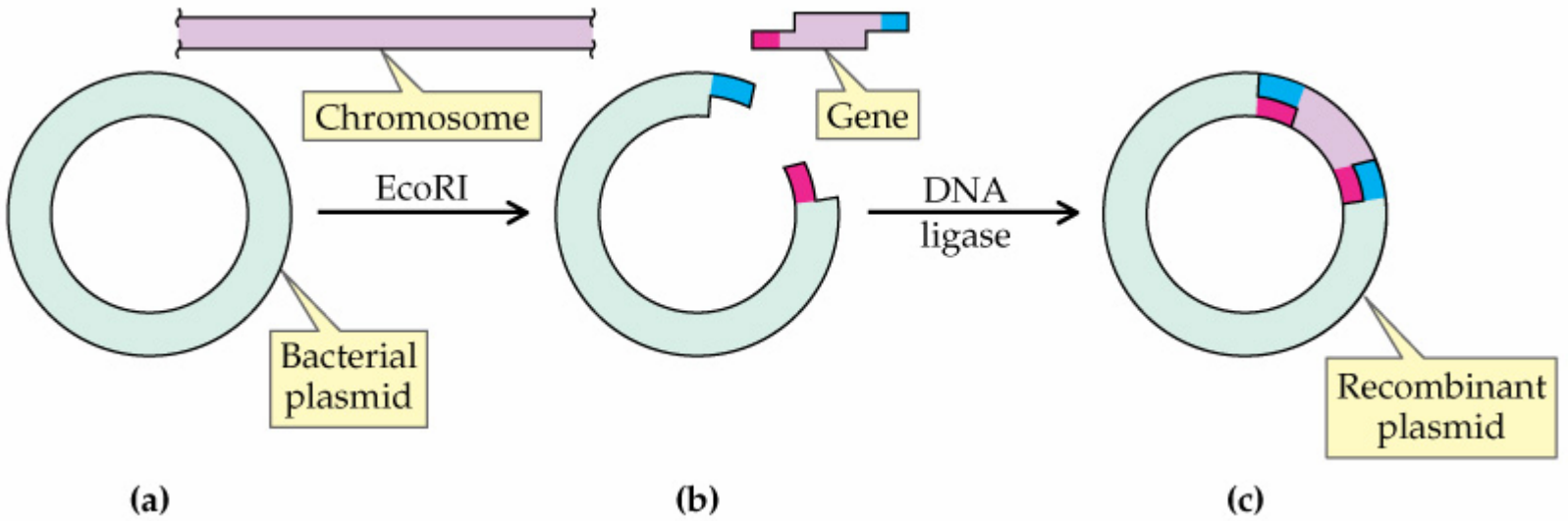
Figure 7.15. Amplification of a DNA sequence using the polymerase chain reaction.

Consider a gene fragment that has been cut from human DNA is to be inserted into a bacterial plasmid. The gene and the plasmid are both cut with the same enzyme that produces sticky ends.



The sticky ends on the gene fragment are complementary to the sticky ends on the opened plasmid.

The two are mixed together in the presence of a DNA ligase enzyme that joins them together by forming their phosphodiester bonds and reconstitutes the now-altered plasmid. The altered plasmid is then inserted into a bacteria cell where the normal transcription and translation take place to synthesize the protein encoded by the recombinant DNA.



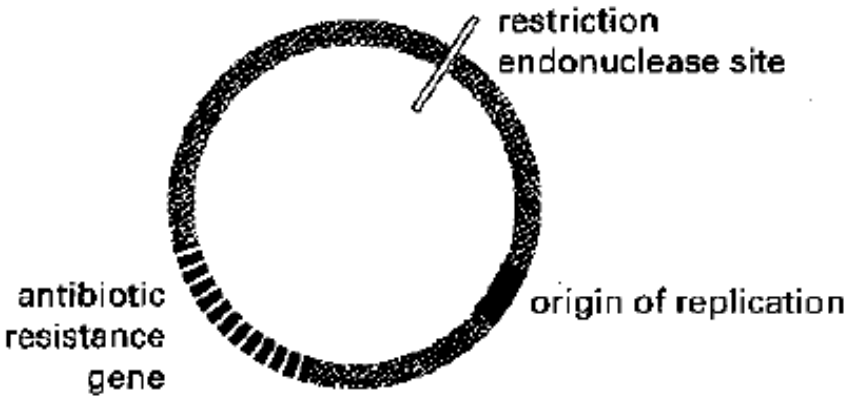
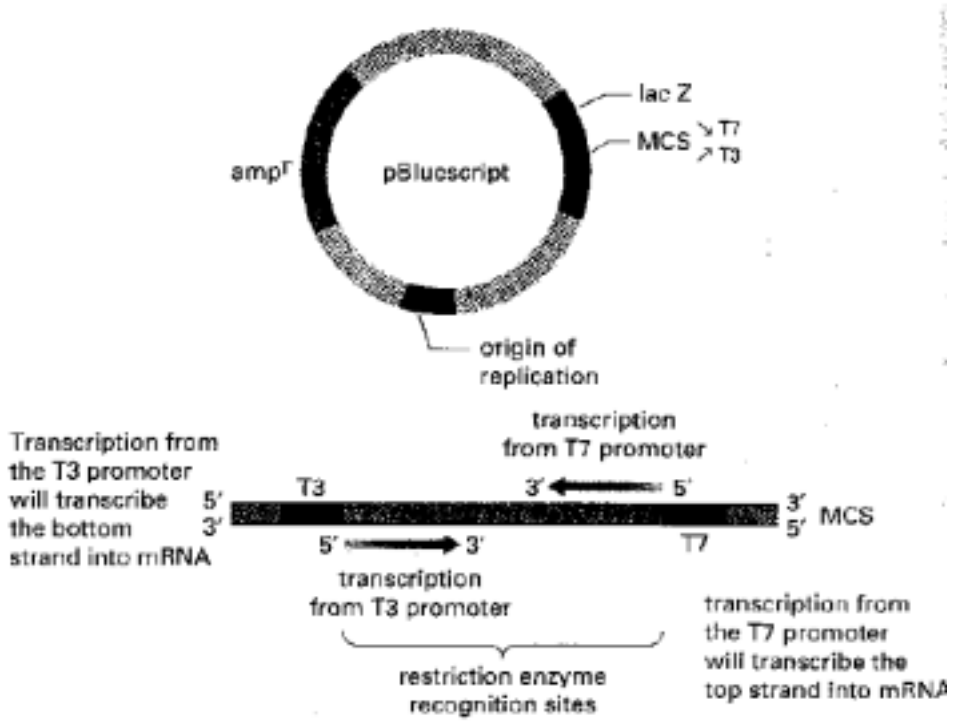


Figure 7.2. A plasmid cloning



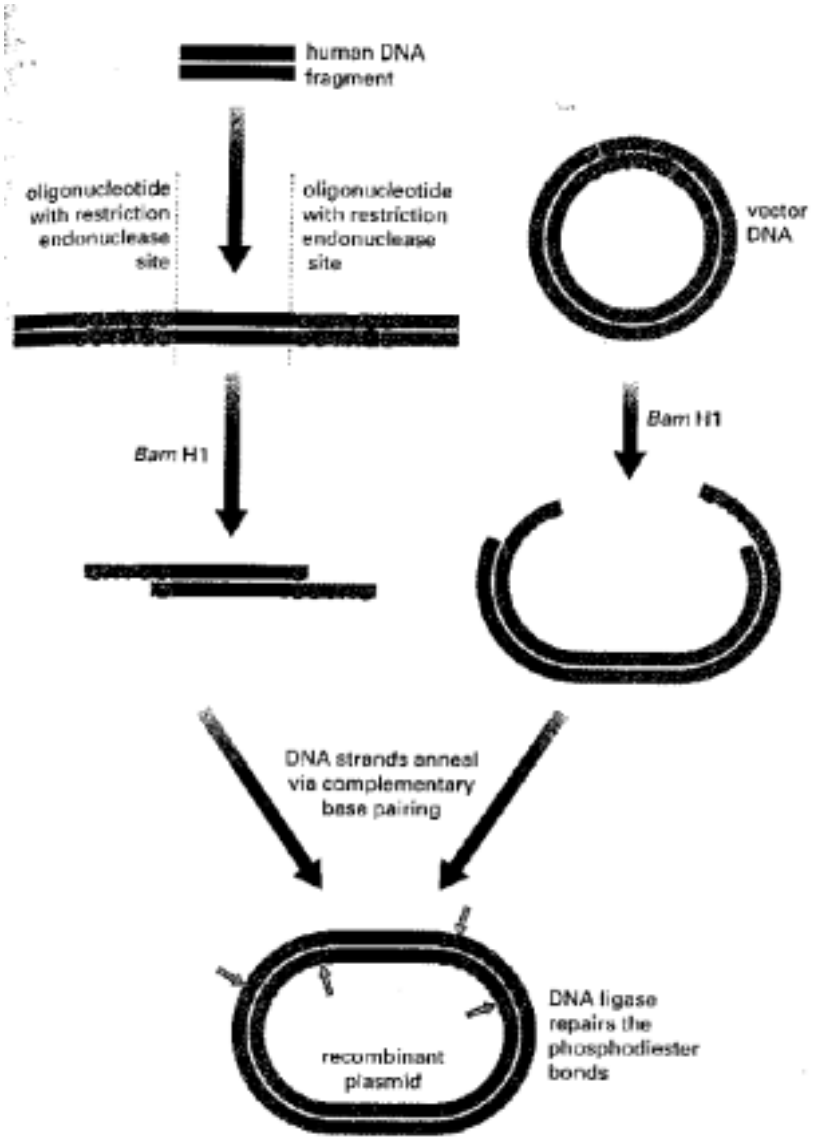


Figure 7.5. Generation of a recombinant plasmid.

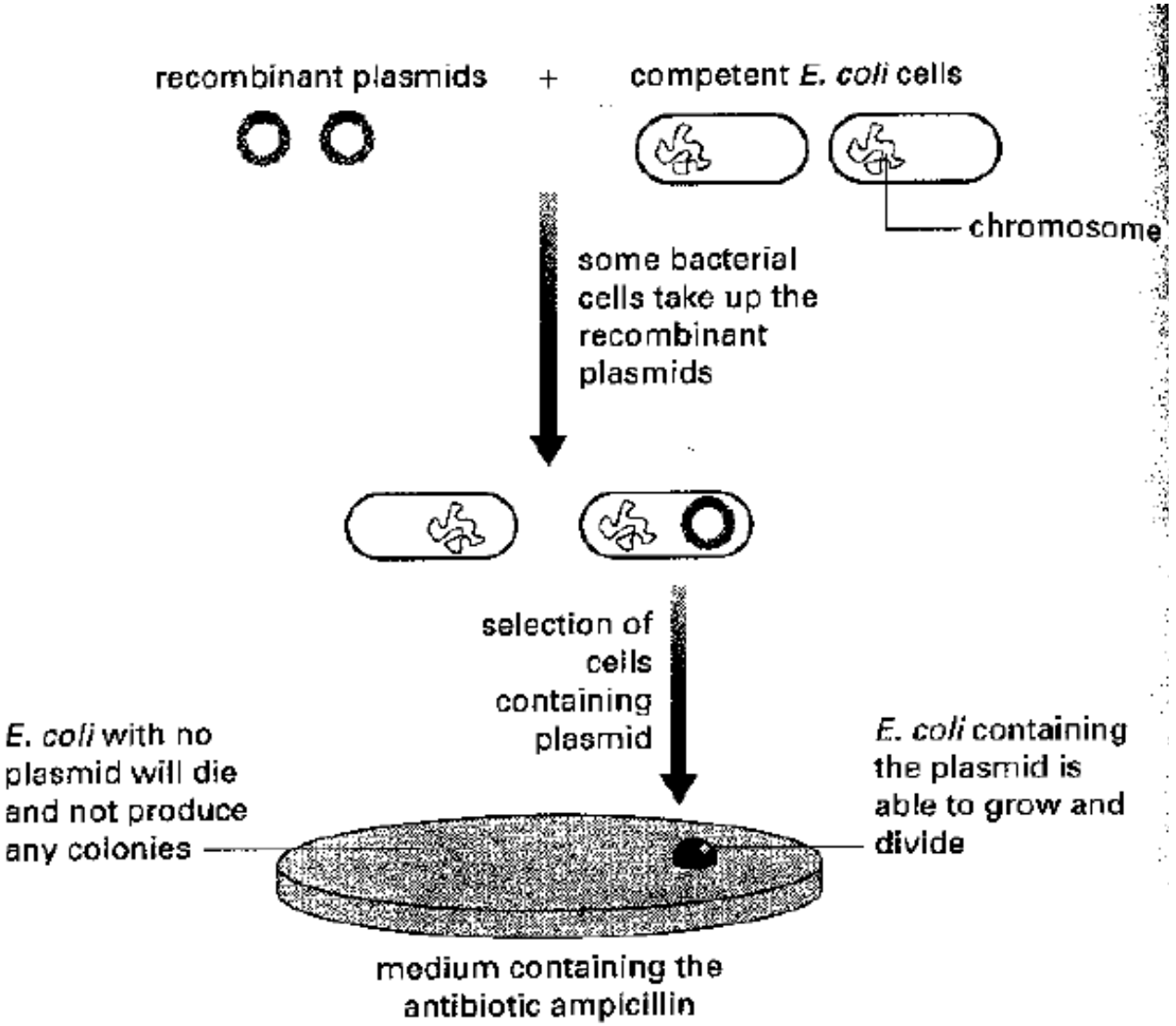


Figure 7.6. Introduction of recombinant plasmids into bacteria.

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Designer Materials for Nucleic Acid Delivery

Theresa M. Reineke and Mark W. Grinstaff,
Guest Editors

MRS BULLETIN • VOLUME 30 • SEPTEMBER 2005

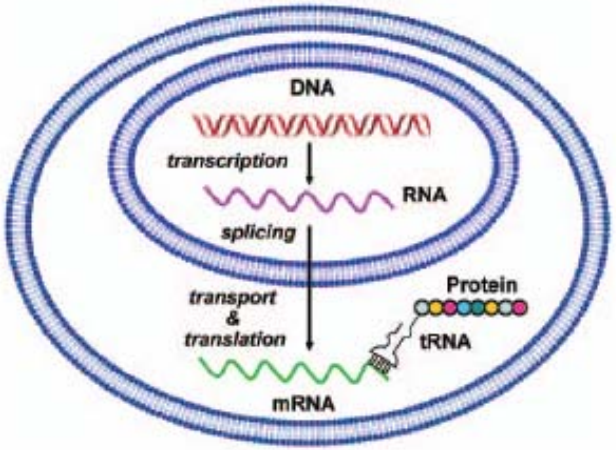


Figure 1. The process of gene expression within a cell. A specific region of DNA (a gene) in the cellular nucleus is transcribed to RNA, which is further processed to messenger RNA (mRNA) and then transported to the cytoplasm of the cell. Transfer RNA (tRNA) decodes the mRNA transcript to synthesize specific protein sequences (translation).

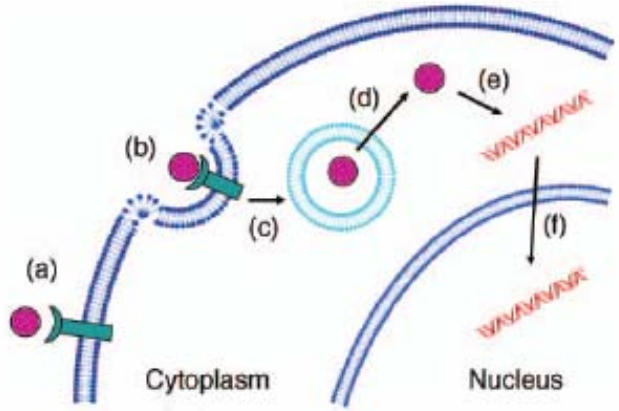


Figure 2. Various materials can bind with and compact nucleic acids into nanoparticles that can be taken up into cells through the process of endocytosis. This process first involves (a) the binding of the nanoparticles to cell-surface glycoproteins. (b) The binding event triggers part of the cellular membrane to envelop the nanoparticle and bring it into the cytoplasm with a membrane-bound vesicle (endosome). (c) Endosomes fuse with other cellular endosomes and mature into degradative lysosomes. (d) The nanoparticles must escape these vesicles to prevent degradation of their therapeutic payload. (e) The material must then release the nucleic acid to perform its therapeutic function in either the cytoplasm, or (f) the genetic material must traverse the nuclear membrane for gene regulation.

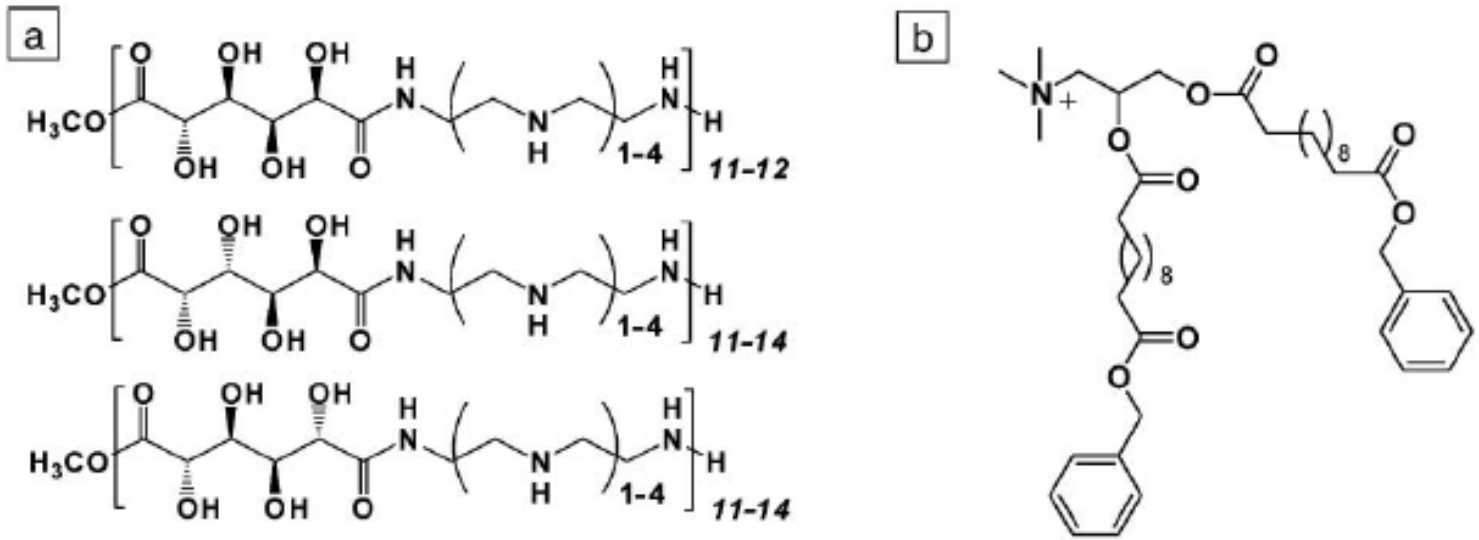


Chart 1. Examples of materials used to deliver nucleic acids. (a) Poly(glycoamidoamine)s synthesized by Reineke et al. exhibit high DNA delivery efficiency and low toxicity in vitro.[16,17] *(b) Functional lipids created by Grinstaff et al. undergo an electrostatic transition to promote the release of DNA from the lipoplex.*[18]

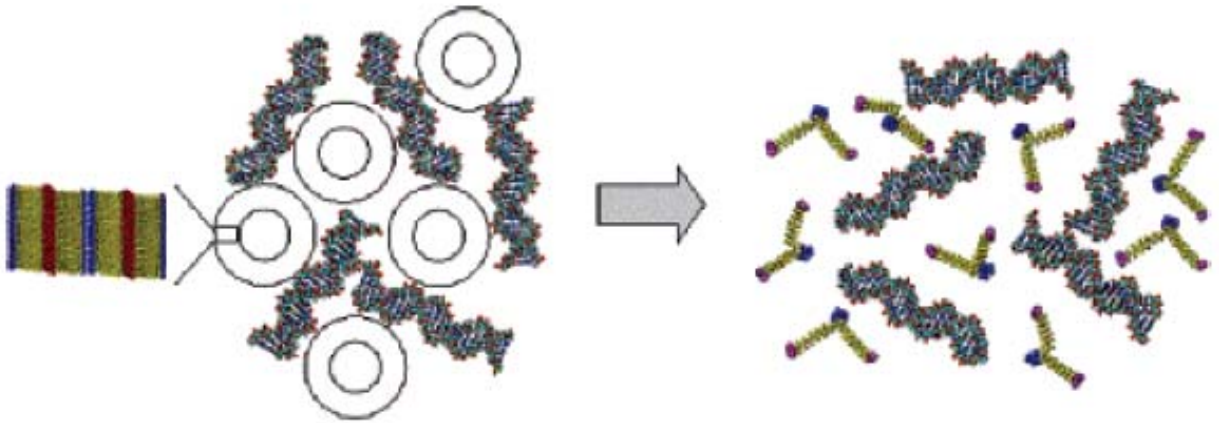


Figure 1. Proposed mechanism for release of DNA from the charge-reversal amphiphiles. A supramolecular assembly is formed between the DNA and the multiwalled vesicles of the amphiphile. Upon enzymatic hydrolysis of the terminal esters of this amphiphile, the DNA is released from the assembly by the newly formed anionic amphiphiles. Not drawn to scale.

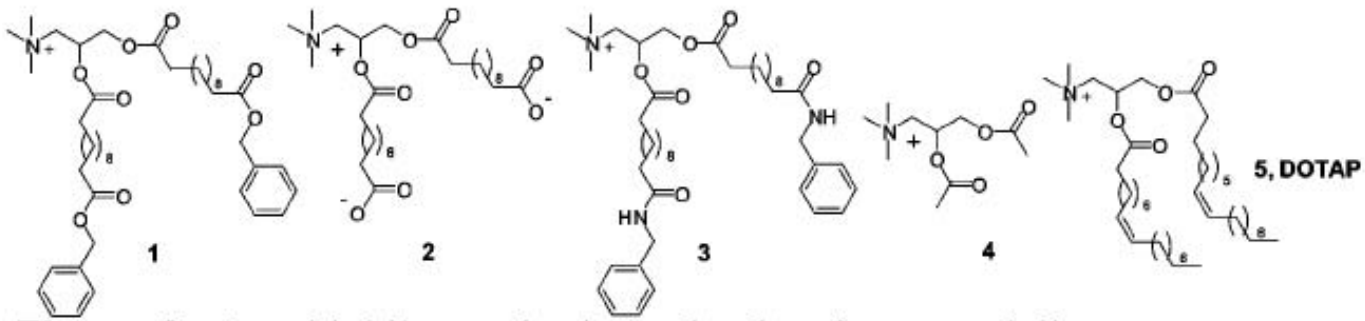


Figure 2. Amphiphiles under investigation for gene delivery.

Polymeric Controlled Nucleic Acid Delivery

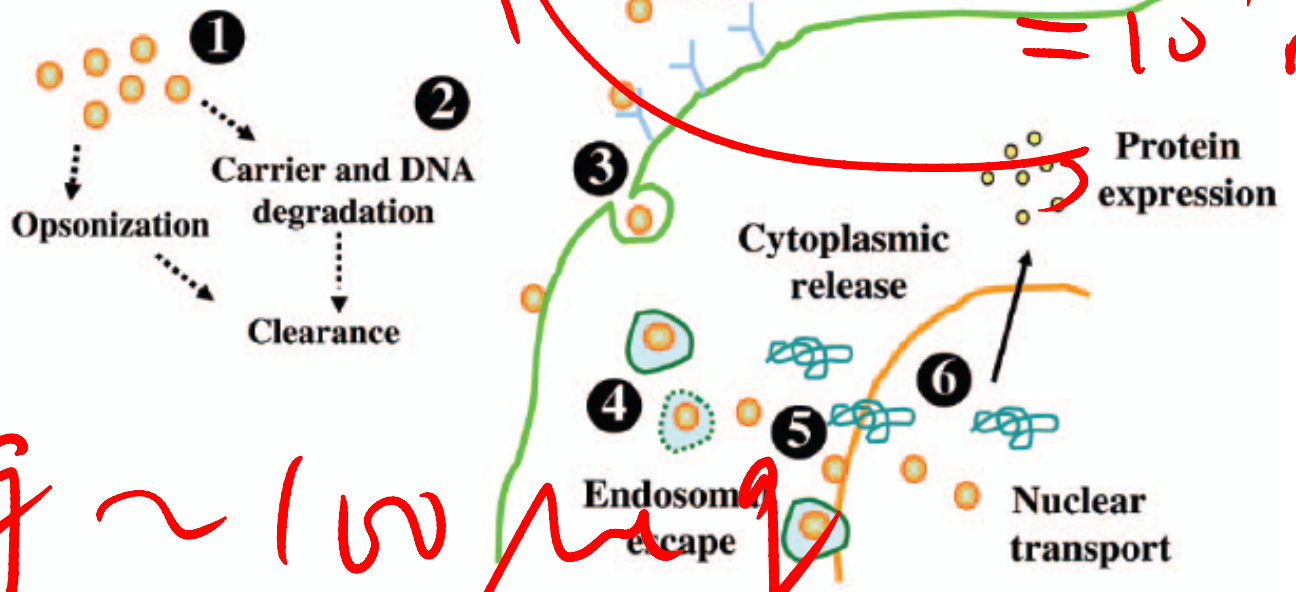


Figure 1. Barriers (1–6) to nonviral gene transfer.

Table I: Representative Polymers Investigated for Gene Delivery.

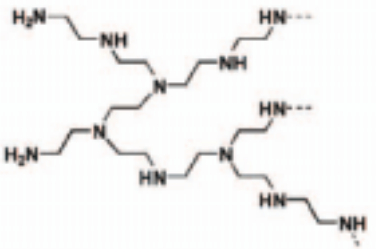
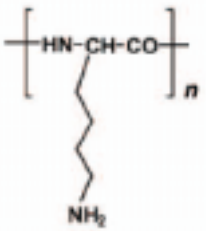
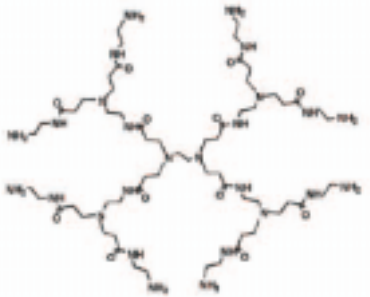
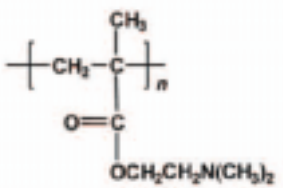
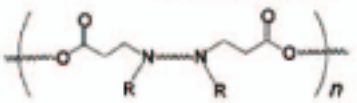
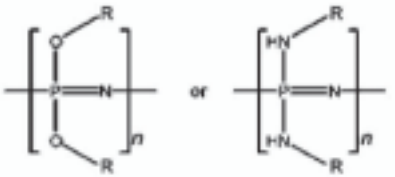
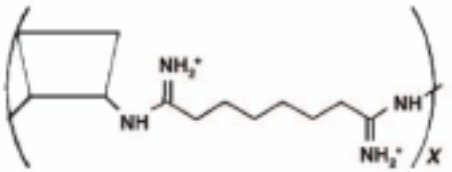
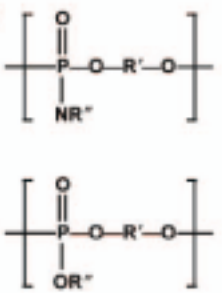
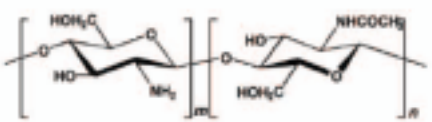
Polymer or Class of Polymer and Reference	Chemical Structure	General Description
Polyethylenimine (Refs. 12–24)		Available in linear and branched forms, polyethylenimine (PEI) is one of the most potent synthetic gene carriers and the most widely studied. It can mediate gene transfer in a variety of cells *in vitro* and tissues *in vivo*. The major drawback of PEI is its cytotoxicity. Numerous attempts have been made to reduce the toxicity by reducing the molecular weight, cross-linking low-molecular-weight segments by biodegradable bonds, PEGylation, removing the *N*-acyl moieties, and conjugating saccharides to PEI.
Polylysine (Refs. 25–32)		One of the earliest investigated gene carriers because of its excellent DNA condensation ability and efficient protection of DNA from nuclease digestion, polylysine is as toxic as PEI but not as potent in transfection efficiency.
Polyamidoamine dendrimer (Refs. 36–39)		Starburst polyamidoamine dendrimers with either ammonia or ethylenediamine as core molecules are highly defined in terms of molecular weight and geometry. With the primary amine groups on the surface to condense DNA, these dendrimers can transfect a wide variety of cells *in vitro*, with efficiency matching that of PEI under optimal conditions, but high cytotoxicity is still a drawback.
Poly[(2-dimethyl-amino)ethyl methacrylate] (Ref. 40)		The high transfection efficiency of PDMAEMA has been postulated to derive from its high buffering capacity to destabilize endosomes, combined with its ability to release the DNA in the cytosol and/or the nucleus. Its strength and shortcomings are similar to those of PEI.

Table I: Representative Polymers Investigated for Gene Delivery. *(Continued)*

Polymer or Class of Polymer and Reference	Chemical Structure	General Description
Poly(β-amino ester) (Refs. 12, 25, 41, 42)		Poly(β-amino esters) can be synthesized by a combinatorial synthetic strategy, via the conjugation addition reaction of diamines and diacrylates. The wide variety of commercially available diamines and diacrylates facilitates parallel synthesis of a large number of polymers and study of their structure–property relationships. High-throughput, cell-based screening has identified many new poly(β-amino ester)s that can transfect with a higher efficiency than PEI.
Polyphosphazene (Ref. 43)		Polyphosphazene can be biodegradable and can exhibit a wide range of physicochemical properties by varying the structures of its side chains. However, only a tertiary amine structure can be used in the side chain, because the use of primary and secondary amines would lead to a cross-linked polyphospazene in the substitution reaction of poly(dichloro)phosphazene.
Cyclodextrin-containing polycation (Refs. 9, 44–50)		The linear water-soluble polymers are generally synthesized by the polycondensation of a difunctionalized cyclodextrin monomer (either hydroxyl- or amine-derivatized) and a difunctionalized comonomer. The structure shown is only one of many possible variations. The structure–property relationships of a large number of these polymers has been established and many of them show promising transfection potency.
Polyphosphoramidate (Refs. 59–63) and polyphospate Refs. 52–58)		The polyphosphoramidates and polyphosphates are series of polymers with a phosphoester backbone containing different charge groups in the side chain connected to the backbone through a phosphoramide (P–N) or a phosphate (P–O) bond, respectively. These gene carriers have different charge groups, side-chain lengths, and branching structures, but they are structurally related to allow a systematic investigation of their structure–property relationships, including DNA binding capacity, cytotoxicity, DNA protection, biodegradability, DNA release kinetics, and transfection efficiency. Some of them are as potent as PEI but show much lower cytotoxicity and better tissue biocompatibility.
Chitosan (Refs. 57, 67–76)		Chitosan is a biodegradable polysaccharide comprised of D-glucosamine repeating units and is extracted from crustacean shells. It becomes soluble at pH < 6 to allow complexation with DNA. The properties of chitosan nanoparticles are sensitive to the molecular weight and degree of deacetylation of the polysaccharides. It is the first polymeric gene carrier investigated for delivery of DNA through the oral route.

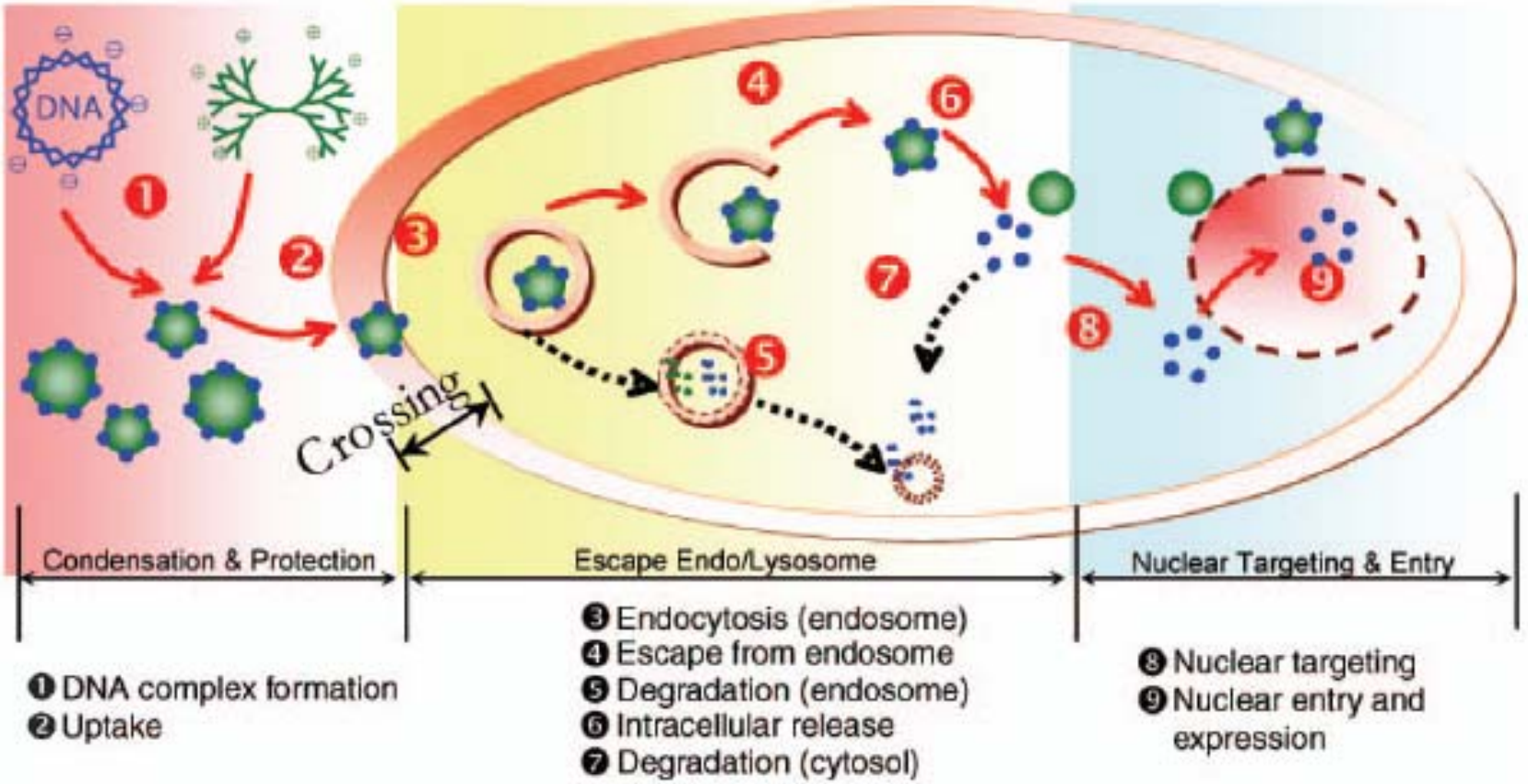


Figure 2. Four major cellular barriers (modified from Reference 1).

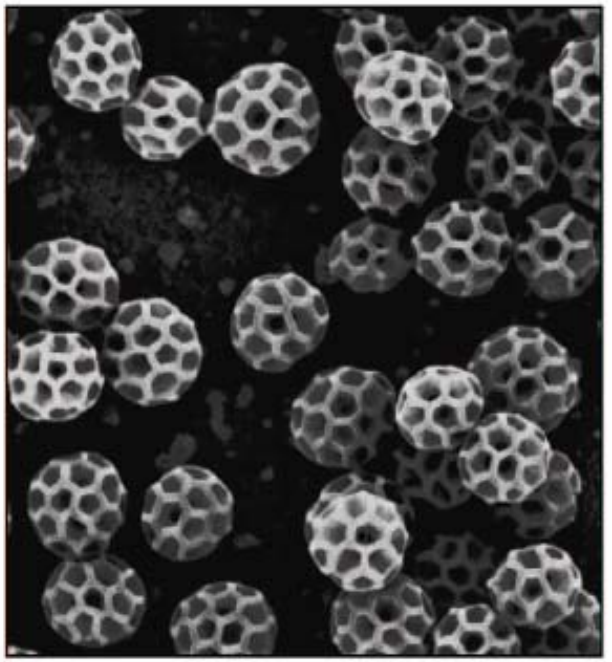


Figure 3. Scanning electron micrograph of nanoscale hollow buckyballs self-assembled from dendrimer-like DNA-based hybrid molecules.[60] The buckyballs are ~400 nm in diameter.

Table I: DNA Delivery Systems at Different Scales.

Scale	Size	Features
Macroscopic (milliscale)	>100 μm	Localized at site of placement Immobilized within tissue DNA released into interstitial space
Microscale	μm	Targetable to anatomical regions (e.g., Peyer's patches in gut, capillaries in tissue) Intracellular by phagocytosis
Nanoscale	<1 μm	Intracellular Targetable to subcellular compartments

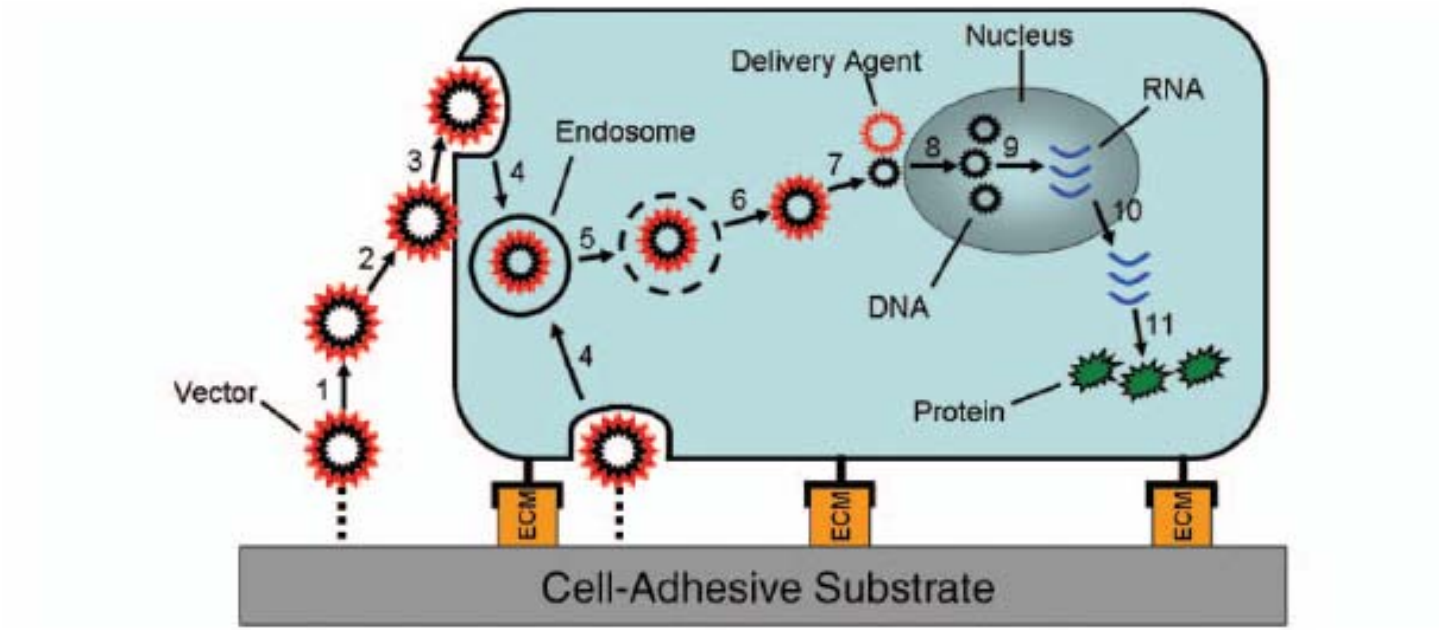


Figure 1. Steps leading to gene expression: (1) Release of vector from substrate, (2) association of vector with cell membrane, (3) endocytosis, (4) formation of early endosome, (5) transport in late endosome, (6) escape from endosome, (7) transport to nucleus and dissociation of delivery agent, (8) entry into nucleus, (9) transcription into RNA, (10) transport of RNA to cytoplasm, and (11) translation of RNA into protein.

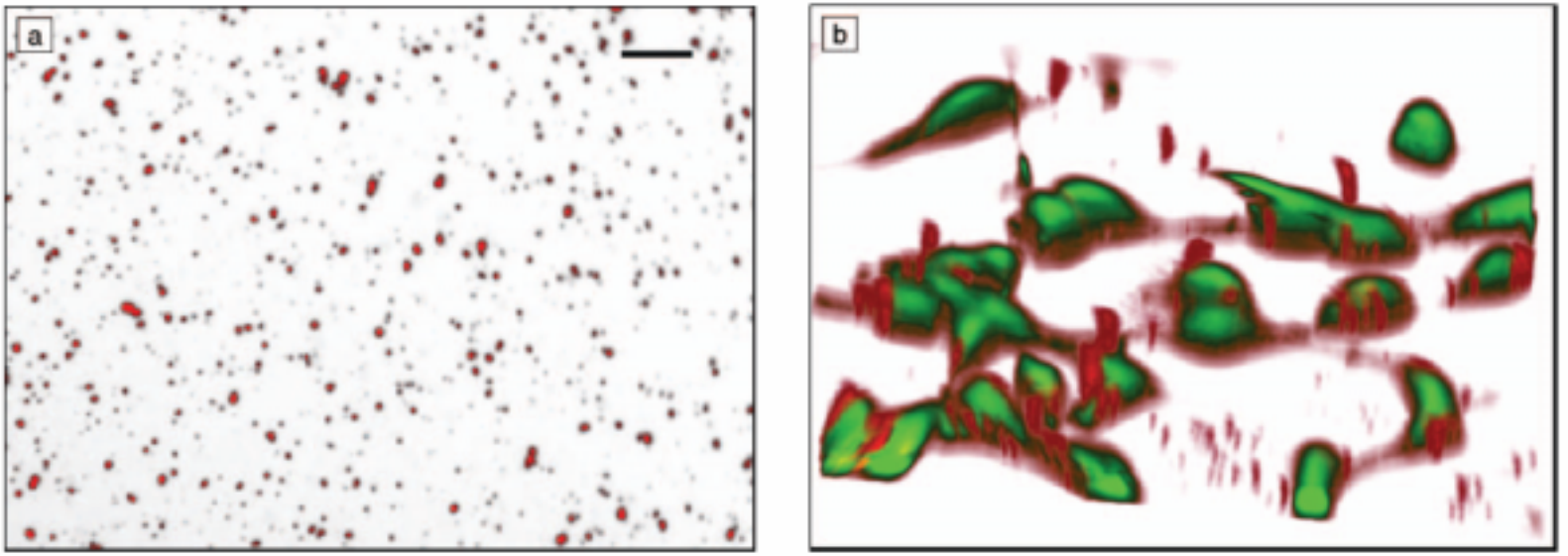


Figure 2. (a) Polyethylenimine–DNA complexes homogenously distributed across a polystyrene substrate. Scale bar is 10 μm. (b) Redistribution of complexes to cell surface on a serum-coated substrate. Cells (green) have an average width of ~15–20 μm.

Peptide-Enhanced Nucleic Acid Delivery

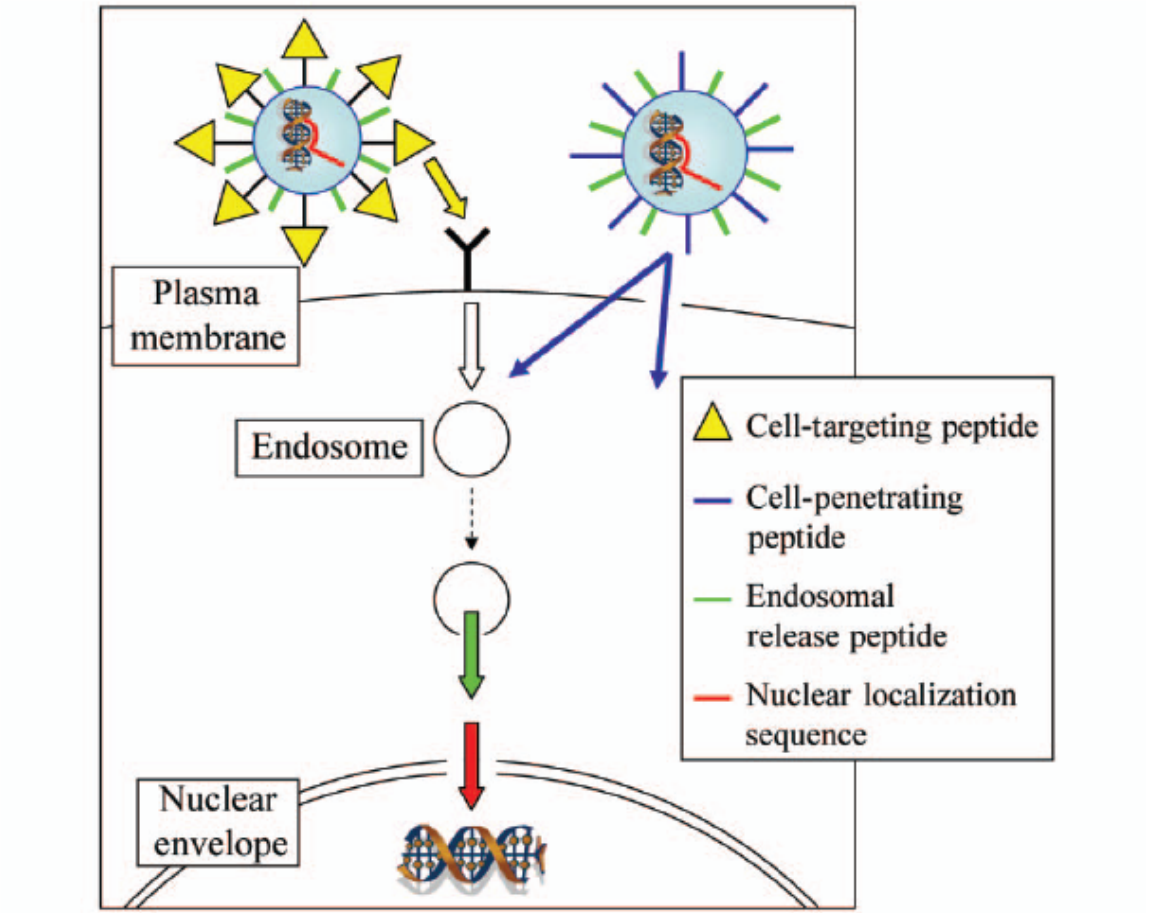


Figure 1. Peptide-enhanced nucleic acid delivery. The incorporation of peptides into nonviral delivery systems can improve cell targeting and entry, endosomal escape, and nuclear entry.

Table I: Cell-Penetrating Peptides (CPPs) Studied for Nucleic Acid Delivery.

CPP	Sequence	Origin	Cargo	References
TAT	YGRKKRRQRRR	HIV-1 TAT	pDNA, asODN	16–21
Penetratin	RQIKIWFQNRRMKWKK	AntpHD	asODN, PNA, siRNA	5, 22–24
Transportan	GWTLNSAGYLLKINLK ALAALAKKIL	Galanin and mastoparan fusion	PNA, siRNA	5, 23, 25
Polyarginine	Stearyl-R_8, various arginine-rich sequences	Engineered	pDNA	26–28

TAT = transactivator of transcription; pDNA = plasmid DNA; asODN = antisense oligodeoxynucleotide; AntpHD = Antennapedia protein homeodomain; PNA = peptide nucleic acid; siRNA = short interfering RNA.

Table II: Nuclear Localization Sequence (NLS) Peptides Incorporated Into Gene Delivery Systems.

NLS Peptide	Sequence	Mechanism	Origin	Reference(s)
SV40 large T-antigen	PKKKRKV	Binds importin α	Derived from simian virus 40 large tumor antigen	49, 51, 52, 54, 57–66
M9	NQSSNFGPMKGGNFGG RSSGPYGGGGQYFAKP RNQGGY	Recognized by the importin β-like shuttle protein transportin 1	From heterogeneous nuclear ribonucleo-protein A1	50
Vpr	DTWTGVEALIRILQQLL FIHFRIGCRHSRIGIIQQR RTRNGA	Independent of importin α/β or transportin pathways	Derived from C-terminal residues of HIV-1 viral protein	67
Ad3	AKRARLSTSFNPVYPYE DES	Distinct domains for endocytosis and nuclear entry	Derived from Ad3 fiber protein	68
IBB	AARLHRFKNKGKDSTE MRRRRIEVNVELRKAK KDDQMLKRRNVSC	Binds importin β	Derived from hSRP1α	69
HTLV	MPKTRRRRRRSQRKRPP TWAHFPGFGQGSLC	Binds importin β	Derived from human T cell leukemia virus type 1 Rex protein	56

Note: Not all studies reported NLS-mediated, sequence-specific enhancement of transgene expression.