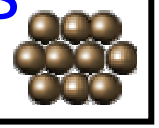
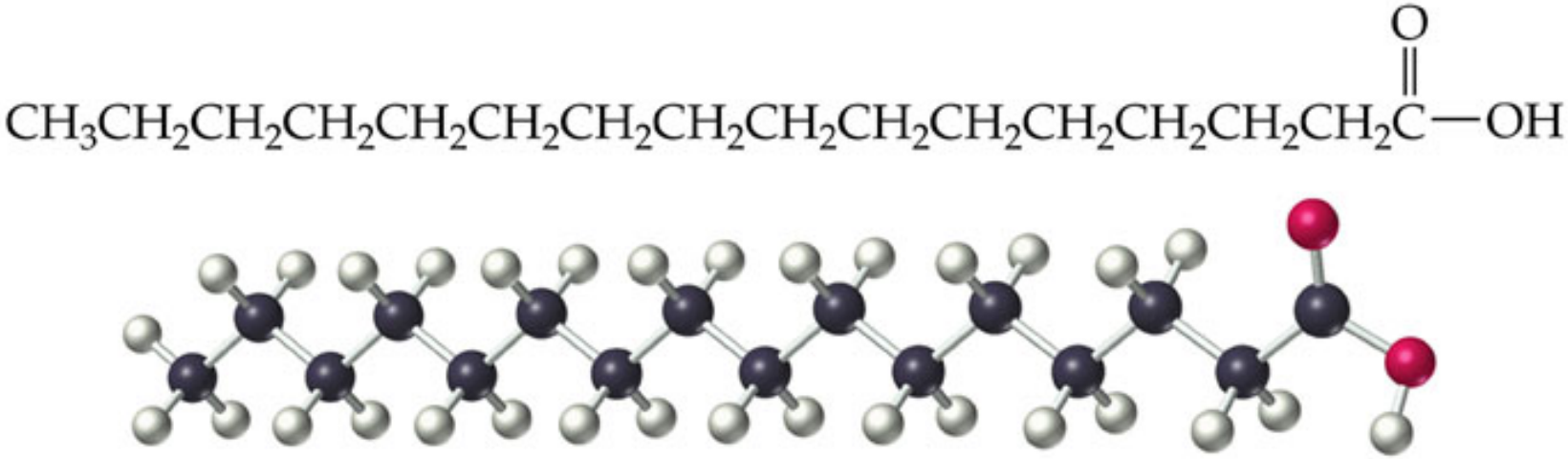
Review of Cell Biology

Lipid

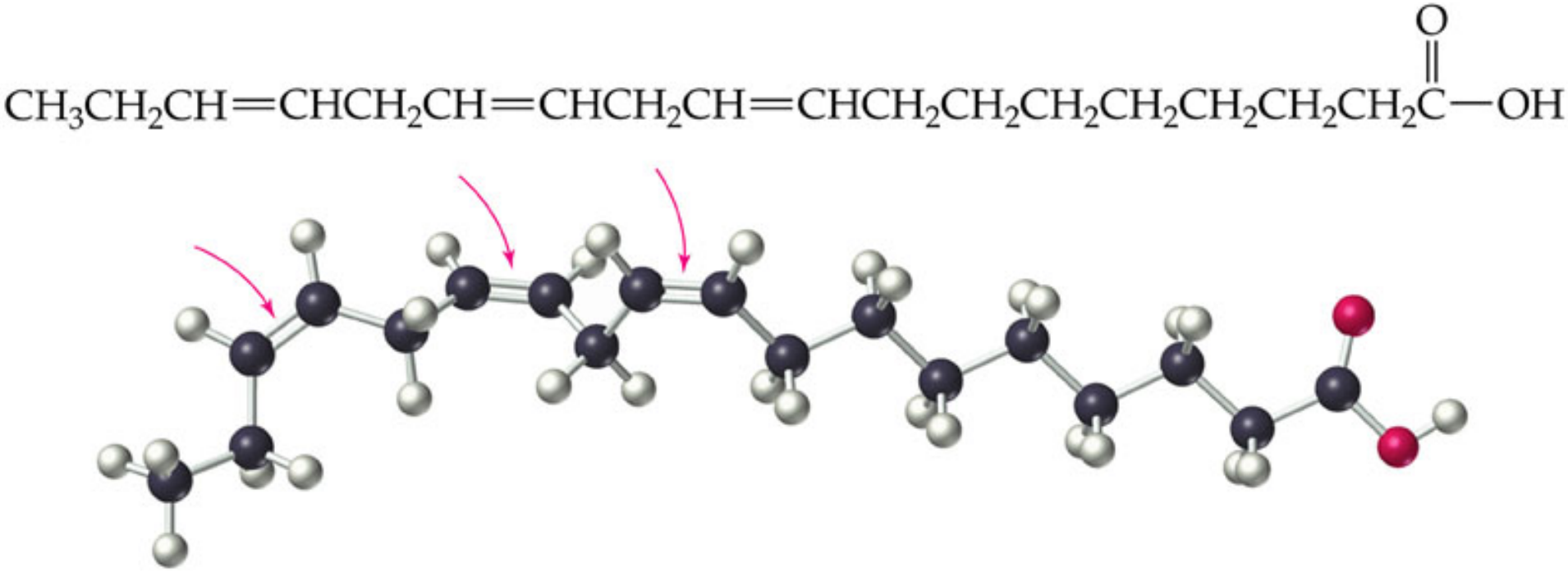
- ***Lipids*** are naturally occurring molecules from plants or animals that are soluble in nonpolar organic solvents.
- Lipid molecules contain large hydrocarbon portion and not many polar functional group, which accounts for their solubility behavior.

- *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.

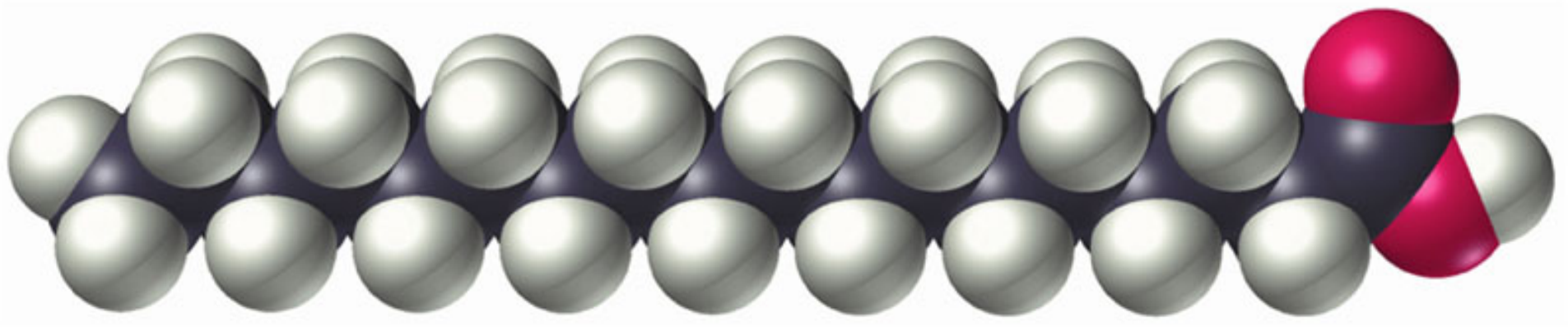




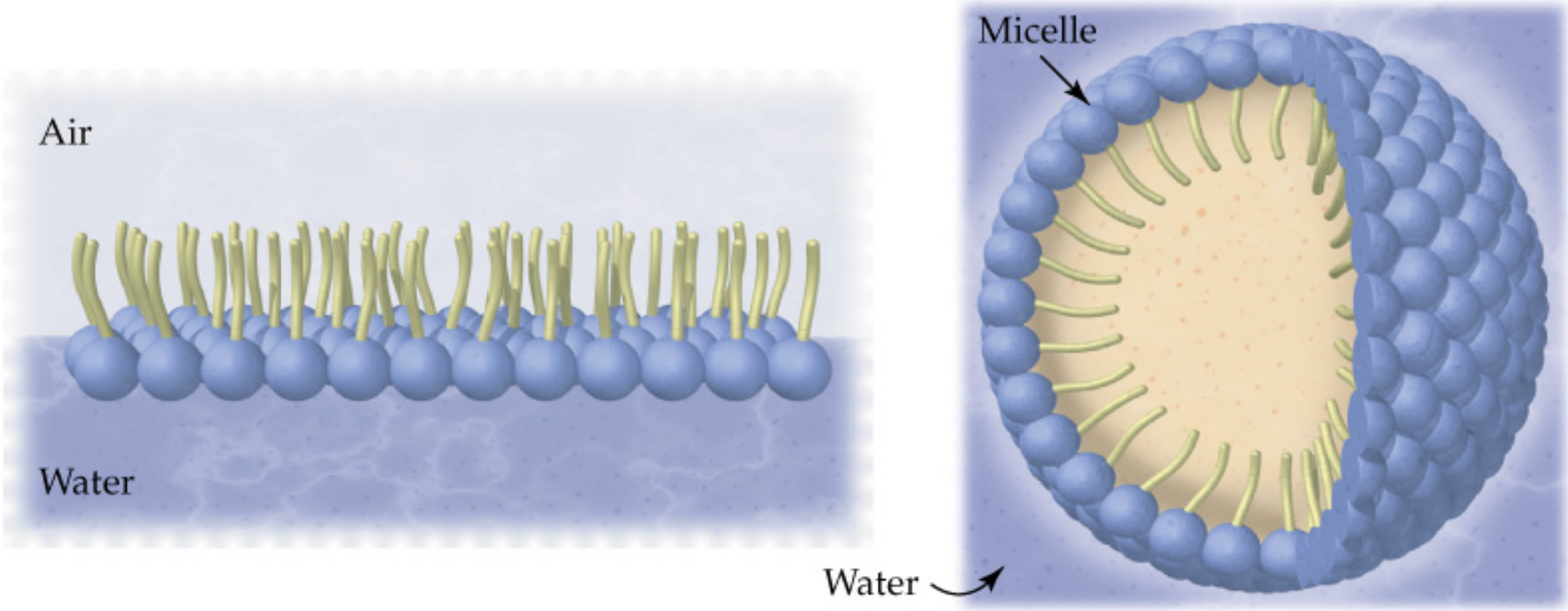
A saturated fatty acid
(palmitic acid)

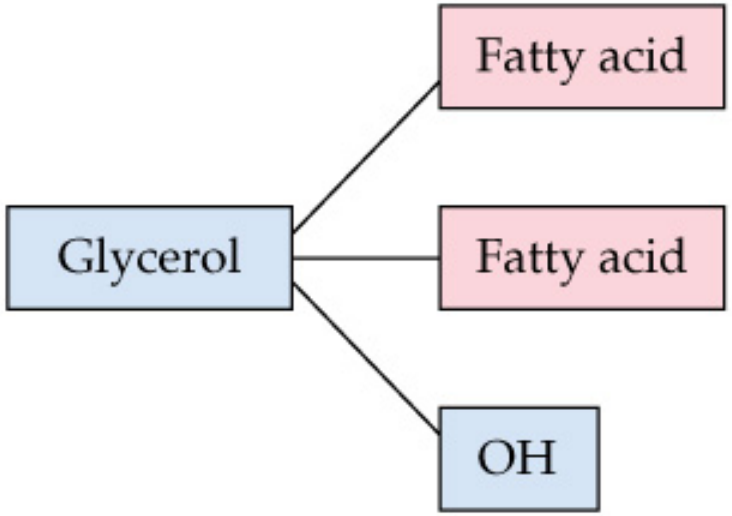


A *cis* unsaturated fatty acid
(linolenic acid)

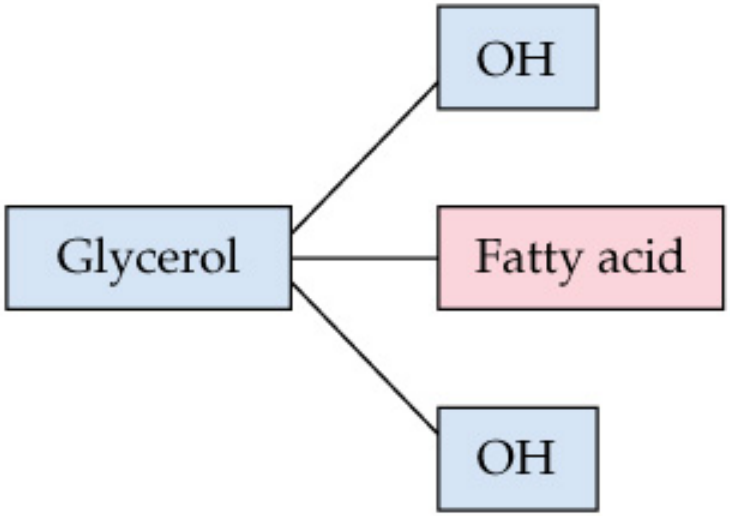


Stearic acid, an 18-carbon saturated fatty acid

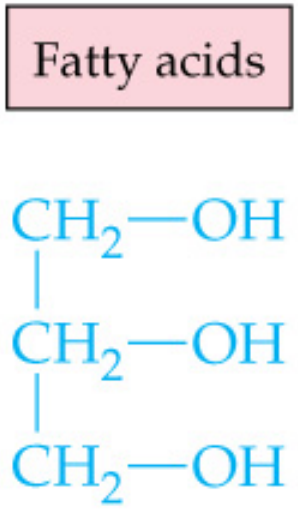

Air
Water
Micelle
Water



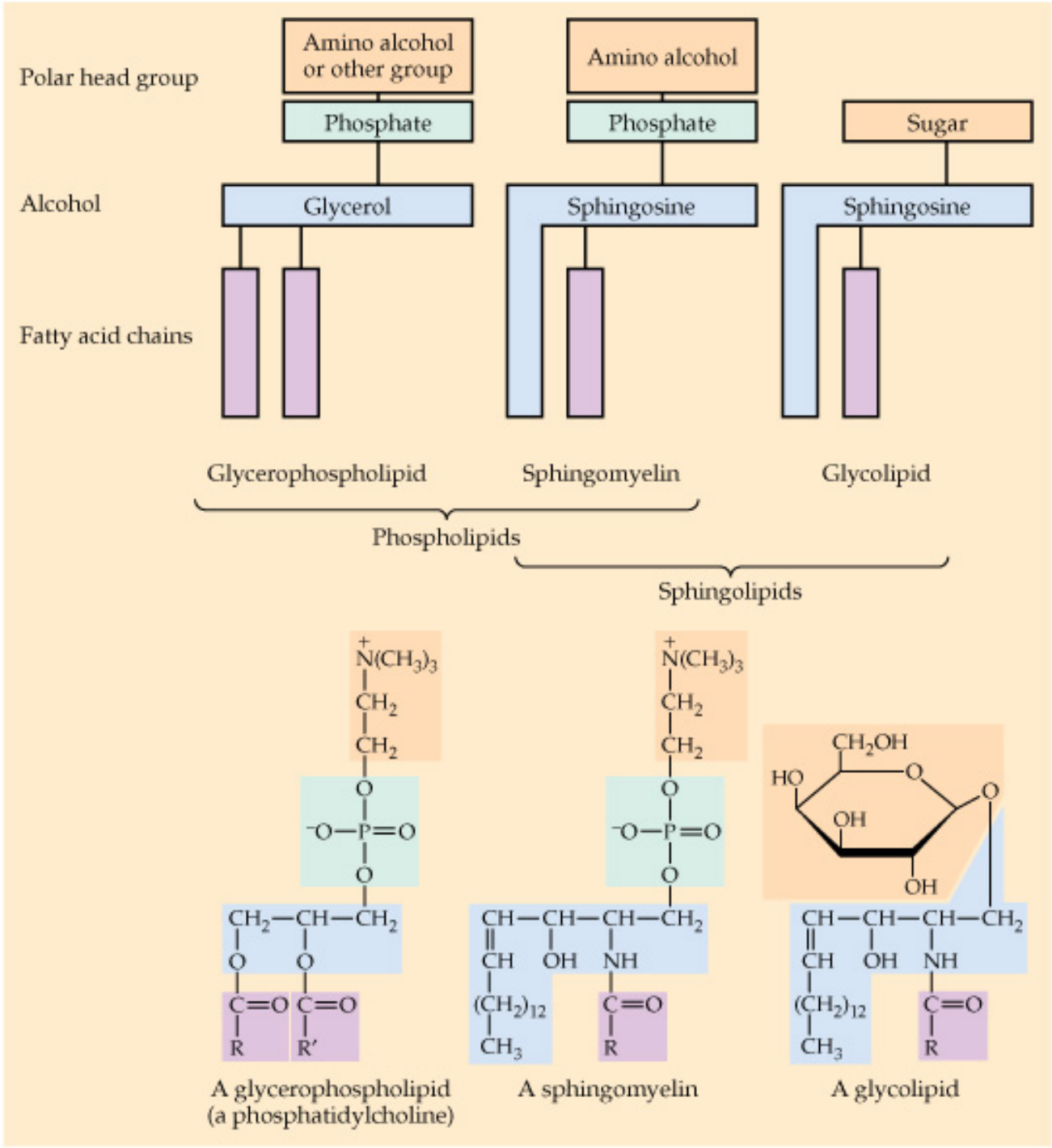
Diacylglycerol

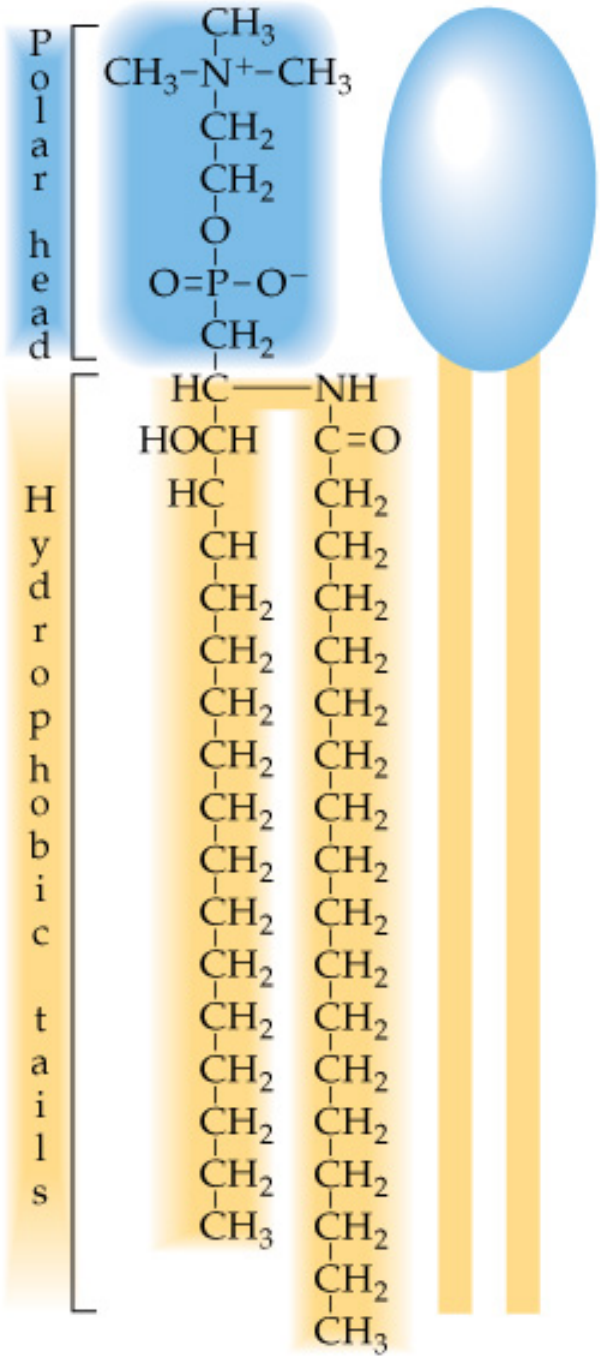


Monoacylglycerol

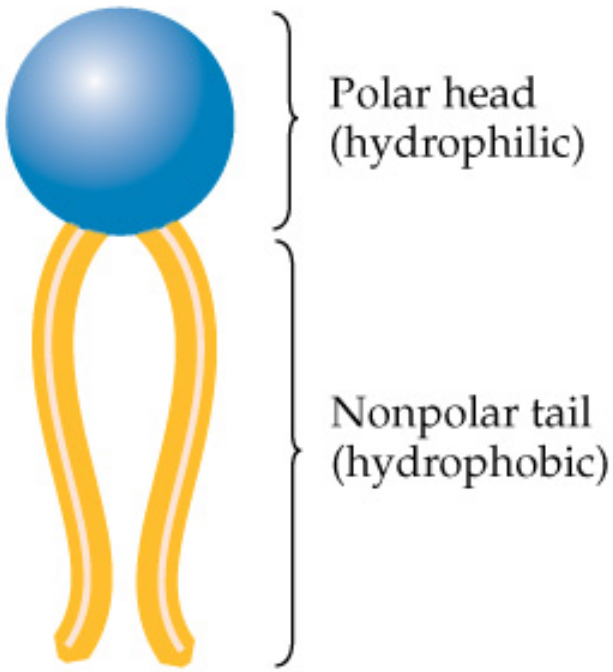


Glycerol

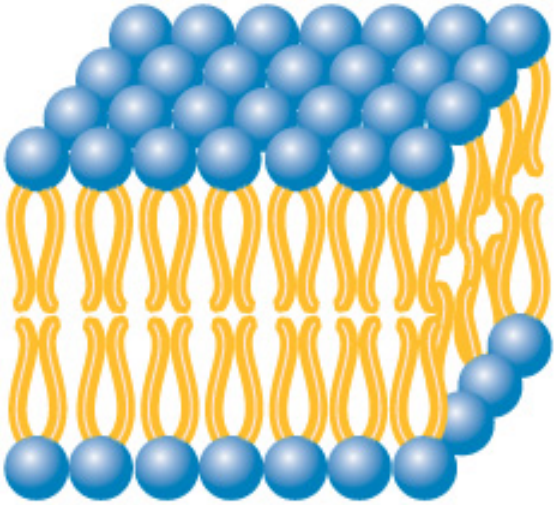

Polar head group
Alcohol
Fatty acid chains
Amino alcohol or other group
Phosphate
Glycerol
Amino alcohol
Phosphate
Sphingosine
Sugar
Sphingosine
Glycerophospholipid
Sphingomyelin
Glycolipid
Phospholipids
Sphingolipids
A glycerophospholipid (a phosphatidylcholine)
A sphingomyelin
A glycolipid



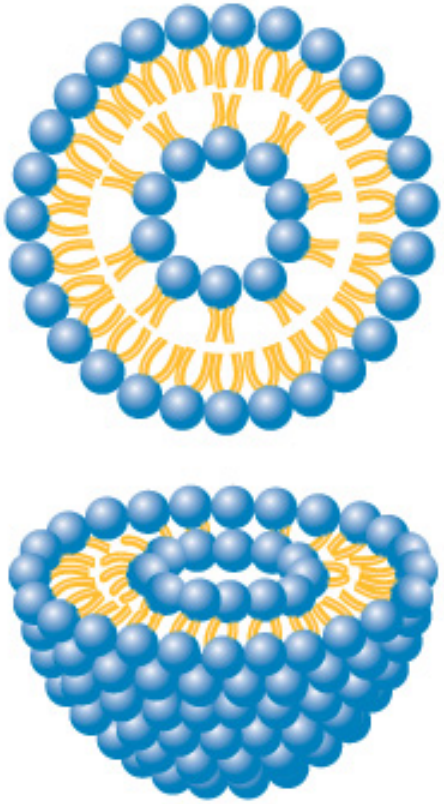
A sphingomyelin



Membrane lipid



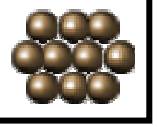
Lipid bilayer



Liposome

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.



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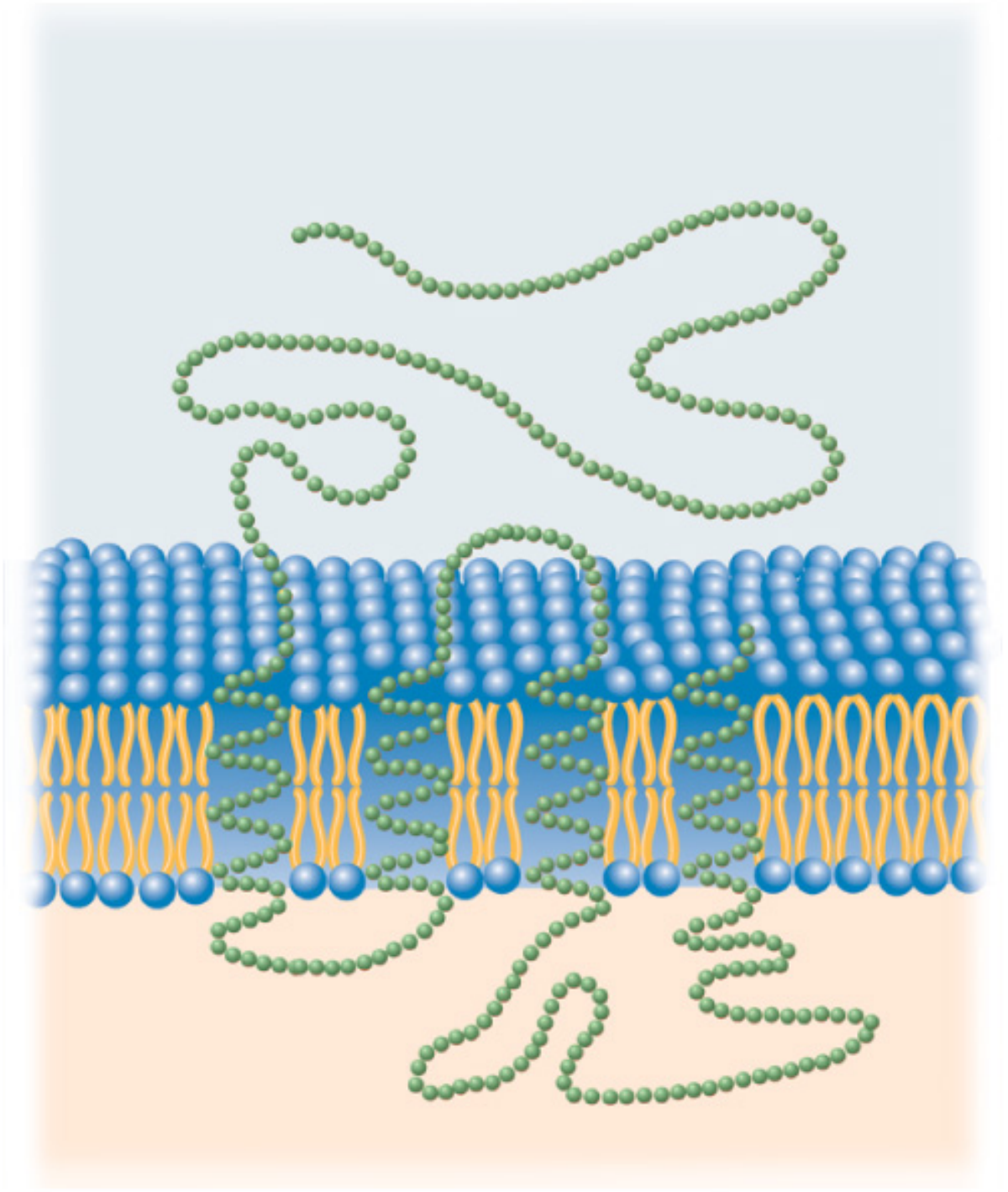
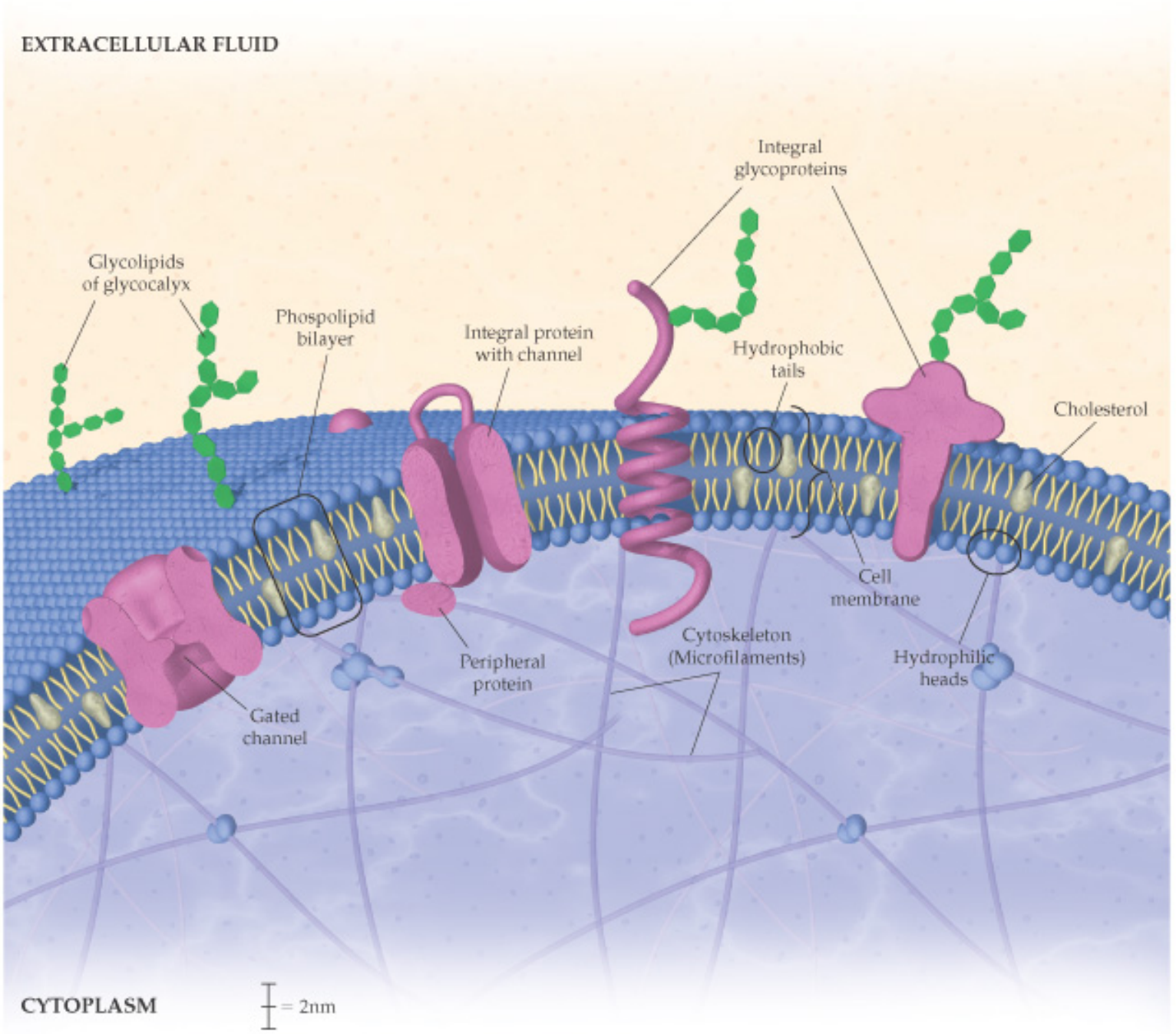
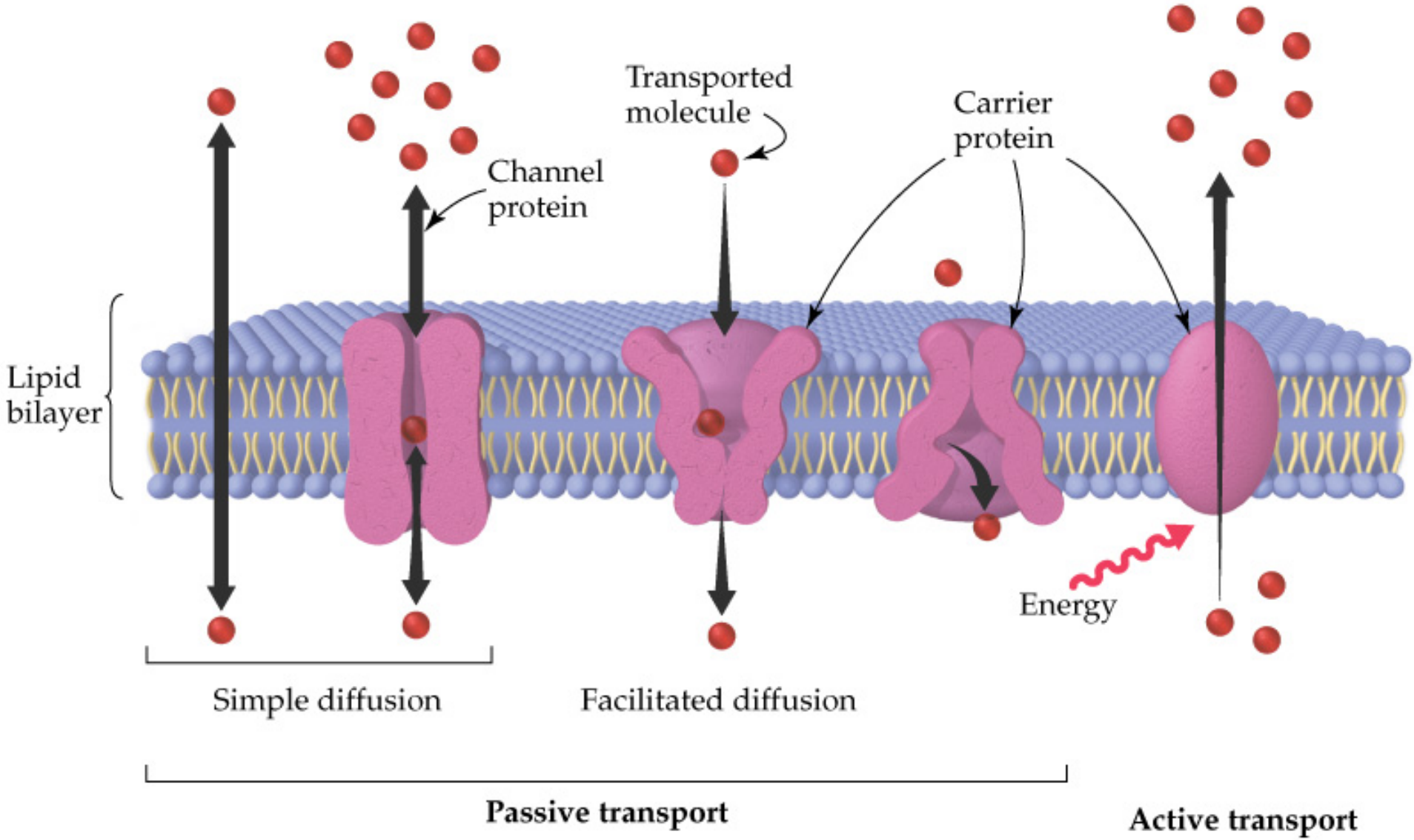
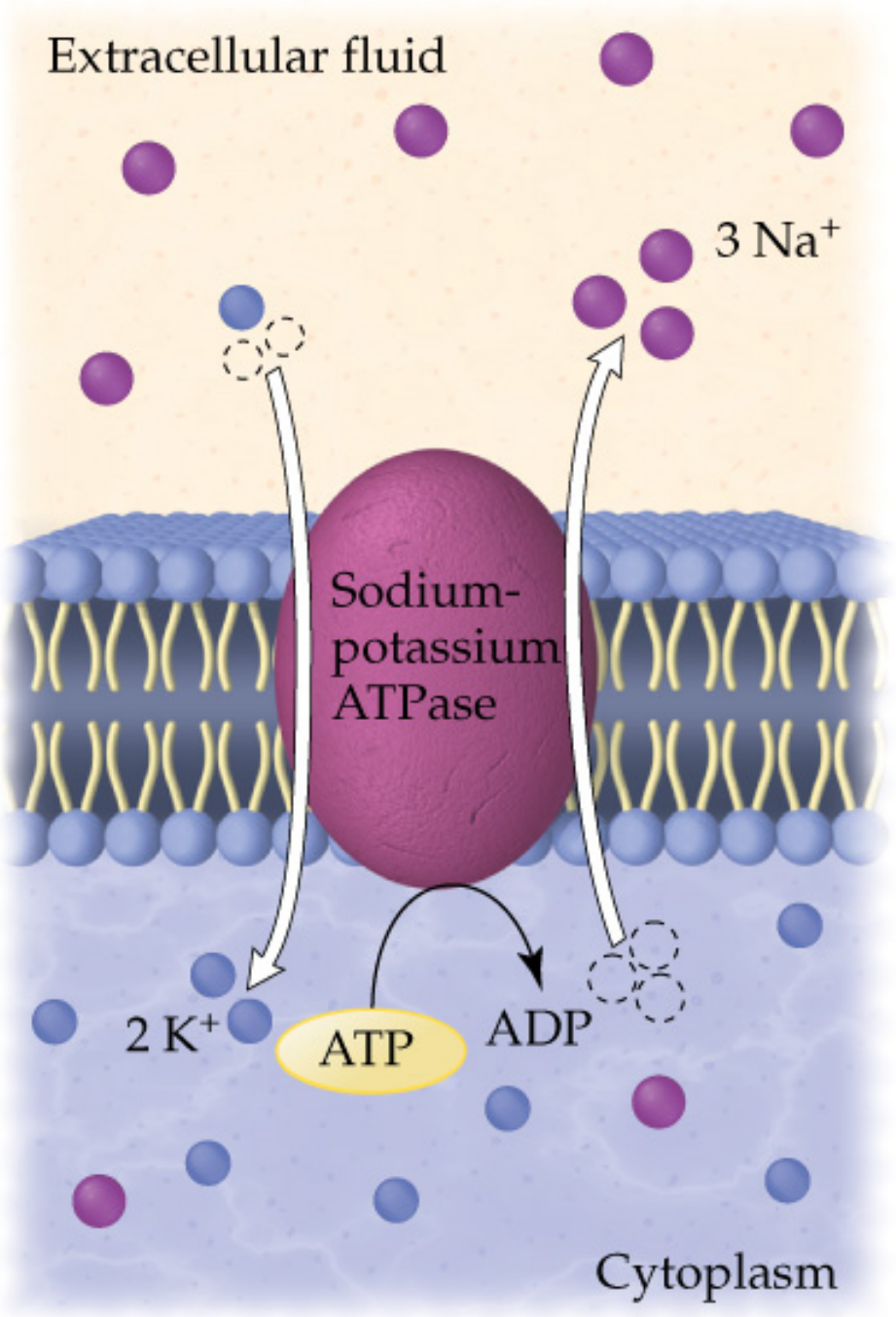
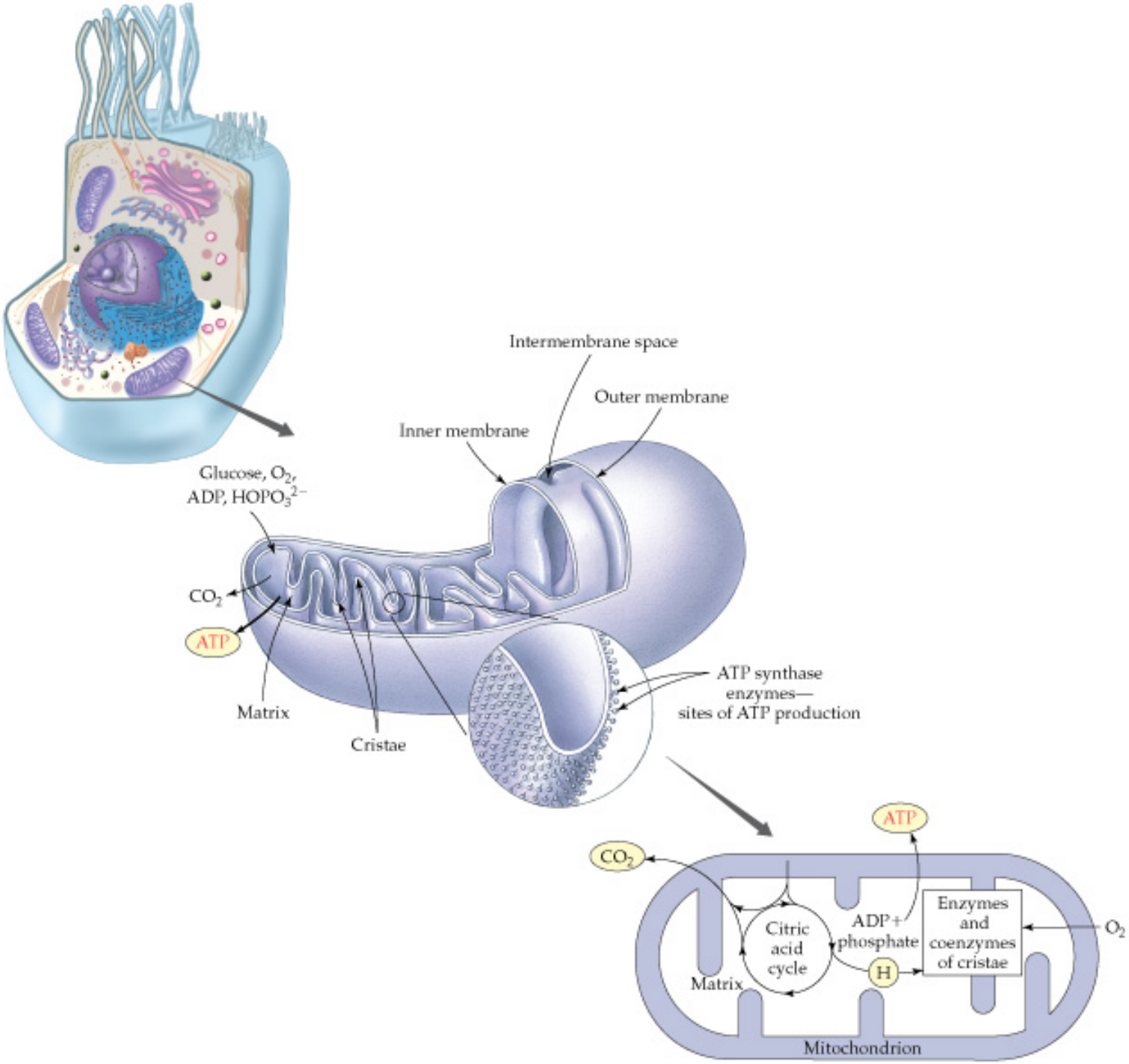



EXTRACELLULAR FLUID
Integral glycoproteins
Glycolipids of glycocalyx
Phospolipid bilayer
Integral protein with channel
Hydrophobic tails
Cholesterol
Cell membrane
Cytoskeleton (Microfilaments)
Peripheral protein
Hydrophilic heads
Gated channel
CYTOPLASM
= 2nm

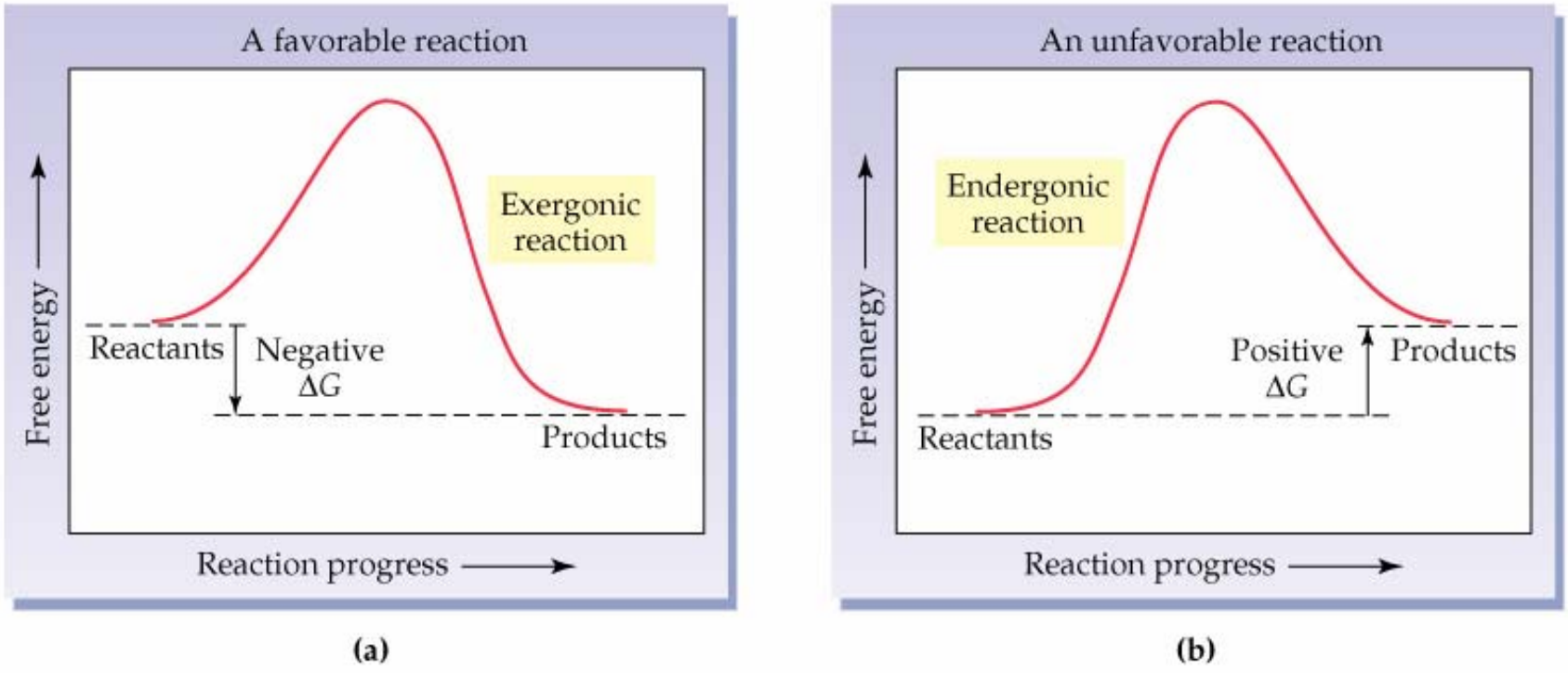

Transported molecule
Carrier protein
Channel protein
Lipid bilayer
Energy
Simple diffusion
Facilitated diffusion
Passive transport
Active transport


Extracellular fluid
3 Na+
Sodium-
potassium
ATPase
2 K+
ATP
ADP
Cytoplasm


Intermembrane space
Outer membrane
Inner membrane
Glucose, O_2, ADP, $HOPO_3^{2-}$
CO_2
ATP
Matrix
Cristae
ATP synthase enzymes— sites of ATP production
ATP
CO_2
Citric acid cycle
ADP + phosphate
H
Enzymes and coenzymes of cristae
O_2
Matrix
Mitochondrion

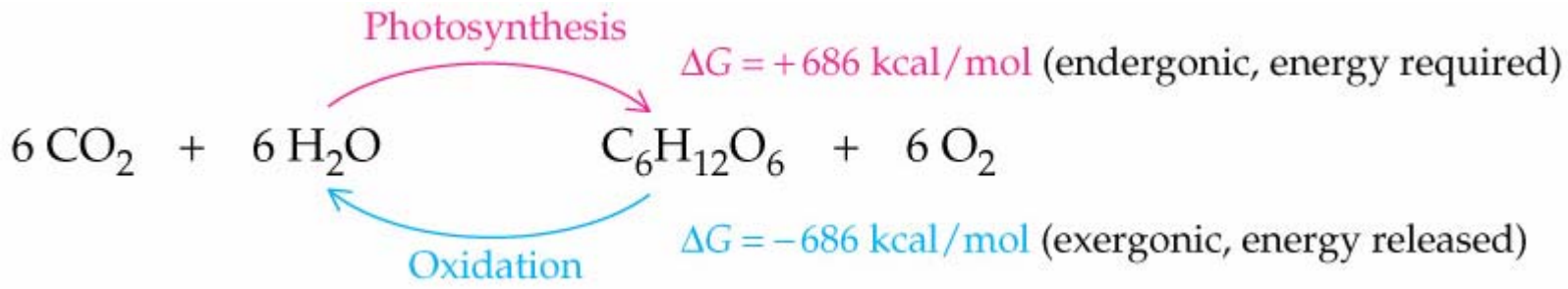
Energy and Biochemical Reactions

- Reactions in living organisms are similar to reactions in a chemical laboratory.
- Spontaneous reactions, those are favorable in the forward direction, release free energy and the energy released is available to do work.
- Spontaneous reactions , also known as *exergonic* reactions, are the source of our biochemical energy.
- Products of exergonic reactions are more stable than the reactants and the free energy change ΔG has a negative value.

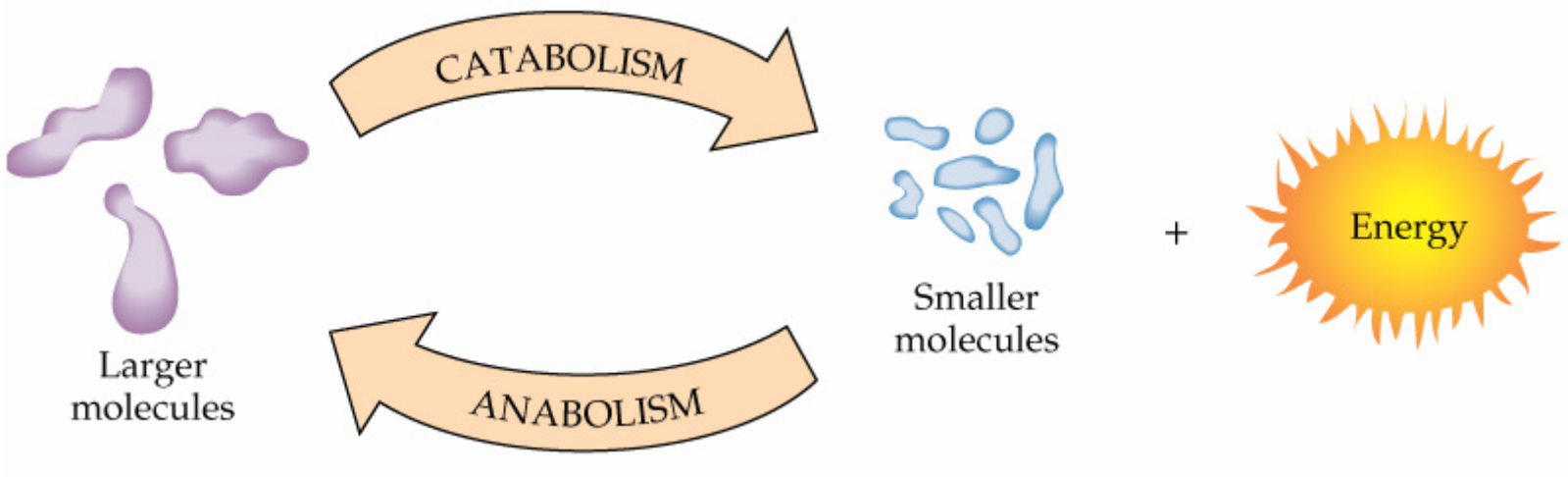


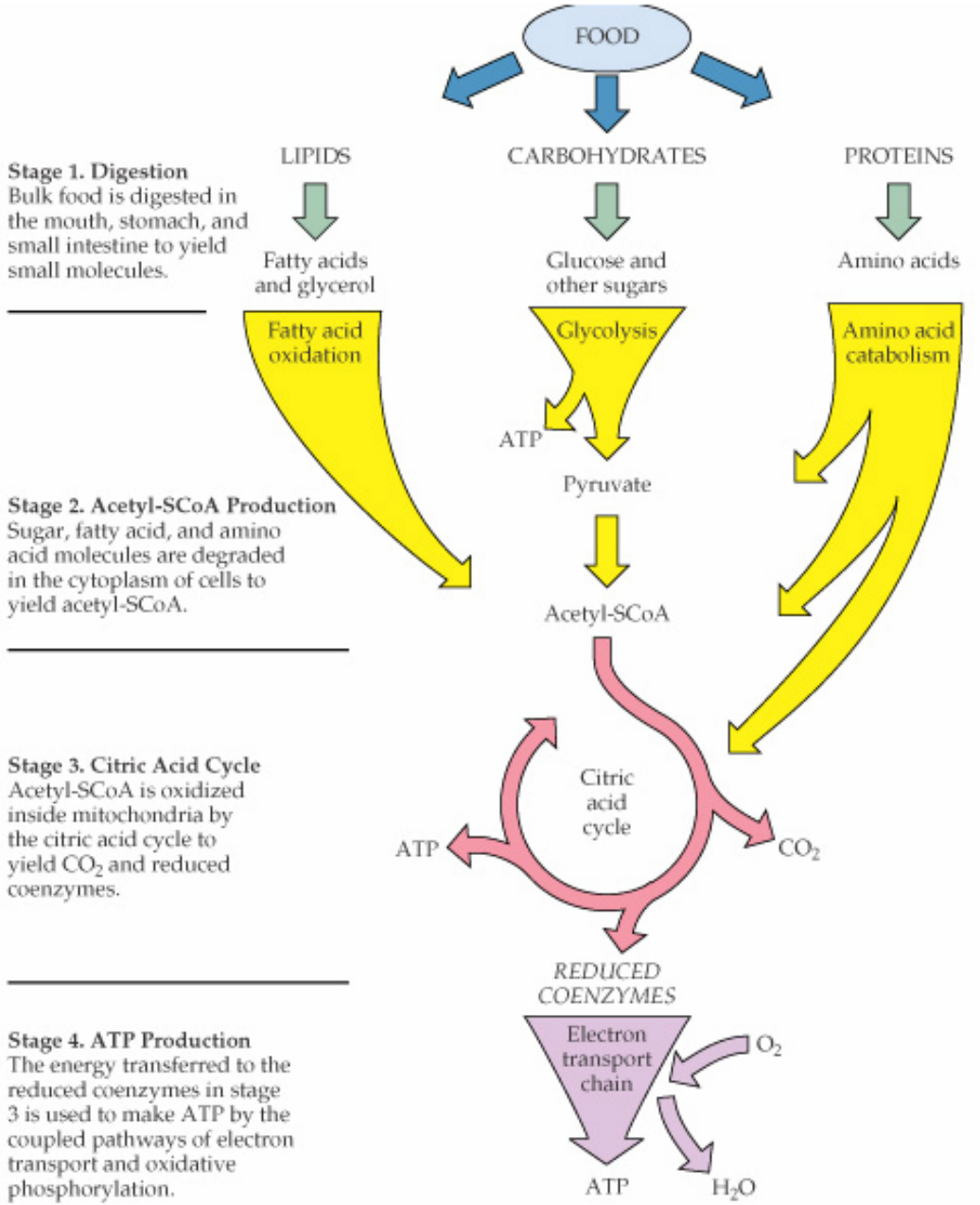
•Energy diagram of an (a) exergonic and (b) endergonic reaction

- *Photosynthesis* in plants, converts CO_2 and H_2O to glucose plus O_2 which is the reverse of oxidation of glucose. The sun provides the necessary external energy for photosynthesis (686 kcal of free energy per mole of glucose formed).



•The *mitochondria* is often called the cell's power plants. Within the mitochondria, small molecules are broken down to provide the energy for an organism and also the principle energy carrying molecule adenosine triphosphate (ATP) is produced.


CATABOLISM
Larger molecules
Smaller molecules
+
Energy
ANABOLISM


FOOD
LIPIDS
CARBOHYDRATES
PROTEINS
Fatty acids and glycerol
Glucose and other sugars
Amino acids
Fatty acid oxidation
Glycolysis
Amino acid catabolism
ATP
Pyruvate
Acetyl-SCoA
Citric acid cycle
ATP
CO_2
REDUCED COENZYMES
Electron transport chain
O_2
ATP
H_2O
Stage 1. Digestion
Bulk food is digested in the mouth, stomach, and small intestine to yield small molecules.
Stage 2. Acetyl-SCoA Production
Sugar, fatty acid, and amino acid molecules are degraded in the cytoplasm of cells to yield acetyl-SCoA.
Stage 3. Citric Acid Cycle
Acetyl-SCoA is oxidized inside mitochondria by the citric acid cycle to yield CO_2 and reduced coenzymes.
Stage 4. ATP Production
The energy transferred to the reduced coenzymes in stage 3 is used to make ATP by the coupled pathways of electron transport and oxidative phosphorylation.

ATP and Energy Transfer

- Adenosine triphosphate (ATP) transport energy in living organisms.
- ATP has three $-PO_3^-$ groups.
- Removal of one of the $-PO_3^-$ groups from ATP by hydrolysis produces adenosine diphosphate (ADP). Since this reaction is an exergonic process, it releases energy.
- The reverse of ATP hydrolysis reaction is known as phosphorylation reaction. Phosphorylation reactions are endergonic.

•Biochemical energy production, transport, and use all depends on the ATP $\rightleftharpoons$ ADP interconversions.

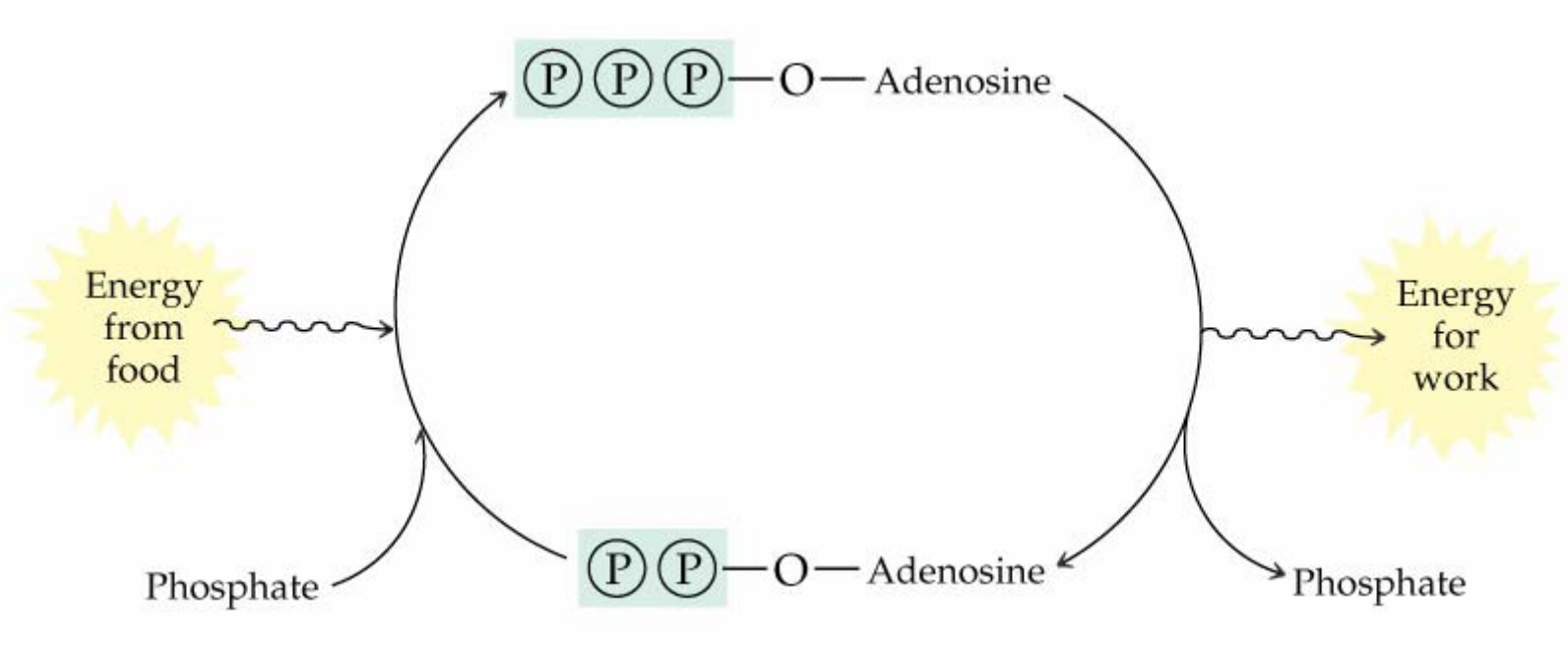
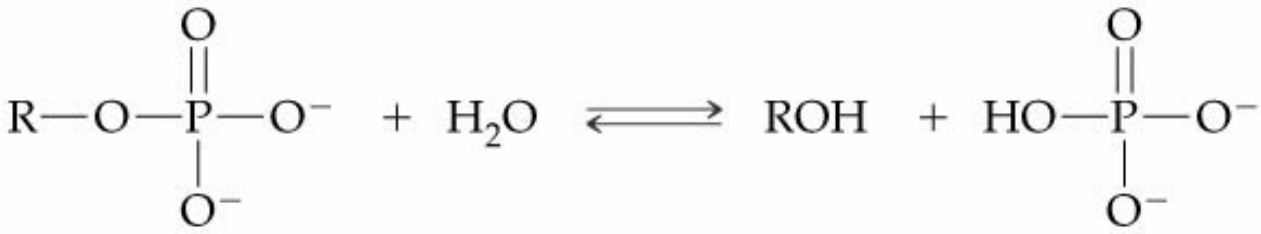


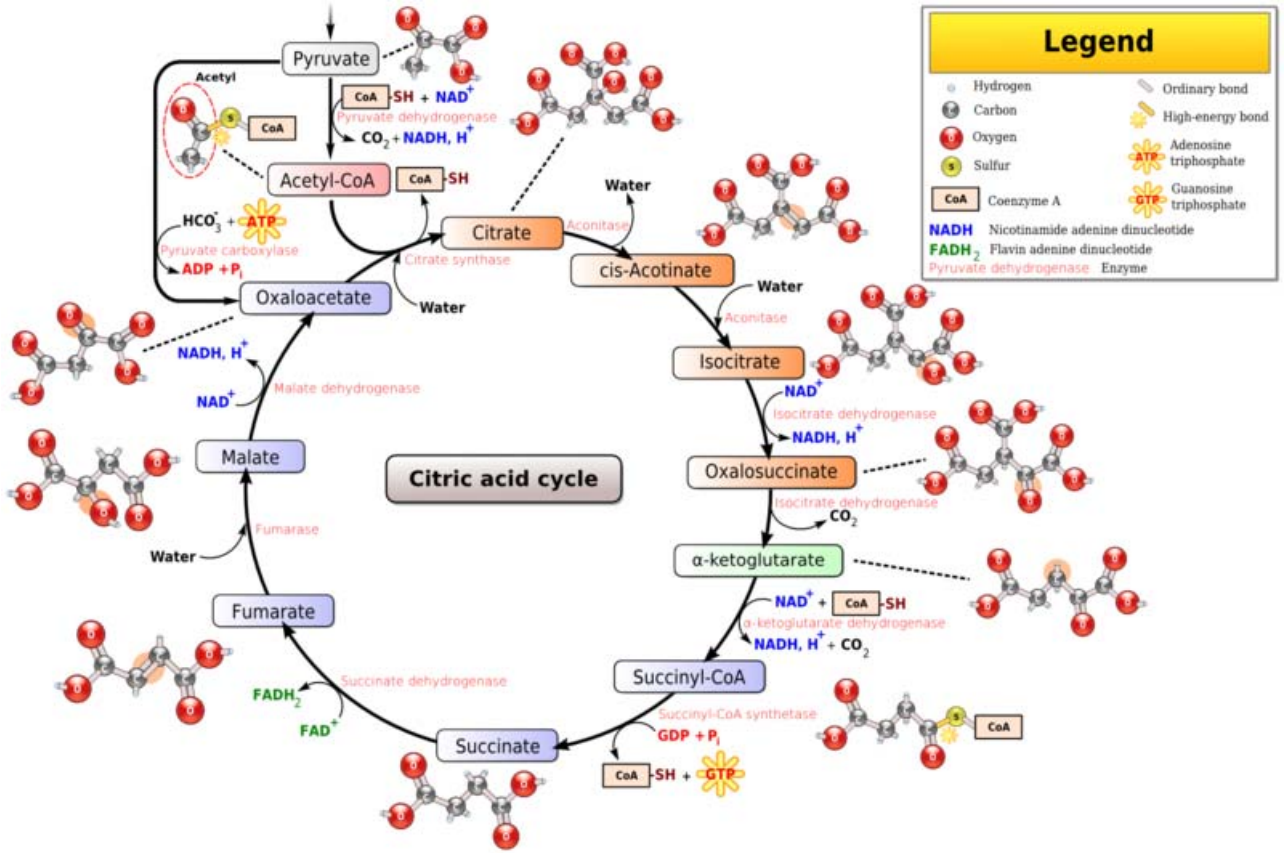
TABLE 21.1 Free Energies of Hydrolysis of Some Phosphates



Compound Name	Function	ΔG (kcal/mol)
Phosphoenol pyruvate	Final intermediate in conversion of glucose to pyruvate (glycolysis)—stage 2, Figure 21.5	−14.8
1, 3-Bisphosphoglycerate	Another intermediate in glycolysis	−11.8
Creatine phosphate	Energy storage in muscle cells	−10.3
ATP (⟶ ADP)	Principal energy carrier	−7.3
Glucose 1-phosphate	First intermediate in breakdown of carbohydrates stored as starch or glycogen	−5.0
Glucose 6-phosphate	First intermediate in glycolysis	−3.3
Fructose 6-phosphate	Second intermediate in glycolysis	−3.3

- A few enzymes continuously cycle between their oxidized and reduced forms.

Coenzyme	As Oxidizing Agent	As Reducing Agent
Nicotinamide adenine dinucleotide	NAD^+	$NADH/H^+$
Nicotinamide adenine dinucleotide phosphate	$NADP^+$	$NADPH/H^+$
Flavin adenine dinucleotide	FAD	$FADH_2$
Flavin mononucleotide	FMN	$FMNH_2$

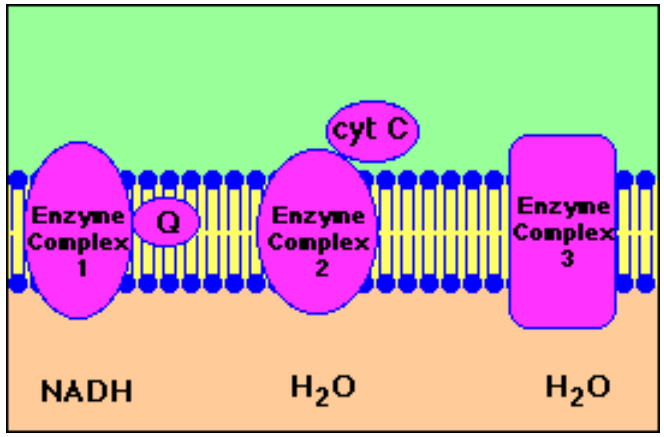


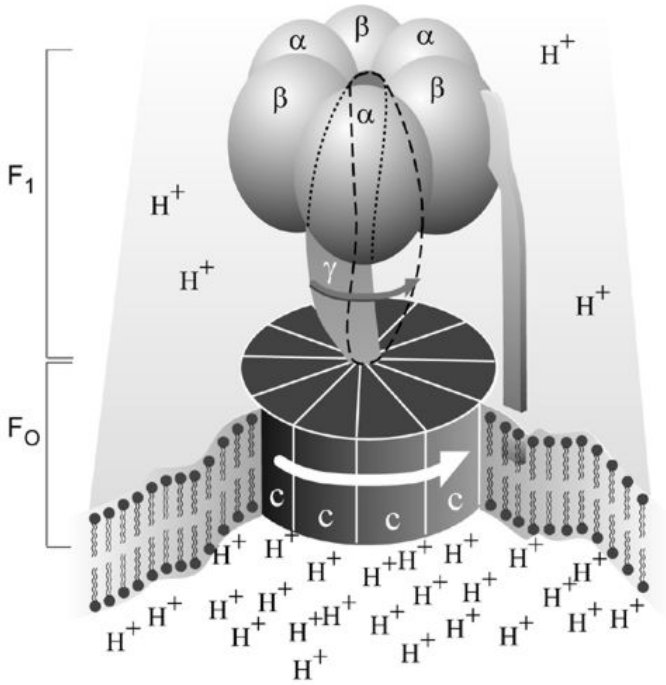
Acetyl-CoA + 3 NAD+ + FAD + GDP + Pi + 2H20
<==> CoASH + 3 NADH + FADH2 + GTP + 2CO2 + 3H

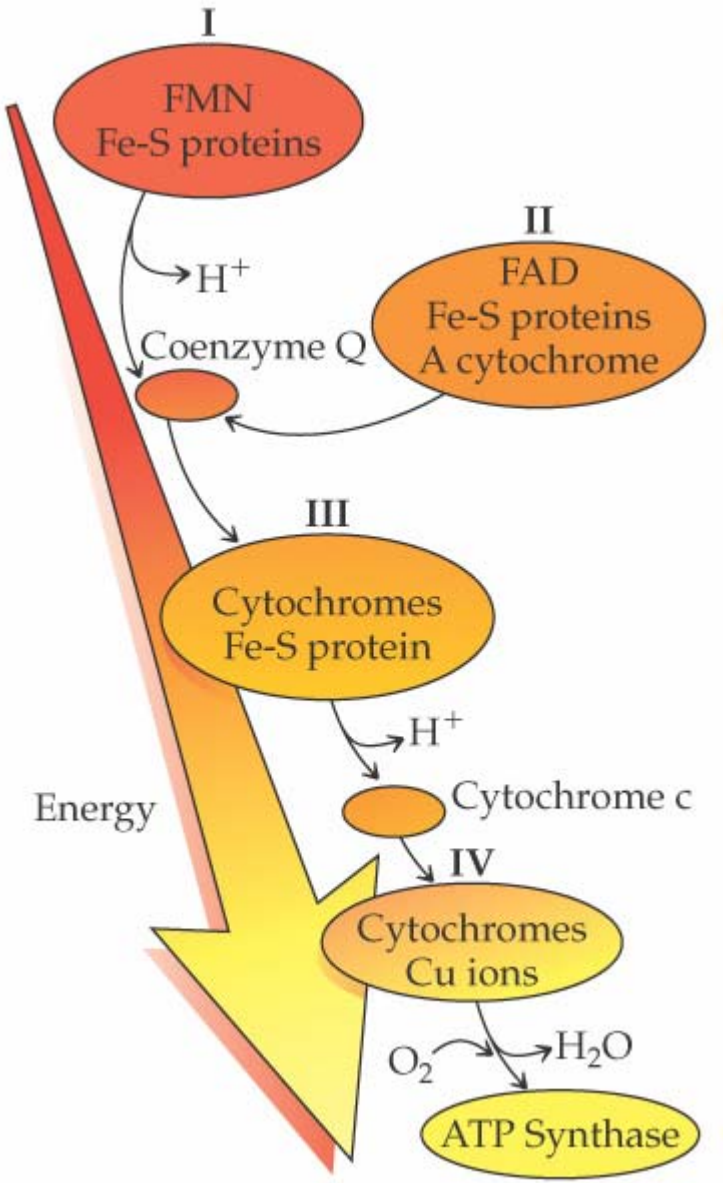
The citric acid cycle

The Electron-Transport Chain and ATP Production

- Electron transport chain: The series of biochemical reactions that passes electrons from reduced coenzymes to oxygen and is coupled to ATP formation. The electrons combine with the oxygen we breathe and with hydrogen ions from their surrounding to produce water.
- Electron transport involves four enzyme complexes held in fixed positions within the inner membrane of mitochondria and two electron carriers move from one complex to another.



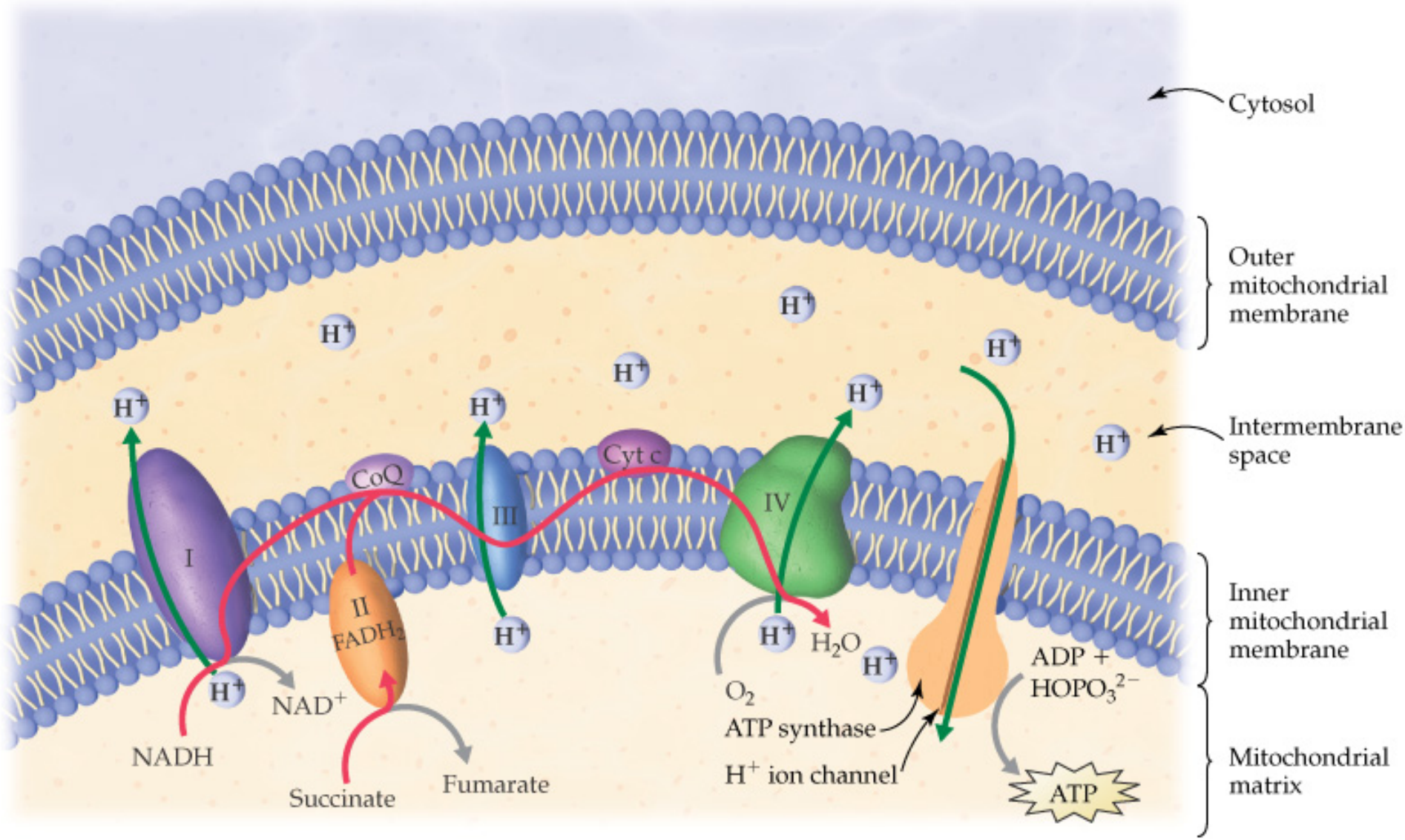


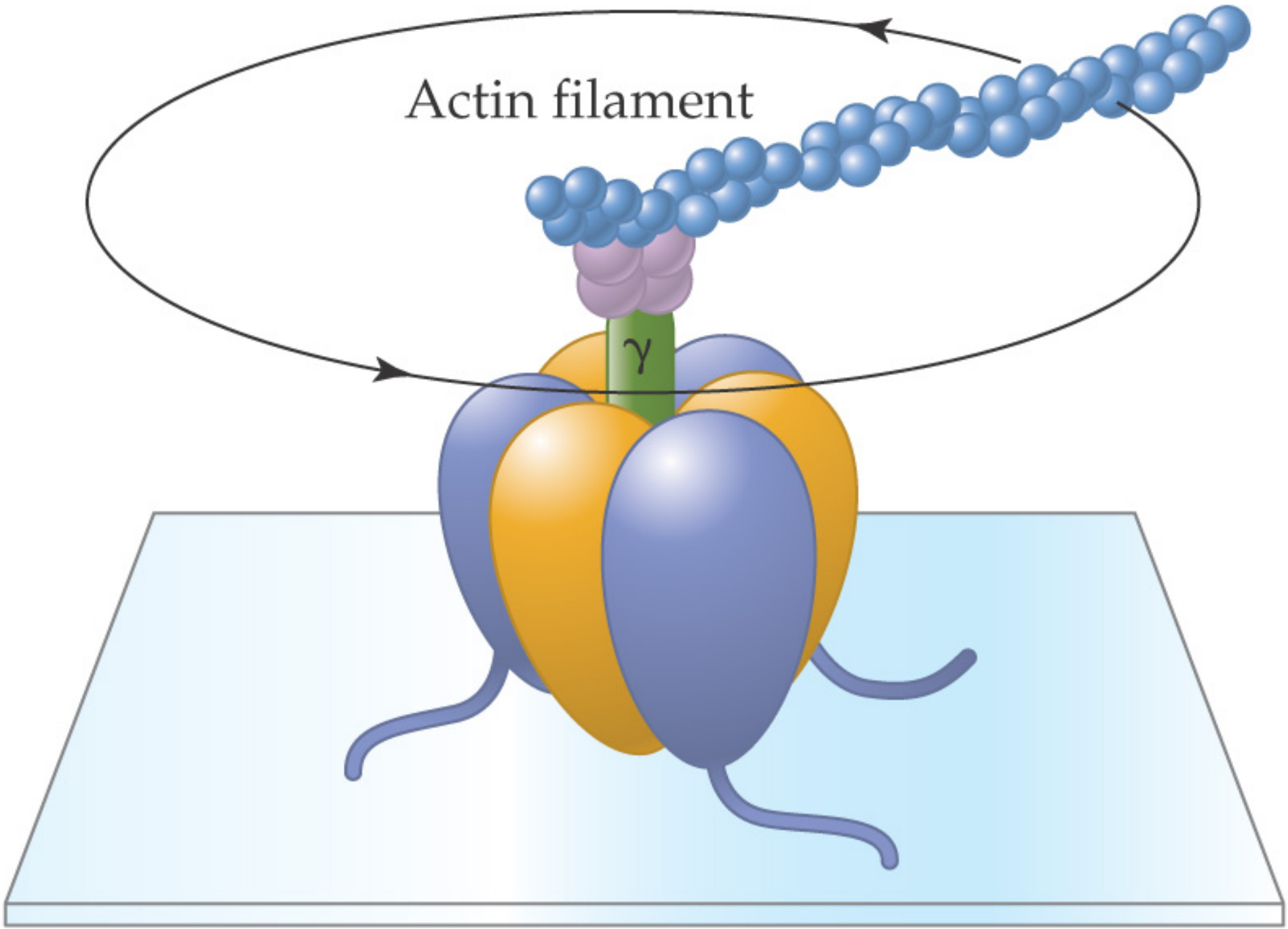


- Pathway of electrons in electron transport

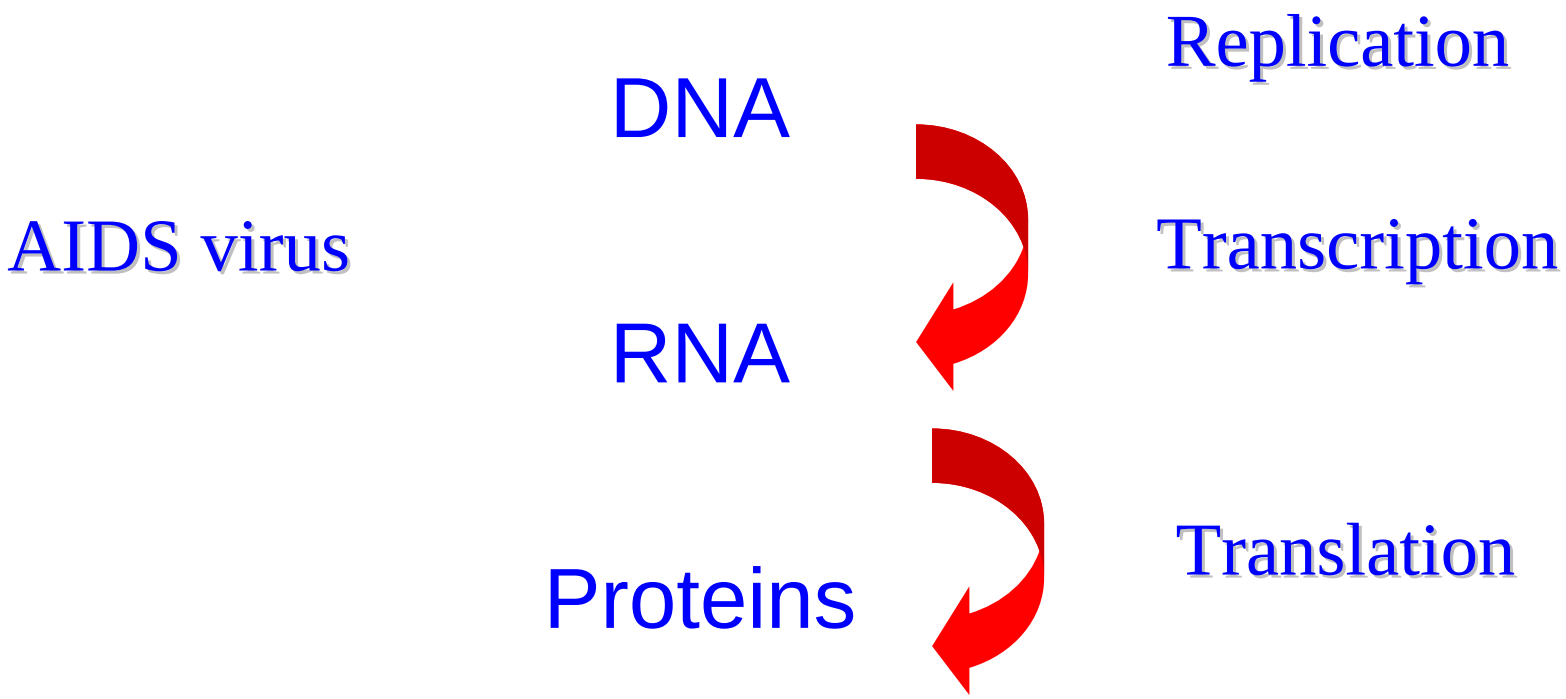
- **ATP Synthesis**
- ADP is converted to ATP by a reaction between ADP and hydrogen phosphate ion. This is both an oxidation and phosphorylation reaction. Energy released in the electron transport chain drives this reaction forward.

Vedio: http://www.iubmb-nicholson.org/swf/ATPSynthase.swf

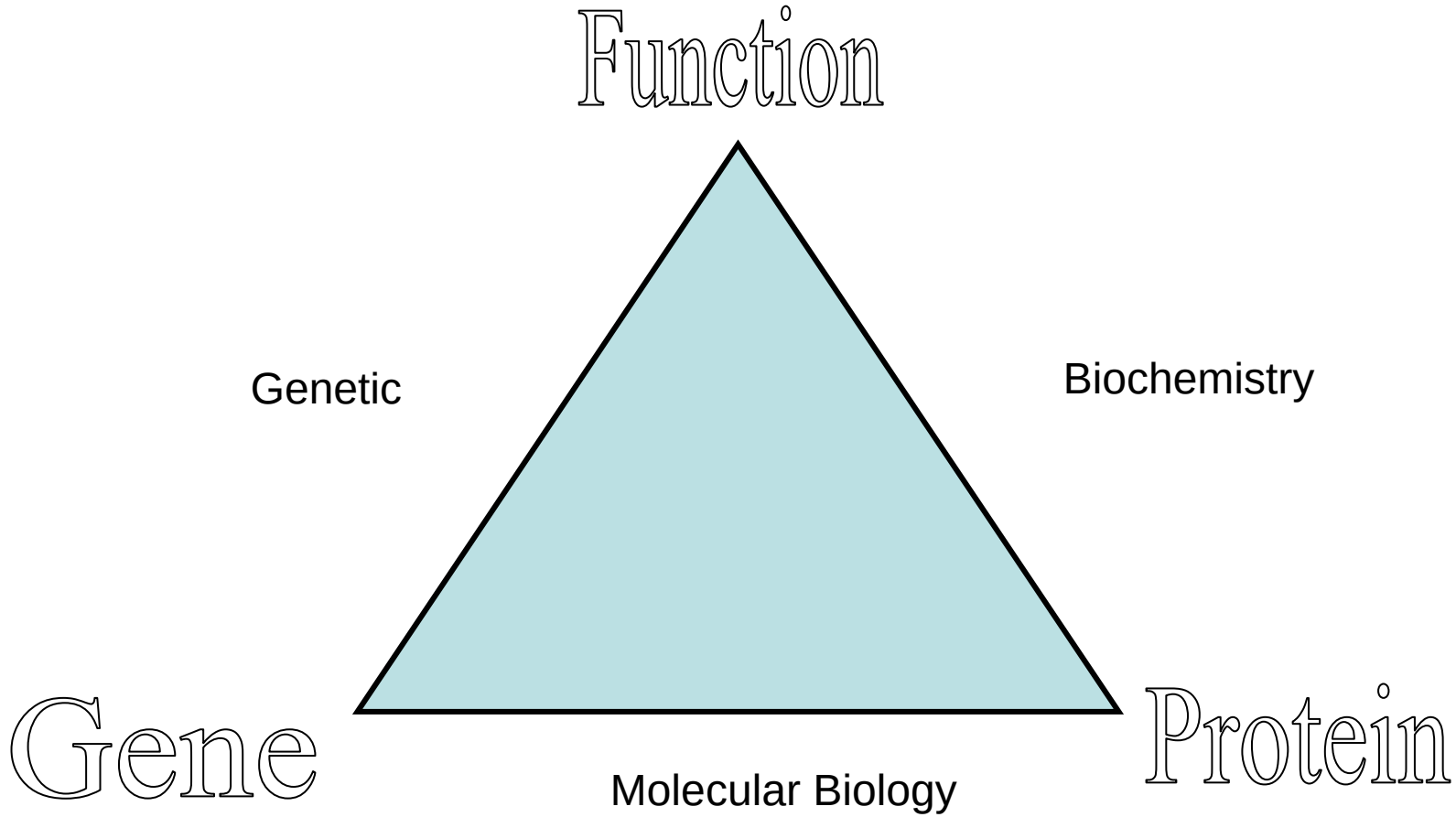

Cytosol
Outer mitochondrial membrane
Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
H+
CoQ
Cyt c
I
II
FADH2
III
IV
NADH
NAD+
Succinate
Fumarate
O2
H2O
ATP synthase
H+ ion channel
ADP + HOPO3 2−
ATP

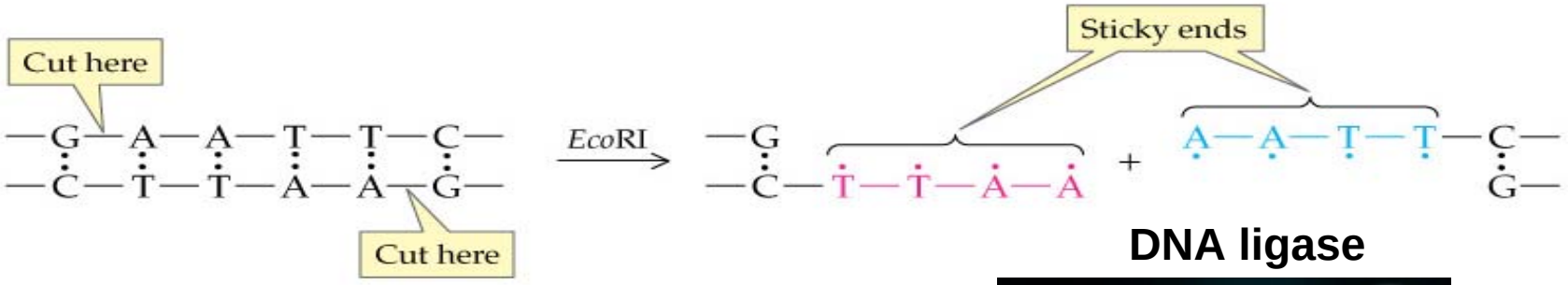

Actin filament
γ

Central Dogma

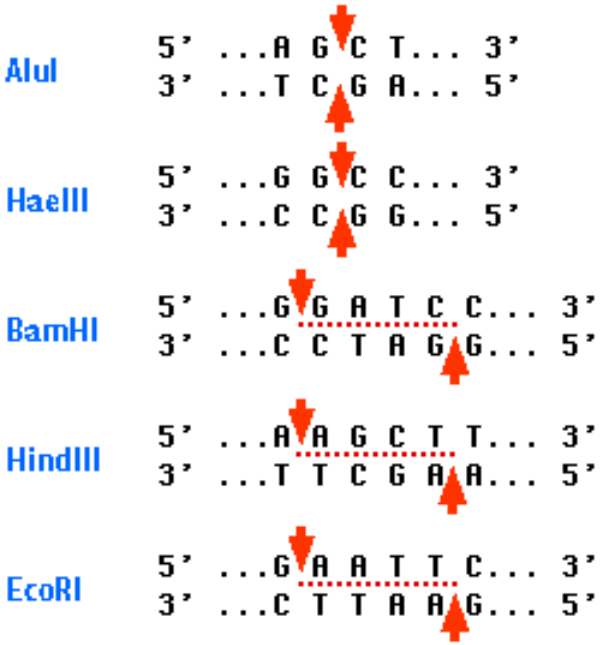


Recombinant DNA





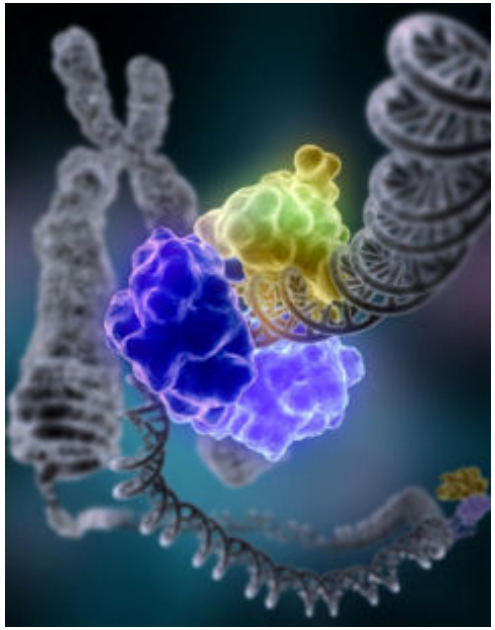
Restriction Enzyme

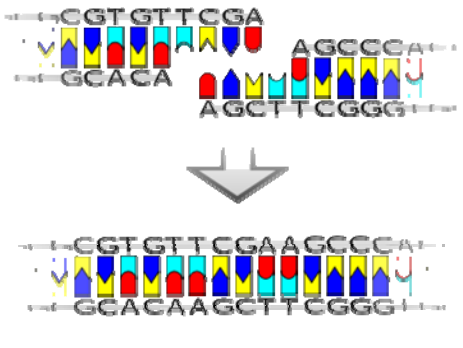


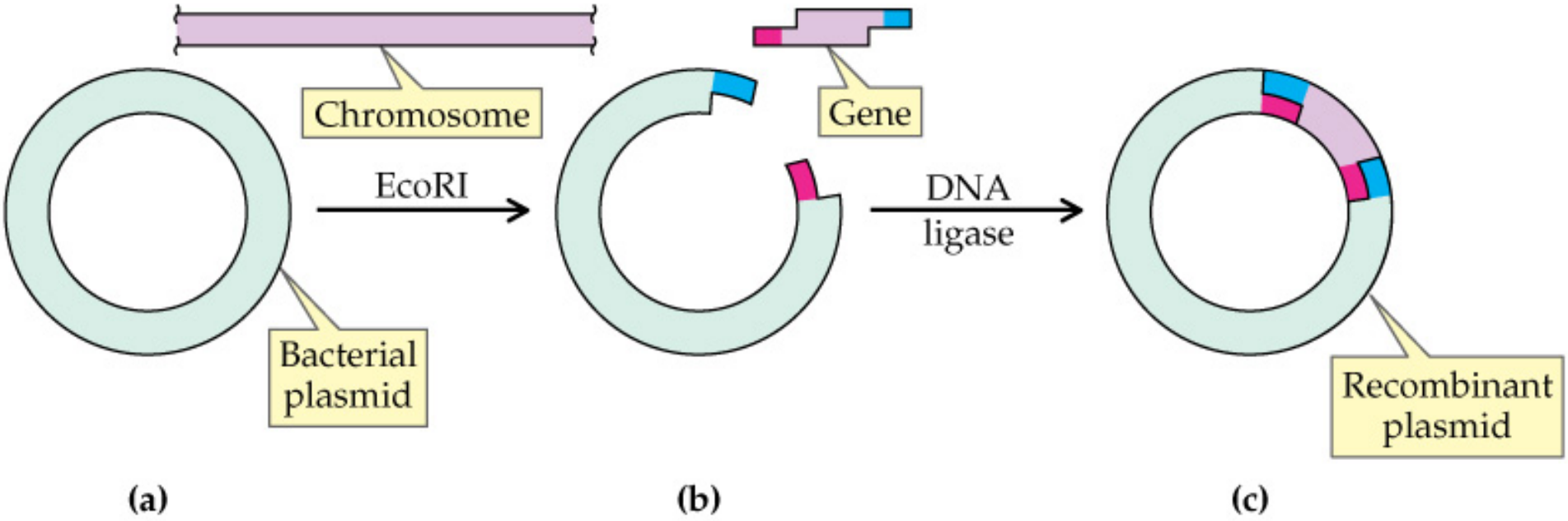
AluI and **HaeIII** produce blunt ends

BamHI **HindIII** and **EcoRI** produce "sticky" ends

DNA ligase



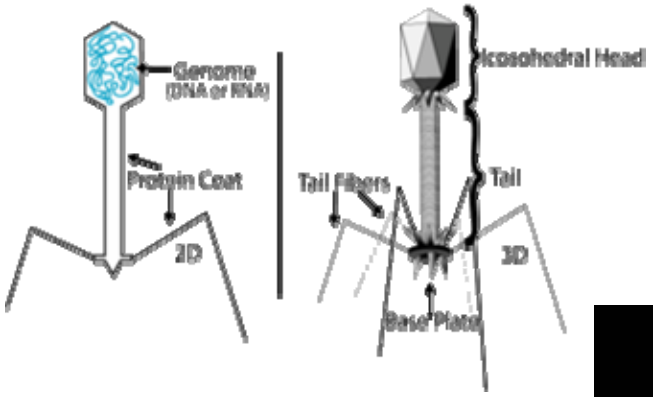


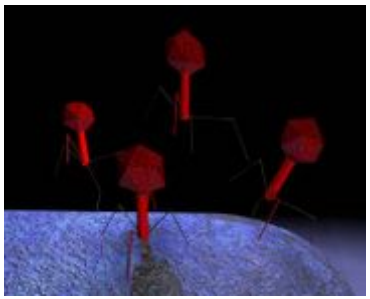

Chromosome
Gene
EcoRI
DNA
ligase
Bacterial
plasmid
Recombinant
plasmid
(a)
(b)
(c)

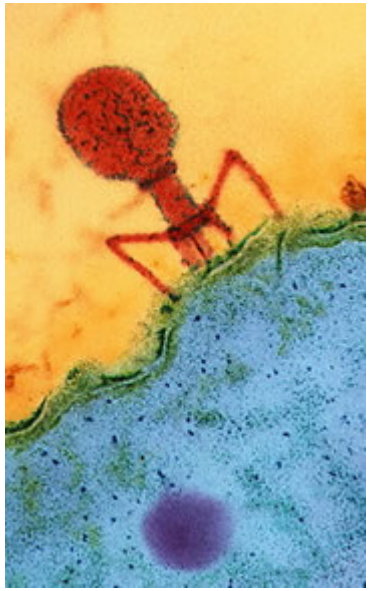
Life

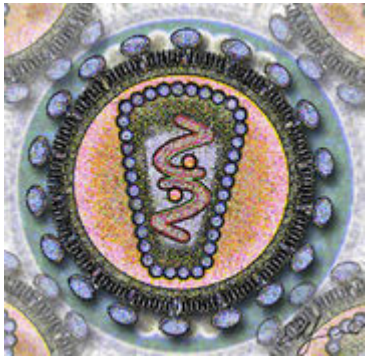
- Replication: reproduction
- Function: catalytic functions
- RNA world:
- Virus is not alive

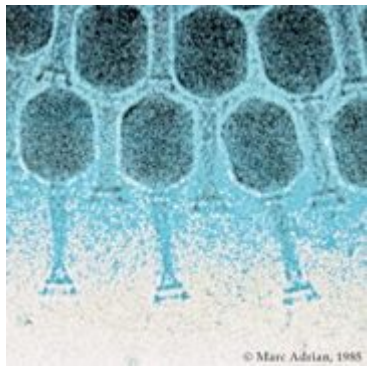
Virus


Genome
(DNA or RNA)
Protein Coat
2D
Icosohedral Head
Tail Fibers
Tail
3D
Base Plate





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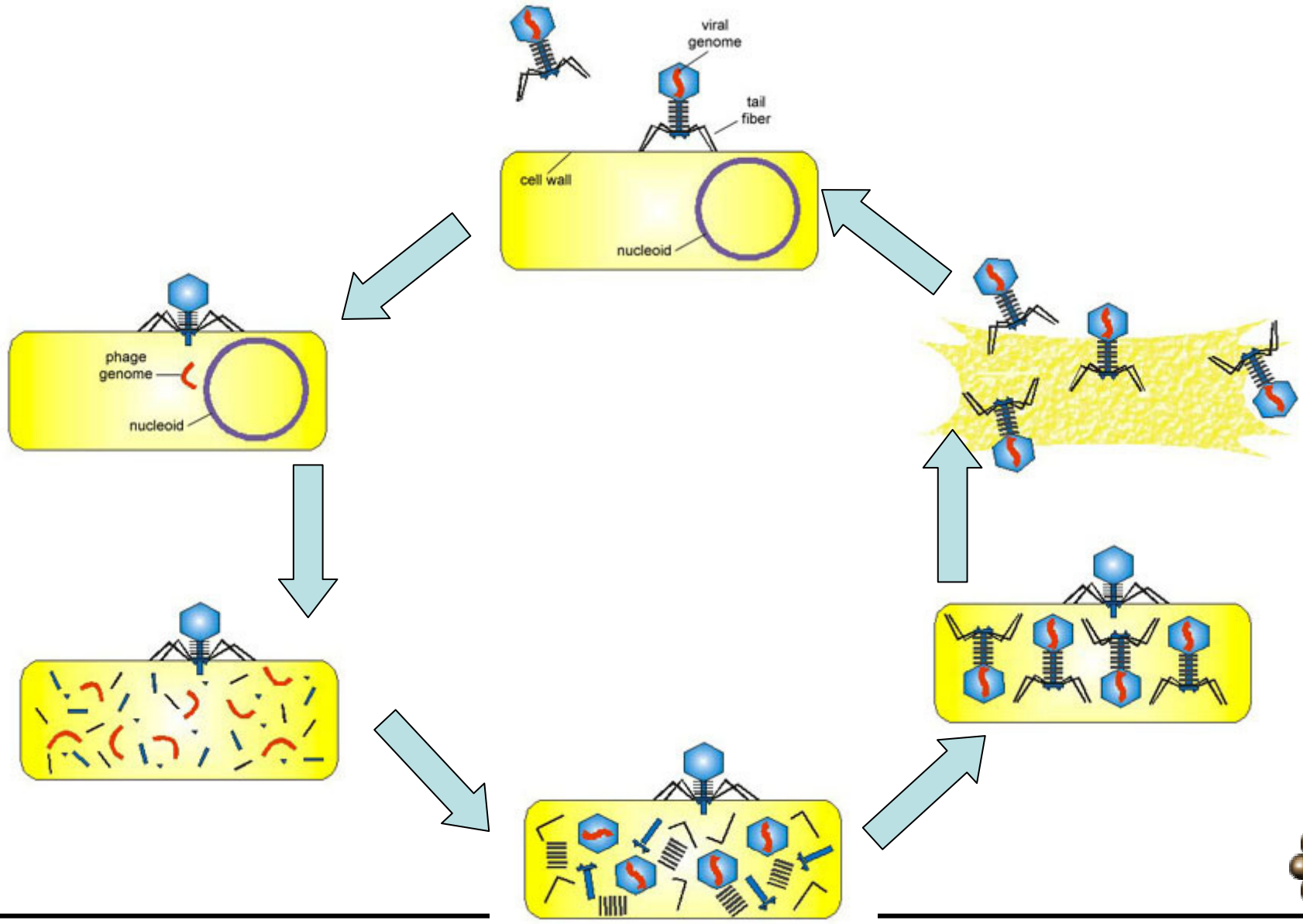
Virus Reproduction

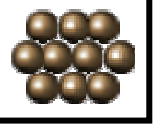


Gene Therapy

- Gene therapy is a technique for correcting defective genes responsible for disease development. Researchers may use one of several approaches for correcting faulty genes:
  - **A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common.**
  - **An abnormal gene could be swapped for a normal gene through homologous recombination.**
  - **The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.**
  - **The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.**

How Gene Therapy Works?

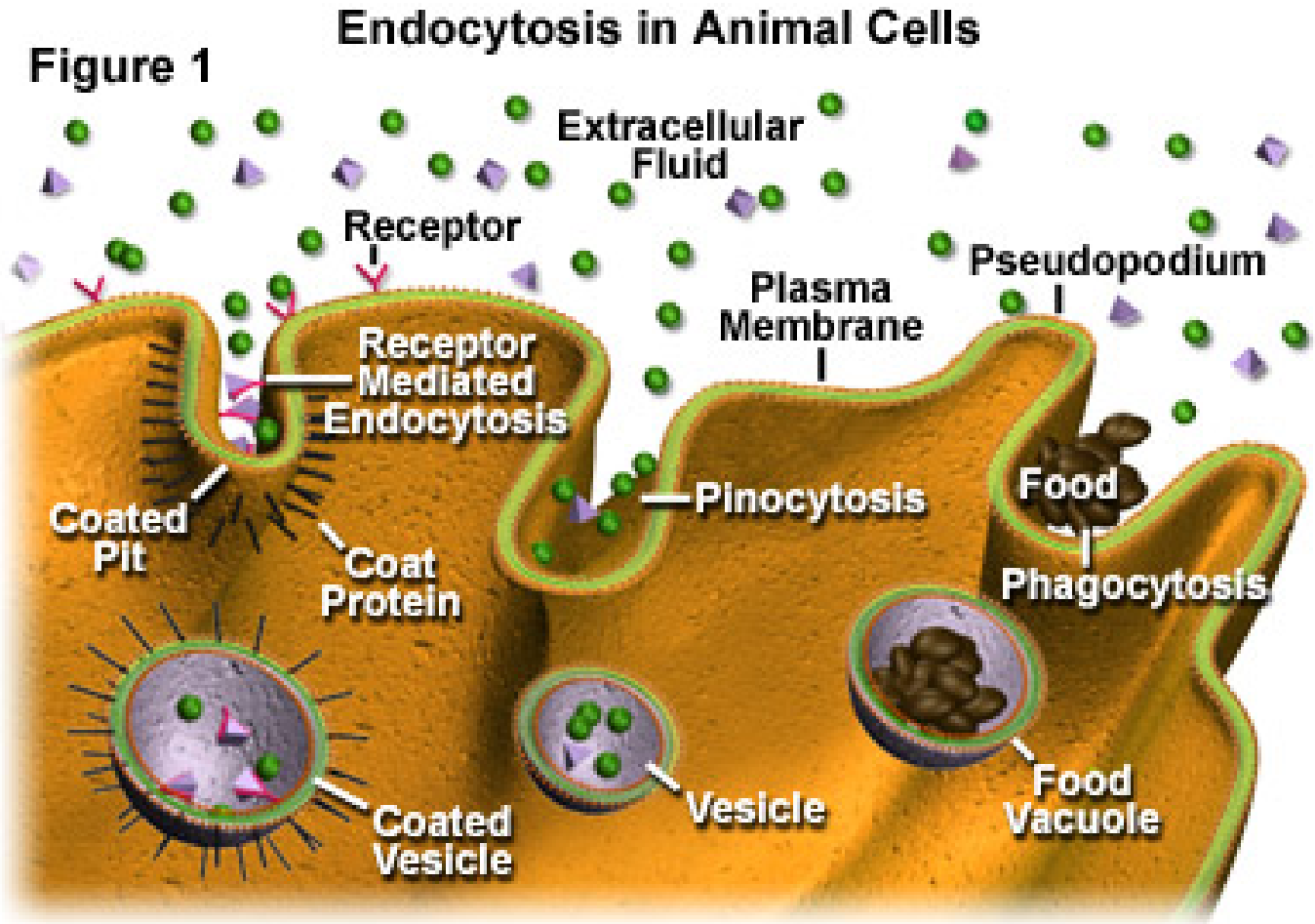
- In most gene therapy studies, a "normal" gene is inserted into the genome to replace an "abnormal," disease-causing gene. A carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vector is a virus that has been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove disease-causing genes and insert therapeutic genes.
- Target cells such as the patient's liver or lung cells are infected with the viral vector. The vector then unloads its genetic material containing the therapeutic human gene into the target cell. The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state.



Gene Delivery

- Transfection- the delivery of foreign molecules such as DNA and RNA into eukaryotic cells
- Naked DNA is not suitable for in-vivo transport of genetic materials-> degradation by serum nucleases
- Ideal gene delivery system
  - Biocompatible
  - Non-immunogenic
  - Stable in blood stream
  - Protect DNA during transport
  - Small enough to extravagate
  - Cell and tissue specific

Endocytosis



Endocytosis

- Phagocytosis is the process by which cells ingest large objects, such as cells which have undergone apoptosis, bacteria, or viruses. The membrane folds around the object, and the object is sealed off into a large vacuole known as a phagosome.
- Pinocytosis is a synonym for endocytosis. This process is concerned with the uptake of solutes and single molecules such as proteins.
- Receptor-mediated endocytosis is a more specific active event where the cytoplasm membrane folds inward to form coated pits. These inward budding vesicles bud to form cytoplasmic vesicles.

http://highered.mcgraw-hill.com/olc/dl/120068/bio02.swf

Endocytosis pathways

- Macropinocytosis is the invagination of the cell membrane to form a pocket which then pinches off into the cell to form a vesicle filled with extracellular fluid (and molecules within it). The filling of the pocket occurs in a non-specific manner. The vesicle then travels into the cytosol and fuses with other vesicles such as endosomes and lysosomes.
- Clathrin-mediated endocytosis is the specific uptake of large extracellular molecules such as proteins, membrane localized receptors and ion-channels. These receptors are associated with the cytosolic protein clathrin which initiates the formation of a vesicle by forming a crystalline coat on the inner surface of the cell's membrane.
- Caveolae consist of the protein caveolin-1 with a bilayer enriched in cholesterol and glycosphingolipids. Caveolae are flask shaped pits in the membrane that resemble the shape of a cave (hence the name caveolae). Uptake of extracellular molecules are also believed to be specifically mediated via receptors in caveolae.

Endocytic pathway in mammalian cells

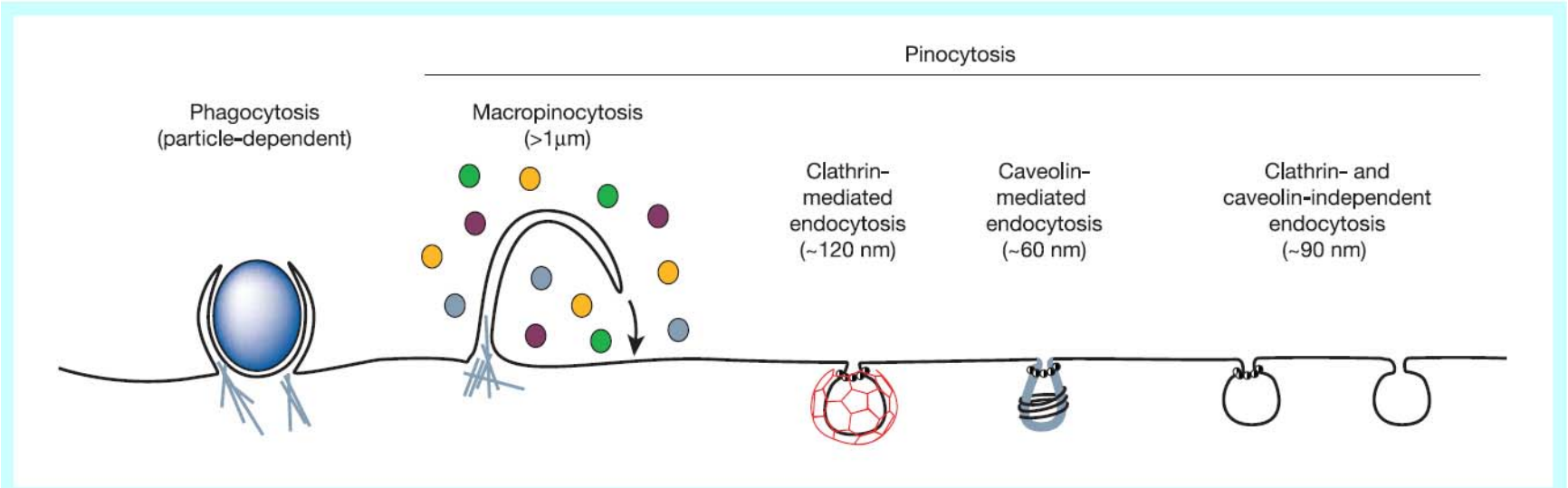


Figure 1 Multiple portals of entry into the mammalian cell. The endocytic pathways differ with regard to the size of the endocytic vesicle, the nature of the cargo (ligands, receptors and lipids) and the mechanism of vesicle formation.

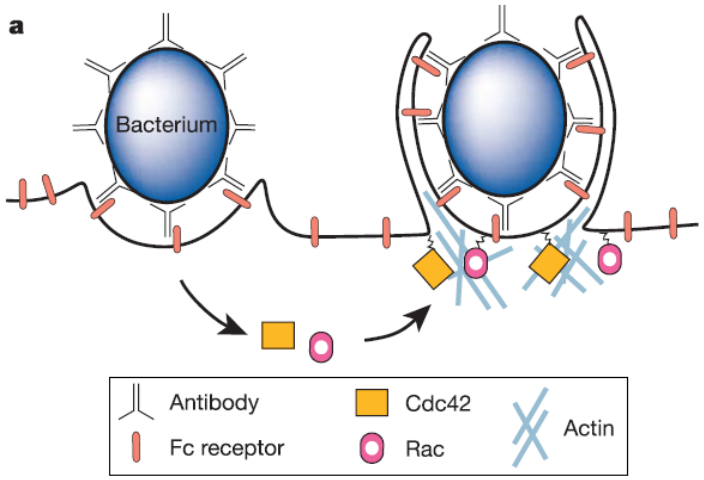


Figure 2 Cargo-stimulated signalling pathways induce uptake by phagocytosis and caveolae. **a**, Fc receptors on the surface of macrophages are activated by immunoglobulin-γ molecules bound to a bacterium. A signalling cascade that involves Rac, Cdc42 and downstream kinases triggers actin rearrangements, protrusion of the membrane around the bacterium, and its engulfment into a phagosome. **b**, Albumin binds to and presumably clusters its receptor, gp60, in caveolae to activate $G_{i\alpha}$ and Src kinases, triggering caveolae endocytosis.

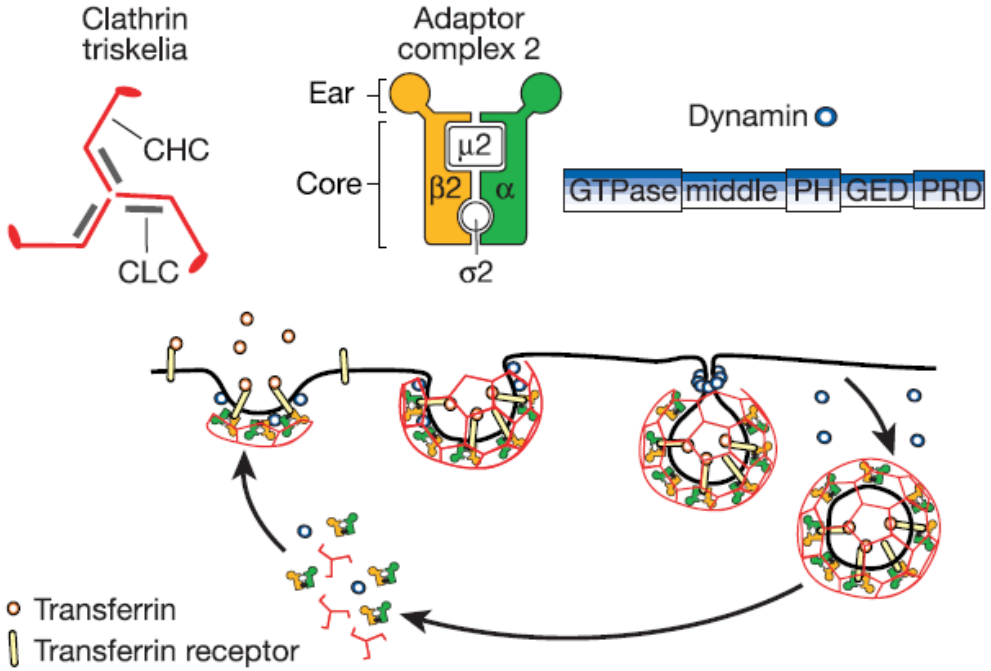
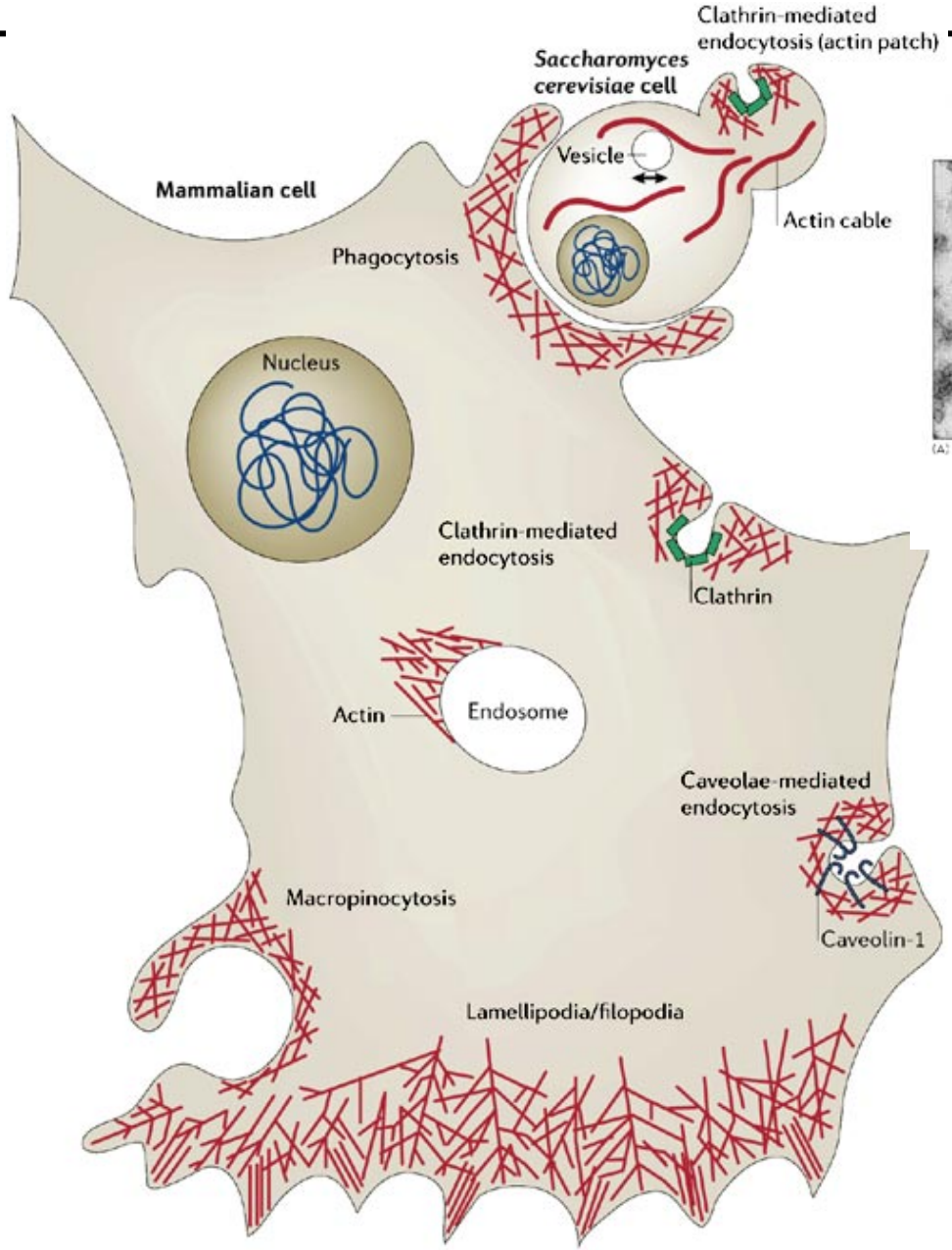
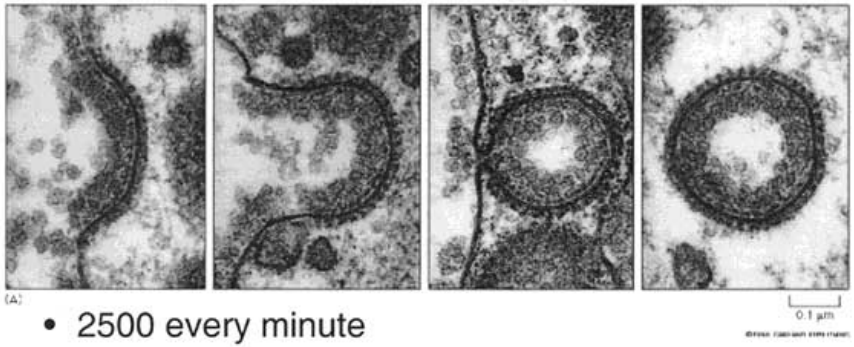


Figure 3 Core components of the machinery driving clathrin-mediated endocytosis. Clathrin triskelions, composed of three clathrin heavy chains (CHC) and three tightly associated light chains (CLC), assemble into a polygonal lattice, which helps to deform the overlying plasma membrane into a coated pit. Heterotetrameric AP2 complexes are targeted to the plasma membrane by the α-adaptin subunits, where they mediate clathrin assembly through the β2-subunit, and interact directly with sorting motifs on cargo molecules through their μ2 subunits. Dynamin is a multidomain GTPase that is recruited to the necks of coated pits, where it can assemble into a spiral or 'collar' to mediate or monitor membrane fission and the release of CCVs (see text for details). A subsequent uncoating reaction recycles the coat constituents for reuse.

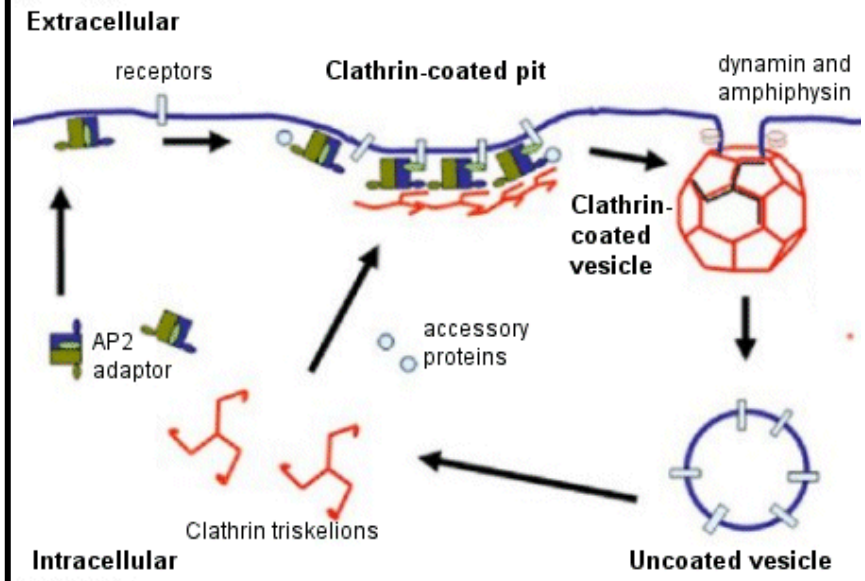


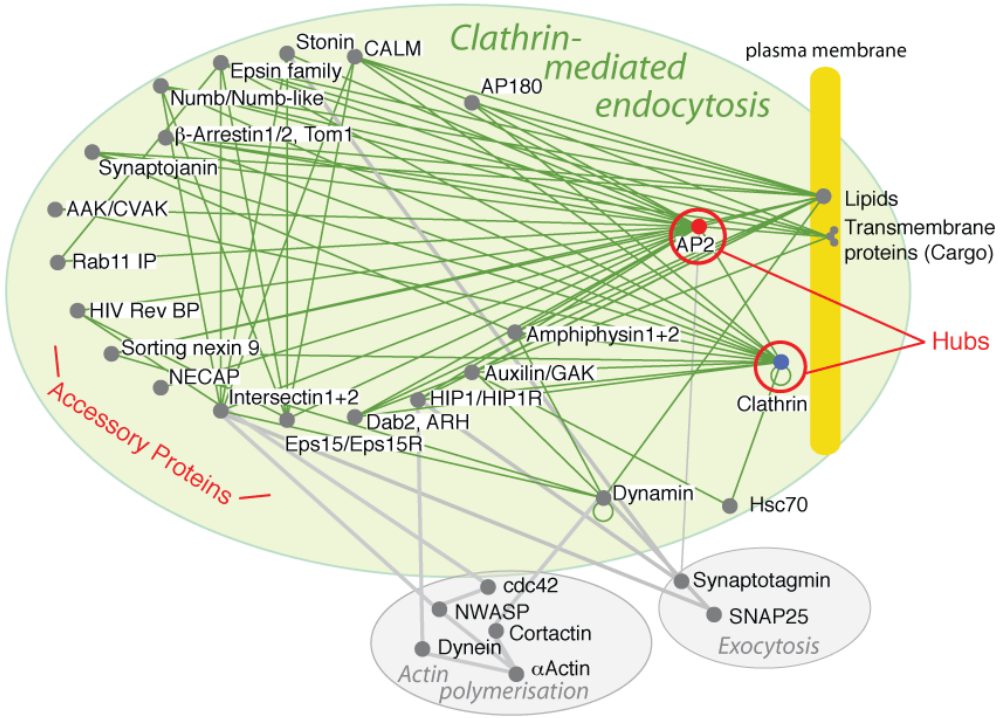
Formation of Clathrin-Coated Vesicles



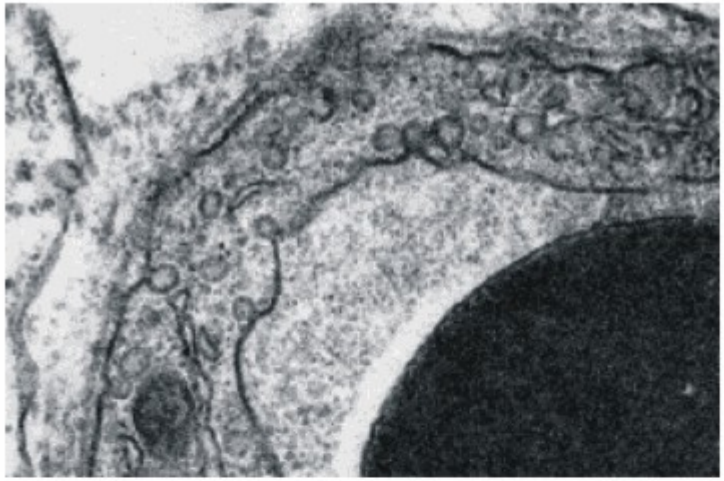
- 2500 every minute
- CCV uncoat within seconds

Clathrin-mediated endocytosis



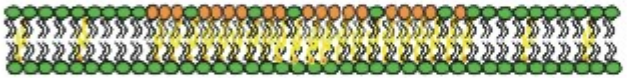


A

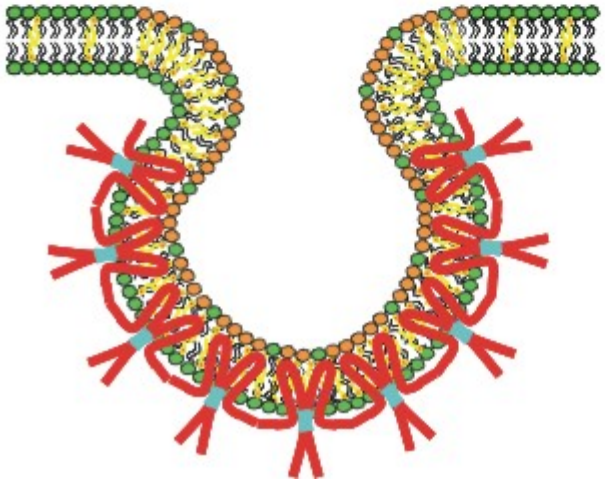


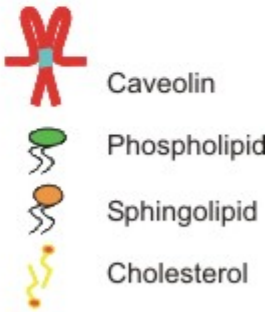
B

Lipid Rafts



Caveolae





Barrier to non-viral gene delivery

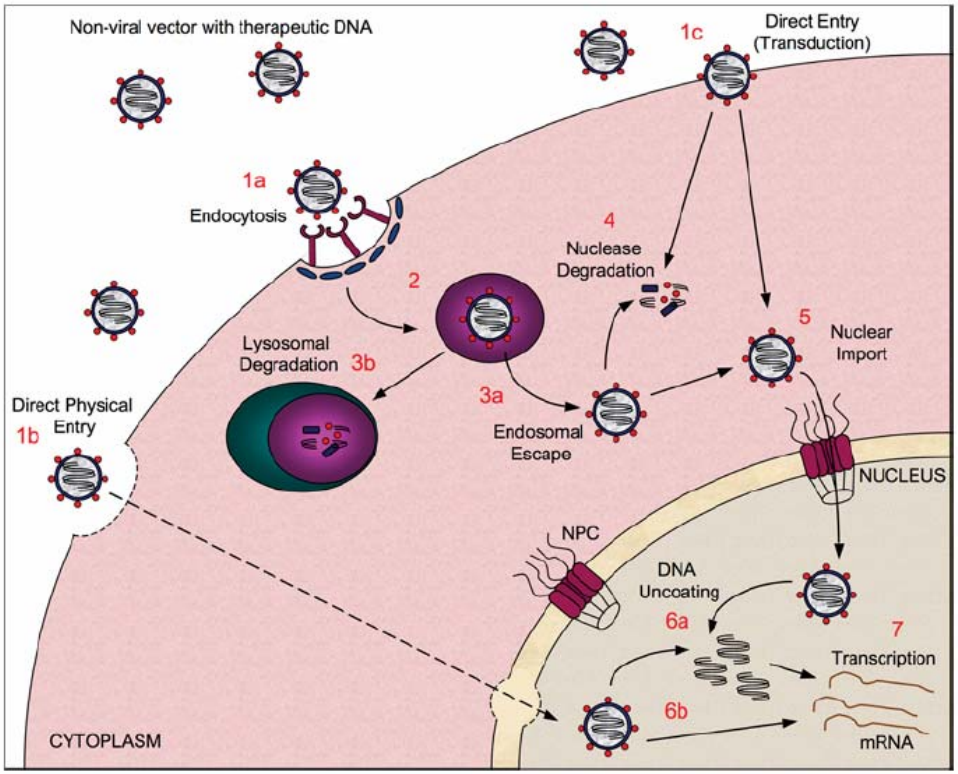


Figure 1 Barriers to non-viral gene delivery

Representation of the route travelled by a non-viral gene-delivery vector carrying therapeutic DNA to the nucleus. A non-viral vector, formed by interaction of the DNA with a carrier compound, must cross the plasma membrane to enter the cell. This can be via several routes, including endocytosis-based entry (1a), direct physical entry routes, such as electroporation or ballistic delivery (1b), or direct entry via protein transduction (1c). Depending on the mode of cellular entry, the vector may become encapsulated in an endosome (2), from which it must escape (3a) or it will become degraded when the endosome fuses with a lysosome (3b). The DNA will at some point be subjected to degradation by cytosolic nucleases (4), as it traverses through the cytoplasm to reach the nucleus. Finally, the vector must undergo nuclear transport (5) through NPCs embedded in the NE in order to gain access to the nucleoplasm. Once in the nucleus, the DNA may (6a) or may not (6b) need to be uncoated, depending upon the vector used, before it can ultimately be transcribed (7).

Biochem. J. (2007) **406**, 185–202 (Printed in Great Britain) doi:10.1042/BJ20070505

NLS-mediated nuclear import

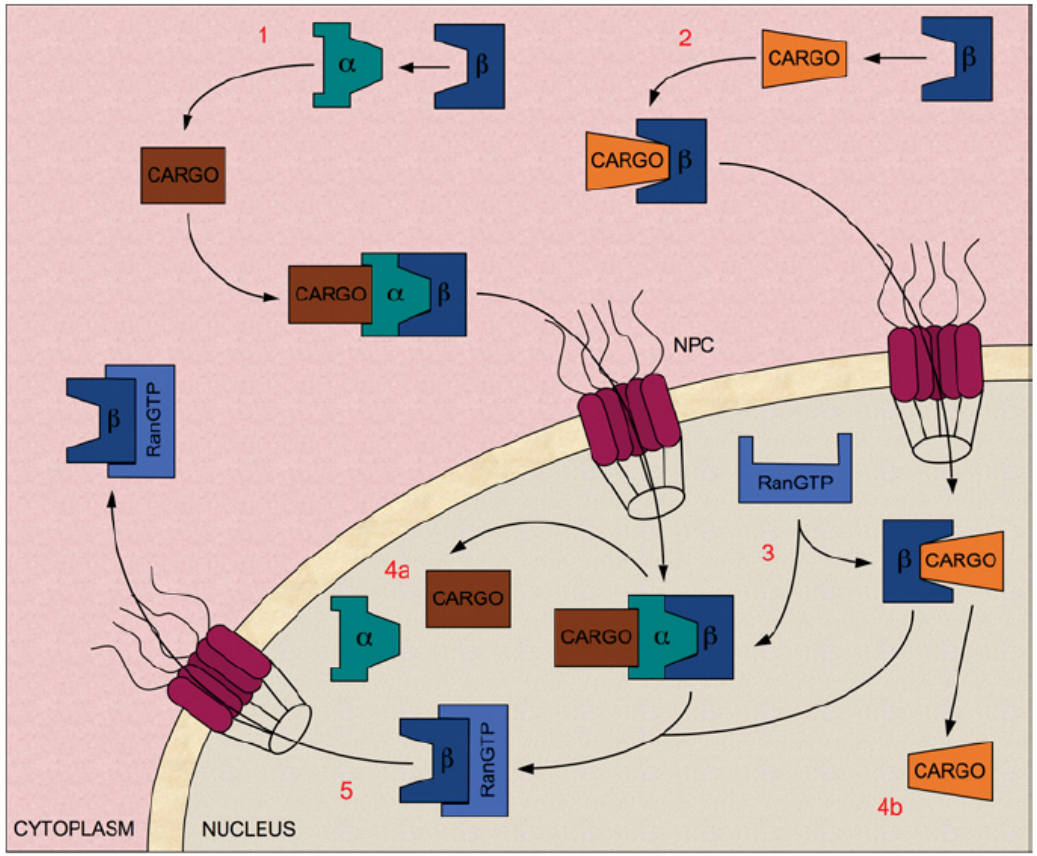
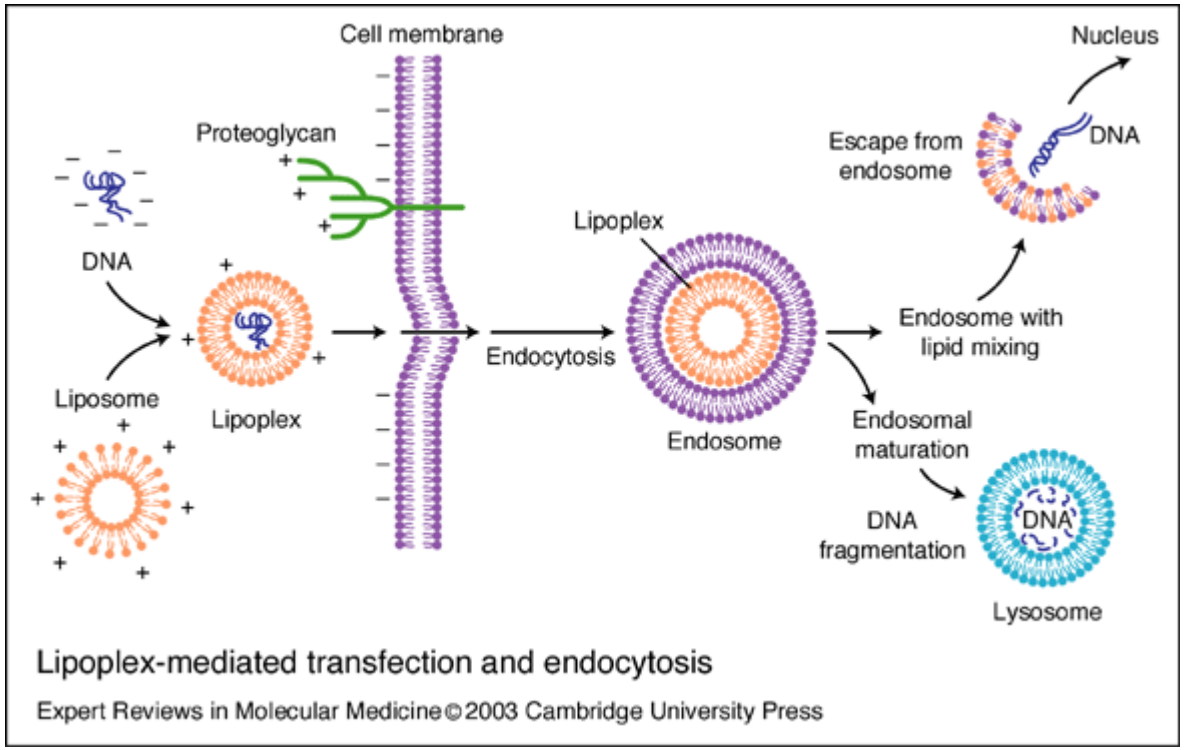


Figure 2 NLS-mediated nuclear import pathways

In classical nuclear import, the NLS found in cargo bound for the nucleus is recognized by the Imp α subunit of the Imp α/β heterodimer (1). However, there are also many examples where Imp β or one of its many homologues can mediate nuclear import or cargo proteins independently of Imp α (2). In both cases, transient interactions between the Imp β and the nucleoporin proteins that line the NE-embedded NPCs mediate translocation into the nucleus. Once inside, RanGTP binds to Imp β (3), releasing Imp α and the cargo into the nucleoplasm (4a and 4b). RanGTP itself is then recycled back to the cytoplasm (5), where it is converted into its RanGDP state (not shown). An animated version of this Figure can be found at http://www.BiochemJ.org/bj/406/0185/bj4060185add.htm

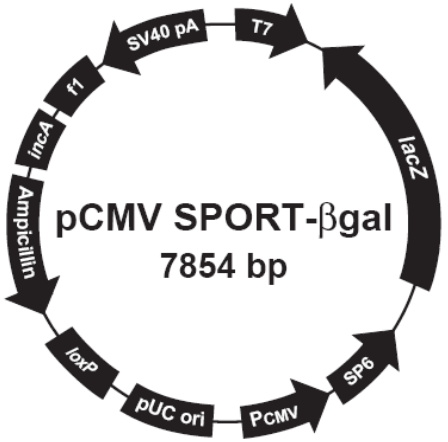
Transfection



Lipoplex-mediated transfection and endocytosis

Transient and Stable Transfection

- Transient
  - No chromosome integration
  - Expression 24-96 Hr
  - Super-coiled plasmid
- Stable
  - Chromosome integration
  - Linear DNA
  - 1 in 10^4
  - Selection



Challenges

- Cell targeting
- Transport through the cell membrane
- Uptake and degradation in endolysome
- Intracellular trafficking of plasmid to nucleus

Transfection Technology

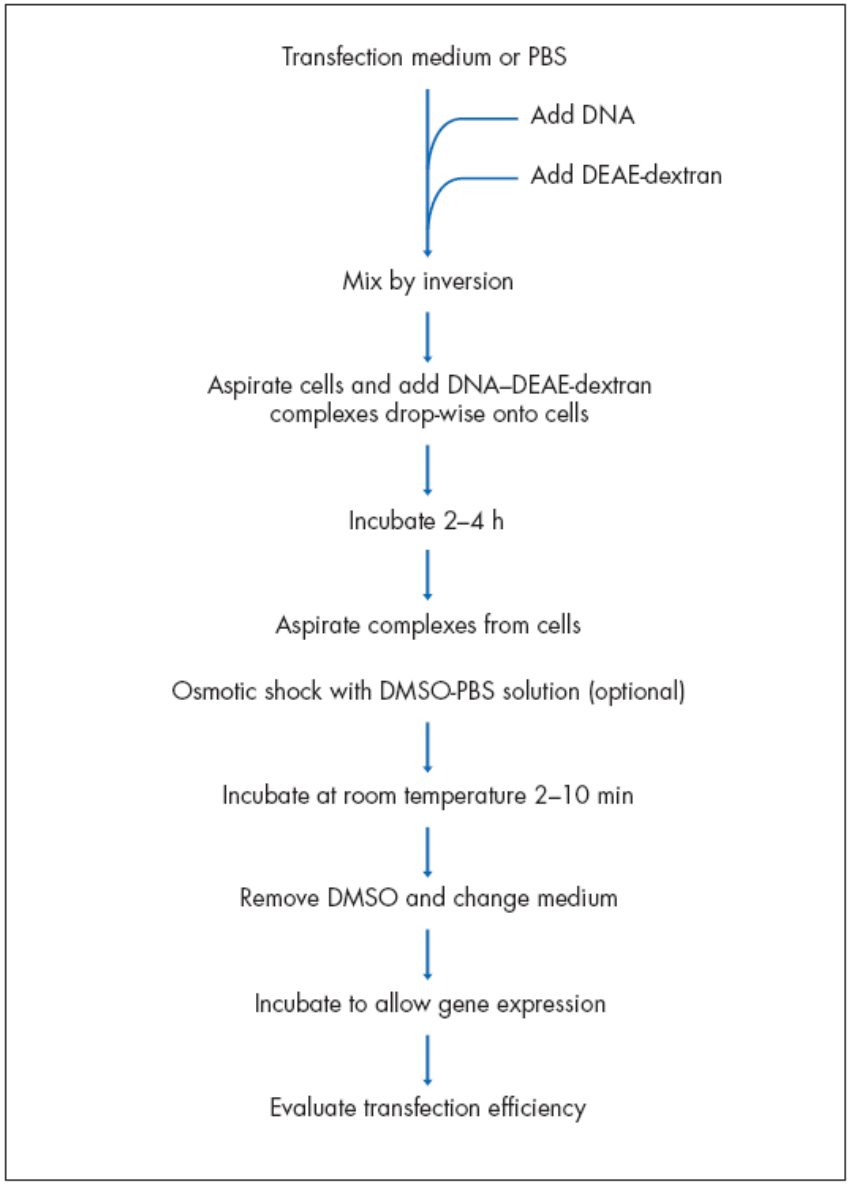
- DEAE dextran
- Calcium phosphate
- Electroporation
- Microinjection
- Ballistic particle
- Nanoparticles
  - Cationic liposome
  - Cationic polyermer
  - Activated dendrimer
  - Gold nanoparticles
  - Chitosan

DEAE-dextran

- Diethylaminoethyl (DEAE)-dextran was introduced in 1965 (5) and is one of the oldest methods for introducing nucleic acids into cultured mammalian cells. The positively charged DEAE-dextran molecule interacts with the negatively charged phosphate backbone of the nucleic acid. The DNA–DEAEdextran complexes appear to adsorb onto the cell surface and be taken up by endocytosis. The advantages of this technique are its relative simplicity and reproducibility of results. Disadvantages include cytotoxic effects and the fact that the amount of serum in the culture medium must be temporarily reduced during the transfection procedure. In addition, the DEAE-dextran method is best suited for transient transfection only.

Dextran is a complex branched polysaccharide made of many glucose molecules joined into chains of varying lengths.

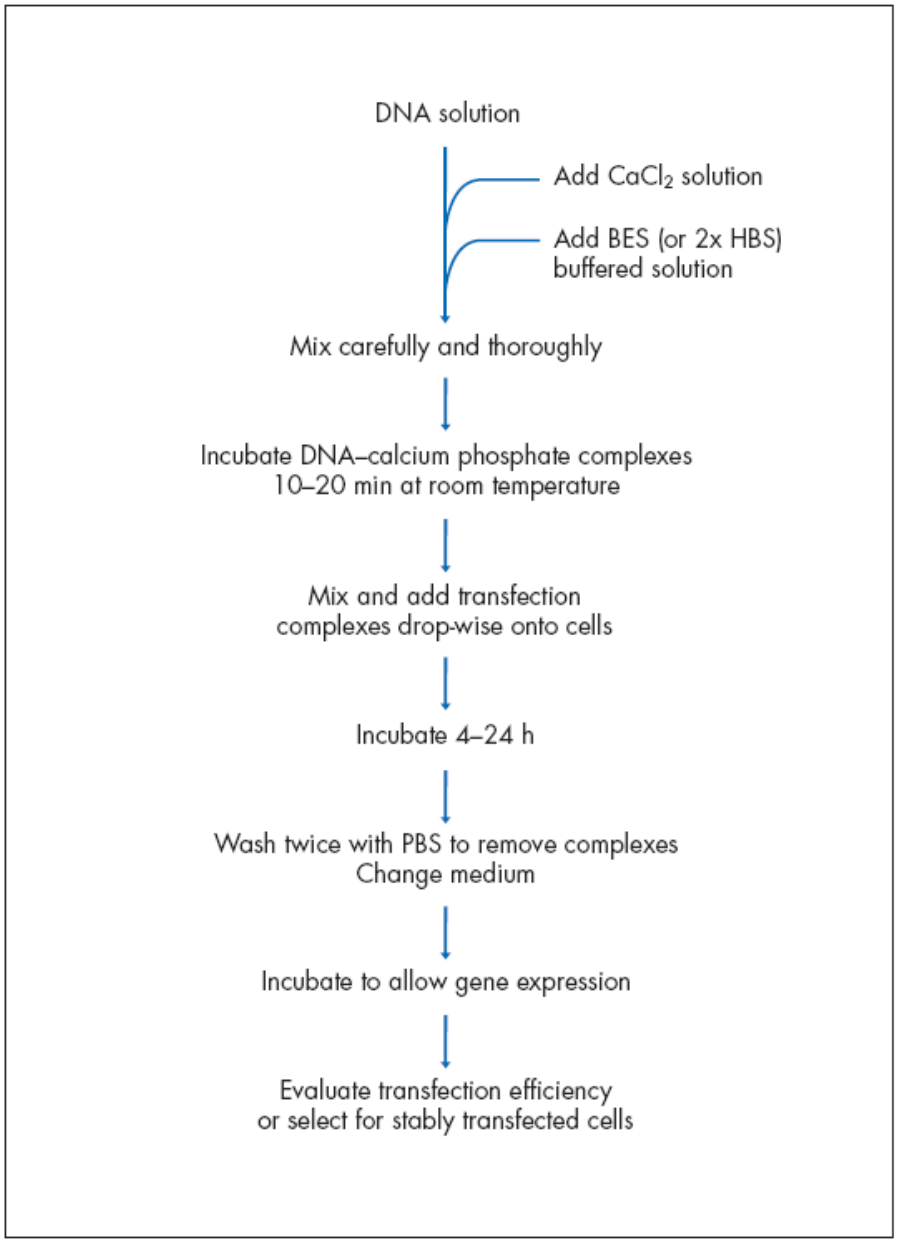
DEAE-Dextran Method*



Calcium Phosphate

- The calcium-phosphate method was first used in 1973 to introduce adenovirus DNA into mammalian cells (6). The principle involves mixing DNA in a phosphate buffer with calcium chloride. The resulting calcium-phosphate–DNA complexes adhere to the cell membrane and enter the cytoplasm by endocytosis. Advantages of calcium-phosphate–based transfection are its easy handling and, compared with the DEAE-dextran method, its much higher suitability for stable transfections. However, a common disadvantage is low reproducibility, which is mainly caused by variation in transfection complex size and shape. These variations can be caused by minor changes in the pH of the solutions used for the transfection, as well as the manner in which these solutions are combined. A further drawback of the calcium-phosphate method is that some cell types, including primary cells, may resist this form of DNA transfer.

Calcium-Phosphate Method*



Nanocontainers

- Liposomes
- Dendrimers
- Layer by Layer Deposition
- Block Copolymer
- Shell Cross-Link

Cationic Liposome

Liposomes were first introduced in 1987 by Felgner and coworkers (9). The liposomes currently in use typically contain a mixture of cationic and neutral lipids organized into lipid bilayer structures. Transfection-complex formation is based on the interaction of the positively charged liposome with the negatively charged phosphate groups of the nucleic acid. The uptake of the liposome–DNA complexes may be mediated by endocytosis. Compared to the DEAE-dextran and calciumphosphate methods, liposomes often offer higher transfection efficiency and better reproducibility. However, one drawback of liposome-mediated transfection is that the presence of serum during the transfection procedure often lowers the transfection efficiency. For this reason, serum is often omitted when transfecting with liposomes. In many cases, the absence of serum from the medium increases the cytotoxicity of the liposome. Another drawback of classical liposome-mediated transfection is that results

- Stability

Liposomes

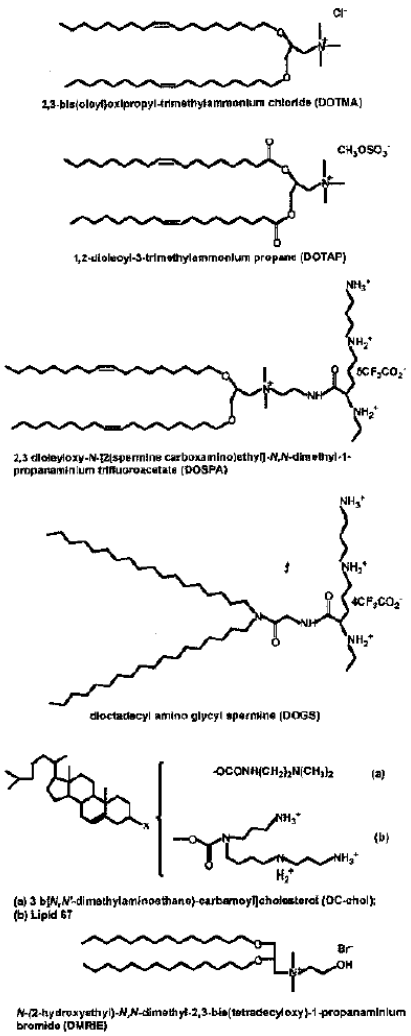


Figure 21.7 Structures of some cationic lipids commonly used in gene therapy.

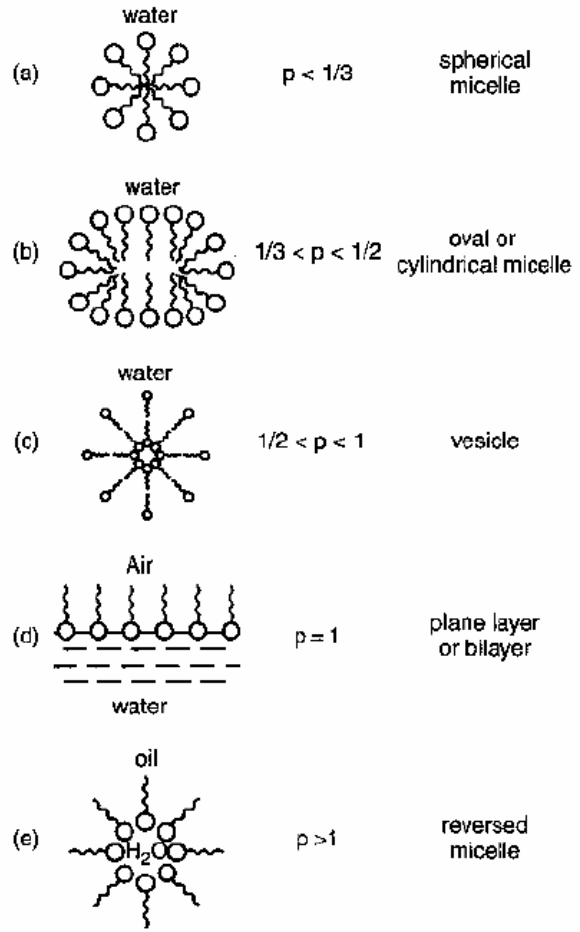
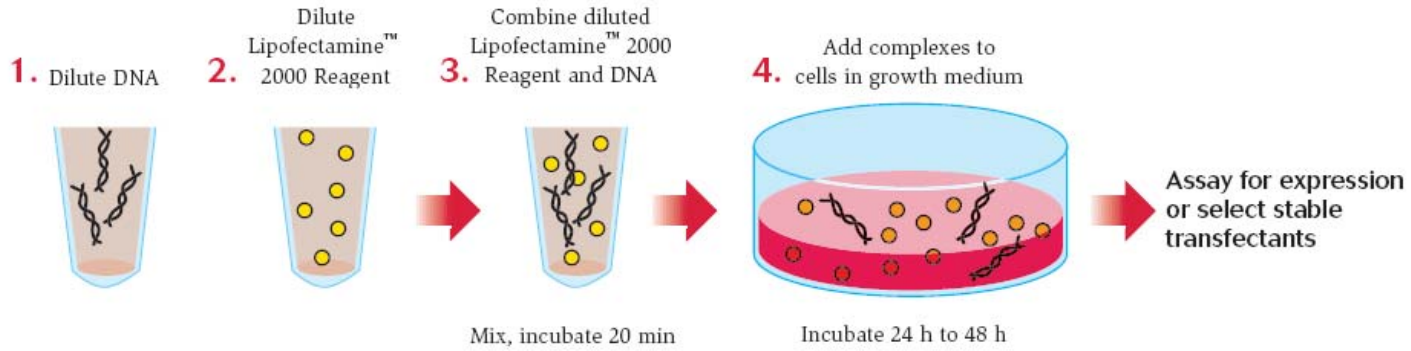
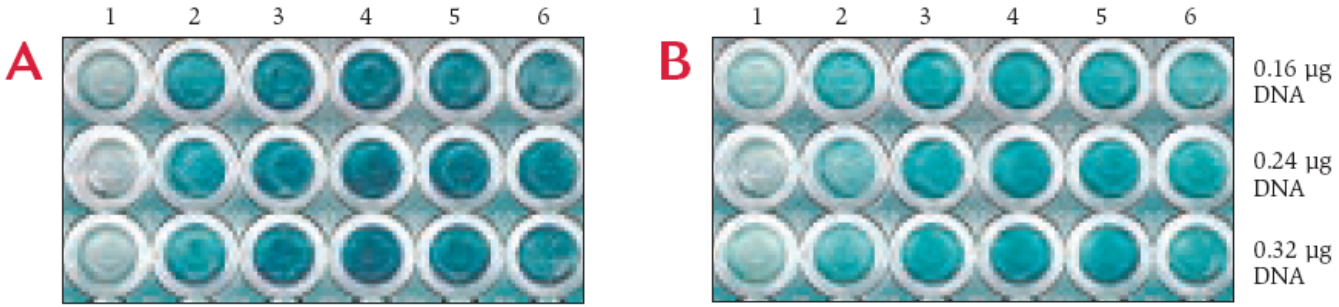


Figure 12.14. Sketch of structures formed by amphiphilic molecules at water–oil or water–air interfaces for various values of the packing parameter p of Eq. (12.6). [Adapted from E. Nakache et al., in Nalwa (2000), Vol. 5, Chapter 11, p. 580.]

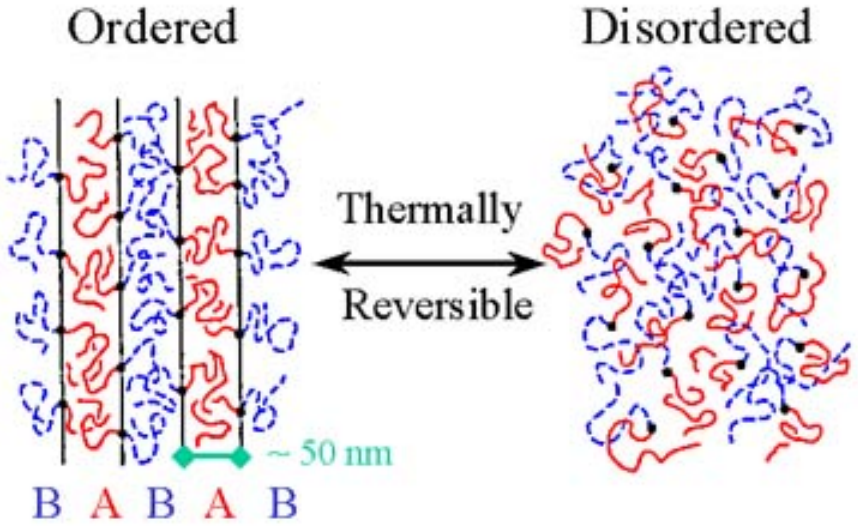




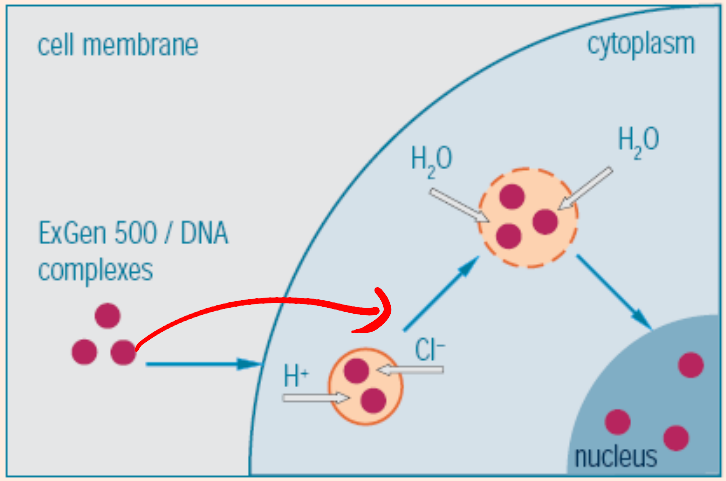
CHO-S cells were transfected with pCMV•SPORT-βgal DNA (0.16 µg to 0.32 µg) and Lipofectamine™ 2000 Reagent (0.2 µl to 1.2 µl, columns 1-6 respectively) in 96-well plates. After 24 hours, cells were stained with X-gal. **Panel A:** Cells (2 x 10^4) were plated the day before transfection in growth medium containing serum. **Panel B:** The day of transfection, cells were trypsinized, counted, and 5 x 10^4 cells were added directly to the wells containing the complexes.

Cell Line	Cell Type	Transfection efficiency (%)
293-F	Human kidney	99
293-H	Human kidney	99
CHO-S	Hamster ovary	96
COS-7L	Monkey kidney	99
BE(2)C	Human neuroblastoma	77
SKBR3	Human breast cancer	49
MDCK	Dog kidney	43
HT1080	Human fibrosarcoma	81
Human fibroblasts	Primary passaged	48
HeLa	Human cervical carcinoma	94
CV-1	Monkey kidney	70
Vero	Monkey kidney	86
PC12	Rat pheochromocytoma	85
Murine ES	Mouse embryonic stem	75
Rat Hepatocytes	Primary liver	50
E18 Cortical Neurons	Rat primary	25
E18 Hippocampal Neurons	Rat primary	30

Block Copolymers

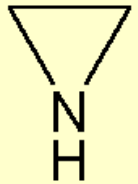


Polymer



1. ExGen 500 interacts with DNA to form small, stable, highly diffusible complexes which are readily endocytosed.
2. "Proton-sponge" effect of ExGen 500 buffers endosomal pH by provoking massive proton accumulation and passive chloride influx.
3. Rapid osmotic swelling causes endosomal rupture, allowing translocation of DNA to the nucleus without DNA degradation.

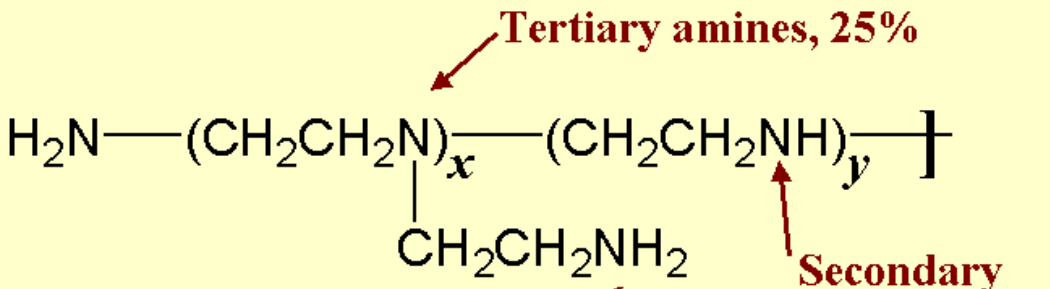
Synthesis of Poly-ethylenimine



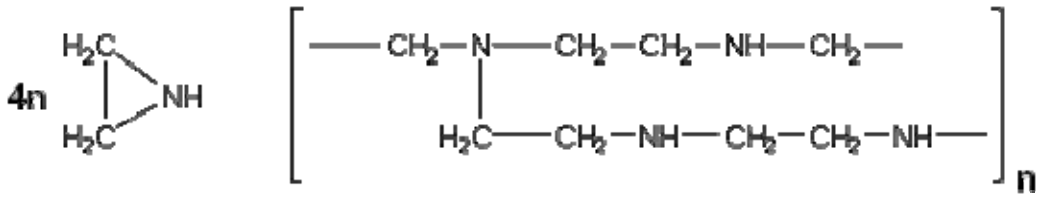
Ethylene imine

Catalyst

PEI



M. Hubbe



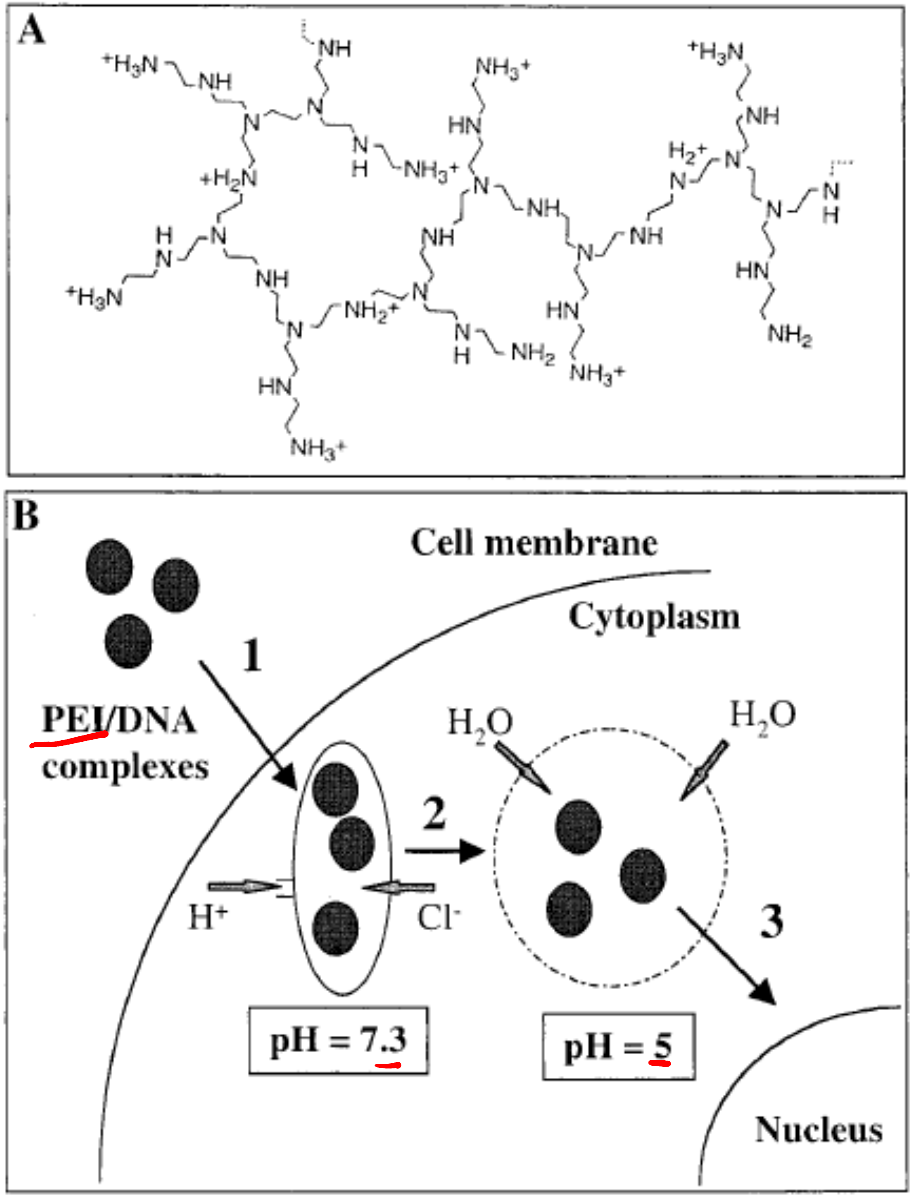
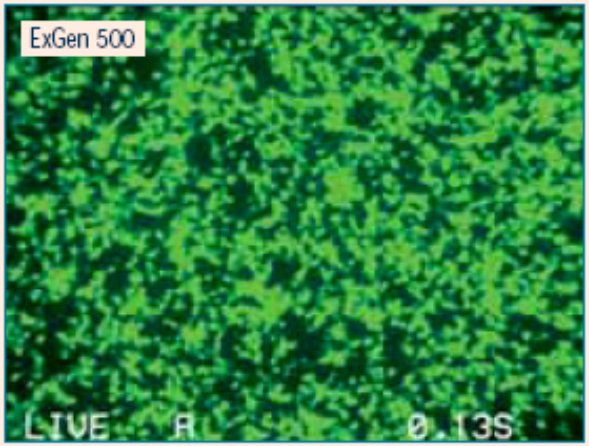
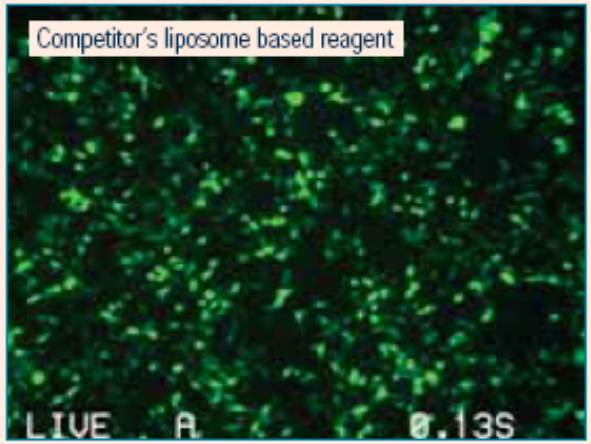


Figure 1. (A) Structure of PEI with a random topology. (B) The 'proton sponge effect': after endocytosis of the cationic complexes (1), acidic endosome buffering (2) leads to increased osmotic pressure and finally to lysis (3)

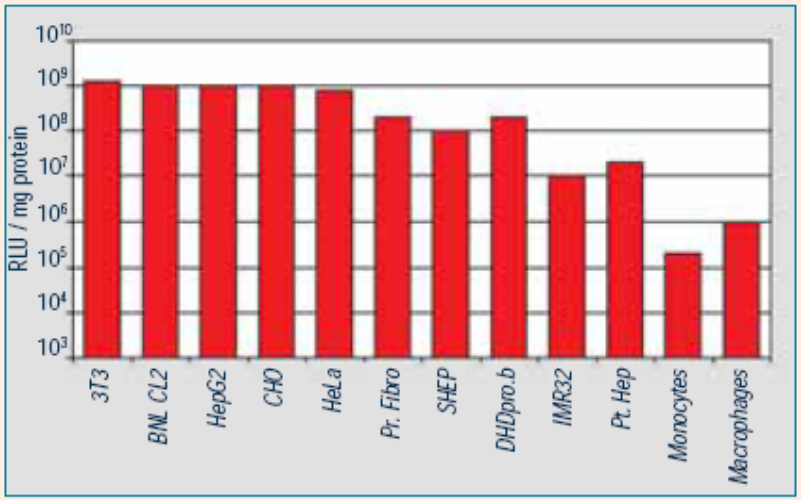
ExGen 500 performs when other transfection reagents fail





Expression of Green Fluorescent Protein (GFP) in 293 cells.
Cells were transfected with a vector containing the GFP coding sequence using ExGen 500 and competitor's liposome based reagent.

ExGen 500 transfects a wide variety of cell types



Dendrimers

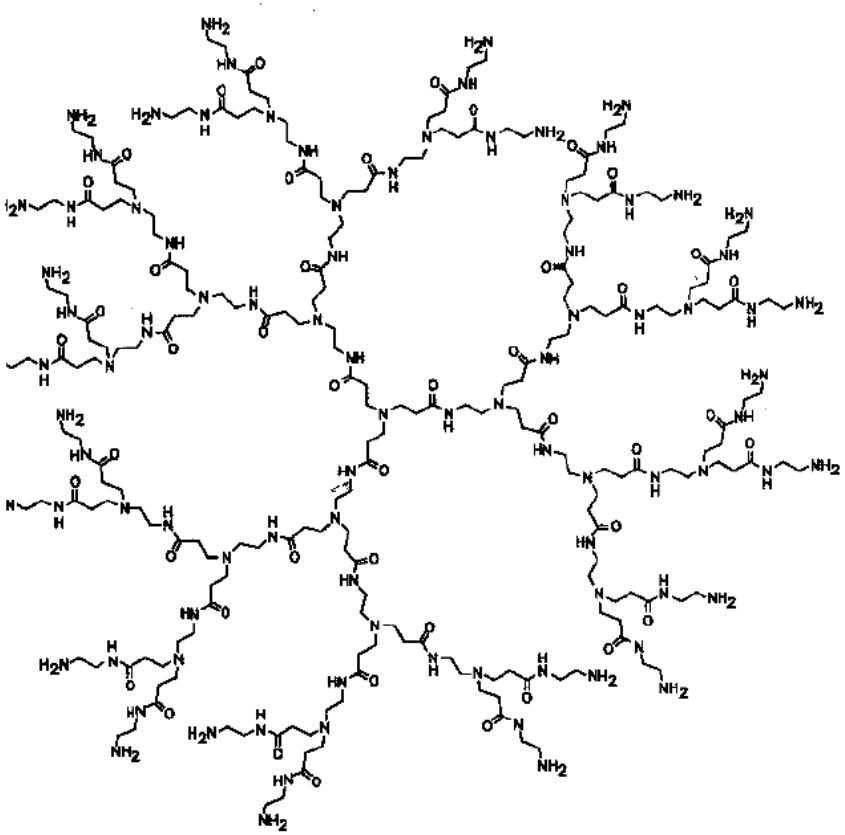


Figure 11.16. Fifth-generation polyaminoamine (PAMAM) dendrimer. [Prepared by D. A. Tomalia, H. Baker, J. R. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, and P. Smith, *Polym. J.* **17**, 117 (1985).]

Dendrimer

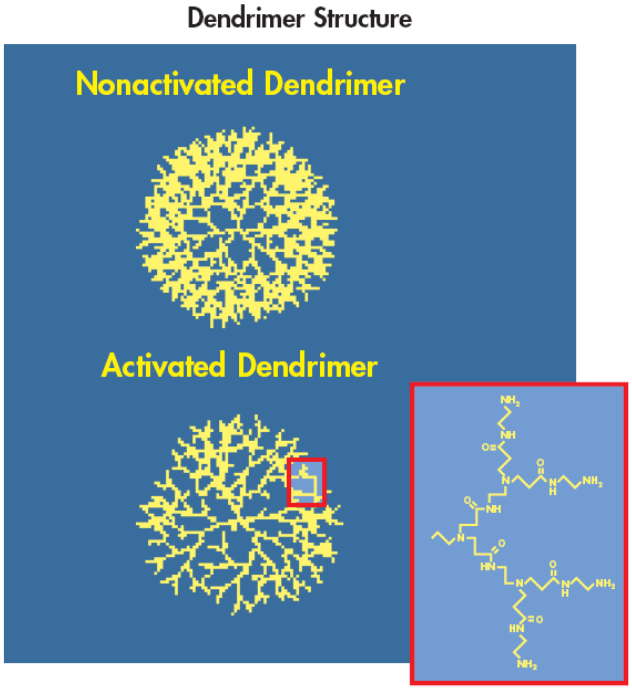
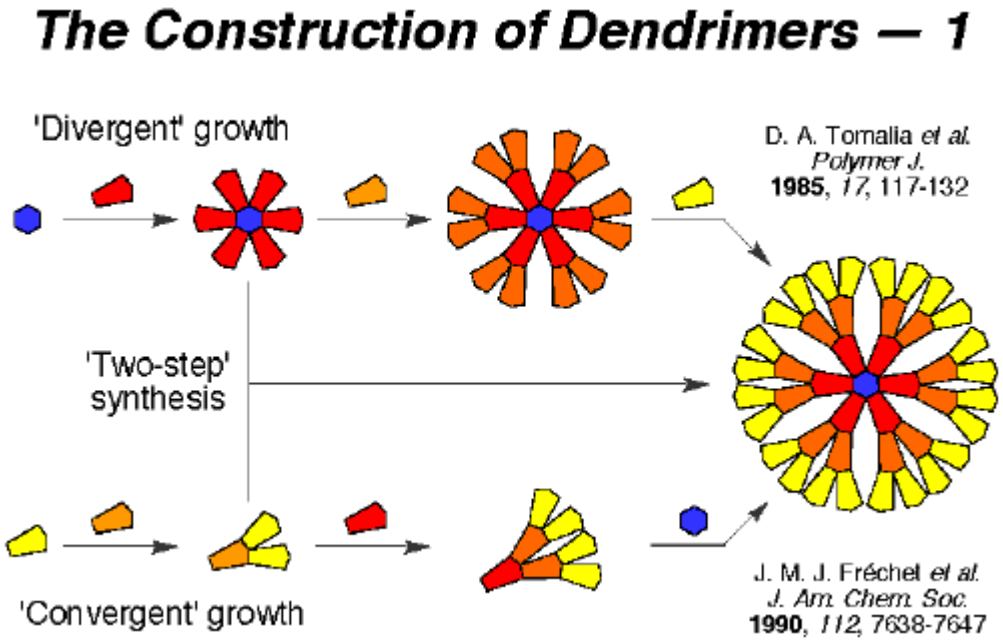
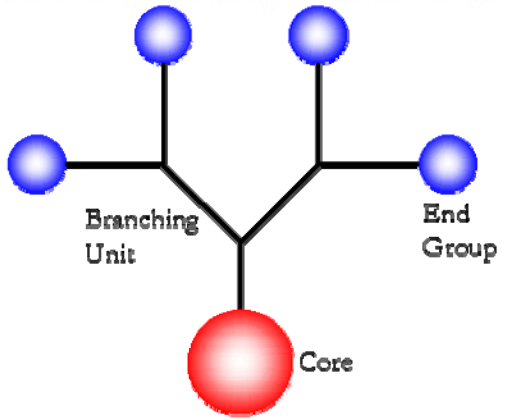
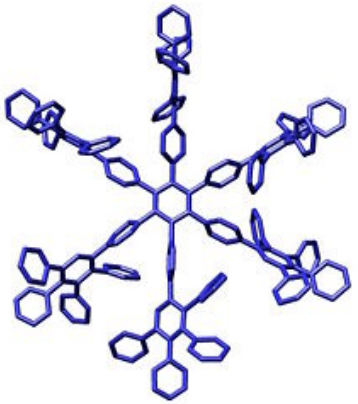


Figure 1. *Schematic diagram of an activated and non-activated dendrimer. A portion of the activated dendrimer molecule is enlarged to show the chemical structure of the molecular branches.*







Activated-Dendrimer–DNA Interaction

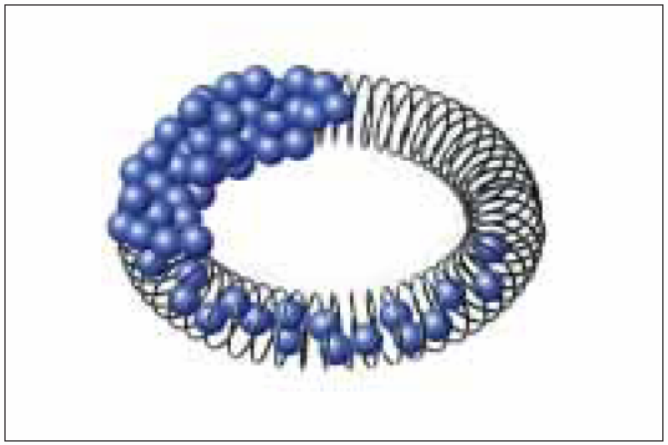


Figure 2. *Model of the activated-dendrimer–DNA complex. Activated dendrimers (purple spheres) interact with DNA (black) to form a ring-like (toroid-like) structure. The upper right section of the illustration shows naked DNA, the lower section shows the interaction between dendrimers and DNA inside the complex, and the upper left section shows the final complete coverage of DNA within the complex.*

PolyFect Reagent with HeLa Cells

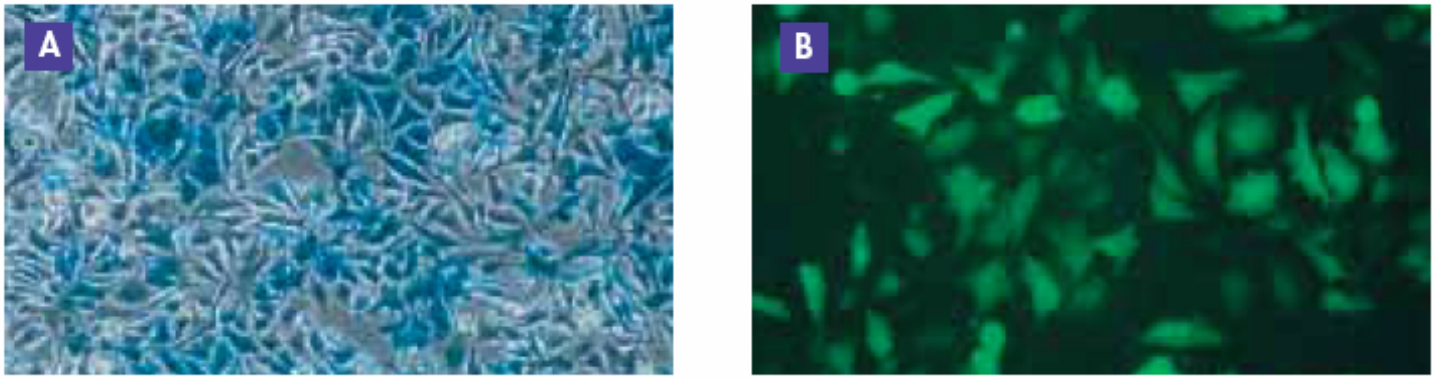


Figure 8. *Expression of* **A** *β-galactosidase and* **B** *green fluorescent protein (GFP) in HeLa cells. Cells were cotransfected in 6-well plates with β-galactosidase and GFP reporter plasmids using PolyFect Transfection Reagent and the HeLa cell protocol. Expression was visualized by X-gal staining or fluorescence microscopy 2 days post-transfection.*

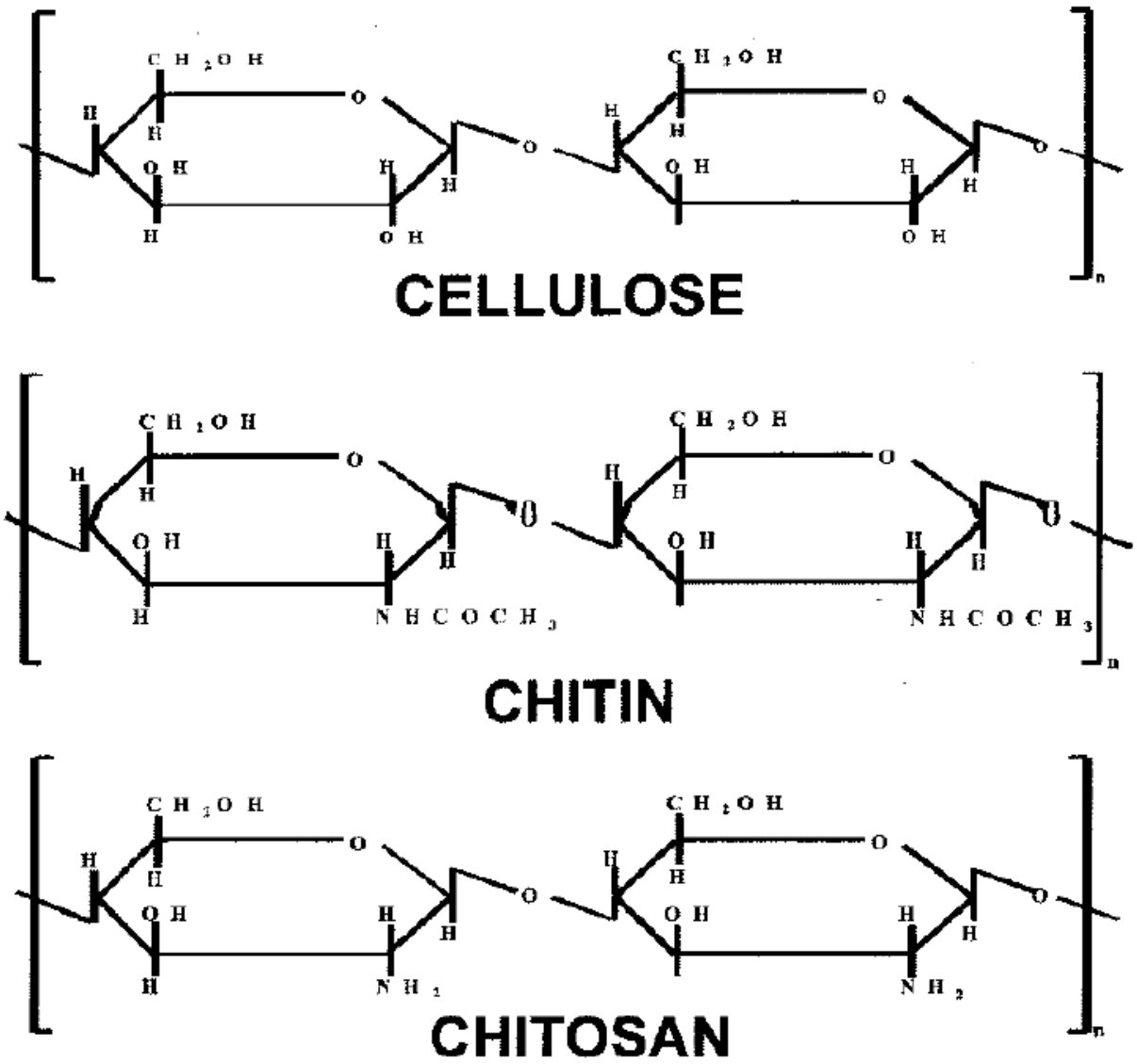
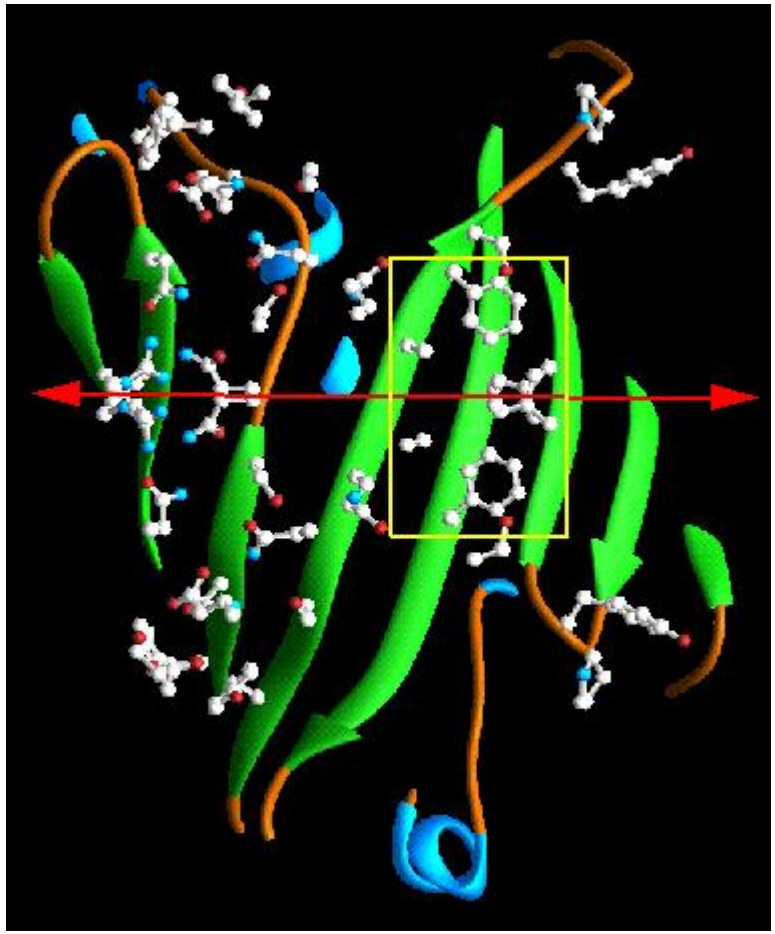
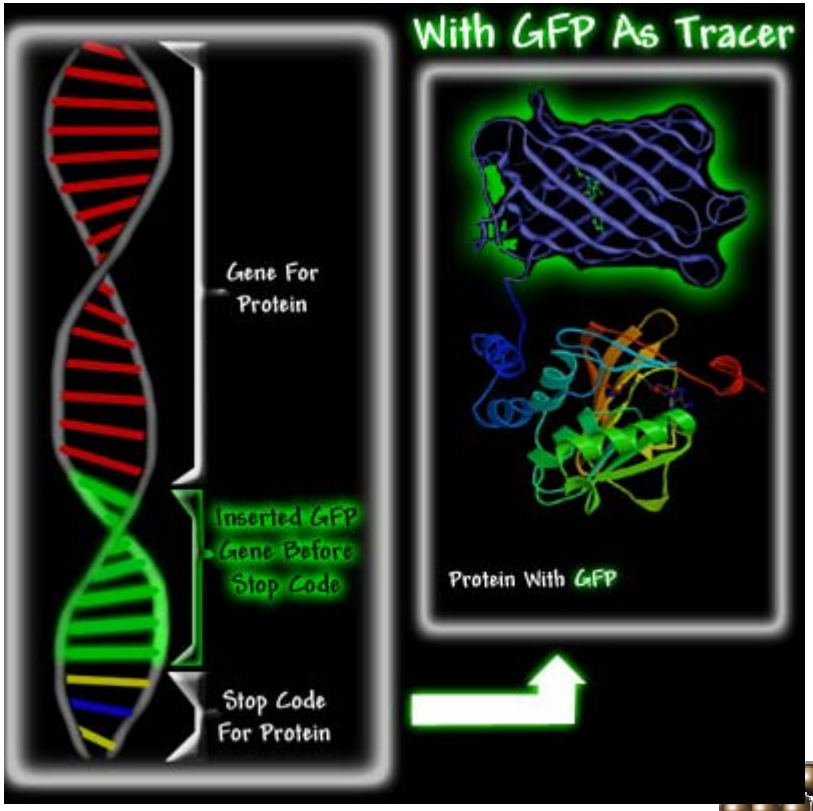


Figure 21.3 Structural similarities between cellulose, chitin, and chitosan.

Green Fluorescent Protein (GFP)



The **green fluorescent protein** (**GFP**) is a protein from the jellyfish *Aequorea victoria* that fluoresces green when exposed to blue light.



GFP Rats

Gold Nanoparticles

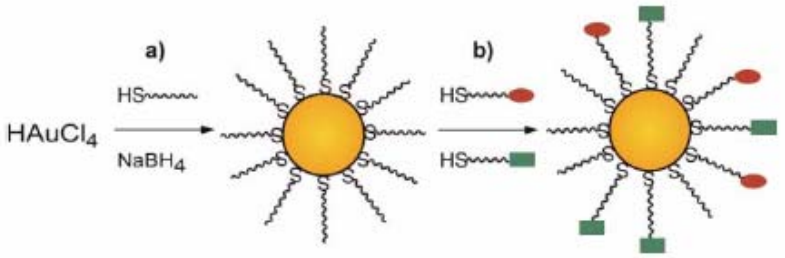
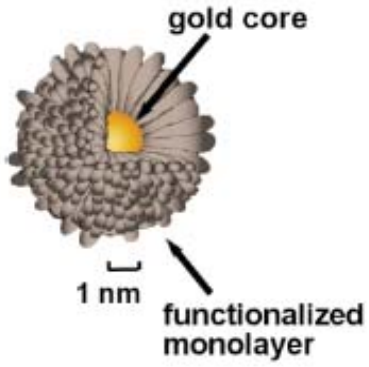
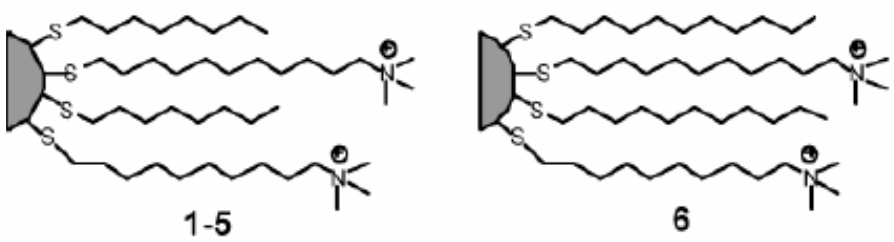
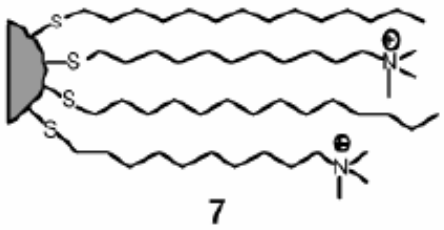


Fig. 2 Synthesis of gold MPCs using (a) the Brust–Schiffrin reaction and MMPCs *via* (b) the Murray place-exchange method.







MMPC	1	2	3	4	5	6	7
% cationic coverage	100	85	68	63	58	77	89

Figure 1. MMPCs used for transfection.

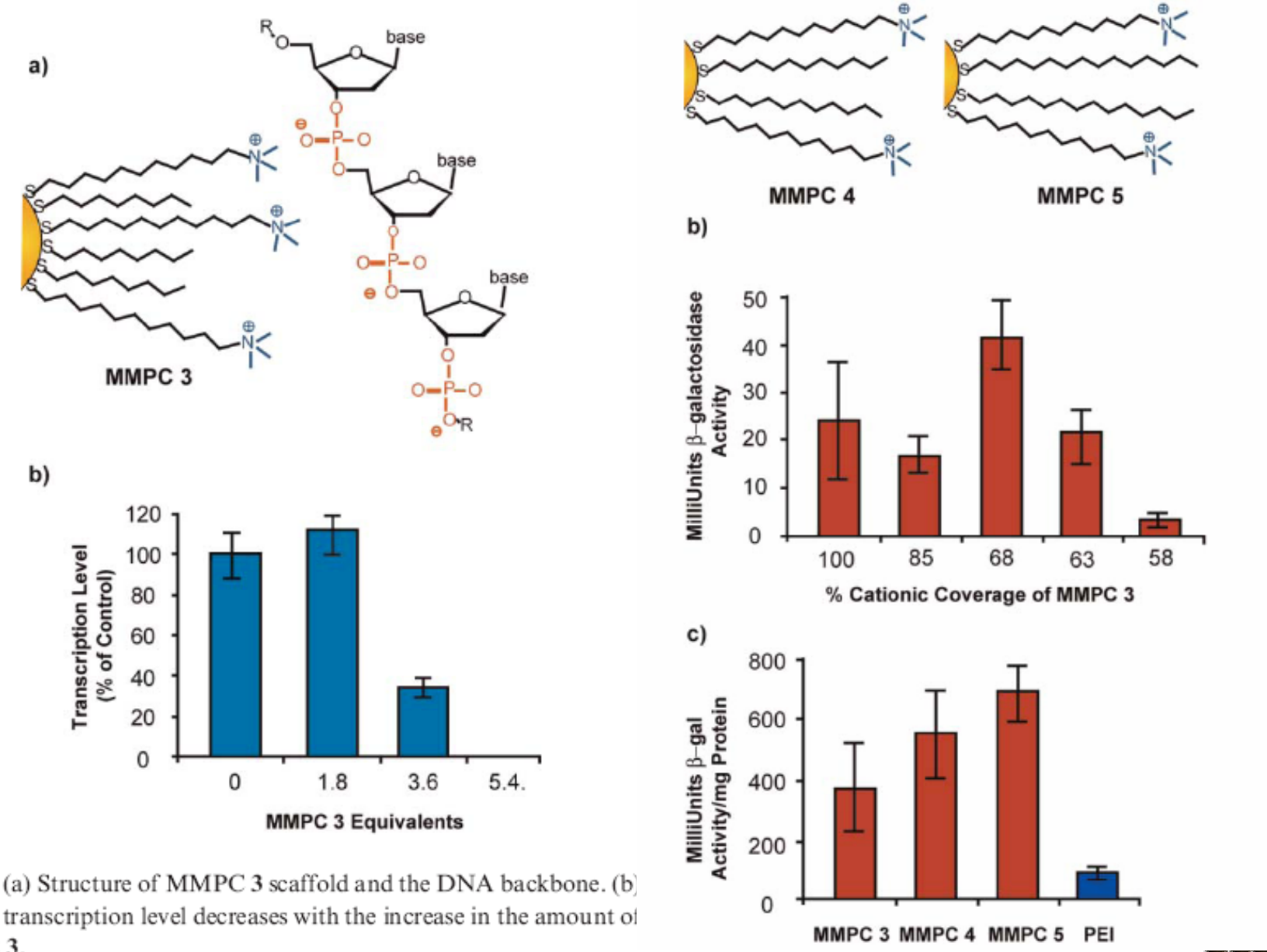
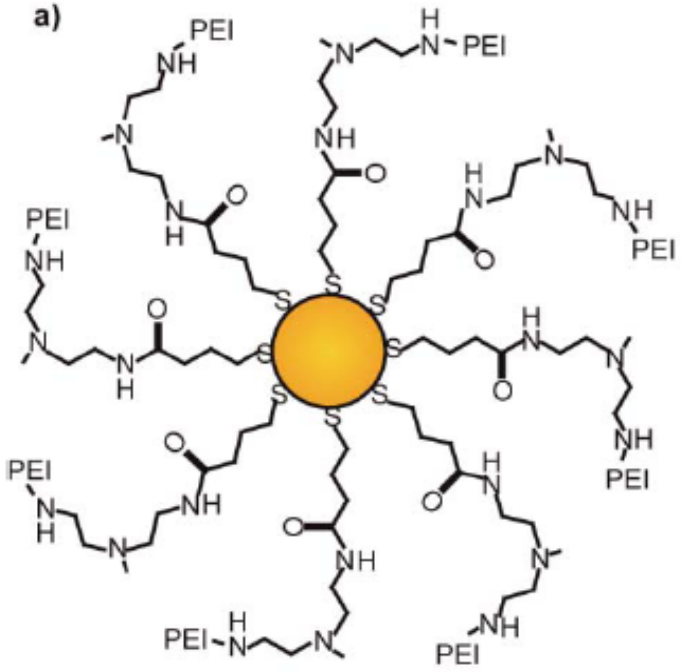


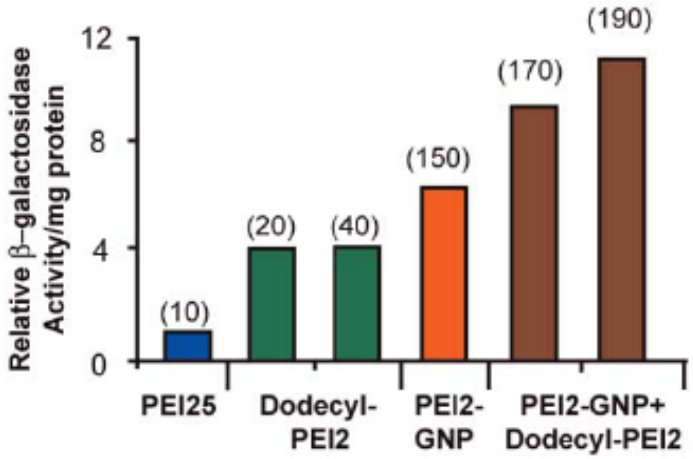
Fig. 5 (a) Structure of MMPC **3** scaffold and the DNA backbone. (b) Percent transcription level decreases with the increase in the amount of MMPC **3**.

a)


PEI
NH
N
H
O
S

MPC 6

b)


Relative β–galactosidase Activity/mg protein
12
8
4
0
(10)
(20)
(40)
(150)
(170)
(190)
PEI25
Dodecyl-PEI2
PEI2-GNP
PEI2-GNP+ Dodecyl-PEI2

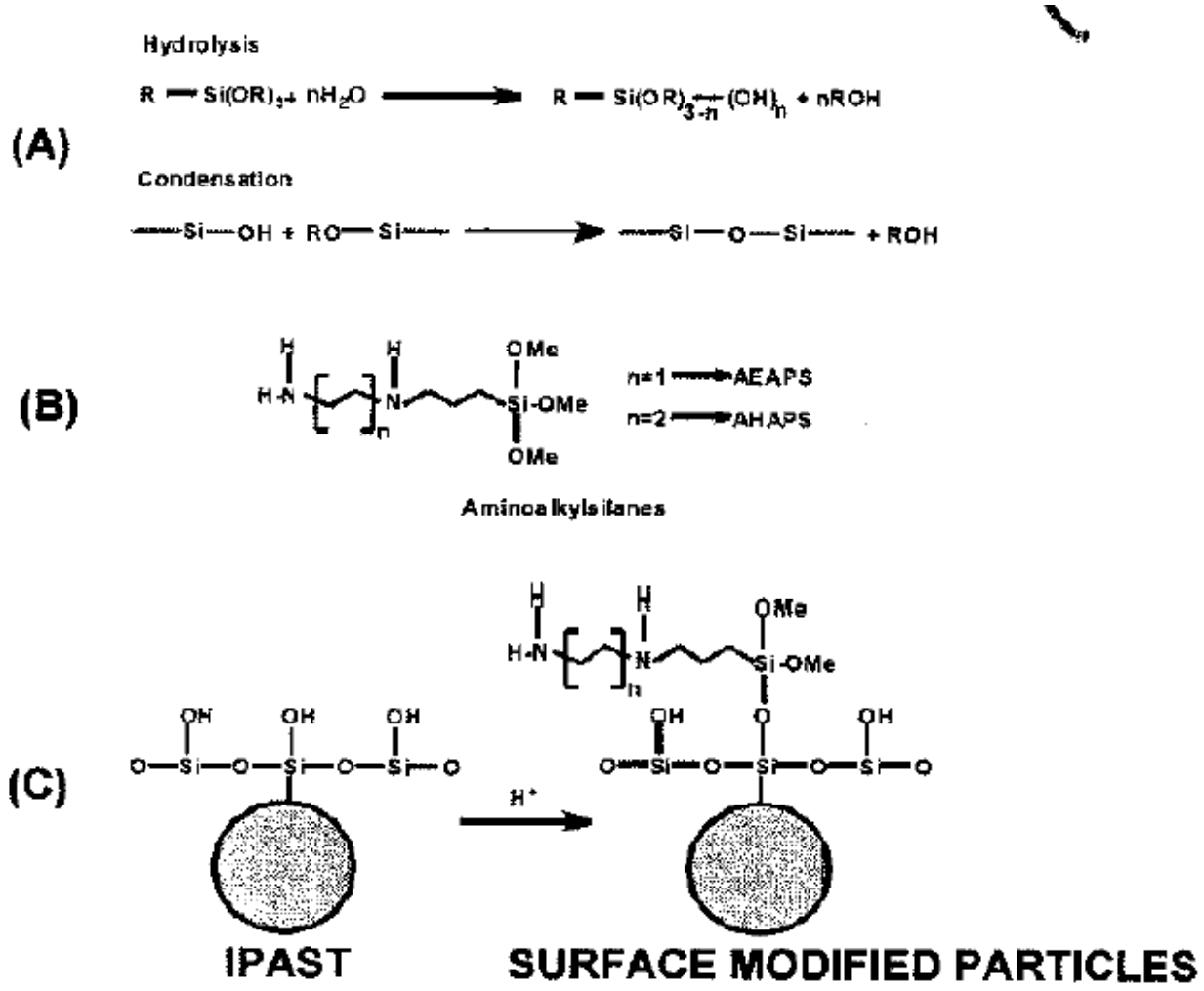


Figure 21.11 (A) Hydrolysis and condensation of unmodified particles. (B) Alkylaminoalkanes. (C) Modification scheme.

Parameters

- Cell density
- Amount of DNA
- Transfection reagent to DNA ratio
- Incubation period with DNA complex
- Incubation time following transfection

•Eukaryotic cells are about 1000 times larger than bacteria cells and also have a membrane enclosed nucleus containing their DNA, and several other internal structures known as organelles.

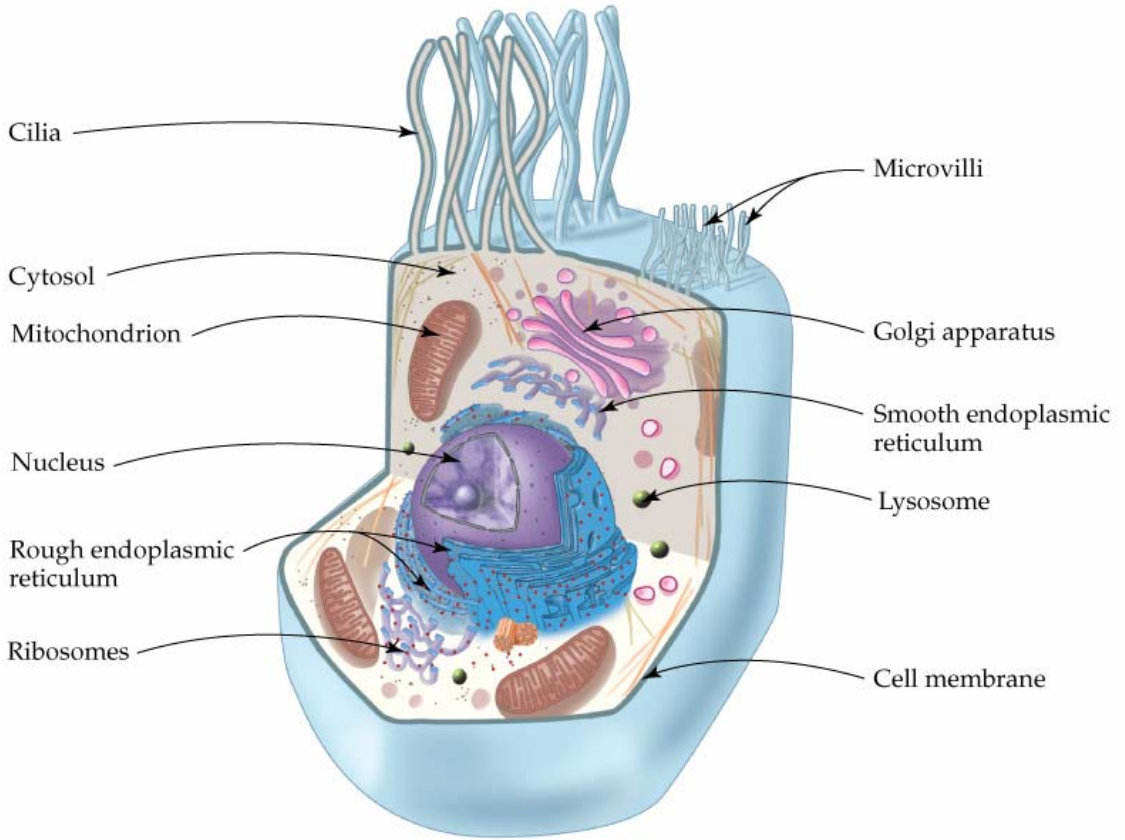
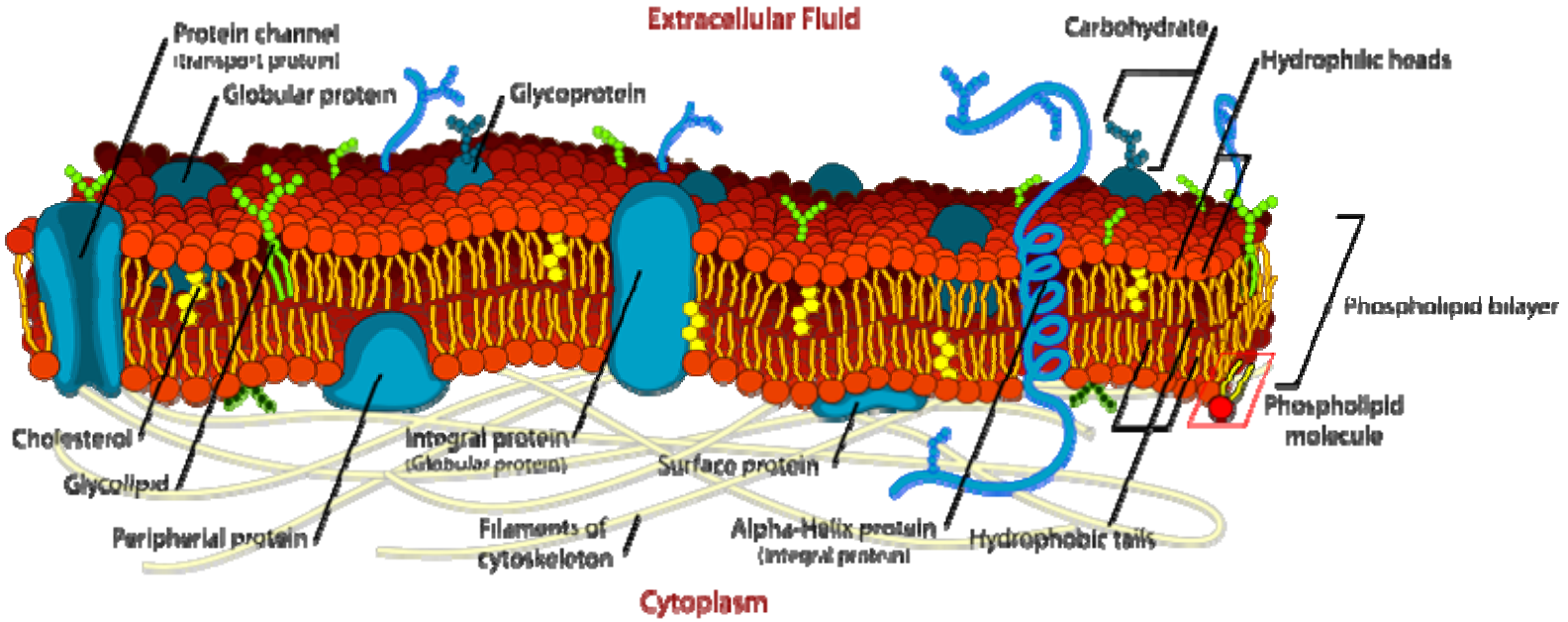


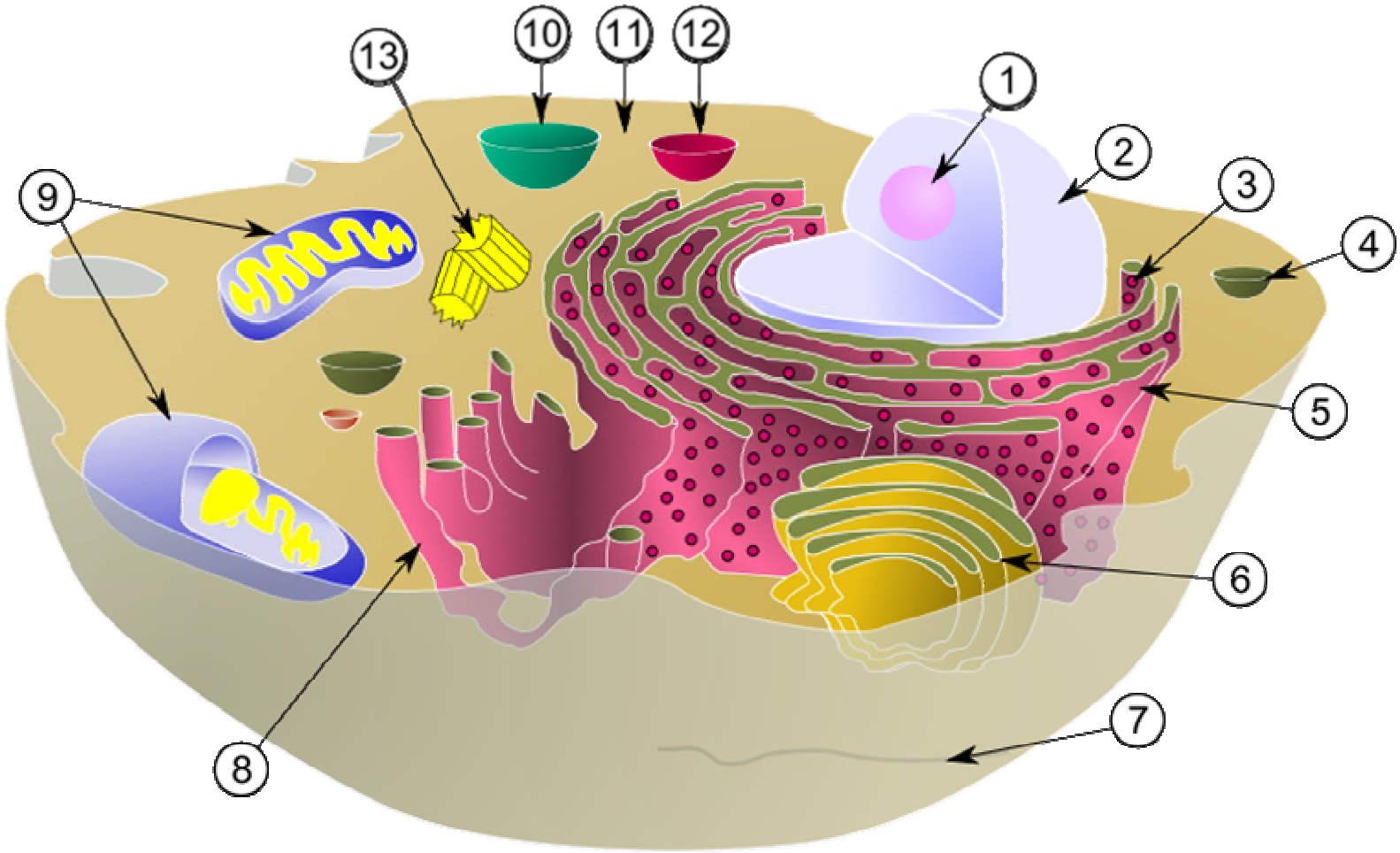
Fig 21.3 A generalized eukaryotic cell.

Cell Membrane

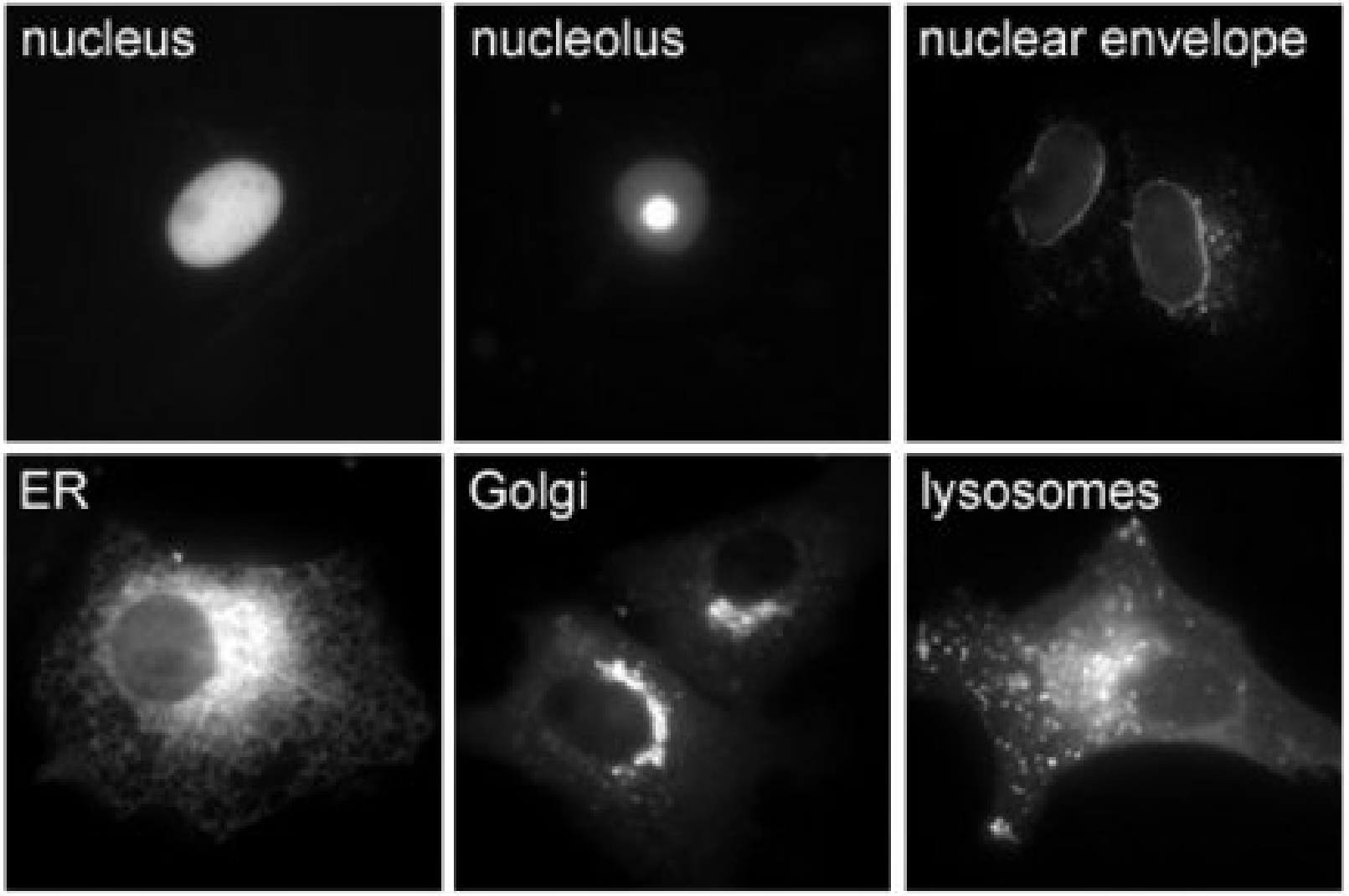


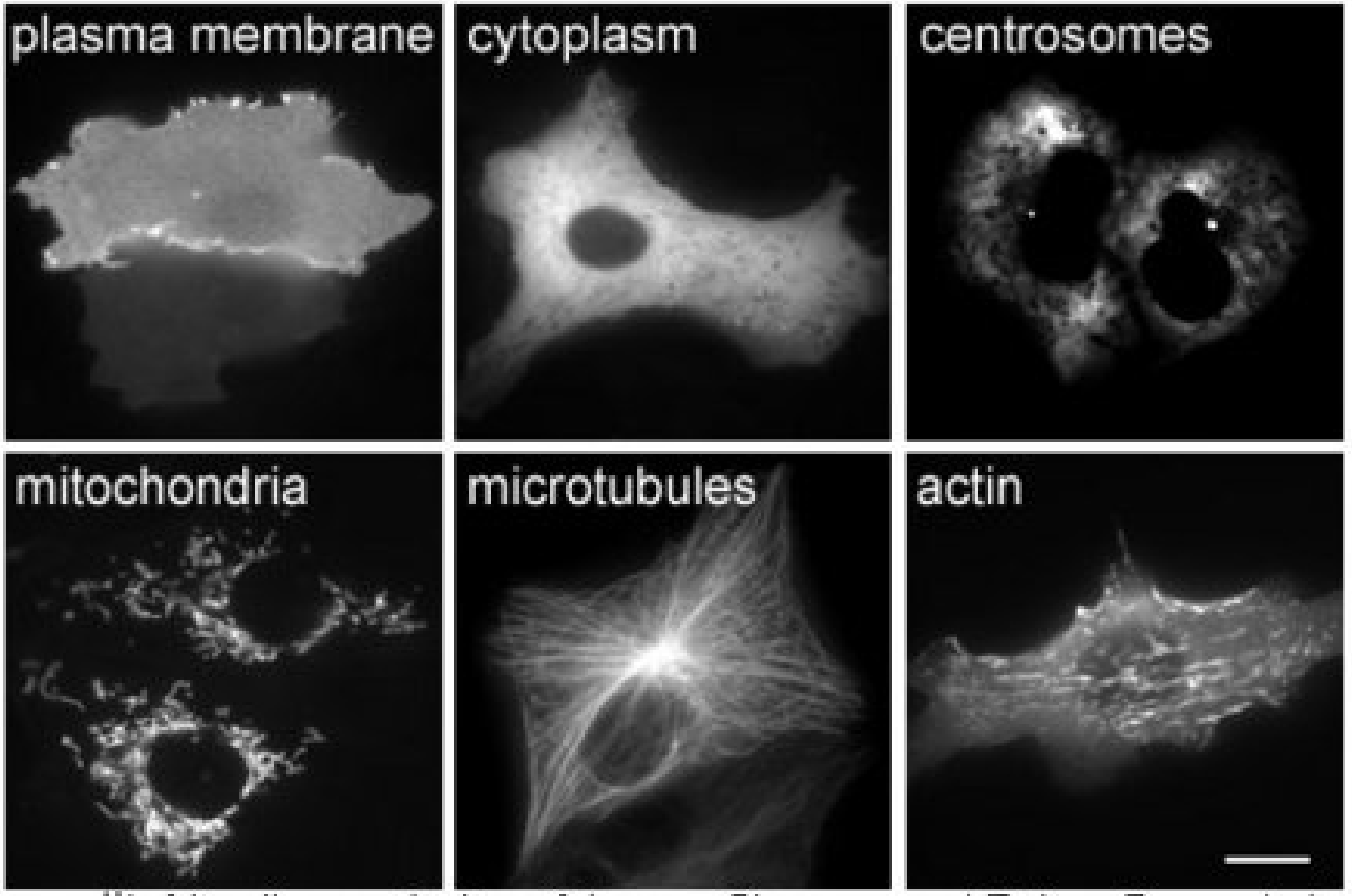
Characteristic diffusivities

Particle	Typical size	Diffusion constant
Solute ion	10^{-1} nm	$2\times10^{3}\ \mu m^2/s$
Small protein	5 nm	$40\ \mu m^2/s$
Virus	100 nm	$2\ \mu m^2/s$
Bacterium	1 μm	$0.2\ \mu m^2/s$
Mammalian/human cell	10 μm	$0.02\ \mu m^2/s$



•Schematic showing the cytoplasm, with its components (or *organelles*), of a typical animal cell. Organelles: (1) nucleolus (2) nucleus (3) ribosome (4) vesicle (5) rough endoplasmic reticulum (6) Golgi apparatus (7) cytoskeleton (8) smooth endoplasmic reticulum (9) mitochondria (10) vacuole (11) cytosol (12) lysosome (13) centriole.


nucleus
nucleolus
nuclear envelope
ER
Golgi
lysosomes



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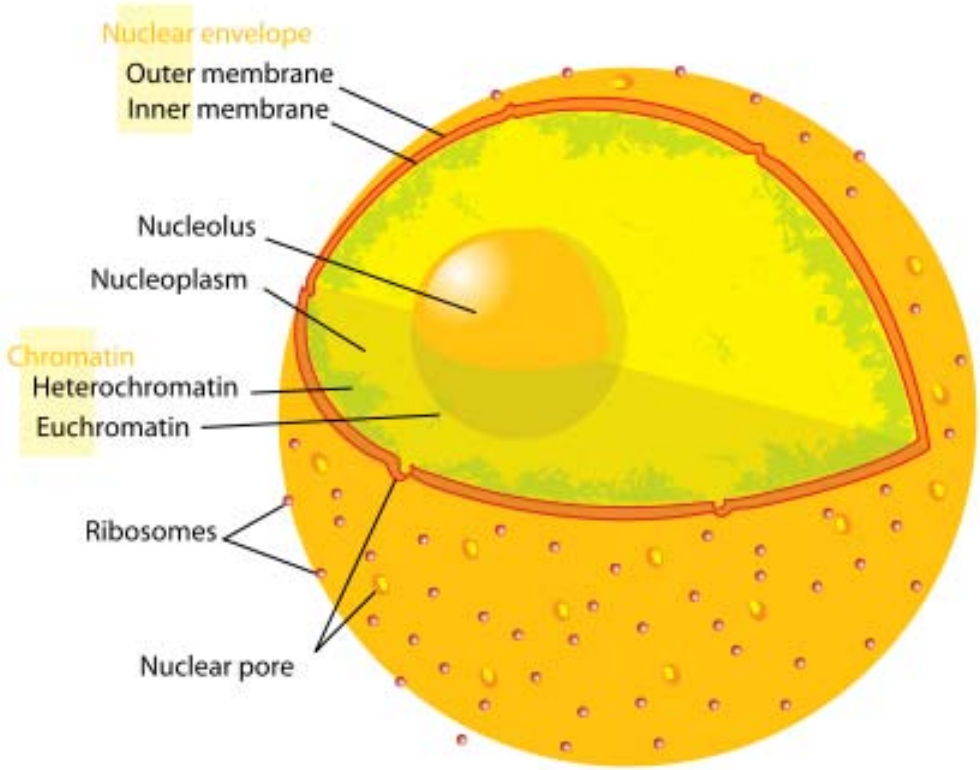
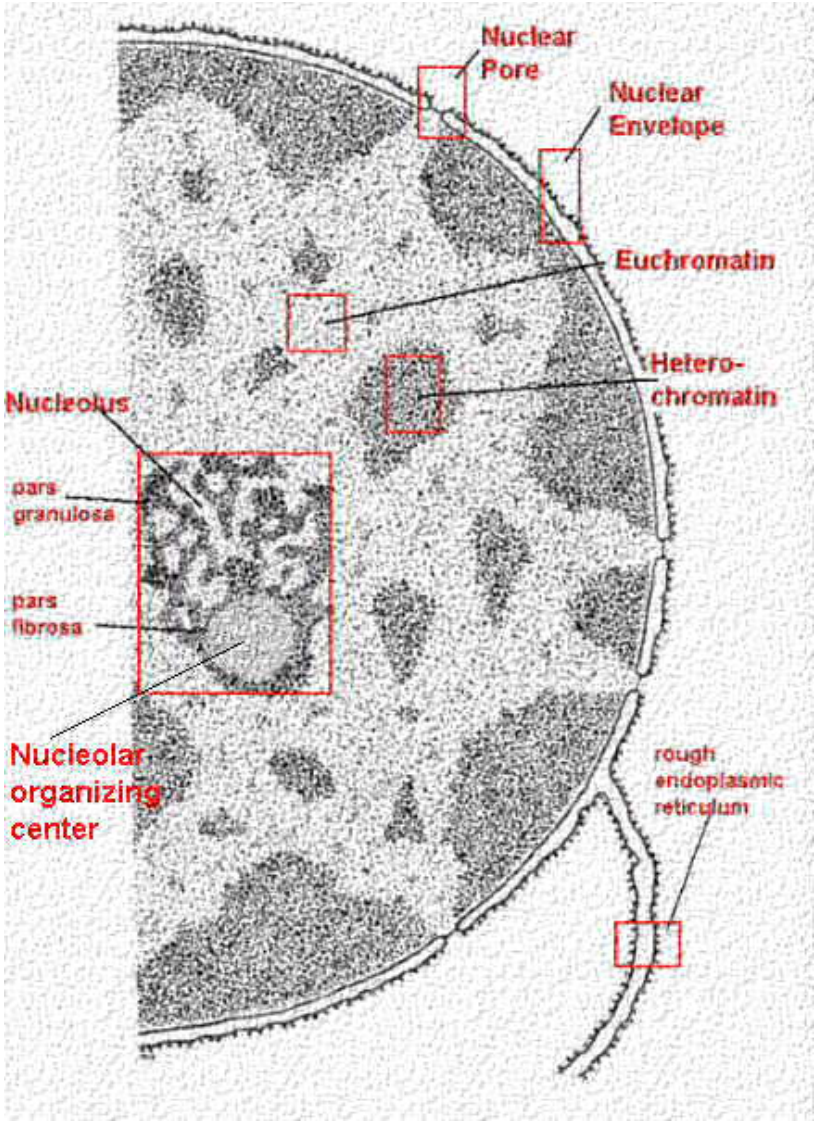
Nucleus



•In cell biology, the **nucleus** is a membrane-enclosed organelle found in most eukaryotic cells. It contains most of the cell's genetic material, organized as multiple long linear DNA molecules in complex with a large variety of proteins such as histones to form chromosomes. The genes within these chromosomes make up the cell's nuclear genome. The function of the nucleus is to maintain the integrity of these genes and to control the activities of the cell by regulating gene expression.

In cell biology, the **nucleolus** (plural *nucleoli*) is a "sub-organelle" of the cell nucleus, which itself is an organelle. A main function of the nucleolus is the production and assembly of ribosome components



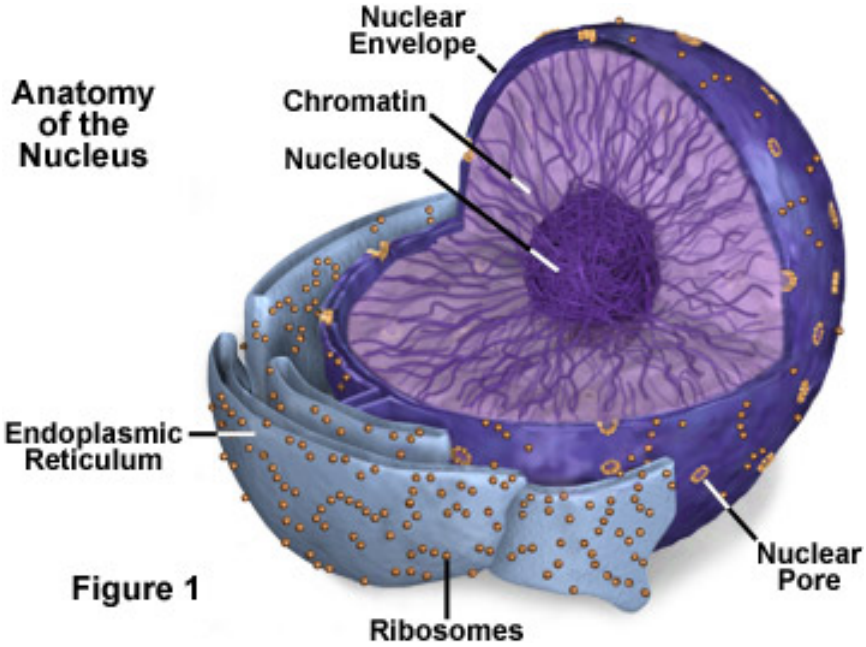


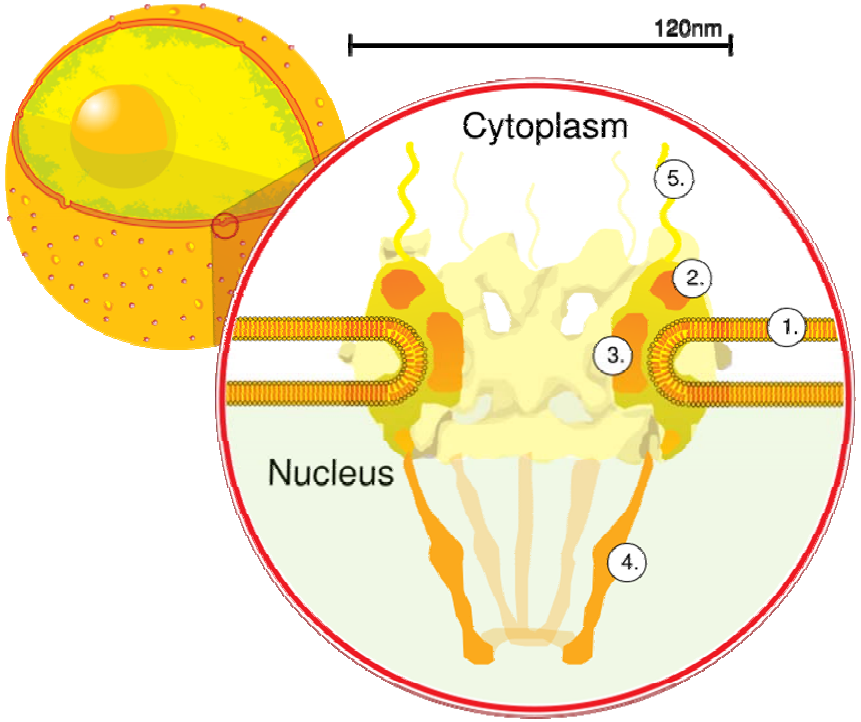
Figure 1

The main roles of the nucleolus are to synthesize rRNA and assemble ribosomes

The main function of the cell nucleus is to control gene expression and mediate the replication of DNA during the cell cycle

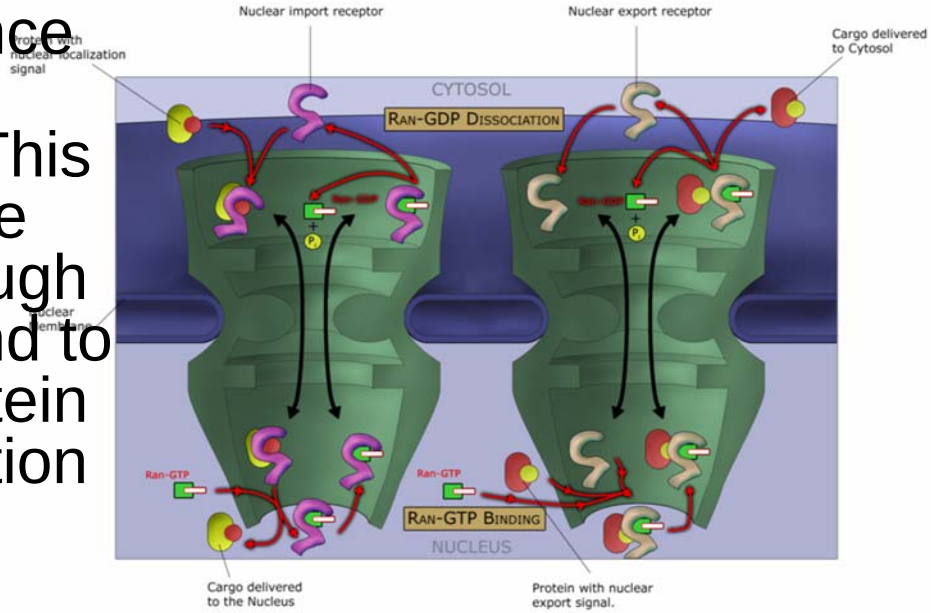
Nuclear pores

Nuclear pores, which provide aqueous channels through the envelope, are composed of multiple proteins, collectively referred to as nucleoporins. The pores are 100 nm in total diameter; however, the gap through which molecules freely diffuse is only about 9 nm wide, due to the presence of regulatory systems within the center of the pore. This size allows the free passage of small water-soluble molecules while preventing larger molecules, such as nucleic acids and proteins, from inappropriately entering or exiting the nucleus. These large molecules must be actively transported into the nucleus instead. The nucleus of a typical mammalian cell will have about 3000 to 4000 pores throughout its envelope



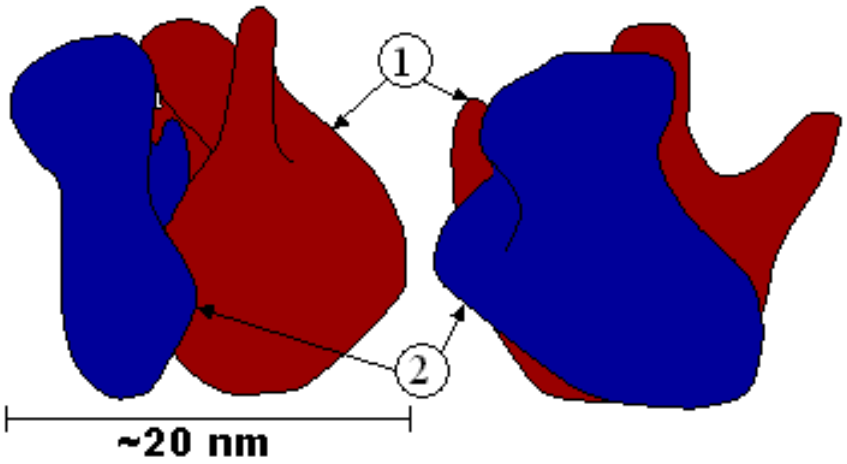
Nuclear localizing sequence (NLS)

- A **nuclear localizing sequence** (**NLS**) is an amino acid sequence which acts like a 'tag' on the exposed surface of a protein. This sequence is used to confine the protein to the cell nucleus through the **Nuclear Pore Complex** and to direct a newly synthesized protein into the nucleus via its recognition by cytosolic nuclear transport receptors. Typically, this signal consists of a few short sequences of positively charged lysines or arginines. Typically the NLS will have a sequence (NH2)-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-(COOH).



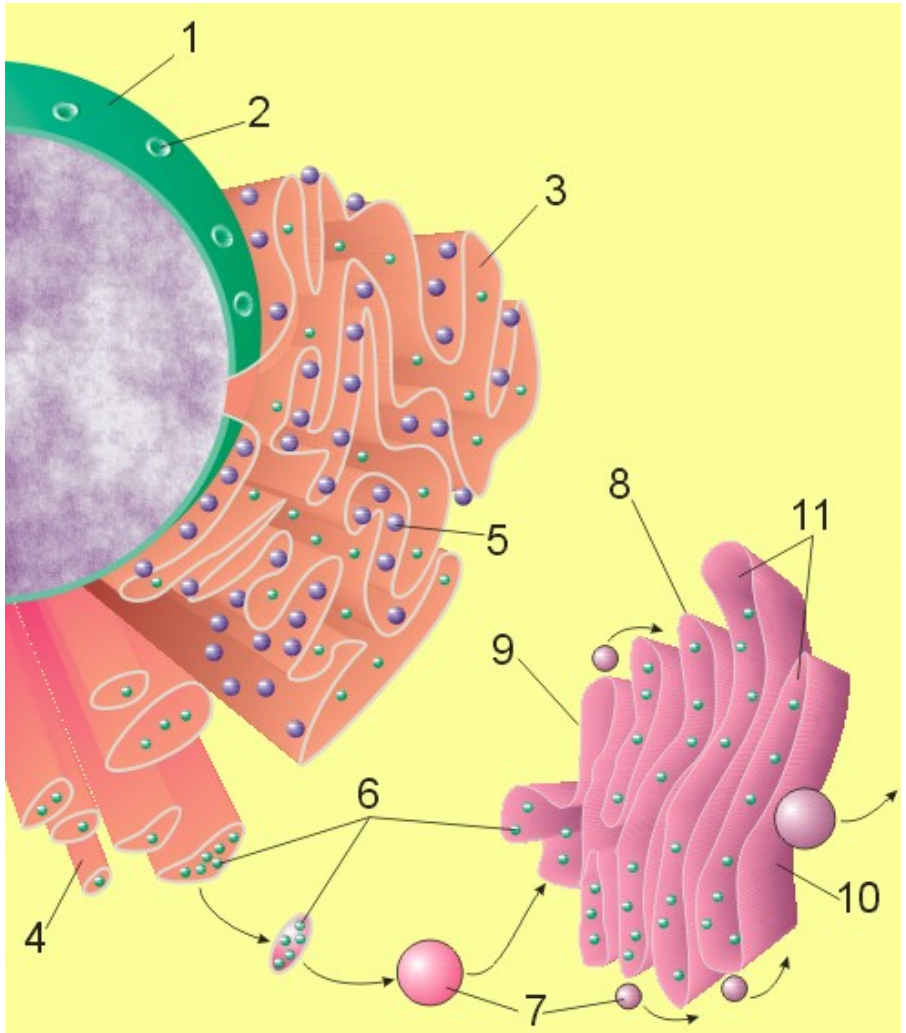
Ribosome

A **ribosome** is a small, dense organelle in cells that assembles proteins. Ribosomes are about 20nm in diameter and are composed of 65% ribosomal RNA and 35% ribosomal proteins (known as a Ribonucleoprotein or RNP). It translates messenger RNA (mRNA) to build a polypeptide chain (e.g., a protein) using amino acids delivered by Transfer RNA (tRNA). It can be thought of as a giant enzyme that builds a protein from a set of genetic instructions. Ribosomes can float freely in the cytoplasm (the internal fluid of the cell) or bound to the endoplasmic reticulum, or to the nuclear envelope.



Endoplasmic Reticulum

The **endoplasmic reticulum** or **ER** is an organelle found in all eukaryotic cells that is an interconnected network of tubules, vesicles and cisternae that is responsible for several specialized functions: Protein translation, folding, and transport of proteins to be used in the cell membrane (e.g., transmembrane receptors and other integral membrane proteins), or to be secreted (exocytosed) from the cell (e.g., digestive enzymes); sequestration of calcium; and production and storage of glycogen, steroids, and other macromolecules.[1] The endoplasmic reticulum is part of the endomembrane system. The basic structure and composition of the ER membrane is similar to the plasma membrane.



Rough endoplasmic reticulum

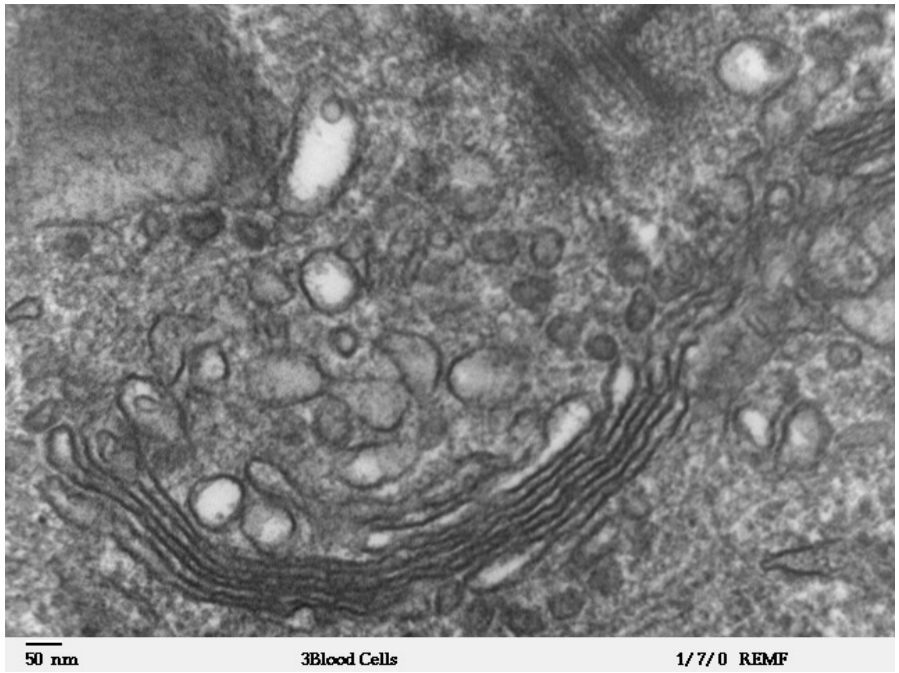
- The surface of the rough endoplasmic reticulum is studded with protein-manufacturing ribosomes giving it a "rough" appearance. But it should be noted that these ribosomes are not resident of the endoplasmic reticulum incessantly. The ribosomes only bind to the ER once it begins to synthesize a protein destined for sorting. The membrane of the rough endoplasmic reticulum is continuous with the outer layer of the nuclear envelope. Although there is no continuous membrane between the rough ER and the Golgi apparatus, membrane bound vesicles shuttle proteins between these two compartments. The rough endoplasmic reticulum works in concert with the Golgi complex to target new proteins to their proper destinations

Smooth endoplasmic reticulum

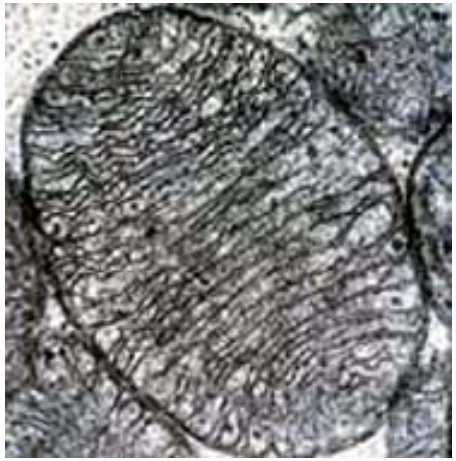
- The smooth endoplasmic reticulum has functions in several metabolic processes, including synthesis of lipids, metabolism of carbohydrates and calcium concentration, and attachment of receptors on cell membrane proteins. It is connected to the nuclear envelope. Smooth endoplasmic reticulum is found in a variety of cell types (both animal and plant) and it serves different functions in each. It consists of tubules and vesicles that branch forming a network. In some cells there are dilated areas like the sacs of rough endoplasmic reticulum. The network of smooth endoplasmic reticulum allows increased surface area for the action or storage of key enzymes and the products of these enzymes. The smooth endoplasmic reticulum is known for its storage of calcium ions in muscle cells.

Golgi apparatus

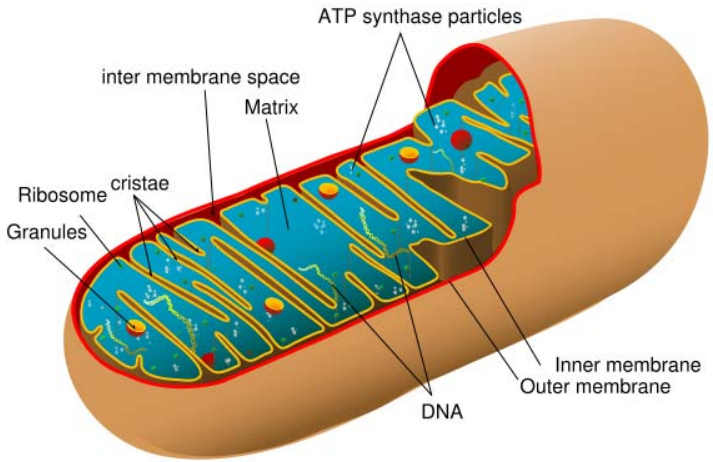
The **Golgi apparatus** (also called the **Golgi body**, **Golgi complex**, or **dictyosome**) is an organelle found in typical eukaryotic cells. It was identified in 1898 by the Italian physician Camillo Golgi and was named after him. The primary function of the Golgi apparatus is to process and package macromolecules synthesised by the cell, primarily proteins and lipids. The Golgi apparatus forms a part of the endomembrane system present in eukaryotic cells.



Mitochondrion



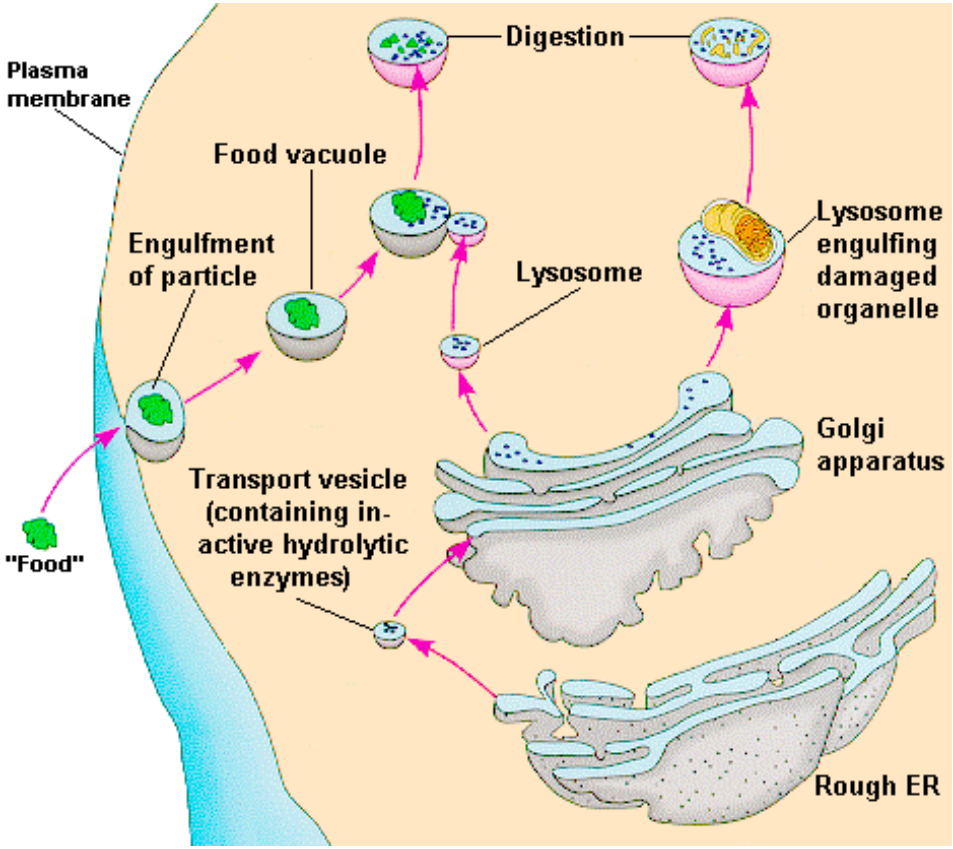
- In cell biology, a **mitochondrion** is a membrane-enclosed organelle, found in most eukaryotic cells.Mitochondria are sometimes described as "cellular power plants," because they convert NADH and NADPH into energy in the form of ATP via the process of oxidative phosphorylation. A typical eukaryotic cell contains about 2,000 mitochondria, which occupy roughly one fifth of its total volume. Mitochondria contain DNA that is independent of the DNA located in the cell nucleus. According to the endosymbiotic theory, mitochondria are descended from free-living prokaryotes.



Lysosomes

- **Lysosomes** are organelles that contain digestive enzymes (acid hydrolases). They digest excess or worn out organelles, food particles, and engulfed viruses or bacteria. The membrane surrounding a lysosome prevents the digestive enzymes inside from destroying the cell. Lysosomes fuse with vacuoles and dispense their enzymes into the vacuoles, digesting their contents. They are built in the Golgi apparatus. The name *lysosome* derives from the Greek words *lysis*, which means dissolution or destruction, and *soma*, which means body. They are frequently nicknamed "suicide-bags" or "suicide-sacs" by cell biologists due to their role in autolysis.

Lysosome



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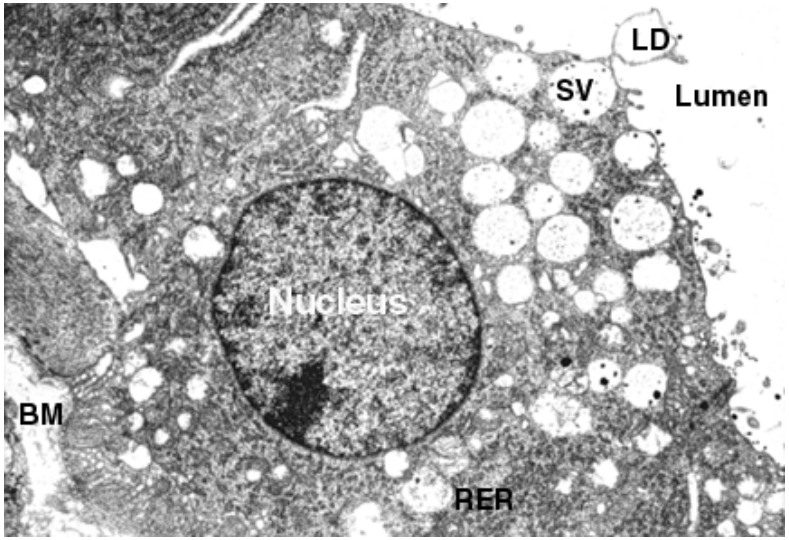
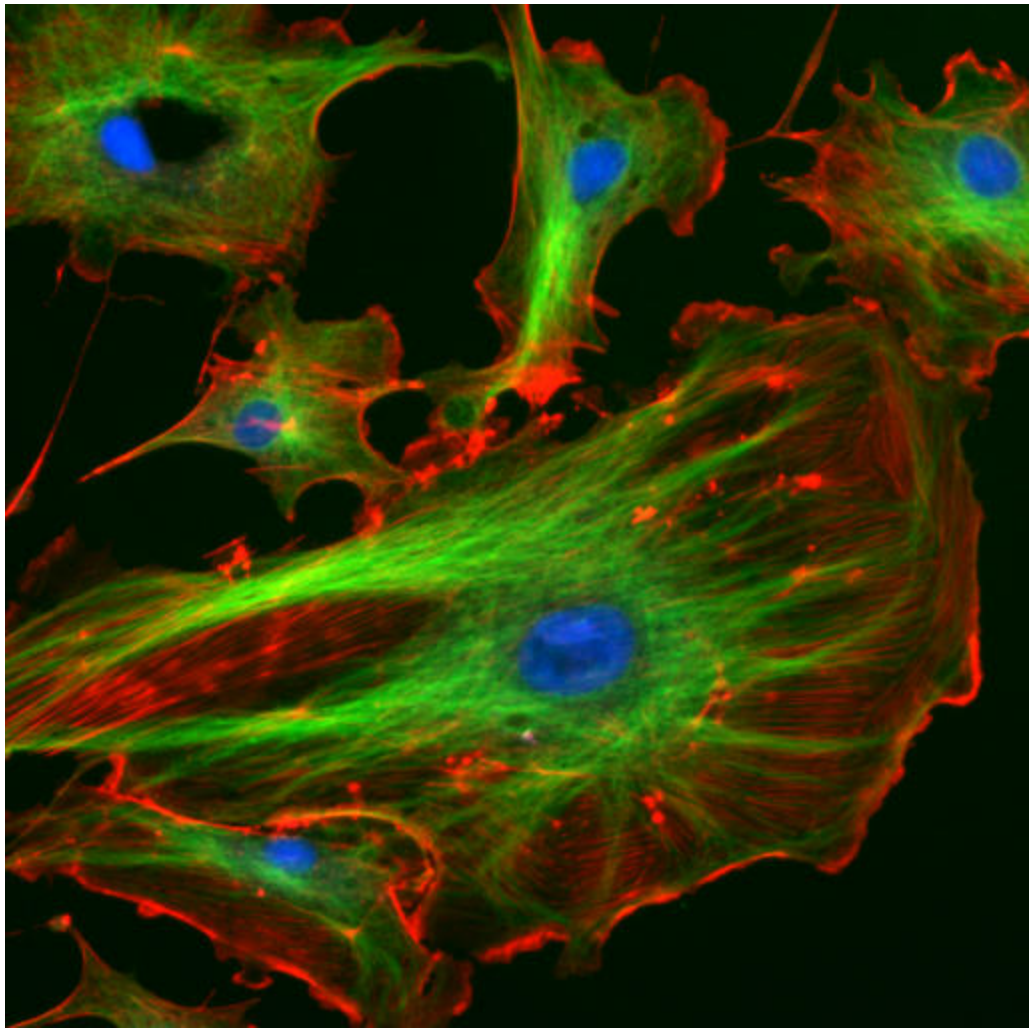
Vesicle

In cell biology, a **vesicle** is a relatively small and enclosed compartment, separated from the cytosol by at least one lipid bilayer. If there is only one lipid bilayer, they are called *unilamellar* vesicles; otherwise they are called *multilamellar*. Vesicles store, transport, or digest cellular products and waste.

This biomembrane enclosing the vesicle is similar to that of the plasma membrane. Because it is separated from the cytosol, the intravesicular environment can be made to be different from the cytosolic environment. Vesicles are a basic tool of the cell for organizing metabolism, transport, enzyme storage, as well as being chemical reaction chambers. Many vesicles are made in the Golgi apparatus, but also in the endoplasmic reticulum, or are made from parts of the plasma membrane.

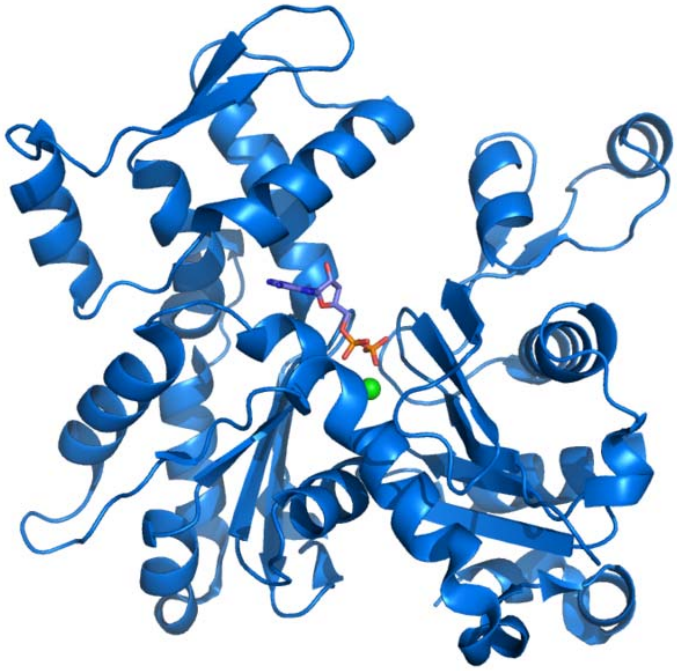


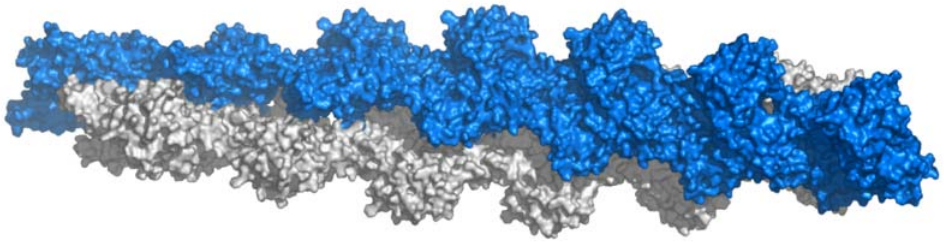
Cytoskeleton



The eukaryotic cytoskeleton. Actin filaments are shown in red, microtubules in green, and the nuclei are in blue.

Actin

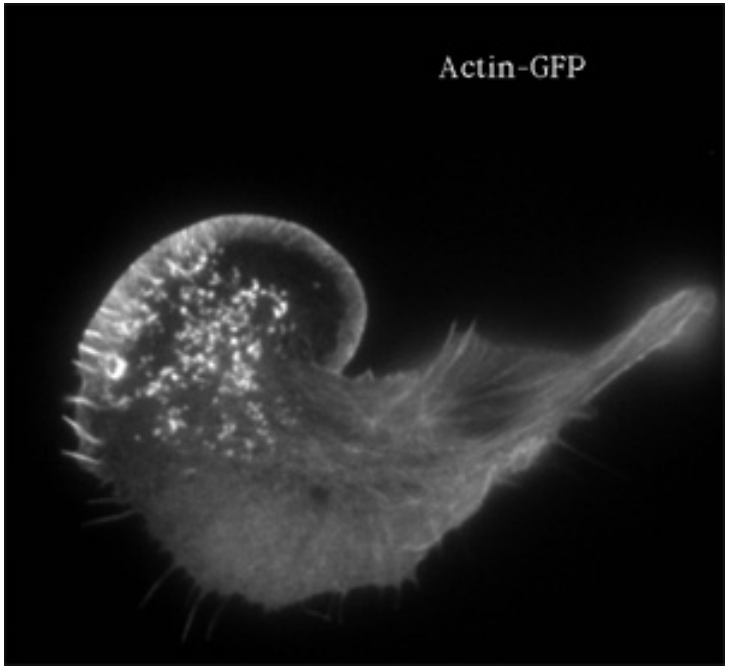


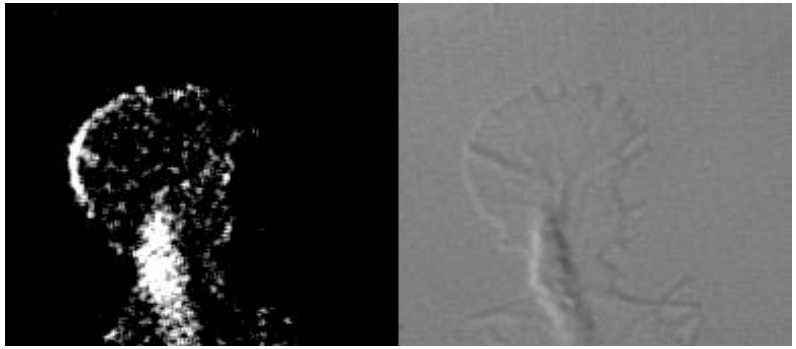


- **Actin** is a globular structural, 42 kDa, protein that polymerizes in a helical fashion to form **actin filaments** (or **microfilaments**). These form the cytoskeleton, a three-dimensional network inside the eukaryotic cell. Actin filaments provide mechanical support for the cell, determine its shape, and enable movement of the cell through lamellipodia, filopodia, or pseudopodia. Actin filaments, along with myosin, have an essential role in muscular contraction. In the cytosol, actin is predominantly bound to ATP, but can also bind to ADP. An ATP-actin complex polymerizes faster and dissociates slower than an ADP-actin complex.

Lamellipodia

- The **lamellipodium** is a cytoskeletal actin projection on the mobile edge of the cell. It contains a two-dimensional actin mesh; the whole structure pulls the cell across a substrate. Within the lamellipodia are ribs of actin called microspikes, which, when they spread beyond the lamellipodium frontier, are called filopodia (Small, et all, 2002). The lamellipodium is born of actin nucleation in the plasma membrane of the cell (Alberts, et al, 2002) and is the primary area of actin incorporation or microfilament formation of the cell. Lamellipodia range from 1 μm to 5 μm in breadth and are approximately 0.2 μm thick.Lamellipodia are found primarily in very mobile cells, crawling at a speeds of 10-20 μm/minute over epithelial surfaces..
- The tip of the lamellipodium is the site where exocytosis occurs in migrating mammalian cells as part of their clathrin-mediated endocytic cycle.

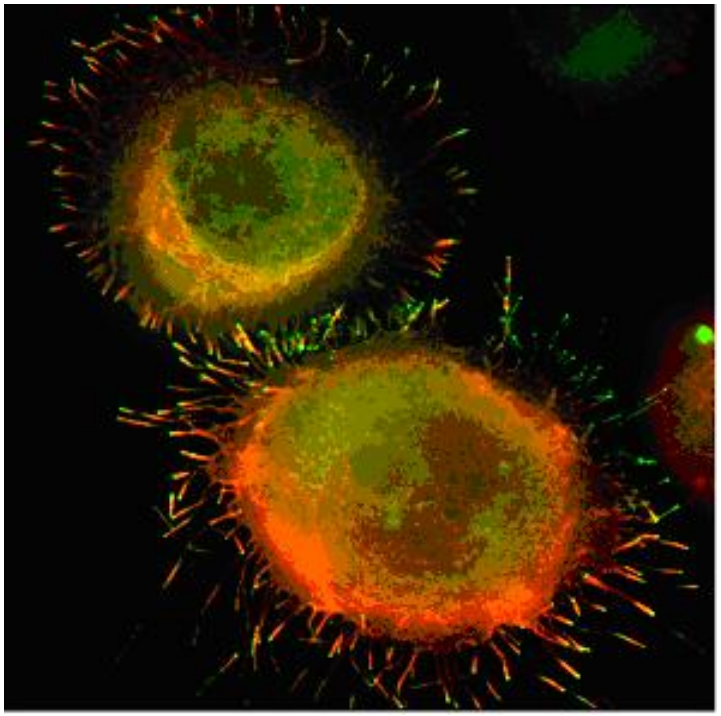




http://www.microscopyu.com/moviegallery/livecellimaging/3t3/t1/3t3-dslwmp1.html

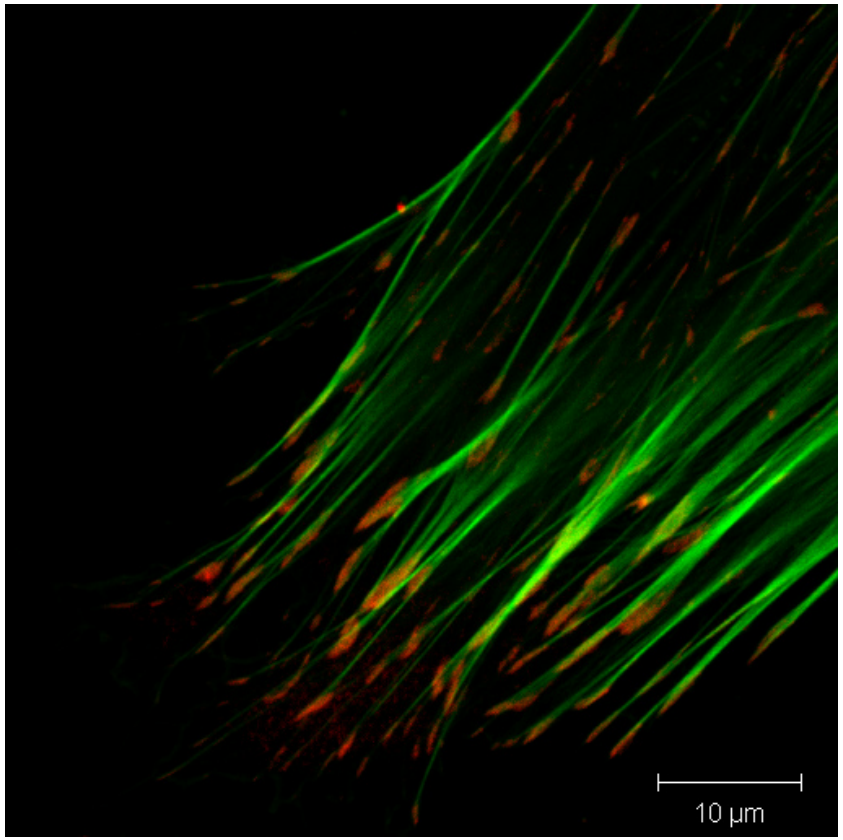
Filopodia

The **filopodia** are slender cytoplasmic projections, similar to lamellipodia, which extend from the leading edge of migrating cells. They contain actin filaments cross-linked into bundles by actin-binding proteins, e.g. fimbrin. Filopodia form focal adhesions with the substratum, linking it to the cell surface. A cell migrates along a surface by extending filopodia at the leading edge. The filopodia attach to the substratum further down the migratory pathway, then contraction of stress fibres retracts the rear of the cell to move the cell forwards.



Focal adhesion

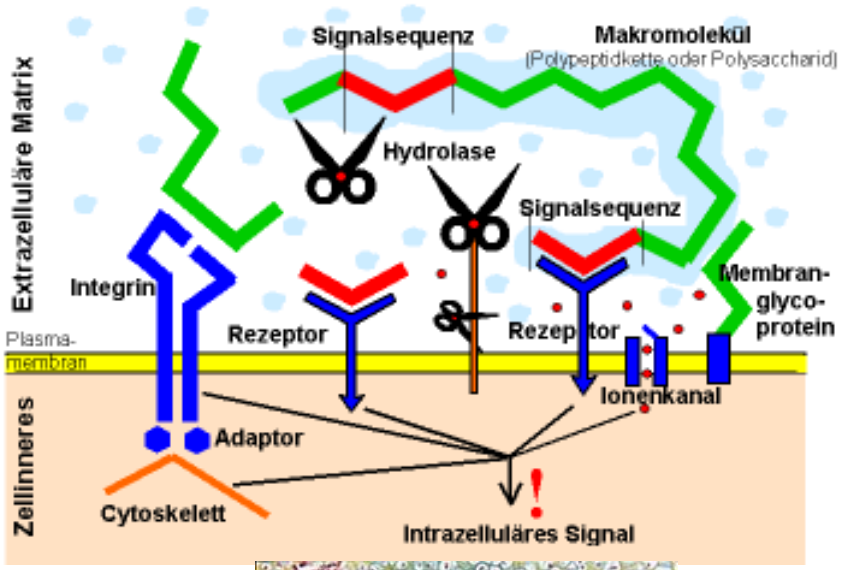
- In cell biology, '**Focal Adhesions'** are specific types of large macromolecular assemblies through which both mechanical force and regulatory signals are transmitted. More precisely, **FAs** can be considered as sub-cellular macromolecules that mediate the regulatory effects (e.g. cell anchorage) of extracellular matrix (ECM) adhesion on cell behavior.

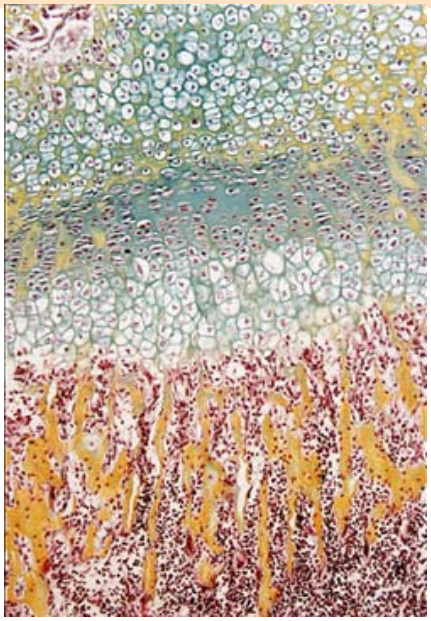


Extra Cellular Matrix

The ECM's main components are various glycoproteins, proteoglycans and hyaluronic acid. In most animals, the most abundant glycoproteins in the ECM are collagens.

ECM also contains many other components: proteins such as fibrin, elastin, fibronectins, laminins, and nidogens, and minerals such as hydroxylapatite, or fluids such as blood plasma or serum with secreted free flowing antigens.



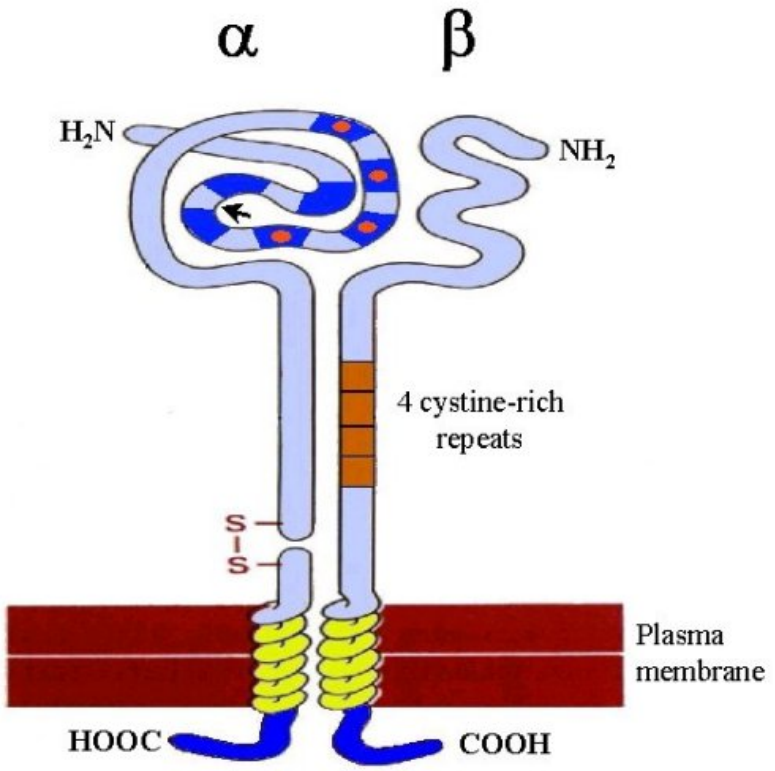


Integrin

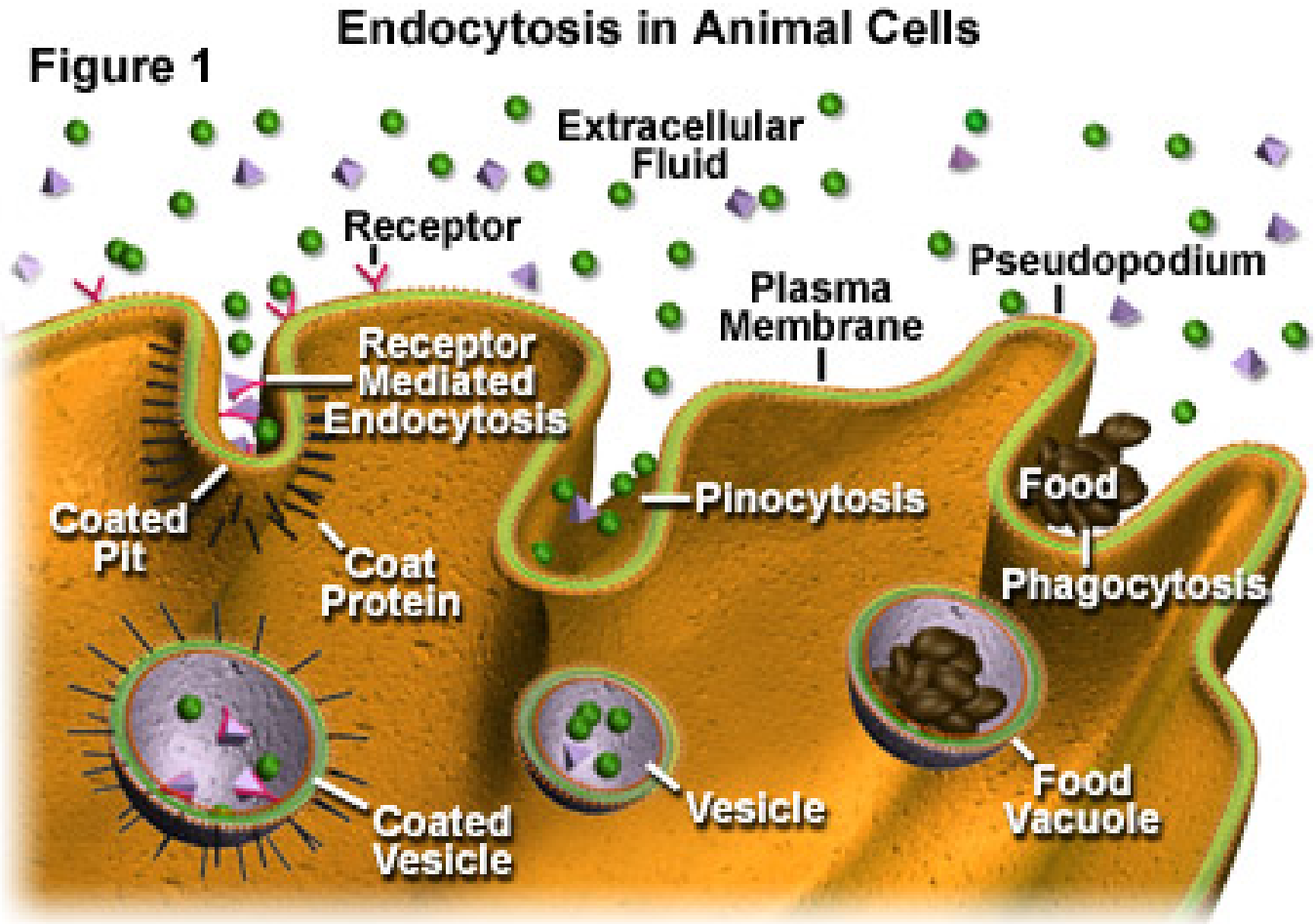
An **integrin**, or **integrin receptor**, is an integral membrane protein in the plasma membrane of cells. It plays a role in the attachment of a cell to the extracellular matrix (ECM) and to other cells and in signal transduction from the ECM to the cell. There are many types of integrin, and many cells have multiple types on their surface. Integrins are of vital importance to all metazoans, from humans to sponges.

Schematic drawing of a typical integrin dimer

Arrow shows the region where an I domain is inserted in some α subunits. Not all α subunits are posttranslationally cleaved. Internal disulphide bonds within subunits are not shown. Dark blue regions in the head segment of the α subunit represent homologous repeats. Those with the EF-hand consensus sequence are marked with red circles to denote binding sites for divalent metal ion.



Endocytosis



Endocytosis

- Phagocytosis is the process by which cells ingest large objects, such as cells which have undergone apoptosis, bacteria, or viruses. The membrane folds around the object, and the object is sealed off into a large vacuole known as a phagosome.
- Pinocytosis is a synonym for endocytosis. This process is concerned with the uptake of solutes and single molecules such as proteins.
- Receptor-mediated endocytosis is a more specific active event where the cytoplasm membrane folds inward to form coated pits. These inward budding vesicles bud to form cytoplasmic vesicles.

http://highered.mcgraw-hill.com/olc/dl/120068/bio02.swf

Endocytosis pathways

- Macropinocytosis is the invagination of the cell membrane to form a pocket which then pinches off into the cell to form a vesicle filled with extracellular fluid (and molecules within it). The filling of the pocket occurs in a non-specific manner. The vesicle then travels into the cytosol and fuses with other vesicles such as endosomes and lysosomes.
- Clathrin-mediated endocytosis is the specific uptake of large extracellular molecules such as proteins, membrane localized receptors and ion-channels. These receptors are associated with the cytosolic protein clathrin which initiates the formation of a vesicle by forming a crystalline coat on the inner surface of the cell's membrane.
- Caveolae consist of the protein caveolin-1 with a bilayer enriched in cholesterol and glycosphingolipids. Caveolae are flask shaped pits in the membrane that resemble the shape of a cave (hence the name caveolae). Uptake of extracellular molecules are also believed to be specifically mediated via receptors in caveolae.