Advanced Cell Biology: BIOL 250
Study guide

Alexey Shipunov

Lectures

Contents

Outline

1 Course in general

1.1 Description

Course description

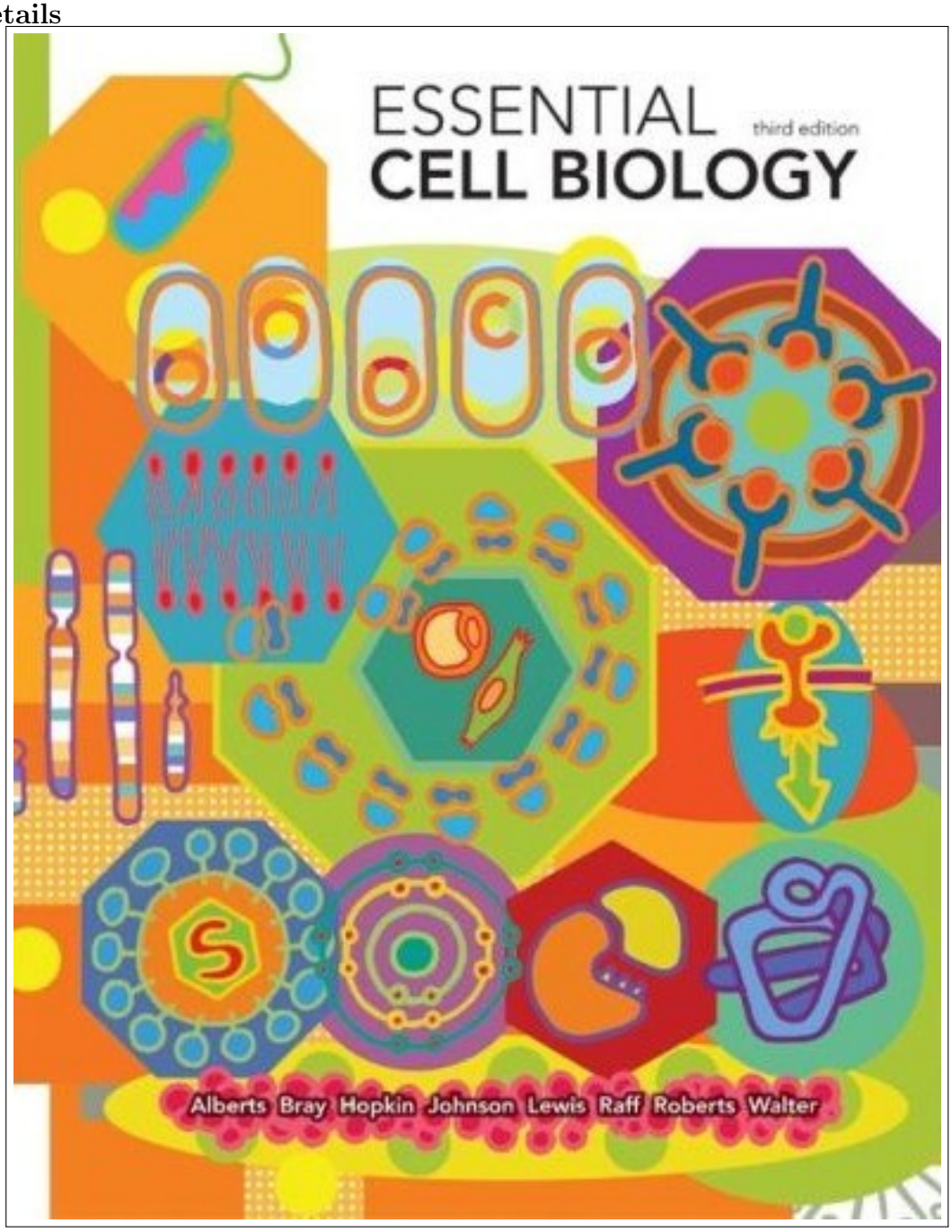
Advanced Cell Biology will penetrate the field of cellular and molecular biological sciences deeper than Introductory Cell Biology. The course is based on the presumption that students already know basics of cell biology and biochemistry. In turn, several higher-level courses are based on Advanced Cell. Therefore, I will concentrate on topics which are most important for understanding cell structure and functions:

- chemical components of cell including DNA and protein structure and interactions;
- genes and genomes: analysis and evolution;
- membrane structure, transport and cell communication.

Instructor

- Dr. Alexey Shipunov
- Office: Moore 229
- Office Hours: Wednesdays and Fridays, 9 a.m. to 11:50 a.m.
- Phone: 858-3116
- E-mail: `alexey.shipunov@minotstateu.edu`

Details



Lectures Mondays, Wednesdays and Fridays, 12:00 a.m. to 12:50 a.m., Moore 210

Laboratories Mondays, Moore 210

Textbook Essential Cell Biology (Alberts et al., 3nd ed., Garland Science publ.)

1.2 Grading

Exams

- Four **equal** exams are given during the semester.
- Only the **three** best exams contribute to the final grade.
- Missed exams count zero points. There are **no make-up** exams.

Labs

- Receiving zero points for **more than one** laboratory results in a failed course.
- Grading of laboratories is based on reports.
- Written reports are prepared and finished during laboratory sessions and passed to the instructor right after the particular laboratory session.
- It is expected that you have reviewed the lecture contents before you come to lab.

Absence

There are five legitimate reasons for absence:

1. emergency situations,
2. attested medical conditions,
3. military duty,
4. participation in MSU sports events,
5. dependent sick leave.

Absence from exams or laboratories needs to be announced to the instructor in advance **via e-mail**. I strongly recommend attending lectures regularly. Statistically, students who achieved best grades are **always attend lectures**.

Lecture tests

- At the end of **every** lecture I will give **one** short test question to answer.
- The question will require 1–3 min to answer and respectively, will give from 1 to 3 points (depending on the complexity).

Points

A total of ≈600 points can be earned and are distributed as follows:

- Three best exams: ≈300 points
- Lecture tests: ≈60 points total
- Laboratory: 240 points (20 points per lab)

Grading points may vary between exams, tests, and labs.

Letter grades

- $A \geq 90\%$
- $B \geq 80\%$
- $C \geq 70\%$
- $D \geq 60\%$
- $F < 60\%$

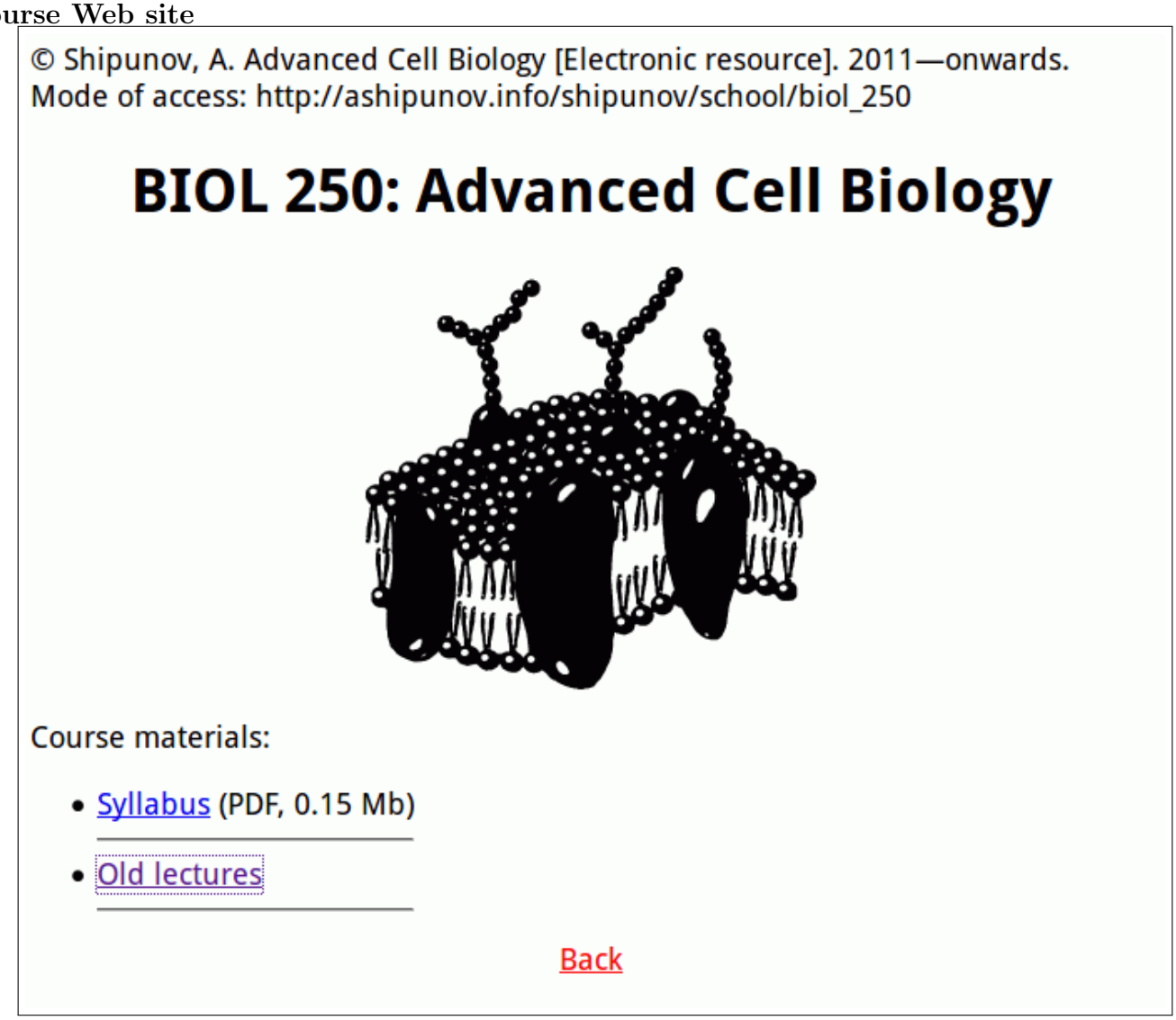
A minimum of one letter grade will be deducted from the grade for academic dishonesty / plagiarism.

1.3 Course schedule

Tentative course sequence

- Introduction to cells, microscopy
- Chemical components of cells
- Proteins and DNA
- Genes and genomes
- Membrane
- Cellular transport and communication
- Cytoskeleton and cell division

Course Web site



`http://ashipunov.info/shipunov/school/biol_250/`

2 What are cells

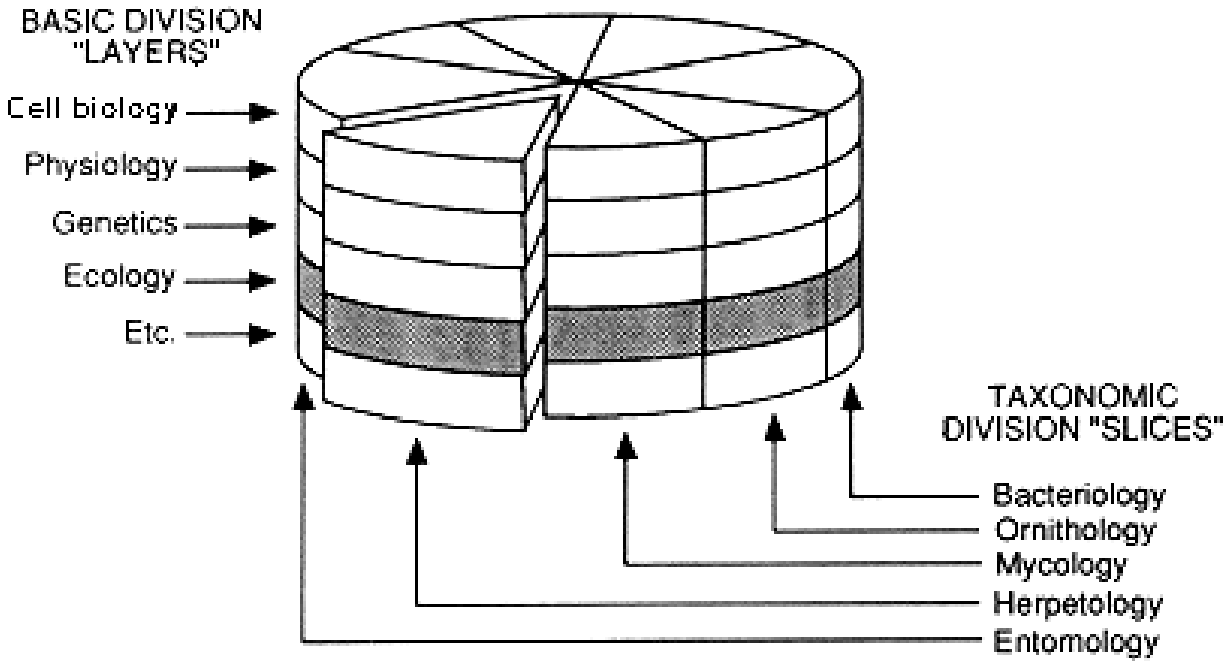
2.1 Biological hierarchy

Levels of organization

- Molecules
- Organelles
- Cells
- Tissues
- Organs
- Organisms
- Populations
- Ecosystems OR Taxonomic groups

Cells are "atoms of life", the least level where organisms are still alive.

Place of cell biology



Layered cake of biology (Odum, 1971): cell biology is a "layer science"

2.2 Cell theory

Cell theory

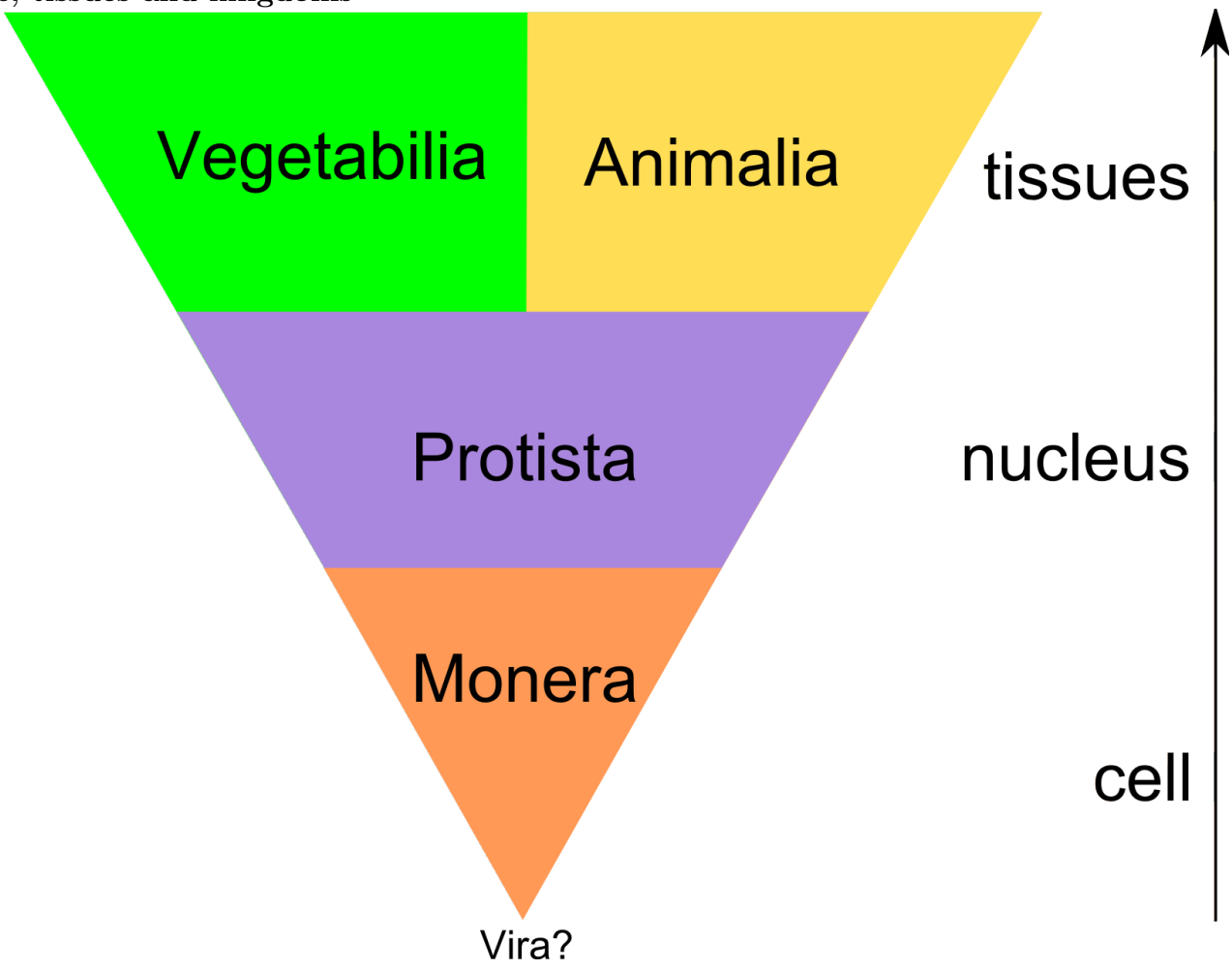
Cells were first discovered in 1665 by Robert Hook. Cell theory was formulated in XIX century:

- All plants and animals are composed of cells (1838, Matthias Schleiden and Theodor Schwann)

- Cells reproduce themselves (1858, Rudolf Virchow)
- All cells arise by reproduction from previous cells (1858, Rudolf Virchow)

3 Unity and diversity of cells

Cells, tissues and kingdoms



Diversity of living things

- Prokaryotes (Monera)
  - Bacteria and Archaea
- Eukaryotes
  - Protists (do not have tissues)
  - Animals
  - Plants (Vegetabilia)

Final question (2 points)

What is that?

- All organisms are composed of cells
- Cells reproduce themselves
- All cells arise by reproduction from previous cells

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

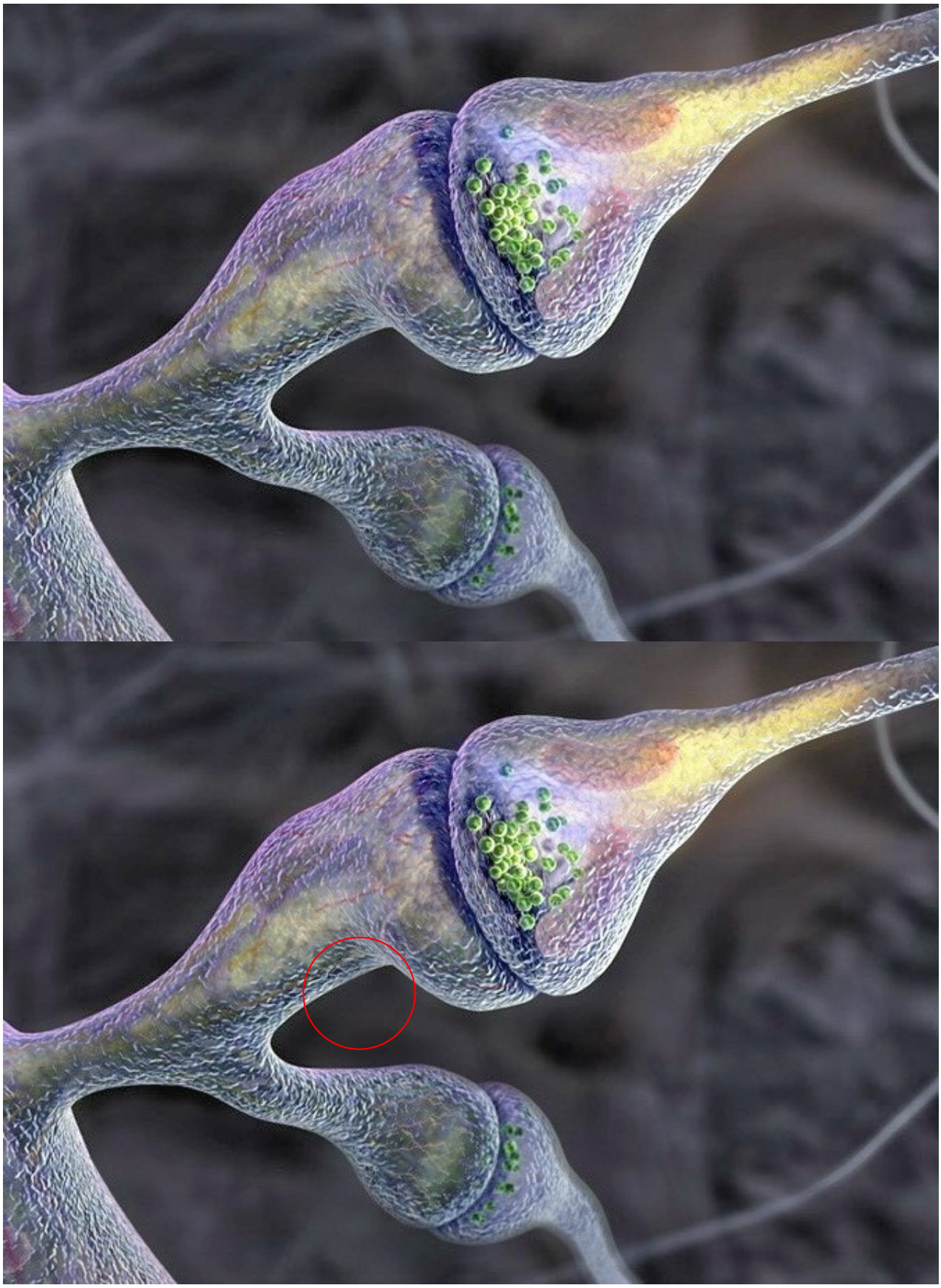
[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 1*: Unity and diversity of cells.

Outline

A Questions and answers

Previous final question: the answer

What is that?



- Synapse—the contact between two neural cells (neurons)

B Introduction to cells

B.1 Microscopy

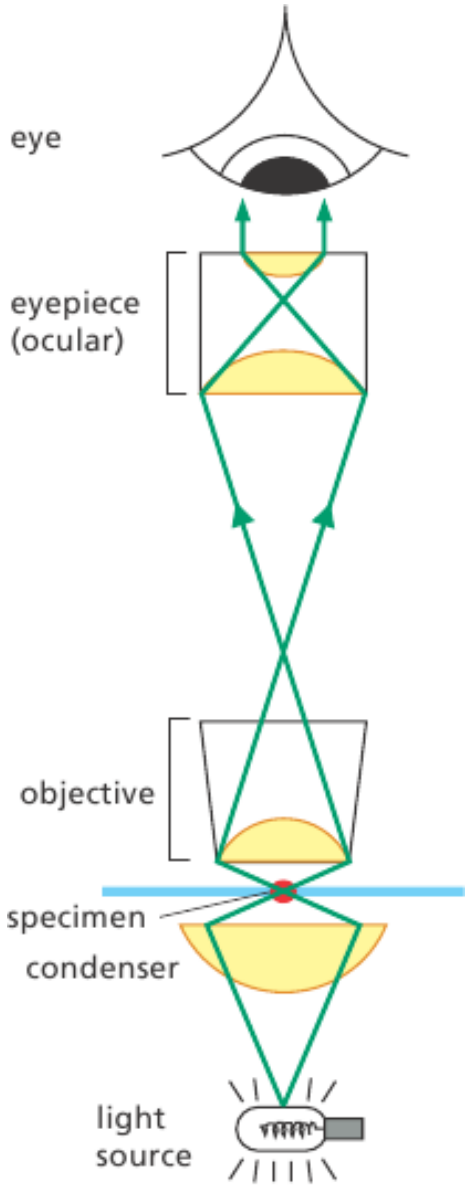
Miscroscopes

Light microscopy based on visible light rays and glass optics, most common are "transparency" microscopes where light goes through object (stained with specific **dyes** or not stained); there are also "reflection" (dissectiscopes) and fluorescent microscopes

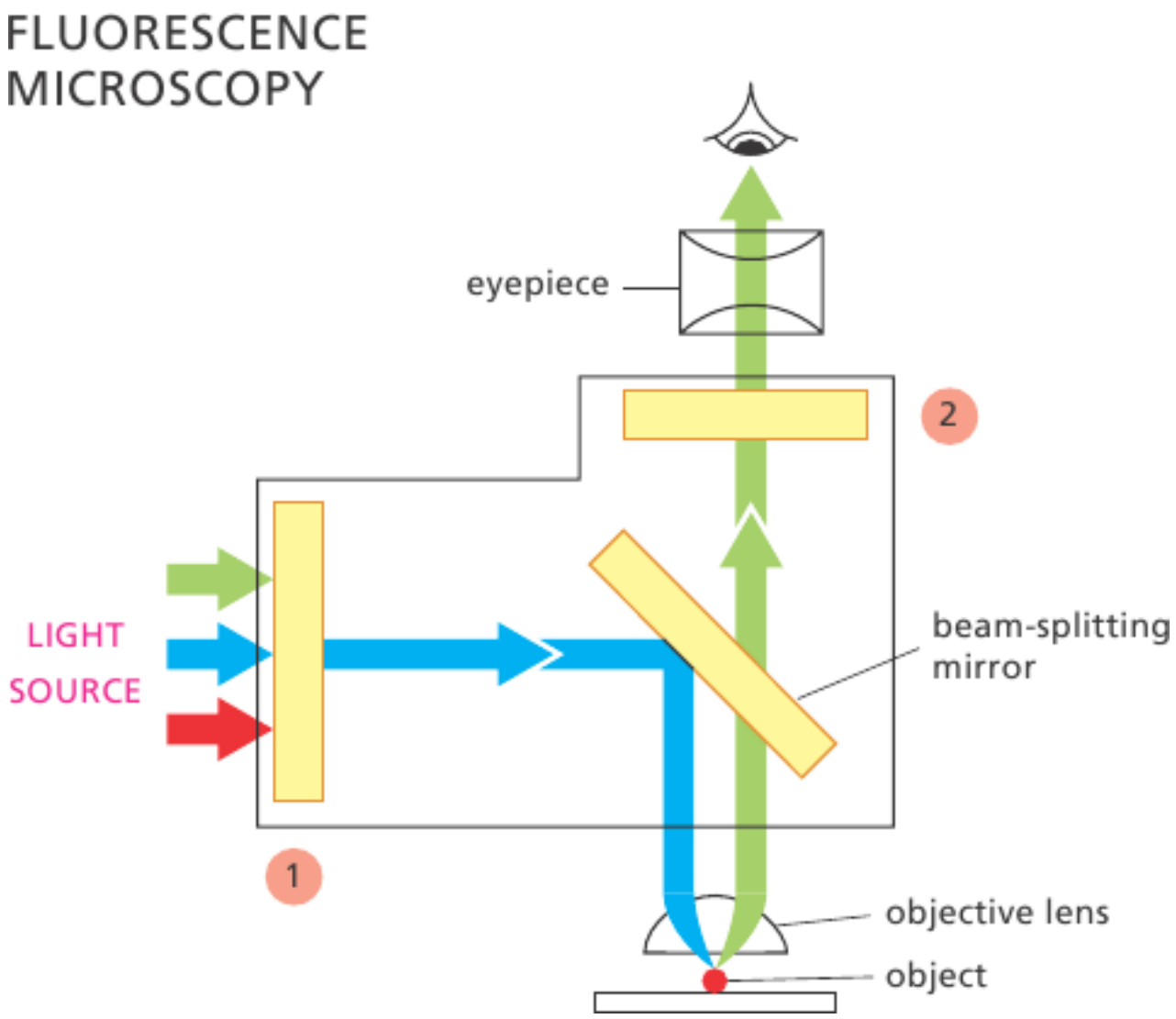
Transmission electron microscopy (TEM) based of the flow of electrons through specially prepared (usually stained with osmium, **Os**), extremely thin object; allows to see the internal organization of cells and organelles

Scanning electron microscopy (SEM) based on the electronic reflection from the surface covered with metals (typically, gold, **Au**) and provides an image of the surface of cells and organisms

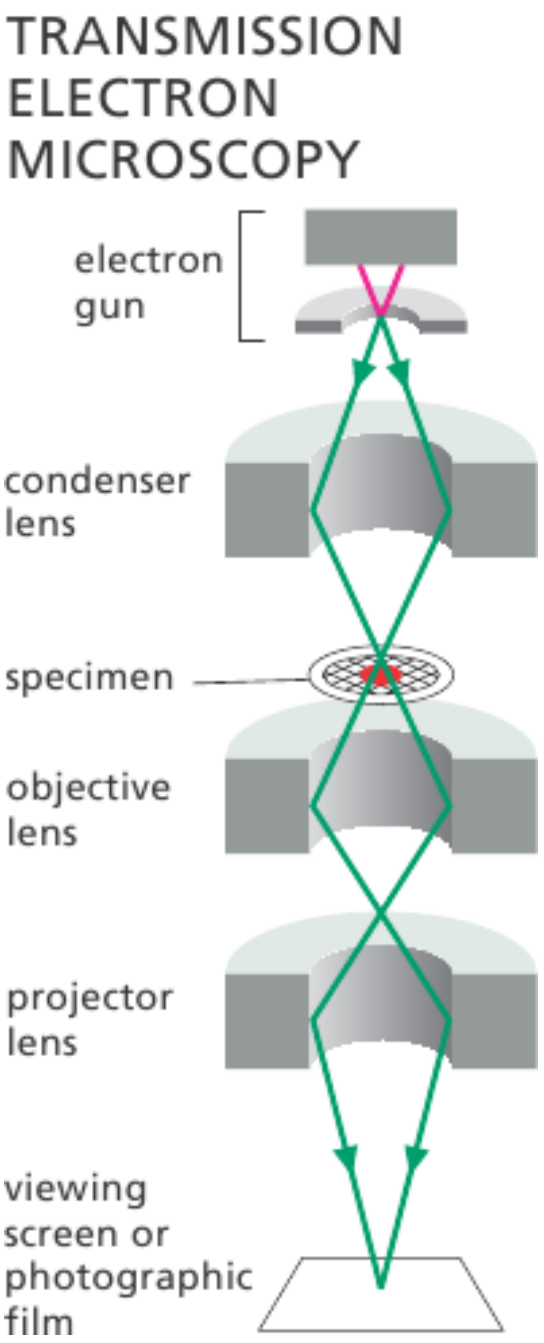
Light microscopy



the light path in a light microscope



Electron microscopy


TRANSMISSION
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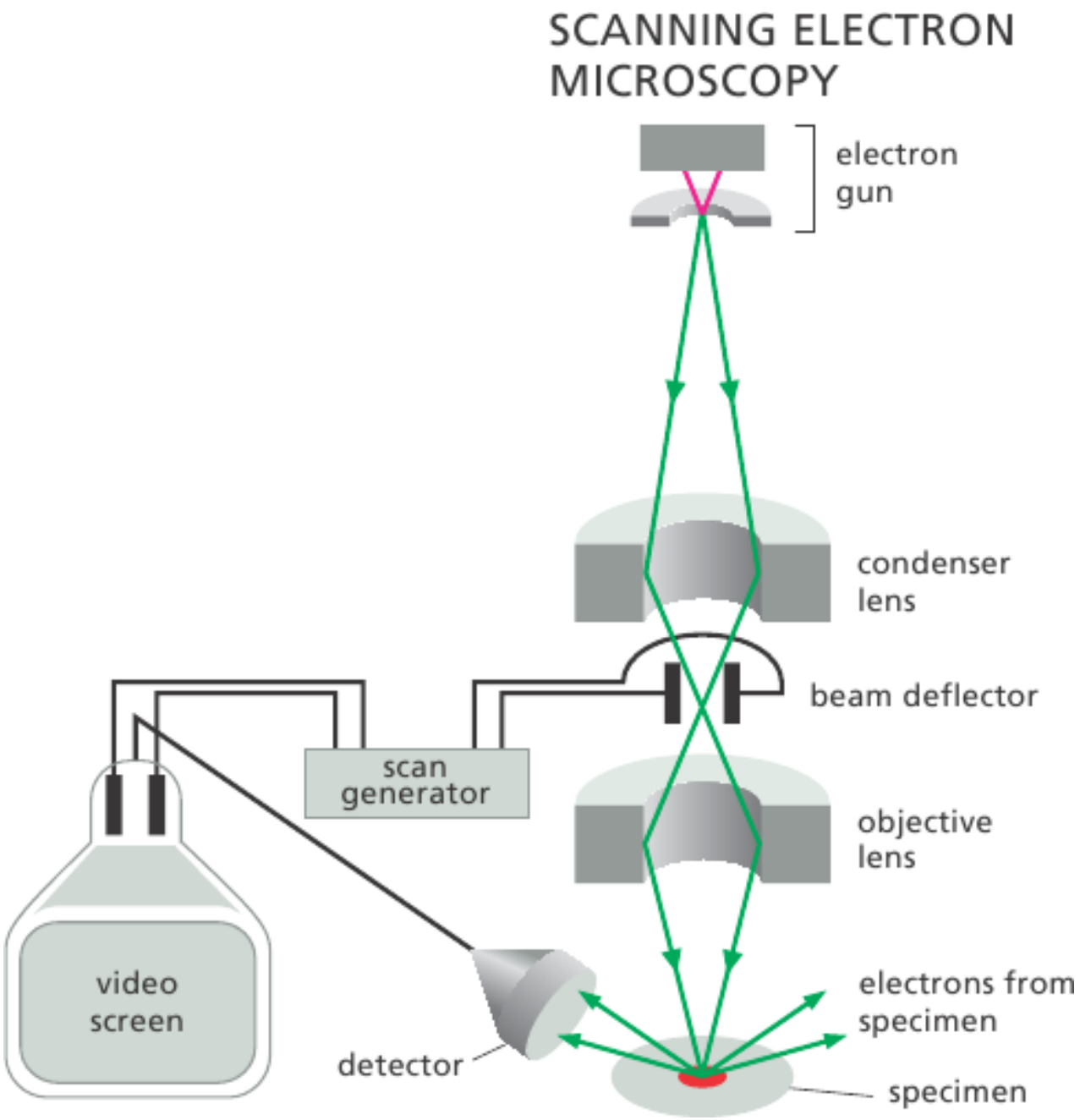
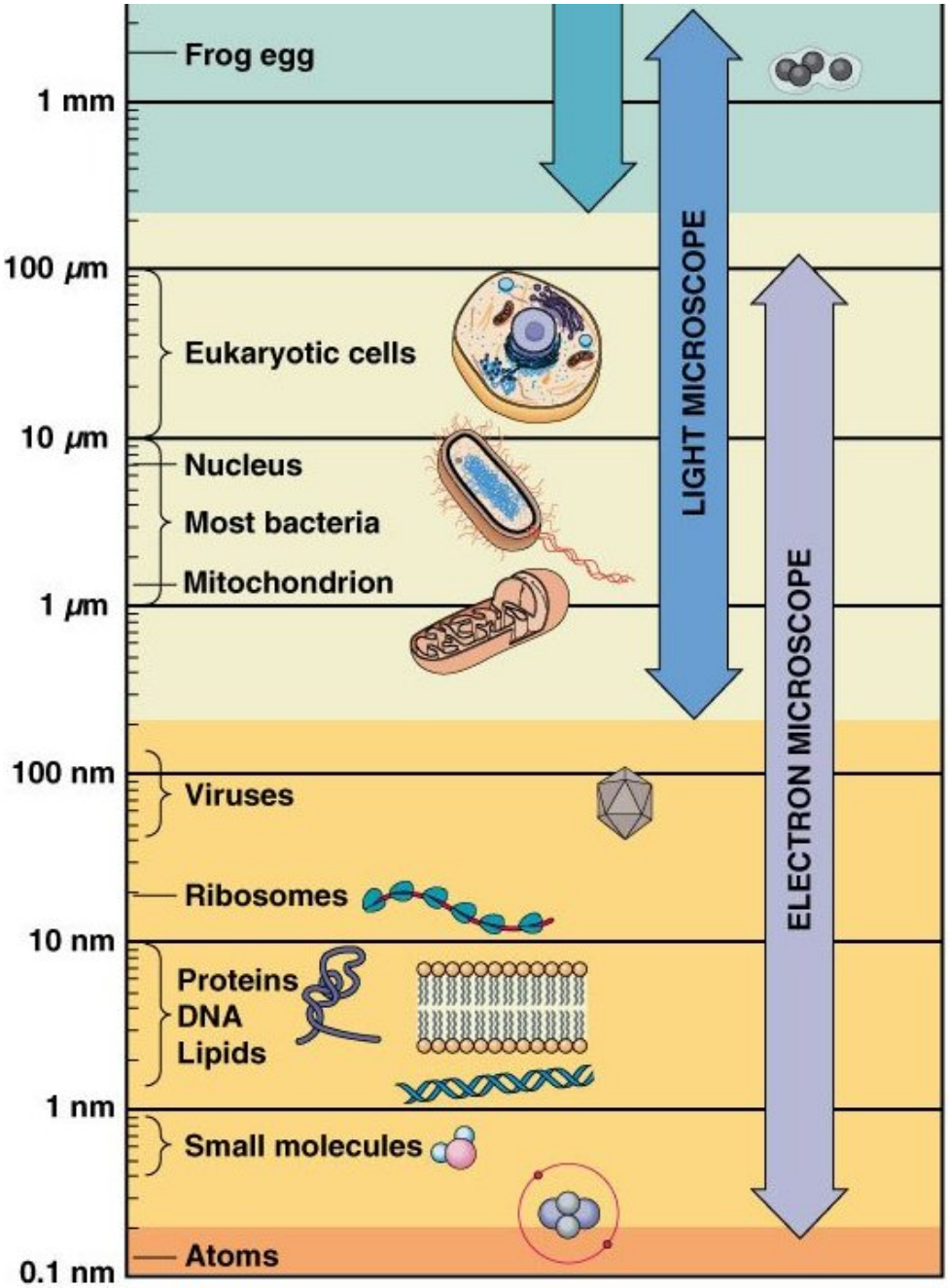


Magnification



B.2 Prokaryotic and eukaryotic cells

Organelles in prokaryotic and eukaryotic cells

- Prokaryotic: [cell wall], plasma membrane, cytosol, [vacuoles], [prokaryotic flagella], nucleoid, [thylakoids]
- Eukaryotic: [cell wall], plasma membrane, cytosol, nucleus, mitochondria, [chloroplasts], endoplasmatic reticulum, [Goldgi apparatus], vesicles (vacuoles, lysosomes etc.), cytoskeleton, [eukaryotic flagella]

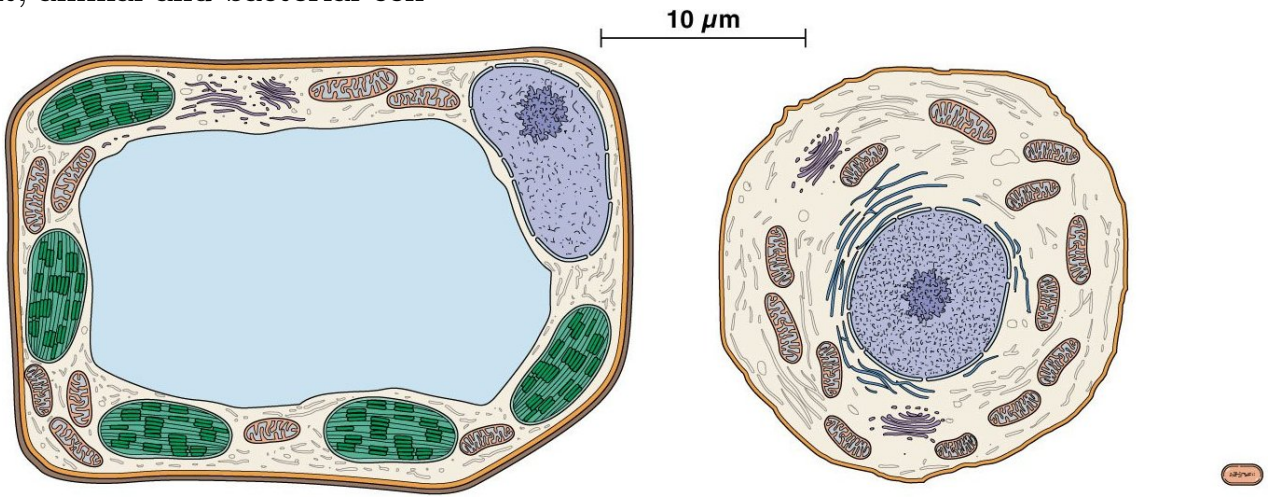
Comparison of prokaryotic and eukaryotic cells

Please copy drawings from a board

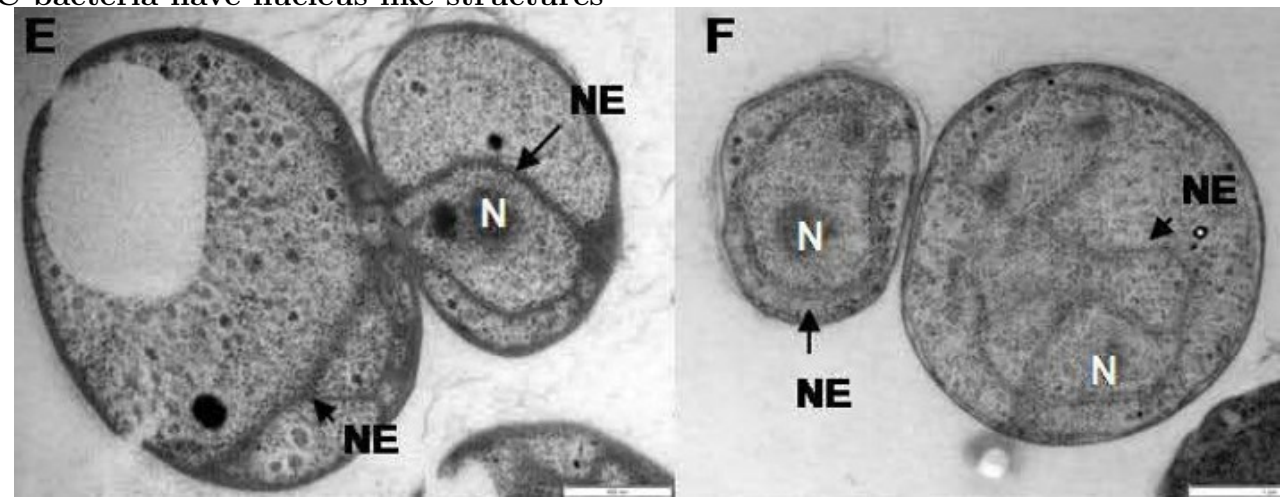
Comparative biology of prokaryotic and eukaryotic cells

- Eukaryotic cells are 10–100 times bigger than prokaryotic
- Eukaryotes have cell motility and capable to endo- and exocytosis
- Prokaryotes have combined RNA and protein synthesis
- Prokaryotes are more diverse chemically, whereas eukaryotes are more diverse morphologically

Plant, animal and bacterial cell



PVC bacteria have nucleus-like structures



[From Lee et al., 2010]

Symbiotic origin of eukaryotic cell

- Double membrane and own DNA are unique features of mitochondria and chloroplasts
- Originally, endosymbiosis was an idea of Russian scientists (Konstantin Merezhkovsky and Boris Kozo-Poljansky), it was revived in 60s by Lynn Margulis (UMass Amherst).
- The host cell was probably predatory archaeon (belongs to Archaea domain) or PVC bacteria (hypothesis of Forterre, 2010)

- Mitochondria were first symbionts, probably proteobacteria
- Chloroplasts appeared later, from cyanobacteria

C Model organisms

Diversity of life and model organisms

- *Escherichia coli*, or *E. coli*: proteobacteria
- *Saccharomyces cerevisae*: fungal protist
- *Arabidopsis thaliana*: flowering plant from cabbage family
- *Drosophila melanogaster*: fly (Diptera) insect
- *Caenorabditis elegans*: round worm (Nematoda)
- *Mus musculus*: common mouse (rodent mammal)

Less common model organisms

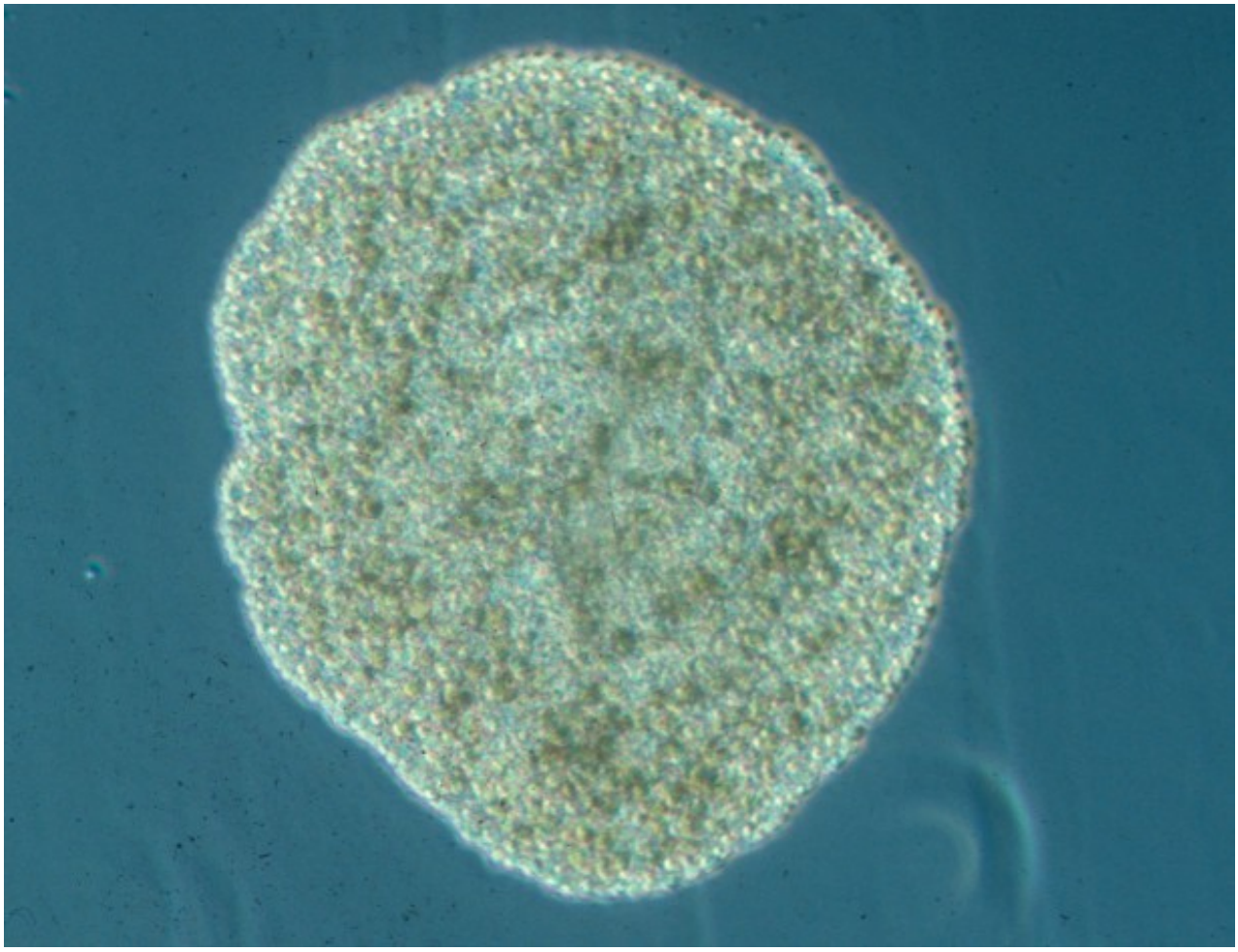
- *Gallus gallus*: chicken (Aves, birds)
- *Danio rerio*: zebrafish (Pisces)
- *Strongylocentrotus purpuratus*: purple sea urchin (Echinodermata)
- *Hydra vulgaris*: freshwater hydra (Cnidaria)
- *Trichoplax adhaerens*: basal animal (Placozoa)
- *Neurospora crassa*: orange bread mold (fungal protist)
- and many others

Zebrafish, *Danio rerio*

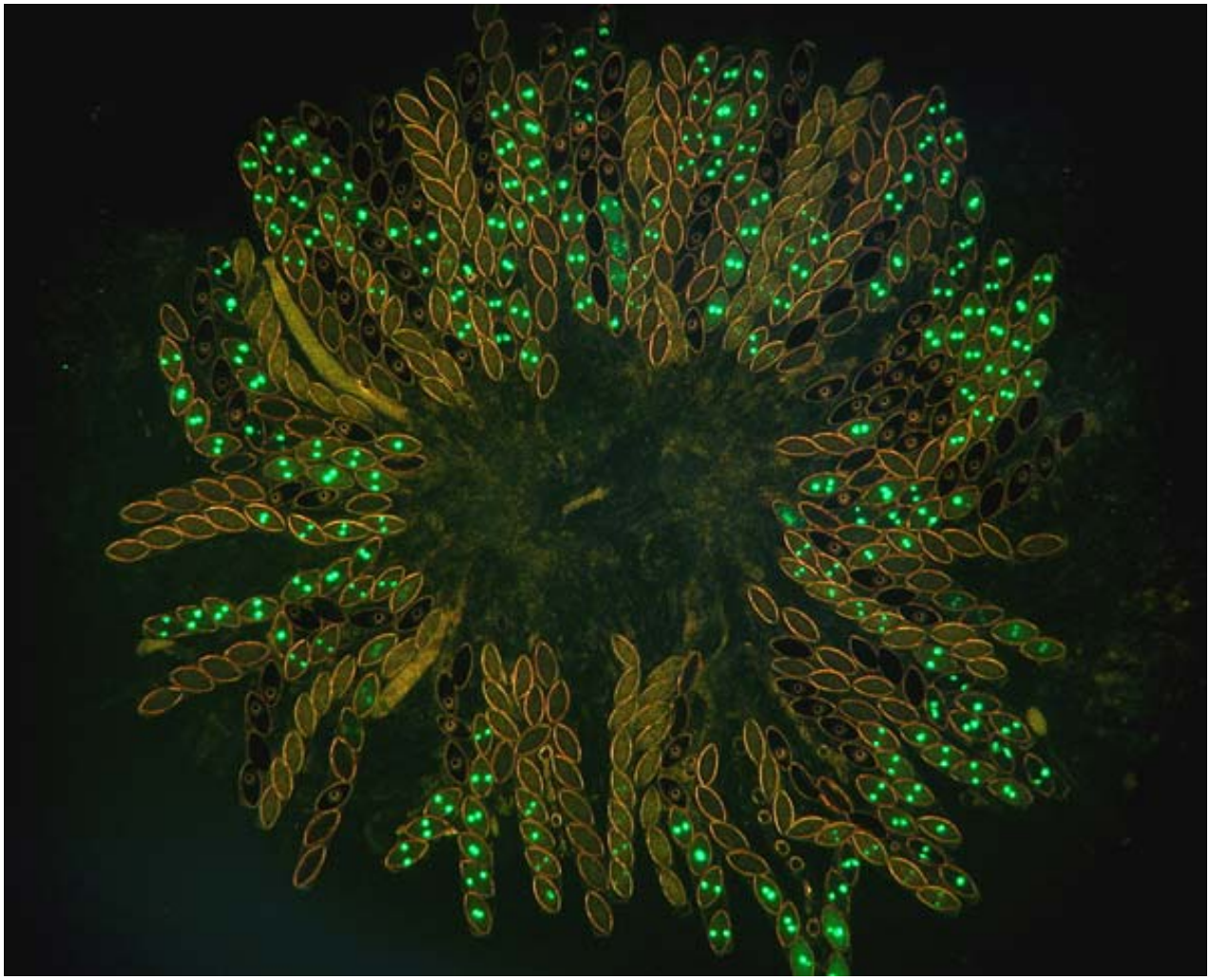
Sea urchin, *Strongylocentrotus purpuratus*

Hydra, *Hydra vulgaris*

Trichoplax adhaerens



Orange bread mold, *Neurospora crassa* under fluorescent microscope



Final question (2 points)

Which organelle is present in most prokaryotic cells and absent in all eukaryotic?

- Electron microscope can only work with dead cells
- Eukaryotic cells are "cells of second level" where part of organelles (mitochondria, chloroplasts) originated from different prokaryotic cells.

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 1*: Cells under the microscope; The prokaryotic cell; The eukaryotic cell; Model organisms.

Outline

D Questions and answers

Previous final question: the answer

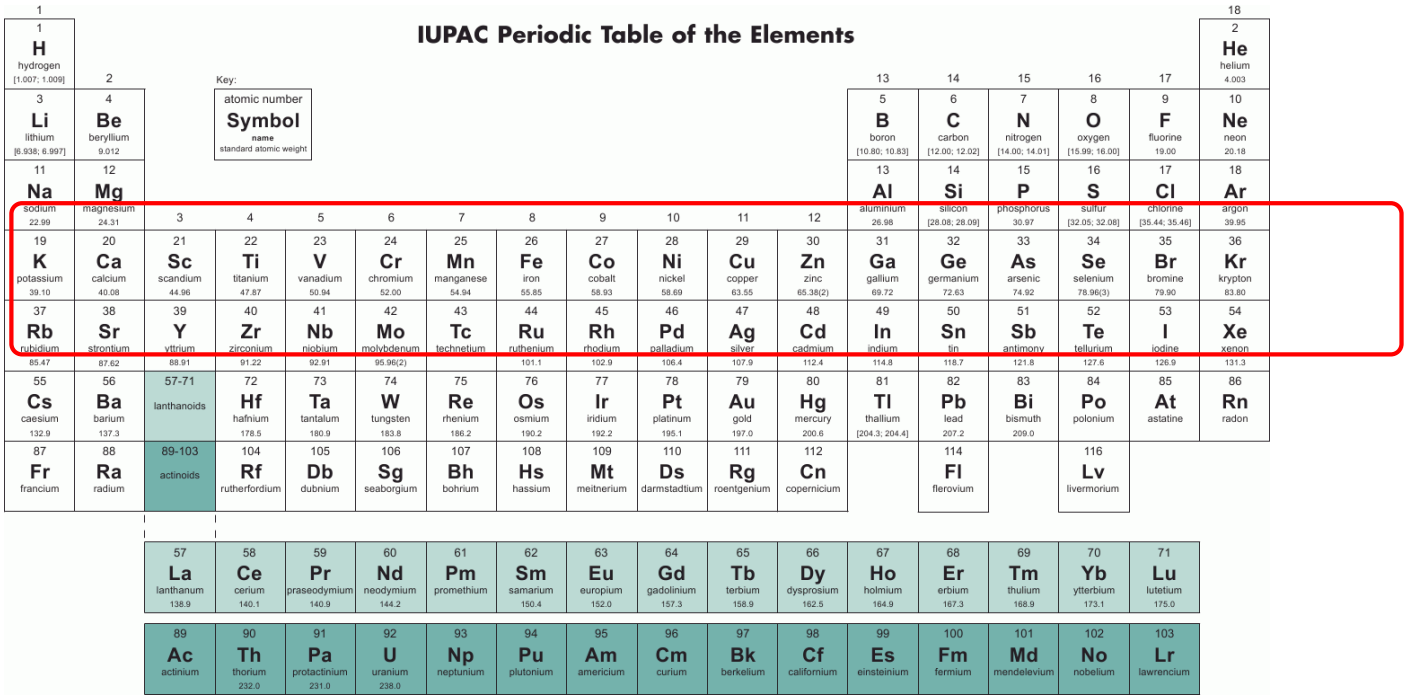
Which organelle is present in most prokaryotic cells and absent in all eukaryotic?

- Flagella$_1$
- Thylakoid
- Nucleoid

E Chemistry of life

E.1 Chemical elements and atoms

Periodic table and atomic features



Terms of atomic chemistry and physics

- Number of protons, neutrons, electrons and periodic table
- Isotopes, radioactivity
- Atomic weight, molecular weight
- Mole, molar solution, Avogadro's number

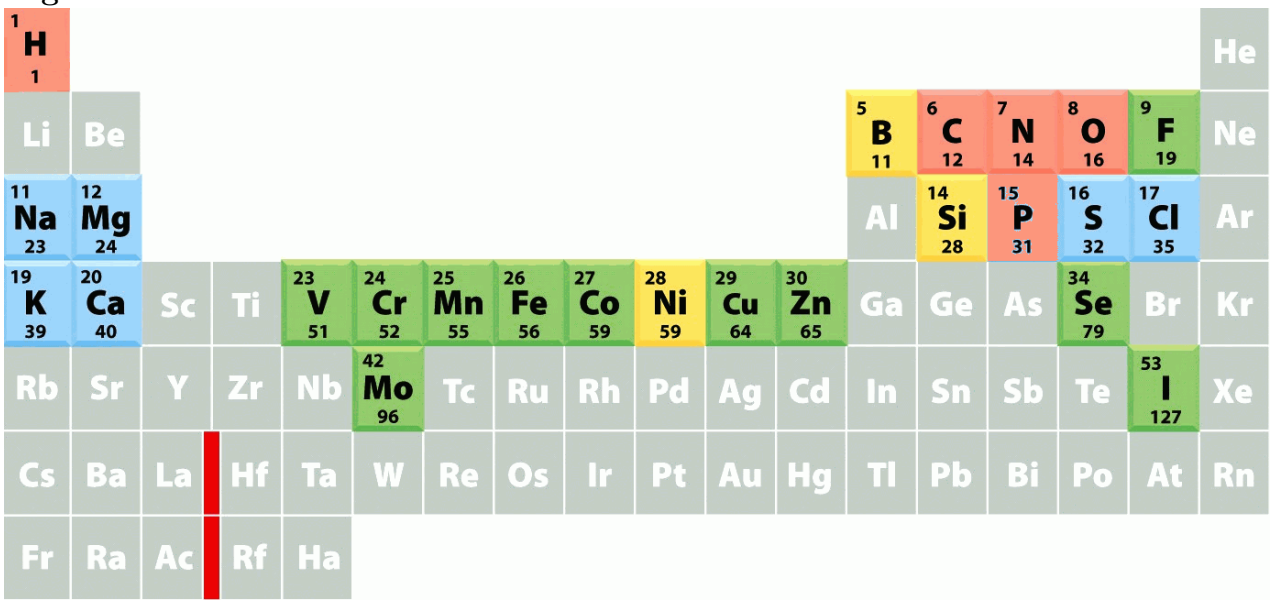
Primary elements

- Main three biogenic elements: carbon (C), hydrogen (H), oxygen (O)
- Slightly less important are nitrogen (N) and phosphorus (P)
- Potassium (K), sodium (Na), calcium (Ca), magnesium (Mg): as cations, e.g. K^+ or Ca^{2+}
- Chlorine (Cl) and sulfur (S): used as anions, e.g. Cl^-

Microelements

- Play a lesser roles and used in lesser amounts (¡ 0.9%)
- These are: iron (Fe), silicon (Si), iodine (I), fluorine (F), selenium (Se), vanadium (V), manganese (Mn), boron (B), molybdenum (Mo), copper (Cu), nickel (Ni), zinc (Zn) and chromium (Cr)

All biogenic elements



Ionic bonds

- Based on electrostatic attraction
- Requires electron transfer from one to another atom
- Molecules with ionic bonds are normally well dissolved in water

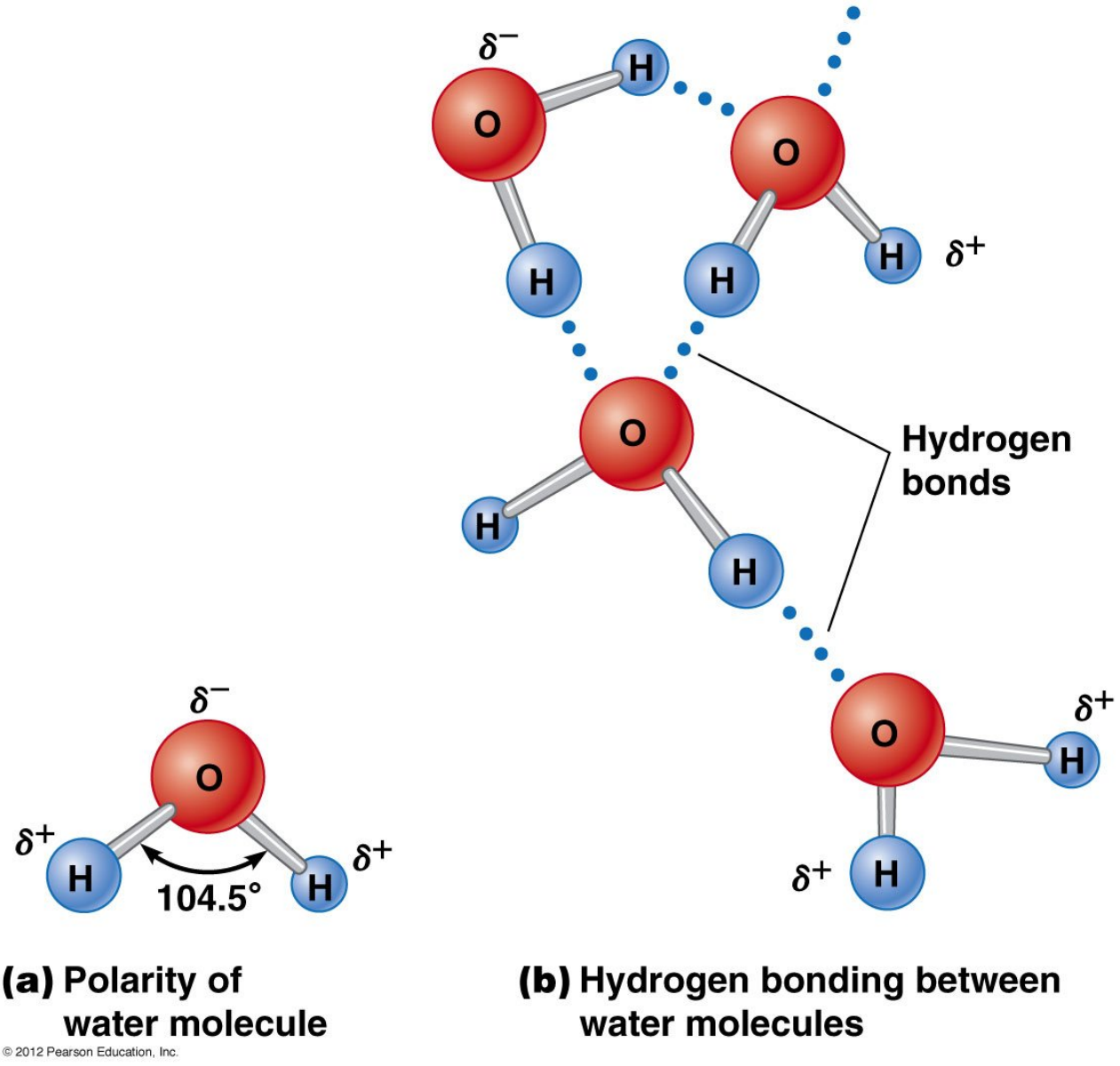
Covalent bonds

- Based on electron sharing
- Depending on strength, may be polar or non-polar

Hydrogen bonds

- Molecule-to-molecule bonds
- Normally occurs between molecules with polar covalent bonds and appropriate size

Water molecule and hydrogen bonds



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Water and its importance

- Universal solvent
- Water molecules are cohesive (water attracts water) and has high surface tension (pond-skaters may skate on water)
- Water molecules are adhesive (water attracts other materials)
- Water has high heat capacity (keeps warmth)
- Water is more dense than ice (water bodies are not completely frozen)

Pond-skater

Acids, bases and pH

- Molecules dissociates with hydrogen ion (or, in other model, hydronium ion) are acids
- Molecules dissociates with hydroxyl ion are bases
- Weak acids/bases have high frequency of reverse reaction
- pH represents the concentration of hydrogen ions, high pH (¿ 7) corresponds with bases, low pH (1–5) corresponds with acids

Hydrophobic "bonds"

- Inside water solutions, hydrophobic (non-solvable) molecules often united in groups
- This process is often called "hydrophobic" bonds

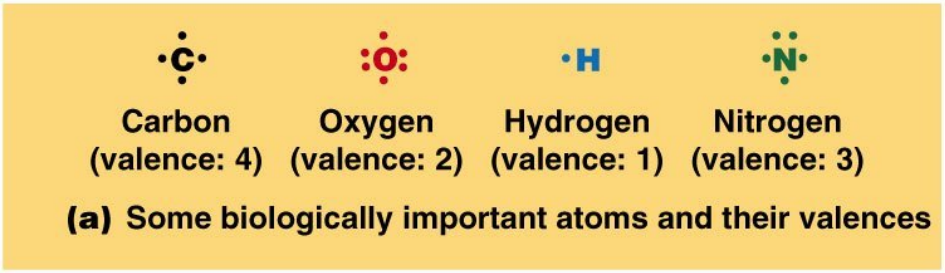
F Organic molecules

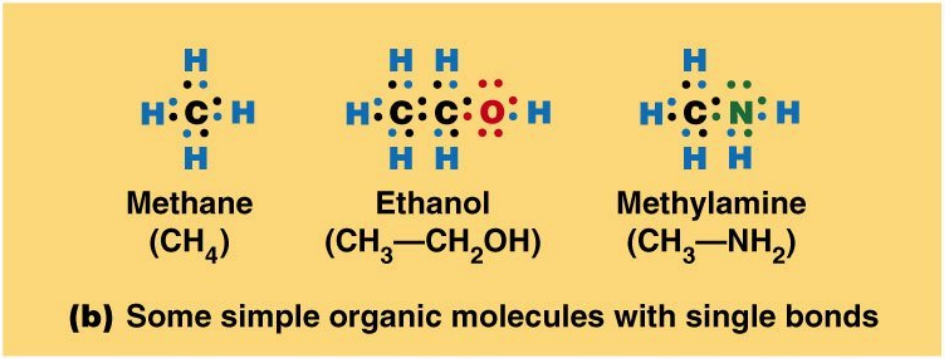
F.1 Basics of organic chemistry

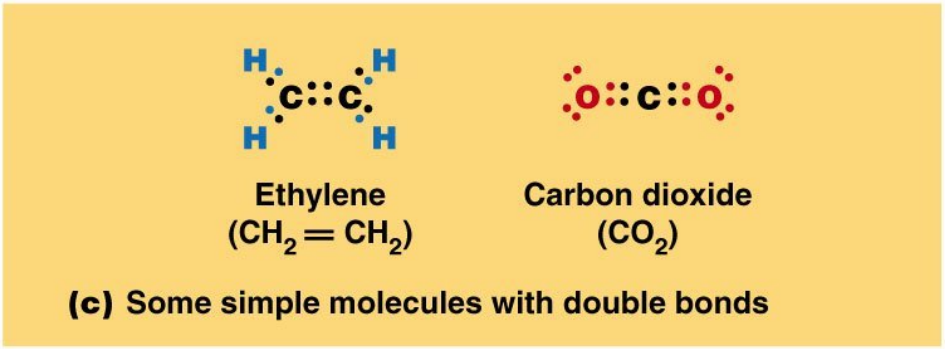
Carbon and carbon skeleton

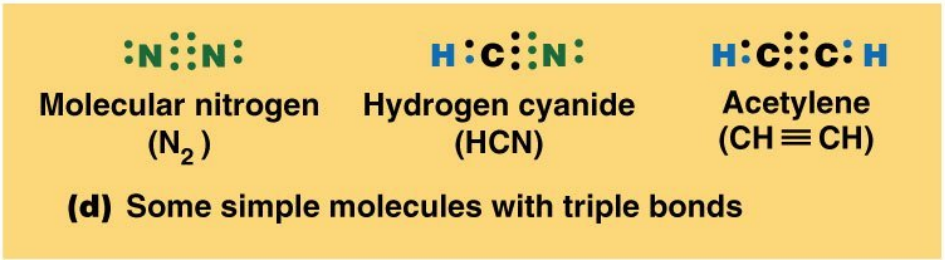
- Carbon atom has a small size and 4 electrons in the outer layer
- Consequently, it can form 4 bonds per atom, double and triple bonds, ad may even form long chains of same element
- Other elements with similar features: silicon (same Group IV!), nitrogen, sulfur

Bonds in organic molecules



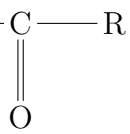
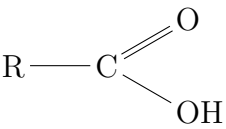






Basic classes of organic molecules

- Hydrocarbons with single, double and triple bonds: C_nH_m
- Aromatic hydrocarbons (arenes): benzene etc.
- Alcohols and phenols: R–OH
- Ethers: R–O–R
- Aldehydes: R–CHO, R—C(=O)H

- Ketones: R–CO–R, 
- Carboxylic acids: R–COOH, 
- Amines: R–NH_2

Basic groups of biochemical compounds

- Mono-, disaccharides (sugars) and polysaccharides: alcolols + ketons / aldehydes
- Fatty acids and lipids: hydrocarbons + carboxylic acids
- Amino acids and proteins: amines + carboxylic acids
- Nucleotides and nucleic acids: sugars + amines + phosphorous acid

Final question (1 point)

Name one chemical element which is NOT biogenic

Summary

- There are five main biogenic elements: carbon (C), hydrogen (H), oxygen (O), nitrogen (N) and phosphorus (P)
- Ionic and covalent bonds are inter-atomic, hydrogen and hydrophilic bonds are inter-molecular
- Organic chemistry is a chemistry of carbon

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 2*: Chemical bonds; Molecules in cells, Panel 2-1.

Outline

G Questions and answers

Previous final question: the answer

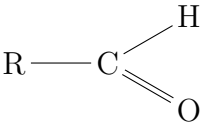
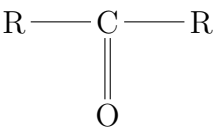
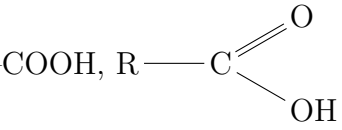
Name one chemical element which is NOT biogenic

- Inert gases: He, Ne, Kr, Xe
- Heavy metals: Au, Os, W, Pb, Hg
- Radioactive atoms: U, Pt

H Organic molecules

H.1 Basics of organic chemistry

Basic classes of organic molecules

- Hydrocarbons with single, double and triple bonds: C_nH_m
- Aromatic hydrocarbons (arenes): benzene etc.
- Alcohols and phenols: R–OH
- Ethers: R–O–R
- Aldehydes: R–CHO, 
- Ketones: R–CO–R, 
- Carboxylic acids: R–COOH, 
- Amines: R–NH_2

Basic groups of biochemical compounds

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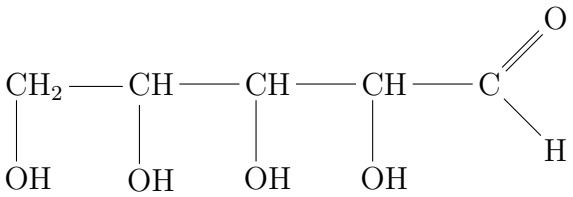
- Nucleotides and nucleic acids: sugars + amines + phosphorous acid

H.2 Carbohydrates

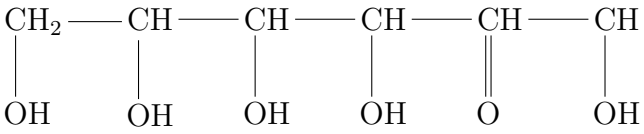
Overview of carbohydrates

- Approximate formula is $C_n(H_20)_m$, but this is only approximation, the real structure has nothing water-related
- Chemically, basic carbohydrates (monosaccharides) are **keto- or aldo- polyalcohols** (poly- starts from 3)
- Polymeric carbohydrates (polysaccharides) are combination of multiple identical monosaccharides, dimeric (disaccharides) contain two monosaccharides

Aldoses and ketoses



aldopentose

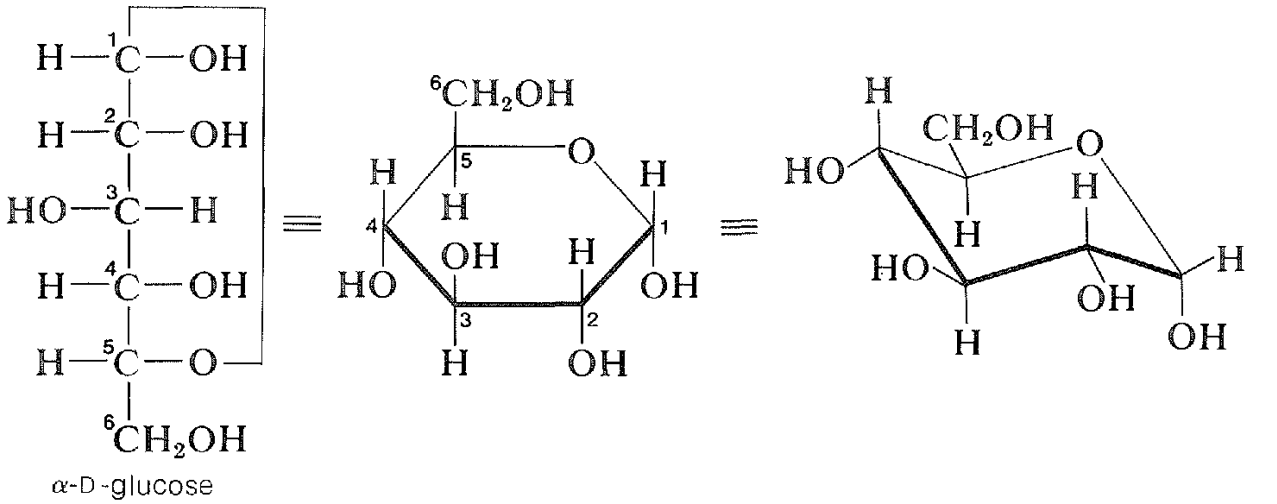


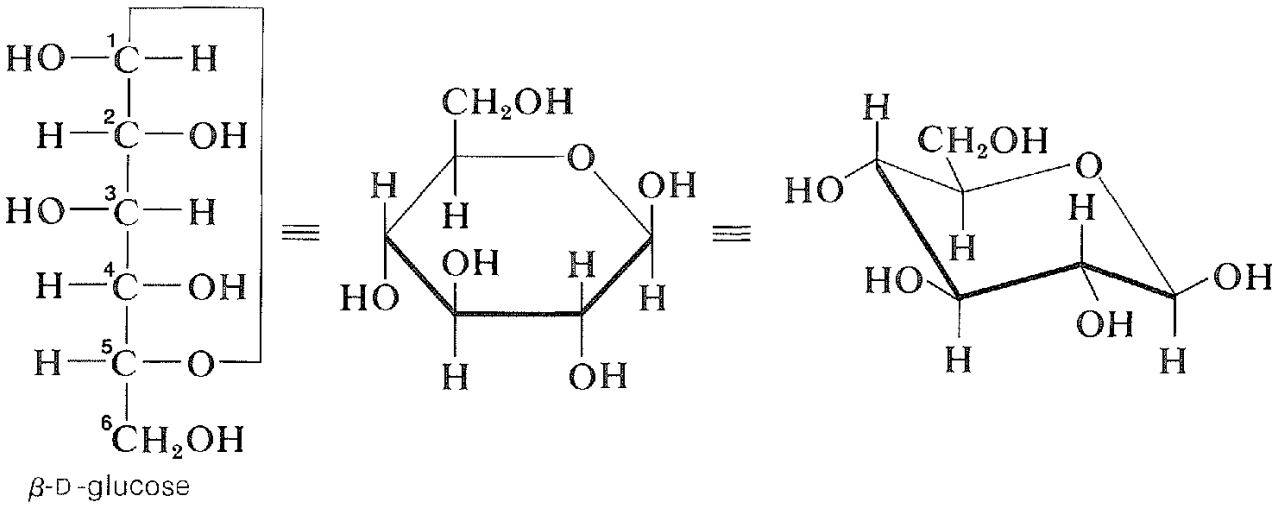
ketohexose

Features of carbohydrates

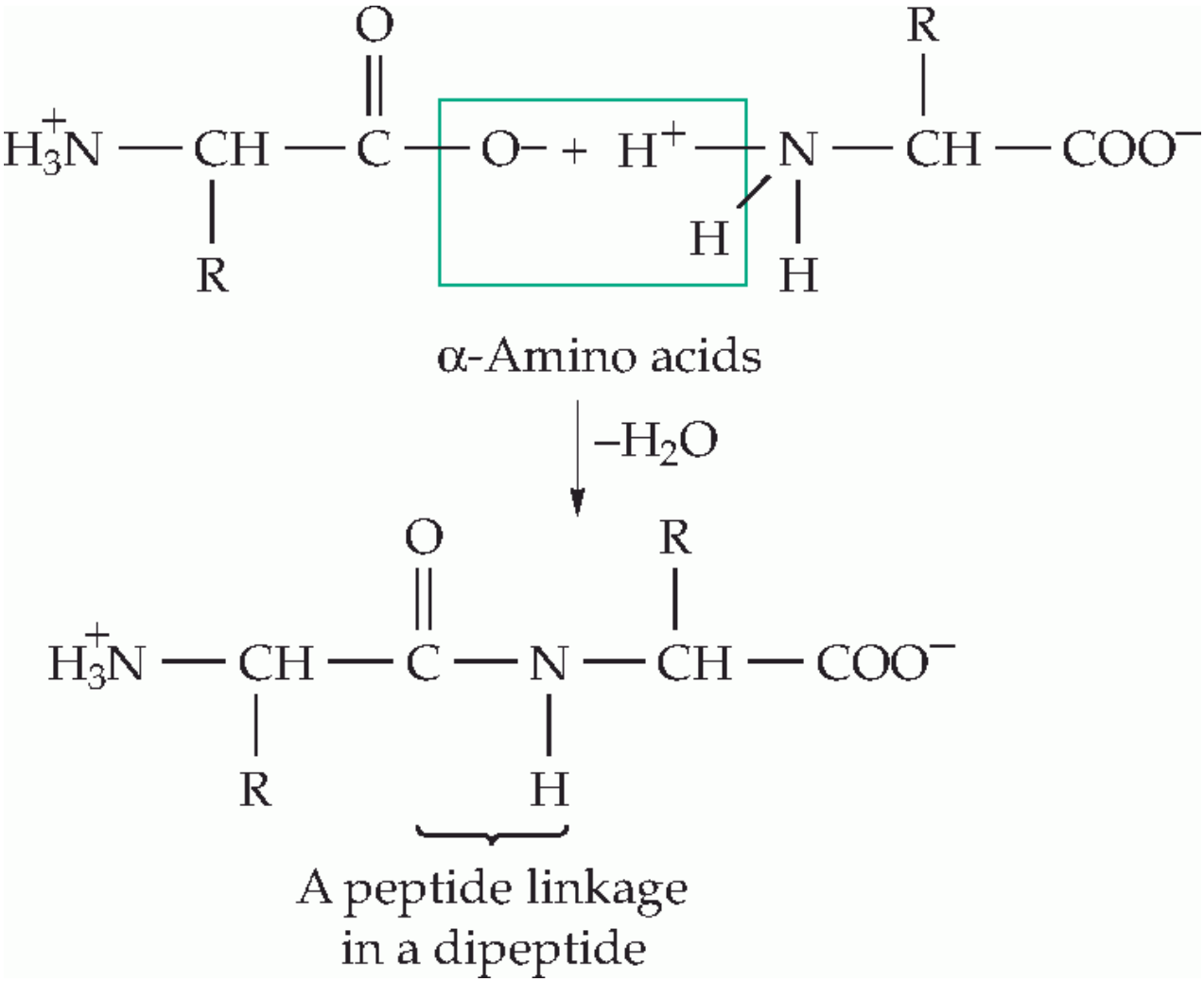
- Monosaccharides could form cyclic structures (rings)
- They have multiple asymmetric carbons, therefore multiple 3D isomers exist
- Moreover, ring may form in two different ways, so there are two additional isomers (α- and β-)
- Reaction of condensation unites monosaccharides in di- and polysaccharides
- When uniting, α- and β- monosaccharides can form different kinds of links

α- and β- glucose





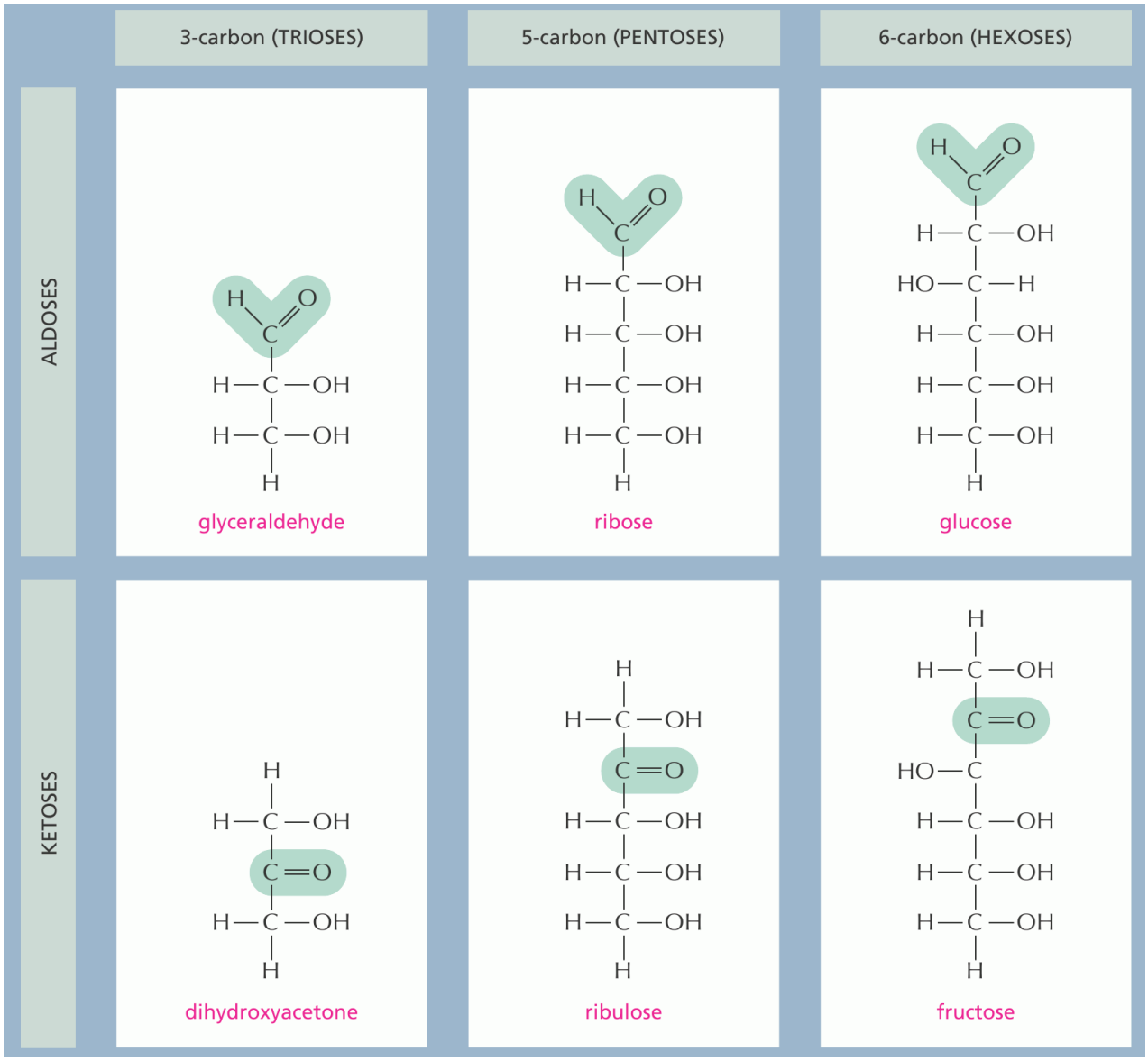
Reaction of condensation



Most important mono-, di and trisaccharides

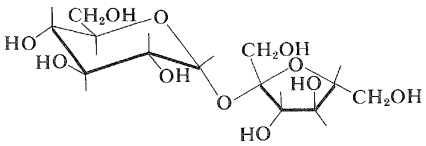
- Pentoses $C_5H_{10}O_5$: ribose, ribulose, xylose (wood sugar)
- Hexoses $C_6H_{10}O_6$: fructose (with five carbons in the ring), glucose and its isomers mannose and galactose (brain sugar)
- Disaccharides $C_{12}H_{20}O_{12}$: sucrose (cane/beet sugar, glucose + fructose); lactose (milk sugar, glucose + galactose); maltose (malt sugar, glucose × 2)
- Trisaccharides $C_{18}H_{30}O_{18}$: raffinose (product of bacterial degrading of polysaccharides)

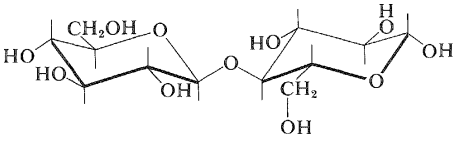
Monosaccharides

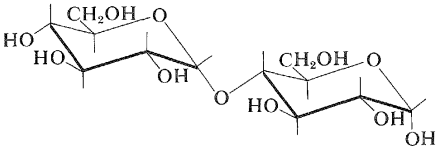


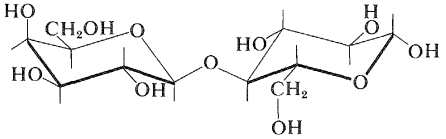
α- and β- disaccharides

α- β-


sucrose


cellobiose


maltose

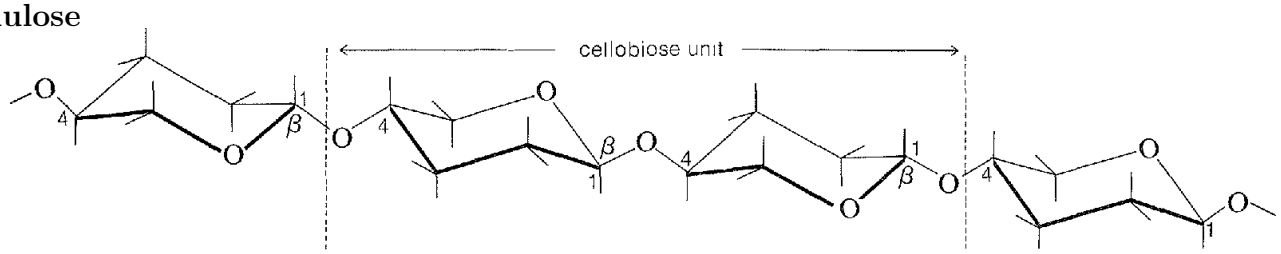

lactose

Most important polysaccharides

- Cellulose (unbranched poly-β-glucose)

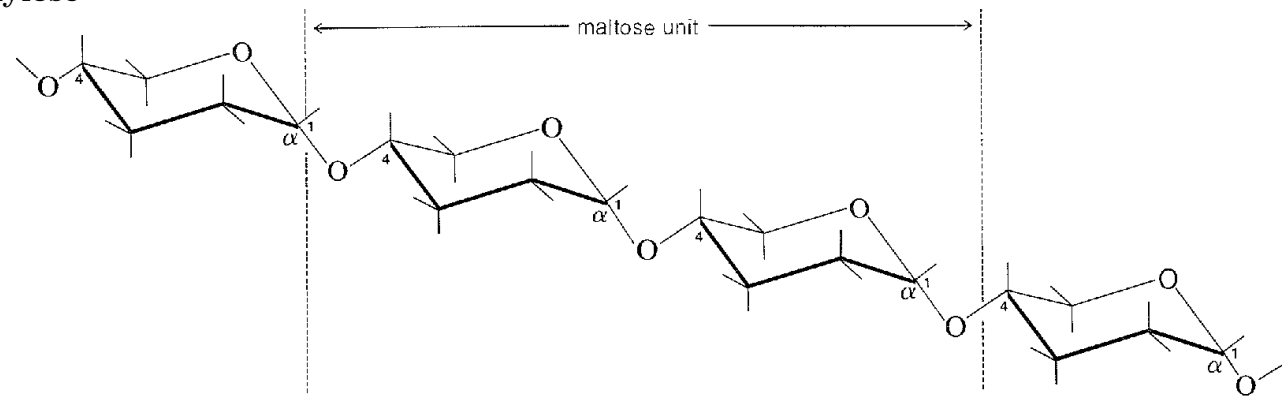
- Amylose and amylopectin (unbranched and branched poly-α-glucose)
- Chitin (amino-poly-β-glucose)
- Hemicelluloses (poly-xyloses)

Cellulose



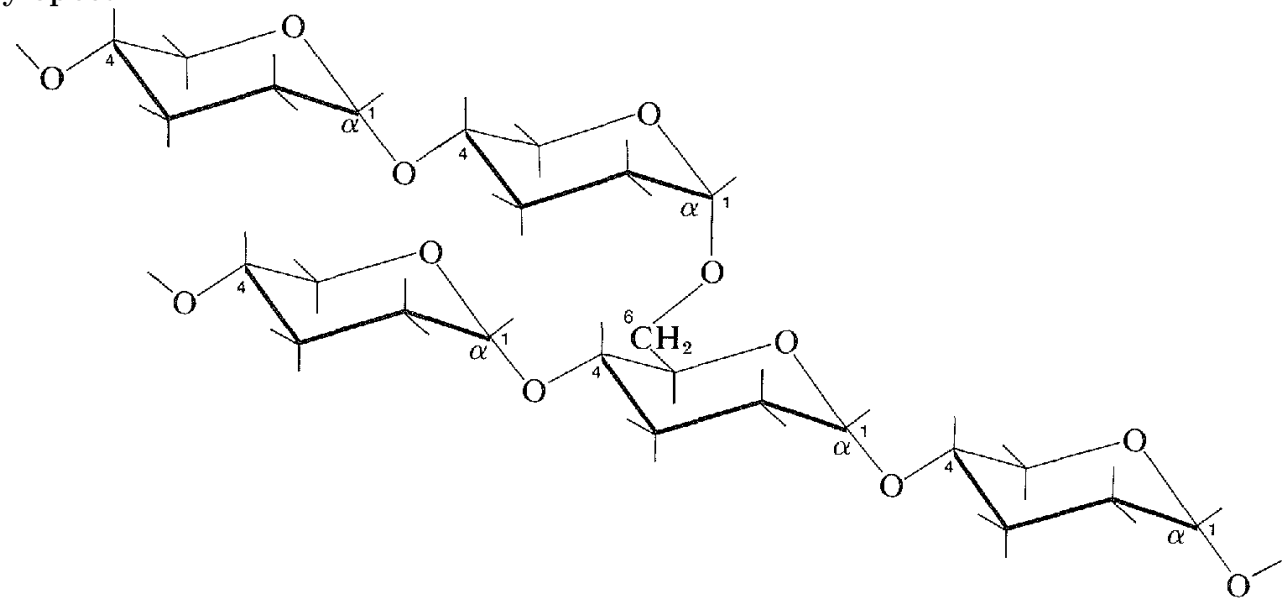
cellulose structure
(the hydroxyls on the rings are omitted for clarity)

Amylose



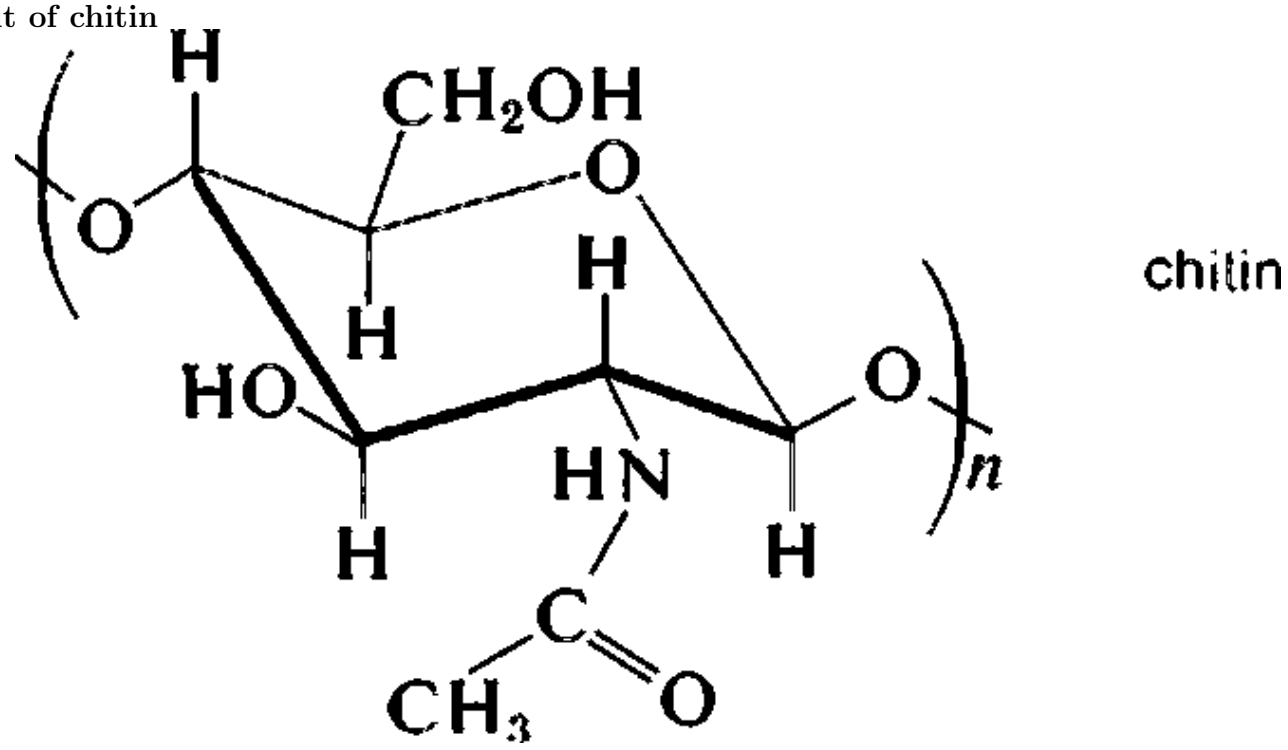
amylose

Amylopectin



amylopectin

Unit of chitin



chitin

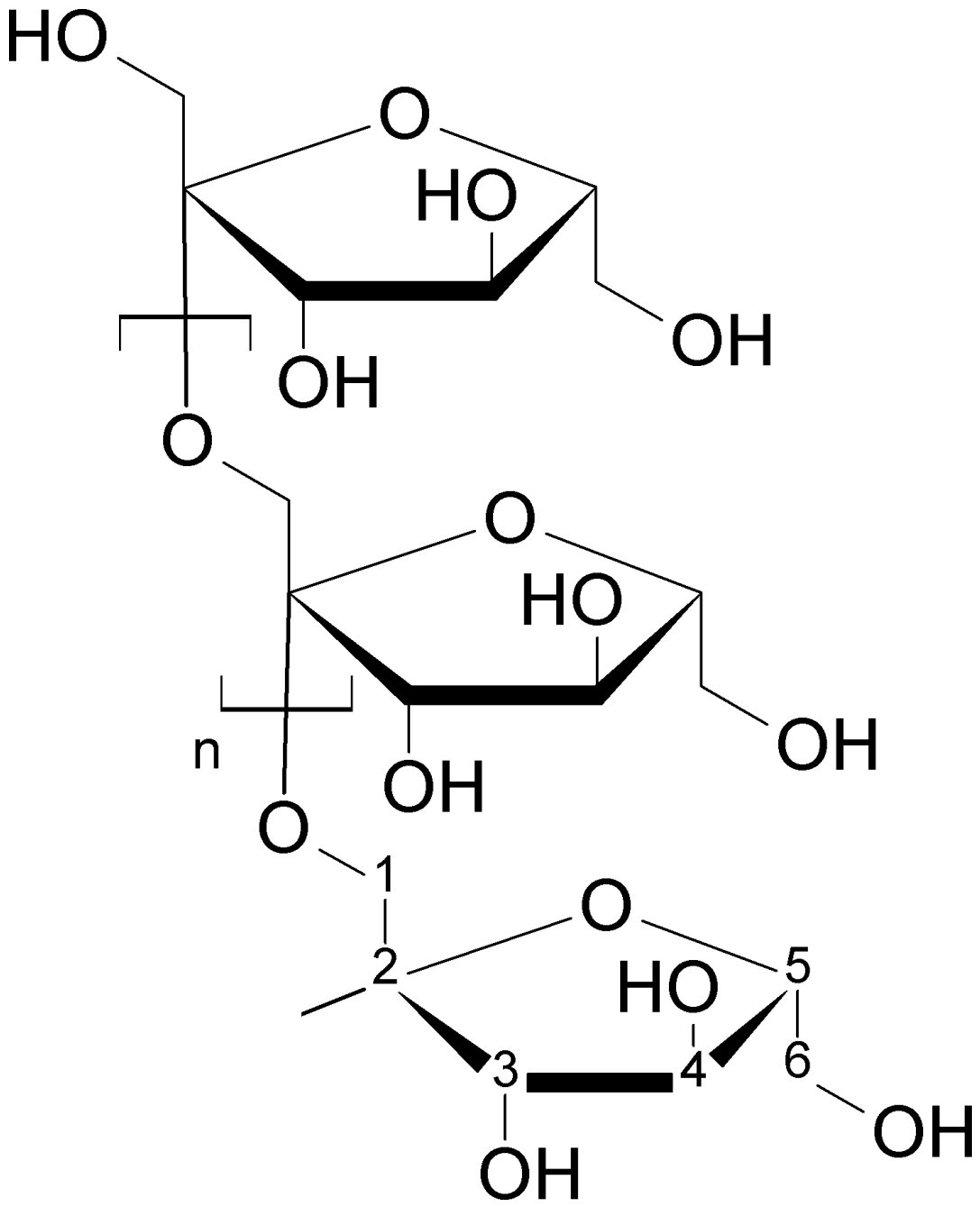
Starch and glycogen

- Starch: amylose + amylopectin
- Glycogen: $\approx$ pure amylopectin

Inulin

- Polymer of fructose (poly-β-fructose), often has a fibrous structure
- Typically, occurs in many plants of sunflower family (e.g., *chicory*, *dandelion* or *Jerusalem artichoke*)

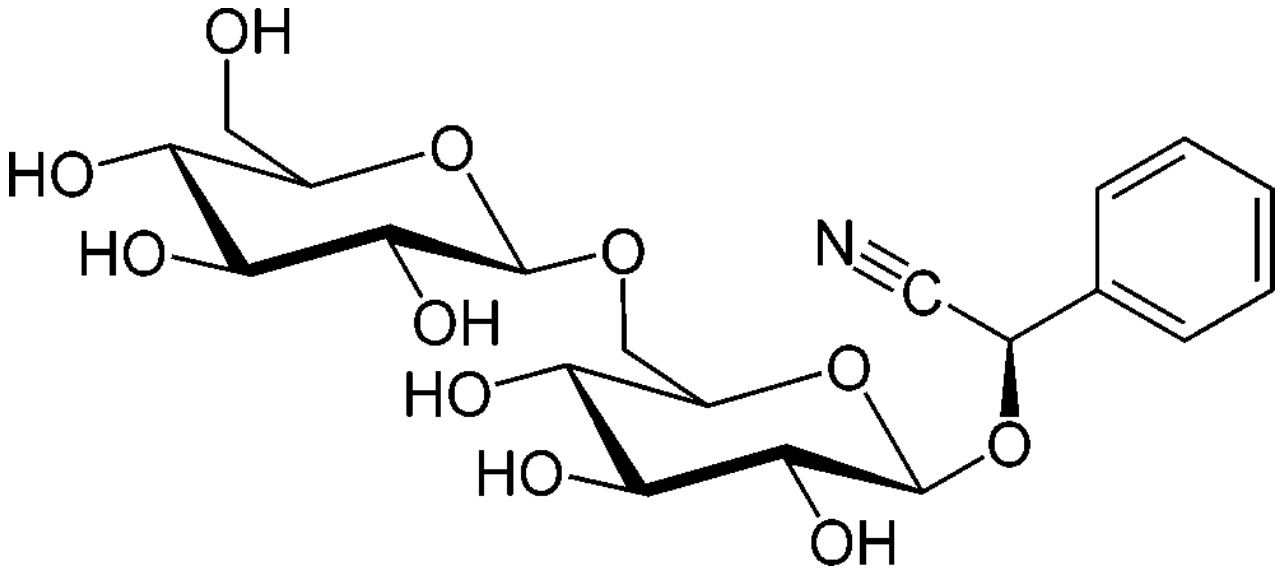
Inulin structure



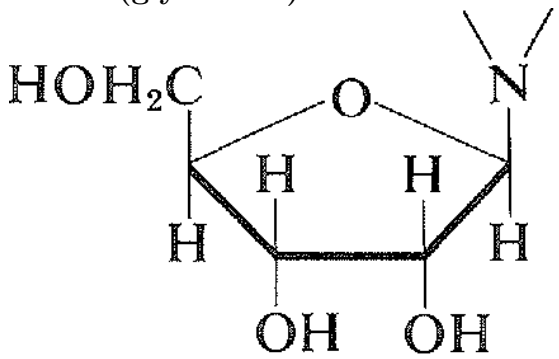
Some important molecules related to carbohydates

- **Glycosides**: monosaccharides bonding trough oxygen to various compounds, including amines (nucleosides)
- **Pectins**: polymers of galacturonic acid (derivative of glucose)
- **Vitamin C** (ascorbic acid): derivative of glucose with acidic properties

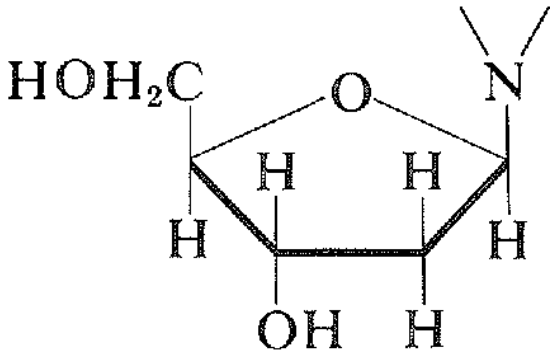
Amygdalin glycoside from almond



Nucleosids (glycosides)

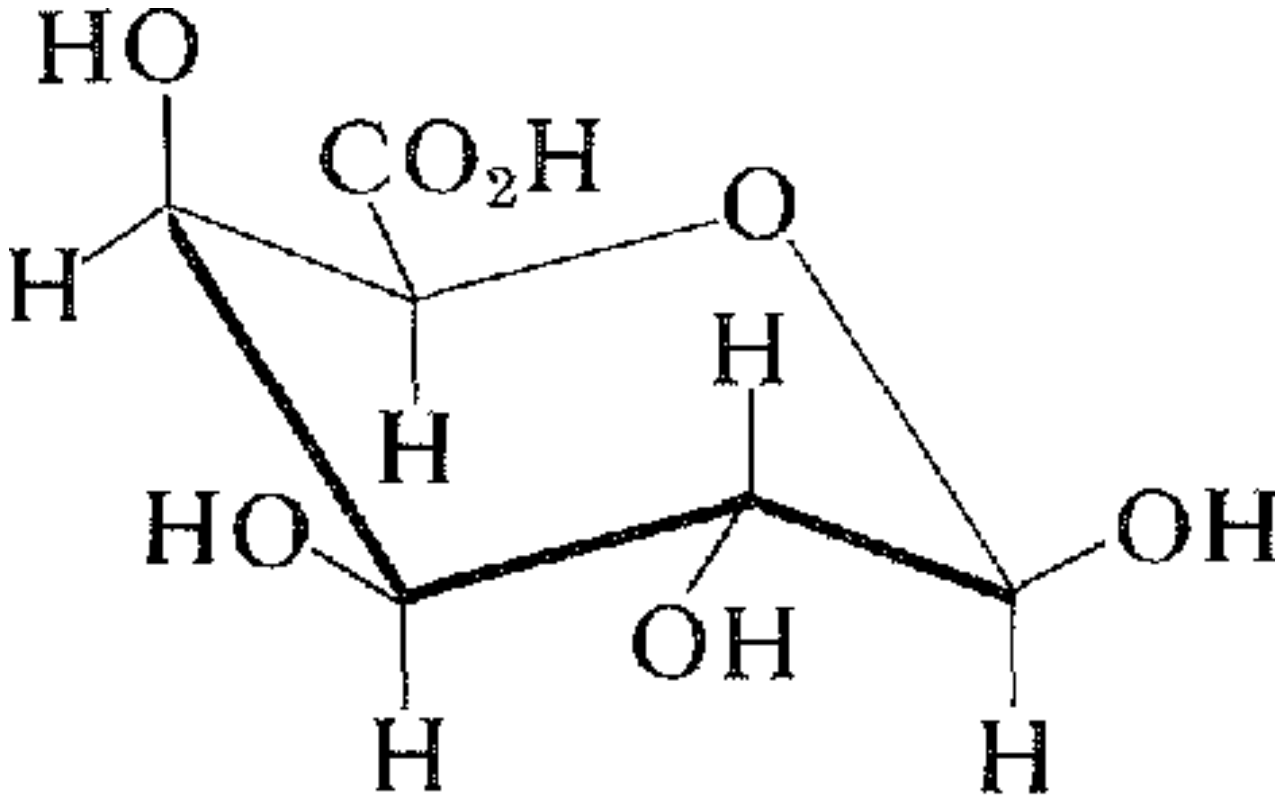


ribonucleoside
(partial structure)



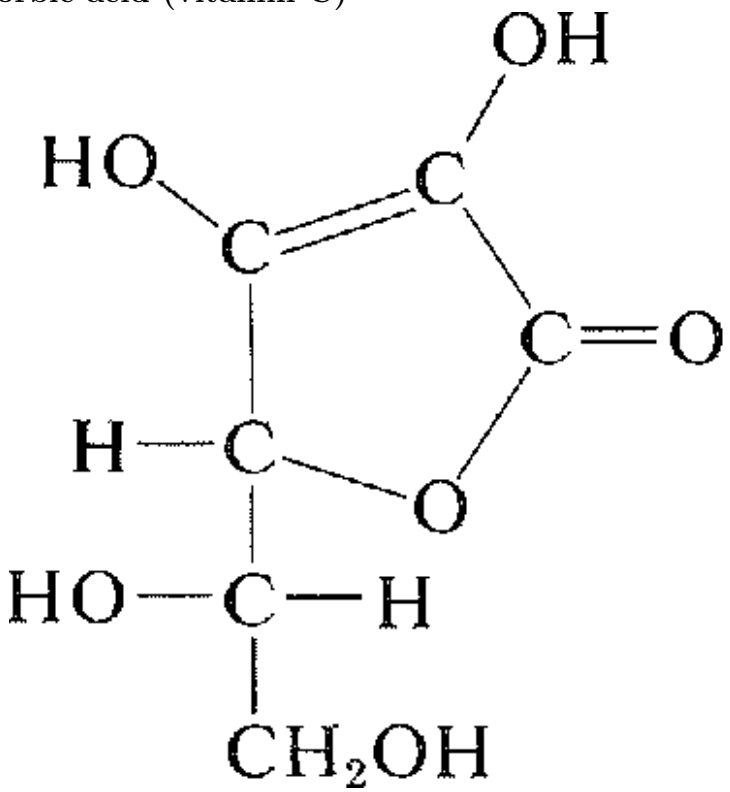
deoxyribonucleoside
(partial structure)

Galacturonic acid (pectins)



β-D-galacturonic acid

Ascorbic acid (vitamin C)



L-ascorbic acid

Final question (2 points)

What is the difference between α- and β- glucose?

Summary

- Carbohydrates are aldo- or keto- polyalcoholes and their polymers; most of them are using as structural molecules or sources of energy

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 2*: Molecules in cells, Panels 2-3, 2-4.

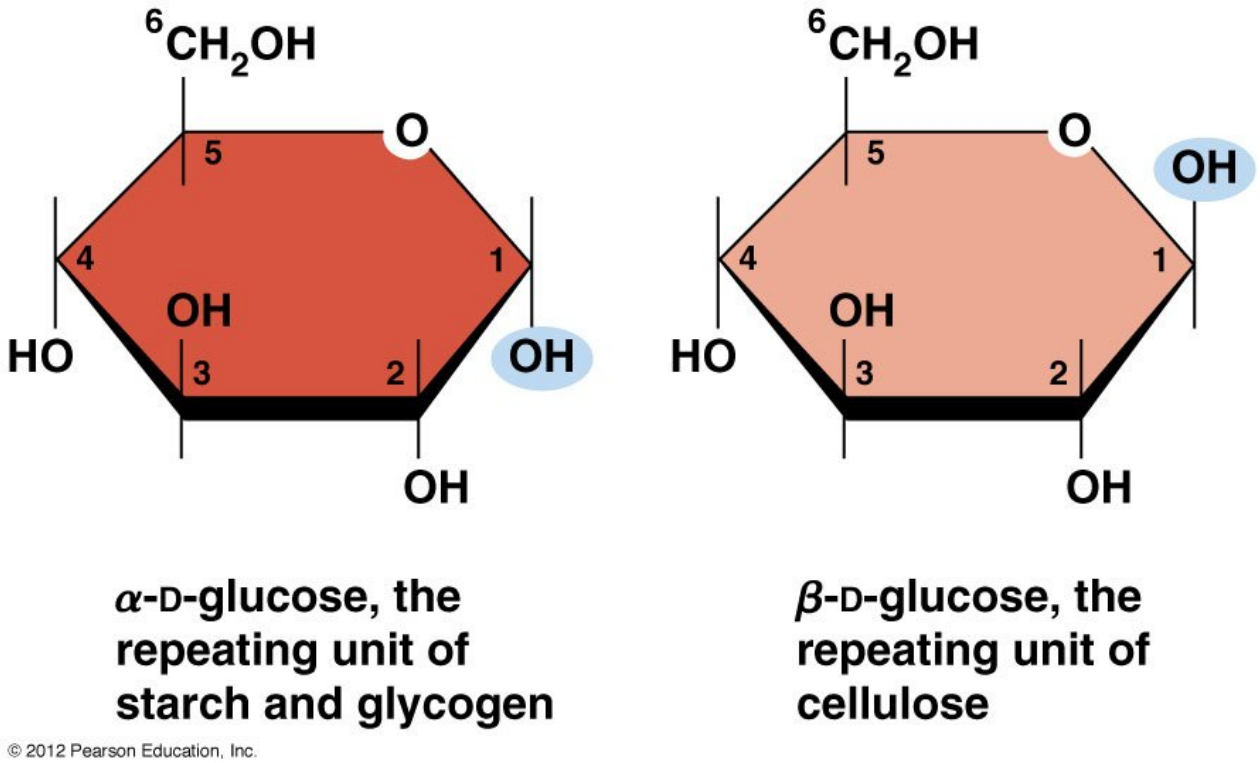
Outline

I Questions and answers

Previous final question: the answer

What is the difference between α- and β- glucose?

α down (axial position), β up (equatorial position)



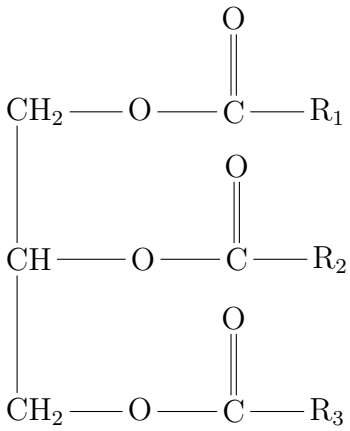
J Fatty acids and lipids

J.1 Storage lipids: oils and fats

Storage lipids: oils and fats

- Fatty acids are massive (C ¿ 15) hydrocarbon acids
- Oils and fats are esters (complex ethers) of glycerol and (often different) fatty acids: **triacylglycerols**, or **triglycerides**
- Stable, hydrophobic and high-energetic molecules

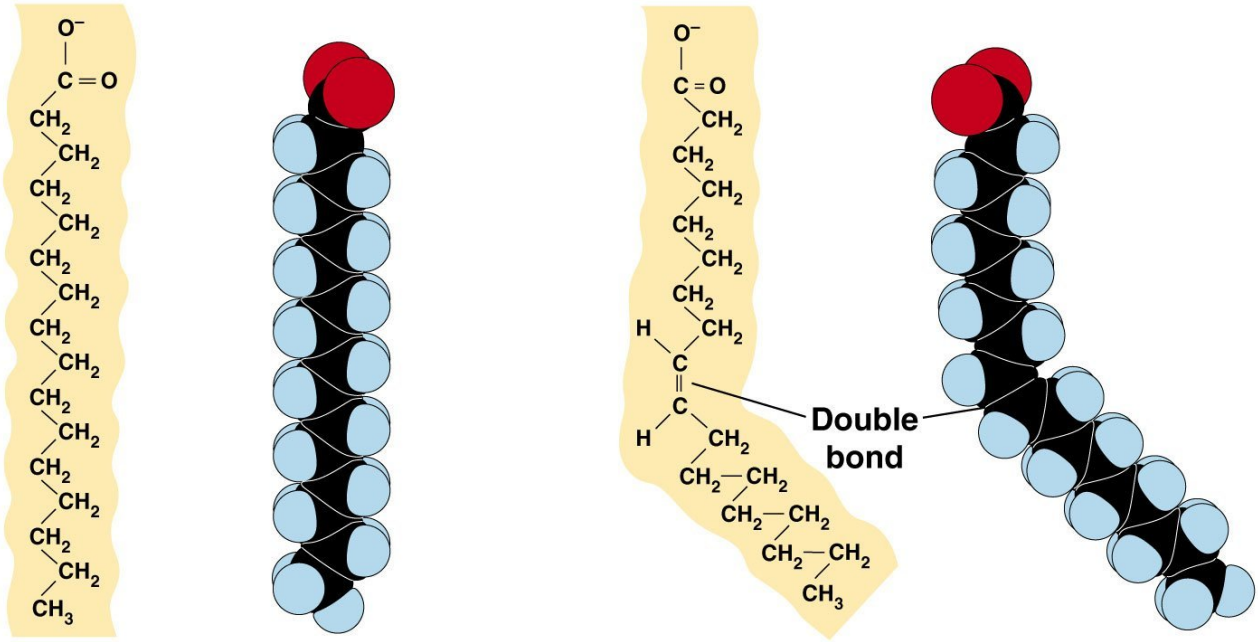
Ttiacylglycerols (triglycerides)



Diversity of fatty acids

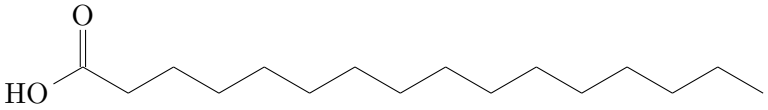
- **Saturated**: contain only single bonds between carbons
- **Unsaturated**: contain also double bonds
- Unsaturated typically have bend chain, and much lower melting temperature
- Trans fats contain hydrogenated unsaturated oils

Saturated and unsaturated fatty acids

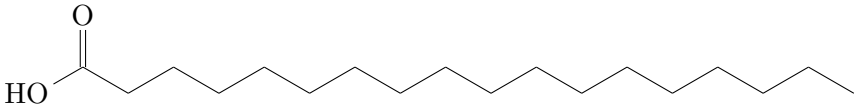


Examples of fatty acids

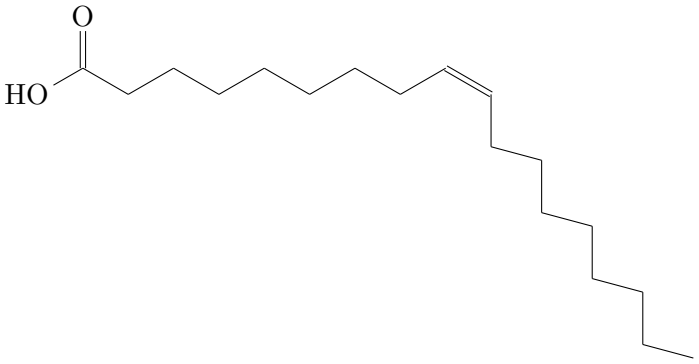
- *Palmitic* acid (COOH–C_{15}): from animal fats



- *Stearic* acid (COOH–C_{17}): from animal fats



- *Oleic* acid (COOH–C_9 =C_8): from olive



- *Linoleic* acid (COOH–C_8 =C_3 =C_6): from flax

EPA, eicosapentaenoic acid (omega-3)

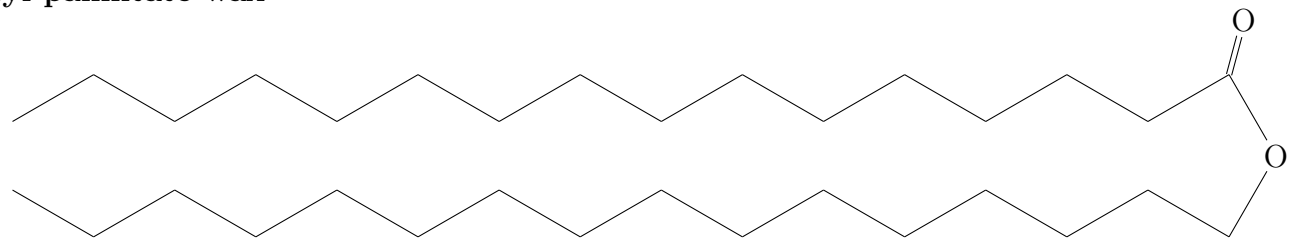
[scale=.85]!z-[:+30]-[:-30]!x!x!x!x!x-[:+30]

Omega-3 fatty acids are considered now as important health factors. Probably, decrease the human depression

Waxes

- Waxes are esters of fatty acids and fatty alcohols (alcohols with long chains)
- Have high melting temperatures
- Use as structural and protective molecules, both in animals and plants

Cetyl palmitate wax

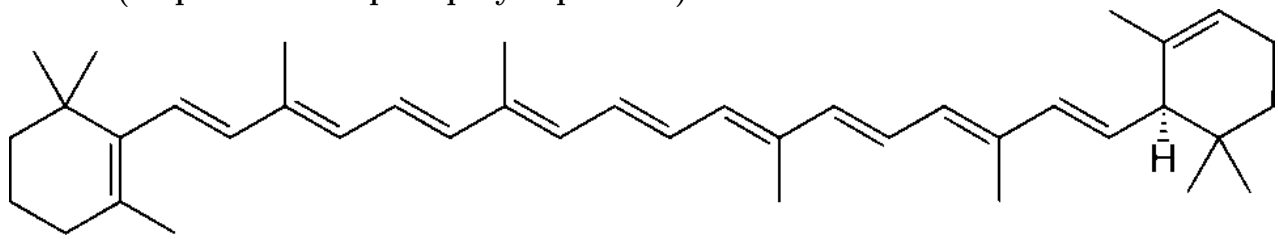


Primary constituent of spermaceti, the wax found in the skull of sperm whales

Plant lipids: isoprenoids

- Derivatives of isoprene, CH_2=CH–C(CH_3)=CH_2
- Simple polyisoprenoids (*terpenes*) form some aroma compounds
- Complex polyisoprenoids (*terpenoids*) are carotenes and other plant pigments, and also components of latex

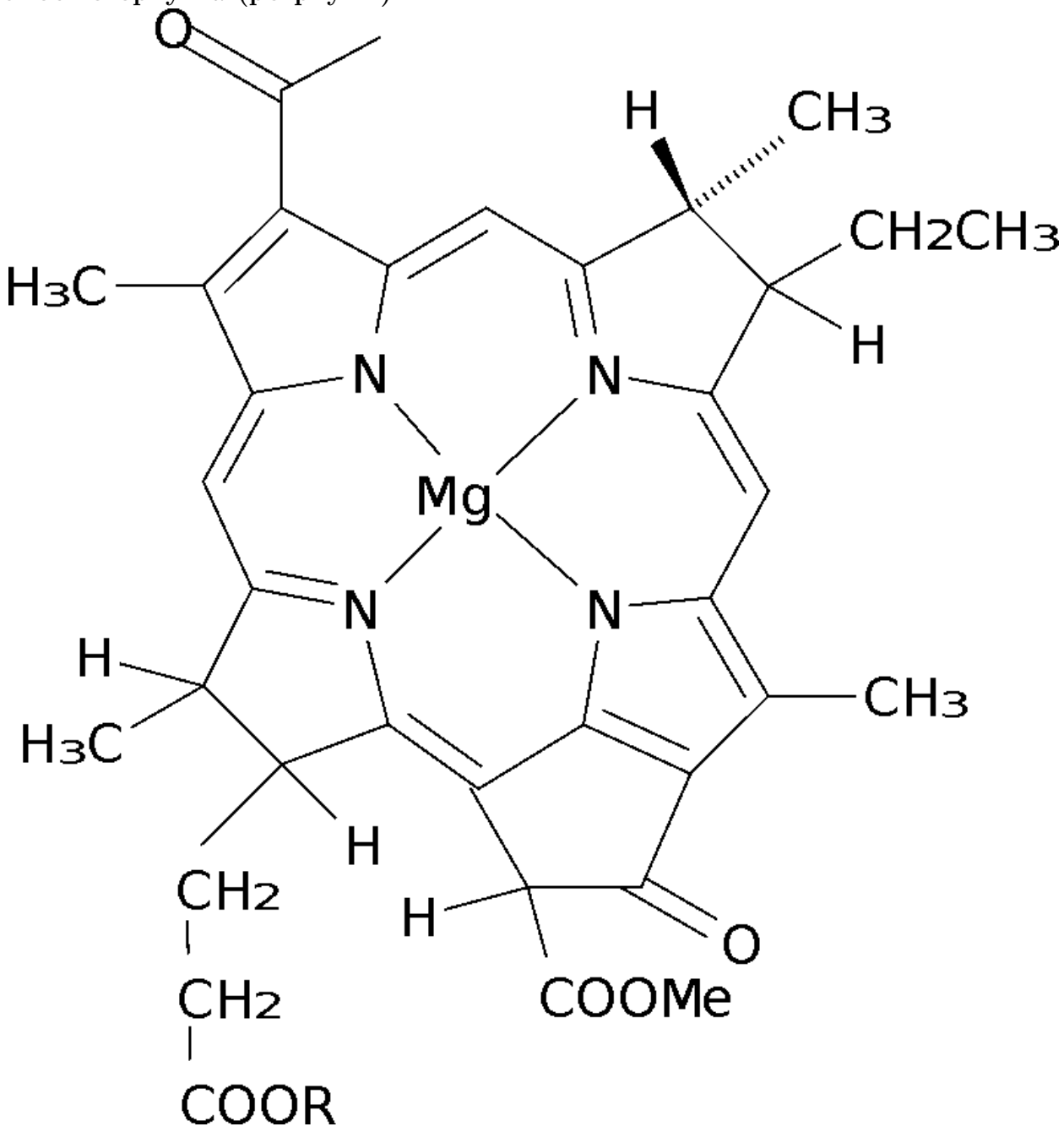
α-carotene (terpenoid: complex polyisoprenoid)



Porphyrins

- Occur in plants and animals
- Easily form complexes with metals and gases
- *Chlorophyll* and *heme* (red blood pigment) are examples of porphyrins

Bacteriochlorophyll *a* (porphyrin)



J.2 Membrane lipids

Membrane lipids

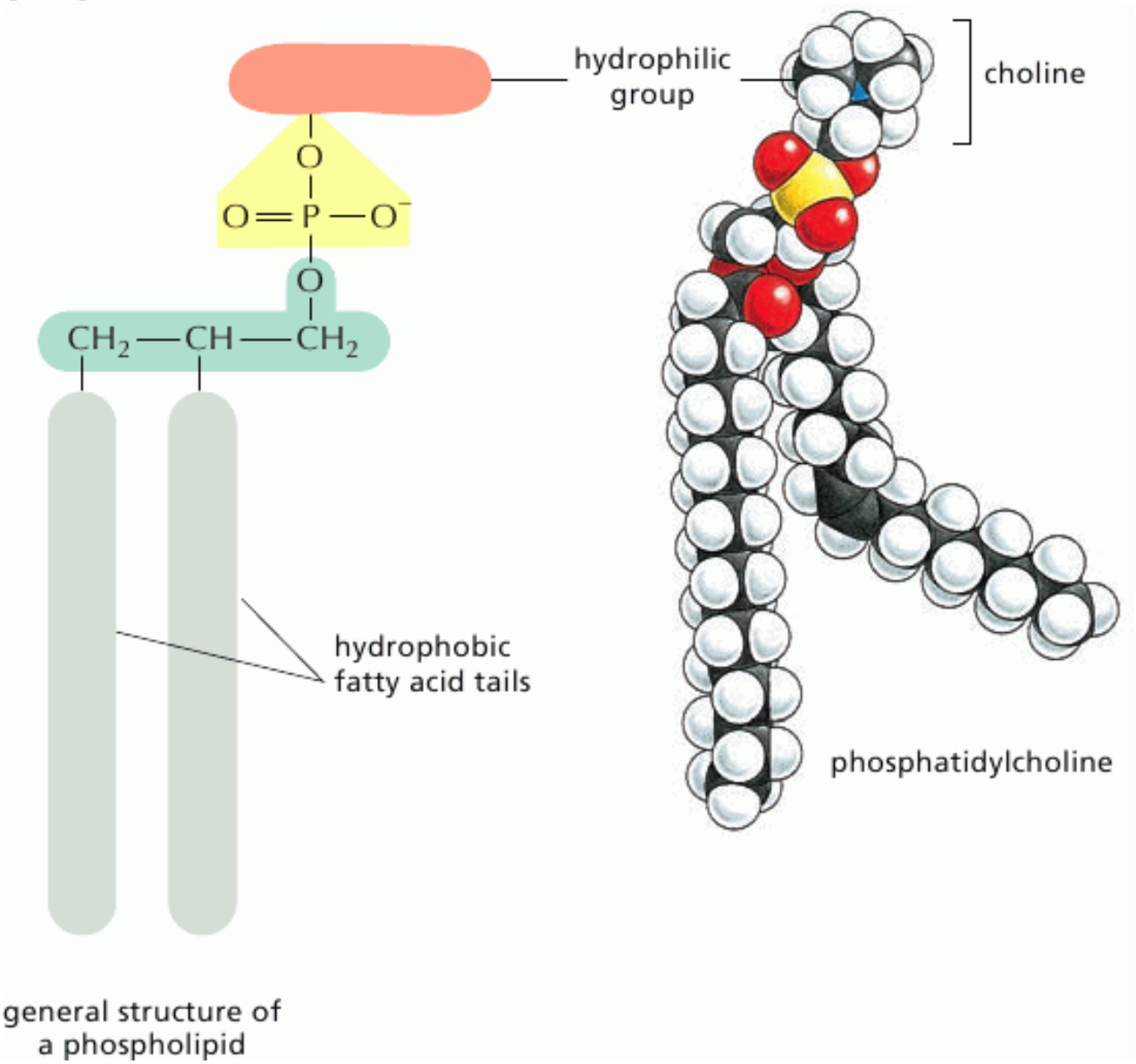
- Membrane lipids are structural units of membrane double layer
- Their chemical structure is similar to triacylglyceroles (fats) but one of fatty acids is replaced with other molecule

Phospholipids

- Phospholipids are esters of glycerol (or sphingoside), fatty acids and phosphorous acid

- Head + two tails structure
- Glycerol + phosphate head is hydrophilic whereas fatty acid tail is hydrophobic

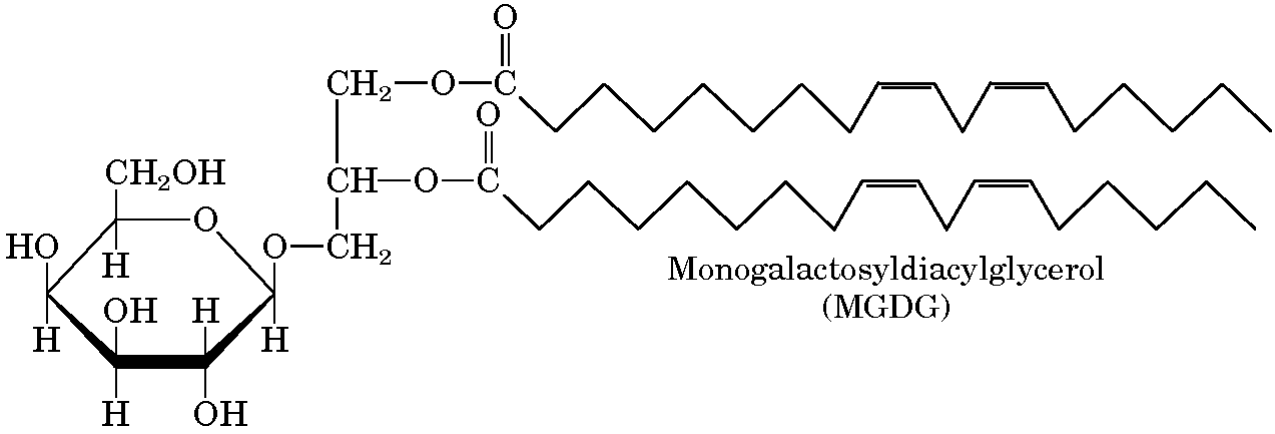
Phospholipids



Glycolipids

- Glycolipids have two hydrocarbon tails and sugar head
- Often occur in plant cells, especially in chloroplasts

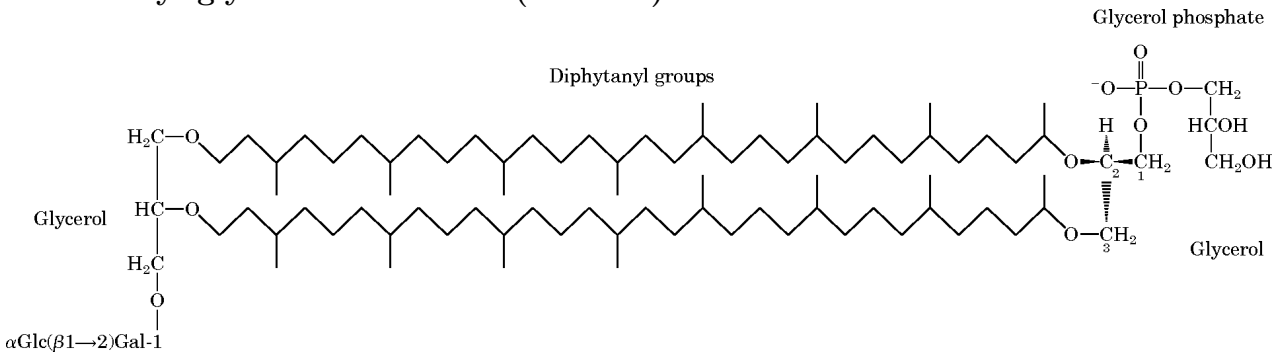
Glycolipids



Archaea membrane lipids

- Archaea (or archebacteria) have highly specific biochemistry
- Their membranes contain glycerol dialkyl glycerol tetraethers (GDGTs) which are double esters (have glycerol from both ends) and span the whole membrane
- These membranes are much more stable to high temperatures and low pH

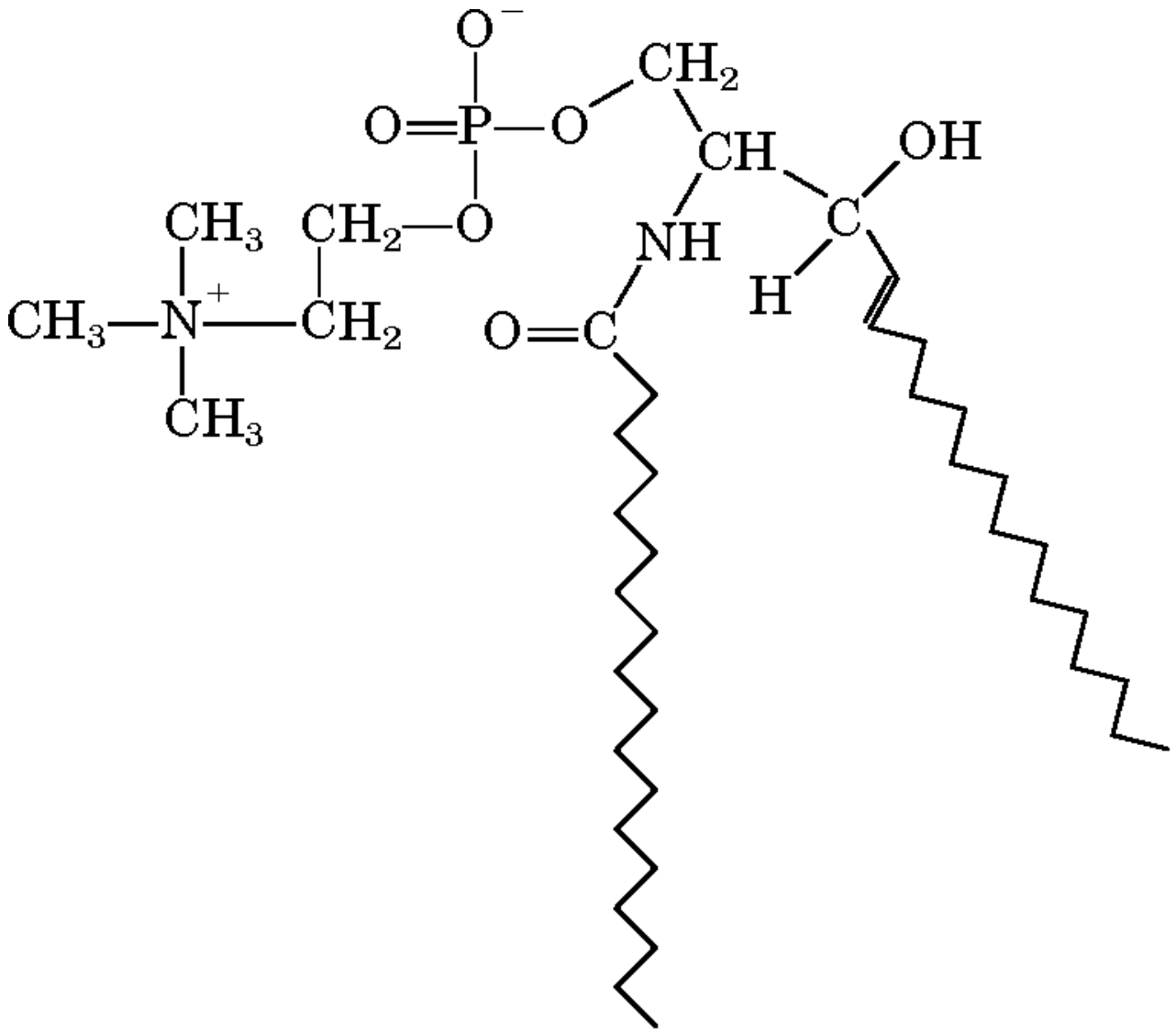
Glycerol dialkyl glycerol tetraethers (GDGTs)



Sphingolipids

- Sphingolipids are composed of one *sphingosine* (long chain amino-alcohol), one polar head and one fatty acid
- Again, head + two tails structure
- Sphingolipids in the membrane are important sites of biological recognition; nervous cells are especially rich of sphingolipids

Sphingomyelin

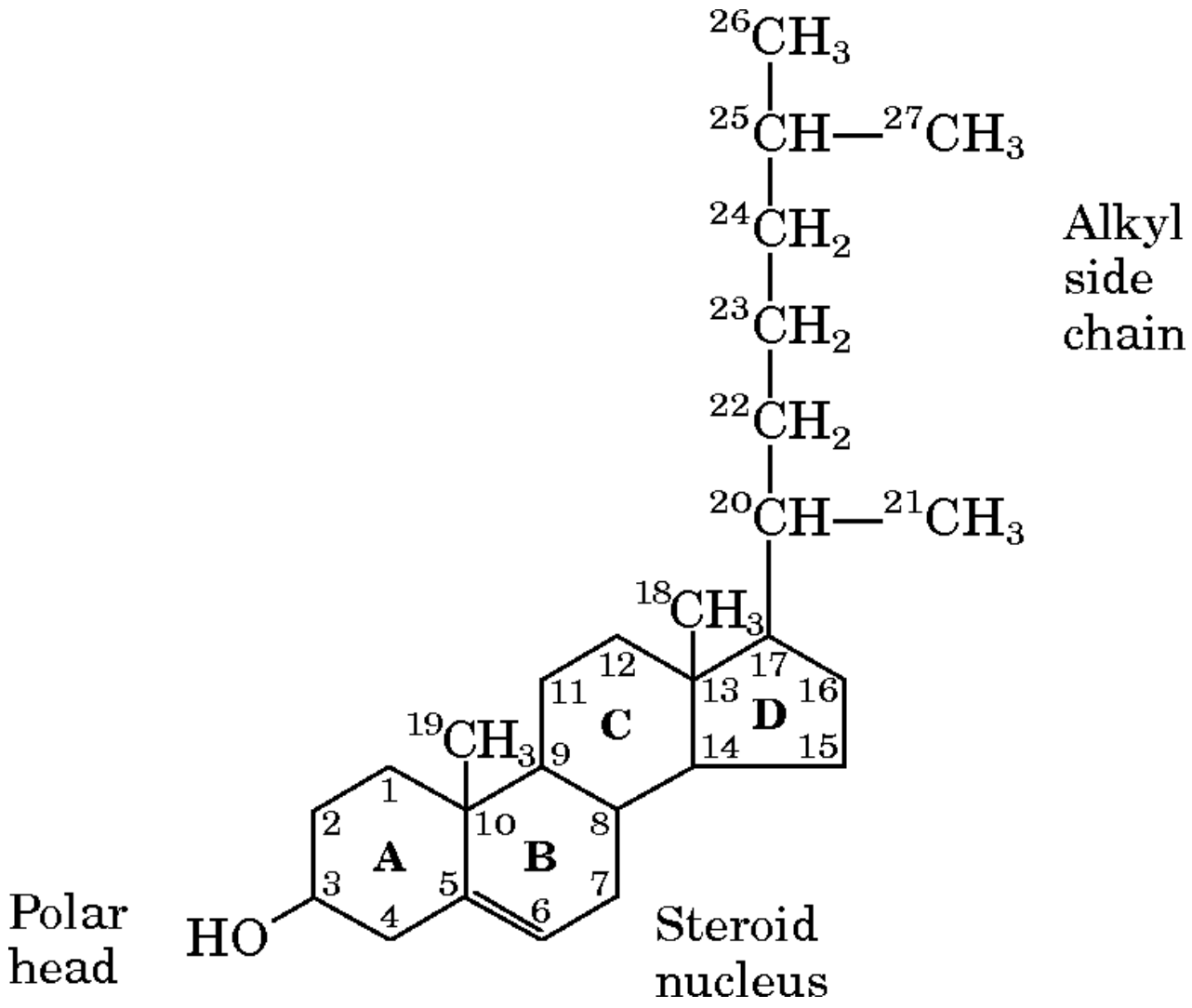


Sphingomyelin

Cholesterol

- Cholesterol is a *sterol*: molecule with four fused carbon rings
- One of main components of membrane, and also precursor to steroid hormones and other molecules
- Coronary disease is directly connected with cholesterol metabolism

Cholesterol

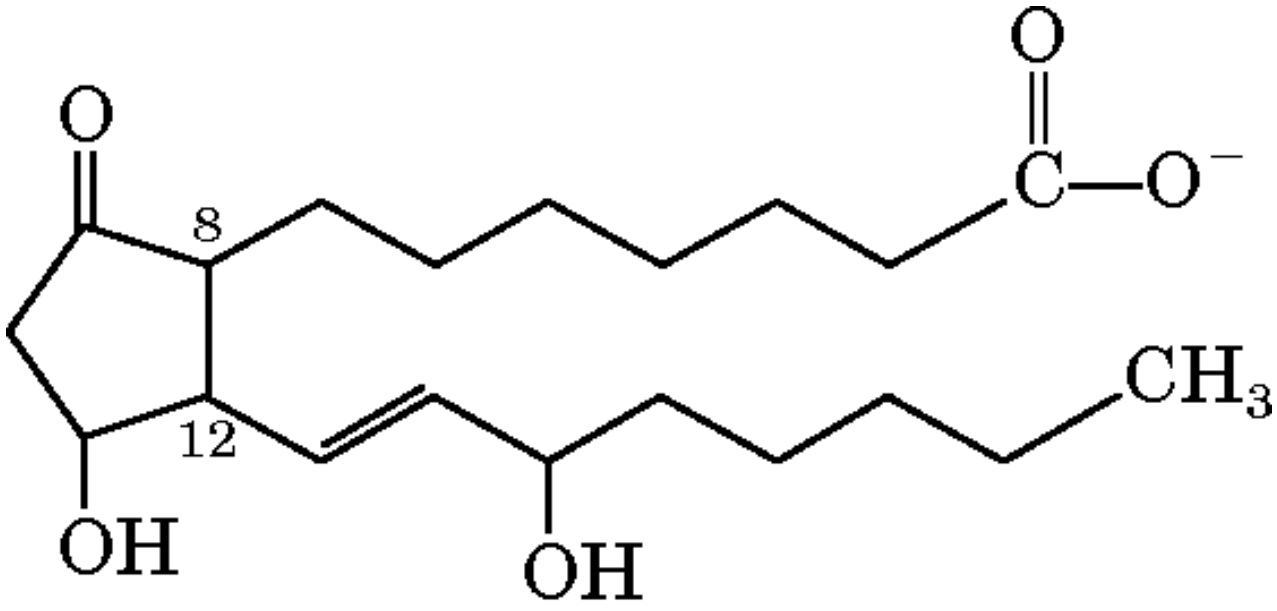


J.3 Signal lipids: sterols and others

Eicosanoids: derivatives of fatty acids

- *Eicosanoids* are hormones—biochemical signals
- They are structurally similar to membrane lipids
- Some of them, e.g., *prostaglandins*, play important physiological roles

Prostaglandin, one of eicosanoids

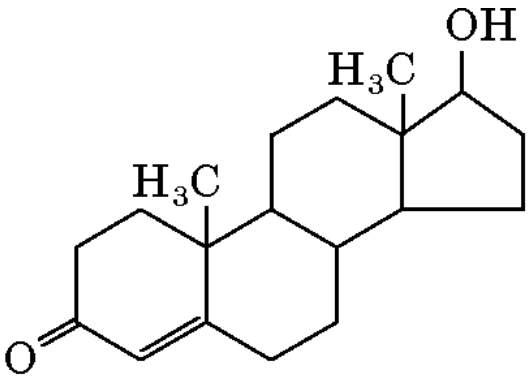


Prostaglandin E_1
(PGE_1)

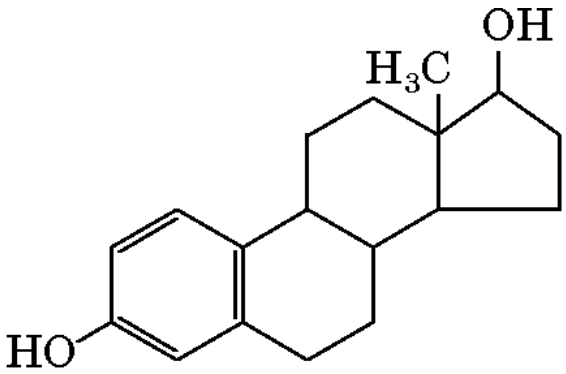
Steroids

- *Steroids* are derivates of *sterols* (mostly cholesterol)
- Occur both in plants and animals
- Have high specificity to receptors and therefore are producing in small quantities
- In vertebrates, play a role of sex hormones

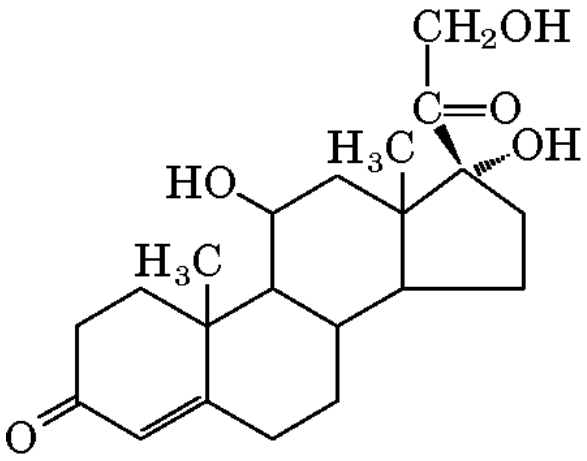
Steroids



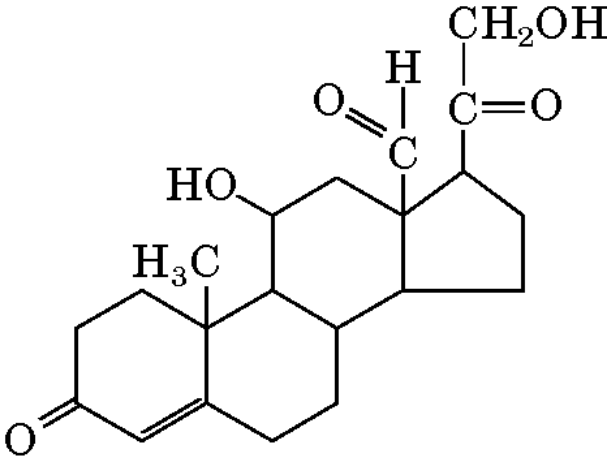
Testosterone



Estradiol



Cortisol



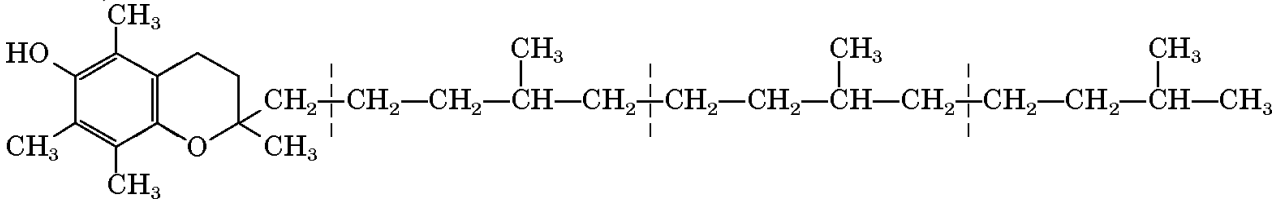
Aldosterone

J.4 Lipid vitamins

Lipid vitamins

- Vitamin **D** (close to sterols) transforms into hormone regulating calcium uptake
- Vitamin **A** (retinol) transforms into retinal which is a main light response pigment of eye
- Vitamin **E** (tokoferol) assists in numerous biosynthetic processes

Vitamin E, tokoferol

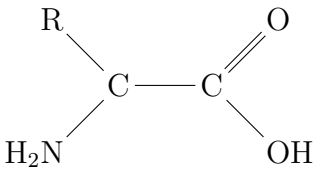


K Amino acids

K.1 Structure and classification

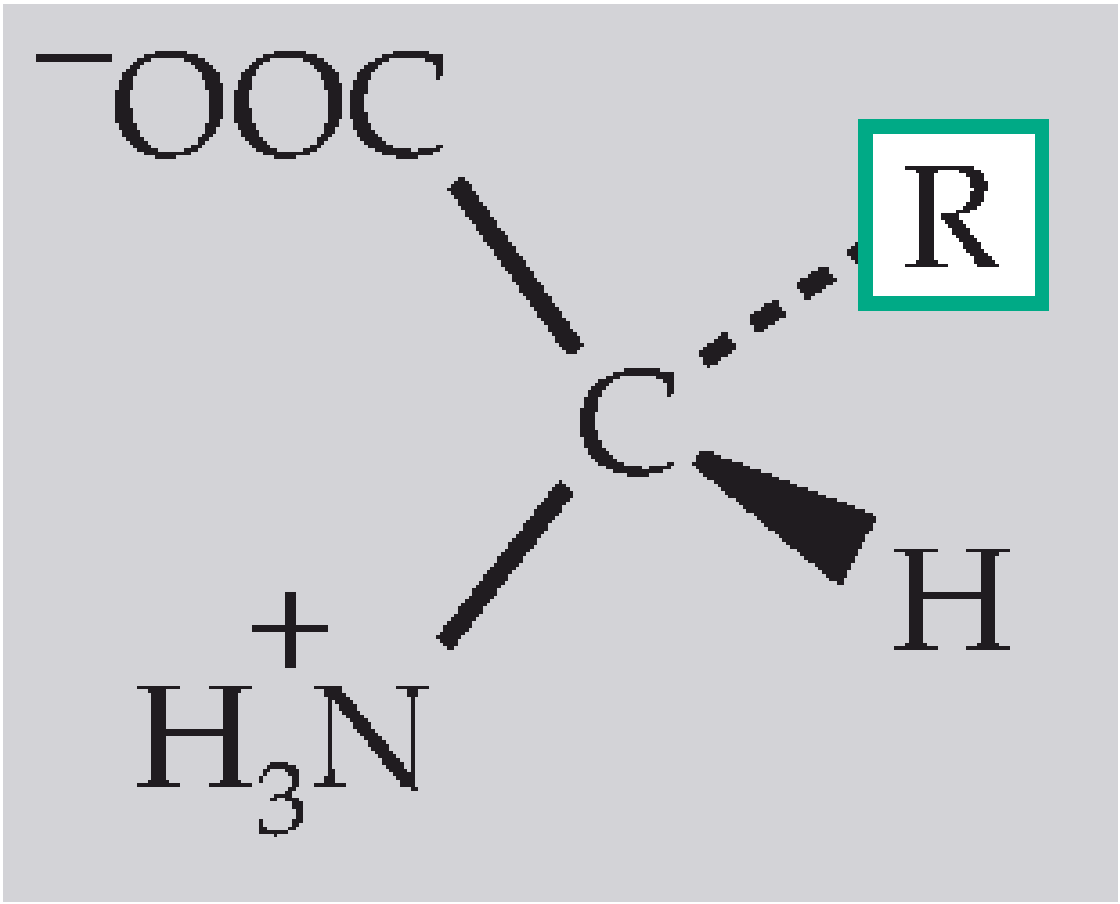
Structure

- Typical formula is $H_2NCHRCOOH$ where R is organic radical:



- Amino group is normally attached to the first carbon in the chain: α-aminoacids

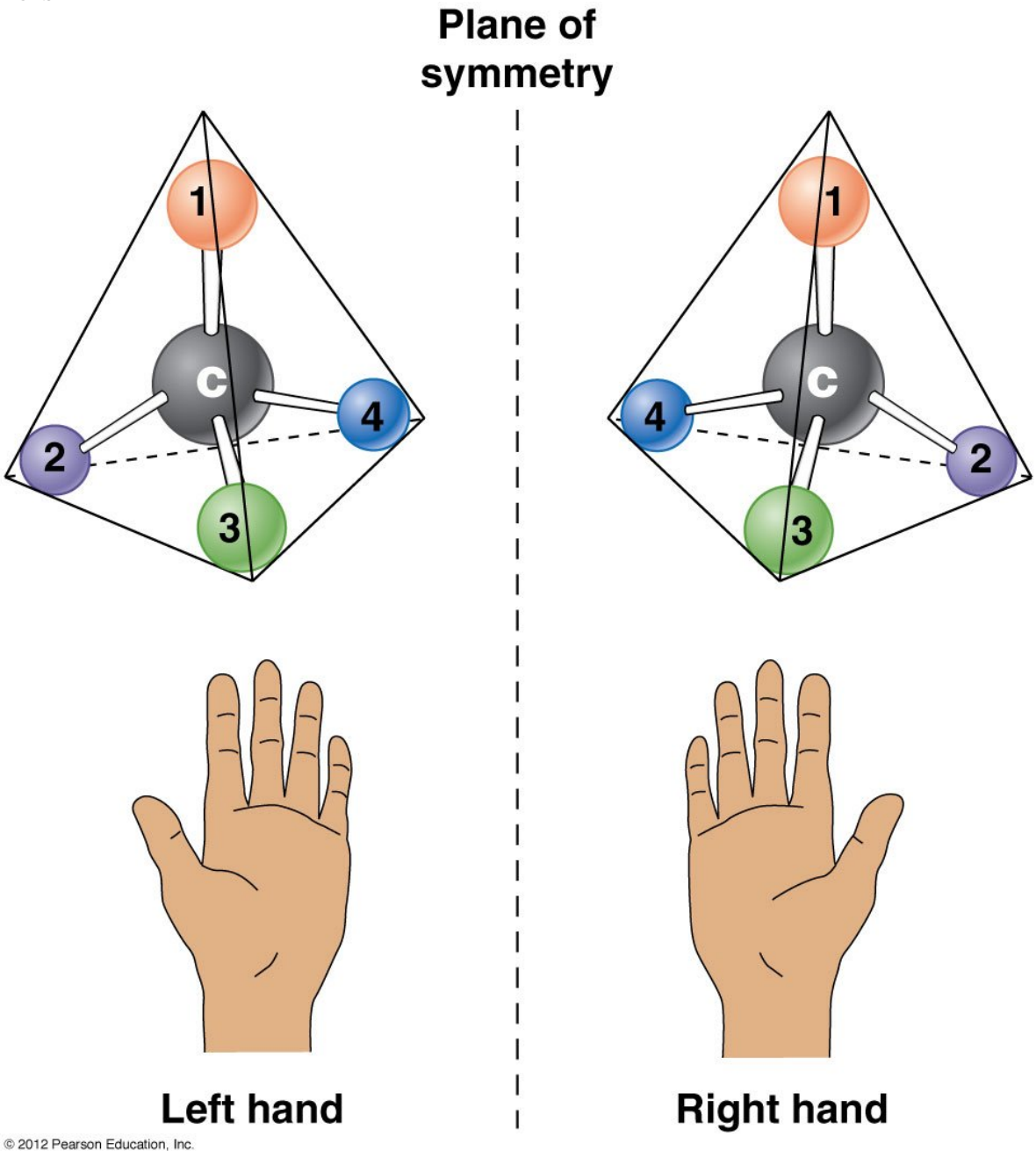
Amino acid



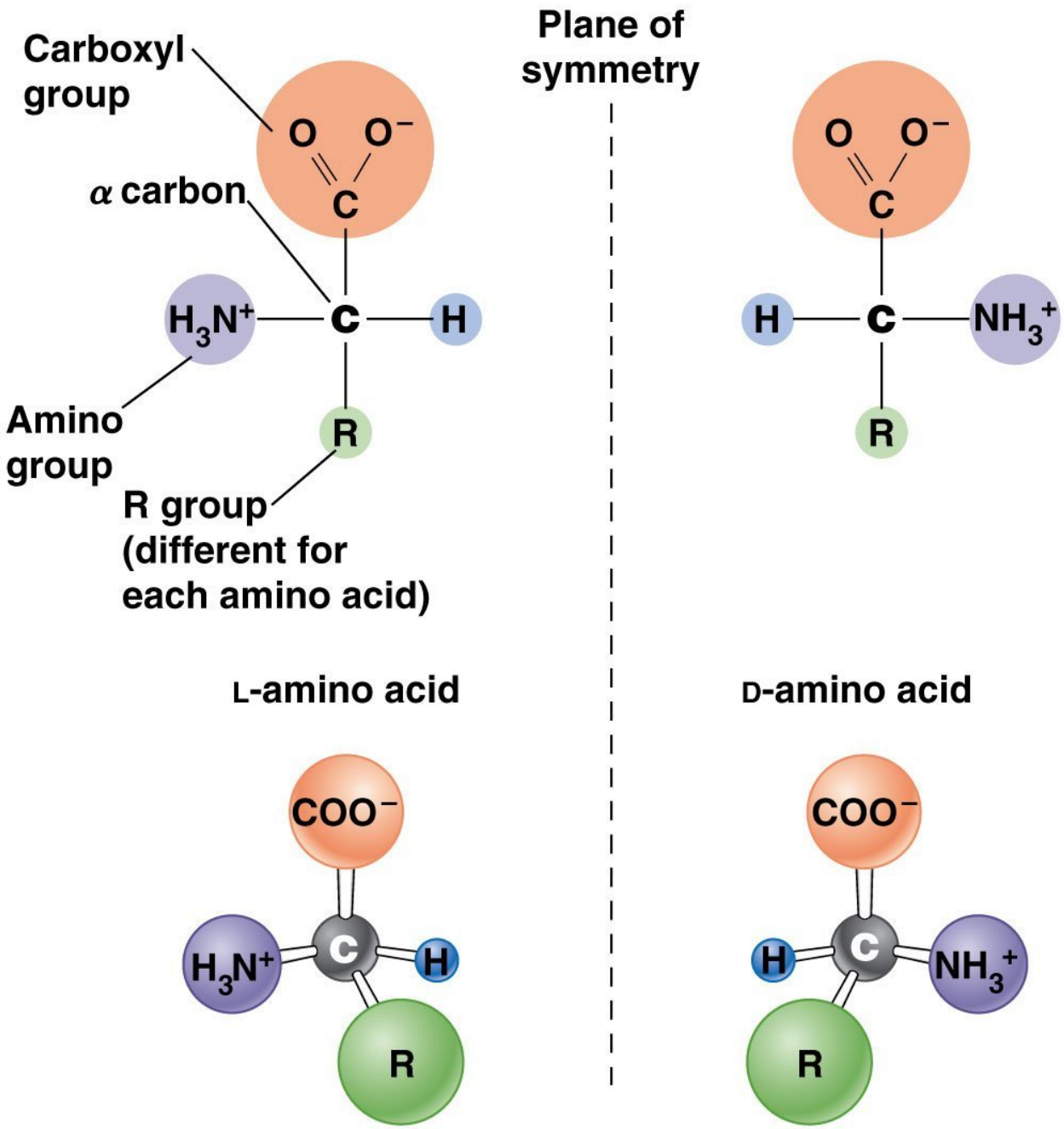
Isomerism

- α-carbon is an asymmetric atom
- Therefore, two optical isomers are possible
- However, in nature only L-isomers occur

3D isomers



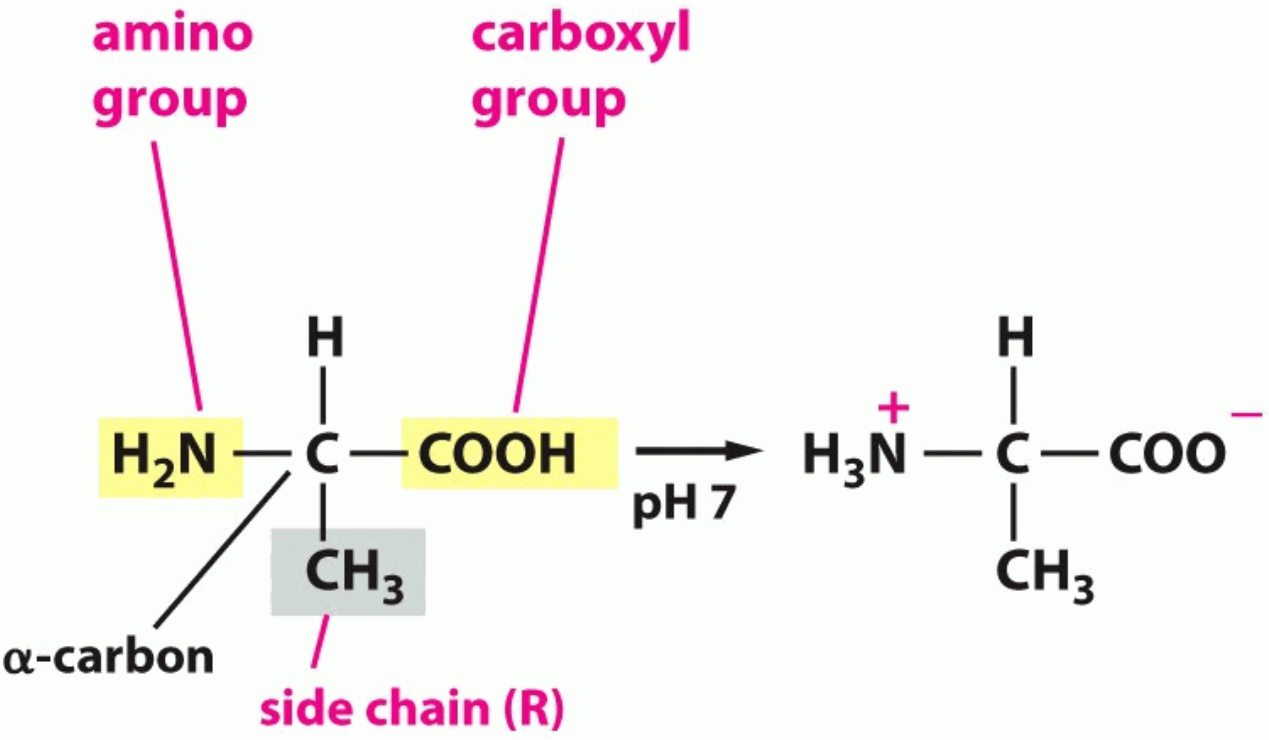
L- and D- isomers



Zwitterions

- If radical is neutral, amino acid could behave as both acid and base
- Many amino acids are present in water solution as **zwitterions**: polar structures similar to both acids and bases

Zwitterion



Diversity: 20 standard and 2 additional

- Standard amino acids are structural units of proteins; they are encoded via DNA triplets
- There are exactly 20 standard amino acids
- In addition, there are two amino acids (selenocysteine and pyrrolysine) which may be coded in deviated genetic codes of some organisms

Essential and non-essential

- Essential amino acids could not be synthesized in human body
- Non-essential amino acids are derivatives of essential
- In all, there are eight essential amino acids:

1. Isoleucine
2. Leucine
3. Lysine
4. Methionine
5. Phenylalanine
6. Threonine
7. Tryptophan
8. Valine

Final question (2 points)

Which role in the cell lipids do NOT play?

Summary

- Lipids are extremely diverse; the only character uniting them is their hydrophobic behavior
- Lipids are extremely diverse; the only character uniting them is their hydrophobic behavior
- There are 20 (+2) standard amino acids classifying in 9 groups

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 2*: : Molecules in cells, Panels 2–5.

Outline

L Questions and answers

Previous final question: the answer

Which role in the cell lipids do NOT play?

- Protein, DNA, RNA synthesis
- Transport
- Cellular respiration
- ~~Making energy~~
- ~~Exo- / endocytosis~~

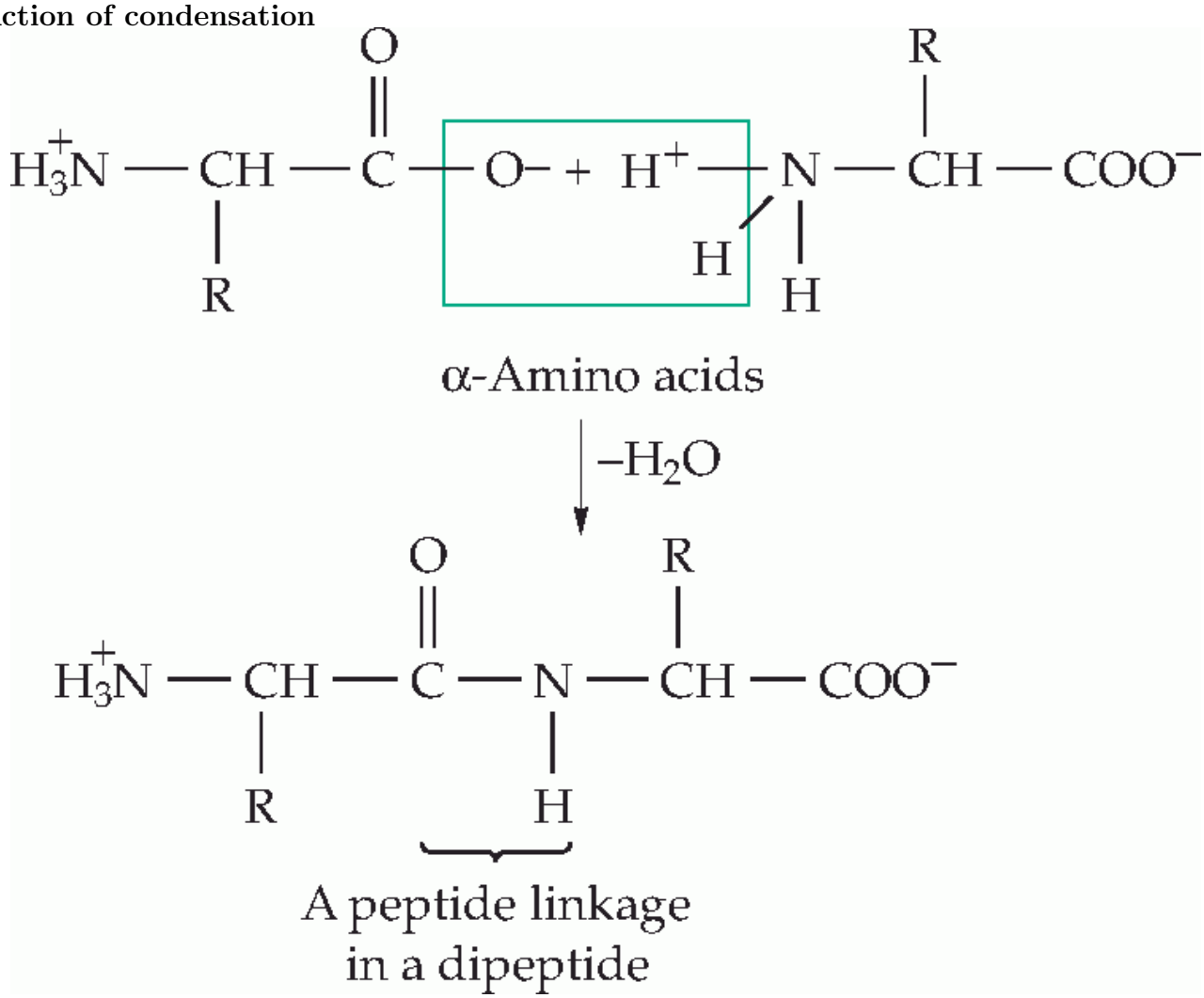
M Amino acids

M.1 Structure and classification

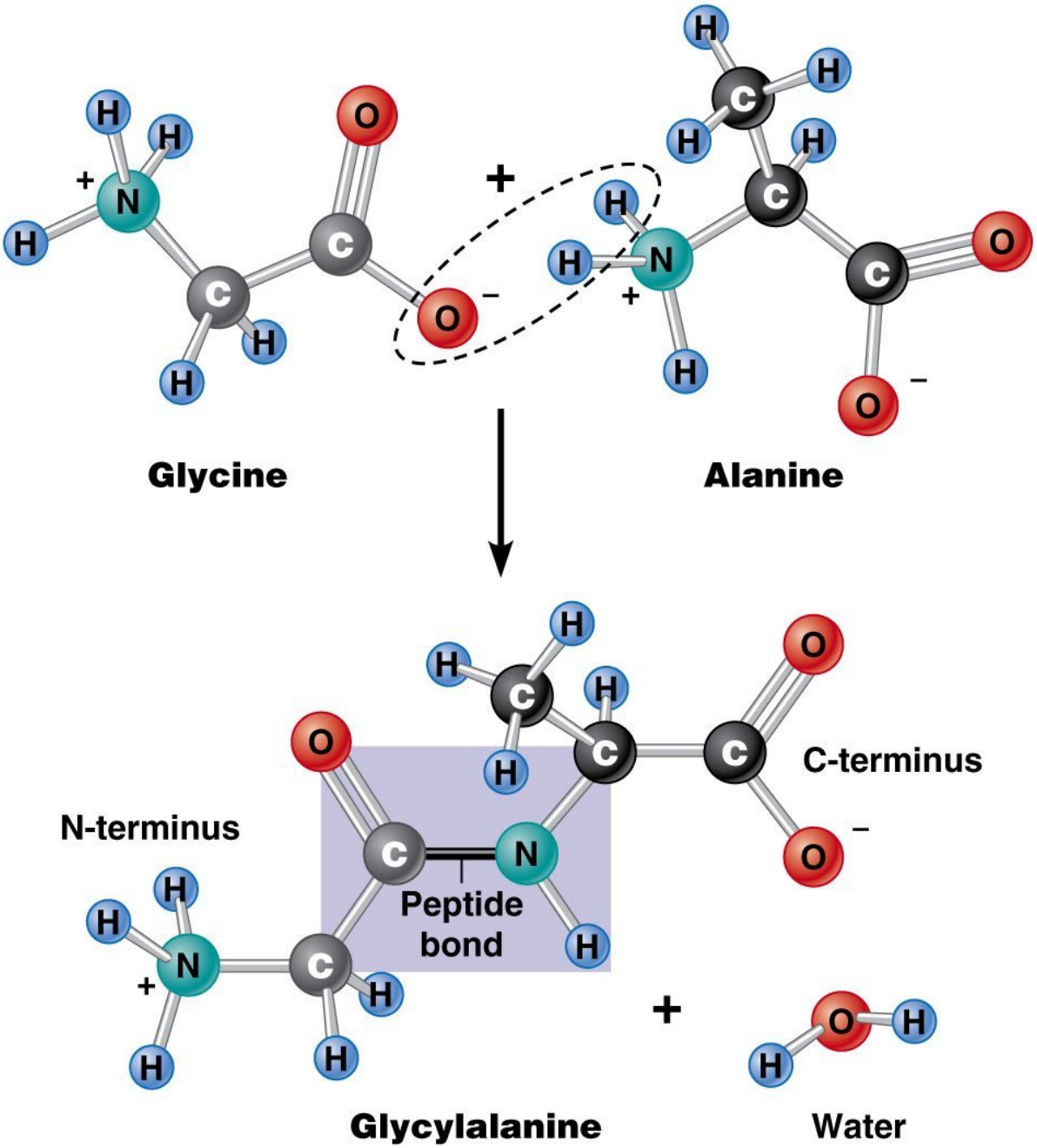
Peptide bonds

- Amino acids may group together in peptides via peptide bonds
- This is **reaction of condensation**, it results also in one water molecule

Reaction of condensation



Reaction of condensation (3D)



Roles

- Most important: components of peptides and proteins
- Many are intermediate stages of different biosynthetic processes, e.g.:
  - *Tryptophan* is a precursor of the neurotransmitter *serotonin*
  - *Aspartate*, *glycine* and *glutamine* are precursors of nucleotides

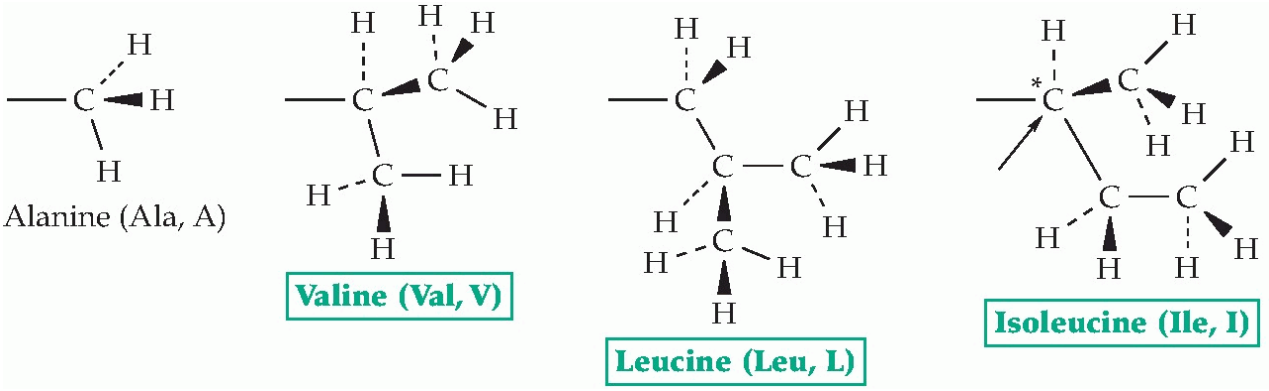
Amino acid diversity by groups: glycine

- Simplest amino acid
- "R" is simply H in case of glycine

Alkyls

- Have hydrocarbon groups as radicals
- Hydrophobic and therefore involving in protein shaping

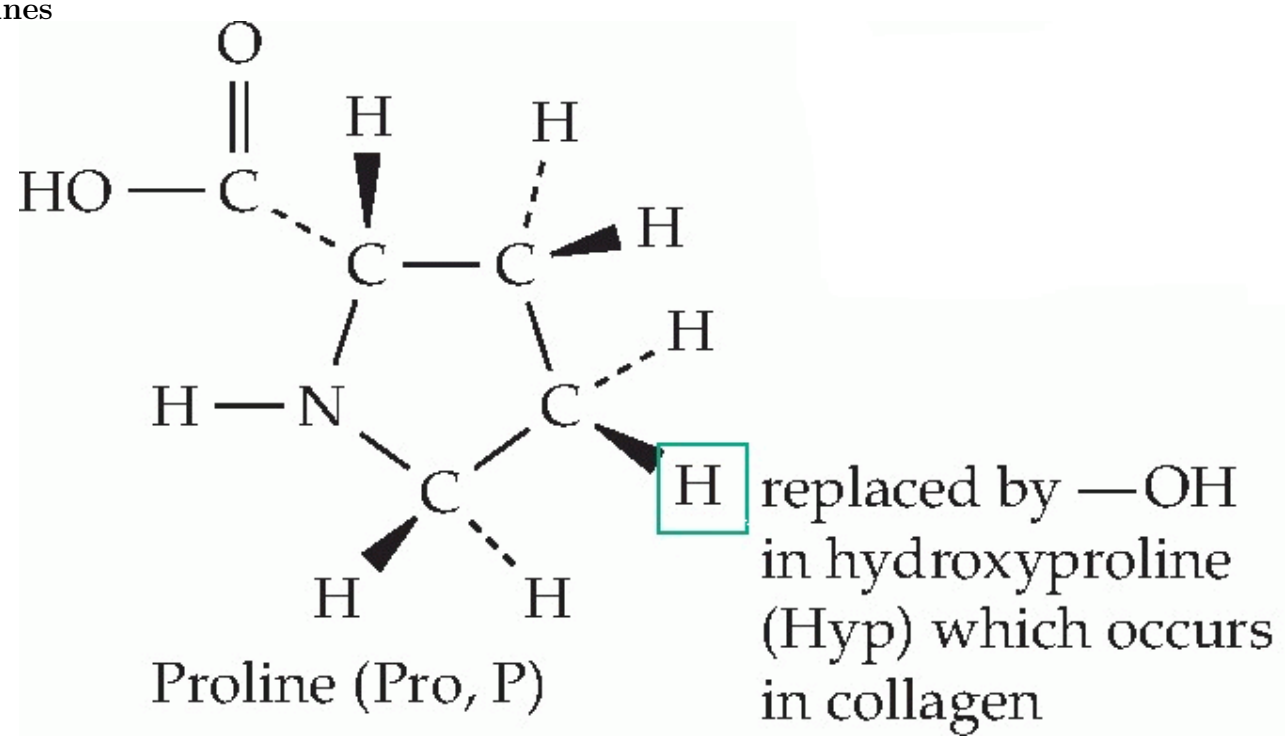
Alkyl amino acids



Imines

- Not an amino acids in a strict sense because there are no α-carbon
- Molecule is rigid and influence protein folding

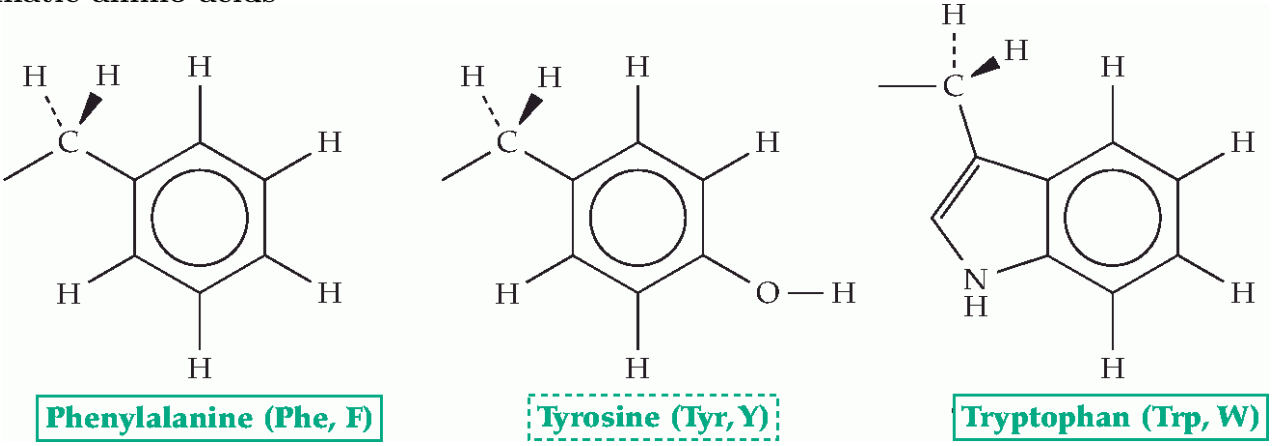
Imines



Aromatic

- Contain benzene groups
- May form hydrophobic bonds

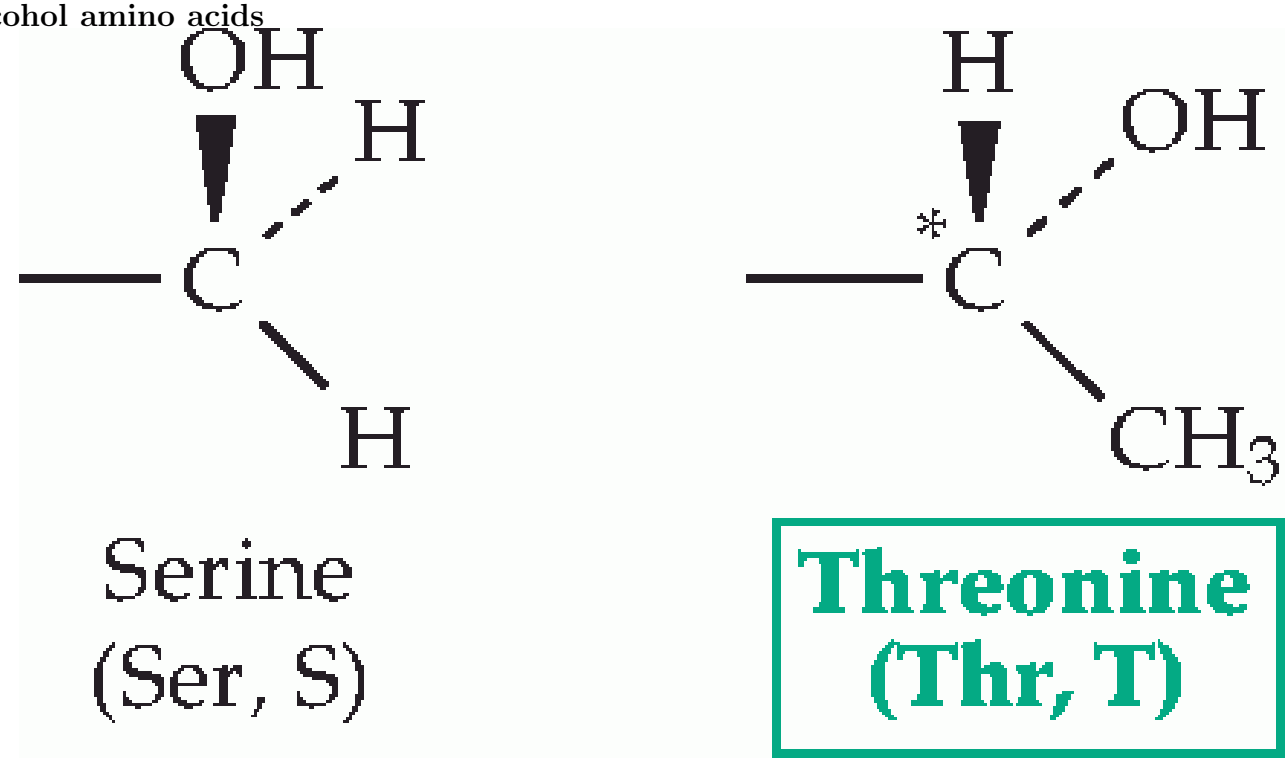
Aromatic amino acids



Alcohols

- –OH group is very weakly acidic
- May form active centers of some enzyme proteins

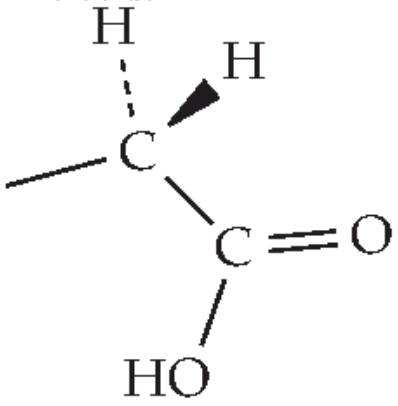
Alcohol amino acids



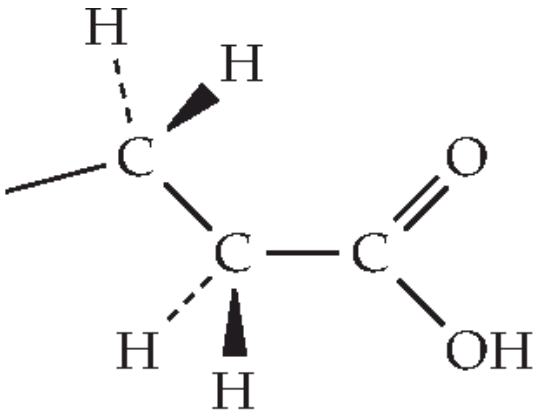
Acidic amino acids

- Weak acids
- Provide anionic (–) groups on the surface of proteins

Acidic amino acids



Aspartic acid (Asp, D)

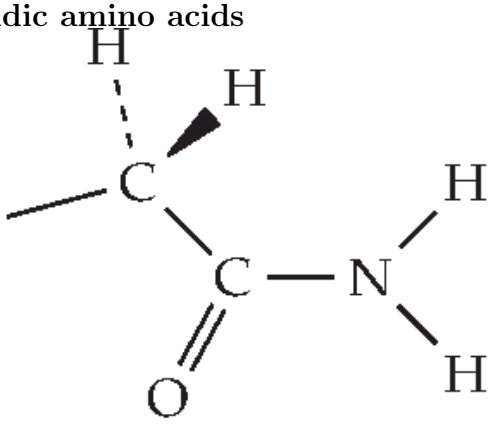


Glutamic acid (Glu, E)

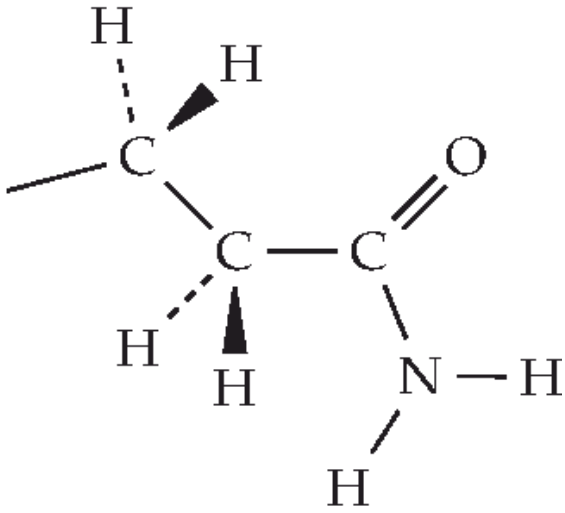
Amidic

- Contain amide group –CONH
- Not acidic, but polar and therefore participate in hydrogen bonding

Amidic amino acids



Asparagine (Asn, N)



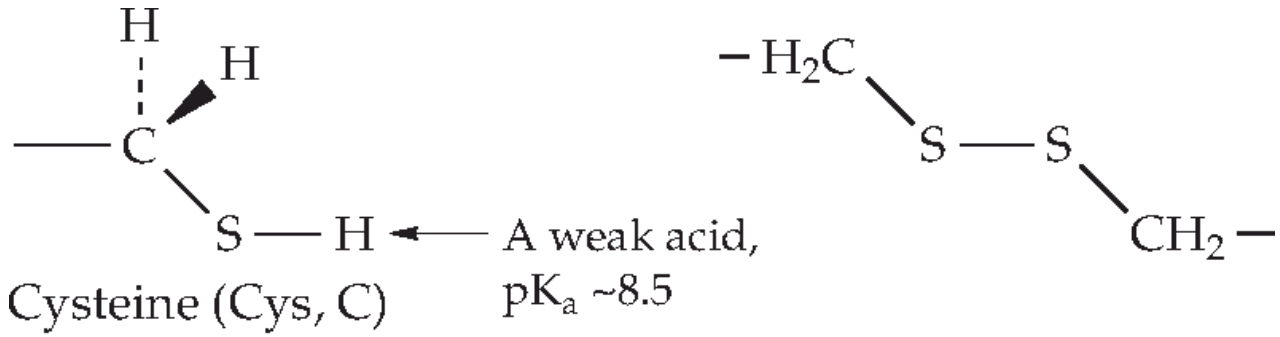
Glutamine (Gln, Q)

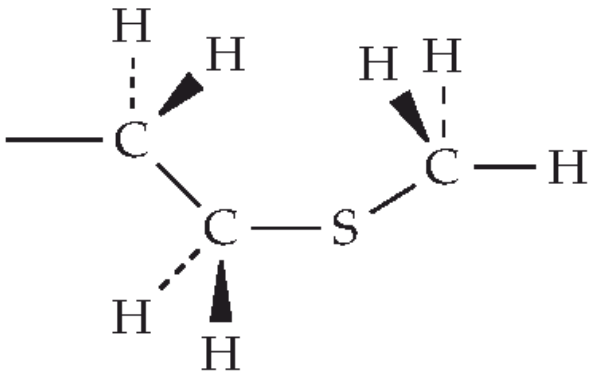
Sulfur-containing

- Two –SH groups of cysteine may form **disulfide bridge** between different parts of protein molecule

Disulfide bonds movie

Sulfur-containing amino acids



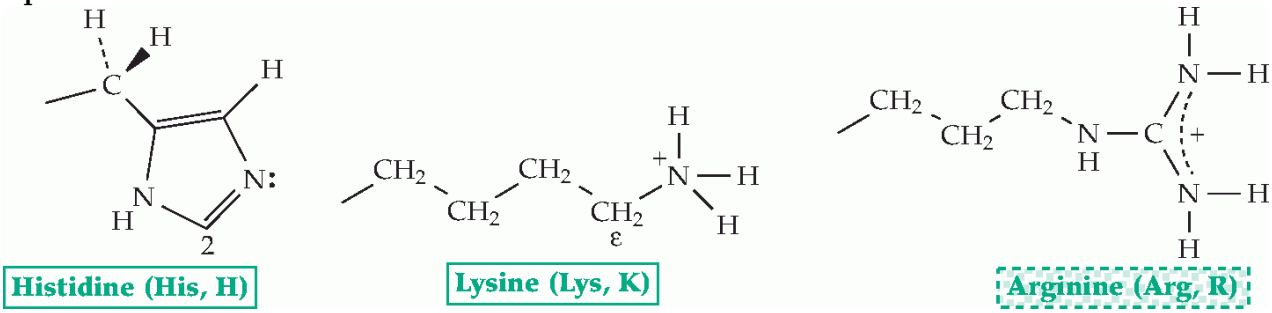


Methionine (Met, M)

Basic amino acids

- Contain different nitrogen basic groups
- Could be strong bases and therefore binds other molecules to proteins

Examples of basic amino acids

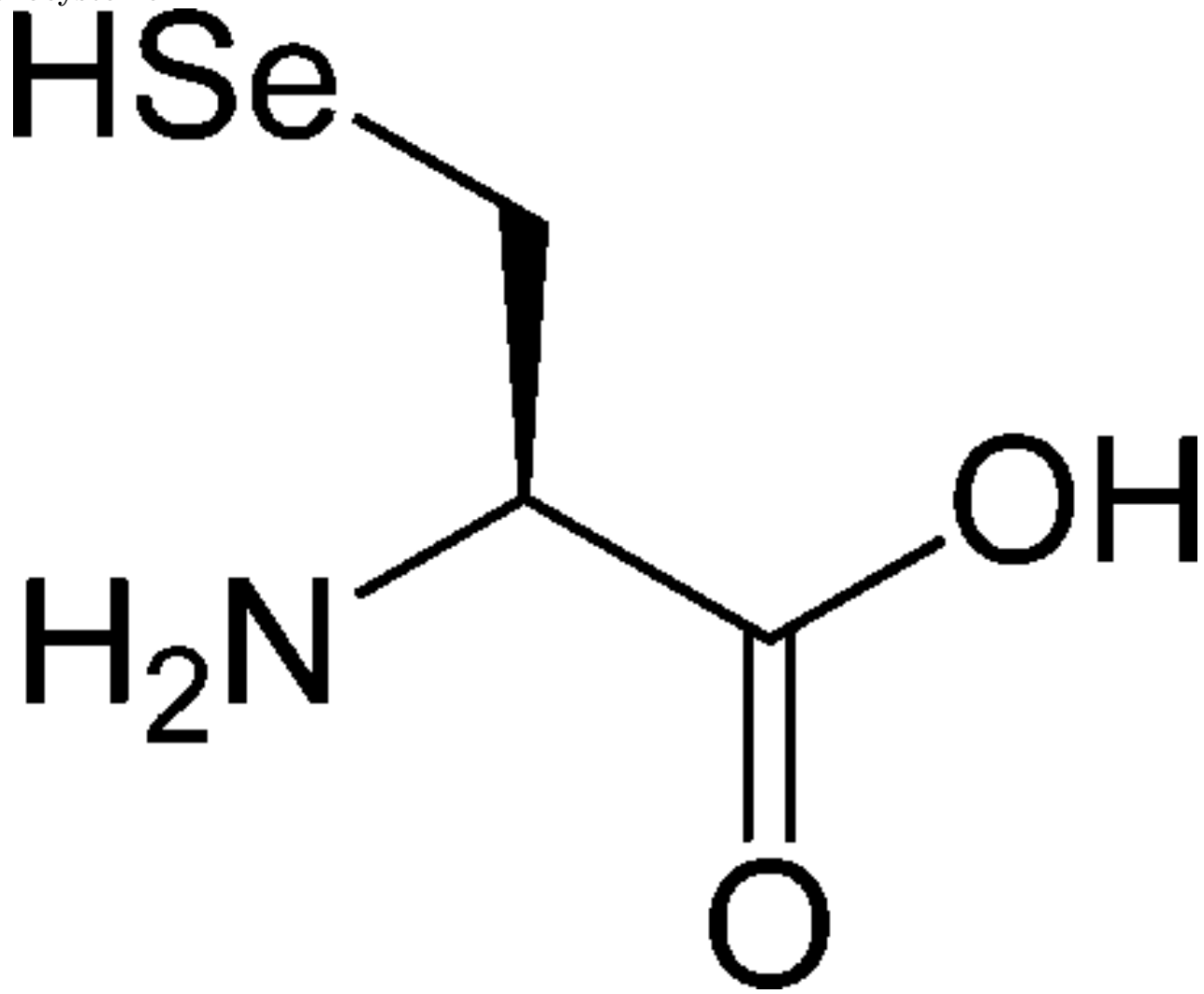


Histidine (His, H) **Lysine (Lys, K)** **Arginine (Arg, R)**

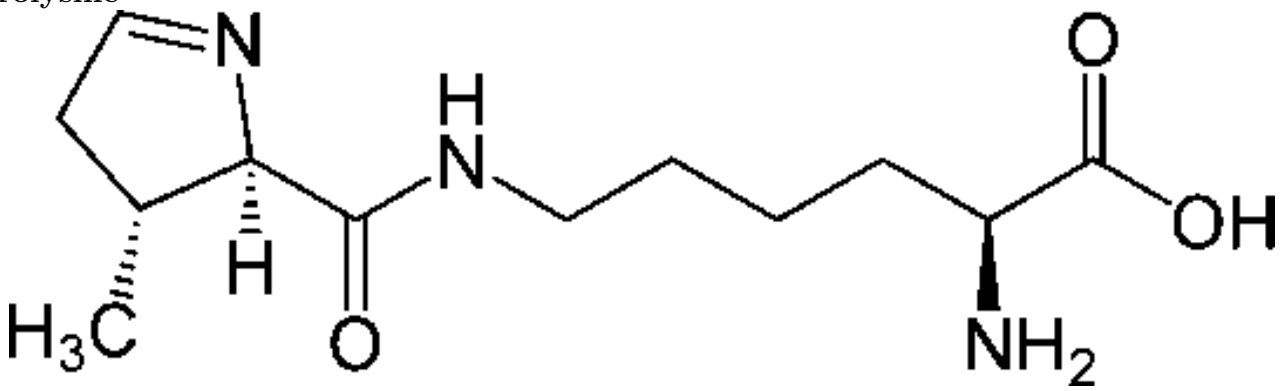
Two extra amino acids

- **Selenocysteine** is similar to cysteine, but selenium instead of sulfur, forming a selenol group and selenoproteins
- **Pyrrolysine** is similar to lysine bu with additional pyrroline ring, it presents in many proteins of archebacteria (archaea)
- They both depend on modified stop codons in RNA (normally, these codons break protein synthesis)

Selenocysteine



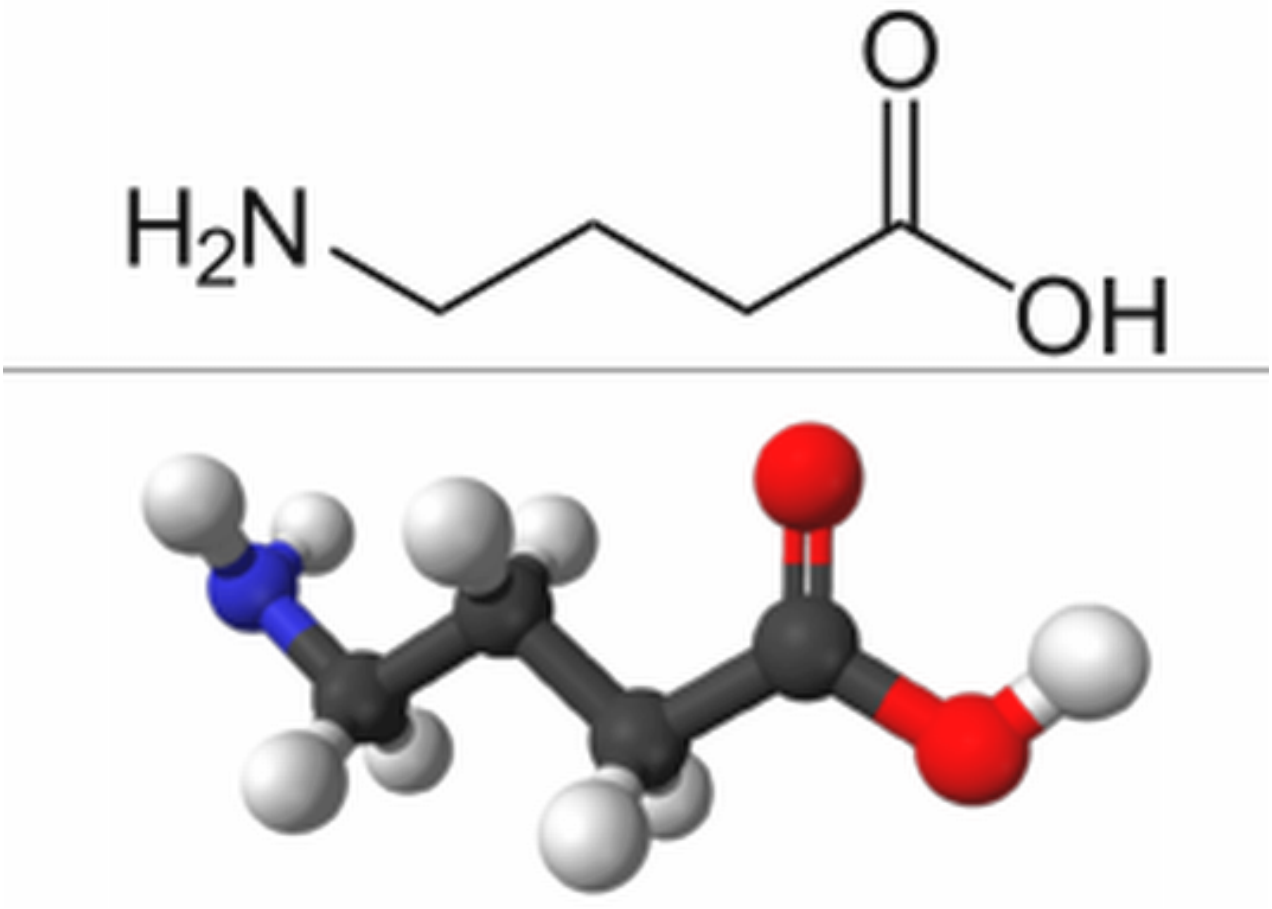
Pyrrolysine



Non-protein amino acids

- **Taurine** abundant in muscular and brain tissues but its functions still not known
- **γ-aminobutyric acid** (GABA) is non-α amino acid; it is one of main neurotransmitters in mammalian nervous system

GABA



N Nucleic acids

N.1 Structure and features

Composition of nucleic acids

- **Nucleic bases**—heterocycles with nitrogen
- **Pentose** in cyclic form
- **Phosphoric acid** H_3PO_4

Phosphate

- Simply H_3PO_4
- Normally, fully dissociated (lost 2 hydrogen ions)

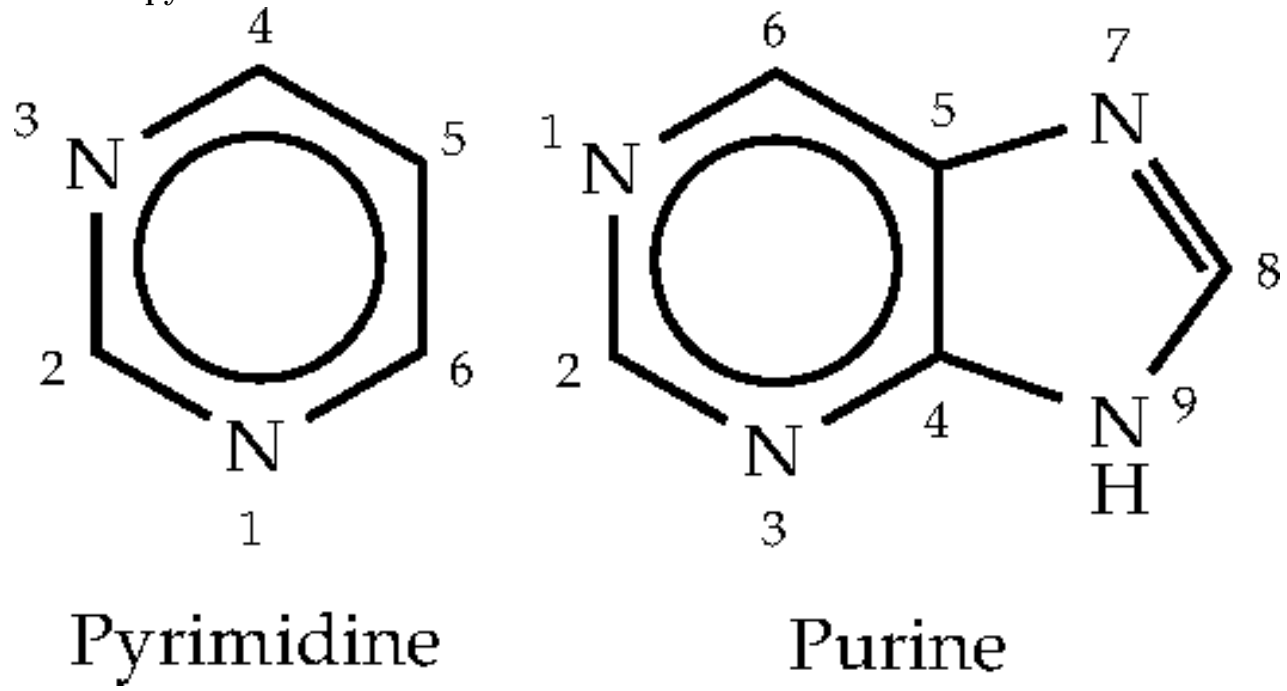
Pentose

- **Deoxyribose** (in DNA)
  OR
- **Ribose** (in RNA)

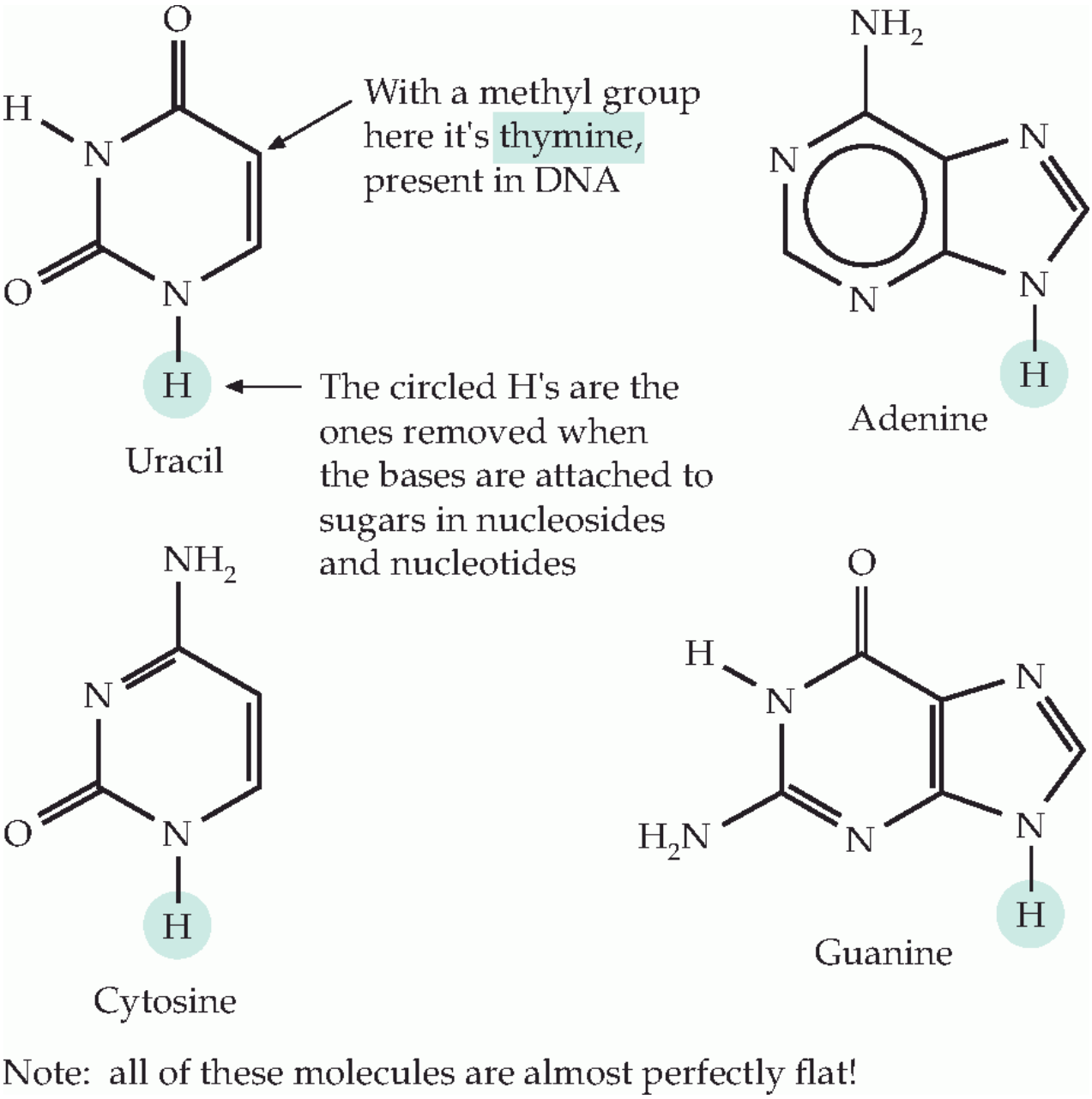
Nucleic bases and nucleosides

- **Pyrimidines** (1-cyclic): **uracil/thymine** or **cytosine**
- **Purines** (2-cyclic + amines): **adenine** or **guanine**
- *Nucleosides* are nucleic bases + pentoses

Purines and pyrimidines



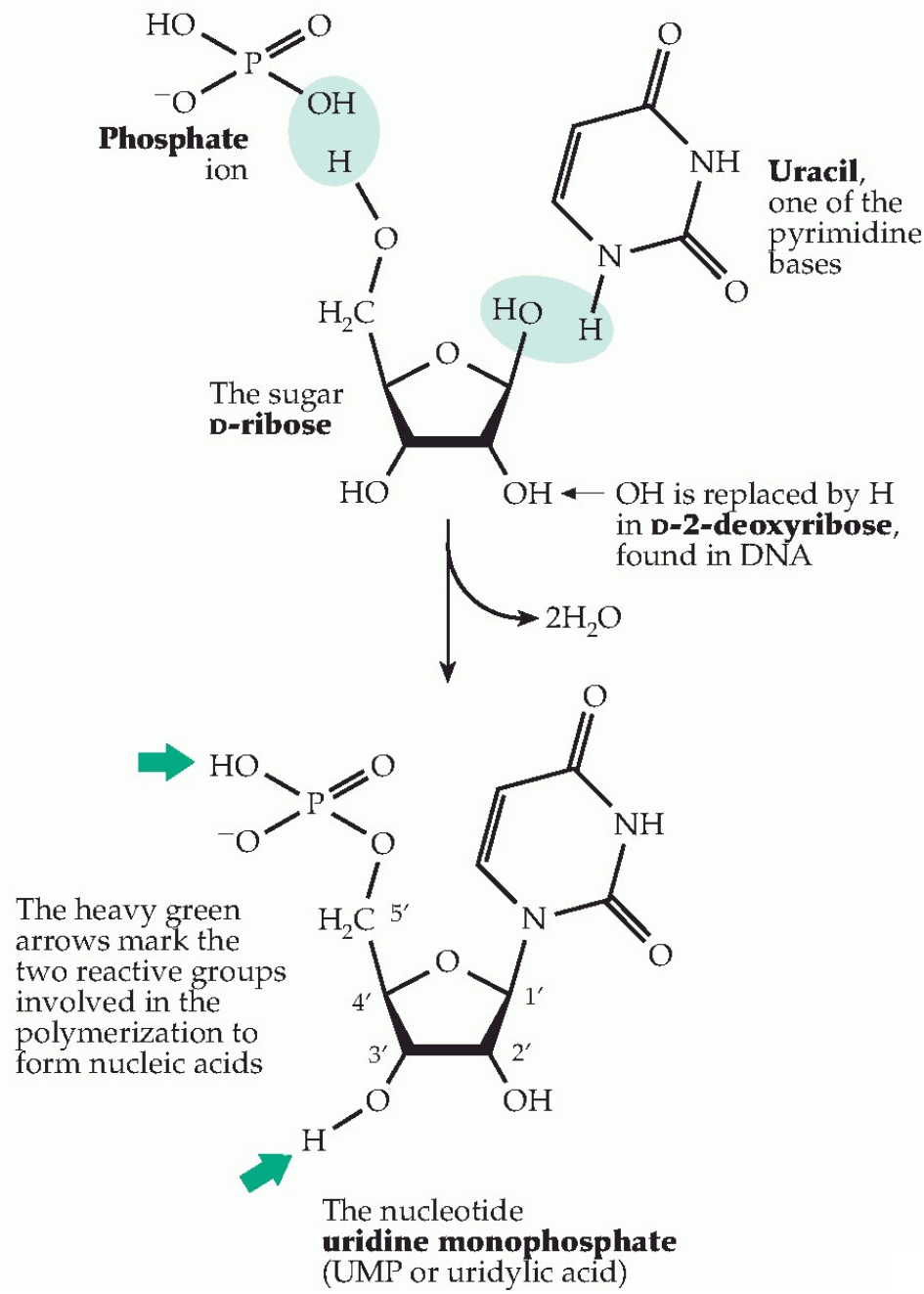
Nucleic bases



Nucleotide synthesis

- Double condensation
- First –OH groups from sugar and phosphoric acid used

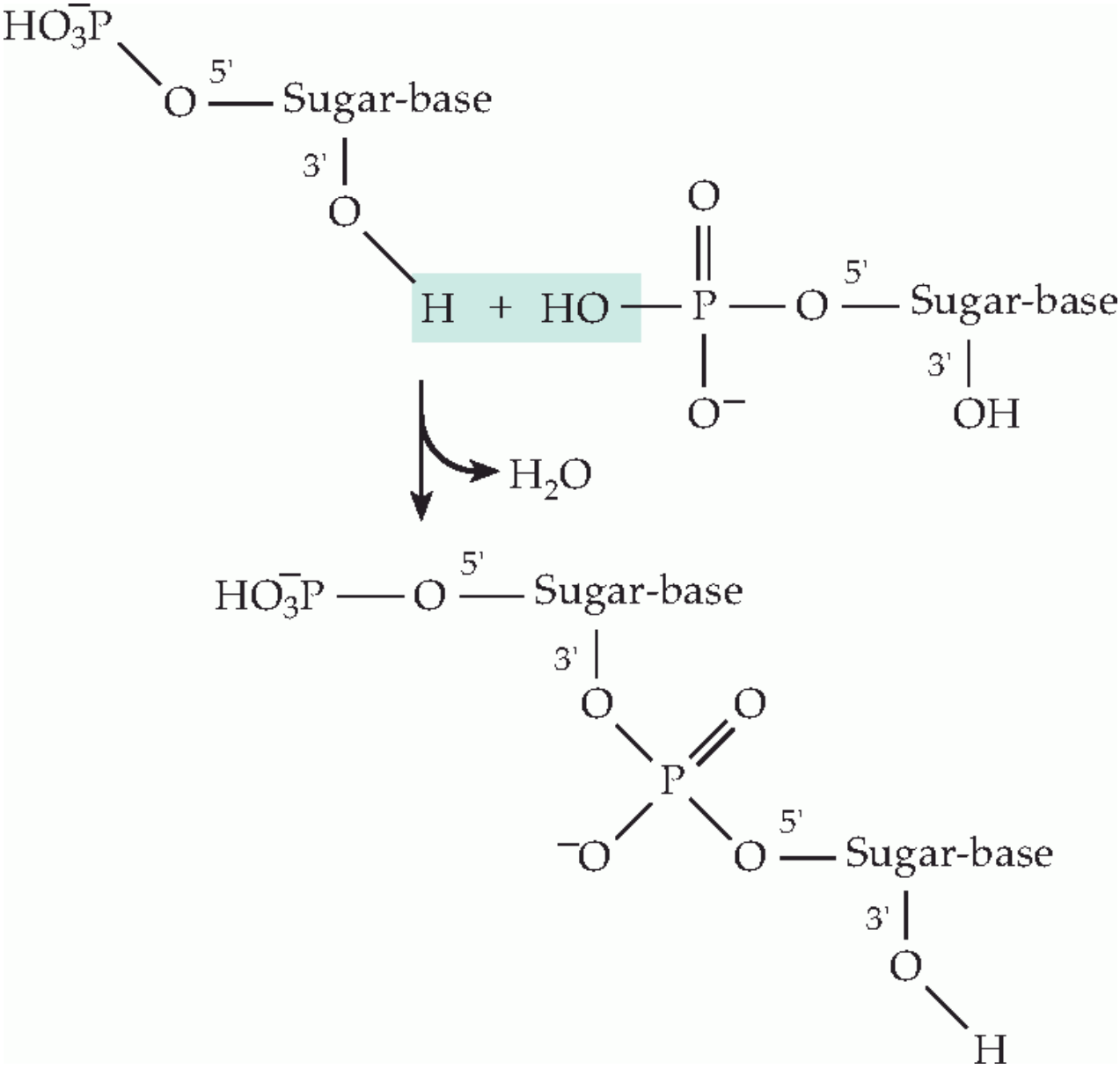
Formation of nucleotide



Nucleic acid synthesis

- Condensation between second free –OH groups of sugar and phosphoric acid
- Resulted polymer may have almost infinite length

Formation of nucleic acid polymers



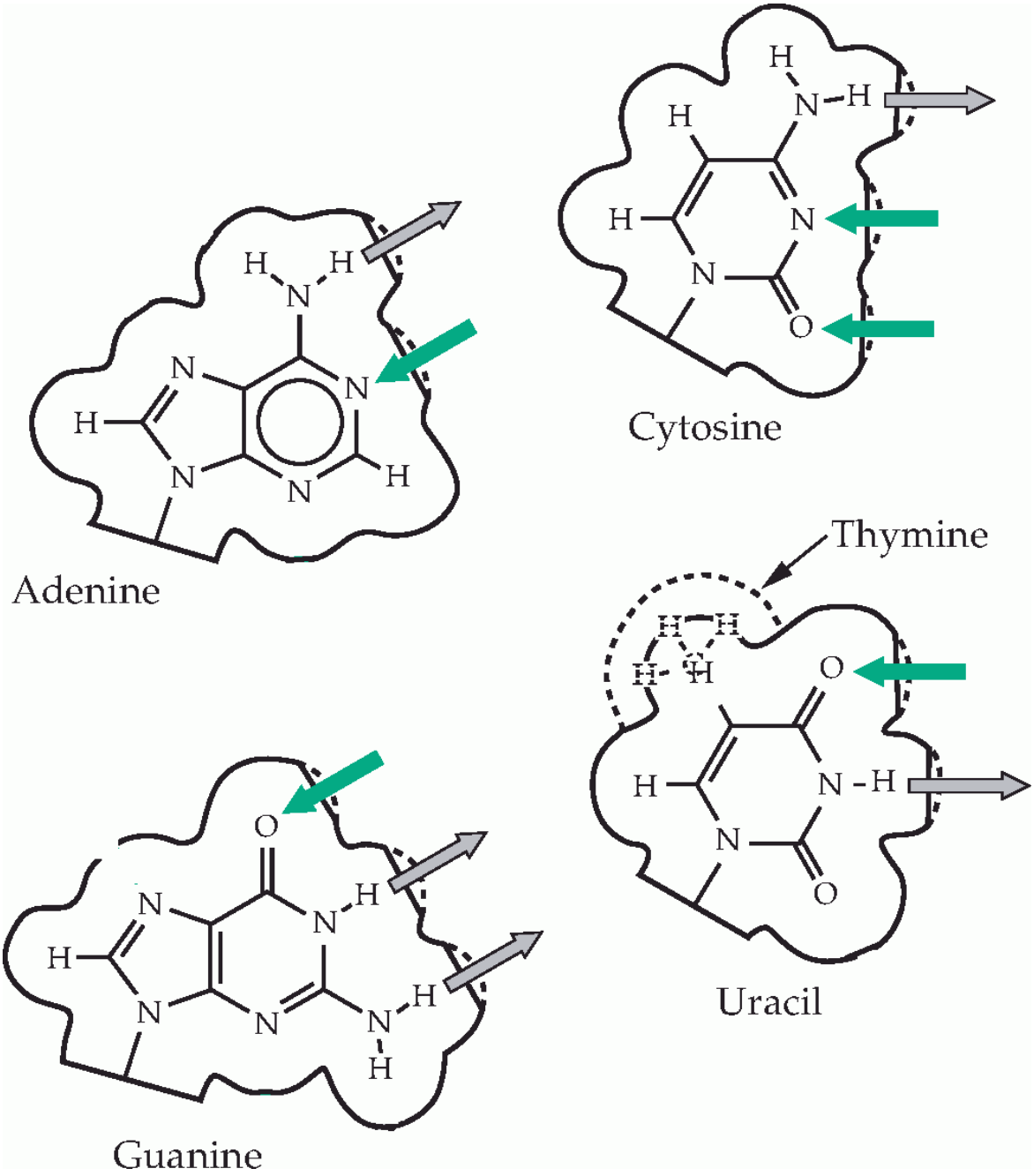
DNA and RNA chemistry

- Deoxyribose vs. ribose
- Thymine vs. uracil

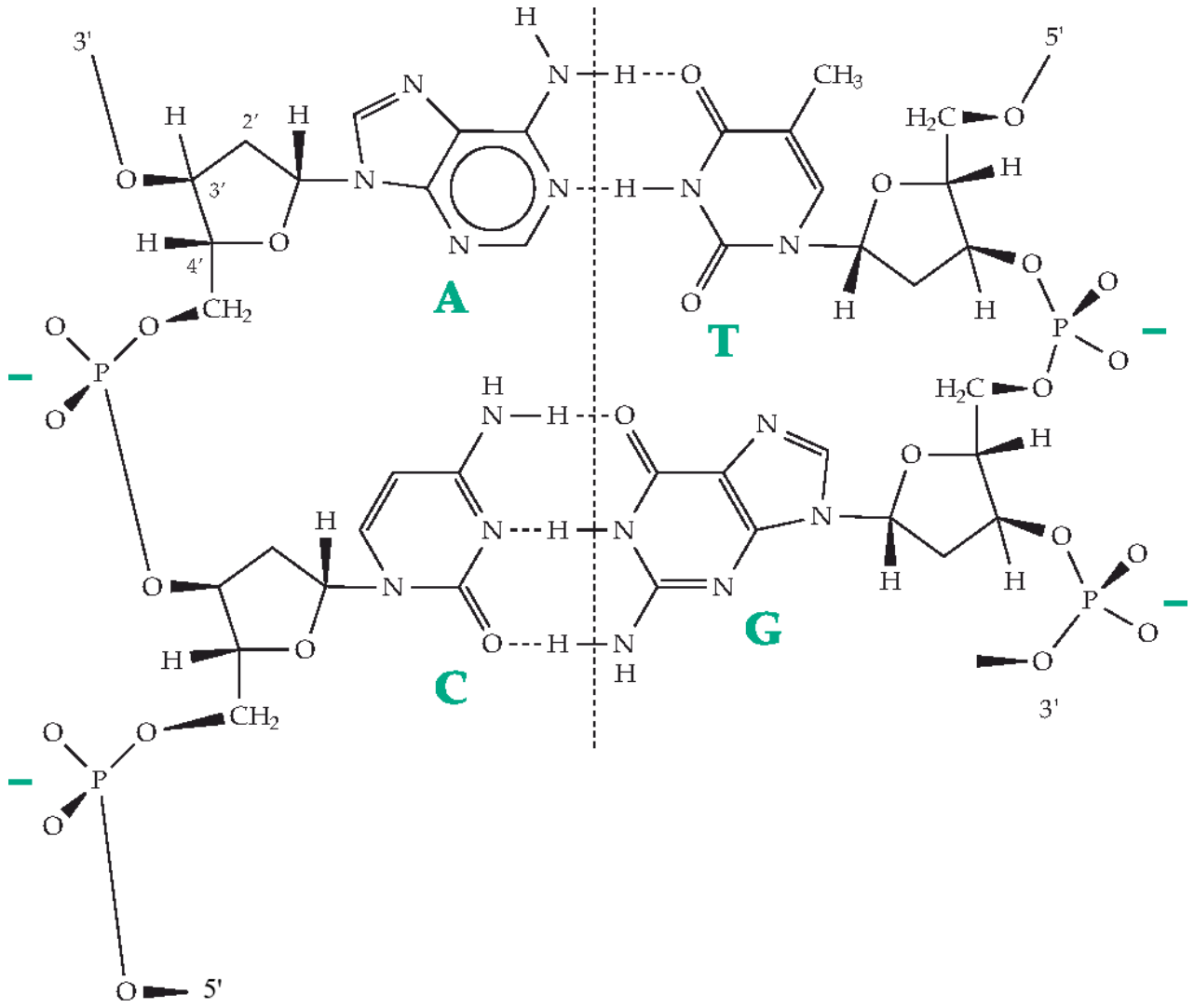
Hydrogen bonds, complementarity and base pairs

- 2 hydrogen bonds (one "in", one "out"): adenine and thymine/uracil
- 3 hydrogen bonds (one "in", two "out" in guanine): guanine and cytosine
- A–T and G–C are base pairs consist of *complementary nucleotides*

Hydrogen bonds between nucleotides



Hydrogen bonds in complementary strands



Final question (3 points)

Write a sequence complementary to **ATTGGAAGC**Is it from DNA or RNA?

Summary

- There are 20 (+2) standard amino acids classifying in 9 groups
- Nucleic acids are composition of purin/pyrimidin base, ribose/deoxyribose and phosphoric acid

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 2*: : Molecules in cells, Panels 2–6.

Outline

O Questions and answers

Previous final question: the answer

Write a sequence complementary to **ATTGGAAGC**Is it from DNA or RNA?

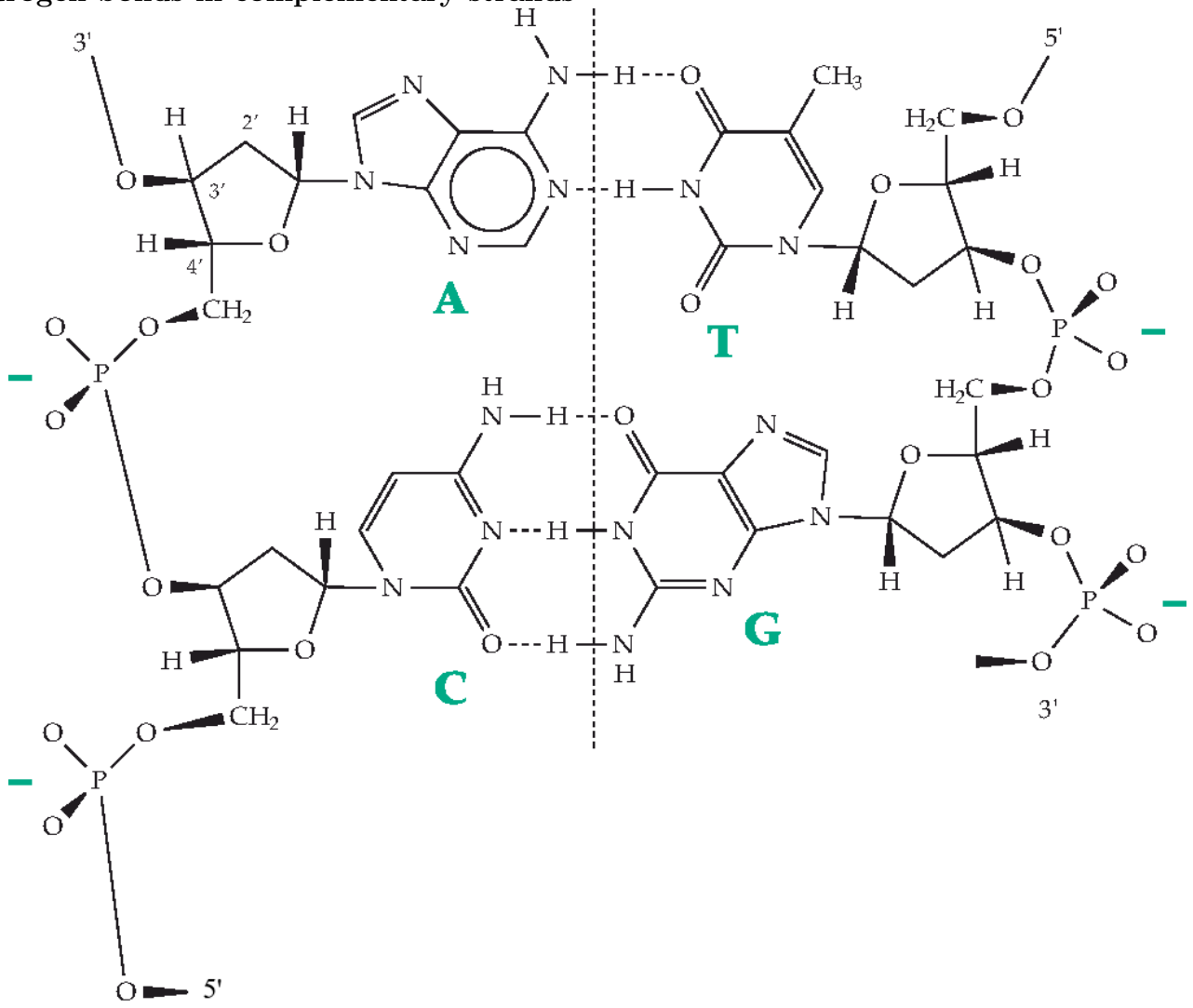
- TAACCTTCG
- DNA

Disulfide bonds movie

P Nucleic acids

P.1 Structure

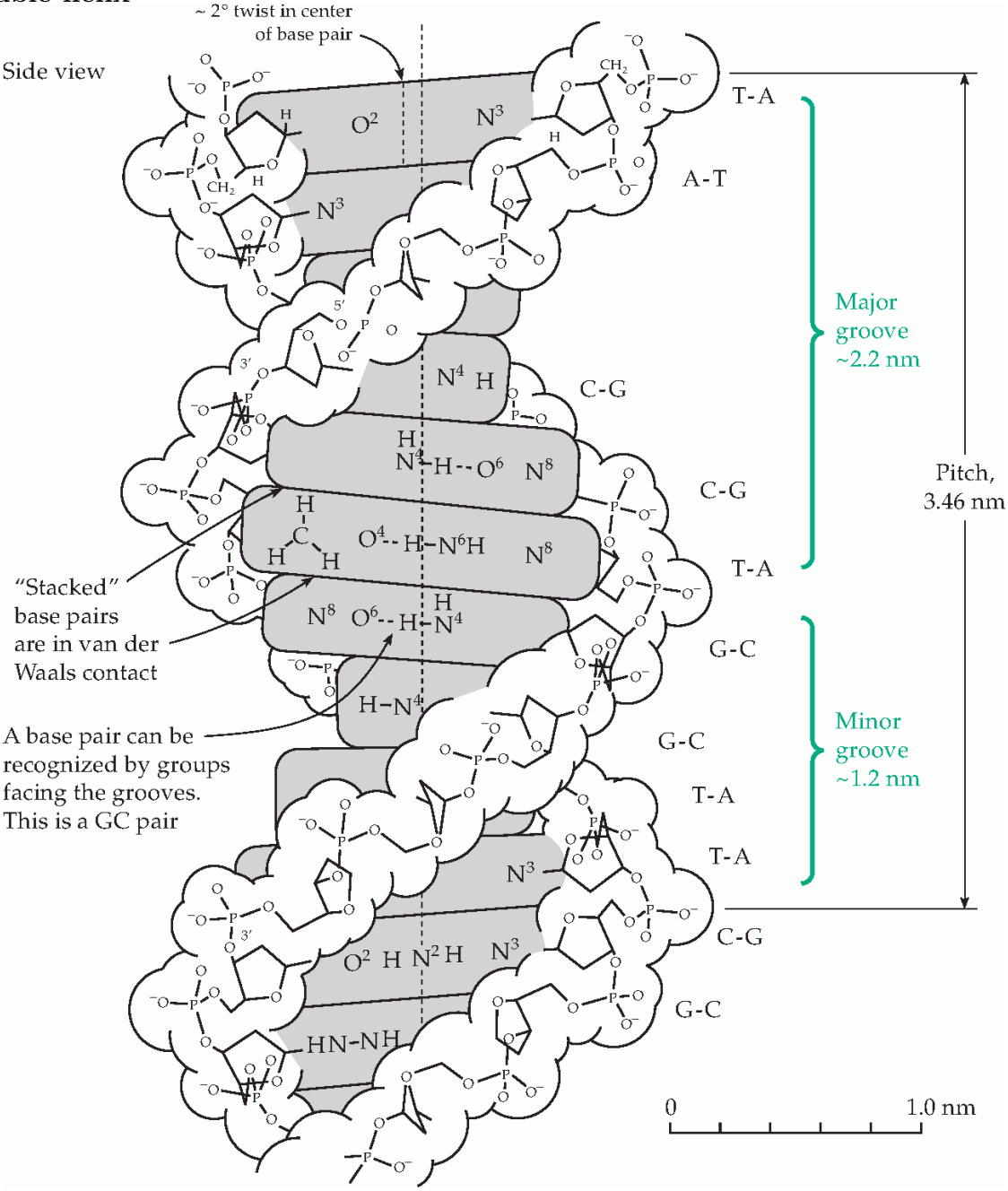
Hydrogen bonds in complementary strands



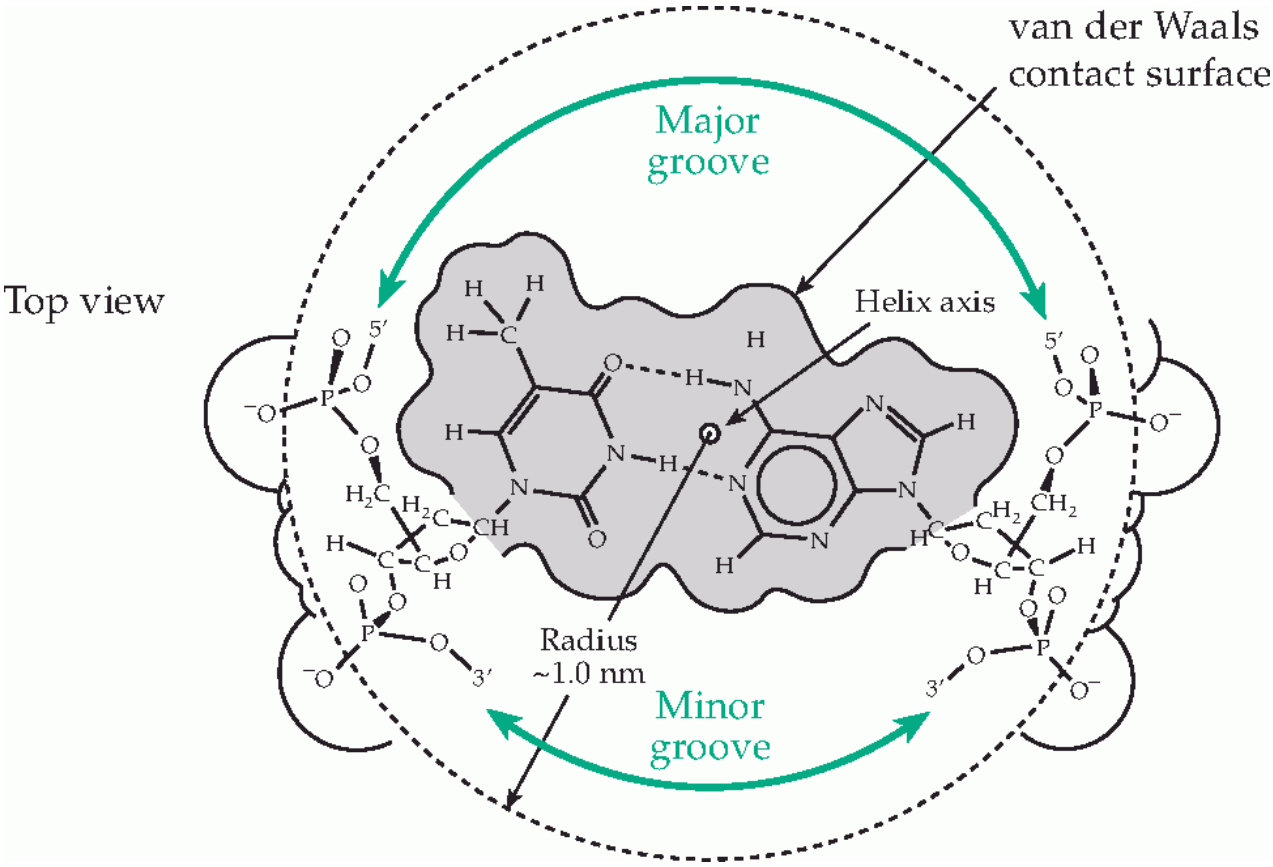
Double helix

- DNA form helical structure where phosphate and sugar form "envelope" and bases form a "core"
- Two grooves: major and minor

DNA double helix



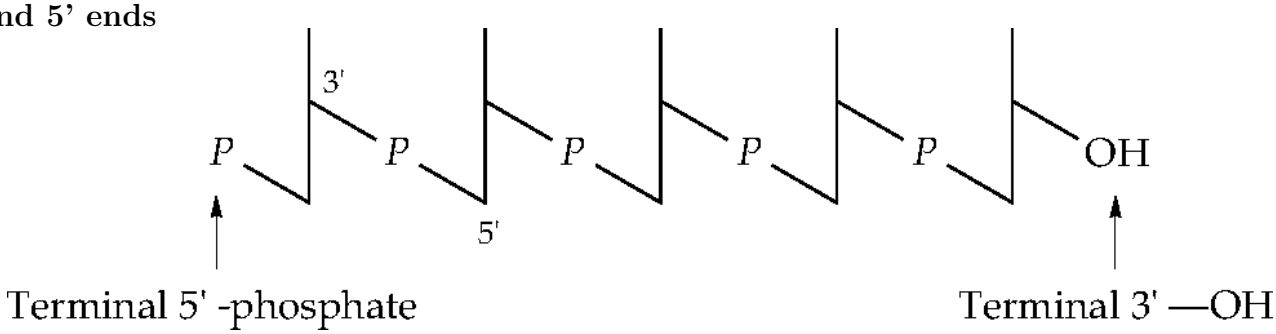
DNA double helix from top



Sequences, ends, abbreviations

- Since nucleotides are complementary, it is usually only one strand listed
- Each strand has 3' (–OH) and 5' ends (phosphate)

3' and 5' ends



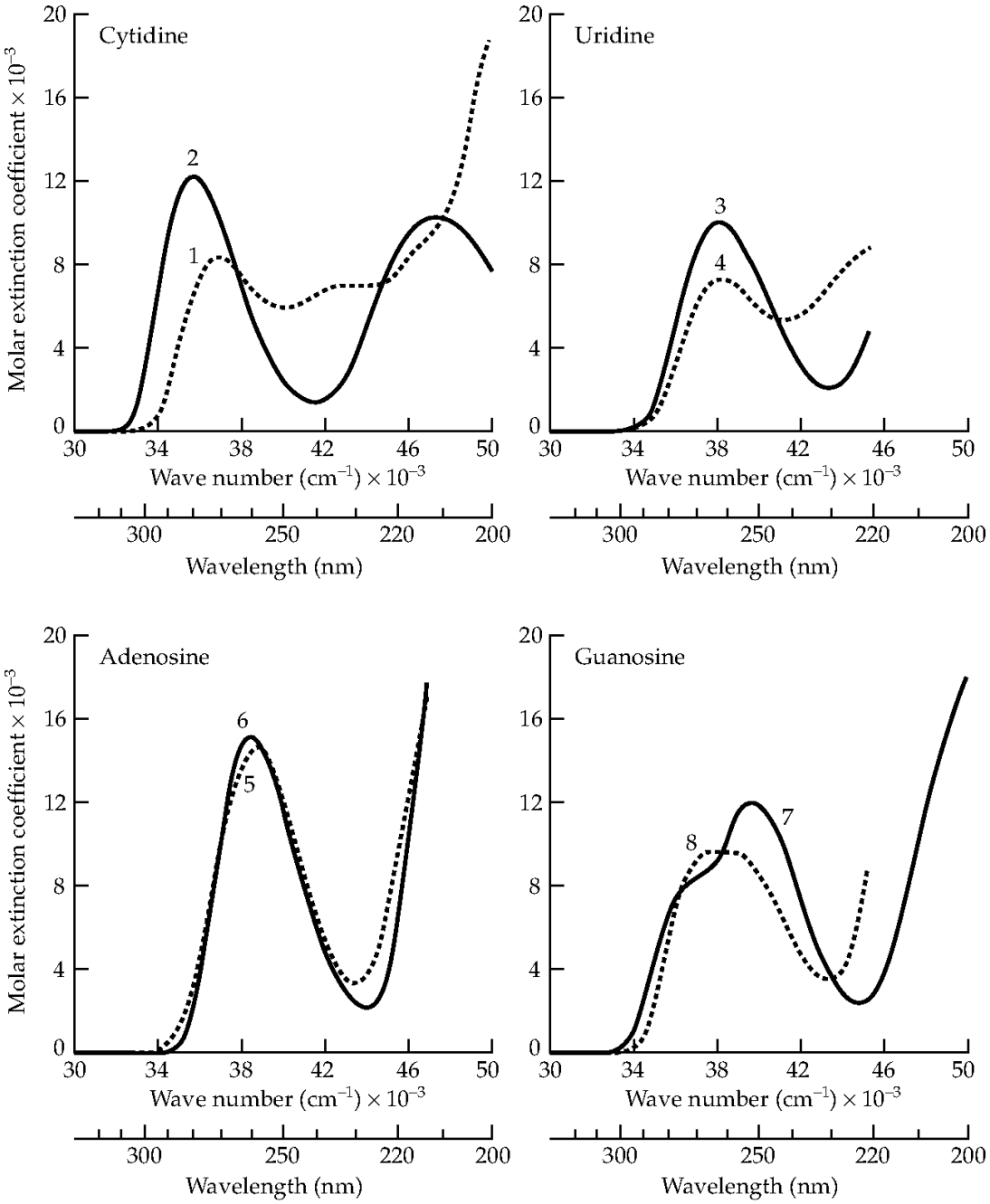
Abbreviations for nucleic acids components

Base:	Uracil (Ura)	Cytosine (Cyt)	Adenine (Ade)	Guanine (Gua)
Nucleoside:	Uridine (Urd or U)	Cytidine (Cyd or C)	Adenosine (Ado or A)	Guanosine (Guo or G)
5'-Nucleotide:	Uridine 5'-phosphate or 5'-uridylic acid (Urd-5'-*P* or UMP)	Cytidine 5'-phosphate or 5'-cytidylic acid (Cyd-5'-*P* or CMP)	Adenosine 5'-phosphate or 5'-adenylic acid (Ado-5'-*P* or AMP)	Guanosine 5'-phosphate or 5'-guanylic acid (Guo-5'-*P* or GMP)

Abbreviations for nucleotide sequences

U,T,C,A,G	Uracil, thymine, cytosine, adenine, guanine
Y or Pyr	Pyrimidine (T or C)
R or Pur	Purine (A or G)
M	Amino base (A or C)
K	Keto base (G or T)
S	Strongly pairing (G or C)
W	Weakly pairing (A or T)
H	Not G (any other base)
B	Not A
V	Not T or U
D	Not C
N	Any base

Nucleotides and UV light

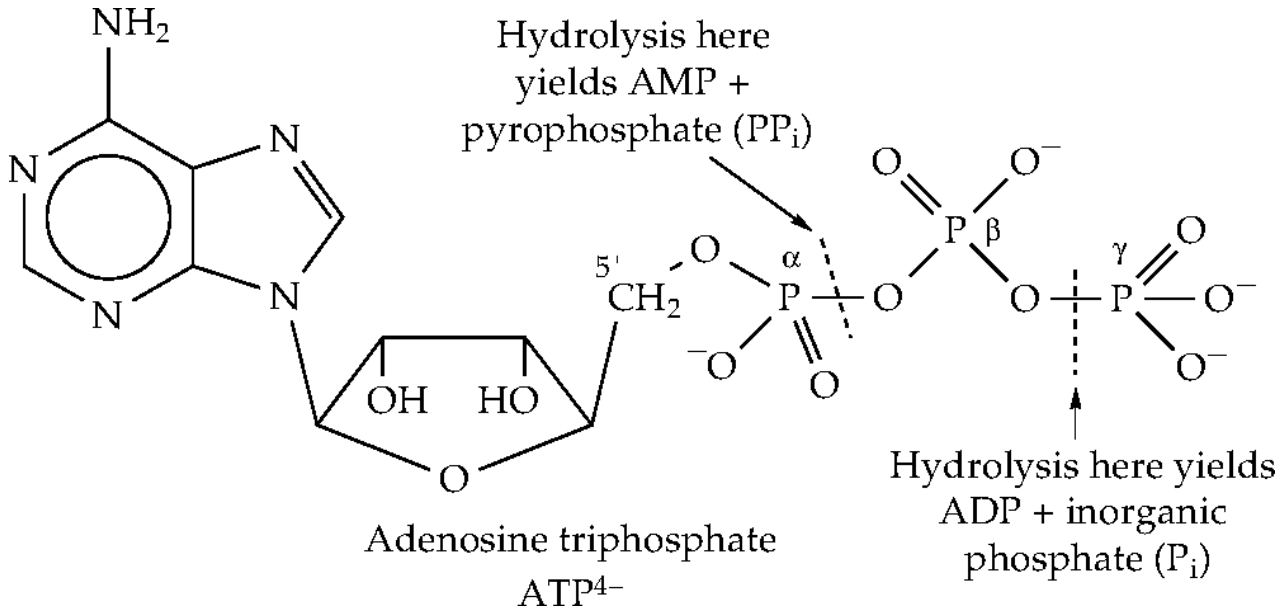


P.2 Other nucleic acids

ATP

- ATP (**adenosine-triphosphate**) is **coenzyme** (ferment helper), derivative of ribose, adenine and three phosphoric acids
- Contain two highly energetic bonds

ATP



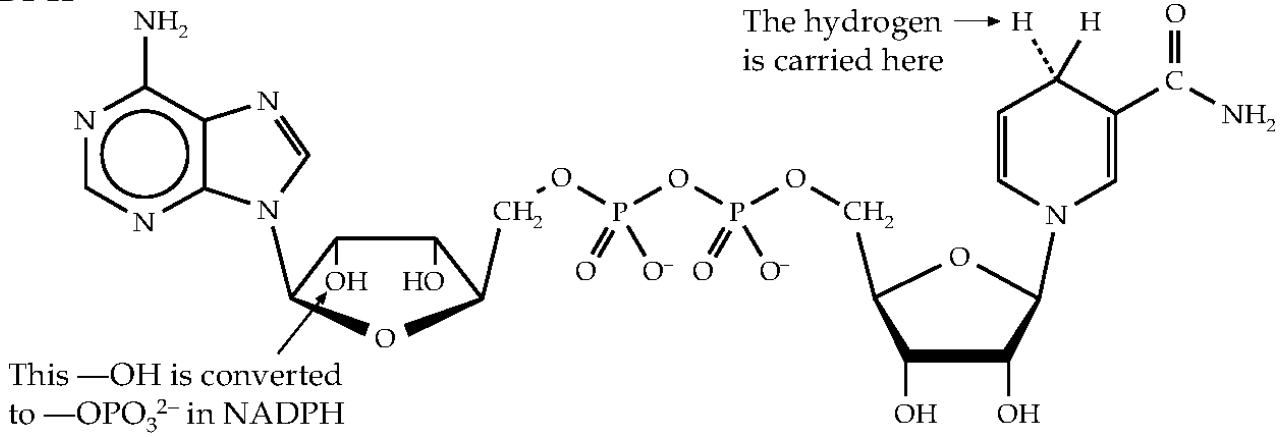
ATP movie

ATP movie

NADP

- NADP, **nicotinamide adenine dinucleotide phosphate** is a coenzyme, derivative of adenine
- Typically, used as hydrogen carrier
- Has a medical name "vitamin B_3"

NADPH

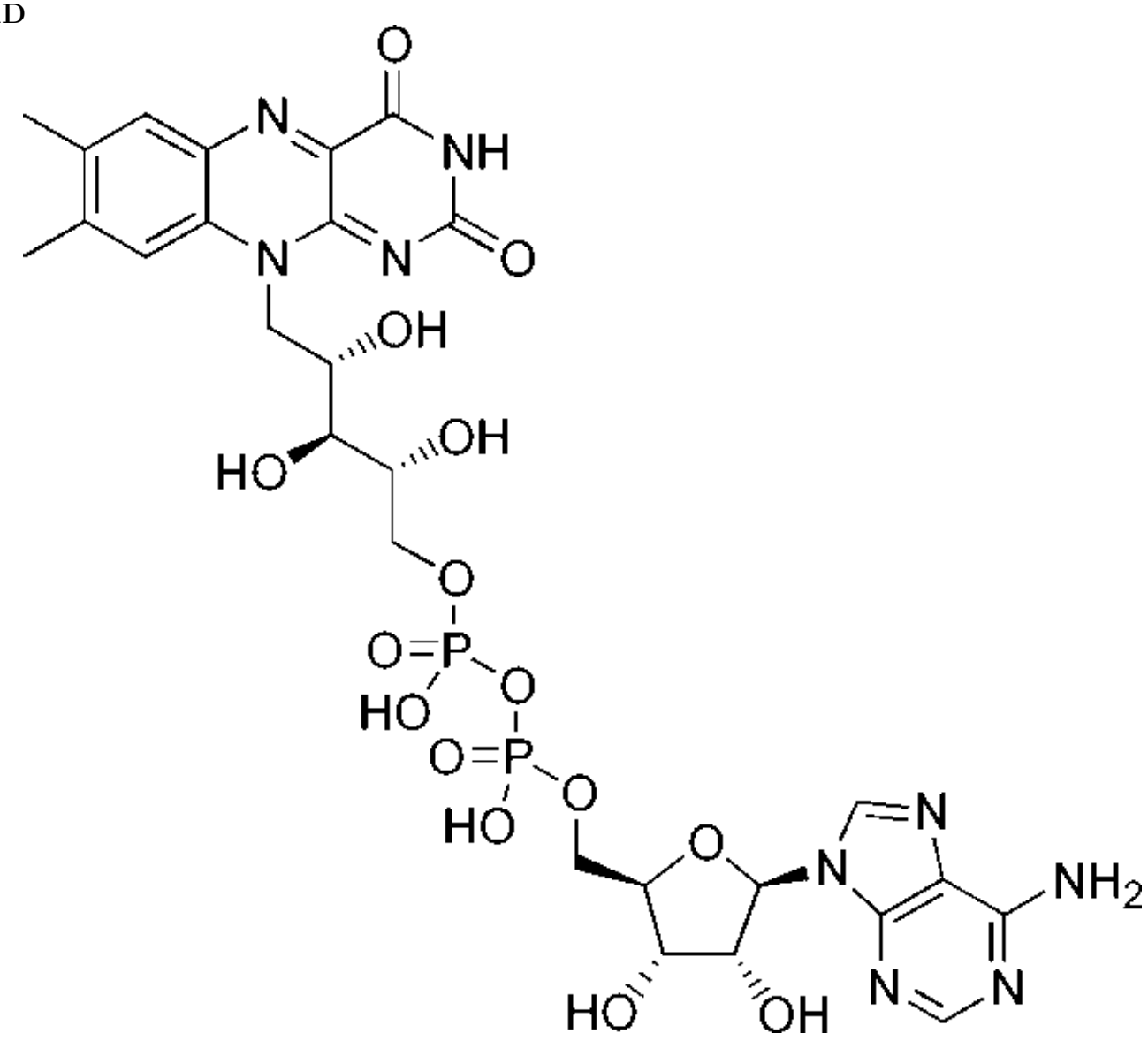


The reduced coenzyme NADH

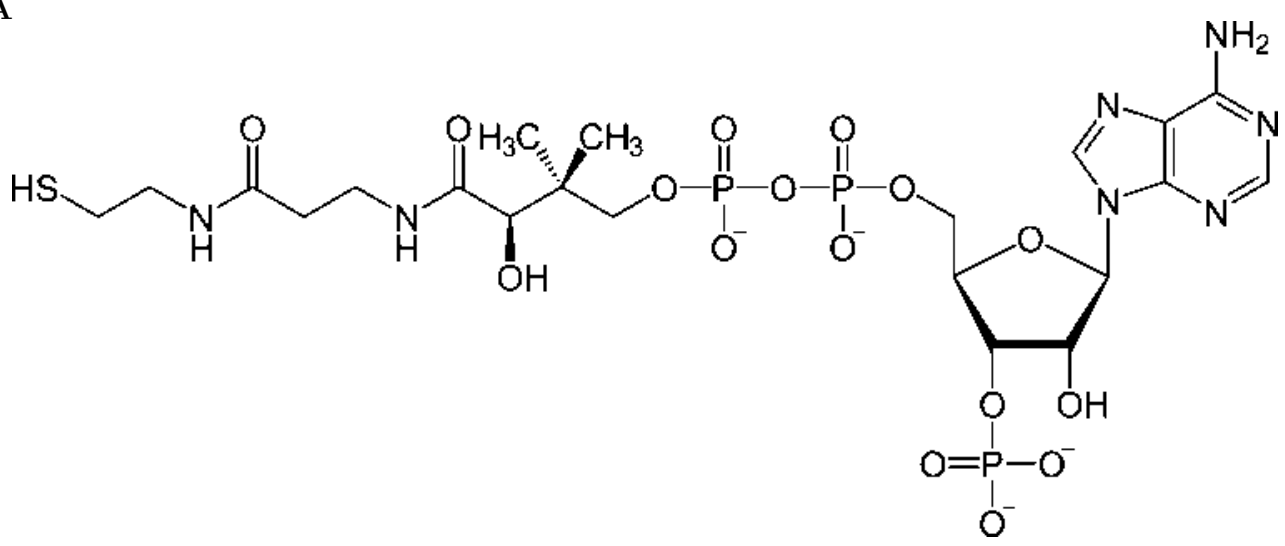
Other nucleotide coenzymes

- FAD, **flavin adenine dinucleotide**, vitamin B_2
- CoA, **coenzyme A**, vitamin B_5
- Both are extremely important for cell respiration

FAD



CoA



Q Macromolecules in cells

Most frequent macromolecules

- Polymer molecules generate most of cell dry weight (30% of total weight)
- Proteins are 15%, nucleic acids 7%, lipids and polysaccharides 2% each

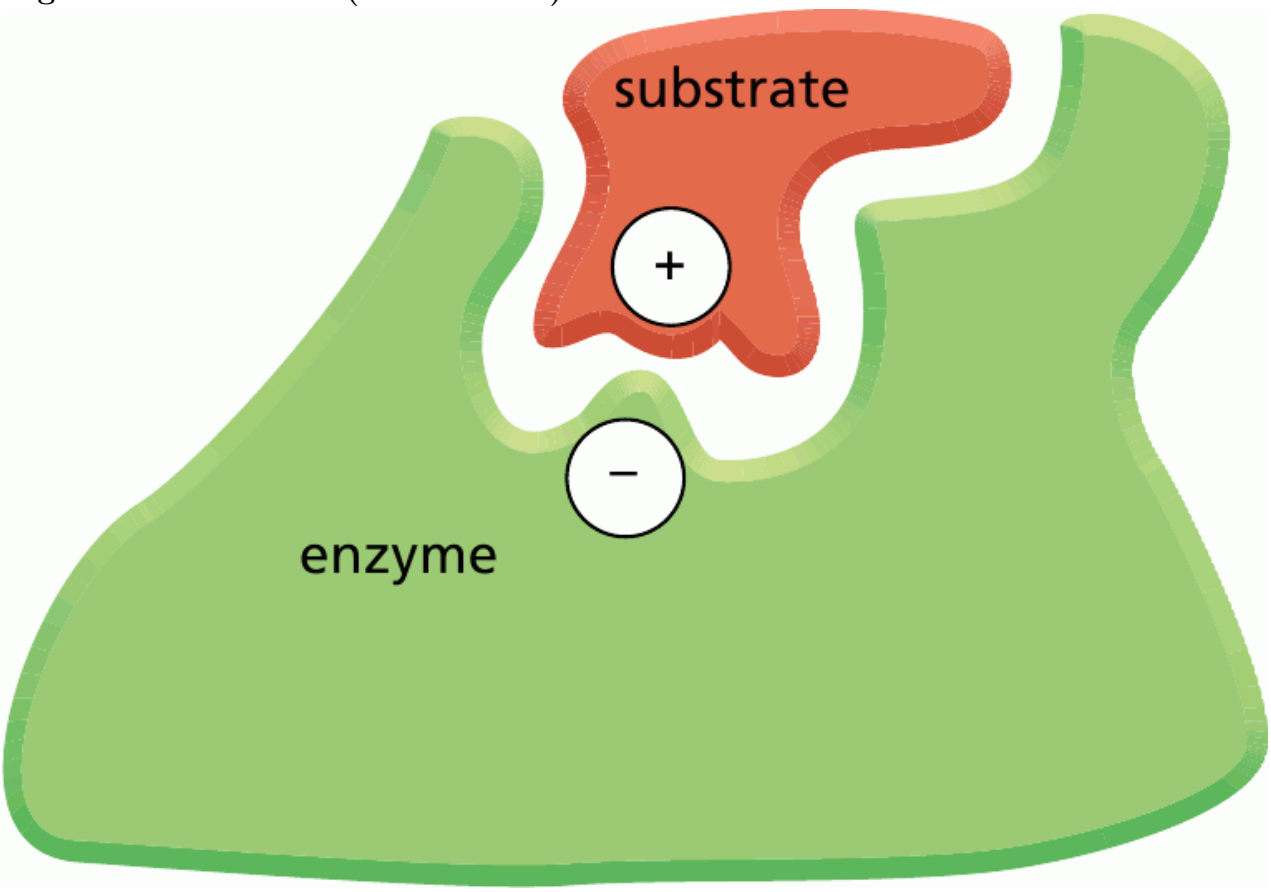
Noncovalent bonds, conformations and binding

- Non-covalent bonds are responsible from shaping of macromolecules
- Almost every macromolecule has different shaping variants—**conformations**
- Intermolecular binding is also due to noncovalent bonds

Types of noncovalent bonds

- Hydrophobic forces
- Van der Waals attractions: due to fluctuation of electric charges
- Electrostatic, including hydrogen

Binding with noncovalent (electrostatic) bonds



R Cells and energy

Metabolism

- **Metabolism** is the sum of all chemical reactions in living organism
- **Catabolism** is the part of metabolism responsible for degrading complex molecules
- **Anabolism** is the opposite part

Metabolism

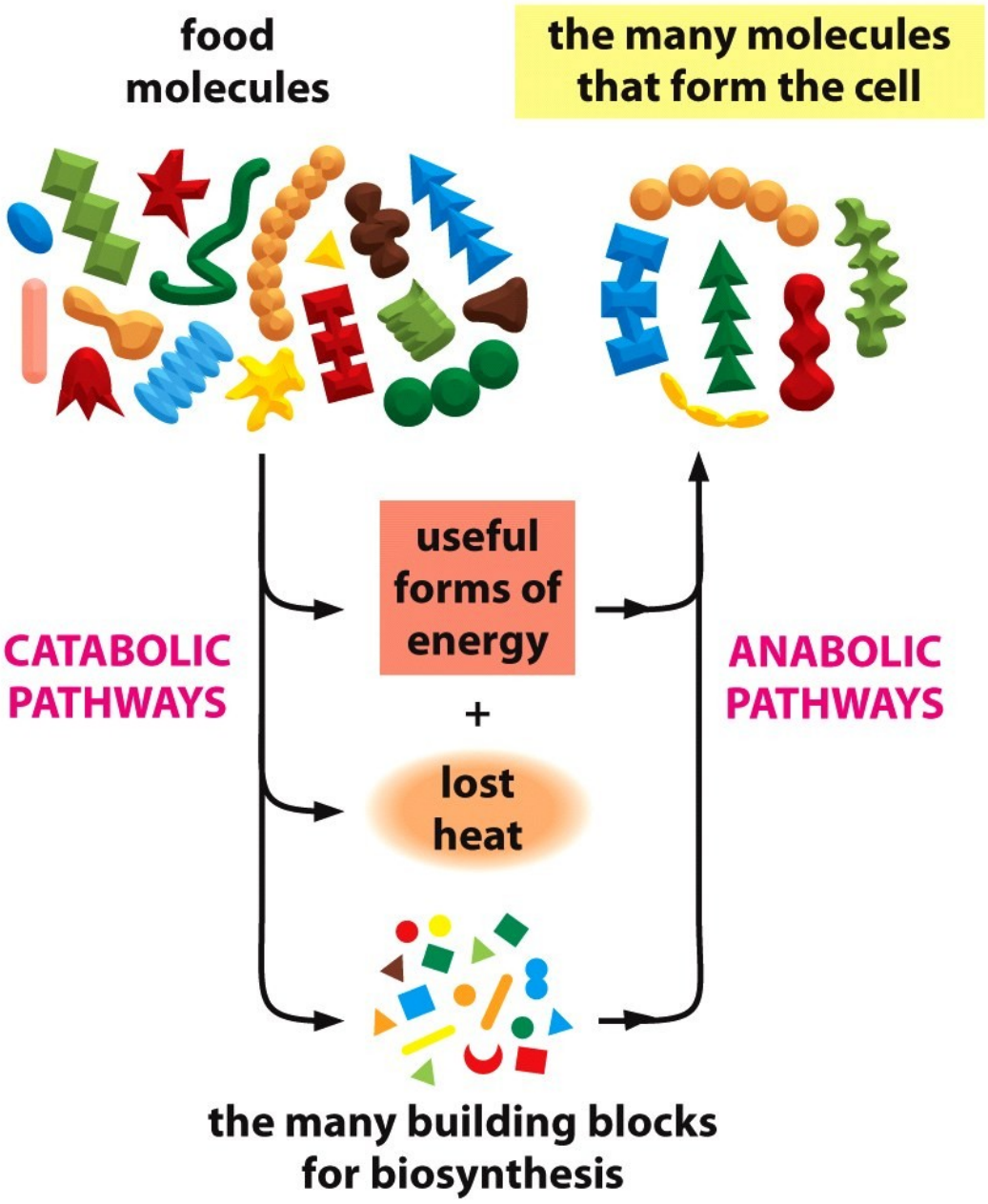


Figure 3-2 Essential Cell Biology 3/e (© Garland Science 2010)

Second law of thermodynamics

- Thermodynamic definition (Rudolf Clausius): *No process is possible whose sole result is the transfer of heat from a body of lower temperature to a body of higher temperature.*

- Simplistic definition: *In isolated system, disorder is always increasing*
- To revert initial order, energy should be spent
- Generally speaking, **entropy** is a measure of disorder (better—measure of randomness)
- In strict sense, entropy is $dS = \frac{\delta Q}{T}$, where Q is amount of heat and T—absolute temperature (constant)

Entropy

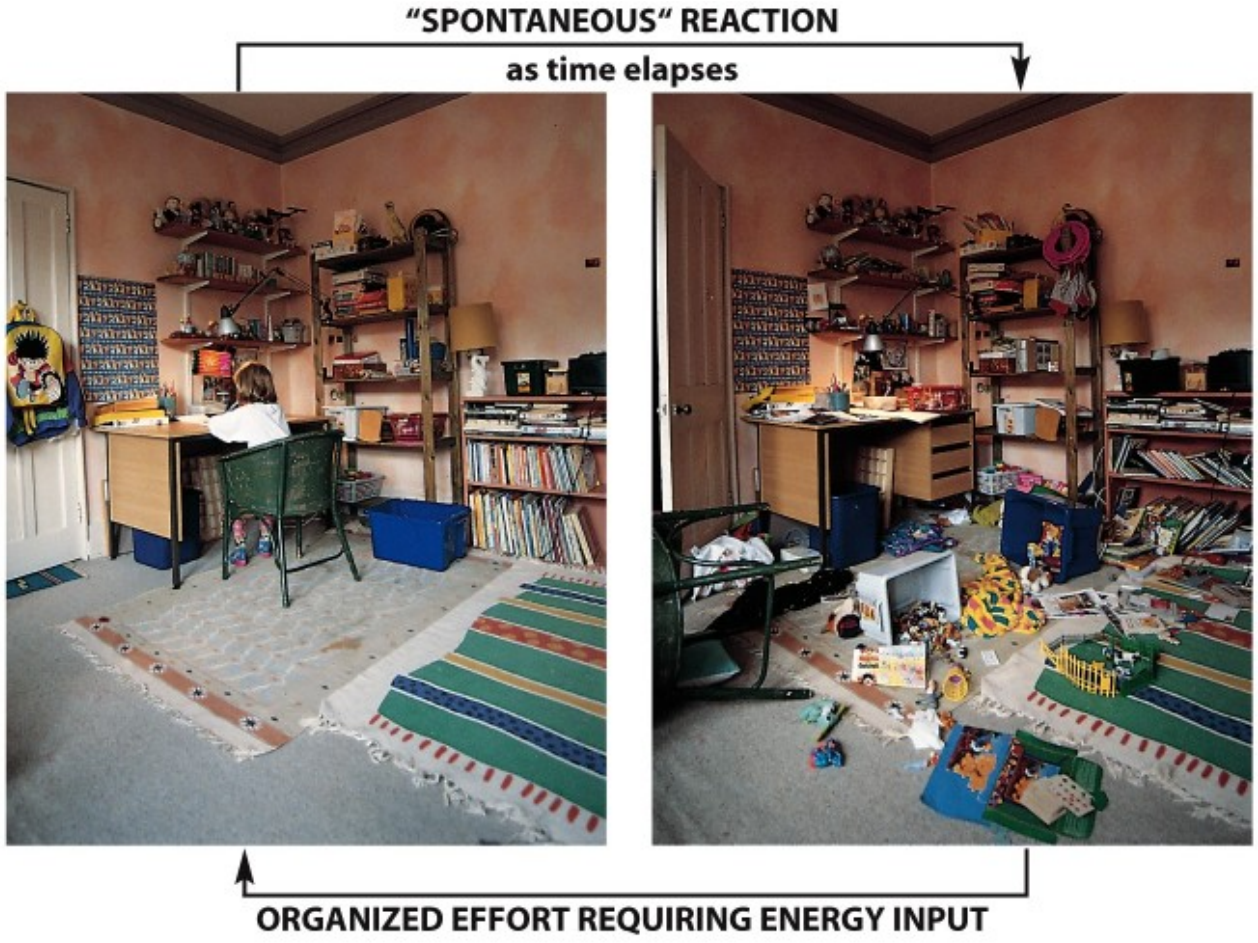


Figure 3-4 Essential Cell Biology 3/e (© Garland Science 2010)

Triumph of entropy: "post-apocalyptic world"

Entropy explained: energy tube

Different forms of energy

- Mechanical energy: potential and kinetic
- Heat energy
- Electromagnetic energy
- All forms are inconvertible; and **first law of thermodynamics** says that *energy never disappears*, it only changes its form

Photosynthesis

- The way of transforming light energy to energy of chemical bonds
- The schematic description is: light energy + CO_2 + H_2O → sugars + O_2 + heat energy
- Part of anabolism

Cellular respiration

- Almost opposite process
- Schematic description: sugars + O_2 → CO_2 + H_2O + chemical energy (ATP)
- Part of catabolism

Oxidation and electron transfer

- Cellular respiration is based on oxidation, taking electrons off
- Converse reaction is reduction; together they are *redox* reactions
- For organic molecules, typical oxidation sequence is: carbohydrates → alcohols → aldehydes → organic acids → CO_2

Enzymes and energy of activation

- Most of processes need the *energy of activation*
- **Enzymes** could lower activation barriers

Energy of activation

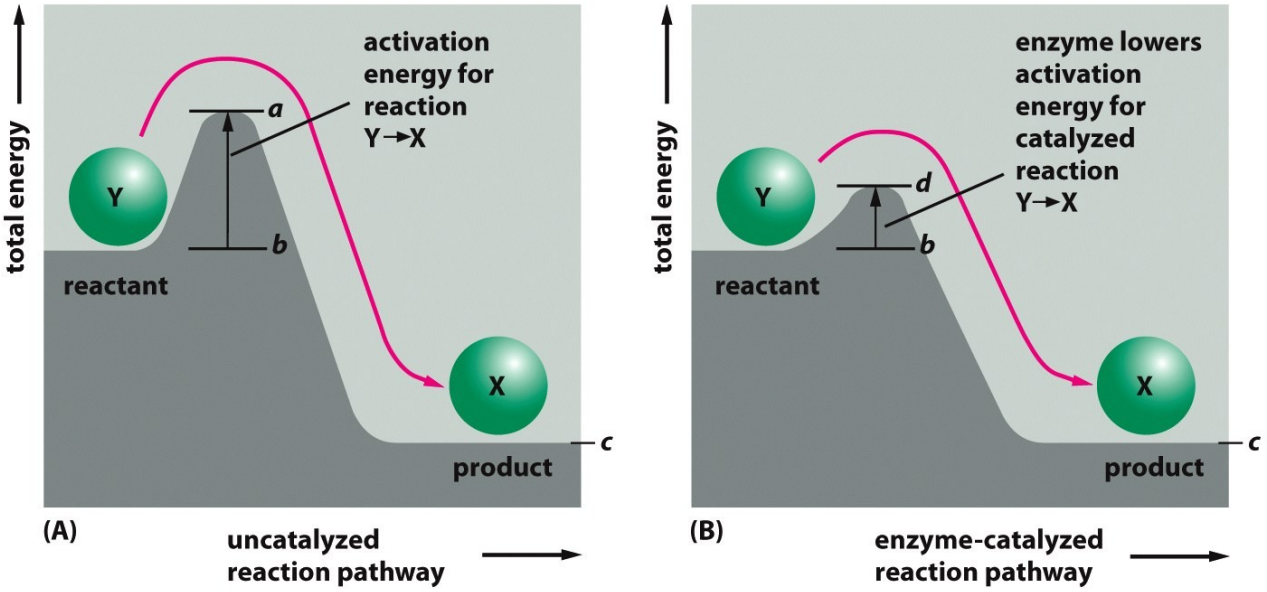


Figure 3-12 Essential Cell Biology 3/e (© Garland Science 2010)

Catalysis movie

Final question (3 points)

How are living organisms working against the second law of thermodynamics?

Summary

- The second law of thermodynamics is about increasing entropy
- All metabolic reactions need energy

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 2, Chapter 3*: 58–100.

Outline

S Questions and answers

Previous final question: the answer

How living organisms are working against the second law of thermodynamics?

- They run catabolic reactions to make an energy for anabolic (synthetic) reactions

Catalysis movie

Enzyme terminology: catalysis and substrate

- Enzyme binds with **substrate**,
- then catalyze conversion of substrate into **product**,
- and returns untouched to the initial state

Catalysis terms

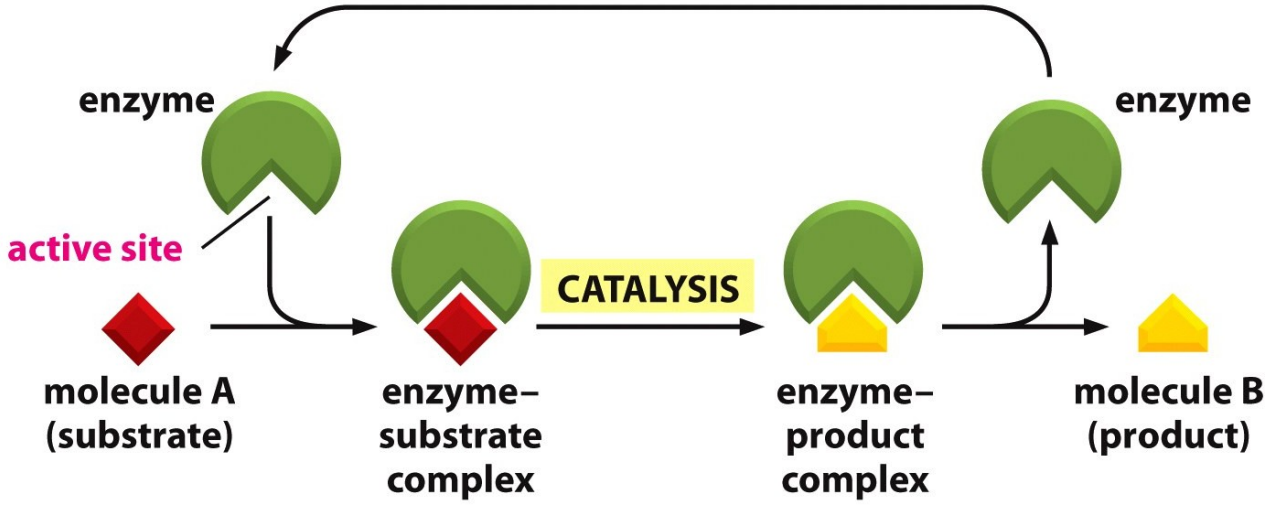


Figure 3-15 Essential Cell Biology 3/e (© Garland Science 2010)

Enzyme performance

- Two numbers are using for measuring enzyme performance: V_{max} and K_M
- V_{max} is maximal available reaction rate
- K_M is the concentration of substrate when the rate is $V_{max}/2$

Enzyme performance

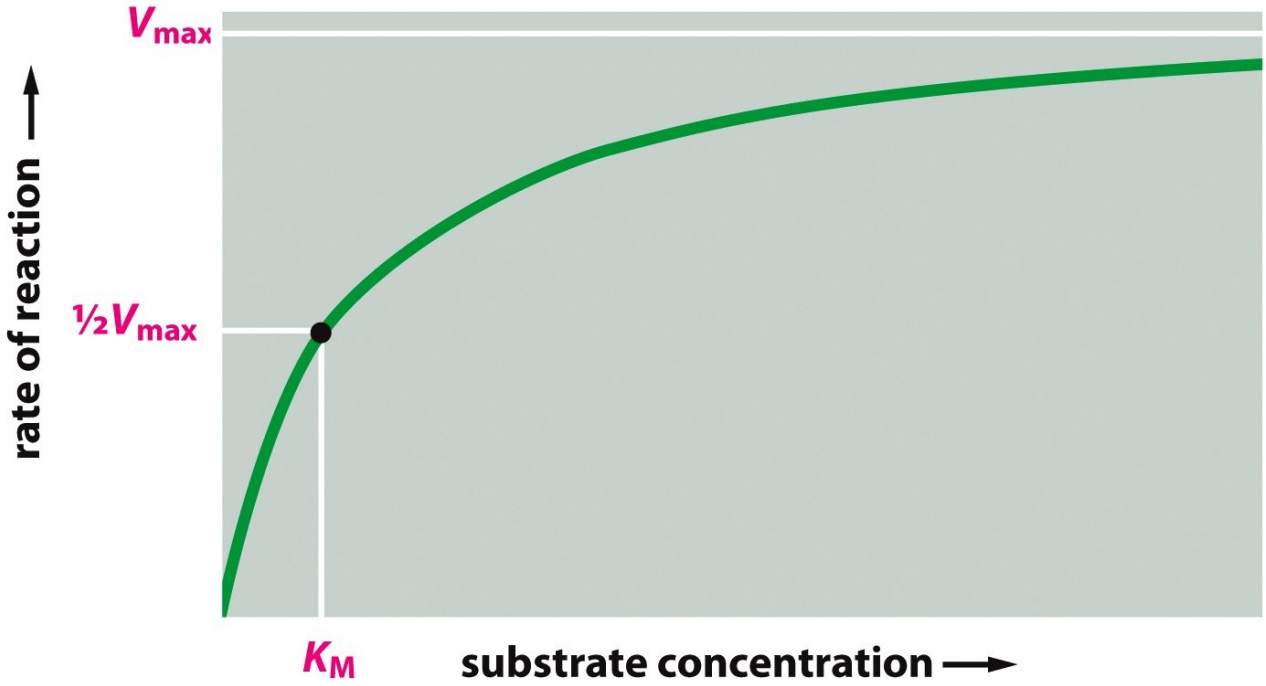


Figure 3-24 Essential Cell Biology 3/e (© Garland Science 2010)

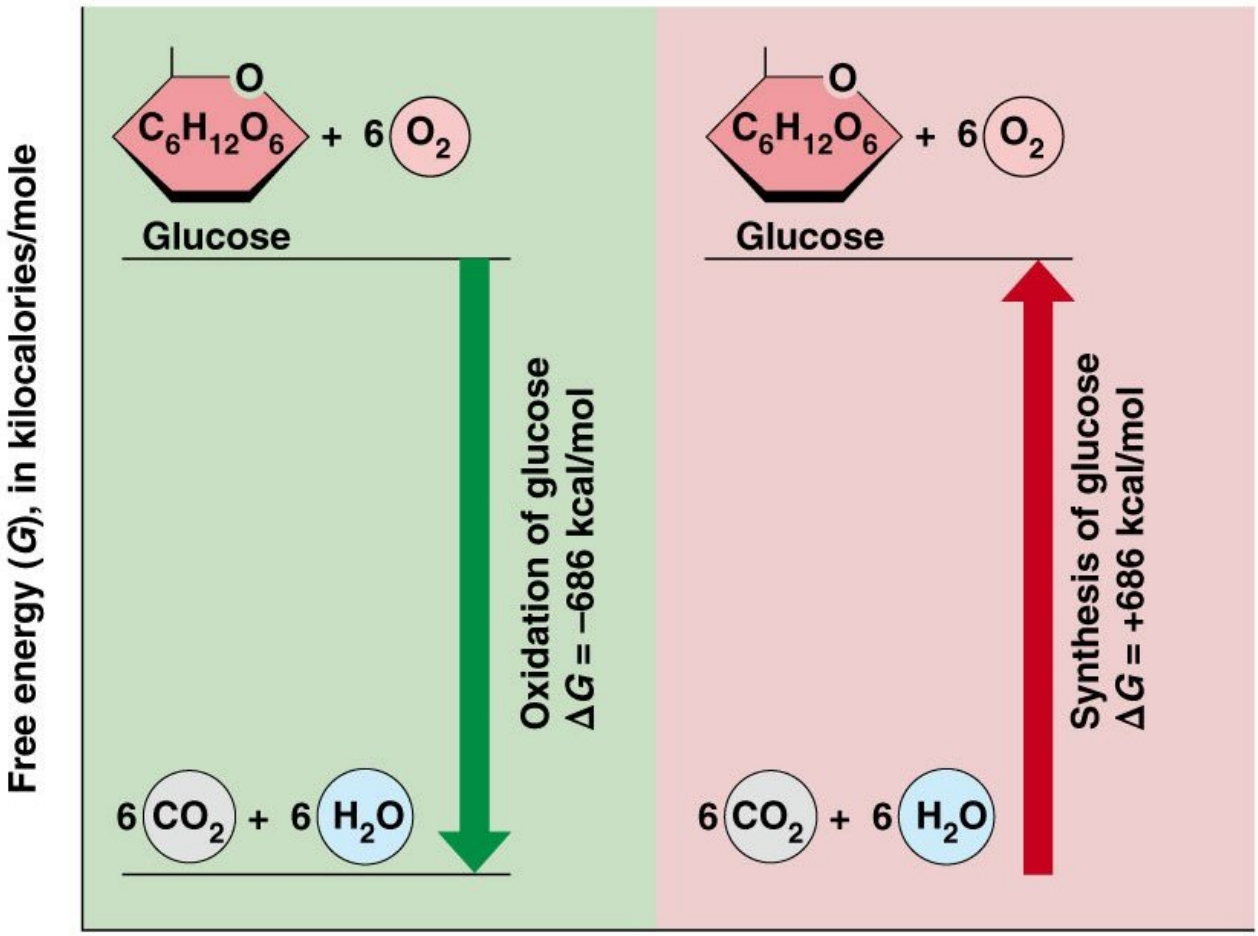
T Energy and chemistry

T.1 Free energy

Free energy

- Gibbs energy, or G, is a chemical analog of potential energy
- If G increases ($\Delta G > 0$), the chemical reaction is non-favorable
- If G decreases ($\Delta G < 0$), chemical reaction is favorable

Delta G



Standard free-energy change

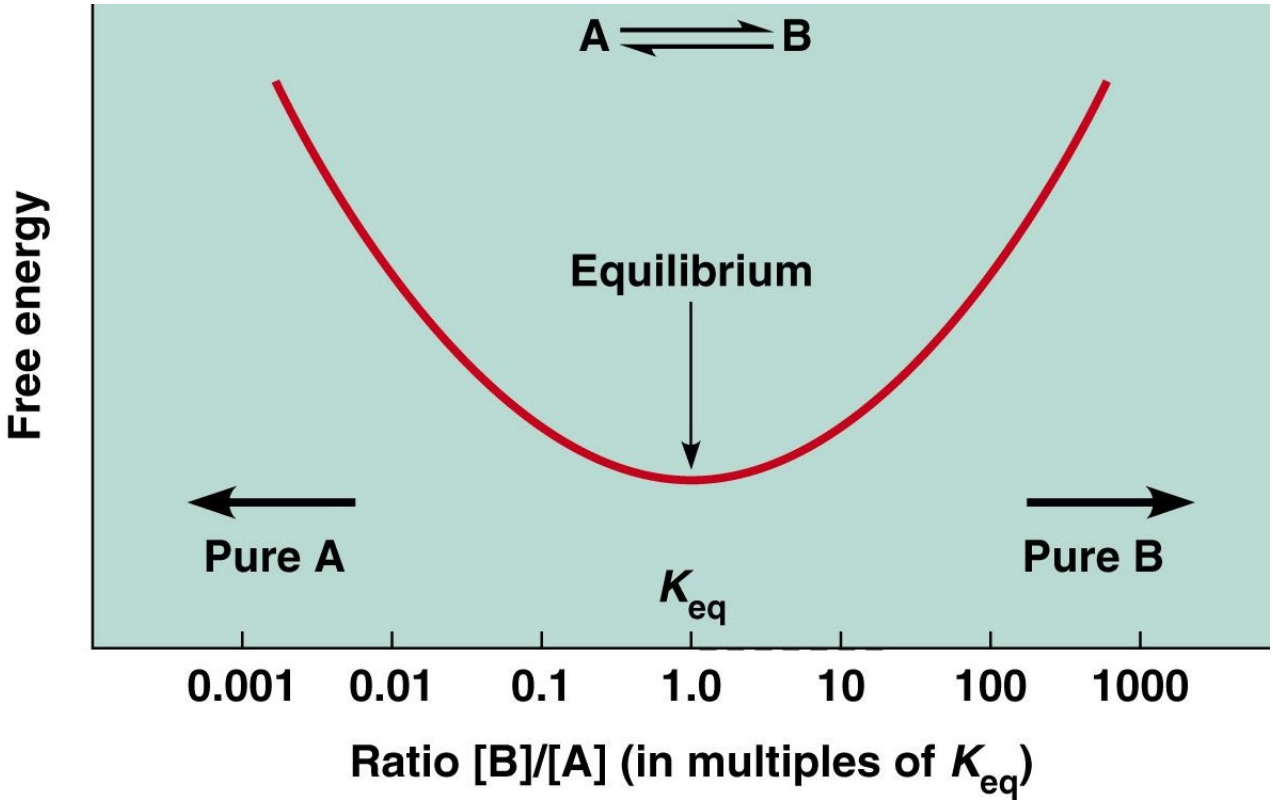
- Simple chemical reaction like $X \to Y$ depends on reagent concentrations, $[X]$ and $[Y]$
- Free energy (Gibbs energy) will also depend on concentration
- To standardize G, we are using $\Delta G^\circ = \Delta G - RT \ln \frac{[X]}{[Y]}$, where R (gas constant) and T (absolute temperature) are constants

Chemical equilibrium

- Chemical reactions are going on until they reach a state of **chemical equilibrium**
- In the equilibrium, reaction is going in both directions without changing concentration of participating chemicals:

  $X \rightleftharpoons Y$

Equilibrium



Equilibrium constant

- On the stage of equilibrium, **equilibrium constant** $K = \frac{[X]}{[Y]}$, and $\Delta G = 0$ (why?*)

  *Because equilibrium reaction does not take of give any energy

- Consequently, standard free-energy on the stage of equilibrium is:

$$\Delta G^\circ = \Delta G - RT \ln \frac{[X]}{[Y]} = -RT \ln K = -0.616 \ln K = -1.42 \log K$$

Equilibrium constant in complex reactions

- In complex reactions, K depends on concentrations of all participants
- E.g., for $A + B \rightleftharpoons AB$: $K = \frac{[AB]}{[A][B]}$
- If reaction has several steps, all changes of free-energy are additive

T.2 Activated carriers: ATP and similar

Activated carrier molecules

- Carriers are used for temporarily storage of both energy and molecular pieces
- Carriers have high diffusion rates

- Carriers work in two steps: (a) activation and (b) condensation
- They are not enzymes!
- For example, ATP and GTP (guanosine triphosphate) store phosphate on the energetic bond

One component and the carrier

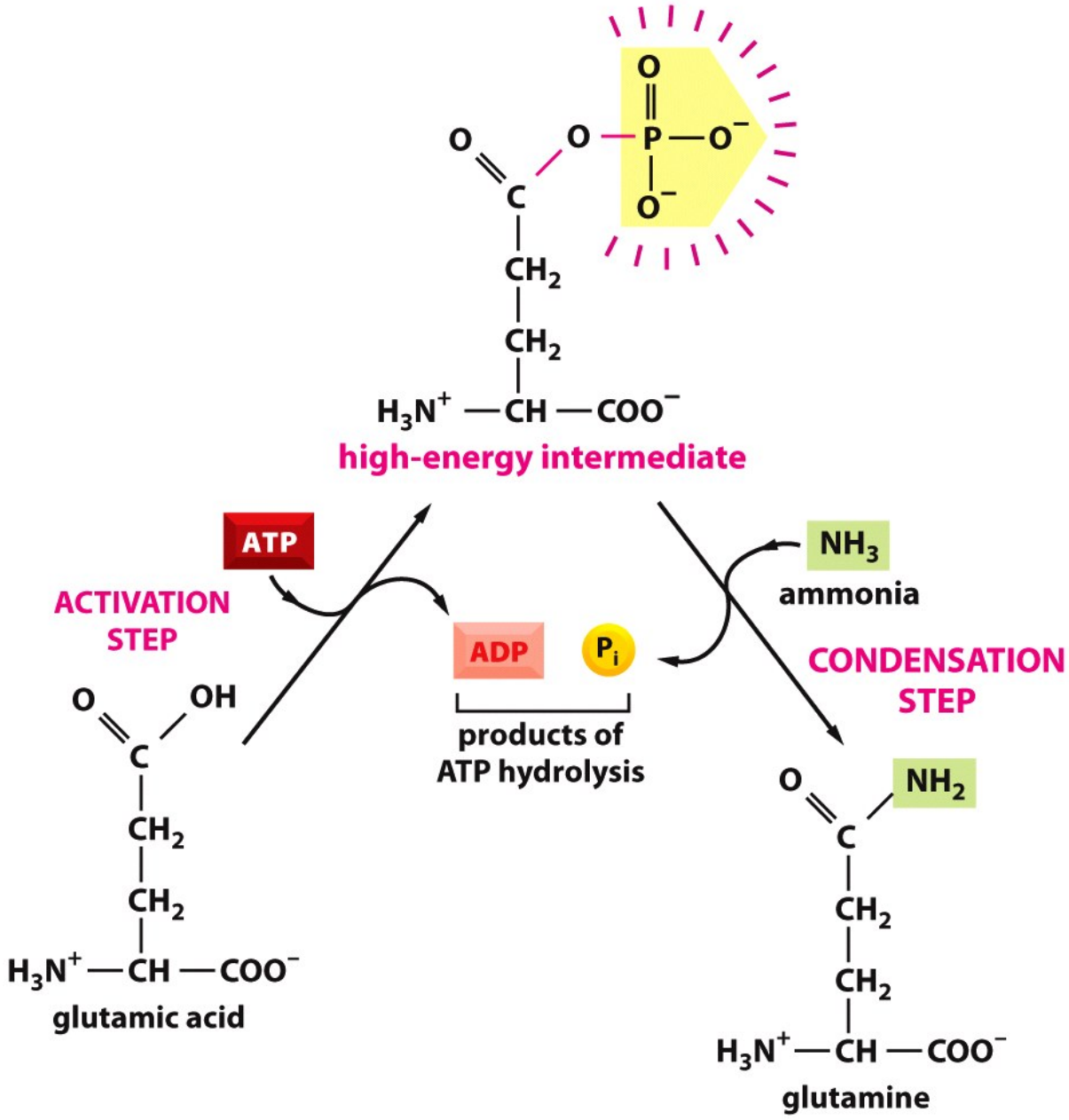


Figure 3-33b Essential Cell Biology 3/e (© Garland Science 2010)

ATP is a carrier of phosphate group, P_i

Two components and the carrier: coupled reactions

There are two variants of coupled reactions:

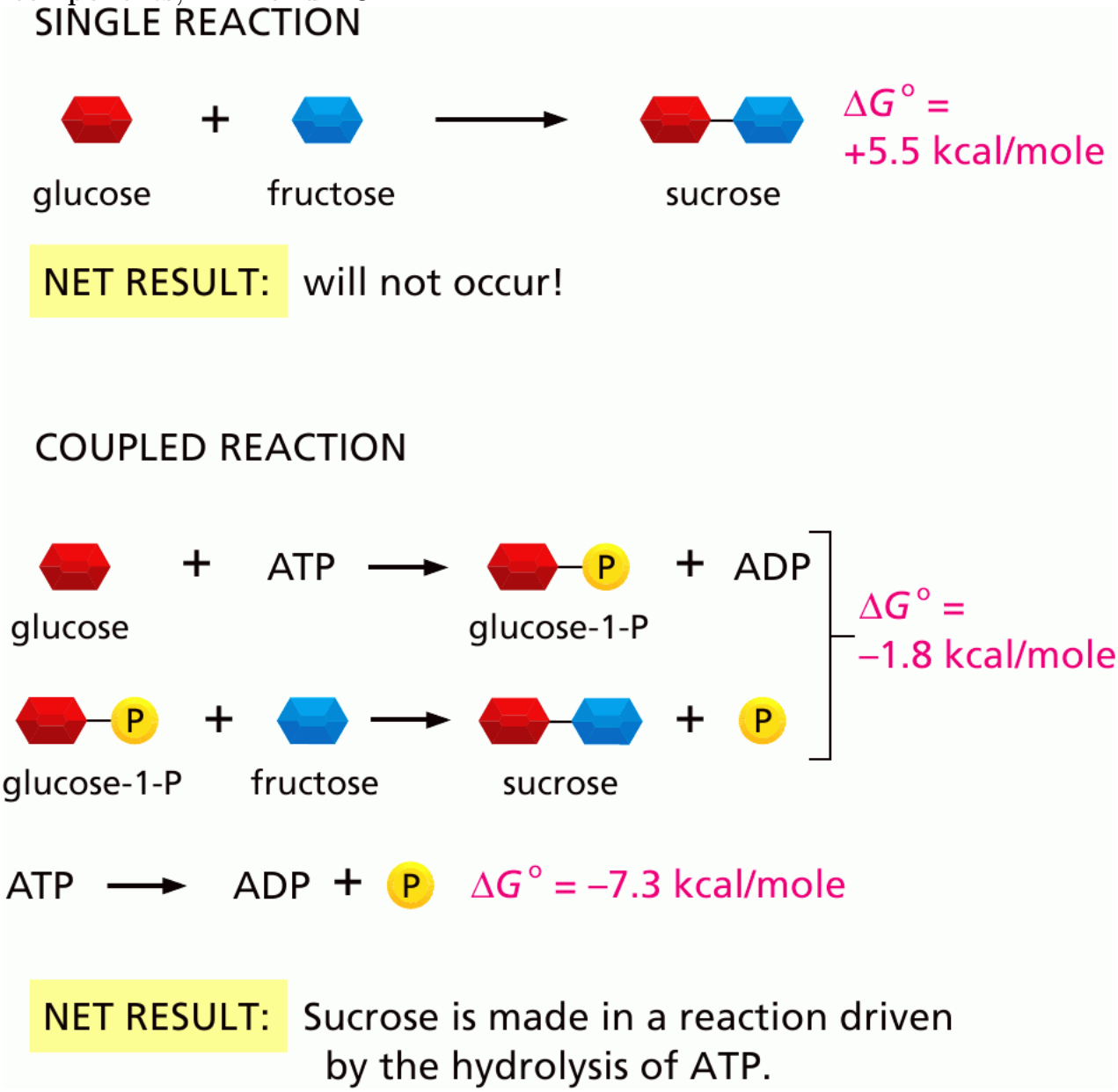
- Breaking (oxidizing) of something AND carrier synthesis
- Or carrier destruction AND synthesis of something (e.g., polymer from monomers)

ATP in coupled reactions

To join A and B, ATP usually acts as intermediate:

- A + ATP $\rightarrow$ A–0–P0$_3$ + ADP
- B–H + A–0–P0$_3$ $\rightarrow$ A–B + P$_i$

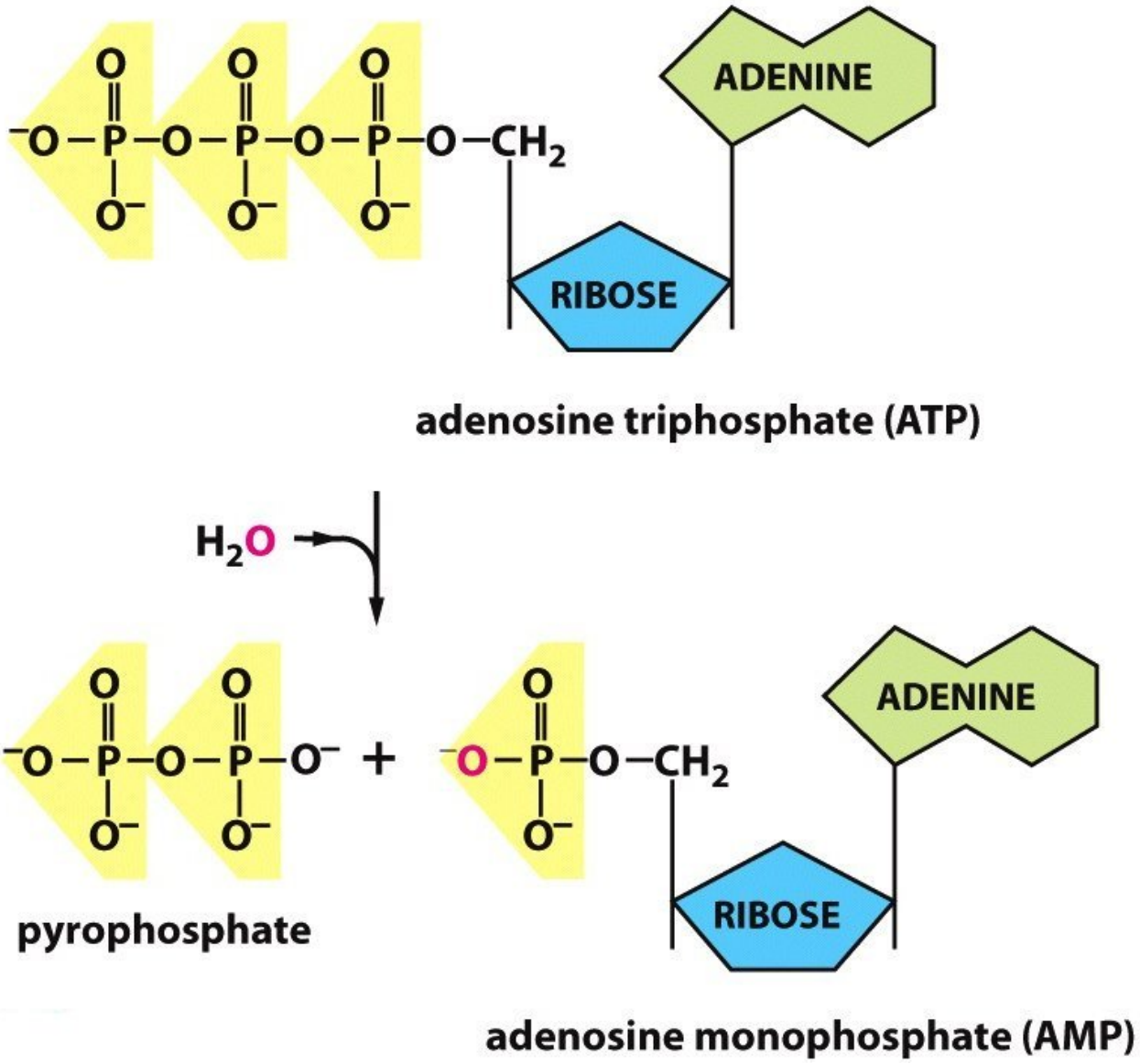
Two components, ATP and ΔG



AMP and pyrophosphate

- Normally, ATP $\rightarrow$ ADP + P$_i$ reaction has $\Delta G^\circ \approx -13$ kcal/mole
- However, some reactions (like synthesis of DNA from nucleotides) need more
- The alternative way: ATP $\rightarrow$ AMP + P$_i$–P$_i$ (pyrophosphate) has $\Delta G^\circ \approx -26$ kcal/mole

How to make pyrophosphate

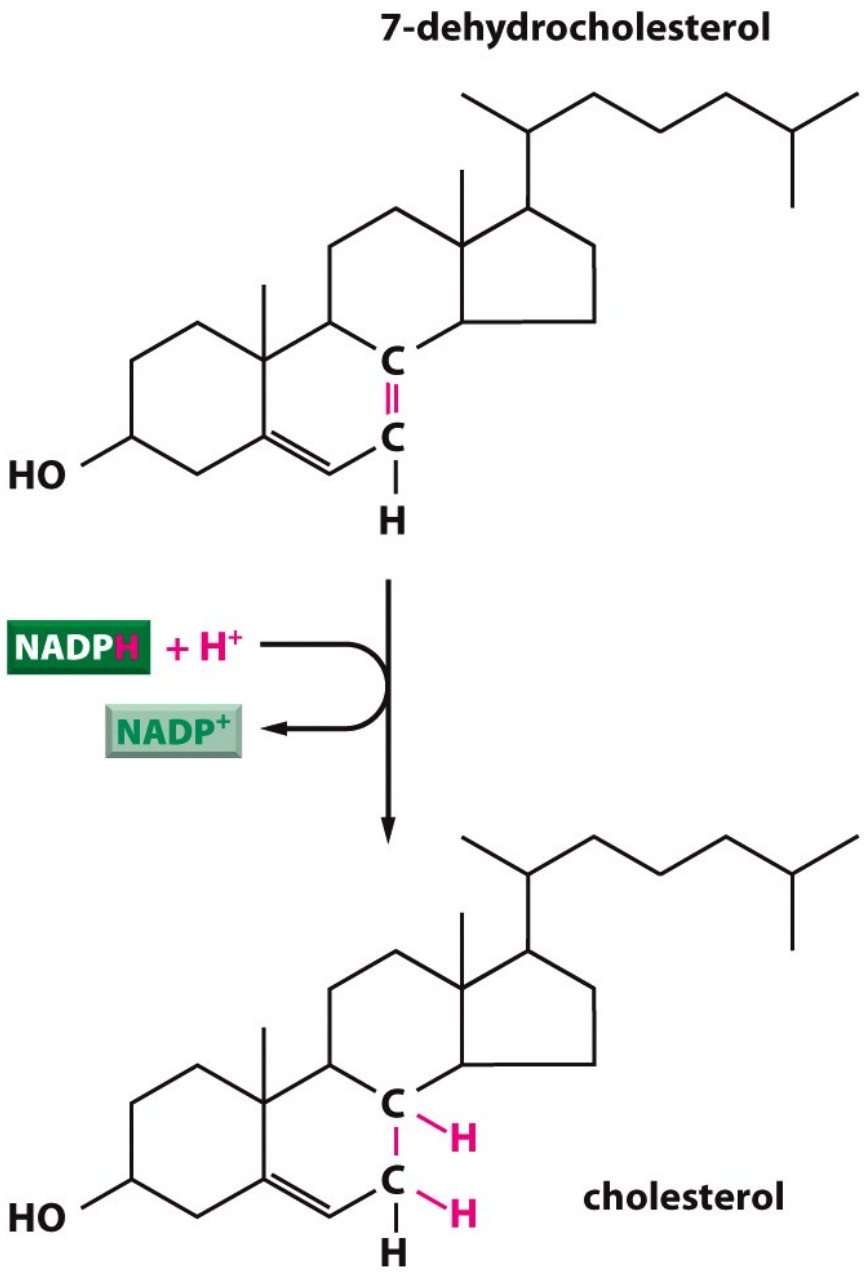


T.3 Other activated carriers

Electron carriers: NAD/NADH, NADP/NADPH

- Both are derivatives of adenine transferring electrons with associated protons (or simply hydrogen, H)
- They are active redox molecules
- NADH typically works in catabolic reactions like cell respiration
- NADPH works mostly in anabolic reactions like synthesis of DNA or synthesis of cholesterol
- FAD belongs to the same group

NADPH gives H to make cholesterol



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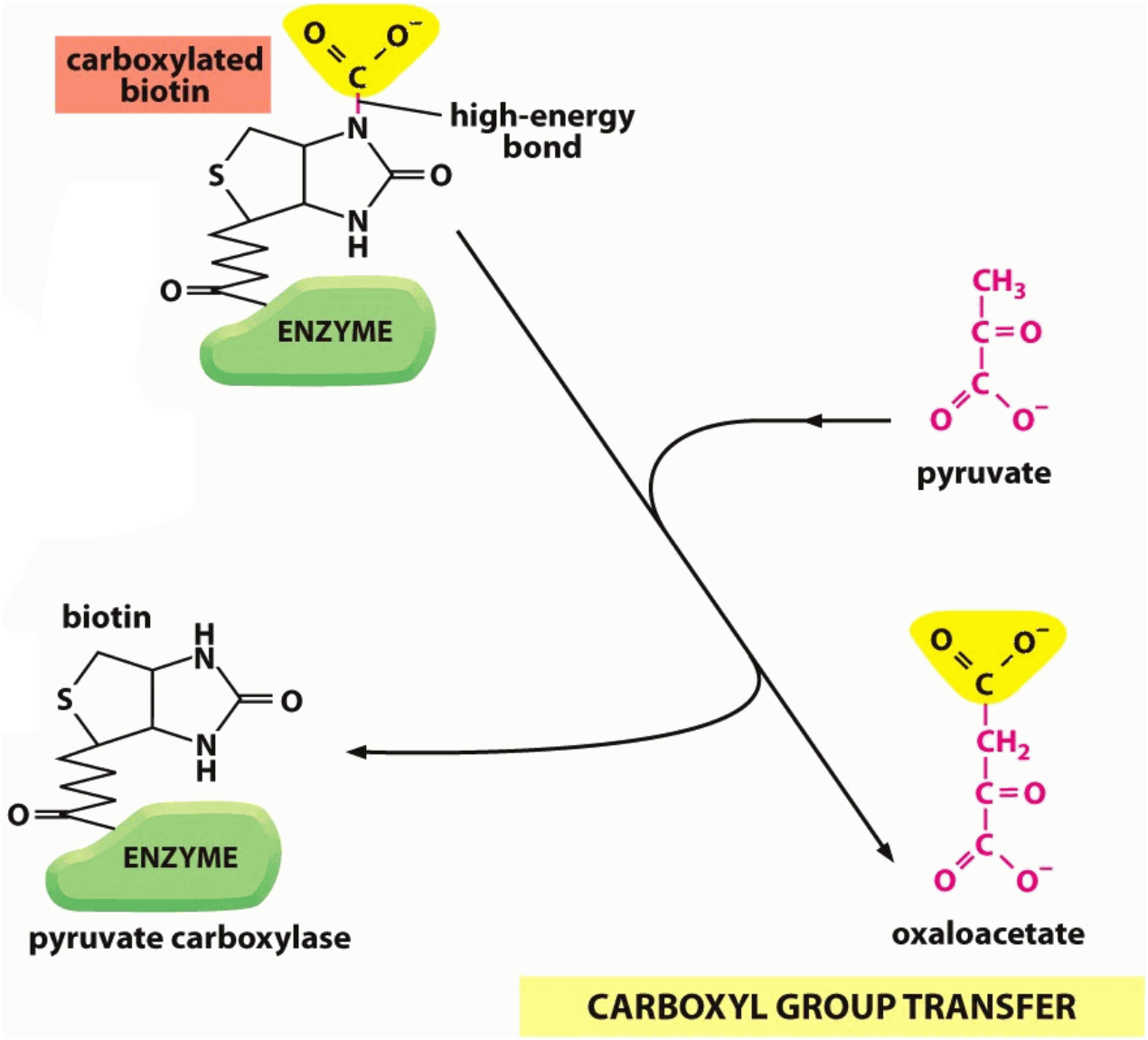
Other carriers

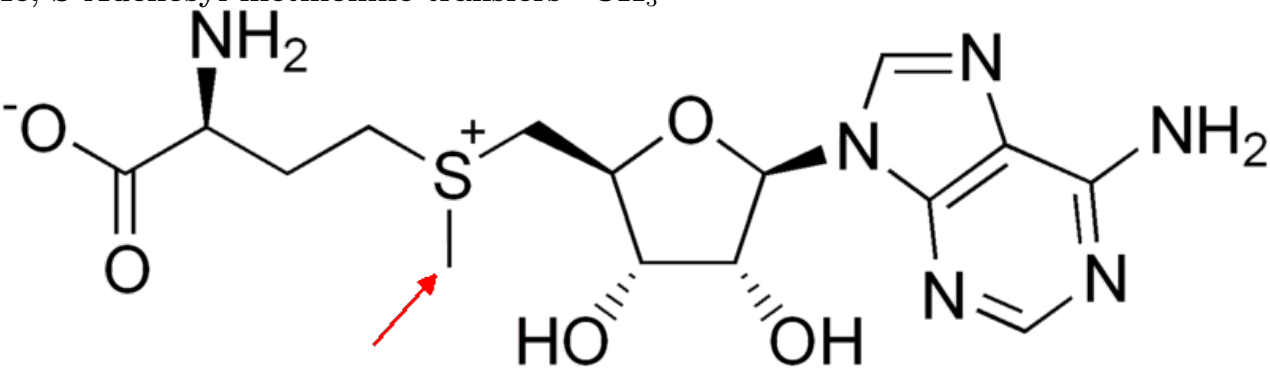
- CoA (or Acetyl–CoA): transfers acetyl group (CH_3–COOH)
- Biotin: carboxyl group (–COOH)
- S-Adenosyl methionine (SAM, SAMe): methyl group (–CH_3)
- Uridine diphosphate glucose (UDP-glucose): whole glucose molecules

The molecular piece which they transfer is attached to their molecules with high-energetic bond

Biotin, vitamin B_7 transfers –COOH



SAMe, S-Adenosyl methionine transfers $-CH_3$



Final question (2 points)

What are enzymes?

- Some reactions are **endoenergetic** ($\Delta G > 0$, some are **exoenergetic** ($\Delta G < 0$)
- On the stage of equilibrium, all reactions at $+37°C$ have $\Delta G° = -1.42 \log K$

- Activated carriers are used in endoenergetic reactions
- Activated carriers may transfer phosphate or different organic groups

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 3*.

Outline

U Questions and answers

Previous final question: the answer

What are enzymes?

- Specific catalysts which lower the activation barrier

V Proteins

V.1 Proteins in general

Proteins

- Unbranched polymers of L-amino acids, normally synthesized on ribosomes
- After synthesis, proteins normally undergo 2D folding, 3D shaping and (sometimes) chemical modification and hyper-polymerization

Functions (review)

- Enzymatic (pepsin, Rubisco)
- Structural (collagen, keratin)
- Transport (hemoglobin, serum albumin)
- Movement (myosin, dinein)
- Storage (ferritin, ovalbumin)
- Signal (insulin, EGF)
- Sensitivity (rhodopsin, acetylcholine receptor)
- Gene regulation (lactose repressor, homeodomain proteins)
- Other (antifreeze, fluorescent etc.)

V.2 Shape and structure of proteins

Amino acid sequence

- Protein = polypeptide backbone + side chains
- Side chains could be negative, positive, uncharged polar and nonpolar

Classification of side chains

AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

POLAR AMINO ACIDS (hydrophilic)

AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

NONPOLAR AMINO ACIDS (hydrophobic)

Figure 4-3 Essential Cell Biology 3/e (© Garland Science 2010)

Folding bonds

- Electrostatic
- Hydrogen
- van der Waals

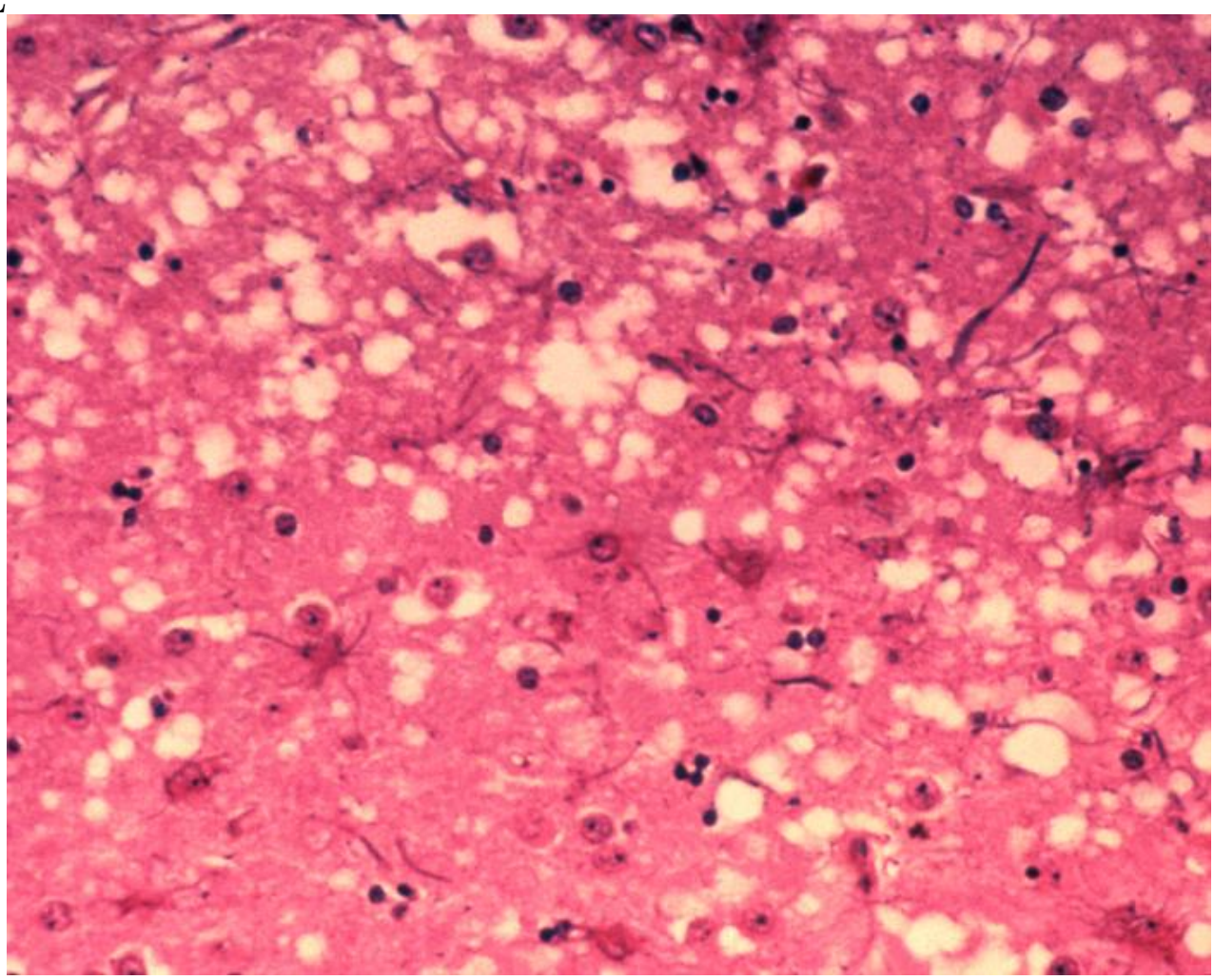
Conformation and denaturation

- Conformation: final 3D structure with minimal Gibbs energy
- At high temperatures, conformations breaks, this is denaturation
- Most proteins may return to previous conformation after renaturation

Prions

- Scrapie and Bovine Spongiform Encelopathy (BSE, "cow madness") caused by proteins
- In humans: Creutzfeldt-Jakob disease (CJD) and Kuru
- These proteins, prions (PrP) induce convertion non-PrP to misfolded form which causes a disease
- Infectious and non-infectious forms have same amino acid sequence but different 3D structure

BSE



Kuru

How prions are working

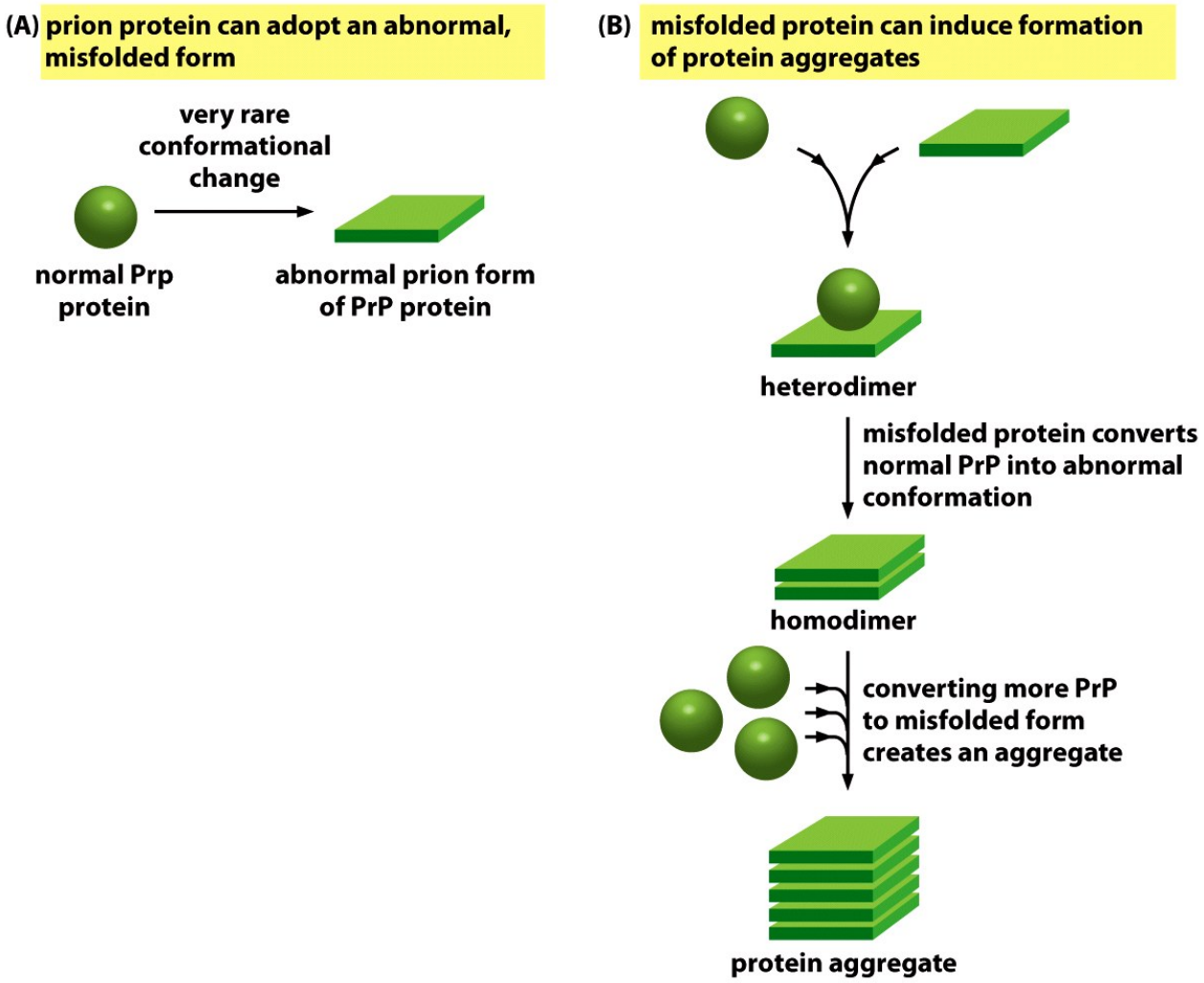
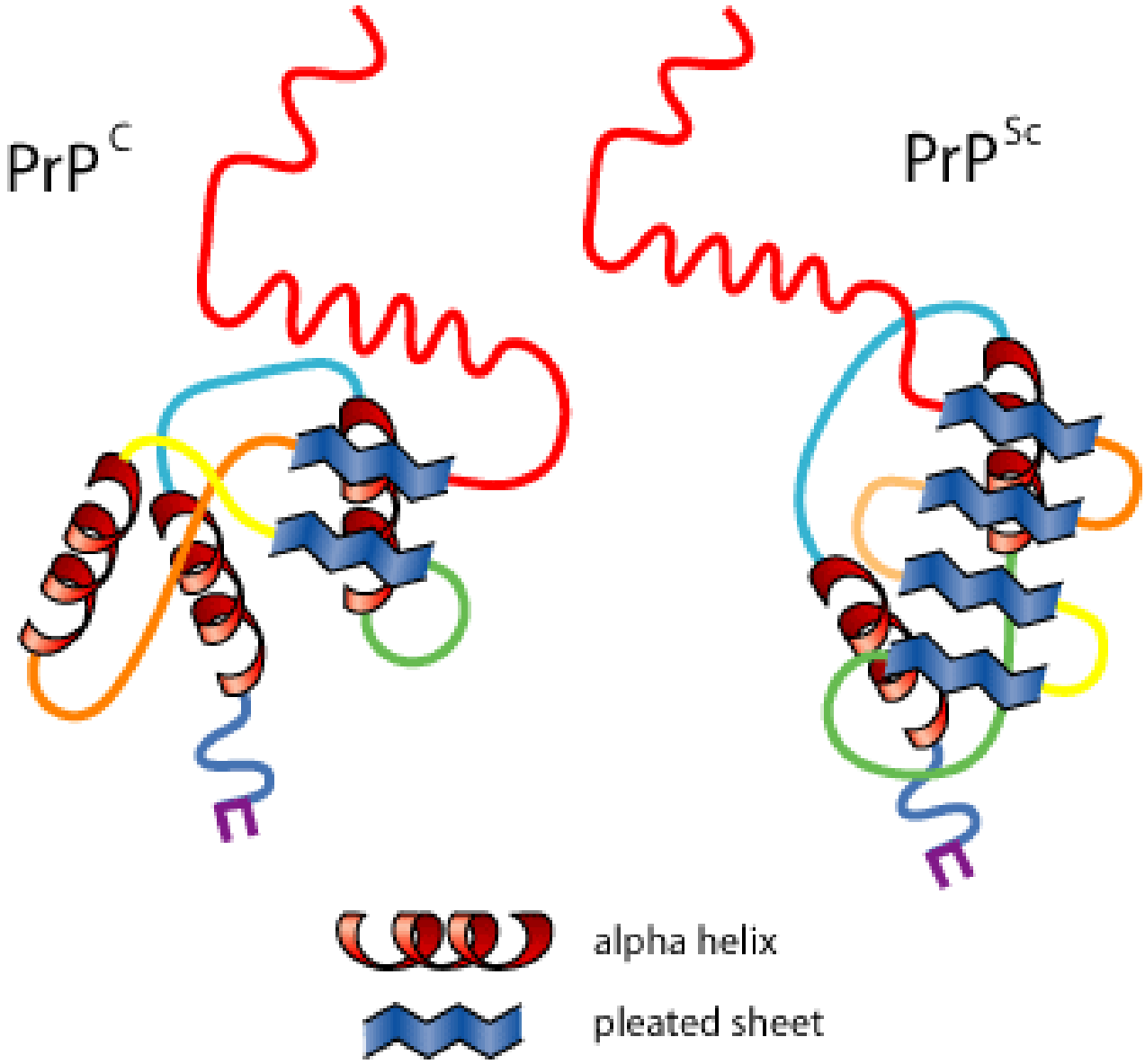


Figure 4-8 Essential Cell Biology 3/e (© Garland Science 2010)

Non-infectious and infectious conformations of prions



Rough classification of 3D forms

- Filaments (collagen)
- Sheets (silk)
- Rings (porin)
- Spheres (myoglobin)

Four ways to depict a 3D structure

- Backbone
- Ribbon
- Wire
- Space-filling

SH2 3D structure movie

V.3 Folding

Folding patterns

- α helix: spiral pattern
- β sheet: linear pattern
- They may coexist in the same molecule

α helix

- Typically, hydrogen bonds connect every fourth amino acid
- Several α helices may coil around each other and form coiled coil, as in collagen and other structural proteins

α helix

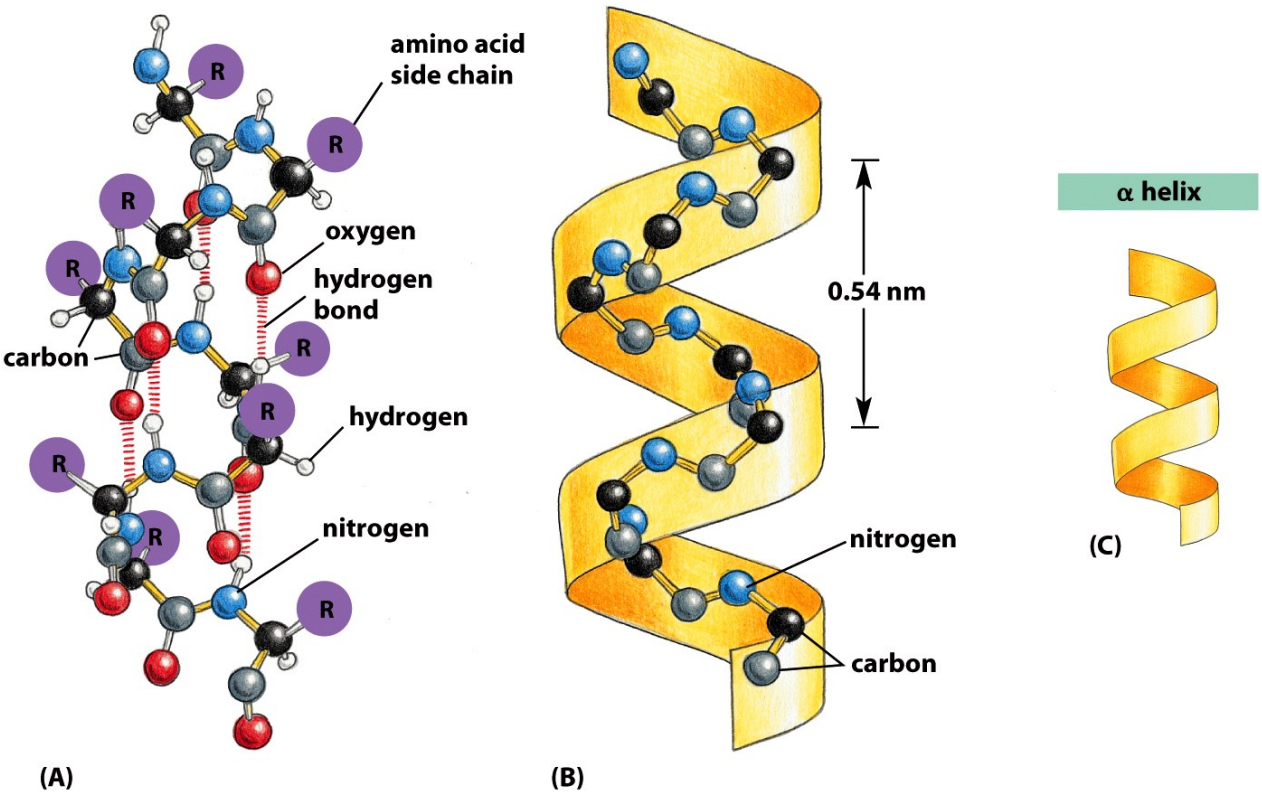


Figure 4-10a-c Essential Cell Biology 3/e (© Garland Science 2010)

α helix movie

β sheets

- Hydrogen bonds connect different sub-chains
- Parallel: amino acid chain keeps the direction (from N to C) and forms loops
- Antiparallel: amino acid chain changes direction and forms completely flat structure

β sheet

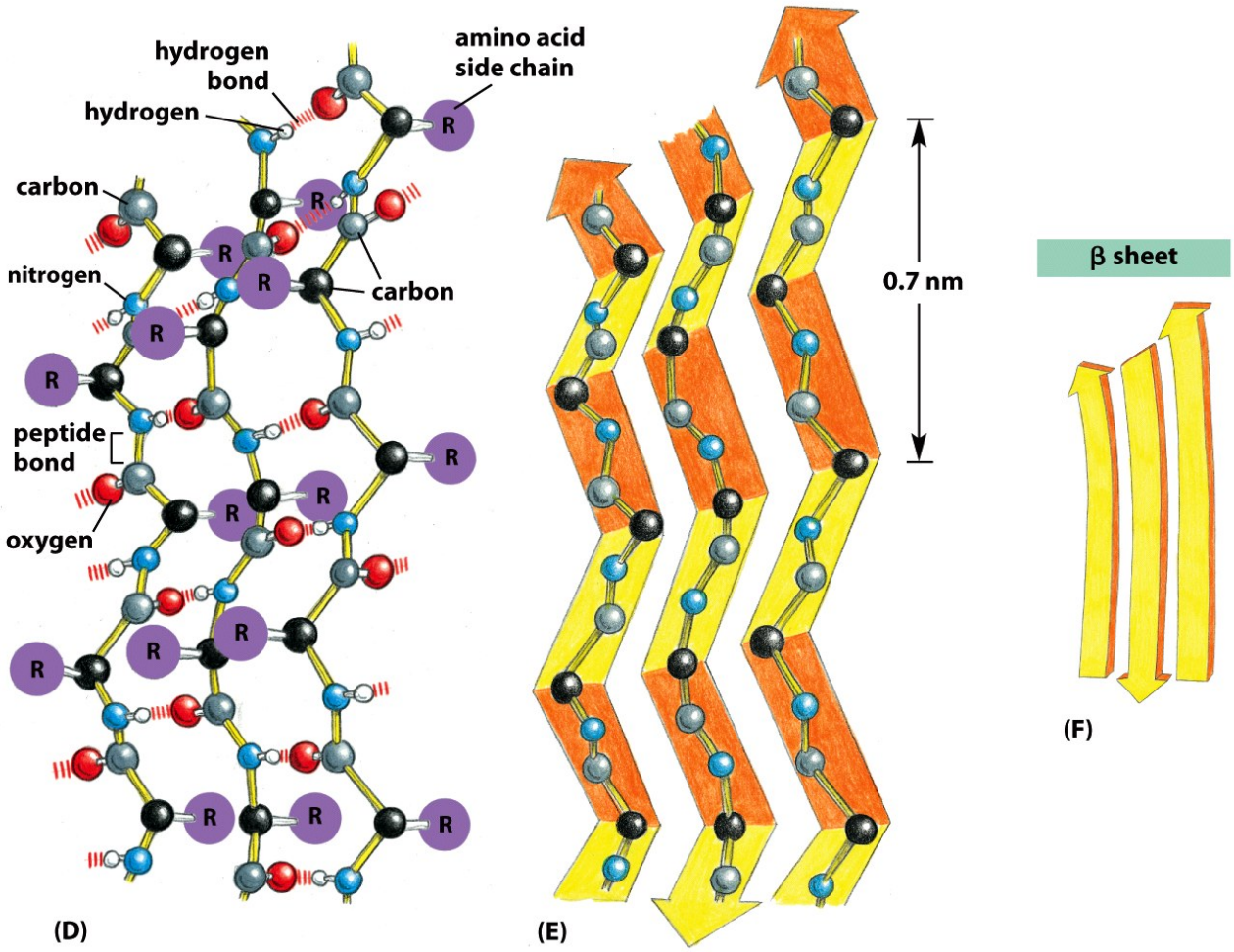


Figure 4-10d-f Essential Cell Biology 3/e (© Garland Science 2010)

Antiparallel and parallel β sheets

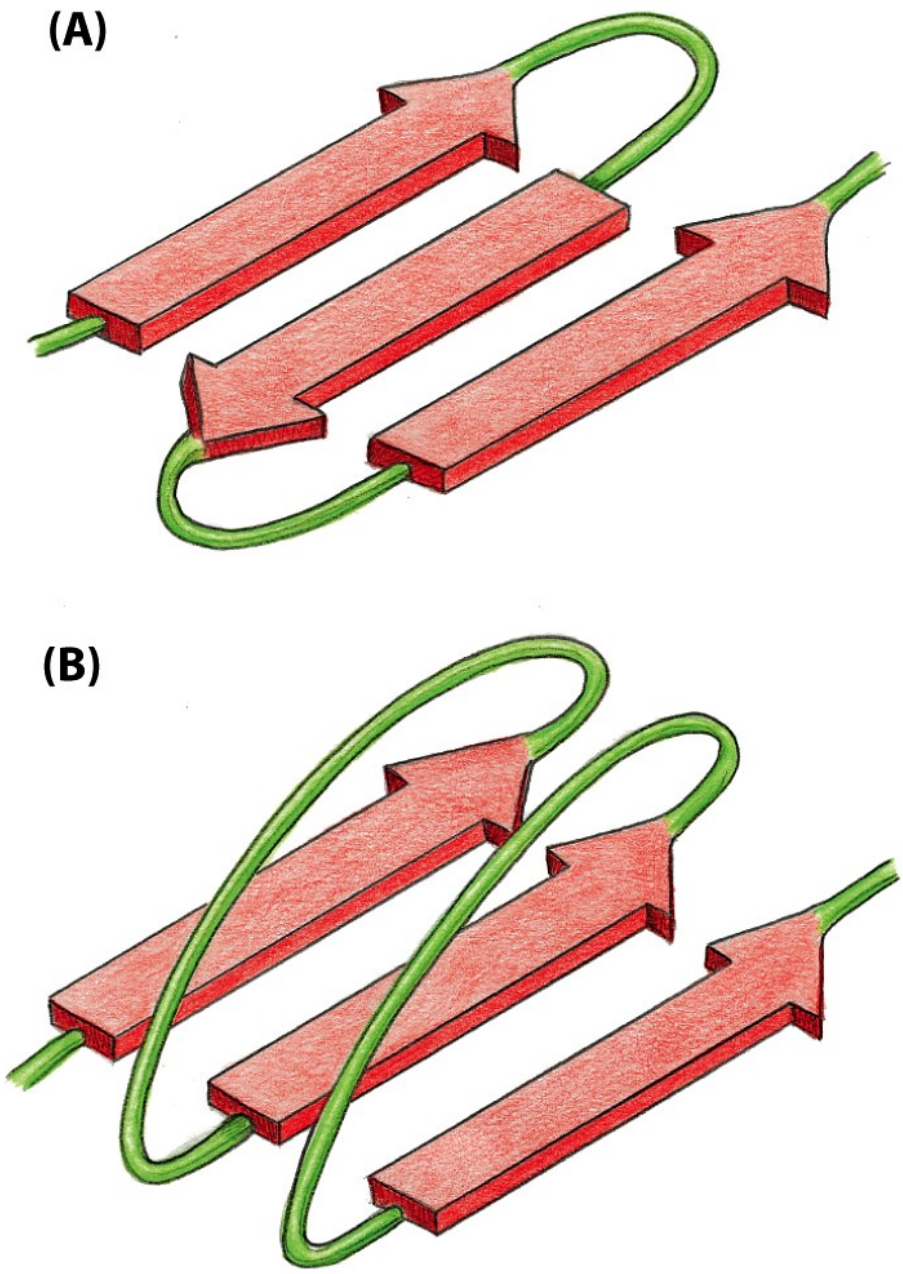


Figure 4-14 Essential Cell Biology 3/e (© Garland Science 2010)

β sheets movie

V.4 Proteins levels of organization

Protein levels of organization

- Primary
- Secondary and tertiary
- OR domain organization, when different part of one molecule fold independently
- Quaternary: subunits which are assembling through binding sites

Domain structure of protein

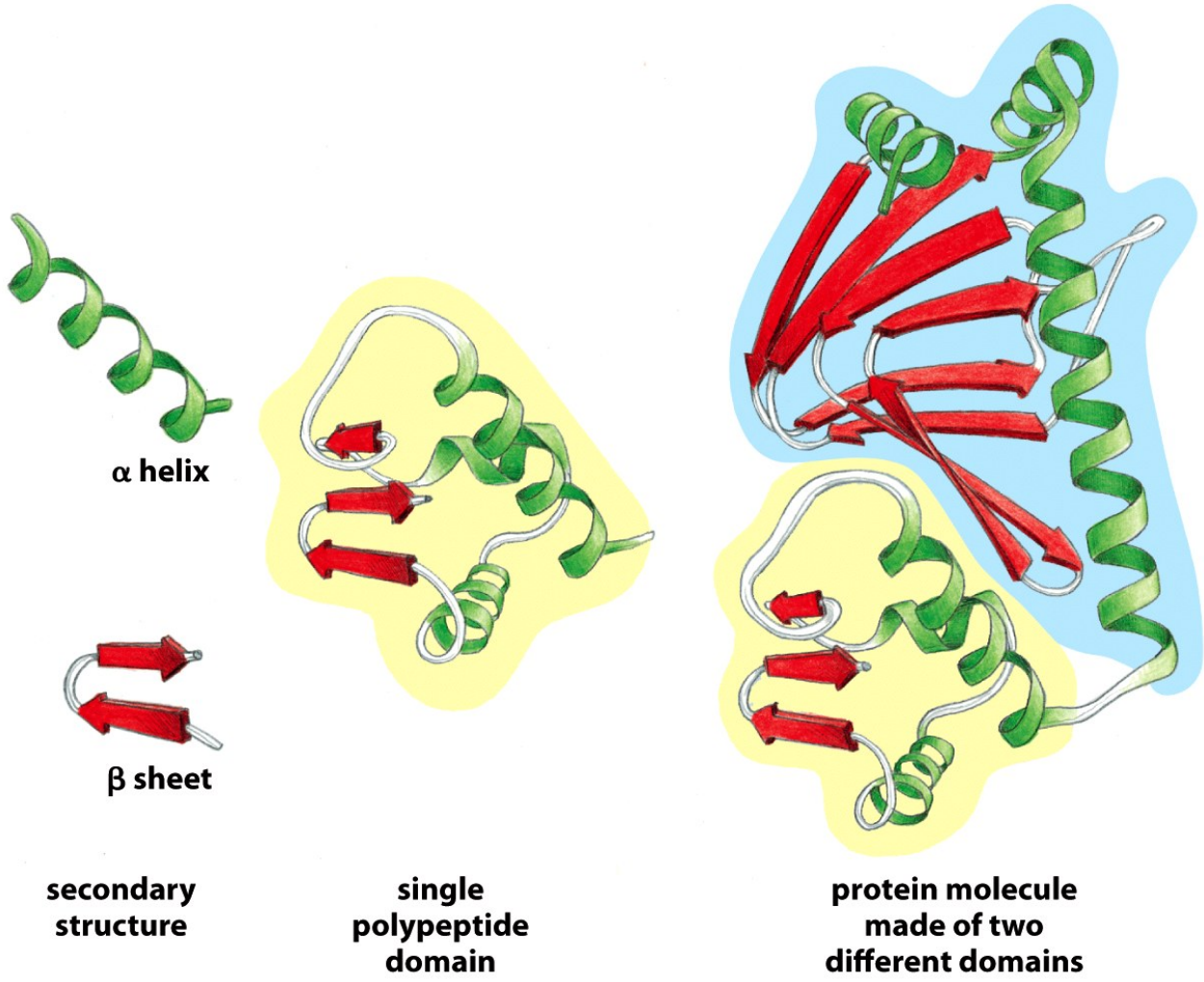


Figure 4-16 Essential Cell Biology 3/e (© Garland Science 2010)

SV40 virus capsid made from multiple protein monomers

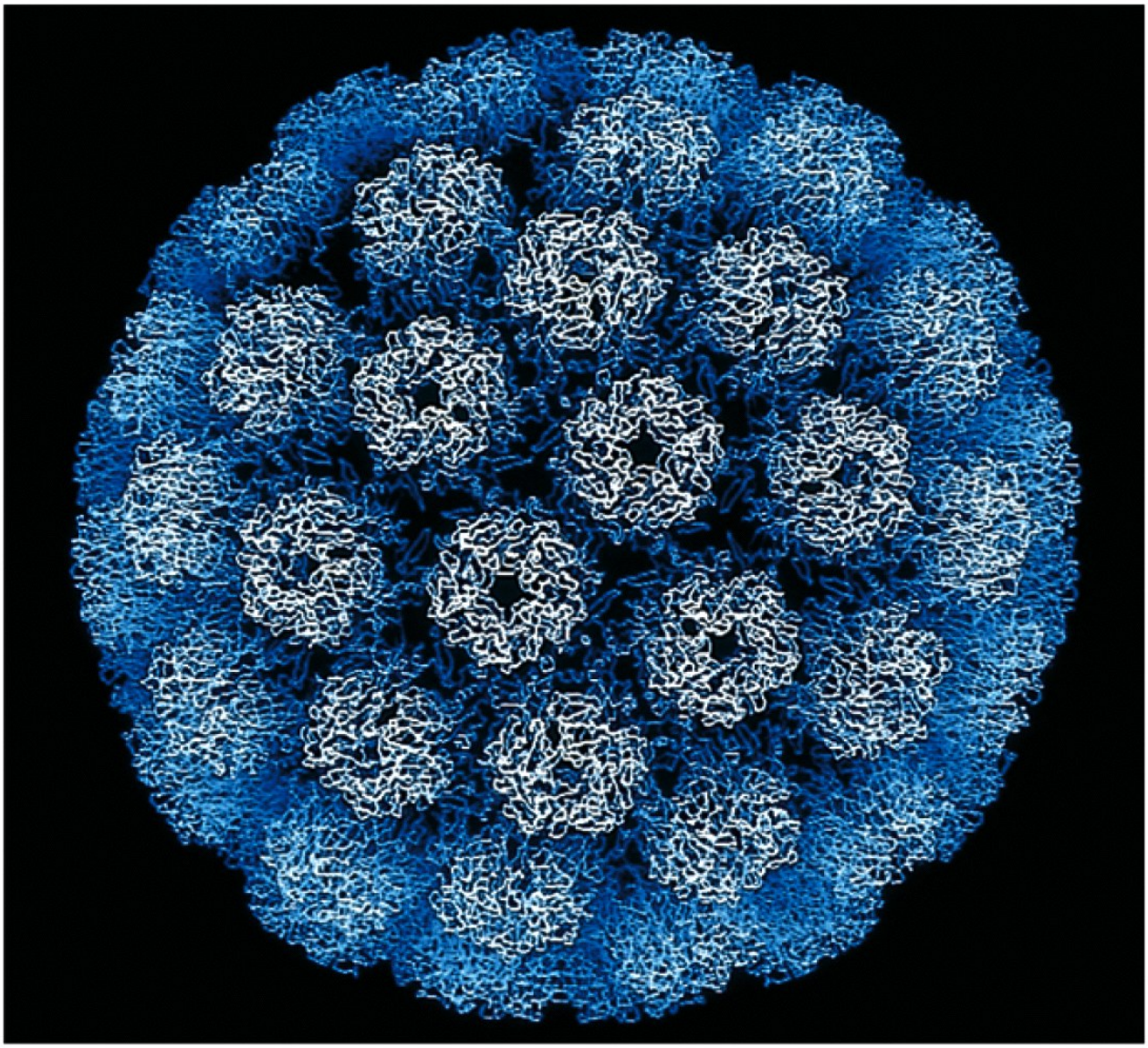


Figure 4-24 Essential Cell Biology 3/e (© Garland Science 2010)

Quaternary structure movie

Stabilization of structure

- Disulfide bonds between cysteins
- Two variants: interchain or intrachain

Inter- and intramolecular disulfide bridges

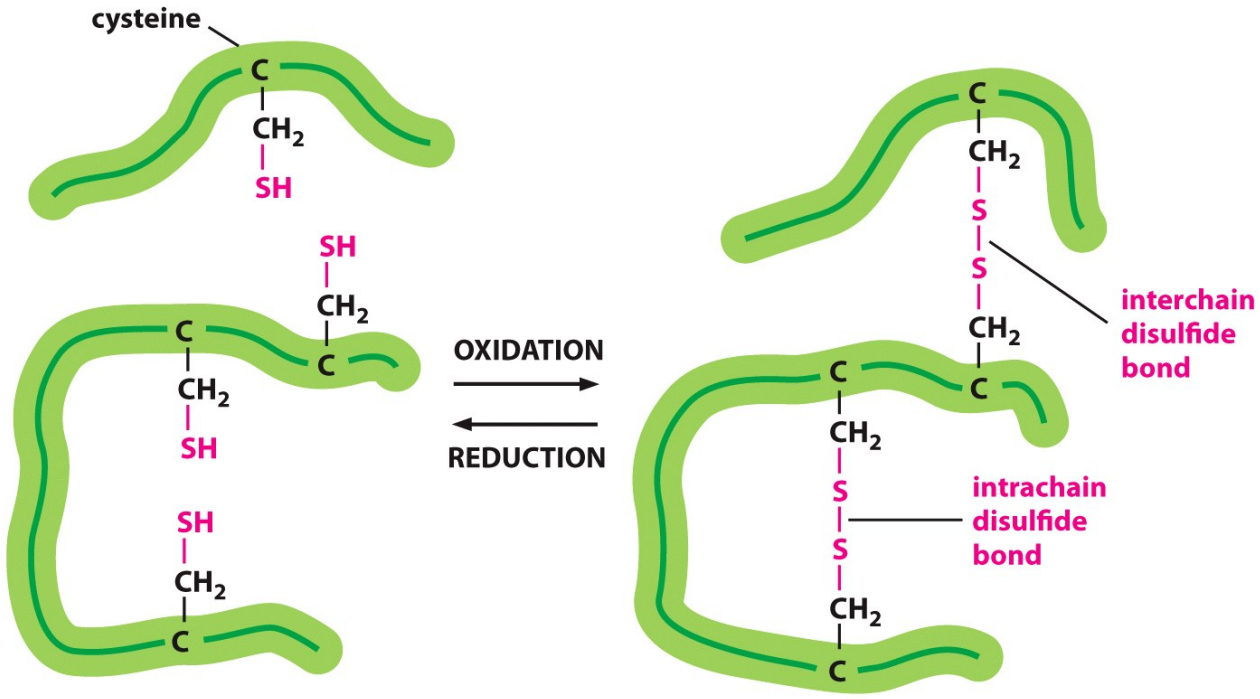


Figure 4-26 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (2 points)

Which secondary structure is more appropriate for collagen: α helix or β sheet?

Summary

- Most important protein secondary structures are α helices and β sheets

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 4*.

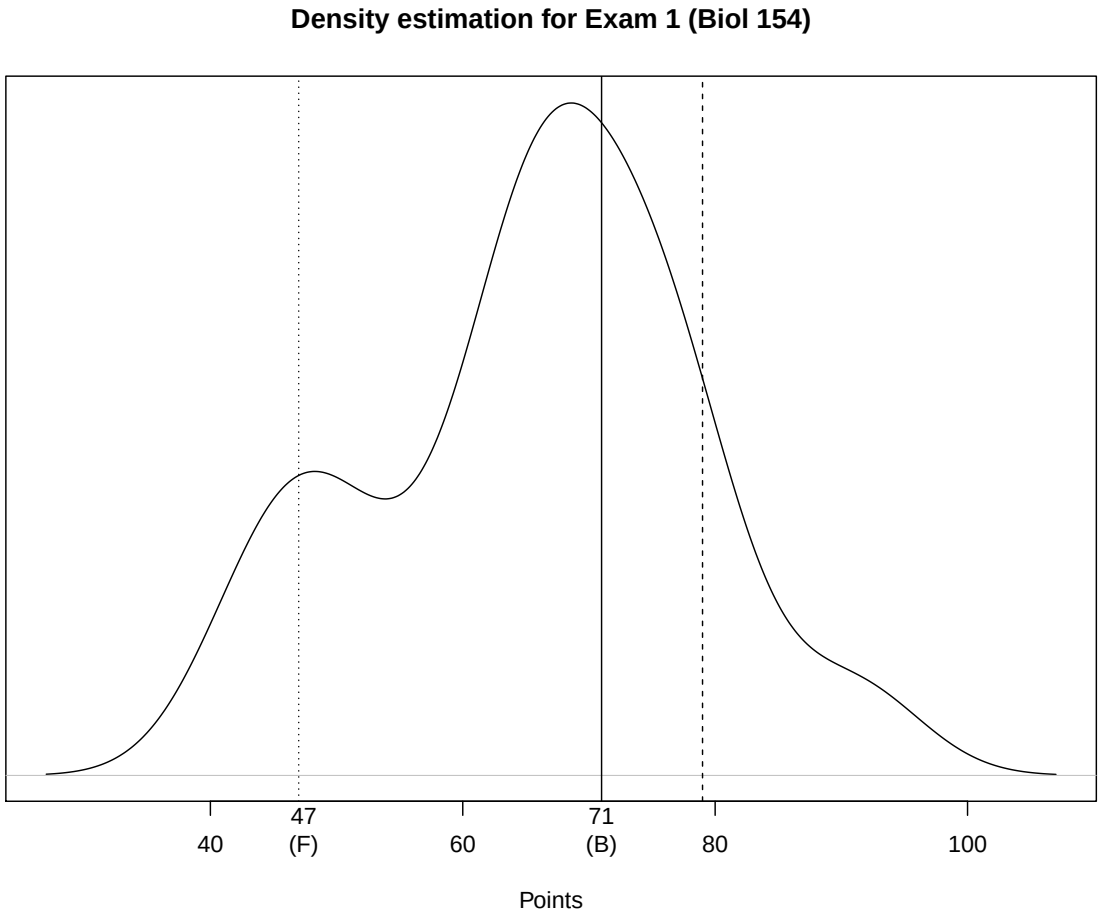
Outline

W Questions and answers

Results of Exam 1: statistic summary

```
Summary:
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  43.00   60.00   66.00   65.76   74.00   91.00
Grades:
  F   D   C   B max
 47  55  63  71  79
```

Results of Exam 1: the curve



Previous final question: the answer

Previous final question: the answer

Which secondary structure is more appropriate for collagen: α helix or β sheet?

- α helix because it is better suited for the long structures

Lab 4

- Go to the Web site and download assignment
- Join one of three review teams, participate in preparing presentation about one of three papers of DNA discovery
- Prepare questions for two other teams
- At the end of lab, everybody will evaluate the each team expertise

X Proteins

X.1 How proteins work

Binding

- Ligand is a molecule binding to protein
- Binding site is a specific region, "keyhole" rehion

CREP protein binds cyclic AMP

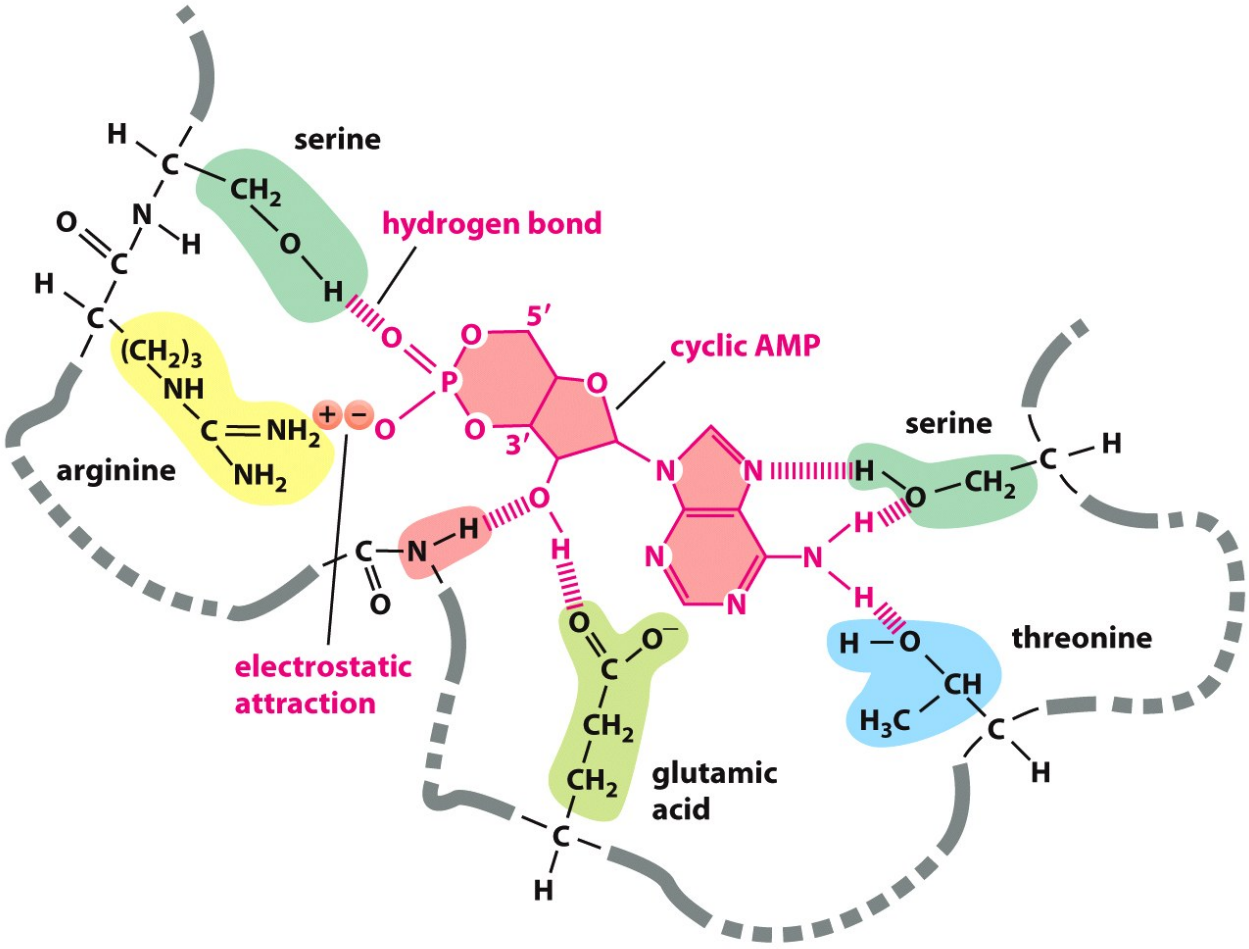


Figure 4-28b Essential Cell Biology 3/e (© Garland Science 2010)

Antibodies

- Antibodies, or γ-immunoglobulins specifically bind almost to every protein possible
- Have two heave and two light protein chains; two top regions are extremely variable in protein sequence
- They are synthesized specifically to external proteins (antigens) after being exposed to them for some time
- Grouping together, antibodies sufficiently isolate antigen and block its functions

Antibody

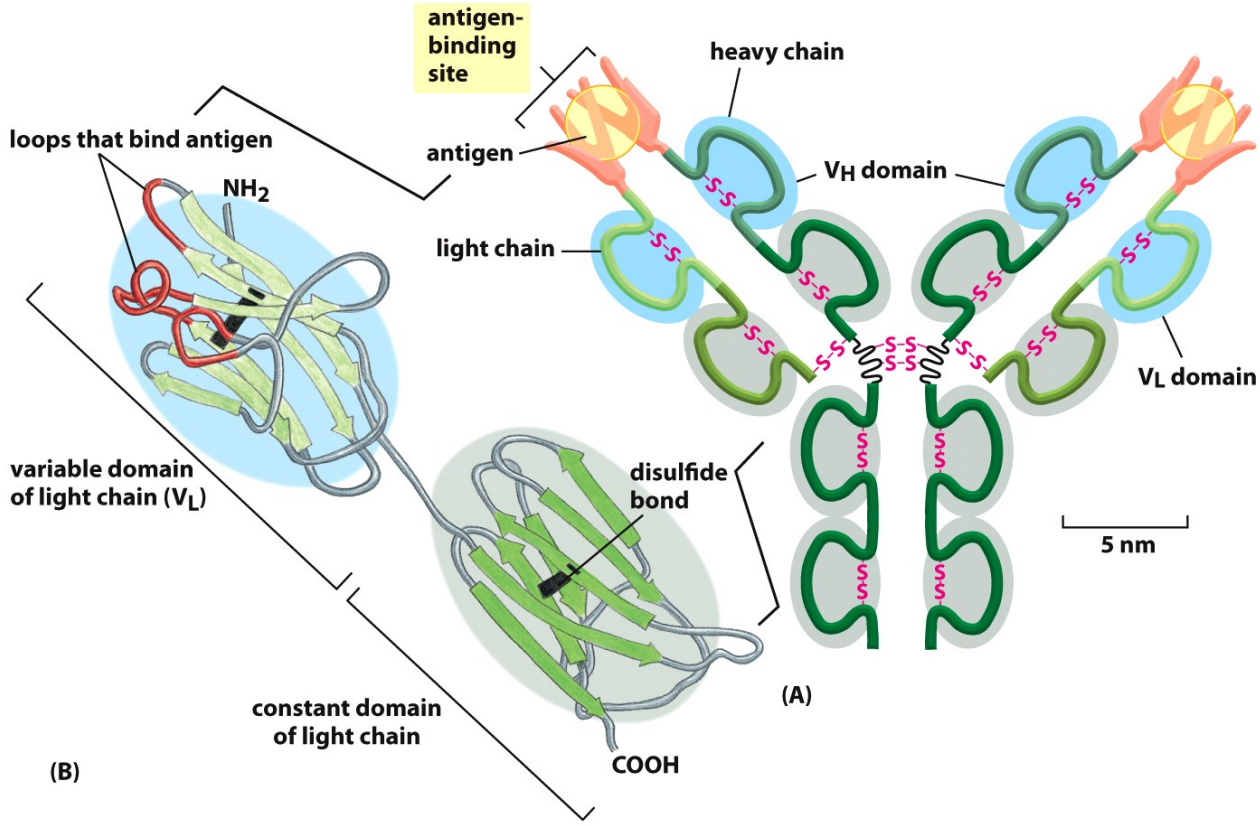


Figure 4-29 Essential Cell Biology 3/e (© Garland Science 2010)

Antibodies movie

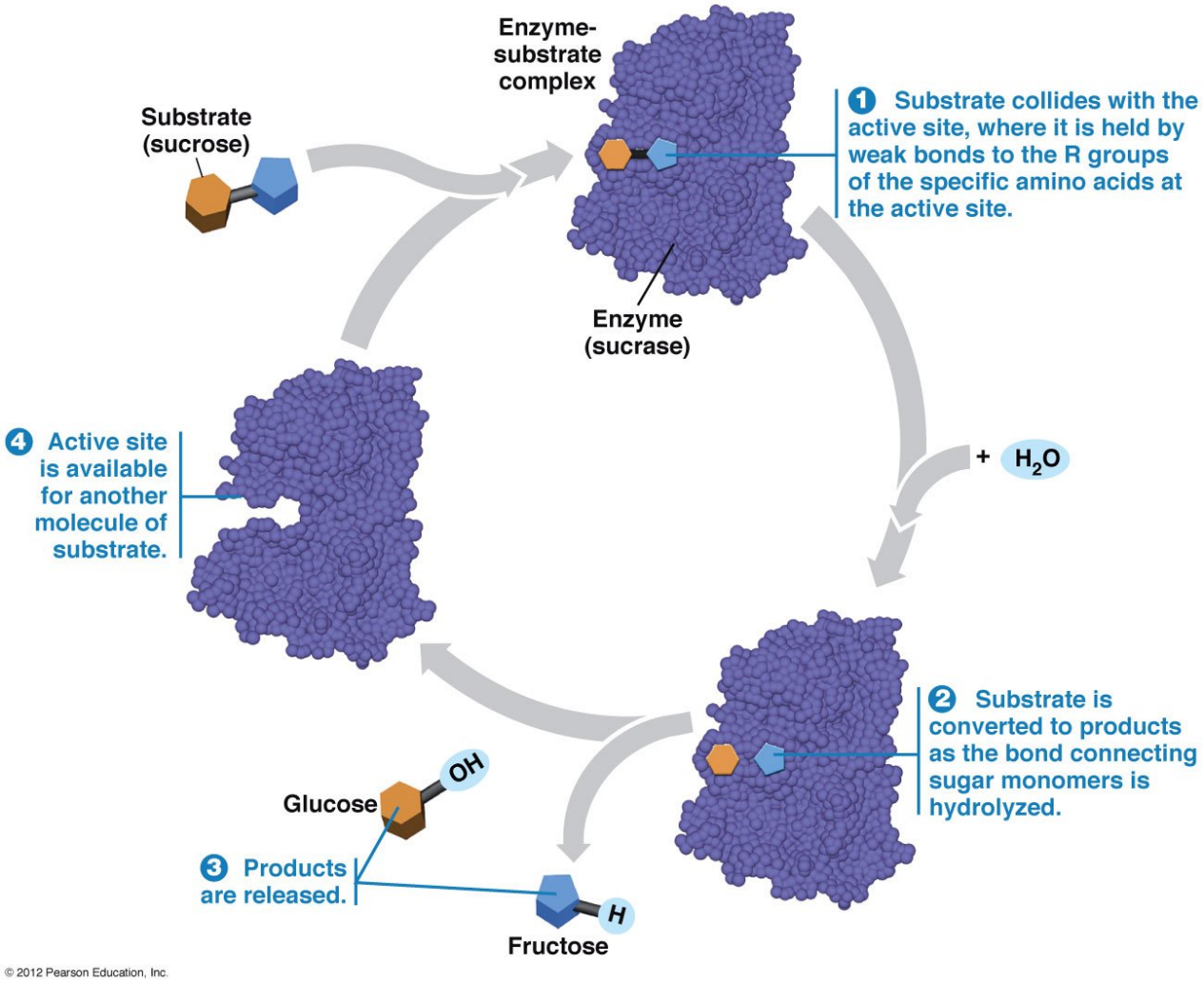
Enzymes

- Enzymes, or organic catalysts, transform the ligand after binding
- All enzymes are highly specific, e.g., to 3D conformation (accept D-glucose and ignore L-glucose etc.)
- Enzymes are often work in groups: there are tandems, chains and even pathways

How lysozyme works

- Main function—non-specific immune reaction; hydrolysis of bacterial cell wall polysaccharides (actually, peptidoglycans)
- Two amino acids works: Glu 35 and Asp 52
- First contributes proton to oxygen "bridge" and polarize water molecule, second attaches to C_1 atom

How lysozyme works I



How lysozyme works II

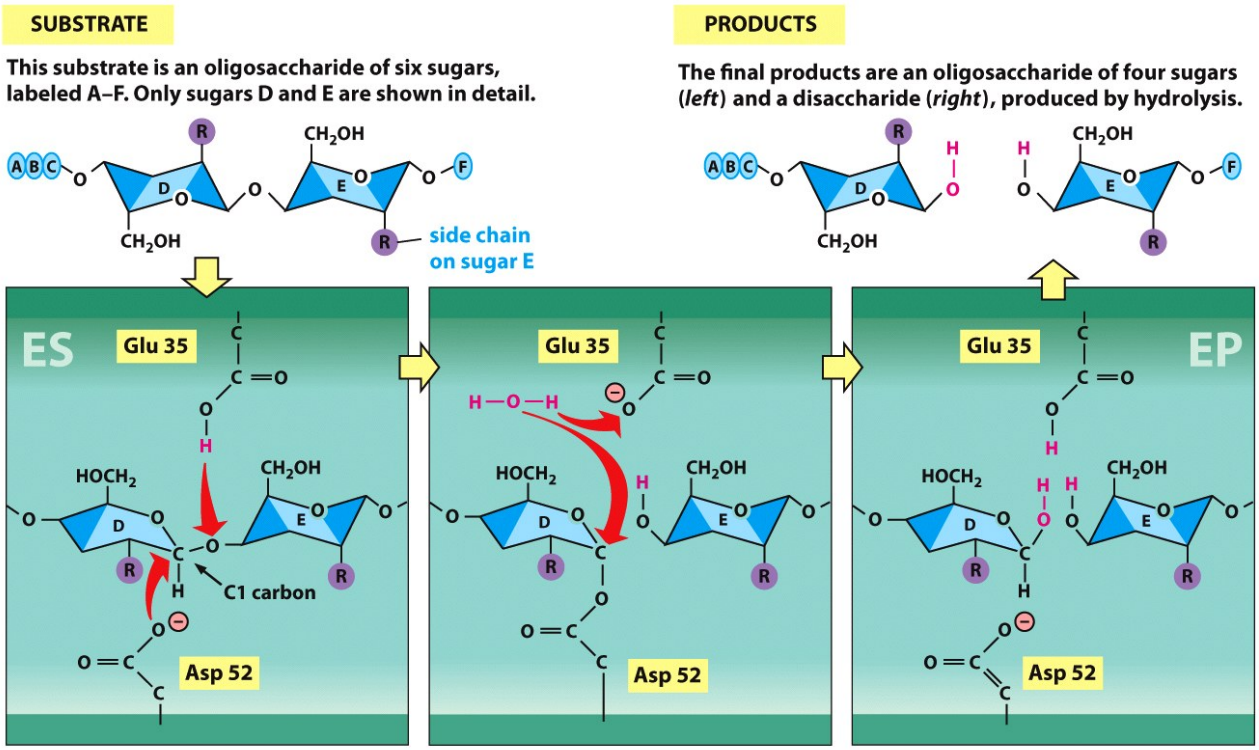


Figure 4-31 Essential Cell Biology 3/e (© Garland Science 2010)

Lysozyme I movie

Lysozyme II movie

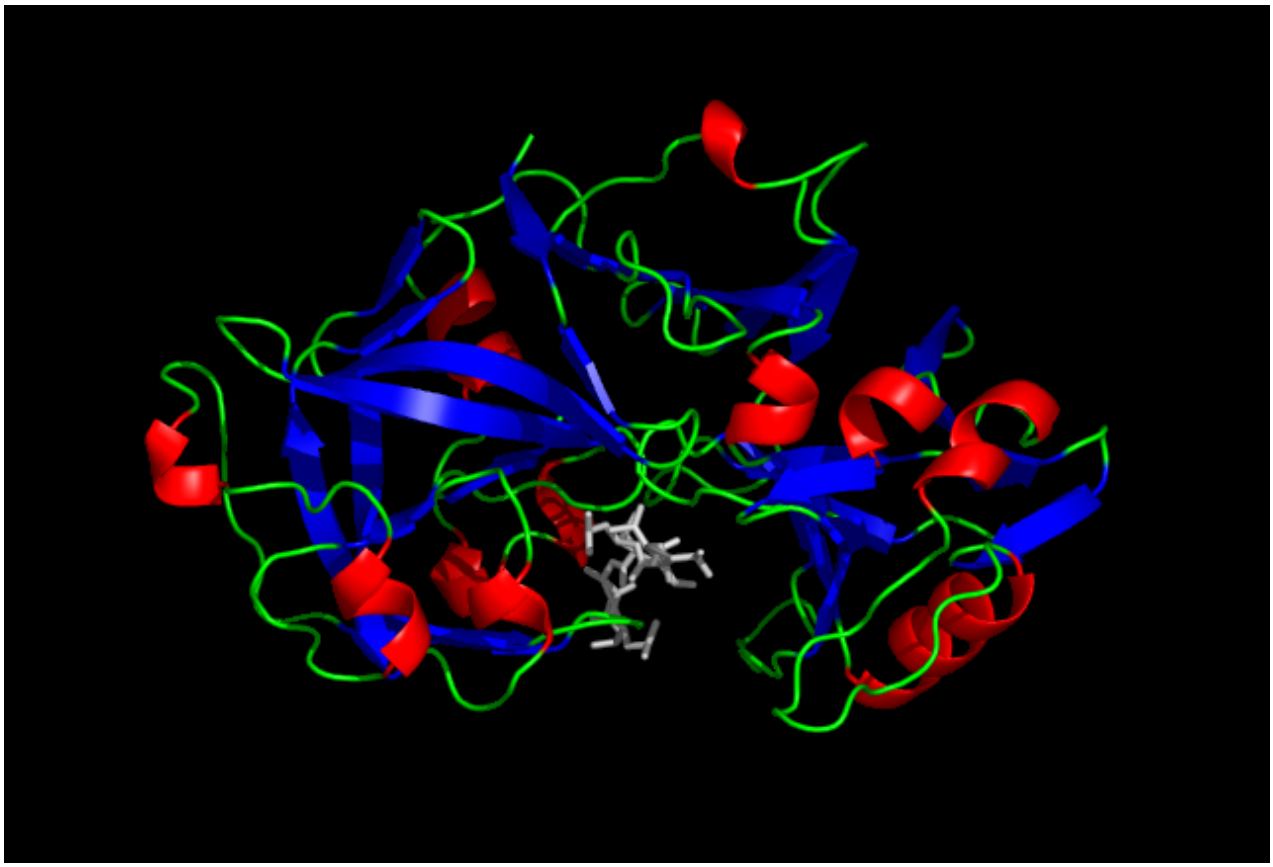
Sites

- Transition site is a place where ligand will be modified
- Active site is a place on enzyme which does main chemical job

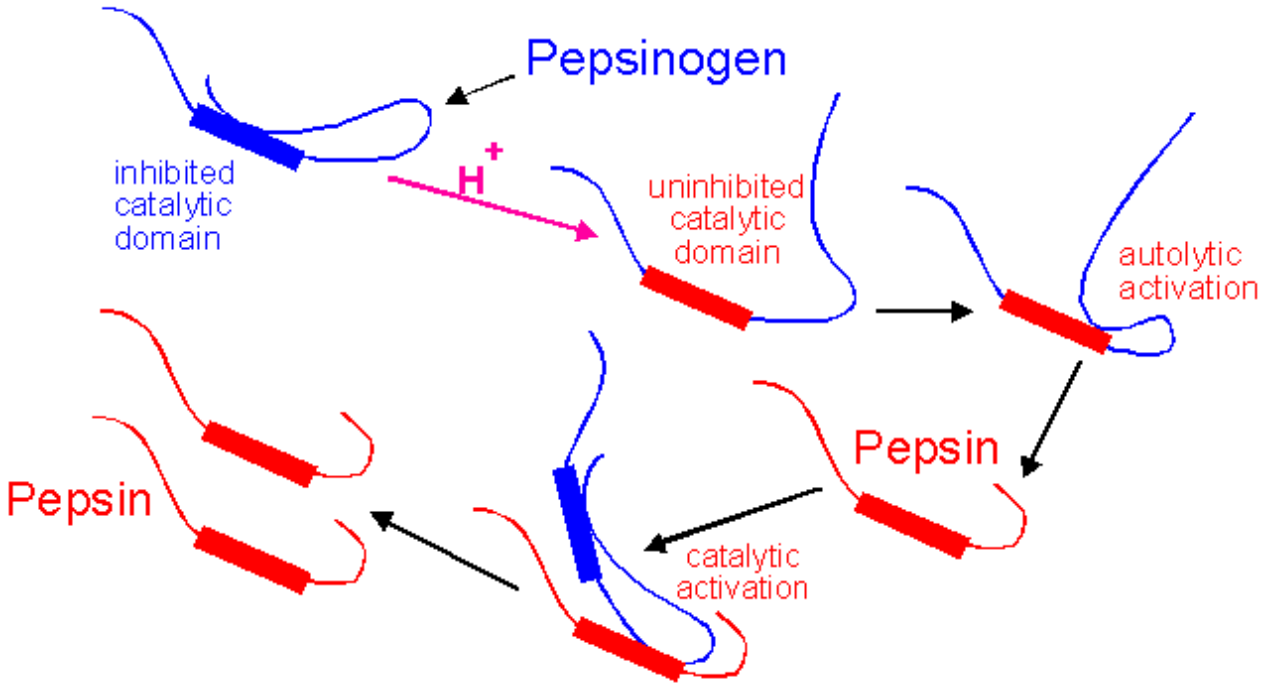
Why pepsin did not digest itself?

- Pepsin catalyze destruction of peptide bonds
- It works only on specific transition sites: cleaving aromatic amino acids like phenylalanine, tryptophan, and tyrosine from N-terminal
- It is poor of these amino acids, but still degrades and needs the constant supply of inactive pepsinogen

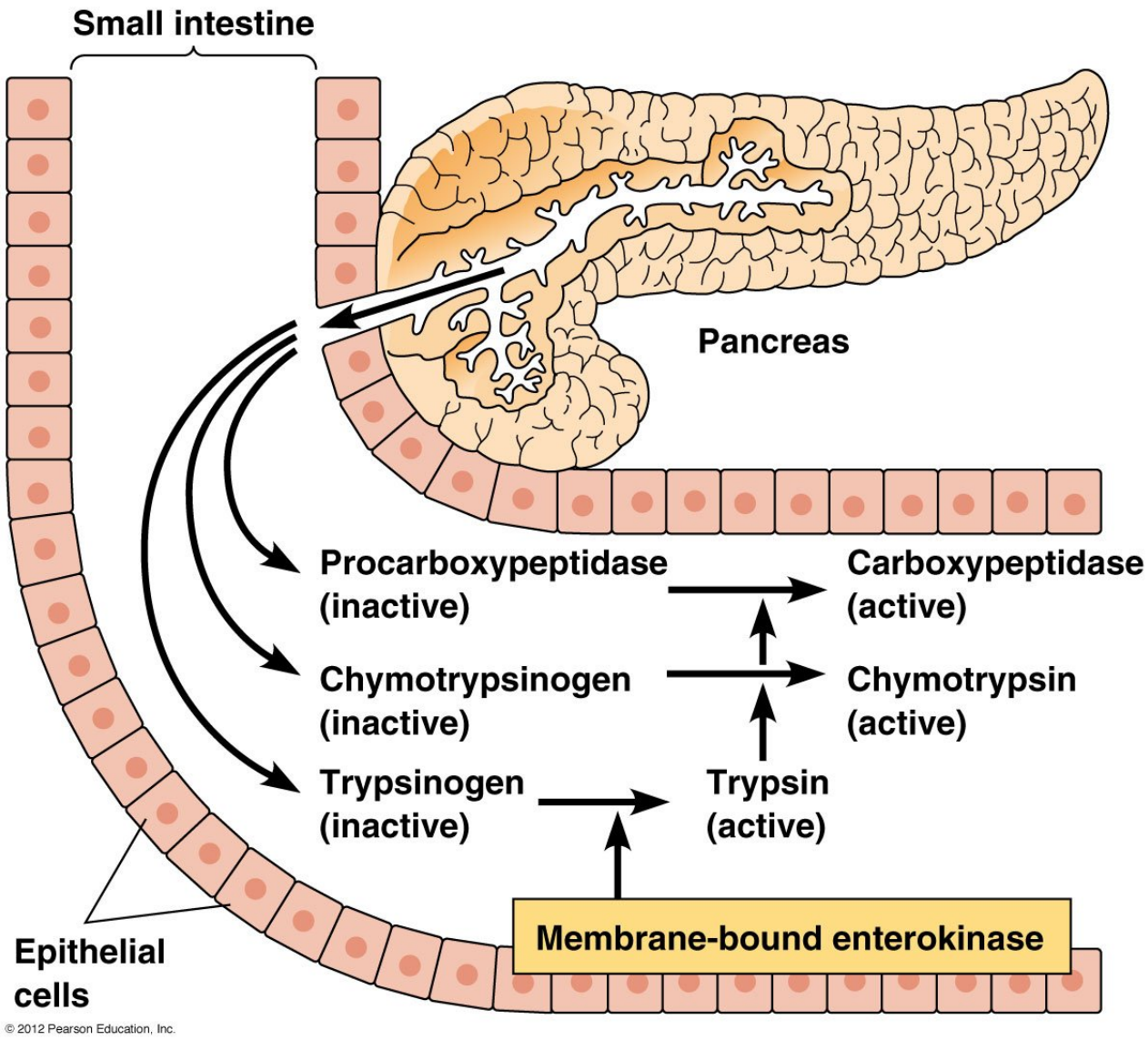
Pepsin



Activation of pepsinogen



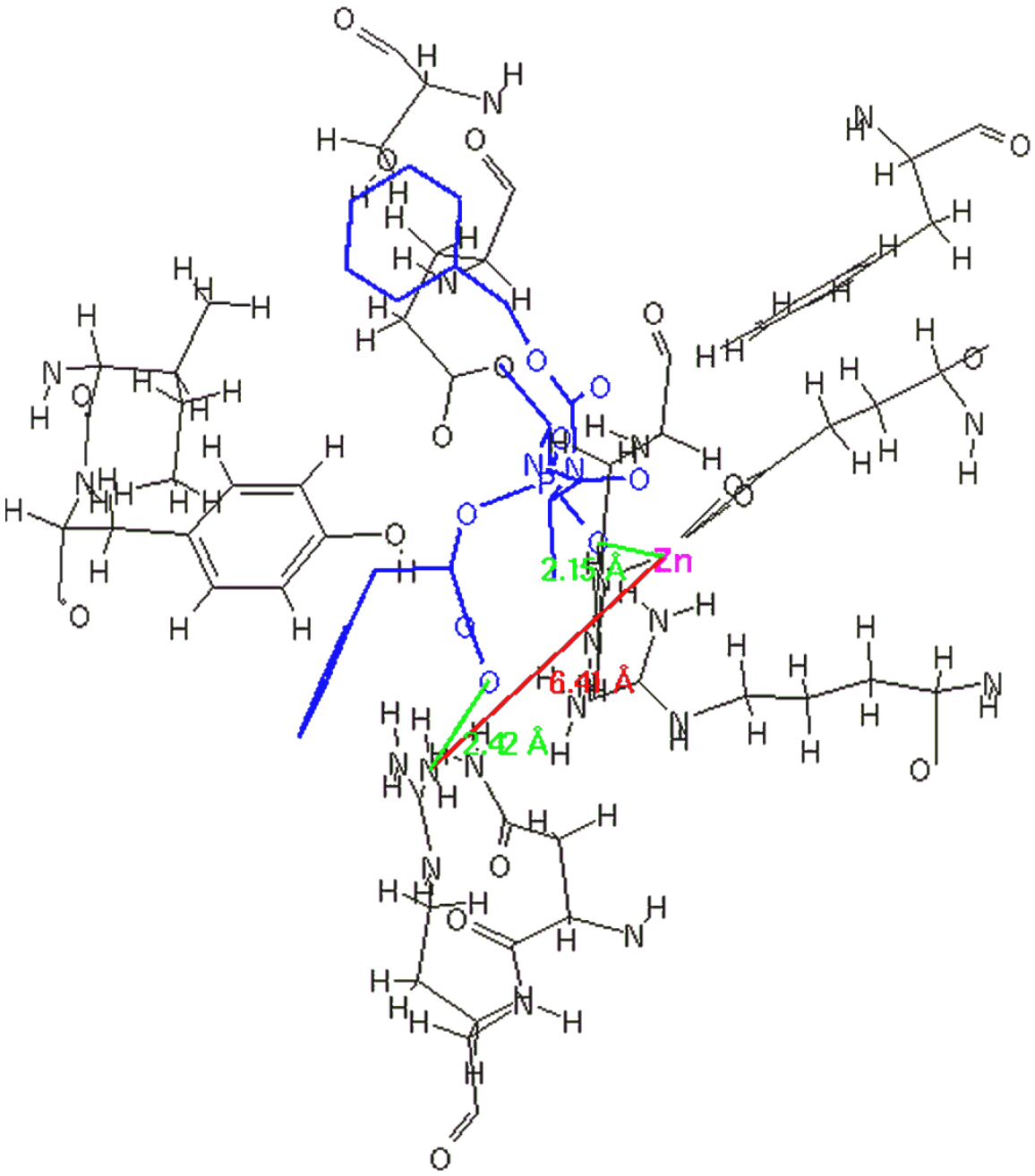
Activation of pancreatic enzymes



Binding of small molecules

- Small molecules bring additional functions to proteins
- Carotenoid retinal binds to rhodopsin (accepts ν); porphyrine heme binds to hemoglobin (accepts O_2/CO_2)
- Carboxypeptidase binds Zn atoms

Carboxypeptidase active site with zinc



X.2 How proteins are controlled

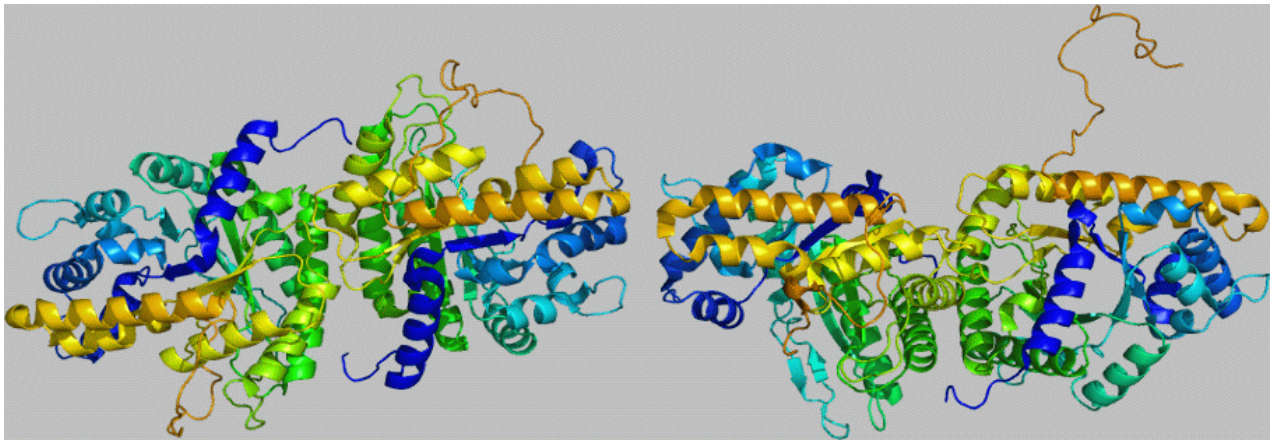
Inhibition

- **Genetic inhibition**: mistakes in primary structure (often caused by recessive mutations) inhibit different enzymes

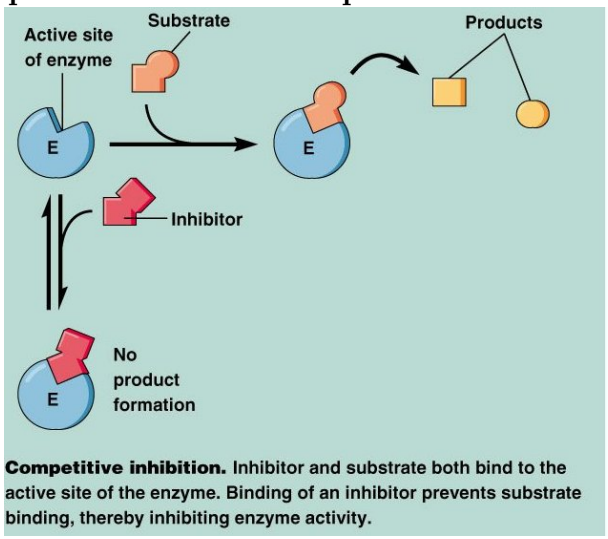
  For example, error in aldolase B gene (which split the fructose molecule) results in HFI (hereditory fructose intolerance), the disease which is often fatal: unprocessed fructose trap phosphates and block many other metabolic reactions

- **Chemical inhibition**: many drugs **reversely** inhibit enzymes chemically

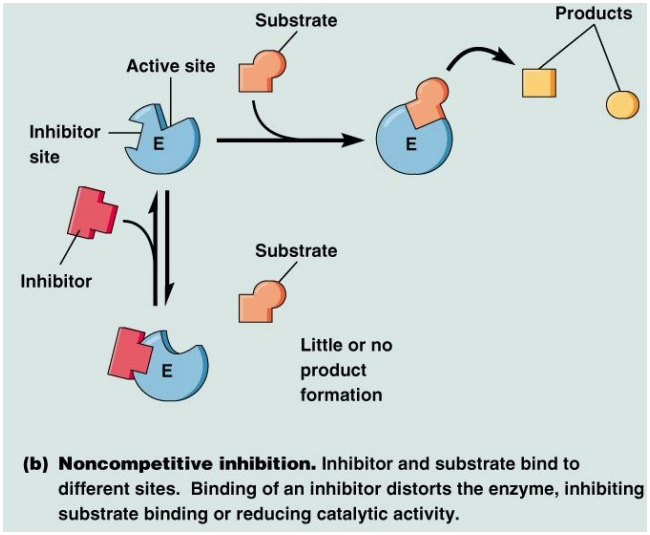
Aldolase B



Competitive and non-competitive inhibition



Competitive inhibition. Inhibitor and substrate both bind to the active site of the enzyme. Binding of an inhibitor prevents substrate binding, thereby inhibiting enzyme activity.



(b) Noncompetitive inhibition. Inhibitor and substrate bind to different sites. Binding of an inhibitor distorts the enzyme, inhibiting substrate binding or reducing catalytic activity.

Final question (2 points)

How many amino acids are in the active site of lysozyme?

Summary

- Almost all proteins bind to other molecules (ligands)
- Enzymes convert ligands
- Some other proteins use ligands as additions to their active sites

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 4*.

Outline

Y Questions and answers

Lab 4: teams and papers

Three teams: ...

Previous final question: the answer

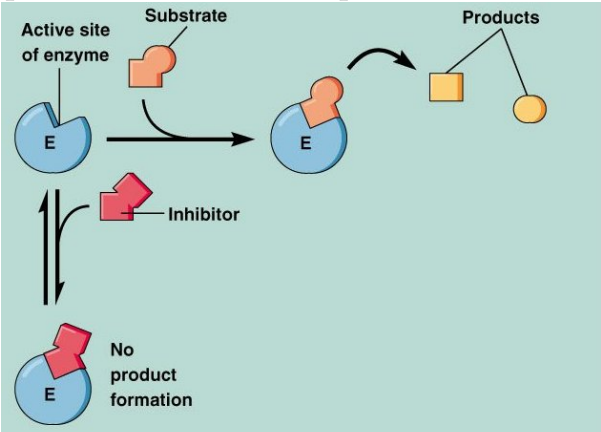
How many amino acids are in the active site of lysozyme?

- 2

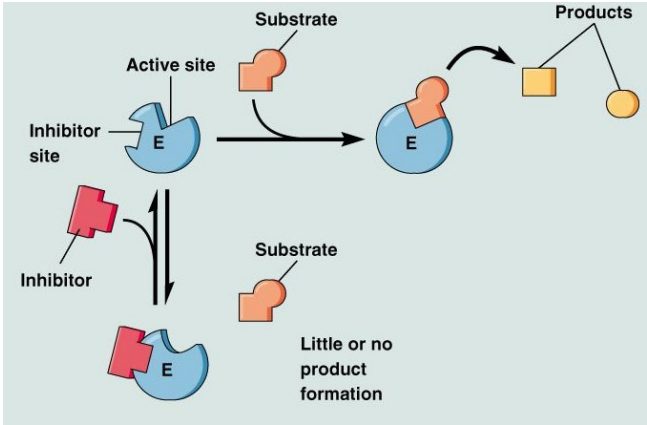
Z Proteins

Z.1 How proteins are controlled

Competitive and non-competitive inhibition



Competitive inhibition. Inhibitor and substrate both bind to the active site of the enzyme. Binding of an inhibitor prevents substrate binding, thereby inhibiting enzyme activity.

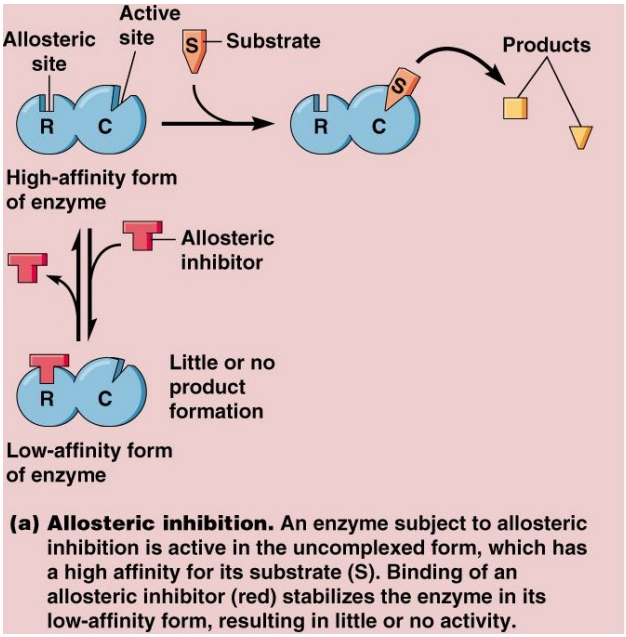


(b) Noncompetitive inhibition. Inhibitor and substrate bind to different sites. Binding of an inhibitor distorts the enzyme, inhibiting substrate binding or reducing catalytic activity.

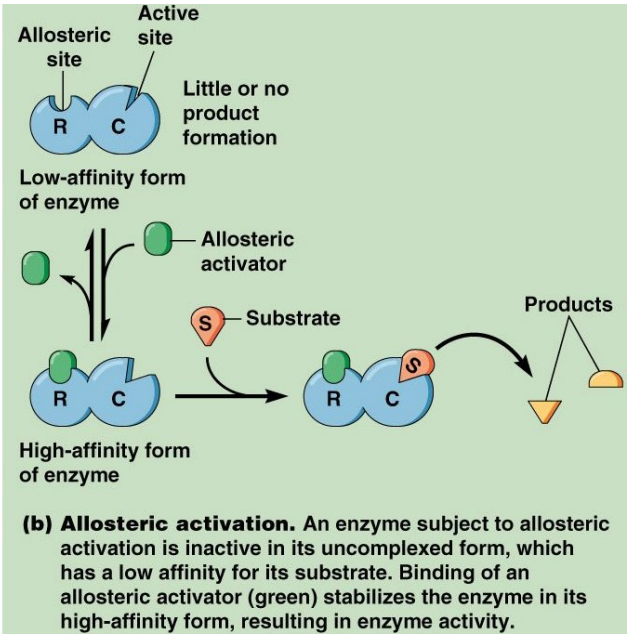
Allosteric enzymes

- Many proteins have at least two binding sites: for substrate and for regulatory molecules
- Depending on the regulatory state, proteins may change conformation
- *Allosteric* proteins have more than one stable conformation (each with different activity)

Allosteric activatin and inhibition

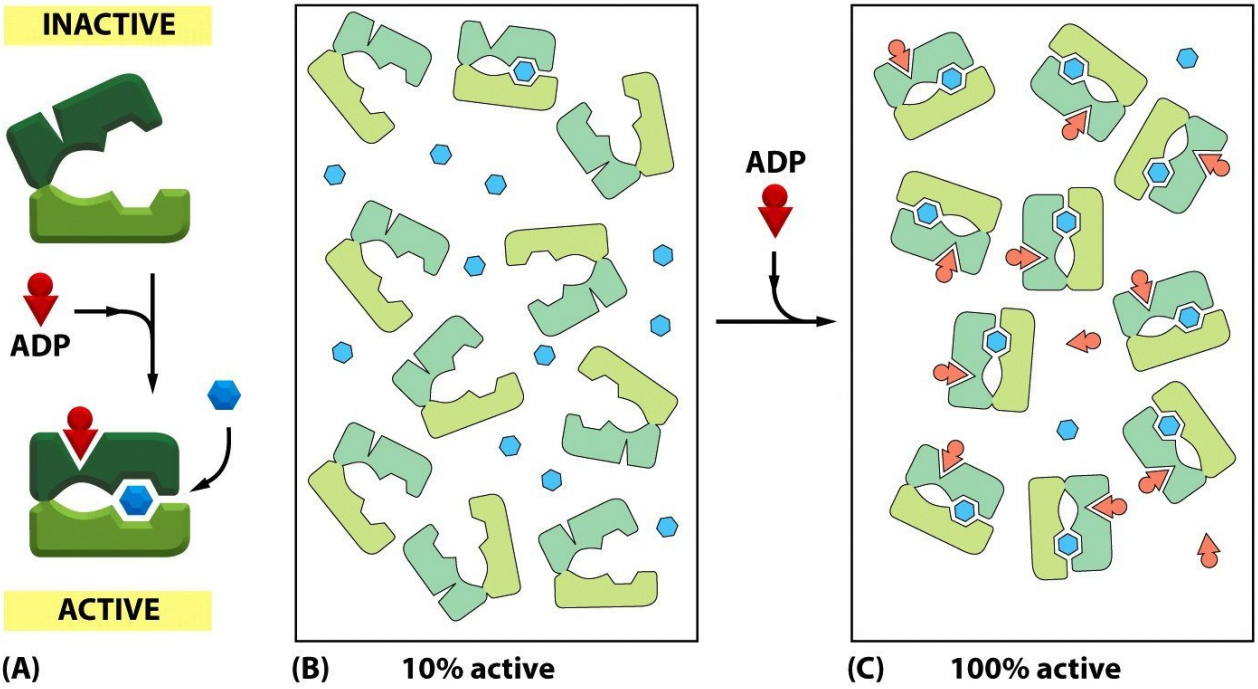


(a) Allosteric inhibition. An enzyme subject to allosteric inhibition is active in the uncomplexed form, which has a high affinity for its substrate (S). Binding of an allosteric inhibitor (red) stabilizes the enzyme in its low-affinity form, resulting in little or no activity.



(b) Allosteric activation. An enzyme subject to allosteric activation is inactive in its uncomplexed form, which has a low affinity for its substrate. Binding of an allosteric activator (green) stabilizes the enzyme in its high-affinity form, resulting in enzyme activity.

Allosteric activatin with ADP



Allosteric inhibiiton: enzyme aspartate transcarbamoylase with CTP

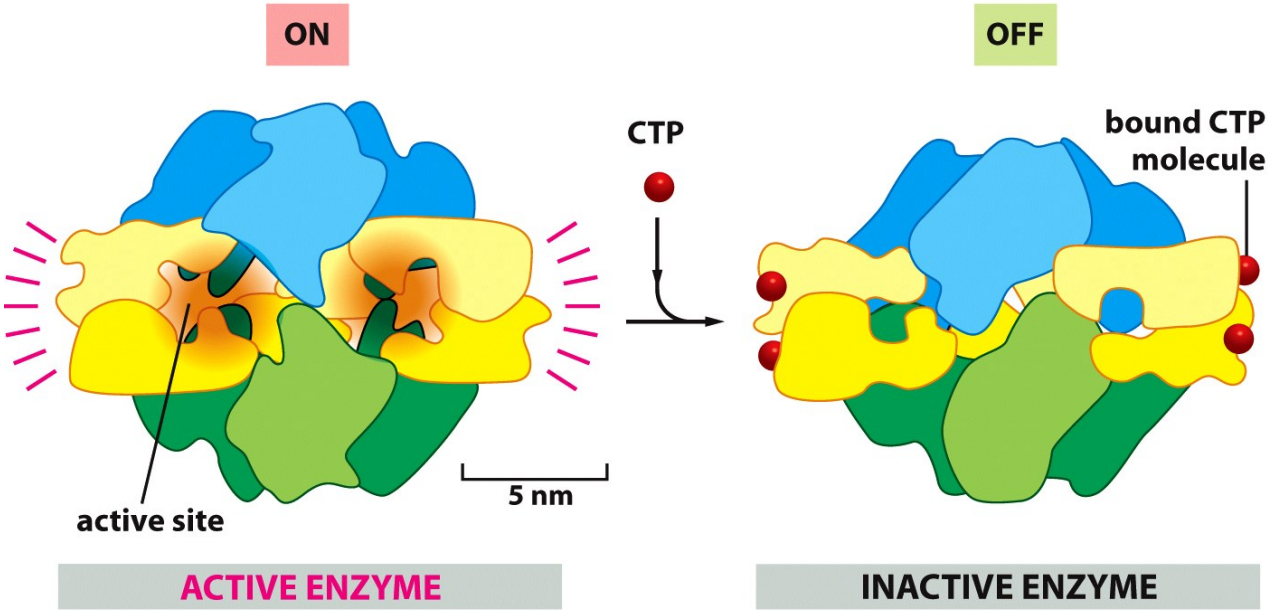


Figure 4-36 Essential Cell Biology 3/e (© Garland Science 2010)

Feedback inhibition

- Feedback inhibition occurs when later product in a pathway suppresses earlier stage of this pathway
- This is a negative regulation

Negative feedback

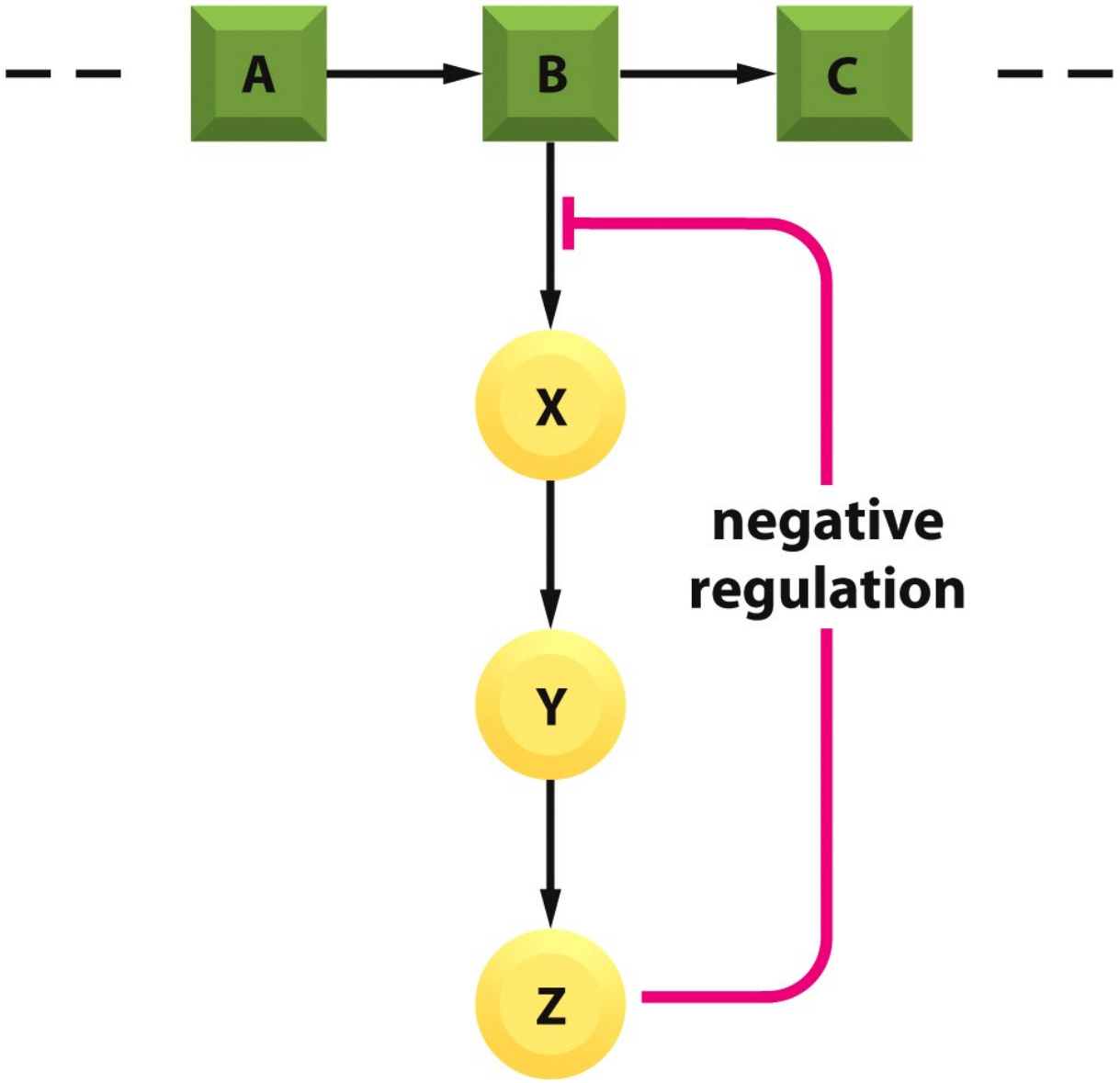


Figure 4-34 Essential Cell Biology 3/e (© Garland Science 2010)

Protein phosphorylation

- Majority of proteins are controlled through *phosphorylation*: attaching a phosphate group to one of side chains (e.g., –OH)
- *Protein kinase* catalyzes phosphorylation
- *Protein phosphataze* catalyzes *dephosphorylation*

Phosphorylation

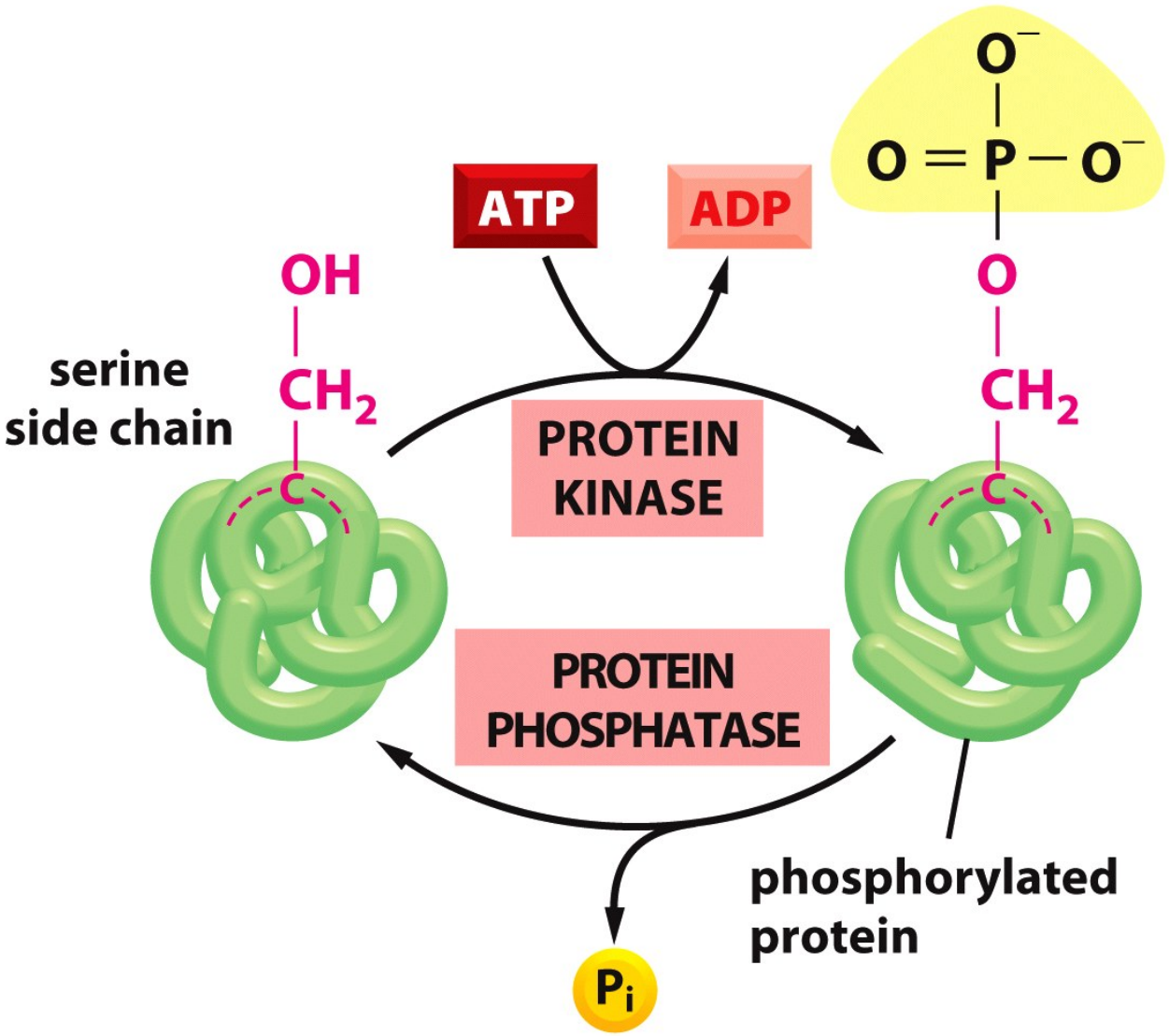
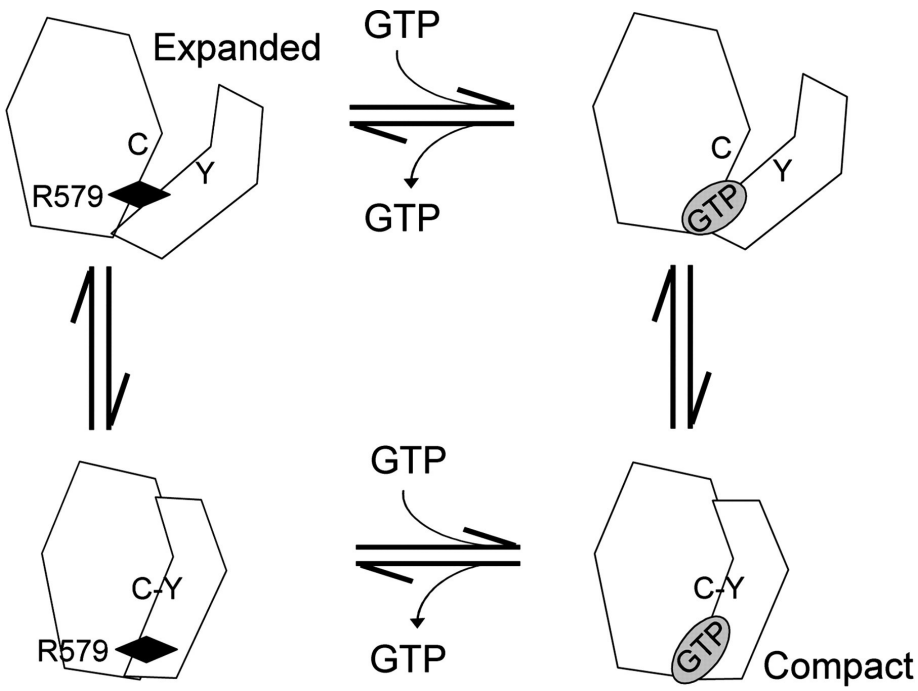


Figure 4-38a Essential Cell Biology 3/e (© Garland Science 2010)

GTP binding

- Instead of phosphate group, some proteins may bind *GTP*, guanosine triphosphate
- Attached GTP releases phosphate and turn in GDP; at the same moment, protein changes its conformation to inactive. The process is reversible.
- Bacterial elongation factor EF-Tu beating transport RNA while activated (with GTP); after GTP hydrolysis it releases tRNA

Model of allosteric inhibition of transglutaminase 2 by GTP.



Begg G E et al. PNAS 2006;103:19683-19688

©2006 by National Academy of Sciences

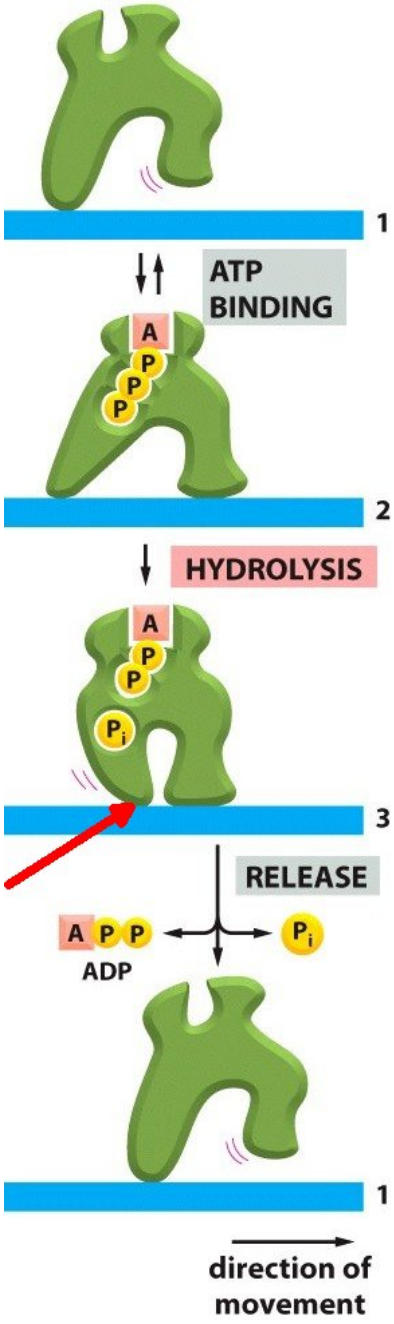
PNAS

EF-Tu movie

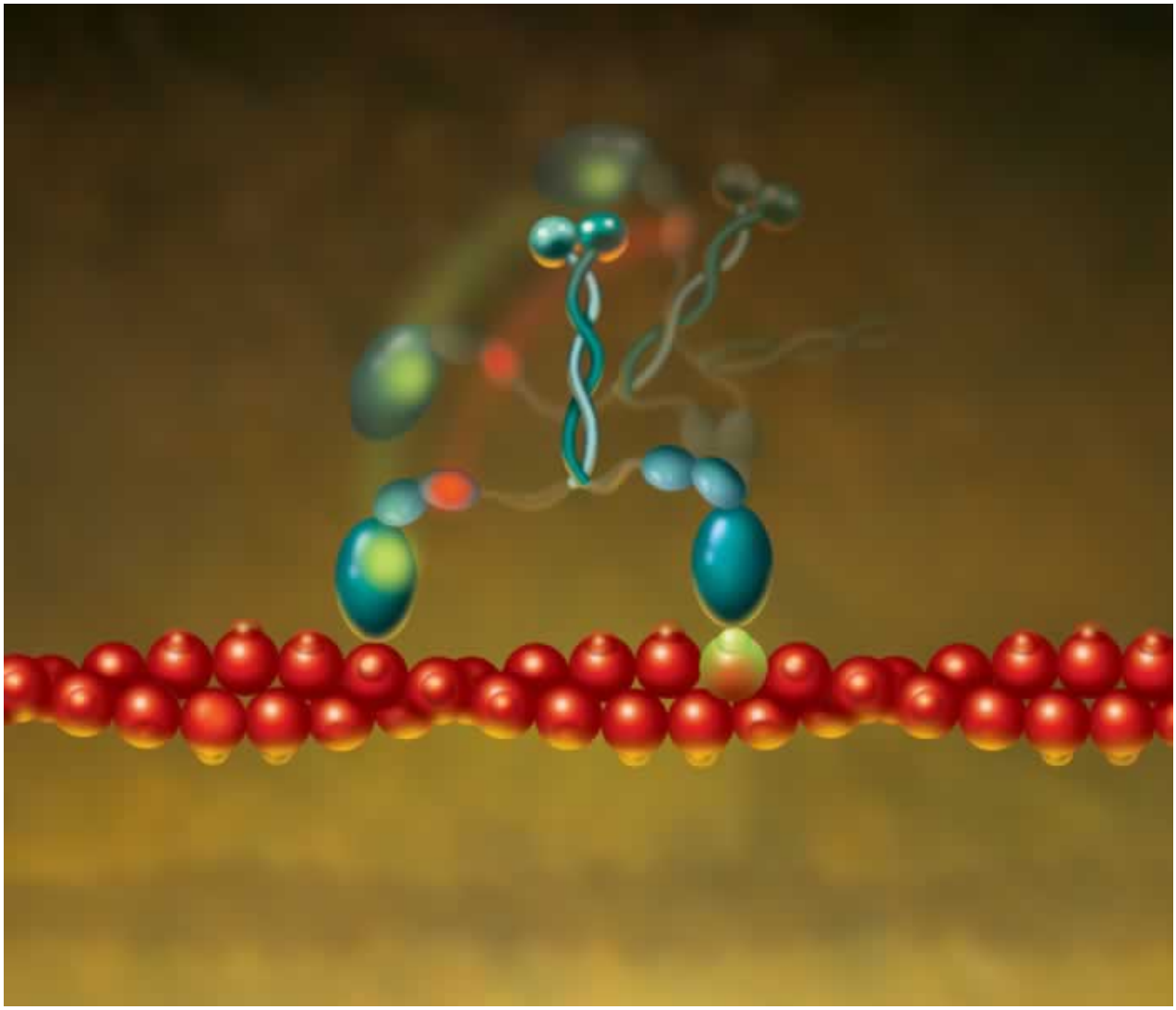
Allosteric motor proteins

- Changes of conformation allow some proteins to "walk" along the surfaces
- As an example, *myosin* runs along *actin* filaments in muscle cells
- Motor proteins are ATP/GTP-binding: ATP (or GTP) provides both change in conformation of motor proteins
- Hydrolysis of ATP/GTP makes reverse reaction practically impossible (only ATP synthetases may reconstruct the ATP): therefore, they walk directionally

Protein walking


1
ATP
BINDING
A
P
P
P
2
HYDROLYSIS
A
P
P
Pi
3
RELEASE
A P P
ADP
Pi
1
direction of
movement

Myosin



Myosin movie

Protein machines

- Protein machine is a linked set of several proteins
- Hydrolysis of ATP or attached GTP drives conformational changes
- Covalent modifications of side chains work as a regulatory code for most protein machines

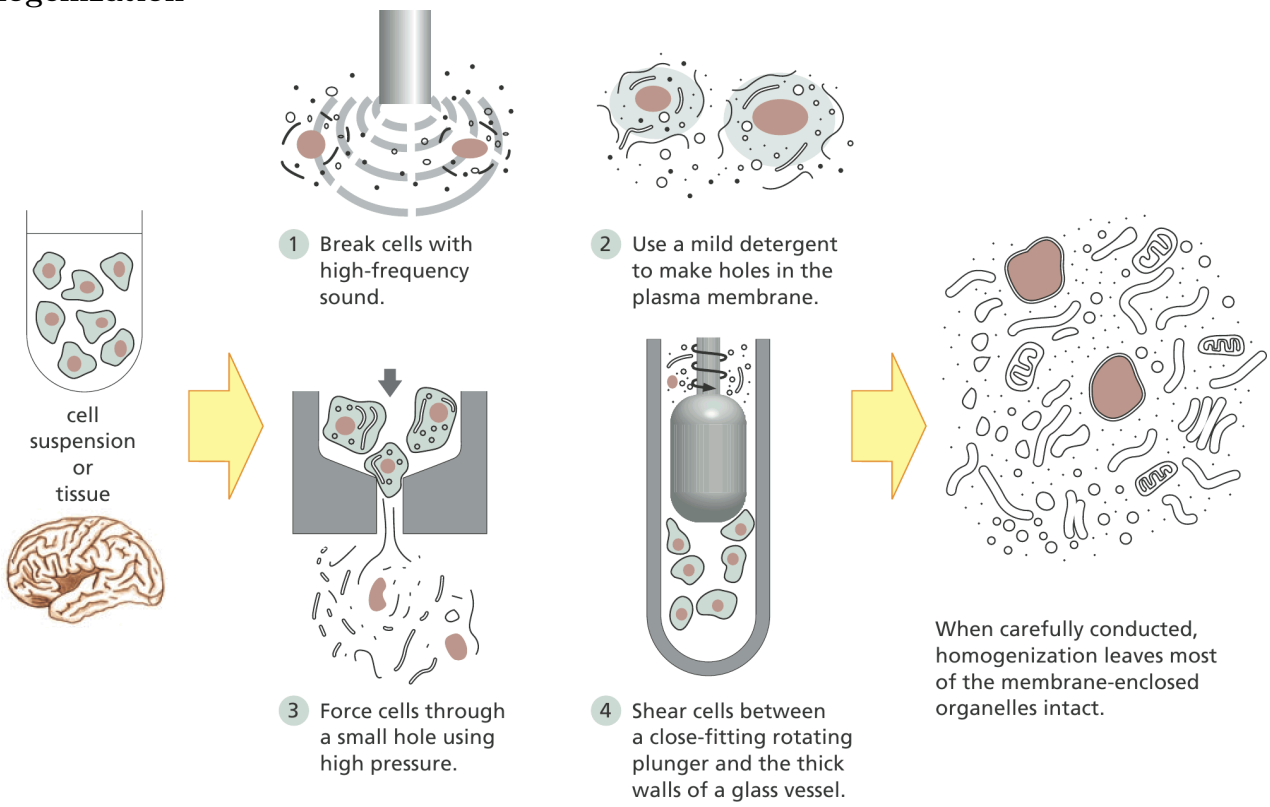
"Protein machine" movie

Z.2 How proteins are studied

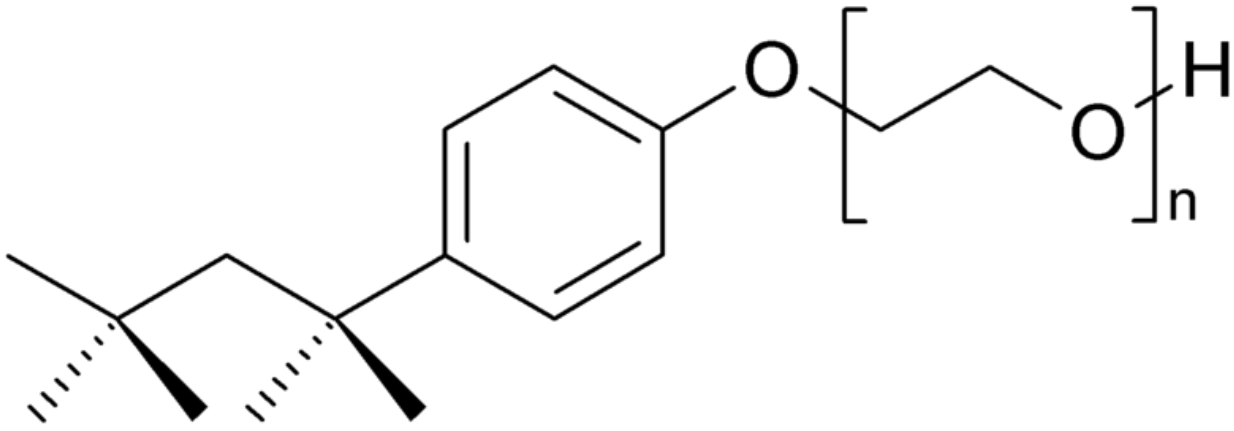
Homogenization

- Breaking cell contacts / cell walls
- Breaking cells: ultrasound, forcing through small holes, blending, using detergent (like Triton X-100) or osmosis
- Resulting product is a homogenate

Homogenization



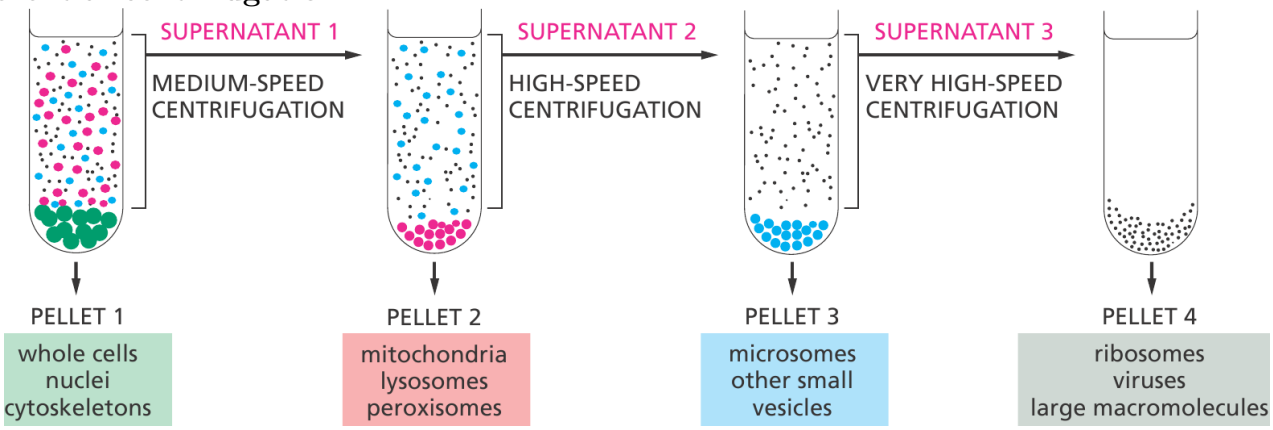
Triton-X-100 molecule



Differential centrifugation

- Step-by-step centrifugation where supernatant is used as a source of next step
- Typical sequence of sediments (pellets) is: nuclei → mitochondria, lysosomes → small vesicles → ribosomes, large macromolecules

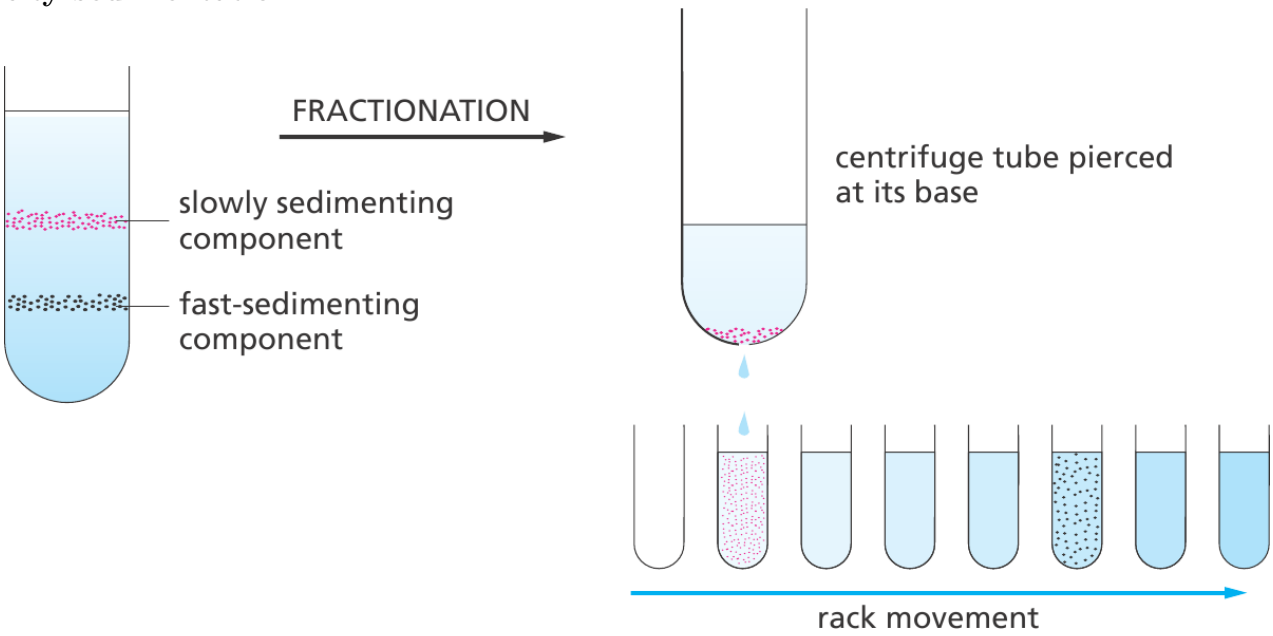
Differential centrifugation



Velocity sedimentation

- When a gradient of something (e.g., sucrose) present inside a tube, different types of molecules have different sedimentation speed depending on their size (larger proteins sediment **faster**)
- Fractionation may be used to remove parts of this gradient: pipetting (usually with cut pipette tip) or puncturing the bottom of a tube

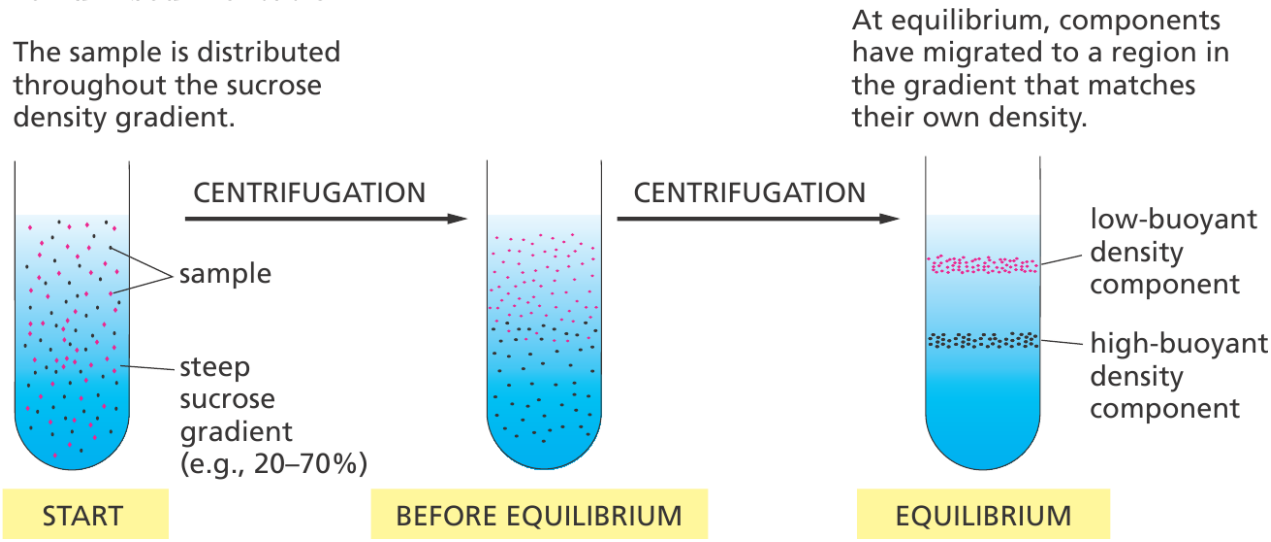
Velocity sedimentation



Equilibrium sedimentation

- Based on buoyant density (floating ability of molecules)
- More thick solution of sucrose or $CsCl_2$ (20–70%) with a gradient is used
- Every component of cell will move down until it reaches some density of surrounding liquid

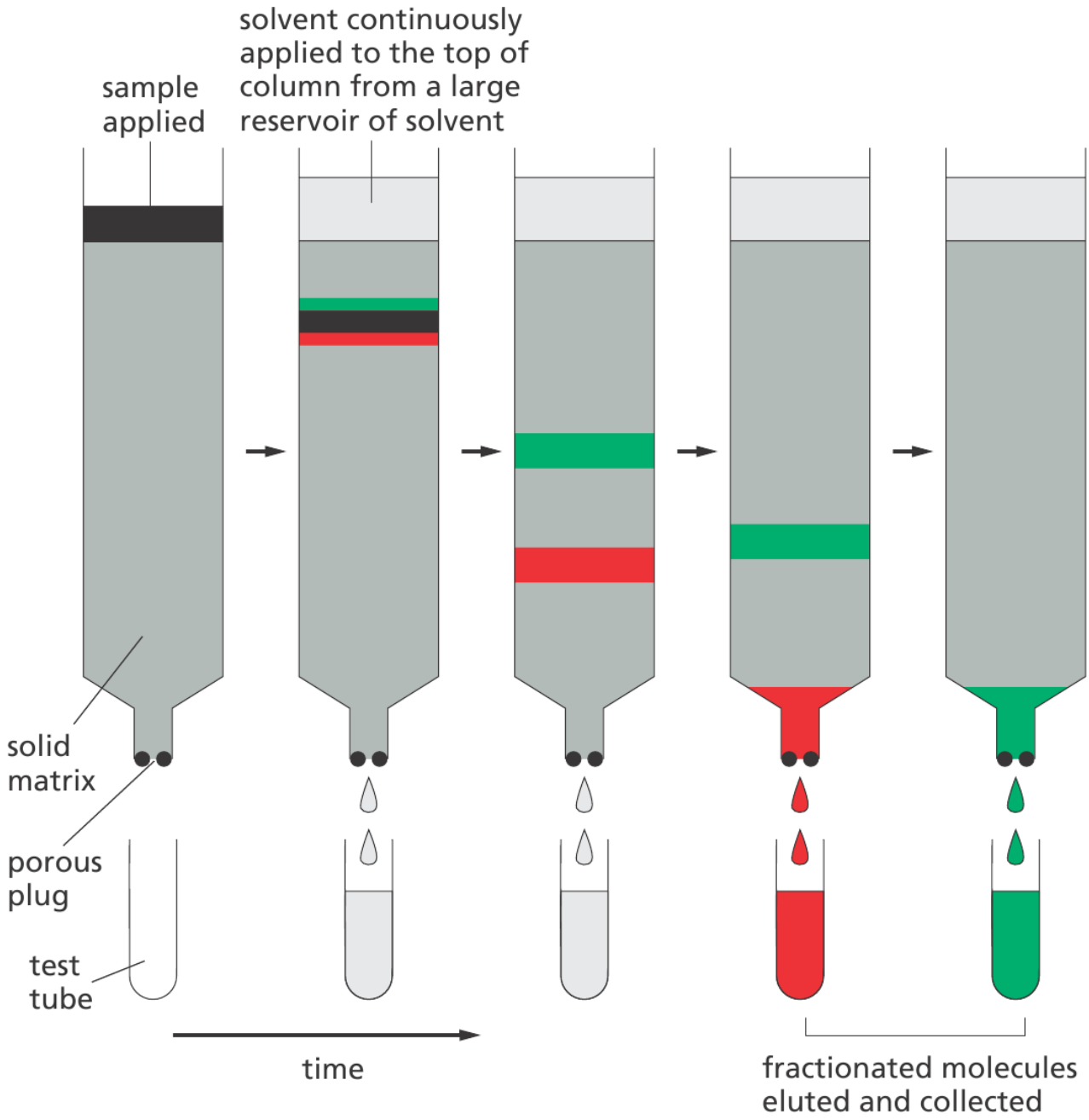
Equilibrium sedimentation



Column chromatography

- Mixture of proteins is pumped through chromatographic column
- There is a matrix inside column which contains chemicals with different affinities to different proteins
- As a result, some proteins will be attached to the matrix
- Then, we wash attached proteins out and therefore separate them from initial mixture
- There are numerous different variants of chromatography

Column chromatography



Different kinds of chromatography

Chromatography may be based on:

- Electric charge: ion-exchange chromatography
- Size: gel-filtration chromatography
- Binding: affinity chromatography

Final question (2 points)

Which way of sedimentation employs differences between molecular sizes?

Summary

- Homogenization produce the initial mixture of proteins

- Separation of this mixture could be done through centrifugation, sedimentation, electrophoresis and chromatography

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 4*.

Outline

Questions and answers

Previous final question: the answer

Which way of sedimentation employs differences between molecular sizes?

- Velocity sedimentation: c proteins sediment faster

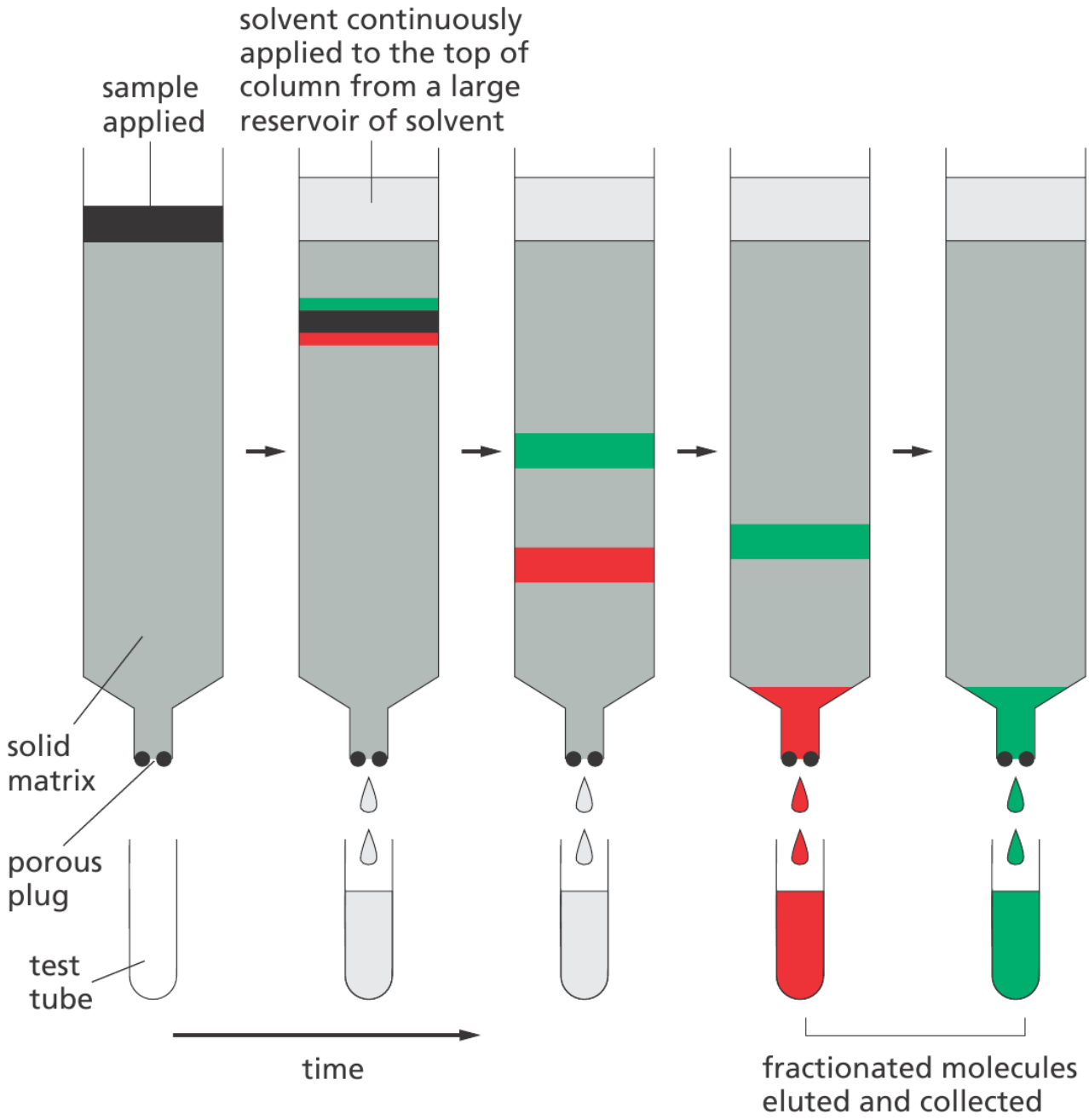
Proteins

.1 How proteins are studied

Column chromatography

- Mixture of proteins is pumped through chromatographic column
- There is a matrix inside column which contains chemicals with different affinities to different proteins
- As a result, some proteins will be attached to the matrix
- Then, we wash attached proteins out and therefore separate them from initial mixture
- There are numerous different variants of chromatography

Column chromatography

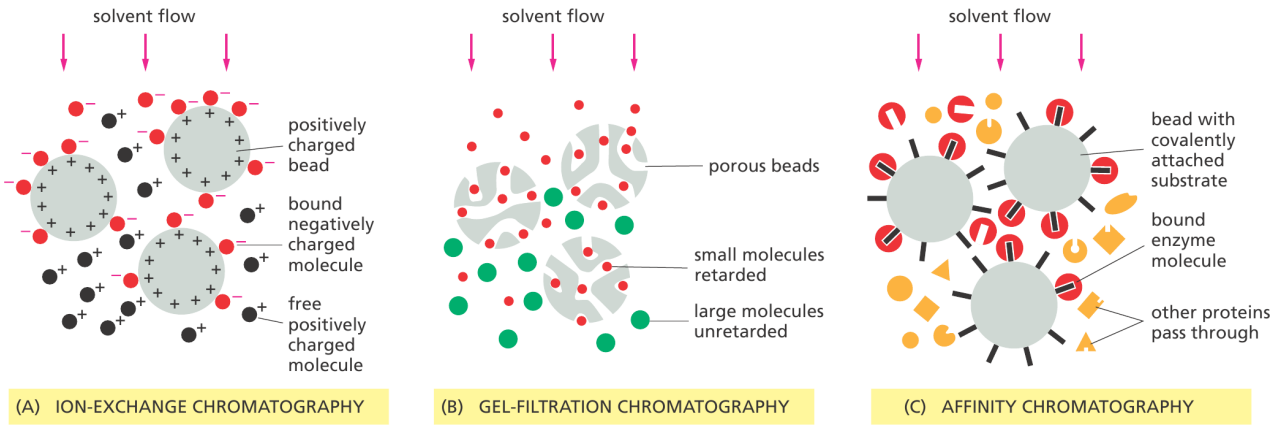


Different kinds of chromatography

Chromatography may be based on:

- Electric charge: ion-exchange chromatography
- Size: gel-filtration chromatography
- Binding: affinity chromatography

Variants of chromatography



Affinity chromatography

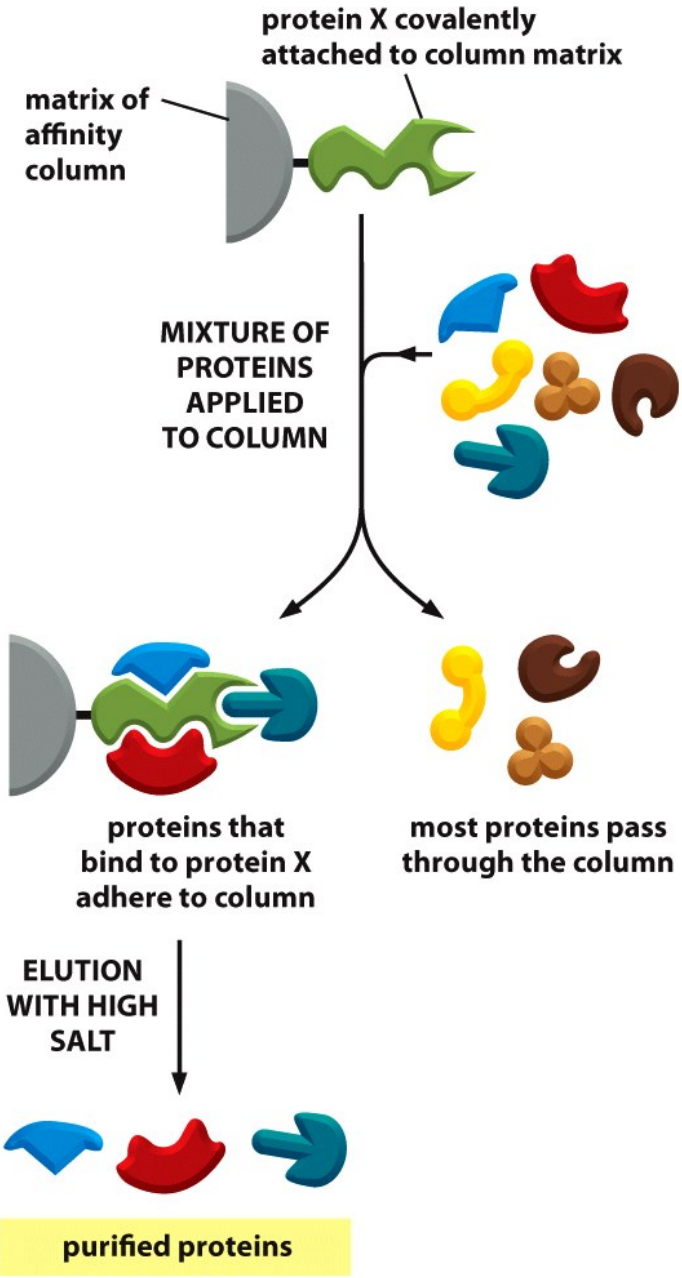
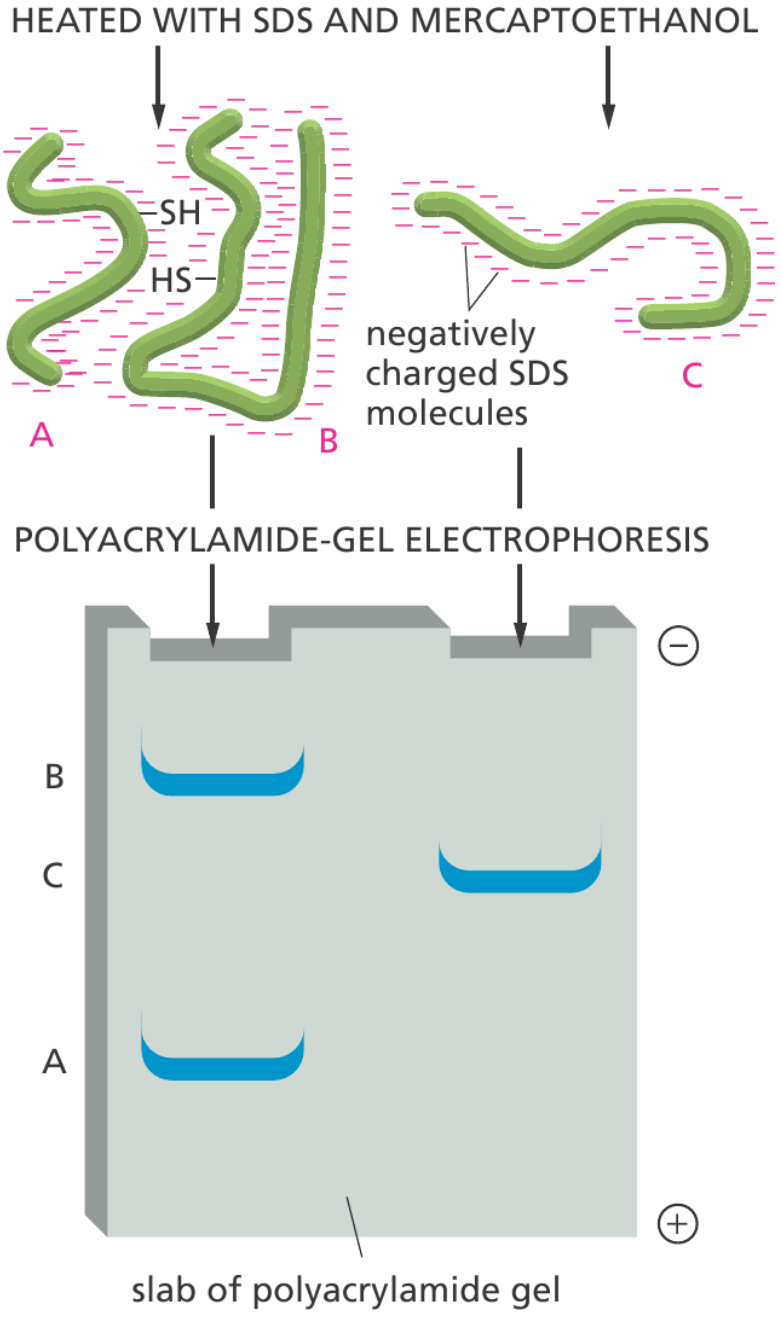


Figure 4-49 Essential Cell Biology 3/e (© Garland Science 2010)

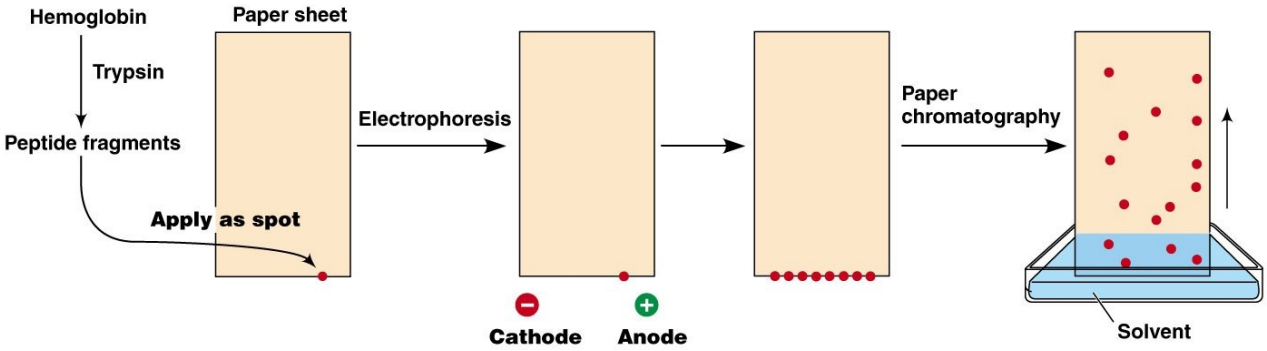
Gel electrophoresis

- Since proteins have different weight and electric charge, they will move at different speed if we apply electric field to initial mixture
- To make speed slower, one needs to use gels (e.g., polyacrylamide) instead of liquids
- Protein molecules usually processed with *sodium dodecyl sulfate* (SDS) to make charge equal and *mercaptoethanol* is used to break disulfide bridges. As a result, they will move only on the basis of their molecular weight.

Polyacrylamide gel electrophoresis



Electophoresis and chromatography combined

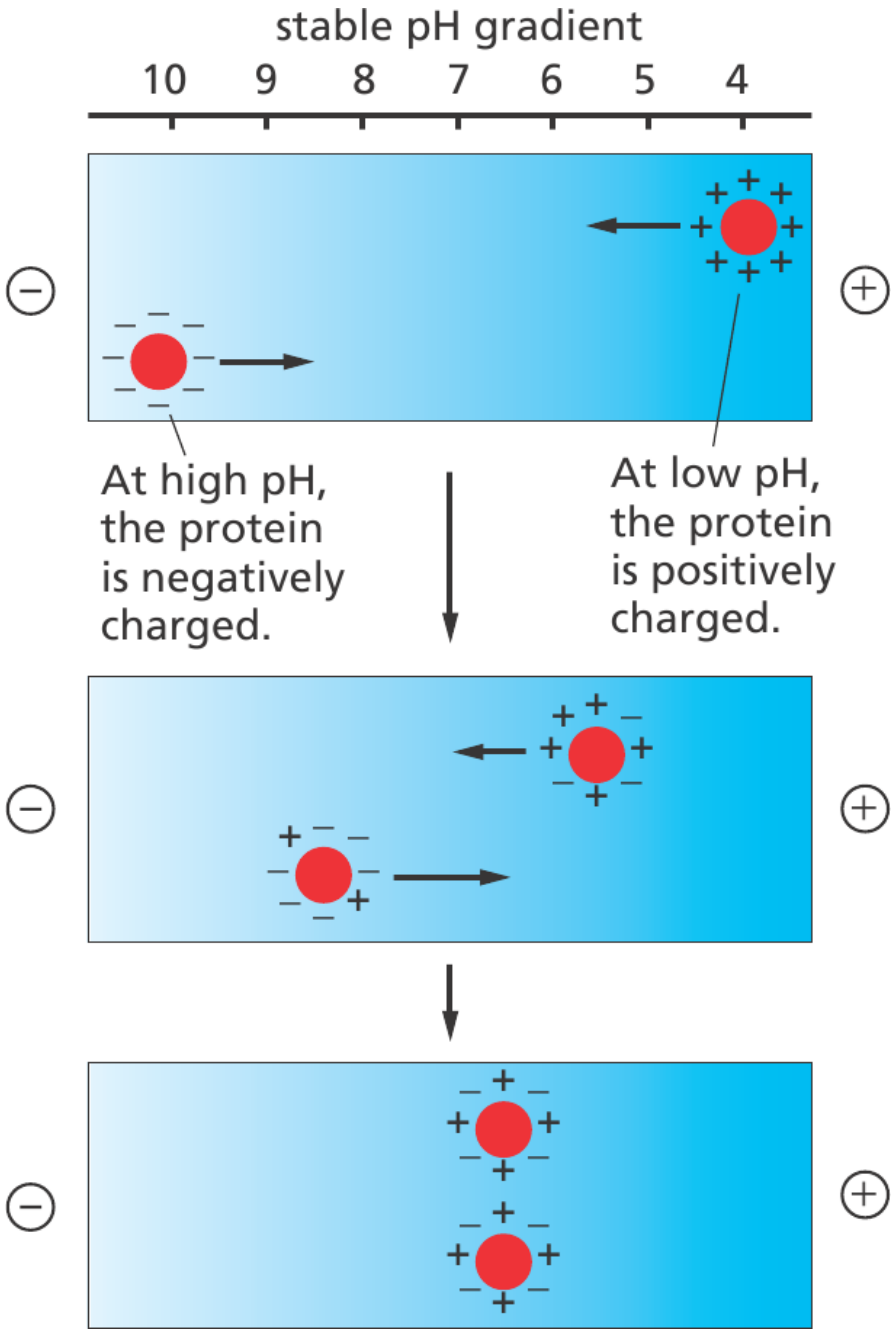


This is used used for sickle cell anemia (and similar) research
Sickle cell anemia movie

Isoelectric focusing

- This is a variant of electrophoresis in narrow tubes with pH gradient
- Proteins will move until it charge will become 0. This is an isoelectric point of protein.

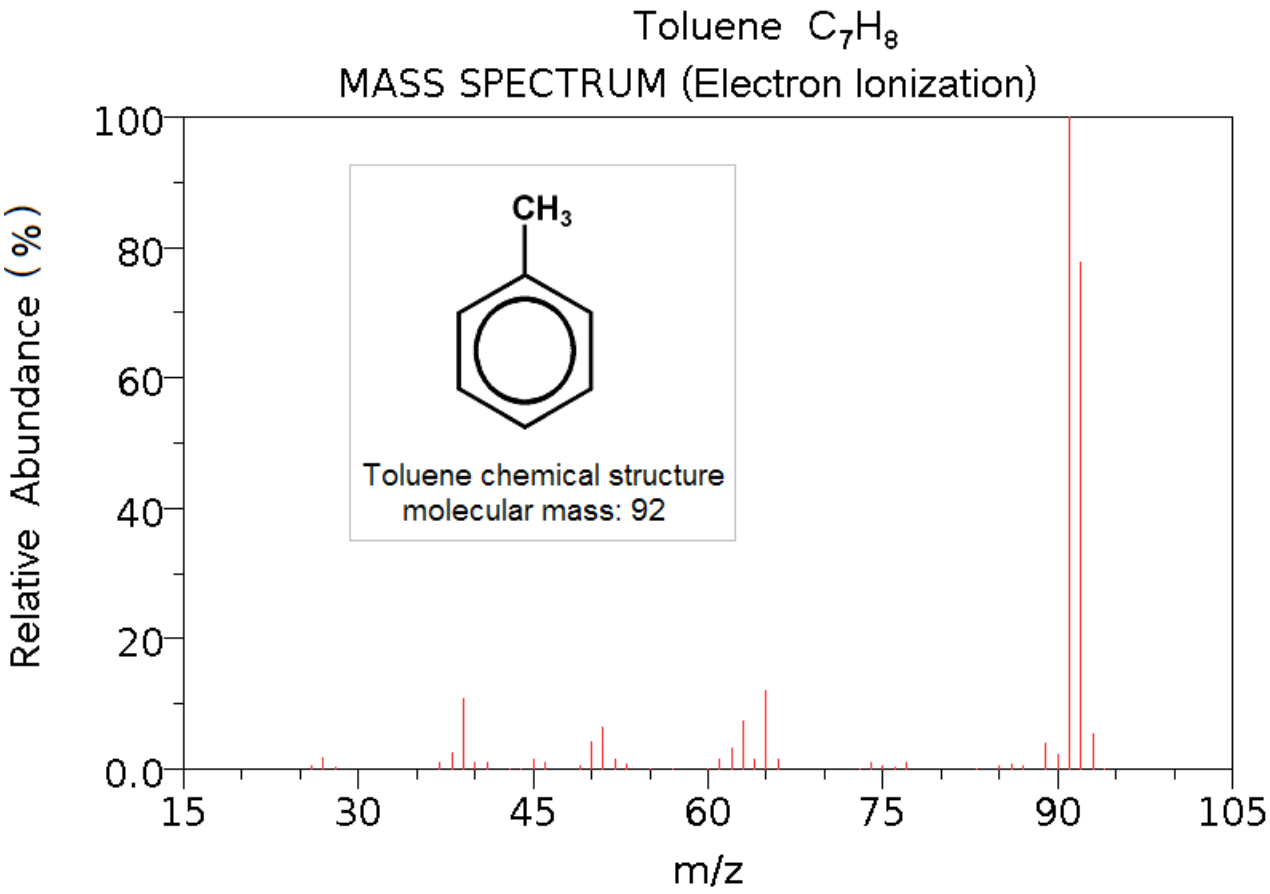
Isoelectric focusing



Mass spectrometry

- Based on measuring mass of protein fragments
- Protein is fragmented, then placed into the engine which calculates mass of every fragment
- This will produce a kind of fingerprint image (mass spectrum) for every protein fragment
- Combination of mass spectra from fragments will give a protein sequence

Mass spectrometry



Mass spectrometry real life example (*Rubisco* protein)

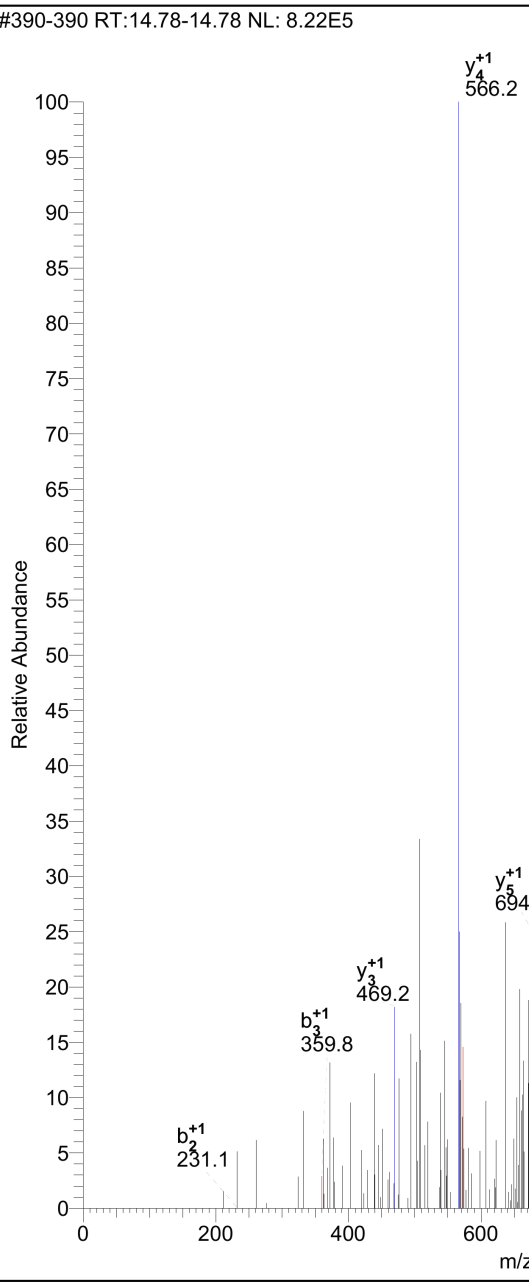
Reference: gi|16326265|dbj|BAB70576.1| ribulose 1,5-bisphosphate carboxylase
Database: C:\Xcalibur\database\ACE.fasta
Number of Amino Acids: 464 Monoisotopic MW: 51406.9 pI: 6.00

Protein:

GFKAGVKDYK **LTYYTPDYET KDTDILAAFR** VTPQPGV**PPE EAGAAVAAES**
STGTWTTVWT DGLTSLDRYK GRCYHIEPVA GEESQFIAYV AYPLDLFEEG
SVTNMFTSIV GNVFGFKALR ALRLEDLRIP PAYSK**TFQGP PHGIQVER**DK
LNKYGRPLLG CTIKPKLGLS AKNYGRAVYE CLR**GGLDFTK DDENVNSQPF**
MRWRDRFLFC AEAIYKAQAE TGEIKGHYLN ATAGTCEEMM KRAVFARELG
VPIVMHDYLT GGFTANTSLA HYCRDNGLLL HIHRAMHAVI DRQKNHGIHF
RVLAKALRMS GGDHIHAGTV VGKLEGER**EI TLGFVDLLRD DYIEKDR**SRG
IYFTQDWVSL PGVLPVASGG IHVWHMPALT EIFGDDSVLQ FGGGTLGHPW
GNAPGAVANR **VALEACVQAR** NEGRDLAREG NEIIREAGKW SPELAAACEV
WK**EIKFEFPA MDTL**

Protein Coverage:

Sequence	MH+	% Mass	AA	% AA
FKAGVKDYKL	1168.67	2.27	2 - 11	2.16
FKAGVKDYKLTYYTPDYET	2302.13	4.48	2 - 20	4.09
KAGVKDYKLTYYTPDYET	2155.06	4.19	3 - 20	3.88
AGVKDYKLTYY	1320.68	2.57	4 - 14	2.37
AGVKDYKLTYYTPDYETK	2155.06	4.19	4 - 21	3.88
GVKDYKLTY	1086.58	2.11	5 - 13	1.94
YKLTYYTP	1048.53	2.04	9 - 16	1.72
KLTYYTPDYETKDTDILAAF	2368.16	4.61	10 - 29	4.31
LTYYTPDYETK	1393.65	2.71	11 - 21	2.37
LTYYTPDYETKDTDILAA	2093.00	4.07	11 - 28	3.88
LTYYTPDYETKDTDILAAFRV	2495.23	4.85	11 - 31	4.53
LTYYTPDYETKDTDILAAFRVTPQPGVPP	3268.64	6.36	11 - 39	6.25
PDYETKDT	968.42	1.88	16 - 23	1.72
PDYETKDTDILAA	1451.69	2.82	16 - 28	2.80
DYETKDTDILAAFRVTP	1954.98	3.80	17 - 33	3.66
ETKDTDILAAFRV	1478.78	2.88	19 - 31	2.80
KDTDILAAFRVTPQPGVPPEEAGAAVAAESST	3195.62	6.22	21 - 52	6.90
DTDILAAFR	1021.53	1.99	22 - 30	1.94
DTDILAAFRVT	1221.65	2.38	22 - 32	2.37
DTDILAAFRVTPQPGVPPEEAGAAVAA	2663.37	5.18	22 - 48	5.82
DILAAFRV	904.53	1.76	24 - 31	1.72
DILAAFRVTPQP	1327.74	2.58	24 - 35	2.59
DILAAFRVTPQPGVPPE	1806.97	3.52	24 - 40	3.66
ILAAFRVTPQP	1212.71	2.36	25 - 35	2.37
ILAAFRVTPQPGVPPE	1691.95	3.29	25 - 40	3.45
LAAFR	577.35	1.12	26 - 30	1.08
LAAFRVTPQPGV	1255.72	2.44	26 - 37	2.59
AAFRVTPQPGVPPEEAGAAVAAESSTG	2567.27	4.99	27 - 53	5.82
AFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWT	3371.65	6.56	28 - 60	7.11
FRVTPQPGVPPEEAGAAVAAES	2180.10	4.24	29 - 50	4.74
RVTPQPGV	853.49	1.66	30 - 37	1.72
RVTPQPGVPPEEAG	1433.74	2.79	30 - 43	3.02
TPQPGVPPEEAGAAVAAESSTGTWTTVWTD	3013.41	5.86	32 - 61	6.47
PQPGVPPEEAGAAVAAESS	1763.84	3.43	33 - 51	4.09
QPGVPPEEAGAA	1122.54	2.18	34 - 45	2.59
QPGVPPEEAGAAVAAESSTGT	1925.91	3.75	34 - 54	4.53
GVPPEEAGAAVAAESSTGTWTT	2088.97	4.06	36 - 57	4.74
GVPPEEAGAAVAAESSTGTWTTVWTD	2590.19	5.04	36 - 61	5.60
VPPEEAGAAVAA	1081.55	2.10	37 - 48	2.59
VPPEEAGAAVAAE	1210.60	2.35	37 - 49	2.80
VPPEEAGAAVAAESSTGTWT	1930.90	3.76	37 - 56	4.31
VPPEEAGAAVAAESSTGTWTTVWTDGLT	2804.33	5.46	37 - 64	6.03
PPEEAGAAVAAESSTGTWTTVWTDGLTSLDR	3176.50	6.18	38 - 68	6.68
PEEAGAAVAAESSTGTWTTV	1934.90	3.76	39 - 58	4.31
PEEAGAAVAAESSTGTWTTVW	2120.98	4.13	39 - 59	4.53
EAGAAVAAESSTGTWTTVWTDGLT	2382.11	4.63	41 - 64	5.17



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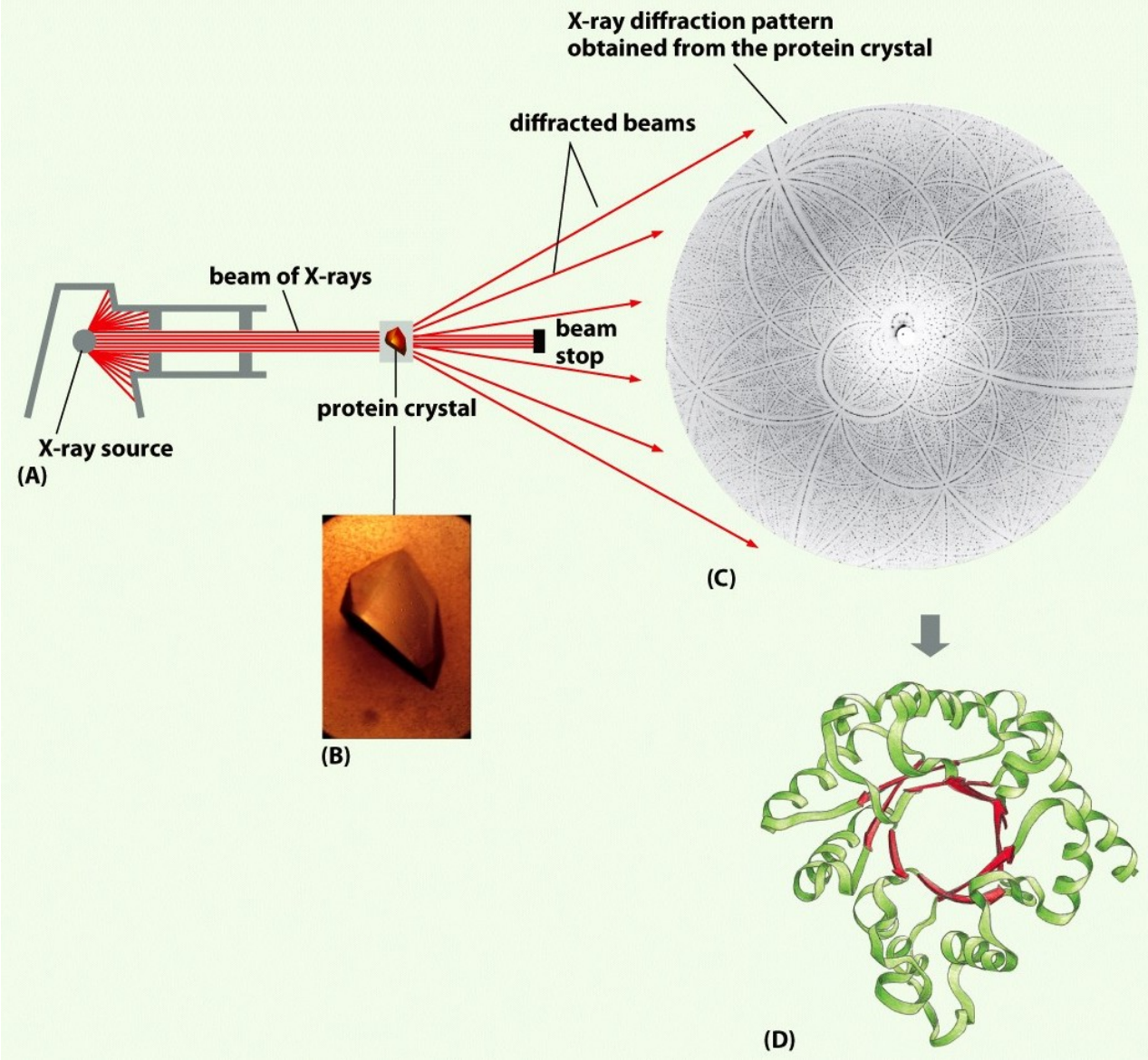
X-rays crystallography

- Based on the ability of proteins to form crystals
- These crystals will scatter X-rays and we will see difraction pattern, different for different proteins
- Useful for prediction of protein 3D structure

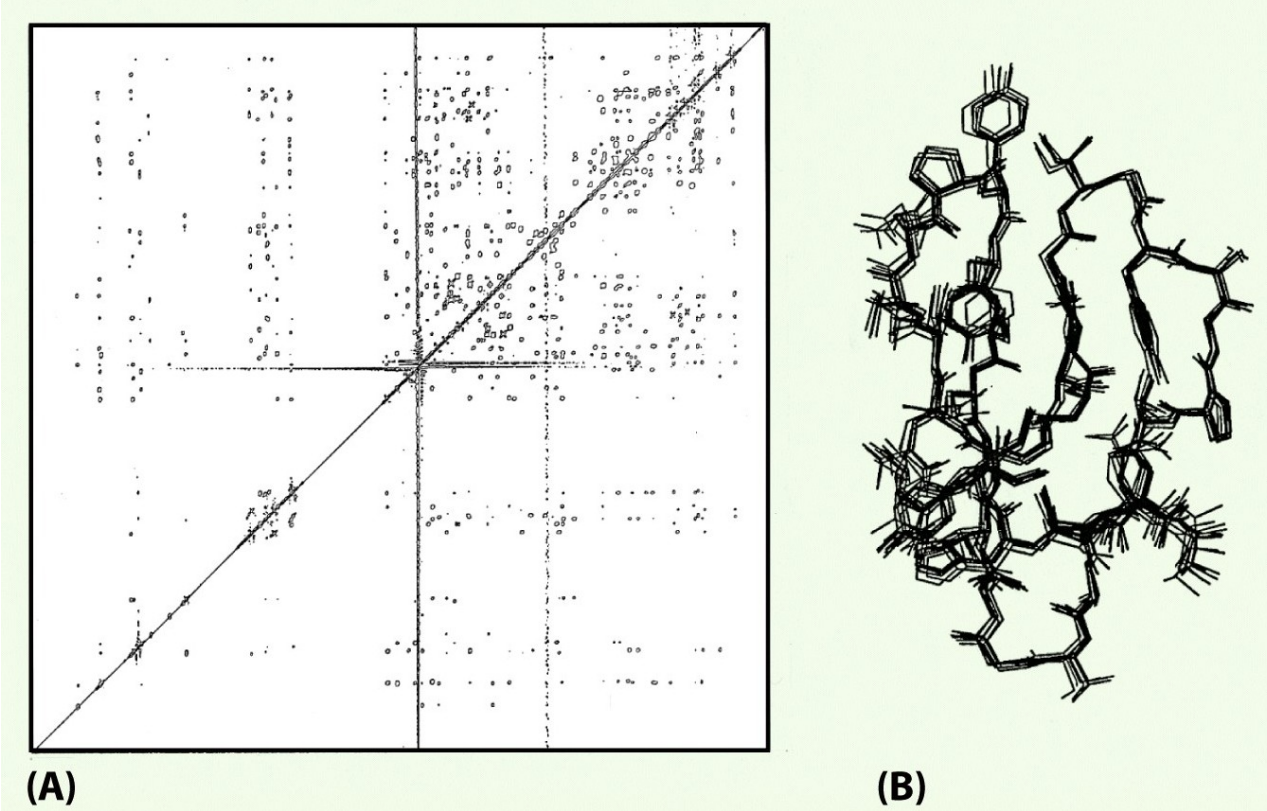
X-rays crystallography



Nuclear magnetic resonance (NMR)

- Unfortunately, not all proteins form crystals and it is not always possible to obtain enough protein for crystallization
- Method is based on the response of nuclei to radio waves
- Again, it is used mostly for understanding conformation of protein

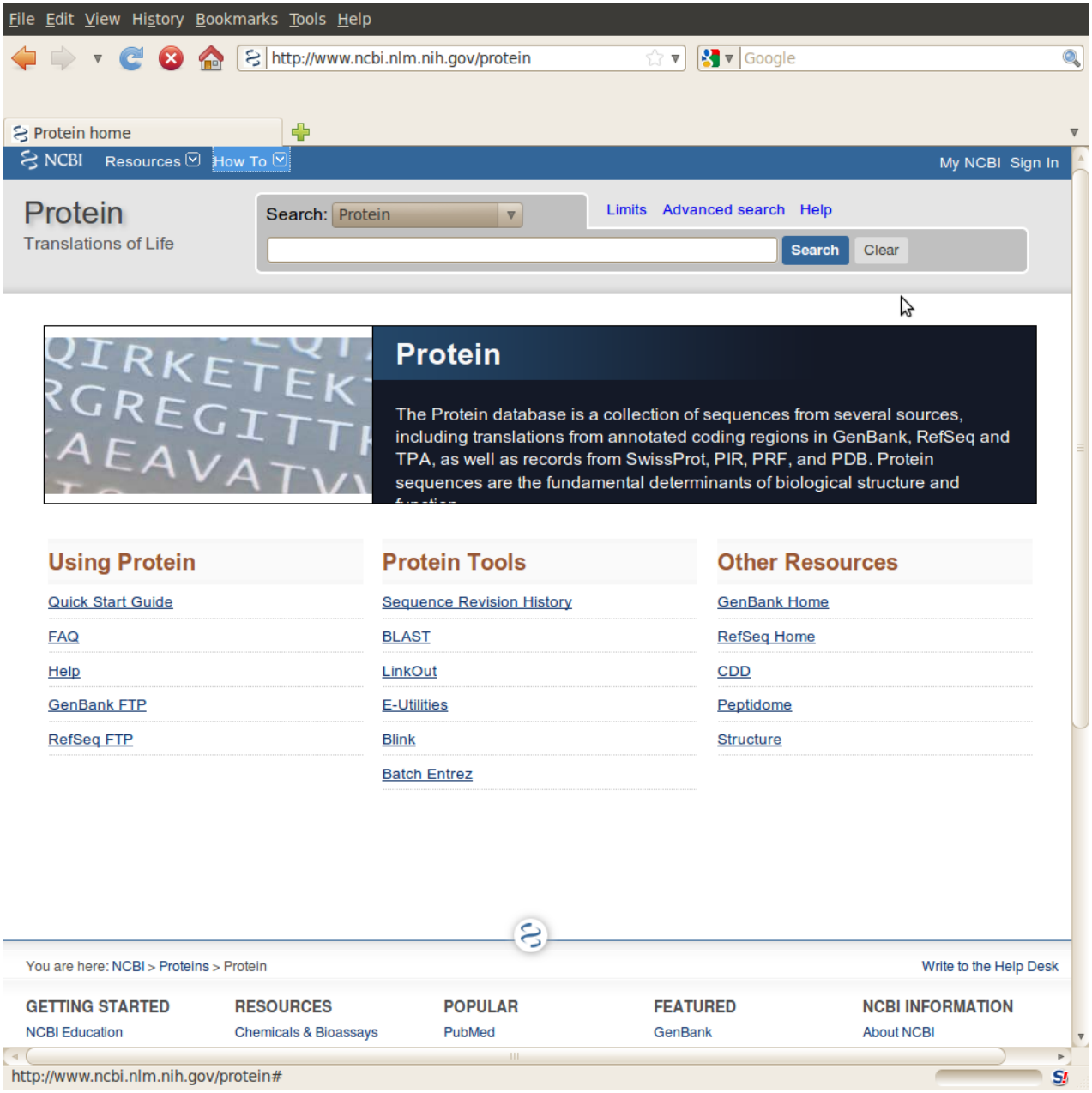
Nuclear magnetic resonance



Protein databases

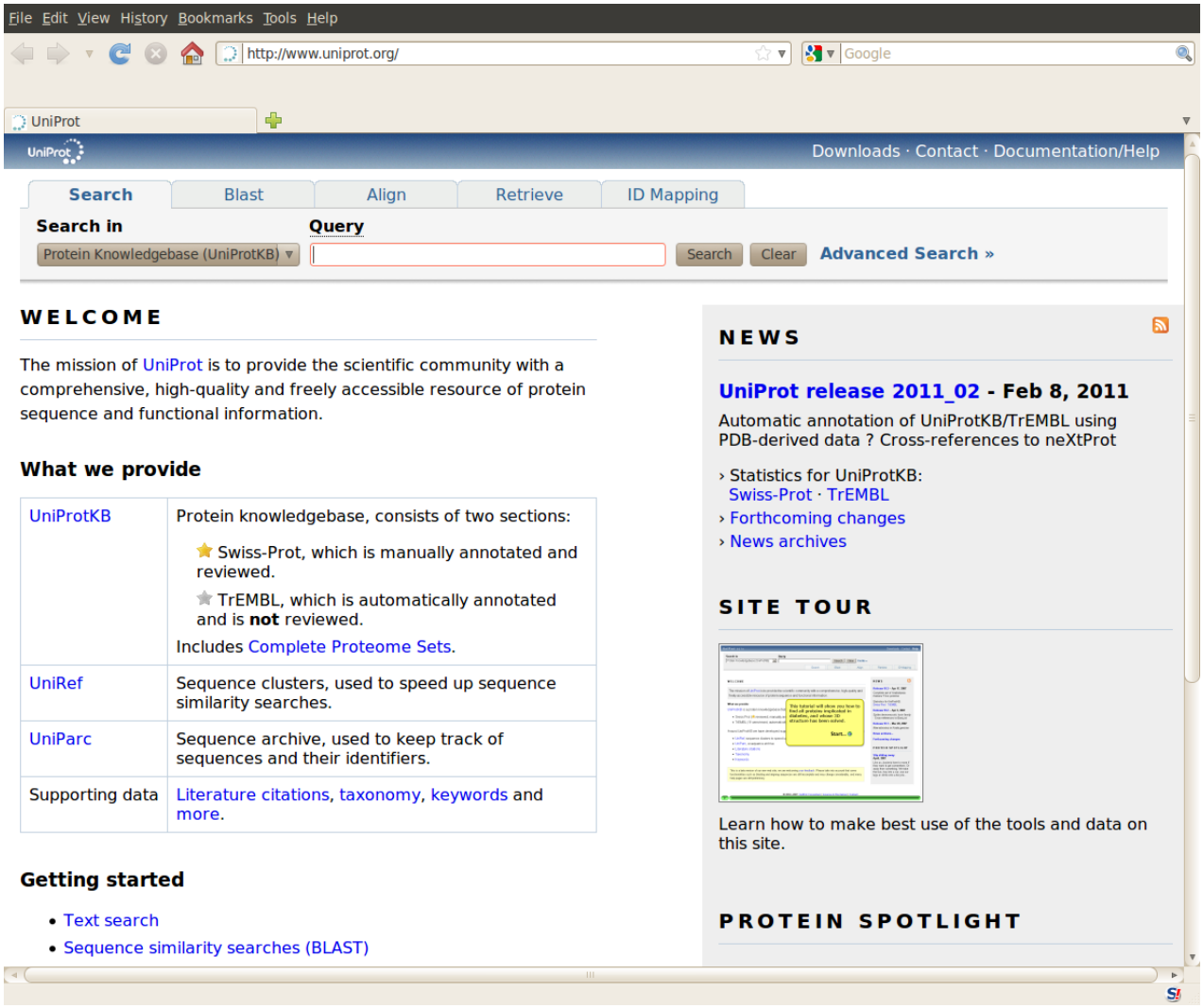
- UniProt
- NCBI protein
- RCSB

NCBI Protein database



RCSB database

UniProt database



Final question (1 point/answer)

Which methods are using for understanding the 3D shape (conformation) of proteins?

Summary

- Multiple methods of protein analysis help to clean proteins, reveal their sequence and understand their conformation
- Databases hold this information for future research

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 4*.

Outline

Questions and answers

Previous final question: the answer

Which methods are using for understanding the 3D shape (conformation) of proteins?

- X-ray crystallography
- NMR
- FoldIt and alike
- Velocity sedimentation

DNA

.1 DNA replication

Template

- Both DNA strands may act as a template for the synthesis of other strand
- Template hypothesis was first expressed by Nikolaj Koltsov in 1927 (but he thought that proteins are template molecules)

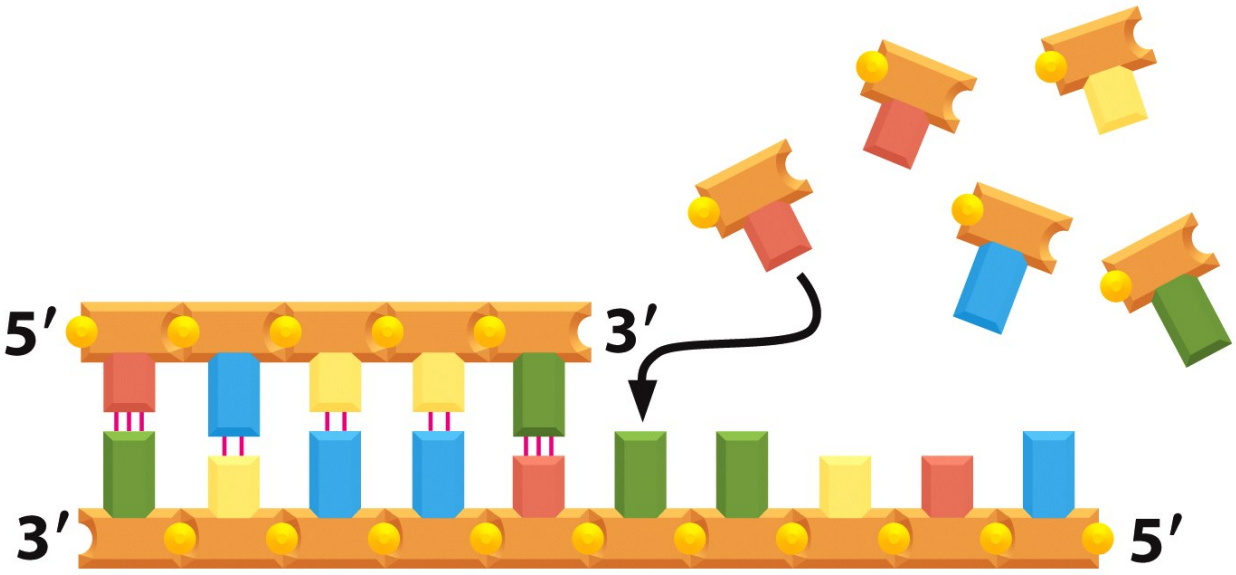


Figure 6-2 Essential Cell Biology 3/e (© Garland Science 2010)

Is replication semiconservative?

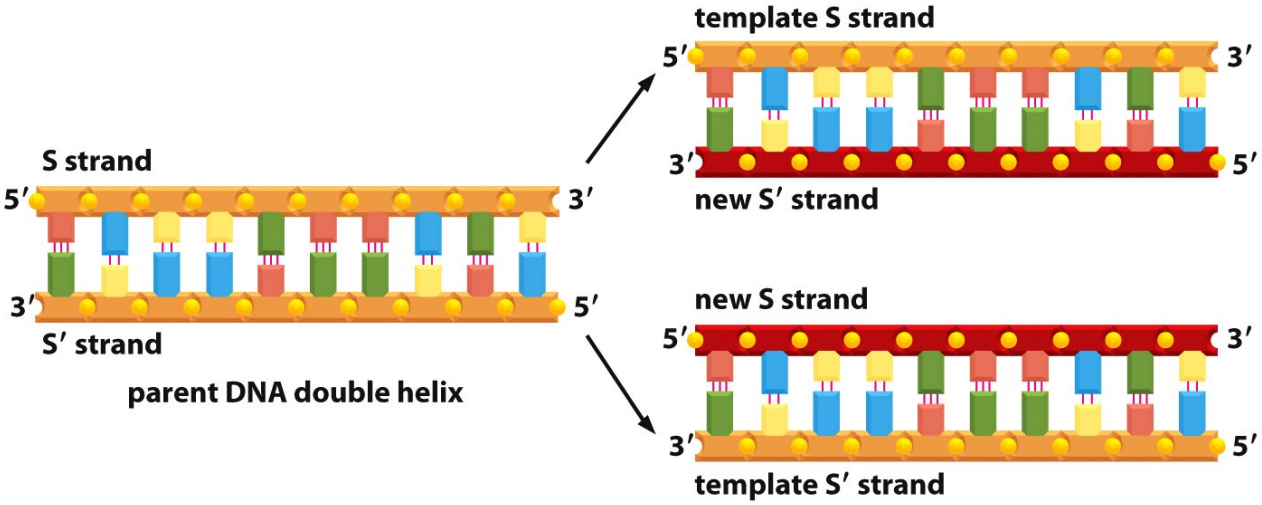


Figure 6-3 Essential Cell Biology 3/e (© Garland Science 2010)

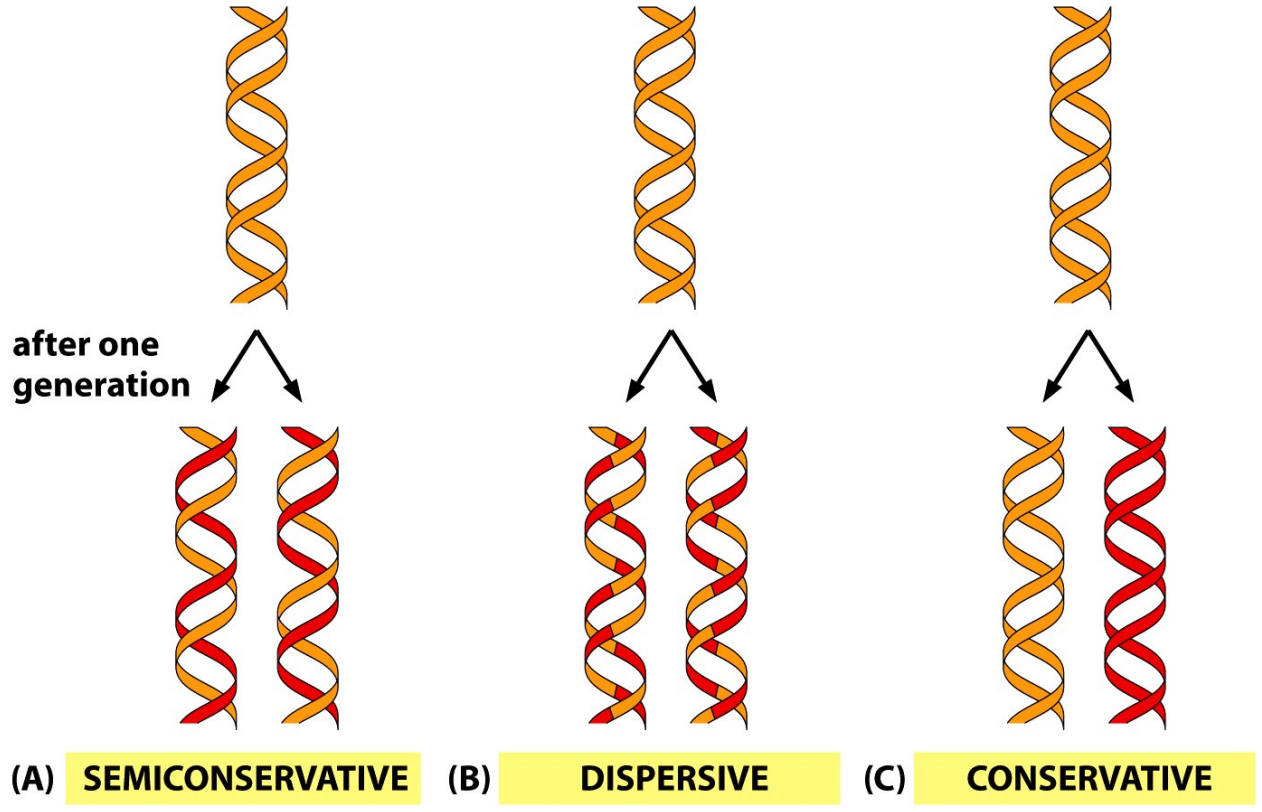


Figure 6-6 Essential Cell Biology 3/e (© Garland Science 2010)

Meselson-Stahl experiment (1958)

- Theoretically, three variants of replications were possible: semiconservative, dispersive and conservative
- Experiment was based on two bacterial cultures which grew on different media: with normal nitrogen ^{14}N, and with heavy nitrogen, ^{15}N
- After growing for 20' on heavy medium, bacteria produce DNA molecules with intermediate weight *only*

- That ruled out conservative hypothesis. How to rule out dispersive hypothesis?

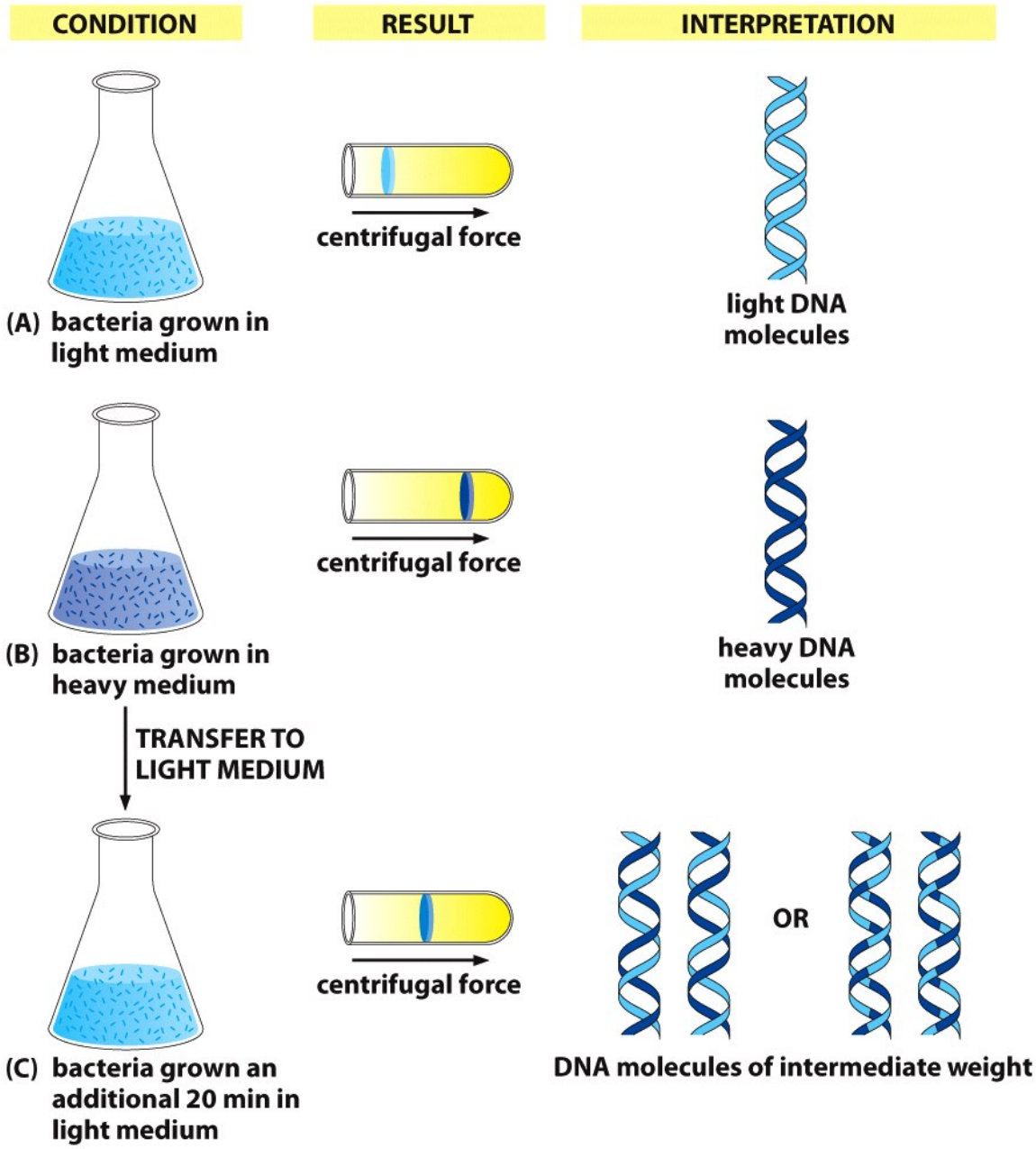


Figure 6-8 Essential Cell Biology 3/e (© Garland Science 2010)

Replication origin and forks

- DNA double helix should open for replication: this is a replication origin place
- When replication starts, these openings will grow and form replication forks which are visible under microscope

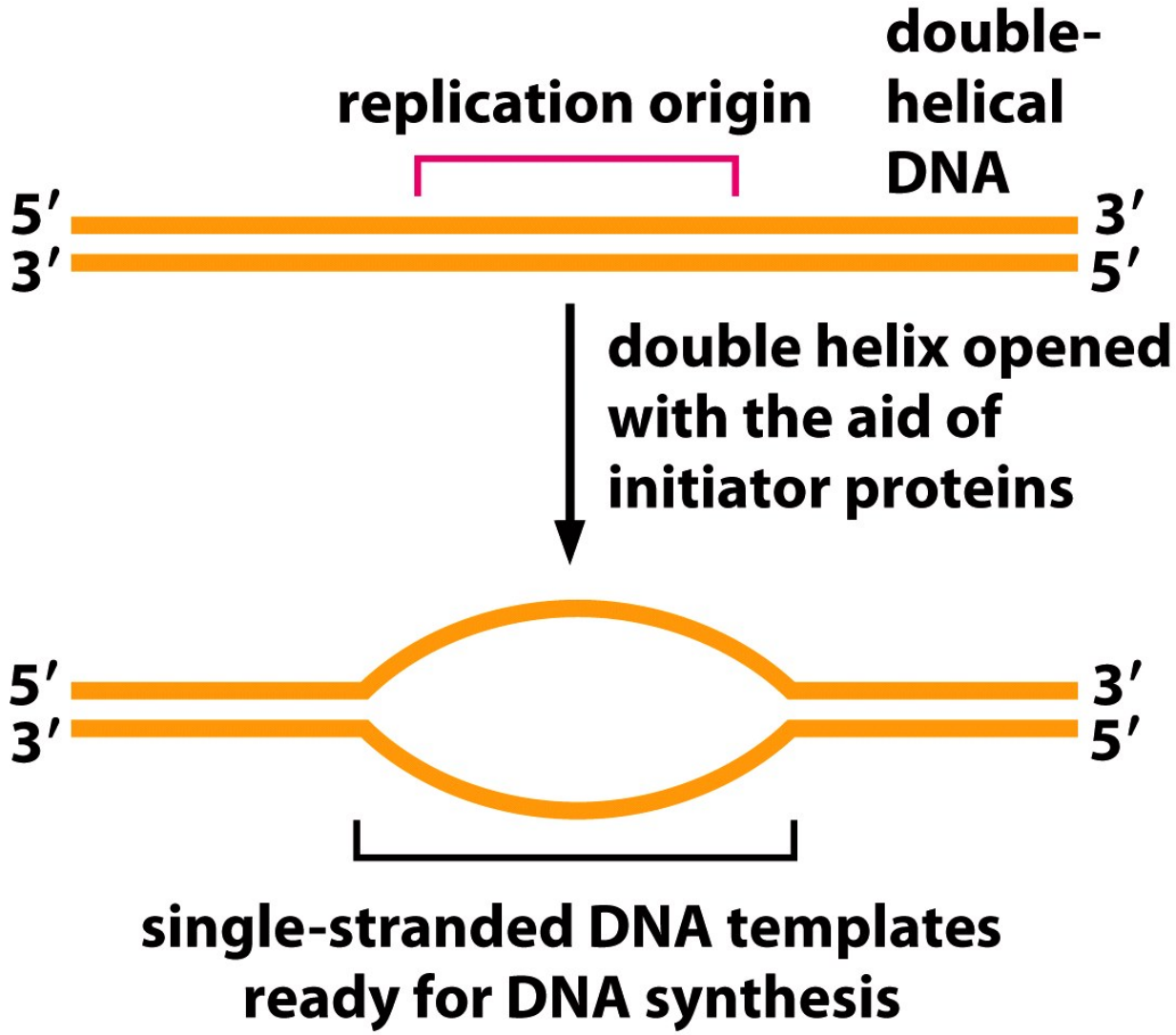


Figure 6-5 Essential Cell Biology 3/e (© Garland Science 2010)

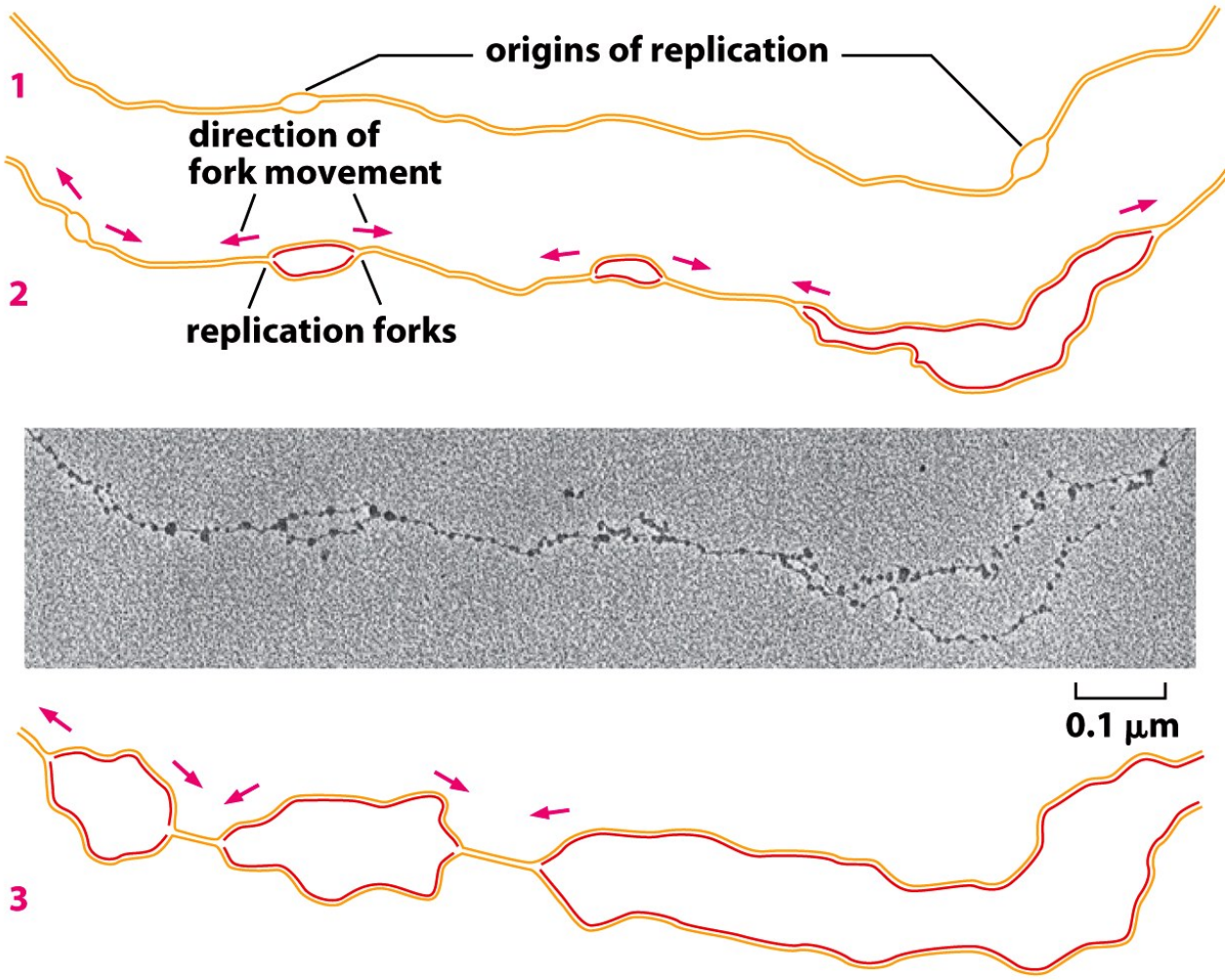


Figure 6-9 Essential Cell Biology 3/e (© Garland Science 2010)

Lagging and leading strands

- **DNA is synthesized only in 5'-to-3' direction**. Structure of DNA and complexity of replication do not allow the other direction.
- Therefore, fork is asymmetrical: one strand is replicating smoothly whereas other strand (lagging) replicated by "leaps", still 5'-to-3'
- Every "leap" produce one Okazaki fragment which are later joined

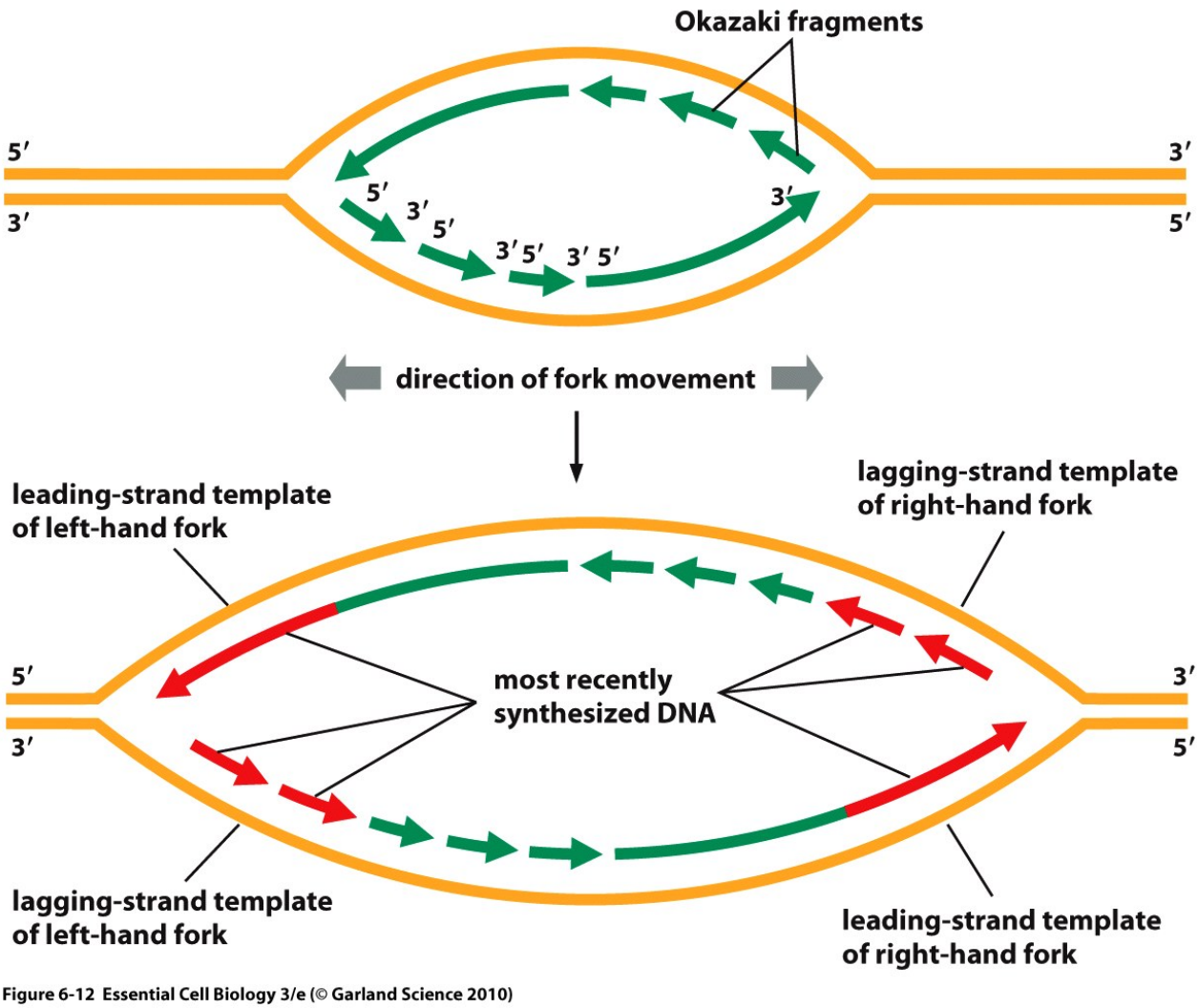


Figure 6-12 Essential Cell Biology 3/e (© Garland Science 2010)

DNA polymerase movie

DNA polymerase: proofreading

- First player in the replication "game": **DNA polymerase**
- Two different binding sites in DNA polymerase work for (1) DNA synthesis and (2) DNA proofreading
- Every time DNA polymerase adds new nucleotide, it checks if previous was correctly placed (if not, it removes the wrong nucleotide)
- Proofreading goes in opposite, 3'-to-5' direction
- As a result, DNA polymerase enzyme error rate became less than ≈0.00000001

Proofreading

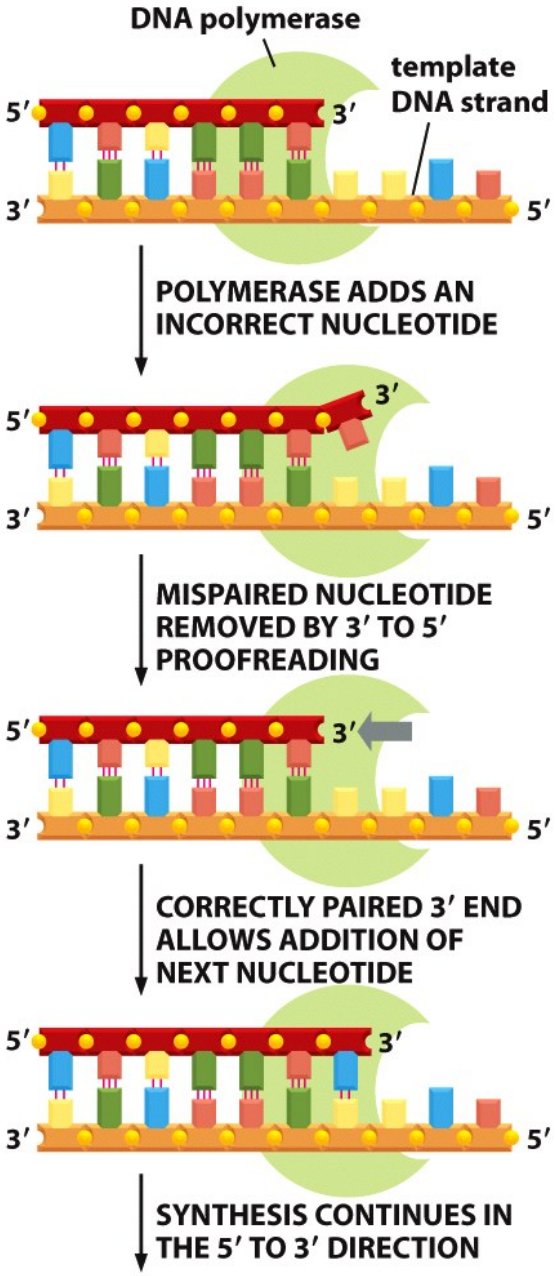


Figure 6-13 Essential Cell Biology 3/e (© Garland Science 2010)

RNA primers

- DNA polymerase cannot start nucleotide chain itself
- Instead, **primase** enzyme synthesize small RNA primer (≈10 nucleotides) which used as a starting point
- Primase error rate is ≈0.0001 (high!)
- In lagging strand, RNA sites are interleaving with DNA fragments
- Then RNA was replaced with DNA, erased, and **DNA ligase** joins fragments together
- Why primer is RNA?

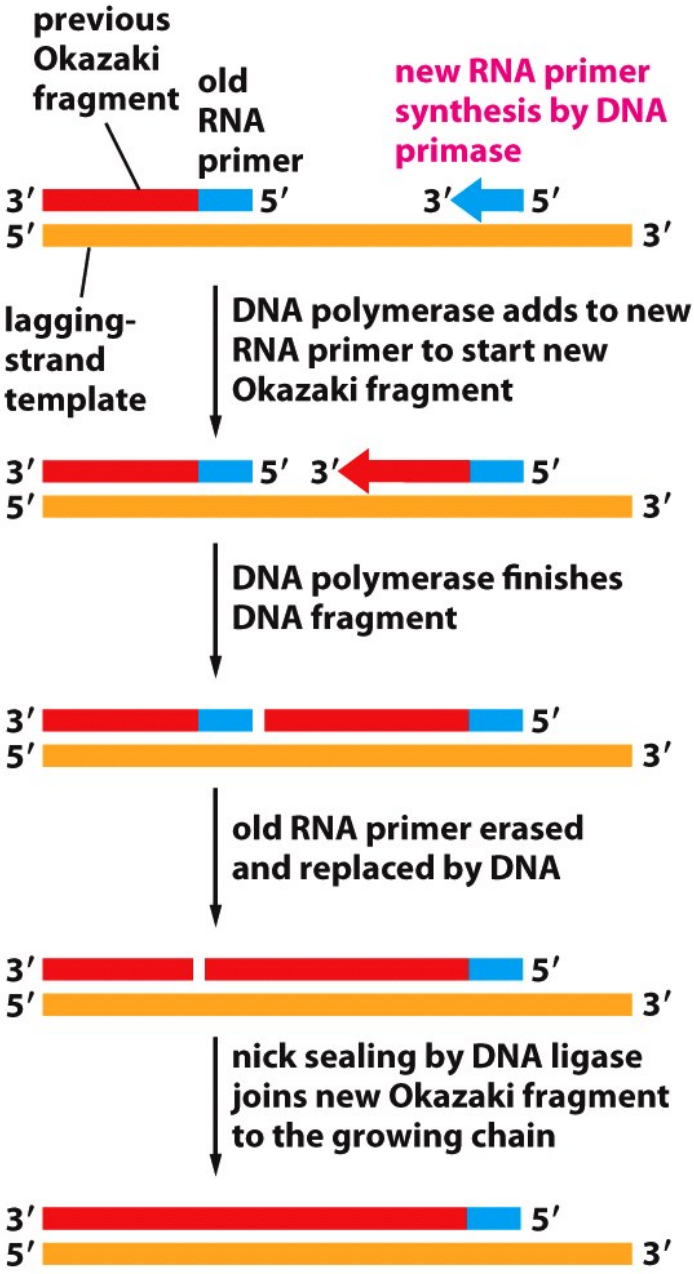
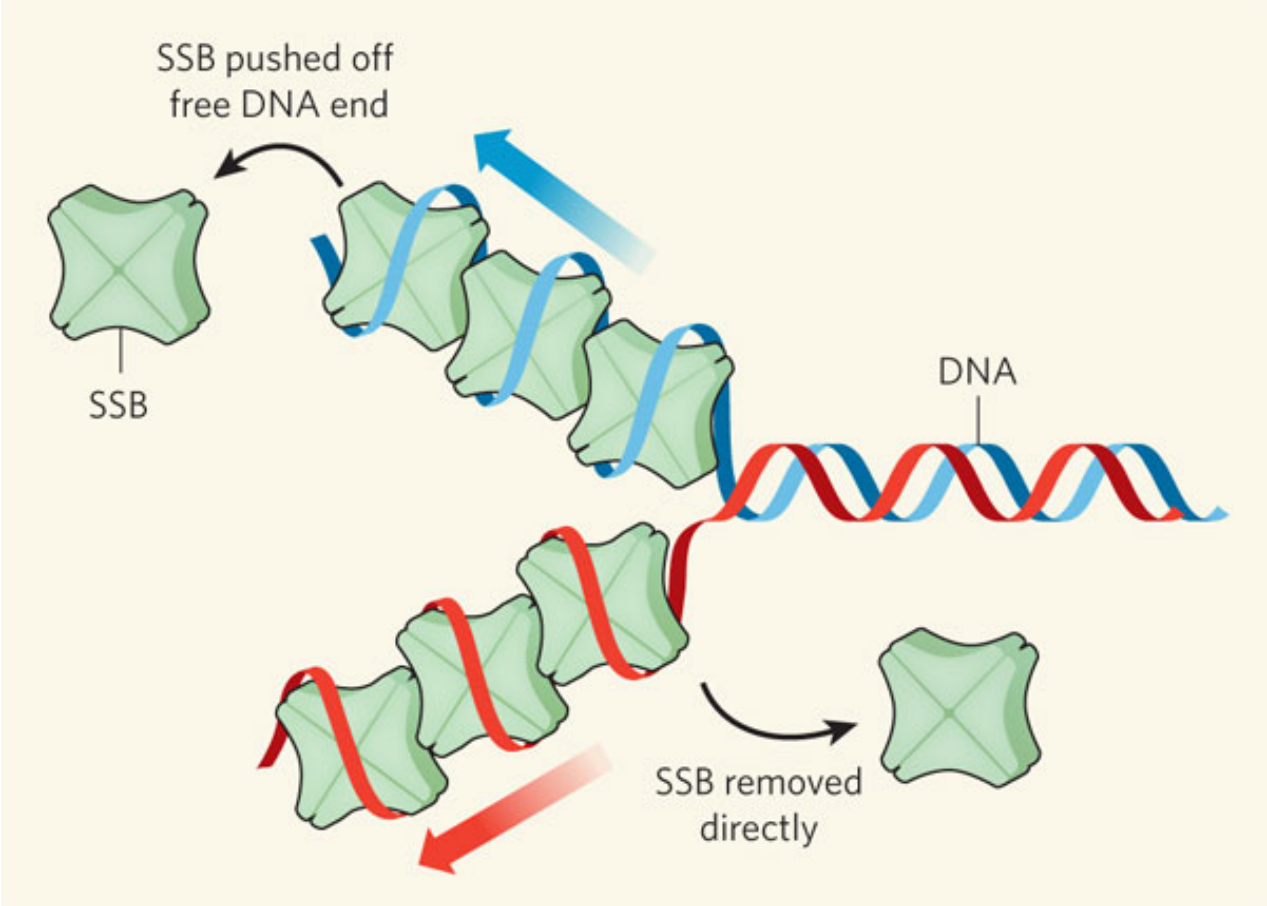


Figure 6-16 Essential Cell Biology 3/e (© Garland Science 2010)

DNA helicases

- DNA helicases are natural zippers
- They use energy of ATP to untangle the double helix
- *Single-strand binding protein* (SSBP) associates with DNA strand to prevent re-forming base pairs

Single strand binding protein, SSBP



DNA helicase movie

Final question (3 points)

Why cells use RNA as DNA replication primers?

Summary

- DNA replication is a semiconservative process
- DNA replication could go only in one direction
- Proofreading and RNA priming are helping in replication

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 6*.

Outline

Questions and answers

Previous final question: the answer

Why cells use RNA as DNA replication primers?

- Primers should be used for starting nucleotide chain, therefore they should be nucleic acids
- Primase is not a perfect replicase and will make errors
- To replace/remove potentially erroneous primers, they need to be distinguishable

DNA

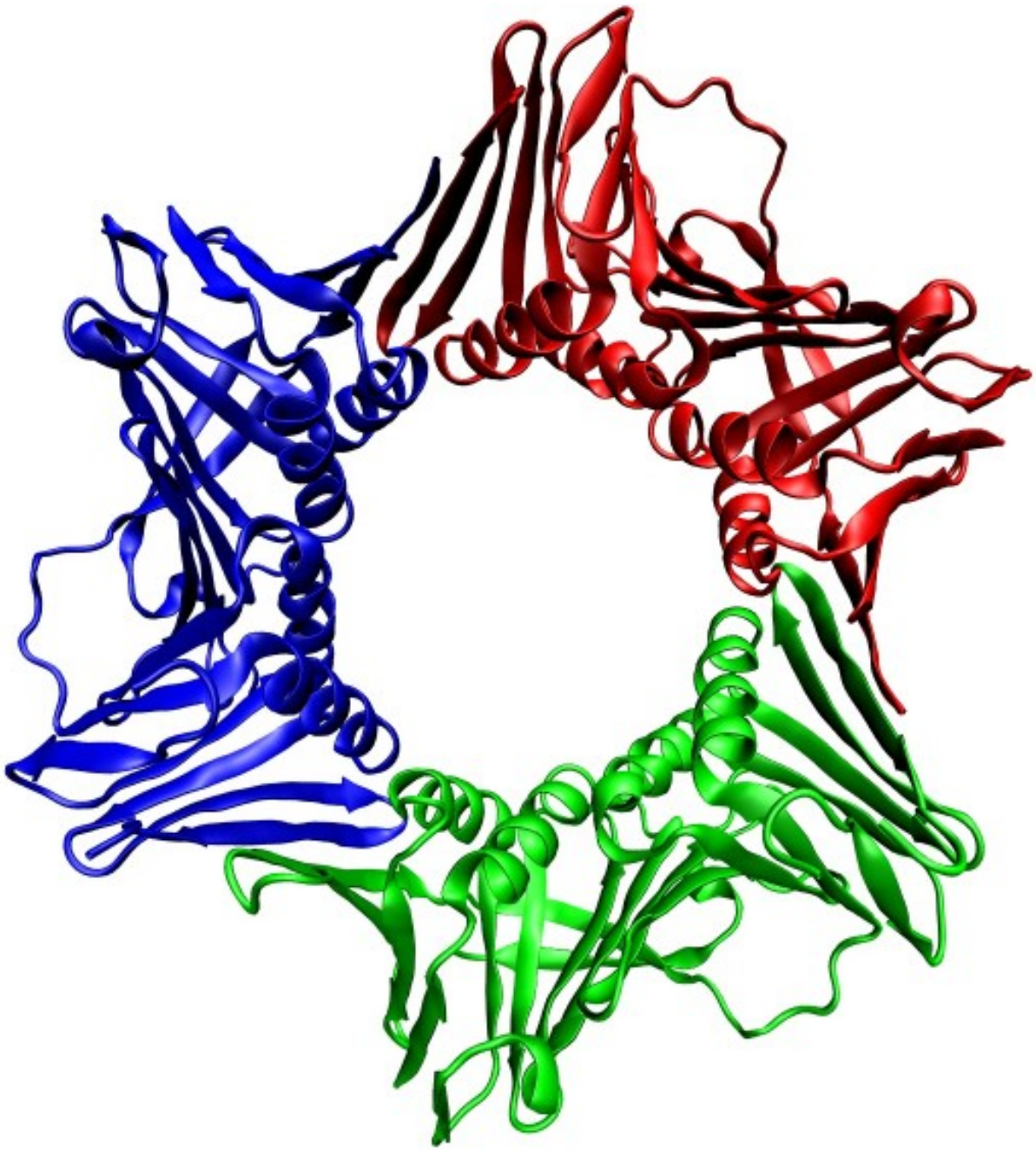
.1 DNA replication

How nucleotides are added in DNA replication

Sliding clamp (DNA clamp)

- Keeps DNA polymerase attached to the template
- Form a ring around different DNA polymerases
- Most are trimers of PCNA proteins

Human DNA clamp protein (trimer of PCNA)



DNA sliding clamp movie

DNA replication complex

1. DNA: old helix, leading strand, lagging strand
2. DNA polymerases
3. DNA helicases
4. SSBP
5. DNA clamp

6. Primase
7. DNA ligase

DNA replication machine (simplified)

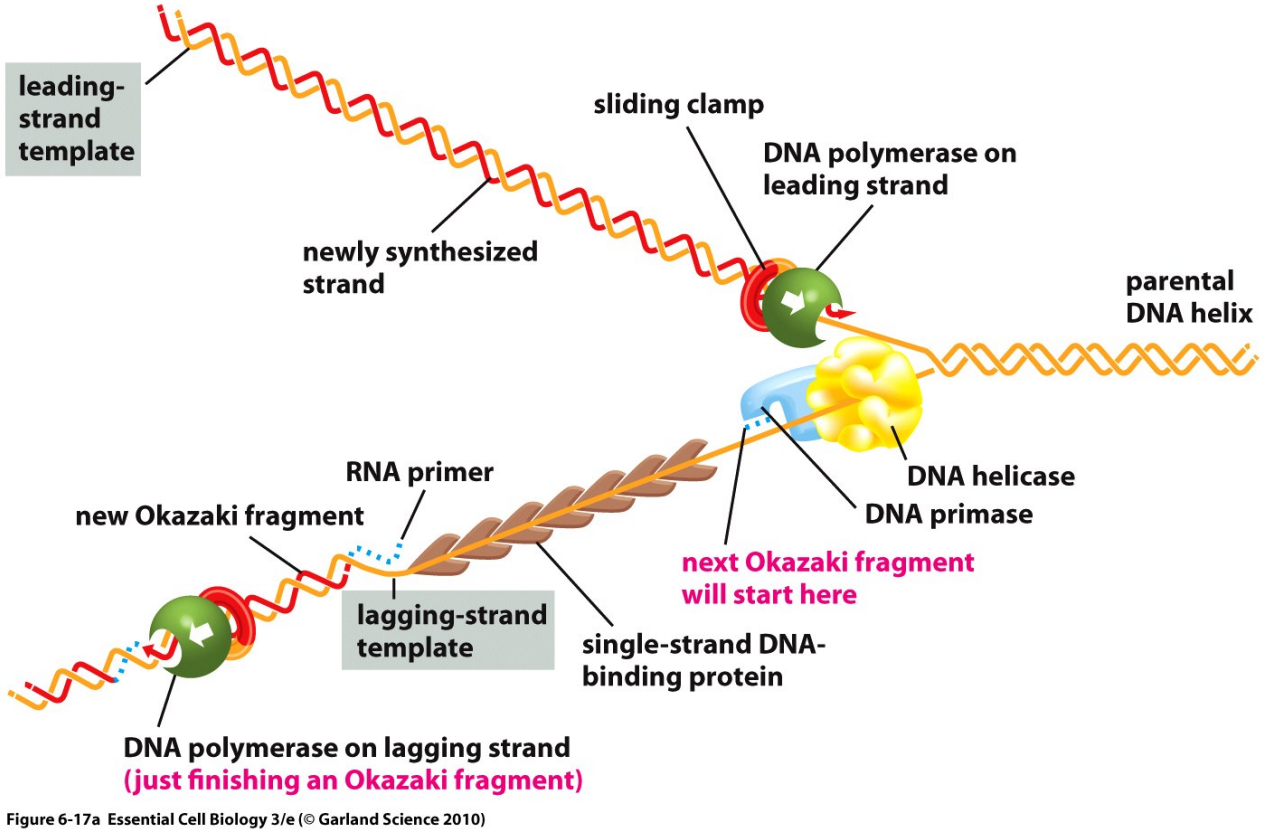


Figure 6-17a Essential Cell Biology 3/e (© Garland Science 2010)

DNA replication machine (more realistic)

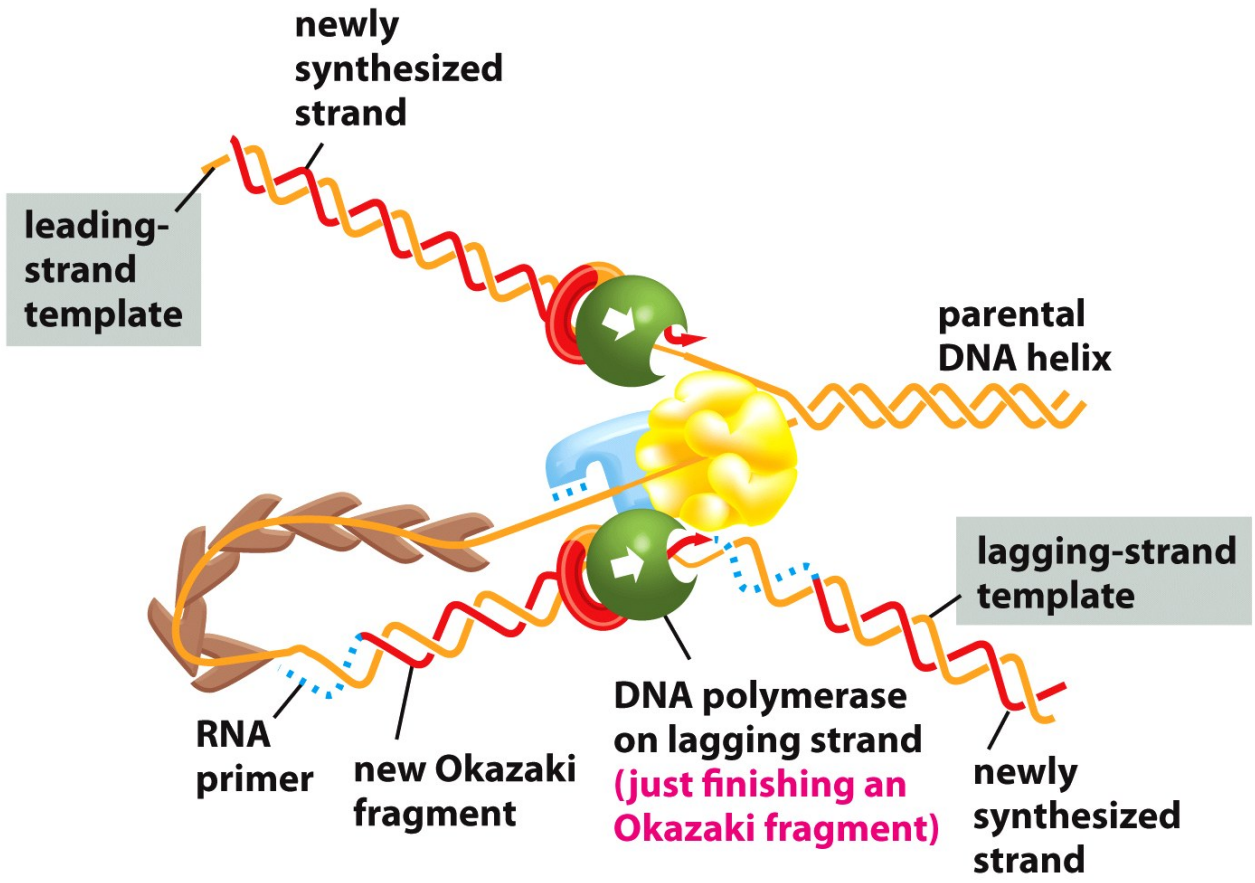


Figure 6-17b Essential Cell Biology 3/e (© Garland Science 2010)

DNA replication sewing machine movie

DNA replication in general: movie I

.2 Telomere problem

Telomerase

- Lagging strand cannot reach the end of DNA molecule
- Every replication cycle chromosome lost parts of telomeres from its ends
- To prevent a loss on meaningful DNA fragments, telomerase extends chromosome with new telomere sequences
- In humans, telomeres are several thousands of TTAGGG sequences
- Telomeres are also recognizable ends of chromosomes

Telomerase and telomere

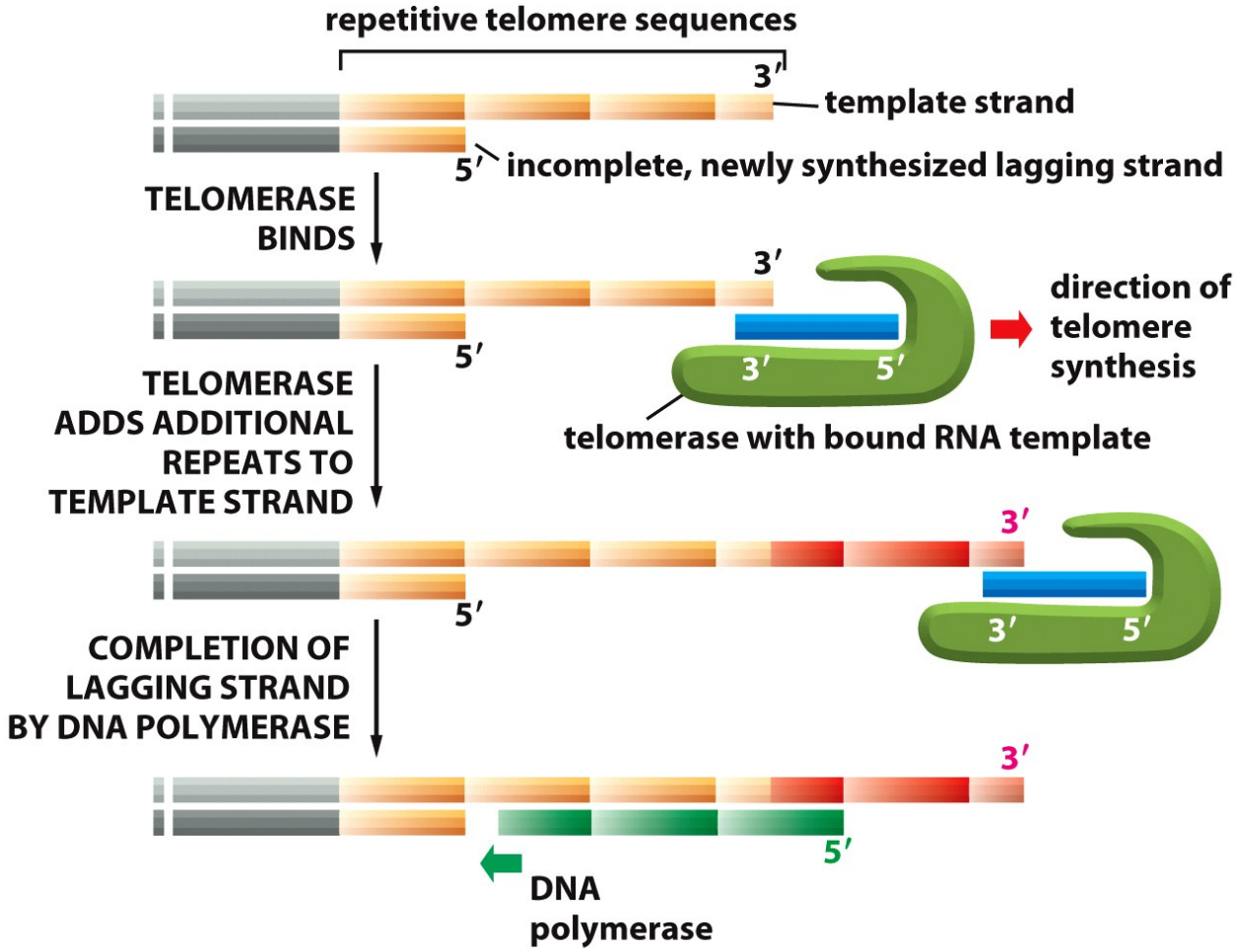


Figure 6-18 Essential Cell Biology 3/e (© Garland Science 2010)

Telomere movie

Telomere theory of aging

- In humans, telomerase is only active in germ cells, stem cells and certain white blood cells
- Olovnikov (1971) postulated that lost of DNA ends will eventually stop division of cells and may stimulate senescence of cells
- There is a strong support of this hypothesis with some cell types (e.g., blood vessels wall cells); however, mice with knocked-out telomerase gene do not show significantly less lifespan

.3 DNA reparation

Mutation theory of ageing and/or cancer

- Accumulation of mutations will result in a constant loss of functions
- Cells will either degrade or start to go out of control (cancer)

DNA mismatch repair

- Normally, error rate of DNA polymerase + proofreading is $\approx 10^{-7}$
- DNA mismatch repair proteins decrease it to $\approx 10^{-9}$
- They react on DNA conformation deviations; recognize newly synthesized strand by nicks, and remove wrong fragments which are later replaced with DNA polymerase and ligase
- Some cancers are results of mutations in DNA mismatch protein genes

DNA mismatched repair system

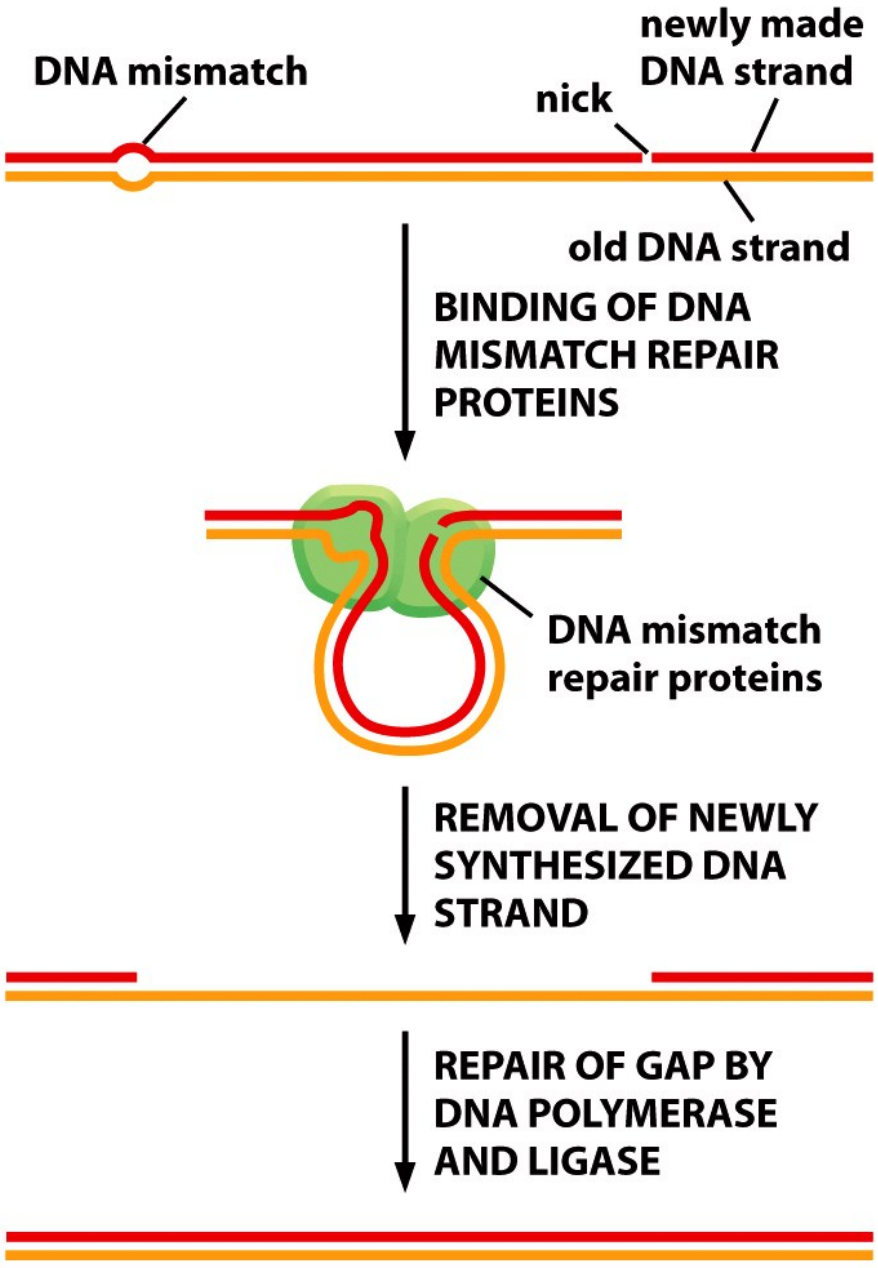


Figure 6-22 Essential Cell Biology 3/e (© Garland Science 2010)

Depurination and deamination

- Depurination is detaching A and G from its sugar; it results in deletion*

- Deamination is C to U conversion; it results in substitution*

Depurination and deamination

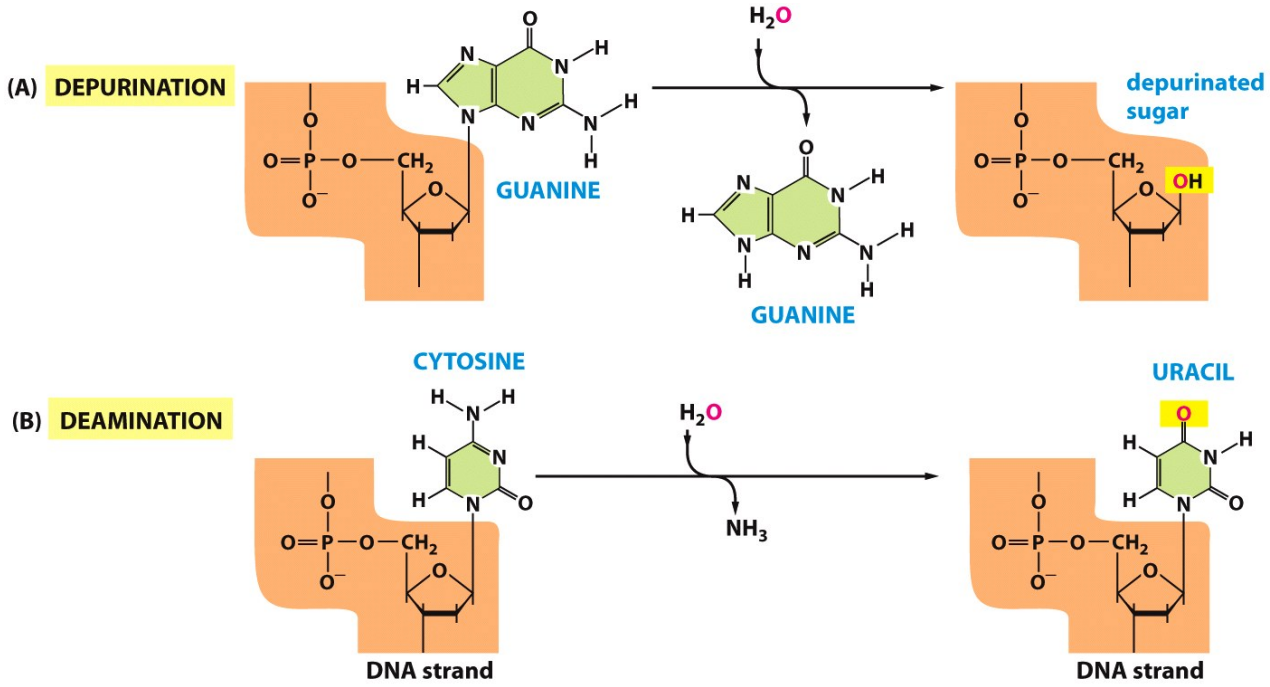


Figure 6-23 Essential Cell Biology 3/e (© Garland Science 2010)

Pyrimidine dimers

- UV radiation often results in forming of pyrimidine (T or C) dimers
- Dimers will stop DNA replication and transcription
- The final result is often a melanoma type of skin cancer

Pyrimidine dimers

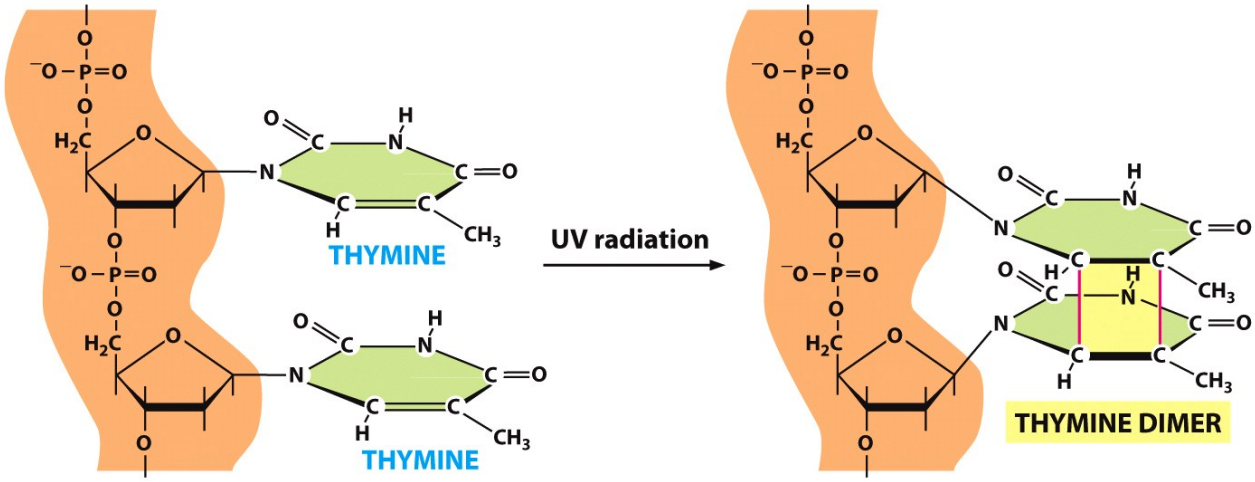
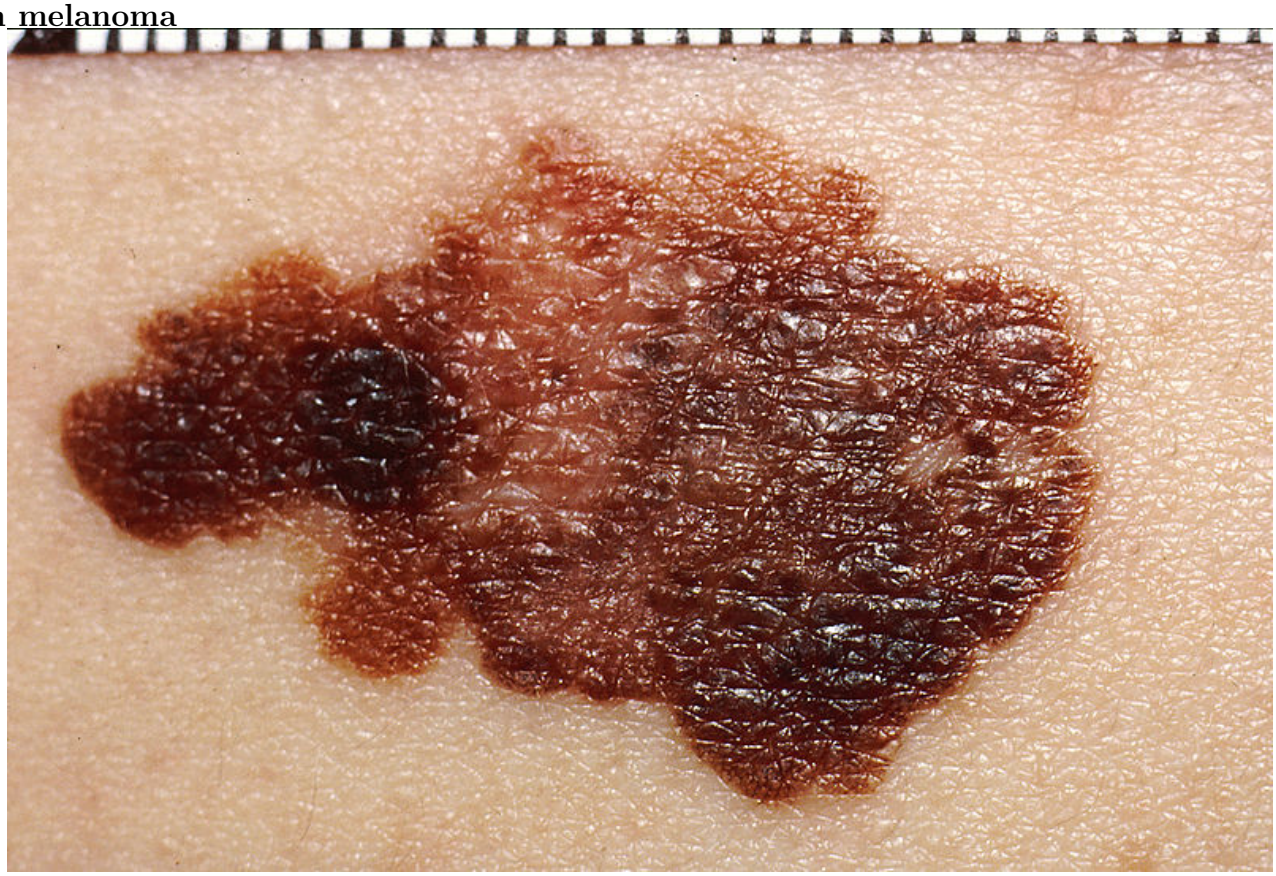


Figure 6-24 Essential Cell Biology 3/e (© Garland Science 2010)

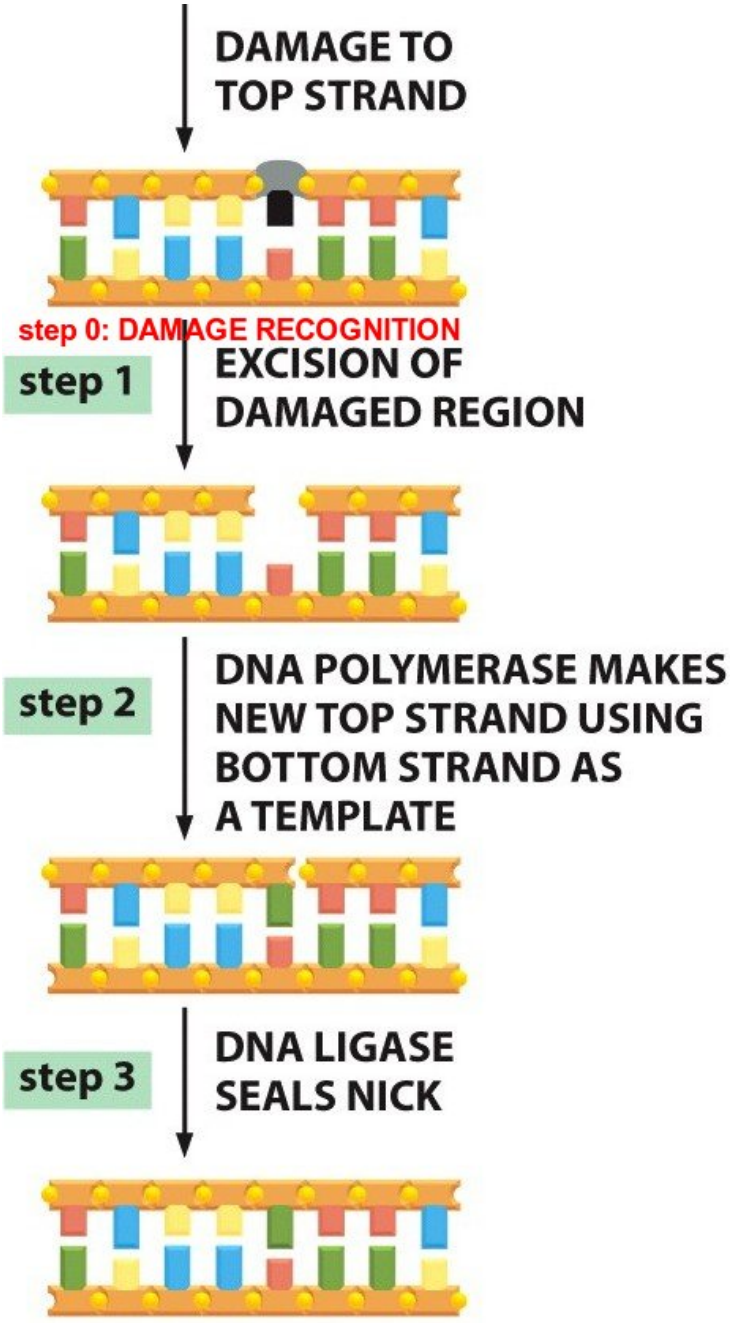
Skin melanoma



Basic way of DNA repair

1. Damage recognition
2. Excision
3. Resynthesis
4. Ligation

DNA repair flow



Nonhomologous end-joining

- The quick-and-dirty mechanism of reparation when both strands are broken
- The reparation will result in deletion

Nonhomologous end-joining

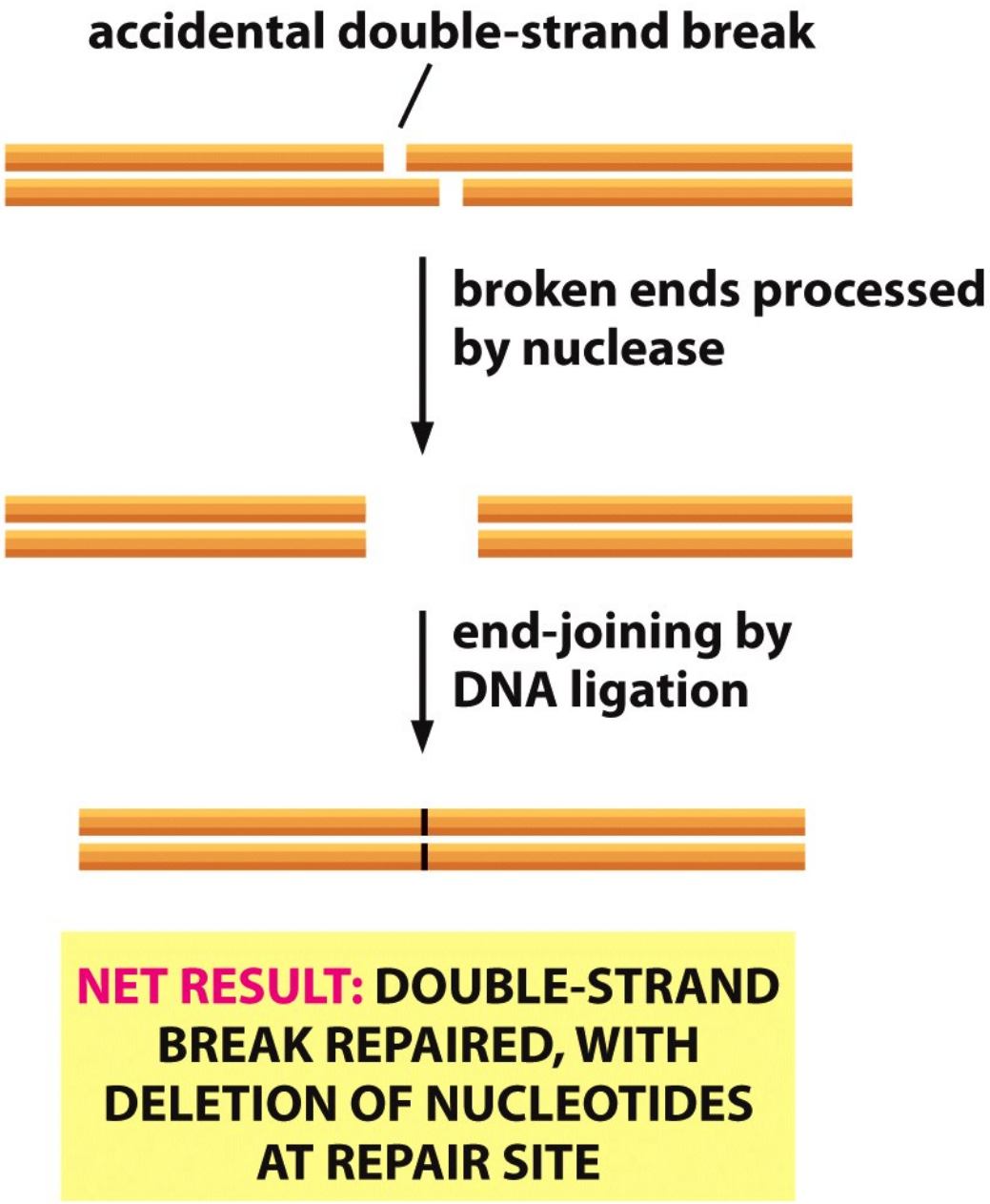


Figure 6-27 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (1 point)

What is the difference between deletion and substitution?

Summary

- Ends of chromosomes are constantly shortening and extending with new telomeres
- DNA replication system is a multienzyme complex
- Ends of chromosomes are constantly shortening and extending with new telomeres
- DNA suffers from multiple damaging events; multiple reparation systems are trying to lower mutation risks

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 6*.

Outline

Questions and answers

Previous final question: the answer

What is the difference between deletion and substitution?

- Deletion is nucleotide **removal** whereas substitution in nucleotide *replacement*
- What could be the third variant?

DNA

Nonhomologous end-joining

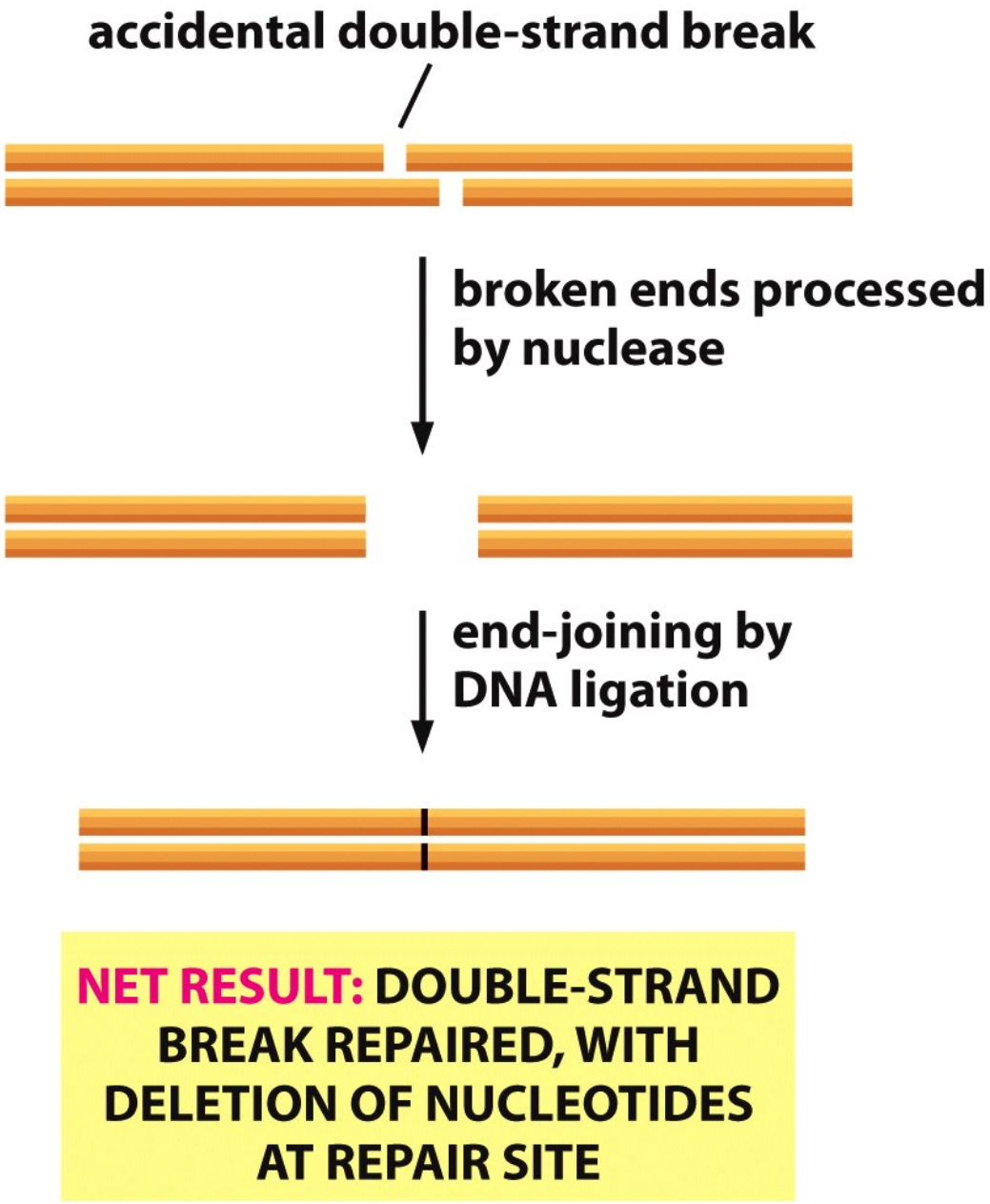


Figure 6-27 Essential Cell Biology 3/e (© Garland Science 2010)

.1 DNA recombination

Homologous recombination/reparation

- Occurs between two different DNA duplexes
- Uses the availability of homologous copies of DNA (e.g., homologous chromosomes)
- Requires breakage of both DNA strands

Homologous reparation, part I

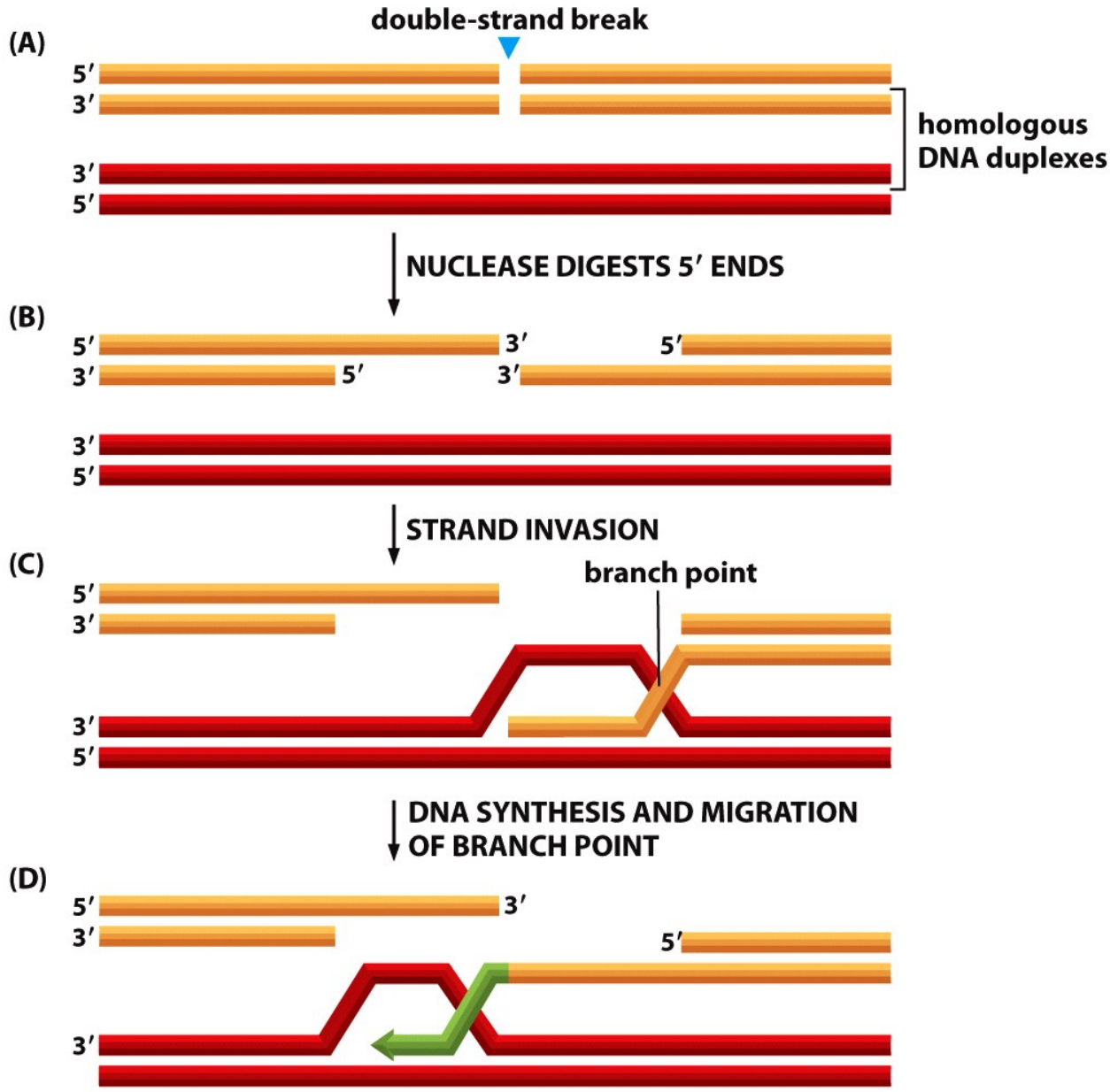


Figure 6-29 part 1 of 2 Essential Cell Biology 3/e (© Garland Science 2010)

Branch point and invading strand

- Enzymes take off two DNA chunks from each strand of one duplex
- *Invading strand* will cross with the strand of other duplex
- Place of crossing between two DNA strands from different duplexes is a *branch point*
- Process is finished with DNA ligation

Homologous reparation, part II

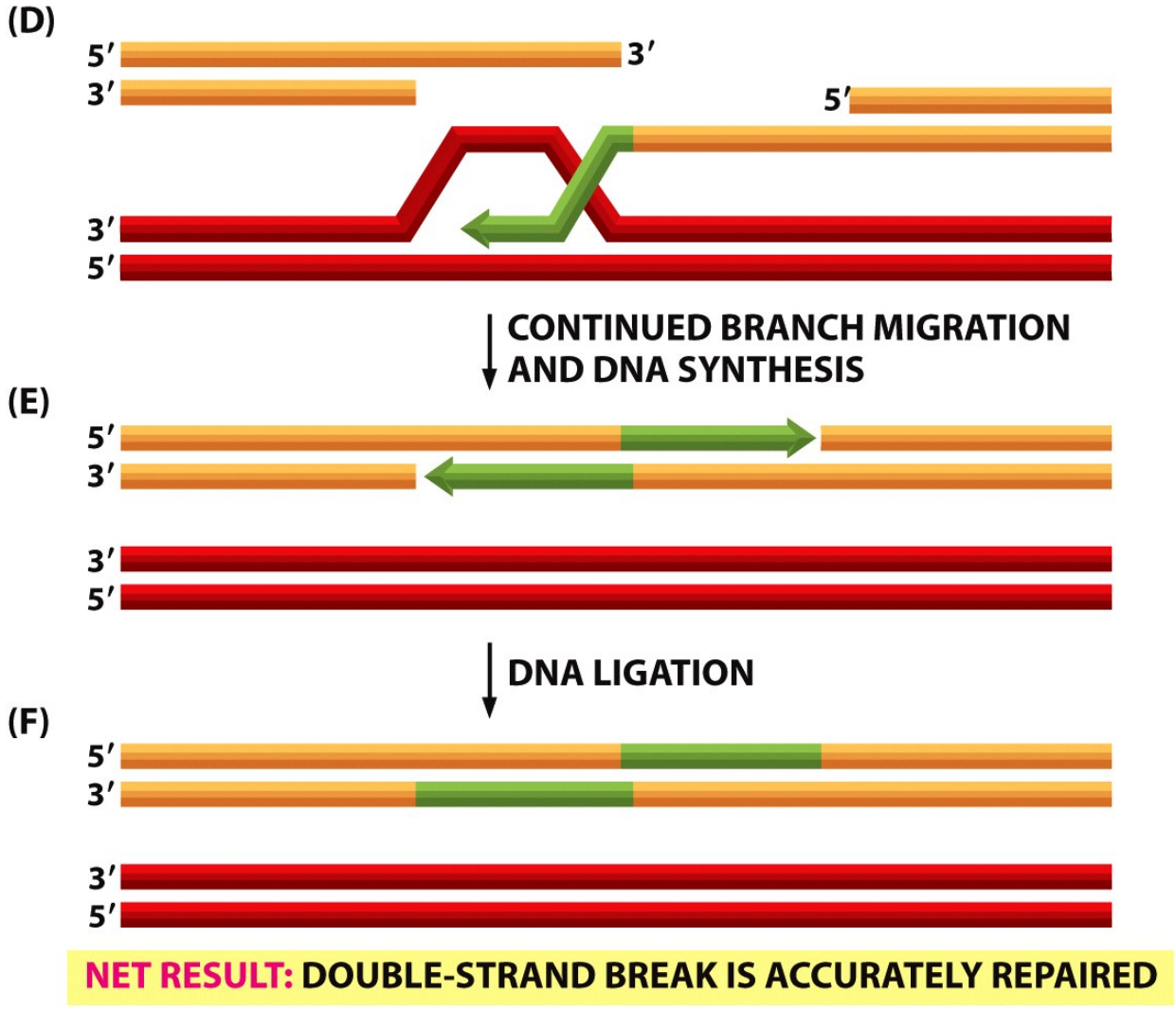


Figure 6-29 part 2 of 2 Essential Cell Biology 3/e (© Garland Science 2010)

Homologous reparation movie

Holliday junctions in meiosis

- In meiosis prophase, DNA duplexes are crossing and changing parts in the way similar to homologous reparation
- Two resulted strands will contain newly synthesized fragments, and all four will cross to form a hybrid molecules

Recombination, part I

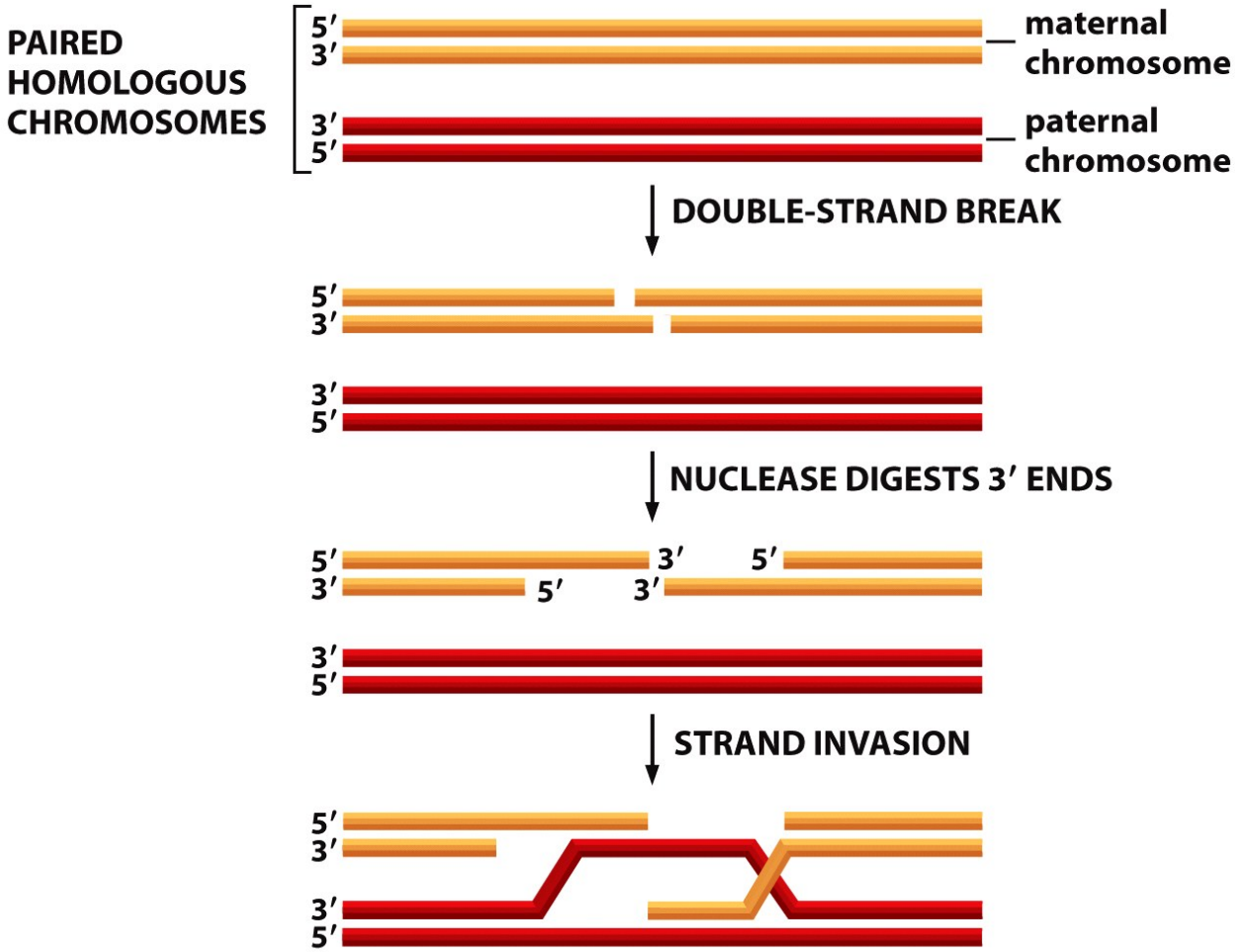


Figure 6-31 part 1 of 2 Essential Cell Biology 3/e (© Garland Science 2010)

Recombination, part II

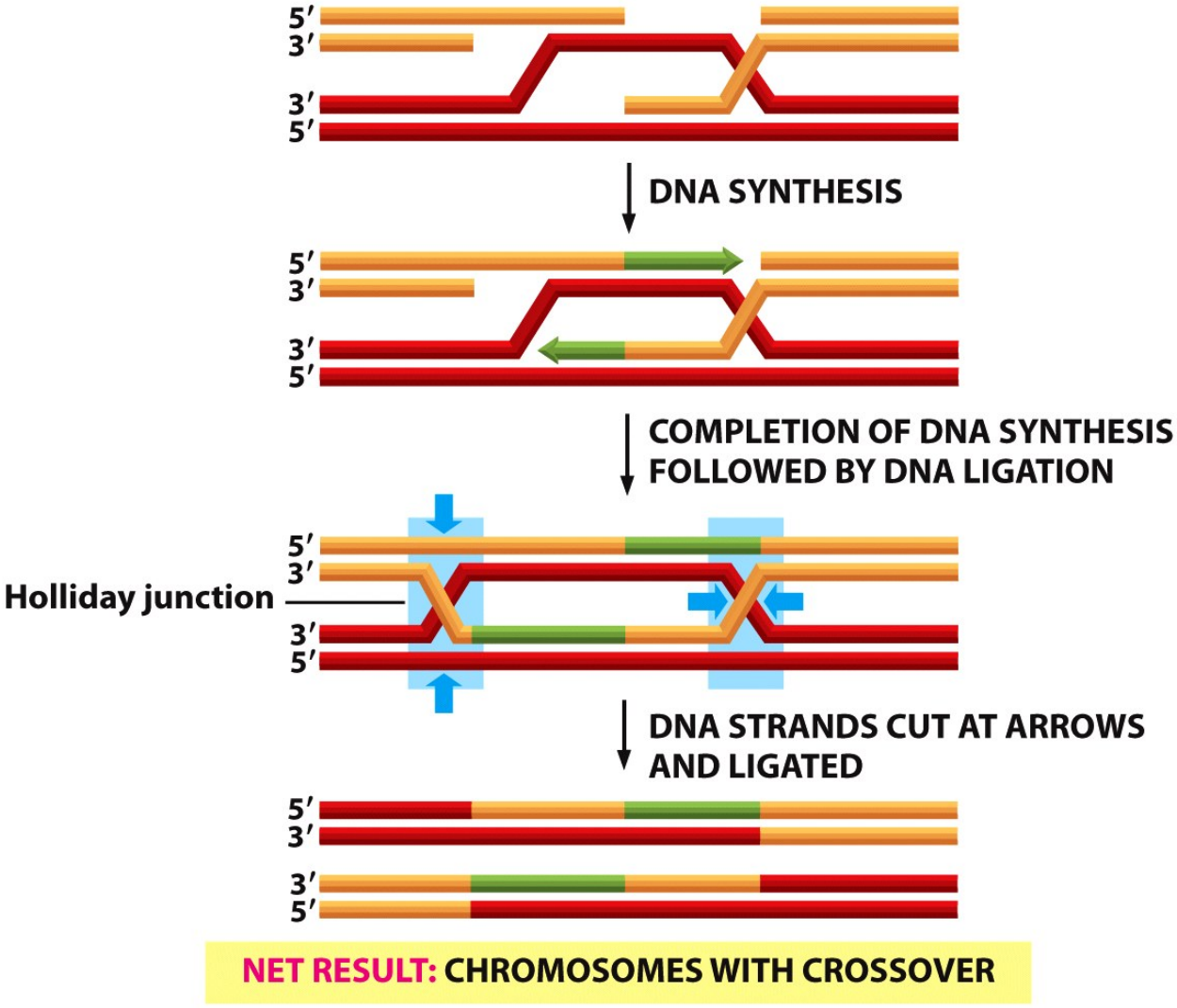


Figure 6-31 part 2 of 2 Essential Cell Biology 3/e (© Garland Science 2010)

Recombination movie

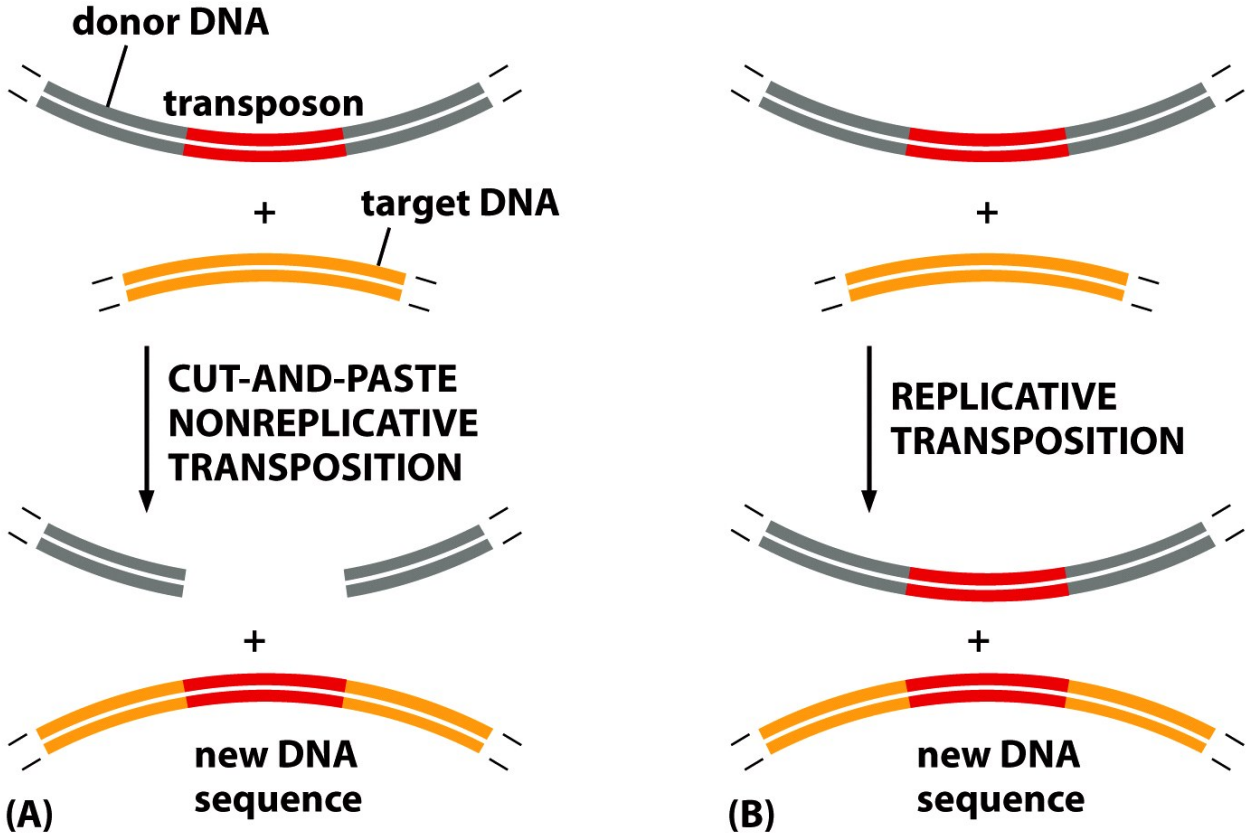
Recombination movie II

Mobile elements

Recombination through mobile elements

- Some parts of DNA molecules can migrate between duplexes, they are transposons
- DNA-only transposons may be cut and pasted (migrate), or replicate across DNA duplexes
- Some transposons replicate through RNA with the help of reverse transcriptase—retrotransposons

DNA only transposons



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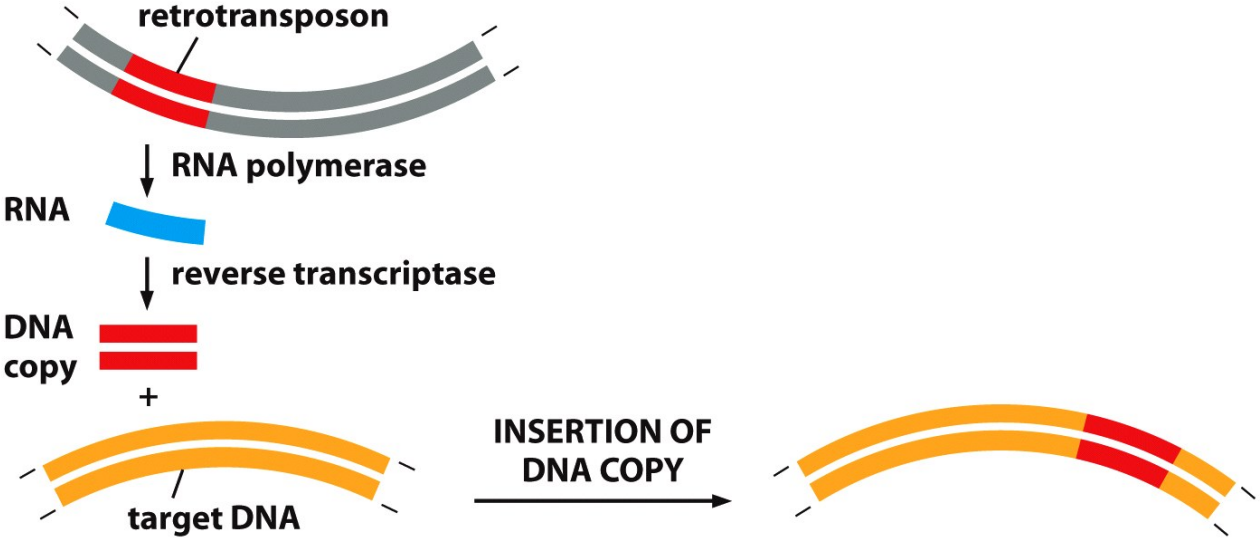
Retrotransposon



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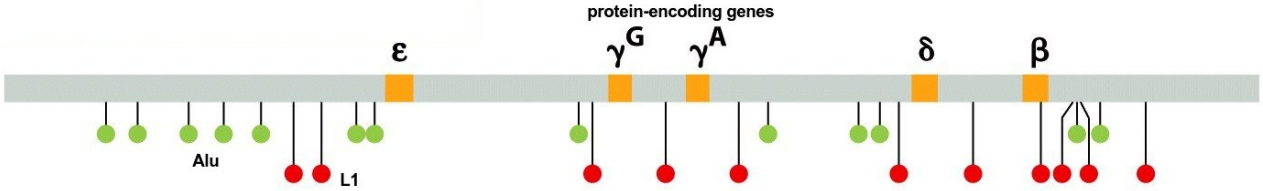
Human retrotransposons

- Long *L1 elements* (15% of human genome) encode reverse transcriptase themselves
- Short *Alu elements* (11% of genome) depend on external enzymes

Human retrotransposons



L1 red, *Alu* green

Alu sequence

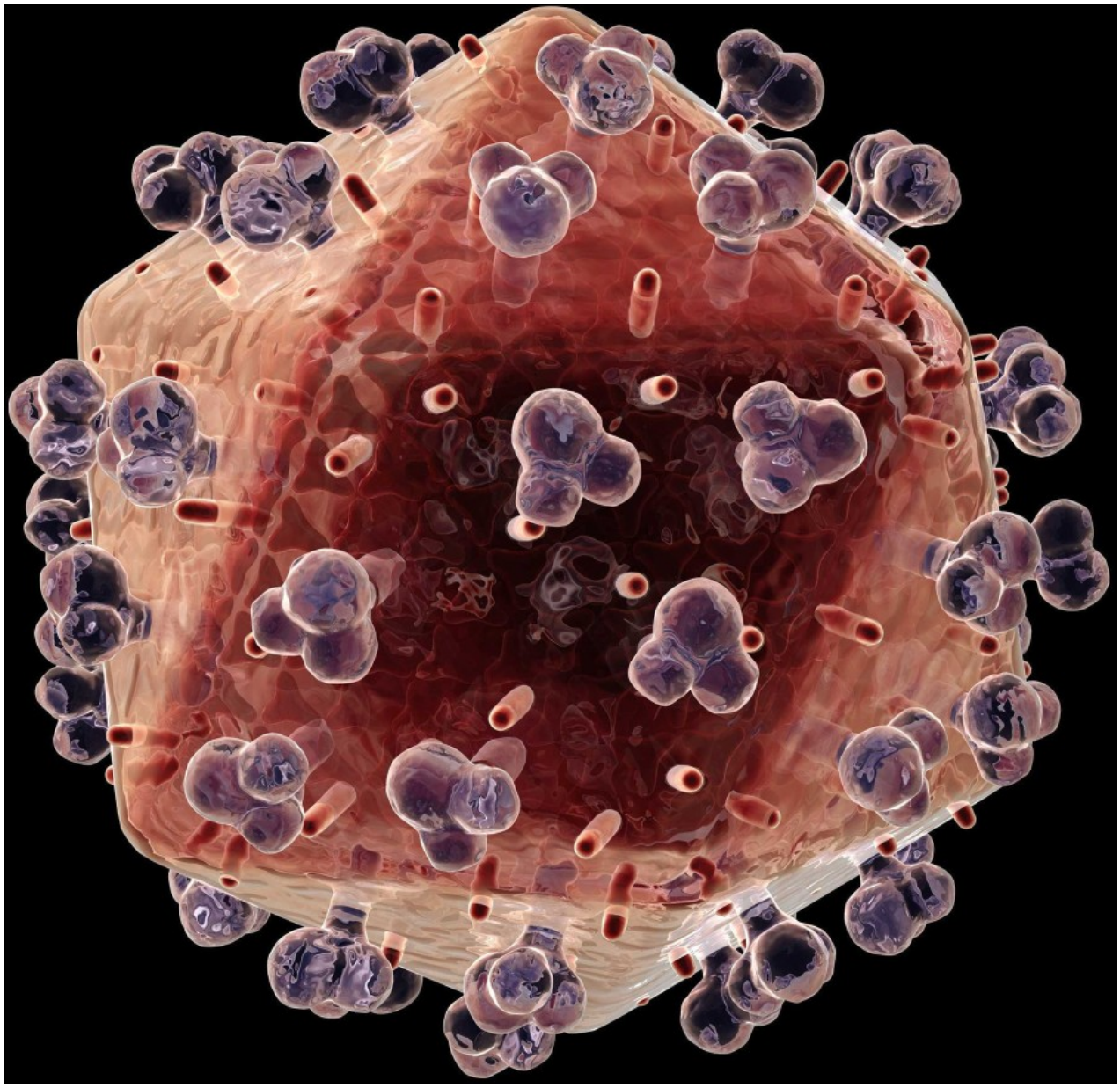
GCCGGGCGCGGTGGCGCGTGCCTGTAGTCCC**AGCT**ACTCG GGAGGCTGAGGCTGGAGGATCGCTTGA
GTAGTGCGCTATGCCGATCGGGTGTCCGCACTAAGTTCGGCATCA ATATGGTGACCTCCCGGGAGCGGGGC
CACCAGGTTGCCTAAGGA GGGGTGAACCGGCCCAGGTCGGAAACGGAGCAGGTCAAAACTCCC GT-
GCTGATCAGTAGTGGGATCGCGCCTGTGAATAGCCACTGCACTC CAGCCTGGGCAACATAGCGAGAC-
CCCGTCTCT

AGCT is the *recognition site*

Viruses and retroviruses

- If transposon acquires the protein shell (capsid) and goes outside cell, it becomes a virus
- Retroviruses (like HIV) use reverse transcriptase and may even insert themselves into cell DNA for a while

HIV retrovirus



Final question (2 points)

What is better for the cell, nonhomologous or homologous reparation? Why?

Summary

- DNA suffers from multiple damaging events; multiple reparation systems are trying to lower mutation risks
- Homologous recombination is used when two similar DNA duplexes (homologous DNAs) are available
- Some viruses could be transposons escaped from cells

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 6*.

Outline

Questions and answers

Previous final question: the answer

What is better for the cell, nonhomologous or homologous reparation? Why?

- Homologous reparation is better if there is enough time. If not, nonhomologous reparation will do the job.

DNA

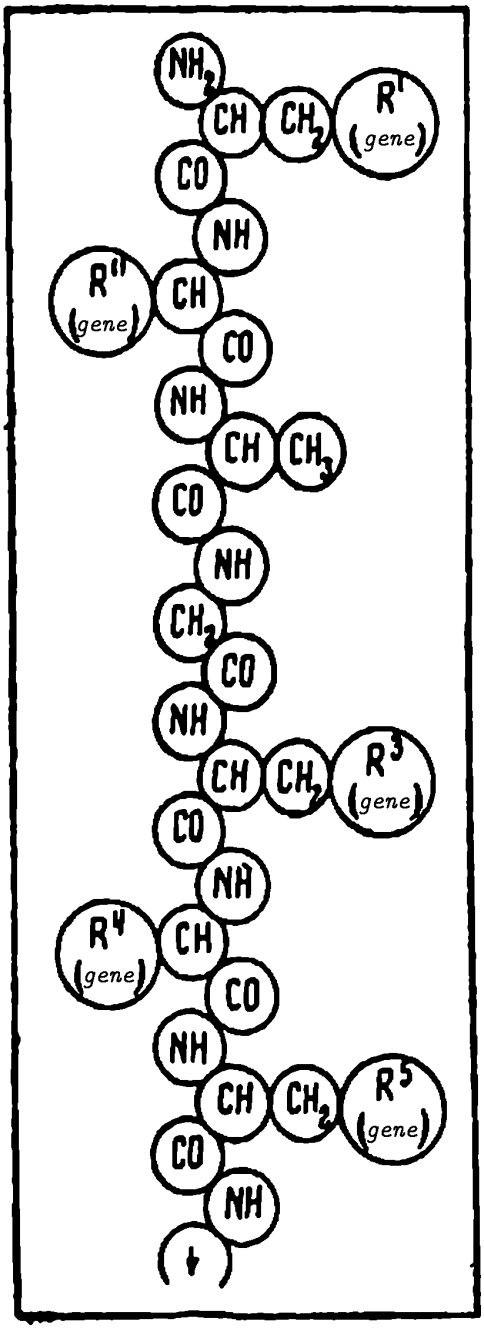
.1 DNA and chromosomes

Structure of DNA movie

Koltsov's template hypothesis

- Koltsov (1928, 1936) hypothesized that hereditary molecule should be polymer with radicals (genes)
- Radicals of daughter molecules will be positioned on the same places (molecule is a template to itself)
- However, Koltsov thought that this should be a protein, because nucleic acids seems to be "too simple"

Koltsov's hypothesis



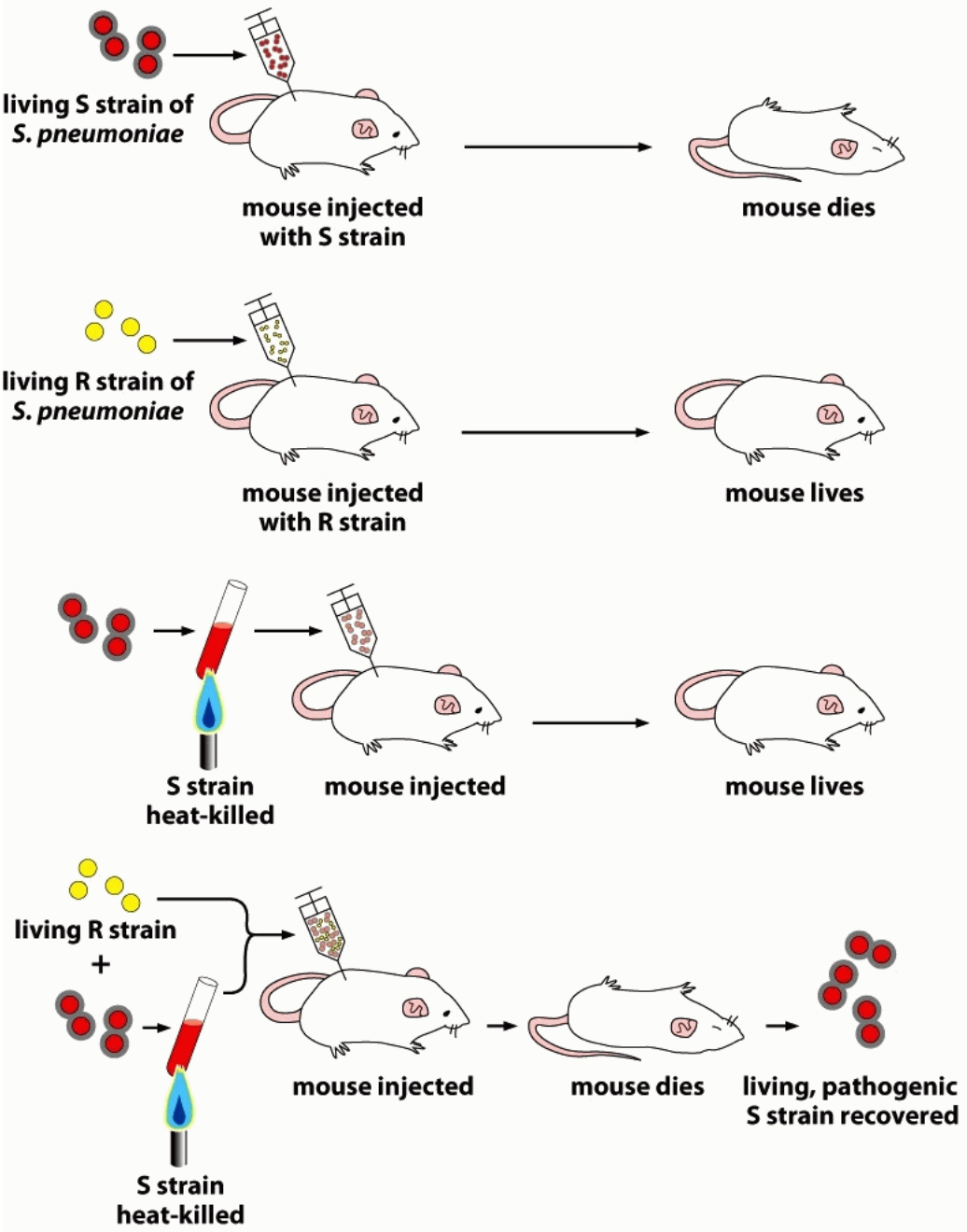
Griffith experiment (1928)

- He used pathogenic and non-pathogenic strains of pneumococci
- Showed that killed pathogenic strain could modify non-pathogenic
- The nature of modification agent was not yet discovered

Rough (nonvirulent) and smooth (virulent) pneumococci

The smooth strain has a polysaccharide capsule that protects it from the host's immune system, while the rough strain doesn't have that protective capsule and is defeated by the host's immune system.

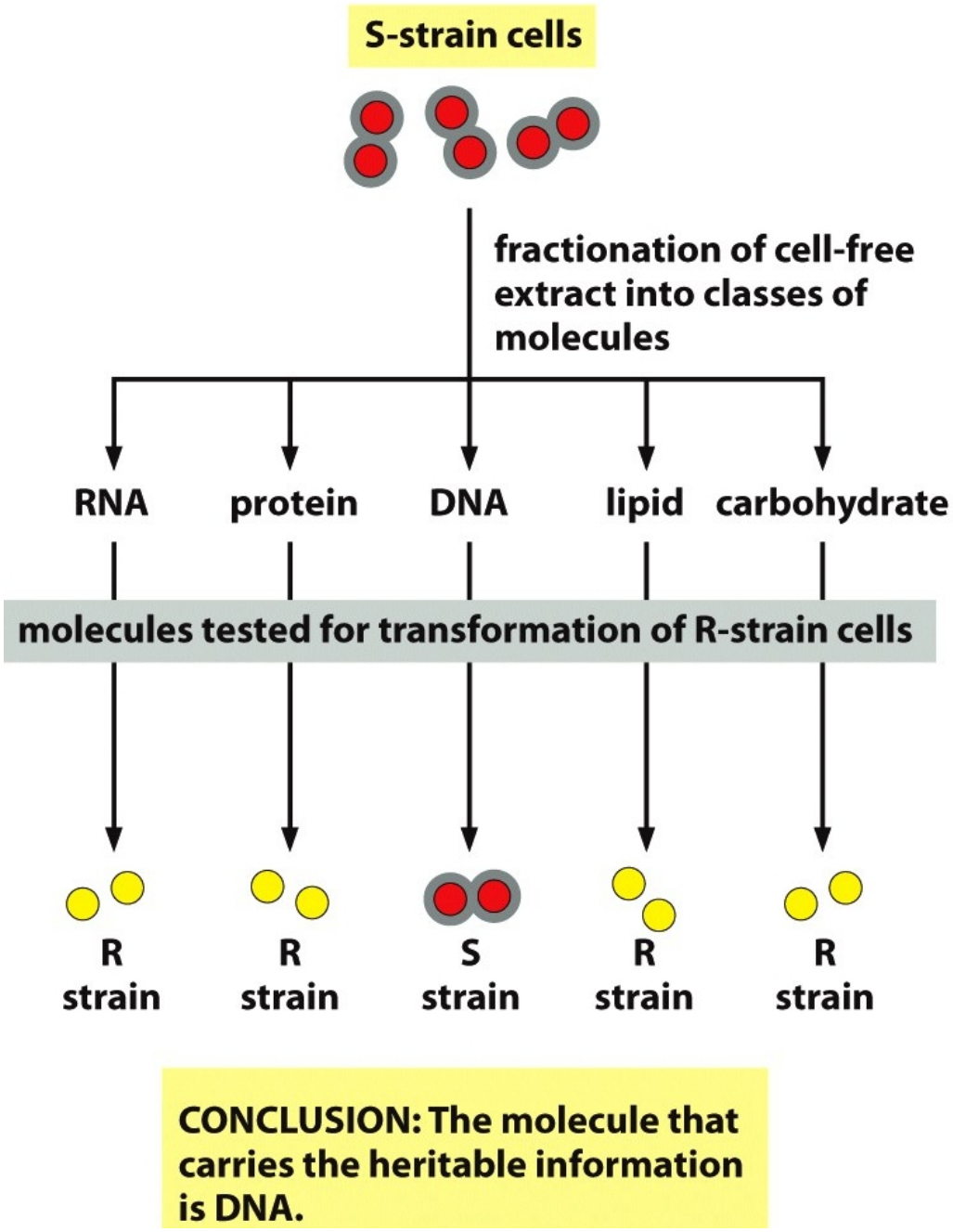
Griffith's experiment



Avery et al. experiment (1944)

- In all, Avery group demonstrated that DNA, not proteins is a modification agent
- They tried also lipids and carbohydrates

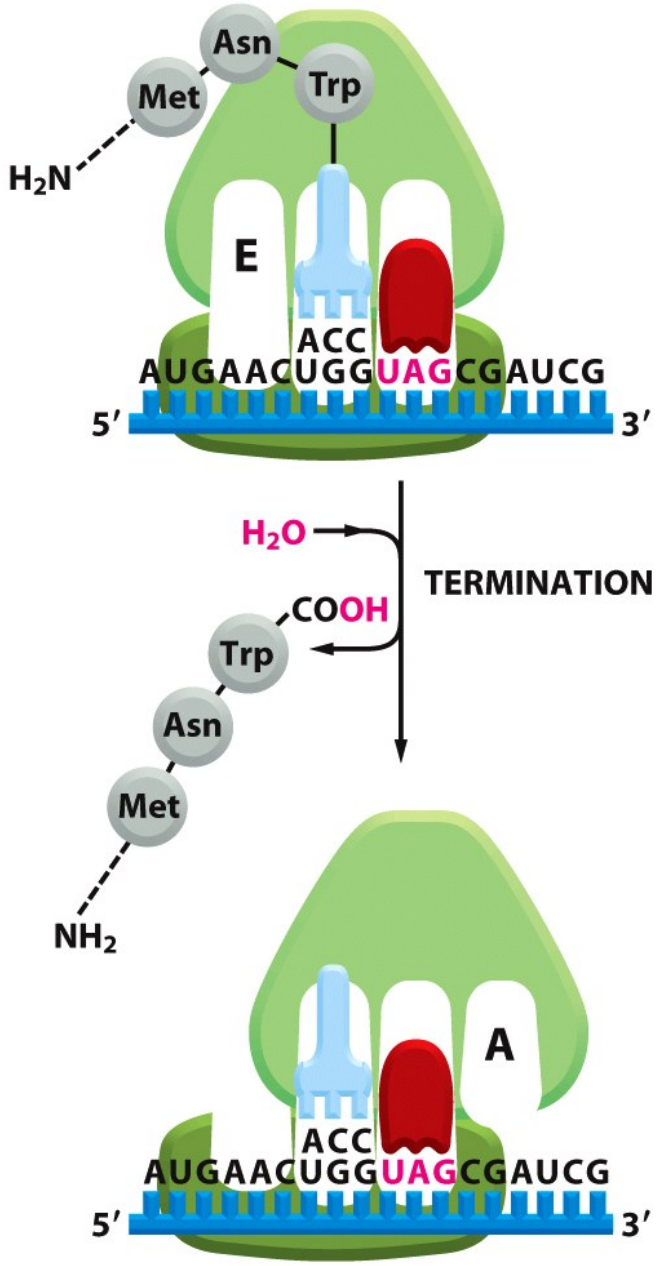
Avery et al. experiment



Hershey and Chase experiment (1952)

- They used bacterial virus (bacteriophage) T2 where capsid (protein envelope was marked with ^{35}S) whereas virus DNA was marked with ^{32}P
- Infected bacteria contain only ^{32}P
- Therefore, infection was due to DNA

T2 bacteriophage



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Hershey & Chase experiment

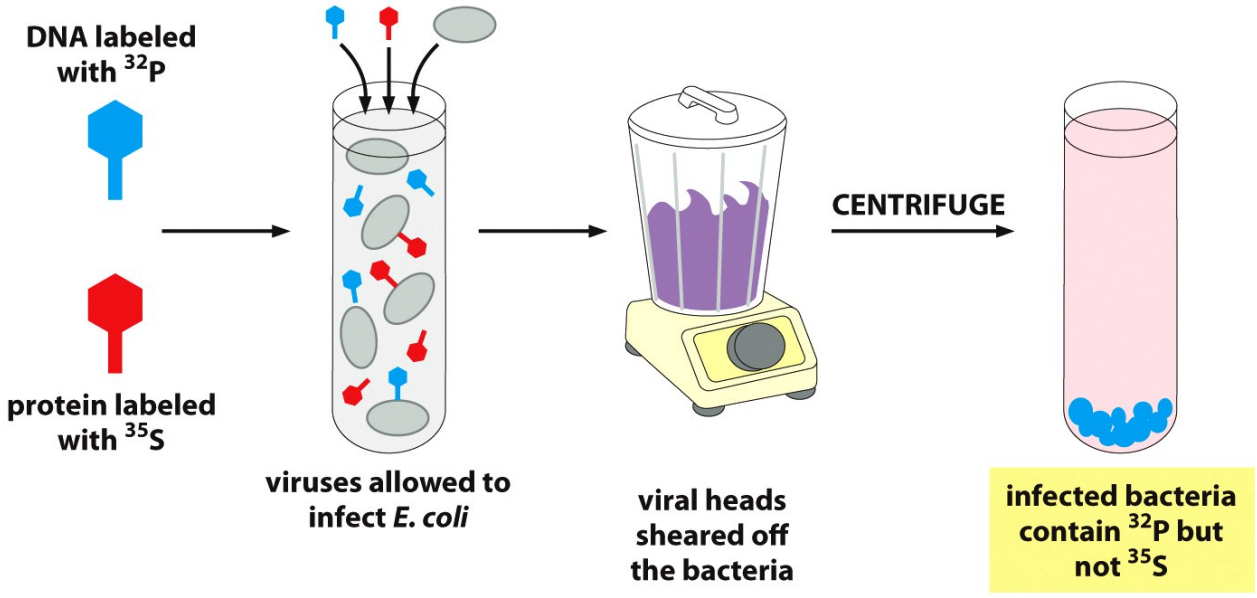
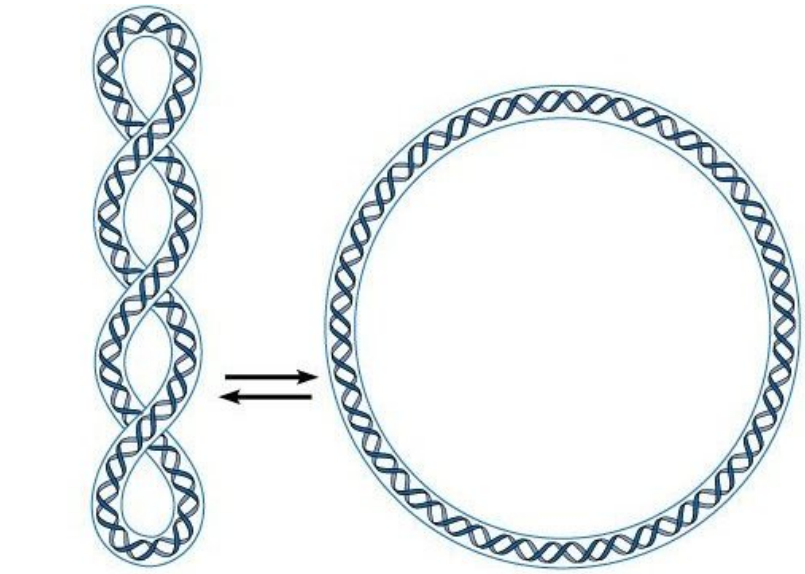


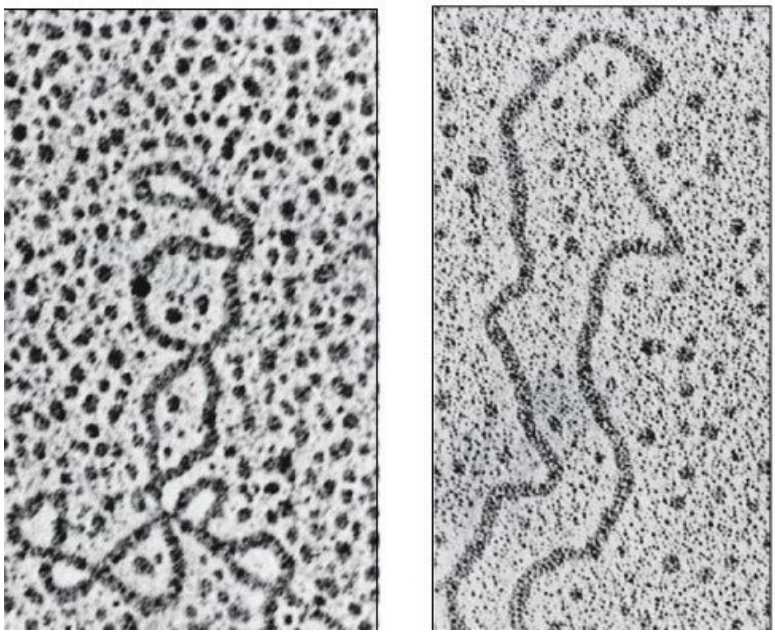
Figure 5-5b Essential Cell Biology 3/e (© Garland Science 2010)

Interphase DNA and chromosome condensation

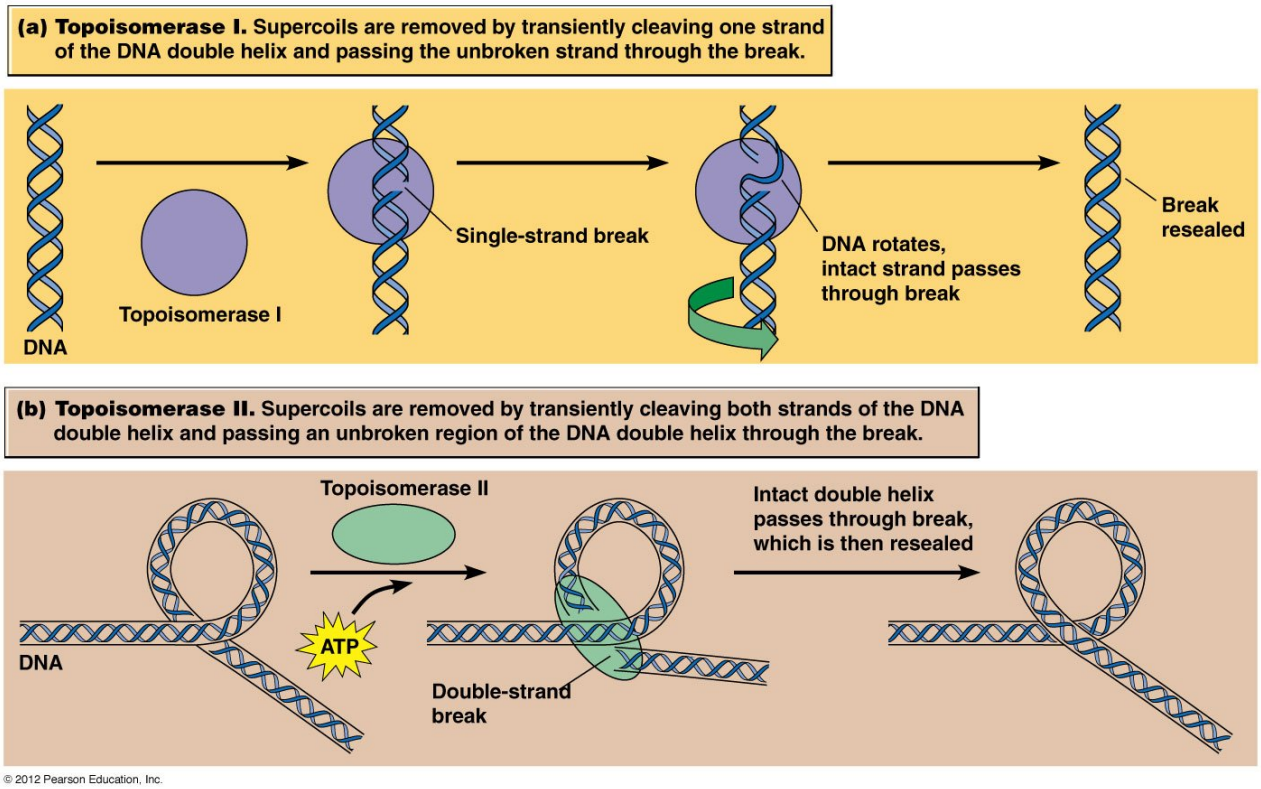
- In eukaryotes, DNA comprises of multiple long filaments which are condensed during cell division (mitosis or meiosis)
- These filaments are interphase DNA, or "interphase chromosomes", they are ≈500 times shorter than completely non-condesed DNA (which is not exist in eukaryotic cells)
- Interphase DNA is normally **super-coiled** (process controlled by **topoisomerases**)
- Chromosomes normally compacted in 10^5 times comparing with non-condensed DNA

Supercoils





Topoisomerases



Chromosomes in the interphase nucleus

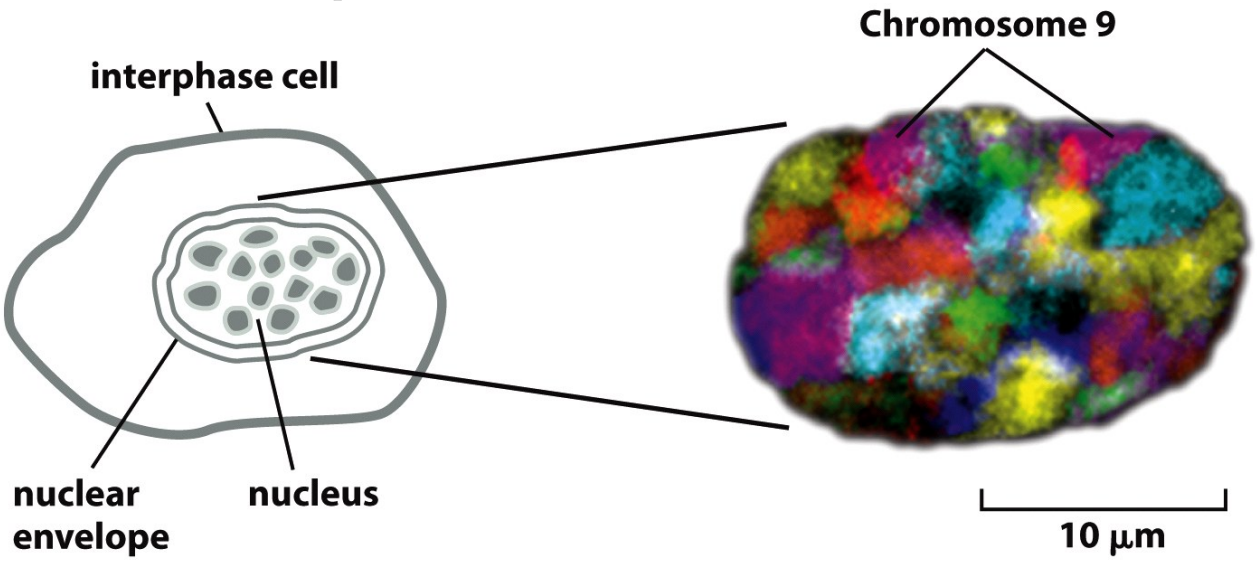


Figure 5-18 Essential Cell Biology 3/e (© Garland Science 2010)

Histones, chromatin and nucleosomes

- 50% of chromosome mass are different histone proteins
- Histones + non-histones + DNA = chromatin
- Histones are responsible for nucleosome and 30 nm fiber levels of packing

30 nm fibers and nucleosomes

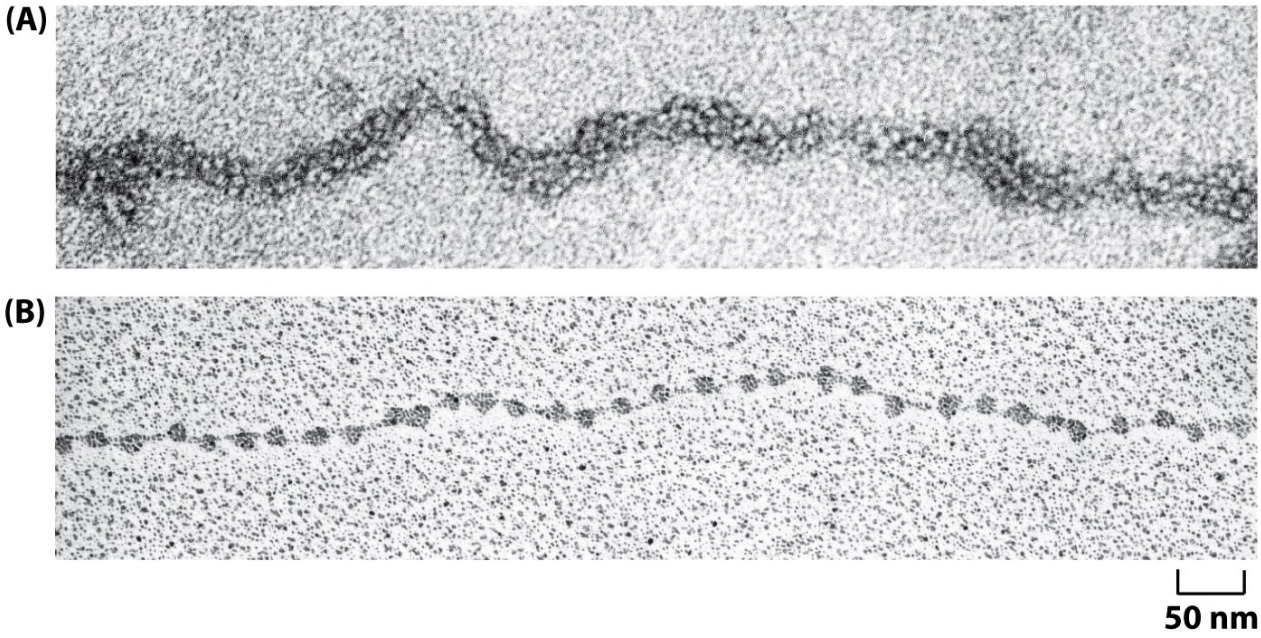
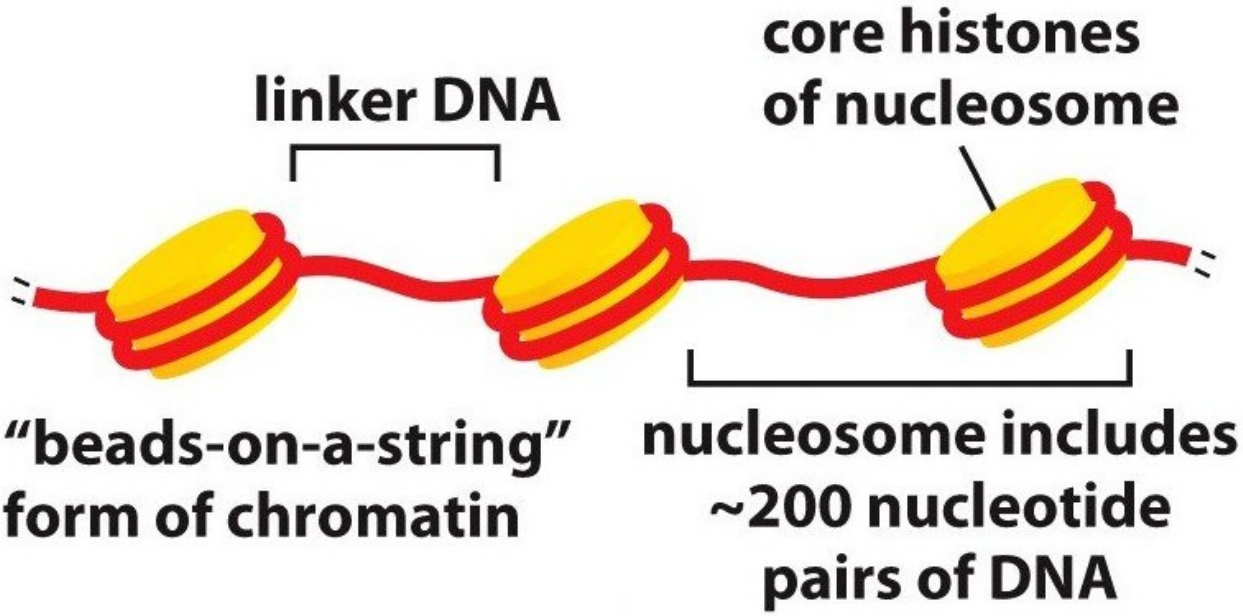


Figure 5-21 Essential Cell Biology 3/e (© Garland Science 2010)

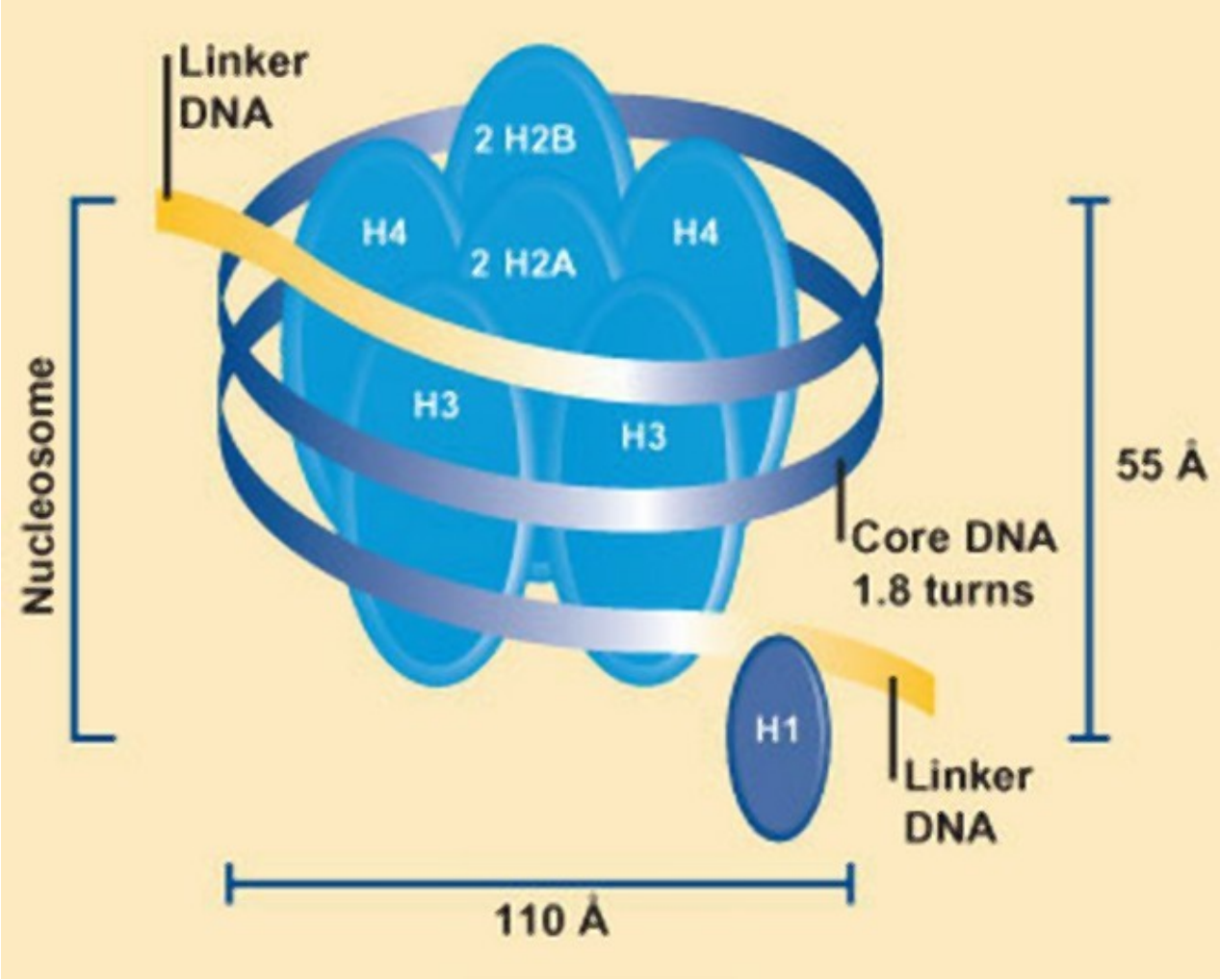
Beads on a string



Diversity of histones

- Histone octamer consists of H2A, H2B, H3 and H4 histones
- Linker histone H1 helps to create a 30 nm fiber

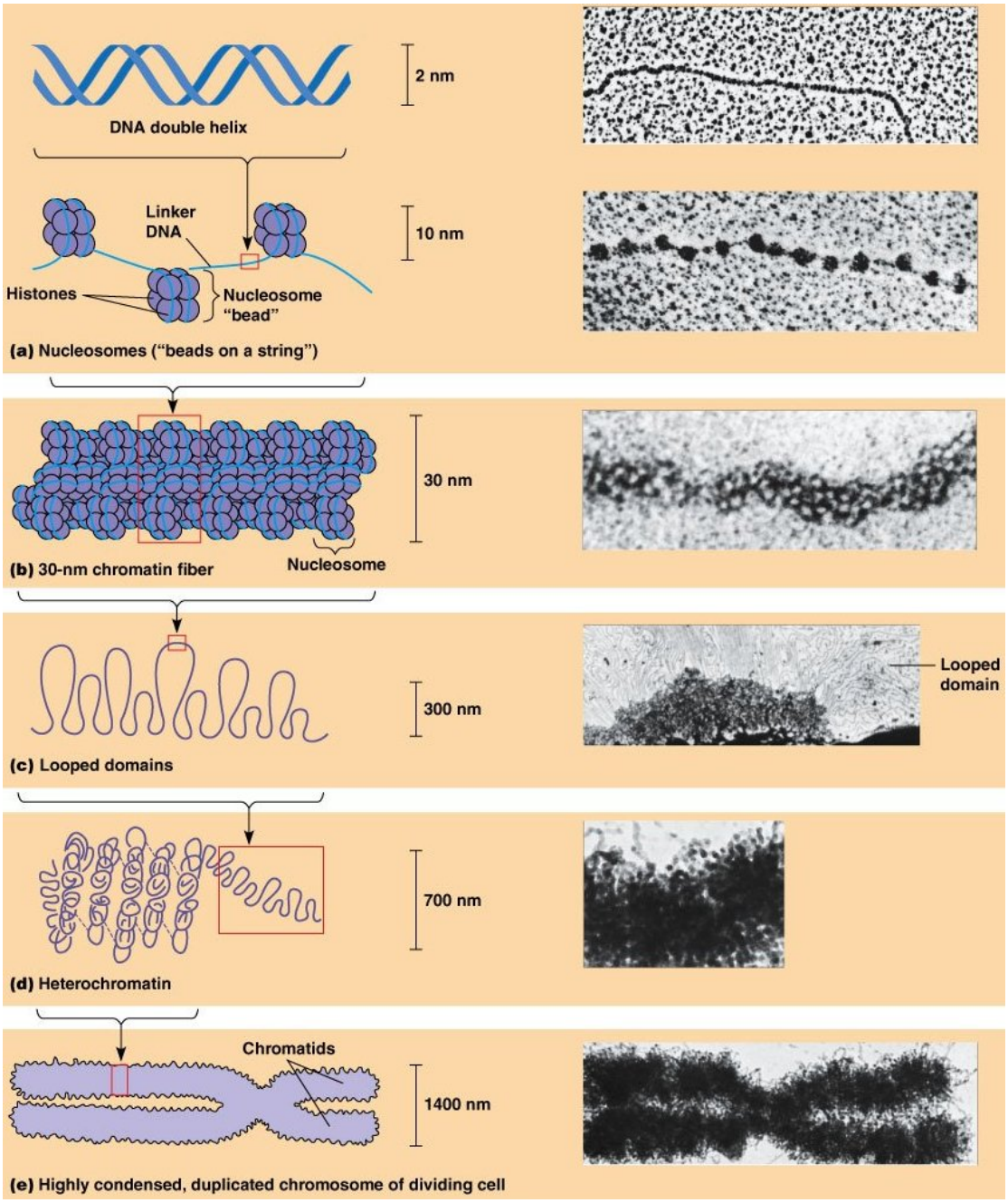
Histones



Five levels of chromosome packing

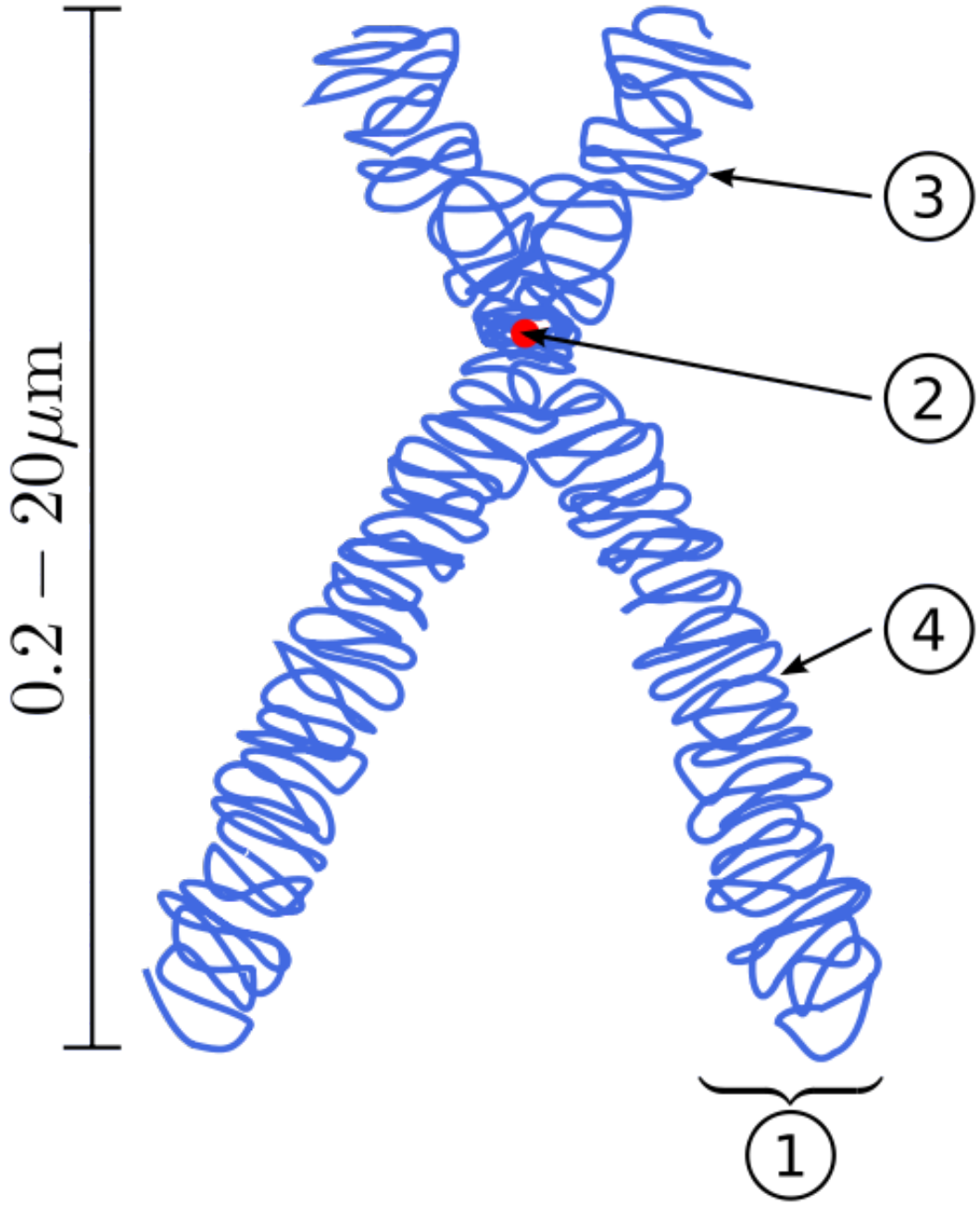
- Nucleosome
- Chromatin 30 nm fiber
- Chromatin loops
- Condensed chromatin loops
- Chromosome

Five levels of compactization



Chromosome coiling movie

Telomeres, centromeres and chromatids



- Telomeres (1) and centromeres (2) are specialized, meaningless DNA sequences which mark centers and ending regions of chromosome
- Chromatids are DNA molecules in one chromosome (normally two)
- Every chromatid has two arms (3, 4) (again, in most cases)

Karyotype

- Chromosomes are different in size and form
- Standard set of chromosomes is a karyotype
- Banding patterns allow to distinguish between chromosomes through the location of A-T rich parts (dark bands)

Banding patterns in human karyotype

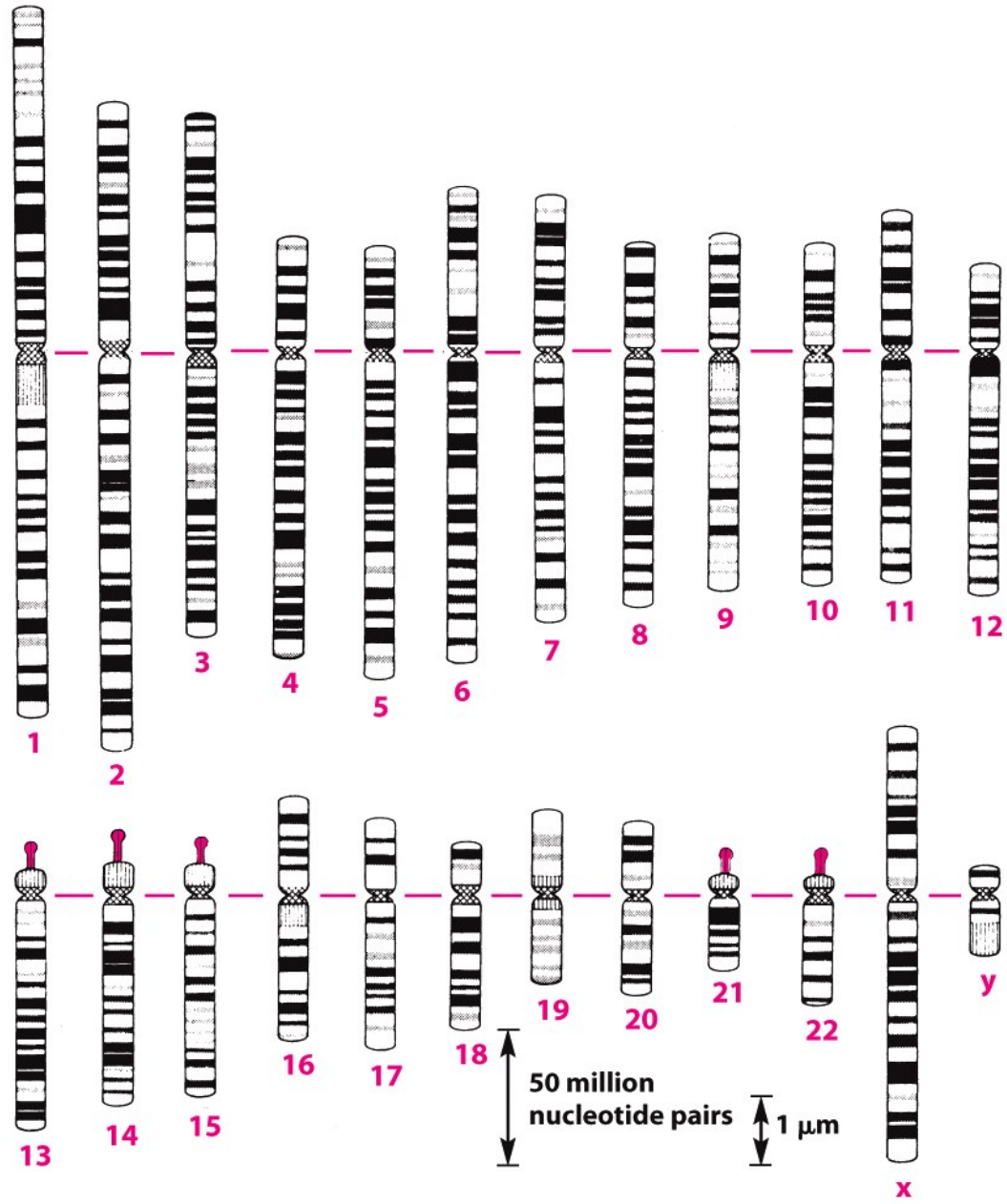


Figure 5-11 Essential Cell Biology 3/e (© Garland Science 2010)

Chromatin remodeling complexes

- To make DNA readable, it should be detached from histones
- Chromatin-remodeling proteins "move" DNA alongside histone octamers and/or compactize/decompactize nucleosomes

Chromatin-remodeling complexes

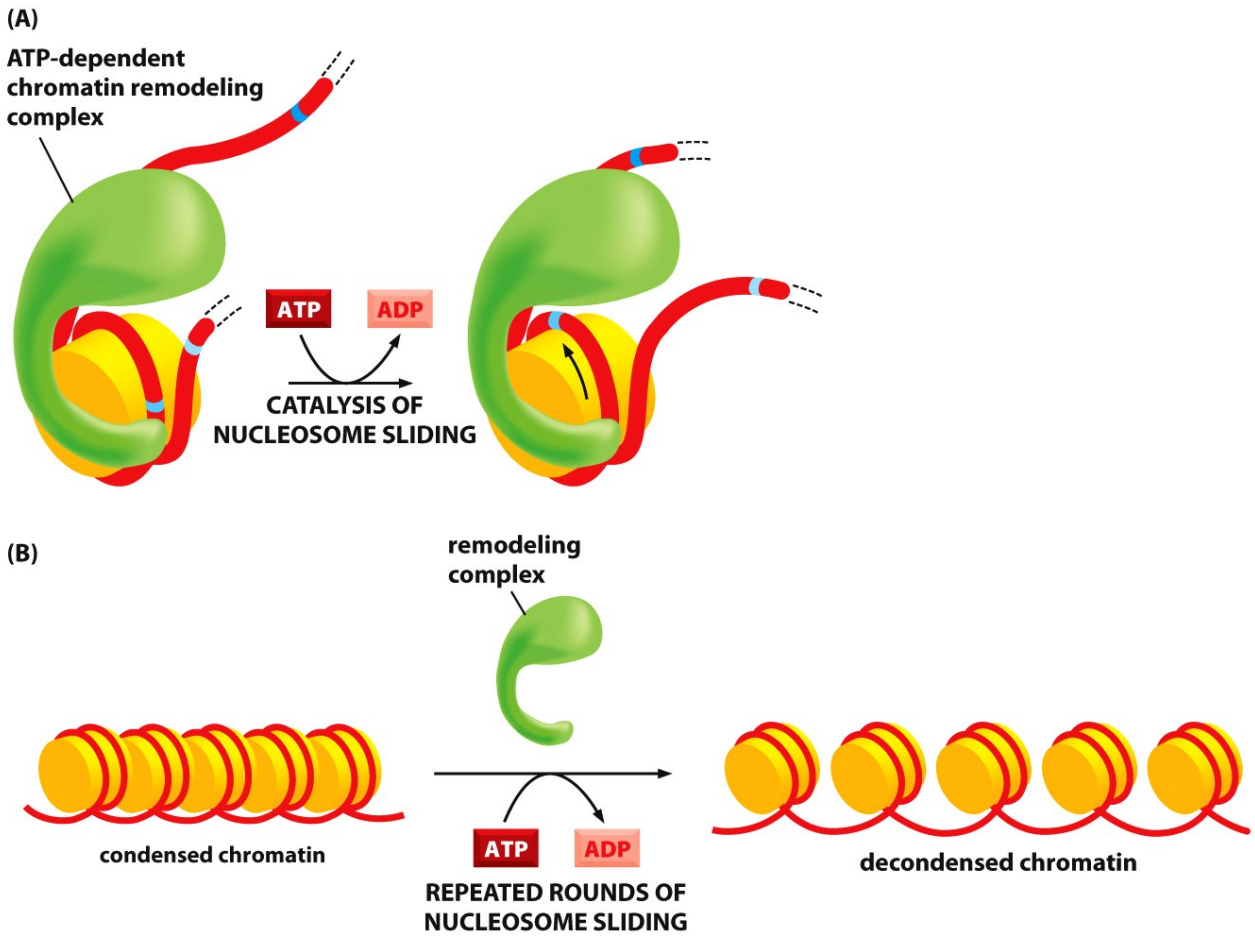


Figure 5-27 Essential Cell Biology 3/e (© Garland Science 2010)

Heterochromatin and euchromatin

- Folded DNA with condensed nucleosomes are restricted from being expressed
- This DNA is a main component of heterochromatin
- Expression will decrease even if gene is too close to heterochromatin part
- Non-condensed DNA is euchromatin

DNA inversion near heterochromatin

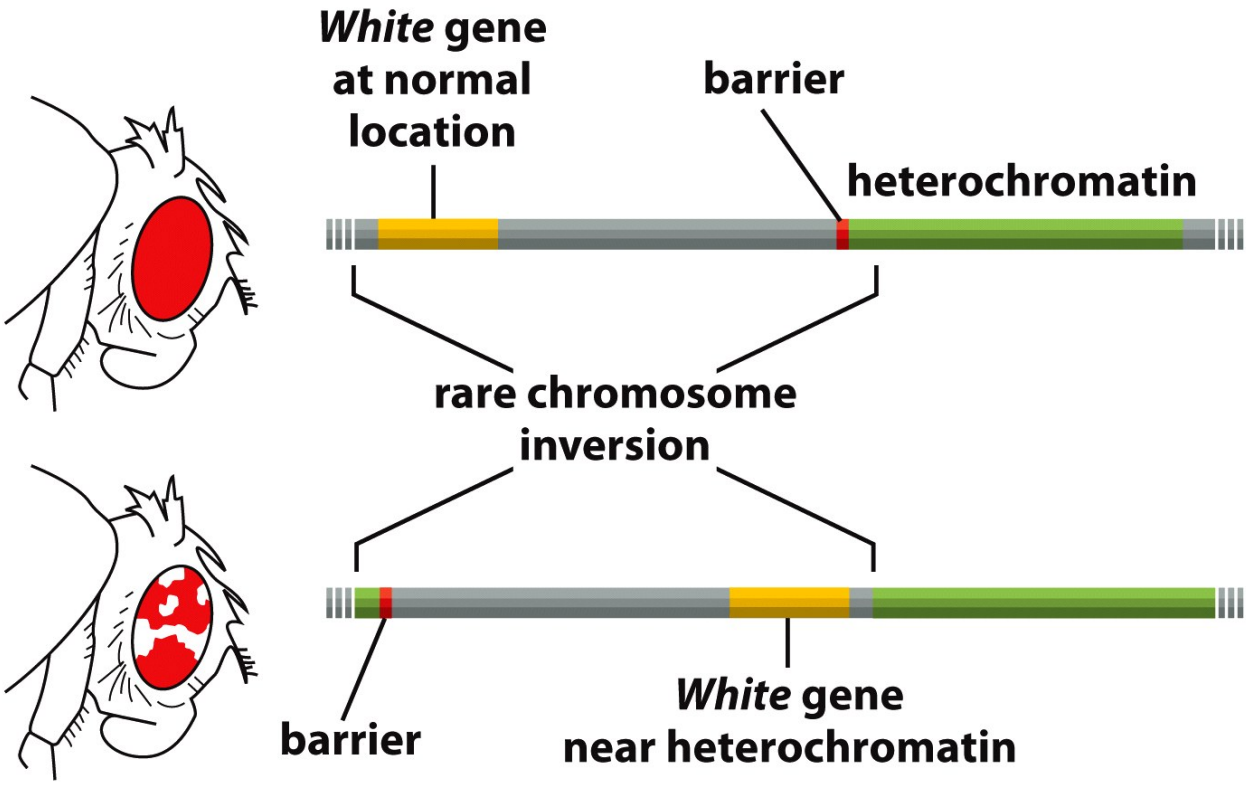
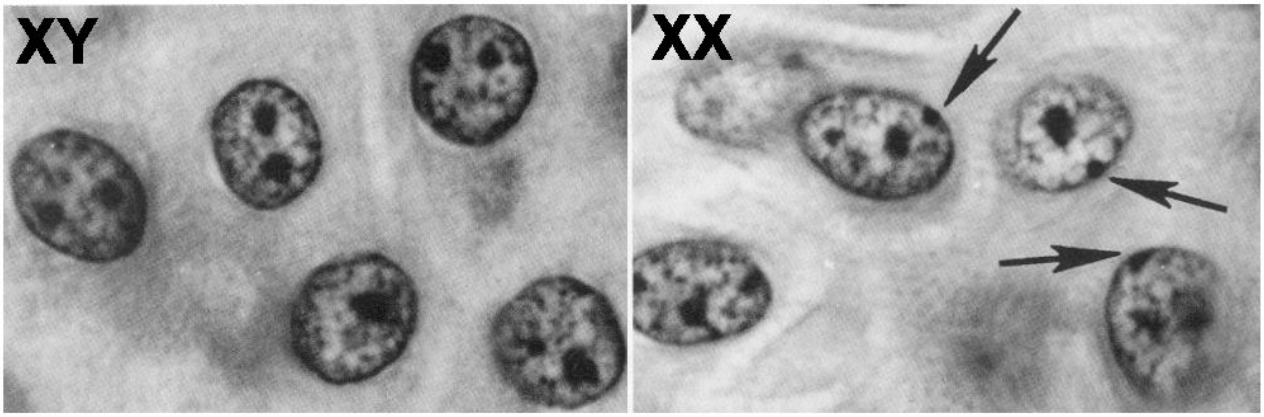


Figure 5-29 Essential Cell Biology 3/e (© Garland Science 2010)

Inactivation of X chromosome

- One of two female X chromosomes in mammals should be deactivated
- It is normally deactivated via DNA silencing with DNA folding (making heterochromatin)
- During the development, every cell lineage will inherit the pattern of deactivation (which X chromosome, #1 or #2 is deactivated)
- In human cells, deactivated X chromosomes are visible as Barr bodies—this is the way of cytological determination of sex

Barr bodies



Final question (1 point)

What is the transformation of bacteria?

Summary

- DNA in cells often have two states: unpacked (interphase DNA, "interphase chromosomes") and packed (chromosomes)
- Histones are proteins responsible for first two levels of chromosome packing

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 5*.

Outline

Questions and answers

Previous final question: the answer

What is the transformation of bacteria?

- Lateral/horizontal gene transfer (LGT/HGT) from bacterium to bacterium.

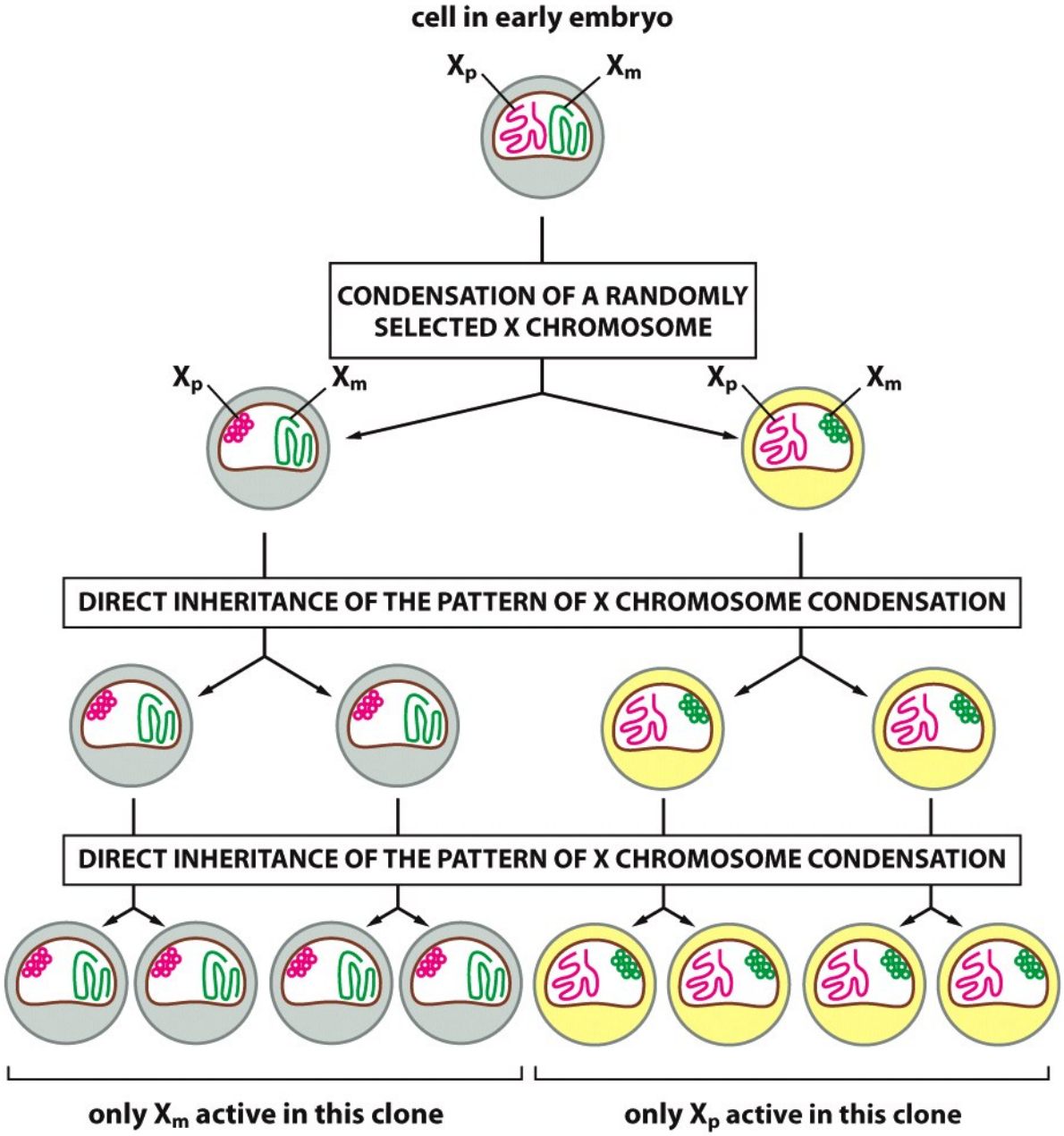
DNA

.1 DNA and chromosomes

Inactivation of X chromosome

- One of two female X chromosomes in mammals should be deactivated
- It is normally deactivated via DNA silencing with DNA folding (making heterochromatin)
- During the development, every cell lineage will inherit the pattern of deactivation (which X chromosome, #1 or #2 is deactivated)
- In human cells, deactivated X chromosomes are visible as Barr bodies—this is the way of cytological determination of sex

Inactivation of X chromosome



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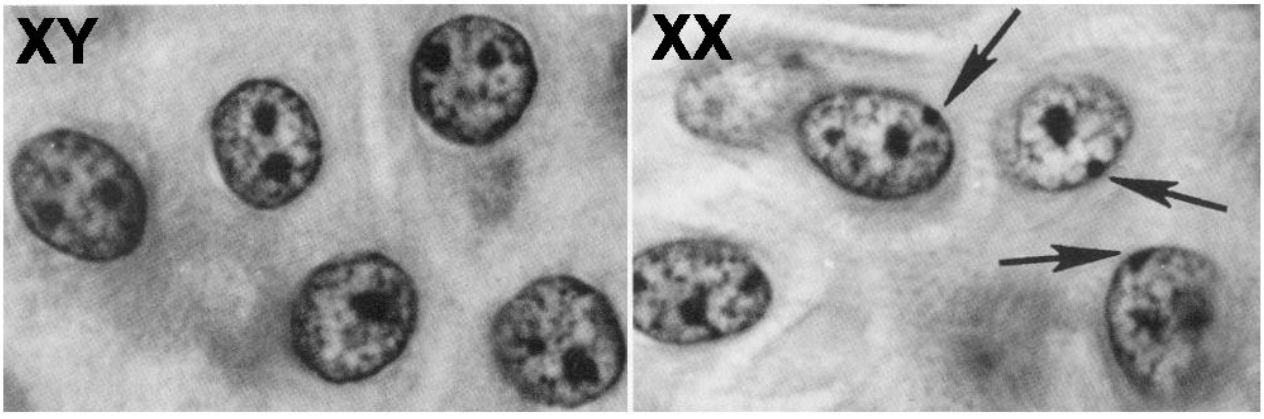
Barr bodies

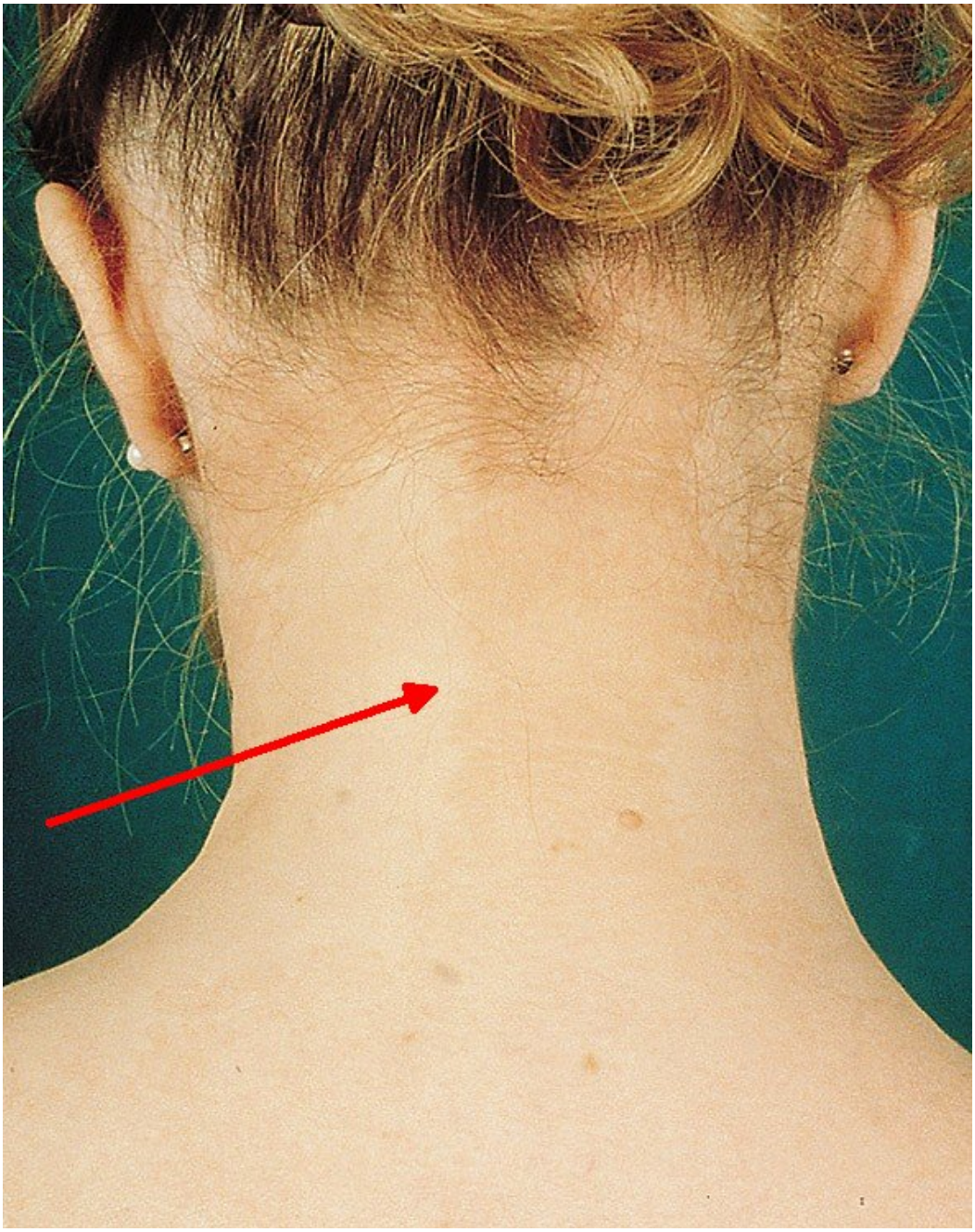


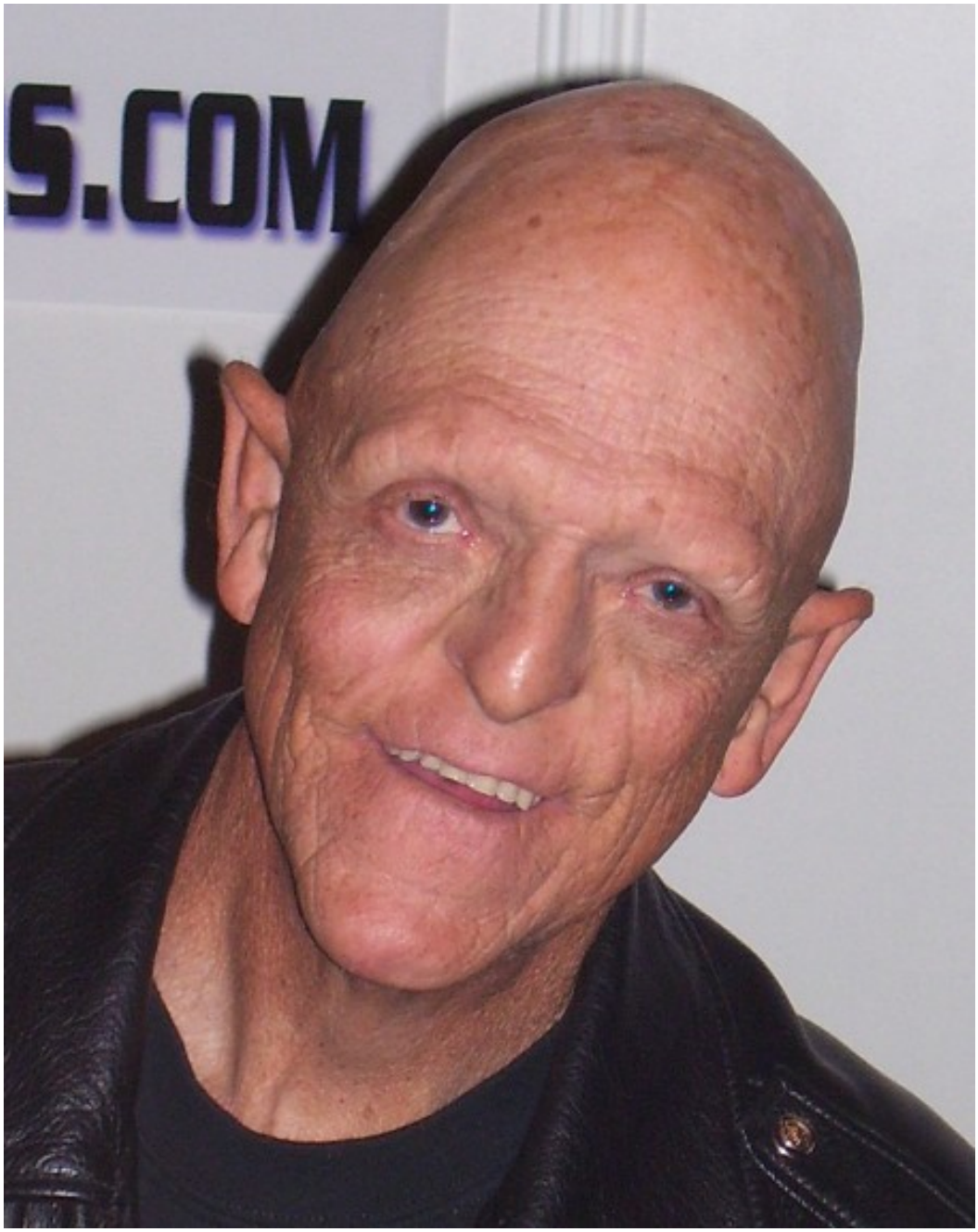
Tri-colored (tortoiseshell and calico) cats

- Cats have three alleles of same gene which correspond with color, and the gene is located in X chromosomes
- X^o will produce white color, homozygote X^OX^O produce white with orange spots, X^BX^B—white and black. But X^OX^B will be tricolor cats, and a size of spots will depend on the time of X chromosome inactivation
- Males have genotype $X^?Y$ and therefore cannot be tricolored (with an exception of chromosome aberrant XXY Klinefelter's syndrome etc.)

Calico cat (left) with early and tortoiseshell cat (right) with late X chromosome inactivation

Human hypohidrotic ectodermal dysplasia has the same pattern of inheritance





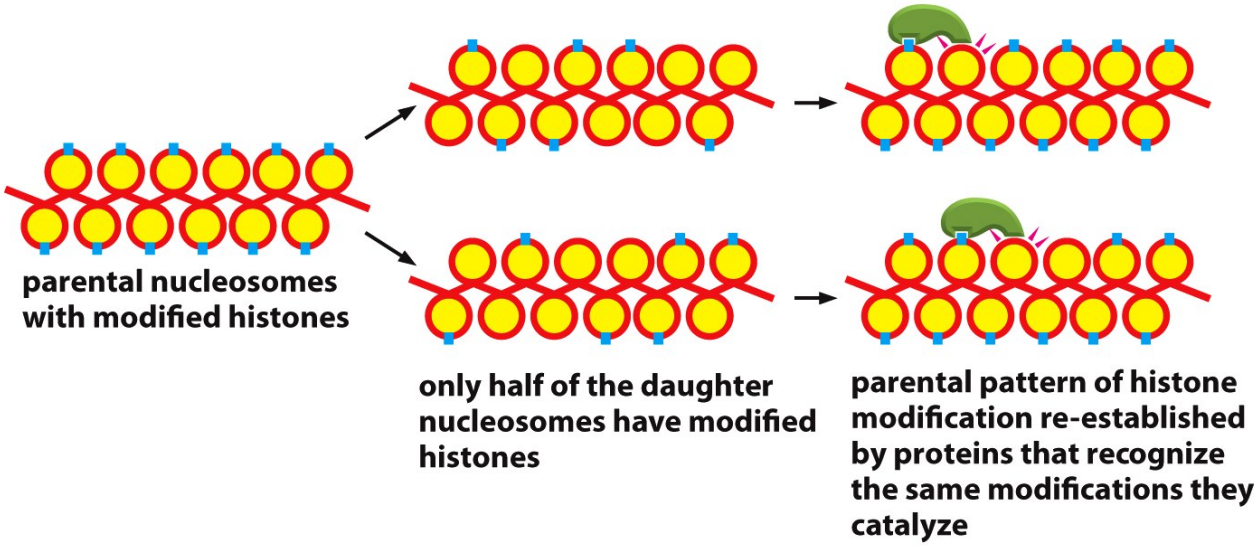
Histone modification

- Histones' amino acids may bind methyl (1–3 groups per lysine), phosphate, acetyl or other groups
- Every modification will have its own "meaning" and will allow or restrict the work with specific DNA sequence

Epigenetic inheritance

- Modifications of histones may be inherited
- This is because modified old histones have templating ability for newly attached histones
- Inherited histone modification and other similar processes are called epigenetic inheritance

One of ways of epigenetic inheritance



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From DNA to RNA

.1 Prokaryotic transcription

Basic dogma of molecular biology

- DNA $\xrightarrow{\text{transcription}}$ RNA $\xrightarrow{\text{translation}}$ Protein
- However, reverse transcriptase does DNA $\xleftarrow{\text{reverse transcription}}$ RNA

Transcription basics

- RNA polymerase(s) copies the chunk of one DNA strands into RNA
- Gene expression depends on intensity of this process (which is generally much faster than DNA synthesis)

Transcription in prokaryotes

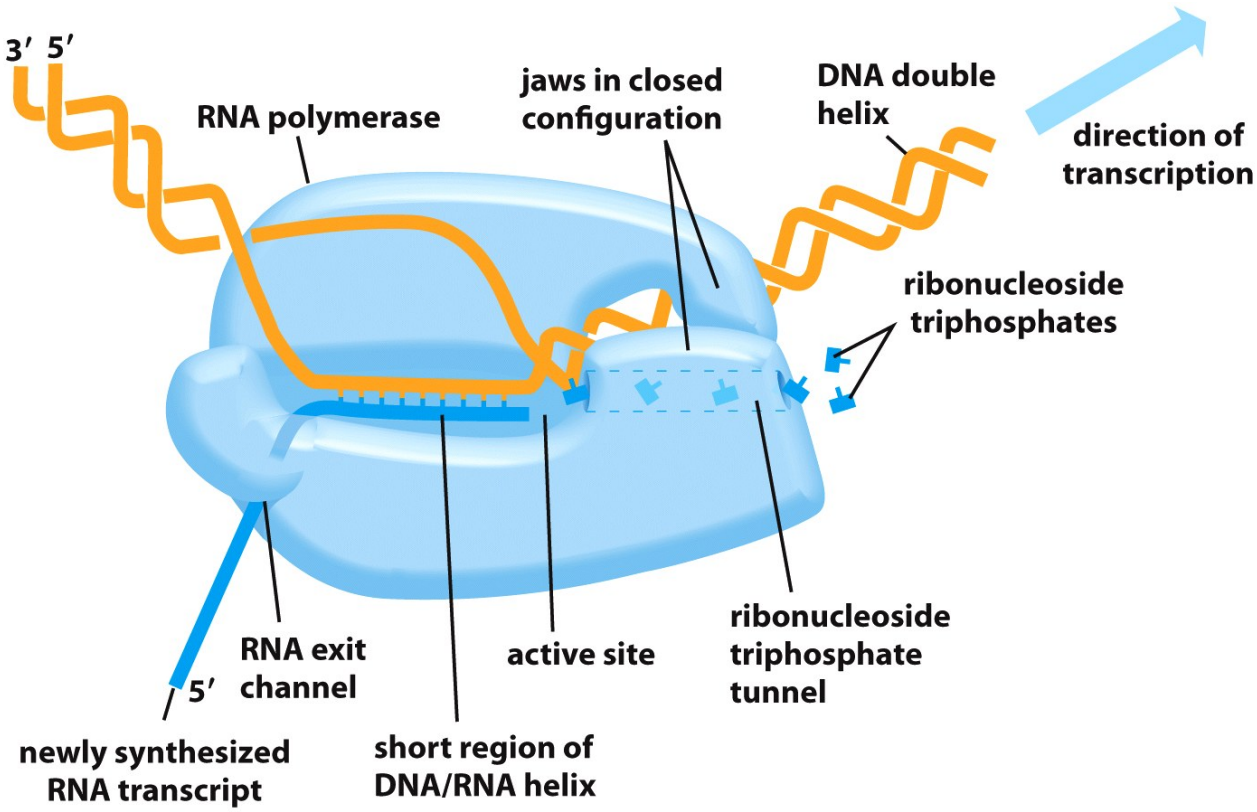


Figure 7-7 Essential Cell Biology 3/e (© Garland Science 2010)

Transcription movie

Types of RNA

- mRNA
- rRNA
- tRNA
- miRNA

RNAs

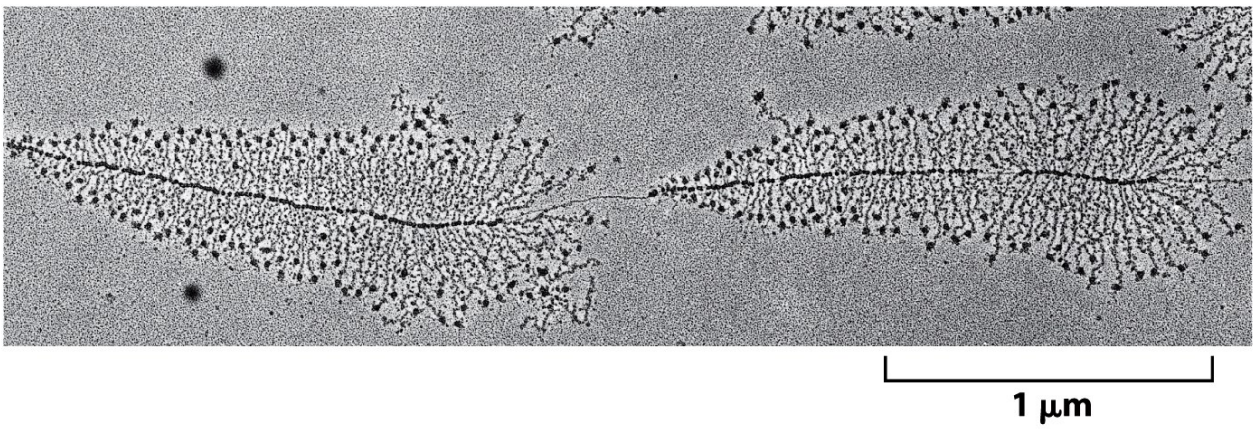


Figure 7-8 Essential Cell Biology 3/e (© Garland Science 2010)

Template and coding strands

- RNA polymerase works with template strands
- RNA is synthesized in 5'→3' direction
- Resulted RNA will copy the other, coding strand

Promoter and terminator

- Promoter will specify the binding place for RNA polymerase
- Terminator specifies unbinding place
- σ factor of RNA polymerase recognizes promoter

Promoter and terminator

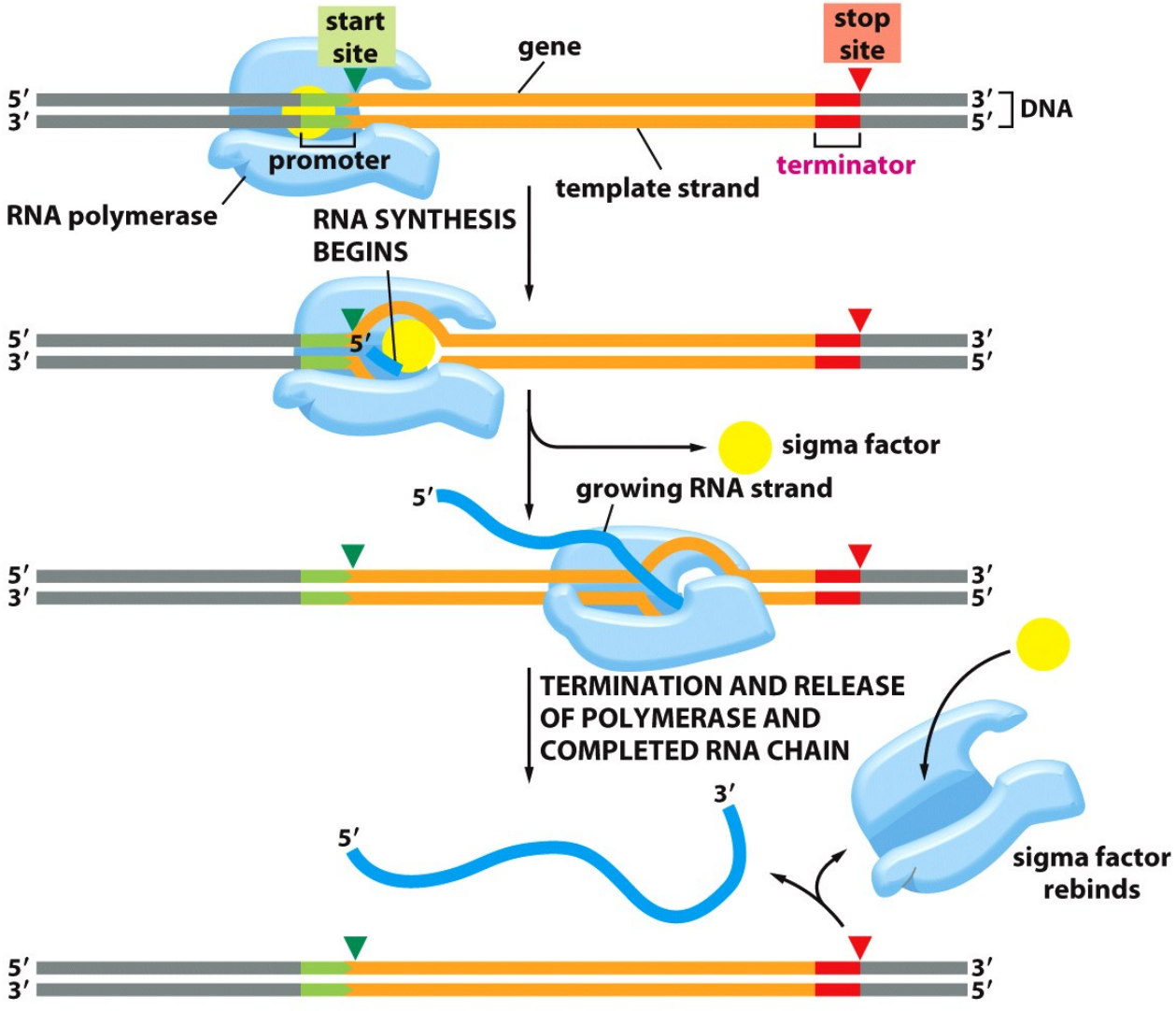


Figure 7-9 Essential Cell Biology 3/e (© Garland Science 2010)

Promoter and terminator sequences

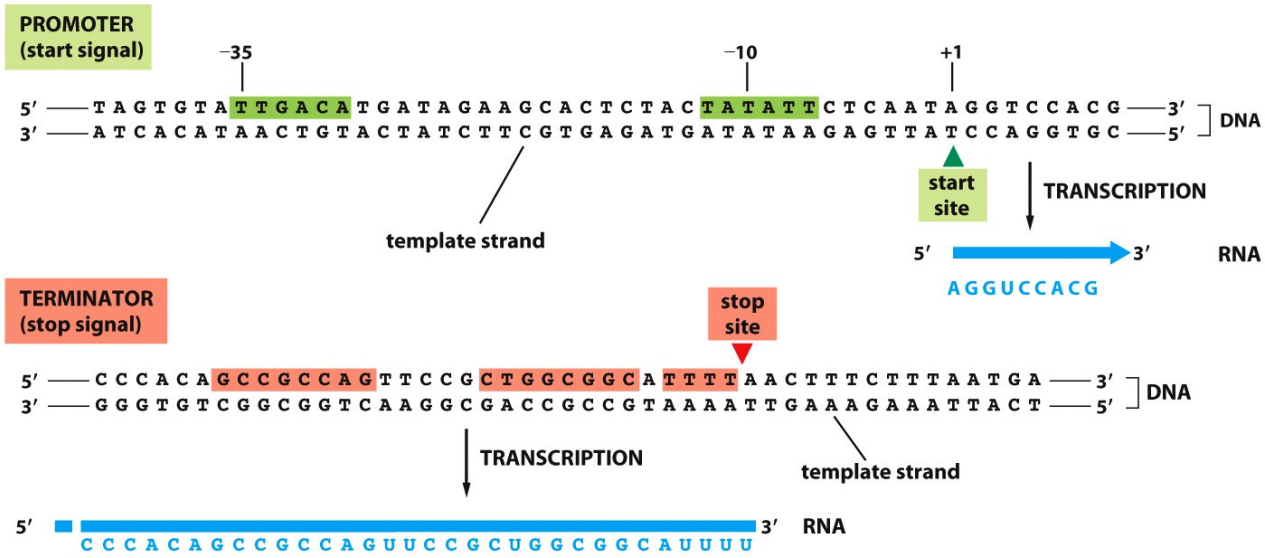


Figure 7-10 Essential Cell Biology 3/e (© Garland Science 2010)

Direction of transcription

- Promoter is asymmetric, therefore RNA polymerase may proceed in only one direction (= work with only one strand)
- Both strands may be templates for different genes

Directions of transcription

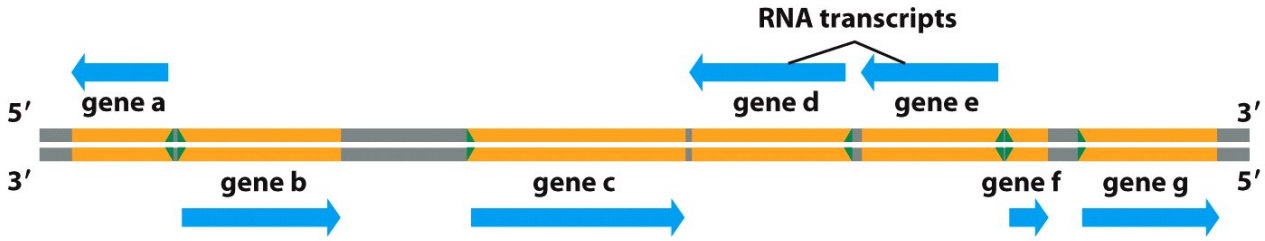


Figure 7-11 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (2 points)

How does RNA polymerase recognizes the "proper" strand of DNA?

Summary

- Chromatin deactivation may be inherited in epigenetic way
- Asymmetric promoters determine which DNA strand will be template and in with direction transcription will go

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 7*.

Outline

Questions and answers

Previous final question: the answer

How RNA polymerase recognizes the "proper" strand of DNA?

- It uses asymmetric nature of promoters

From DNA to RNA

.1 Eukaryotic transcription

Eukaryotic transcription: differences

- Multiple polymerases: I (rRNA genes), II (tRNA genes, 5S rRNA gene) and III (other genes)
- General transcription factors
- DNA is much bigger and more compactized

Eukaryotic RNA polymerase II movie

Transcription factors

- TFIID recognizes TATA box
- TFIIB binds to it as well
- TFIIE, TFIIF and especially TFIIH will help RNA polymerase II to start transcription

Transcription factors movie

Transcription factors (1)

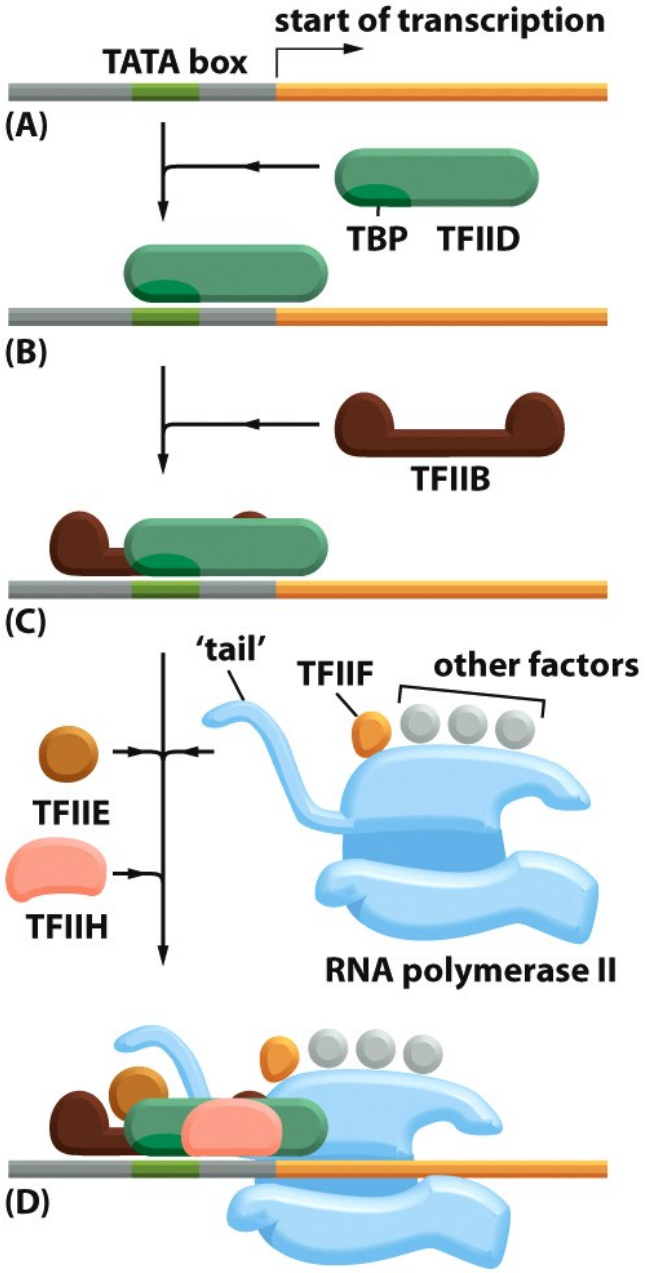


Figure 7-12 part 1 of 2 Essential Cell Biology 3/e (© Garland Science 2010)

Transcription factors (2)

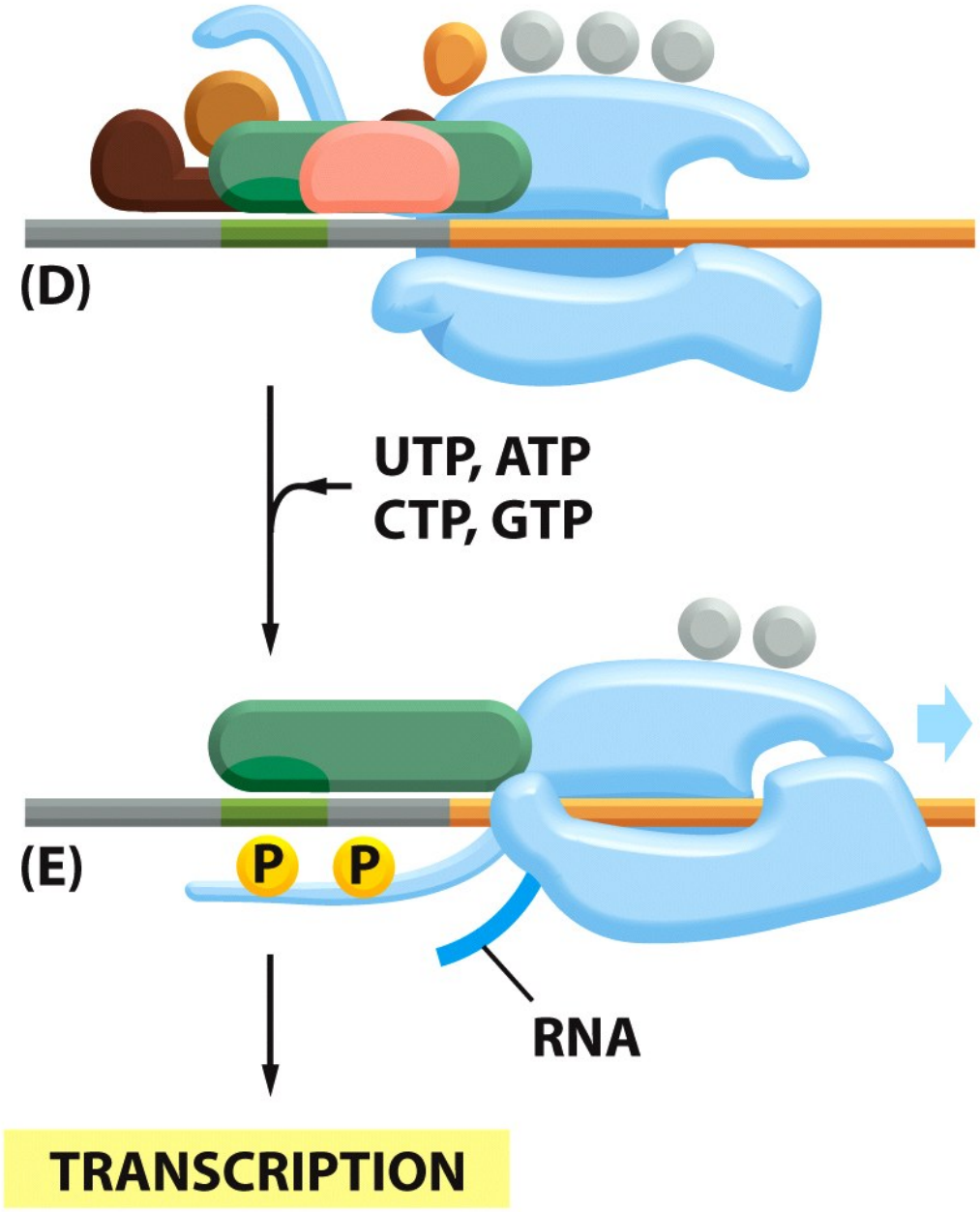


Figure 7-12 part 2 of 2 Essential Cell Biology 3/e (© Garland Science 2010)

.2 RNA processing

RNA processing

- RNA capping: adds methylated G to 5' end of RNA (occurs before transcription completes)
- RNA polyadenilation: adds poly-A tail to 3' end of mRNA
- Increase stability, make mRNA recognizable from other RNAs

RNA processing

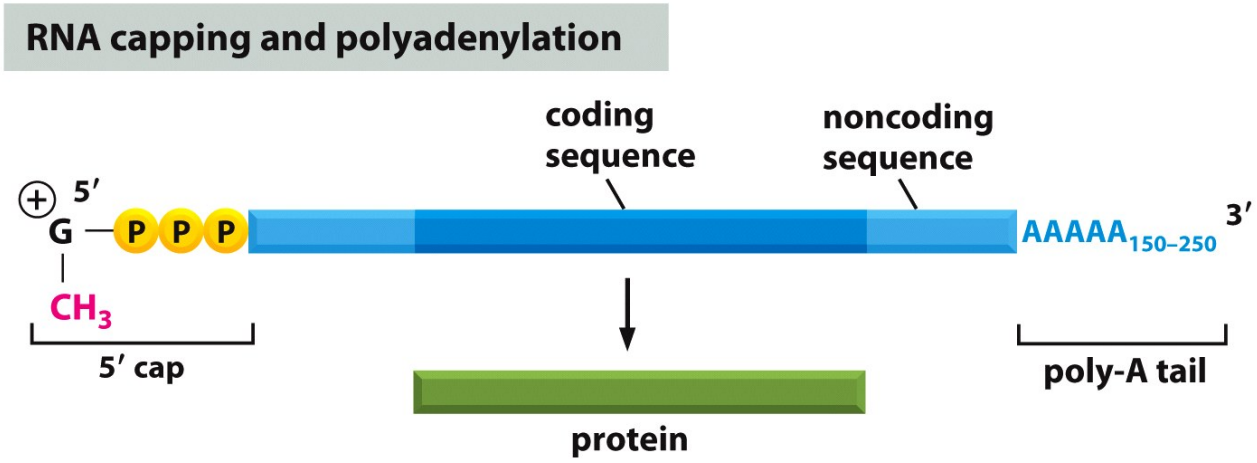


Figure 7-16a Essential Cell Biology 3/e (© Garland Science 2010)

.3 RNA splicing

Introns and exons

- Non-coding sequences are introns (vary from 1 to 10,000 bp)
- Other are exons

Introns and exons

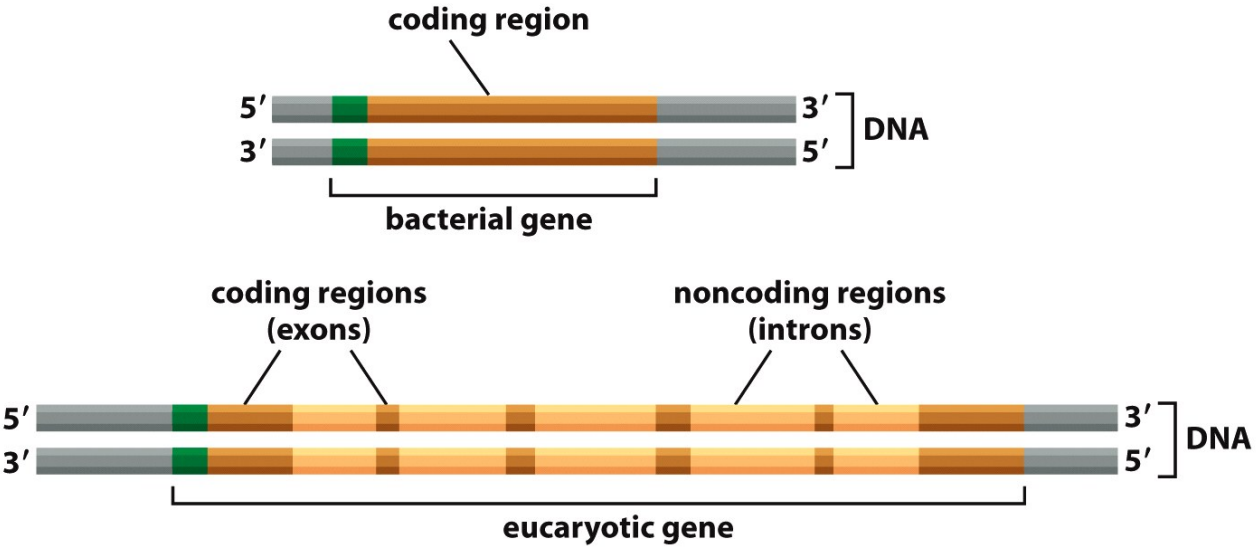


Figure 7-17 Essential Cell Biology 3/e (© Garland Science 2010)

RNA splicing

- Introns should be removed from RNA: this is splicing
- RNA-protein complexes snRPNS ("snurps") recognize the starts and ends of introns
- Snuprs are core part of spliceosome
- Introns form lariat structures when spliced

RNA splicing

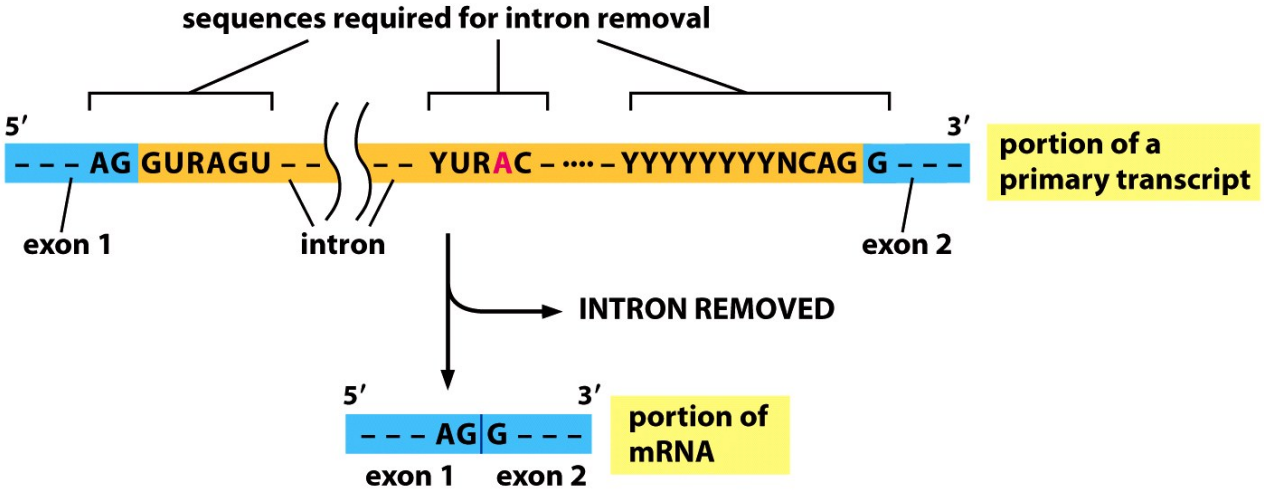


Figure 7-19 Essential Cell Biology 3/e (© Garland Science 2010)

RNA splicing movie

Alternative splicing

- One RNA may be spliced differently
- Every single result of splicing will be the different protein
- This is one more level of "epigenetic freedom"

Alternative splicing

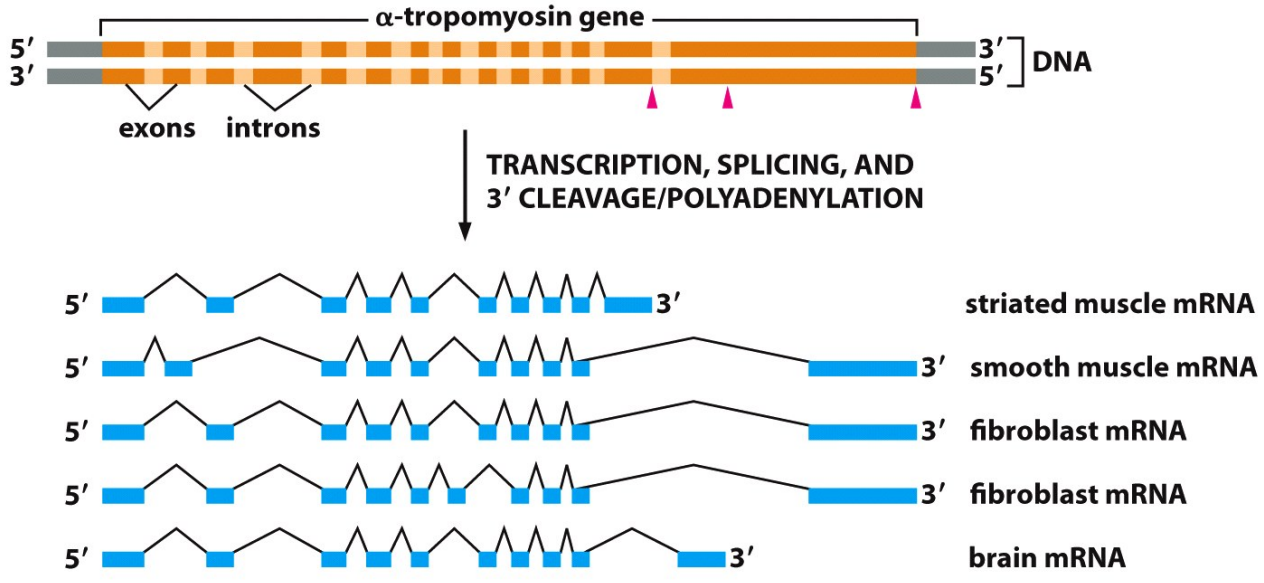


Figure 7-21 Essential Cell Biology 3/e (© Garland Science 2010)

Origin of introns

- Introns will increase the flexibility of genome, but lower the speed of cell replication
- It is therefore possible that prokaryotes are secondary intronless

- Introns were found in some Archaea

.4 RNA export

Selective export of RNA

- Nuclear pore will allow only "ready" mRNA to be exported into cytoplasm
- That will not allow the unprocessed RNA to be translated into protein

RNA export from nucleus

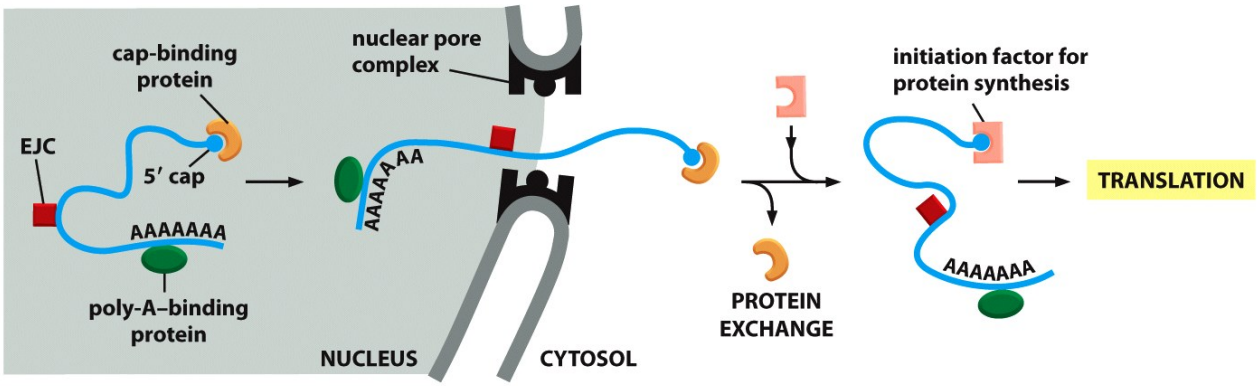


Figure 7-22 Essential Cell Biology 3/e (© Garland Science 2010)

Degrading of RNA

- Bacterial mRNAs live ≈3 min
- In eukaryotes, mRNA lives longer, and the lifespan depends on how RNA was processed

Transcription: eukaryotes vs. prokaryotes

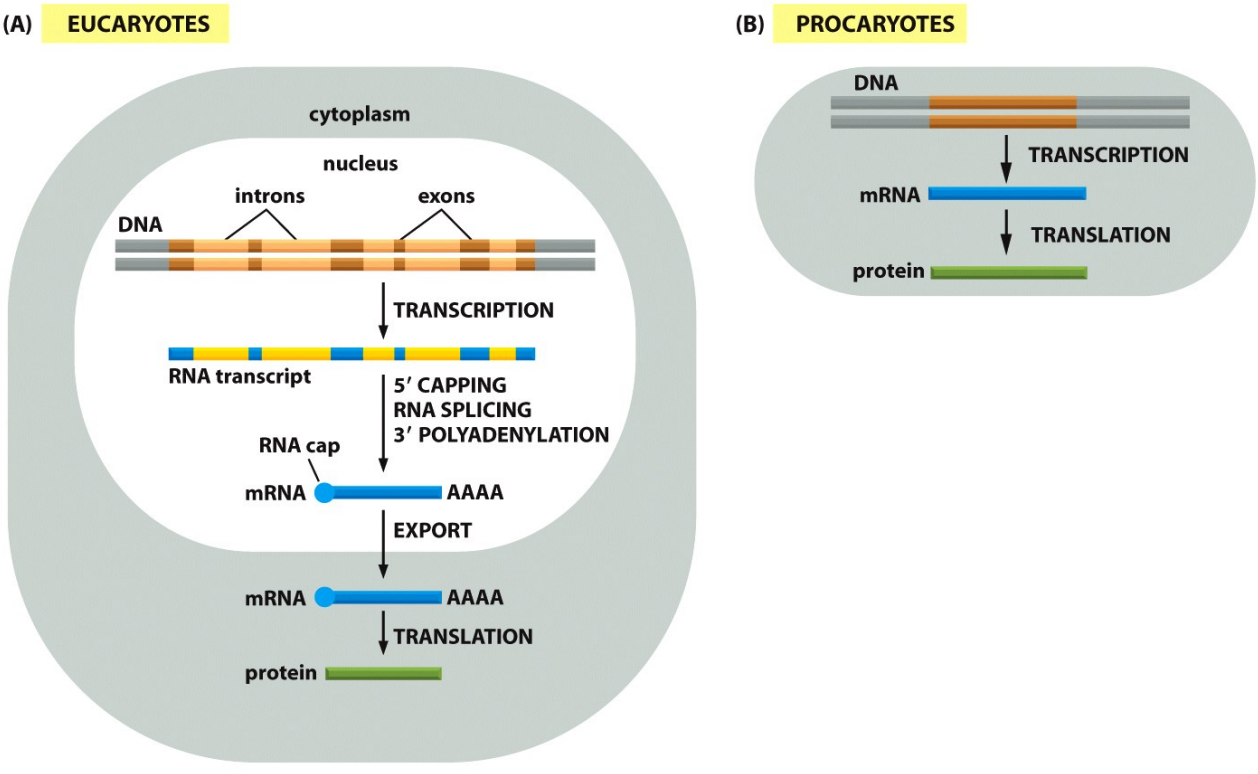


Figure 7-23 Essential Cell Biology 3/e (© Garland Science 2010)

Questions?

.5 Ribosomes

Ribosome

- Prokaryotes: 70S ribosomes
  - Small subunit: 30S
    - 16S RNA
    - 21 proteins
  - Large subunit: 50S
    - 5S RNA + 23S RNA
    - 34 proteins
- Eukaryotes: 80S ribosomes
  - Small subunit: 40S
    - 18S RNA
    - 33 proteins
  - Large subunit: 60S
    - 5S RNA + 28S RNA + 5.8S RNA
    - 49 proteins

Structure of eukaryotic ribosome

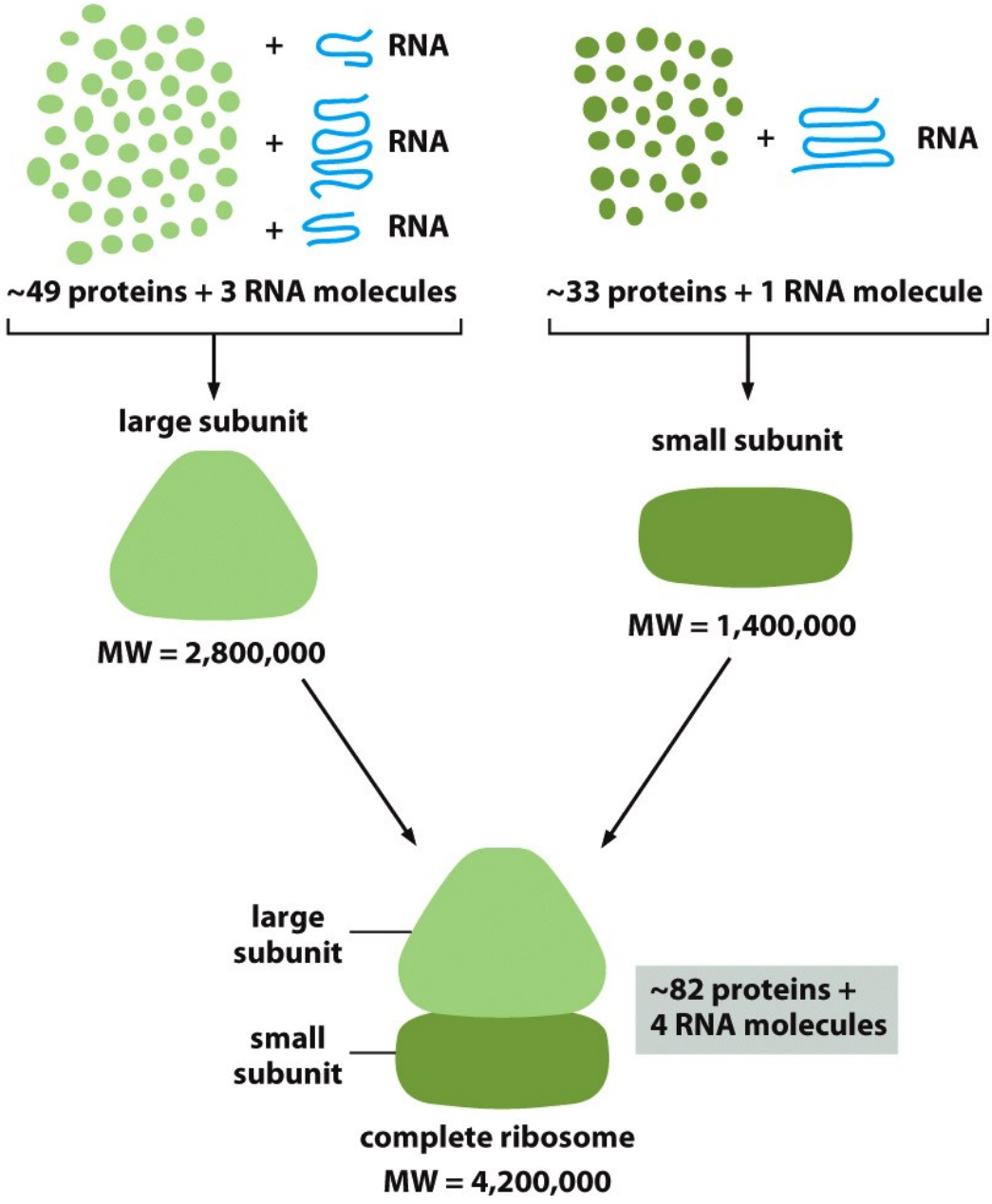


Figure 7-31 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (2 points)

Name at least one most important difference between prokaryotic and eukaryotic transcription

Summary

- Transcription processes are seriously different between prokaryotes and eukaryotes
- Alternative splicing is one of sources of "epigenetic freedom"

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 7*.

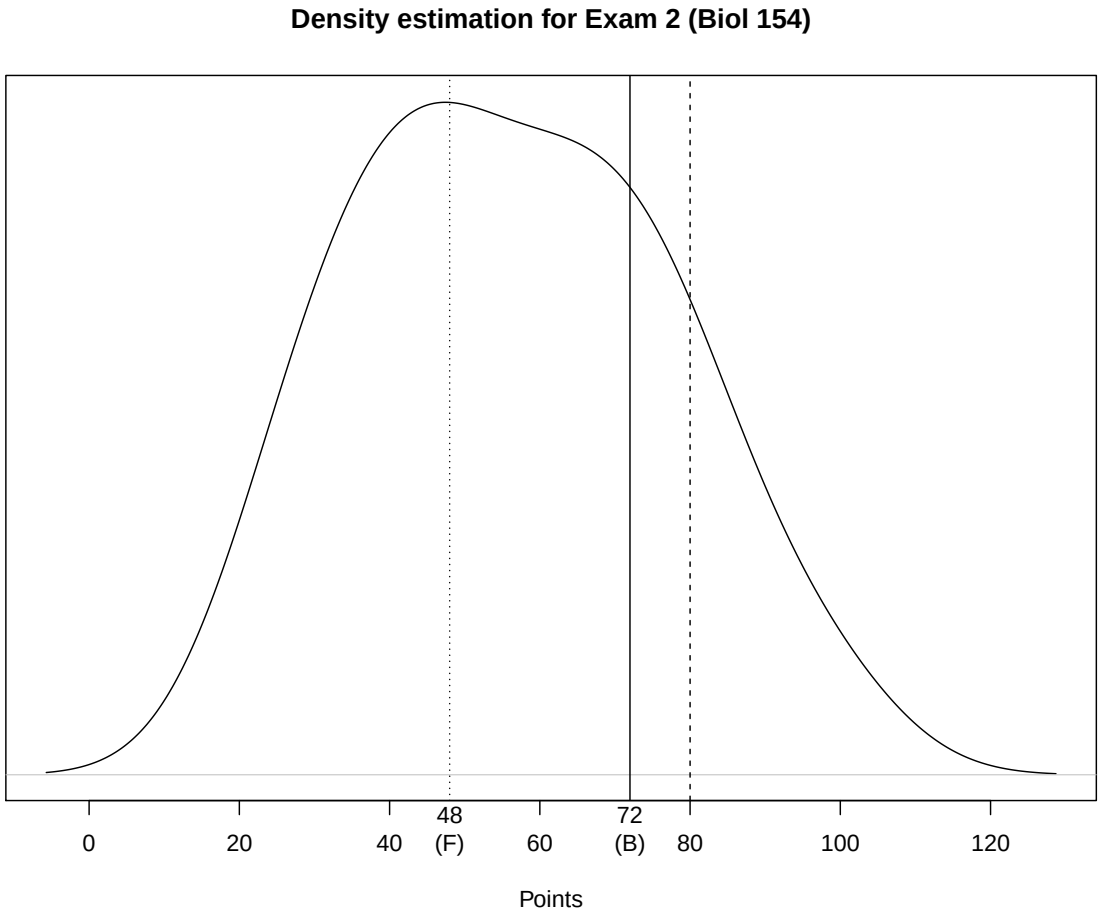
Outline

Questions and answers

Results of Exam 2: statistic summary

```
Summary:
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  26.00   39.00   54.00   56.41   69.00   97.00
Grades:
  F   D   C   B max
 48  56  64  72  80
```

Results of Exam 2: the curve



Question 33

- Are introns always spliced out of mRNA?
    1. Yes
    2. **No**

Previous final question: the answer

Name at least one most important difference between prokaryotic and eukaryotic transcription

- Multiple transcription factors
- TATA box
- Separation of transcription and translation by nuclear envelope (RNA export)
- RNA processing and splicing

From RNA to protein: translation

.1 Genetic code

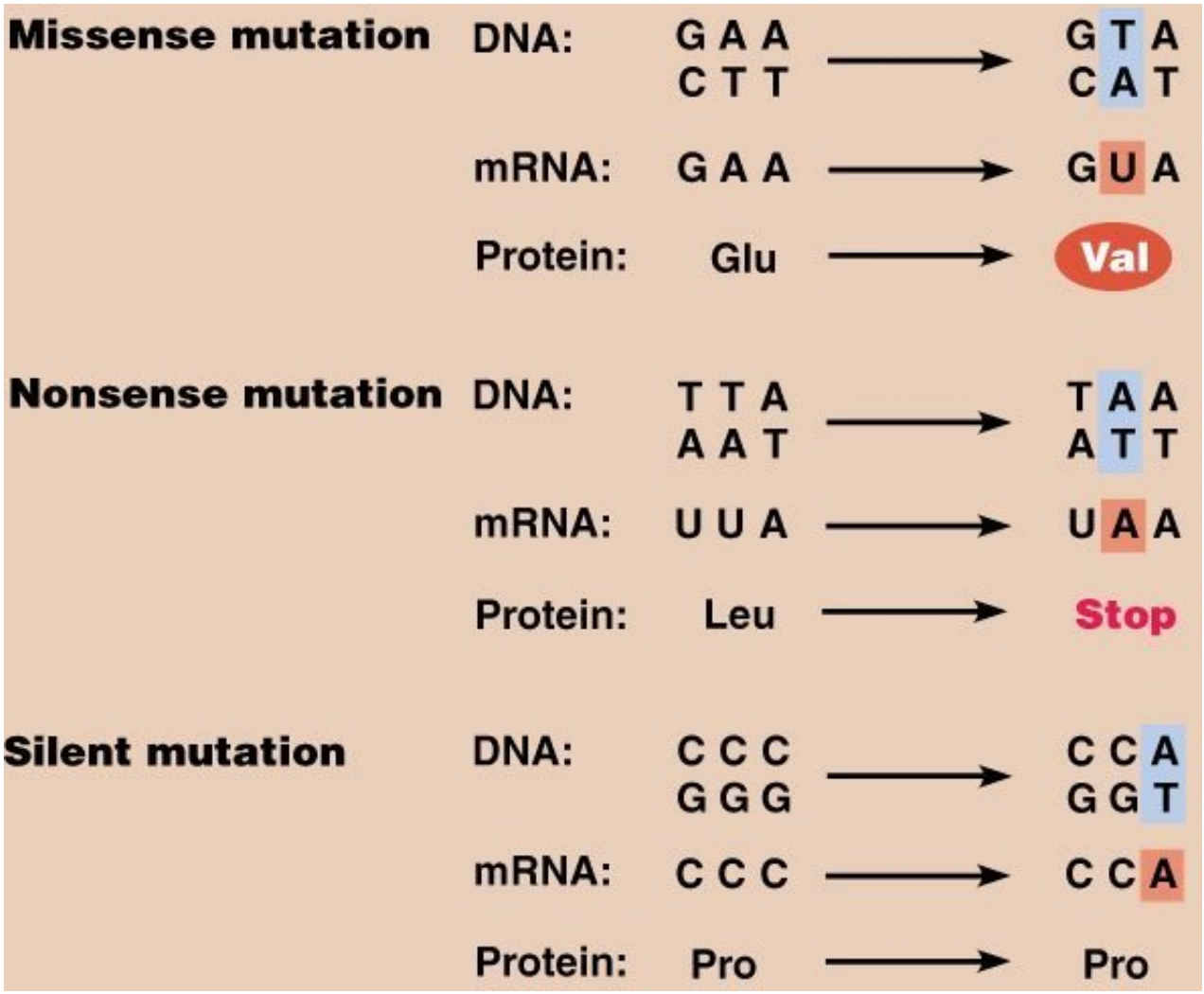
Redundance of genetic code

- Translation is decoding of codons into amino acids
- Four nucleotides may encode 64 amino acids
- As a result, the 3rd position may vary without change of amino acid

Codons vs. amino acids

GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

Code and mutations



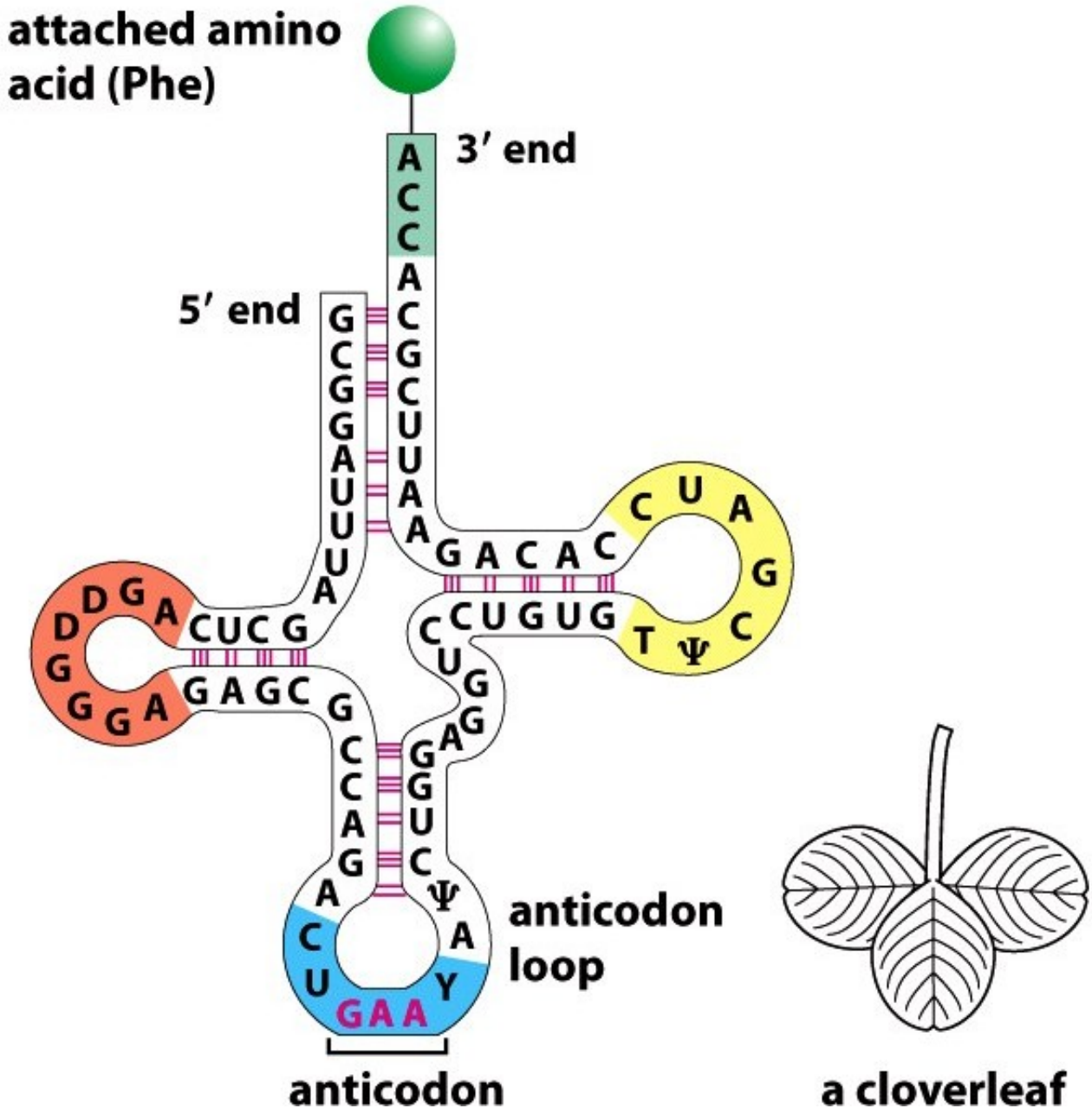
Decifering the code

- First, artificial translation systems (taken from *E. coli* RNAs and enzymes) were created
- In 1960s, Nirenberg and Leder discovered that trinucleotides may be attached to ribosome and activate the transfer RNAs
- They produced big amounts of same codons and then analyzed complexes of ribosome-trinucleotide-aminoacyl-tRNAs.

Transfer RNAs

- "Cloverleaf" of ≈80 nucleotides
- Three loops and (optionally) amino acid on 3' end (the arm, which also contains specific nucleotides)
- Top loop contain anticodon
- There are 31 kinds of tRNAs because many of them can tolerate a mismatch in 3rd position (wobble)

Cloverleaf and tRNA



Transfer RNA movie

Linkage between amino acid and tRNA

- Aminoacyl-tRNA-synthetase is specific to every kind of amino acid
- Aminoacyl-tRNA-synthetase can recognize tRNA arm and anticodon
- Bond between amino acid and tRNA is highly-energetic

amynoacyl-tRNA-synthetase

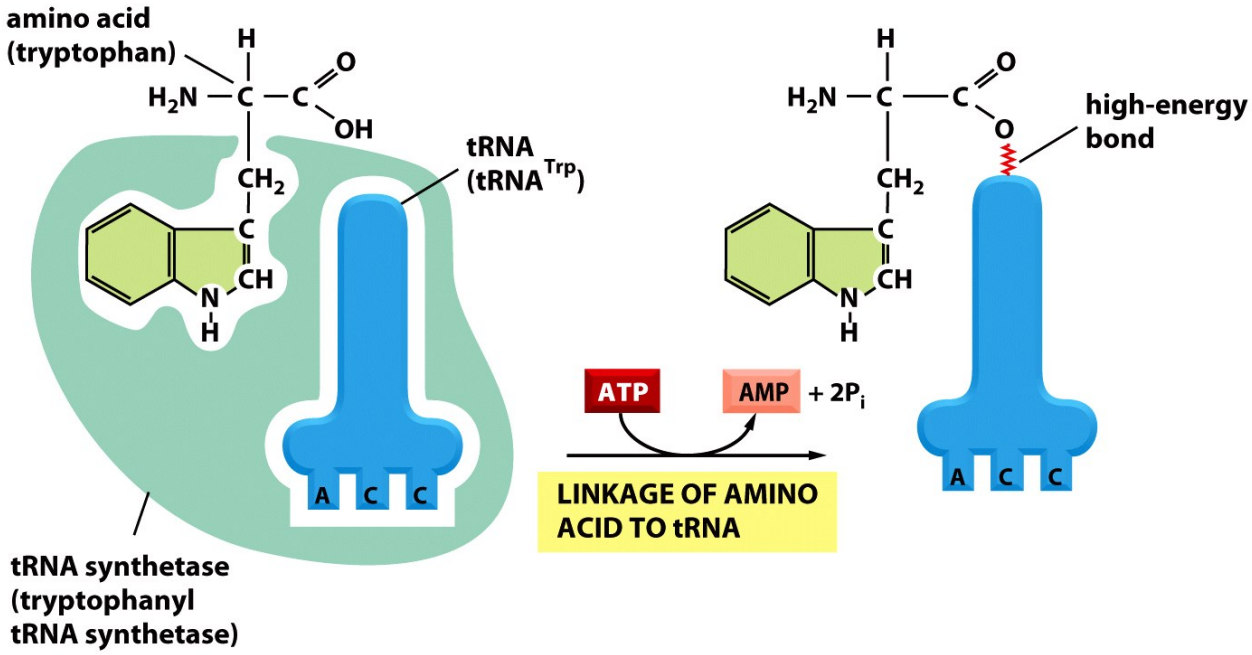


Figure 7-29 part 1 of 2 Essential Cell Biology 3/e (© Garland Science 2010)

Ribozyme

- Ribosome has three binding sites, A, P and E (for aminoacyl-tRNA, peptidyl-tRNA and exit)
- RNAs, not proteins are responsible for ribosome conformation and activity
- Ribosome is a RNA-enzyme, ribozyme

A-P-E binding sites of ribosome in work

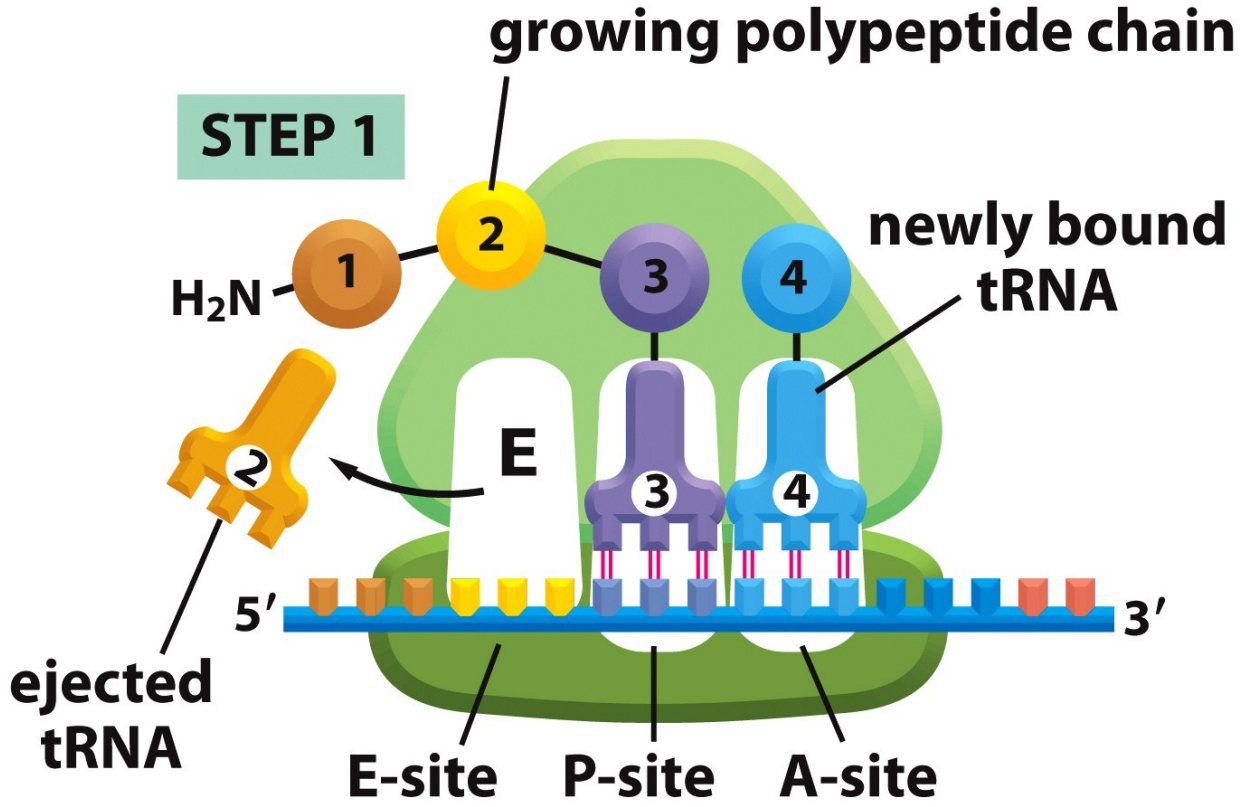


Figure 7-33 part 1 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Four steps of translation cycle

1. Binding of tRNA
2. Moving protein chain
3. Translocation of large subunit
4. Translocation of small subunit

Translation

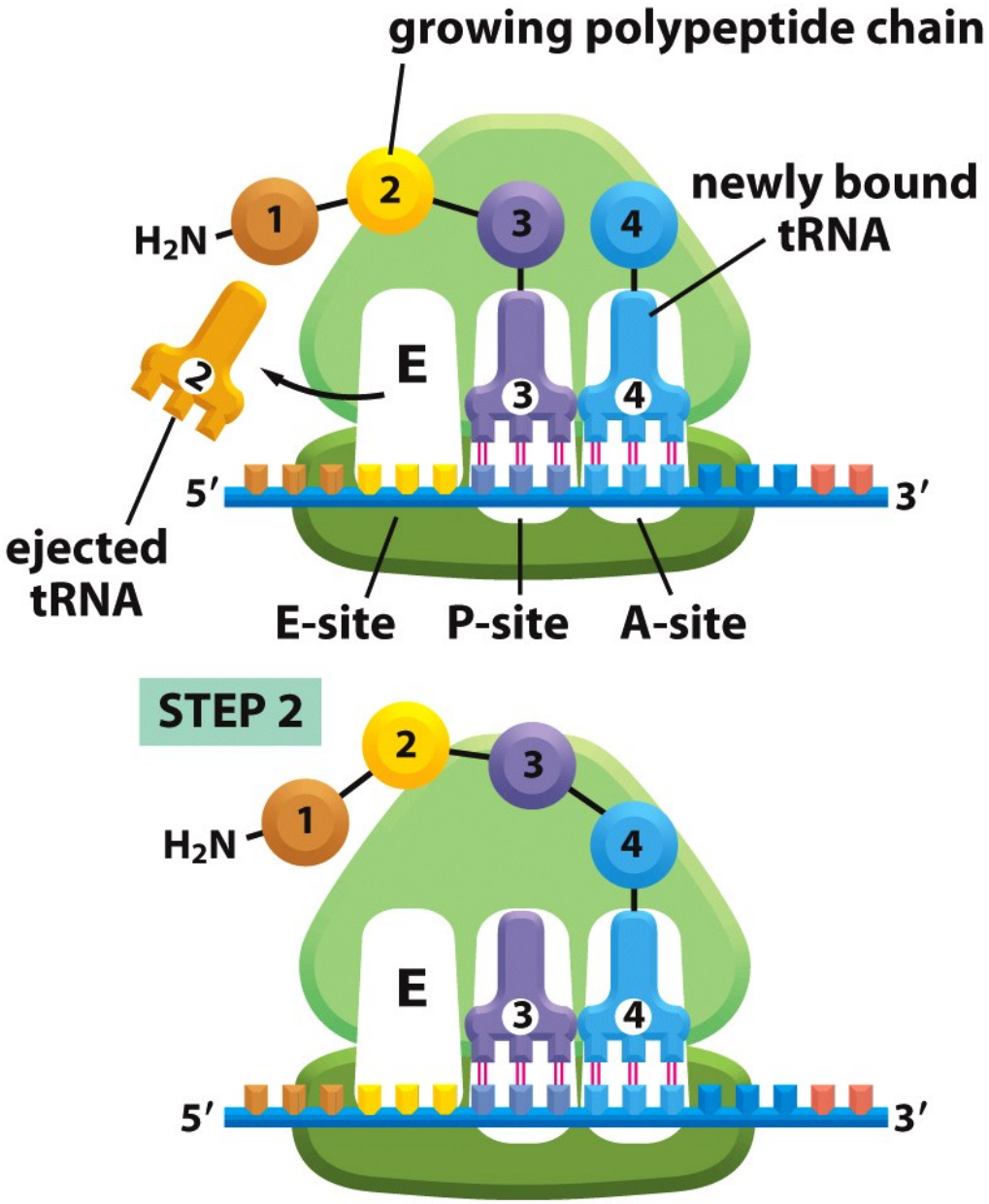
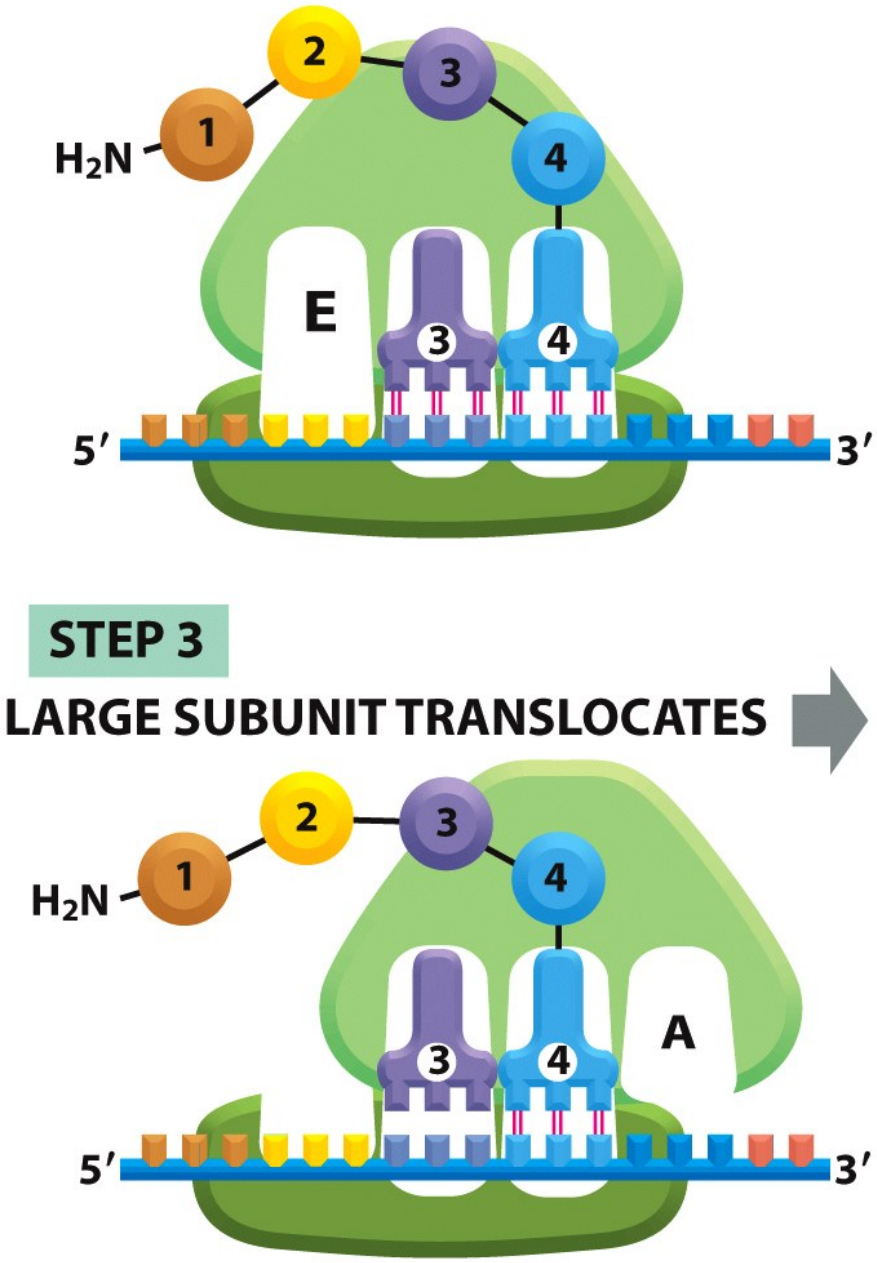


Figure 7-33 part 2 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Translation



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Translation

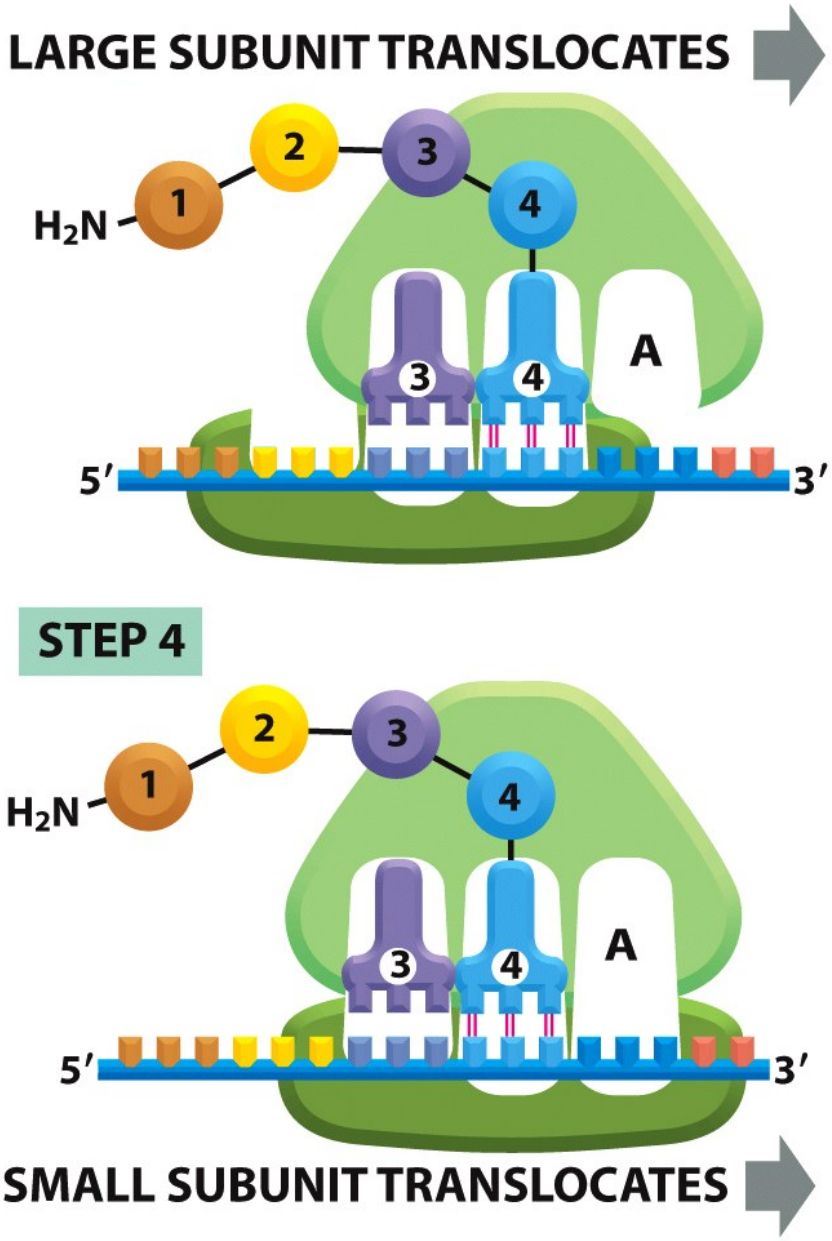


Figure 7-33 part 4 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Translation

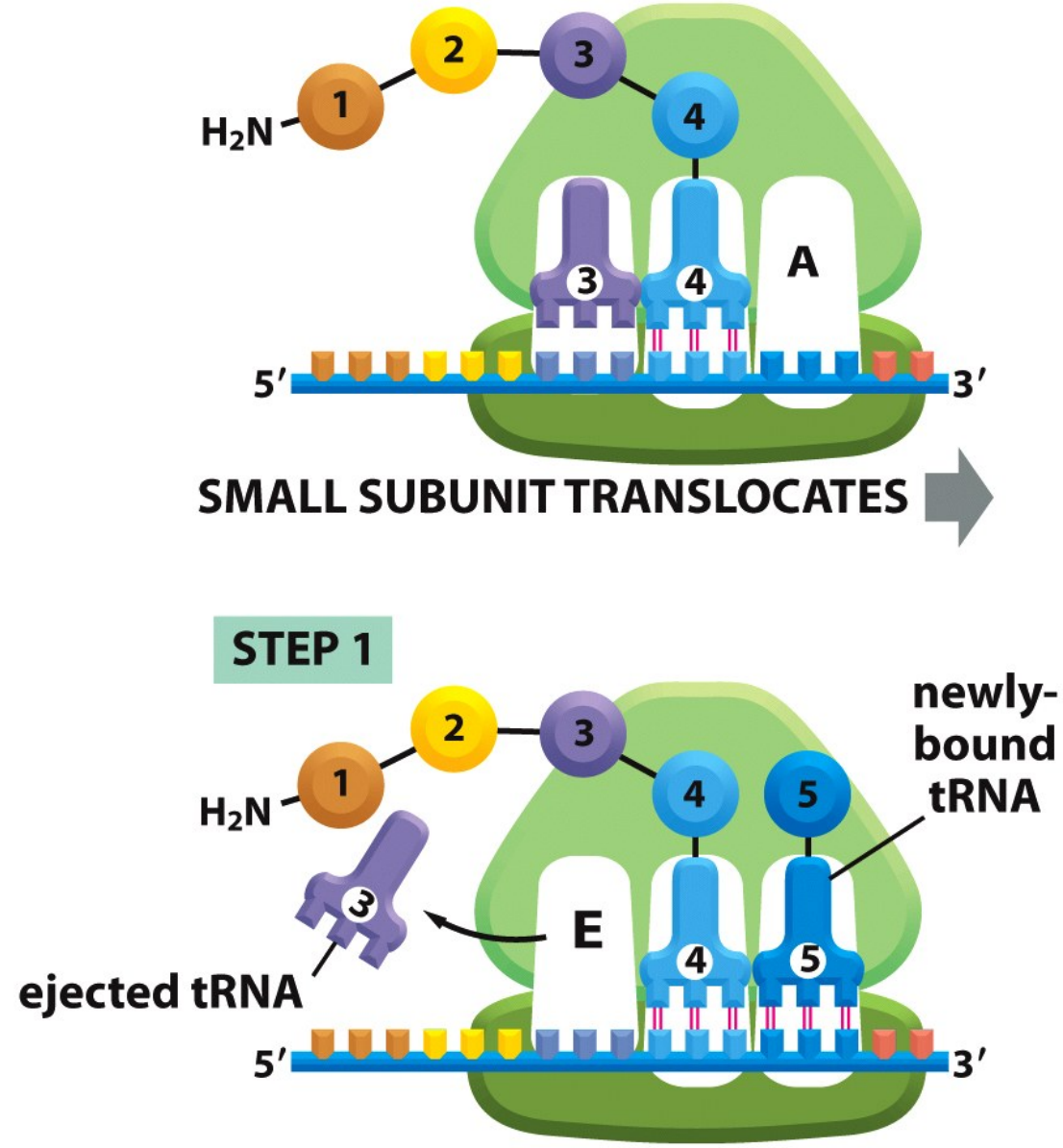


Figure 7-33 part 5 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Translation movie I

Translation movie II

Initiation of translation

- Initiator tRNA carries methionine and establishes complex with small subunit (at P site) and translation initiation factors
- This complex founds AUG start codon, releases factors and attaches large subunit

Initiation of translation

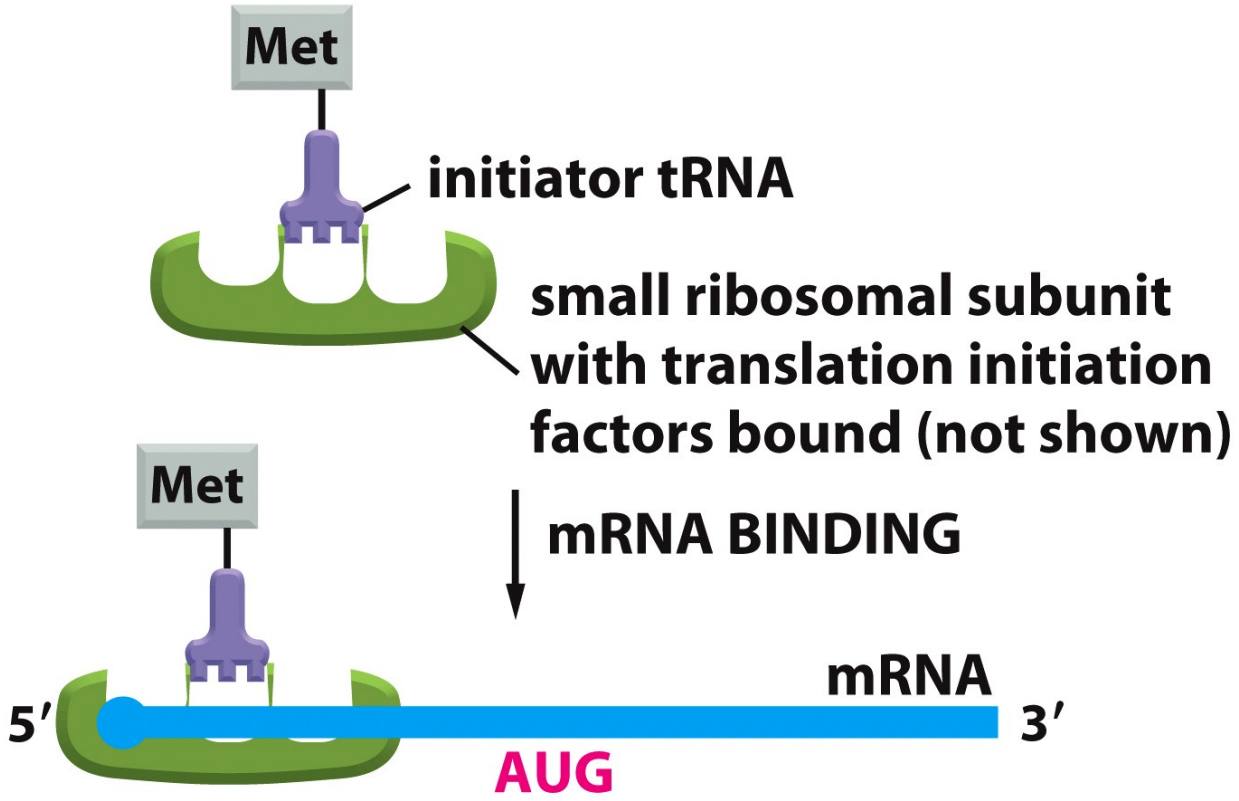


Figure 7-35 part 1 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Initiation of translation

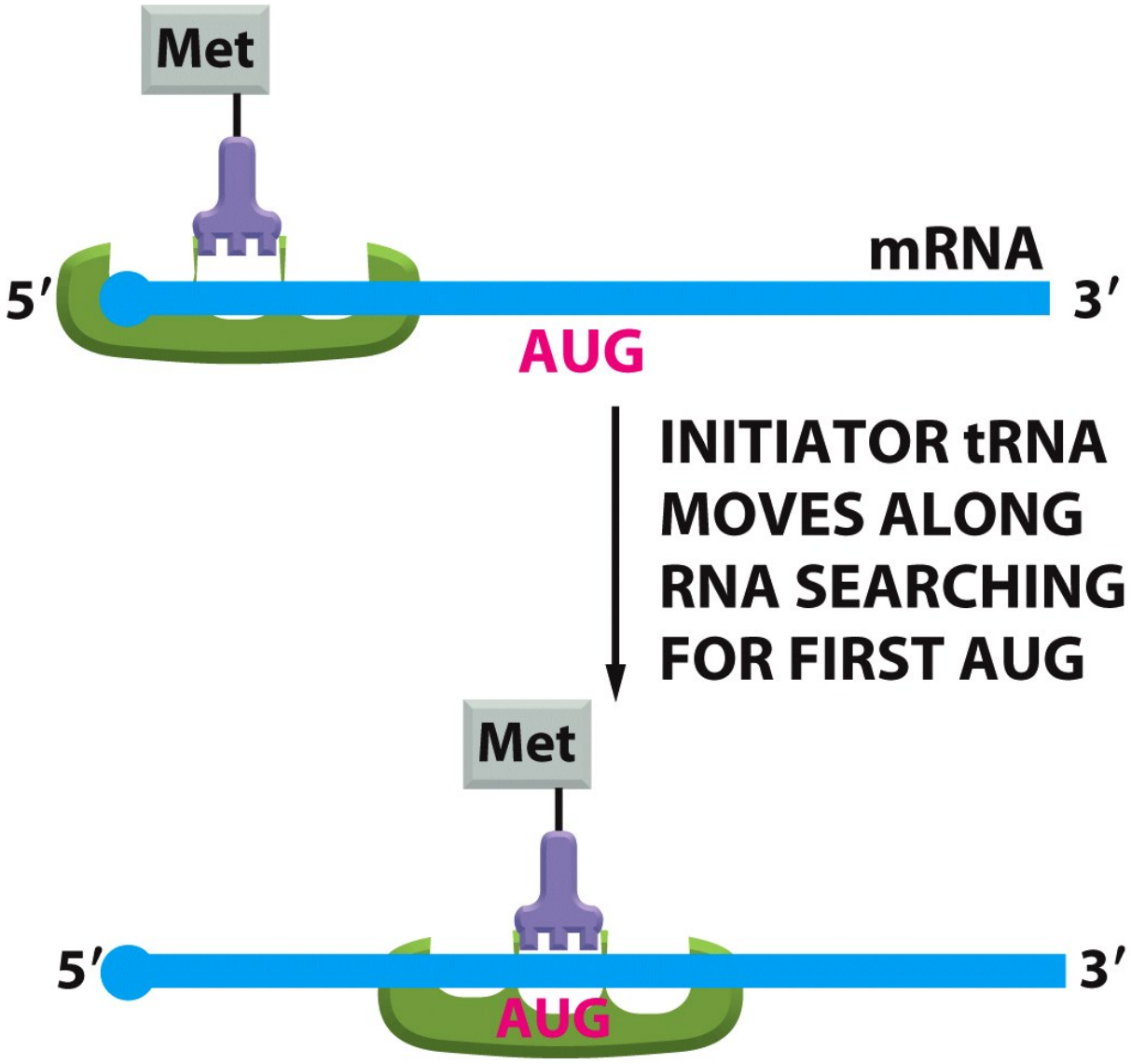


Figure 7-35 part 2 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Initiation of translation

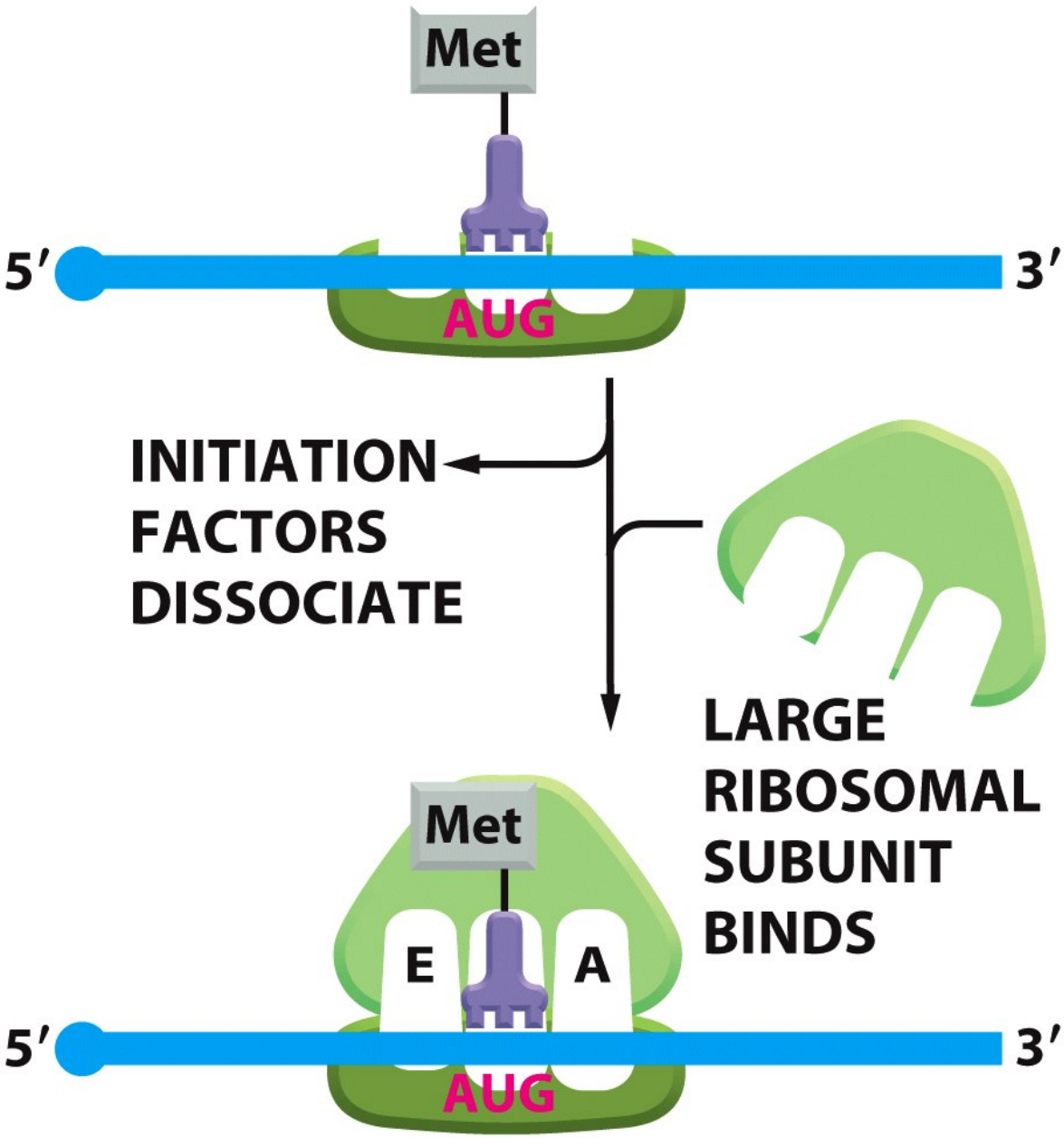


Figure 7-35 part 3 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Initiation of translation

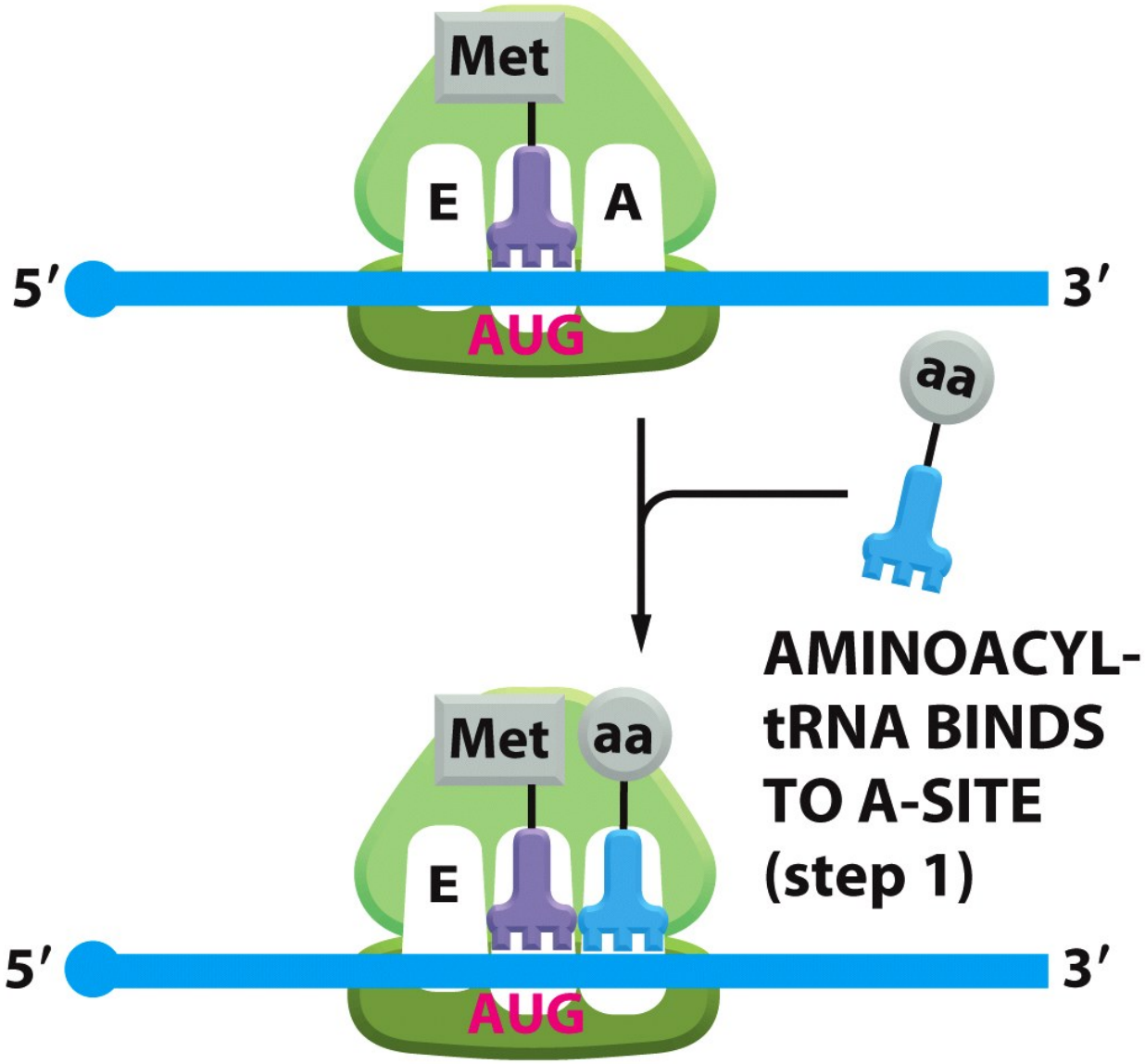


Figure 7-35 part 4 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Initiation of translation

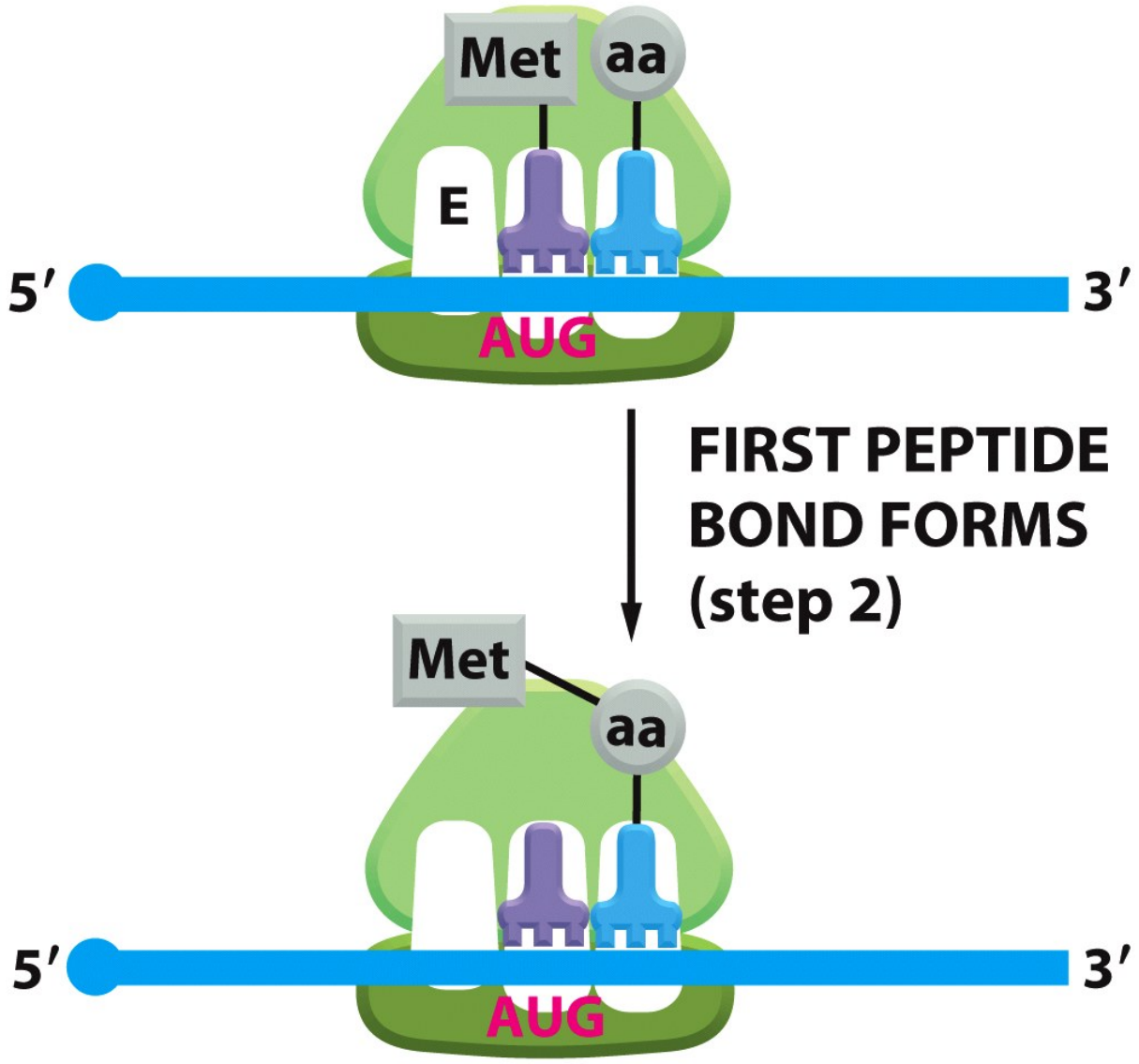
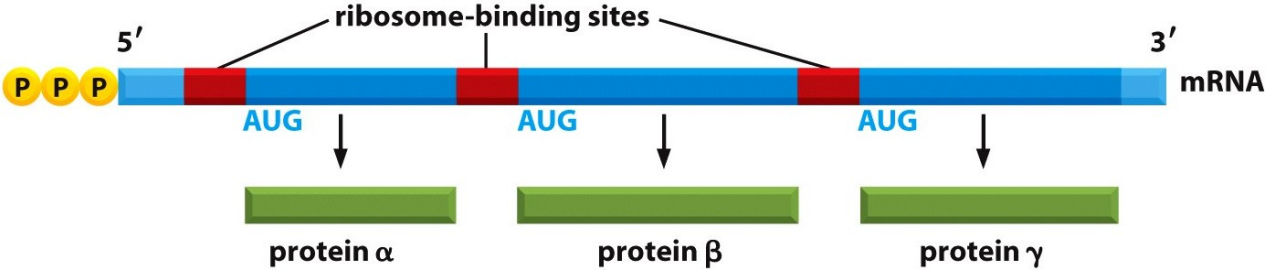


Figure 7-35 part 5 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Polycistronic mRNA



In prokaryotes, polycistronic mRNAs bear multiple starting and stopping points

Termination of translation

- Stop codon(s) binds release factor(s)
- Release factor moves to P site deattaching protein chain from last peptydil-tRNA
- Translation complex is dissolving

Termination of translation

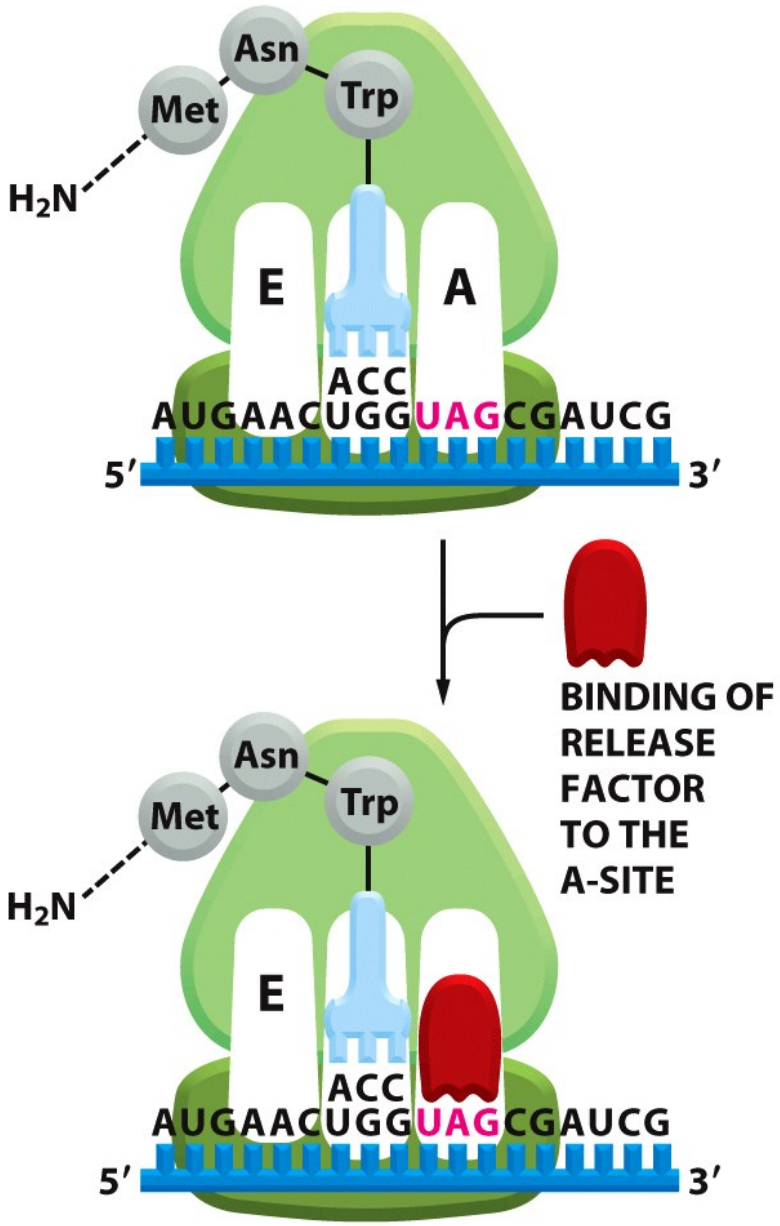


Figure 7-37 part 1 of 3 Essential Cell Biology 3/e (© Garland Science 2010)

Termination of translation

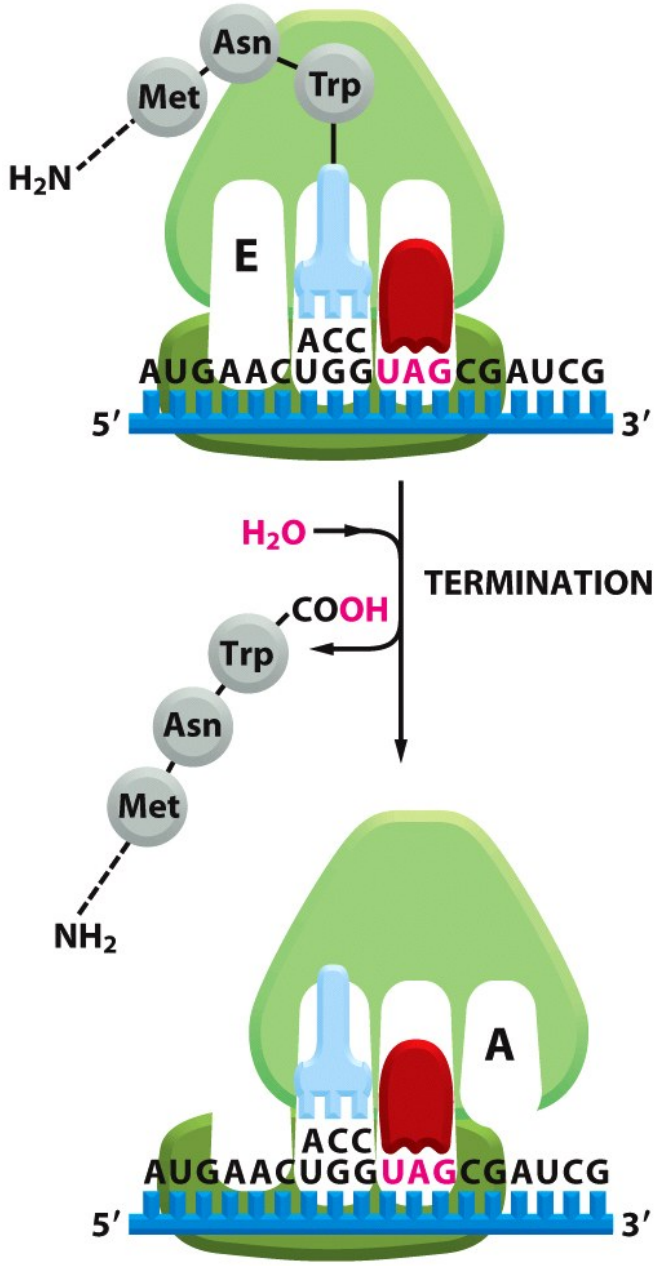


Figure 7-37 part 2 of 3 Essential Cell Biology 3/e (© Garland Science 2010)

Termination of translation

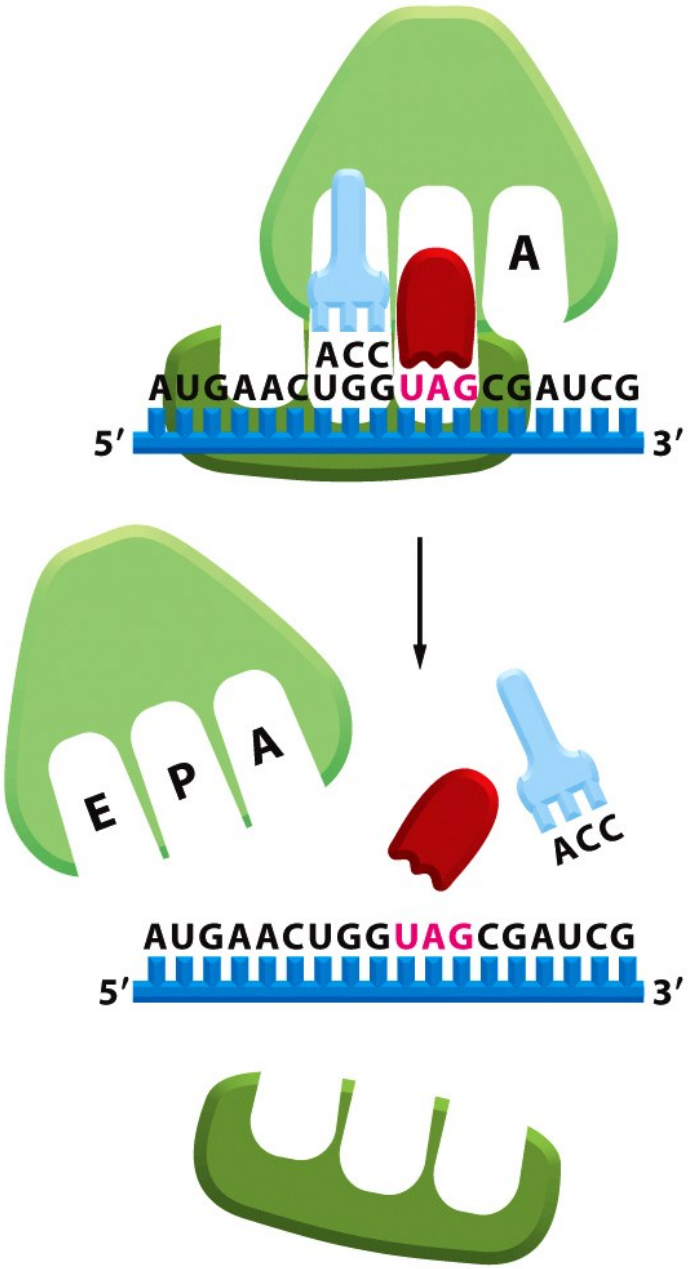


Figure 7-37 part 3 of 3 Essential Cell Biology 3/e (© Garland Science 2010)

Polyribosomes

- Ribosomal complexes which increase the rate of protein synthesis
- The other name is polysomes
- Why ribosomes are located somewhat loosely on mRNA?

Polysome

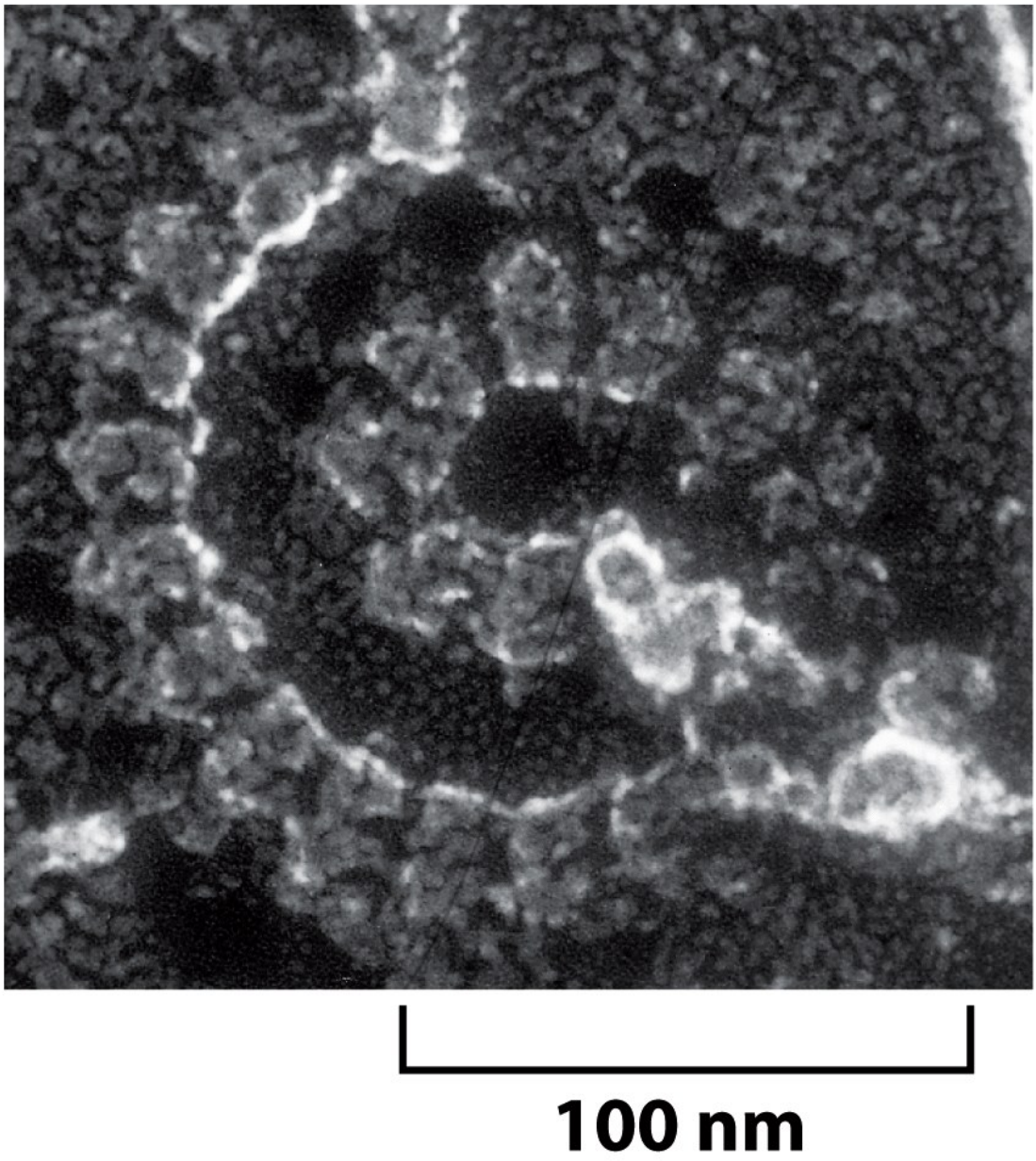


Figure 7-38b Essential Cell Biology 3/e (© Garland Science 2010)

Antibiotics

- Most of antibiotics break parts of translation machinery (and others—cell wall synthesis)
- This is the result of competition between bacteria and fungi, and simplification of bacterial transcription and translation
- Antibiotics often have influence on chloroplasts and/or mitochondria

Antibiotics

TABLE 7–3 ANTIBIOTICS THAT INHIBIT PROTEIN OR RNA SYNTHESIS

ANTIBIOTIC	SPECIFIC EFFECT
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome (step 1 in Figure 7–33)
Streptomycin	prevents the transition from initiation complex to chain-elongating ribosome (see Figure 7–35); also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 7–33)
Cycloheximide	blocks the translocation reaction on ribosomes (step 3 in Figure 7–33)
Rifamycin	blocks initiation of RNA chains by binding to RNA polymerase

Table 7-3 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (2 points)

What is a ribozyme?

Summary

- Ribosome is a ribozyme
- Translation machinery is more similar between pro- and eukaryotes than transcription

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 7*.

Outline

Questions and answers

Previous final question: the answer

What is a ribozyme?

- RNA which works like an enzyme

From RNA to protein

Antibiotics

- Most antibiotics break parts of translation machinery (and others—cell wall synthesis)
- This is the result of competition between bacteria and fungi, and simplification of bacterial transcription and translation
- Antibiotics often have influence on chloroplasts and/or mitochondria

Antibiotics

TABLE 7–3 ANTIBIOTICS THAT INHIBIT PROTEIN OR RNA SYNTHESIS

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Table 7-3 Essential Cell Biology 3/e (© Garland Science 2010)

Protein maturation

- Chaperones spent ATP to help proteins fold correctly
- Phosphorylation and cofactor binding modify proteins further
- Quaternary integration produce protein complexes where separate proteins are subunits

Protein degradation

- Proteases destroy misfolded and broken proteins
- Ubiquitin marks proteins for the destruction
- Proteasome recognized ubiquitin and takes proteins inside for complete degradation

Proteasome

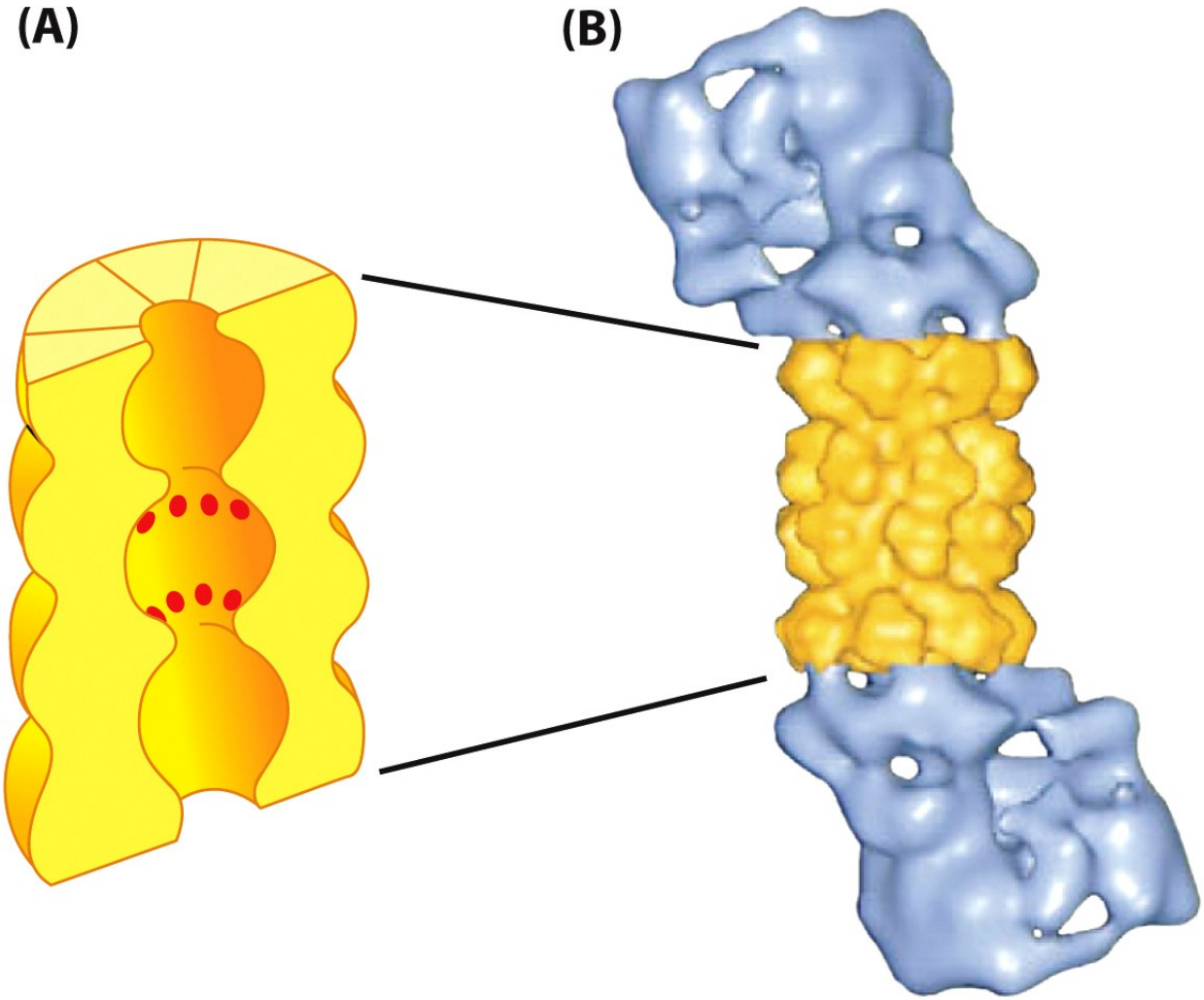


Figure 7-39 Essential Cell Biology 3/e (© Garland Science 2010)

Life cycle of protein

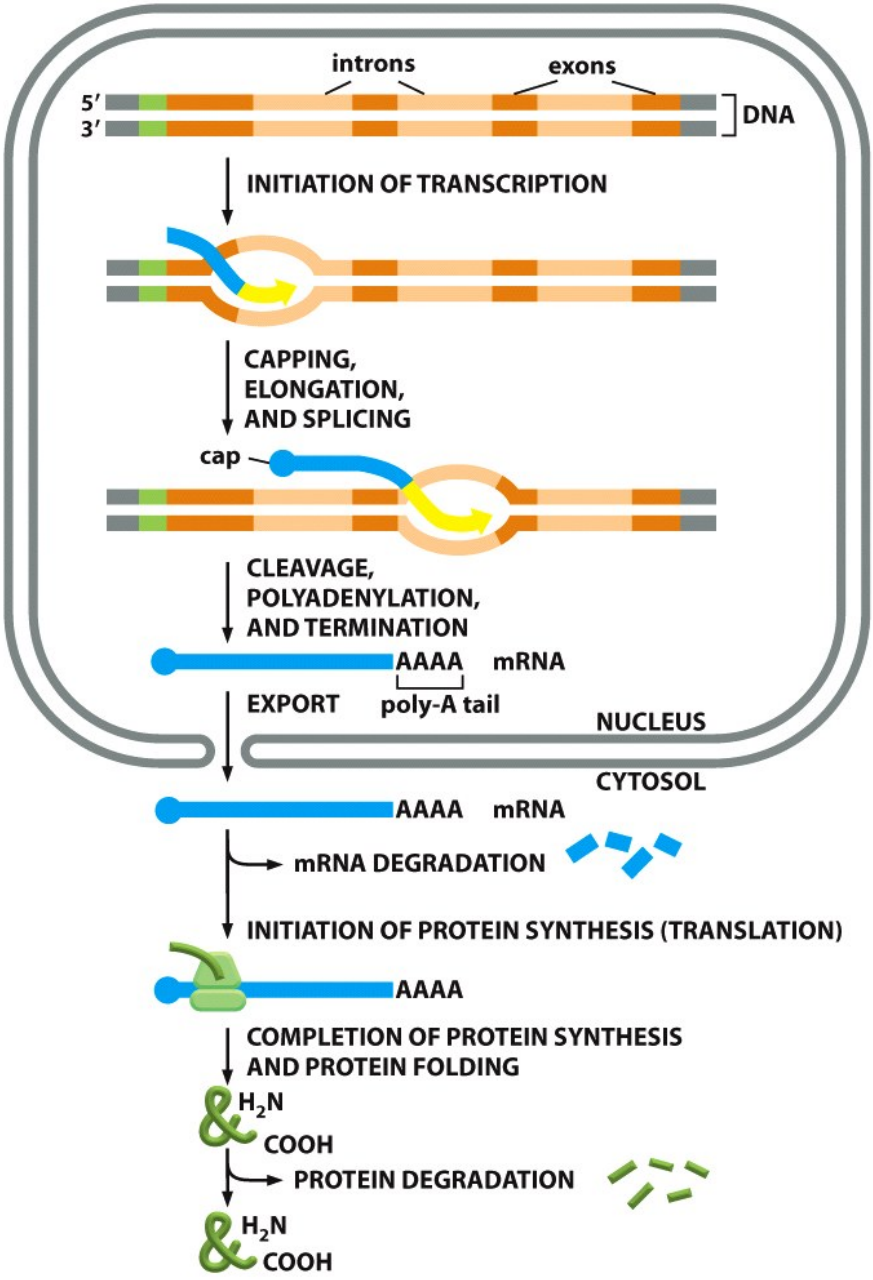
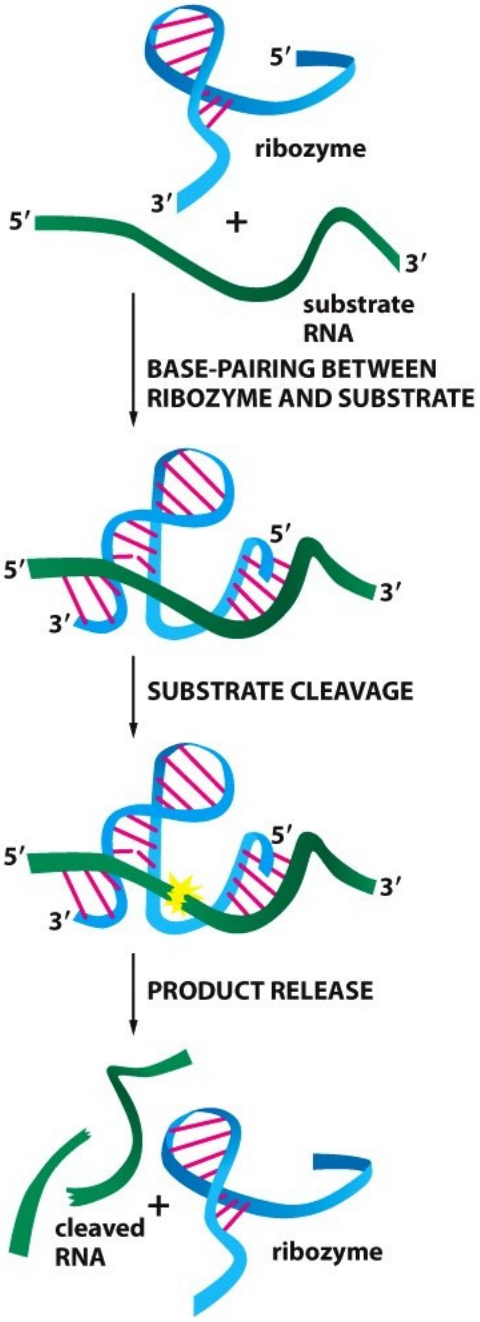


Figure 7-40 Essential Cell Biology 3/e (© Garland Science 2010)

RNA world

- The first living things should be autocatalytic systems
- RNAs bear both information an catalytic activity

Ribozyme



RNA world evolution

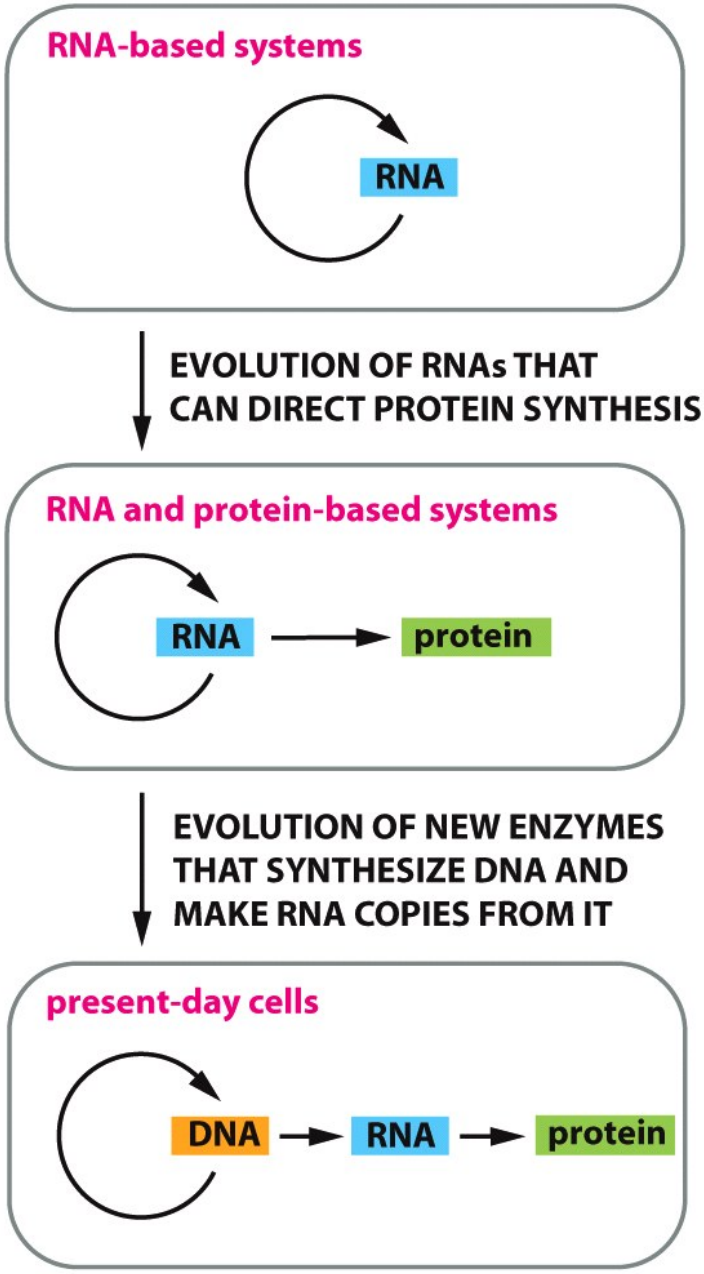


Figure 7-46 Essential Cell Biology 3/e (© Garland Science 2010)

Expression

.1 The basics of expression

Gene expression and cell differentiation

- Different cells will produce different sets of proteins (except housekeeping proteins)
- Differentiated cell expressed 20–50% of existing genes

Cloning of multicellular organisms

- Organisms of "higher" evolutionary position are cloned with more and more difficulties

- Whereas plants are capable to develop new organism almost from any cell, mammals will need extremely complicated technique including transition of nucleus

Making new mammal from a cell

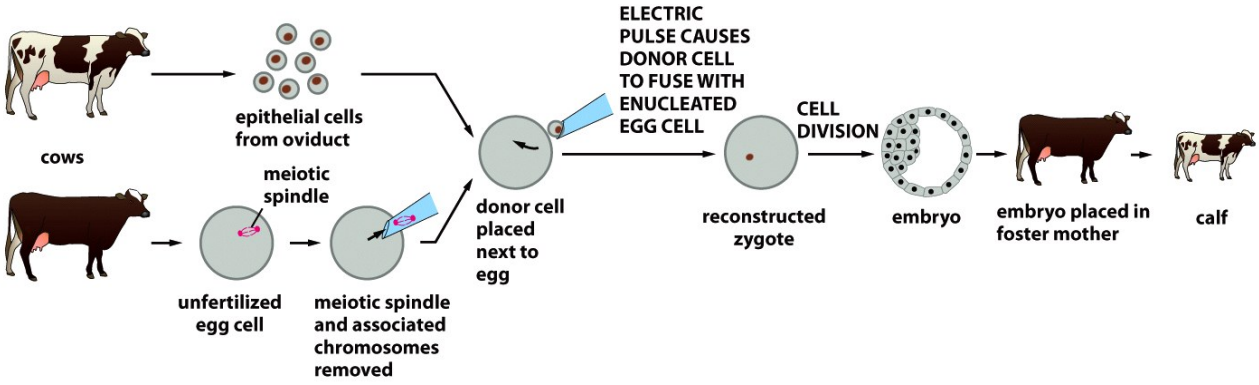


Figure 8-2c Essential Cell Biology 3/e (© Garland Science 2010)

Places of expression regulation

1. Transcription
2. RNA processing
3. RNA transport
4. RNA degradation
5. Translation
6. Protein

Control of gene expression

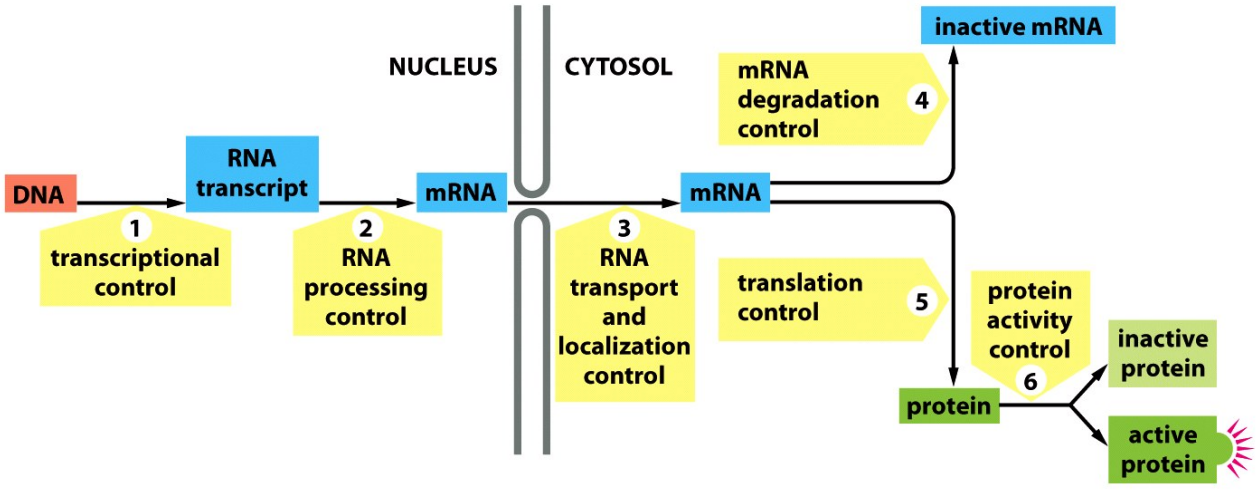


Figure 8-3 Essential Cell Biology 3/e (© Garland Science 2010)

Regulatory sequences

- Promoters + initiation sites upstream to the actual gene
- Regulatory DNA sequences upstream to the promoters
- Transcription regulators to bind with regulatory DNA sequences

DNA-binding motifs

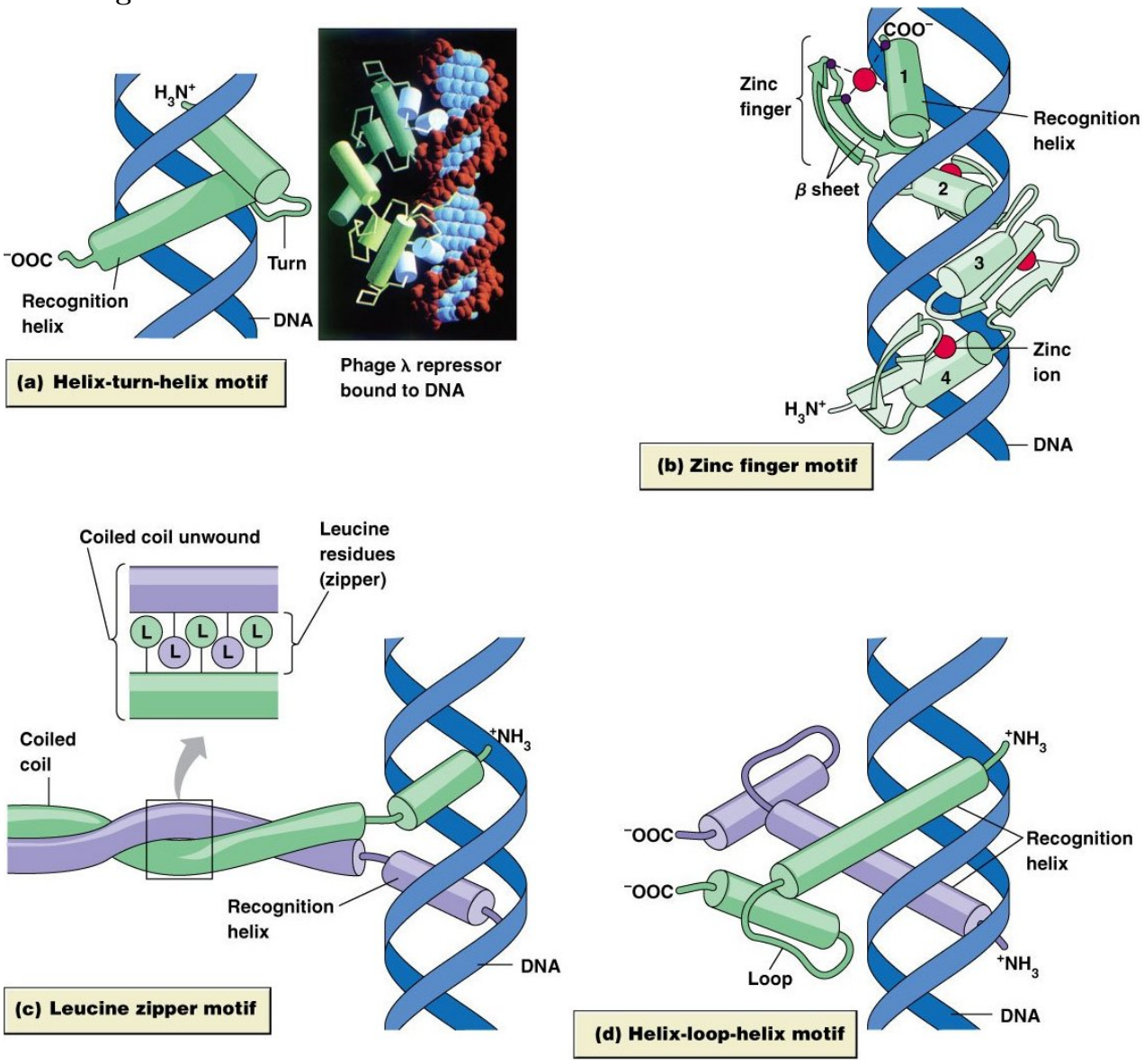
- Homeodomains (helix-turn-helix and helix-loop-helix)
- Zinc finger
- Leucine zipper

Homeodomain movie

Zinc finger movie

Leucine zipper movie

DNA binding motifs



Summary

- Ancient RNAs were probably capable of auto-catalysis and therefore may be basic to first living systems
- Cell differentiation is a result of nonuniform gene expression

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapters 7 and 8*.

Outline

Questions and answers

Previous final question: the answer

What is a chaperone?

- Protein which helps other proteins to fold correctly

Expression

.1 Repressors and activators

Repressors

- Repressor binds to operator and blocks access of RNA polymerase to promoter
- Tryptophan repressor of *E. coli* works when concentration of tryptophane is high (activated by tryptophane)
- However, it will allow small transcription even in "off" position (constitutive expression)

Tryptophane repressor

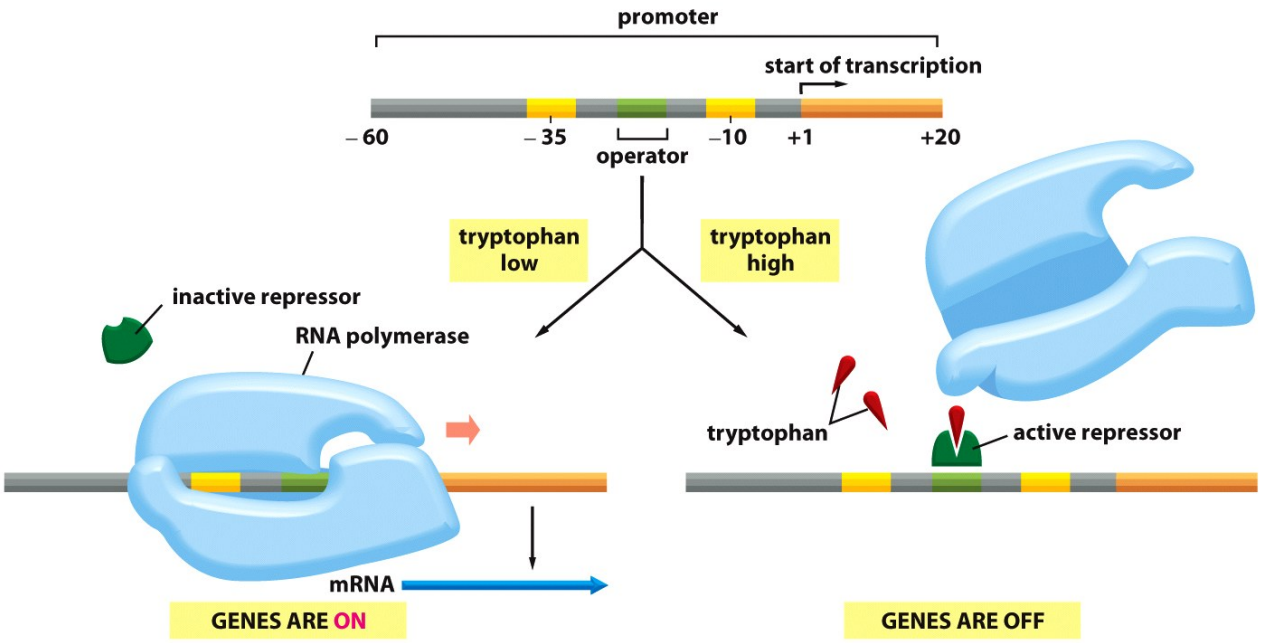


Figure 8-7 Essential Cell Biology 3/e (© Garland Science 2010)

Activators

- Activator sequences are "sleeping promoters", they work as promoters only if activator protein is bound to them
- *E. coli* activator CAP is activated by cyclic AMP (cAMP) which is a signal of lacking glucose; CAP activate proteins which utilize other sugars

Activator

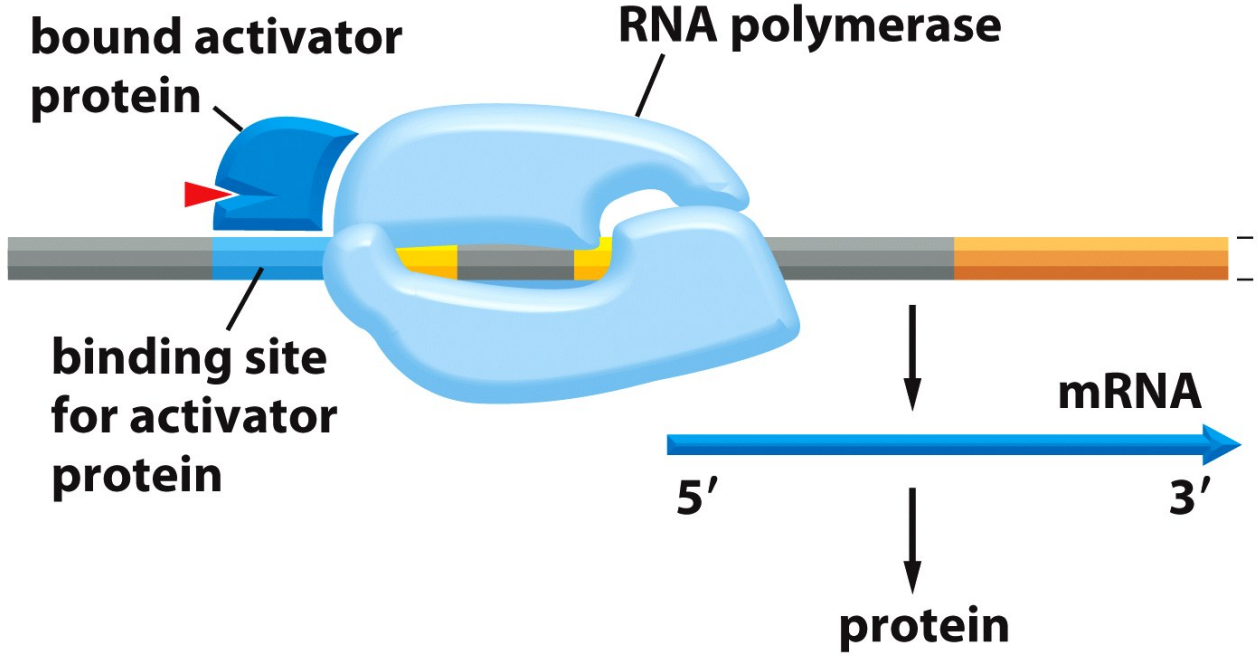


Figure 8-8 Essential Cell Biology 3/e (© Garland Science 2010)

Repressors and activators: *Lac* operon

- LacZ gene encodes β-galactosidase protein which breaks lactose to galactose and glucose
- Only if **lactose is present *AND* glucose is absent**, Lac repressor detaches from operator, and CAP activator promotes RNA polymerase to bind with promoter

Lac operon

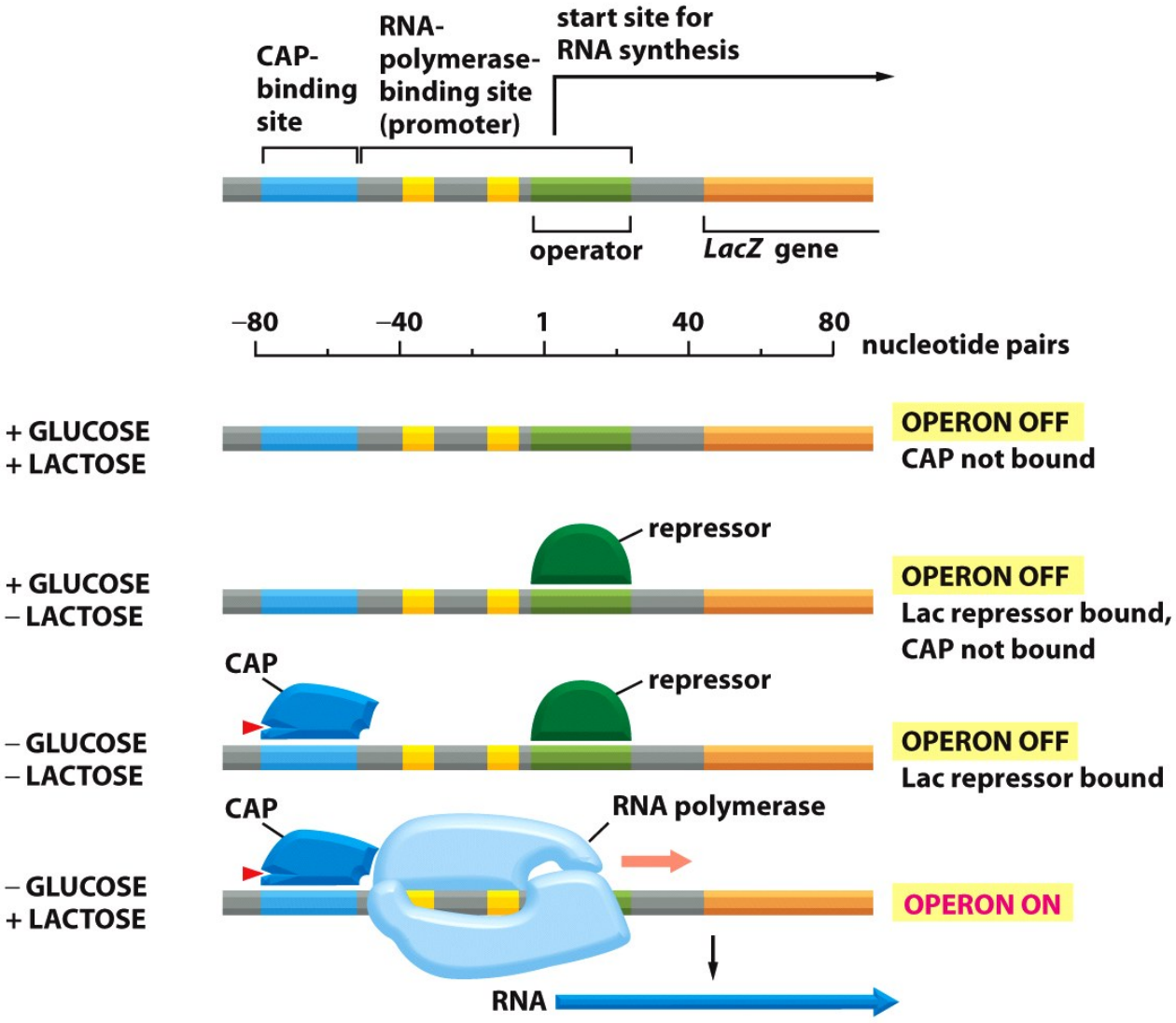


Figure 8-9 Essential Cell Biology 3/e (© Garland Science 2010)

Eukaryotic transcription regulators

- Enhancers may be located upstream or downstream from activated gene
- They work through mediators when DNA form a loop

Enhancer and mediator

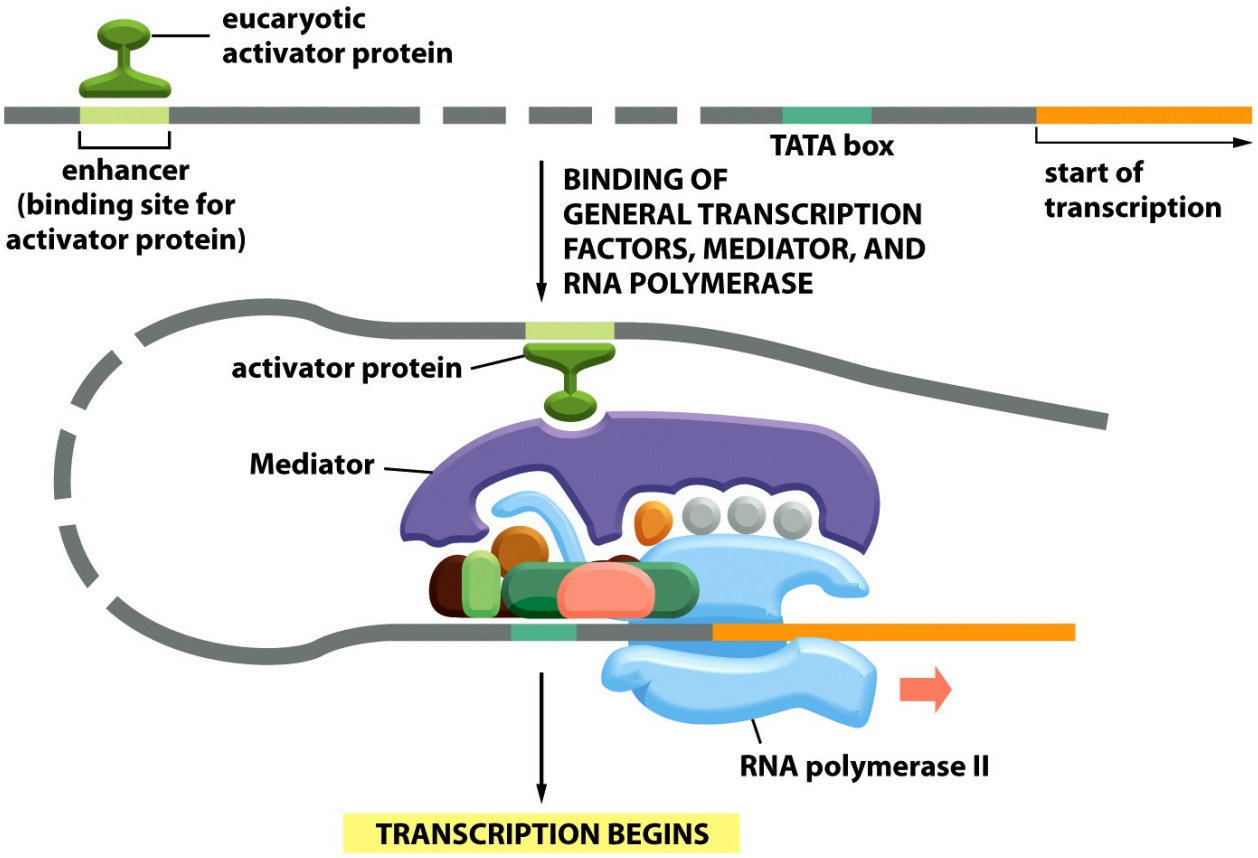


Figure 8-10 Essential Cell Biology 3/e (© Garland Science 2010)

Histones and transcription regulation

- Eukaryotic repressors and activators may work even more "distantly", through histones and chromatin-remodeling complex
- Activators may attract histone acetylases which alter the 3D structure of histones and allow an access to some DNA parts; repressors may attract histone deacetylases

Transcription committee

- Combinatorial control means that multiple regulatory proteins will determine an expression of single gene
- In eukaryotes, each gene is controlled by dozens of regulators

Eukaryotic transcription regulators

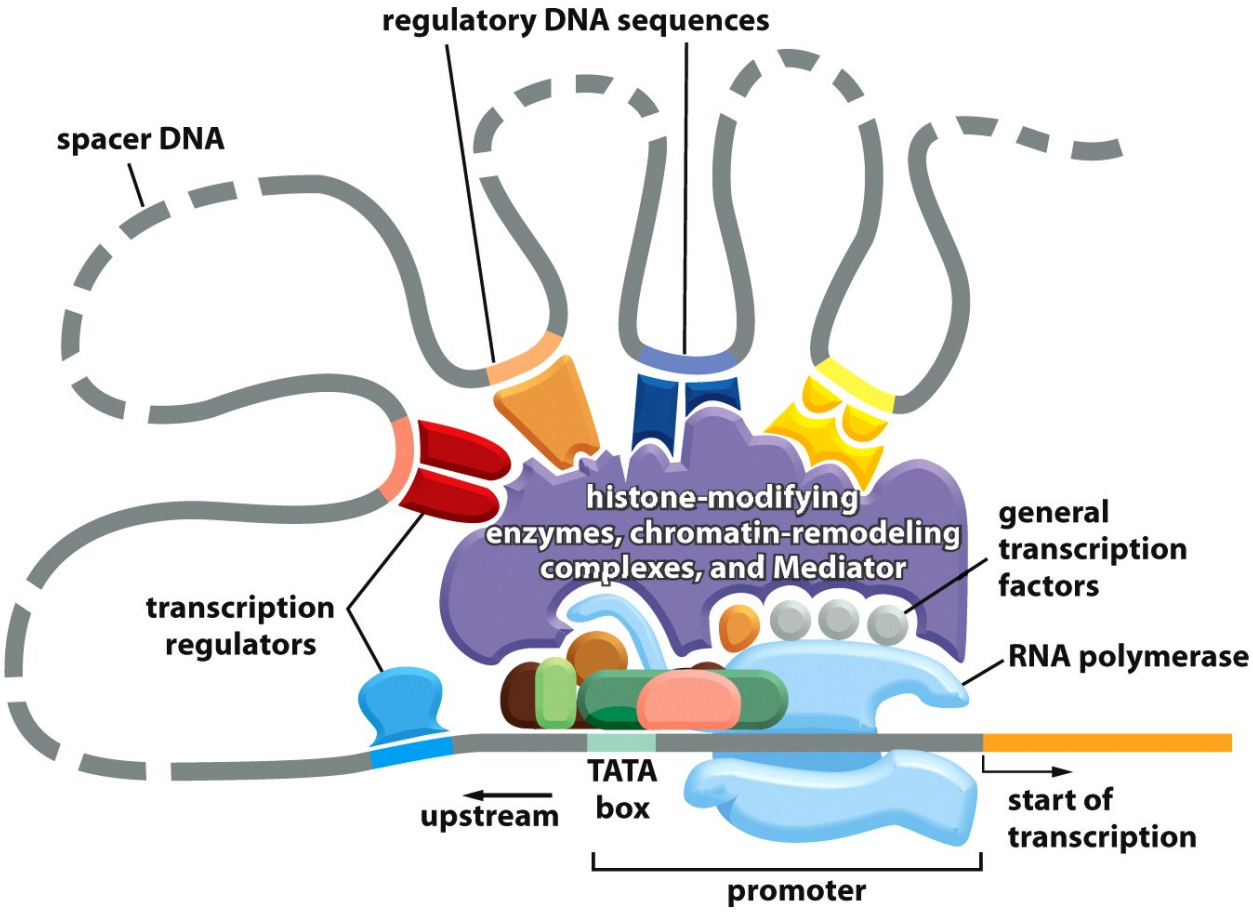


Figure 8-12 Essential Cell Biology 3/e (© Garland Science 2010)

One-to-many regulators

- Glucocorticoid receptor protein forms a complex with hormone (e.g., cortisol)
- This complex works simultaneously as activator for many genes

Glucocorticoid receptor protein

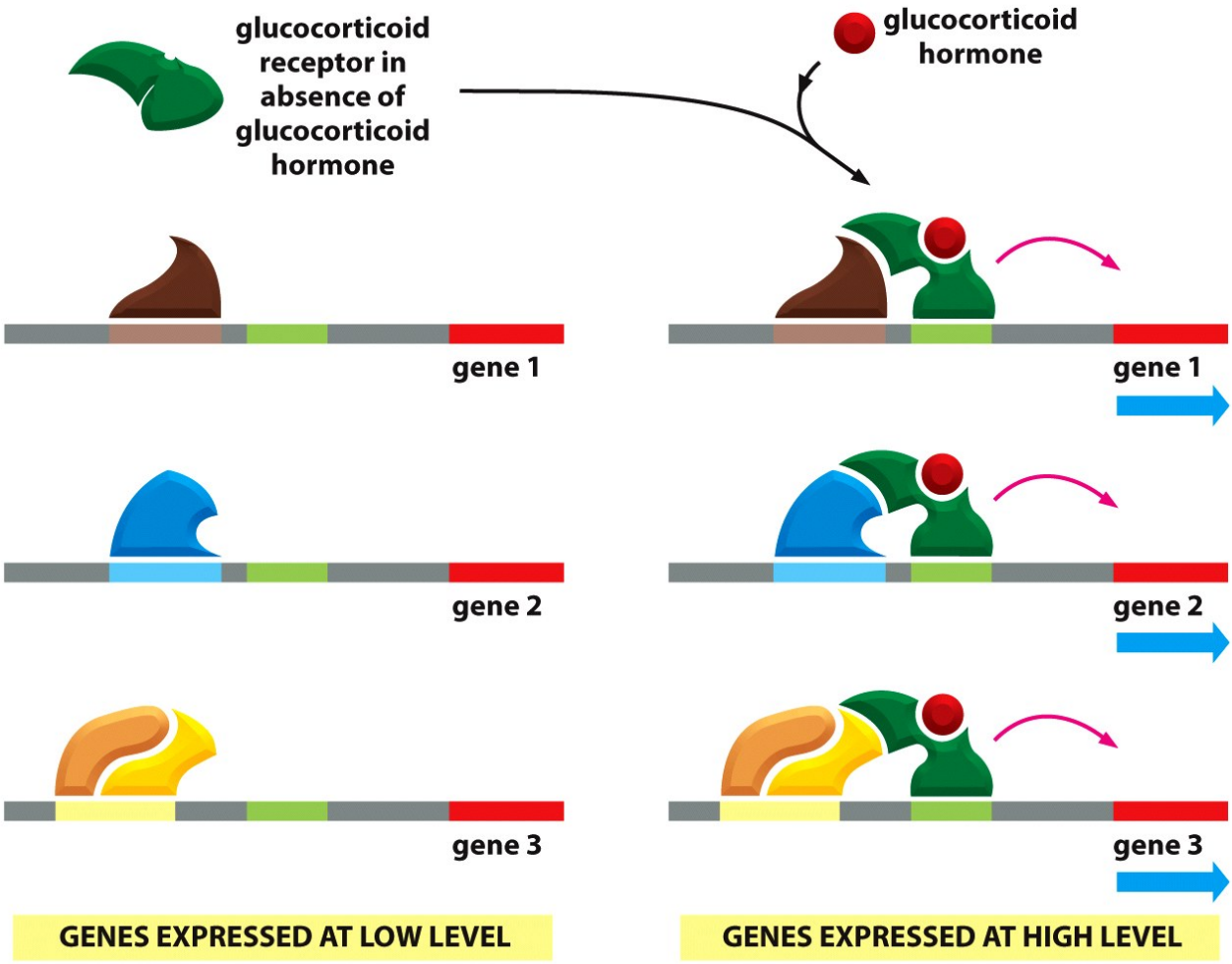


Figure 8-17 Essential Cell Biology 3/e (© Garland Science 2010)

Combinatorial control and cell differentiation

- Skin fibroblasts could be turned in muscle cells (myoblasts) with MyoD regulator
- Different combinations of regulators will result in different kinds of cells

Differentiation through combination

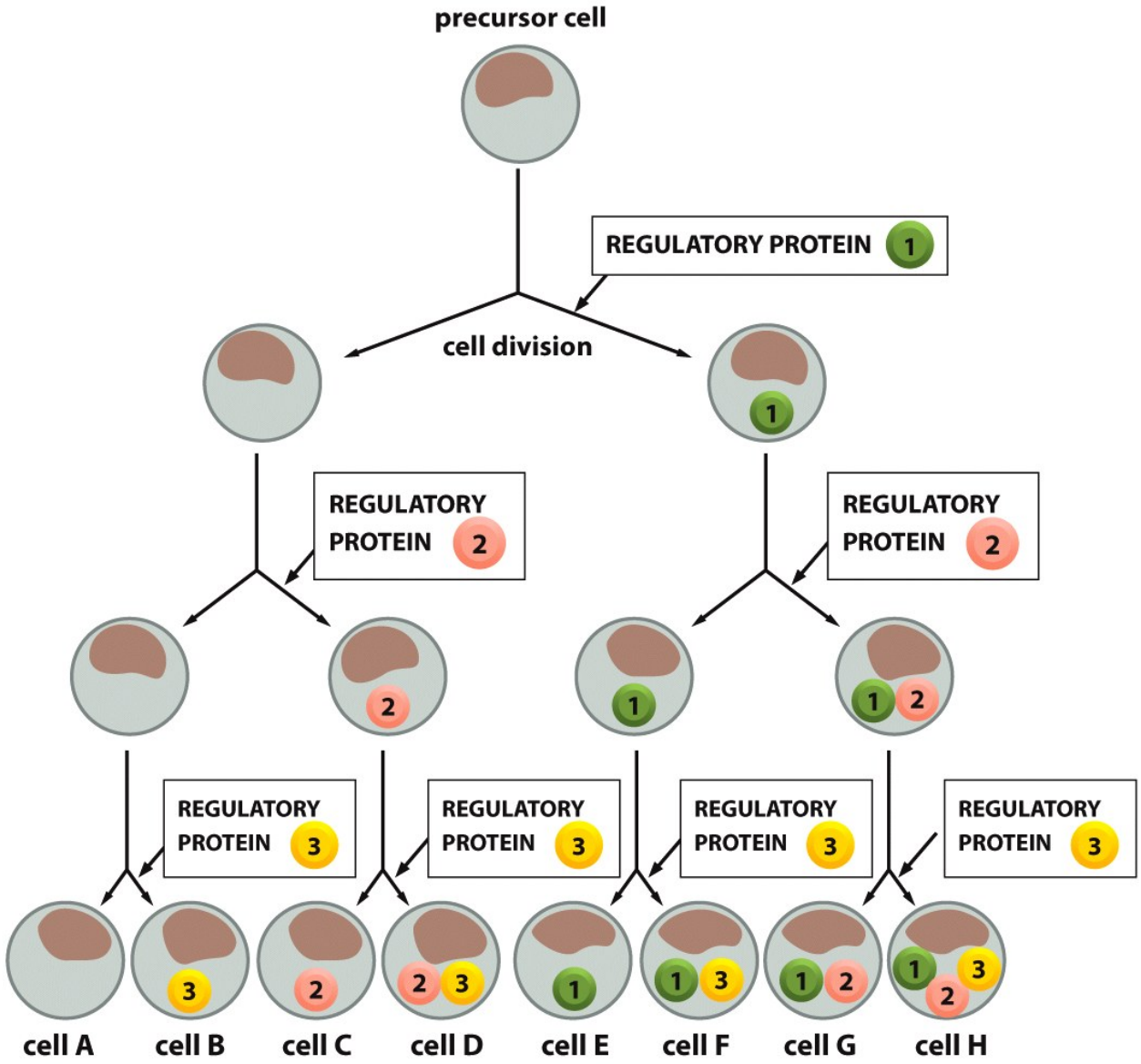


Figure 8-19 Essential Cell Biology 3/e (© Garland Science 2010)

Gene expression and cell differentiation

.1 Positional expression in fly egg

Positional expression in *Drosophila* egg

- In the ovoid fly egg, different proteins are expressed in different parts
- Injection of cytoplasm from posterior end may convert head to the second tail

Double-posterior fly larva

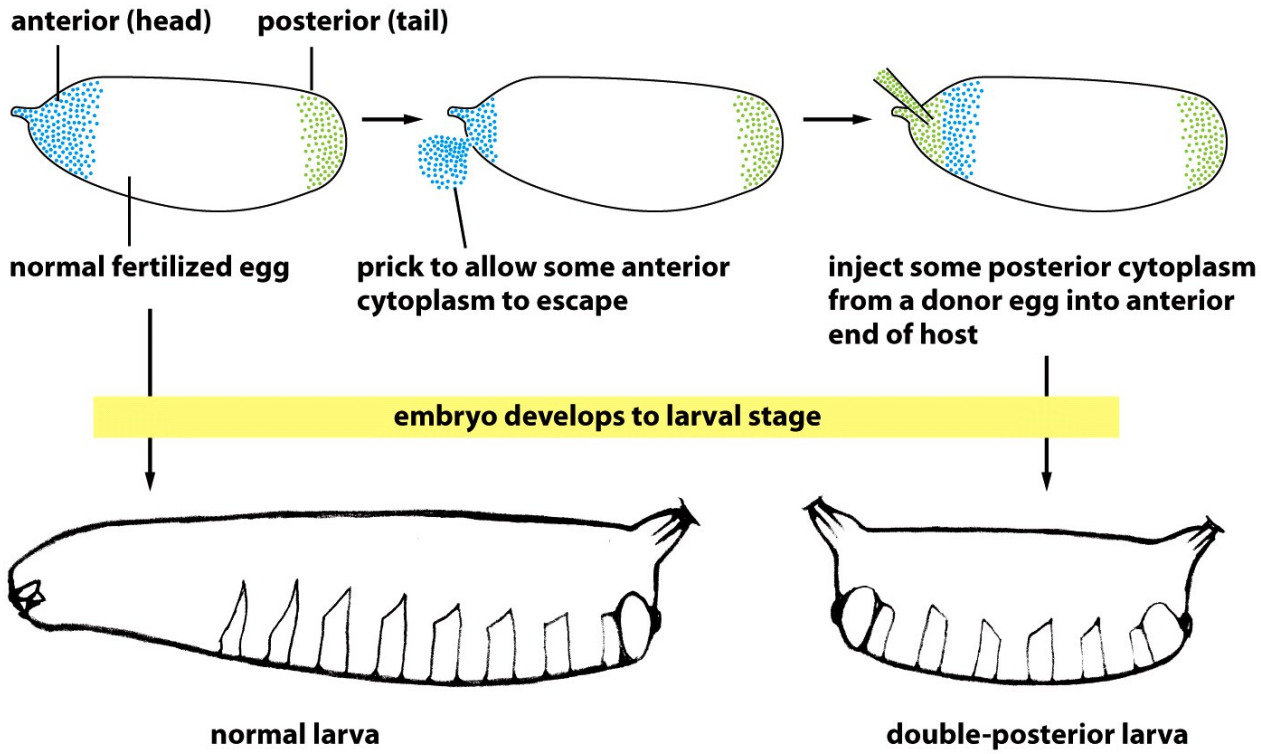


Figure 8-13 Essential Cell Biology 3/e (© Garland Science 2010)

Bicoid, Hunchback, Giant and Krueppel

- Four transcription regulators were discovered using specific antibodies
- Each has its own distribution along the body axis
- They regulate the expression of *Eve* gene

Distributuion of transcription regulators in fly embryo

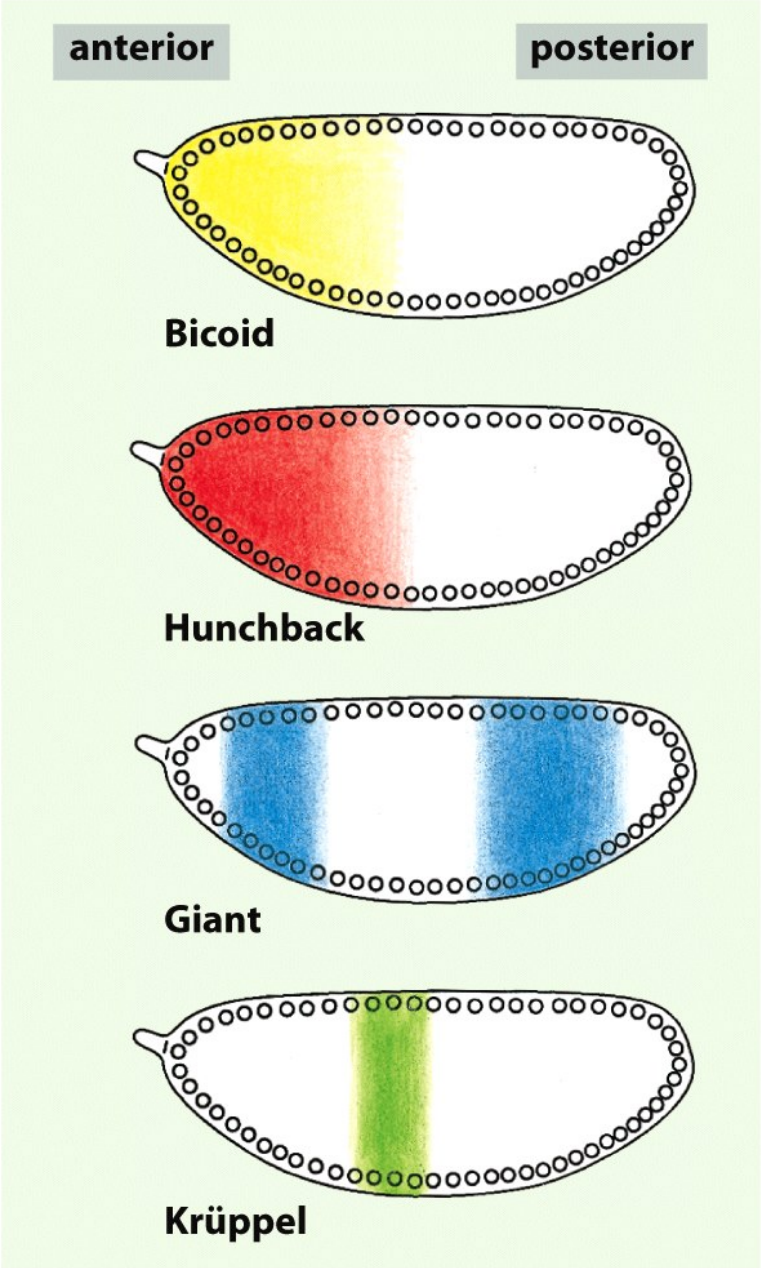


Figure 8-14 Essential Cell Biology 3/e (© Garland Science 2010)

Seven stripes of *Eve*

- Every module will be activated if specific combination of transcription regulators is present
- If module is activated, *Eve* gene expression starts and stripe is formed
- Reporter gene (e.g., LacZ) may help to visualize the role of different activator modules

Stripes

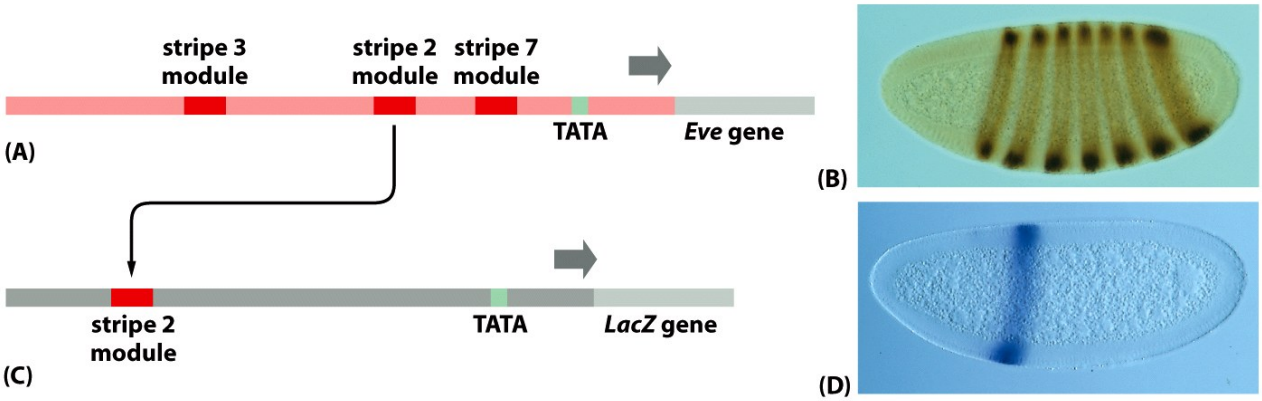


Figure 8-15 Essential Cell Biology 3/e (© Garland Science 2010)

Stripe 2 uses all four for activation

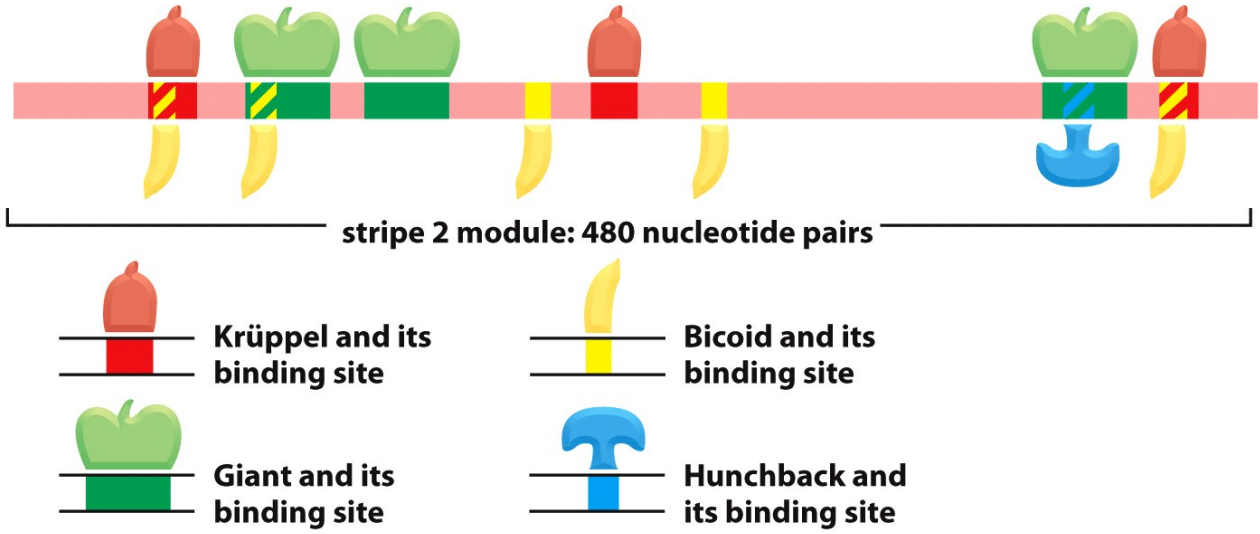


Figure 8-16 Essential Cell Biology 3/e (© Garland Science 2010)

Cell memory

- Positive-feedback loop: transcription activator activated transcription of it own gene
- DNA methylation patterns are inherited in cell lineages

Positive loop inheritance

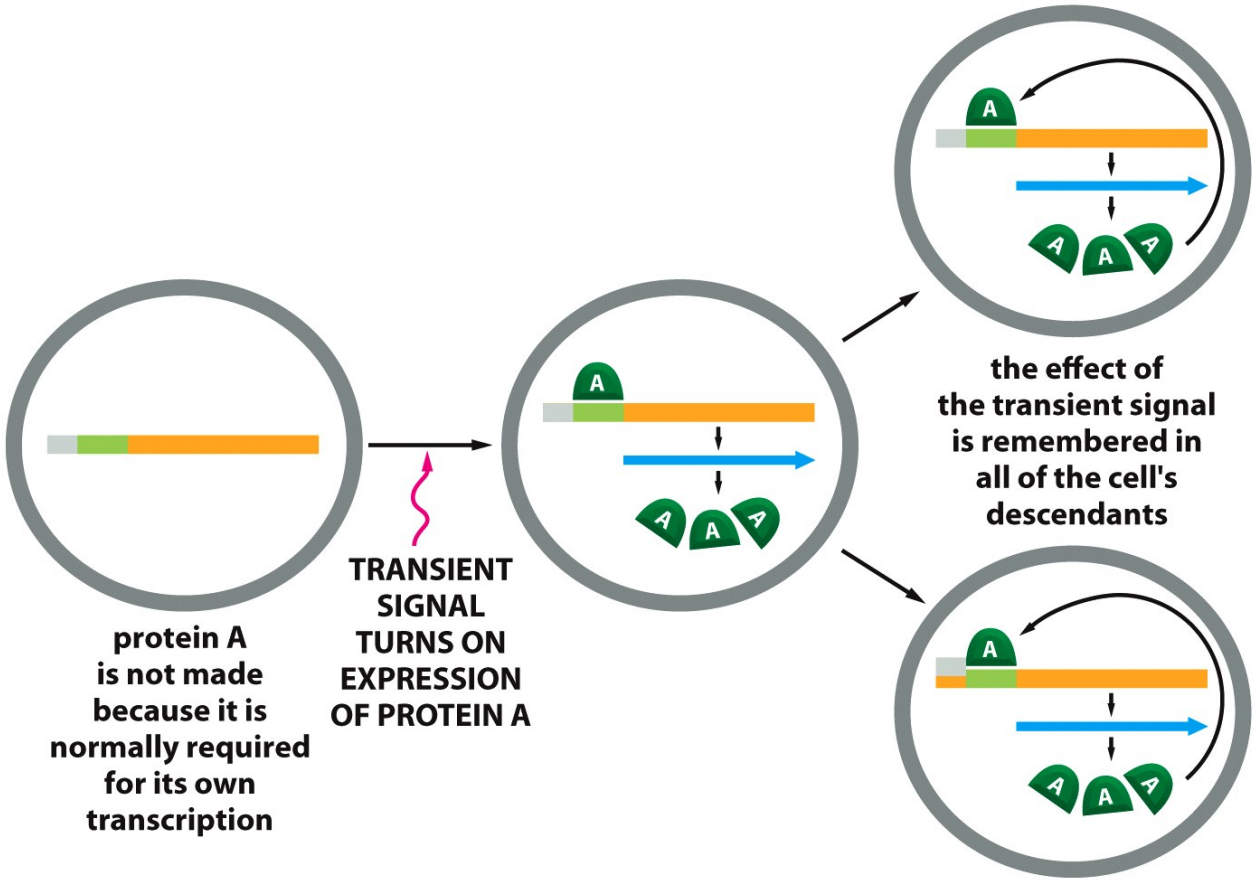


Figure 8-20 Essential Cell Biology 3/e (© Garland Science 2010)

DNA methylation

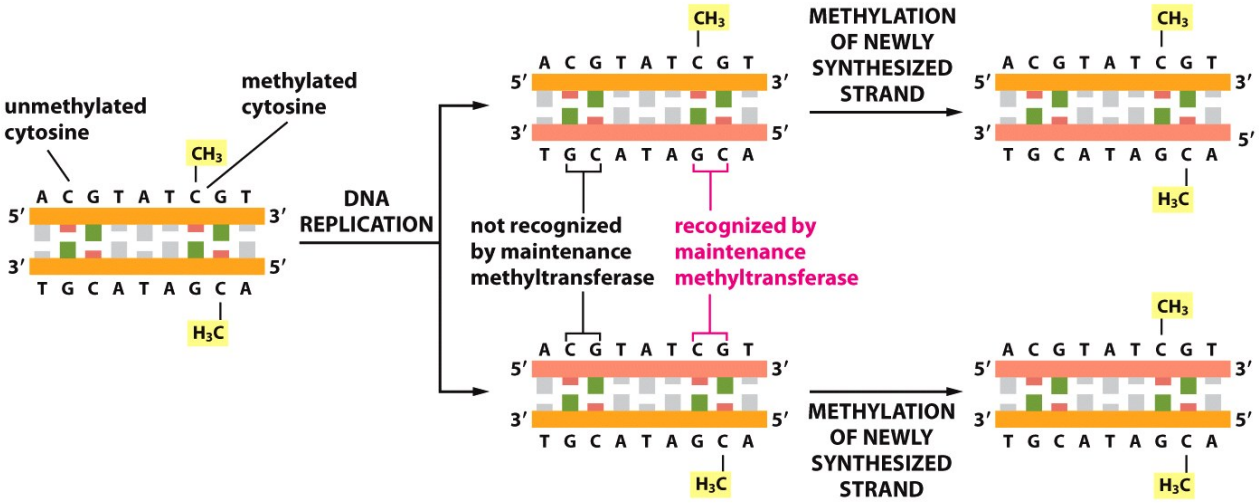


Figure 8-22 Essential Cell Biology 3/e (© Garland Science 2010)

Ey gene

- *Ey*/*Pax*-6 regulators are critical for eye development in insects/vertebrates
- If expression of *Ey* gene will be turned on, eye may appear even on leg!

How to grow eye on the leg

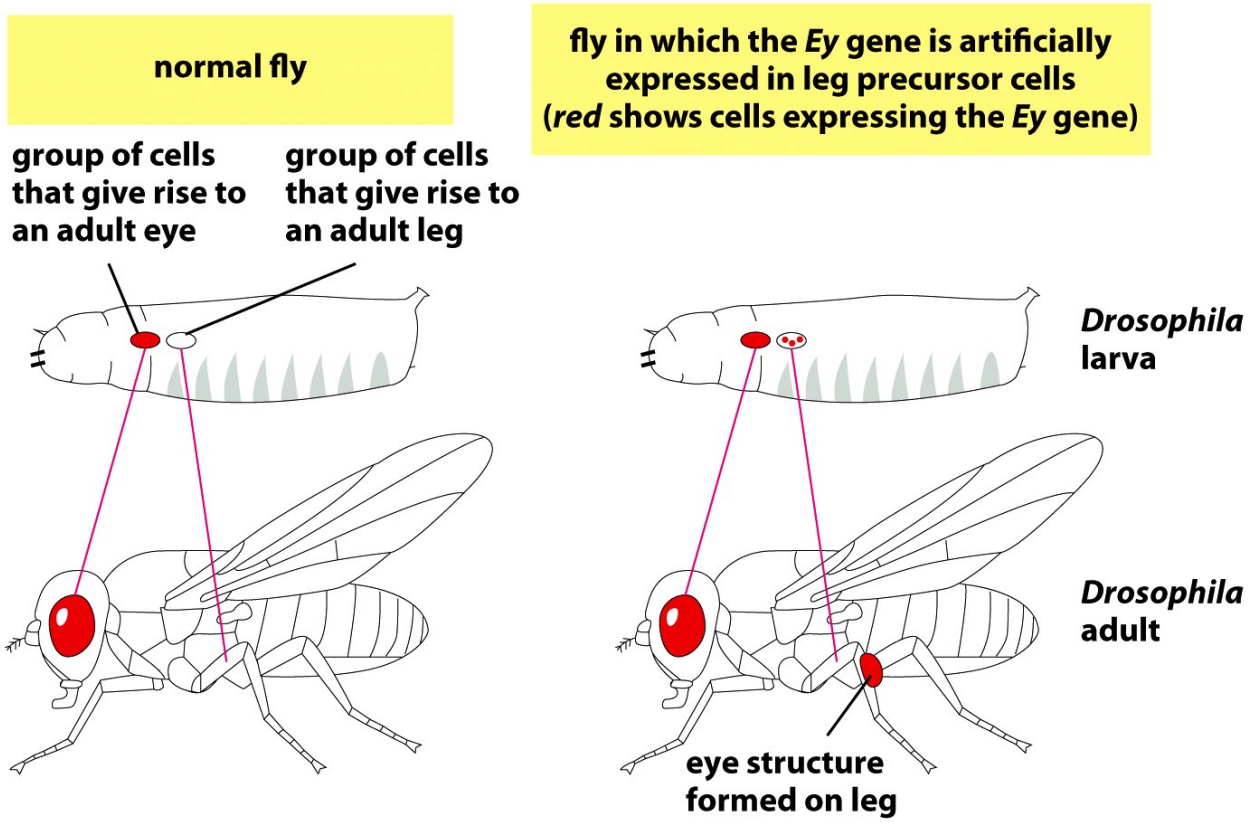


Figure 8-23a Essential Cell Biology 3/e (© Garland Science 2010)

Final question (2 points)

How many signals control the *Lac* operon?

Summary

- Activators and repressors could switch transcription on and off
- Different combinations of transcription regulators activate gene expression in different places and in different ways
- Transcription regulation may be specific to place

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapters 7 and 8*.

Outline

Questions and answers

Previous final question: the answer

How many signals control the *Lac* operon?

- 2

Expression

.1 Post-transcriptional controls

Riboswitches

- mRNAs may control its own synthesis through riboswitches
- Riboswitch is a short sequence which change conformation of mRNA and block/unblock RNA polymerase
- Riboswitches may have small molecules to bind with them

Riboswitch

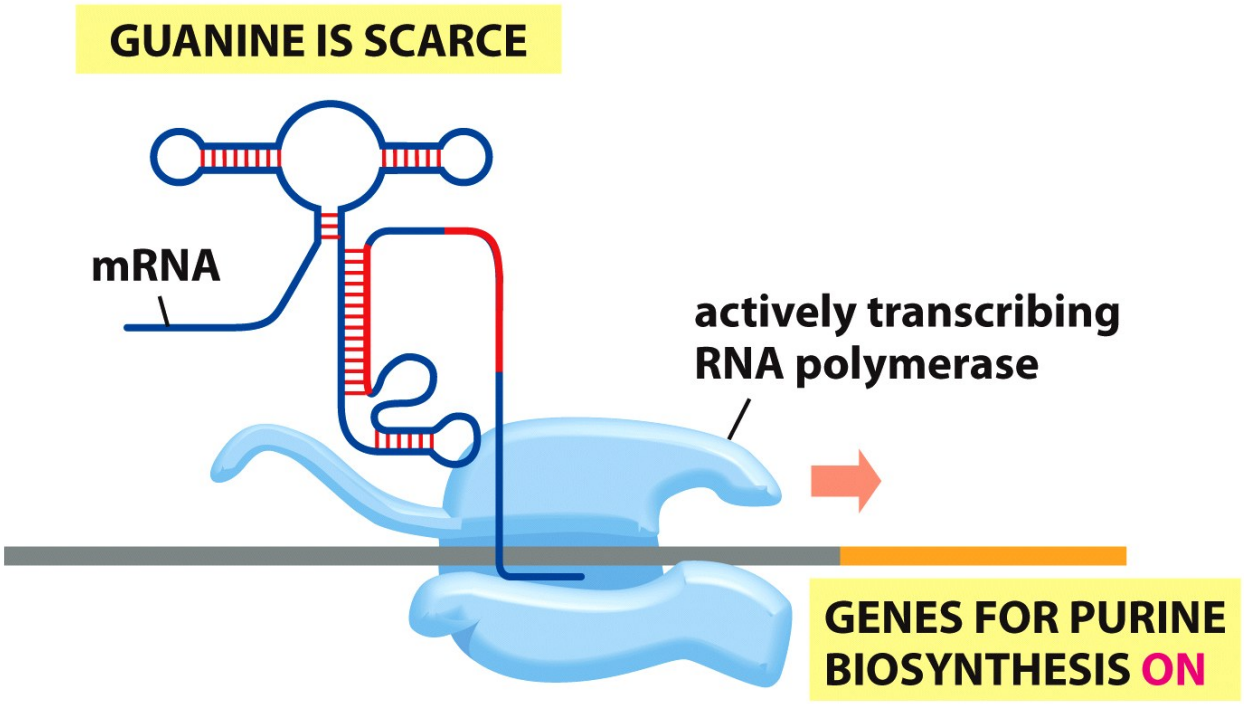


Figure 8-24a Essential Cell Biology 3/e (© Garland Science 2010)

Regulation of translation initiation

Many different kinds of repressors and activators may bind to ribosome-binding site (upstream to AUG codon) and block/unblock translation

- "Thermosensors" change conformation of mRNA and may unblock translation
- Riboswitches may also block/unblock translation
- Antisense complementary RNAs could block translation

mRNA-binding proteins as translation regulators

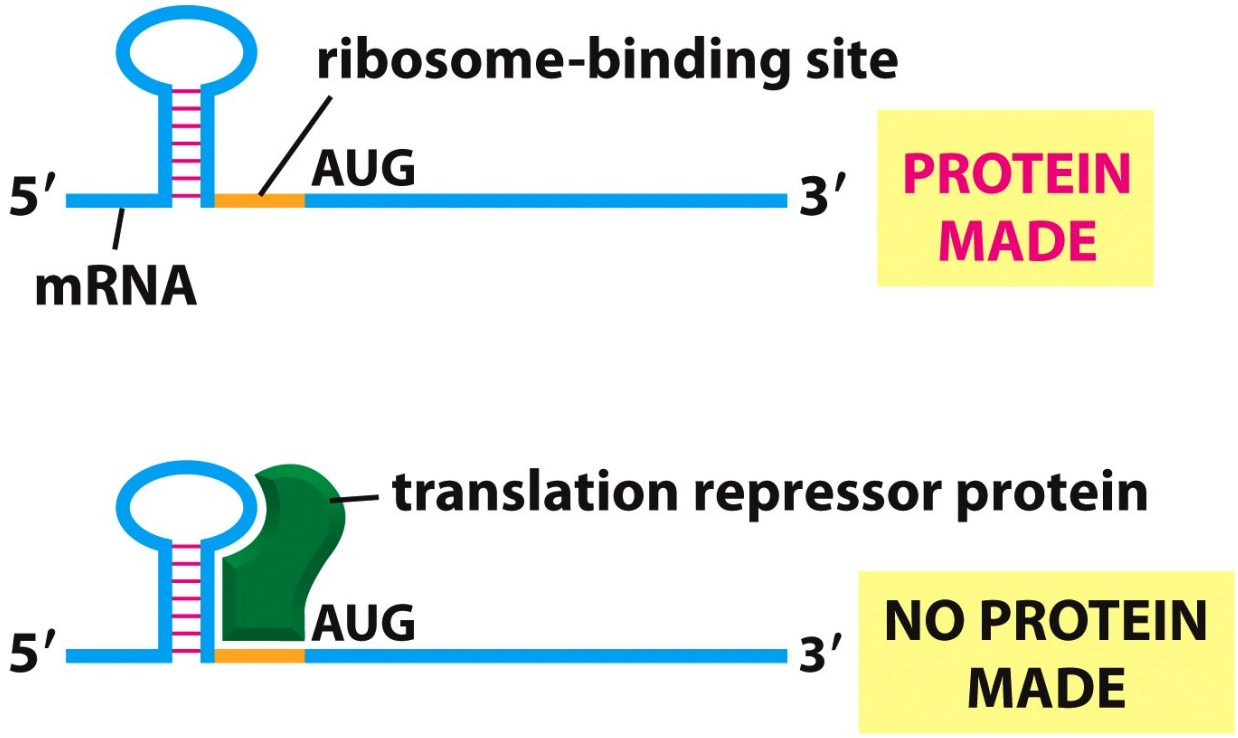


Figure 8-25a Essential Cell Biology 3/e (© Garland Science 2010)

Natural thermosensor RNA as translation regulator

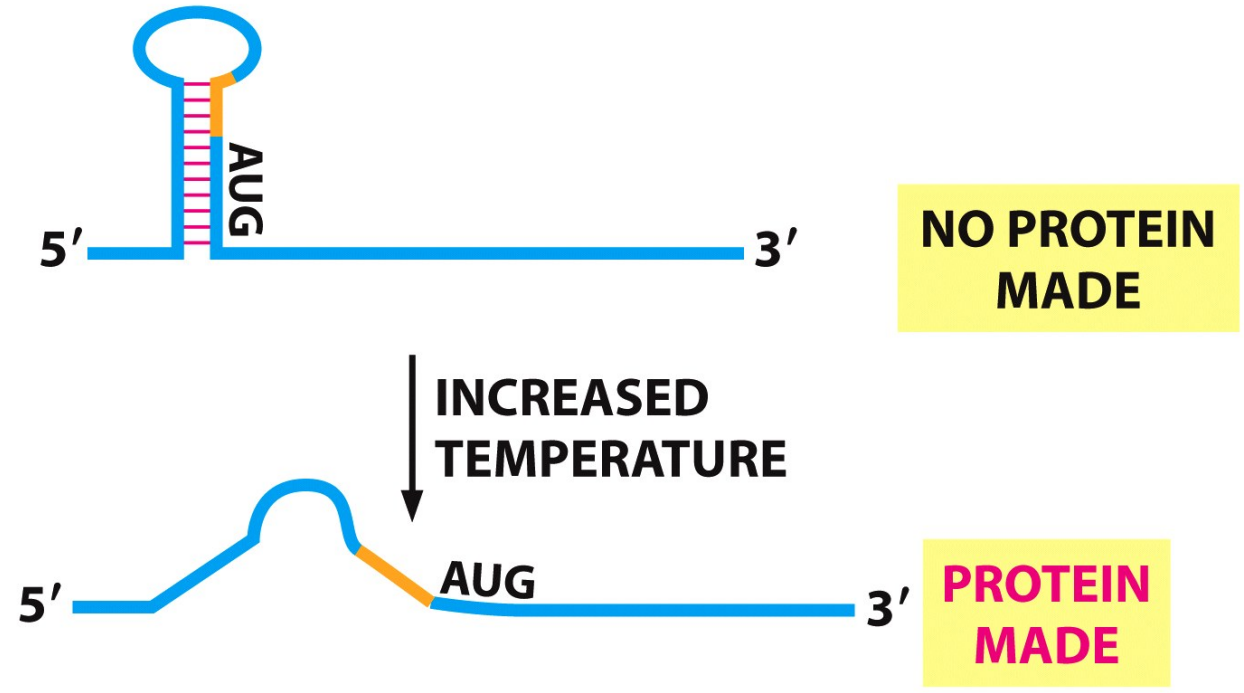
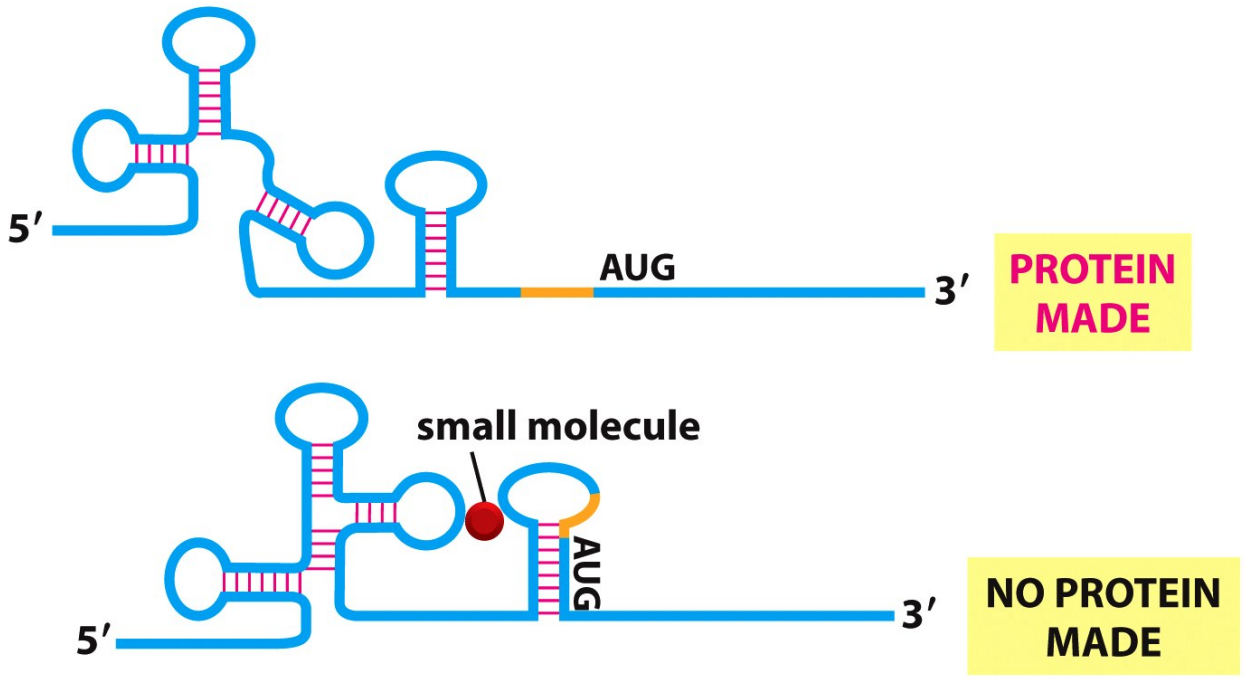


Figure 8-25b Essential Cell Biology 3/e (© Garland Science 2010)

Riboswitches as translation regulators



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Antisense RNAs as translation regulators

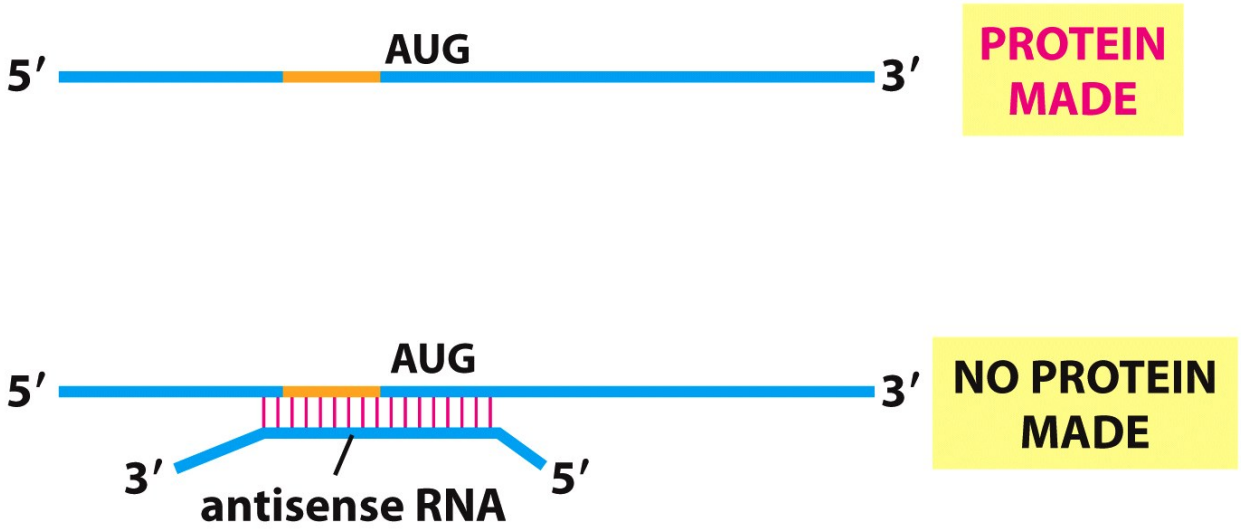


Figure 8-25d Essential Cell Biology 3/e (© Garland Science 2010)

miRNA control

- microRNAs (miRNAs) form a RNA-induced silencing complexes with proteins (RISCs)
- If a RISC finds a complementary mRNA, it blocks translation and destroy this mRNA

miRNA at work

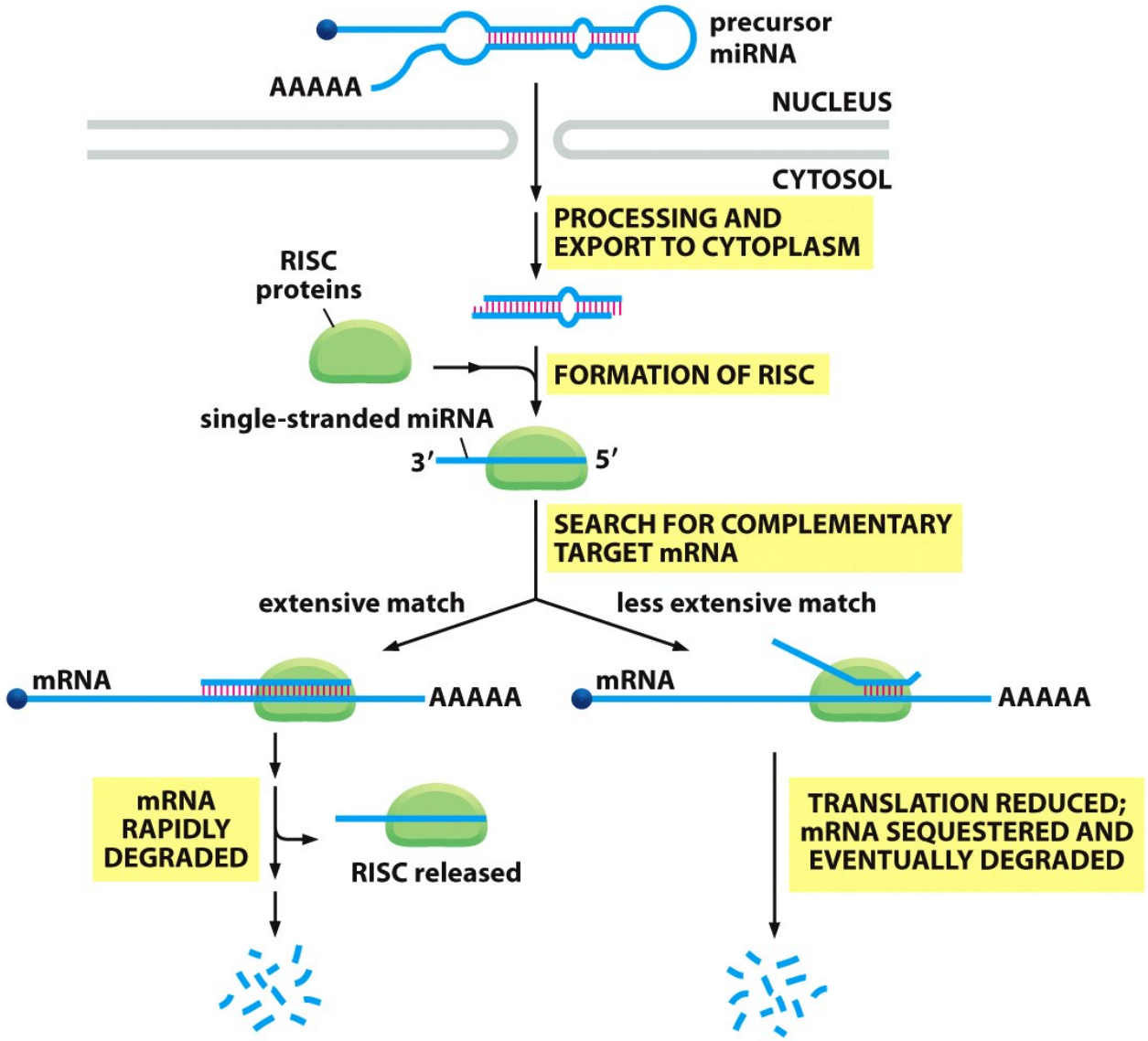


Figure 8-26 Essential Cell Biology 3/e (© Garland Science 2010)

RNA interference

- Dicer nuclease chop dsRNA of virus into fragments
- These fragments (small interfering RNAs, siRNAs) incorporate into RISCs
- Then RISCs become able to "know" invader RNAs
- Prokaryotes have somewhat similar, wonderful CRISPR-CAS system (will be explained later)

siRNAs

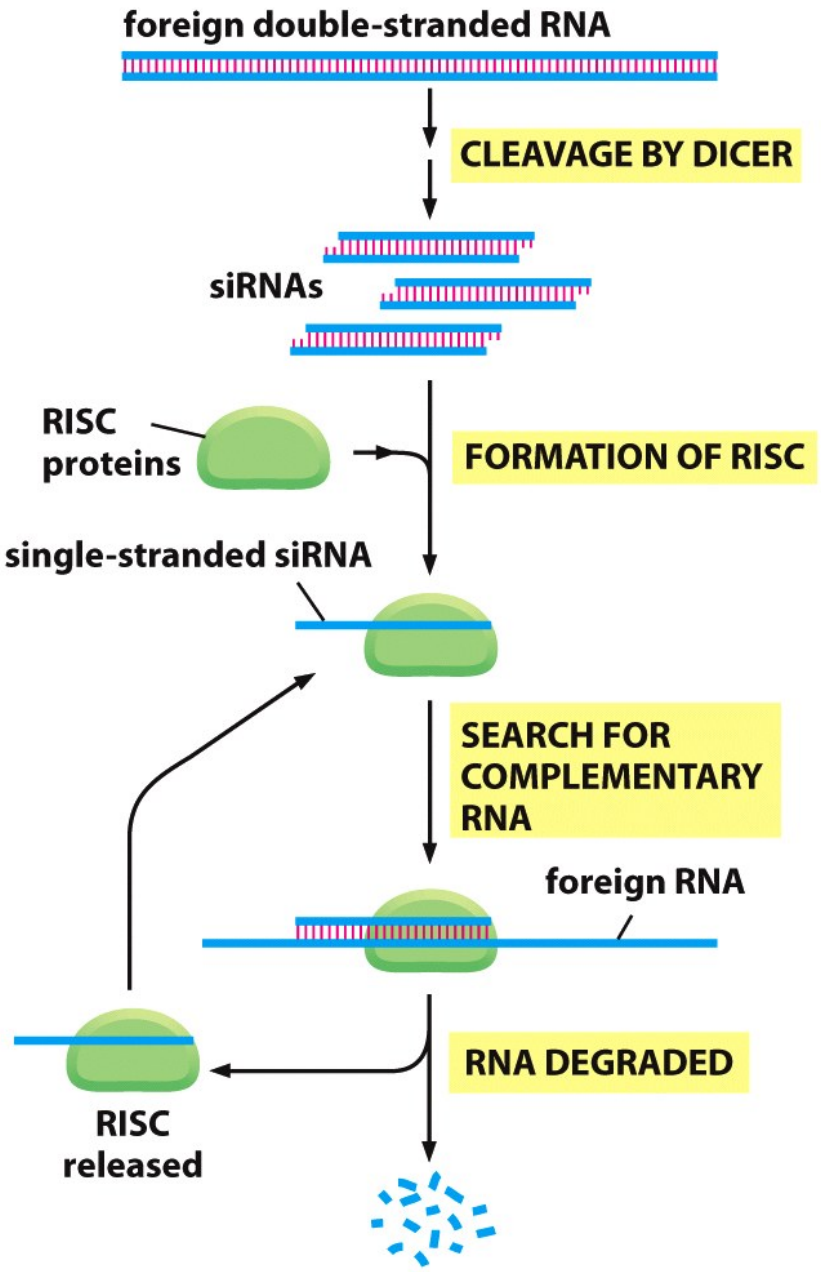


Figure 8-27 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (3 points)

Are riboswitches capable of epigenetic inheritance?

Summary

- Riboswitches, proteins and miRNA can regulate post-transcriptional gene expression
- miRNAs/RISCs are capable to "memorize" invader RNA

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 8*.

Outline

Questions and answers

Previous final question: the answer

Are riboswitches capable of epigenetic inheritance?

- No. They are temporarily repressors/activators.

Genes and genomes

.1 Evolution of genome

Mechanisms of genome changes

- Mutation within gene
- Mutation in regulatory region
- Duplication
- Conversion of meaningless DNA
- Exon shuffling
- Mobile elements and HGT

Types of evolutionary changes in genome

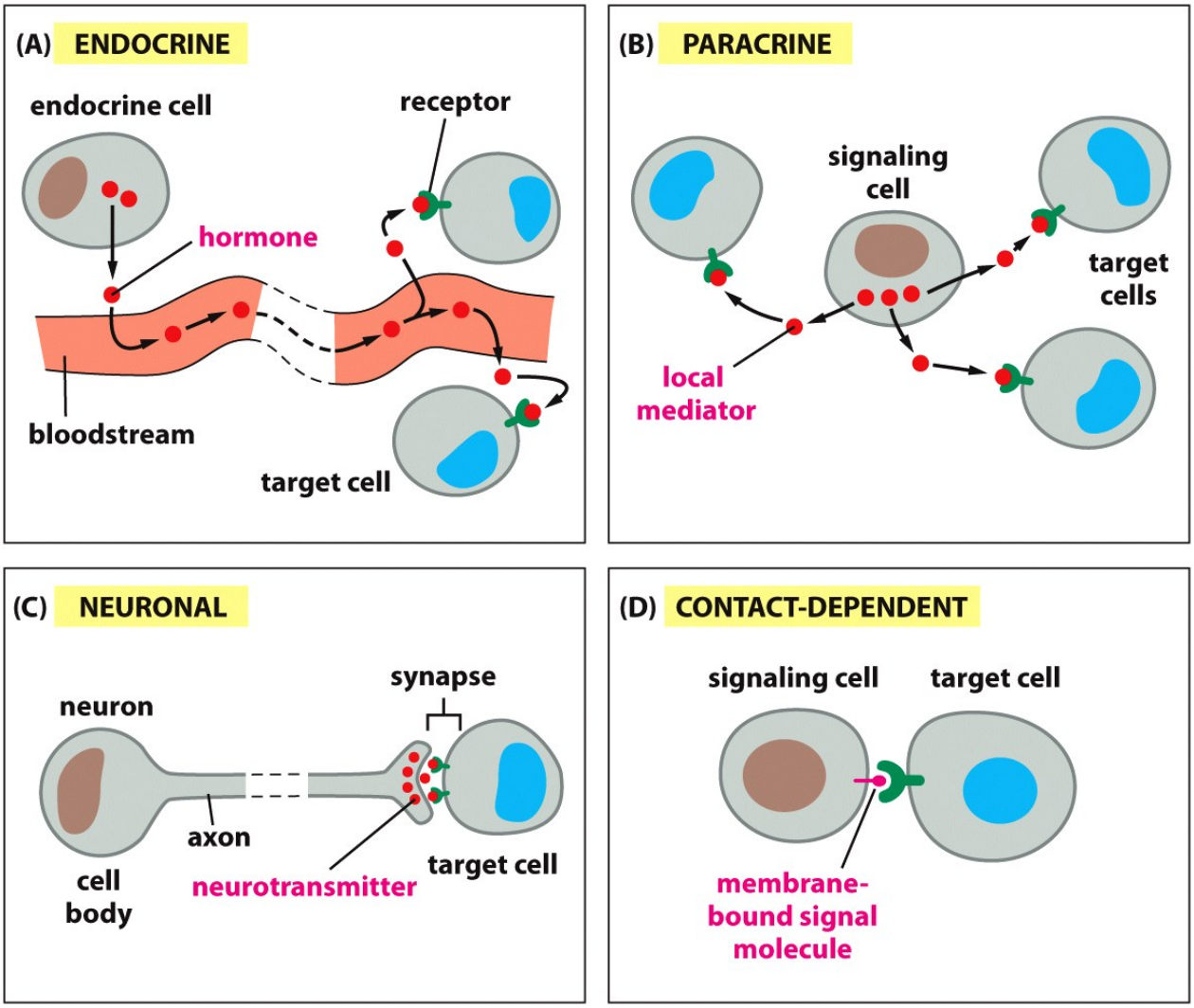
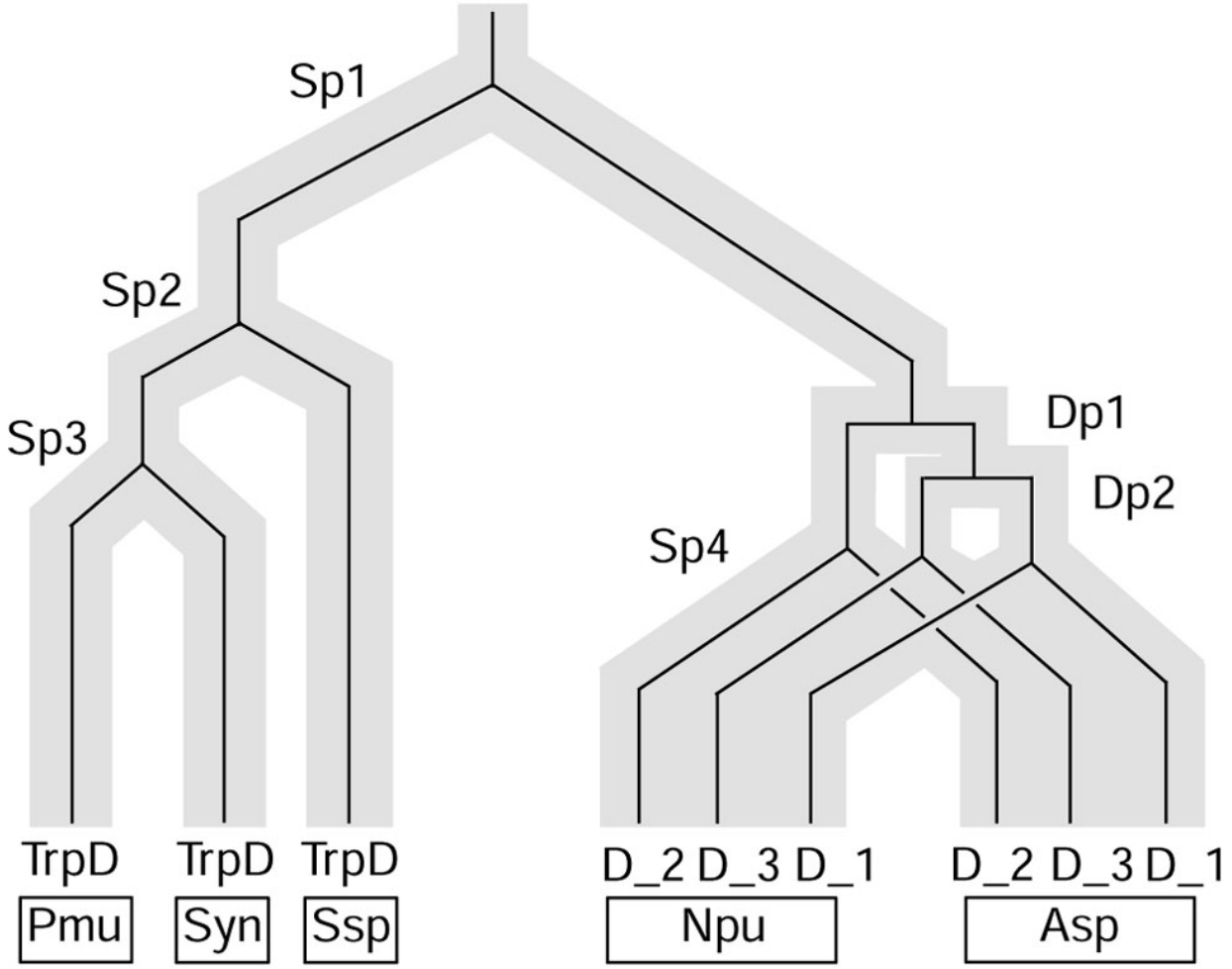


Figure 16-3 Essential Cell Biology 3/e (© Garland Science 2010)

Orthologs and paralogs



Orthologs (left) are specialized variants of the same gene whereas **paralogs** (right) are results of duplication which may evolve in completely different genes.

Germ line and somatic cells

- Germ line: every mutation will change progeny
- Somatic line: mutations have no direct effect on progeny
- However, phenocopies show a way of transition between these two lines

Himalayan rabbit: example of phenocopies

Point mutations: neutral and lethal

- Neutral mutations either will not change protein, or change insignificant part of it
- Lethal mutations will not allow to leave progeny
- Typical mutation rate is 10^{-6}
- Simple mutations could be reversible

Regulatory mutations

- Simple point mutation could block expression of the gene
- Reverse mutation will unblock expression
- Lactose digestion in adults is an example of rapidly spreading mutation of this kind
- Regulatory mutations are simpler to reverse

Evolution through regulatory genes

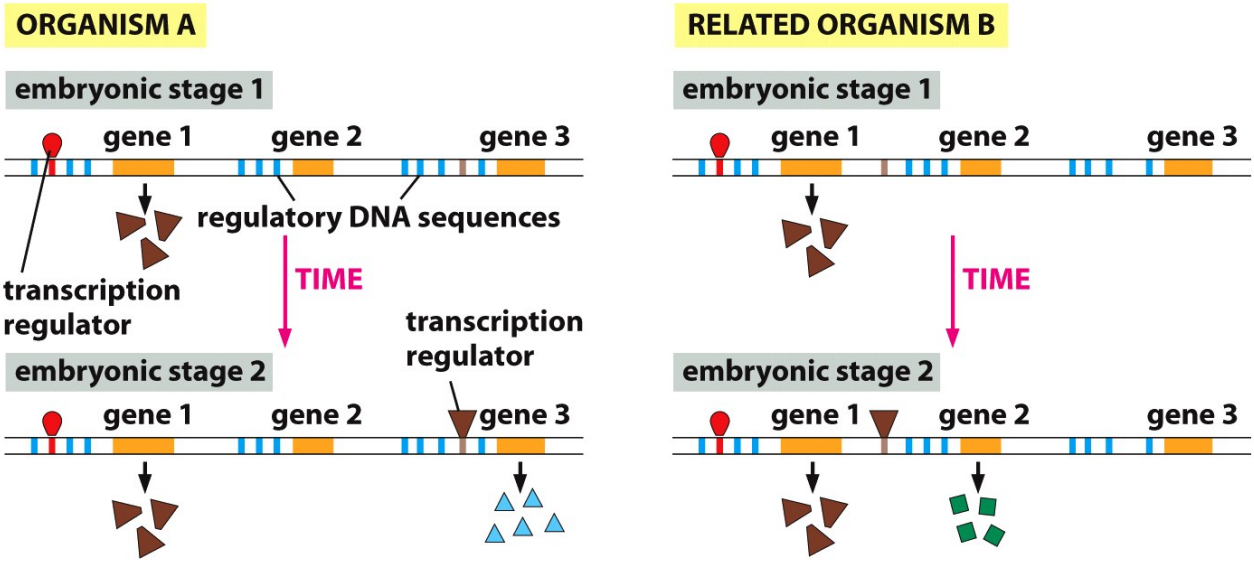


Figure 9-7a Essential Cell Biology 3/e (© Garland Science 2010)

Rise of gene families

- Gene duplication (e.g., in crossover) will ultimately result in accepting of neutral (at first) and non-neutral changes (later)
- This is a gene divergence
- Gene families (e.g., globin family)are mostly results of these divergencies

Gene duplication in crossover

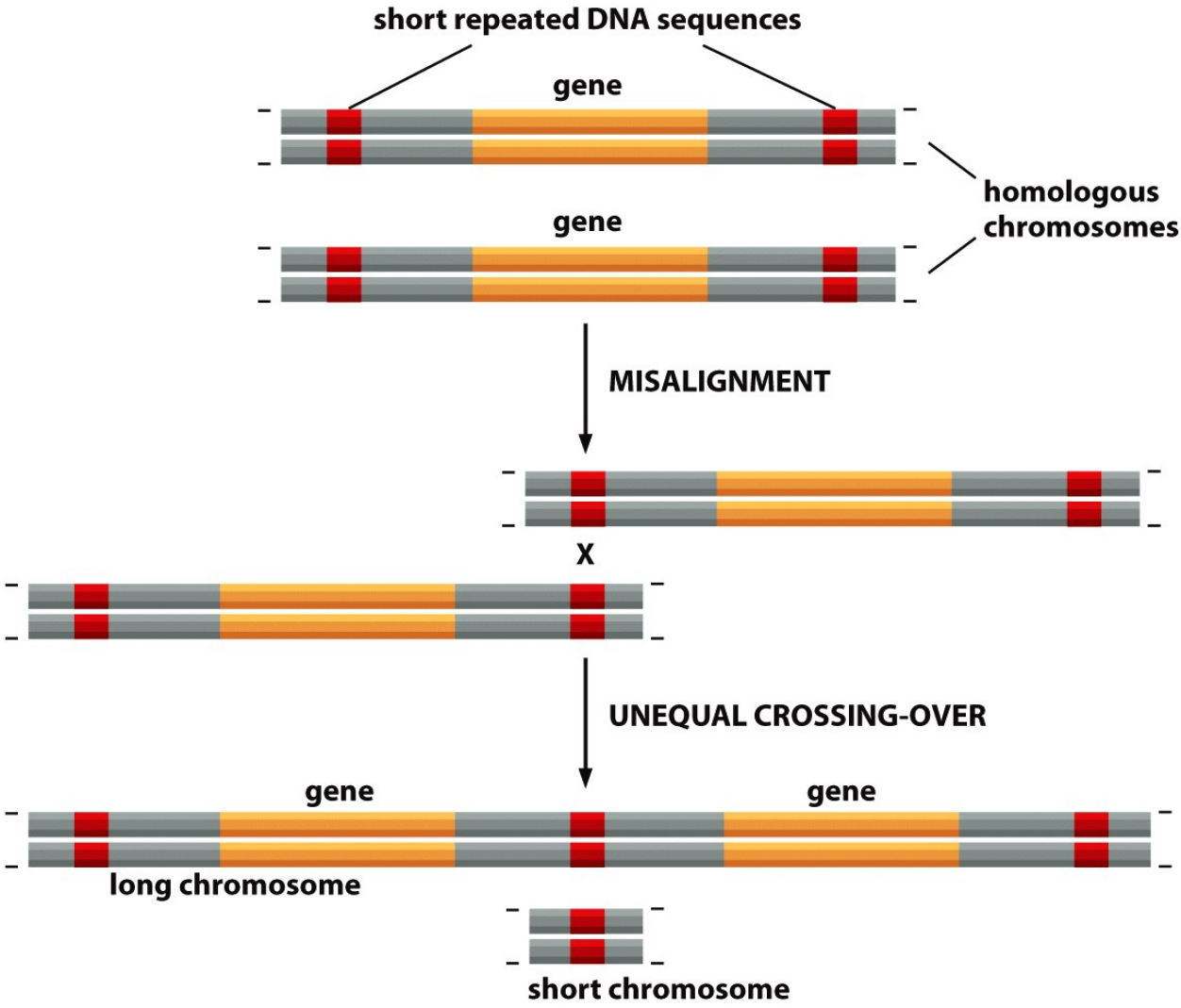


Figure 9-9 Essential Cell Biology 3/e (© Garland Science 2010)

Globine family

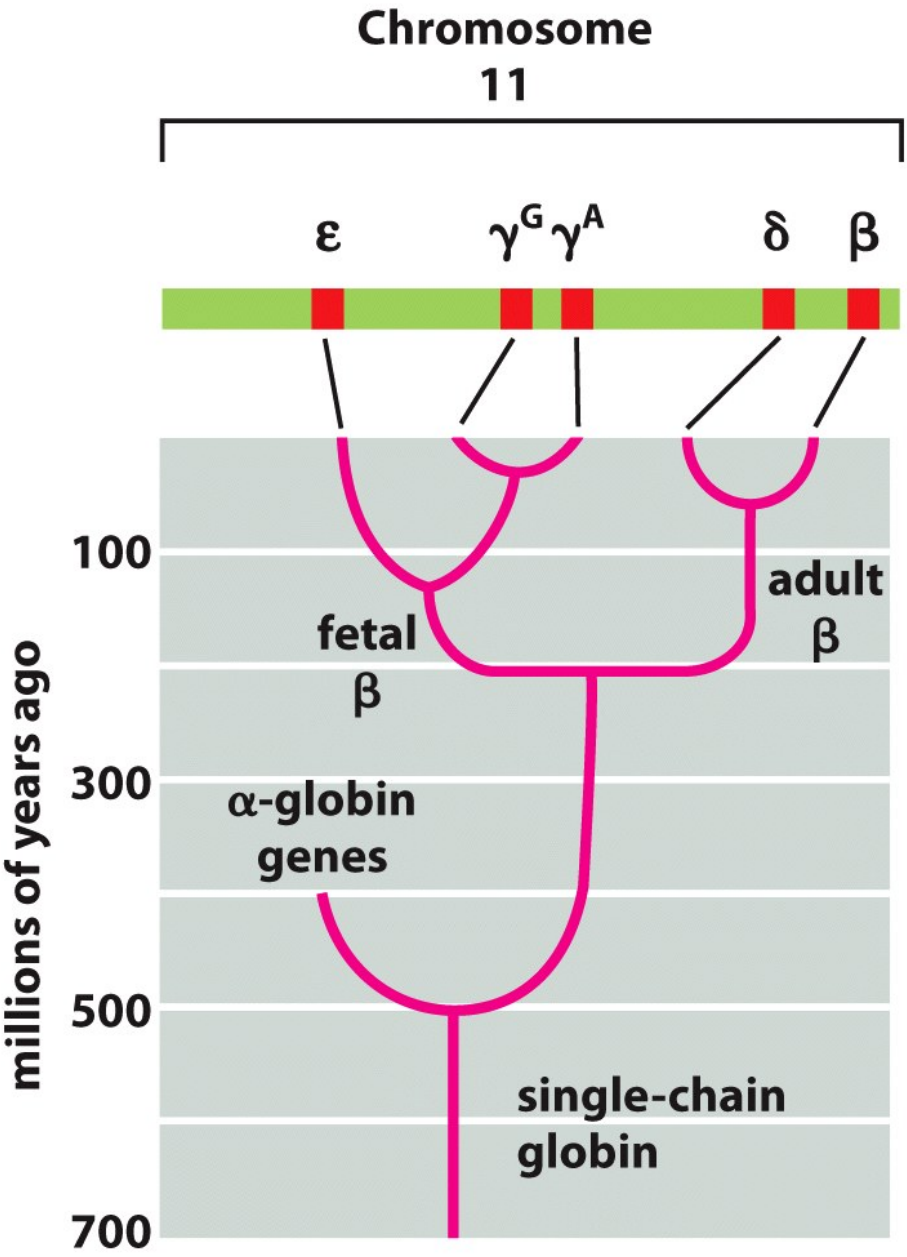
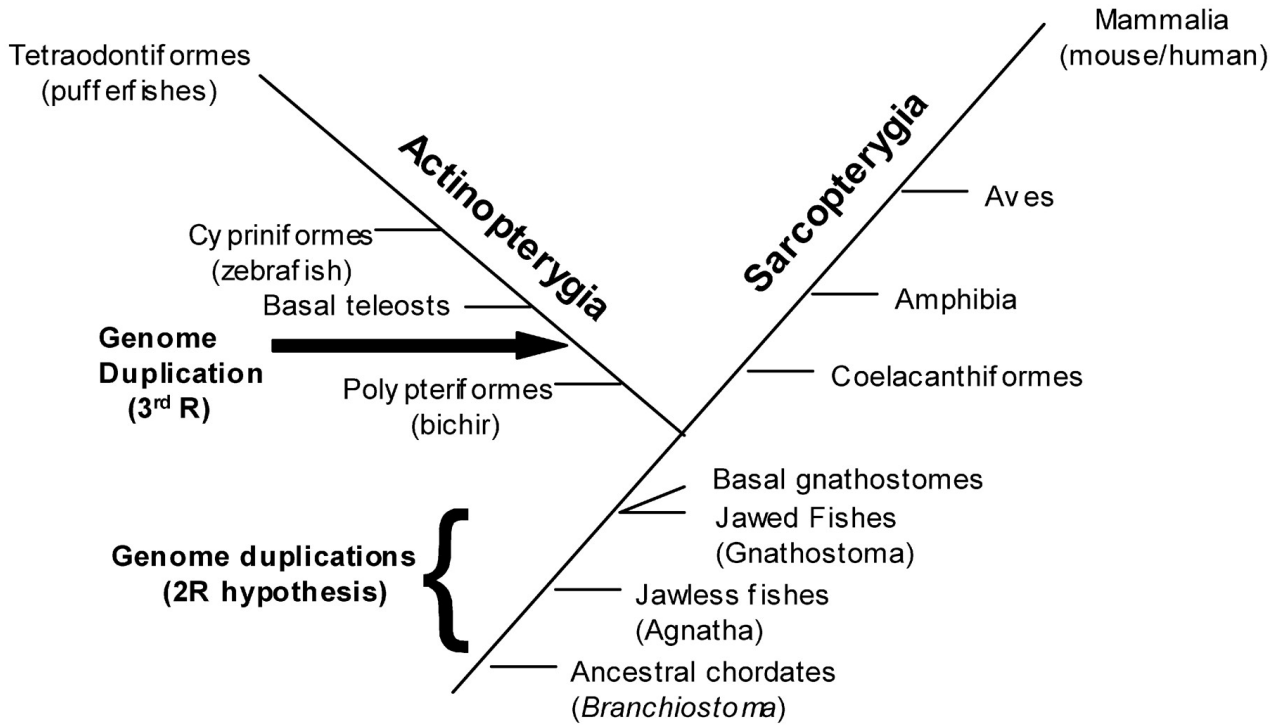


Figure 9-11 Essential Cell Biology 3/e (© Garland Science 2010)

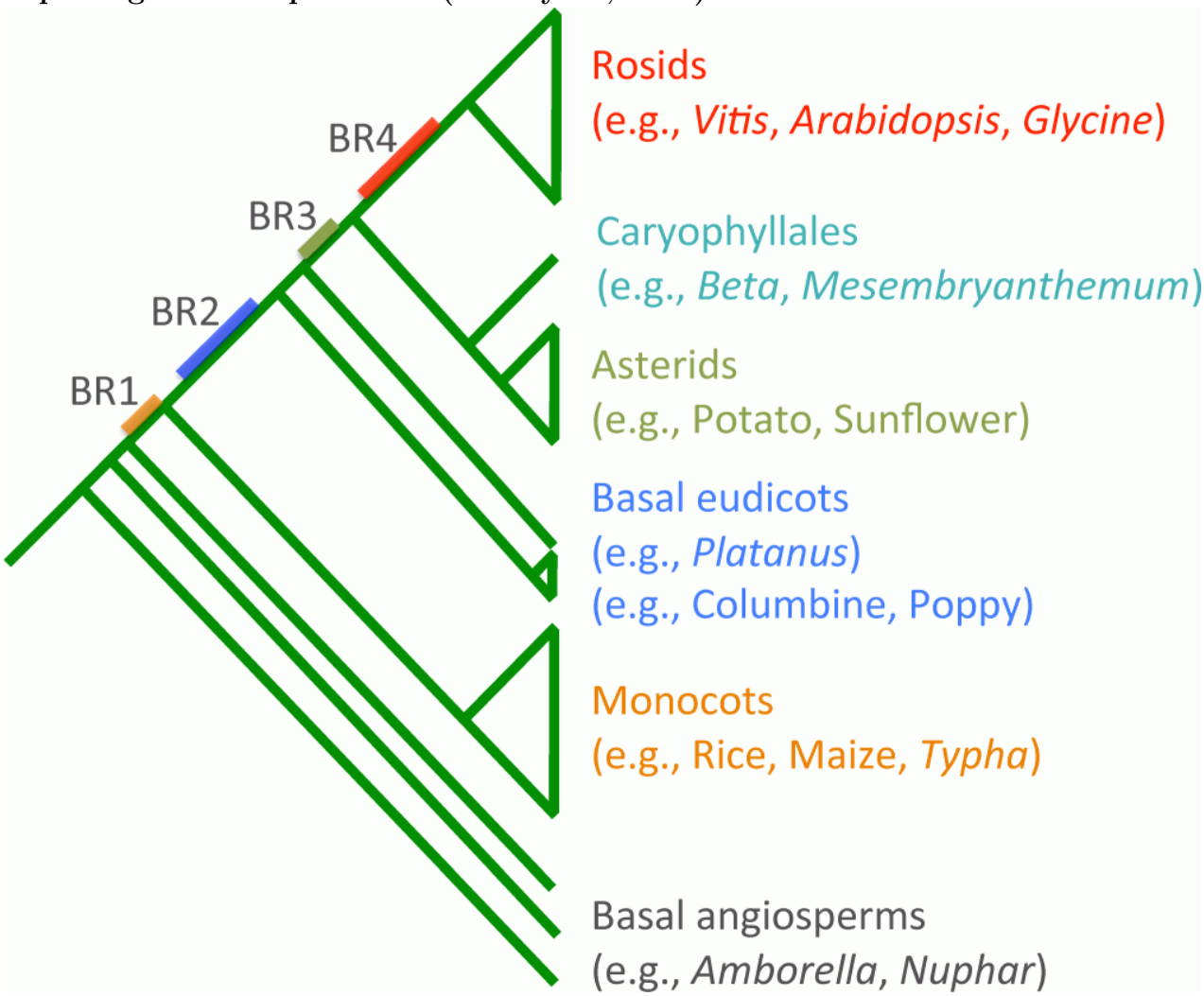
Whole genome duplications

- Whole genome duplication will immediately provide a "space" for new genes and even gene families
- Several major duplications makr evolution of vertebrates and angiosperms
- Duplications also occur in smaller lineages like *Xenopus* frogs or grasses

Chordate genome duplications

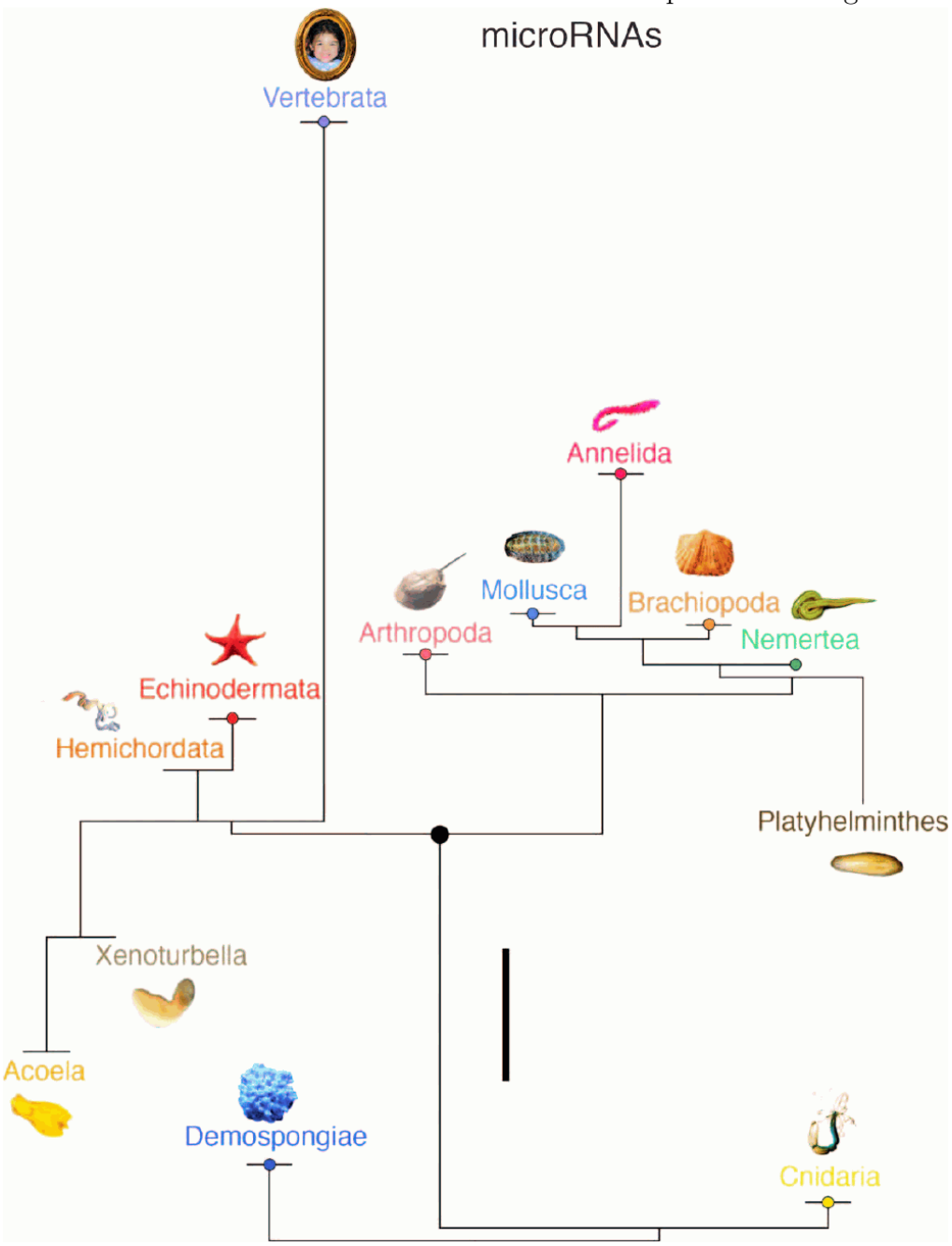


Angiosperm genome duplications (Jiao ey al., 2012)



Acquiring of miRNA in another way of increasing complexity

Increase and decrease of the total amount of miRNA per cell among animal phyla (Erwin et al., 2011)



New exons: recombinant duplications

- In a crossover, exons could be repeated by mistake ("unequal crossover")
- This will modify a gene, making new introns and exons

Exon duplication in crossover

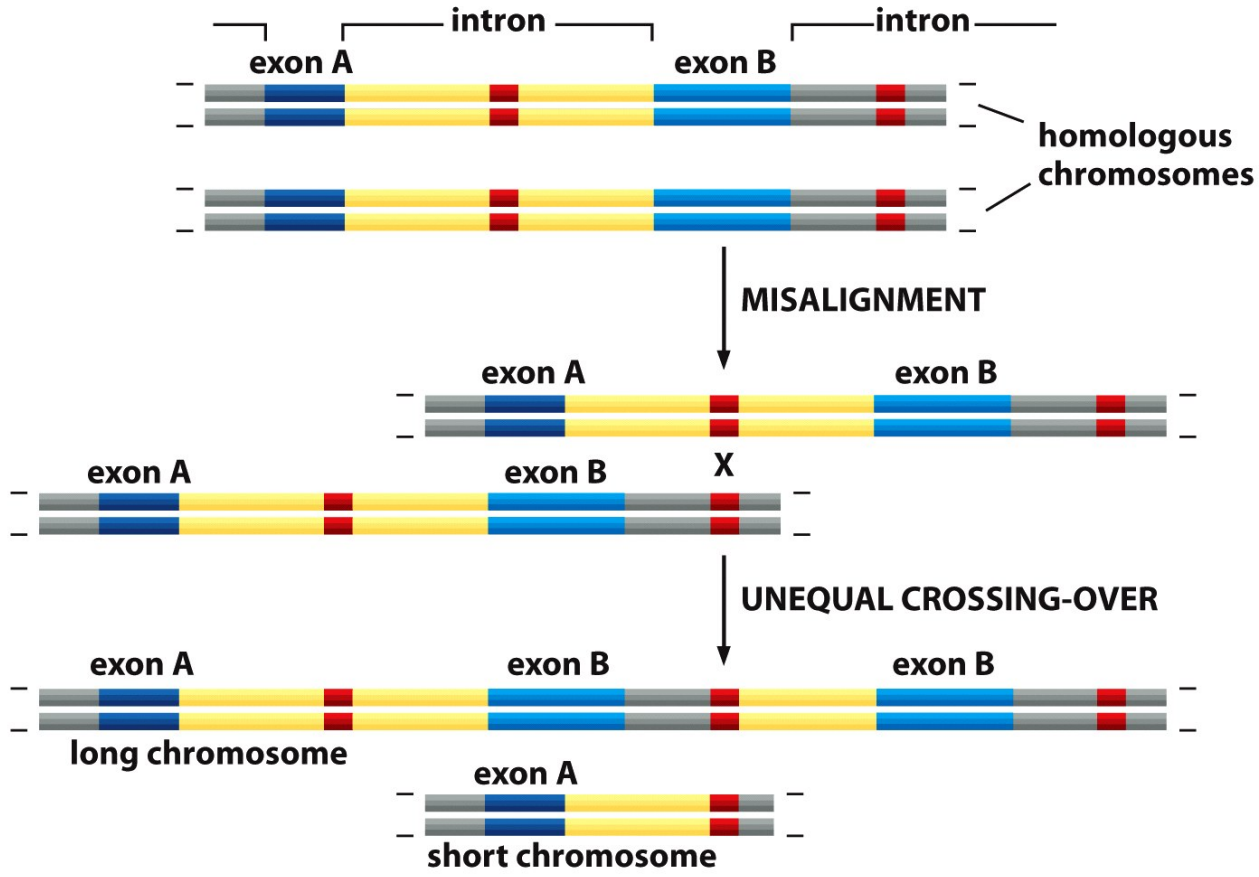


Figure 9-13 Essential Cell Biology 3/e (© Garland Science 2010)

Exon shuffling

- If exons correspond with protein domains, exon shuffling could produce a functional protein with different relative location of domains
- Many proteins arouse in this way

Mobile elements

- Mobile elements could modify existing genes through insertions, deletions and also translocations of bigger fragments
- They can add exons to genes

Mobile elements move exons

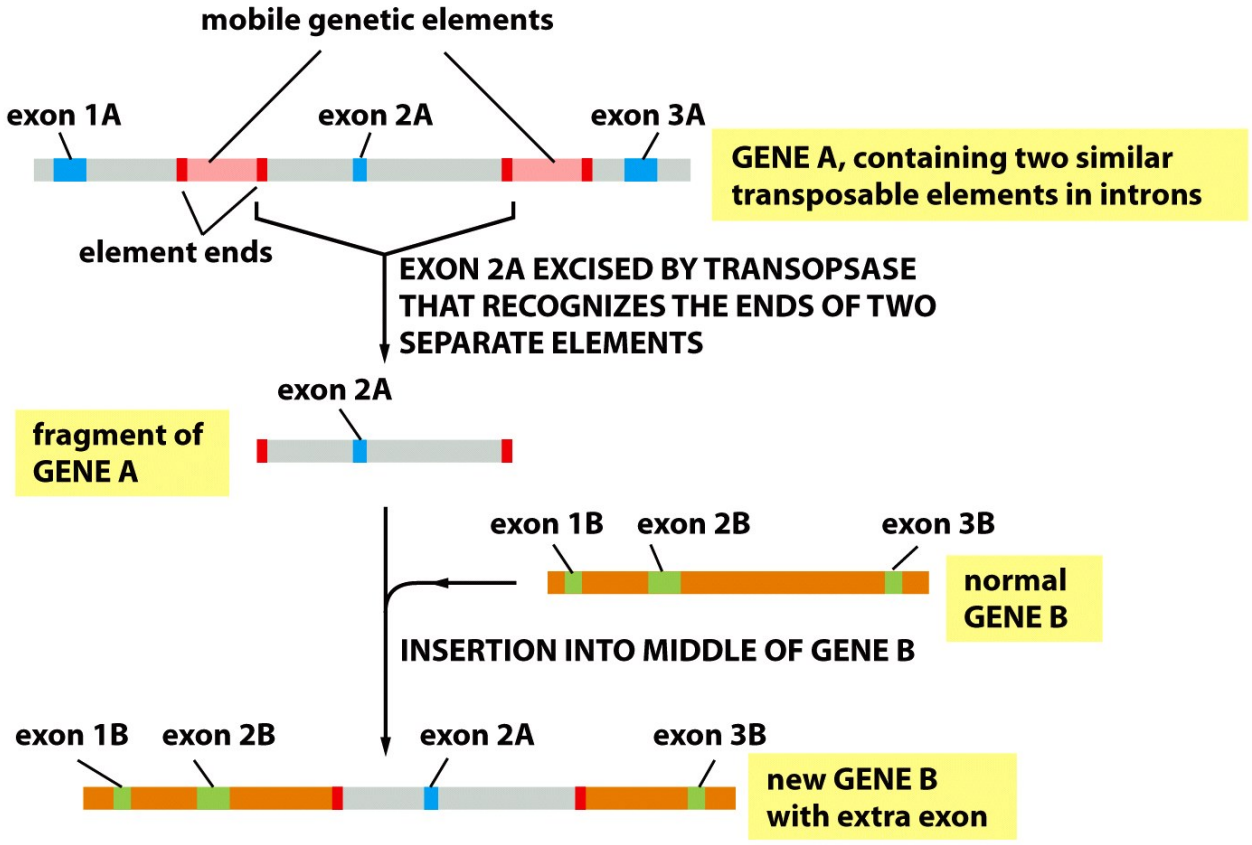
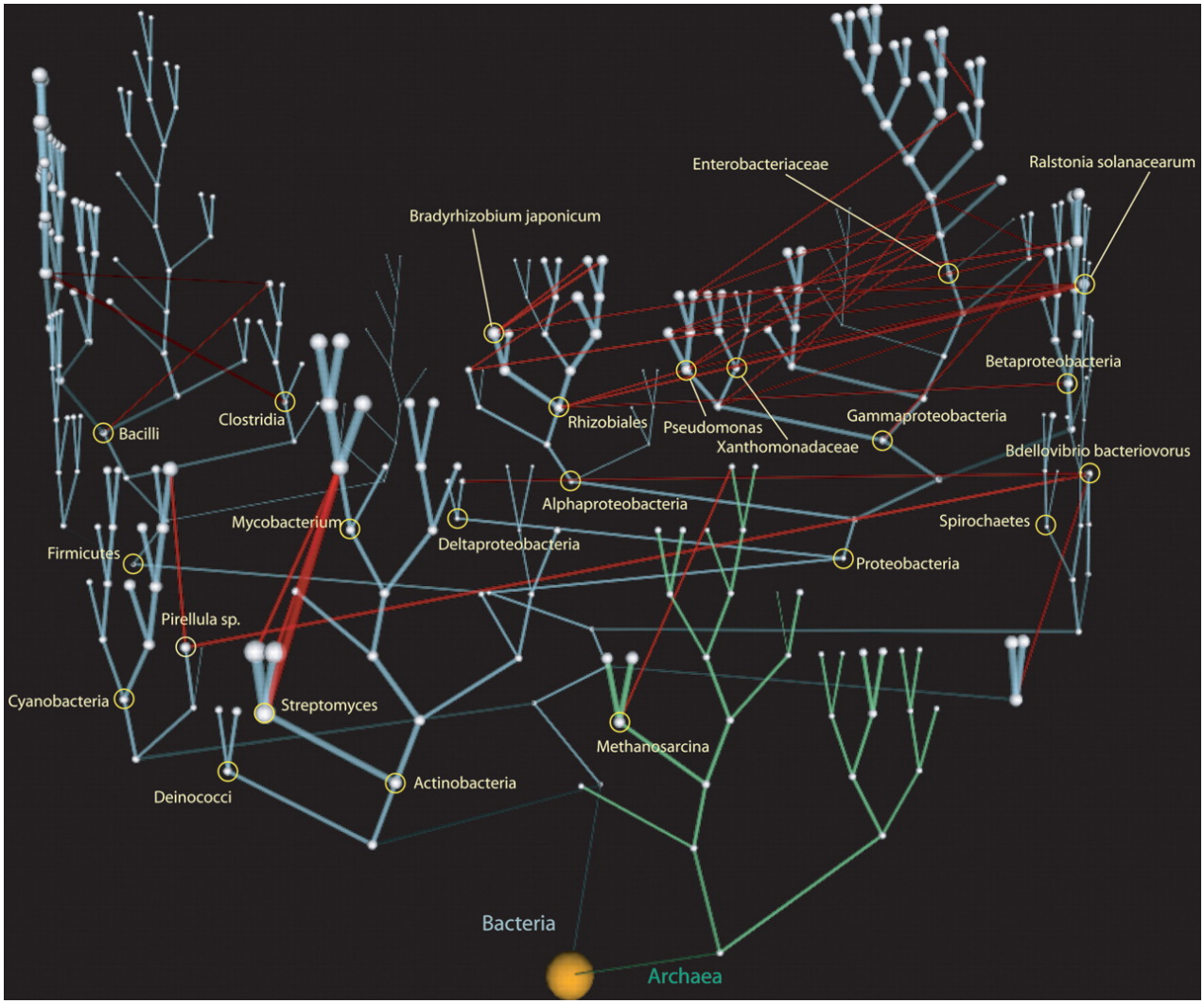


Figure 9-16 Essential Cell Biology 3/e (© Garland Science 2010)

HGT

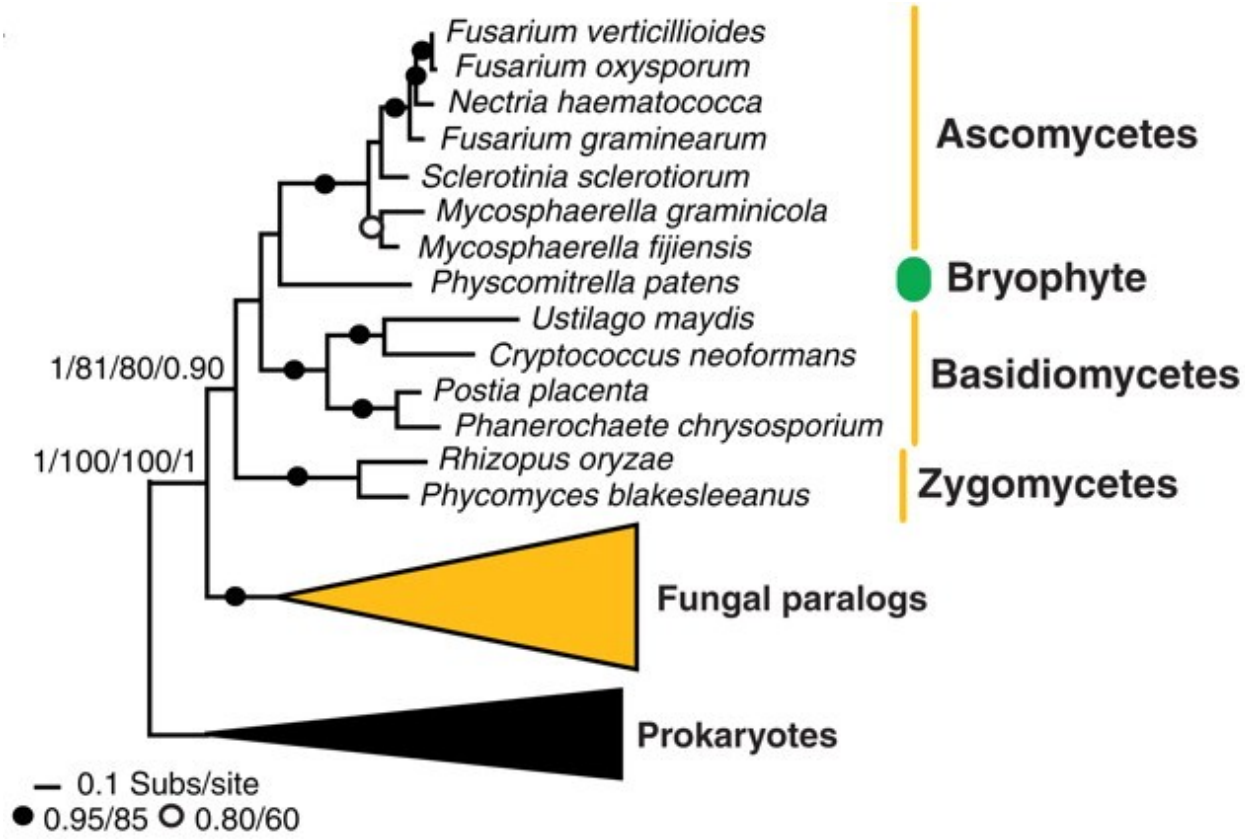
- Horizontal gene transfer occurs mostly in prokaryotes
- However, more in more HGT examples have been found in eukaryotes: plant-fungal HGTs, host-symbiont HGTs etc.

Network of Life concept

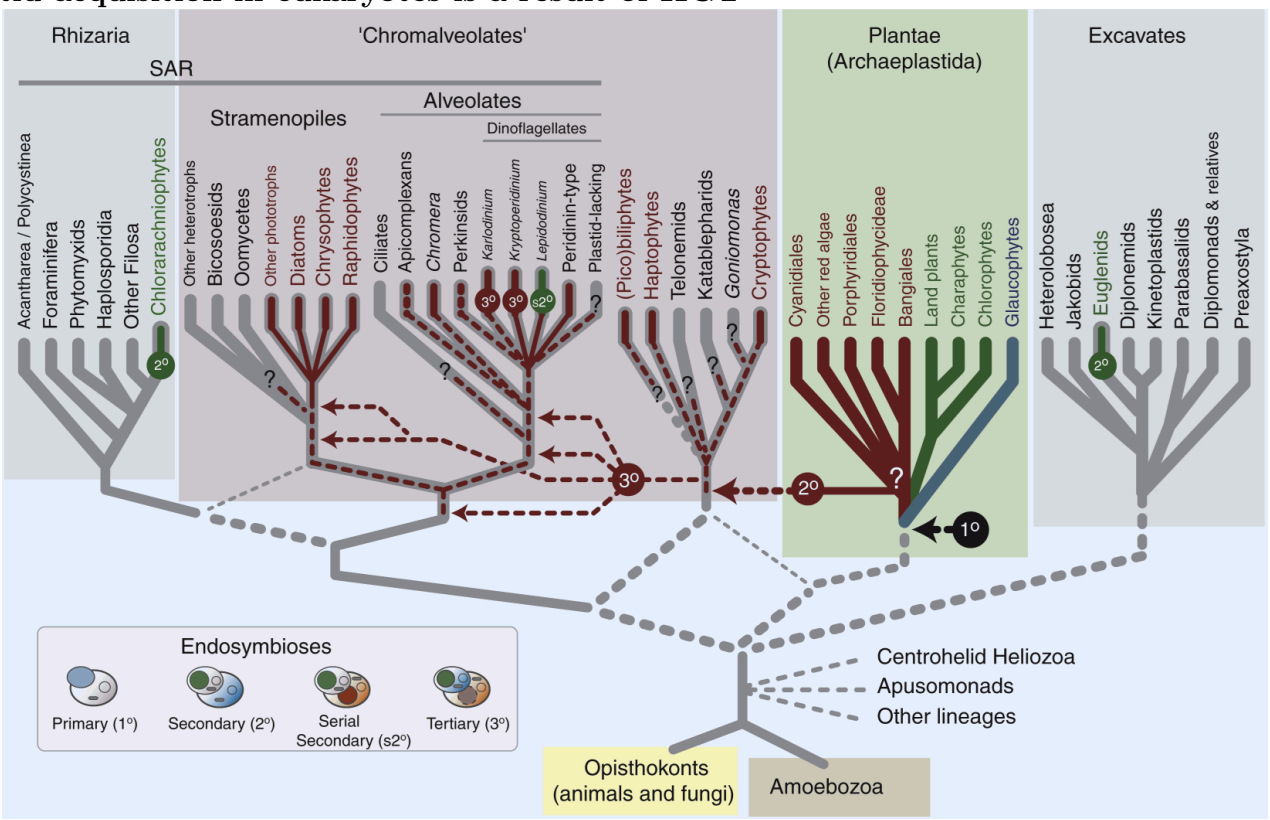


HGT is so frequent in prokaryotes that their ToL (tree of life) is more similar to network. More than 90% of genome maybe result of HGT!

L-fucose permease sugar transporter gene HGT



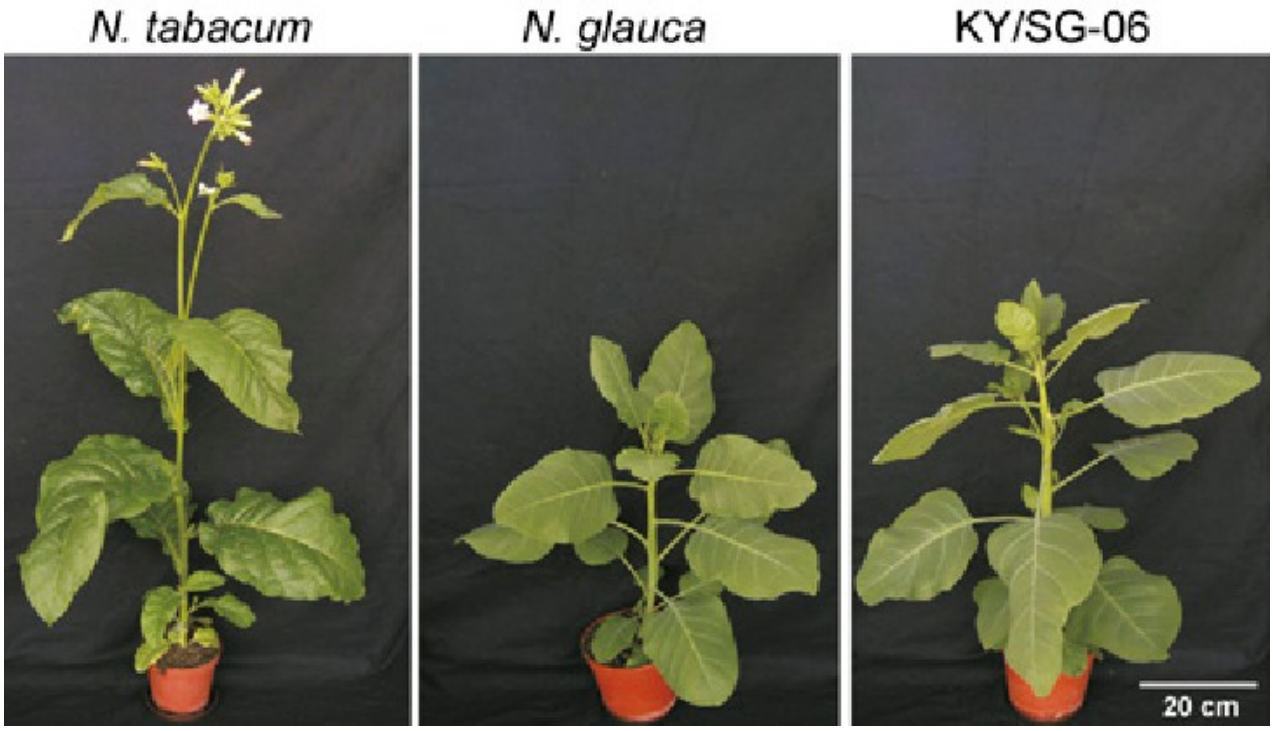
Plastid acquisition in eukaryotes is a result of HGT



Grafted plants may also capture plastids


A


D
1 cm



For explanations, see the Stegemann et al. (2012) paper (`http://www.pnas.org/content/109/7/2434.abstract`).

Final question (2 points)

How gene duplication helps in evolutionary process?

Summary

- Genome evolutionary processes include point mutations, duplications + divergencies, recombinations of gene parts and HGTs

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 9*.

Outline

Questions and answers

Previous final question: the answer

How gene duplication helps in evolutionary process?

- Duplication provides a "room" for the neutral changes and selection

Genes and genomes

.1 Evolution of genome

Making a tree from genes

- To understand, which gene variant is more ancient, we need a reference point (outgroup)
- To obtain a phylogeny, minimum three groups (taxa) are needed
- If we have tree taxa and an outgroup, we can construct a rooted phylogenetic tree

How to find ancestral sequence: using outgroup

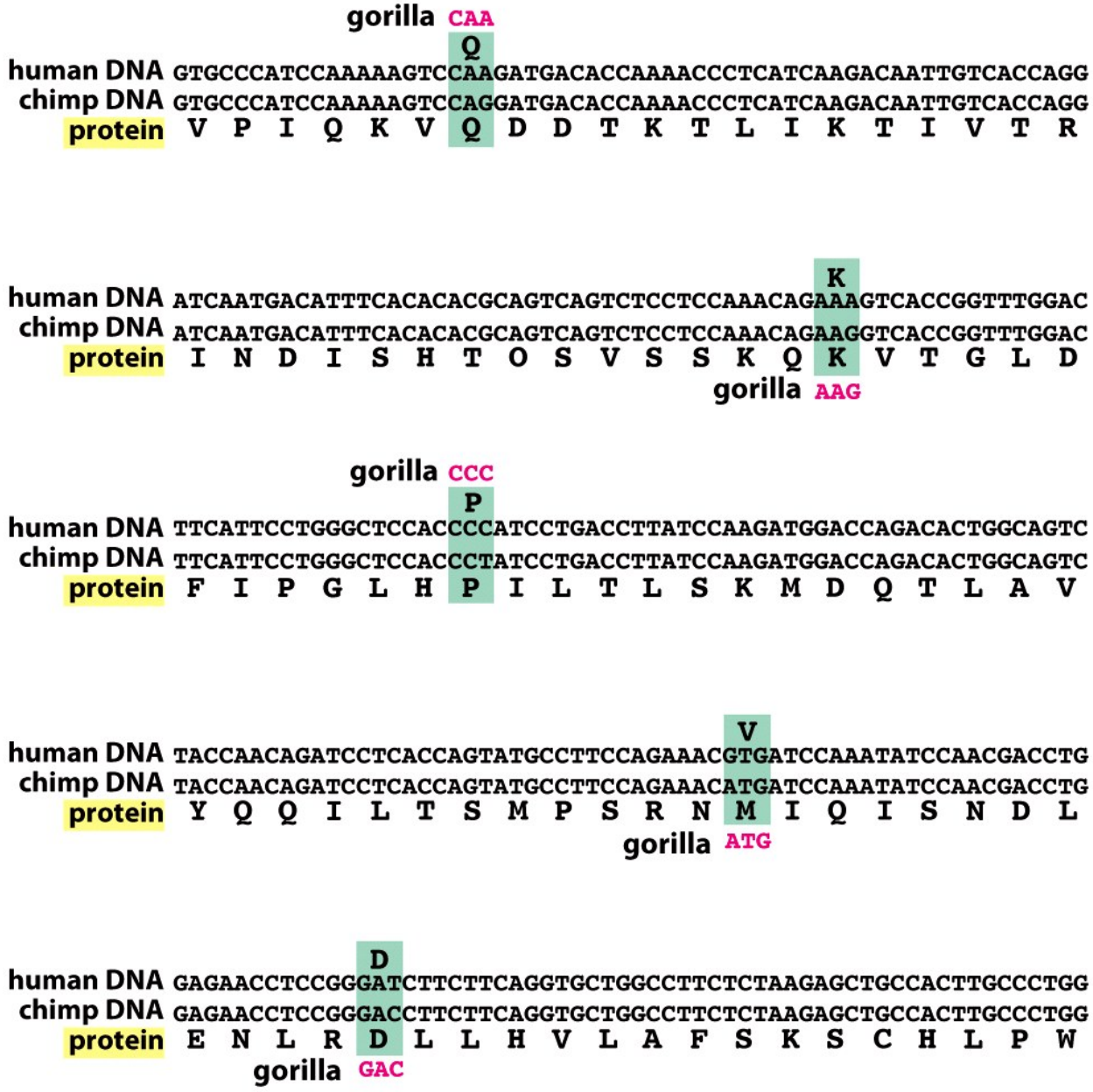
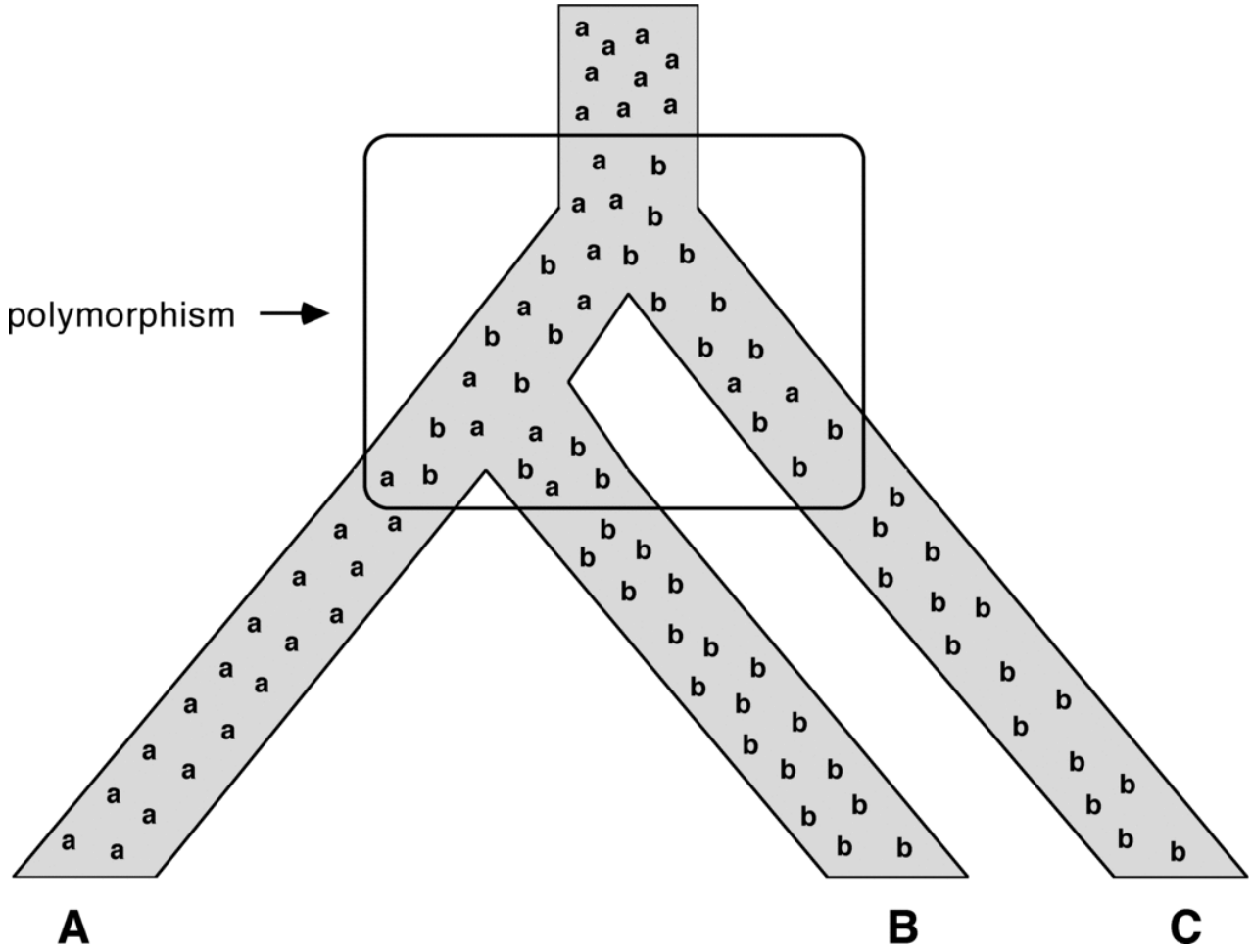


Figure 9-19 Essential Cell Biology 3/e (© Garland Science 2010)

Gene evolution is a mixture



Incomplete lineage sorting results in a mixture of ancestral and advanced genes in sister species.

Conserved synteny

- Some genes and/or gene groups are more prone to mutations
- Typically, there are multiple gene block which appear to be more stable
- These genes are often housekeeping ones, and therefore they are under constant pressure of purifying selection.

Conserved synteny

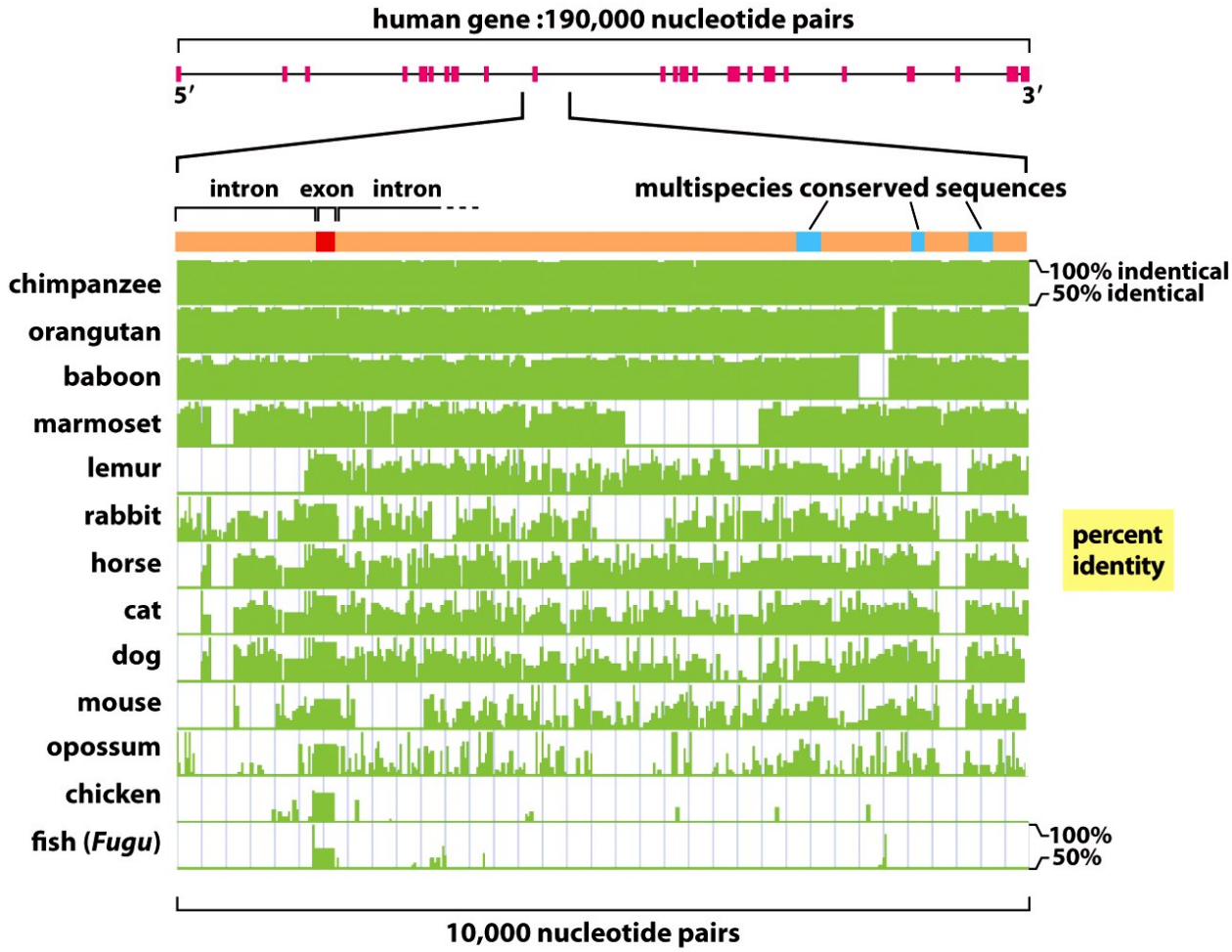
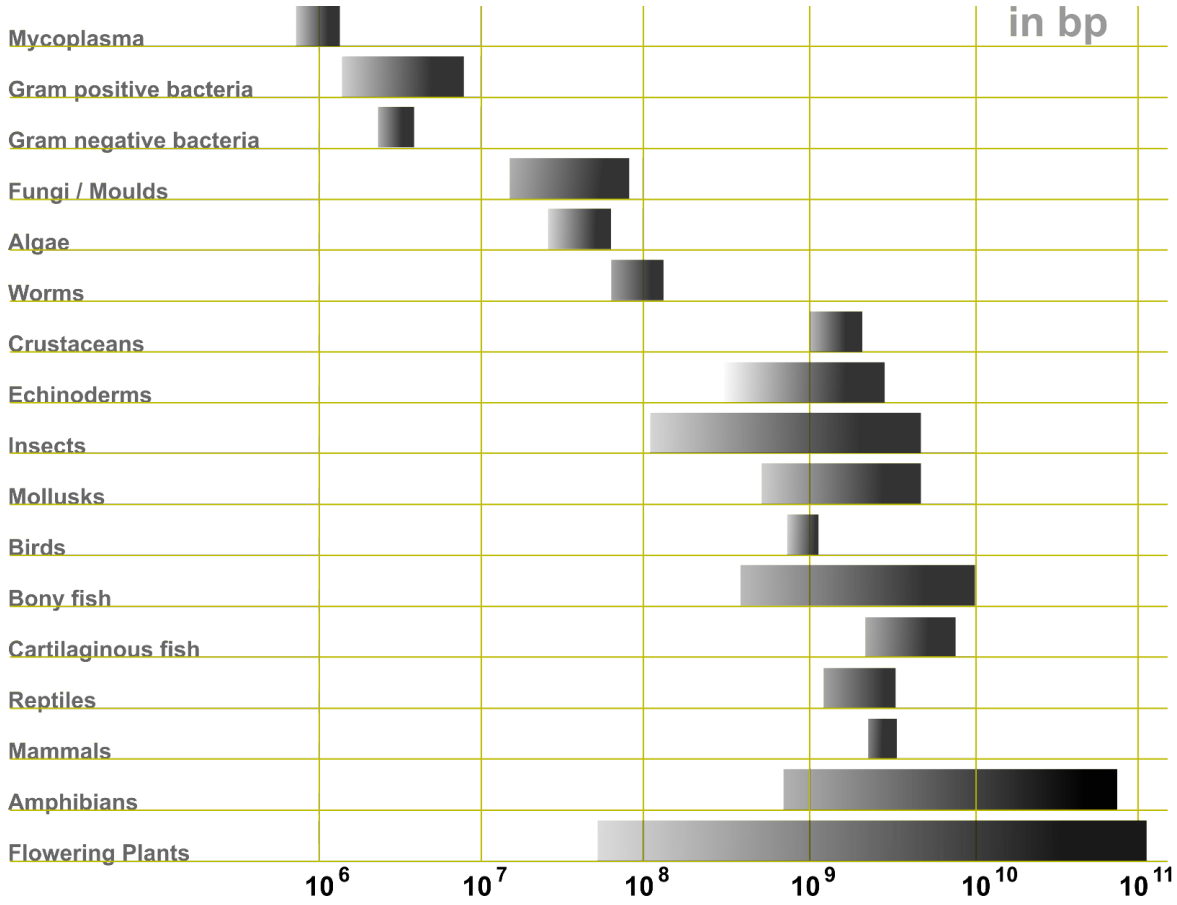


Figure 9-22 Essential Cell Biology 3/e (© Garland Science 2010)

Size differences between genomes

- Genome size typically increased during evolution time, but this is not a absolute rule
- Sometimes genome of "primitive" organisms are much bigger than genomes of "advanced" organisms (like hyge genomes of several amoebas)
- Some genomes experienced extensive compactization (*Fugu* fish, many parasites)

Genome sizes



Three main types of genomes

- Bacteria
- Archaea
- Eukaryotes

Ribosomal 5S RNA in three domains

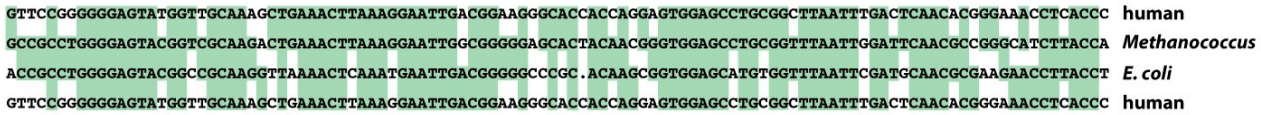


Figure 9-25 Essential Cell Biology 3/e (© Garland Science 2010)

Analyzing genes and genomes

.1 Human genome project

Human genome findings

- Only 25,000 genes
- Only 2% are protein-encoding or regulatory
- Only 1% are unique: 50 sites of human-accelerated changes

Pseudogenes

- 20,000 in human genome
- Closely resemble the functioning gene but are not expressed

Human genome content

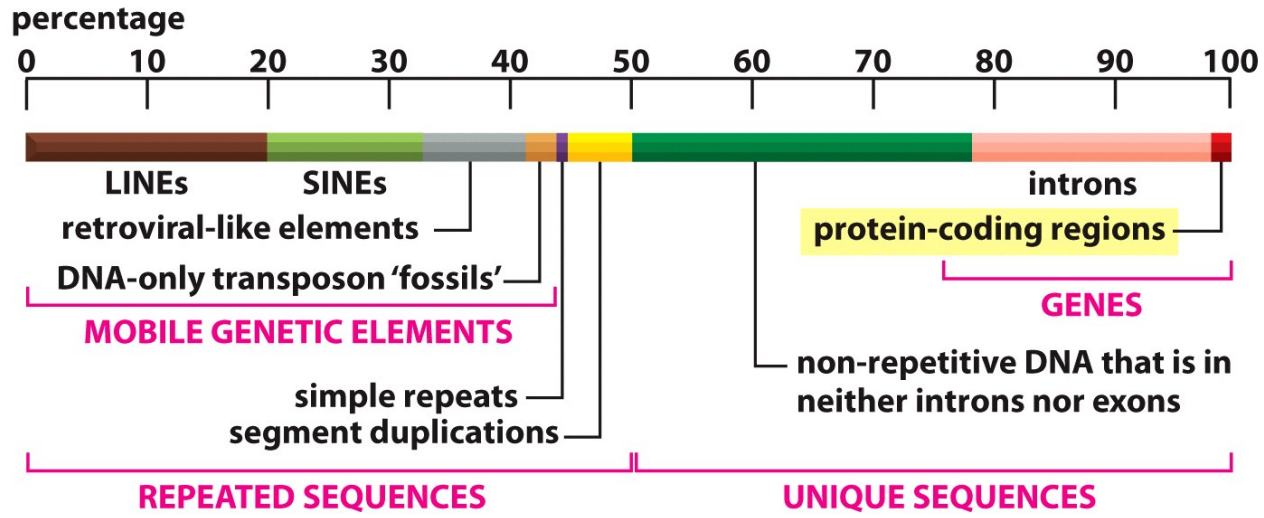


Figure 9-29 Essential Cell Biology 3/e (© Garland Science 2010)

Genome annotation basics

- ORFs (open reading frames): more than 100 bp without stop codons, start with ATG and stops with stop codons
- Examination of ESTs: mRNAs are isolated, converted to cDNAs, then expressed sequence tags (ESTs) from them are sequenced and searched through a genome
- BLAST search through nucleotide databases reveals all similar sequences

SNPs

- Single nucleotide polymorphisms (SNPs) could be individual characters
- CA repeats and other repetitive sequences like microsatellites are freely mutable
- SNPs and CAs and microsats are basics for DNA fingerprinting

Single nucleotide polymorphisms (SNPs)

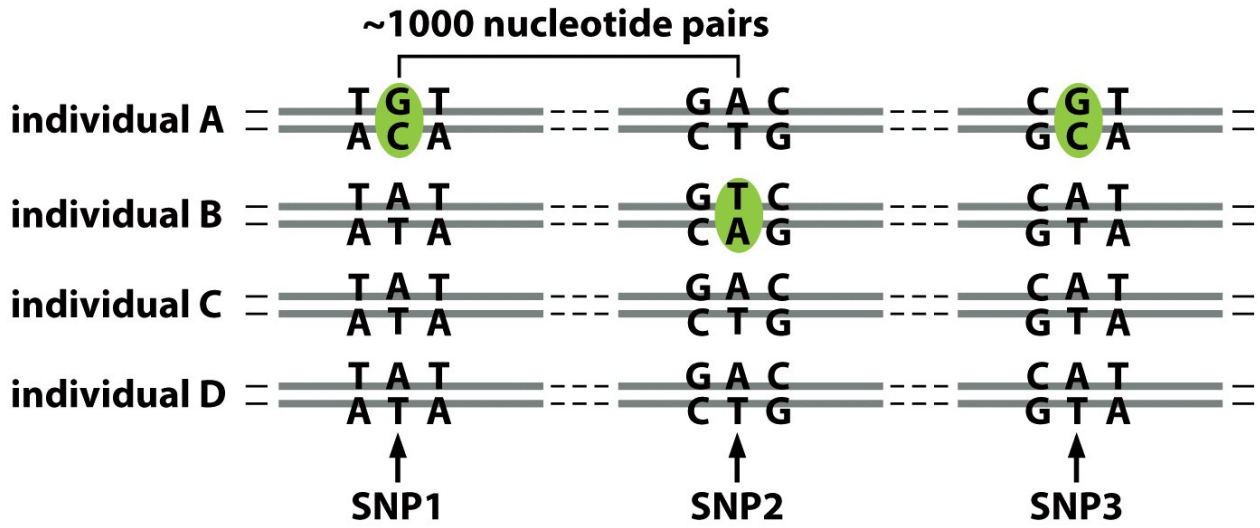


Figure 9-31 Essential Cell Biology 3/e (© Garland Science 2010)

Things which should be deciphered

- Genetic "dark matter"
- Gene content is much more similar across animal groups than was previously thought
- Alternative splicing

Fly *Dscam* gene: ways of alternative splicing

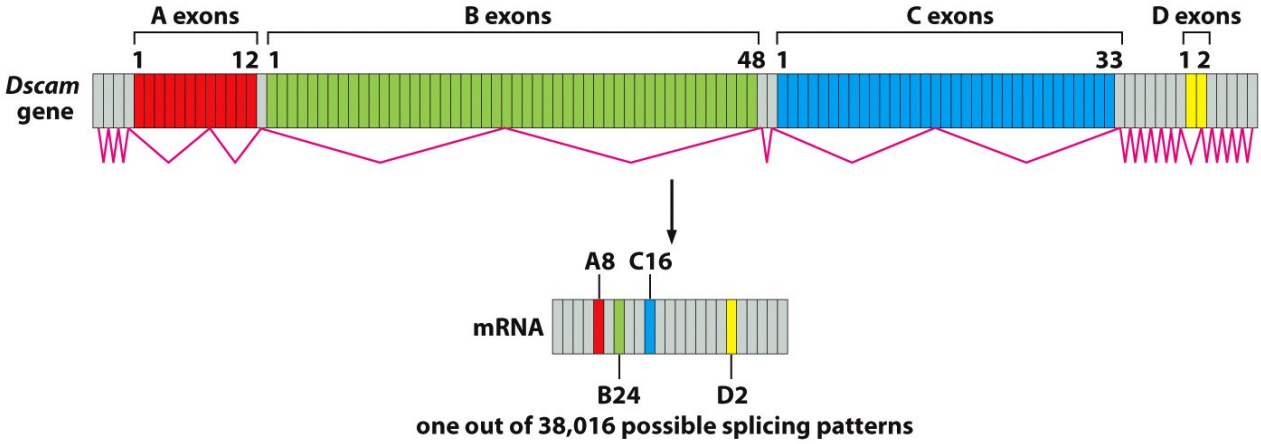


Figure 9-32 Essential Cell Biology 3/e (© Garland Science 2010)

.2 Analysis of DNA

Restriction nucleases

- Often site-specific
- Stable

Restrictases

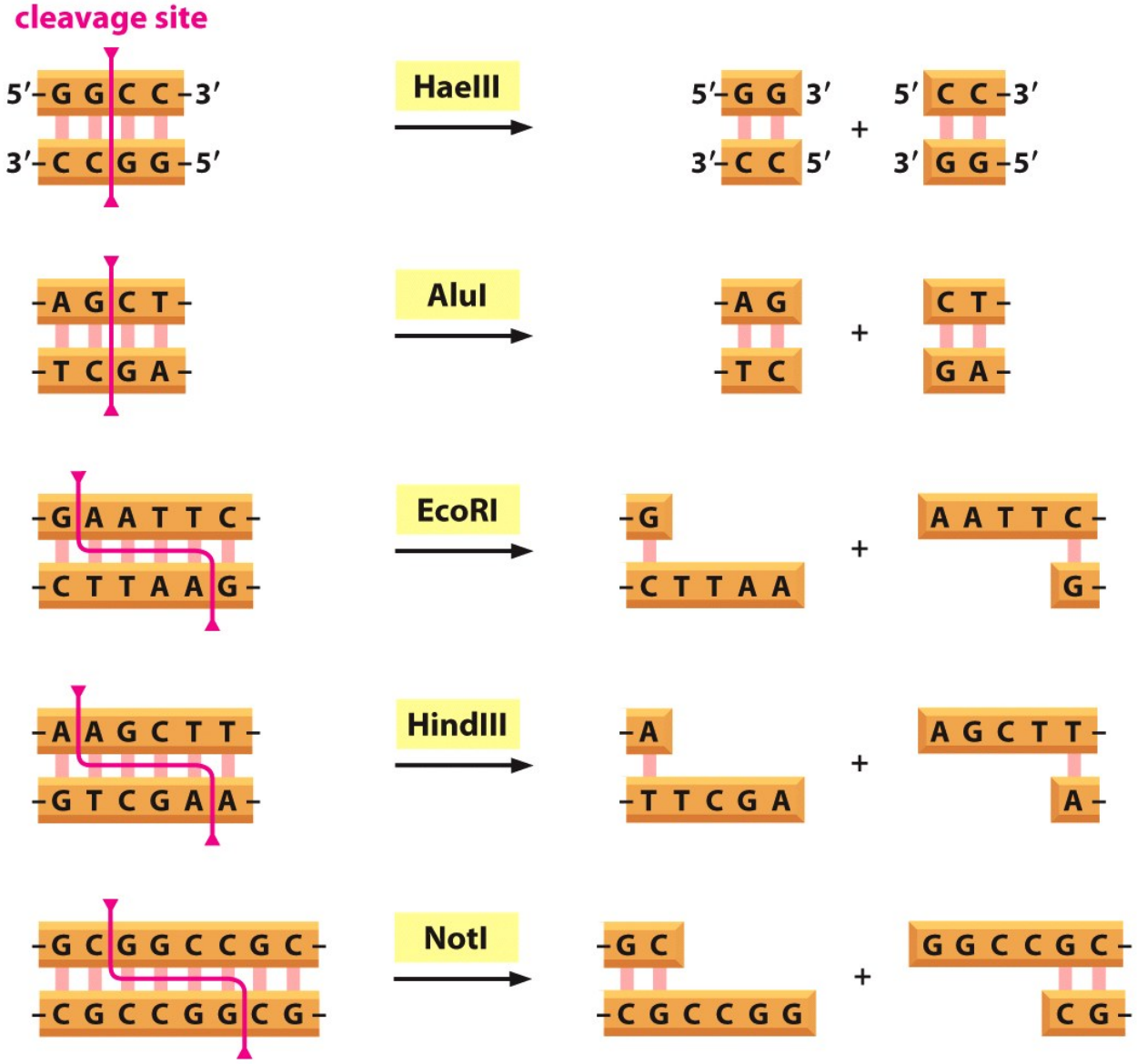
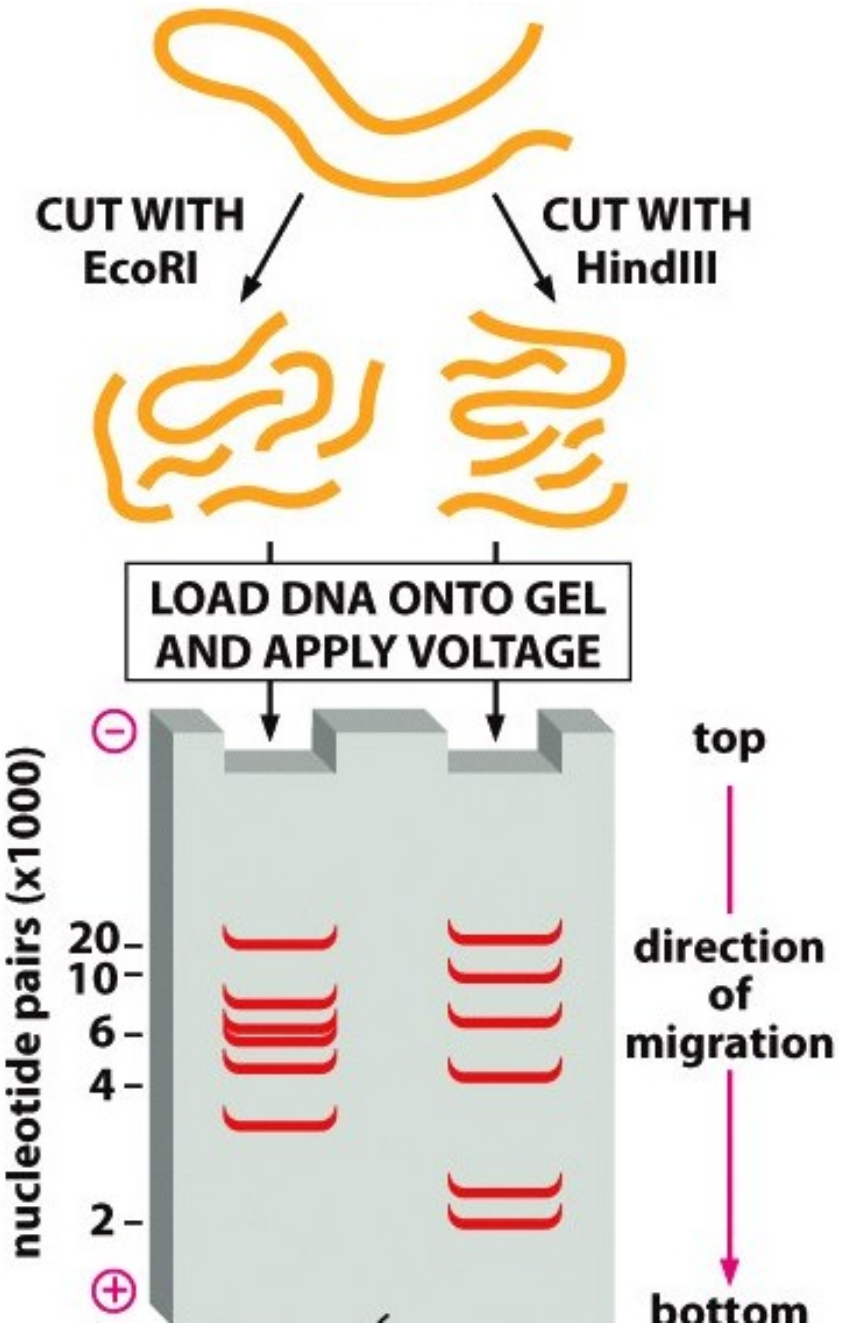


Figure 10-2 Essential Cell Biology 3/e (© Garland Science 2010)

Gel electrophoresis and RFLP

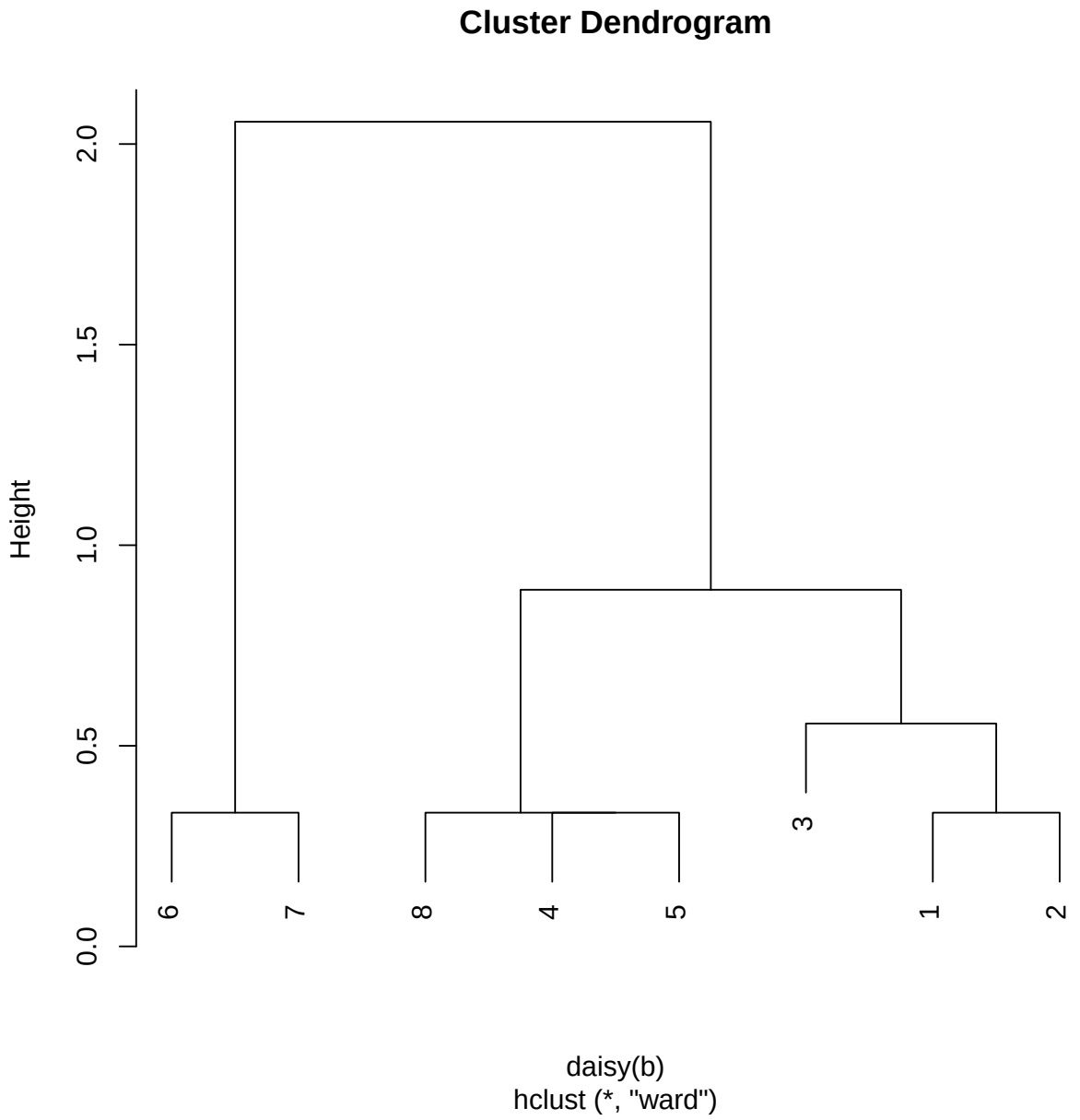
- DNA is normally negatively charged
- With two specific restrictases, we may achieve a specific gel images
- These are a restricted fragment length polymorphisms (RFLP)

Restriction fragments in gel



Restriction fragments polymorphisms, RFLPs

Cluster analysis of RFLP



DNA hybridization

- Denaturation, mixing and renaturation
- May provide a useful information itself: the percentage of hybridized DNA reflects similarity
- More advanced techniques like Southern blotting help to search specific DNA fragments

DNA hybridization

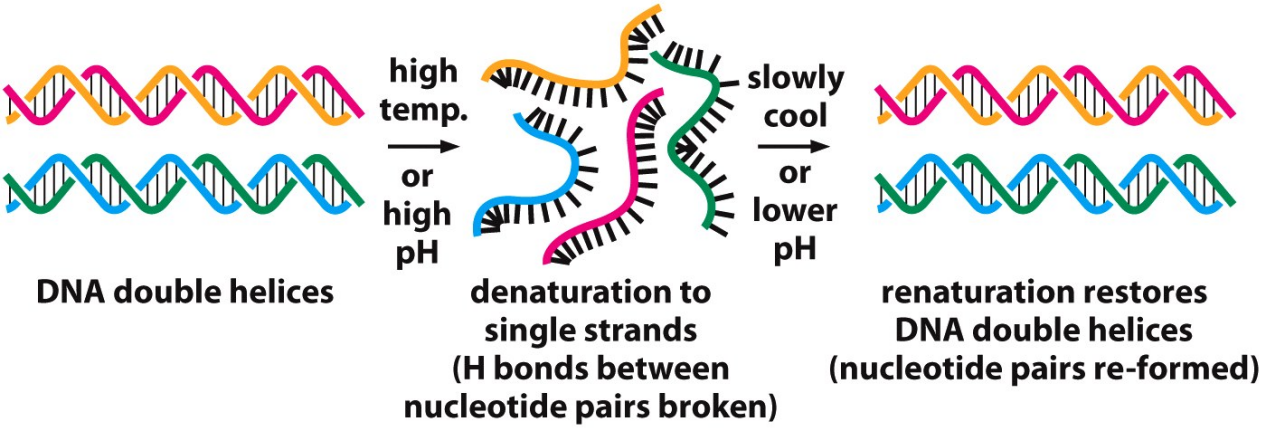
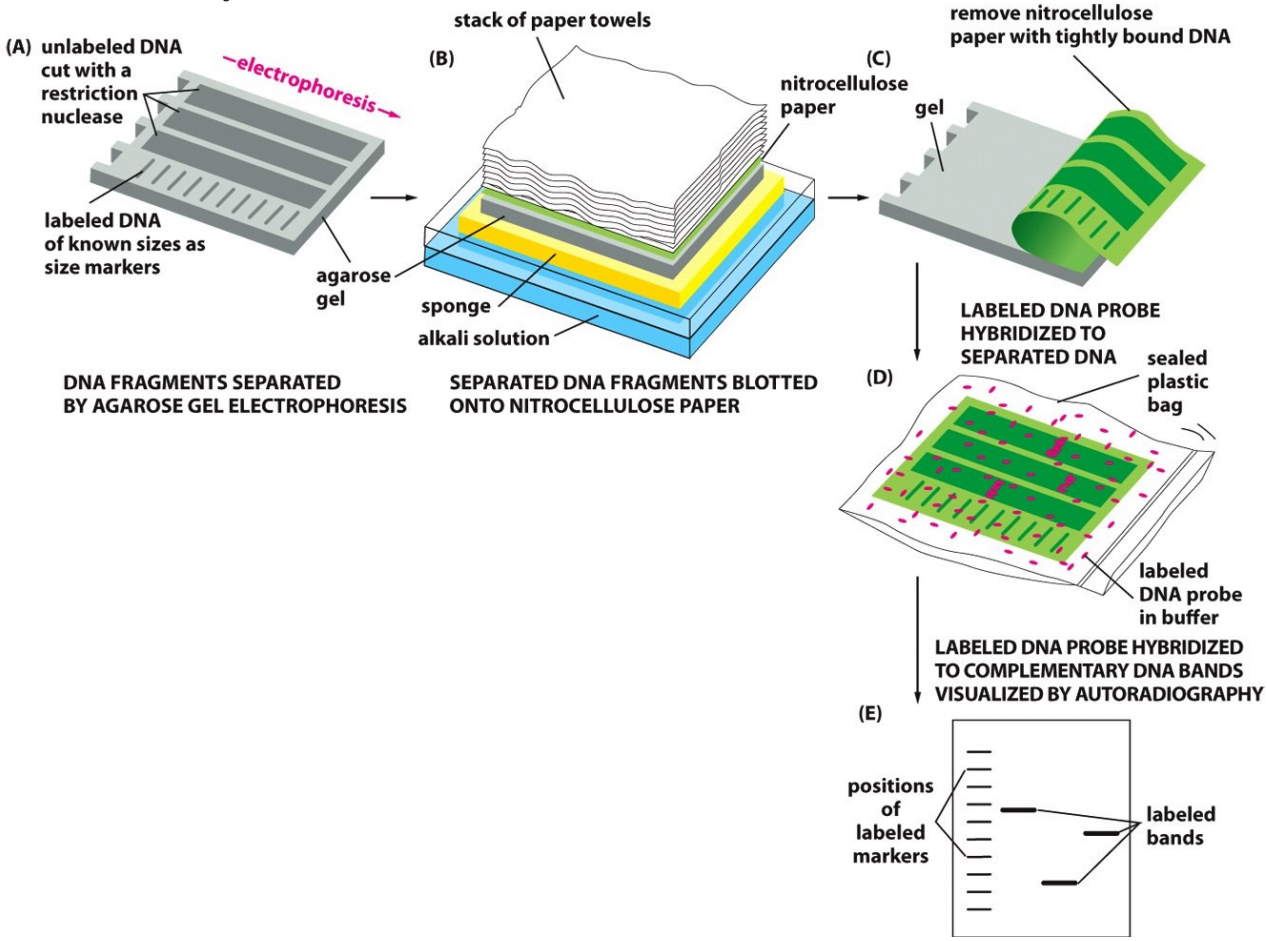


Figure 10-4 Essential Cell Biology 3/e (© Garland Science 2010)

Southern blot analysis: overview



Southern blot

- Southern blot employs both electrophoresis (for DNA fragments separation) and DNA hybridization (for the discovery of given DNA)
- Points of hybridization are visualized using autoradiography

Southern blot, step 1

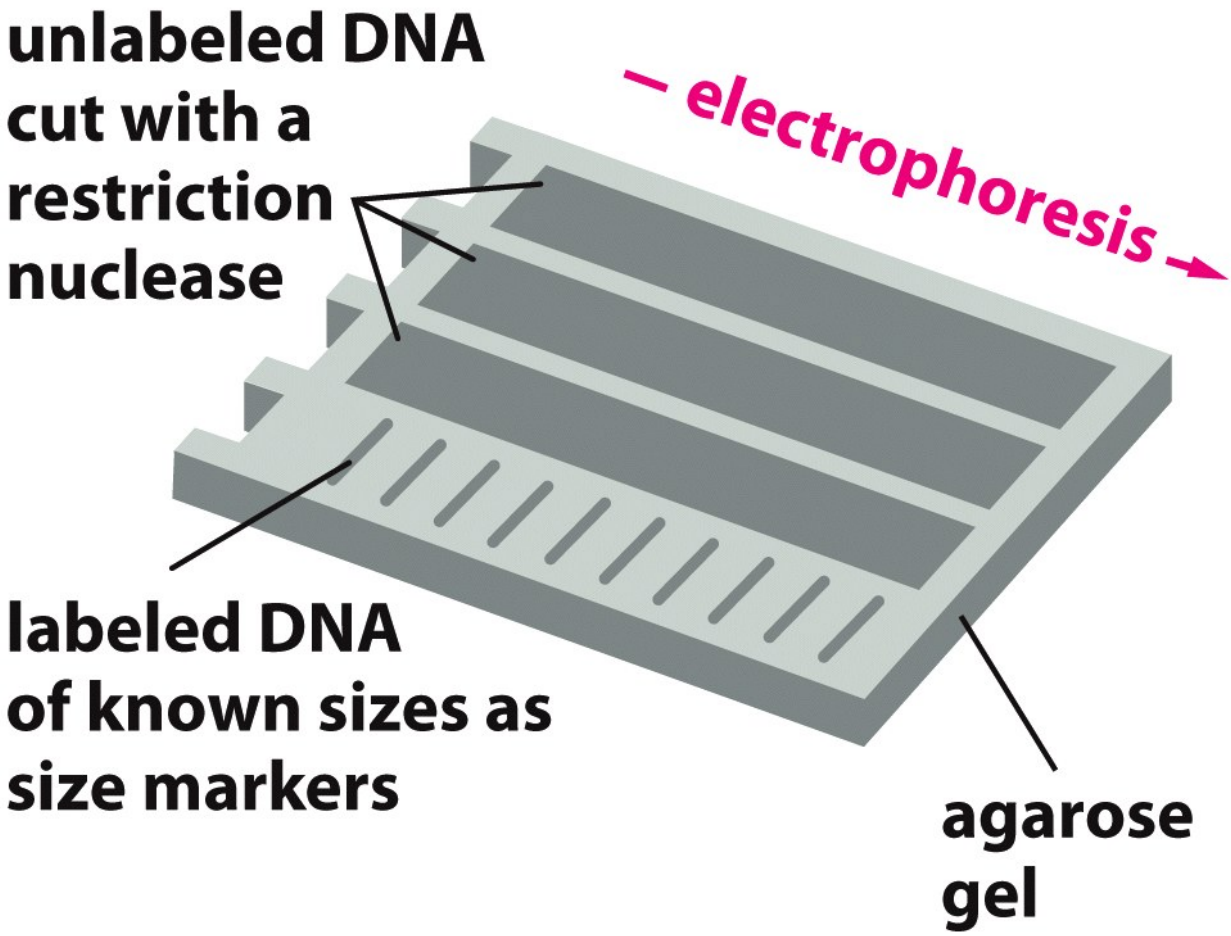


Figure 10-5 part 1 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Southern blot, step 2

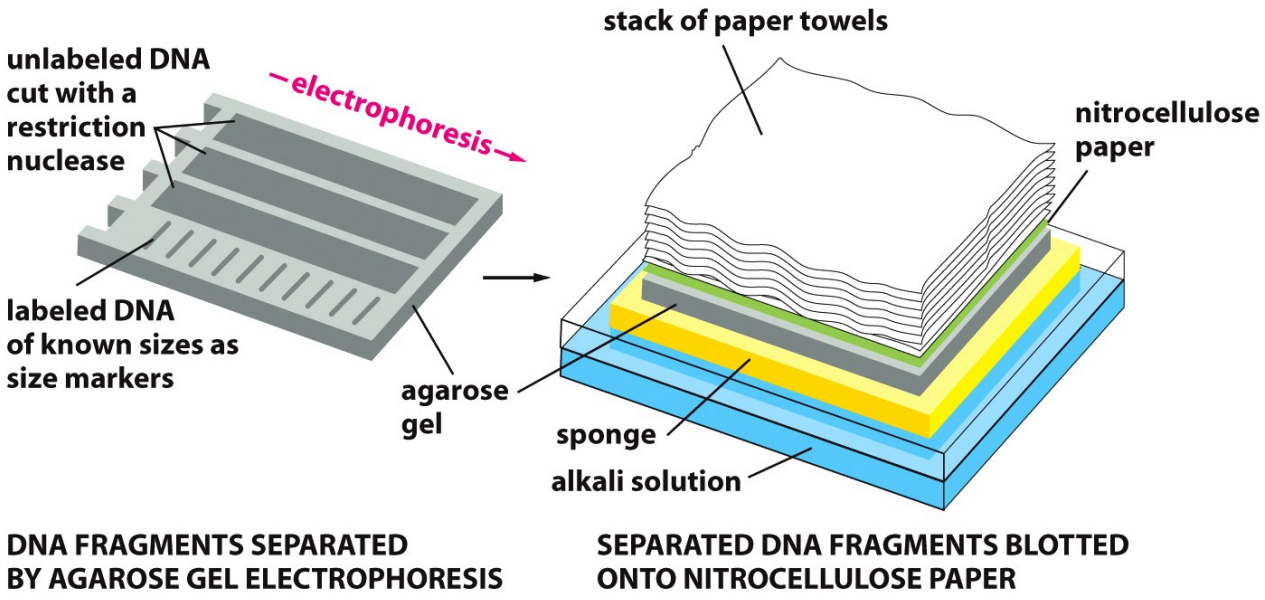


Figure 10-5 part 2 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Southern blot, step 3

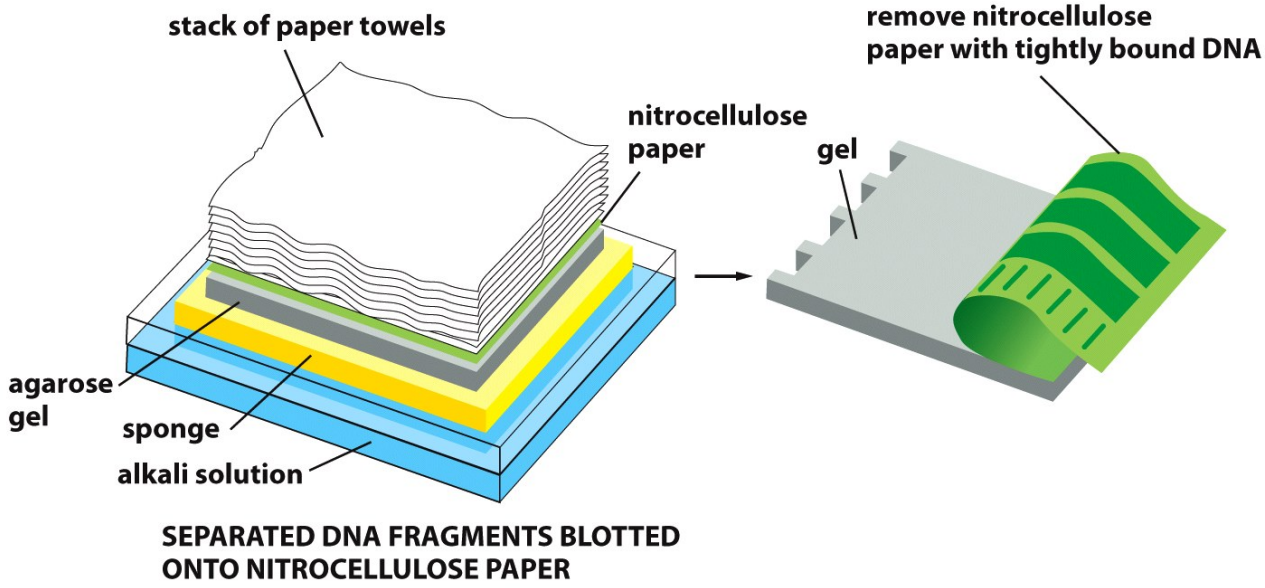


Figure 10-5 part 3 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Southern blot, step 4

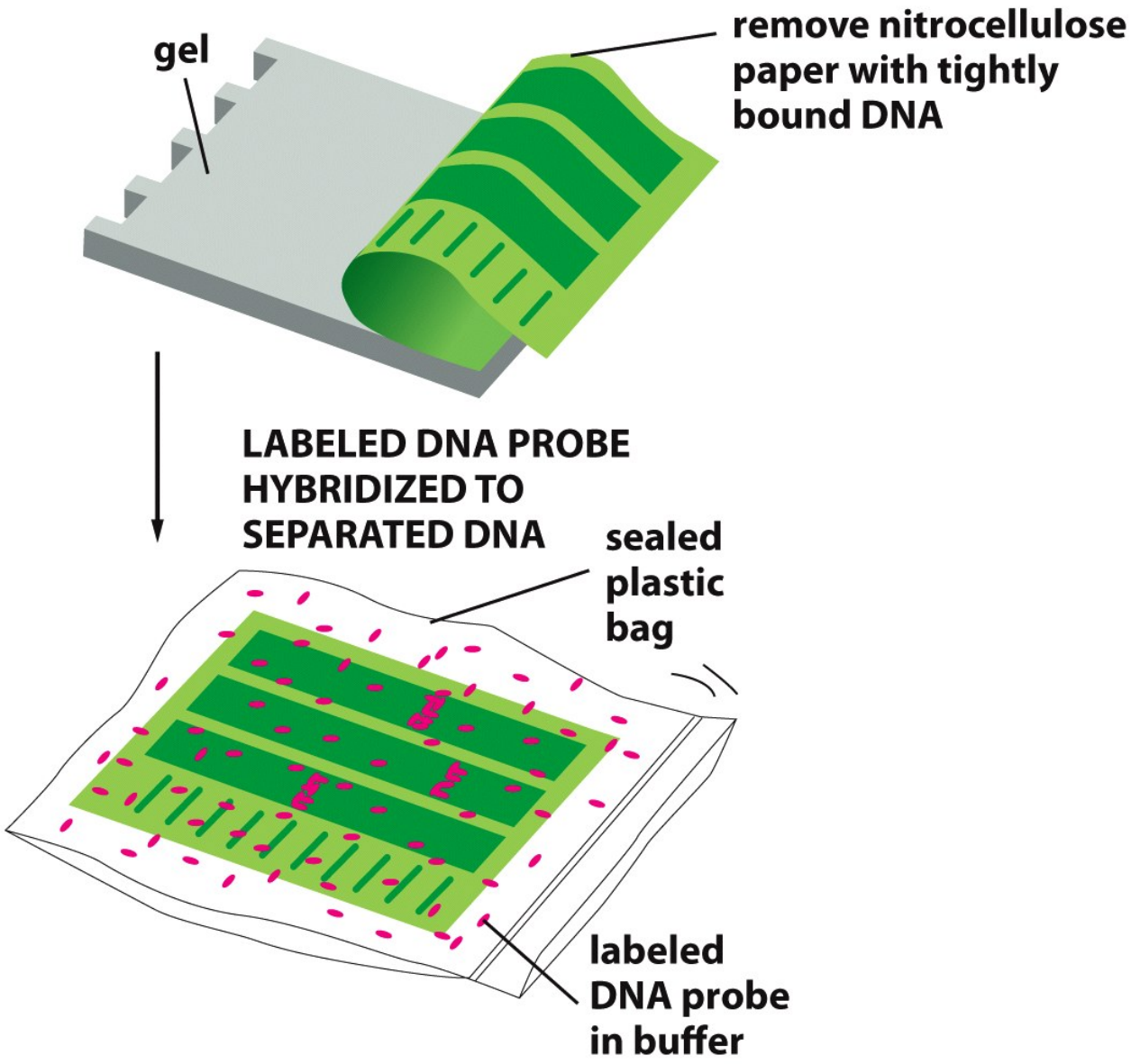


Figure 10-5 part 4 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

Southern blot, step 5

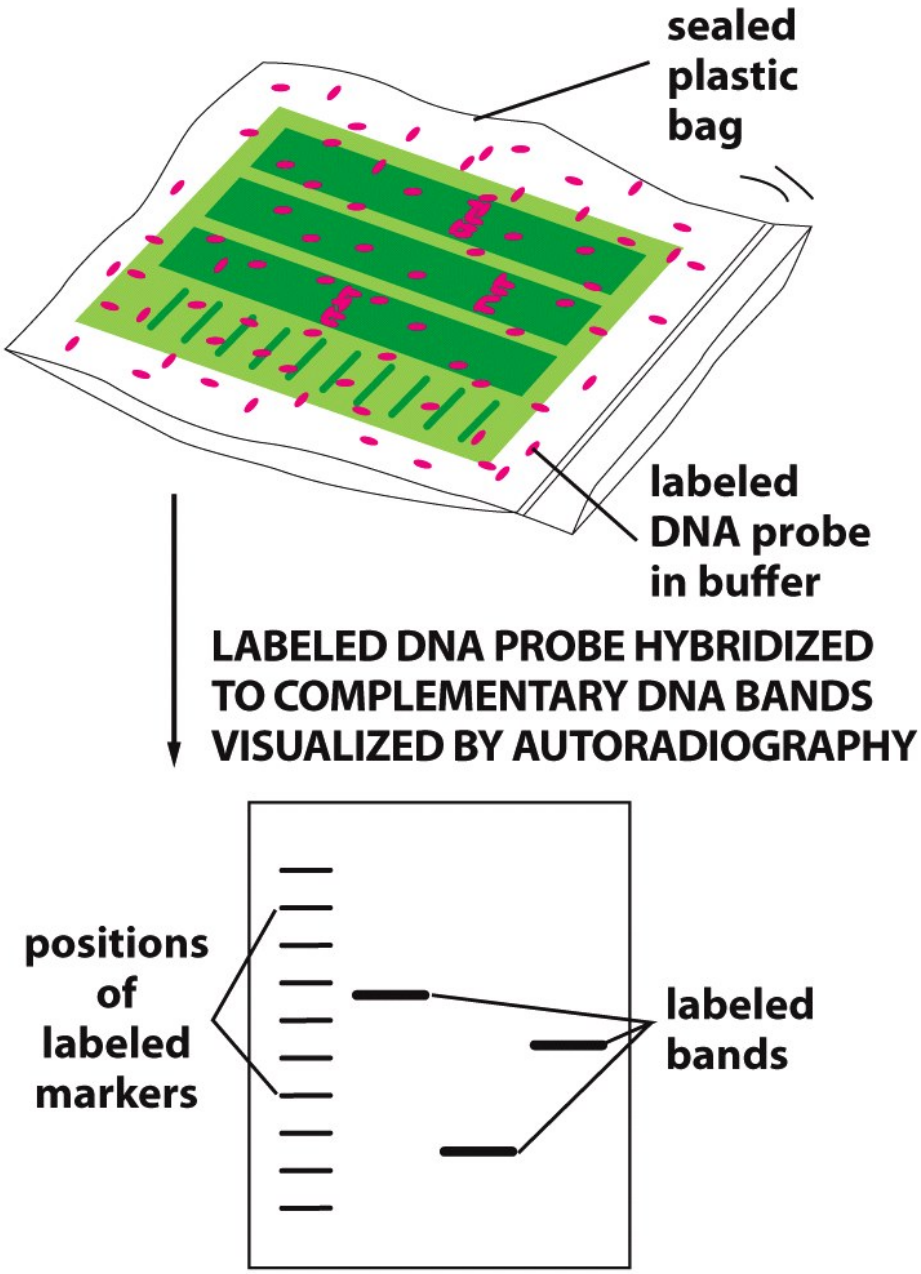


Figure 10-5 part 5 of 5 Essential Cell Biology 3/e (© Garland Science 2010)

.3 DNA cloning

Recombinant molecules

- Restricted fragments may be re-assembled with ligase
- Staggered end needs to be completed with DNA polymerase first, and then molecules will me joined with ligase
- Ligase may insert "foreign" fragment between to others

Ligation after restriction

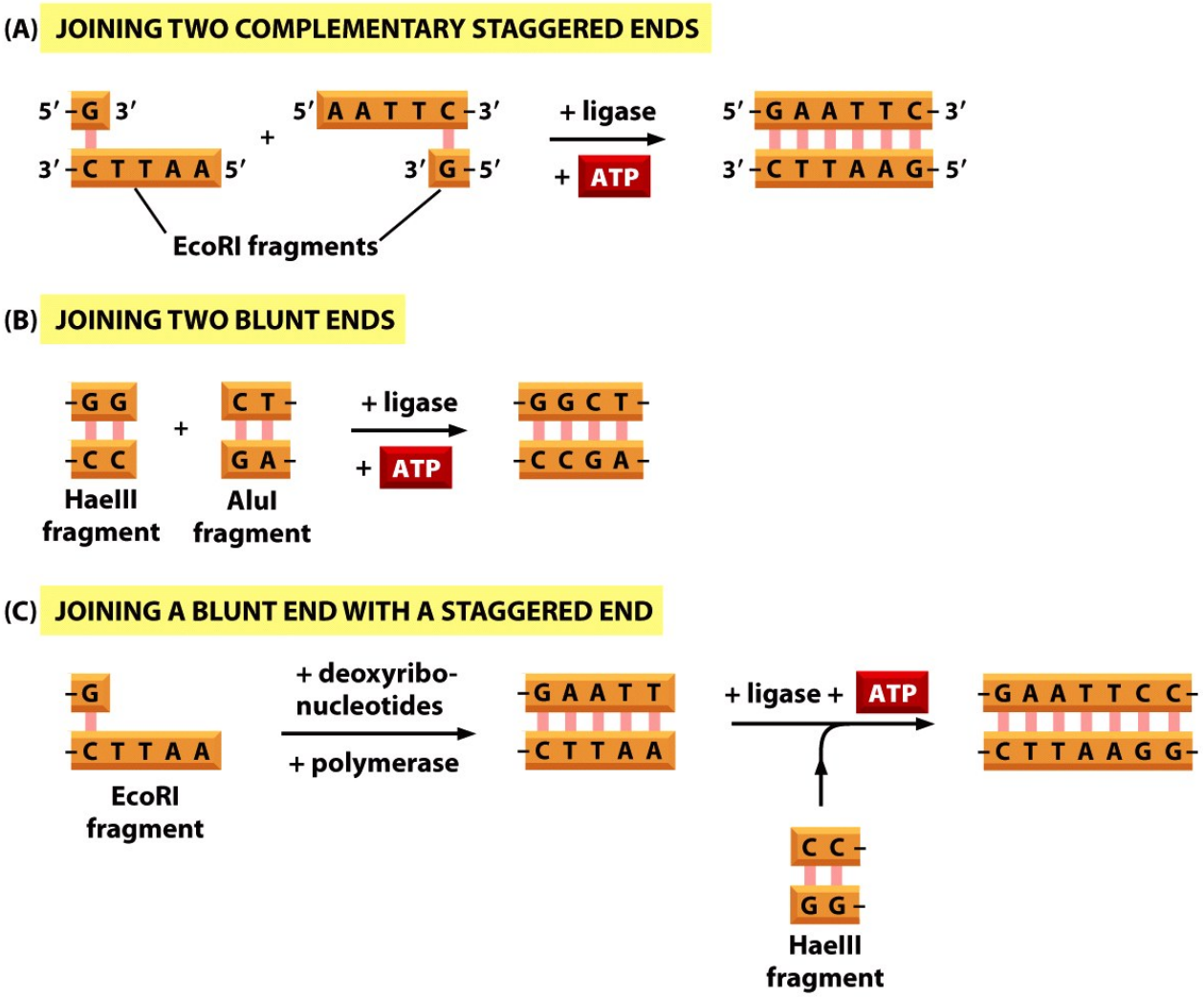


Figure 10-6 Essential Cell Biology 3/e (© Garland Science 2010)

Plasmid vectors

- The best way to transform bacterial cell is to use a plasmid
- Recombinant plasmids are easily taken into bacterial cells and then multiple with bacterial cell divisions

Recombinant plasmid

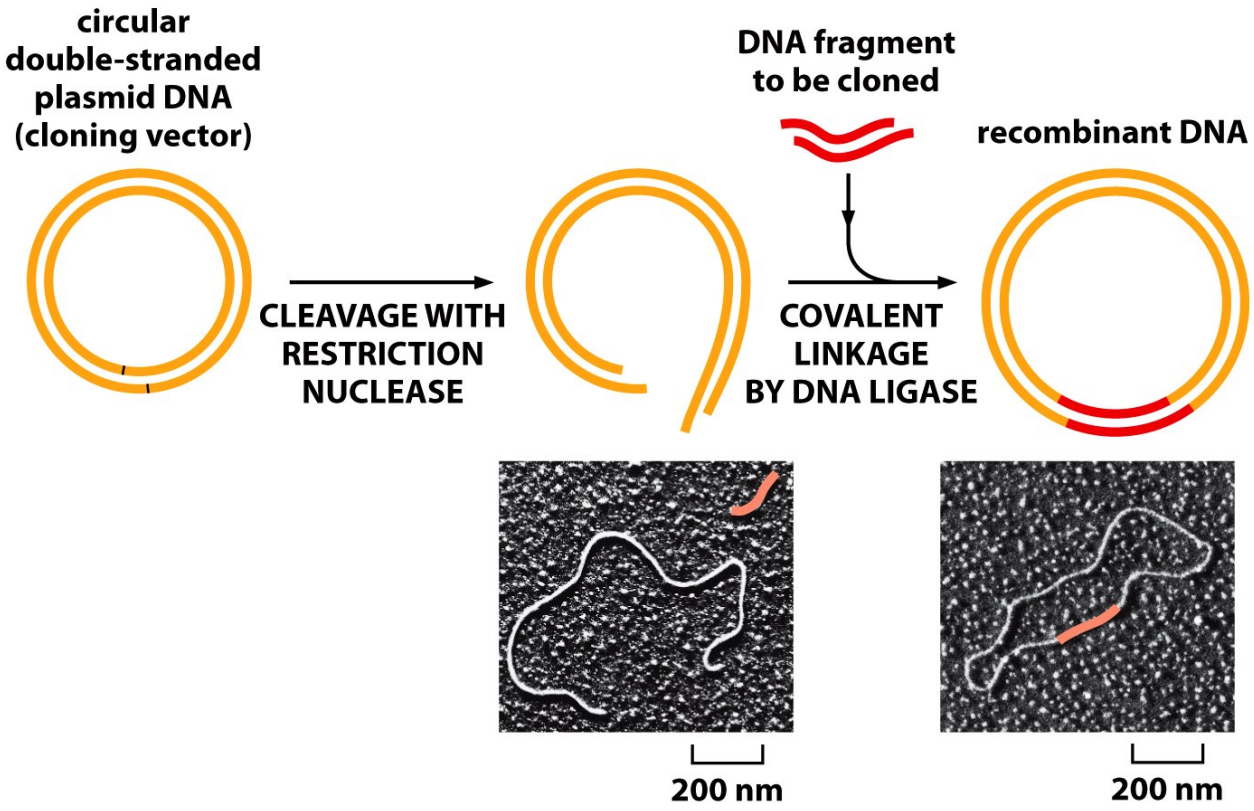


Figure 10-9 Essential Cell Biology 3/e (© Garland Science 2010)

Cloning of plasmid

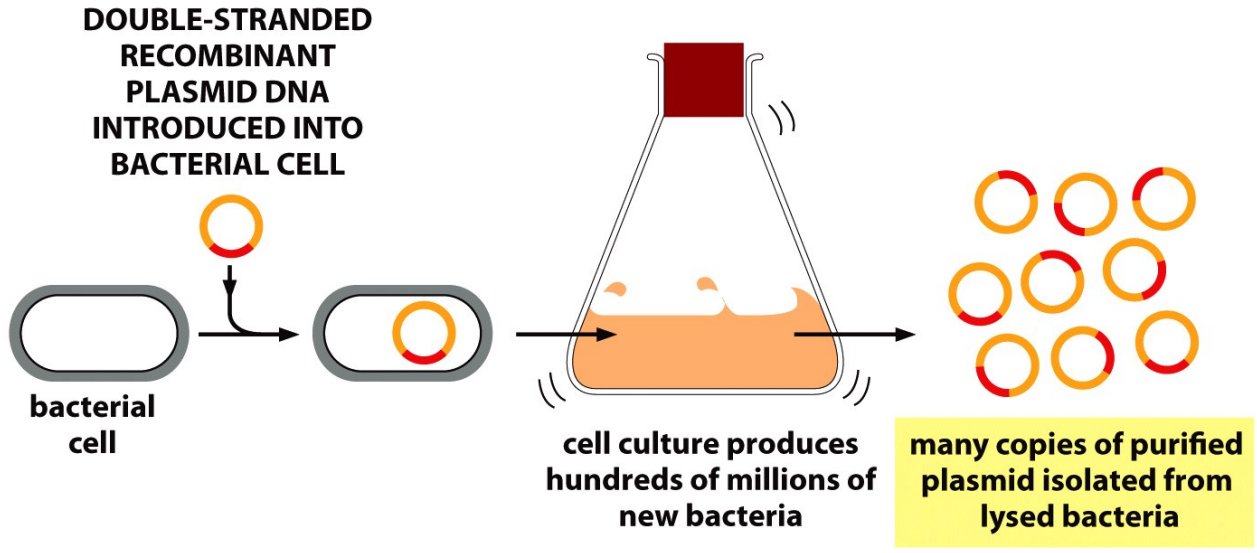


Figure 10-10 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (2 points)

What is a EST?

Summary

- Analysis of genes allows to create phylogenetic trees
- Some parts of genome are more conservative than others

- Genome size is not strictly related with "advancedness" of organism
- Human genome project still needs to reveal translation regulators and alternative splicing
- DNA restriction and DNA hybridization are simple techniques which help to obtain information about DNA polymorphisms

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 10*.

Outline

Questions and answers

Previous final question: the answer

What is a EST?

- Expression Sequence Tag, small fragment of cDNA (DNA which sequence was determined from mRNA) used to identify which parts of genome are actually expressed

Analyzing genes and genomes

.1 Analysis of DNA (and proteins)

Different blots

- Northern blot—similar to southern but applied for RNA
- Western blot—again similar but applied for proteins, and visualization is due to interaction with antibodies interactions (not hybridization, of course)

Southern blot was named for biologist Edwin Southern. Other blots were named just by analogy.

Creation of DNA library

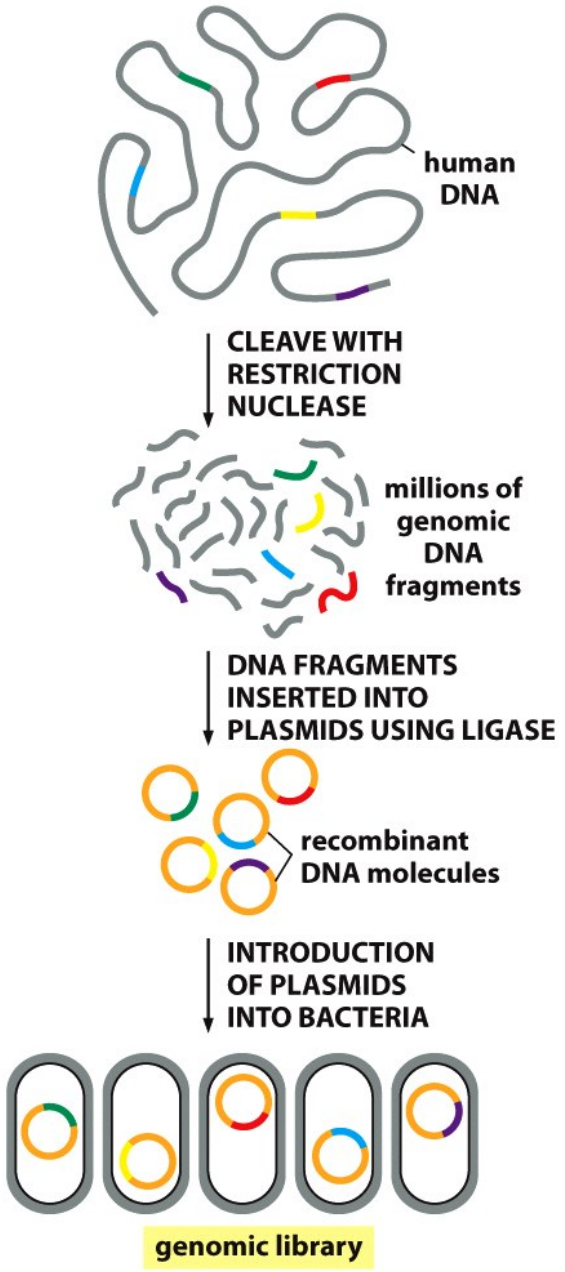


Figure 10-11 Essential Cell Biology 3/e (© Garland Science 2010)

cDNA libraries

- cDNA is a "reversed" DNA (DNA from mRNA)
- cDNA library could be different from genomic library

Genomic library vs. cDNA library

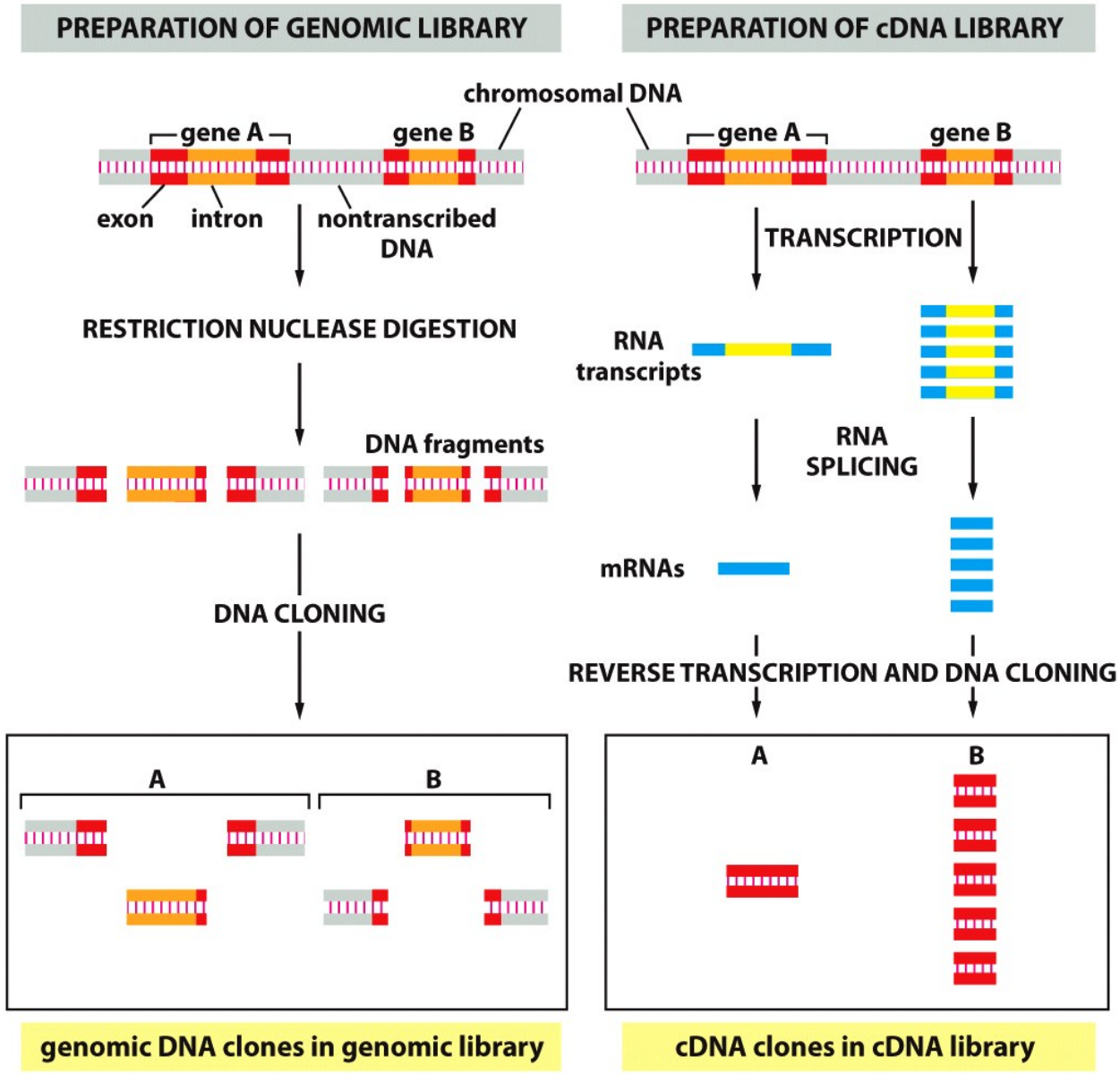


Figure 10-14 Essential Cell Biology 3/e (© Garland Science 2010)

PCR

- PCR is using temperature-specific polymerase, DNA hybridization and thermal annealing instead of helicase
- PCR is a chain reaction because the growth of desired DNA fragments is exponential
- PCR products are almost pure (contain short fragments because primers will eventually amplify short products) and may be used as clones of genomic DNA or cDNA
- Why we normally stop PCR after 25–35 cycles?

  Because with more cycles, result becomes noisy
- PCR is mostly employed as a detection technique; human STR repeats are especially useful here

Small tandem repeats (STRs) for human identification

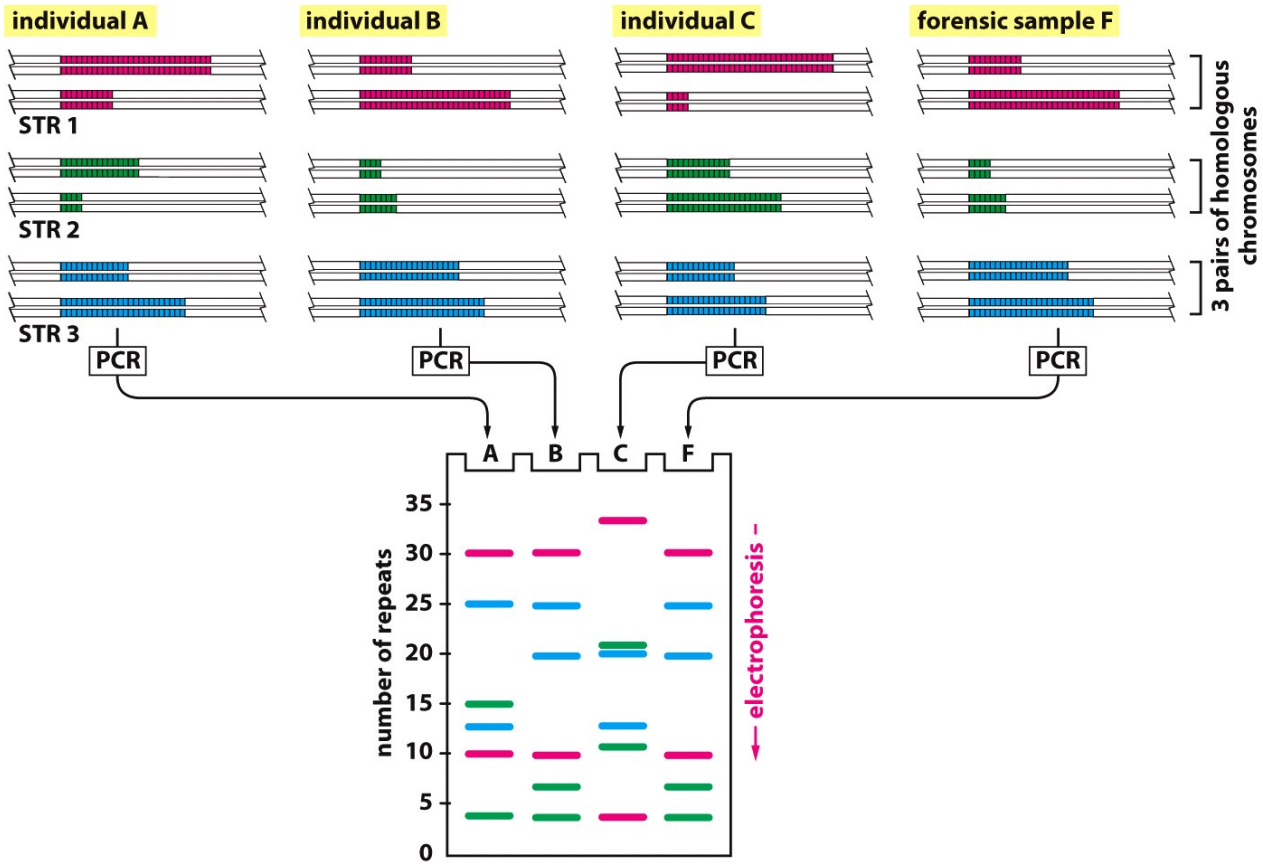


Figure 10-19b Essential Cell Biology 3/e (© Garland Science 2010)

PCR, first steps

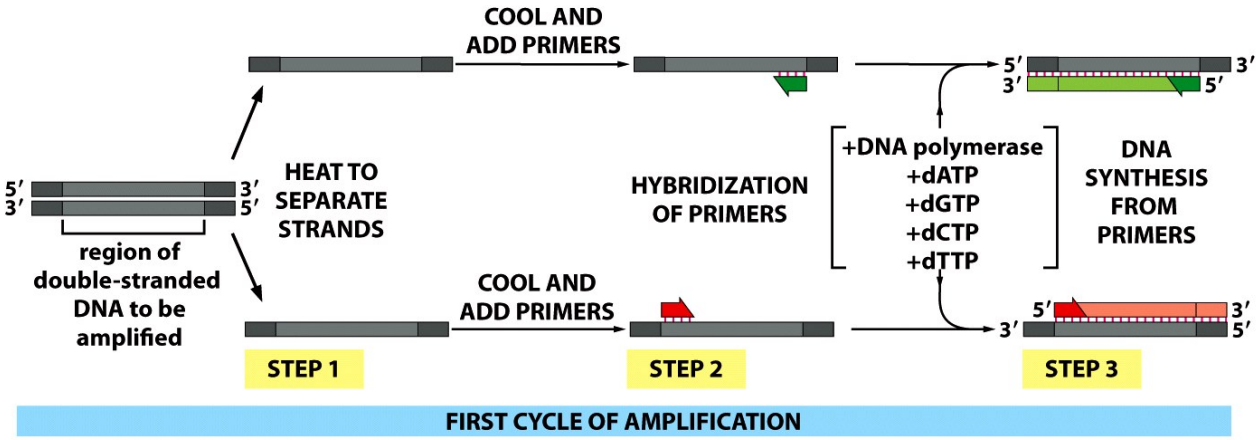


Figure 10-15 Essential Cell Biology 3/e (© Garland Science 2010)

PCR, several cycles

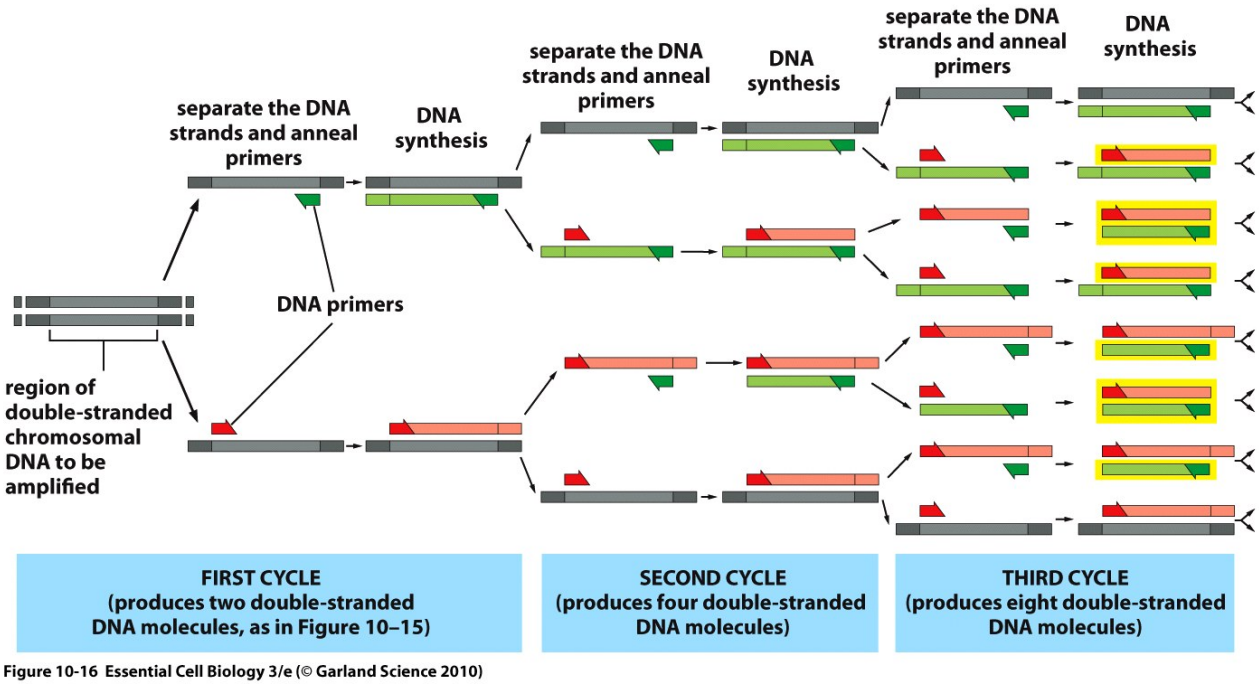


Figure 10-16 Essential Cell Biology 3/e (© Garland Science 2010)

PCR movie

DNA sequencing

- Employs specific way of polymerization used ddNTPs (dideoxy nucleotide triphosphares)
- ddNTPs stop the formation of DNA chain
- ddNTPs could be detected via fluorecsent of radioactive label
- Therefore, we will know length of fragment (because we will do electrophoresis) and which ddNTP is in there (because it is fluorescent or radioactive)
- Then we can calculate a location of specific nucleotide

Dideoxy nucleotide triphposphates (ddNTPs)

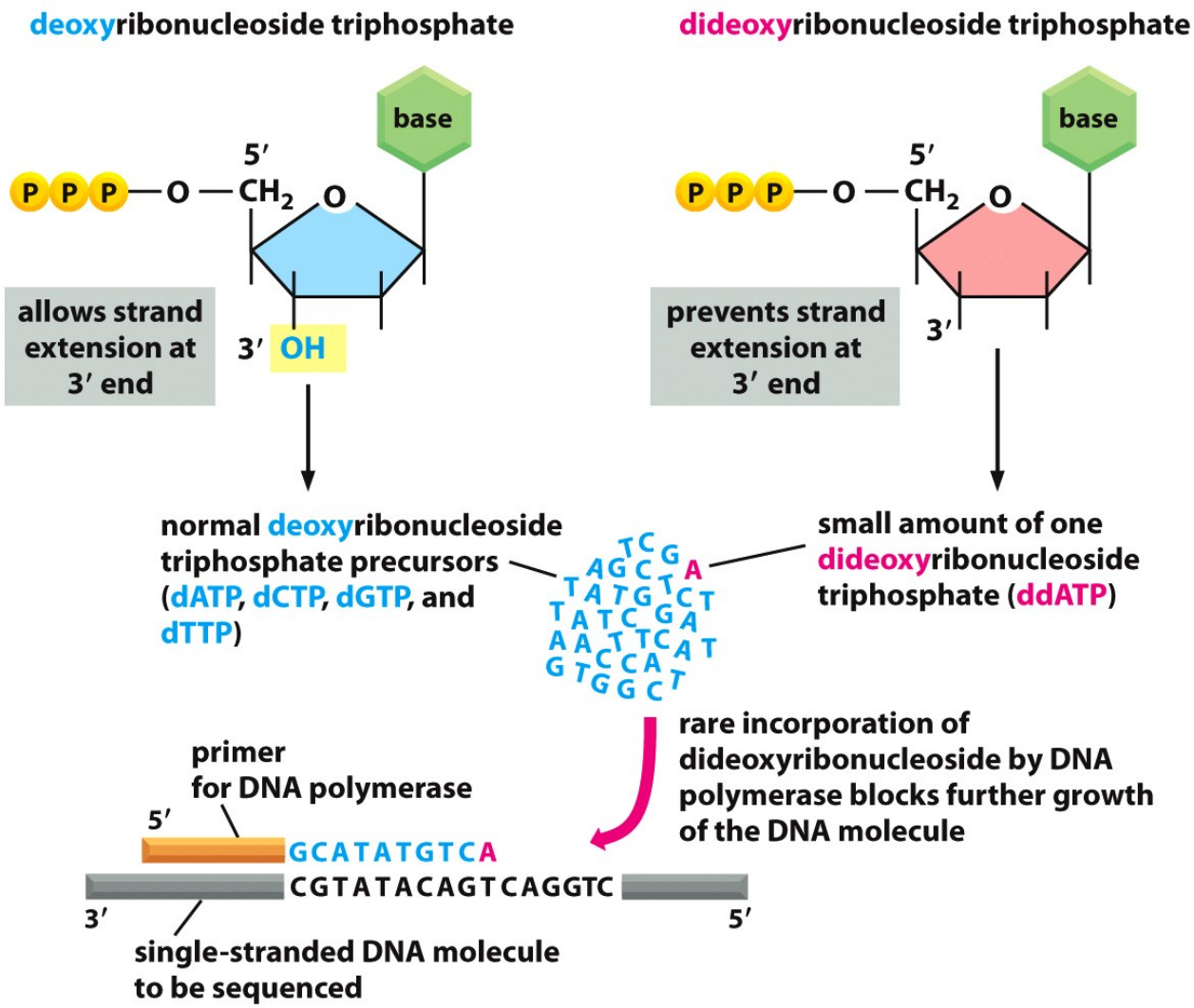


Figure 10-20 Essential Cell Biology 3/e (© Garland Science 2010)

dd-sequencing

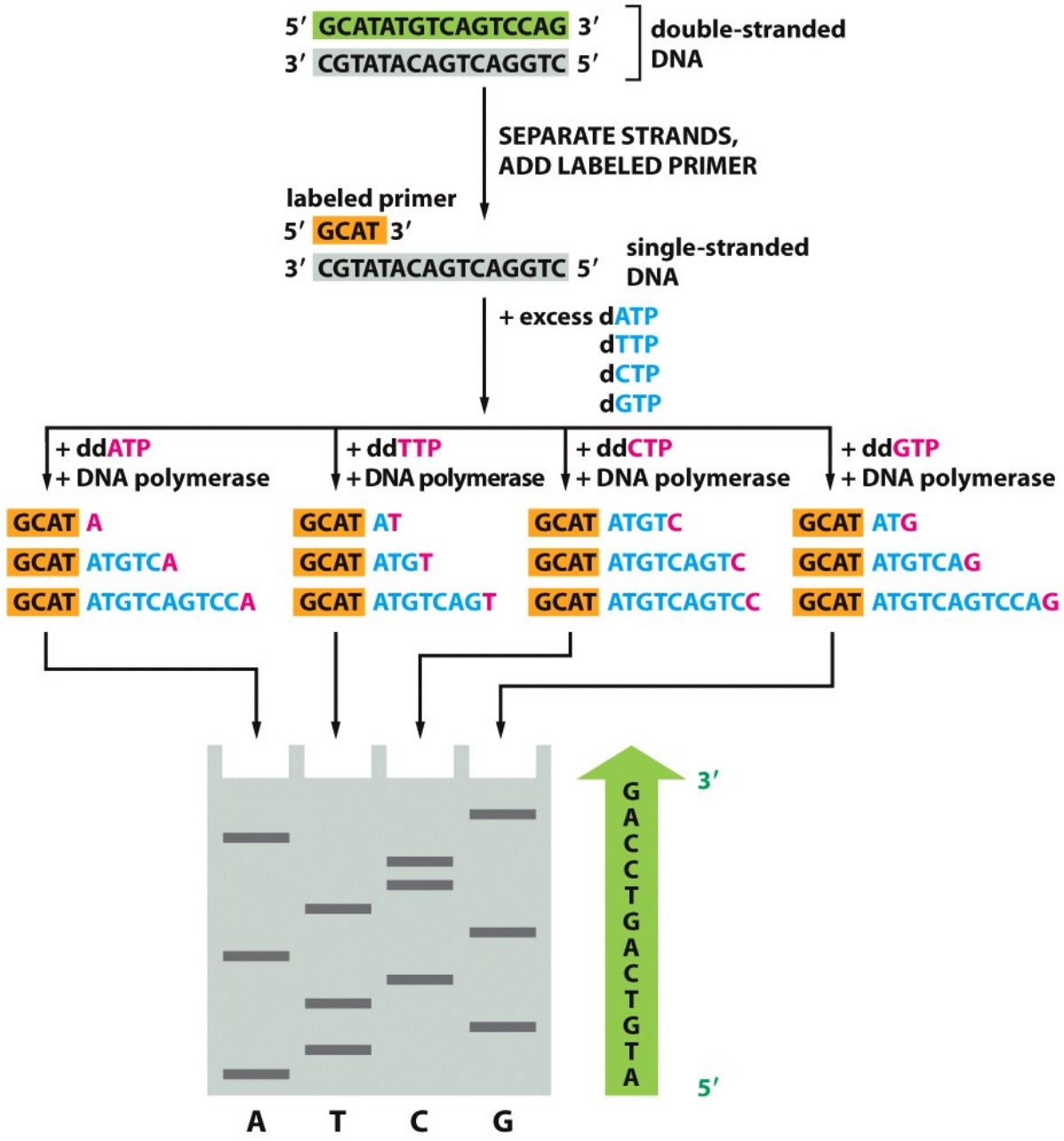


Figure 10-21 Essential Cell Biology 3/e (© Garland Science 2010)

Shotgun sequencing

- This is one of genome sequencing approaches
- DNA fragmented, then sequenced with pre-made library of primers
- Computer re-assembles fragments

Shotgun approach for the genome sequencing

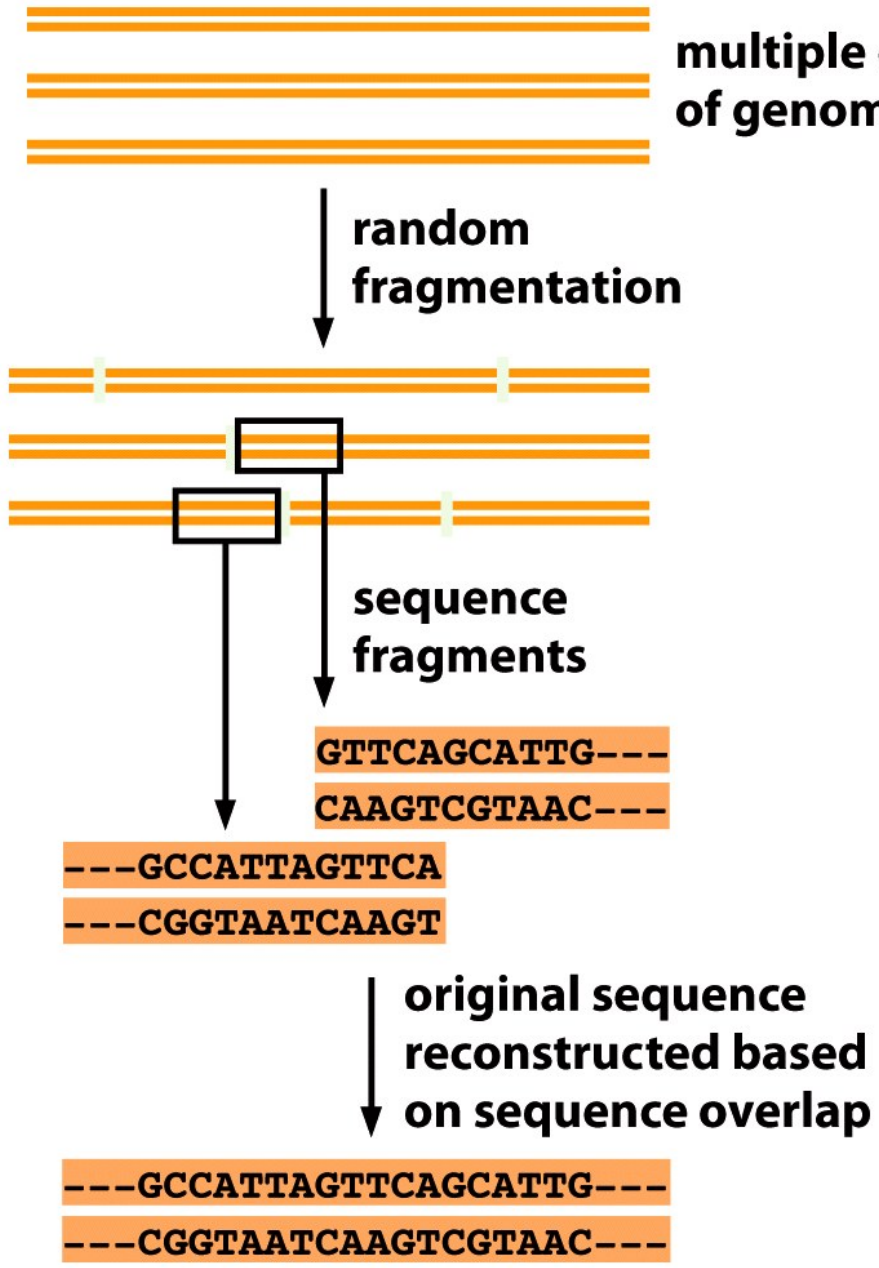


Figure 10-25 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (2 points)

Why DNA sequencing employs ddNTPs?

Summary

- DNA cloning used recombinant DNA and plasmid carriers
- PCR is based on temperature-specific DNA polymerase and DNA hybridization
- DNA Sanger sequencing (dd method) is based on the use of ddNTPs and electrophoresis
- Genome assembly employs programmatic methods

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 10*.

Outline

Questions and answers

Previous final question: the answer

Why does DNA sequencing employ ddNTPs?

- To stop elongation of DNA chain on given length

  AND

- To label nucleotides with specific radioactivity or flourescence

Analyzing genes and genomes

.1 Analysis of DNA (and proteins)

Shotgun approach for the genome sequencing

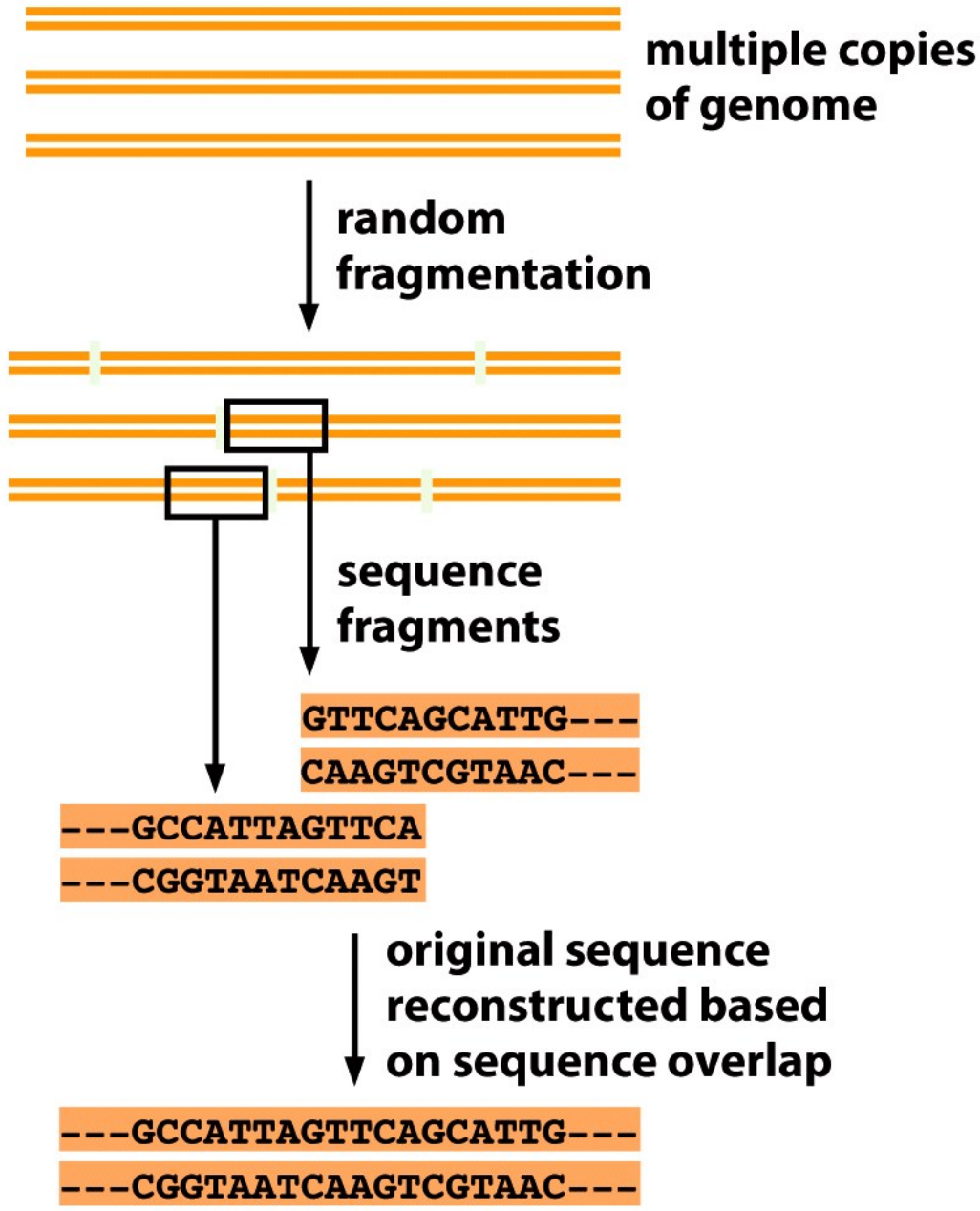


Figure 10-25 Essential Cell Biology 3/e (© Garland Science 2010)

High-throughput methods of sequencing

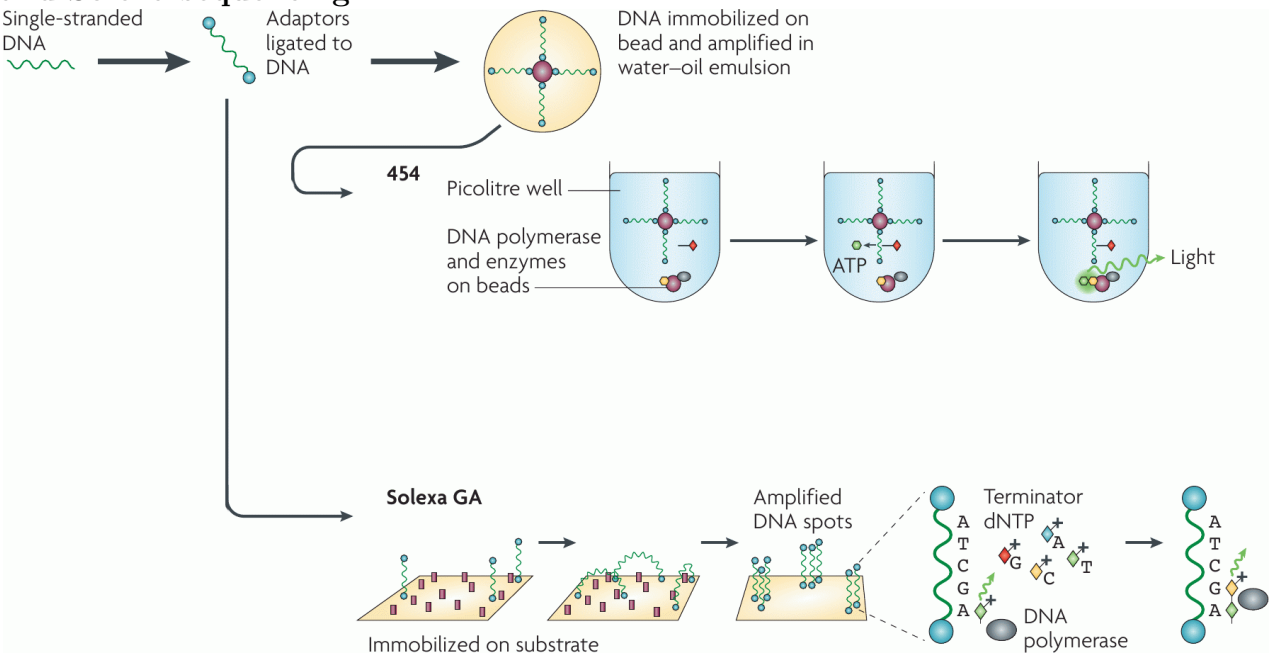
- Pyrosequencing—sequencing by synthesis; complementary strand is sequencing by adding base pairs and observing (by chemoluminescention) which base pair was successfully added

  454 pyrosequencing—a massive variant of pyrosequencing developed in Roche Diagnostics. Emulsion of droplets, each contained small fragment of DNA is studied. Luciferase is using for visualization. SOLiD is a similar technique.

- Illumina (Solexa) sequencing—multiple DNA molecules are attached to slide and amplified; then DNA chains are extended with luminescent reversible terminator bases (RT-bases); then RT bases are cleaved, and next base could be incorporated.

The "default", dd-sequencing has a name "Sanger sequencing"

454 and Solexa sequencing



.2 DNA industry

New DNA

- With multiple (serial) coning, it is possible to create new DNA sequences
- That may result in hybrid proteins or artificial mutations

Making new DNA

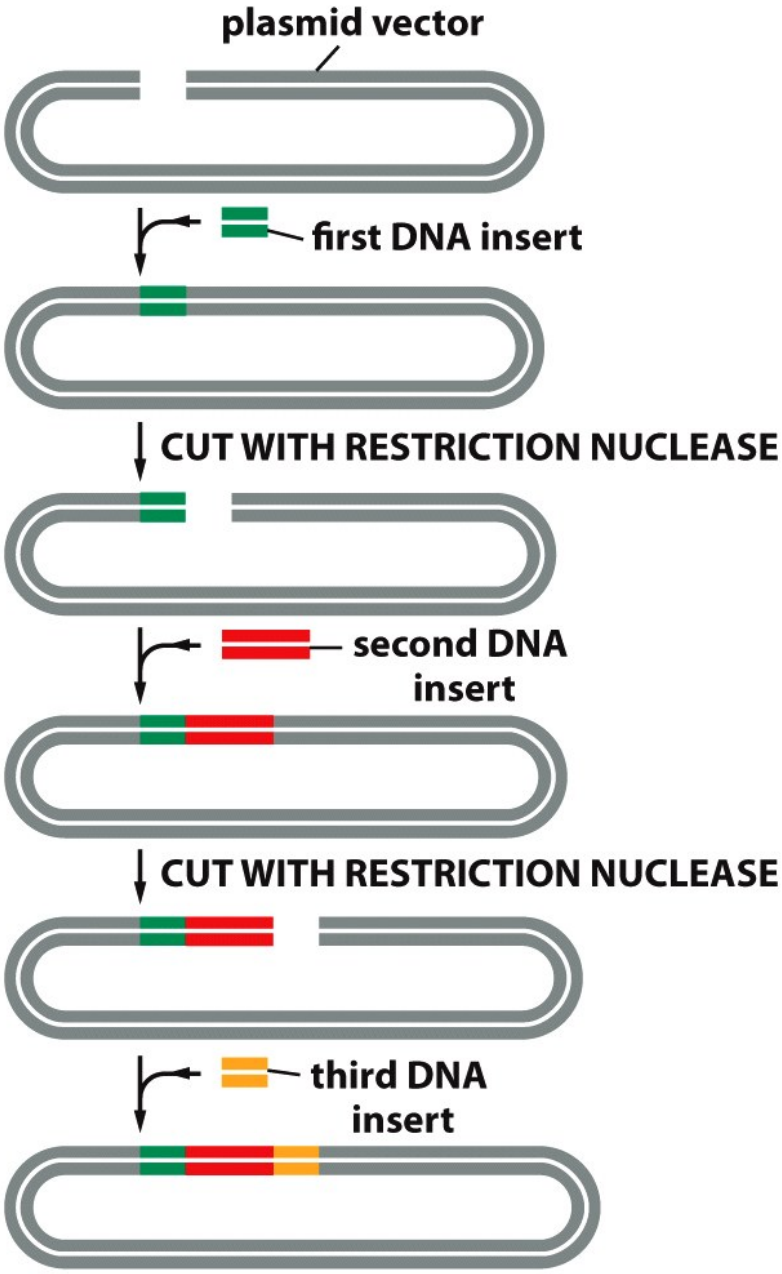


Figure 10-23 Essential Cell Biology 3/e (© Garland Science 2010)

Protein production

- Special expression vectors may include not only genes in strict sense, but also promoters and other regulatory pieces
- In this way, we may achieve gene over-expression
- Commercial proteins are now producing from bacteria with over-expressing DNA insertions

Protein overxepression

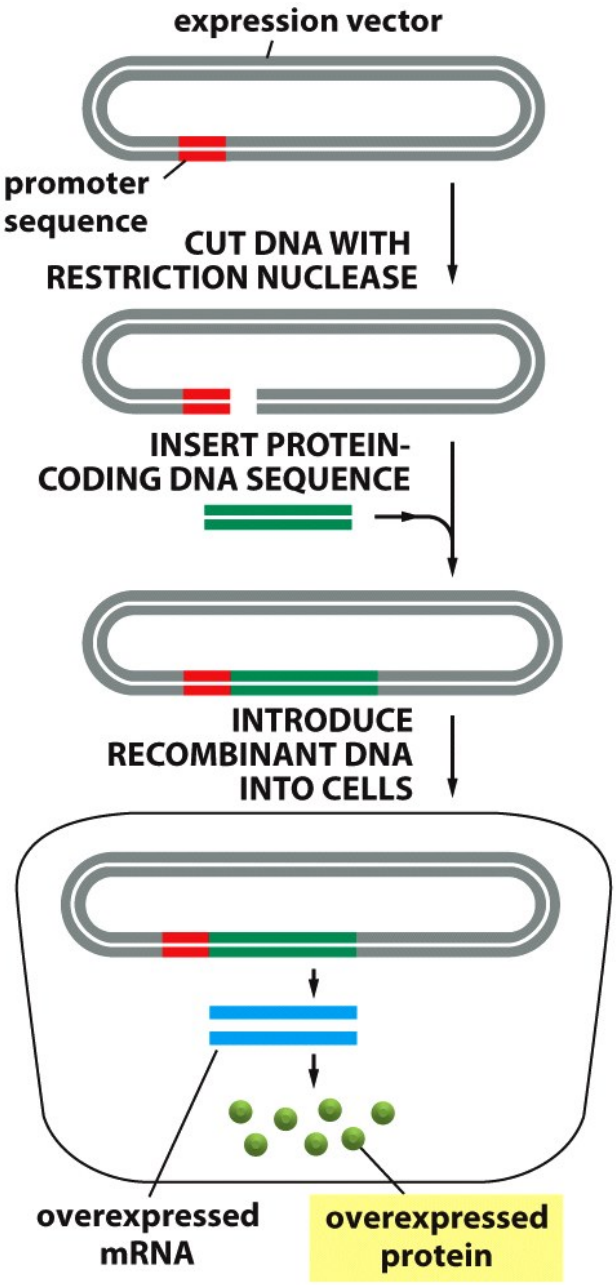


Figure 10-24 Essential Cell Biology 3/e (© Garland Science 2010)

DNA $\longrightarrow$ protein?

- With DNA database search, it is easy to find DNA which may produce a given protein
- Then we may to clone this DNA and use it for protein production

How to "reverse" a Central Dogma

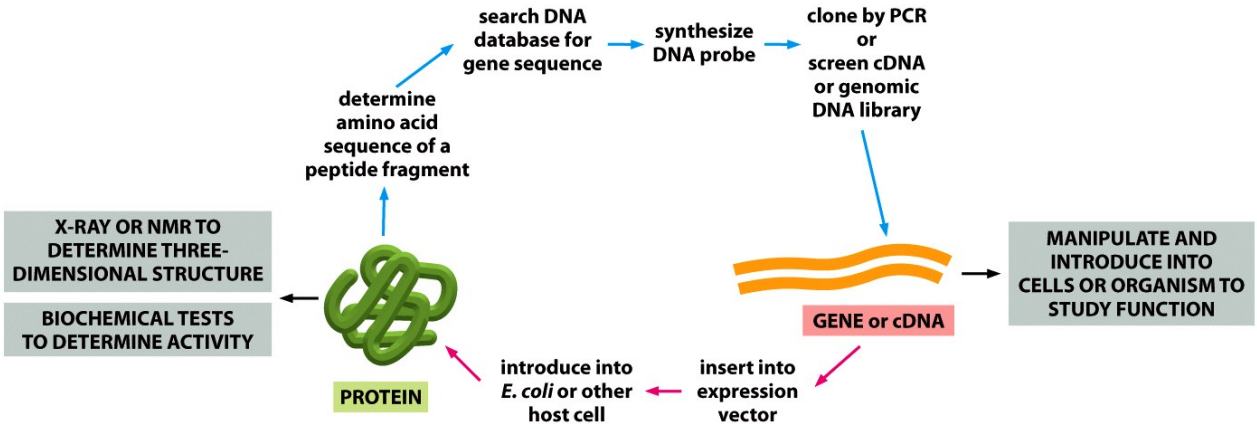


Figure 10-28 Essential Cell Biology 3/e (© Garland Science 2010)

Reporter genes

- If we combine different regulatory sequences and easily traced gene (reporter gene), we may spot different ways of transcription regulation
- It is especially useful with cascades of regulatory sequences (like *Eve* gene from fly)

Reporter gene

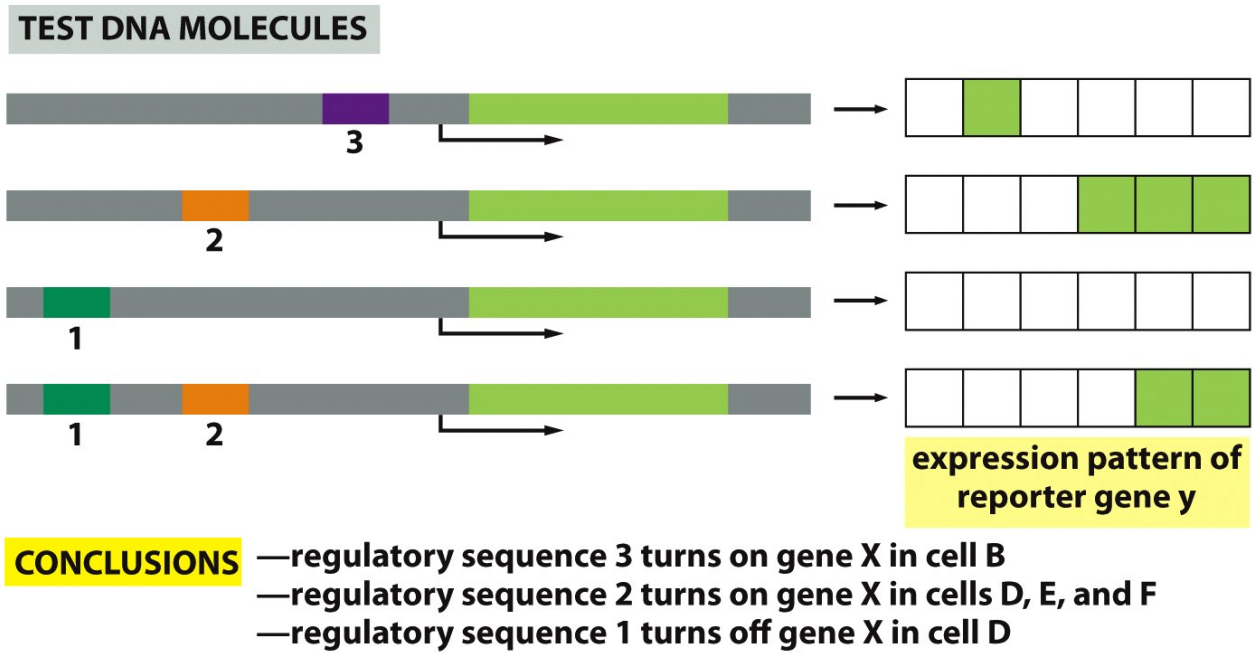


Figure 10-29b Essential Cell Biology 3/e (© Garland Science 2010)

In situ hybridization

- Green fluorescent protein may be fused with protein of interest
- If GFP gene is fused with promoter of interest, we may visualize gene expression
- With other fluorescent or radioactive DNA probes, it is possible to visualize genes in chromosomes etc.

In situ DNA hybridization specific to neurons

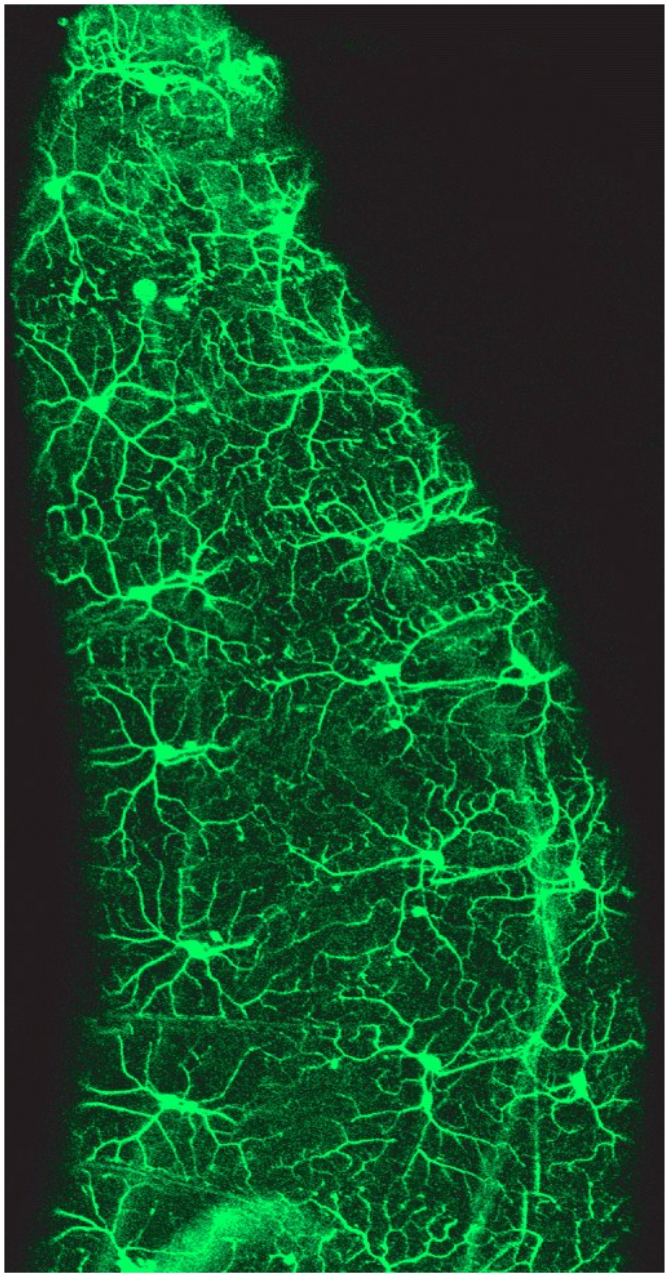
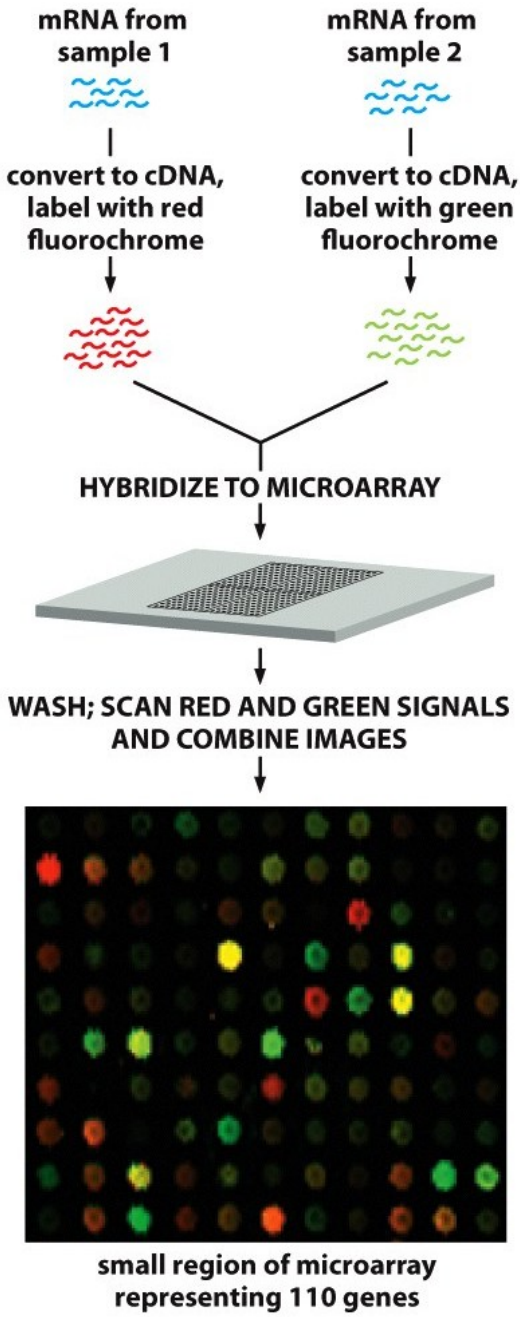


Figure 10-30 Essential Cell Biology 3/e (© Garland Science 2010)

Microarray

- Microarray is a way to simultaneously visualize thousands of genes
- We may, for example, hybridize cDNA from mRNA of cancer and non-cancer cells and understand which mRNAs have been involved in cancer activity

Example of microarray



Site-directed mutagenesis

- Modified primer sequences could initiate the synthesis of modified amplicons
- As a result, we may create proteins with replaced amino acids

Site-directed mutagenesis HOWTO

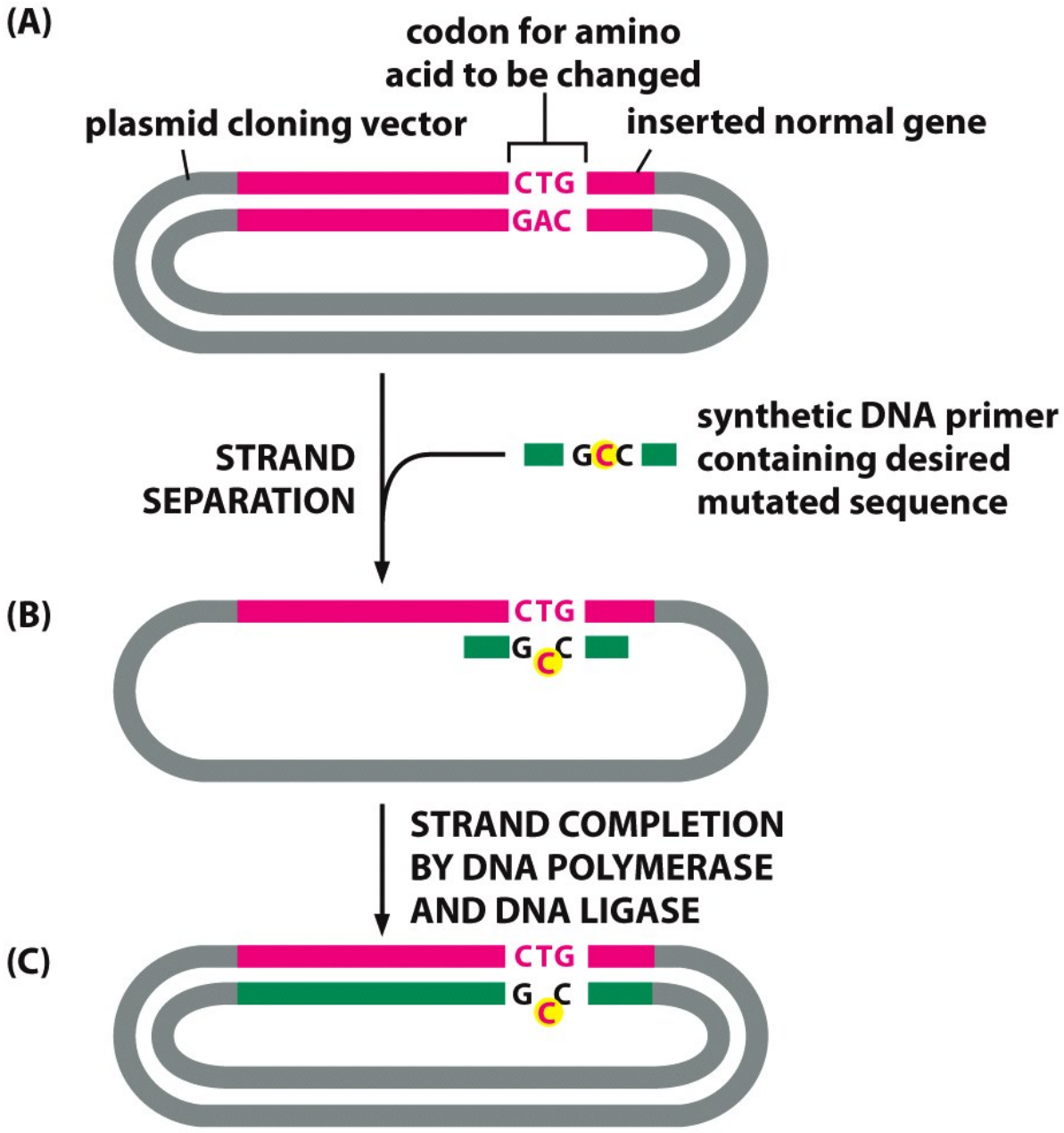


Figure 10-34 part 1 of 2 Essential Cell Biology 3/e (© Garland Science 2010)

GMOs and others

- Gene replacement: recombination between mutant and normal DNA
- Gene knockout: same, but recombinant gene will not work
- Gene addition leads to transgenic organisms (GMOs)

Ways of gene alteration

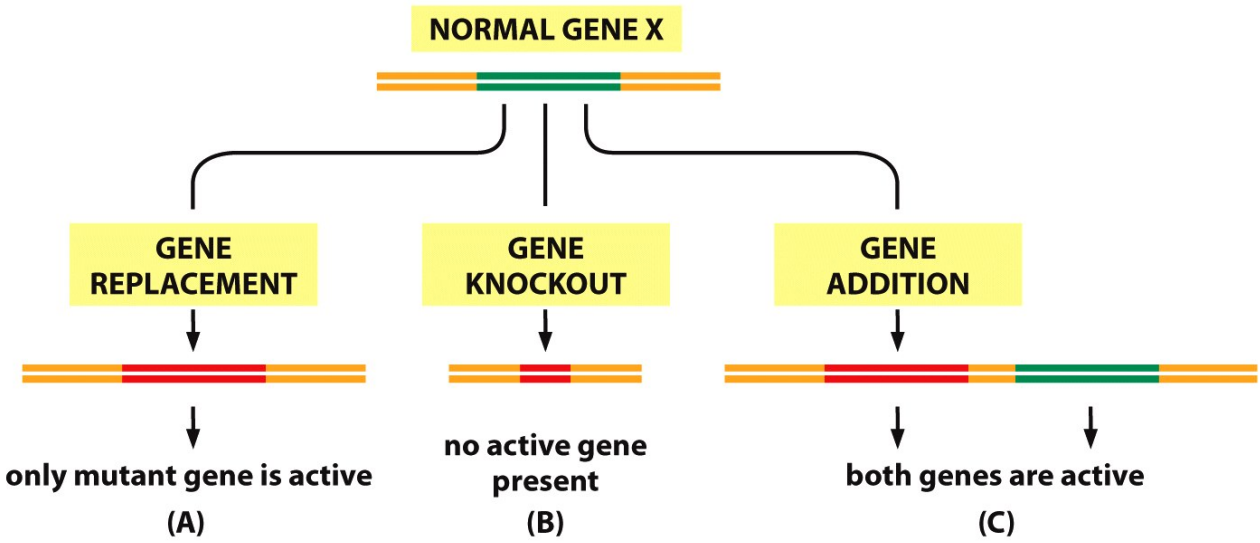
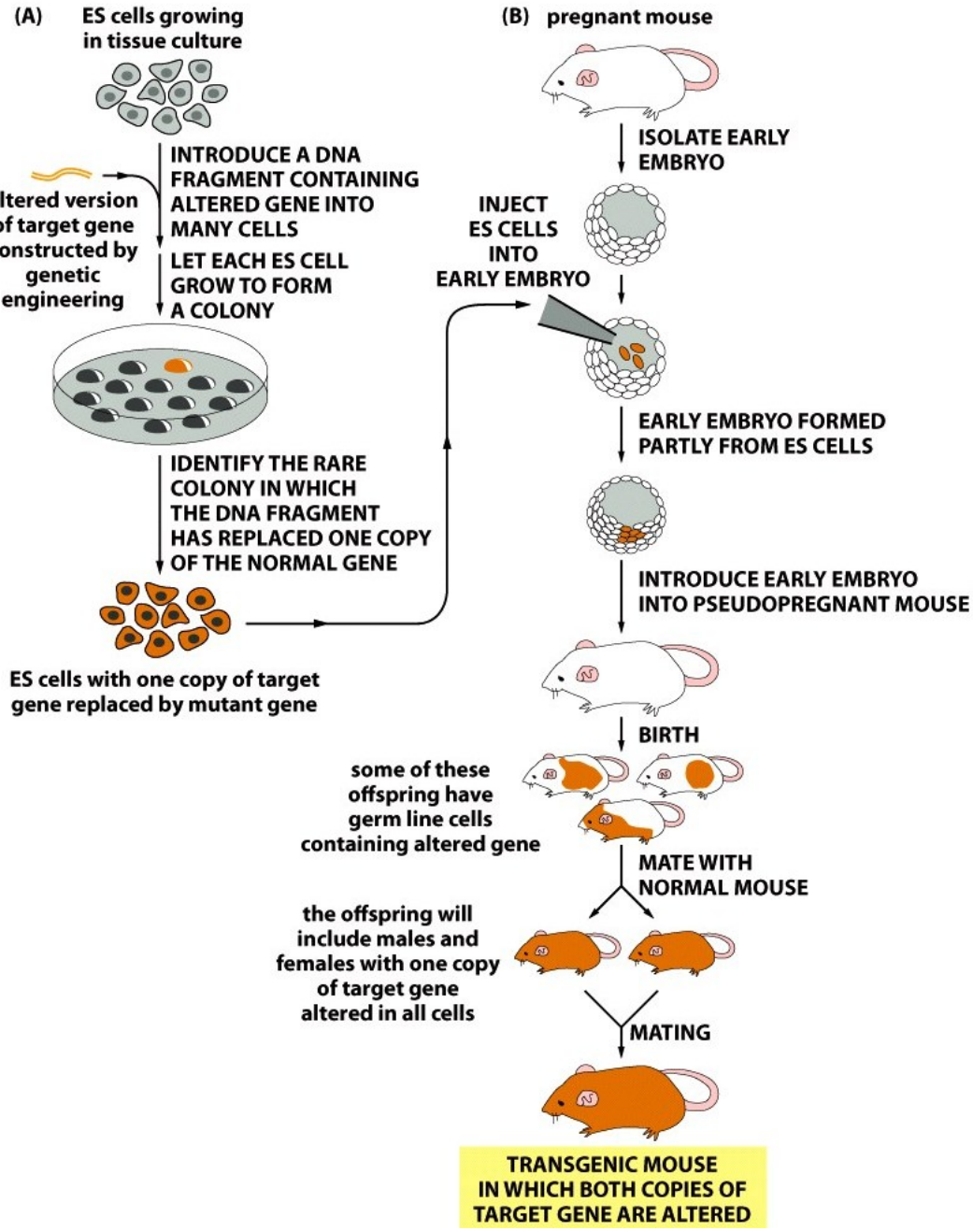


Figure 10-35 Essential Cell Biology 3/e (© Garland Science 2010)

Transgenic mice



Wild (left) and *Xpd* knockout mouse (right)

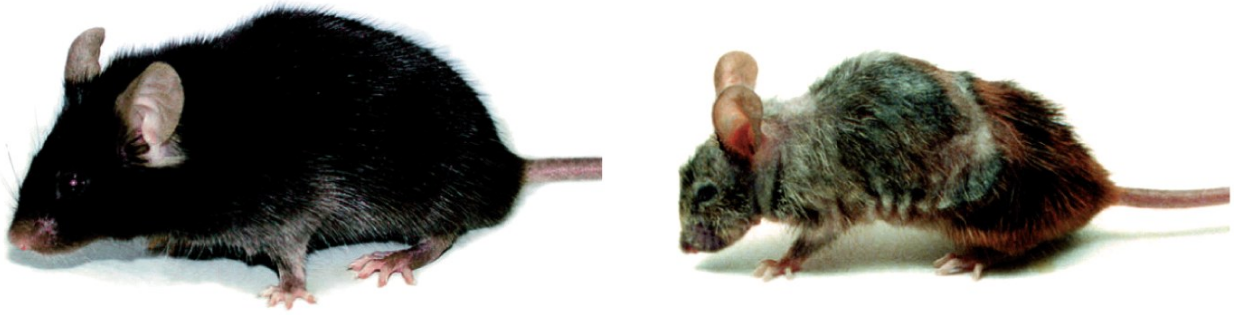


Figure 10-37 Essential Cell Biology 3/e (© Garland Science 2010)

RNA interference

- If we introduce dsRNA of gene which exists in nucleus, we may de-activate its translation through RNA interference
- That is an efficient way to test gene function

Final question (3 points)

How to knockout gene?

Summary

- *In situ* hybridization elucidates regions of gene expression
- Recombinant DNA makes possible to create new organisms

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 10*.

Outline

Questions and answers

Previous final question: the answer

How to knockout a gene?

- Recombine with non-working (plasmid, homologous recombination etc.)
- Suppress
- Use RNA interference

Membranes

.1 Introduction

Selective barriers

Membranes:

- Receive information
- Import and export molecules
- Have a role in moving and expanding cell

Cellular compartments

- Prokaryotes are one-membrane cells
- Eukaryotes have multiple closed and open one- and double-membrane compartments

Eukaryote cell compartments

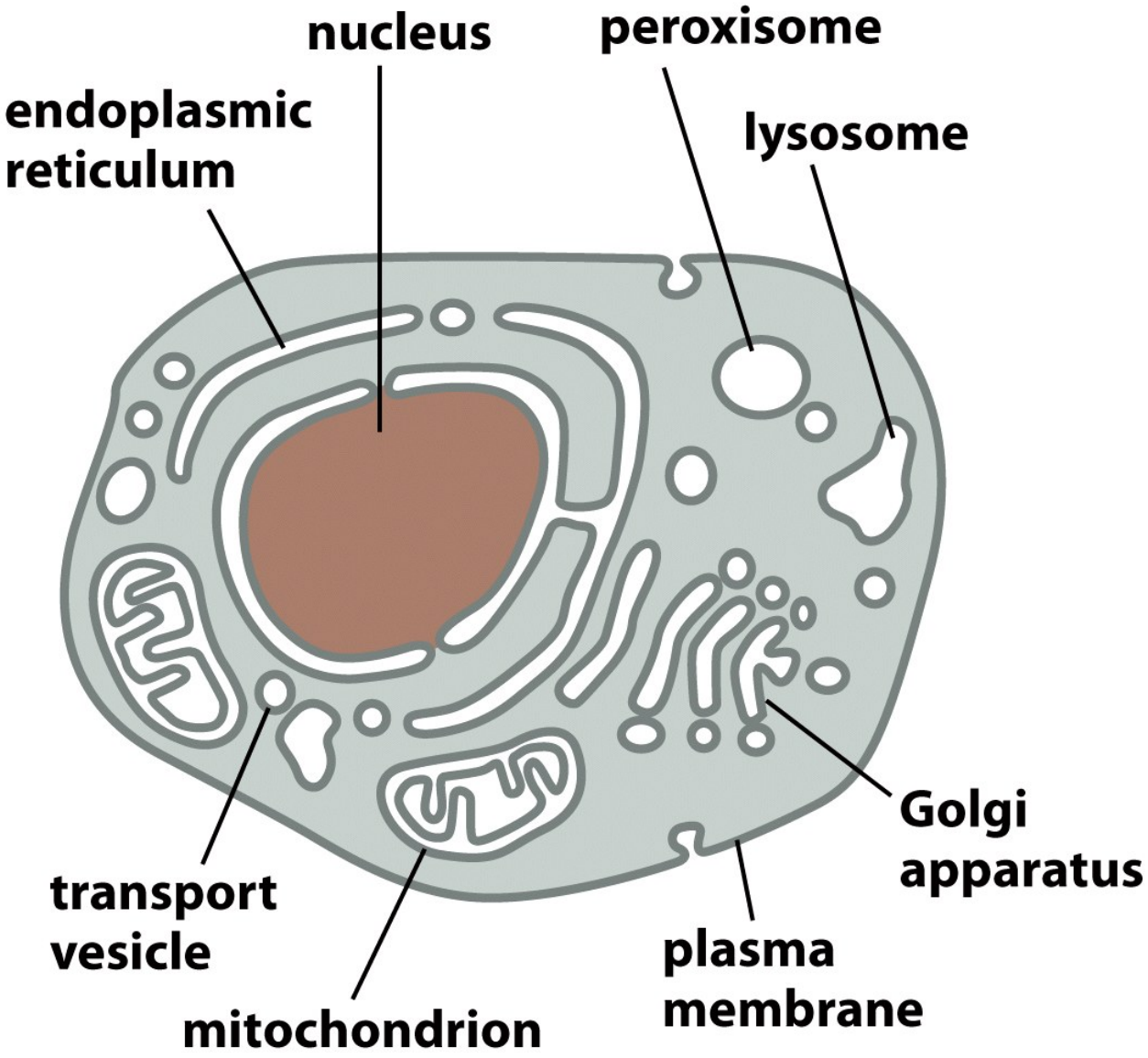


Figure 11-3 Essential Cell Biology 3/e (© Garland Science 2010)

Lipid bilayer

- Two layers of lipids
- Scattered proteins
- Carbohydrates attached to outer surface (cortex)

Amphipathic lipids

- Phospholipids
- Sterols
- Glycolipids

Amphipatic membrane lipids

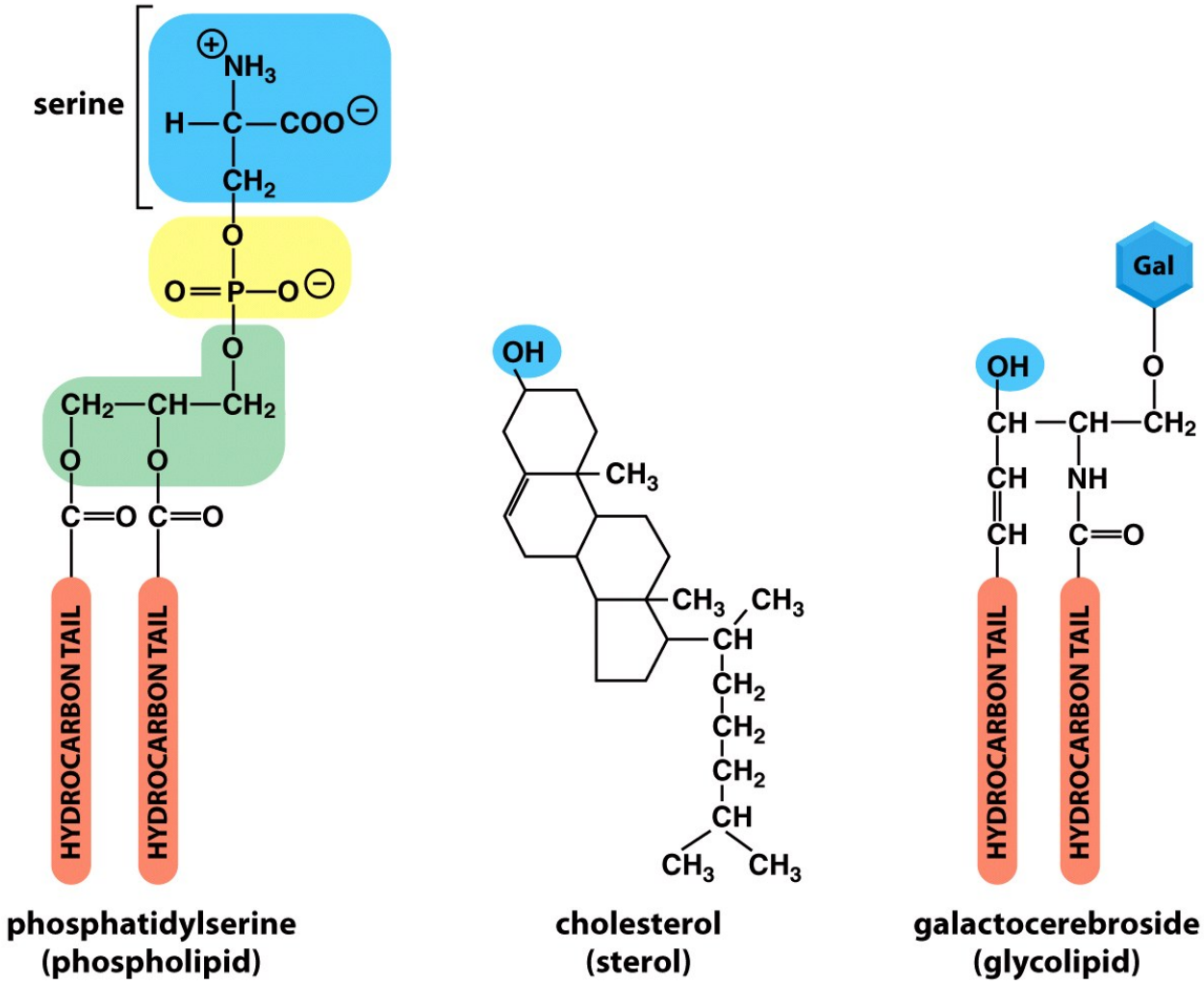


Figure 11-7 Essential Cell Biology 3/e (© Garland Science 2010)

Membrane lipids movie

Fluids

- Physically, membranes are lipid liquid suspension in water
- In artificial conditions, liposomes have structure similar to membranes
- Coacervate theory of life origin based on liposome existence
- Cholesterol can stiff membranes

Membrane fluidity movie

Liposomes

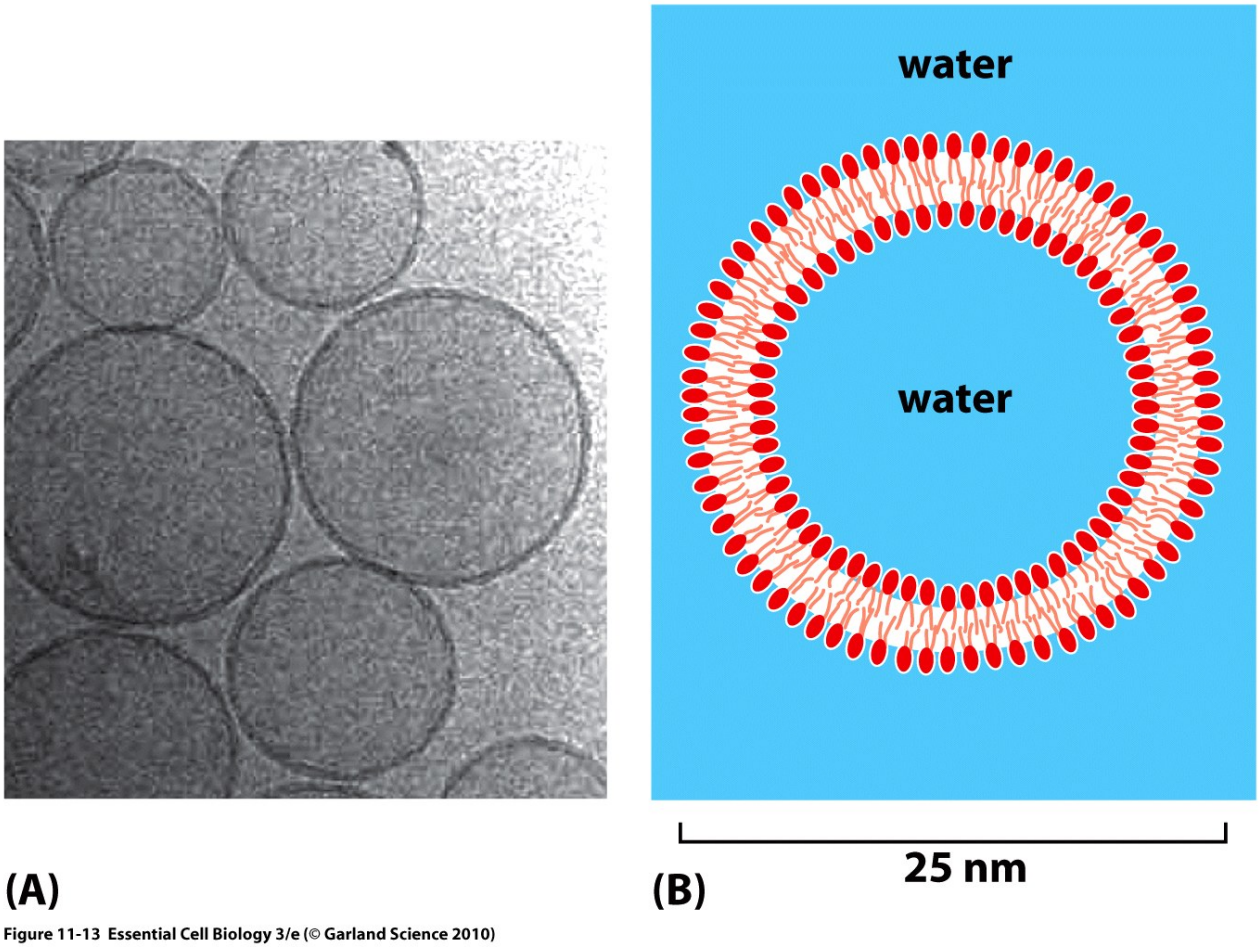


Figure 11-13 Essential Cell Biology 3/e (© Garland Science 2010)

Why bilayers make liposomes

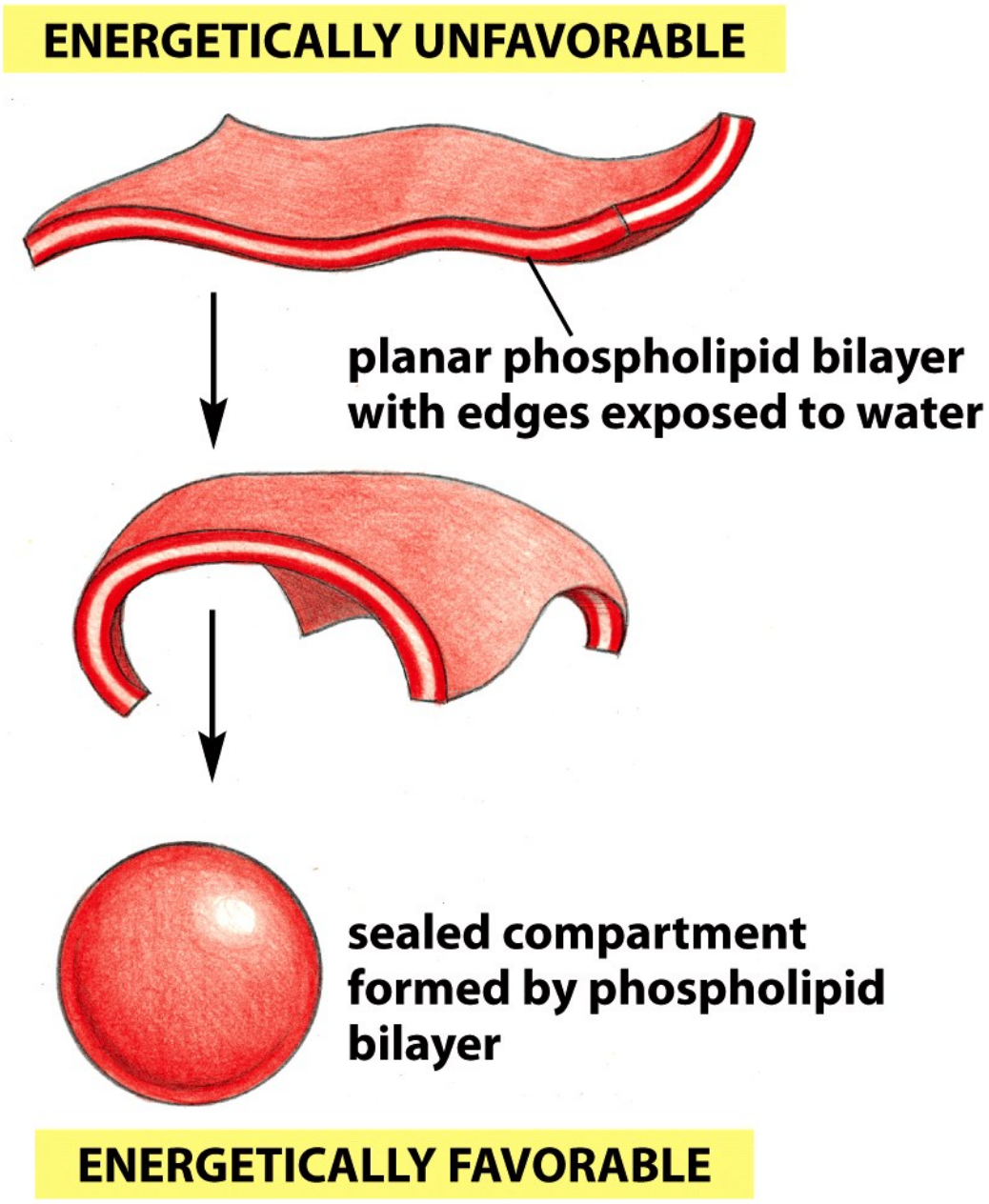


Figure 11-12 Essential Cell Biology 3/e (© Garland Science 2010)

Asymmetry

- Different types of phospholipids located unevenly between two layers
- Enzyme flippase transfer lipids between layers

Saving membrane asymmetry

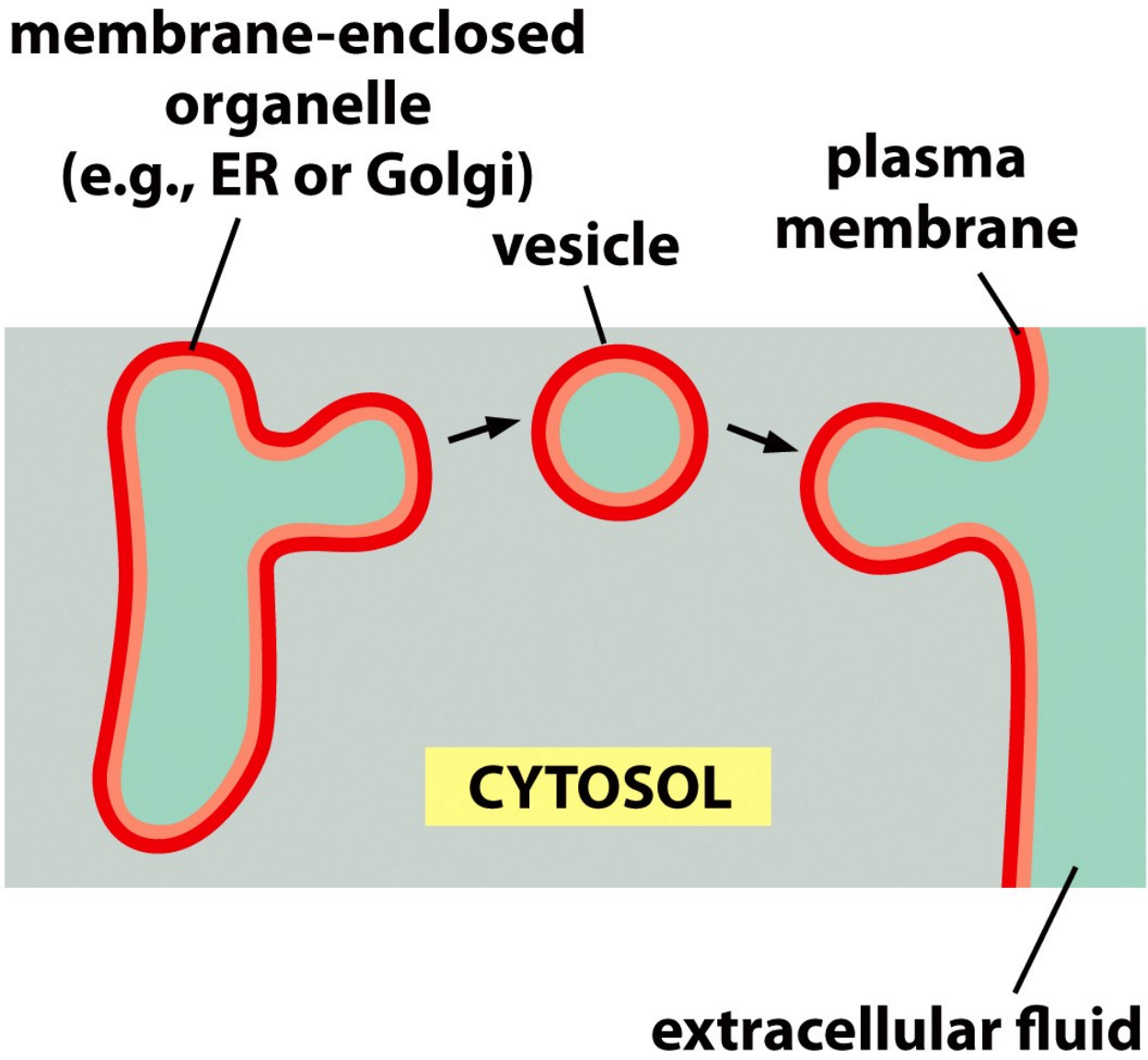


Figure 11-19 Essential Cell Biology 3/e (© Garland Science 2010)

Membrane proteins: functional groups

- Transporters: Na^+ pump
- Anchors: integrines
- Receptors: PDGF (platelet-derived growth factor) receptor
- Enzymes: adenylyl cyclase (catalyze cAMP production)

Functional groups of membrane proteins

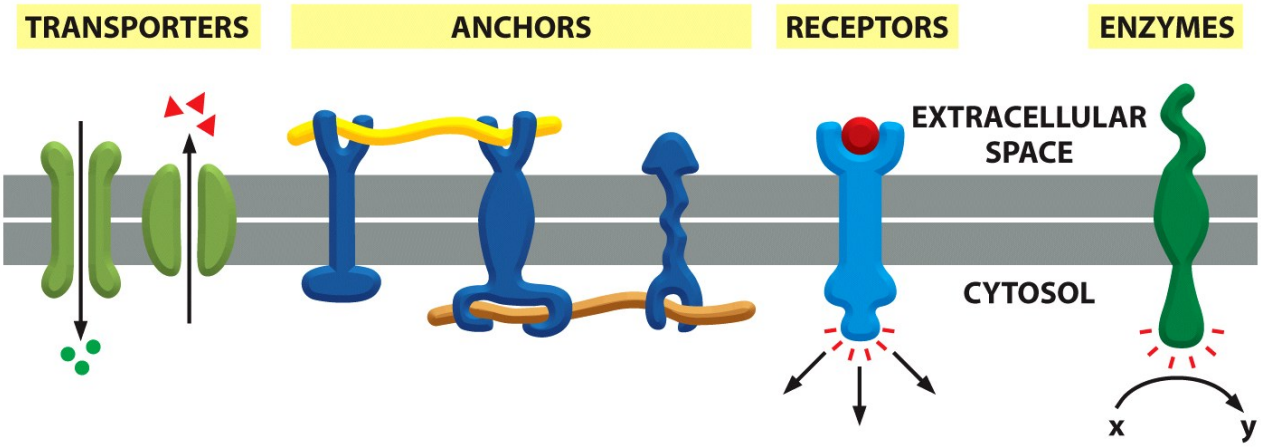


Figure 11-20 Essential Cell Biology 3/e (© Garland Science 2010)

Membrane proteins: positional groups

- Transmembrane (integral, IMP)
- Monolayer-associated (IMP)
- Lipid-linked (IMP)
- Protein-attached (peripheral, PMP)

Positional groups of membrane proteins

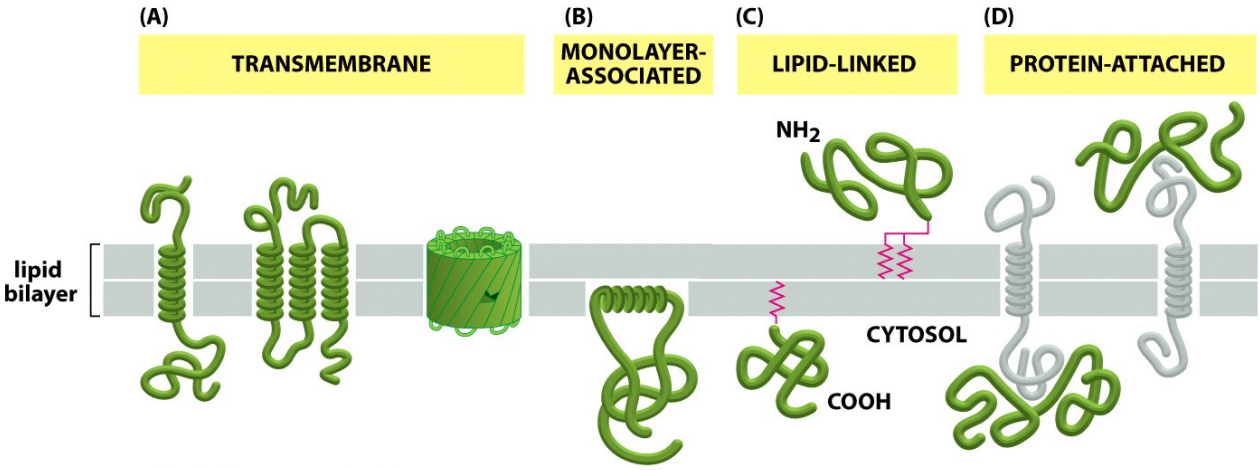


Figure 11-21 Essential Cell Biology 3/e (© Garland Science 2010)

Membrane proteins: secondary structure

- Mostly α-helices
- Sometimes also β-barrels (porins)

Membrane α-helix

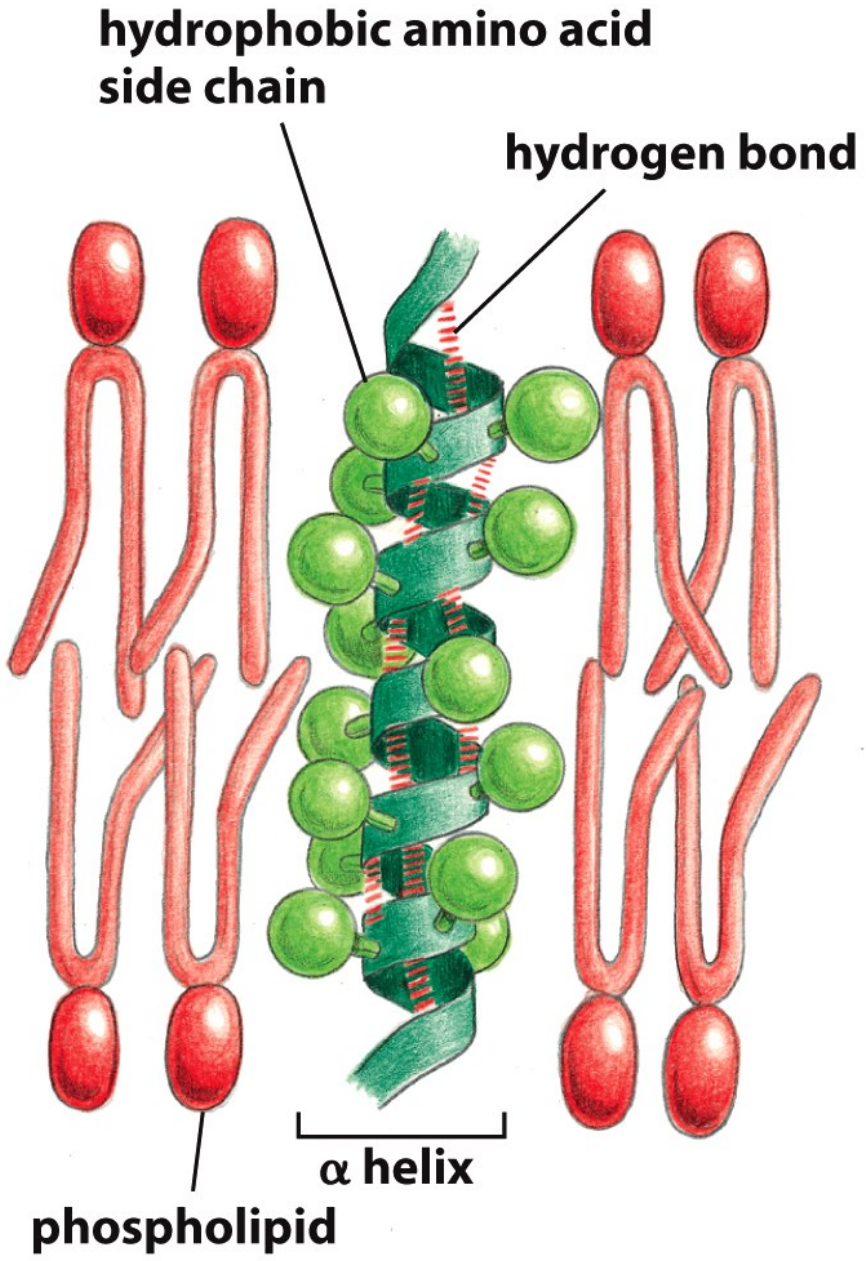


Figure 11-23 Essential Cell Biology 3/e (© Garland Science 2010)

Porin: β-barrel

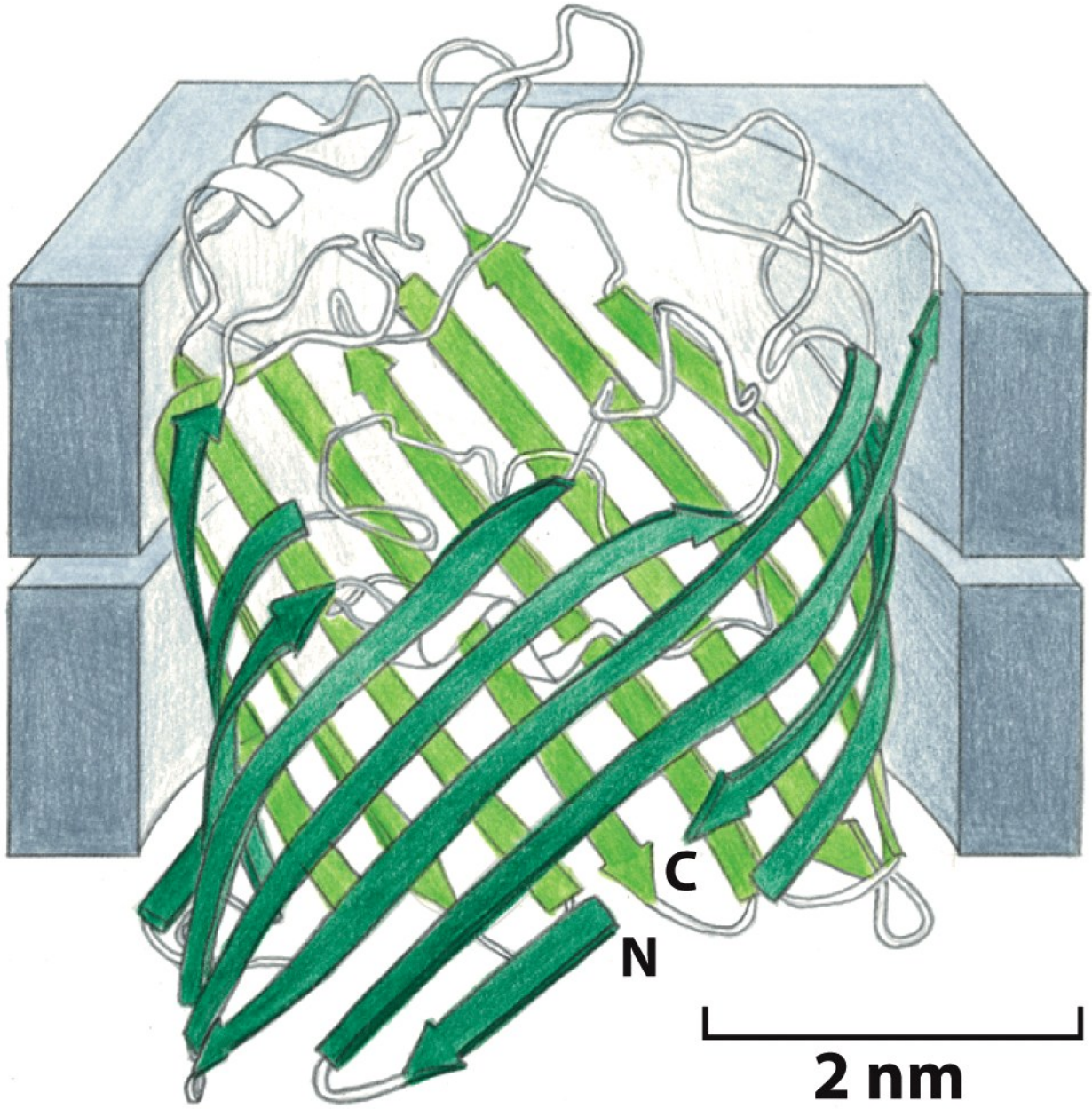


Figure 11-25 Essential Cell Biology 3/e (© Garland Science 2010)

Detergents

- Small lipid-like molecules
- Solubilize phospholipids, leaving proteins intact

Membrane disruption movie

Bacteriorhodopsin

- Small protein of ≈250 amino acids
- Proton pump activated by light

Bacteriorodopsin

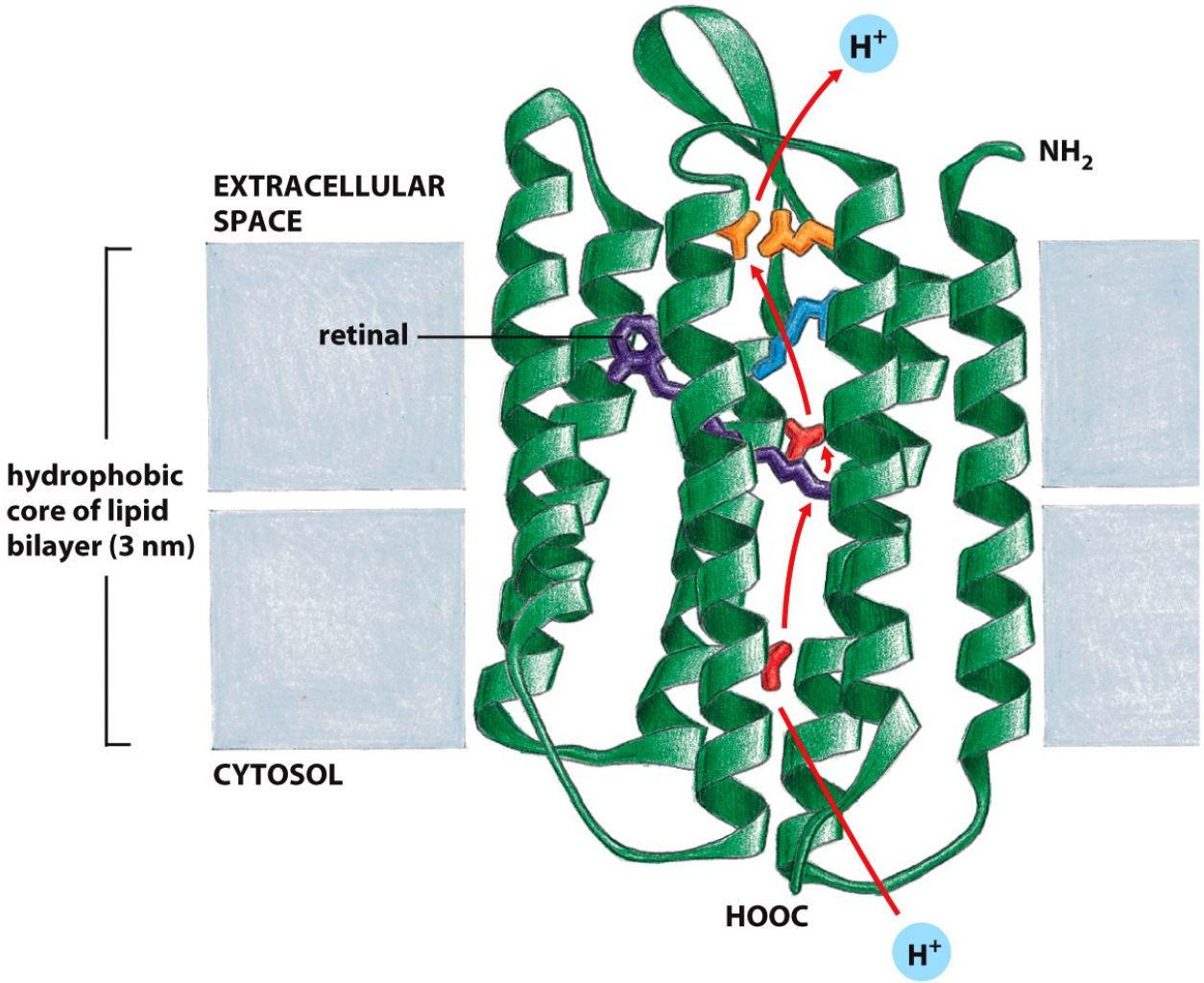


Figure 11-28 Essential Cell Biology 3/e (© Garland Science 2010)

Bacteriorodopsin movie

Cell cortex

- Cell cortex proteins like spectrins form a supportive network
- This network will stabilize a "lipid liquid" movements
- Some peptides may be restricted to particular domains of membrane

Spectrins

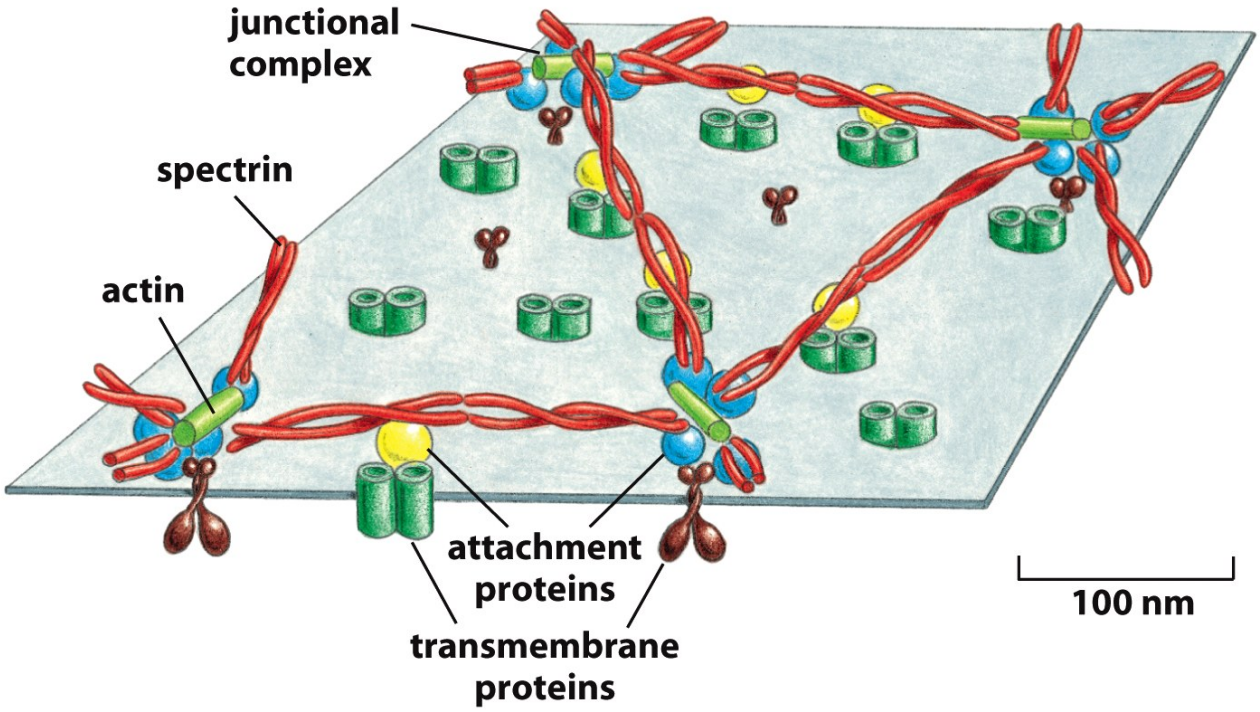


Figure 11-31a Essential Cell Biology 3/e (© Garland Science 2010)

Plasma membrane domains

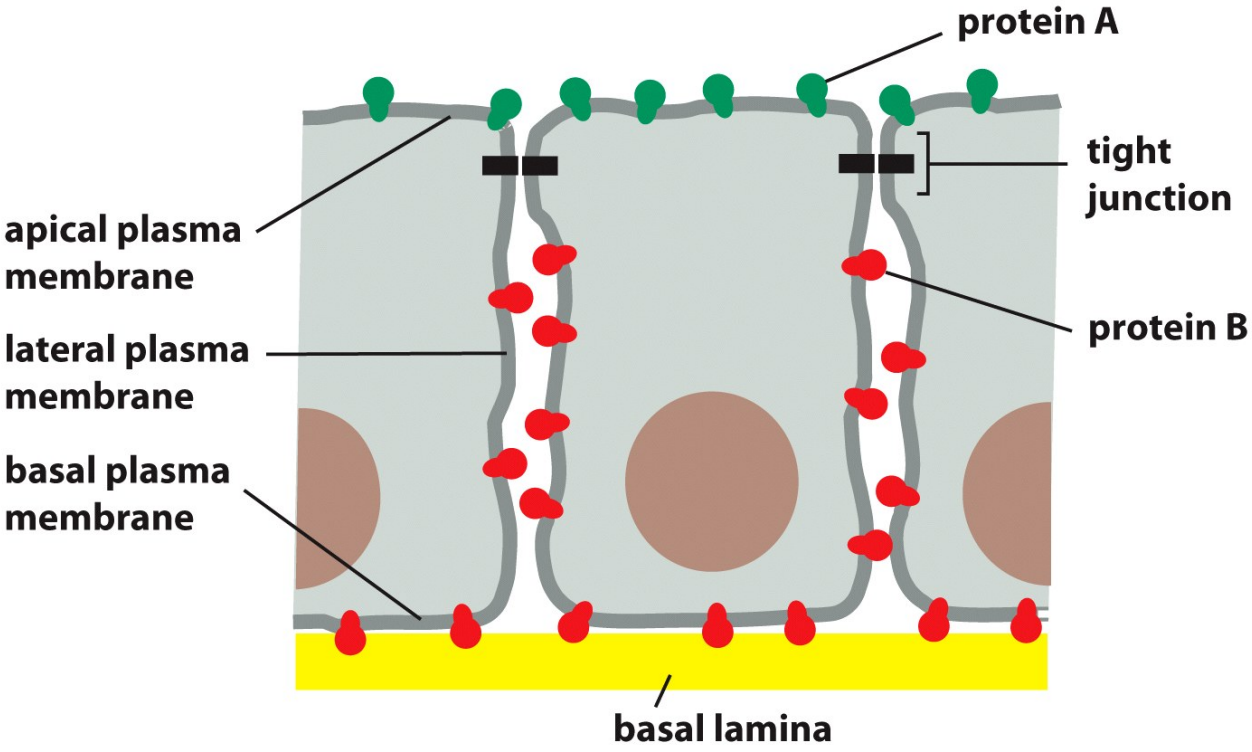


Figure 11-34 Essential Cell Biology 3/e (© Garland Science 2010)

Membrane (cortex) carbohydrates

- Glycoproteins and peptidoglycans
- Form carbohydrate layer

- Protect, lubricate and used as recognition molecules (e.g., for lectins)

Cortex

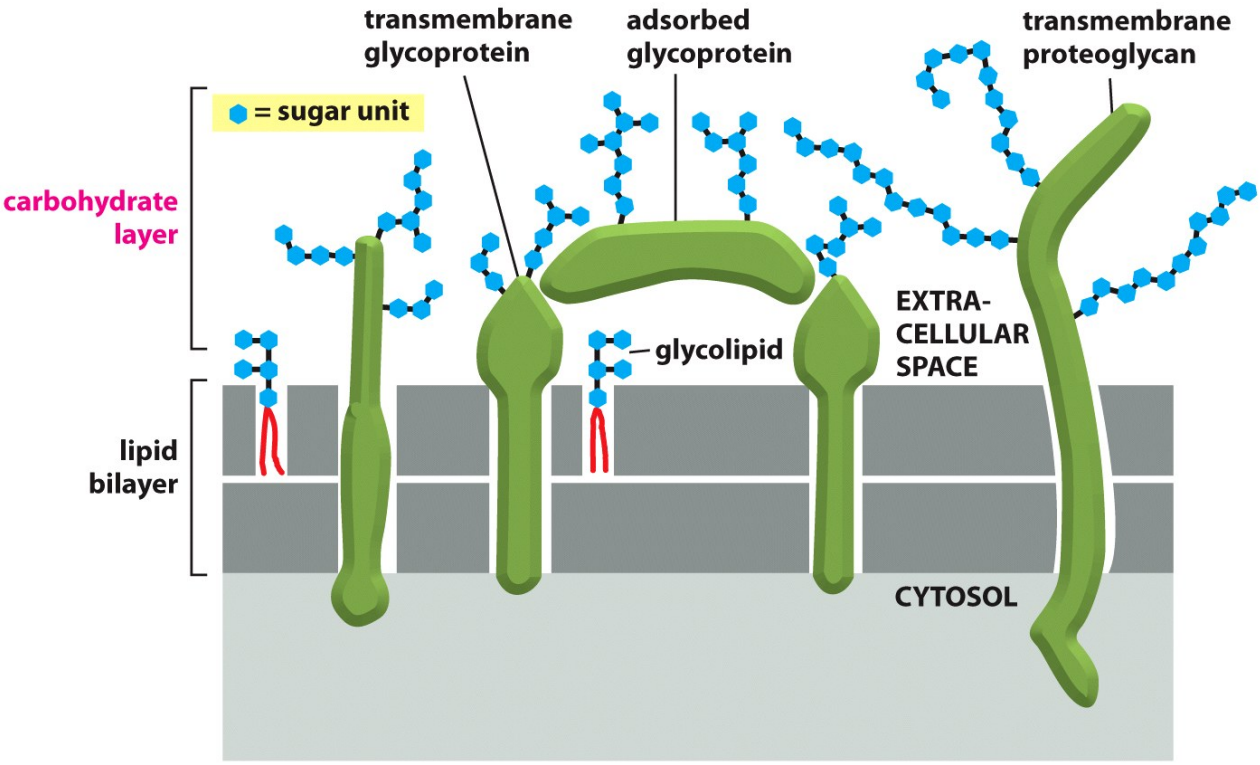


Figure 11-35 Essential Cell Biology 3/e (© Garland Science 2010)

Lectins

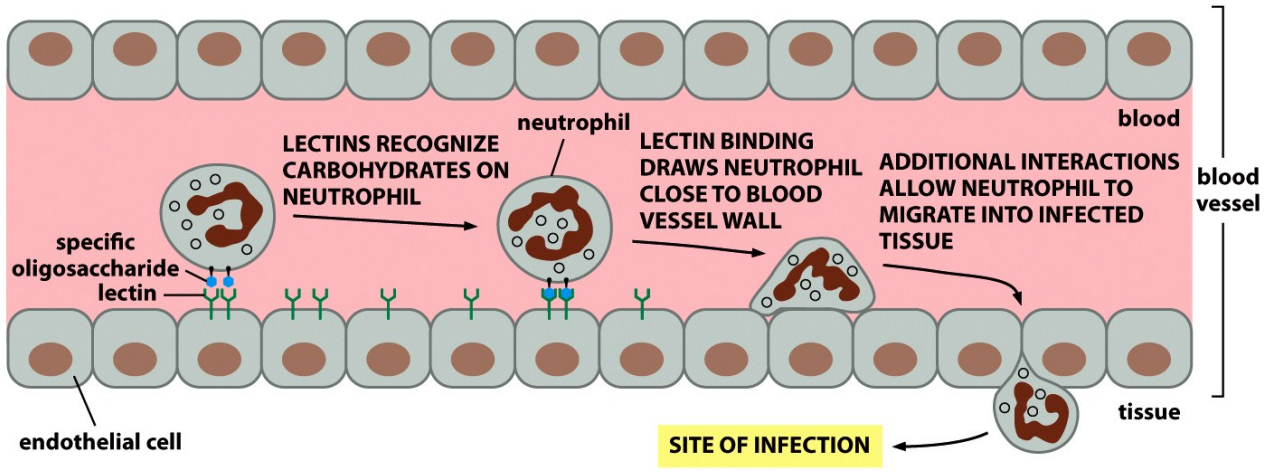


Figure 11-39 Essential Cell Biology 3/e (© Garland Science 2010)

Some methods of membrane research

- FRAP (fluorescence recovery after bleaching)
- SPT (single-particle tracking) microscopy
- Solubilizing and reconstituting of membrane proteins

FRAP movie

Solubilizing and reconstituting

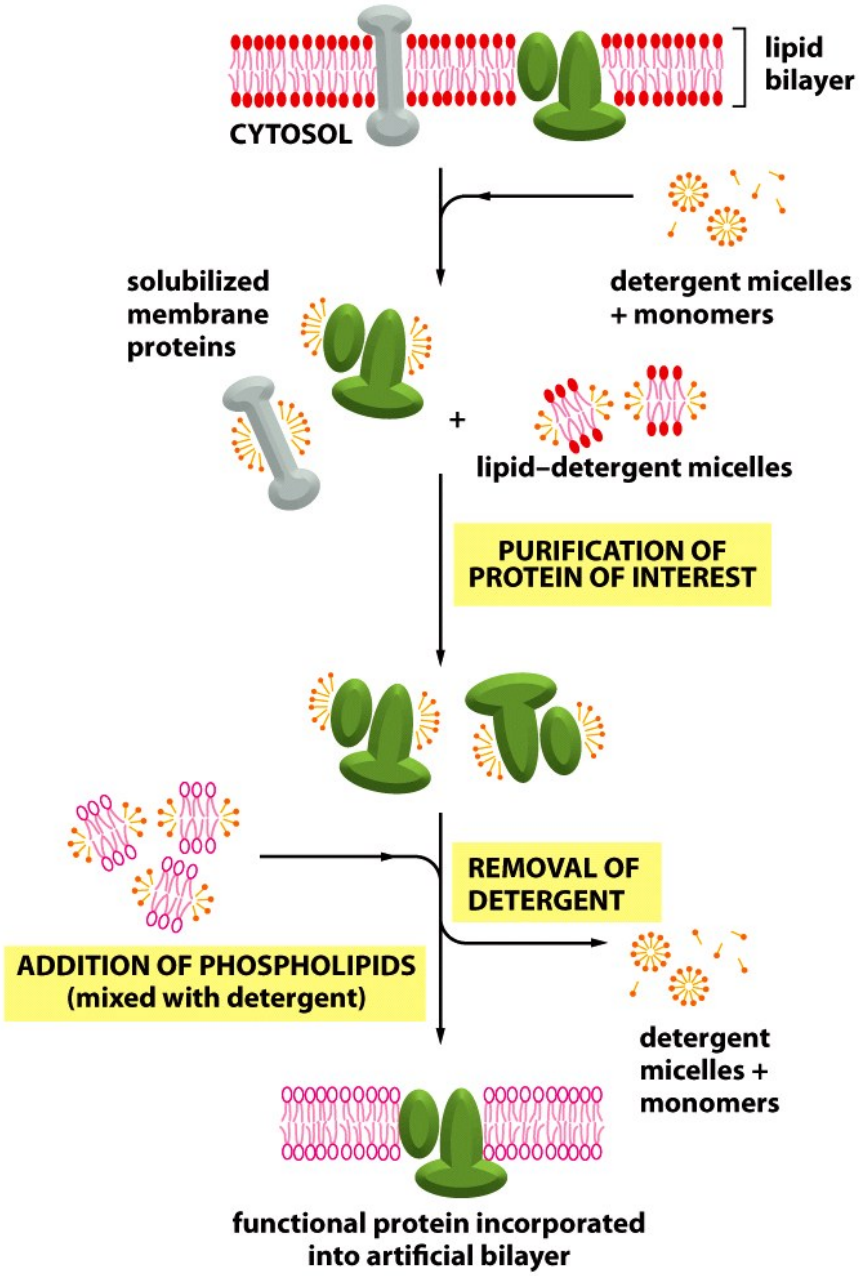


Figure 11-38 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (2 points)

What is the difference between receptor and transporter proteins?

Summary

- Membranes are selective, bilayer, fluid barriers
- Membrane lipids are amphipatic
- Membrane proteins do trans-membrane jobs
- Membrane (cortex) carbohydrates are protectives and "informers"

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 11*.

Outline

Questions and answers

Previous final question: the answer

What is the difference between receptor and transporter proteins?

- Transporter is a "hole" or "revolving door" whereas receptor is a "wall with doorbell". However, it is possible to combine them.

Membranes

.1 Membrane transport

Concentrations

- Unequal between outer and inner spaces
- Higher outside: Na^+, Ca^{2+}, Cl^-
- Higher inside: K^+, H^+

Diffusion

- Easy: small non-polar and uncharged polar
- Impossible: uncharged polar bigger than methanol; all ions

Rates of diffusion

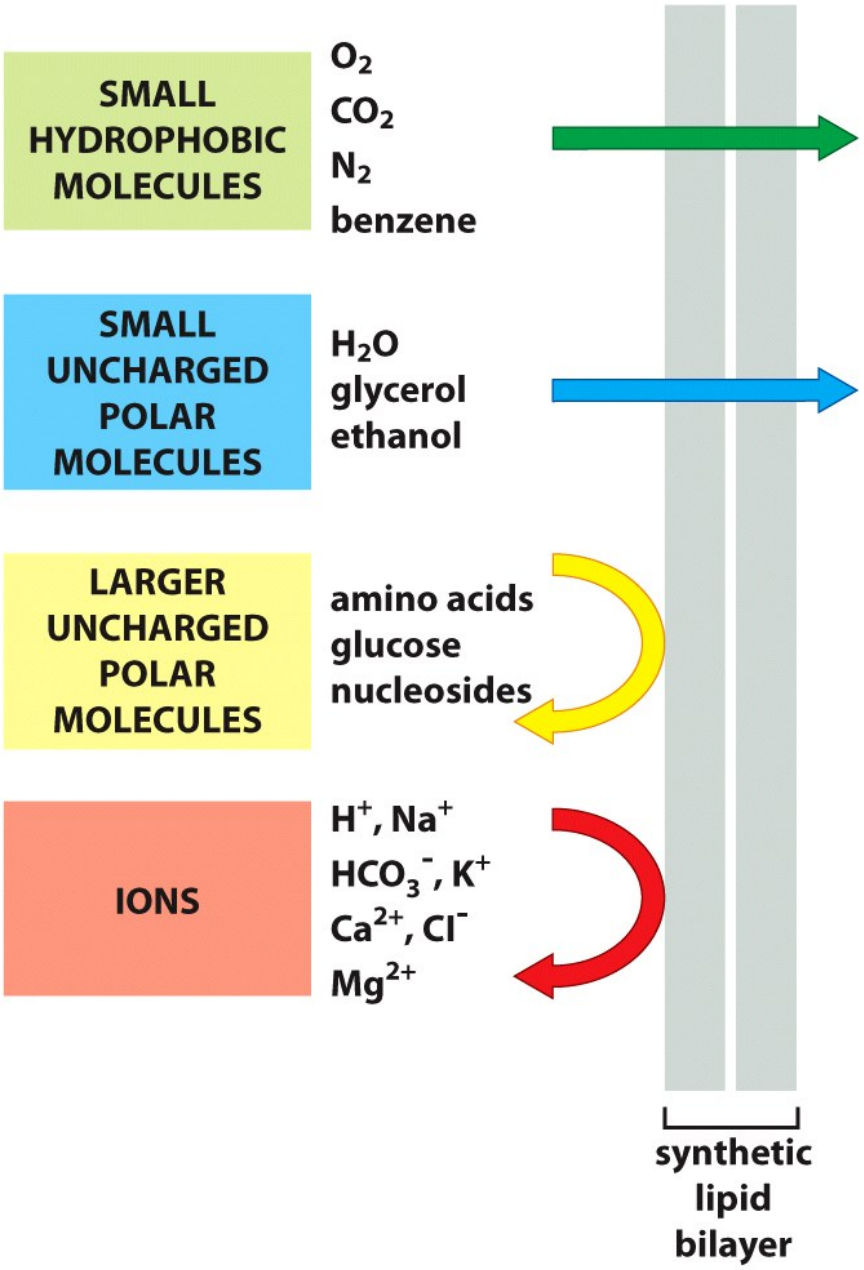


Figure 12-2 Essential Cell Biology 3/e (© Garland Science 2010)

Transporters and channels

- Channels discriminate on the basis of charge and size: "trapdoors"
- Transporters discriminate by binding: "keyholes"

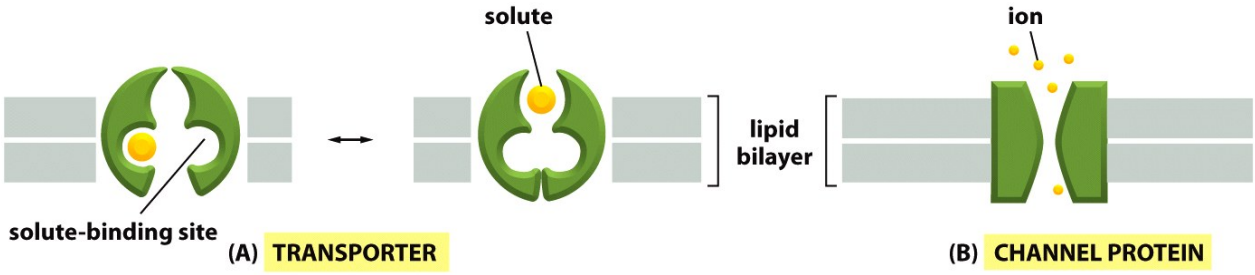


Figure 12-3 Essential Cell Biology 3/e (© Garland Science 2010)

Active and passive transport

- Active: energy spent, against concentration gradient—pumps
- Passive (facilitated diffusion): energy does not spent, along the concentration gradient

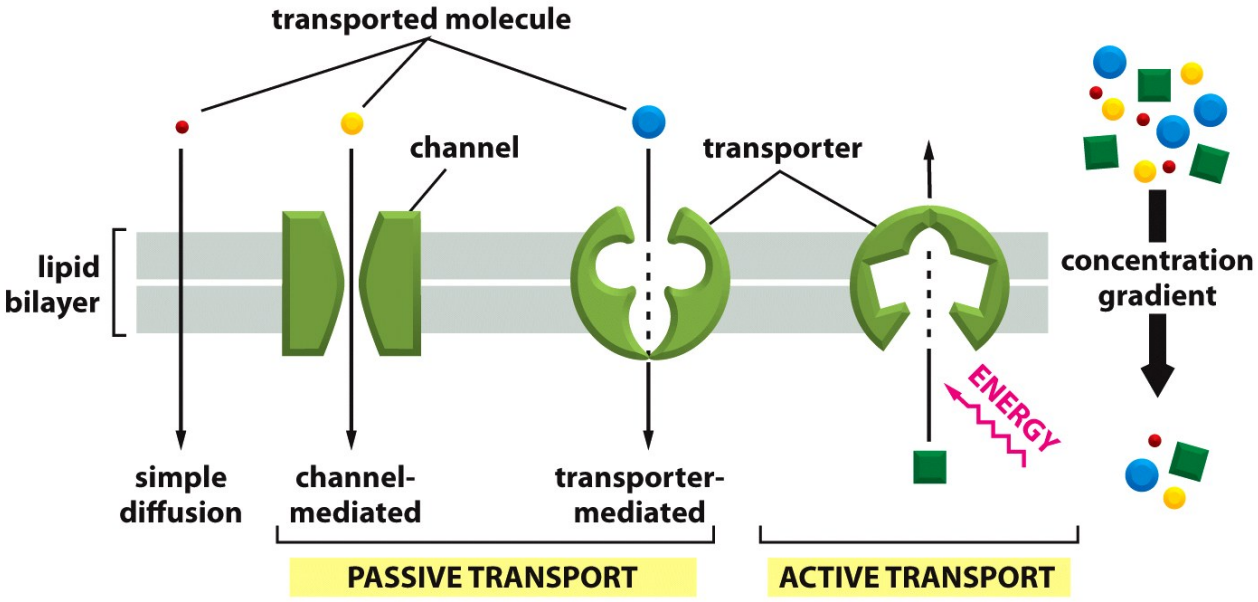


Figure 12-4 Essential Cell Biology 3/e (© Garland Science 2010)

Transporters: passive

- Glucose transporter: binds glucose and transfer it along the concentration gradient
- But only D-glucose
- Chemical + charging gradients = electrochemical gradient

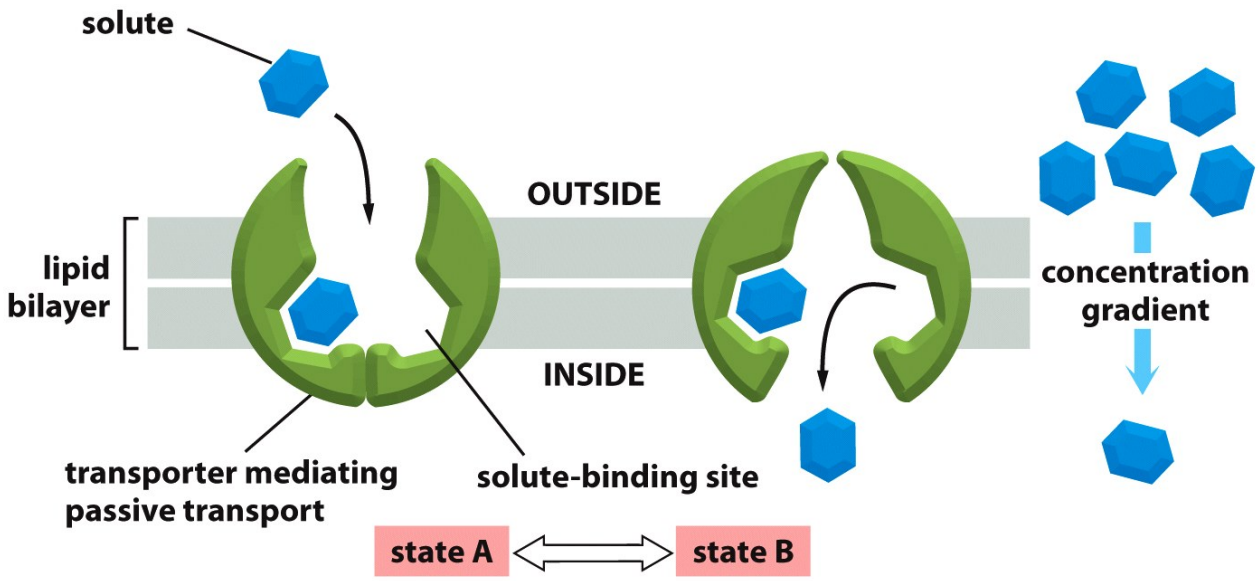


Figure 12-6 Essential Cell Biology 3/e (© Garland Science 2010)

Transporters: active

- Coupled (e.g., with gradient)
- ATP-driven
- Light-driven

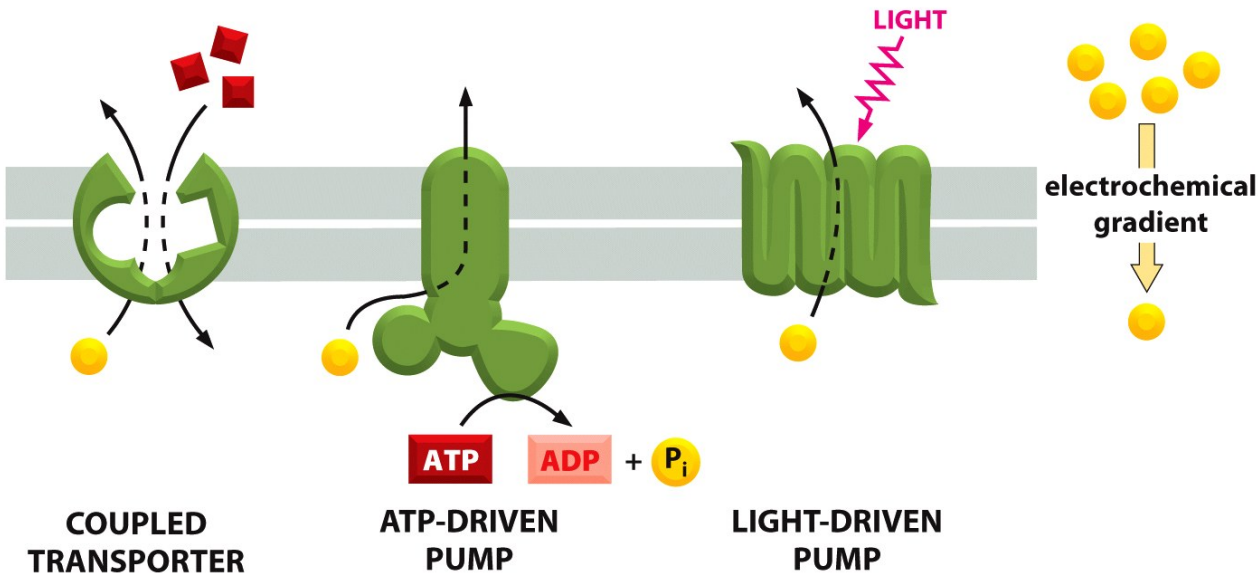


Figure 12-8 Essential Cell Biology 3/e (© Garland Science 2010)

Sodium-potassium pump I

- The other name is Na^+–K^+ ATPase
- ≈30% of ATP consumption in animal cells

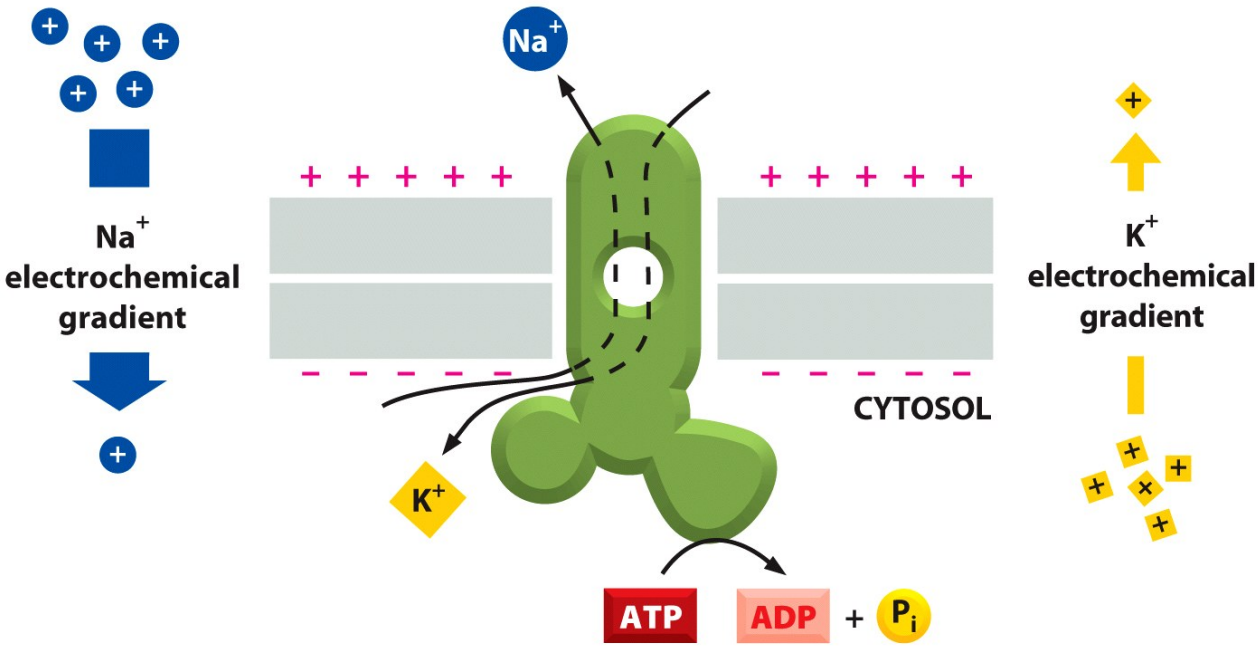


Figure 12-9 Essential Cell Biology 3/e (© Garland Science 2010)

Sodium-potassium pump II

- Sodium-potassium is actually a cycle
- P group is added and released

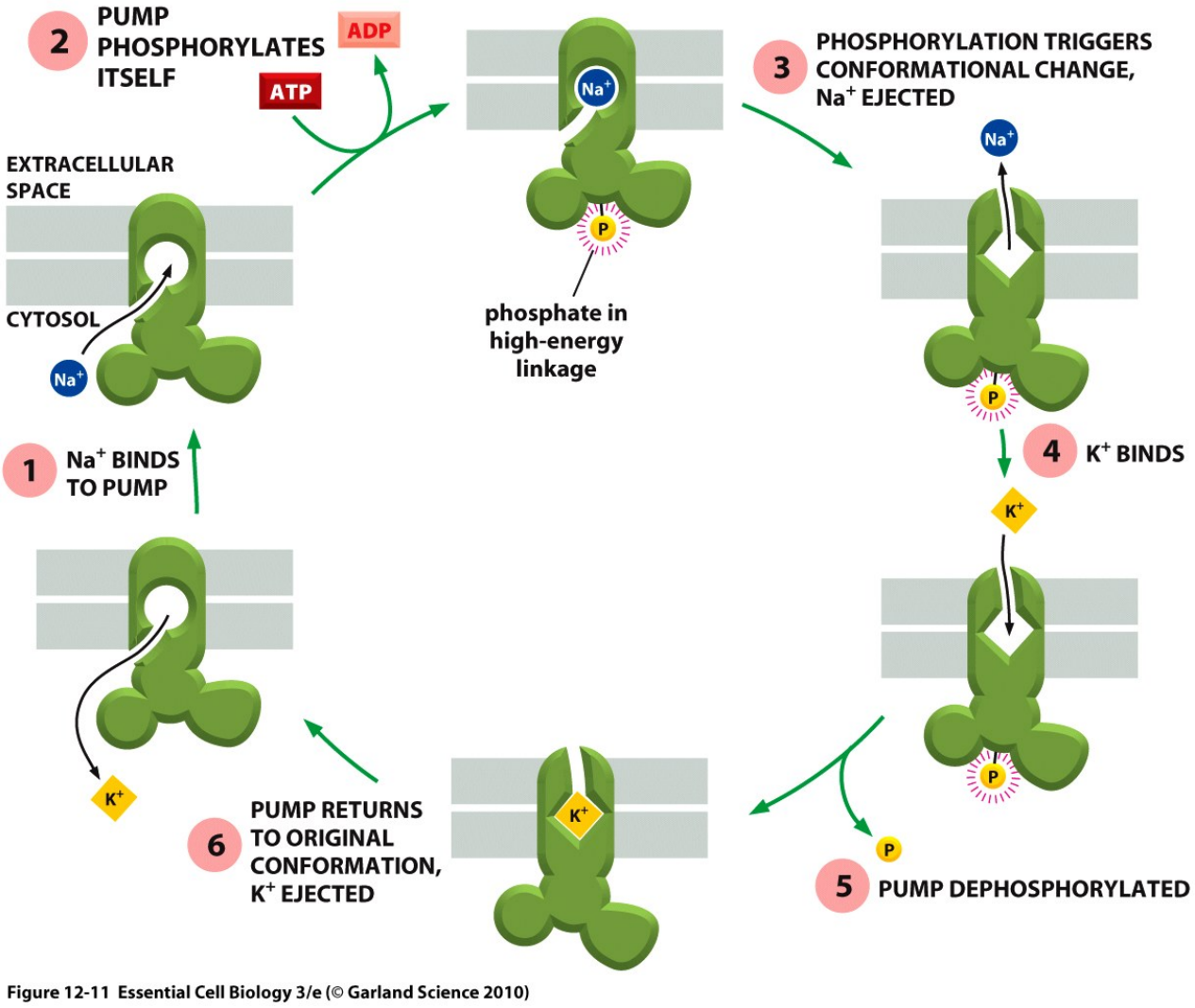


Figure 12-11 Essential Cell Biology 3/e (© Garland Science 2010)

Sodium-potassium movie

Osmotic balance

- Osmosis is the default diffusion of ions in a water across semi-permeable membrane
- Aquaporin channels facilitate the water movement
- Cell walls and contractile vacuoles are used to maintain osmotic pressure, but in animals there is only a sodium-potassium pump (and some other pumps)

Calcium pumps

- The goal is to keep calcium concentration low
- Calcium pump is also an ATPase

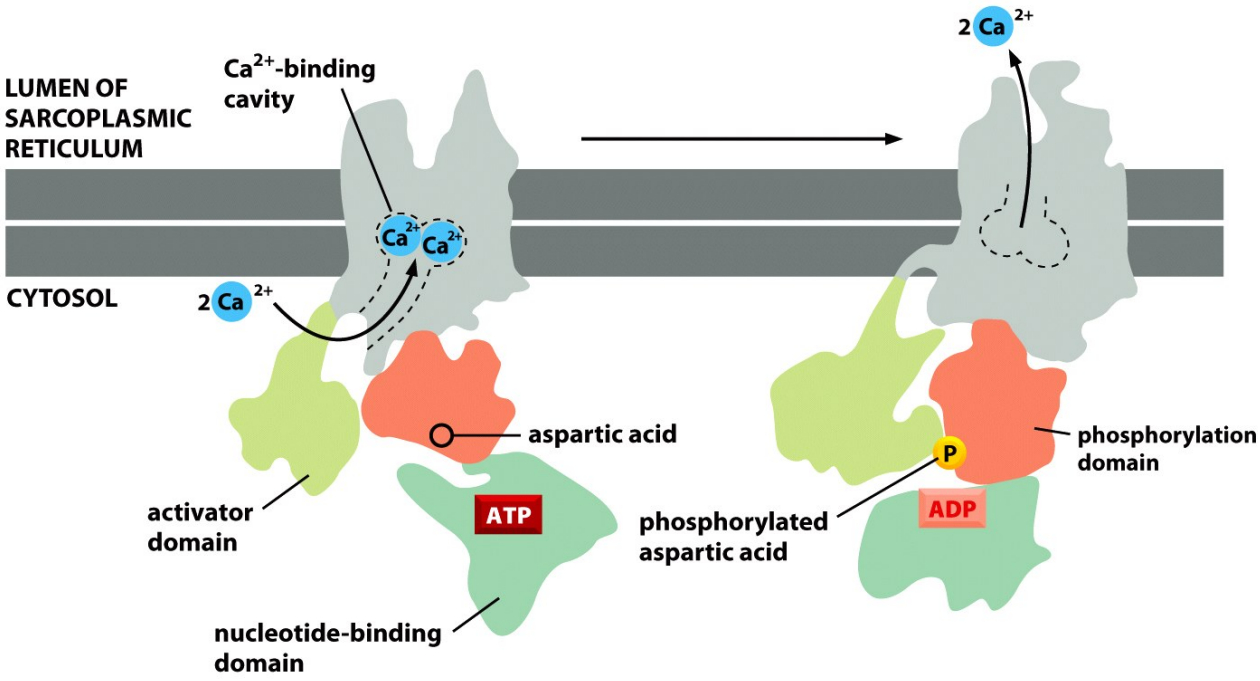


Figure 12-15 Essential Cell Biology 3/e (© Garland Science 2010)

Coupled transporters

- Symports: glucose–Na^+ symport (there is also non-coupled glucose uniport)
- Antiports: Na^+–H^+ exchanger

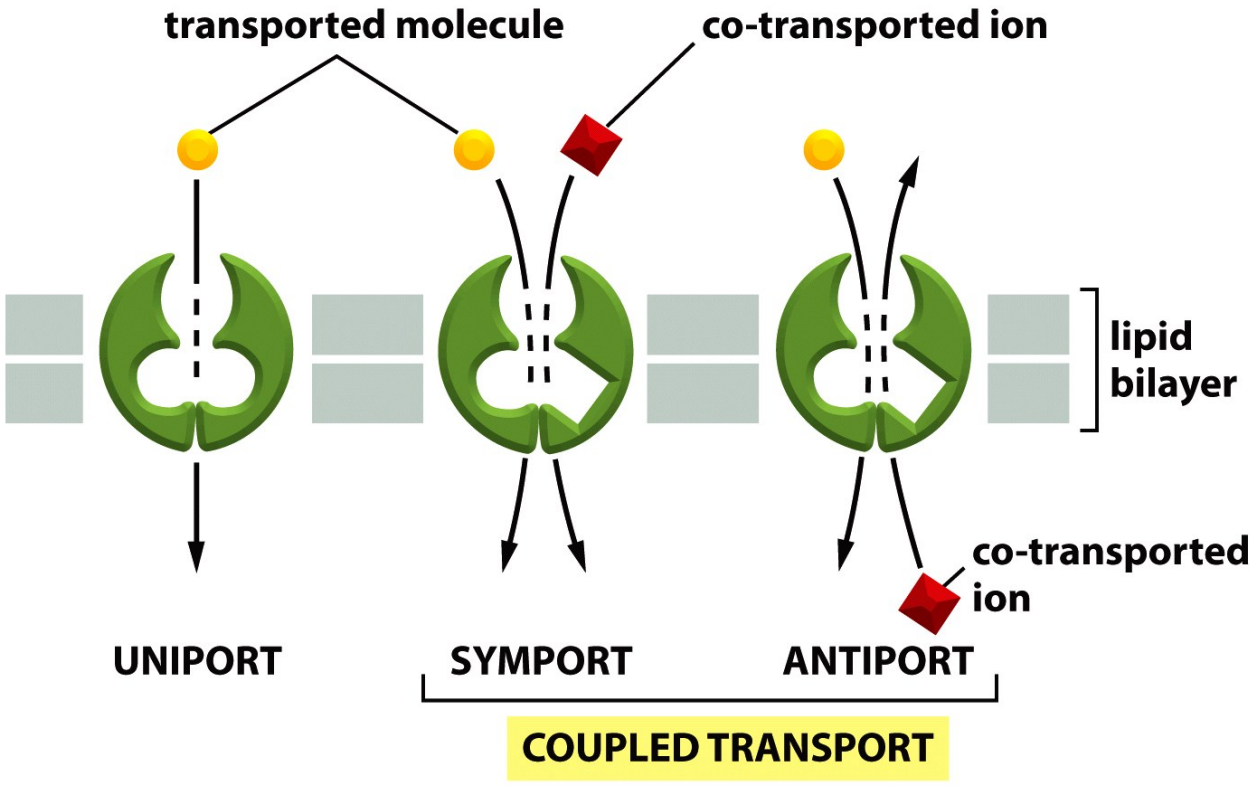


Figure 12-16 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (2 points)

Are channels selective?

Summary

- Both transporters and channels are selective
- Only transporters may be active

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 12*.

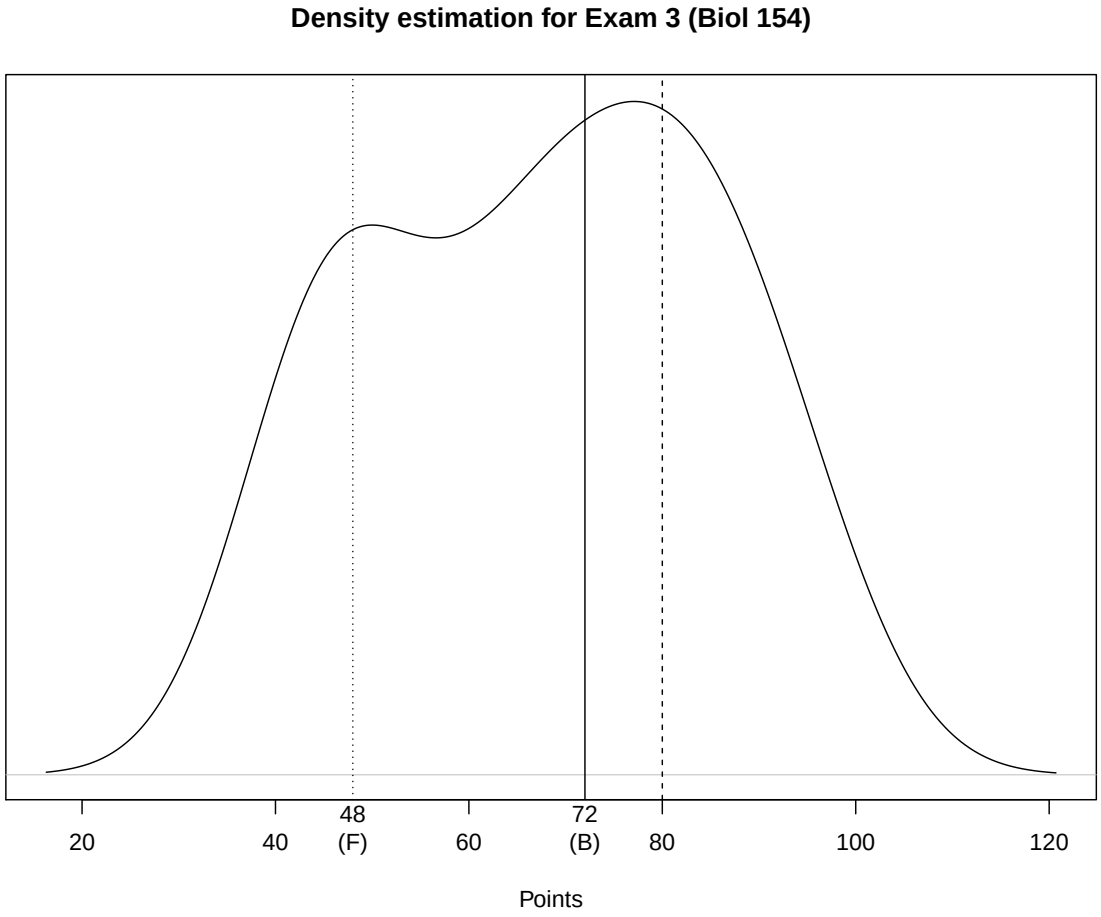
Outline

Questions and answers

Results of Exam 3: statistic summary

```
Summary:
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  43.00   51.00   68.00   67.82   82.00   94.00
Grades:
  F   D   C   B max
 48  56  64  72  80
```

Results of Exam 3: the curve



Question 32

- What is more essential in producing GMO:
    1. Possibility to create a recombinant DNA
    2. High frequency of mutations
    3. **Sexual propagation of progeny**

Previous final question: the answer
Are channels selective?

- Yes!

Membrane transport

.1 Symport and antiport

Coupled transporters

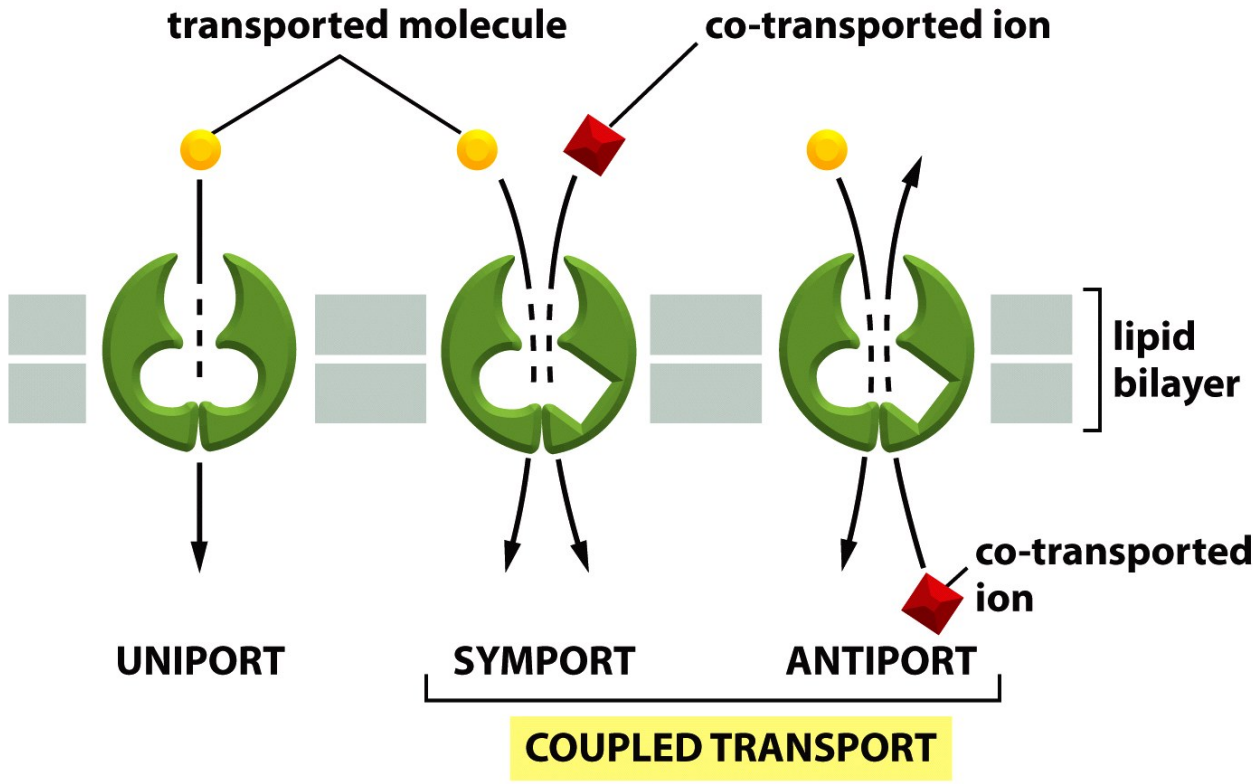
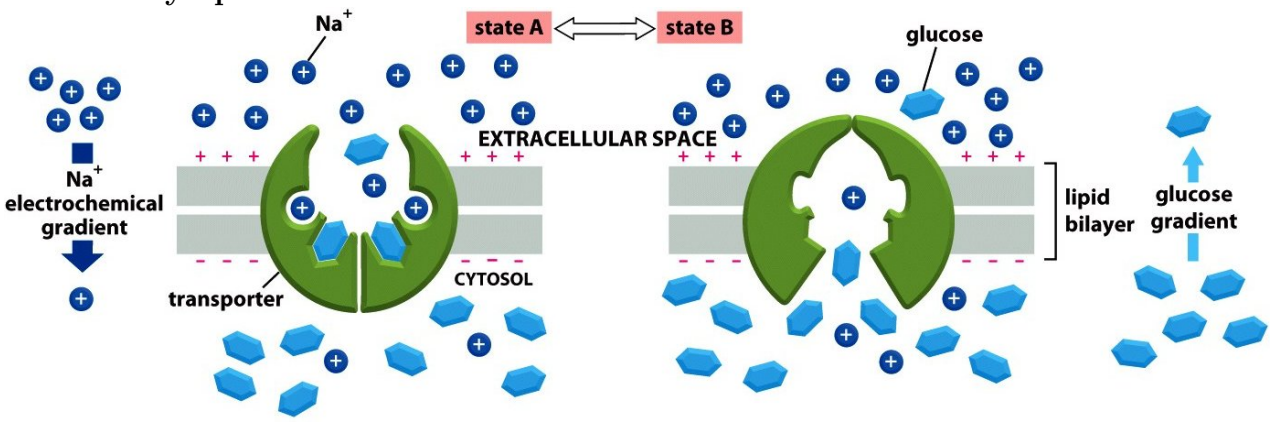


Figure 12-16 Essential Cell Biology 3/e (© Garland Science 2010)

Carrier proteins movie

Glucose–Na^+ symport



It uses electrochemical gradient of Na^+ to transport glucose into cell.

Glucose uptake movie

.2 Transporters

Proton gradients

- Non-animals maintain the osmotic pressure through proton pumps
- Bacteriorodopsin is a light-driven proton pump

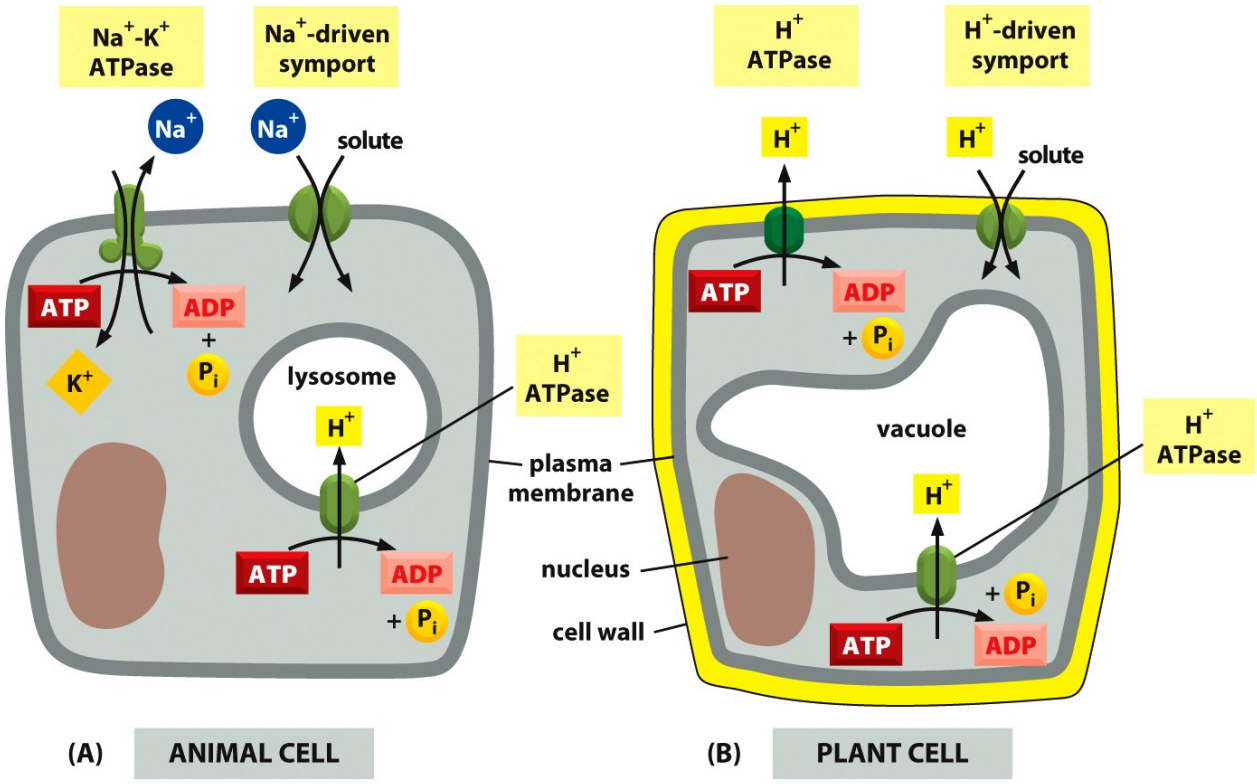


Figure 12-19ab Essential Cell Biology 3/e (© Garland Science 2010)

TABLE 12–2 SOME EXAMPLES OF TRANSPORTERS

TRANSPORTER	LOCATION	ENERGY SOURCE	FUNCTION
Glucose transporter	plasma membrane of most animal cells	none	passive import of glucose
Na^+-driven glucose pump	apical plasma membrane of kidney and intestinal cells	Na^+ gradient	active import of glucose
Na^+-H^+ exchanger	plasma membrane of animal cells	Na^+ gradient	active export of H^+ ions, pH regulation
Na^+-K^+ pump (Na^+-K^+ ATPase)	plasma membrane of most animal cells	ATP hydrolysis	active export of Na^+ and import of K^+
Ca^{2+} pump (Ca^{2+} ATPase)	plasma membrane of eucaryotic cells	ATP hydrolysis	active export of Ca^{2+}
H^+ pump (H^+ ATPase)	plasma membrane of plant cells, fungi, and some bacteria	ATP hydrolysis	active export of H^+
H^+ pump (H^+ ATPase)	membranes of lysosomes in animal cells and of vacuoles in plant and fungal cells	ATP hydrolysis	active export of H^+ from cytosol into vacuole
Bacteriorhodopsin	plasma membrane of some bacteria	light	active export of H^+

Table 12-2 Essential Cell Biology 3/e (© Garland Science 2010)

.3 Ion channels

Ion channels

- Narrow
- Some are wide like gap junction channels, porin channels

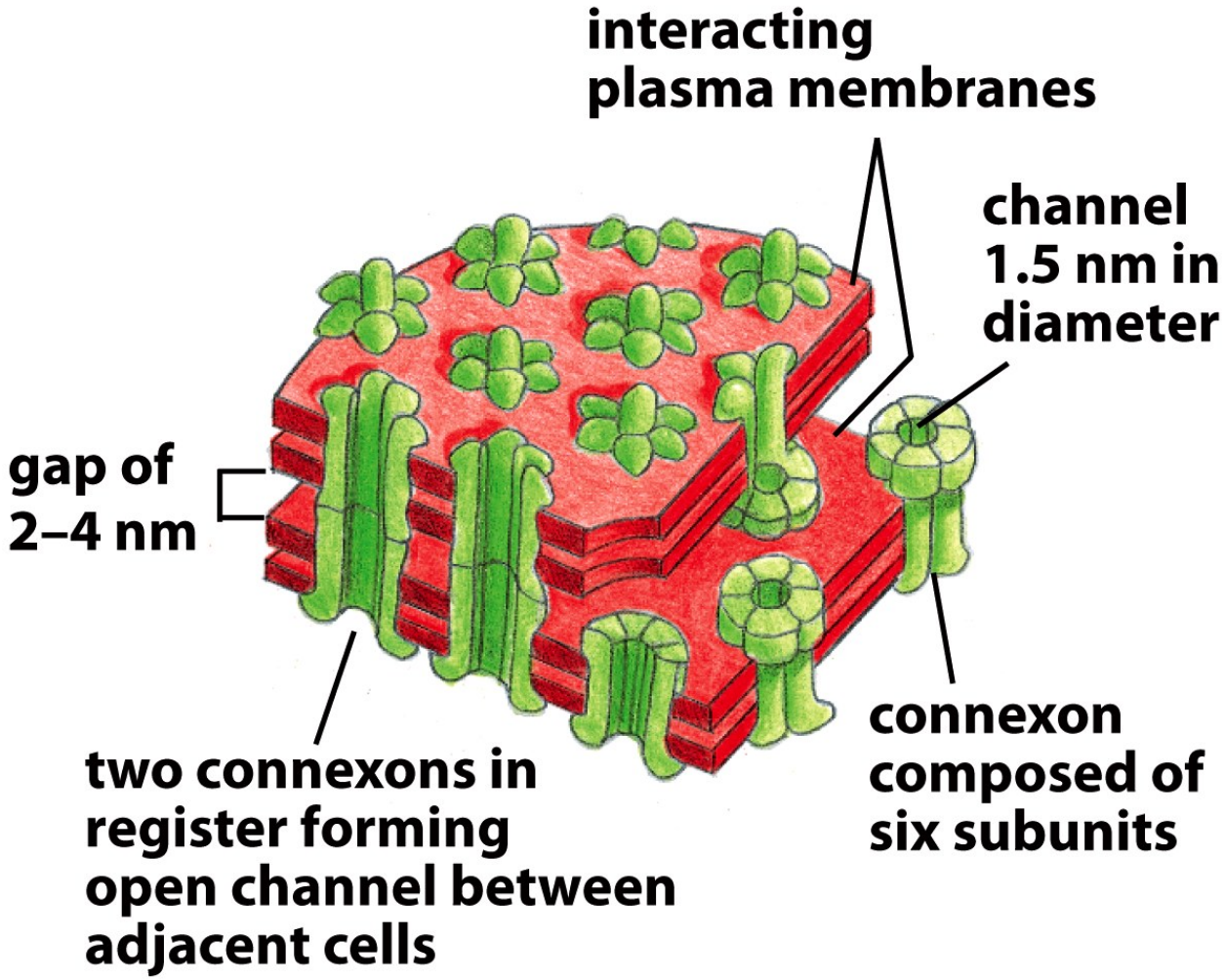


Figure 20-29b Essential Cell Biology 3/e (© Garland Science 2010)

Ion channels: selectivity

- Select ions on the basis of size and charge
- Ion filers facilitate the selectivity of ions
- Differ from simple pores also because channels have two states, open and closed

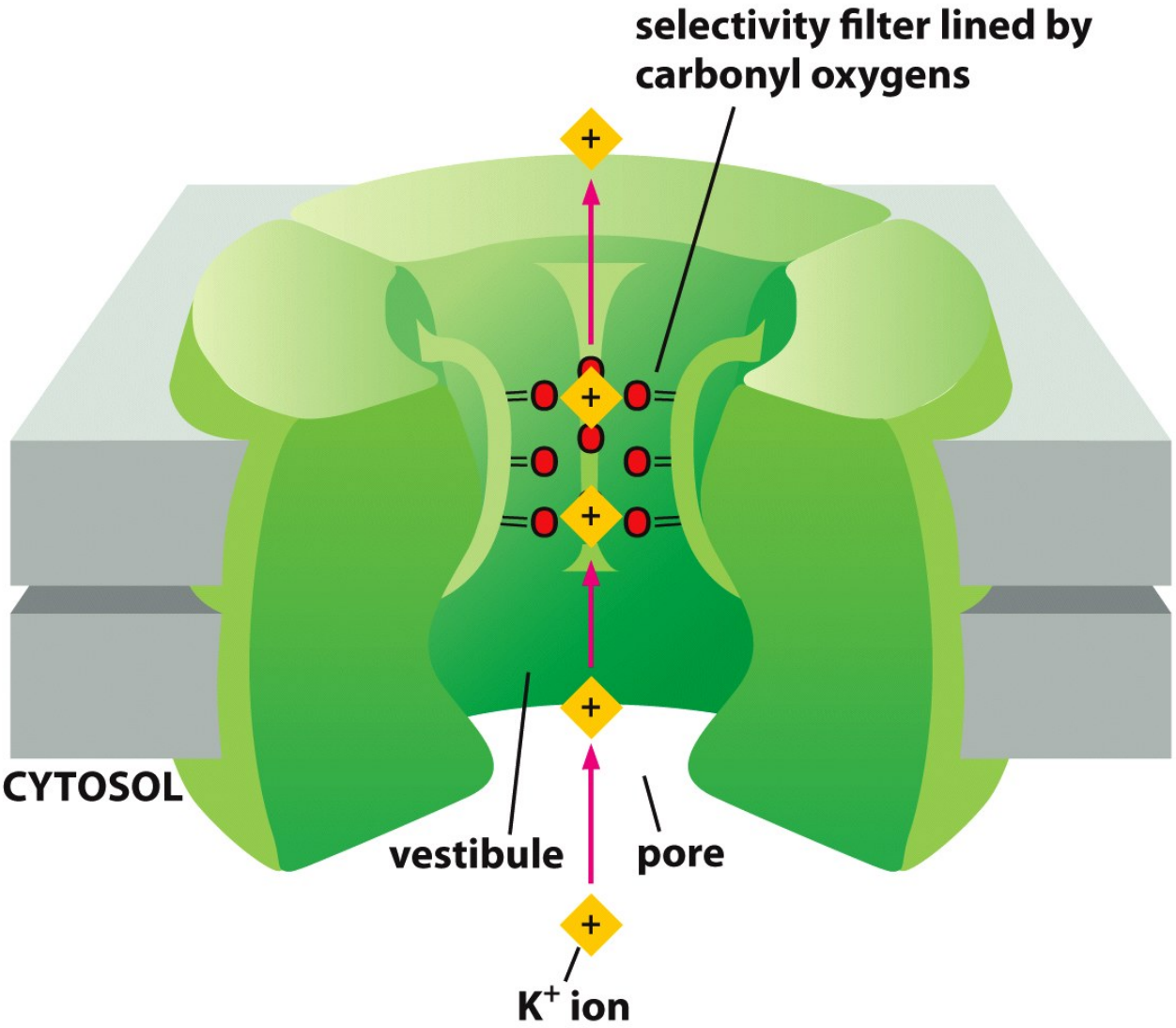


Figure 12-20 Essential Cell Biology 3/e (© Garland Science 2010)

Potassium channel movie

Studying ion channels: patch-clamp recording

- Remove a piece of membrane
- Change ion concentration and voltage round this piece
- Measure changes

Patch-clamp technique

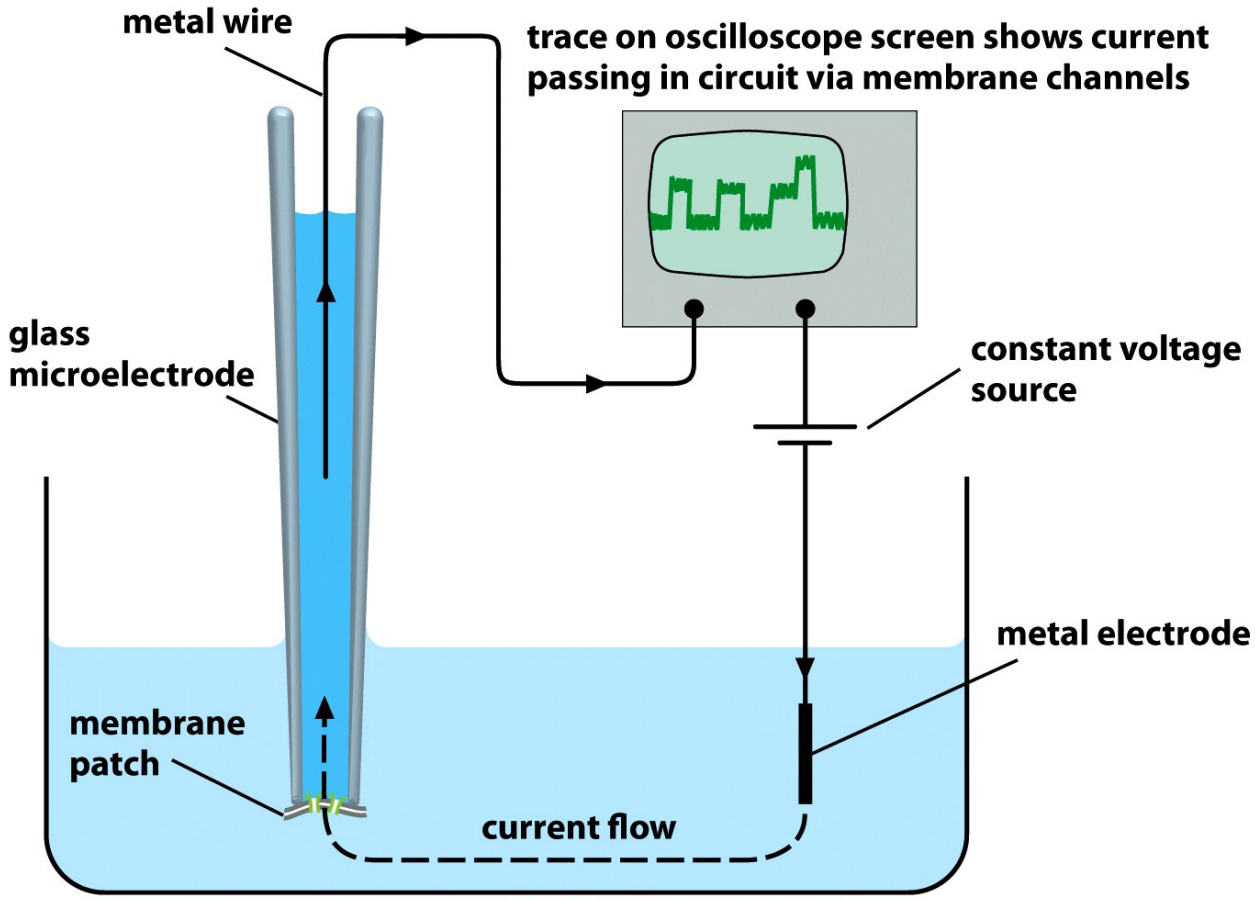


Figure 12-23d Essential Cell Biology 3/e (© Garland Science 2010)

Different types of ion gates

- Voltage-gated
- Ligand-gated
- Stress-gated

Gated channels

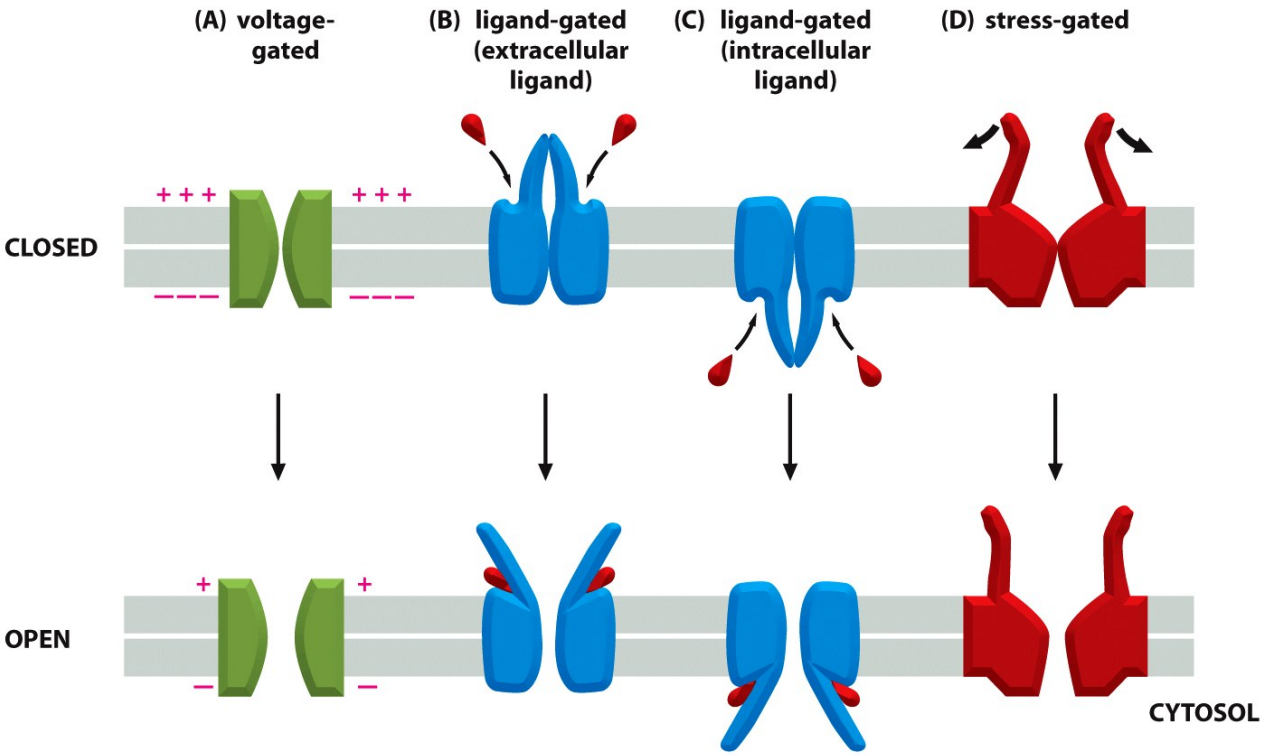


Figure 12-25 Essential Cell Biology 3/e (© Garland Science 2010)

Voltage gates and membrane potential

- Voltage gates will open if electrical (!) membrane potential reaches a given value
- Then, membrane potential changes and channels are closed again
- They are responsible for most cases of sensitivity (except stress)

Mimosa plant

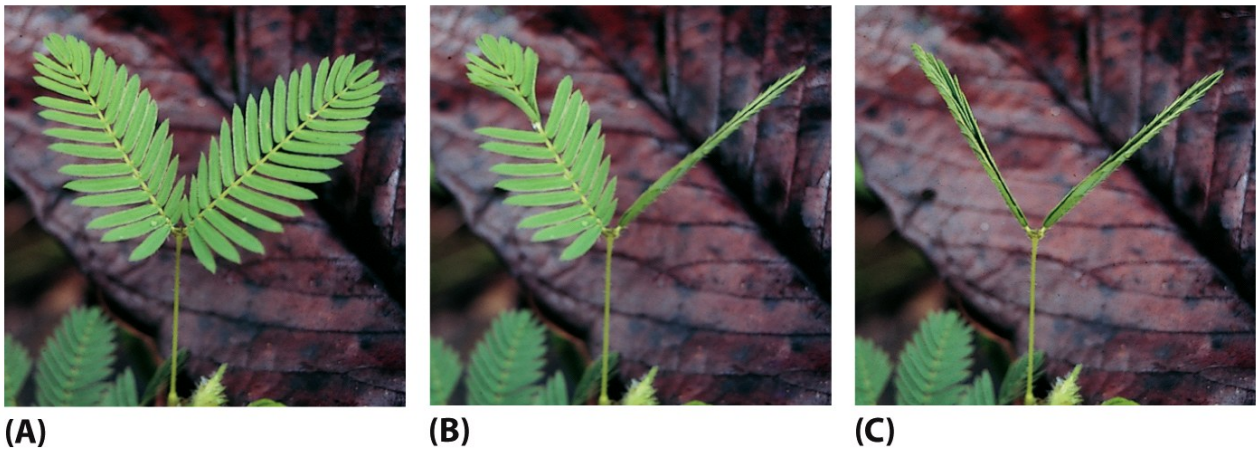


Figure 12-27 Essential Cell Biology 3/e (© Garland Science 2010)

Stress gates

- Stress gates react to mechanical changes
- Present in inner ear of mammals (go back to the lateral line of fishes)

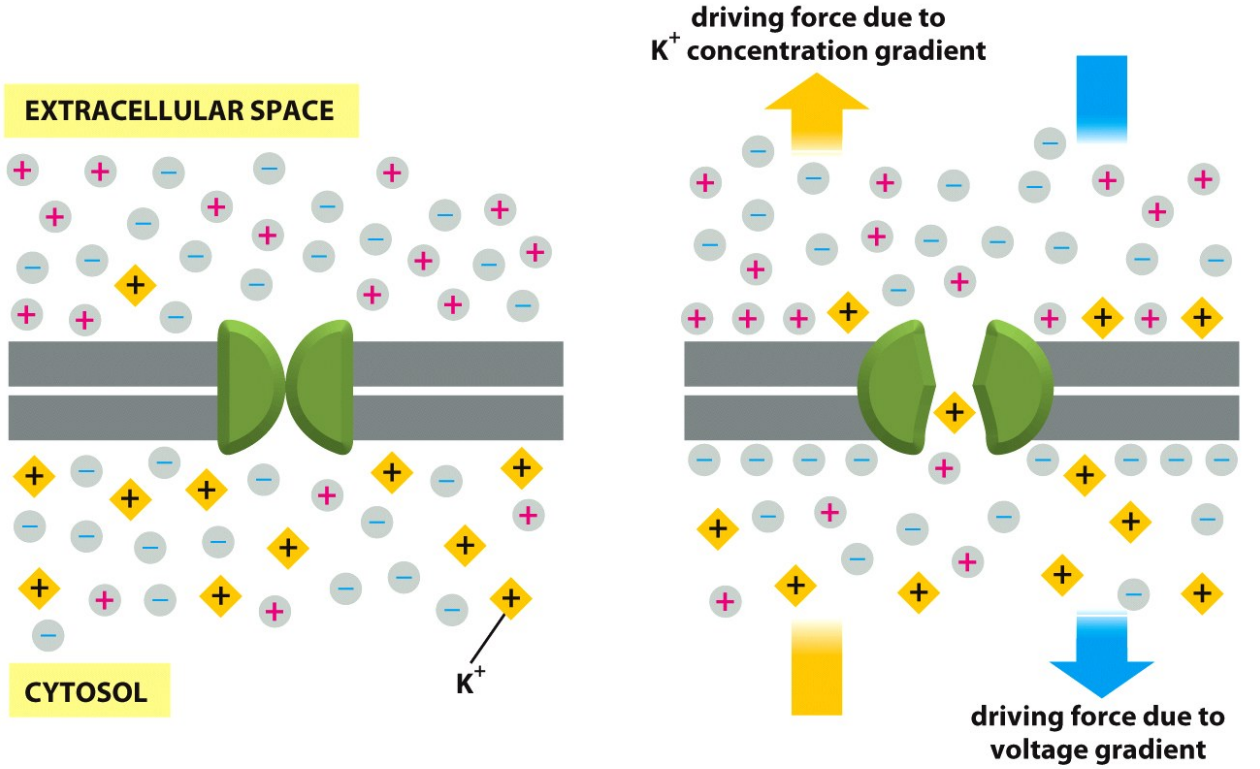
Stress gated channels movie I

Stress gated channels movie II

Origin of membrane potential

- Normally, K^+ leak channels are open randomly and let K^+ to move freely
- However, there are still much more K^+ inside than outside because when it leaks out, electric force will stop this process at some point
- This point is a resting potential, ≈–20–200 mV

K^+ channels



(A) K^+ channel closed, membrane potential = 0; more K^+ inside the cell than outside, but zero *net* charge on each side (positive and negative charges balanced exactly)

(B) K^+ channel open; K^+ moves out, leaving negative ions behind, and this charge distribution creates a membrane potential that balances the tendency of K^+ to move out

Figure 12-29 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (2 points)

What is a difference between plant and animal cells in concentration of ions outside and inside cell?

Summary

- Both transporters and channels are selective
- Only transporters may be active
- Most ion channels are gated and not open all time
- Membrane potential is a result of unequal distribution of electric charge across a membrane

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 12*.

Outline

Questions and answers

Previous final question: the answer

What is a difference between plant and animal cells in concentration of ions outside and inside cell?

- H^+ inequality *versus* K^+/Na^+ and Ca^{2+} inequalities, respectively

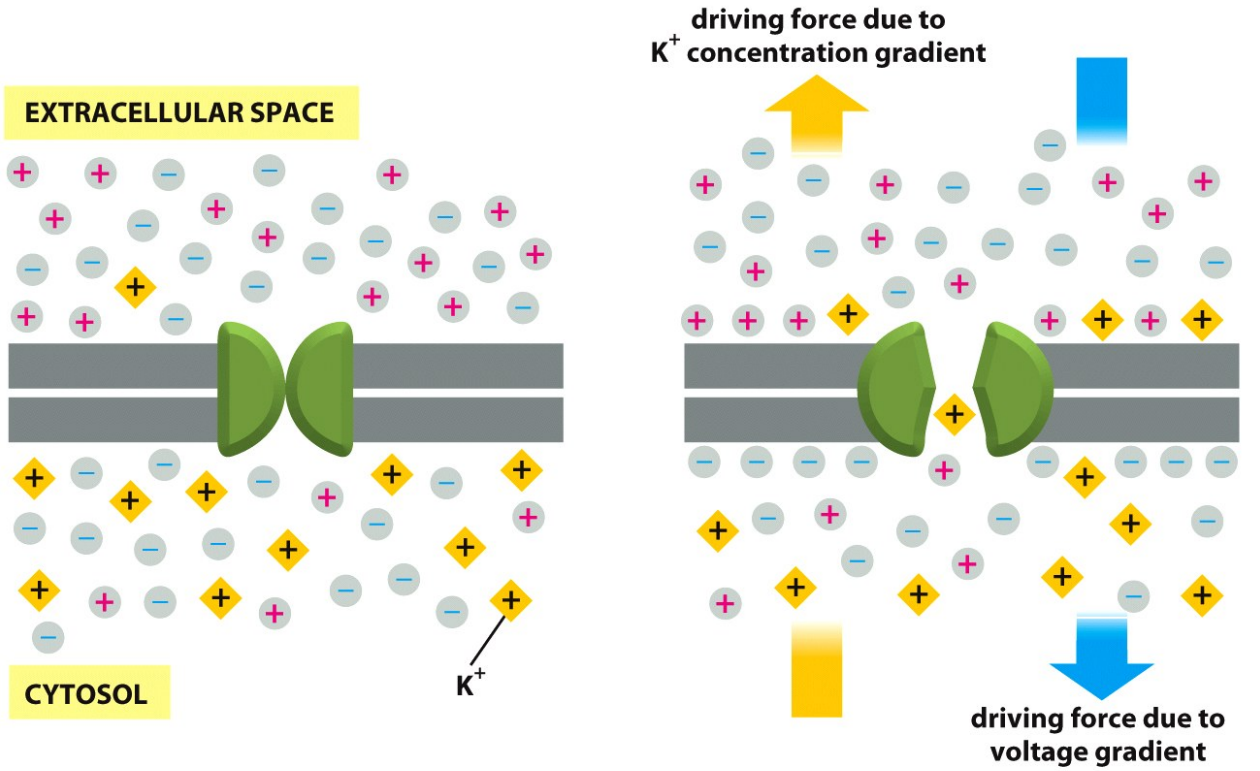
Membrane transport

.1 Membrane potential

Origin of membrane potential

- Normally, K^+ leak channels are open randomly and let K^+ to move freely
- However, there are still much more K^+ inside than outside because when it leaks out, electric force will stop this process at some point
- This point is a resting potential, ≈–20–200 mV

K^+ leak channels support -60 mV resting potential



(A) K^+ channel closed, membrane potential = 0; more K^+ inside the cell than outside, but zero *net* charge on each side (positive and negative charges balanced exactly)

(B) K^+ channel open; K^+ moves out, leaving negative ions behind, and this charge distribution creates a membrane potential that balances the tendency of K^+ to move out

Figure 12-29 Essential Cell Biology 3/e (© Garland Science 2010)

Nernst equation

- Connects chemical and electrical potentials
- $V = 62 \log_{10}(C_o/C_i)$ [at +37°C]
- V is a membrane electrical potential, C_o and C_i are outside and inside concentrations of ion

.2 Signaling in nerve cells

Nerve cells

- Neurons
- Axons distribute signals and ends with nerve terminals
- Dendrites are short branches which receive signals

Giant axons of squids

- Atlantic squid, *Loligo pealei* has extremely long and thick axons (up to 10 cm in length and 1 mm diameter)

- This was an excellent model for studying nerve cells and signals
- Alan Hodgkin and Andrew Huxley received 1963 Nobel Prize for their investigations with giant axons

Atlantic squid

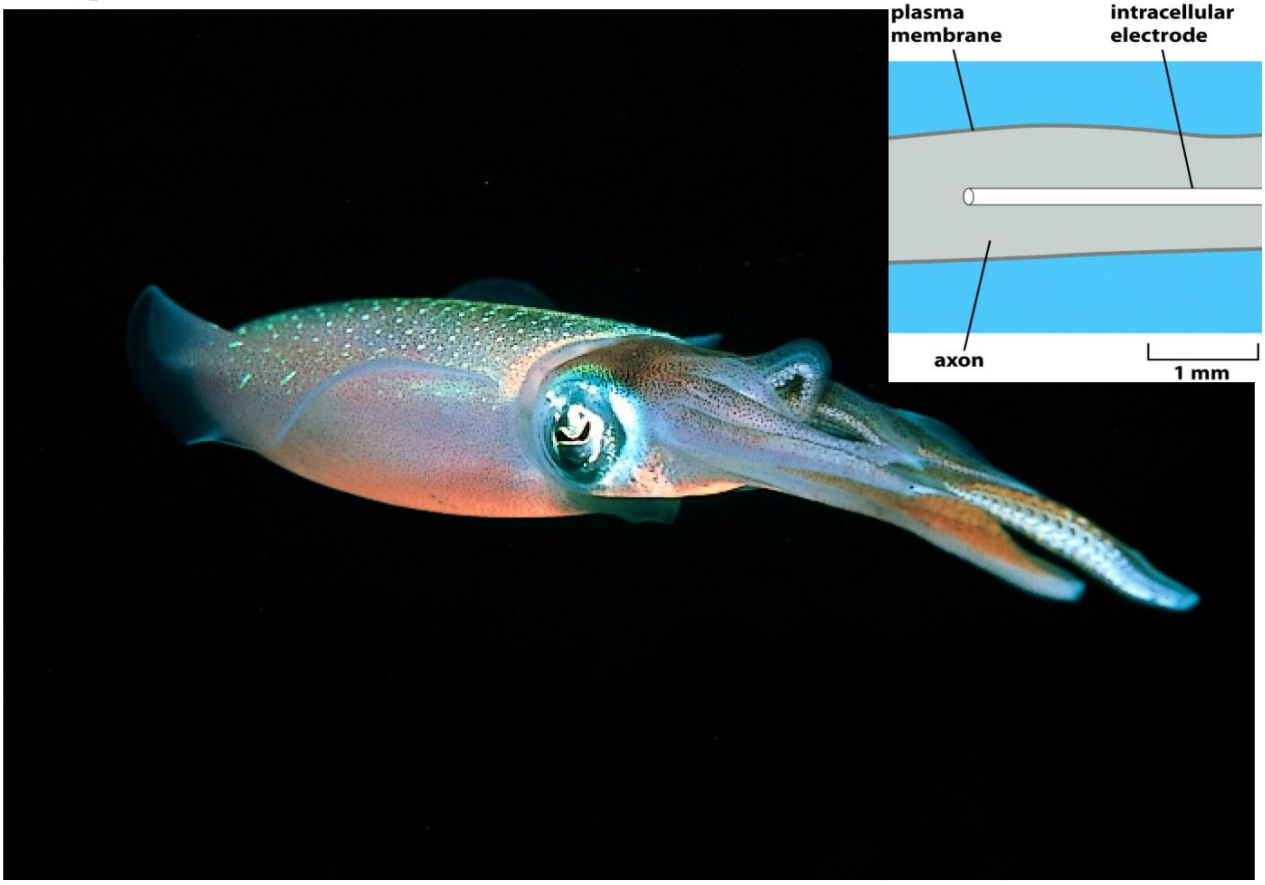
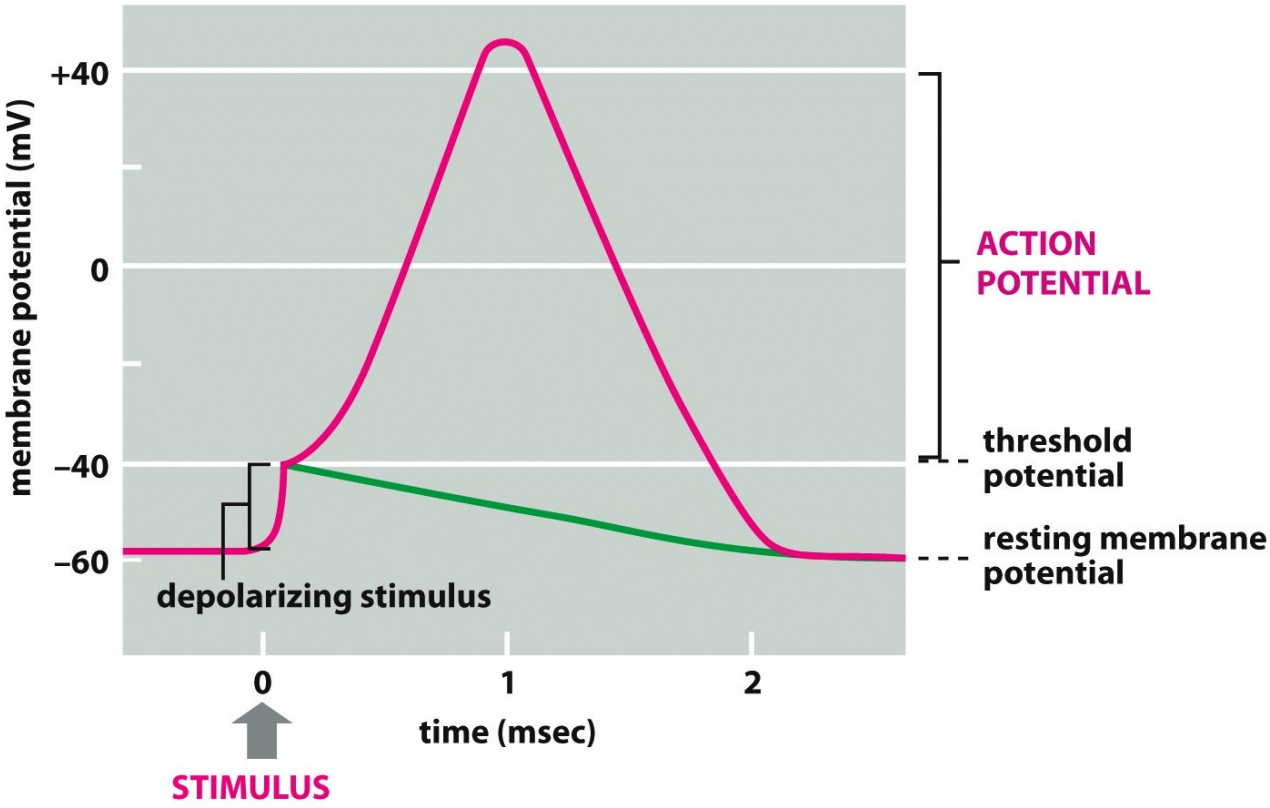


Figure 12-32 Essential Cell Biology 3/e (© Garland Science 2010)

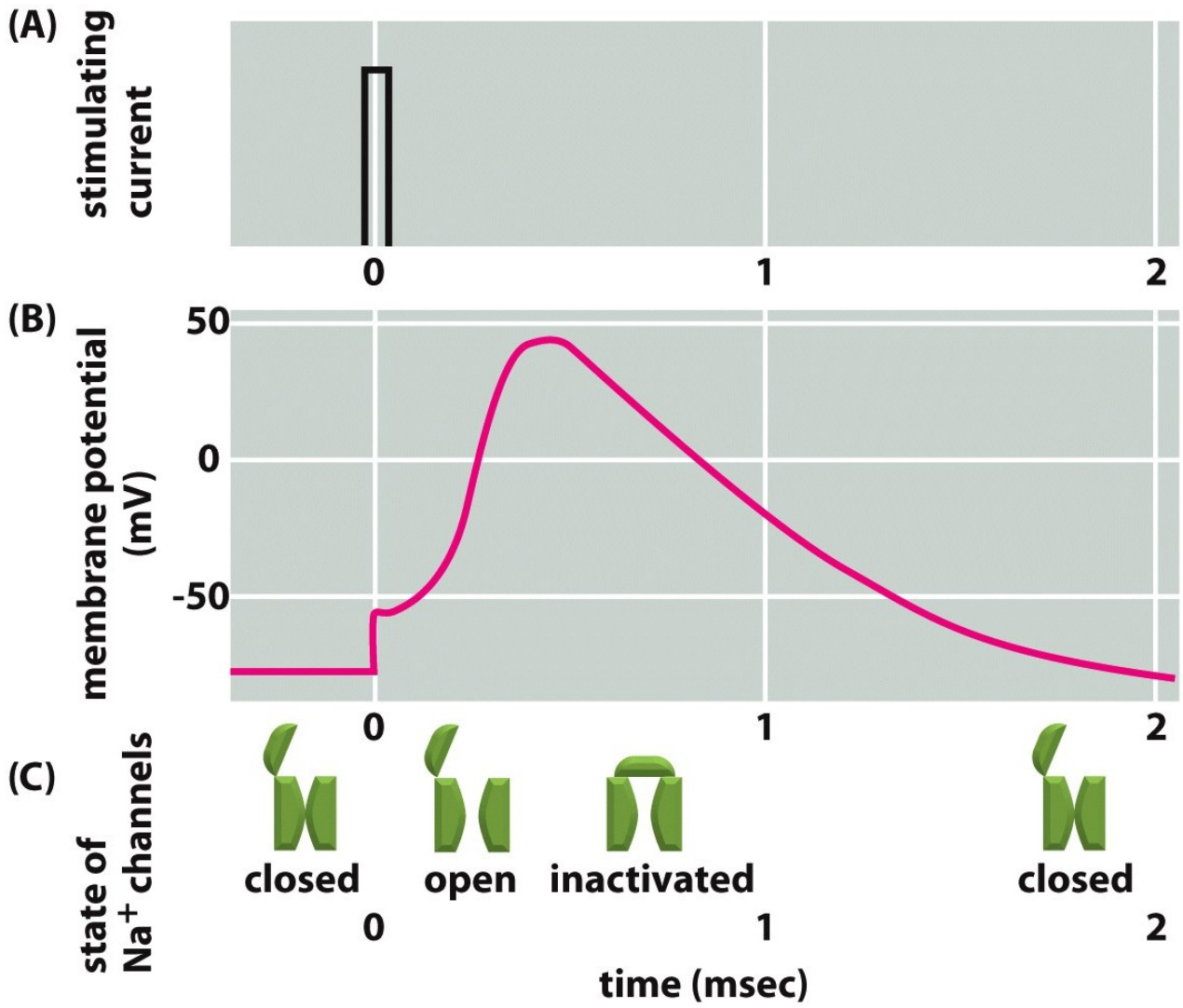
Action potential and Na^+ channels

- Depolarization of neighbor region is a stimulus which opens Na^+ channels and initiate rapid movement of these ions into cell
- Membrane potential changes from ≈–60 to ≈+40 mV and Na^+ channels inactivate
- Then, voltage-gated K^+ channels are open, K^+ leaks out of cell and potential (but not ions!) restores
- Then, Na^+ channels close and Na^+/K^+ pump restore ion concentration to default values

Action potential



Explanation of action potential



Where K^+ channels are open?

Na^+ channel cycle

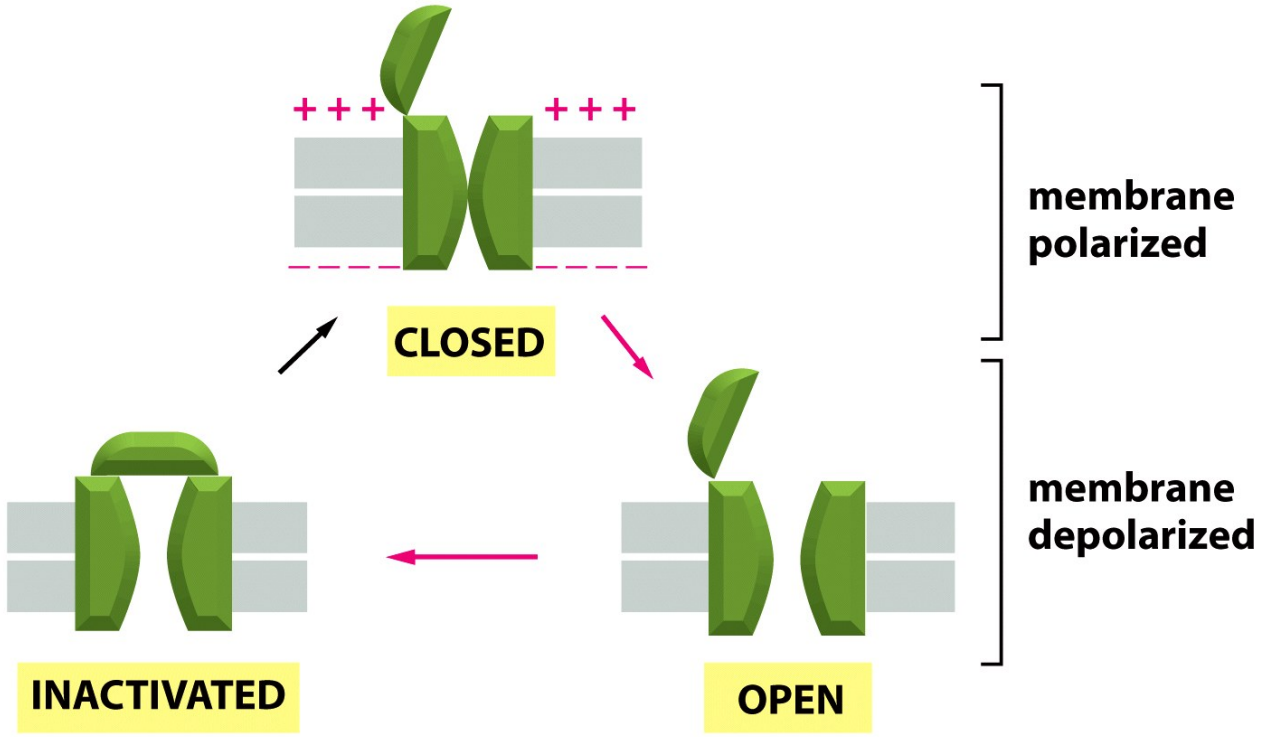


Figure 12-34 Essential Cell Biology 3/e (© Garland Science 2010)

Action potential movie

Propagation of action potential

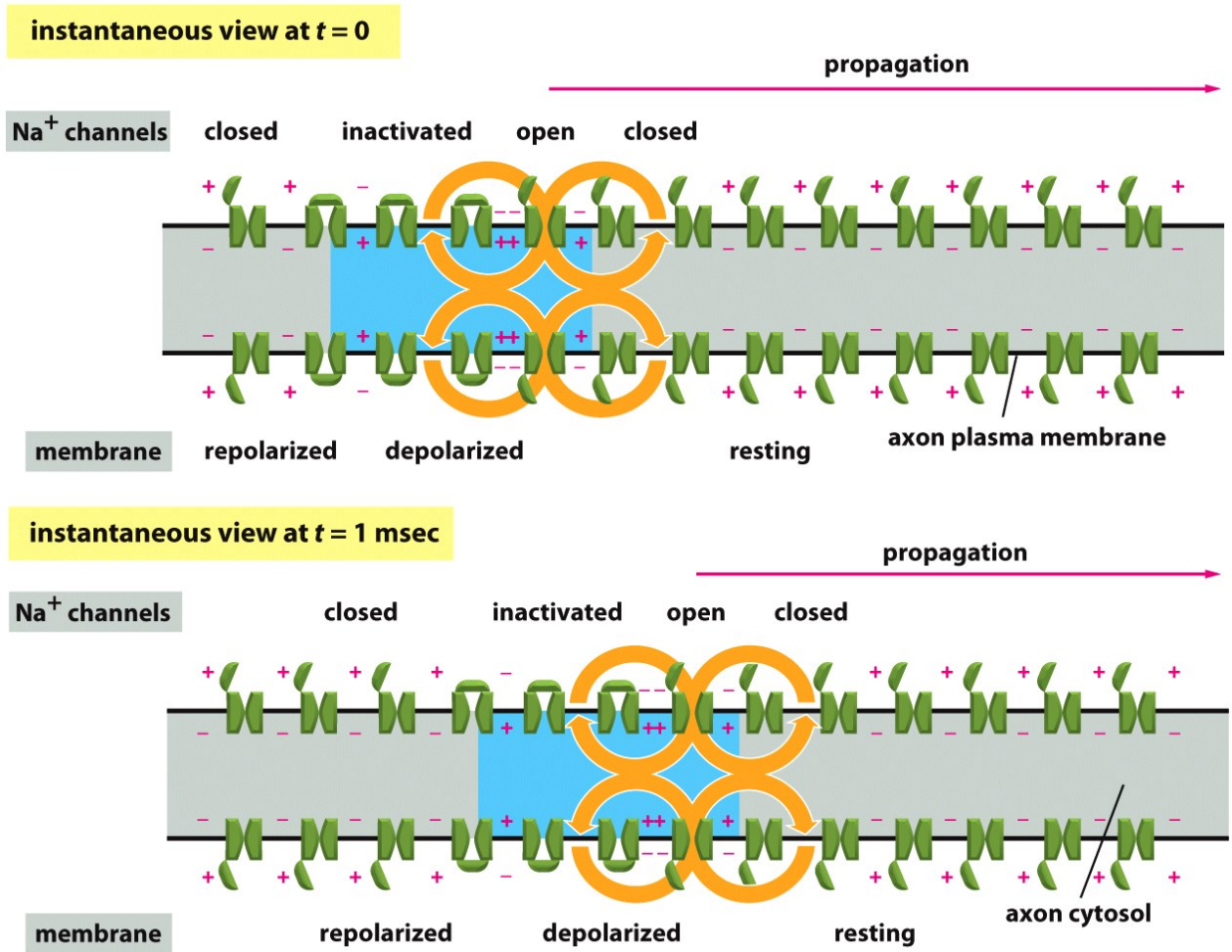


Figure 12-39b Essential Cell Biology 3/e (© Garland Science 2010)

Ca^{2+} channels and synapses

- Synapse converts electrochemical signal to pure chemical
- Depolarization of presynaptic membrane will cause Ca^{2+} channels to open, increase calcium concentration inside cell and initiate fusion of synaptic vesicles to the membrane
- Fusion should be quickly stopped (one of mechanisms is retro-neurotransmitters like endocannabinoids)

Transmitter-gated channels

- Neurotransmitter (small molecule of given configuration) crosses synaptic cleft and binds to ligand-gated receptors
- In synaptic junctions between nerve and muscle cells, acetylcholine neurotransmitter will bind to receprots in postsynaptic muscle cells
- Neurotransmitters need to be quickly destroyed (many poisons like kurare will block this destruction)

Synaptic communication (Part I)

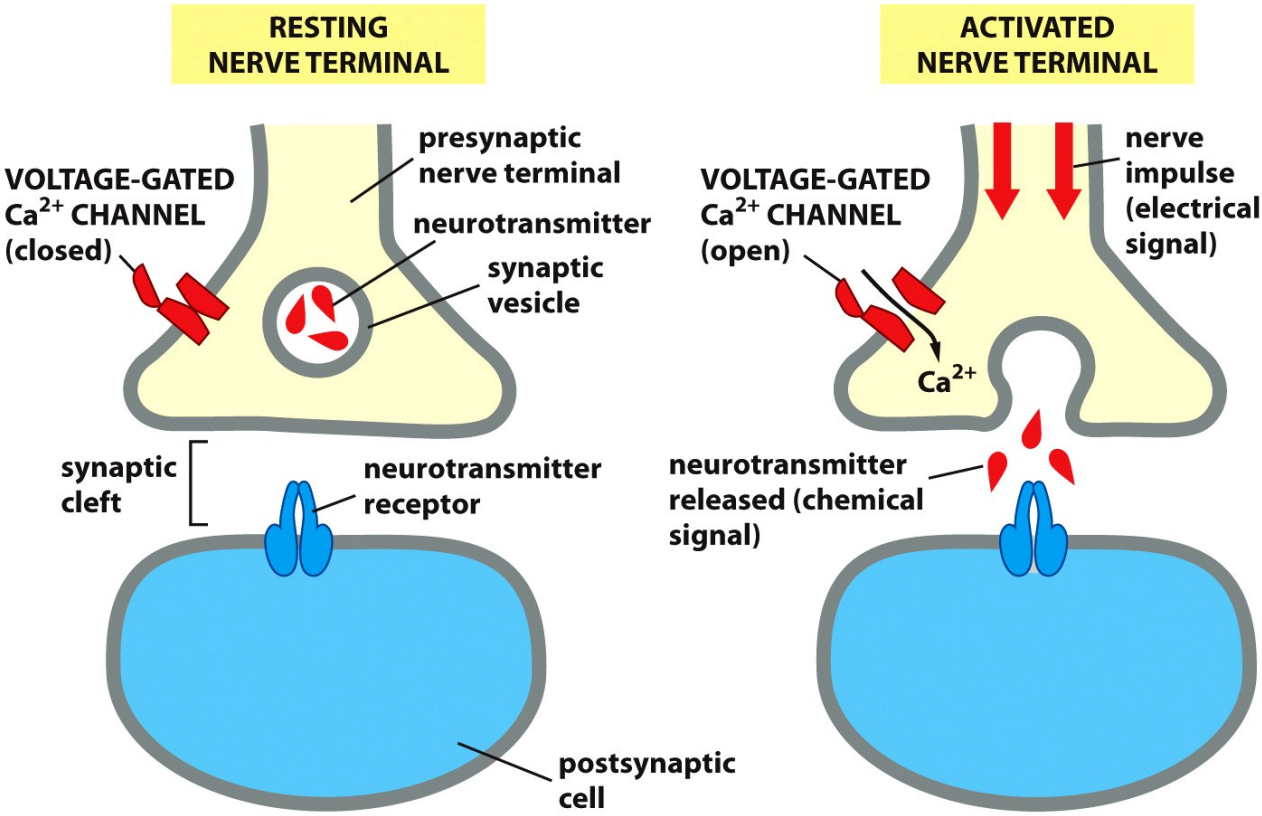


Figure 12-41 Essential Cell Biology 3/e (© Garland Science 2010)

Synaptic communication (Part II)

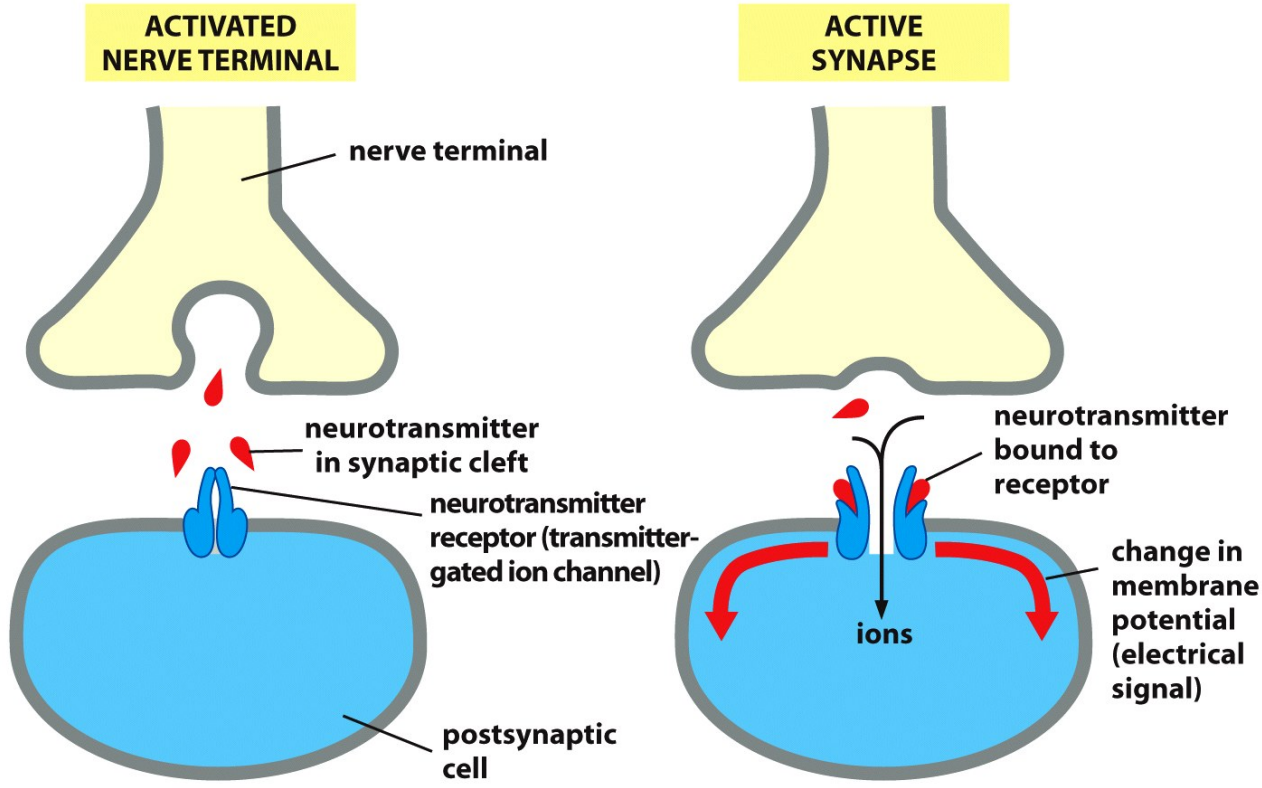
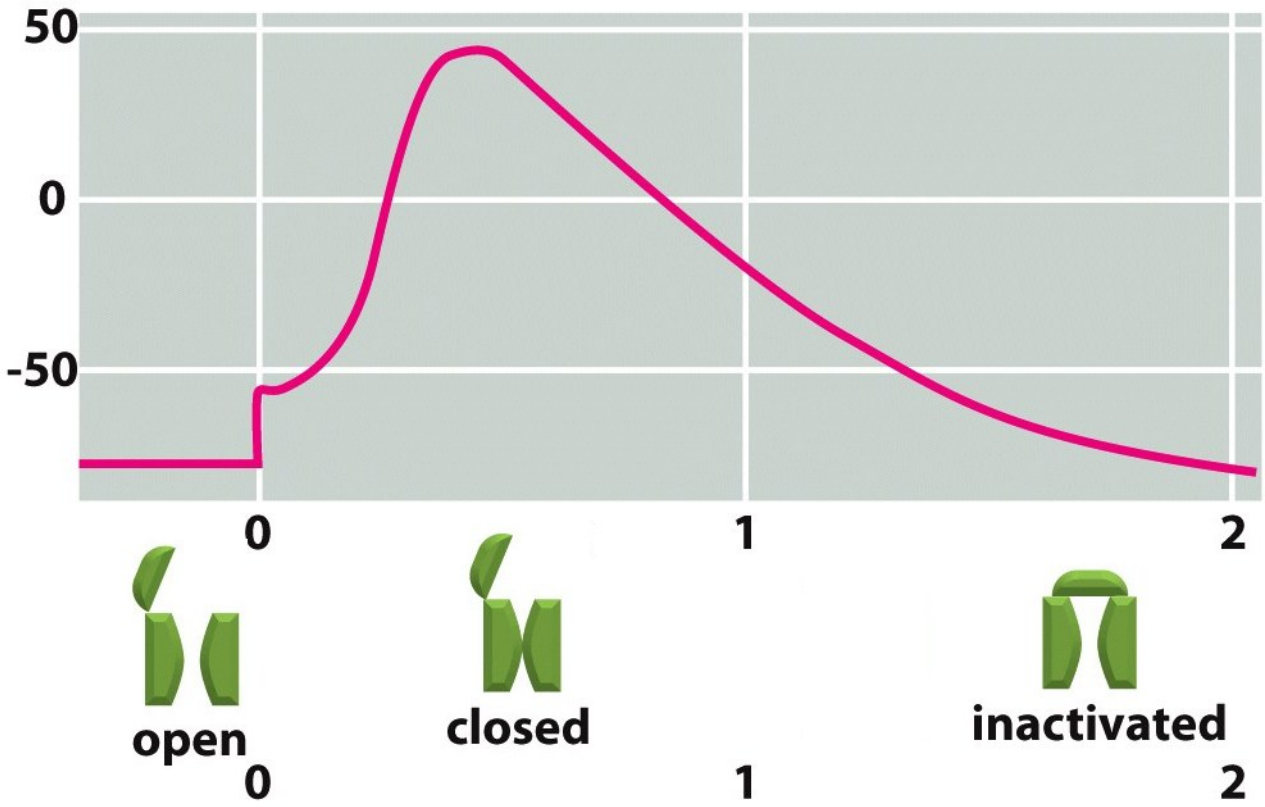


Figure 12-42 Essential Cell Biology 3/e (© Garland Science 2010)

Final question (3 points)

What is wrong in this picture?



Summary

- Membrane potential is a result of unequal distribution of electric charge across a membrane
- Action potential is due to opening, deactivating and closing of Na^+ channels

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

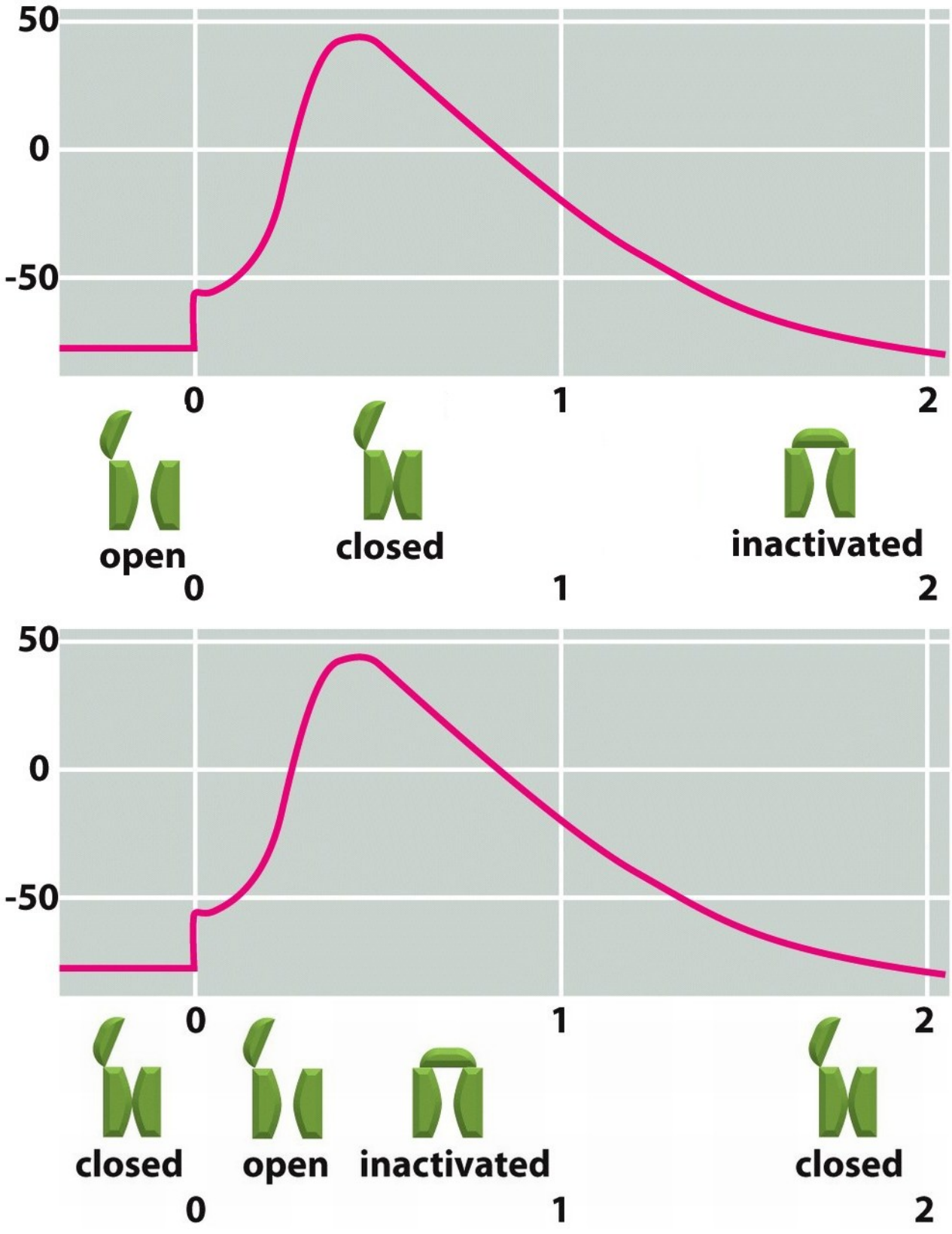
[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 12*.

Outline

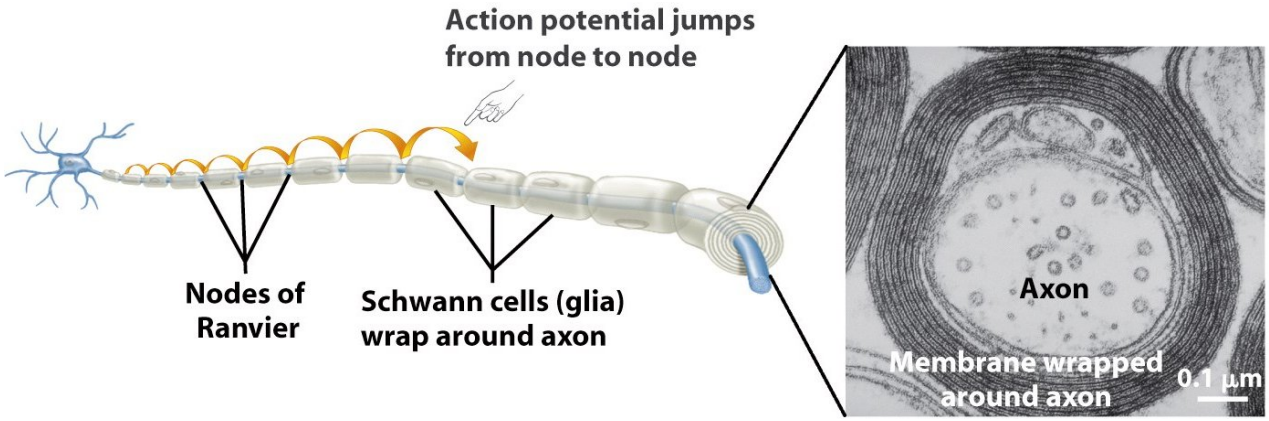
Questions and answers

Previous final question: the answer

What is wrong in this picture?



Myelinated neurons



Membrane transport

.1 Synaptic communication

Synaptic communication (Part I)

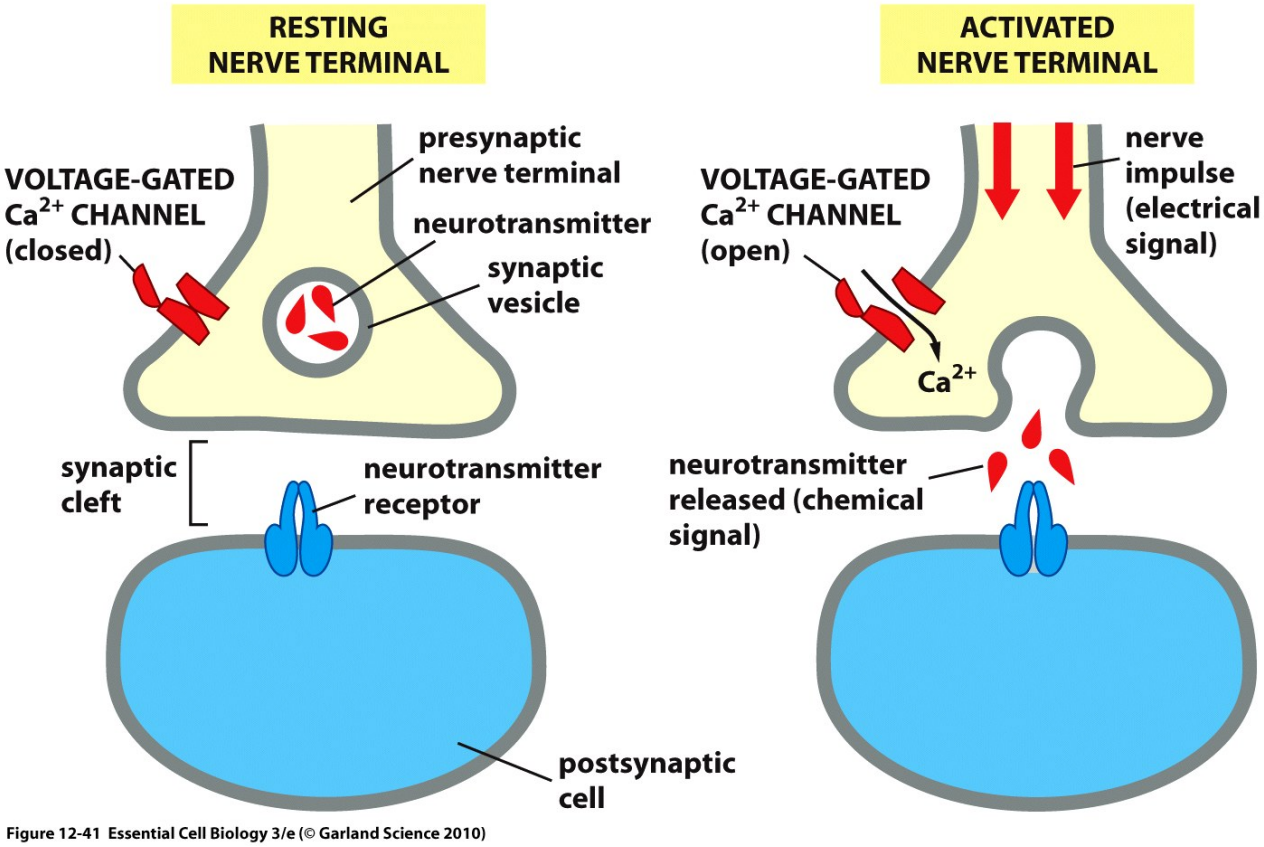


Figure 12-41 Essential Cell Biology 3/e (© Garland Science 2010)

Synaptic communication (Part II)

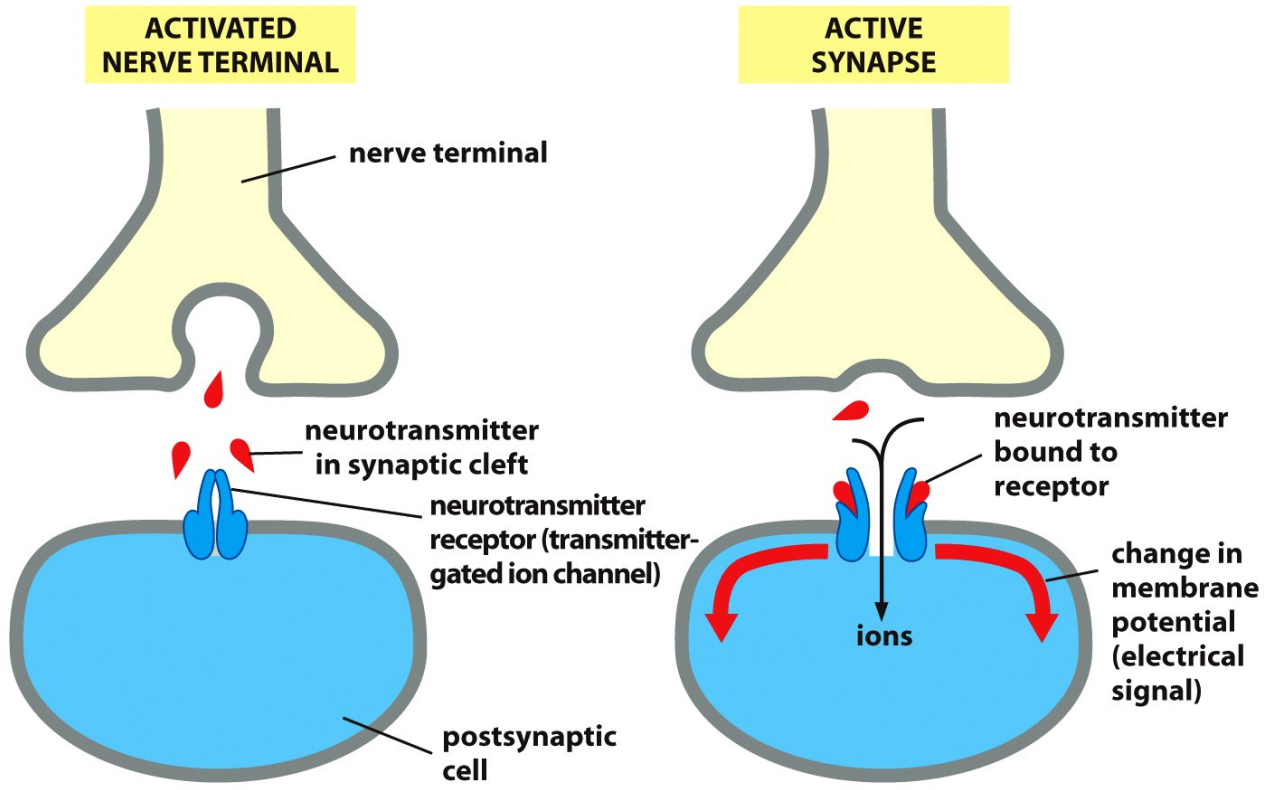


Figure 12-42 Essential Cell Biology 3/e (© Garland Science 2010)

Synaptic communication movie

Excitatory and inhibitory synapses

- Excitatory neurotransmitters (like acetylcholine, glutamate and their substitutes like ephedrine or nicotine) depolarize membrane through ligand-gated Na^+ channels
- Inhibitory neurotransmitters (like glycine or GABA) will bind to ligand-gated Cl^- channels, they open and make membrane even more polarized (hyper-polarized)
- Rich and tangled complexes of inhibition and activation receptors regulate intensity of neural signals

Excitatory and inhibitory synapses

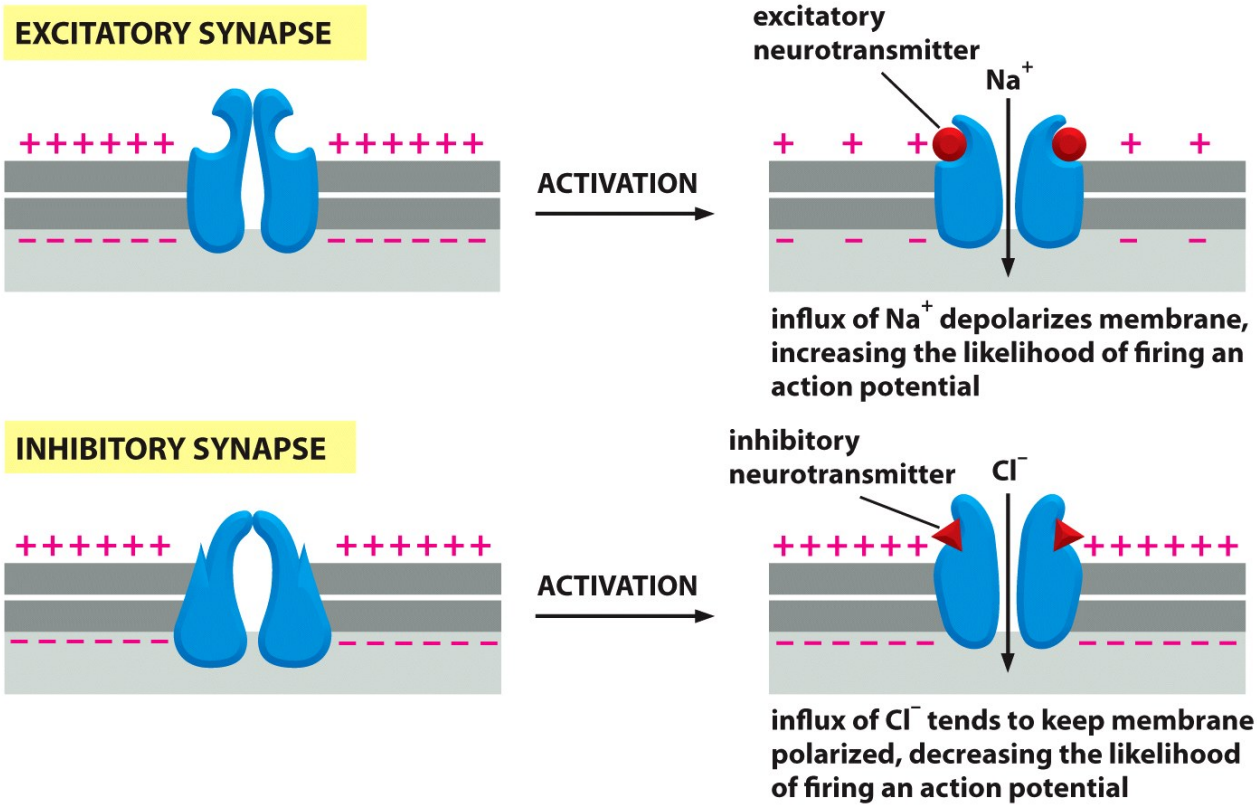


Figure 12-44 Essential Cell Biology 3/e (© Garland Science 2010)

Drunkenness

- Alcohol potentiating the specific γ-2L subunit of the inhibitory GABA receptor
- Inhibits the release of activatory neurotransmitters—glutamate and acetylcholine
- Stimulate release of endorphins (which in turn activate release of dopamine), resulting in suppressing pain and euphoria

Strychnine poisoning

- Strychnine permanently attaches to glycine receptors
- As a result, signals will not be lowered with inhibition

Other neurotransmitters and related drugs

Neurotransmitter	Drug
Acetylcholine	Nicotine (very addictive!)
Norepinephrine	Cocaine
Dopamine	LSD
Serotonin	Mescalin
Glutamate	Ketamine
GABA	Alcohol
Opioids	Heroin
Cannabinoids	Marijuana
Histamine	Diphenhydramine

Synaptic complex

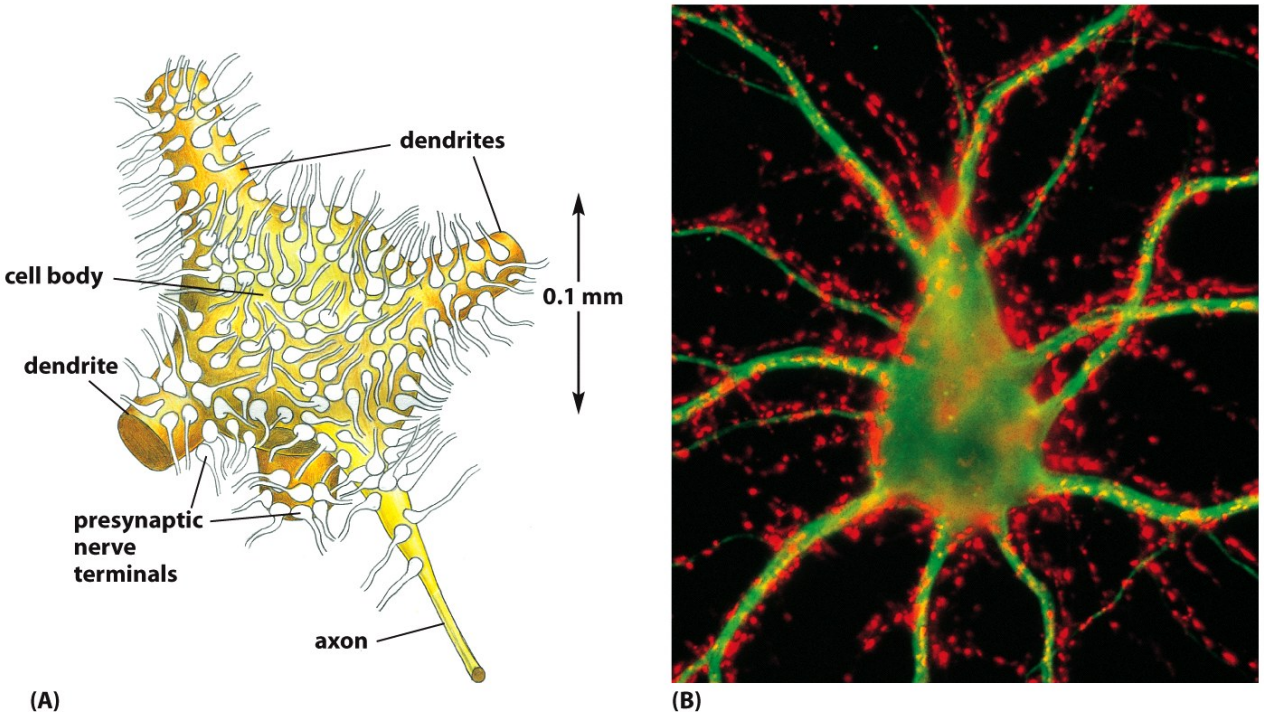
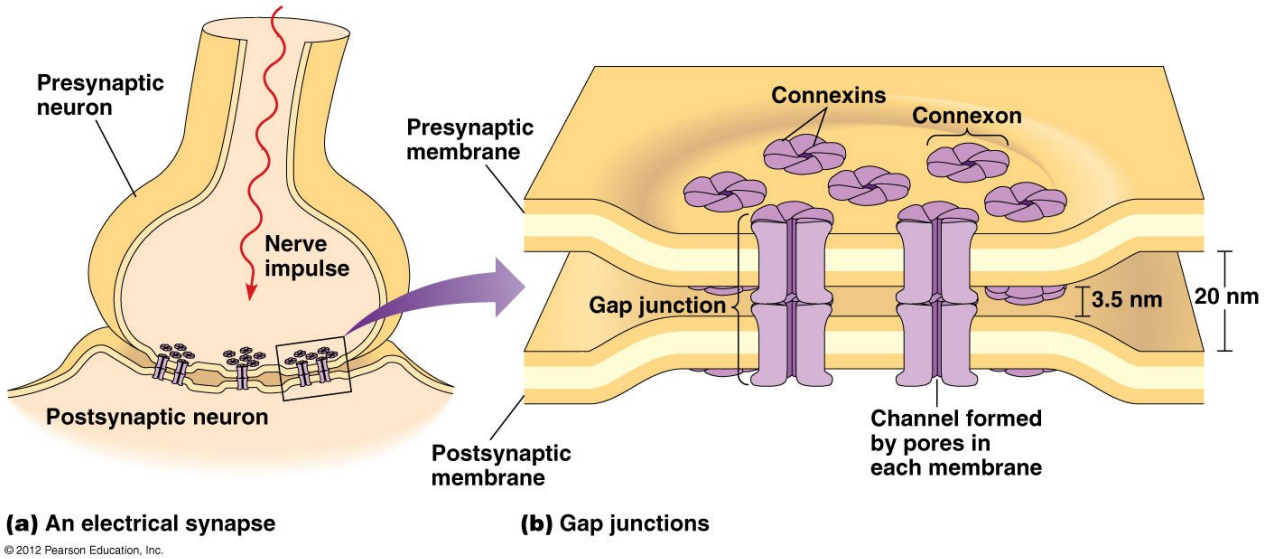


Figure 12-45 Essential Cell Biology 3/e (© Garland Science 2010)

Electrical synapse



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Diversity of ion channels

TABLE 12–3 SOME EXAMPLES OF ION CHANNELS		
ION CHANNEL	**TYPICAL LOCATION**	**FUNCTION**
K^+ leak channel	plasma membrane of most animal cells	maintenance of resting membrane potential
Voltage-gated Na^+ channel	plasma membrane of nerve cell axon	generation of action potentials
Voltage-gated K^+ channel	plasma membrane of nerve cell axon	return of membrane to resting potential after initiation of an action potential
Voltage-gated Ca^{2+} channel	plasma membrane of nerve terminal	stimulation of neurotransmitter release
Acetylcholine receptor (acetylcholine-gated Na^+ and Ca^{2+} channel)	plasma membrane of muscle cell (at neuromuscular junction)	excitatory synaptic signaling
Glutamate receptors (glutamate-gated Na^+ and Ca^{2+} channels)	plasma membrane of many neurons (at synapses)	excitatory synaptic signaling
GABA receptor (GABA-gated Cl^- channel)	plasma membrane of many neurons (at synapses)	inhibitory synaptic signaling
Glycine receptor (glycine-gated Cl^- channel	plasma membrane of many neurons (at synapses)	inhibitory synaptic signaling
Stress-activated cation channel	auditory hair cell in inner ear	detection of sound vibrations

Intracellular transport

.1 Signal sequences

Membrane-enclosed organelles

[We skip chapters 13 and 14!]

- Why eukaryotes need them?
- Cytosol (54% of cell volume), nucleus (12), mitochondria (22), chloroplasts, ER (12), GA (3), lysosomes, endosomes, peroxisomes and small vesicles

Origin of membrane organelles

- Endogenous (e.g., invagination of plasma membrane)
- Symbiotic

Protein sorting: mechanisms of import

- Nuclear pores
- Across membranes
- By vesicles

Signal sequences

- Small sequences of amino acids which indicate where to put this protein
- This works almost exactly like destination block in TCP/IP package!
- Cell enzymes may insert and remove signal sequences

Signal sequences

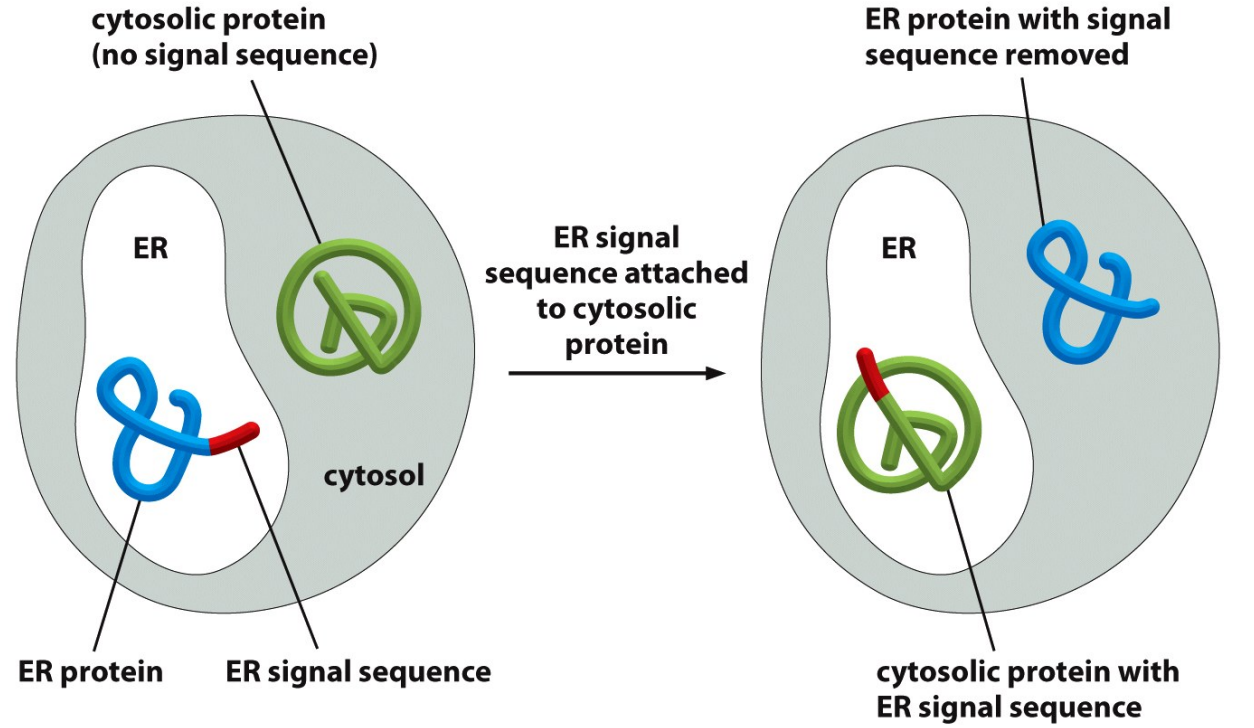


Figure 15-6 Essential Cell Biology 3/e (© Garland Science 2010)

Different signal sequences

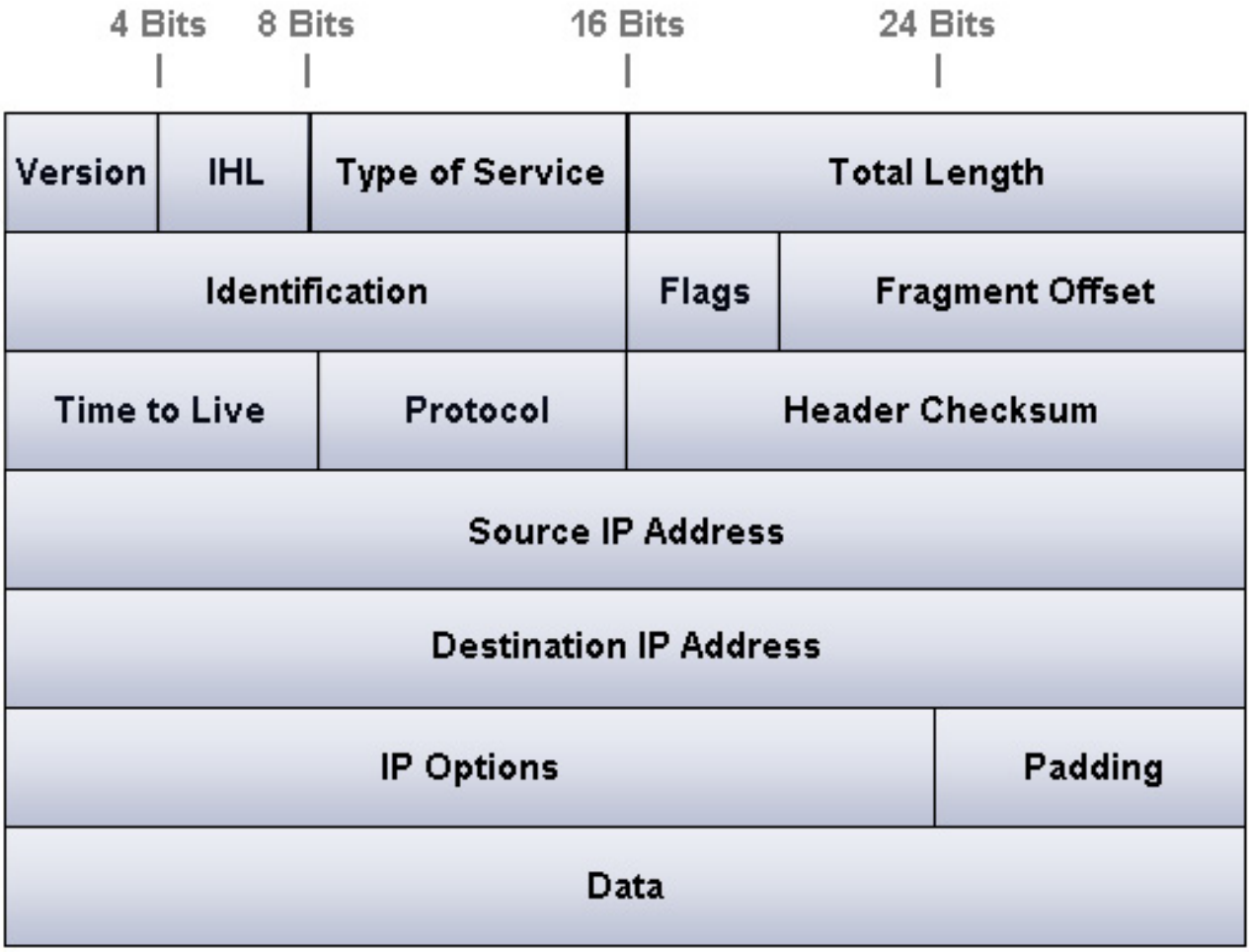
TABLE 15–3 SOME TYPICAL SIGNAL SEQUENCES

FUNCTION OF SIGNAL	EXAMPLE OF SIGNAL SEQUENCE
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Retention in lumen of ER	-Lys-Asp-Glu-Leu-COO^{-}
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Import into peroxisomes	-Ser-Lys-Leu-

Positively charged amino acids are shown in *red*, and negatively charged amino acids in *blue*. An extended block of hydrophobic amino acids is shown in *green*. $^{+}H_3N$ indicates the N-terminus of a protein; COO^{-} indicates the C-terminus. The ER retention signal is commonly referred to by its single-letter amino acid abbreviation, KDEL.

Table 15-3 Essential Cell Biology 3/e (© Garland Science 2010)

TCP/IP package header



.2 Nuclear, mitochondrial and ER import

Nuclear import

- Nucleus needs many proteins which are synthesized in cytoplasm
- Inner nuclear membrane binds chromosome-binding proteins and also nuclear lamina
- Outer membrane is normally continuous with ER

Nuclear pore

- Large, complicated structure of ≈30 proteins
- Some formed passages for small molecules, some participate in floating structures, some form a central channel with meshwork in the middle
- Cytoplasmic fibrils and nuclear basket fibrils are proteins located around the entrance of pore

Nuclear pore

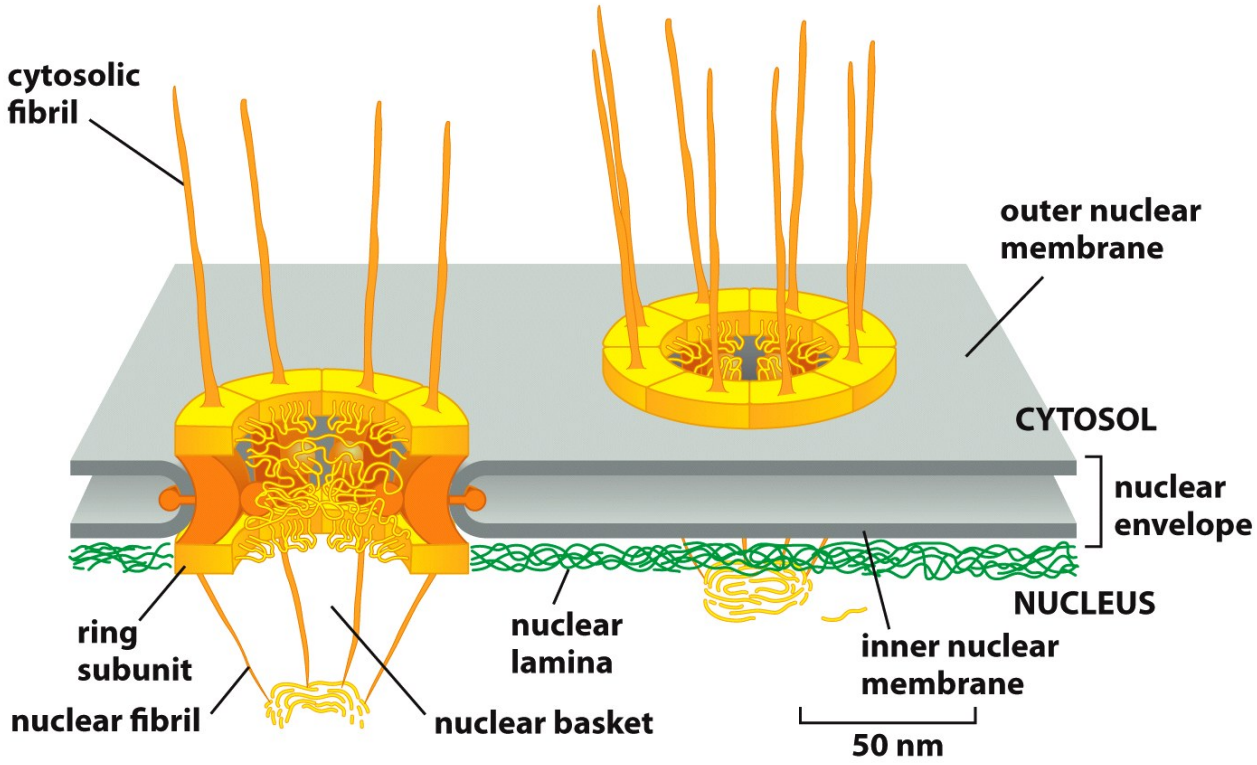


Figure 15-8a Essential Cell Biology 3/e (© Garland Science 2010)

Nuclear transport cycle

- Cargo proteins bind with nuclear transport receptor proteins and go through pore
- Then Ran-GTP protein binds to transport receptor protein, replaces cargo and complex go back to outer cytoplasm
- GTP hydrolyzes to GDP and phosphate, Ran-GDP dissociates, energy used for binding new cargo protein

Nuclear transport: general view

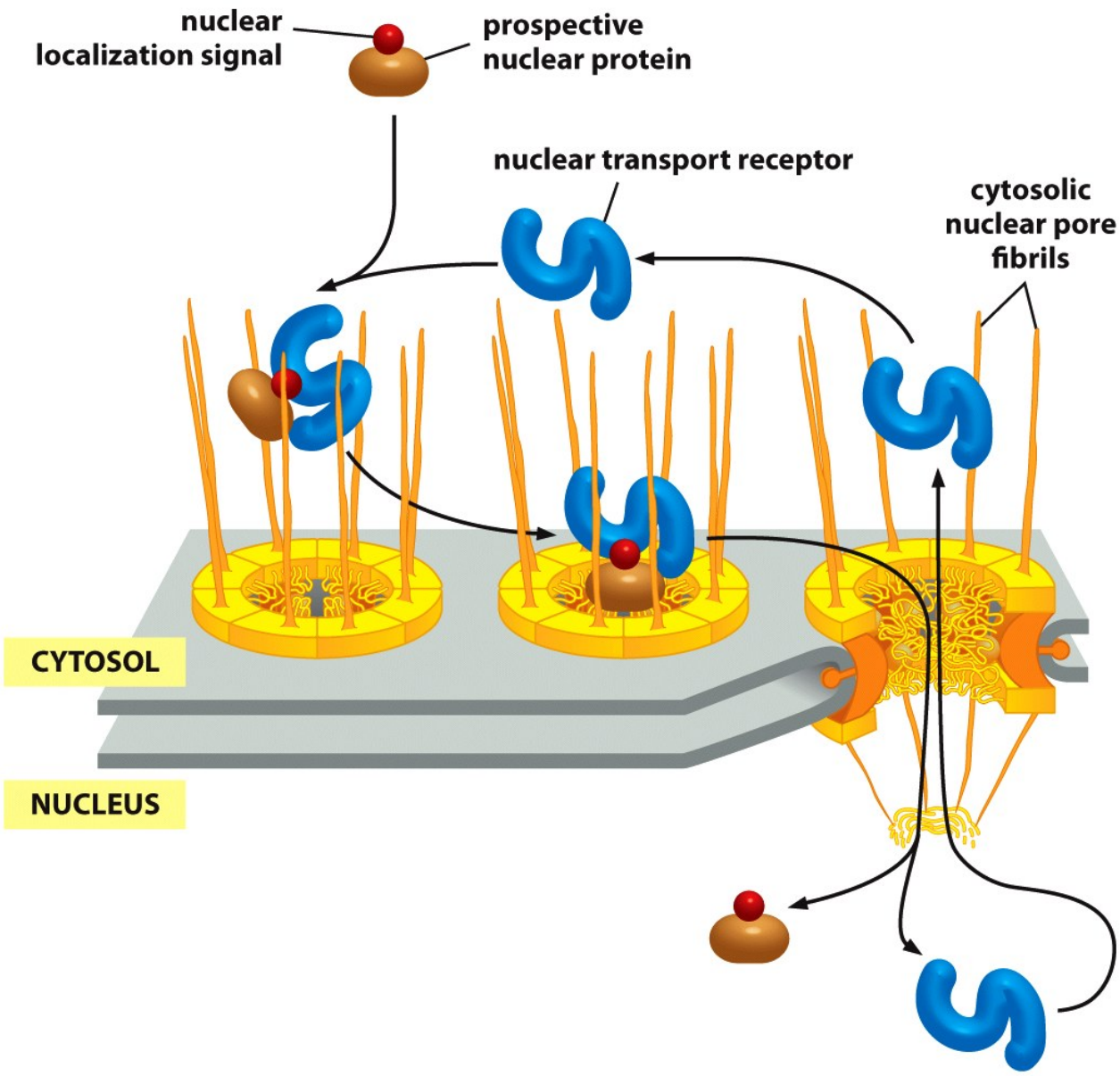


Figure 15-9 Essential Cell Biology 3/e (© Garland Science 2010)

Nuclear transport cycle: detailed view

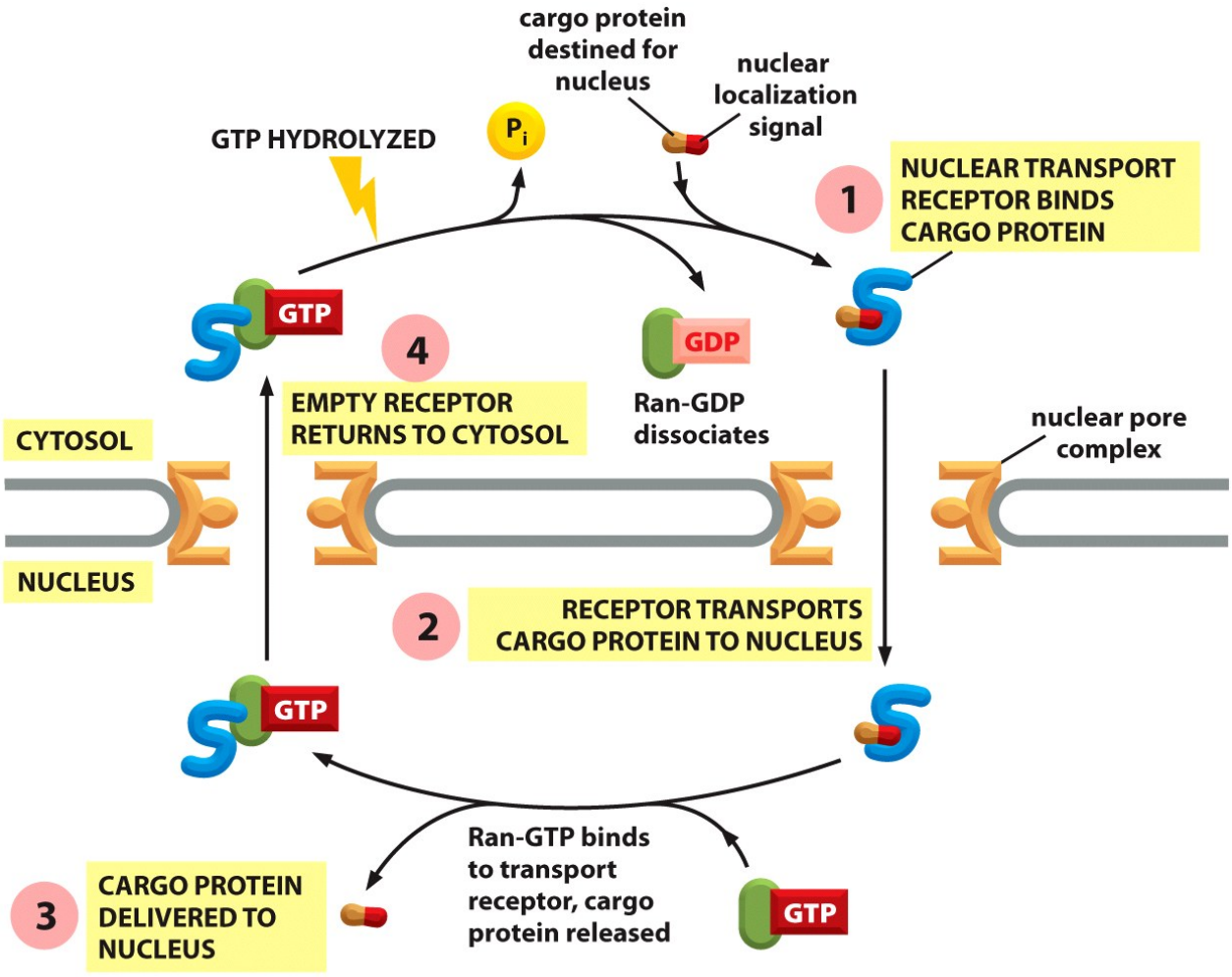


Figure 15-10 Essential Cell Biology 3/e (© Garland Science 2010)

Transport to mitochondria and chloroplasts

- Proteins with appropriate signal sequence squeeze through protein translocators
- They unfold and re-fold later with the help of chaperones
- Proteins also transfer lipids

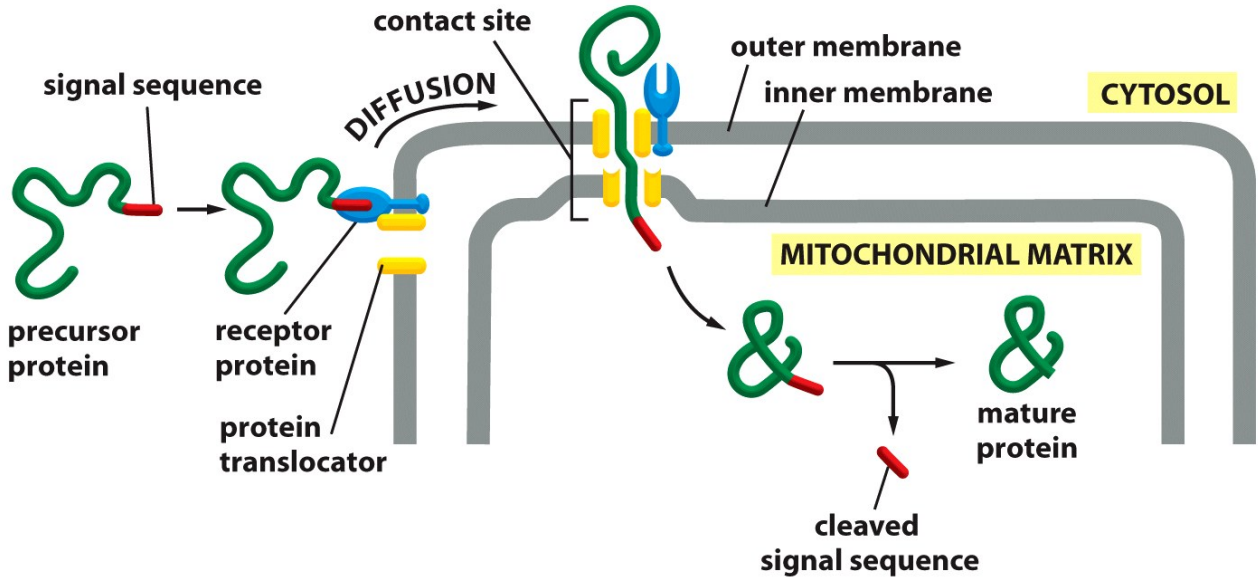


Figure 15-11 Essential Cell Biology 3/e (© Garland Science 2010)

Protein import into mitochondria movie

Final question (2 points)

Why are neurotransmitters small molecules?

Summary

- Ca^{2+}, Cl^{-} channels and neurotransmitters are responsible for synaptic communication
- Effects of drugs and narcotics are often due to complex interaction with different synapses in different synaptic complexes
- Membrane organelles have two different origins
- Moved protein bears signal sequence which corresponds with destination

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 12 and 15*.

Outline

Questions and answers

Previous final question: the answer

What will happen to cytosol protein, if it has no signal sequence?

- Will not move

Membranes

.1 Intracellular transport

Nuclear import

- Nucleus needs many proteins which are synthesized in cytoplasm
- Inner nuclear membrane binds chromosome-binding proteins and also nuclear lamina
- Outer membrane is normally continuous with ER

Nuclear pore

- Large, complicated structure of ≈30 proteins
- Some formed passages for small molecules, some participate in floating structures, some form a central channel with meshwork in the middle
- Cytoplasmic fibrils and nuclear basket fibrils are proteins located around the entrance of pore

Nuclear pore

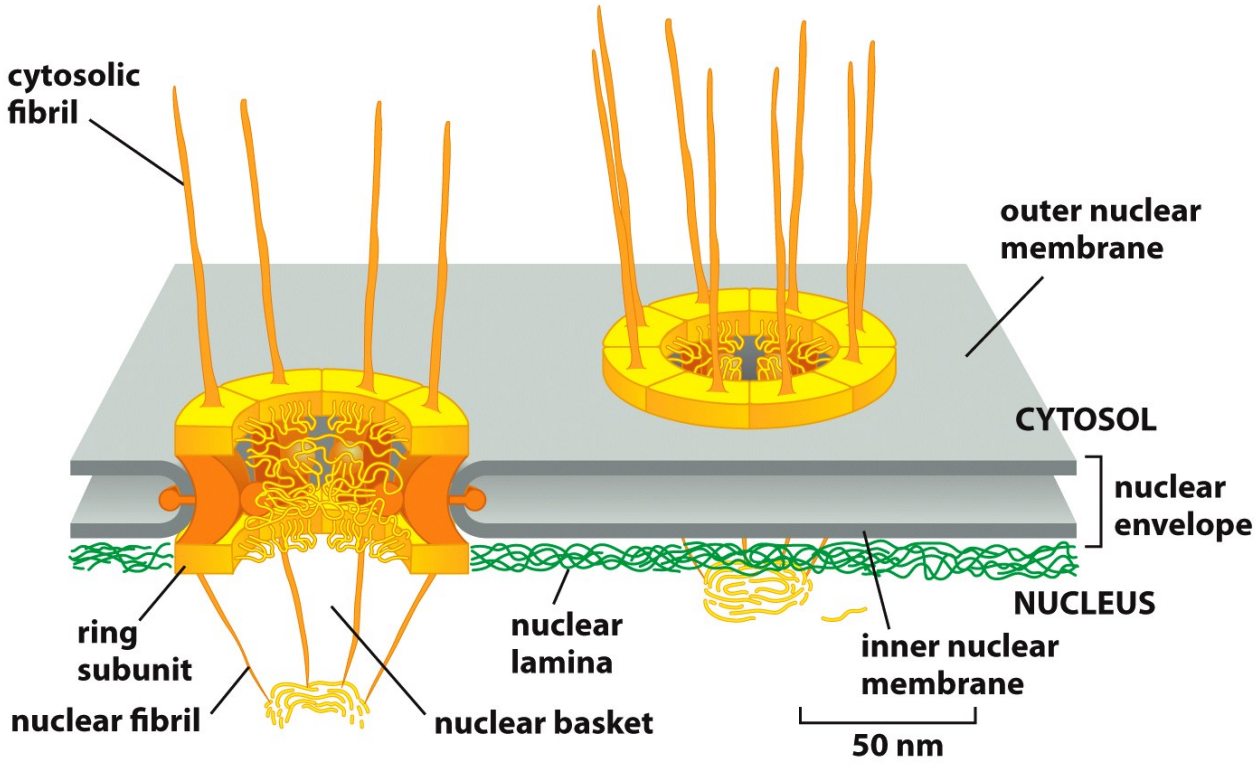


Figure 15-8a Essential Cell Biology 3/e (© Garland Science 2010)

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- Then Ran-GTP protein binds to transport receptor protein, replaces cargo and complex go back to outer cytoplasm
- GTP hydrolyzes to GDP and phosphate, Ran-GDP dissociates, energy used for binding new cargo protein

Nuclear transport: general view

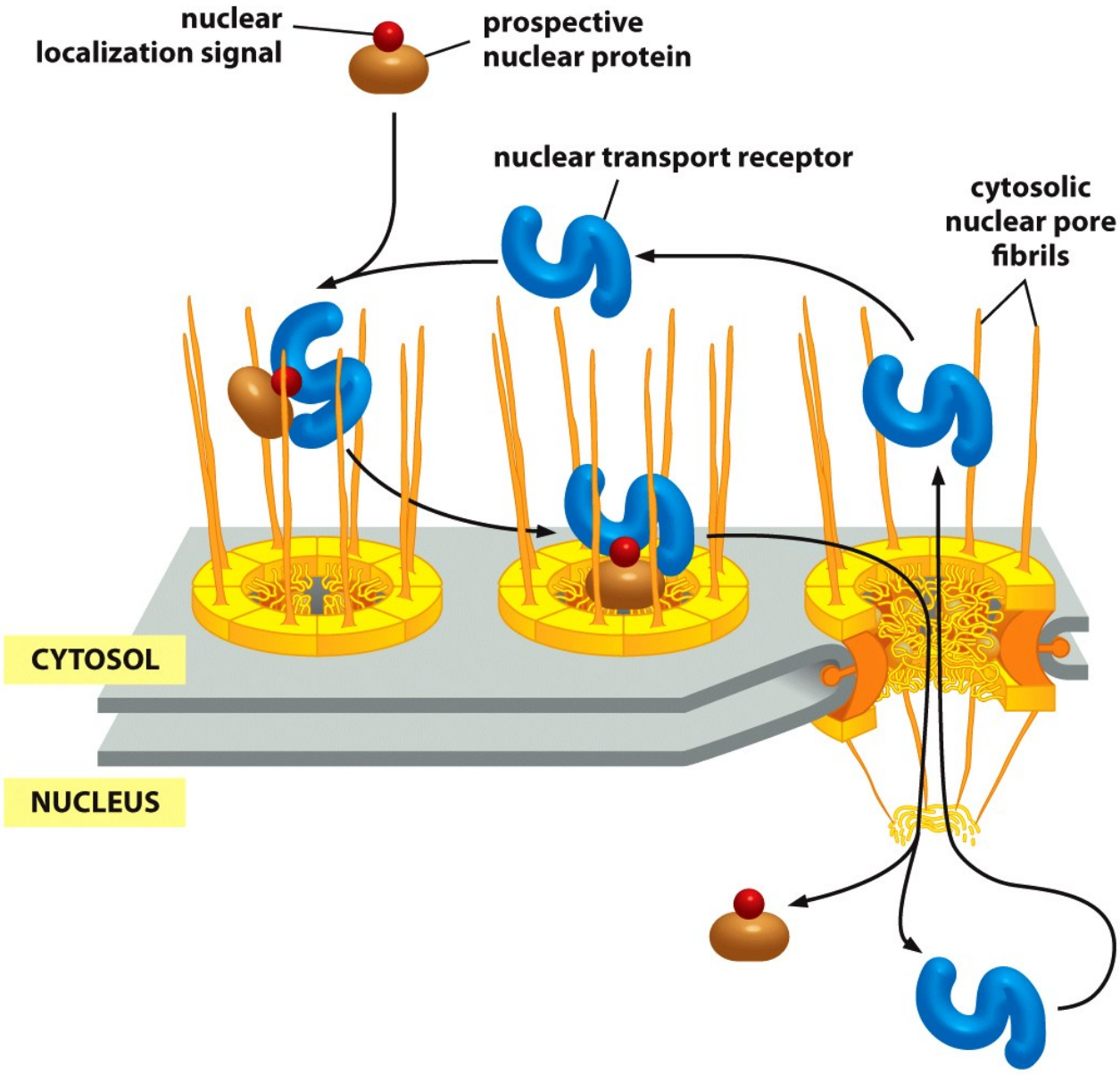


Figure 15-9 Essential Cell Biology 3/e (© Garland Science 2010)

Nuclear transport cycle: detailed view

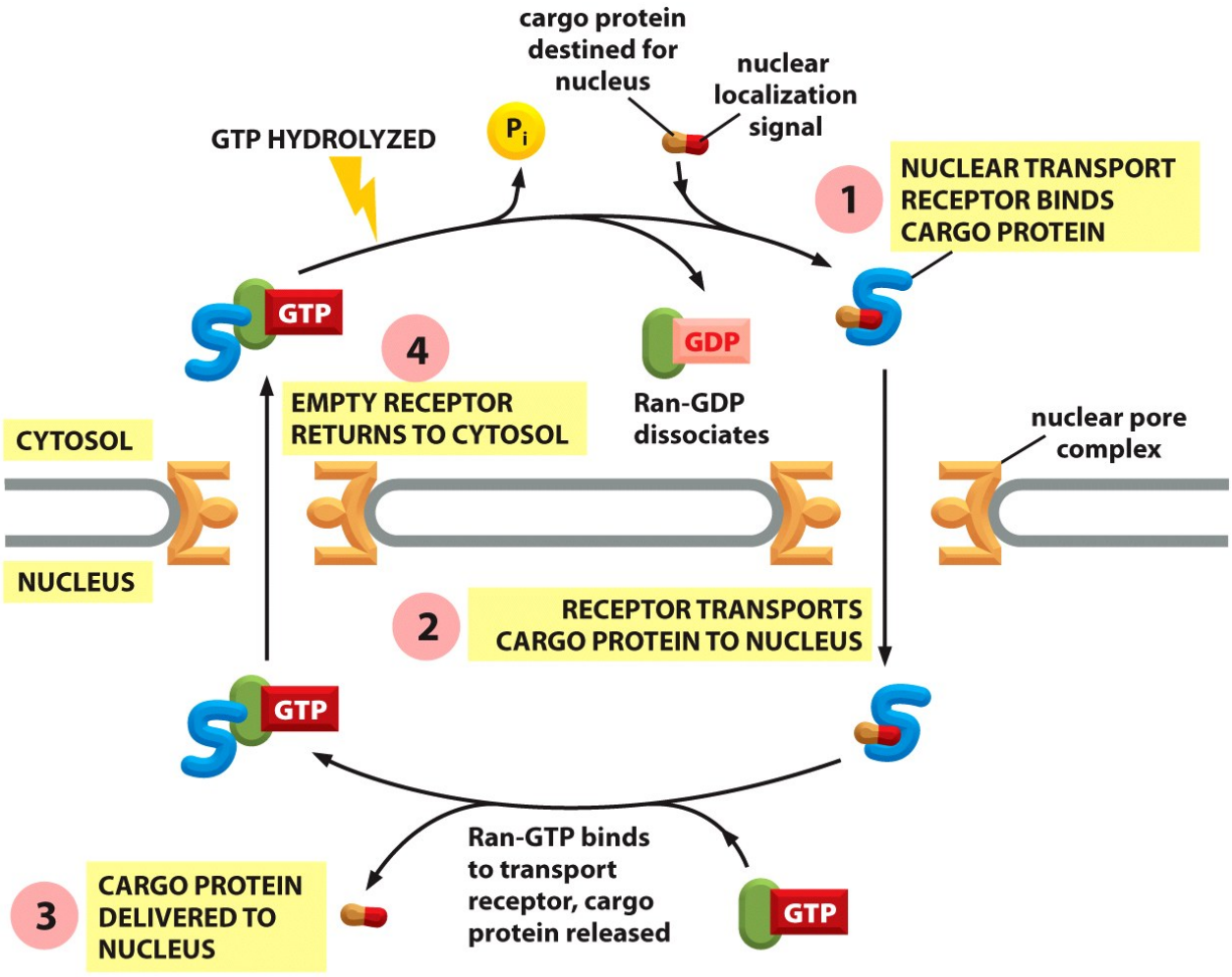


Figure 15-10 Essential Cell Biology 3/e (© Garland Science 2010)

Transport to mitochondria and chloroplasts

- Proteins with appropriate signal sequence squeeze through protein translocators
- They unfold and re-fold later with the help of chaperones
- Proteins also transfer lipids

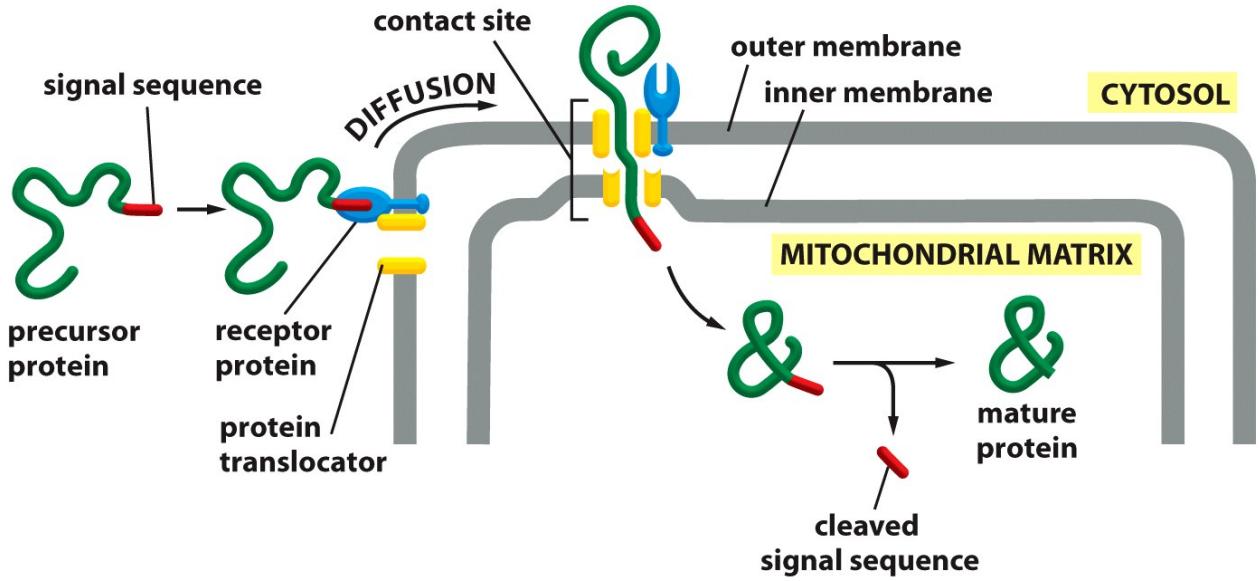


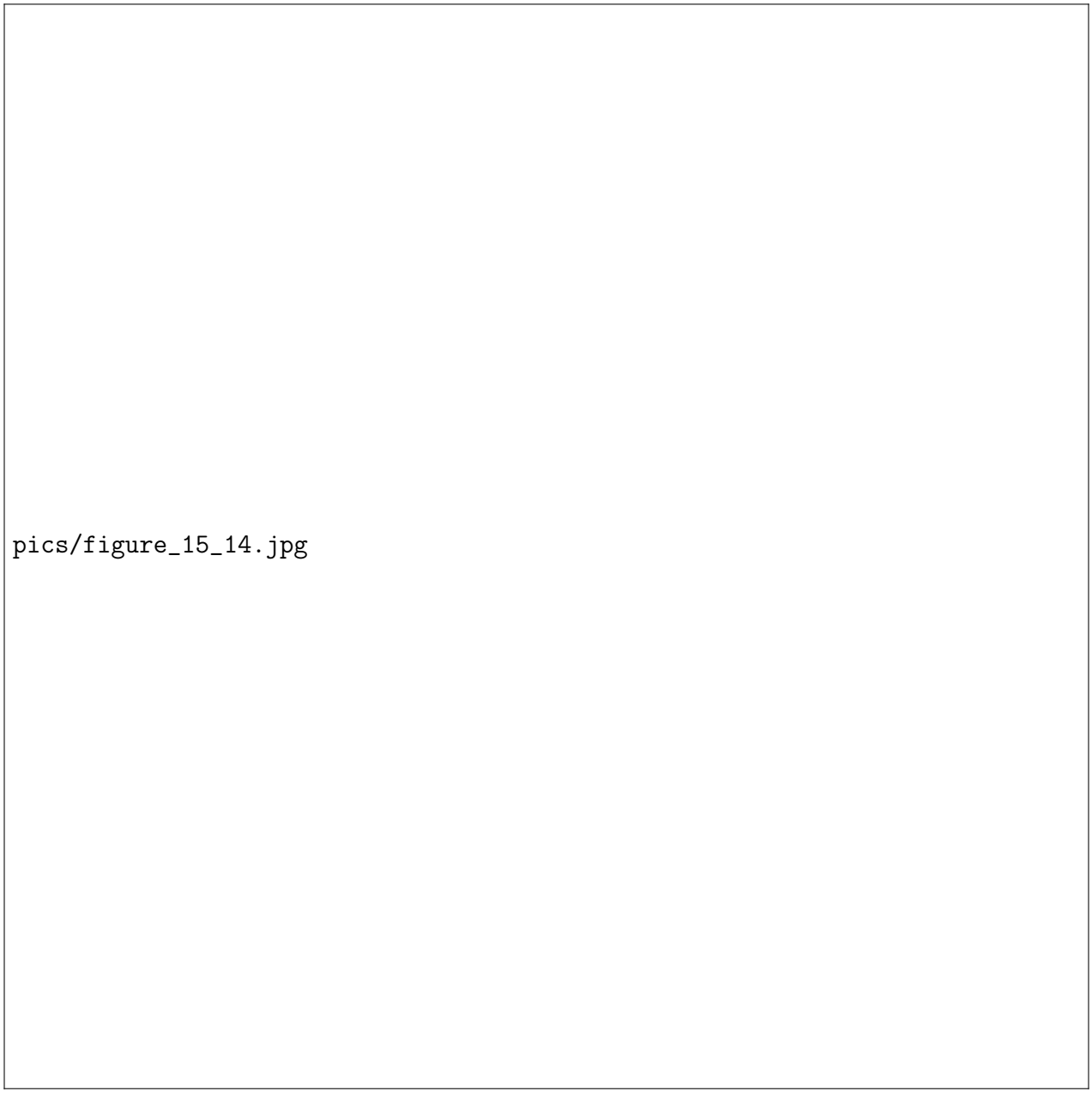
Figure 15-11 Essential Cell Biology 3/e (© Garland Science 2010)

Protein import into mitochondria movie

Transport to ER

- Membrane-bound ribosomes form the rough ER (free ribosomes are in cytosol)
- If the protein contain SRP (signal-recognition particle), SRP receptor on ER membrane will bind ribosome
- New protein will move into ER lumen through translocation channel if it has one signal sequence

ER tubules movie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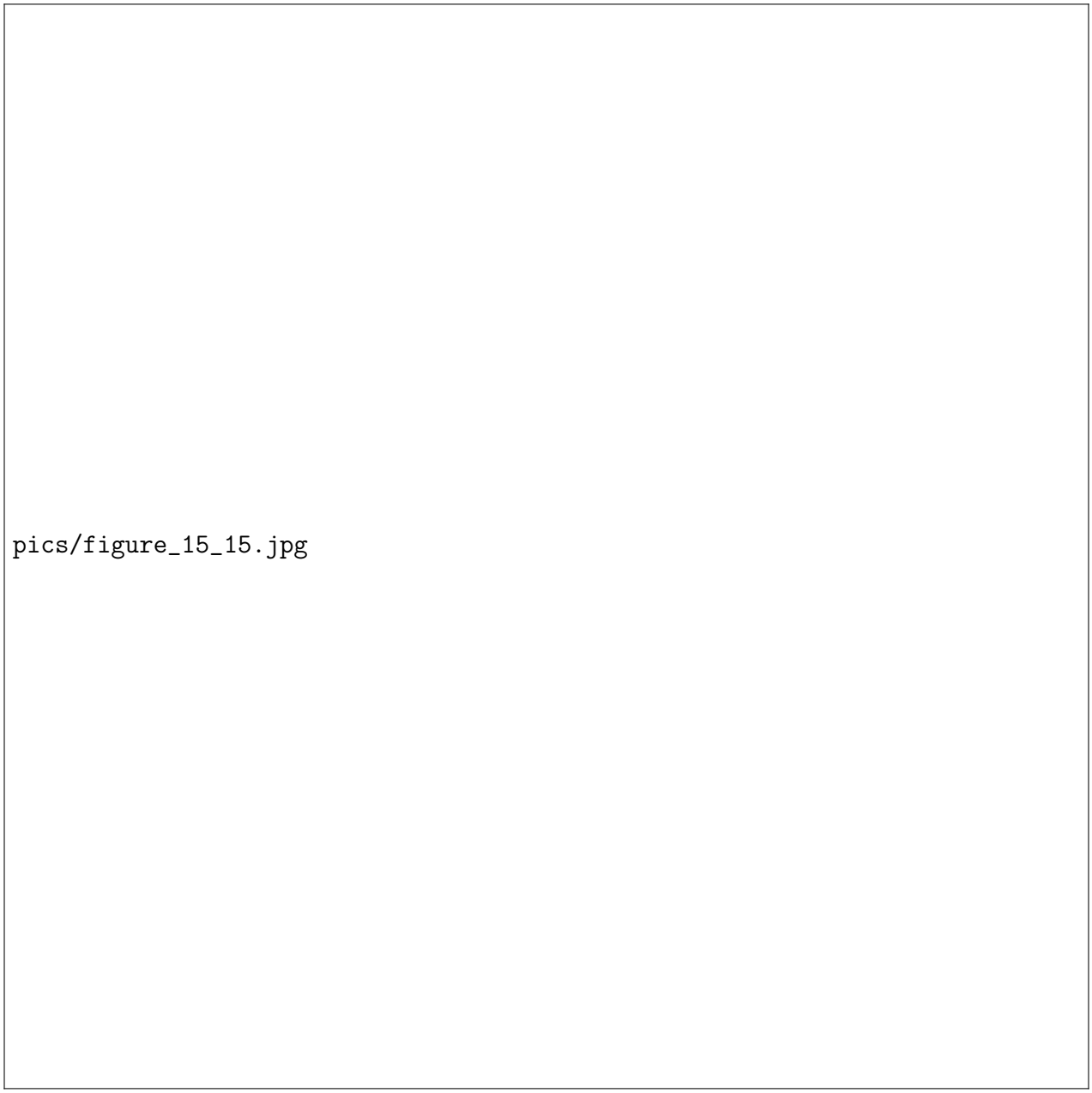
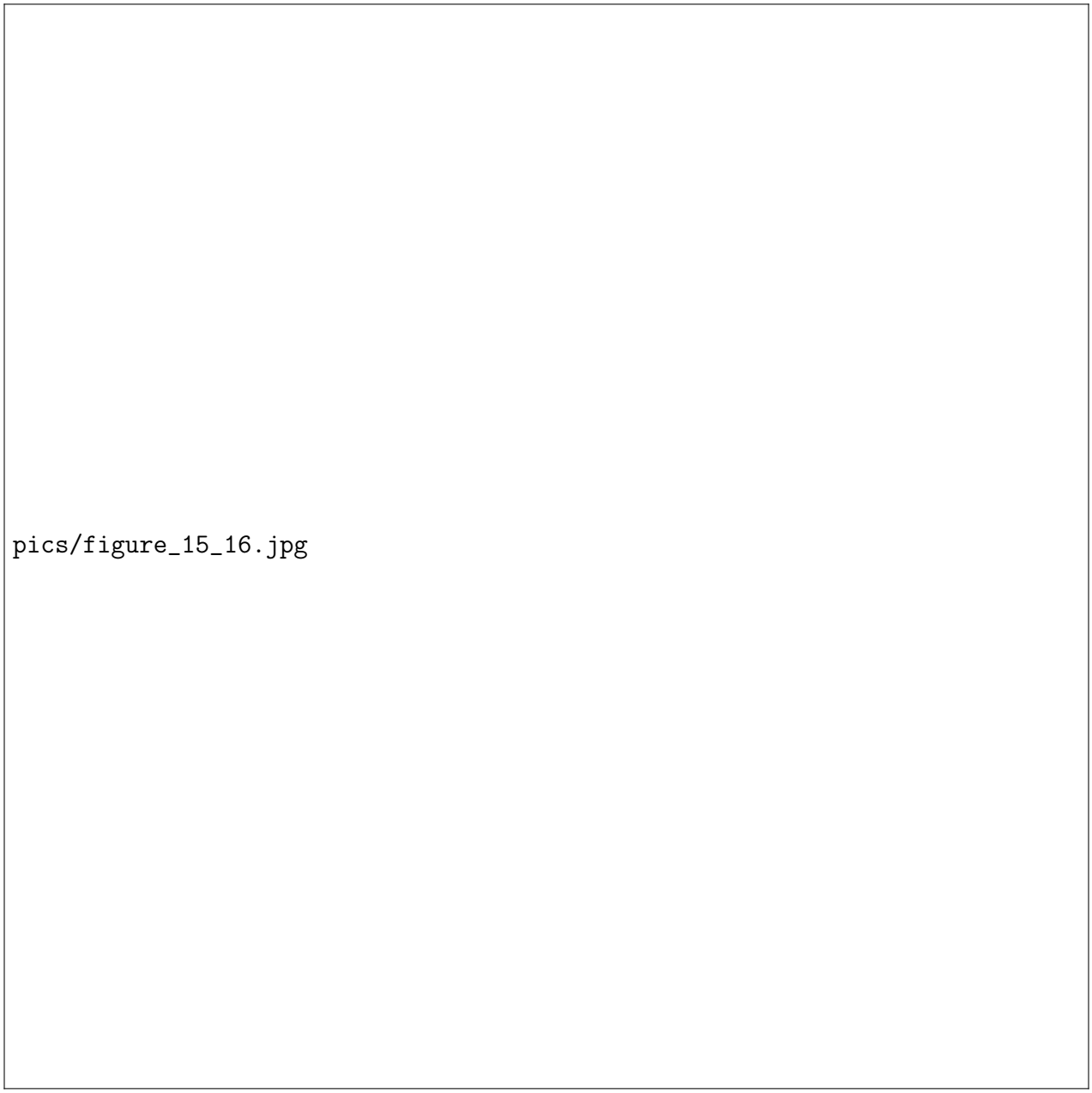


Transmembrane proteins

- Single-pass membrane proteins have start signal on the end and stop signal in the middle; they will stack halfway through the membrane
- Double pass membrane proteins have same signals both located inside molecule; they form U-shape because they double cross a membrane

Protein translocation movie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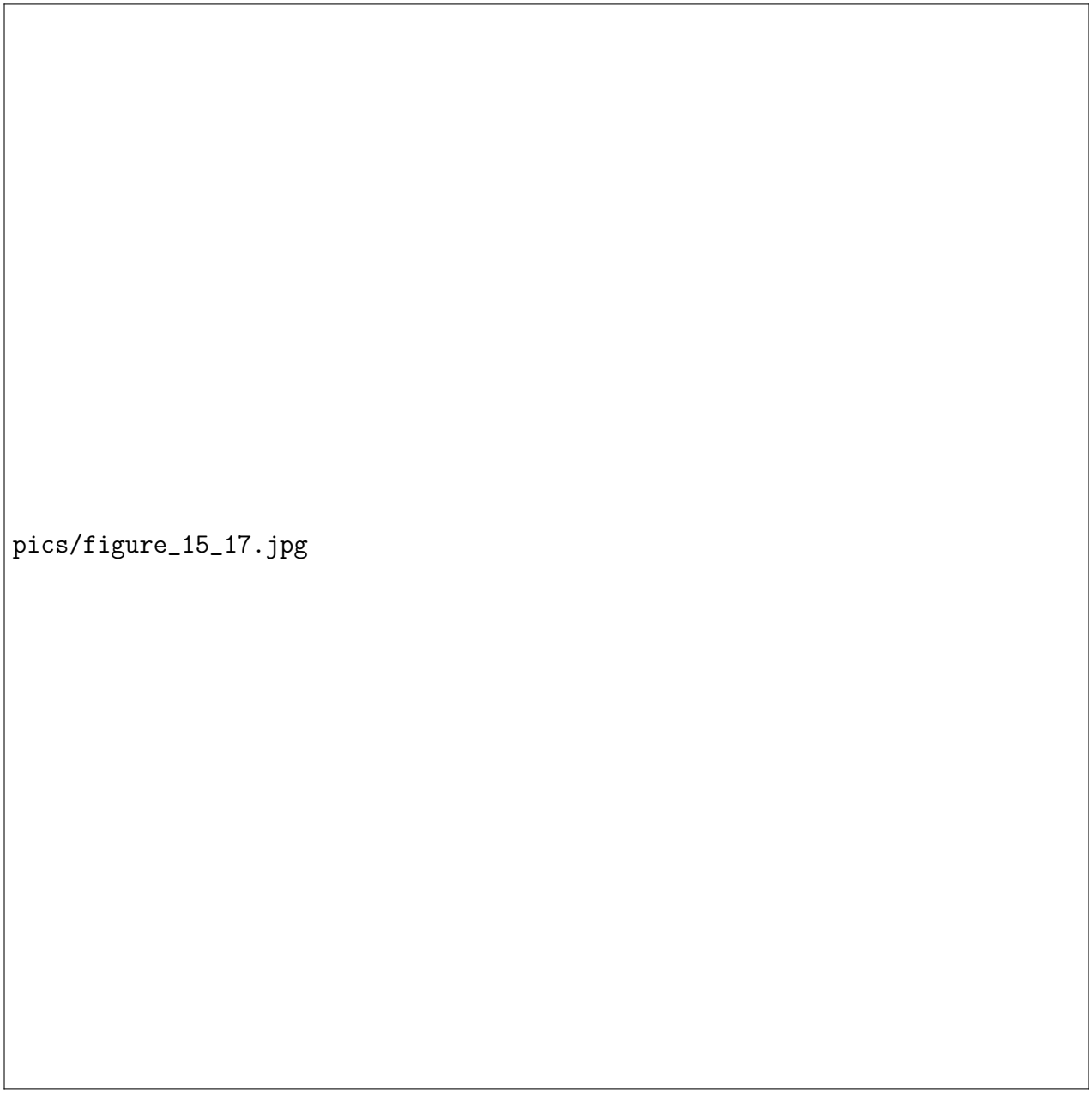


Vesicular transport

- Endocytic pathway: Plasma membrane → endosome + vesicles → lysosome
- Secretory pathway: ER → AG → transport vesicles → plasma membrane

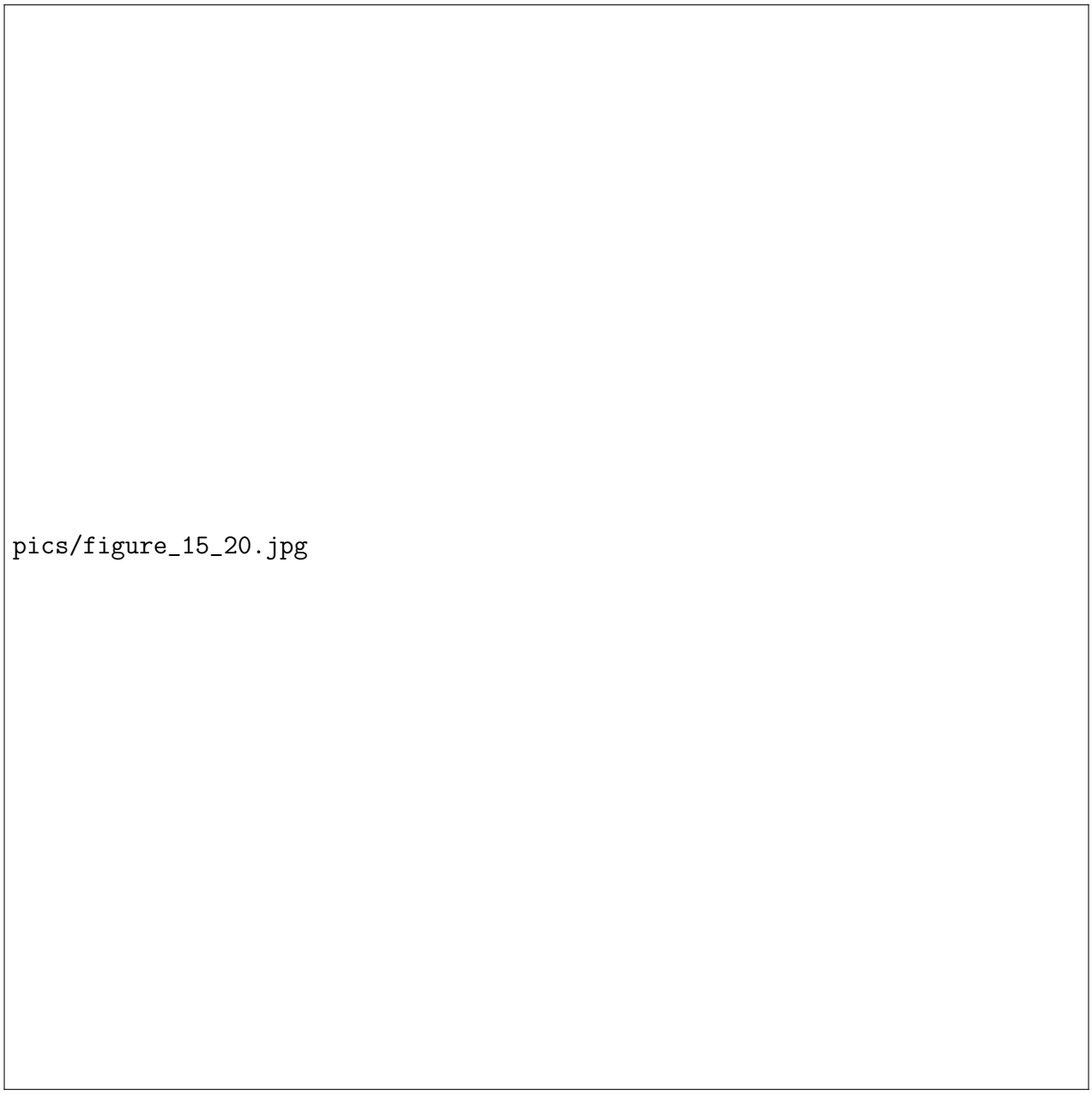
Moving cell compartments movie

Secretory pathway movie

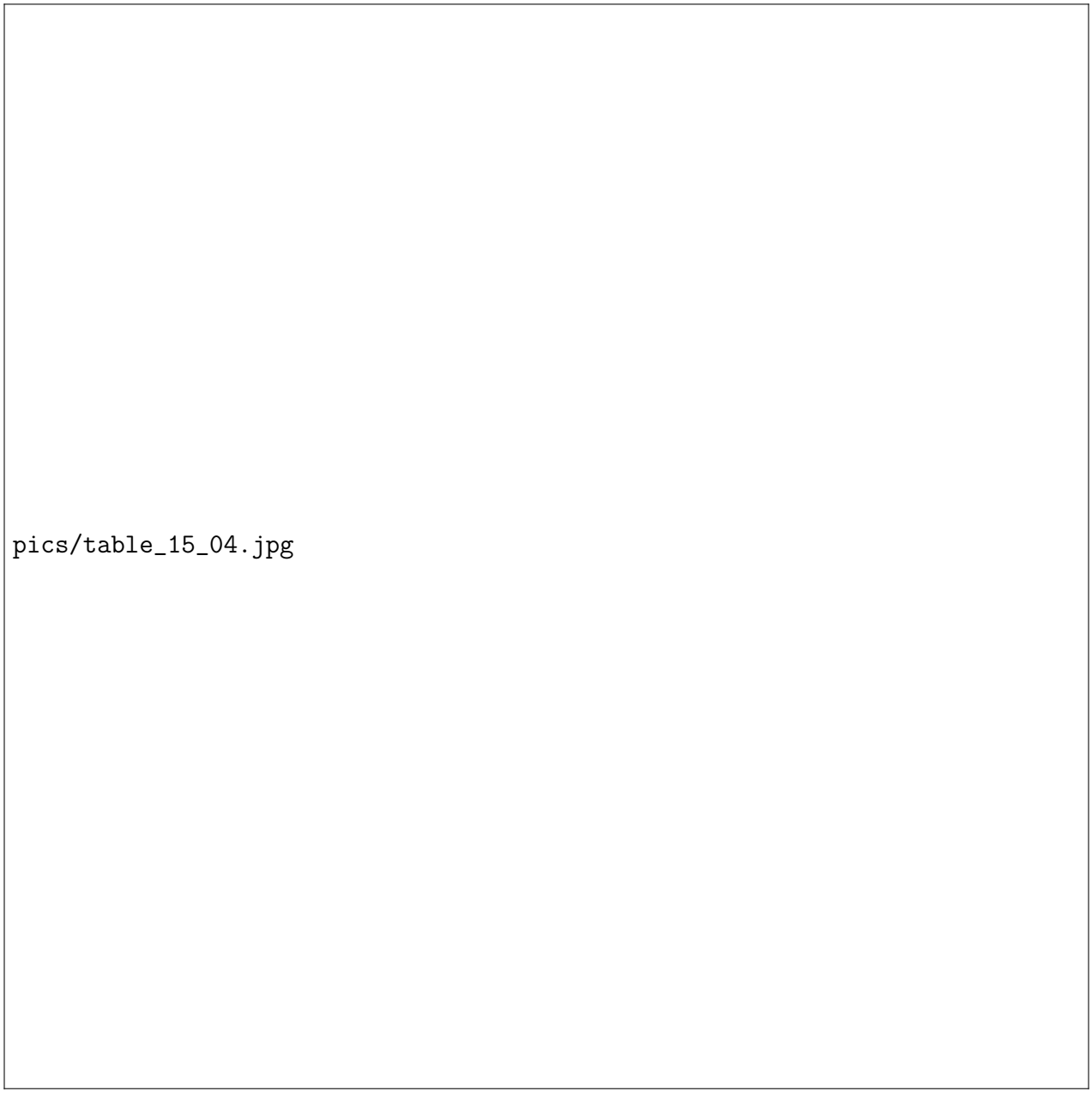
Vesicle budding

- Starts with place covered with adaptin-clathrin complex

- Dynamin punch the vesicle
- Clathrin coat dissolved lately

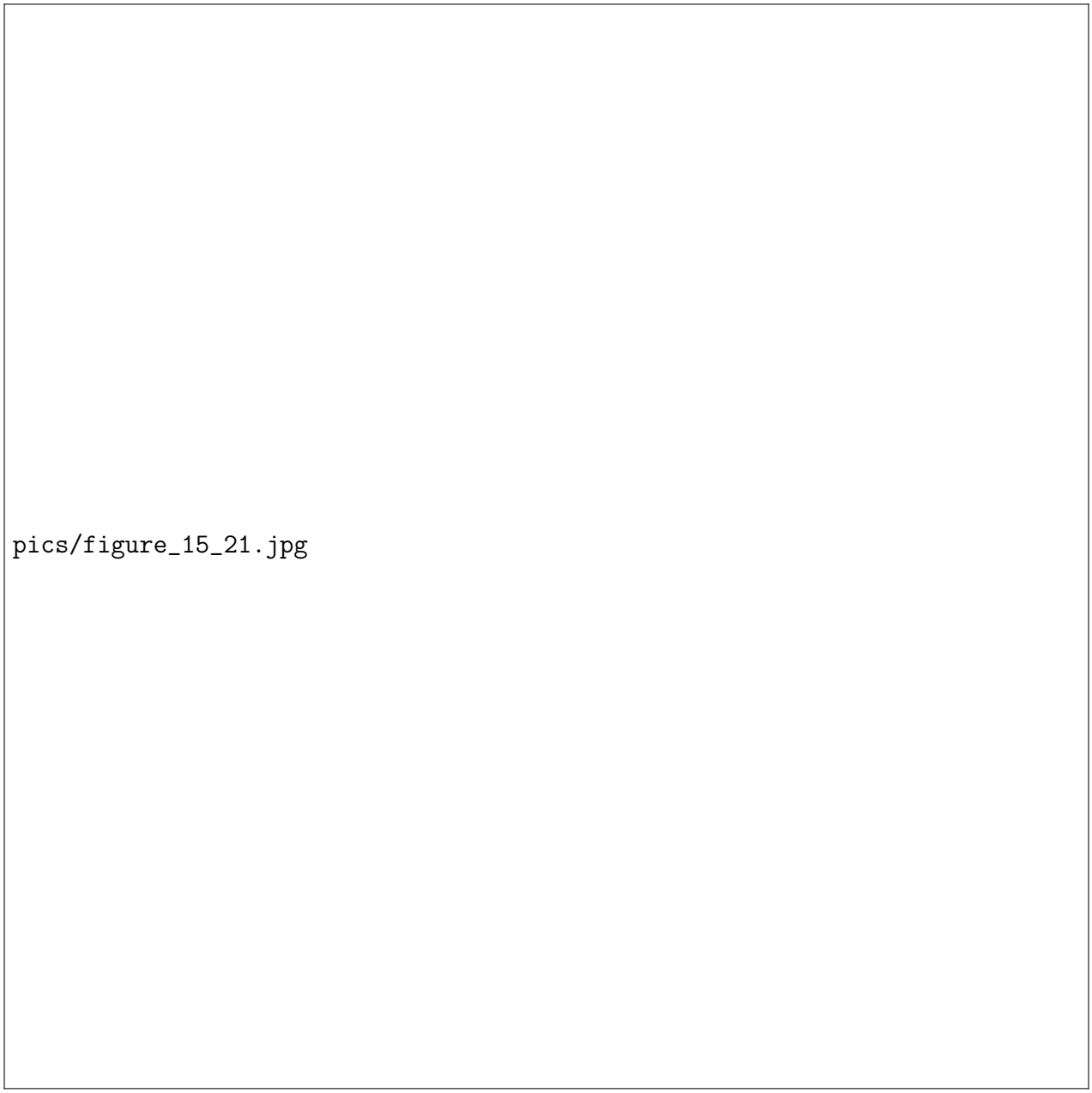


Clathrin movie



Vesicle docking

- Rab and SNARE proteins allow recognition of different types of vesicles
- Rab-binding tethering proteins will lock vesicle nearby the membrane
- If SNAREs are complementary with SNAREs on target membrane, membranes will fuse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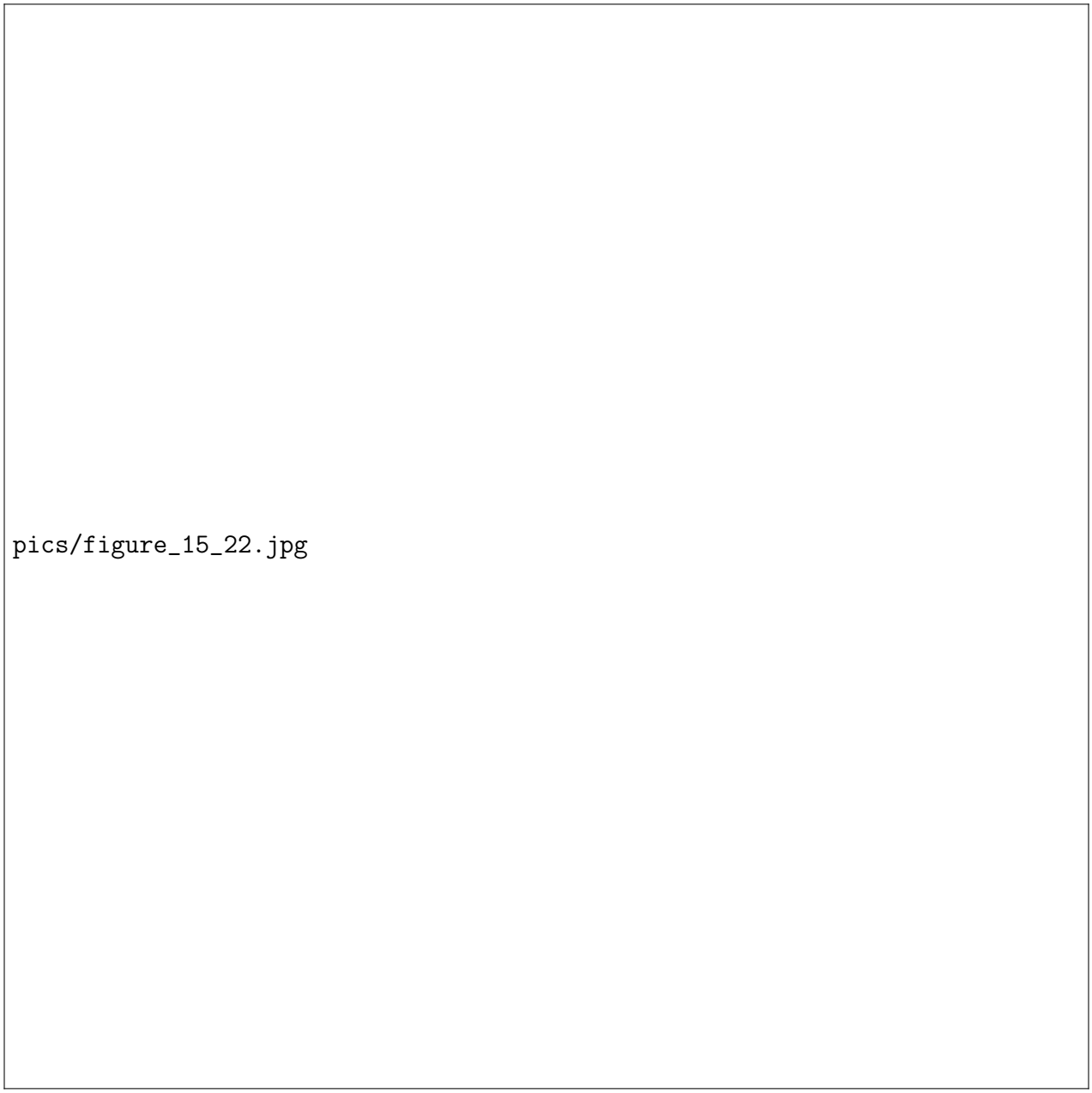


Final question (1 point)

What is the role of chaperones in mitochondria and chloroplasts?

- Nuclear pore transport is a cyclic process involving Ran-GTP and nuclear transport receptor
- ER membrane makes most cell lipids and proteins
- Protein coat is essential for vesicle budding and docking

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 15*.

Outline

Questions and answers

Previous final question: the answer

What will happen to cytosol protein, if it has no signal sequence?

Stay in cytosol, then degrade

Intracellular transport

.1 Endosomes and lysosomes

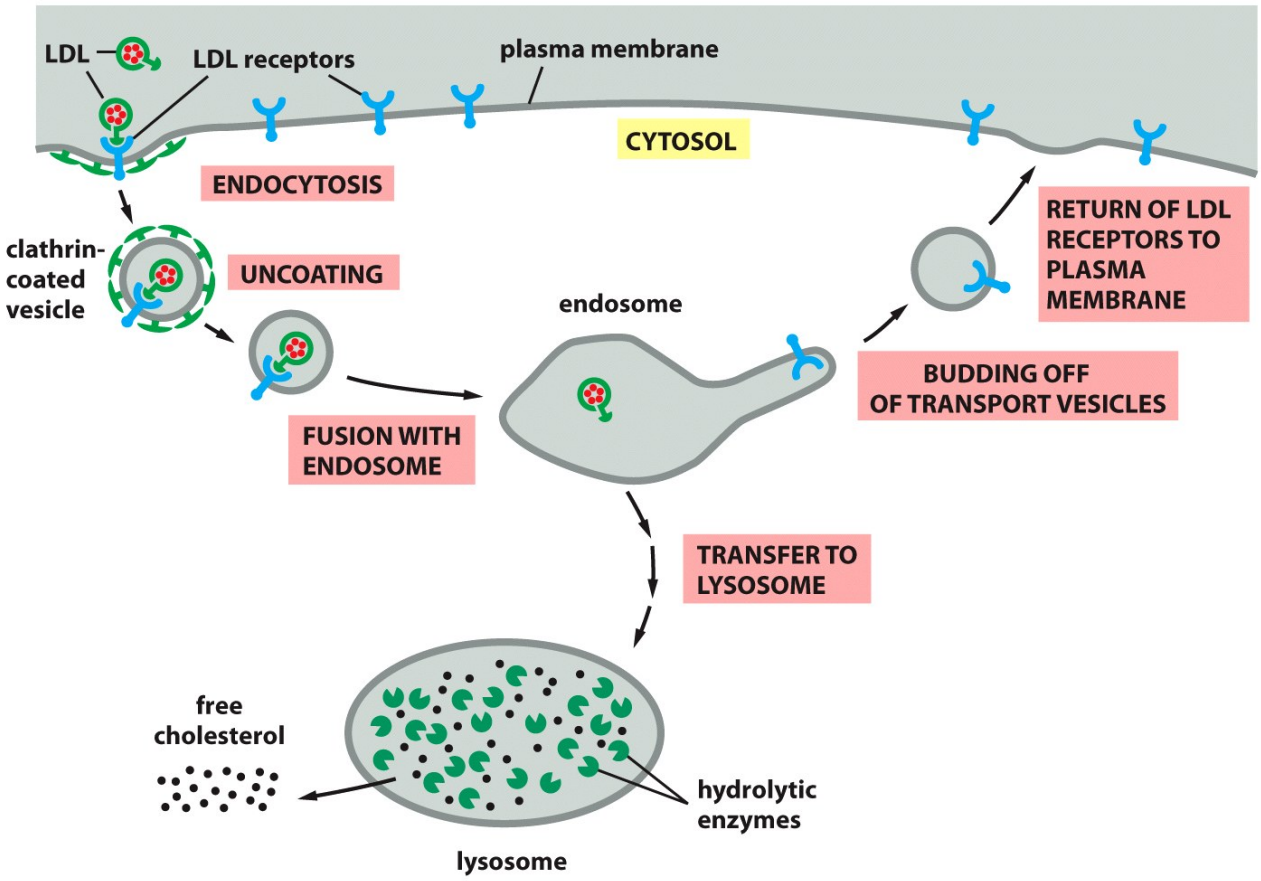


Figure 15-33 Essential Cell Biology 3/e (© Garland Science 2010)

Endosomes

- Initially, early endosomes
- After uncoating and fusion, become late endosomes
- May proceed back, to lysosome or to different place in a membrane

Lysosomes

- Lysosomes are cellular shredders
- Contain multiple enzymes and proton pump
- Also participate in autophagy

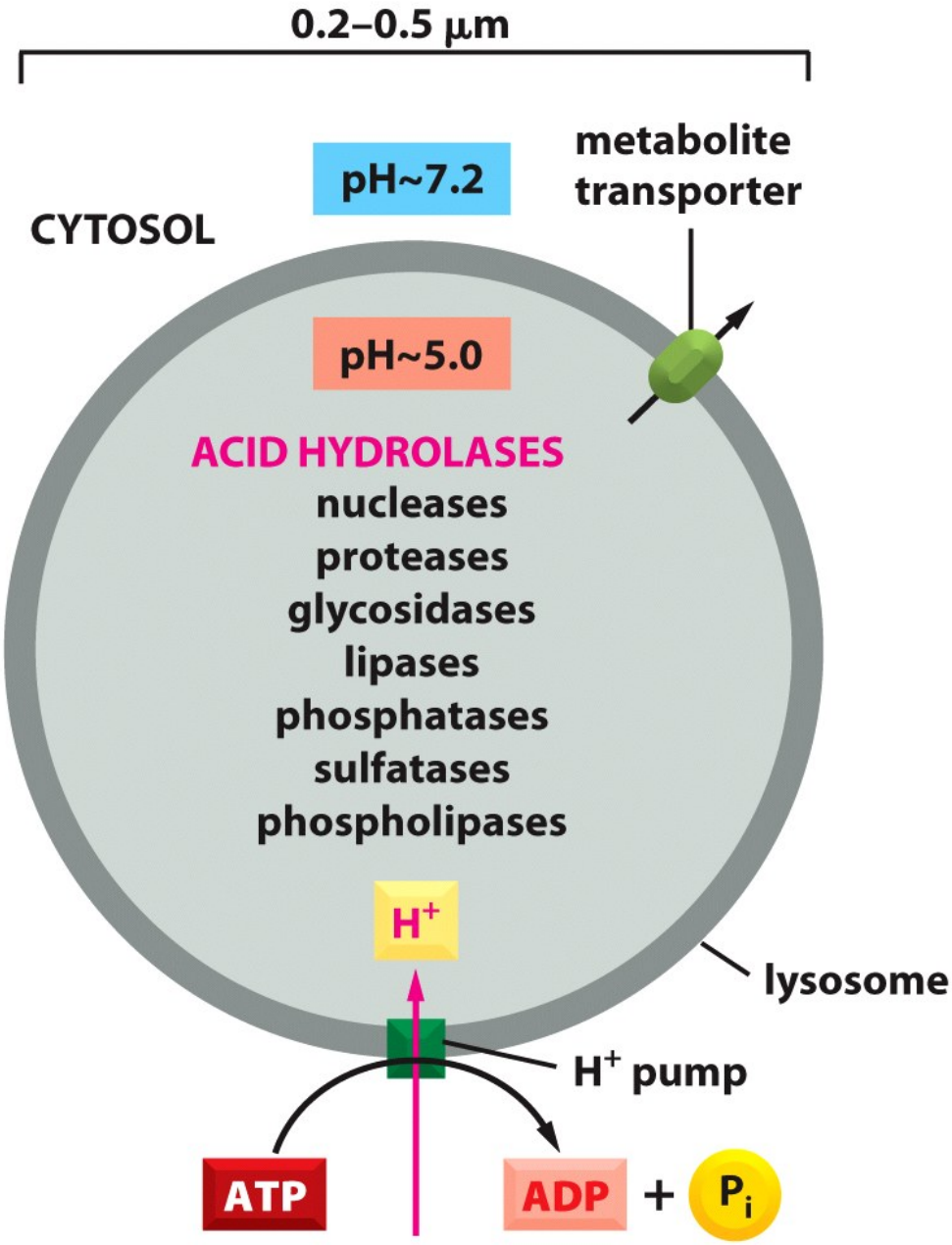


Figure 15-35 Essential Cell Biology 3/e (© Garland Science 2010)

Cell communication

.1 Signals

General principles

- Signal cell → Target cell
- Target cell will take signal molecule (*reception*) and then (not necessary) make *transduction* with receptor protein

Types of signals

- Endocrine: hormones
- Paracrine: local mediators
- Neuronal: neurotransmitters
- Contact-dependent: membrane-bound signal molecules (like Delta-Notch system in fly neurons)

Animal cell signals

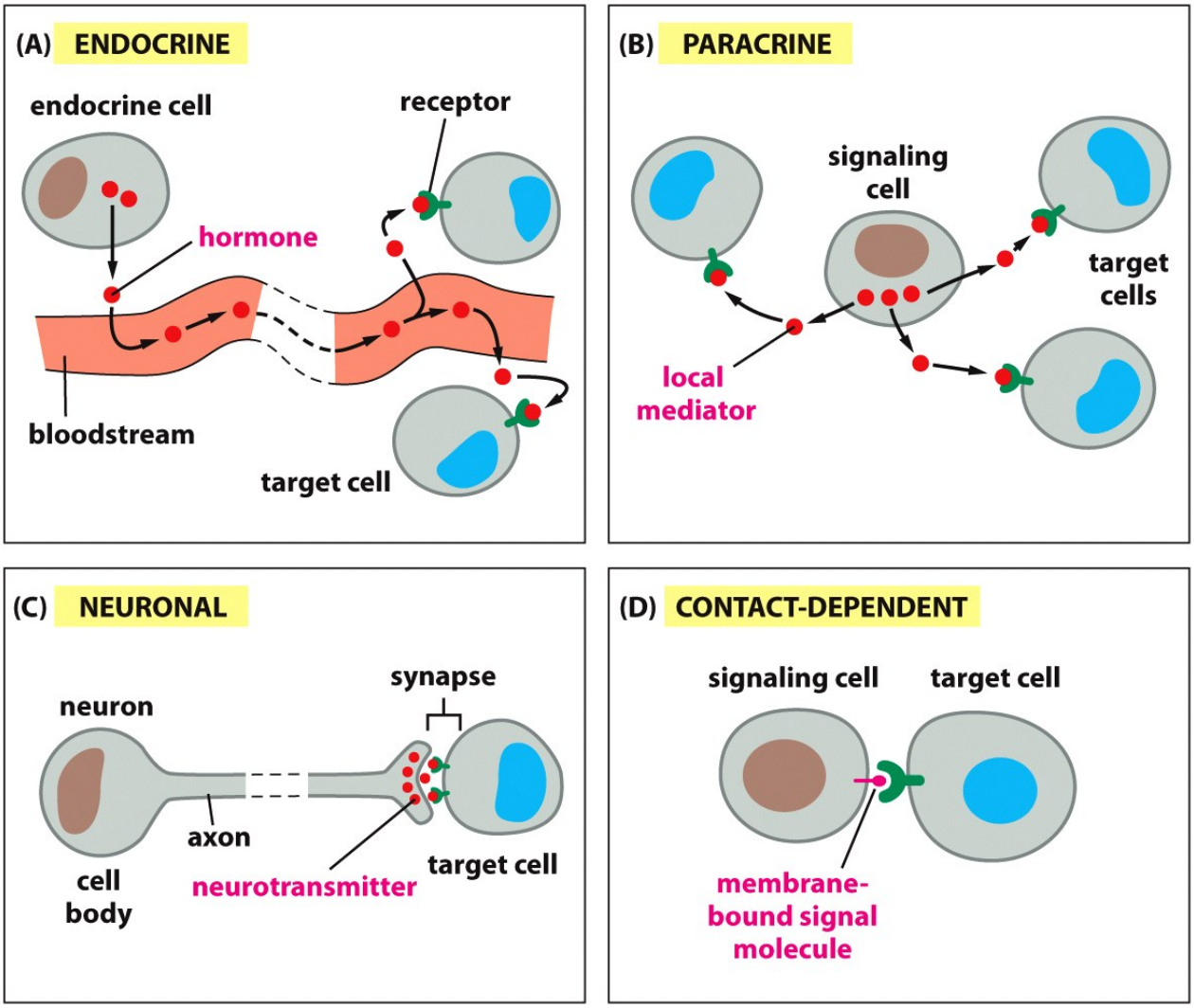


Figure 16-3 Essential Cell Biology 3/e (© Garland Science 2010)

Diversity of responses

- Receptor protein, not a signal molecule, provides a response
- Depending on receptor, response may be completely different (e.g., in case of acetylcholine)

- Generally, there may be: "survive" ("contract", "excrete", "stay still"), "grow/divide", "differentiate", "die" (apoptosis) responses

Acetylcholine effects

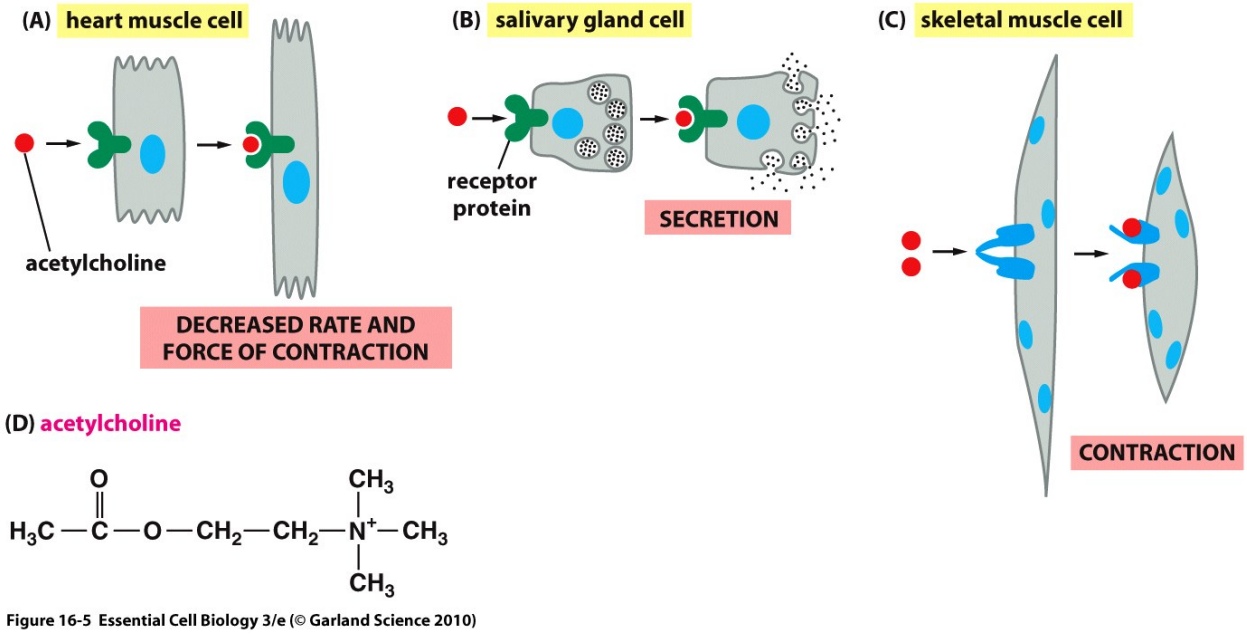


Figure 16-5 Essential Cell Biology 3/e (© Garland Science 2010)

Speed of response

- Fast response: alter protein function
- Slow response: through transcription/translation

Cross-membrane hormone signals

- Steroid hormones are small enough (+ hydrophobic) to go through the membrane
- They bind to receptor proteins which are transcription regulators and may be located in nucleus or in cytoplasm

Cortisol signaling pathway

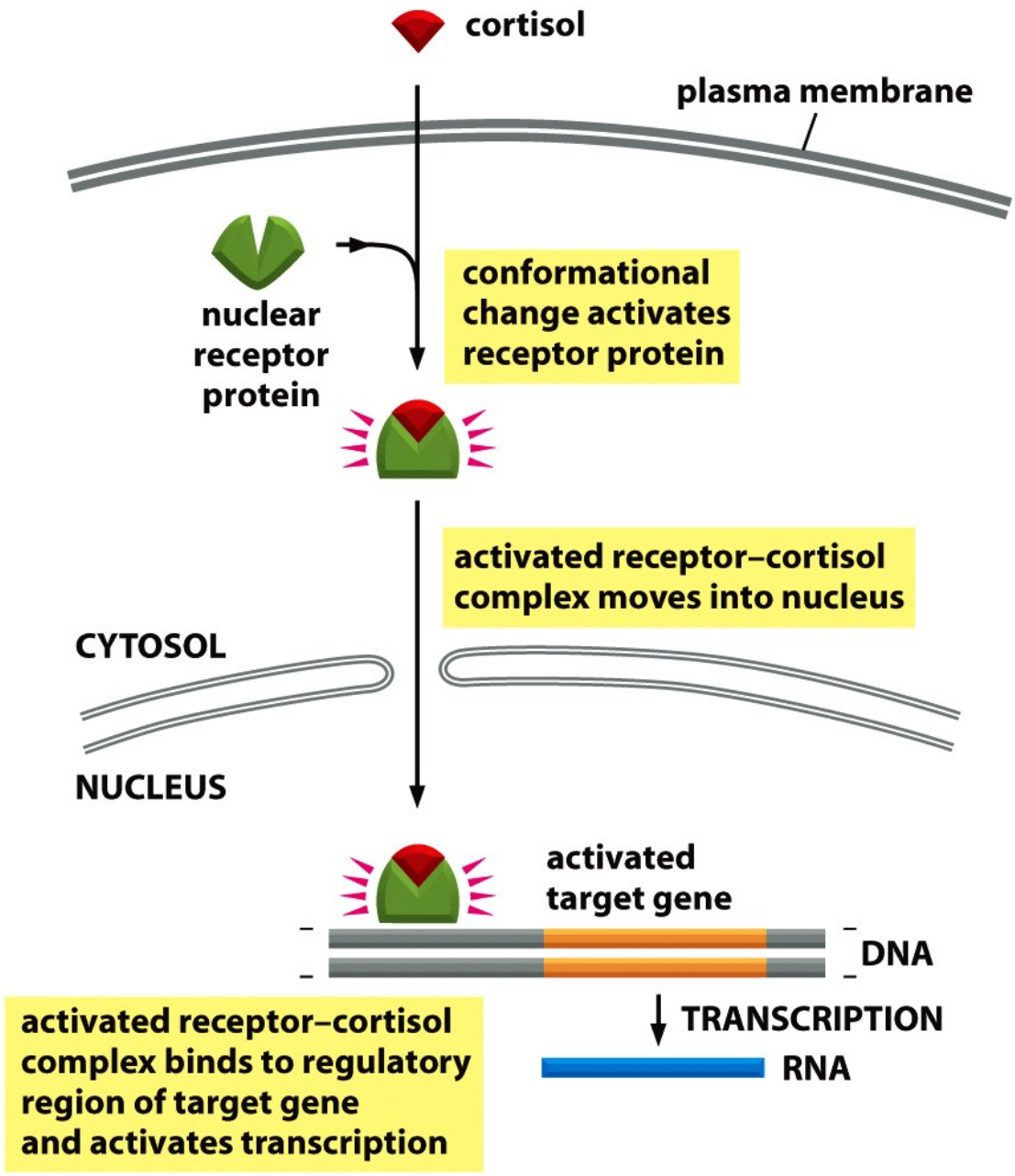


Figure 16-10 Essential Cell Biology 3/e (© Garland Science 2010)

Cross-membrane gas signals

- Nitroglycerin (component if dynamite!) releases nitric oxide, N=O which goes across membrane and activate *guanylyl* enzyme responsible for making *cyclic GMP*
- Smooth and heart muscle cells will relax, blood vessels will dilate
- Viagra drug activates cyclic GMP synthesis and dilation of vessels responsible for erection

Nitric oxide signaling pathway

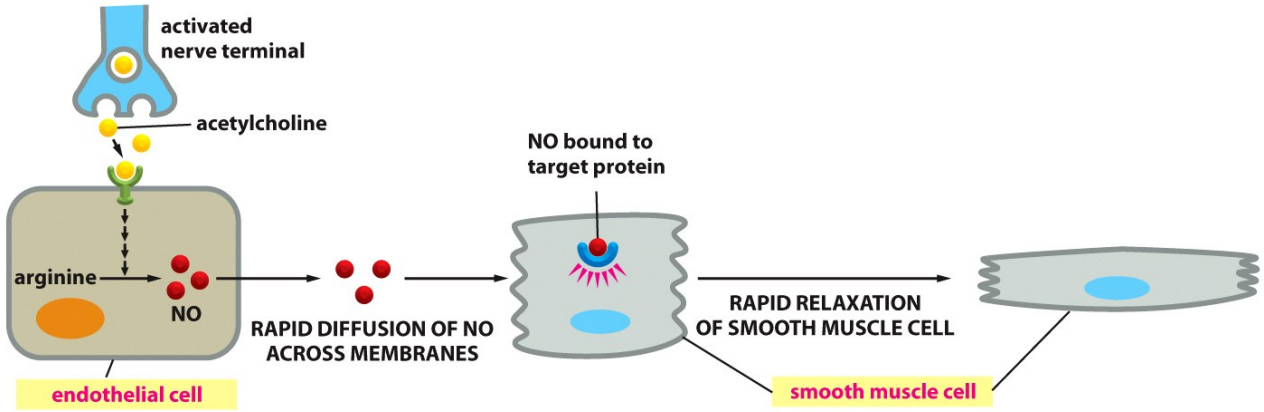
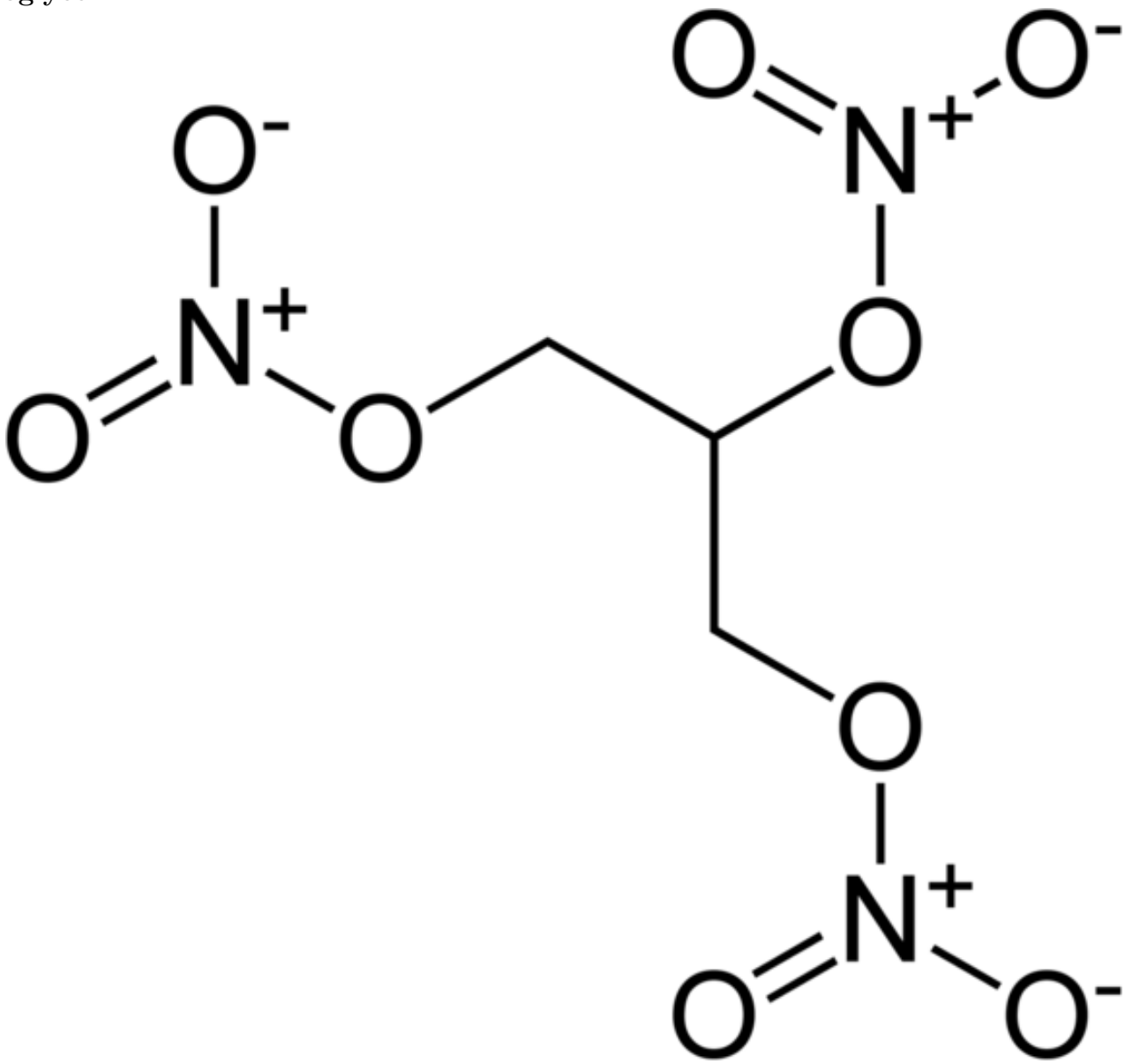


Figure 16-11b Essential Cell Biology 3/e (© Garland Science 2010)

Nitroglycerin



.2 Receptor-bind signals

Receptor-bind signals

- Most of signals are proteins which are larger or not hydrophobic and cannot go through the membrane
- They bind to membrane receptors activating *intracellular signaling molecules* (ISM)
- ISM will cause effector proteins to alter metabolism, change cell shape or movement or alter gene expression

Intracellular signaling pathways (ISP)

- Relay signal forward
- Amplify
- Integrate multiple signals
- Distribute signals to more than one effector

Intracellular signaling pathways

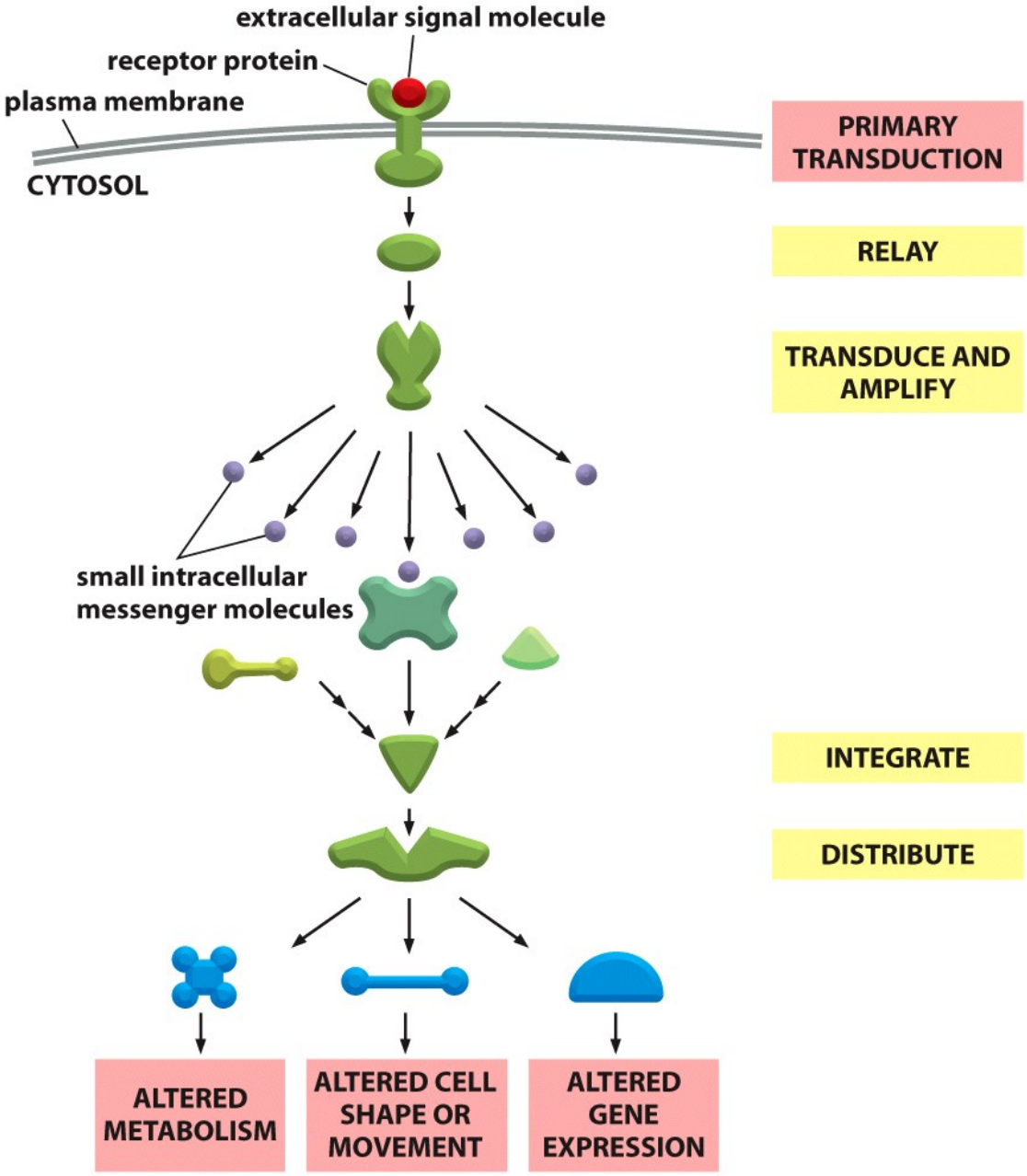


Figure 16-13 Essential Cell Biology 3/e (© Garland Science 2010)

Molecular switches

- Many ISPs have active and inactive states
- The switch between forms is due to phosphorylation of GTP binding
- Kinases (serine/threonine or tyrosine) attach phosphate, phosphatases detatch phosphate

Molecular switches

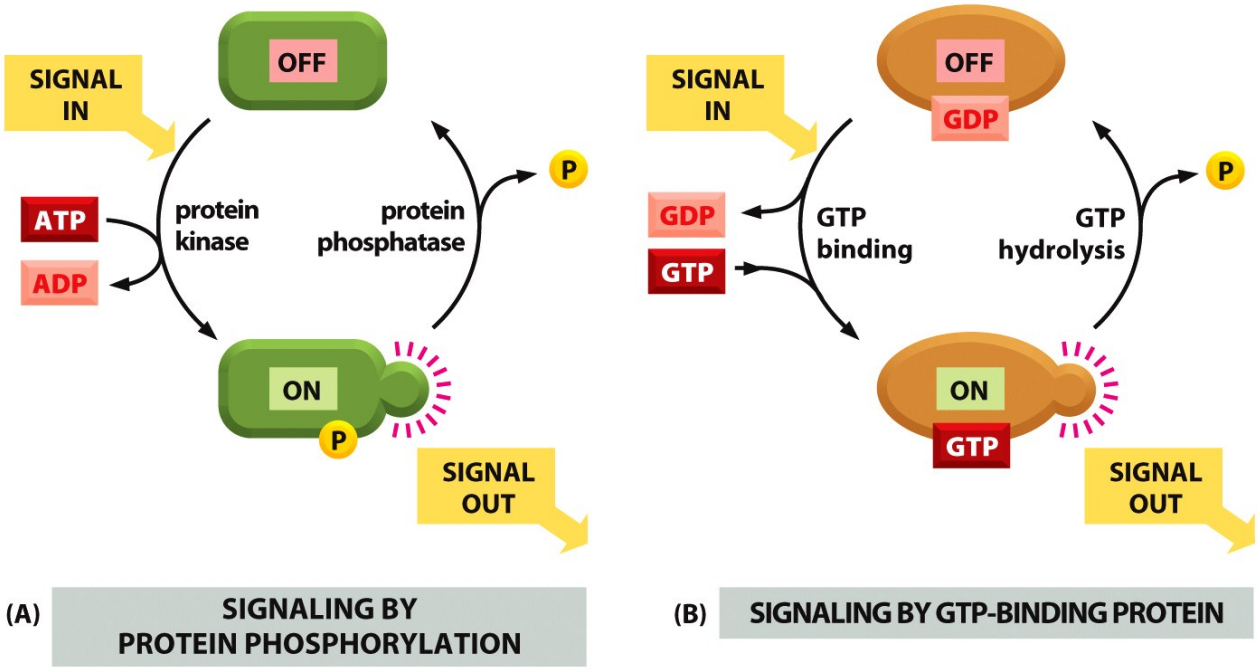


Figure 16-14 Essential Cell Biology 3/e (© Garland Science 2010)

Three classes of receptors

1. Ion-channel-coupled receptors = ligand-gated channels: curare
2. G-protein-coupled receptors (GPCR) have a mediator membrane protein, G-protein: morphine
3. Enzyme-coupled receptors are proteins with enzymatic or enzyme-binding activity: many hormones

Ion-channel-coupled receptors

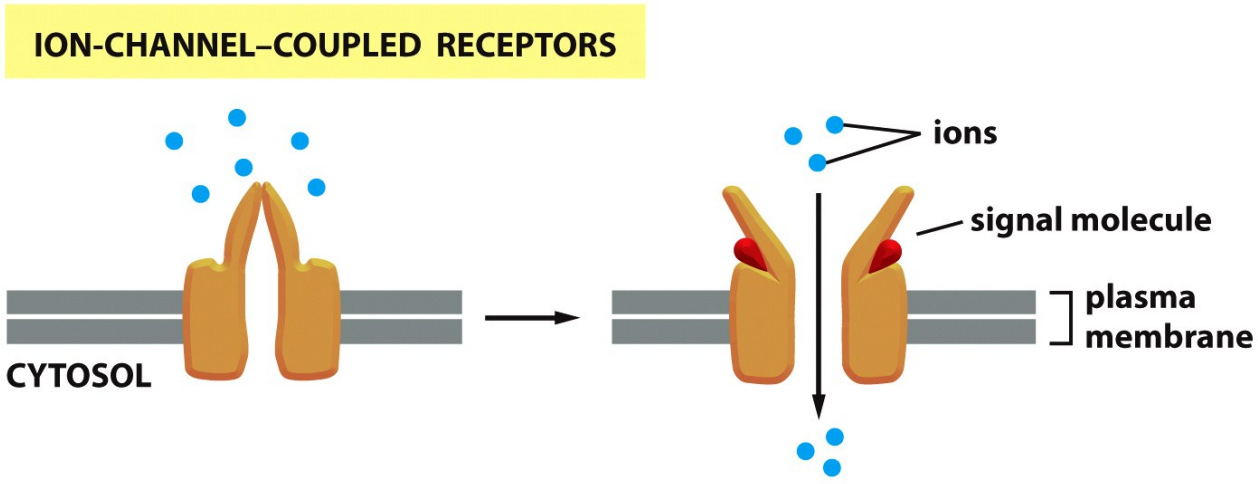


Figure 16-15a Essential Cell Biology 3/e (© Garland Science 2010)

Ca^{2+} receptor movie

GPCRs

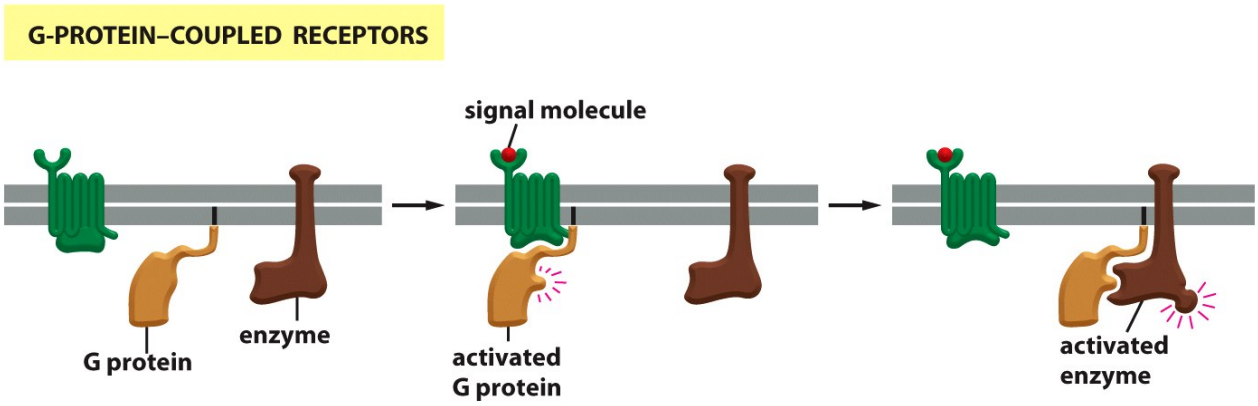
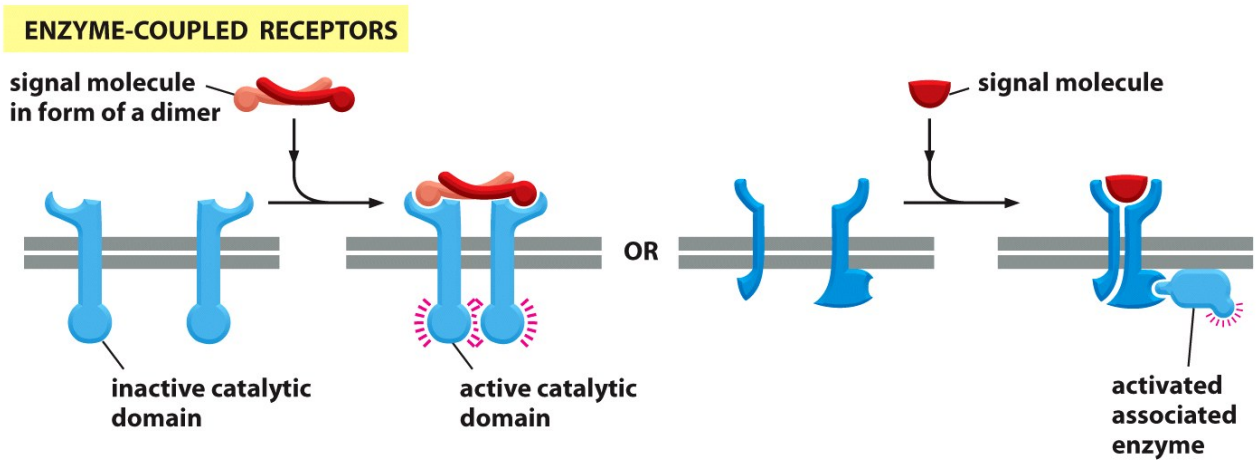


Figure 16-15b Essential Cell Biology 3/e (© Garland Science 2010)

Enzyme-coupled receptors



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.3 G-protein-coupled receptors

G-protein-coupled receptors (GPCRs)

- About 50% of known drugs are working with GPCRs
- They are seven-pass transmembrane proteins structurally similar to bacteriorhodopsin

Stimulation of GPCRs

- Signal facilitate conformational change of receptor
- Receptor will activate G protein
- In activated G protein, α subunit binds GTP, other two form $\beta\gamma$ complex
- α and/or $\beta\gamma$ bind to target protein
- α subunit will finally hydrolyze GTP and deactivate itself plus $\beta\gamma$ complex

Activation of GPCRs

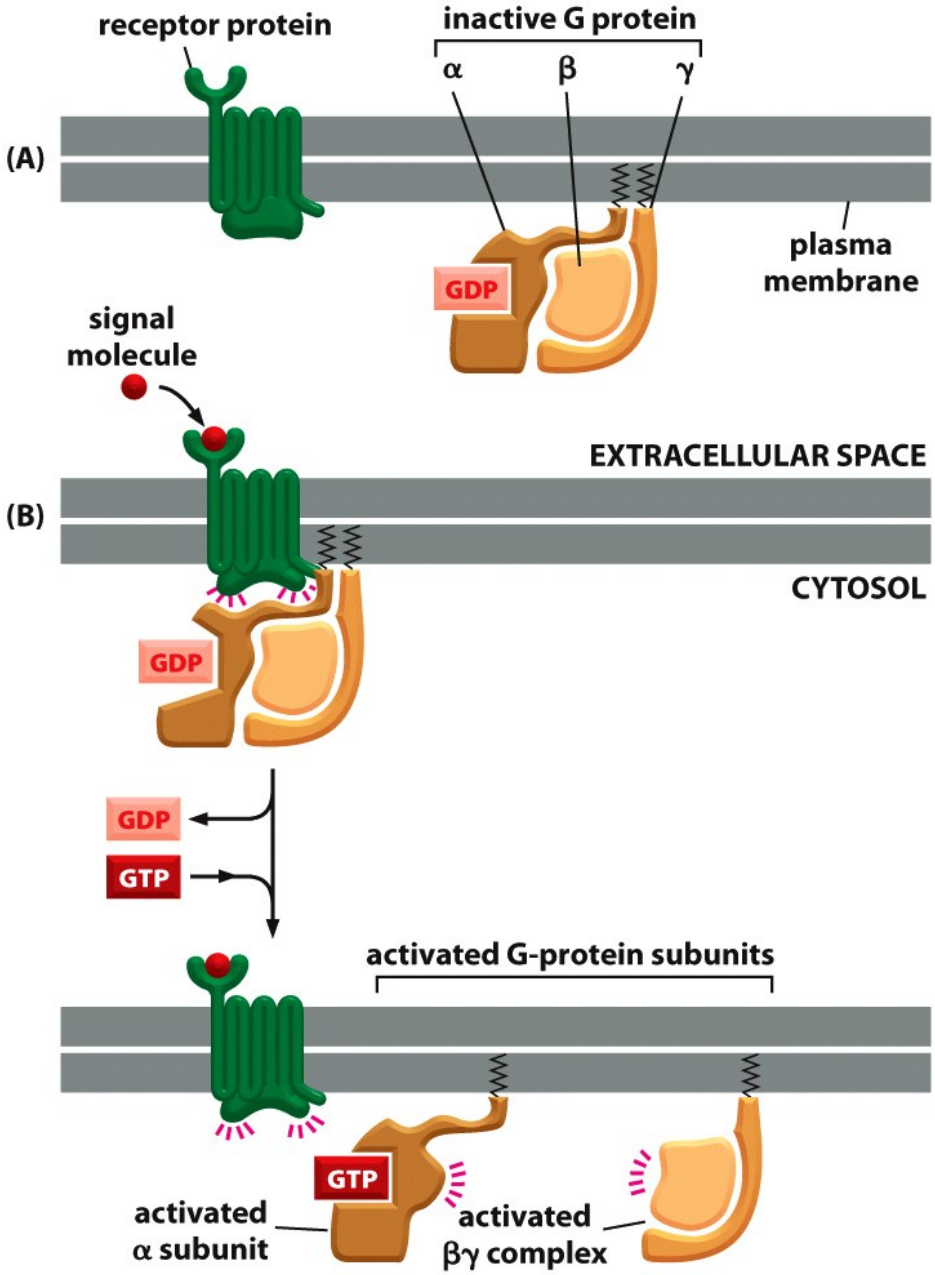


Figure 16-17 Essential Cell Biology 3/e (© Garland Science 2010)

Deactivation of GPCRs

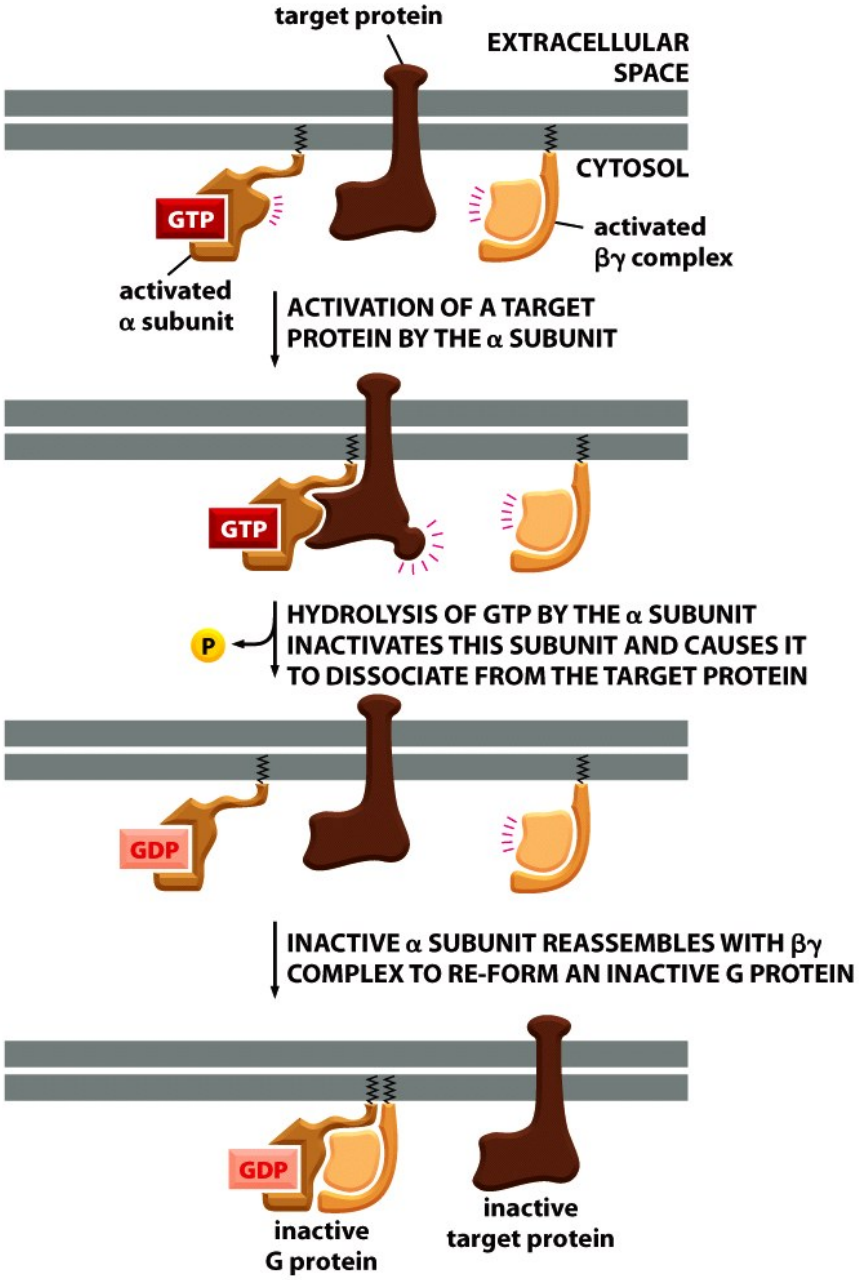


Figure 16-18 Essential Cell Biology 3/e (© Garland Science 2010)

Cholera and whooping cough (pertussis)

- Cholera toxin stops deactivation of particular G-protein; as a result, water and ions will not be absorbed in gut
- Pertussis toxin stops activation of inhibitory G-protein; as a result, prolonged signal is generated resulted in a cough

G-protein signalling movie

GPCRs as ion channels regulators in heart muscle cells

- Acetylcholine signal binds to GPCR of muscle cell membrane

- Activated $\beta\gamma$ complex facilitates K^+ ligand channels to open; as a result exitability is lowered

GPCRs as ion channel regulator

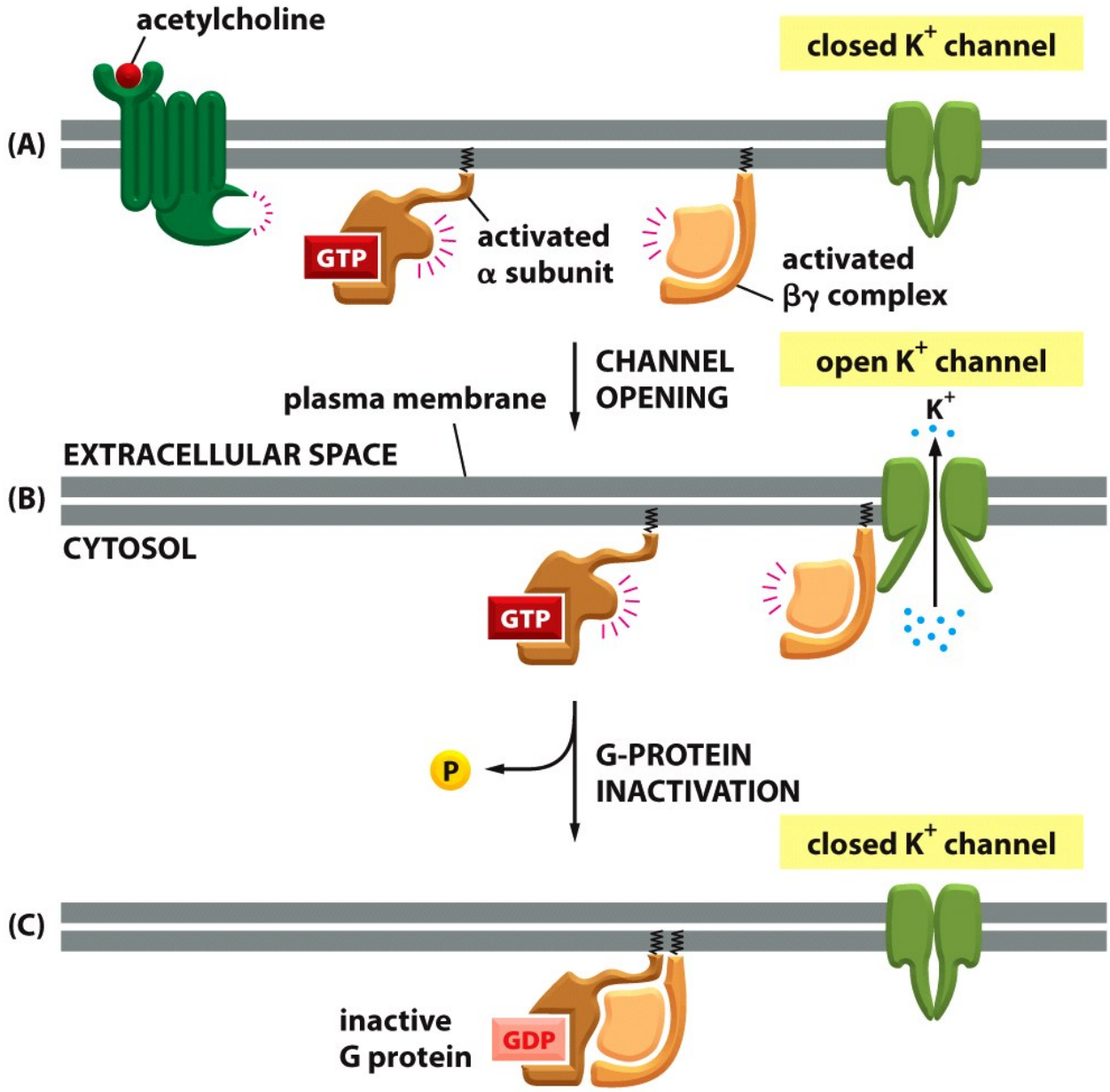


Figure 16-19 Essential Cell Biology 3/e (© Garland Science 2010)

GPCRs as membrane enzyme activators

- G-protein may activate either adenylyl cyclase, or phospholipase C
- Both are enzymes producing small molecules—second messengers
- Phospholipase C produces inositol triphosphate and diacylglycerol

GPCRs and cyclic AMP

- Adenylyl cyclase produces cyclic AMP

- In turn, cAMP activates PKA (AMP-dependent protein kinase)
- PKA may either catalyze cellular reactions (e.g., glycogen breakdown), or activate transcription regulators

cAMP

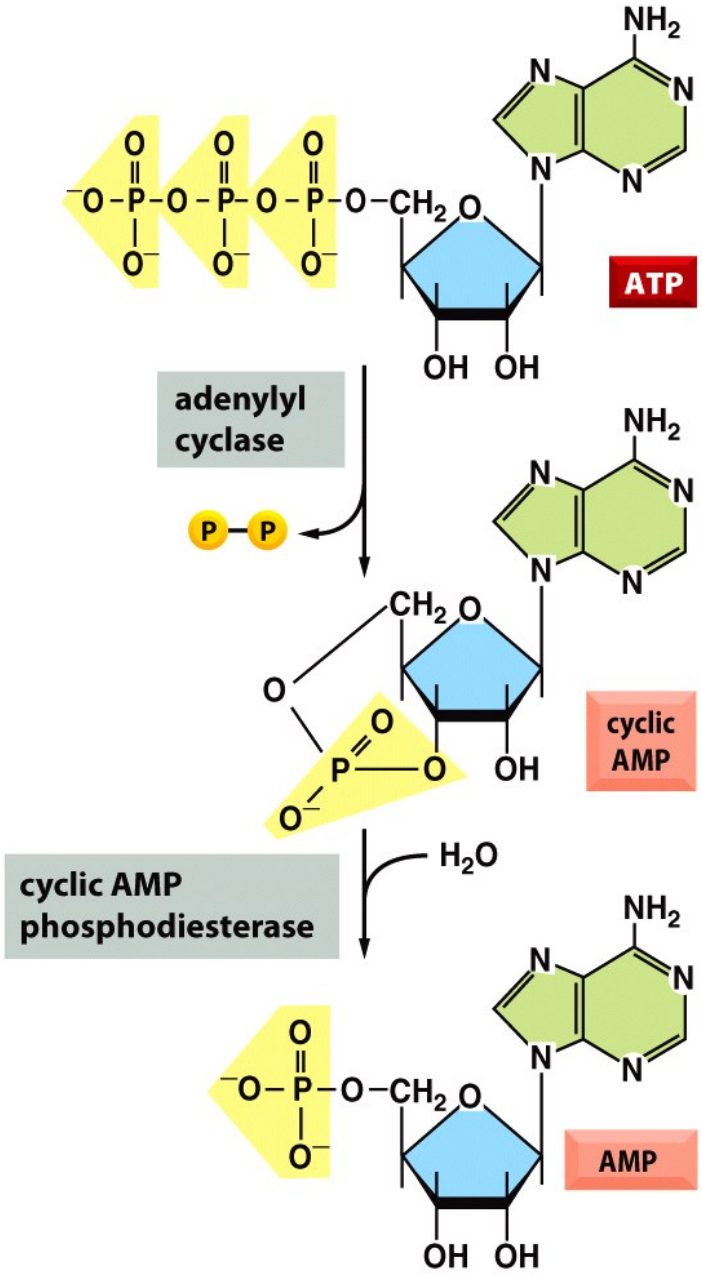


Figure 16-21 Essential Cell Biology 3/e (© Garland Science 2010)

Adrenaline

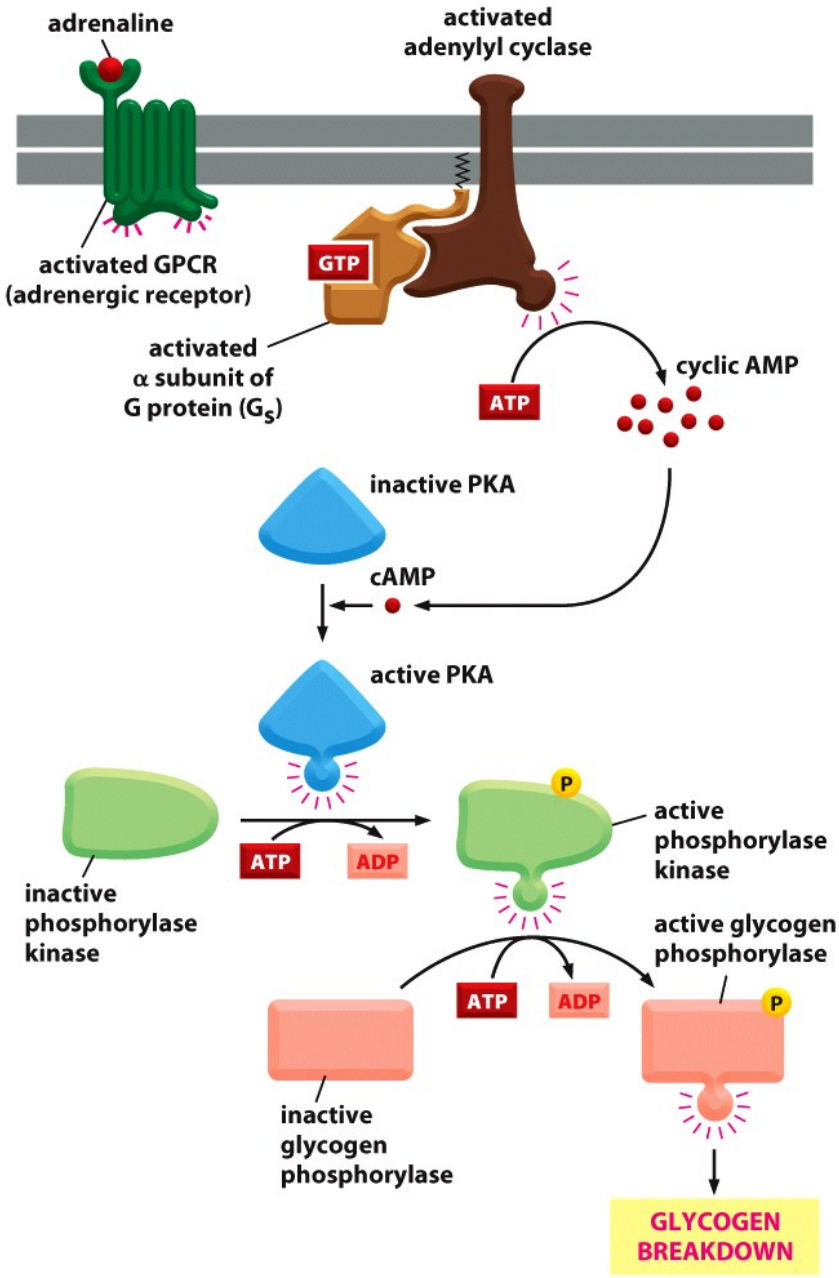


Figure 16-23 Essential Cell Biology 3/e (© Garland Science 2010)

cAMP transcription regulation

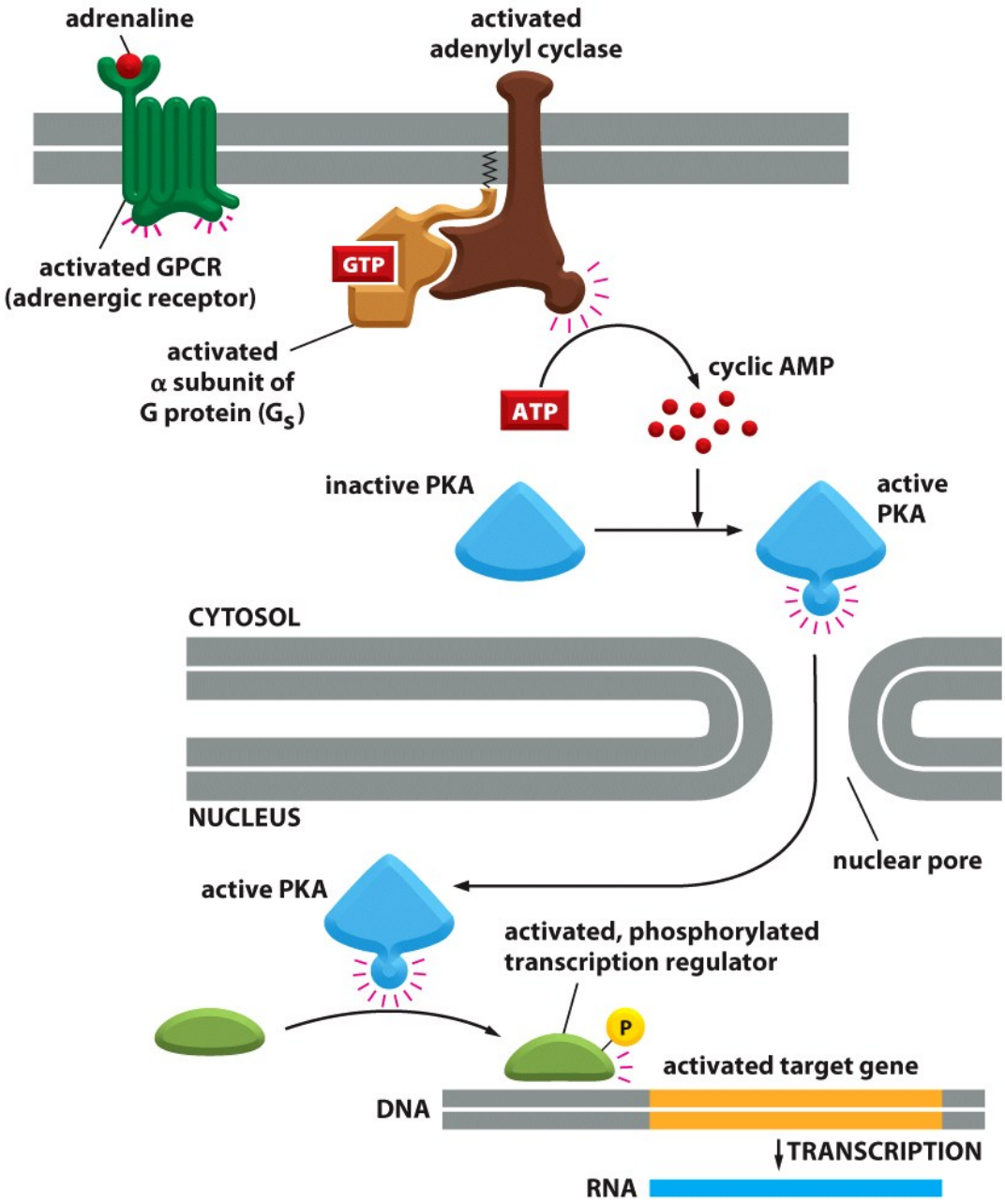


Figure 16-24 Essential Cell Biology 3/e (© Garland Science 2010)

cAMP movie

Inositol phospholipid pathway

- Instead of adenylyl cyclase, some G proteins activate phospholipase C
- Phospholipase C cleaves inositol phospholipid present in the inner layer of membrane
- Vasopressin, acetylcholine and trombin activate this pathway

IP_3, diacylglycerol and PKC

- Phospholipase C produces inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG)

- IP_3 activates Ca^{2+} channels in ER
- DAG activates protein kinase C (Ca^{2+} is needed)
- Both Ca^{2+} and PKC are signals to other proteins

Inositol 1,4,5-triphosphate (IP_3) signal pathway

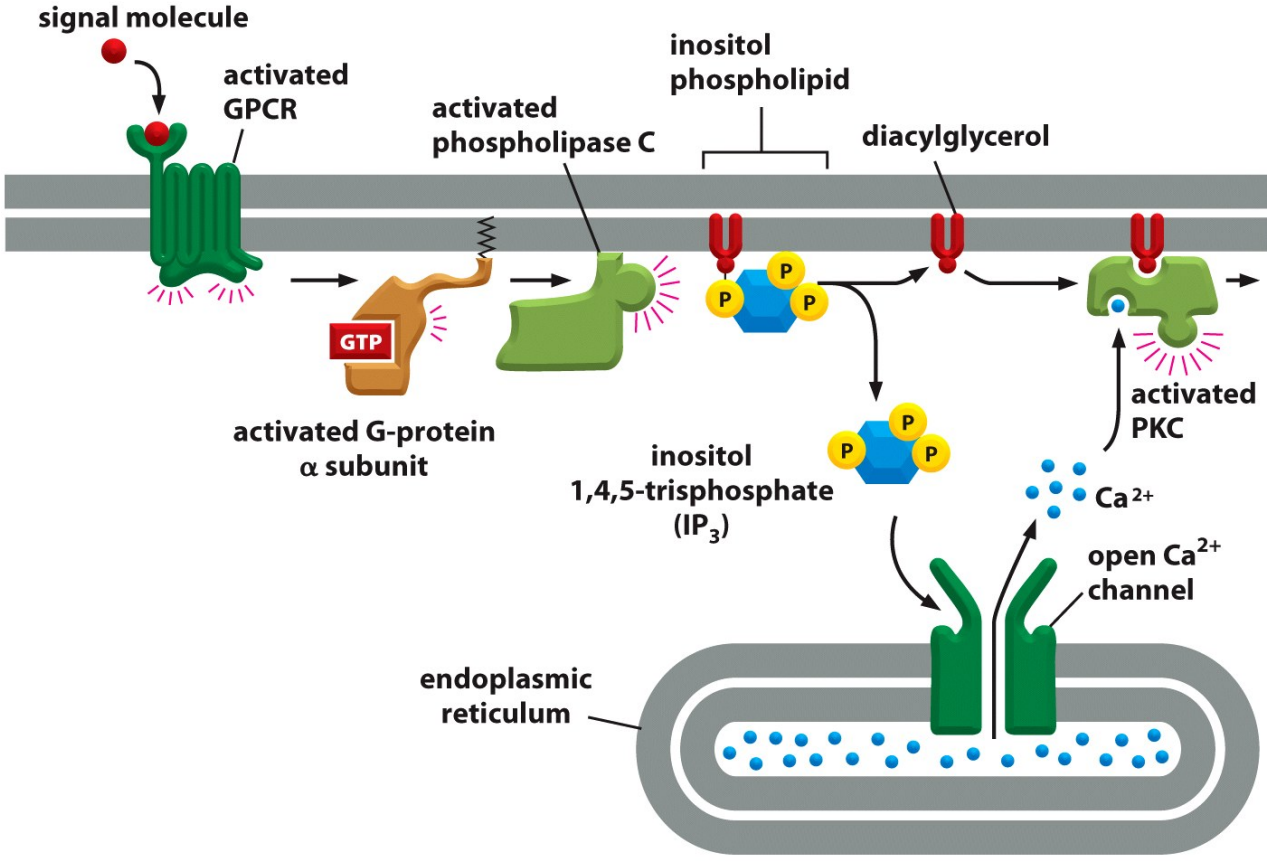


Figure 16-25 Essential Cell Biology 3/e (© Garland Science 2010)

Ca^{2+} signal

- Ca^{2+} is a frequent intracellular signal which signals egg to divide, muscle cell to contract etc.
- Normally, Ca^{2+} concentration is high both in ER and outer space

Calcium wave movie

Calmodulin

- Calmodulin is a Ca^{2+} responsive protein
- It binds four Ca^{2+} ions and changes conformation, wrapping around target proteins
- Ca^{2+}/calmodulin-dependent protein kinases are activated by calmodulin and in turn start to phosphorylate other proteins (some CaMs are probably responsible for location memory)

Calmodulin

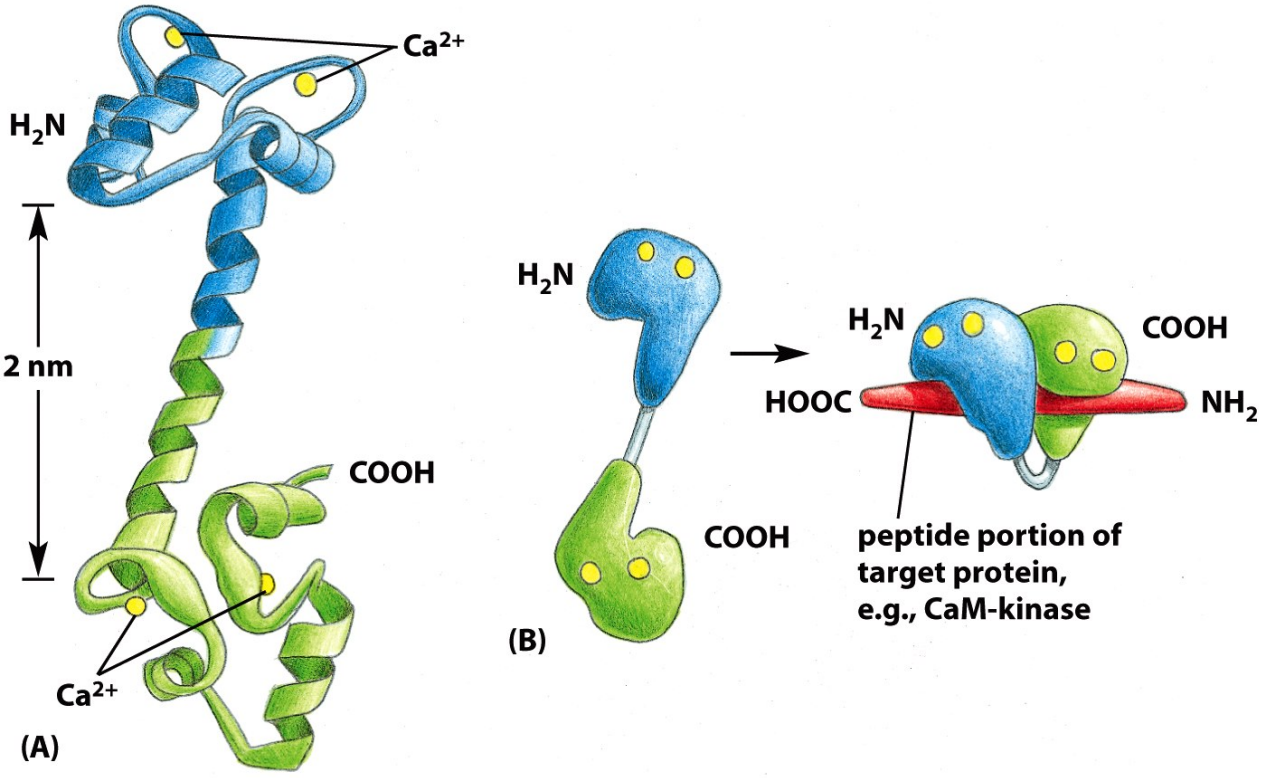


Figure 16-27 Essential Cell Biology 3/e (© Garland Science 2010)

Calmodulin movie

Signaling cascades

- Despite of complexity, could be very fast
- Allow amplification and adaptation

Rhodopsin-transducin signaling cascade

- Rhodopsin receptor activated transducin G protein
- α subunit of transducin activates hydrolysis of cyclic GMP and closing of cation channels
- On the bright light, amplification steps are inhibited and cascade adapts

Amplification in rhodopsin-transducin cascade

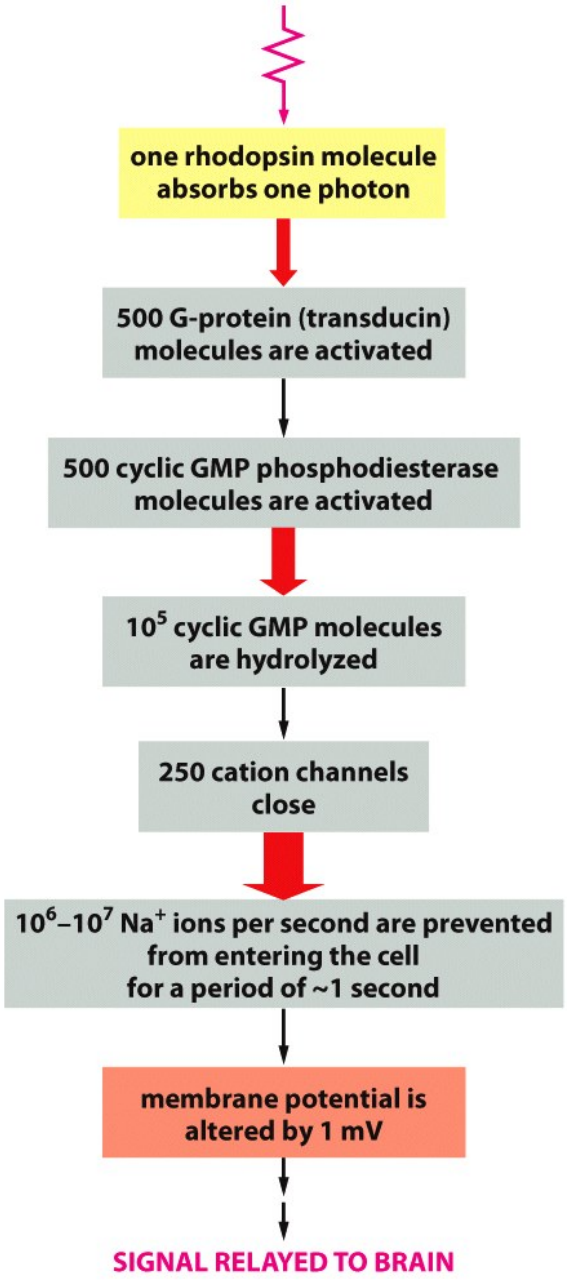


Figure 16-29 Essential Cell Biology 3/e (© Garland Science 2010)

TABLE 16–3 SOME CELL RESPONSES MEDIATED BY CYCLIC AMP

EXTRACELLULAR SIGNAL MOLECULE*	TARGET TISSUE	MAJOR RESPONSE
Adrenaline	heart	increase in heart rate and force of contraction
Adrenaline	skeletal muscle	glycogen breakdown
Adrenaline, ACTH, glucagon	fat	fat breakdown
ACTH	adrenal gland	cortisol secretion

TABLE 16–4 SOME CELL RESPONSES MEDIATED BY PHOSPHOLIPASE C ACTIVATION		
SIGNAL MOLECULE	**TARGET TISSUE**	**MAJOR RESPONSE**
Vasopressin (a peptide hormone)	liver	glycogen breakdown
Acetylcholine	pancreas	secretion of amylase (a digestive enzyme)
Acetylcholine	smooth muscle	contraction
Thrombin (a proteolytic enzyme)	blood platelets	aggregation

Final question (2 points)

How does dynamite relate to Viagra?

Summary

- Membrane receptors, endosomes and lysosomes are parts of endocytic pathway
- Small hydrophobic signals can go directly through the membrane
- Most signals need transduction with membrane receptors of three main classes: ion-channel-coupled, G-protein-coupled and enzyme-coupled
- G-protein-coupled receptors (GPCRs) may activate membrane ion channels or membrane enzyme proteins (e.g., adenynyl cyclase)
- Inositol phospholipid pathway involves PKC and Ca^{2+} instead of PKA; separate Ca^{2+} pathway involves CaM-kinases

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapters 15–16*.

Outline

Questions and answers

Previous final question: the answer

How does dynamite relate to Viagra?

- Dynamite contains nitroglycerin, nitroglycerin releases nitric oxide, nitric oxide facilitates cyclic GMP synthesis
- Viagra also facilitates cyclic GMP synthesis

Cell communication

.1 Enzyme-coupled receptors

Enzyme-coupled receptors: RTKs

- Many of enzyme-coupled receptors have growth-related (growth, proliferation, differentiation) or movement-related functions
- Their signals are paracrine molecules which work at low concentrations
- Most of enzyme-coupled receptors have tyrosine kinase cytoplasmic domain (PKA, PKC and CaM are serine/threonine kinases): receptor tyrosine kinases (RTKs)

Activation and deactivation of RTKs

- Signal causes RTKs to form dimers which start to phosphorylate themselves
- Phosphorylated dimers are binding sites for many proteins, including direct signals and adaptors
- Protein tyrosine phosphatases or endosomes will stop dimers to produce a signal

Activation of RTK

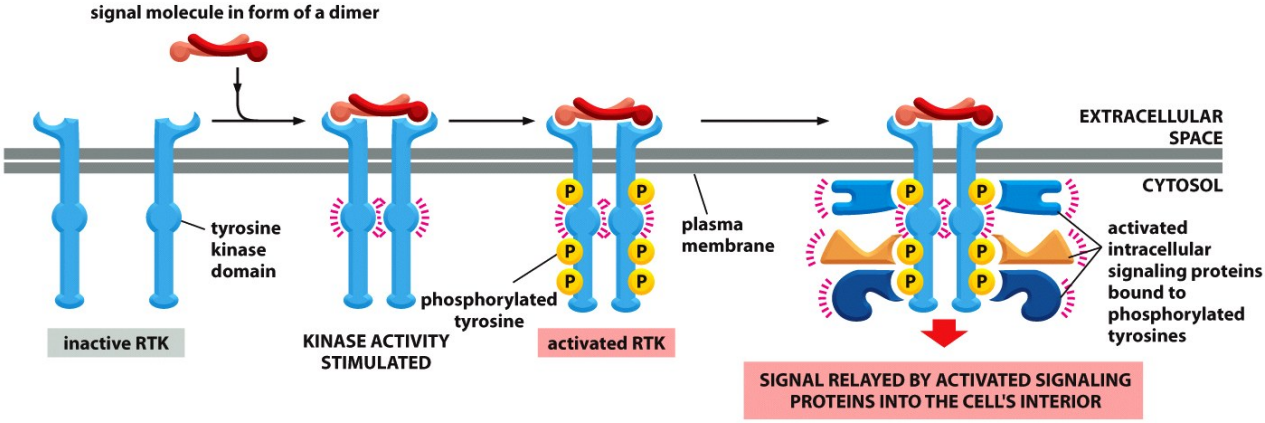


Figure 16-30 Essential Cell Biology 3/e (© Garland Science 2010)

Monomeric GTPases: Ras protein

- Ras is a monomeric GTPase, similar to α subunit of G protein
- Ras is activated through adaptor and Ras-activating proteins which bind GTP to it
- Ras is then hydrolyze GTP into GDP and became inactive again

Activation of Ras

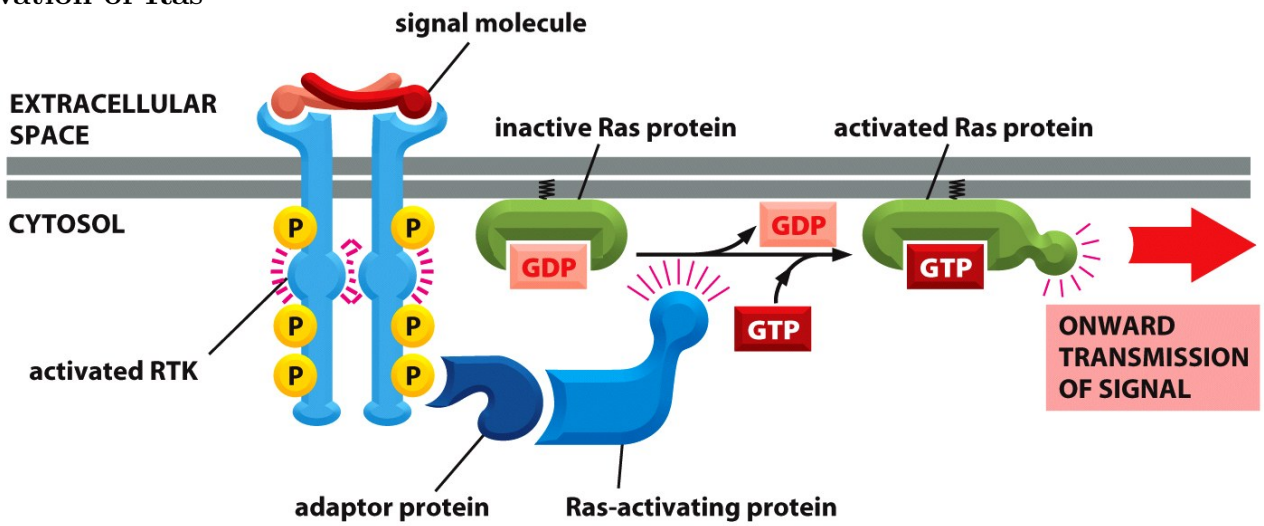


Figure 16-31 Essential Cell Biology 3/e (© Garland Science 2010)

Ras movie

MAP-kinase signaling module

- Active Ras may activate mitogen-activated protein kinase module (MAP-kinase module)
- MAP kinase will change gene expression and protein activity which may result in cell proliferation
- MAP kinase is activated by MAP kinase kinase which is activated by MAP kinase kinase kinase which is activated by active Ras
- Some cancers are related with inability of activated Ras to hydrolyze its own GTP

MAP kinase pathway

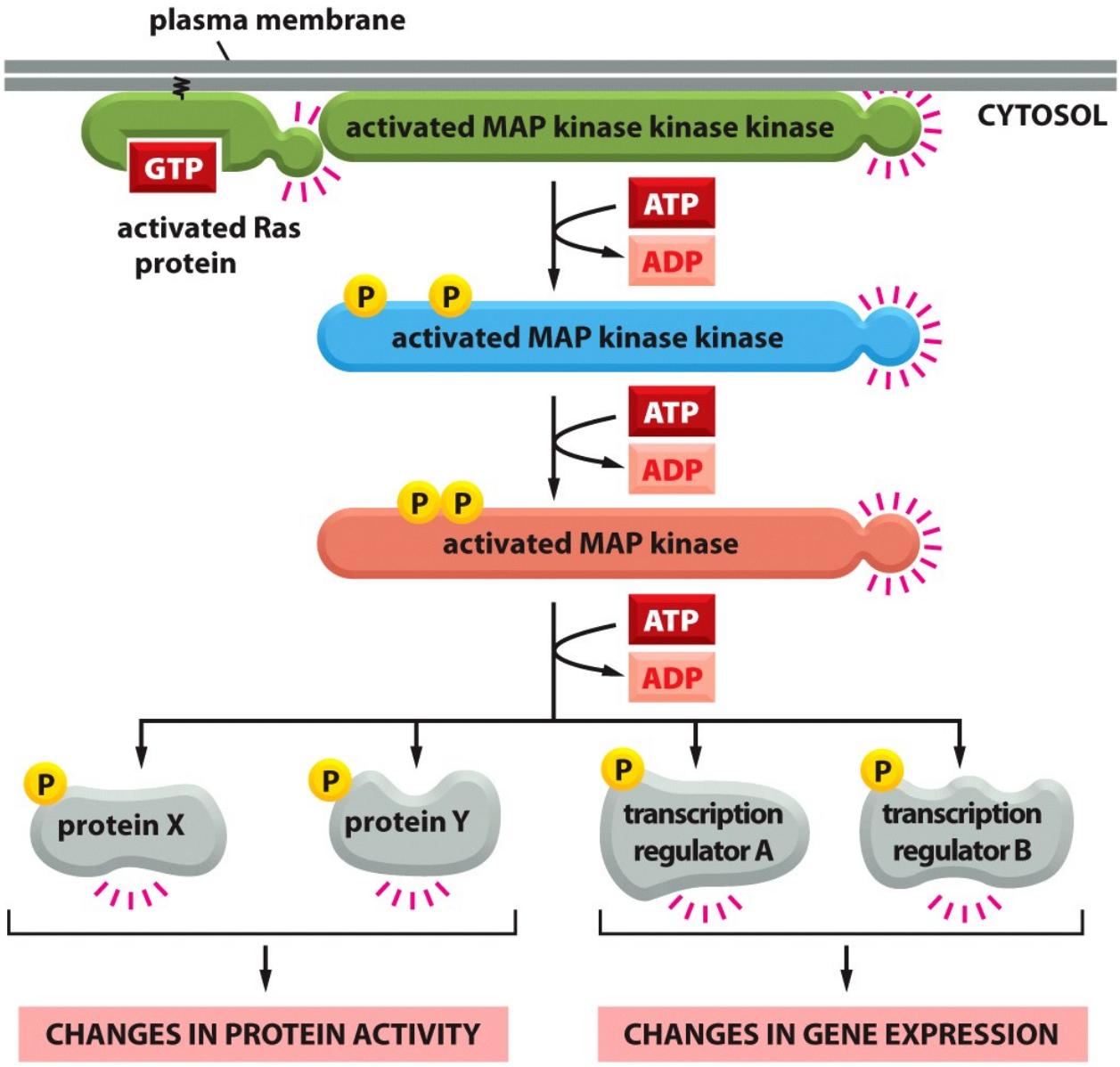


Figure 16-32 Essential Cell Biology 3/e (© Garland Science 2010)

How to study binding places

- Point mutation in target amino acids are used
- Typically, amino acid of interest is replaced with non-polar Ala (alanine)
- Co-immunoprecipitation will help to obtain protein complexes

Mutants and binding sites

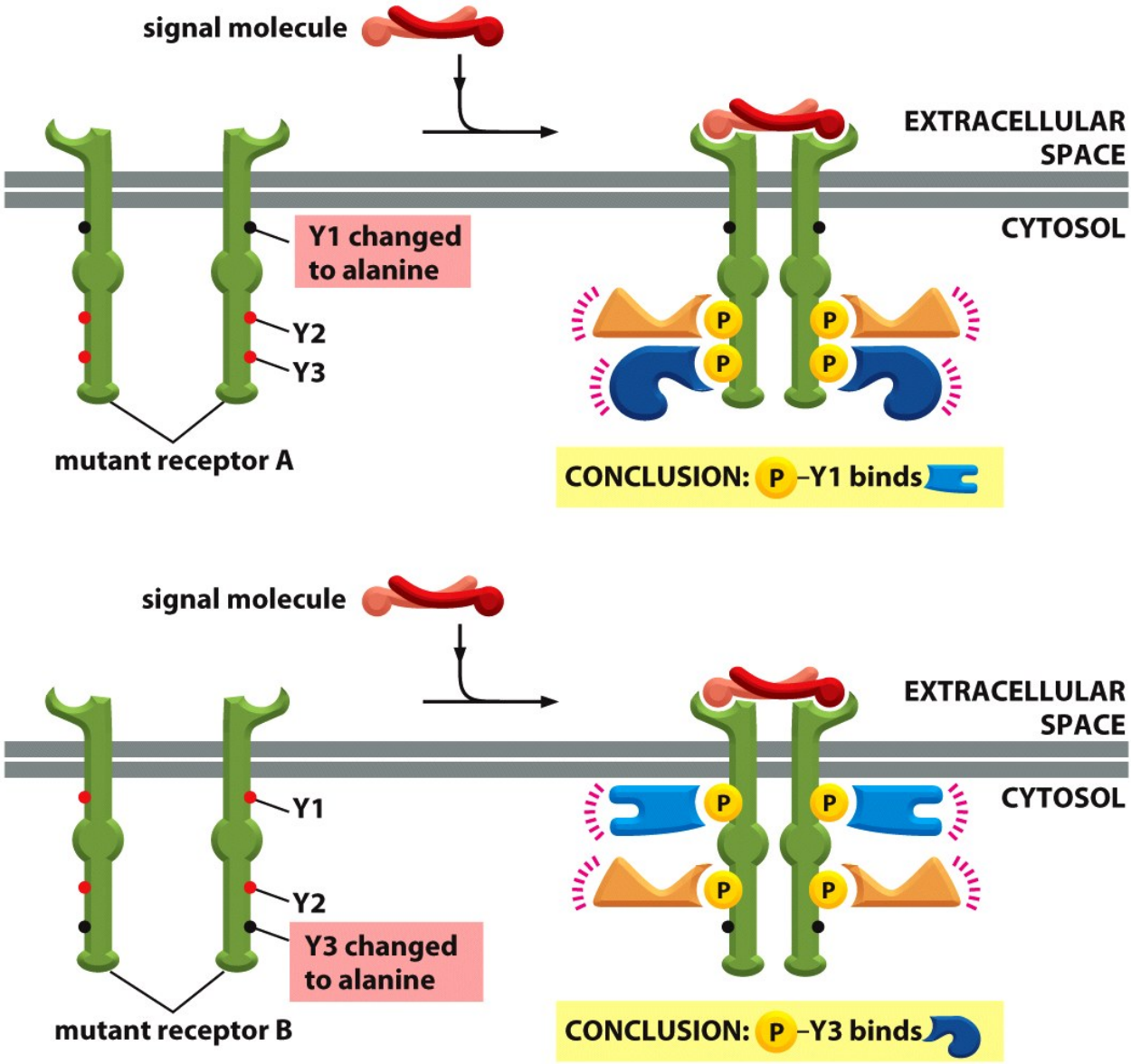


Figure 16-36 Essential Cell Biology 3/e (© Garland Science 2010)

Mutant Ras proteins

- In many cancers, constantly active form of Ras is involved
- There is also dominant-negative form of Ras which blocks pathway
- siRNAs will also help to block the synthesis of particular protein

Constatntly active Ras

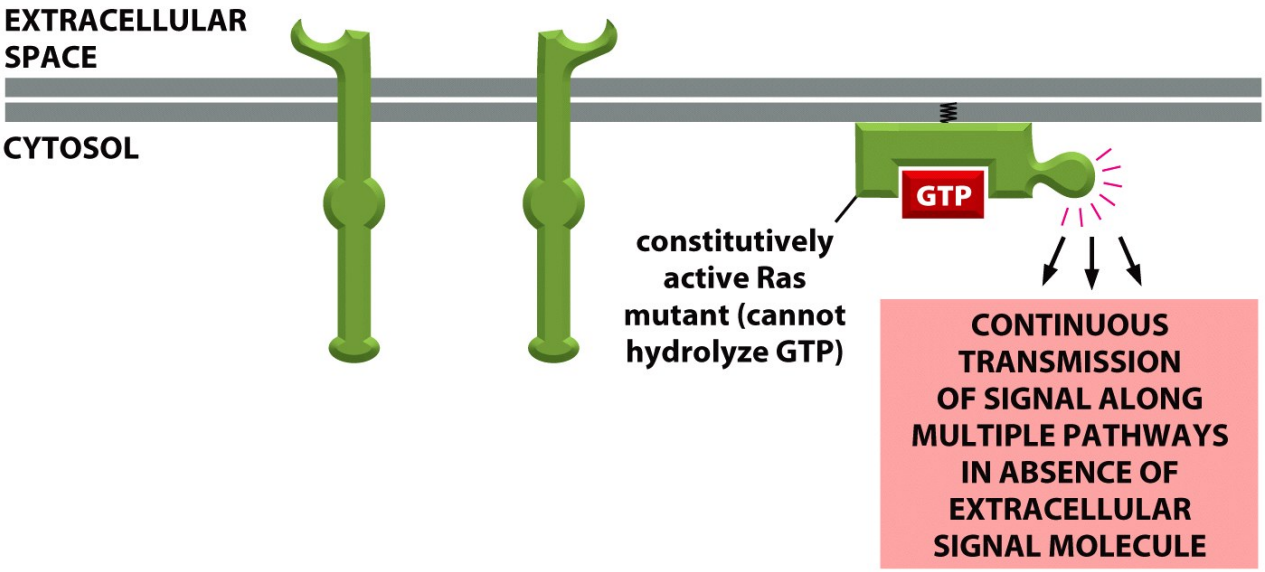


Figure 16-37 Essential Cell Biology 3/e (© Garland Science 2010)

How to determine the pathway sequence

- Mutant forms of each pathway protein are needed
- With combination of constantly active Ras, effects will be different if protein positioned upstream or downstream of Ras

Mutation in upstream protein

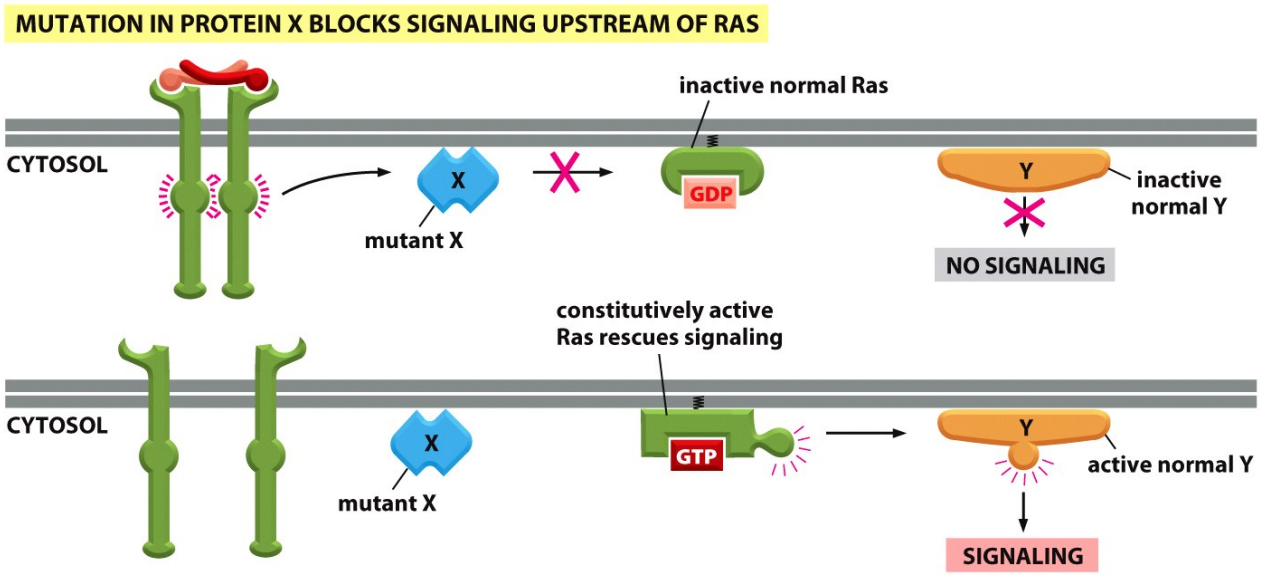


Figure 16-38b Essential Cell Biology 3/e (© Garland Science 2010)

Mutation in downstream protein

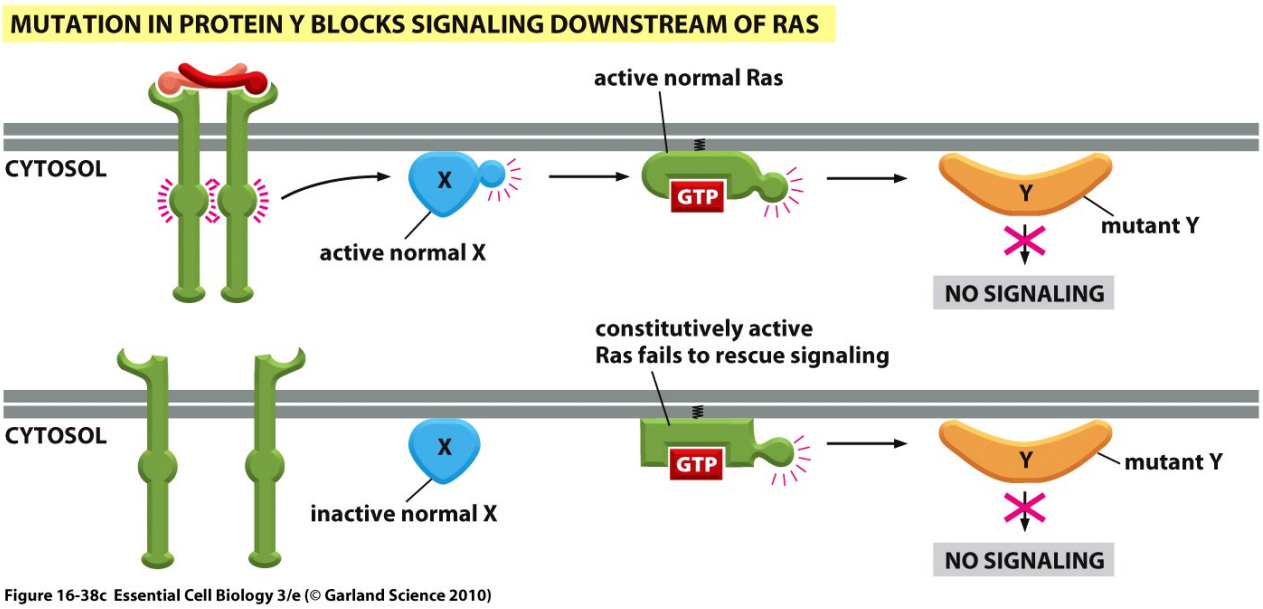


Figure 16-38c Essential Cell Biology 3/e (© Garland Science 2010)

Phosphoinositide 3-kinase (PI 3-kinase)

- RTK activated from IGF (insulin-like growth factor) activate phosphoinositide 3-kinase (PI 3-kinase)
- PI 3-kinase phosphorylates inositol phospholipid which in turn (through other kinases) activated protein kinase B (Akt, or PKB)
- In short, PI 3-kinase creates membrane docking site where different proteins (including Akt) will be phosphorylated

PI 3-kinase pathway

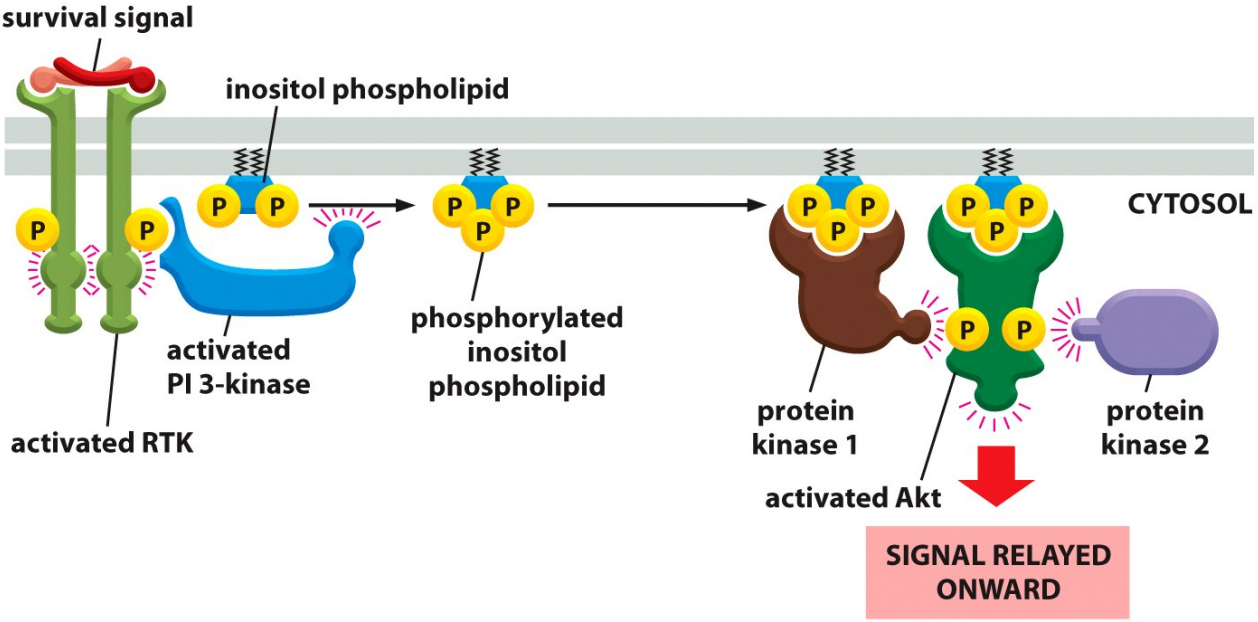


Figure 16-33 Essential Cell Biology 3/e (© Garland Science 2010)

Activated Akt and cell growth and survival

- Activated Akt inactivates apoptosis though dissolving of Bad-Bcl2 complex
- Activated Akt also indirectly activates Tor protein kinase which increase level of protein synthesis and inhibit protein degradation
- Suppressing Akt may stop different cancers

Activated Akt, Bad, Bcl2 and apoptosis

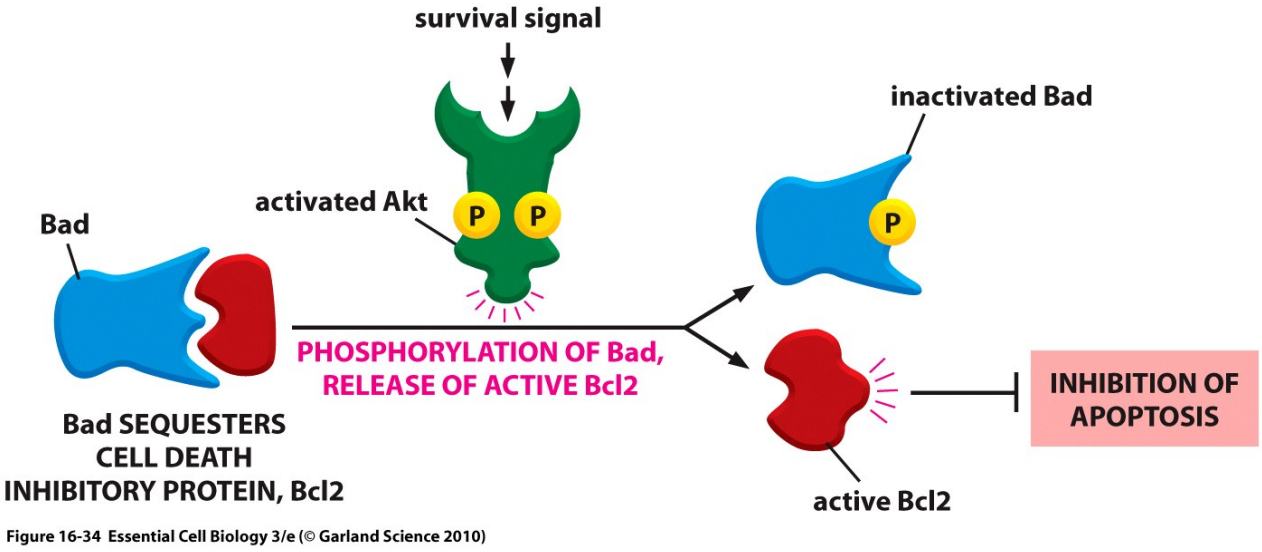


Figure 16-34 Essential Cell Biology 3/e (© Garland Science 2010)

Cytokines

- Cytokines are local mediators which activate transcription regulators (e.g., interferons activate synthesis of viral-resistant proteins)
- These transcription regulators (STATs) may come straight to nucleus

JAK-STAT signaling pathway

- Unlike RTKs, cytokine receptors have no enzymatic activity: instead, they associate with tyrosine kinases JAKs
- JAKs may reciprocally phosphorylate themselves and then phosphorylate STATs
- STATs dissociated from complex, dimerize and migrate to nucleus

JAK-STAT pathway

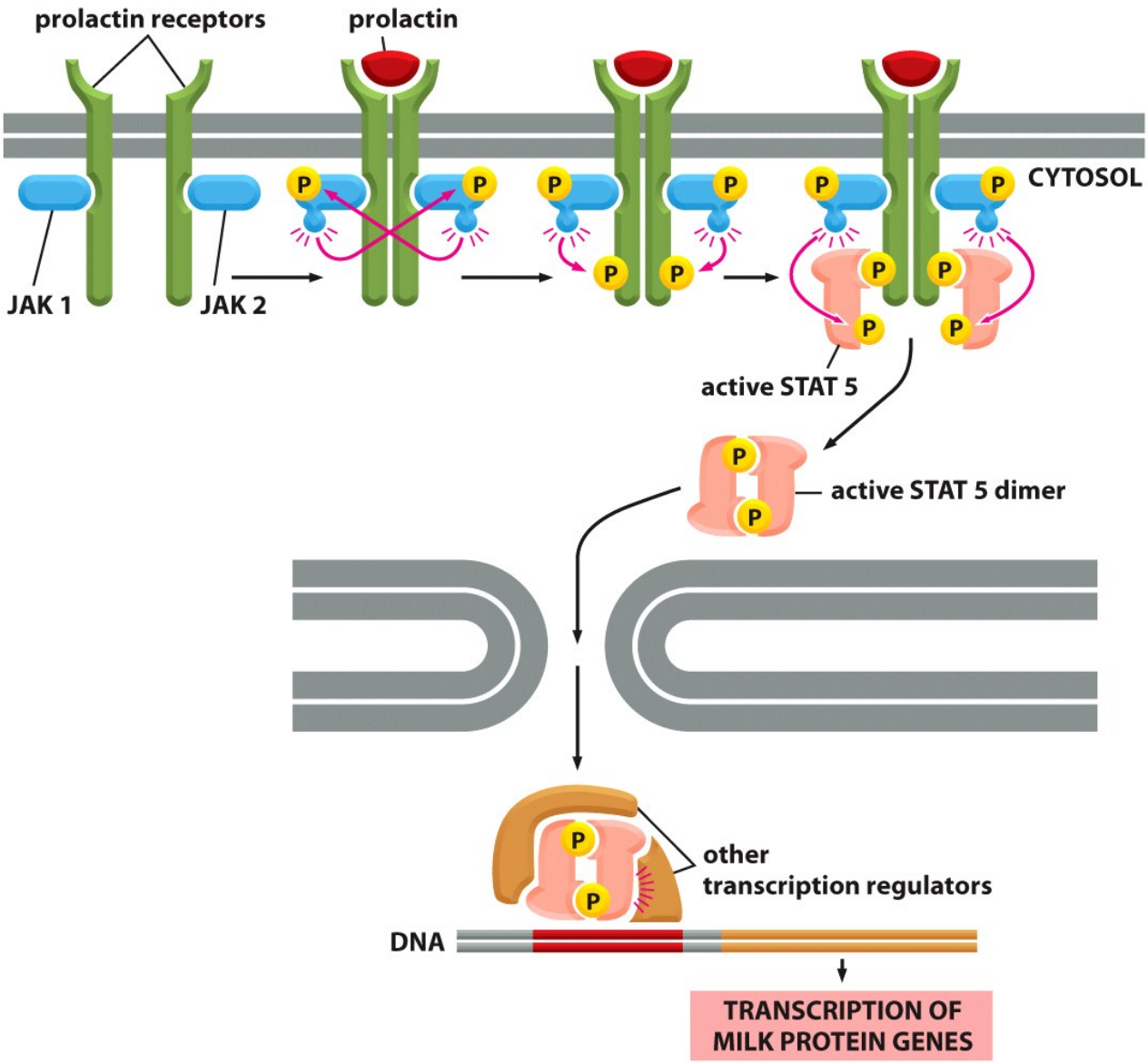


Figure 16-39 Essential Cell Biology 3/e (© Garland Science 2010)

Notch pathway

- In fly neural cells, membrane Delta signal proteins activate Notch receptors
- By activation, Notch cleaved and its tail migrates to nucleus where activates responsible genes

Notch pathway

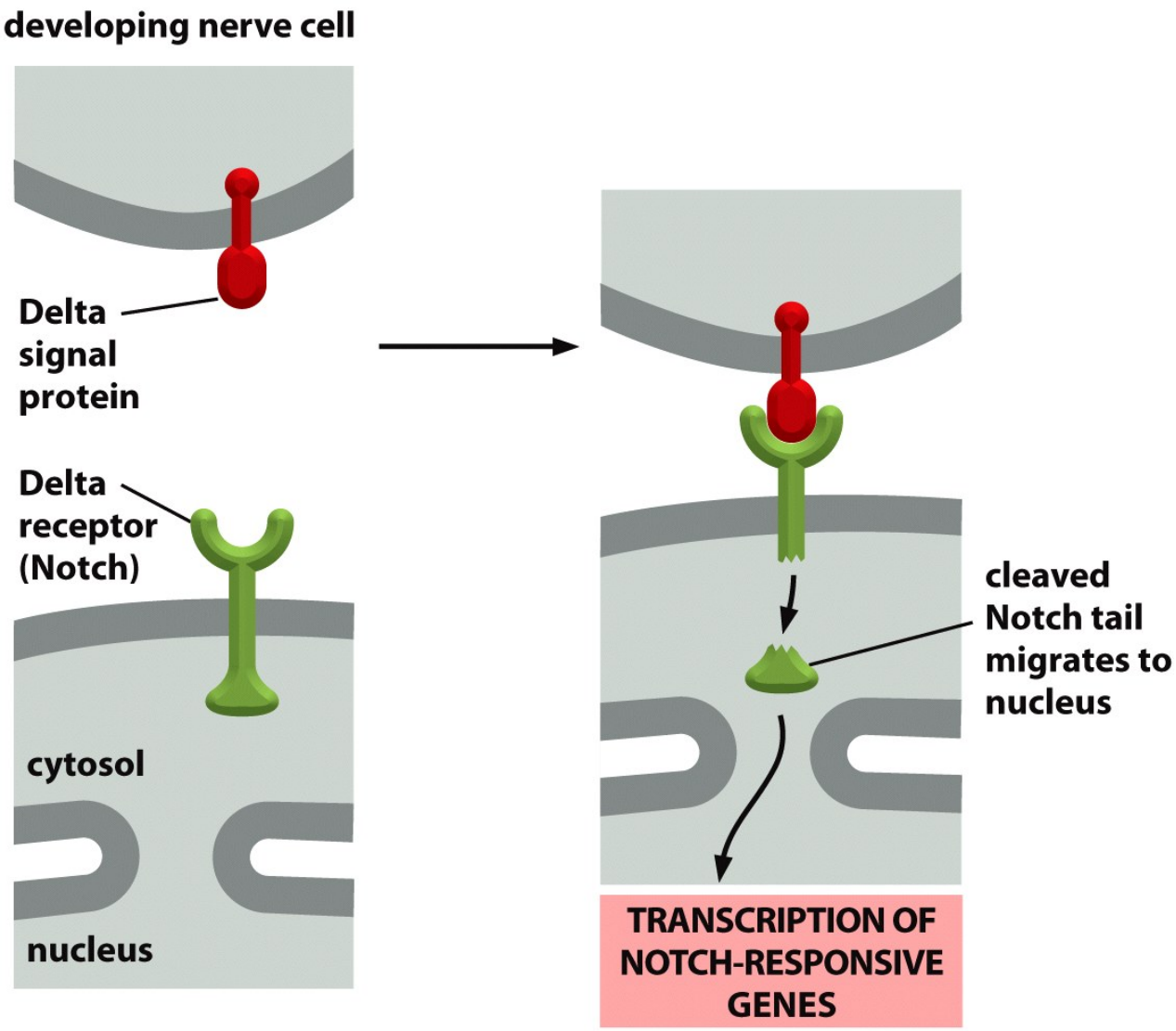


Figure 16-40 Essential Cell Biology 3/e (© Garland Science 2010)

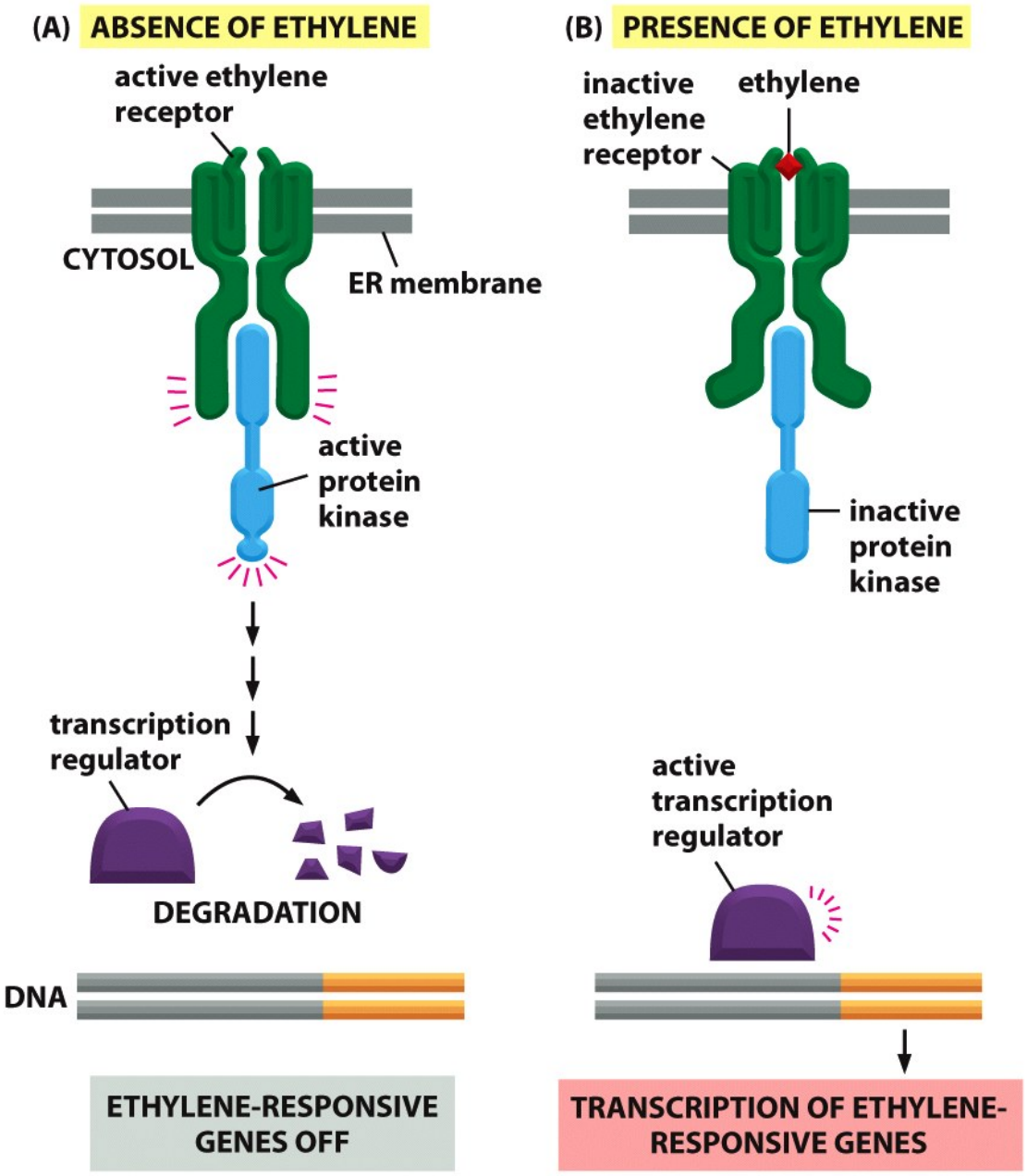
Multicellularity in plants and animals

- Plant cells are not using RTKs, steroid hormones, cyclic AMPs
- GPCRs are known for plants, but there are few of them
- In contrast, plants have specific receptors for specific plant molecules—plant hormones

Ethylene pathway in plants

- Ethylene controls ripening of fruits
- Ethylene receptor is somewhat similar to enzyme-coupled receptors of animals but it is dimeric and active with an absence of signal (!)
- Ethylene presence will make receptor kinase inactive and transcription regulators will not degrade but start to activate transcription

Ethylene signaling pathway



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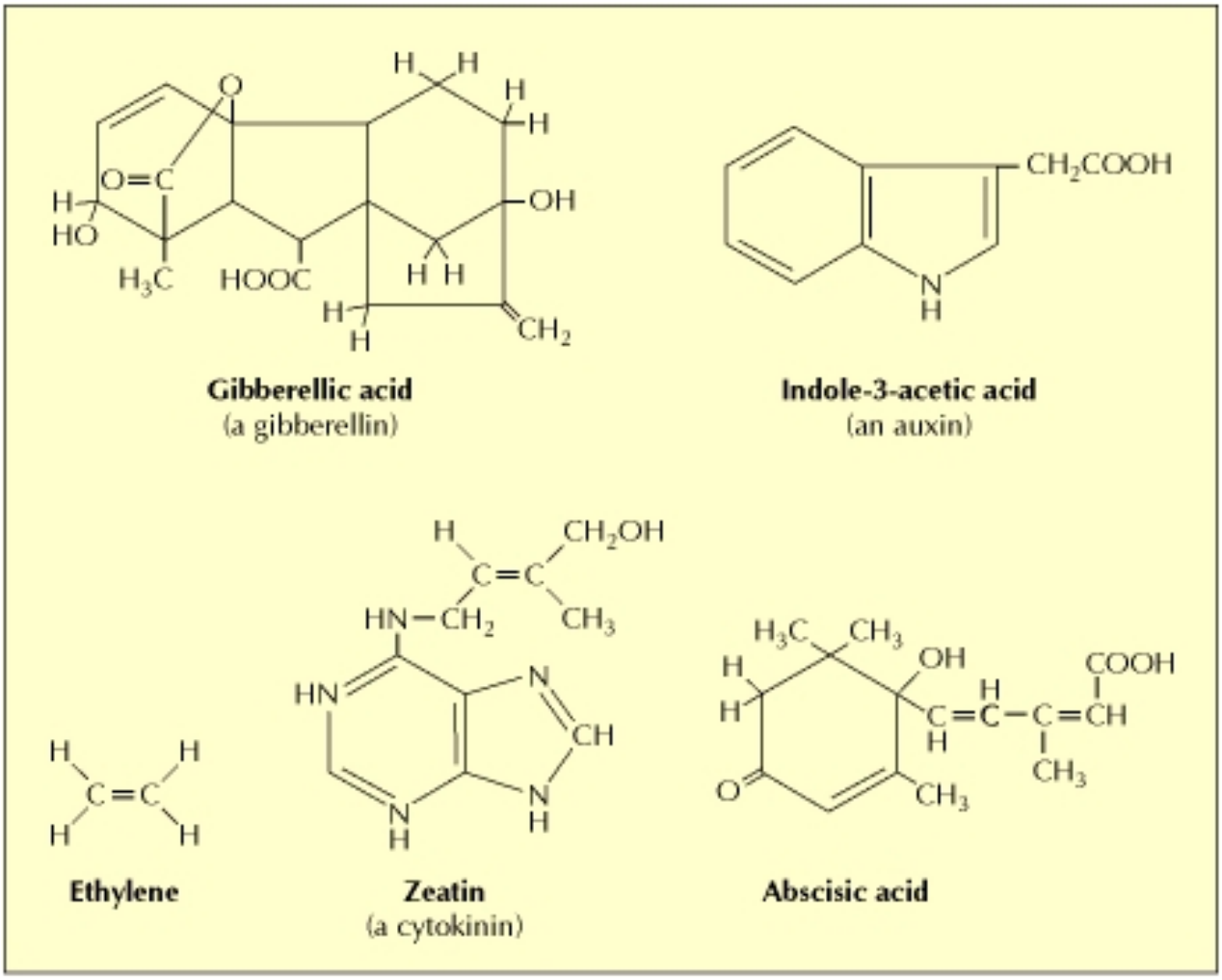
Plant hormones



Interconnections of pathways

- Some pathway components like Ca^{2+} or phospholipase C could participate in both GPCR and RTK pathways
- Pathway may also integrate signal via double phosphorylation or activating complementary proteins

Interconnections of pathways

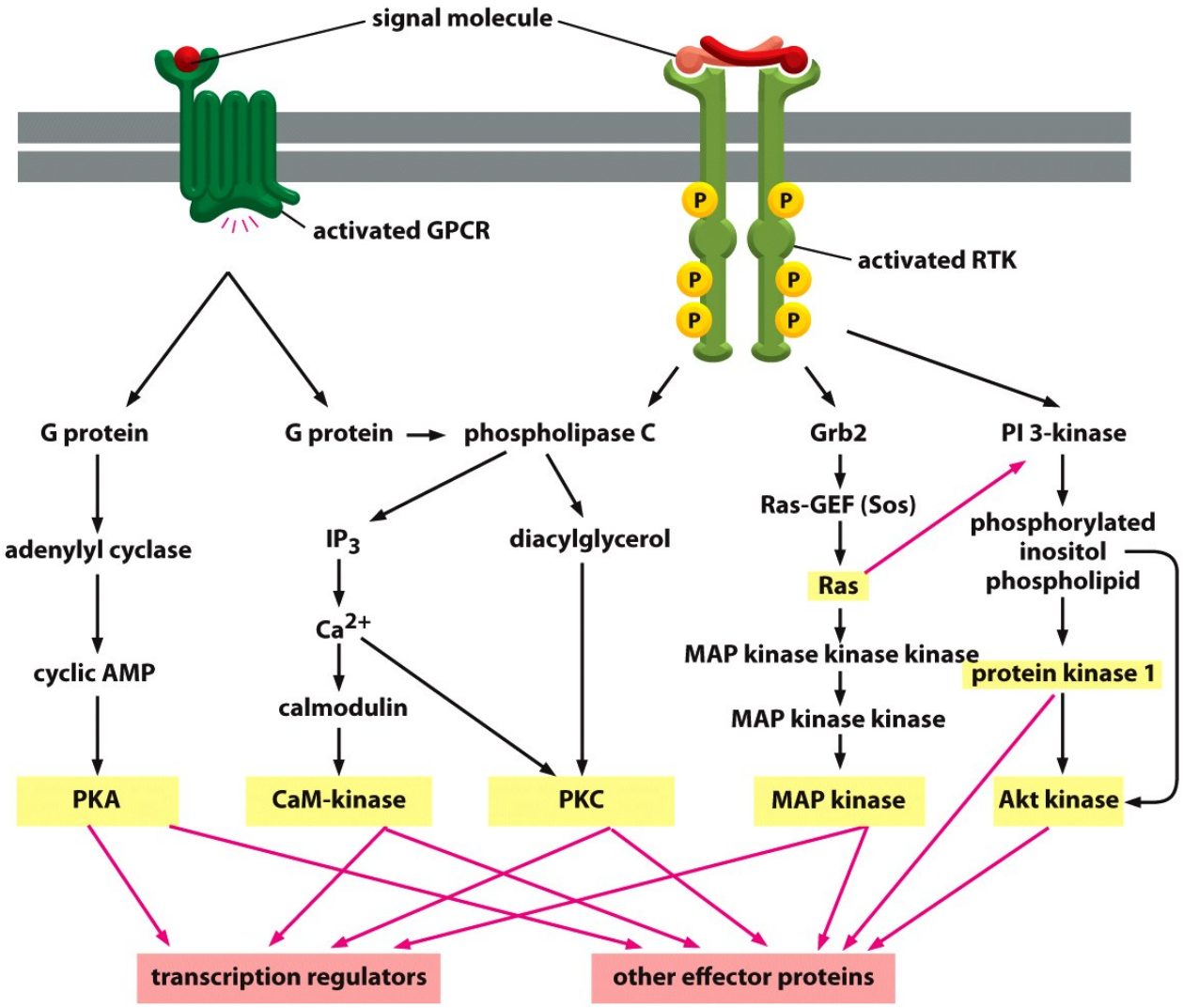


Figure 16-42 Essential Cell Biology 3/e (© Garland Science 2010)

Integration of signal

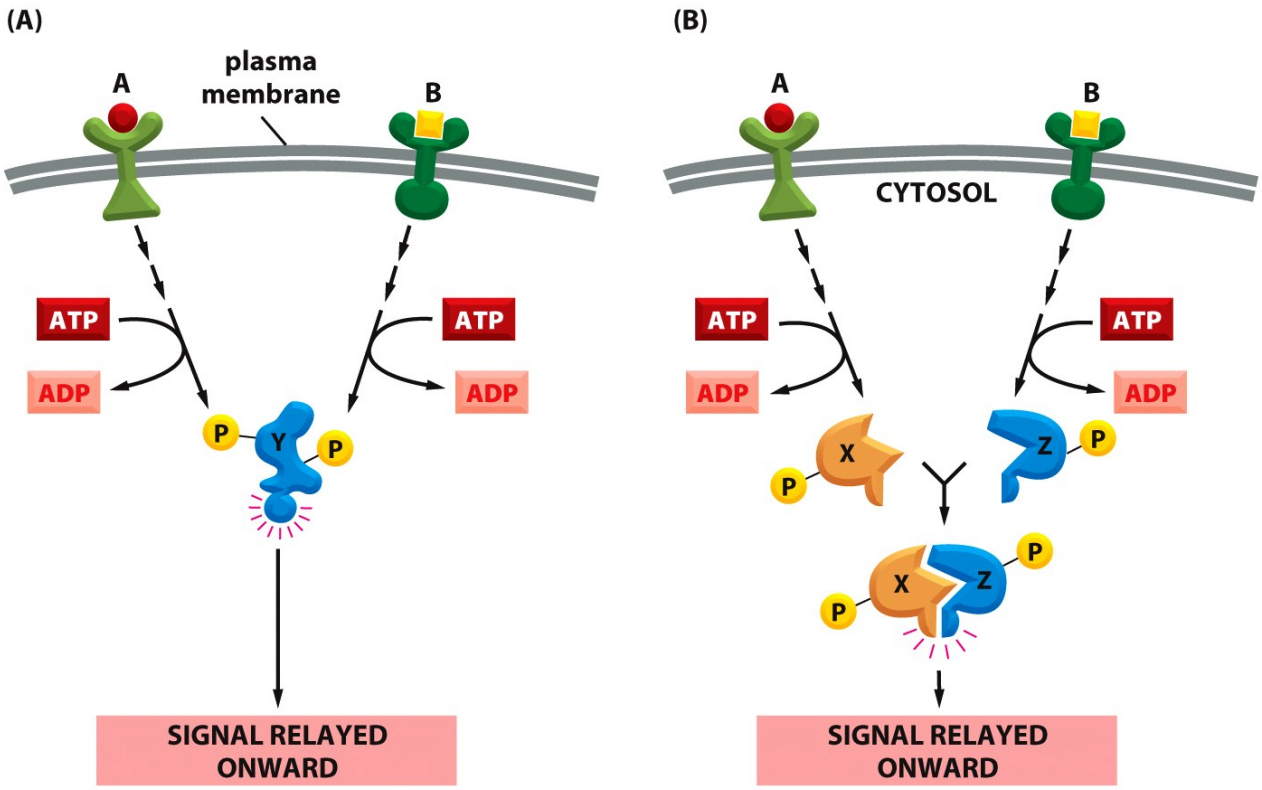


Figure 16-43 Essential Cell Biology 3/e (© Garland Science 2010)

Chemotaxis movie

Neutrophil movie

Lymphocyte movie

Final question (2 points)

How do researchers use constantly active Ras protein?

Summary

- Many enzyme-coupled receptors are tyrosine kinases (RTKs) which phosphorylate themselves
- Constant activation of MAP-kinase signaling module by Ras leads to many human cancers
- PI 3-kinases will activate cell growth (similarly to MAP kinases) via creation of membrane docking sites
- Notch and cytokine receptors activate a direct pathway into nucleus
- Plant signaling systems are different from animal

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 16*.

Outline

Questions and answers

Previous final question: the answer

How do researchers use constantly active Ras protein?

- For determining sequence of proteins in a signal pathway

Cytoskeleton

Structure of cytoskeleton

Cytoskeleton

- Filament-like: intermediary filaments and actin filaments
- Microtubules
- All are polymers of proteins

Intermediate filaments

- Keratin in epithelial cells
- Vimentin in connective-tissue cells
- Neurofilaments
- Nuclear lamins
- Strengthening cell

Strengthening of cell layer

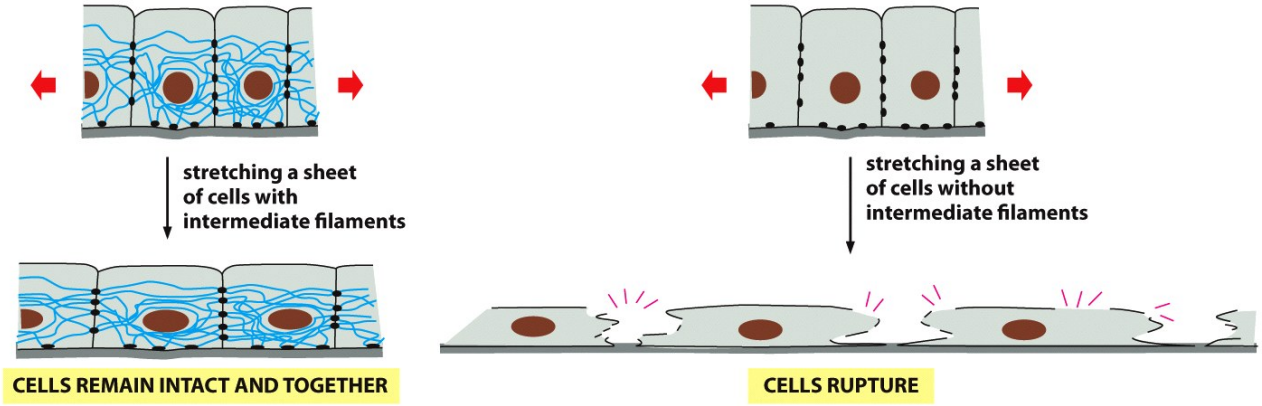


Figure 17-4 Essential Cell Biology 3/e (© Garland Science 2010)

Microtubules

- Grow from centrosome
- Form flagella or cilia
- Form mitotic spindle
- Organize interior of cell
- Drive intracellular transport

Microtubules

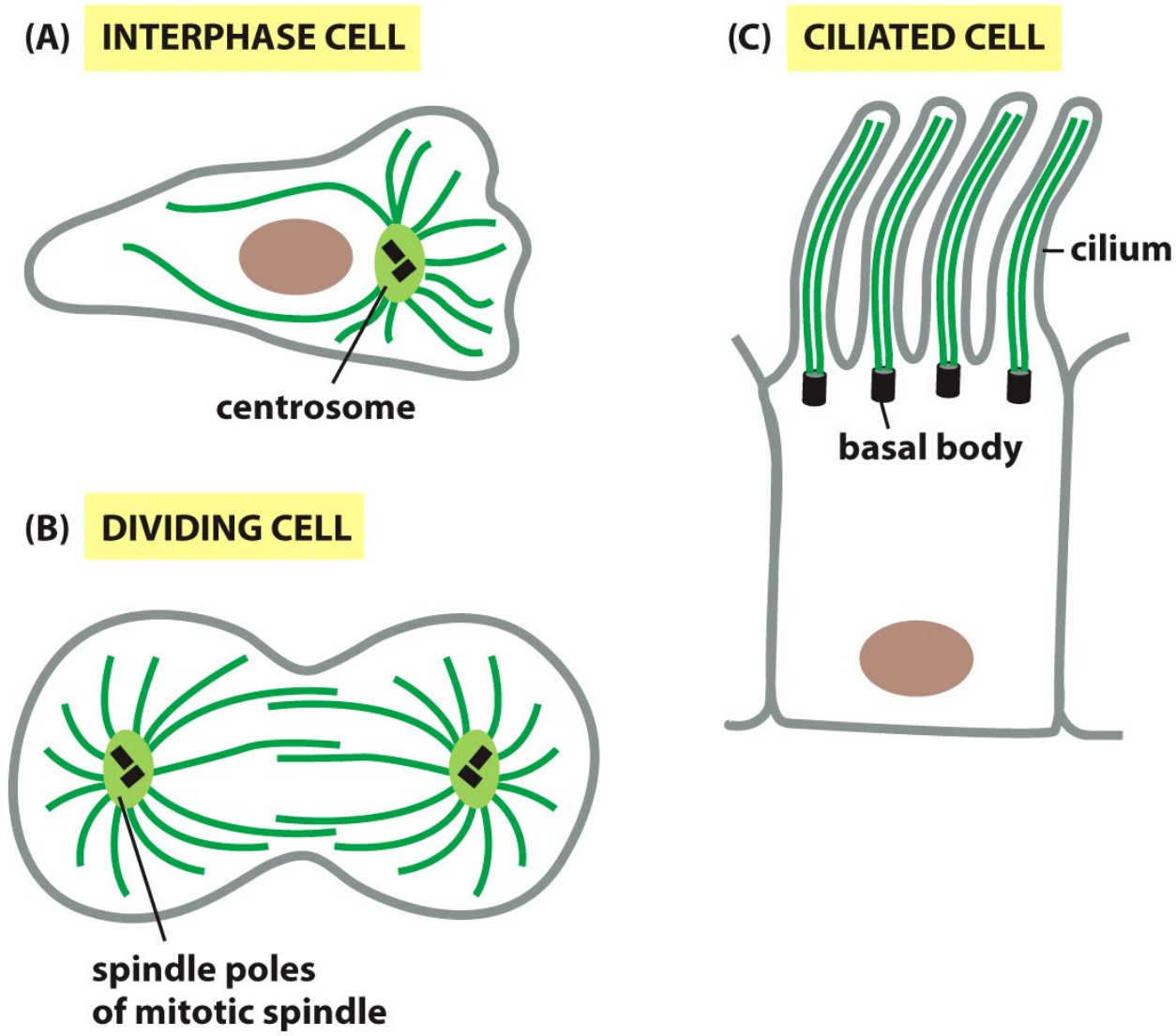


Figure 17-8 Essential Cell Biology 3/e (© Garland Science 2010)

Growth and shrinking of microtubules

- Microtubule is made of 13 tubulin microfilaments, each contain pairs of β-tubulin (– end) and α-tubulin (+ end)
- Tubulin dimers bind GTP and form a growing GTP cap of microtubule; if GTP cap is lost, microtubules start to shrink
- Capturing the plus end will stabilize microtubule

Growing and shrinking of microtubules

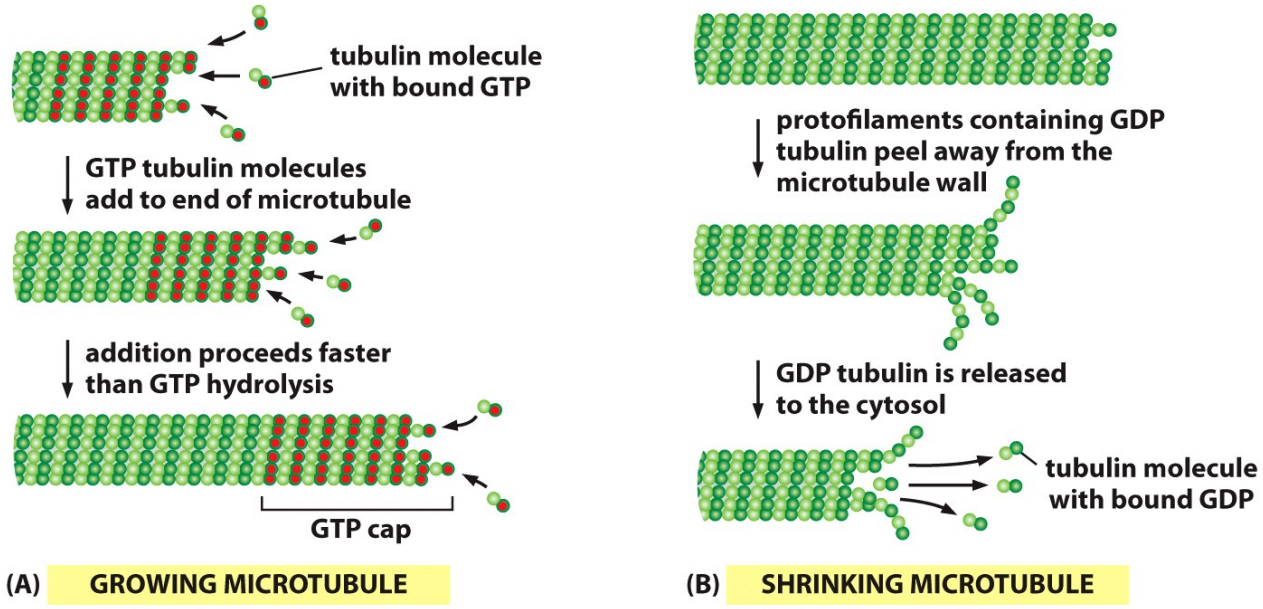


Figure 17-12 Essential Cell Biology 3/e (© Garland Science 2010)

Microtubule-specific drugs

	Action
Microtubule-specific drugs	
Taxol	binds and stabilizes microtubules
Colchicine, colcemid	binds subunits and prevents polymerization
Vinblastine, vincristine	binds subunits and prevents polymerization

Centrosomes

- γ-tubulin rings: starting places of microtubule growth
- Two perpendicular centrioles (they are similar to basal bodies of flagella)
- Cetrosome has a fisherman-like behavior: microtubules are constantly growing out of it, then degrading, but some are stabilizing

Motor proteins

- Kinesins and dyneins are dimers that hydrolyze ATP and move
- They can move molecules and even whole organelles

Kinesin and dynein

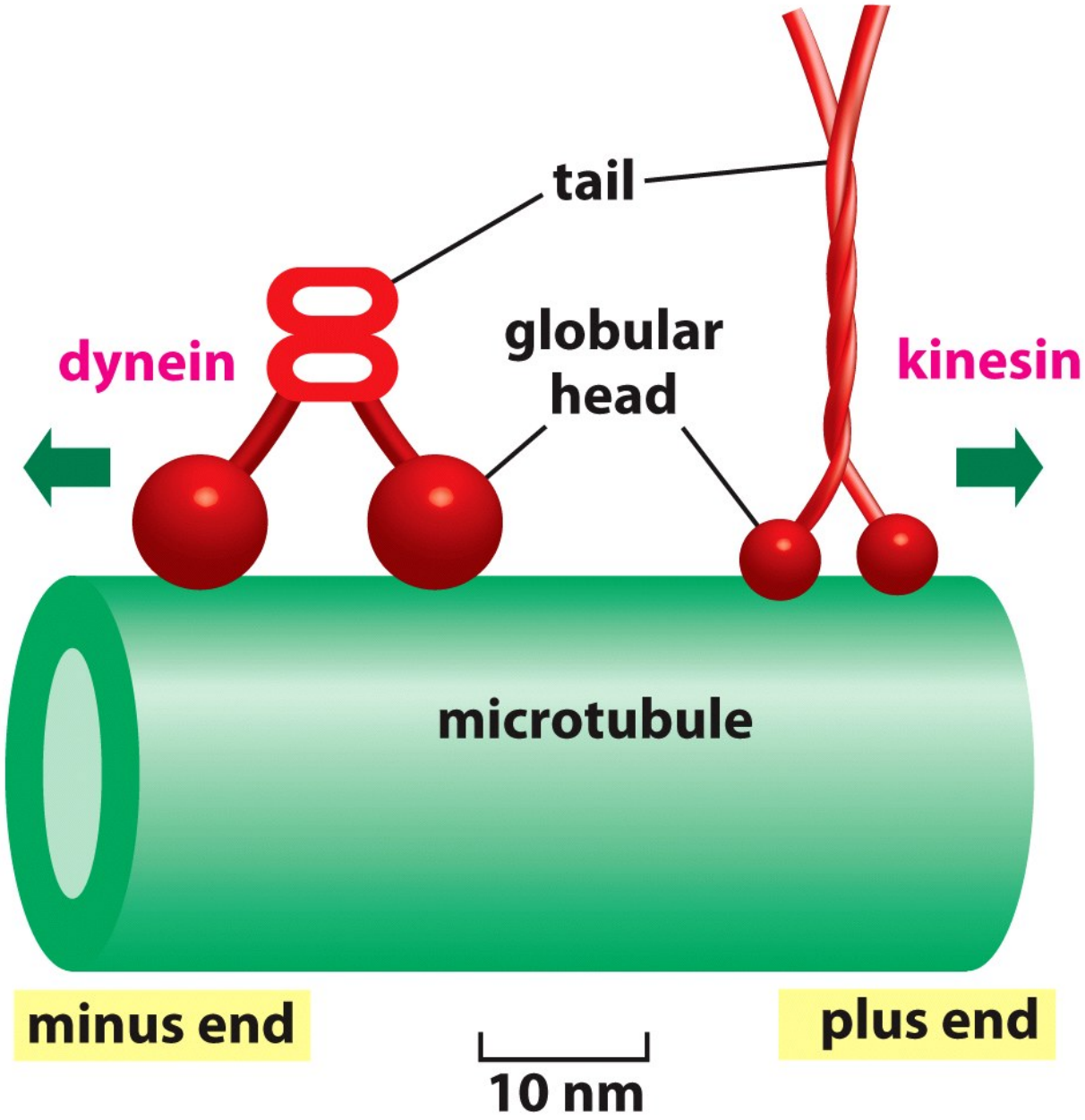


Figure 17-16a Essential Cell Biology 3/e (© Garland Science 2010)

Kinesin movie

Organelle movement movie

Flagella/cilia

- Hairlike structures growing from cytoplasmic basal bodies
- Contain 9×2 + 2 microtubules connected by dynein arms
- They are natural oars

$9 \times 2 + 2$ structure of flagella

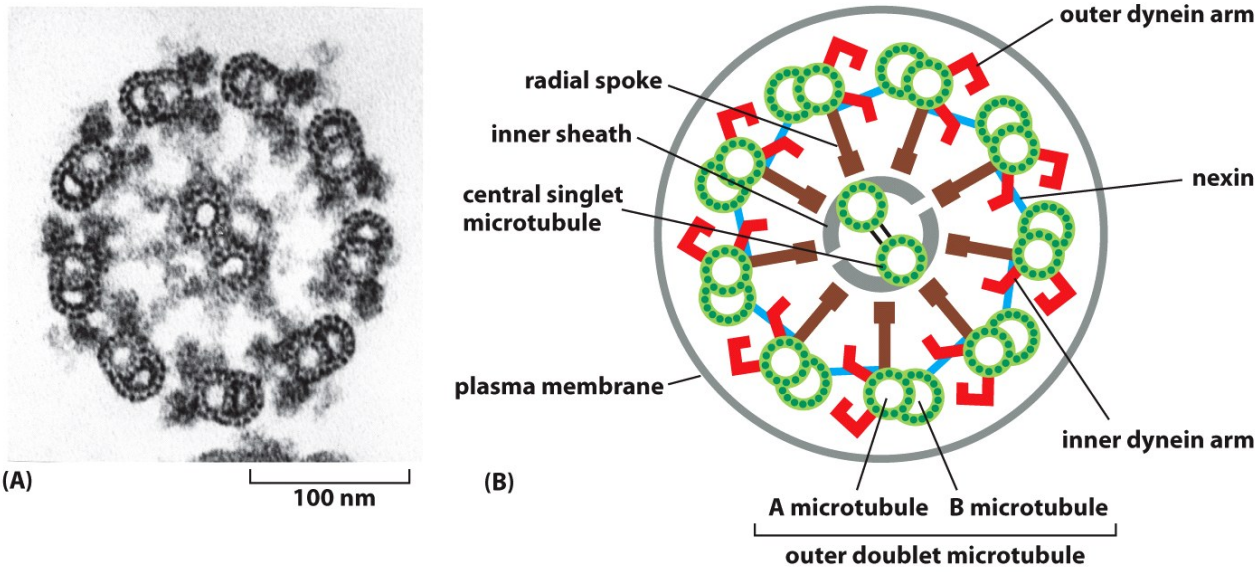


Figure 17-26 Essential Cell Biology 3/e (© Garland Science 2010)

Flagella bending

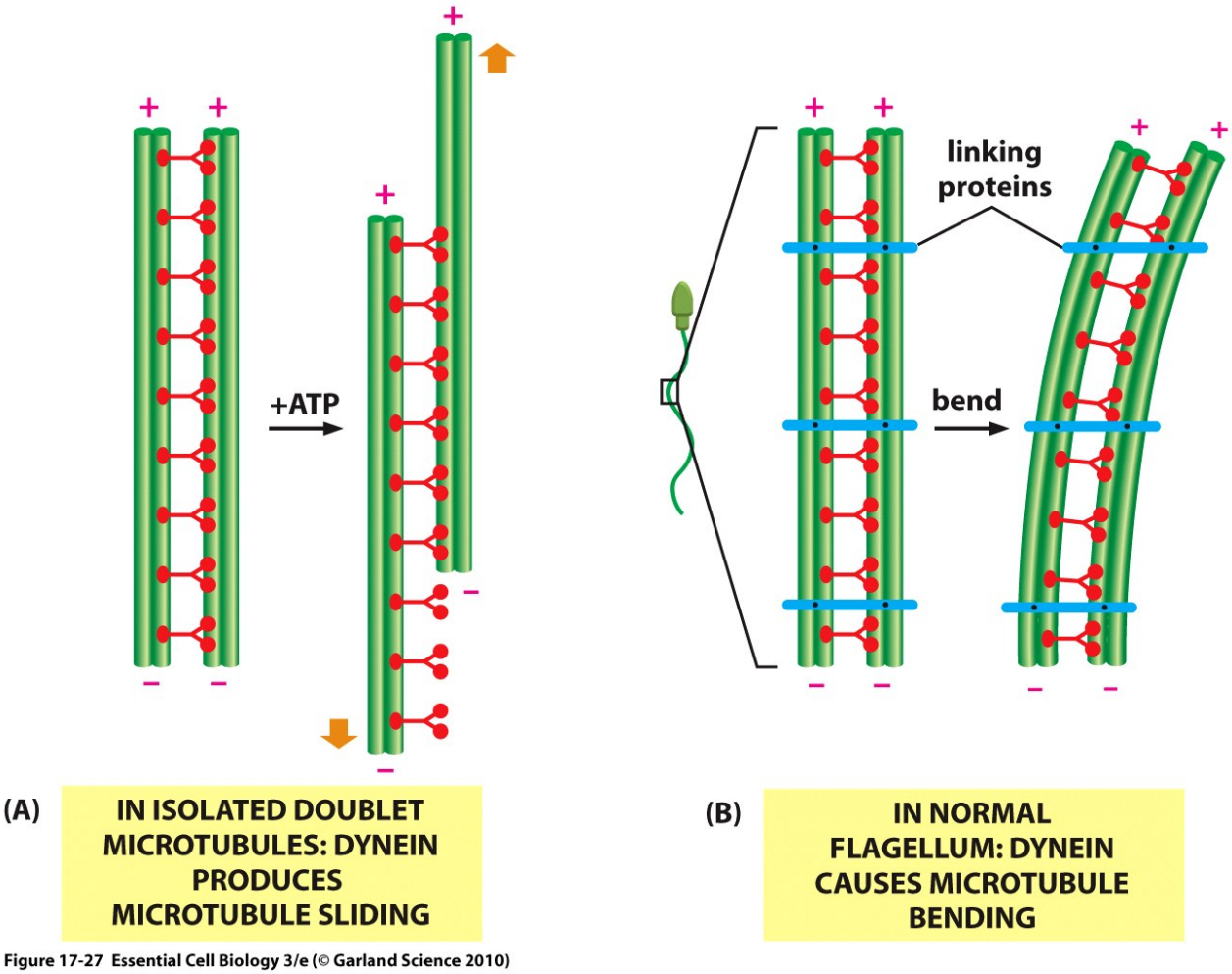


Figure 17-27 Essential Cell Biology 3/e (© Garland Science 2010)

Actin filaments

- Fast-growing and unstable, they need to contact with multiple protein types (e.g., capping proteins stabilize actin ends)
- Actin filaments are polar, but thinner and shorter than microtubules, ATP-binding
- Allow cell to change its form

Growing and shrinking of actin filaments

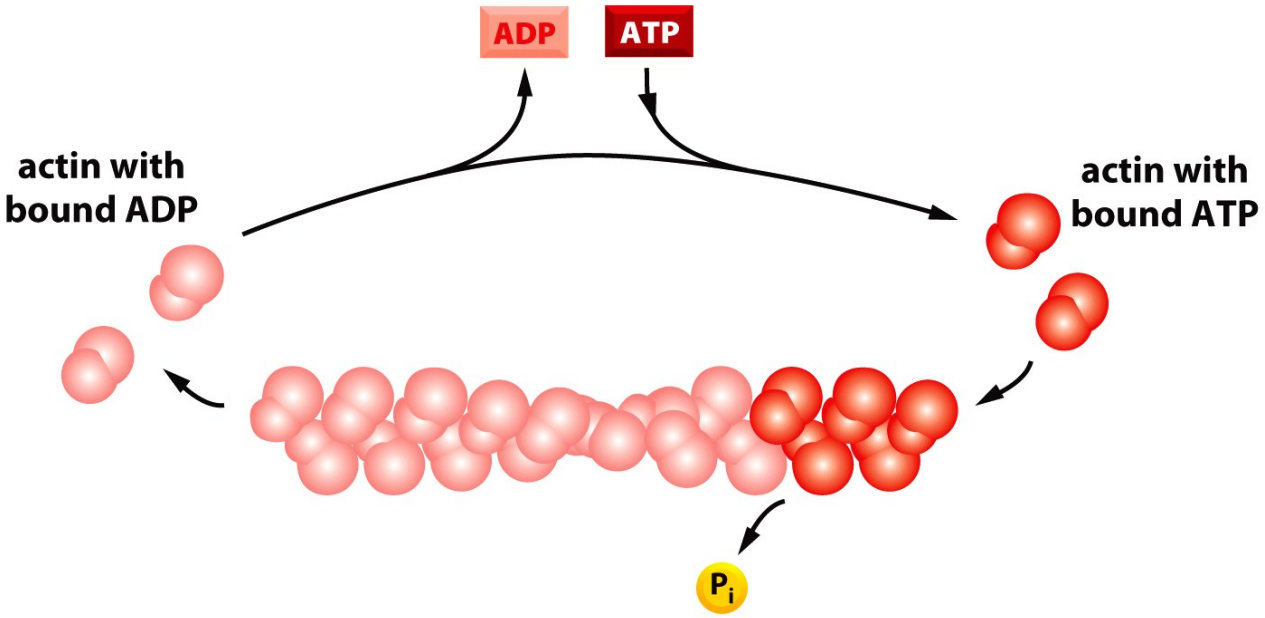
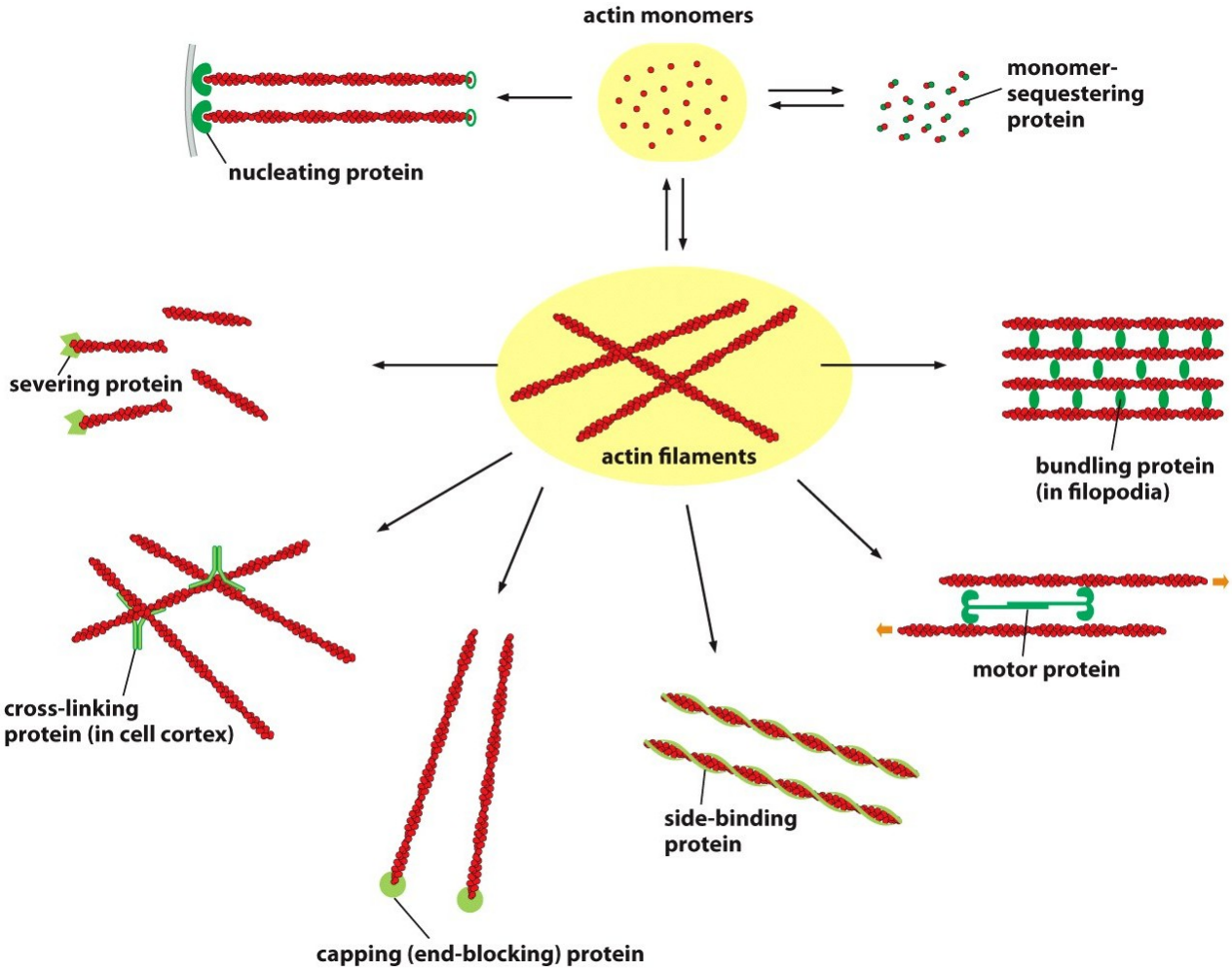


Figure 17-30 Essential Cell Biology 3/e (© Garland Science 2010)

Actin binding proteins



Cell crawling

- Web of growing actin filament will push the leading edge of pseudopodium forward
- ARPs are starting poins of new filaments, formins promote filament growing

Formation of pseudopodium

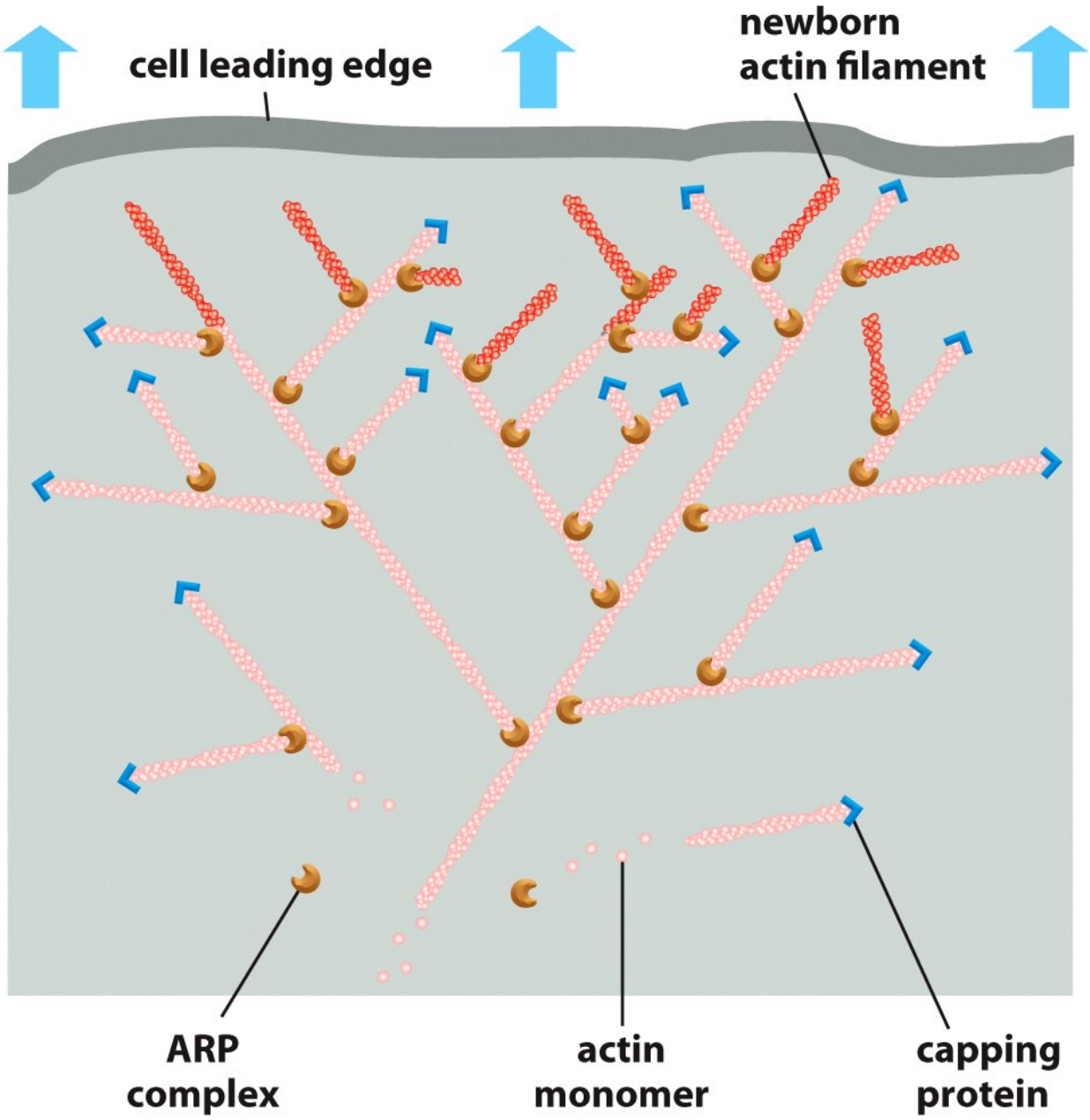


Figure 17-34b Essential Cell Biology 3/e (© Garland Science 2010)

Crawling actin movie

.1 Myosin and muscle contraction

Myosin

- Two subfamilies of ATP-binding motor proteins
- Myosin-I have one head and one tail, myosin-II (in muscle cells) are two-headed
- GTP-binding Rho proteins activate actin polymerization and subsequently the movement of cell

Myosin-I

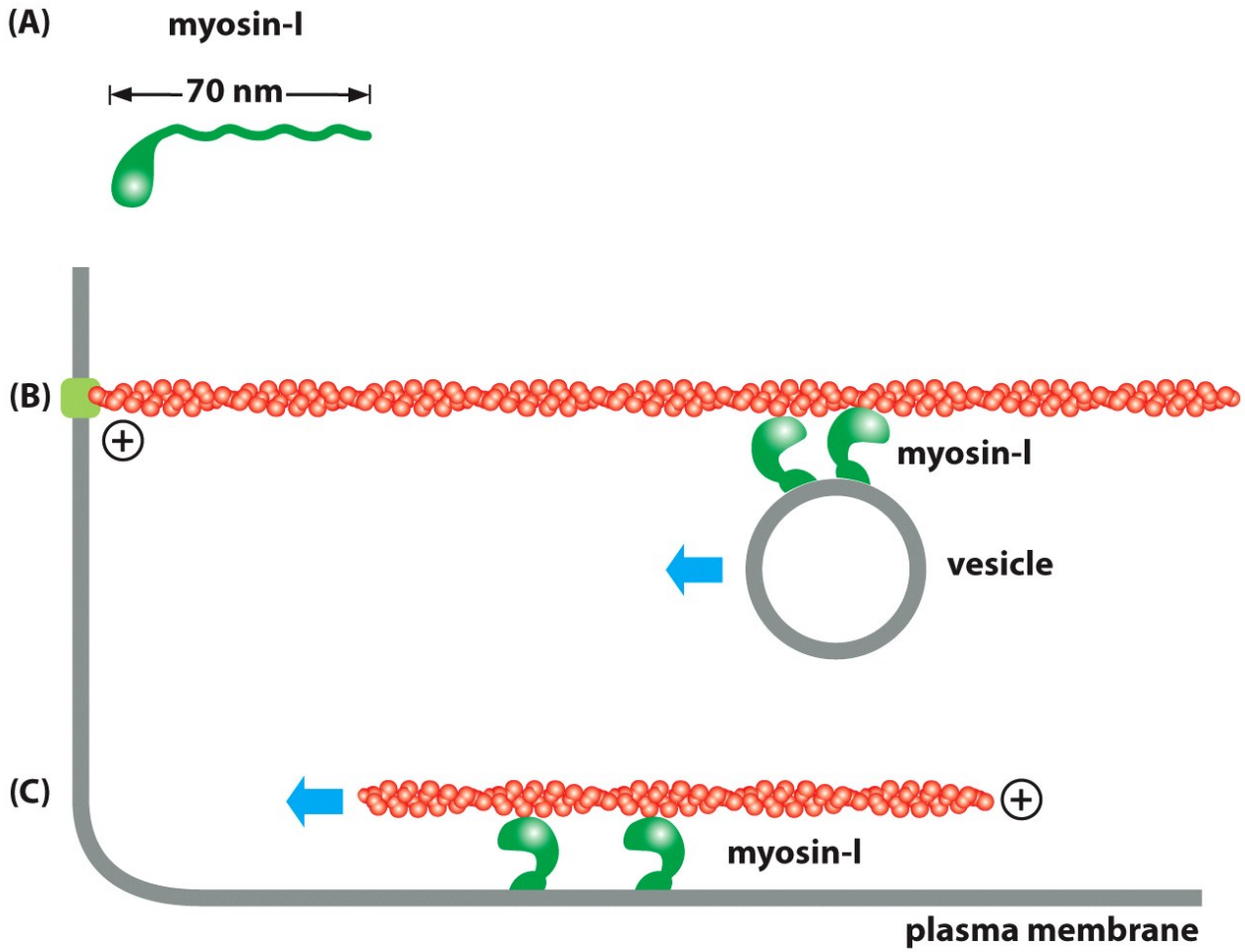


Figure 17-36 Essential Cell Biology 3/e (© Garland Science 2010)

Myosin-II

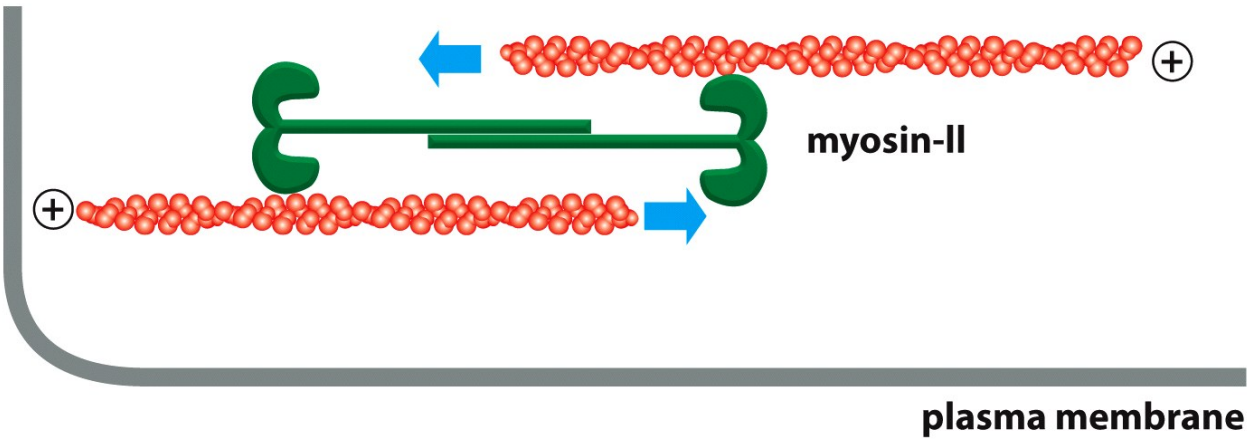


Figure 17-39 Essential Cell Biology 3/e (© Garland Science 2010)

Myosin movie

Actin-myosin muscle contraction

- Myofibrils consist of sarcomeres (2 nm long), sarcomeres consist of actin and myosin II
- Myosin filaments start to pull actin filaments; this results in muscle contraction

Muscle cells

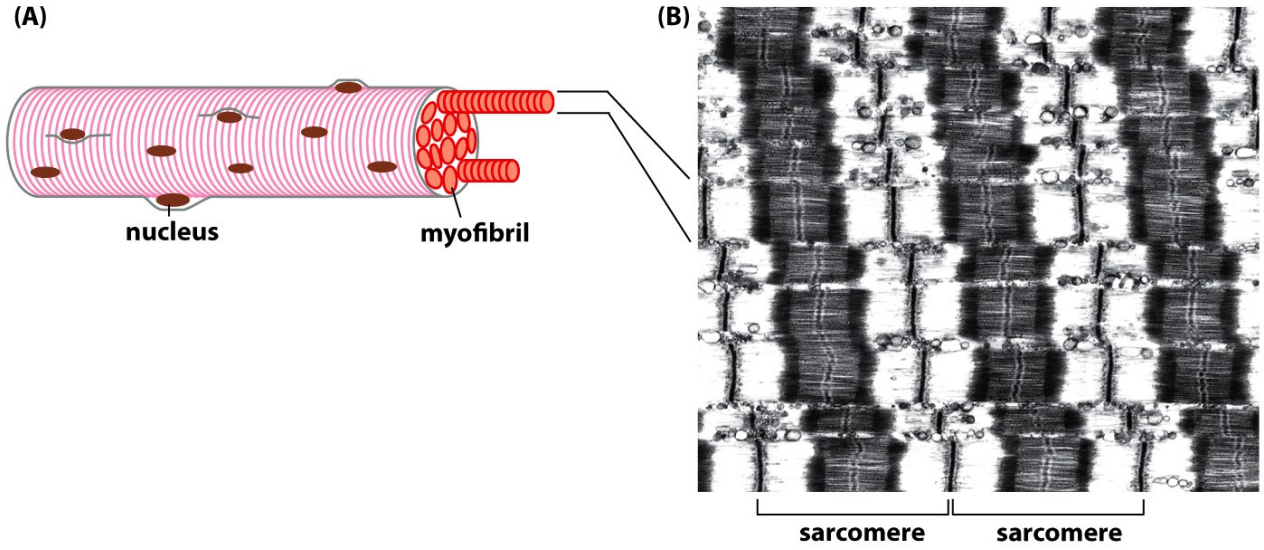


Figure 17-40 Essential Cell Biology 3/e (© Garland Science 2010)

Sarcomeres

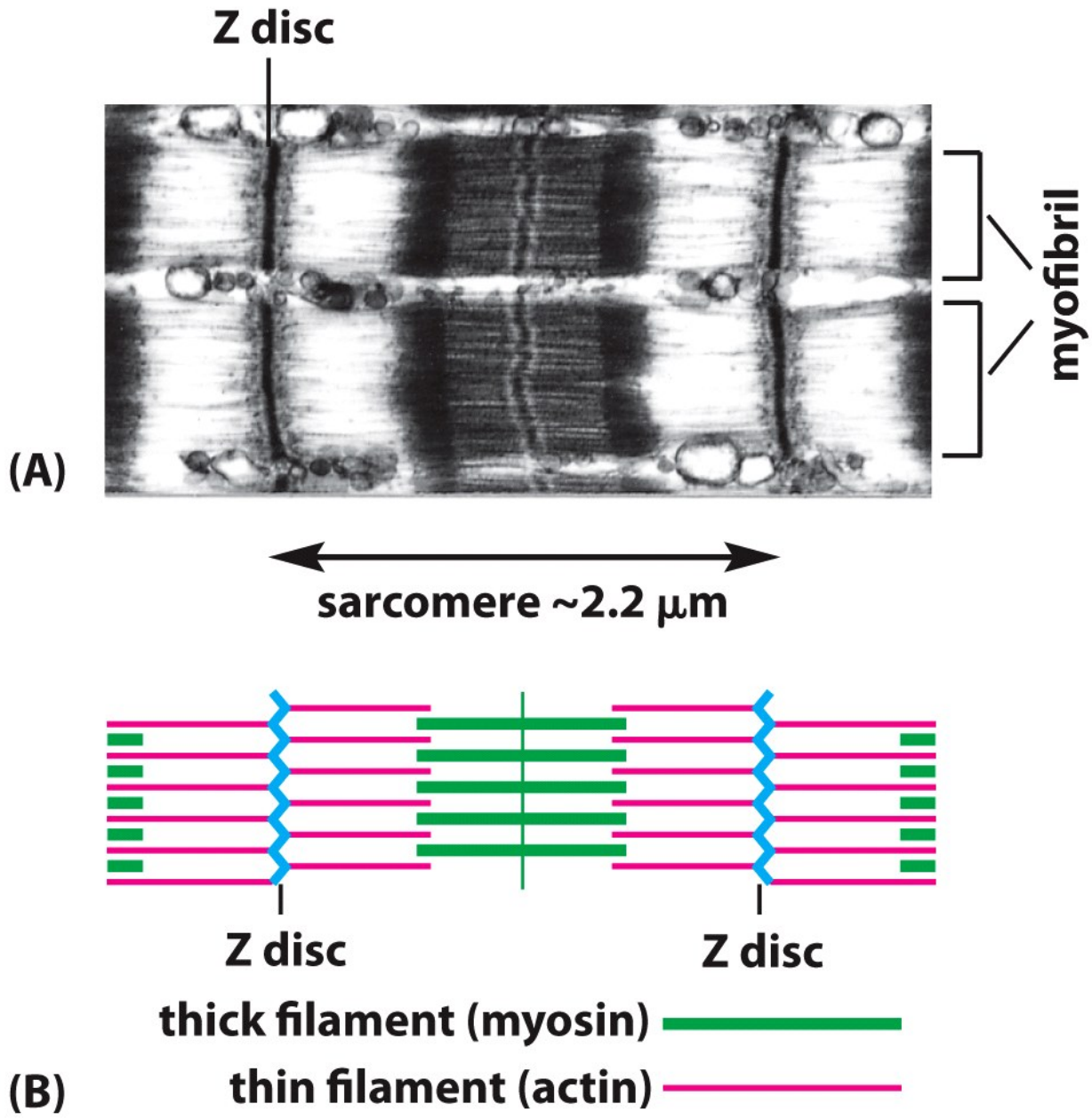


Figure 17-41 Essential Cell Biology 3/e (© Garland Science 2010)

Muscle contraction

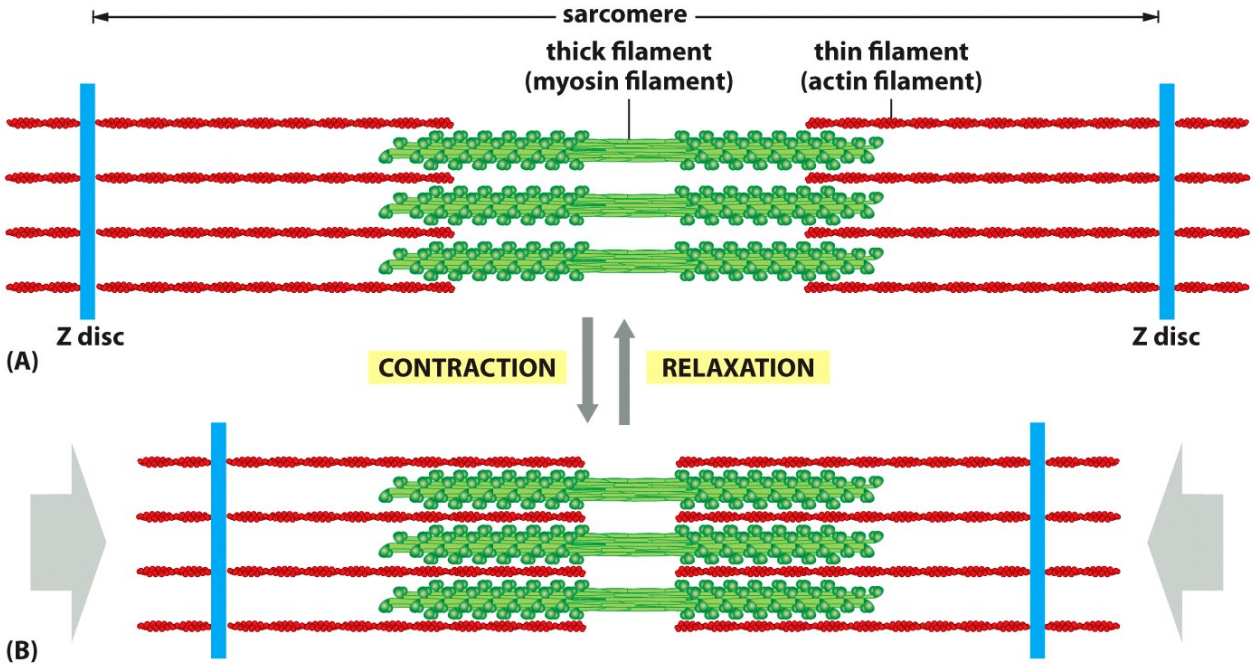


Figure 17-42 Essential Cell Biology 3/e (© Garland Science 2010)

Role of Ca^{2+}

- Calcium signals are released in contacts between neural and muscle cells, and are going into muscle cells via T-tubules
- In a places where T-tubules are close enough to ER (sarcoplasmatic reticulum), action potential "jumps" from T-tubule membrane to ER membrane and activate the calcium rush from ER into cytosol
- This is a contraction signal: calcium ions will interact with troponin-tropomyosin system which unblocks the myosin II

Muscle contraction movie

Final question (2 points)

What will happen with cell if microtubules will not be able to grow?

Summary

- Intermediary filaments are polymers of ropelike polymers of fibrous proteins
- Microtubules are labile hollow tubes of tubulin, kinesins and dyneins move along microtubules
- Actin filaments are helical polymers of actin; myosins move along actin

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 16*.

Outline

Questions and answers

Previous final question: the answer

What will happen with cell if microtubules will not be able to grow?

- Organelle movement will stop
- Mitosis will stop
- Microtubules will shrink
- New flagella/cilia will not appear

Cellular communities

.1 Cell connection

Extracellular matrix in plants

- Primary and secondary cell walls
- Both contain cellulose microfibrils; secondary cell walls also contain polyphenols (like lignin)

Plant cell wall

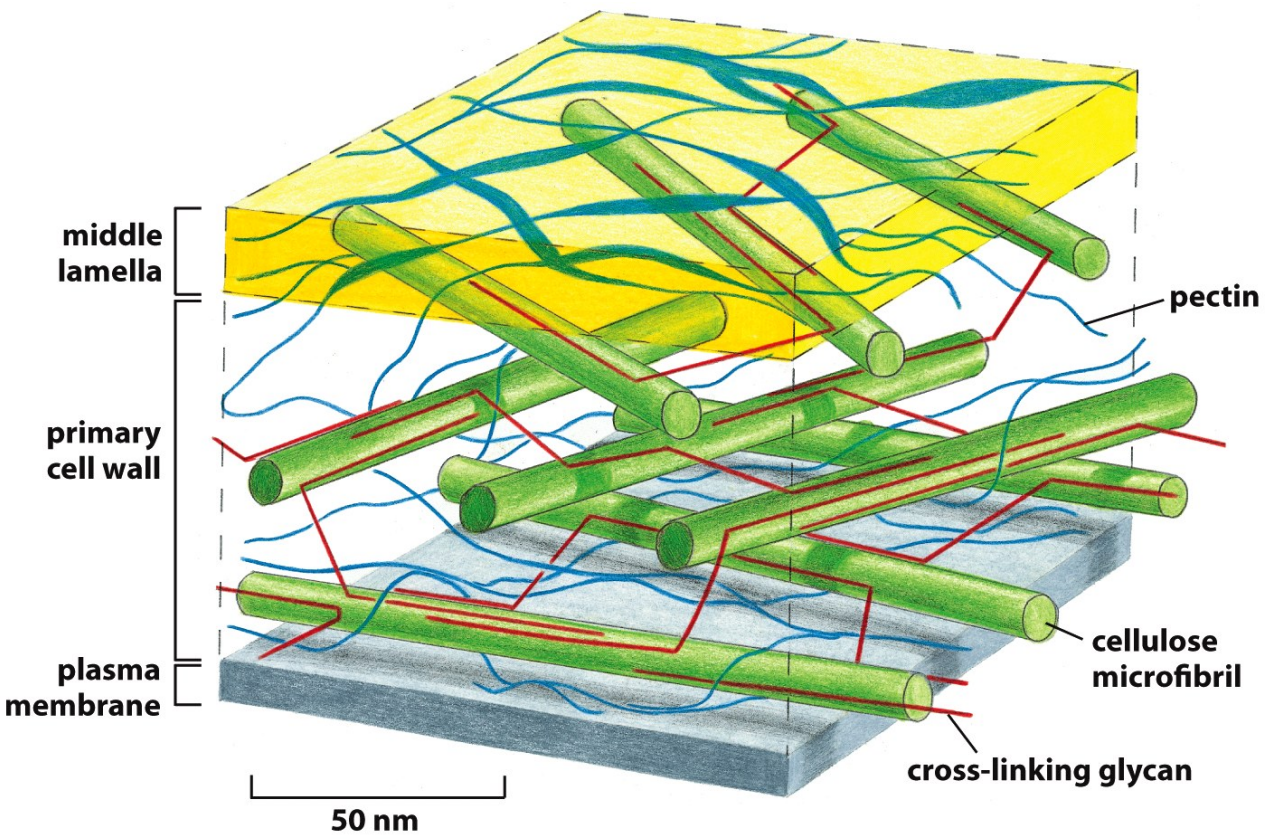


Figure 20-5 Essential Cell Biology 3/e (© Garland Science 2010)

Extracellular matrix in animals

- Occurs mostly in connective tissues
- Contains fibrous protein **collagen**

Integrins

- Fibronectin-integrin complex bind cytoskeleton (actin filaments) to the extracellular matrix
- Activated integrin will bind more effectively to the matrix

Integrins and fibronectins

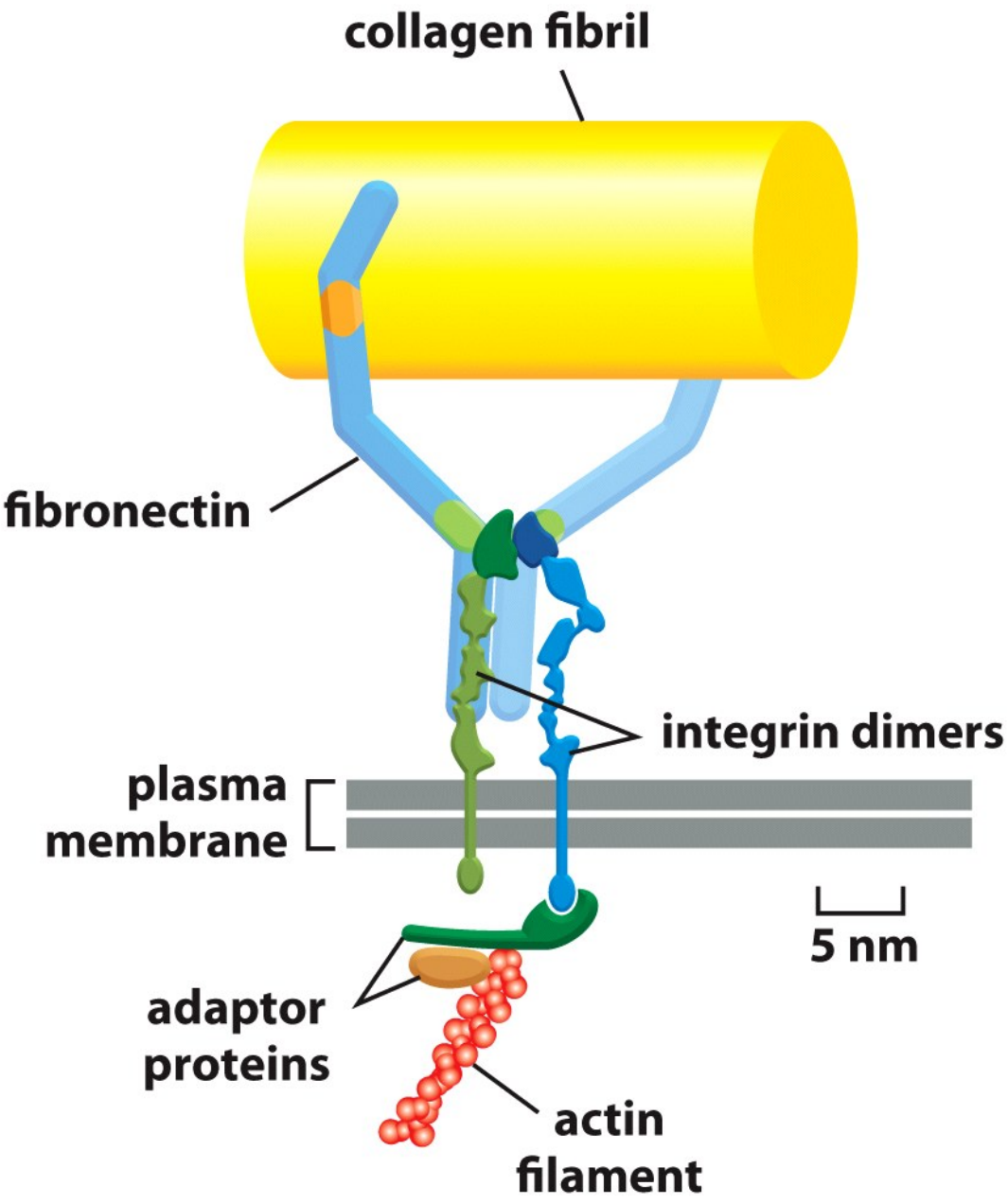


Figure 20-14c Essential Cell Biology 3/e (© Garland Science 2010)

Activation of integrins

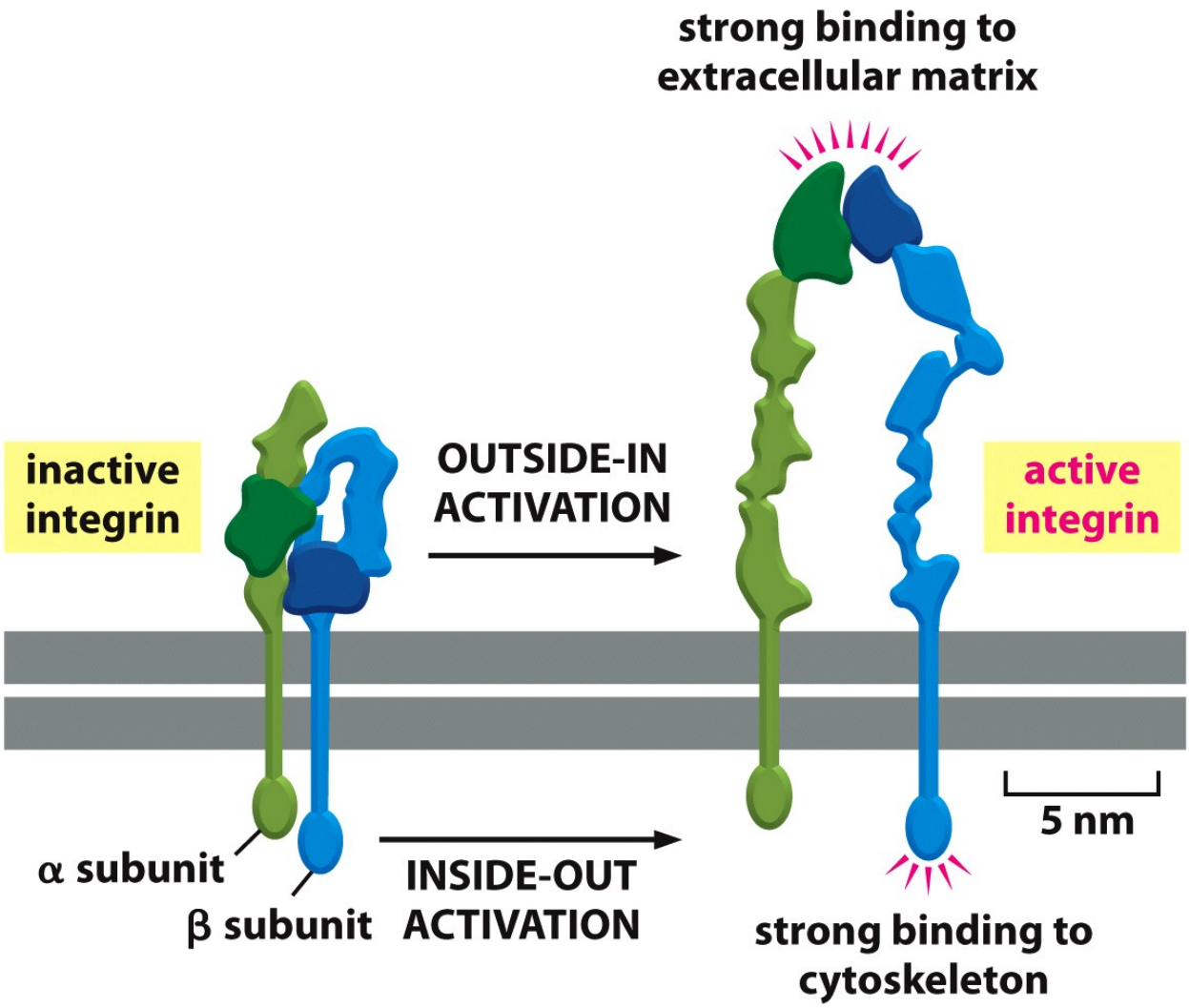


Figure 20-15a Essential Cell Biology 3/e (© Garland Science 2010)

Role of proteoglycans and other derivatives of polysaccharides

- Proteoglycans will provide a space-filling and compression resistance
- Tissues rich of proteoglycans are jelly-like

Basal lamina, tight and cytoskeleton junctions

- Epithelial cells have the tight layer of extracellular matrix: basal lamina
- On the apical side, these cells are closely packed together with **tight junctions** using scotch tape proteins **occludin** and **claudin**
- In addition, **adherens junctions** will join actin fibrils of neighbor cells via Velcro **caldherin** protein; and **desmosome junctions** join intermediate filaments via multiple caldherins
- **Hemidesmosomes** attach cells to the basal lamina

Tight junctions

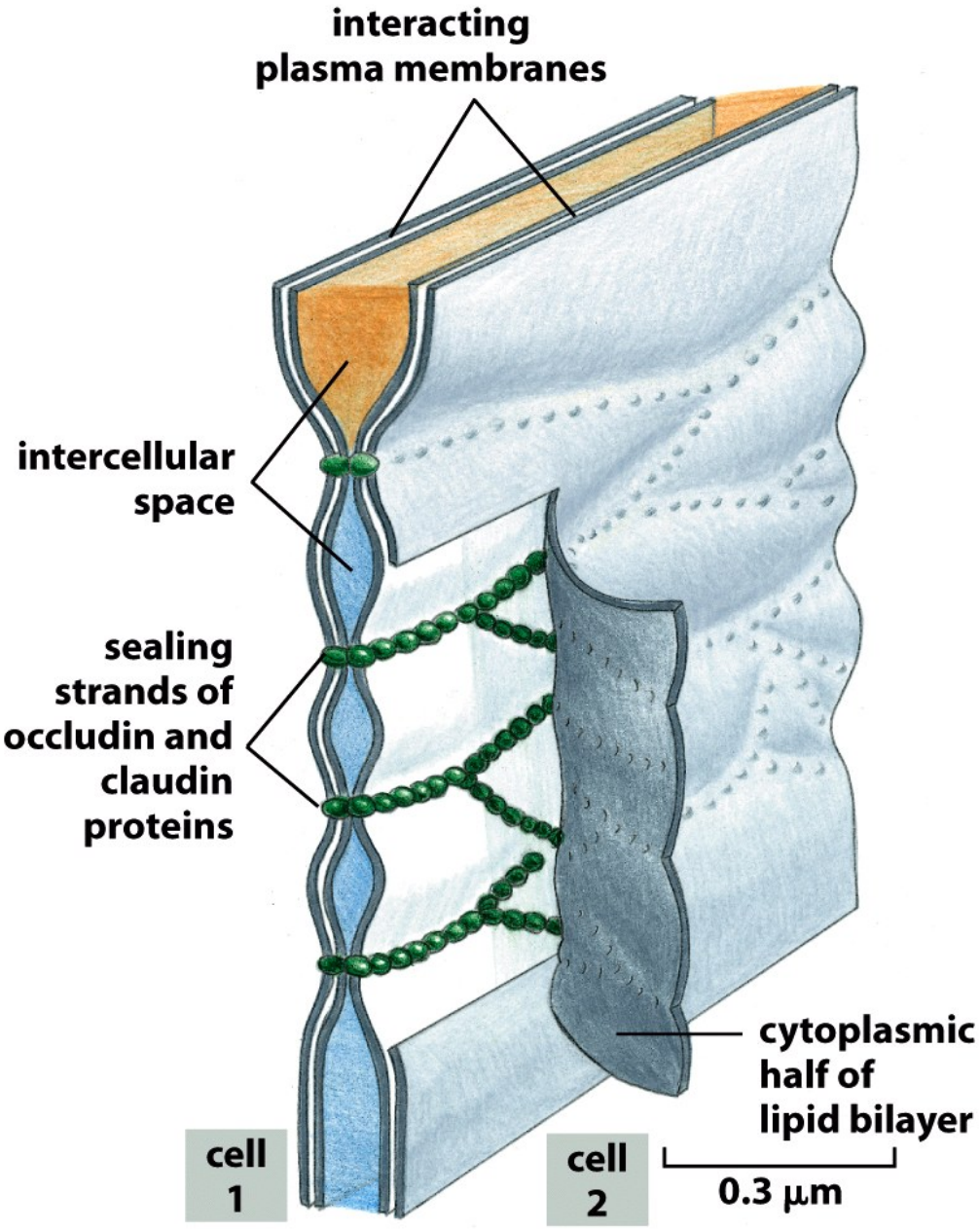


Figure 20-23c Essential Cell Biology 3/e (© Garland Science 2010)

Tight junctions and grass seeds

- Grass seeds normally contain proteins (**glutens**) which (a) have only few of useful amino acids and (b) inhibit carbohydrate digestion. The latter could be avoided by heating (cooking bread)
- Gliadin (component of glutens) also split into polypeptides which *weakens tight cell junctions*: this will cause celiac disease. 10% of humans are sensitive to glutens.

Adherens junction

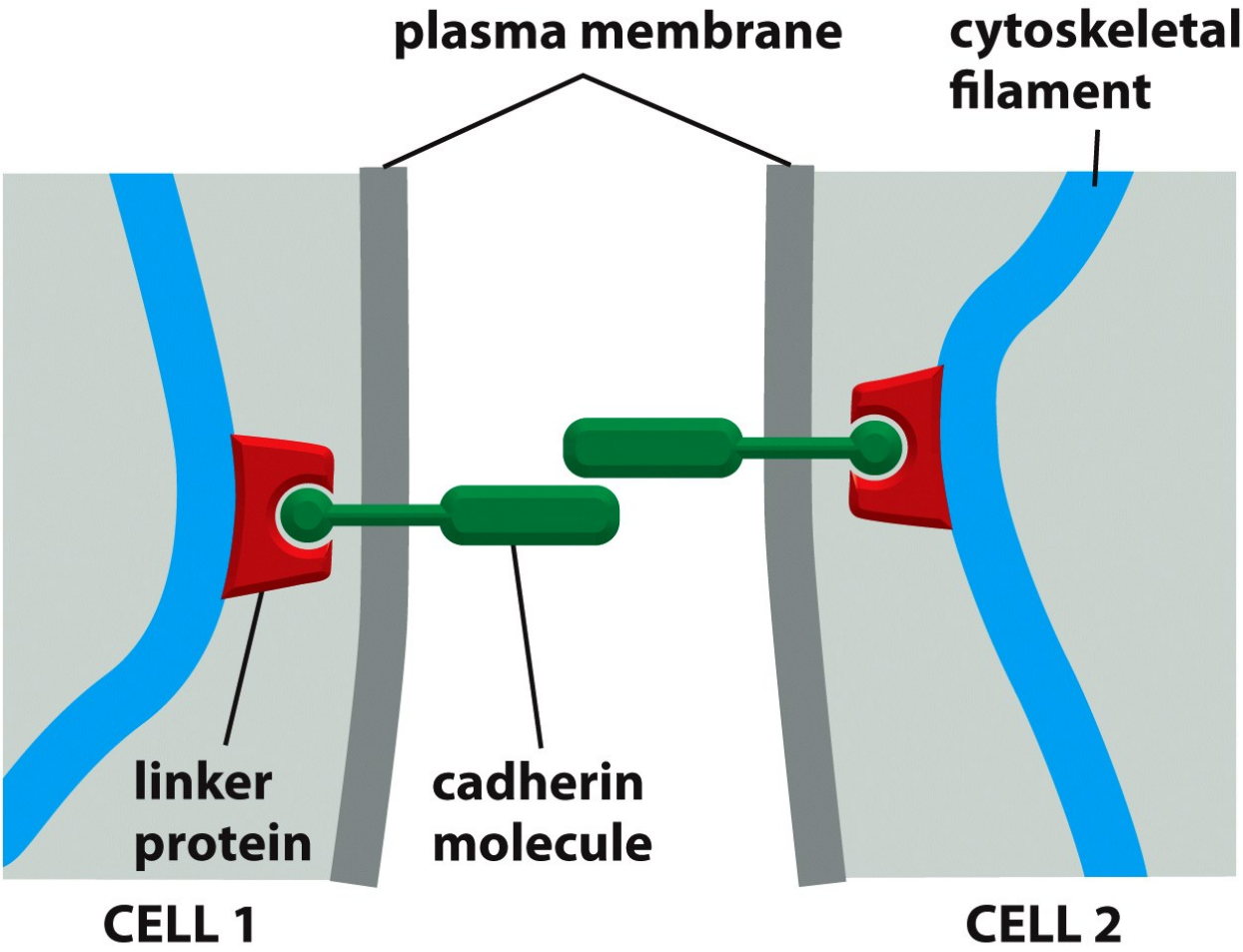


Figure 20-24 Essential Cell Biology 3/e (© Garland Science 2010)

Desmosome

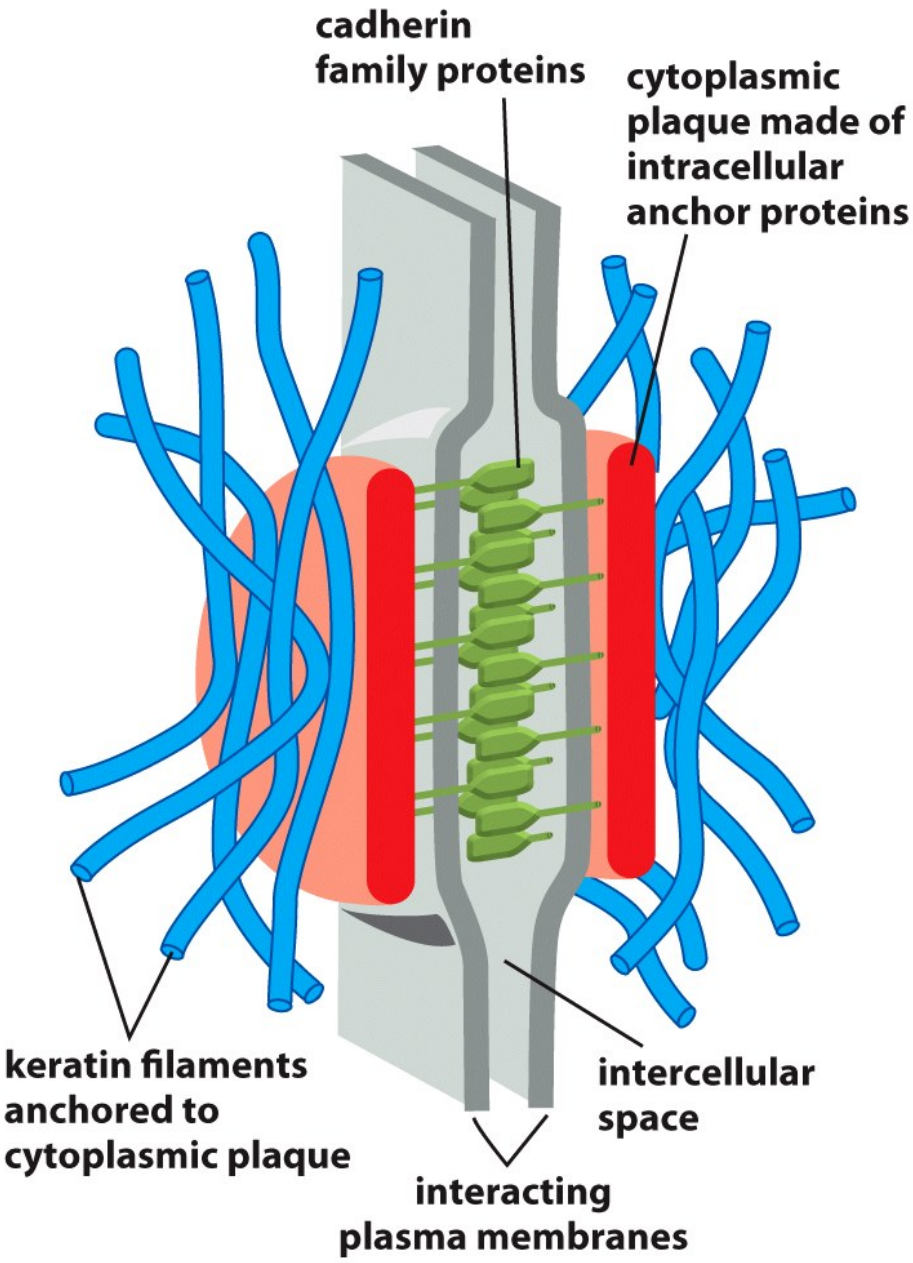


Figure 20-27b Essential Cell Biology 3/e (© Garland Science 2010)

Hemidesmosome

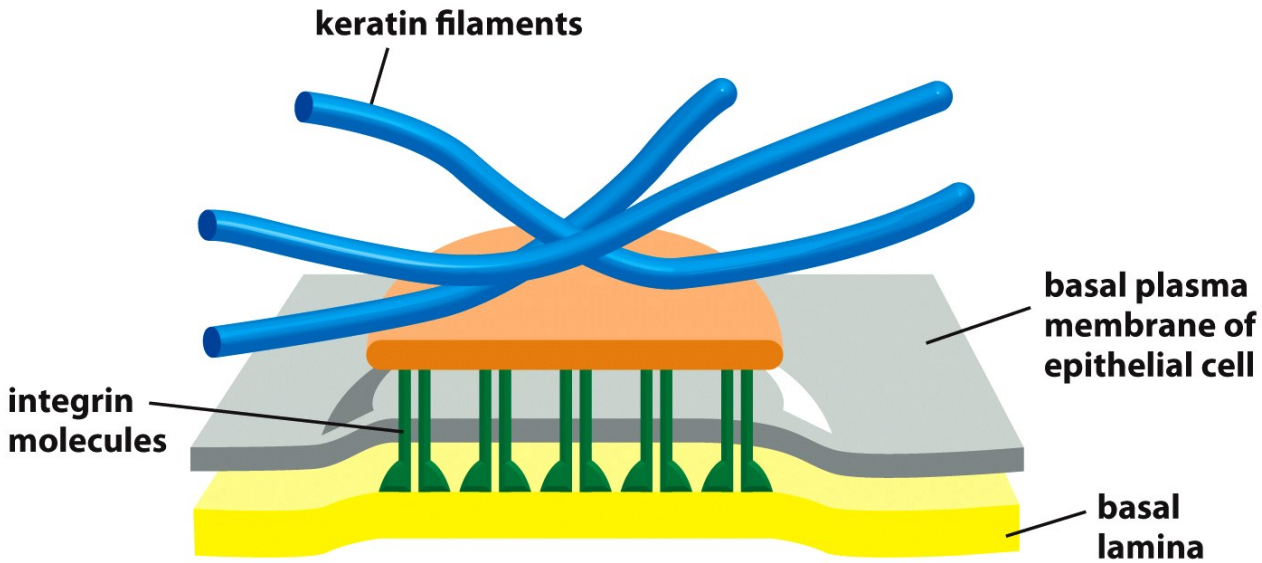


Figure 20-28 Essential Cell Biology 3/e (© Garland Science 2010)

Junctions movie

Gap junctions

- **Gap junctions** are somehow similar to plasmodesmata in plants; they will allow cells to exchange ions and small molecules
- **Connexons** are protein assembles which regulate the gap junction transfer

Gap junctions

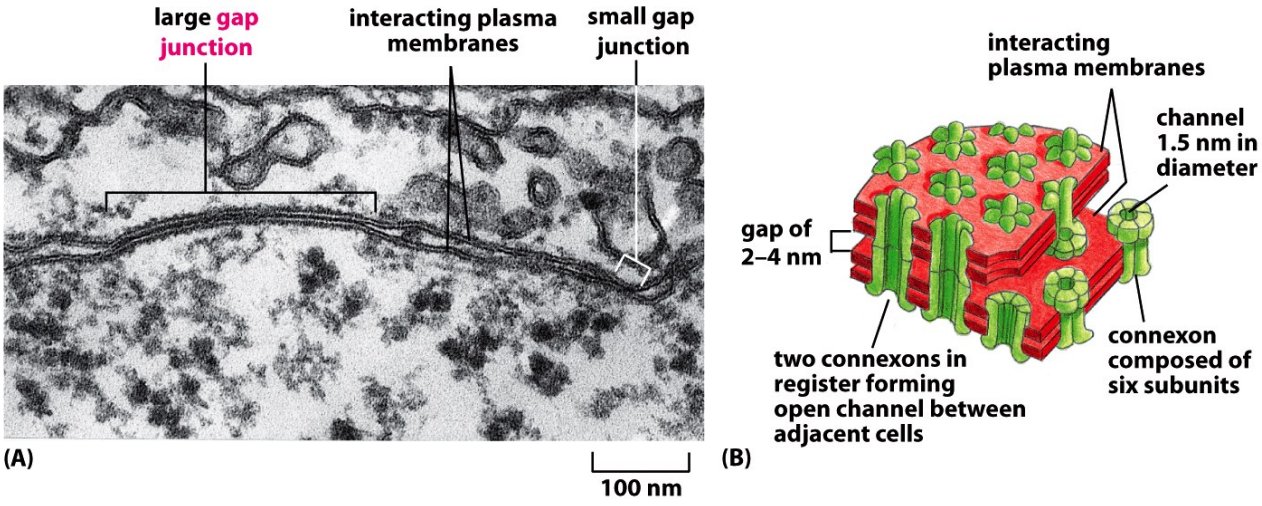


Figure 20-29 Essential Cell Biology 3/e (© Garland Science 2010)

Types of junctions

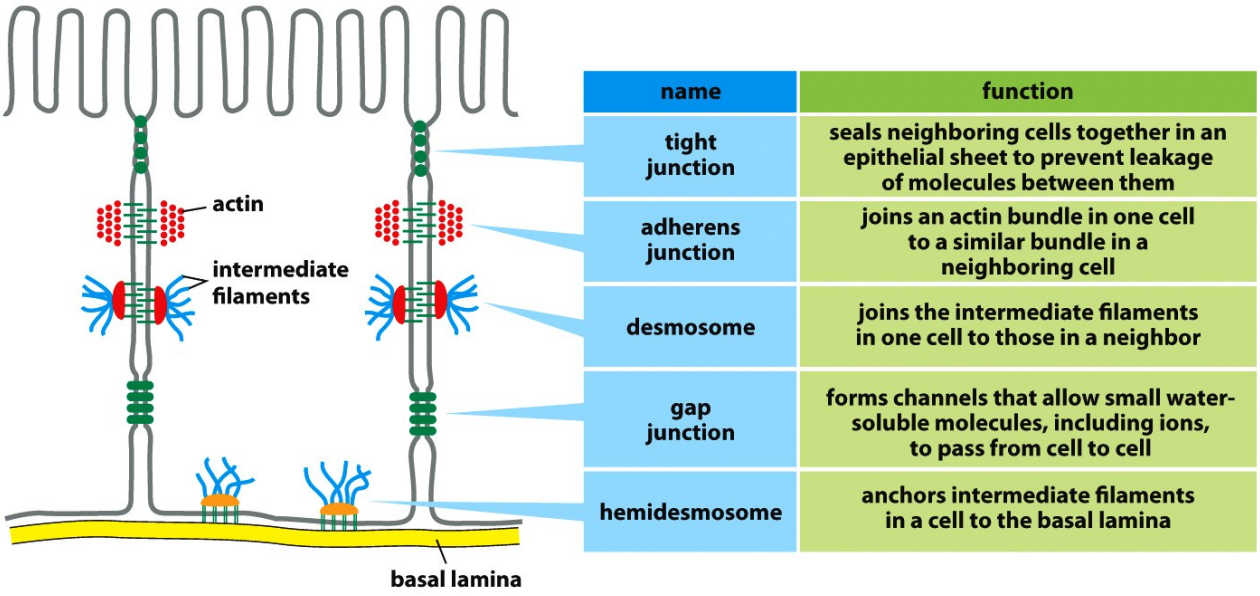


Figure 20-22 Essential Cell Biology 3/e (© Garland Science 2010)

.2 Stem cells

Stem cells

- All tissues need to be renewed, but specialization do not allow for cell division
- Stem cells are cells specialized for production of new cells

Stem cells

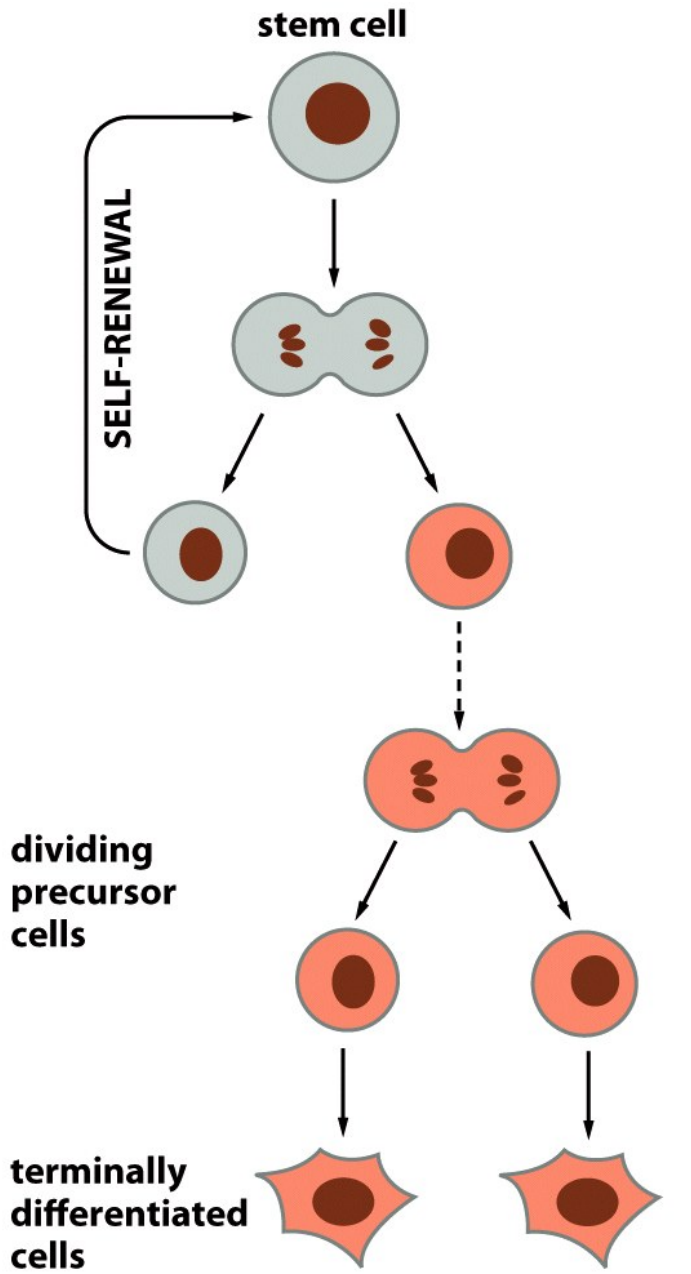


Figure 20-35 Essential Cell Biology 3/e (© Garland Science 2010)

Wound healing movie

Megakaryocyte movie

Using of stem cells

- Cloning (via unfertilized egg): reproductive and therapeutic
- New tissue generation via iPS (induced pluripotent stem cells)

Cloning

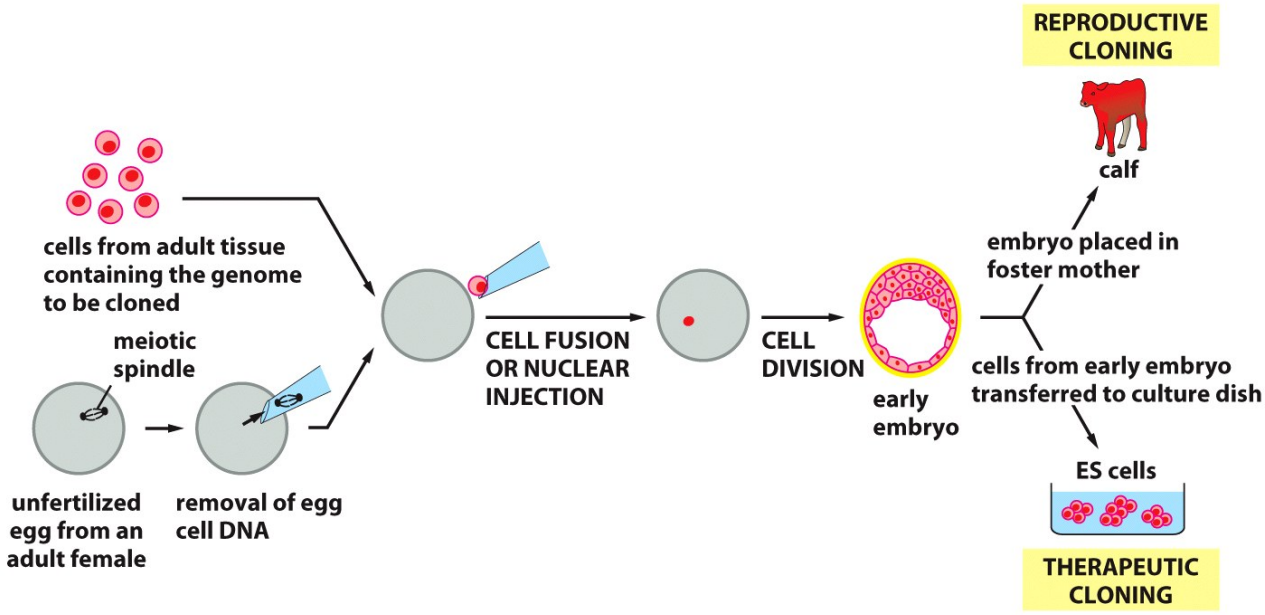


Figure 20-42 Essential Cell Biology 3/e (© Garland Science 2010)

iPS cells

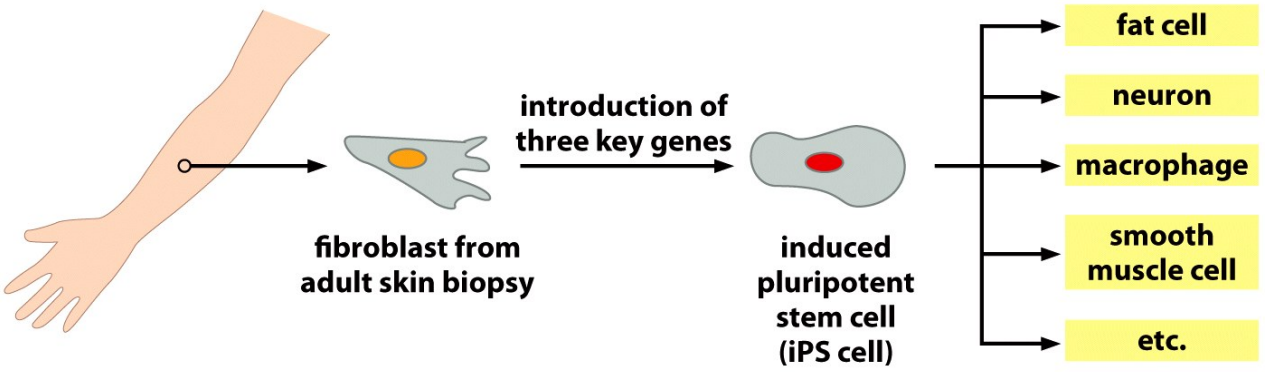


Figure 20-43 Essential Cell Biology 3/e (© Garland Science 2010)

Apoptosis

- Apoptosis is a regulated cell death (not a necrosis!)
- Bcl2 proteins block the conversion of protocaspases into caspases which activate a proteolytic cascade

Bcl2 pathway

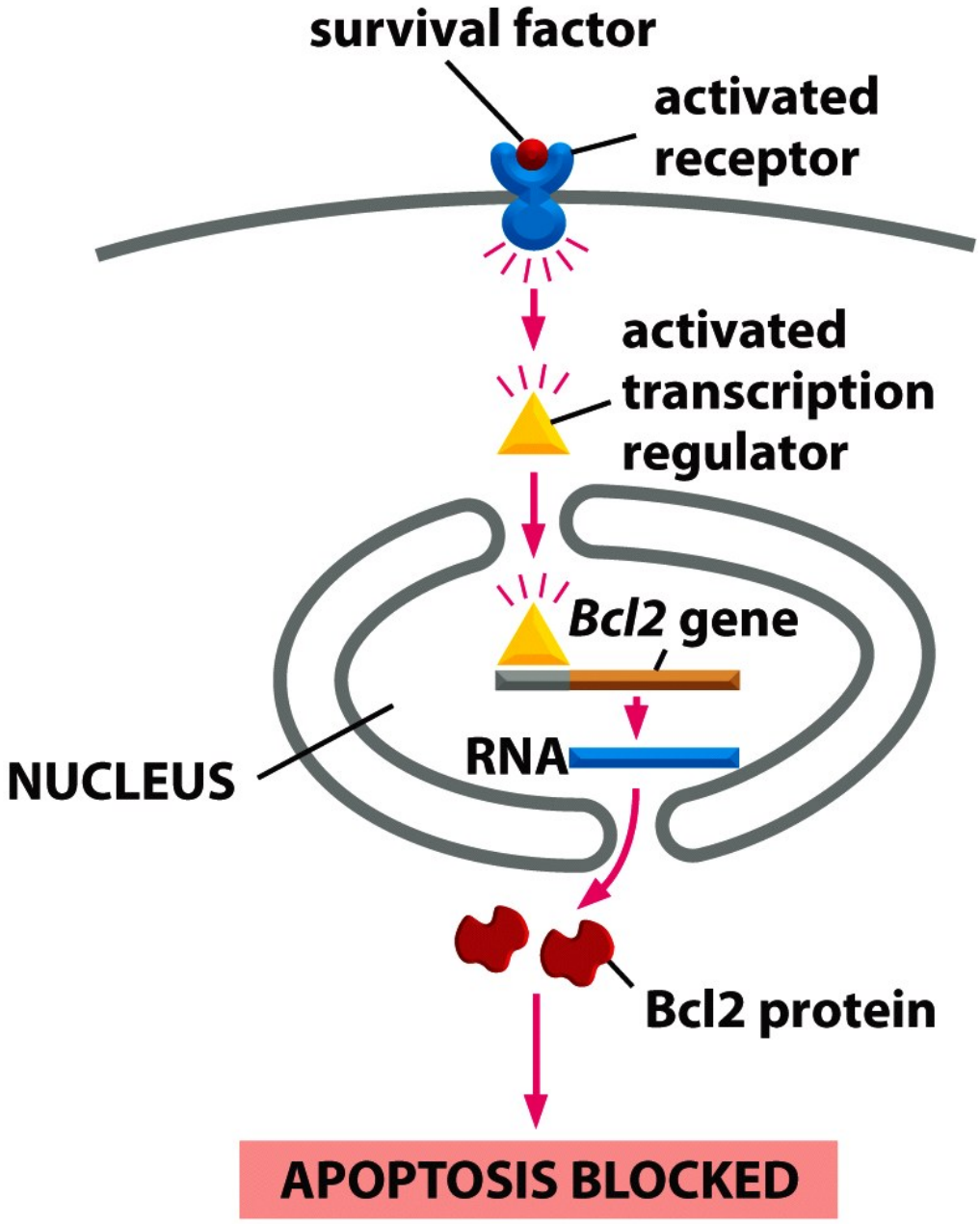


Figure 18-42 Essential Cell Biology 3/e (© Garland Science 2010)

Apoptosis movie

.3 Cancer

Cancer cells

- Proliferate (both benign and malignant)
- Invade (malignant)
- Metastasize

Breast cancer cells movie

Causes of cancer

- Viruses
- Mutation accumulation
- Genetic instability

Genes critical to cancer

- Proto-oncogenes (mutation is typically dominant)
- Tumor suppressor genes (mutation is typically recessive)

Colorectal cancer

- Colorectal cancer is often starts due to inactivation of the second (non-mutated) copy of APC gene
- When active, this gene inactivates TCF proliferation complex

Colorectal cancer

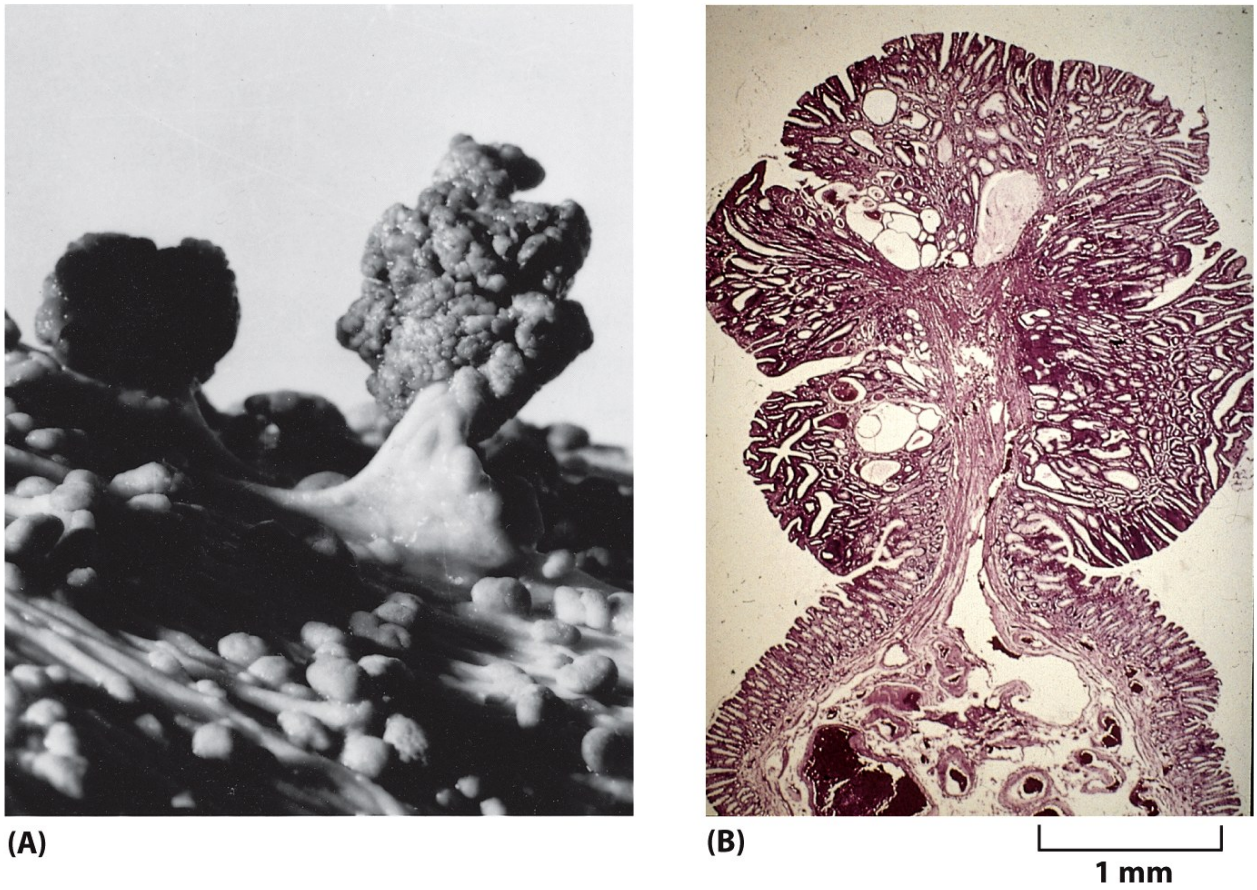
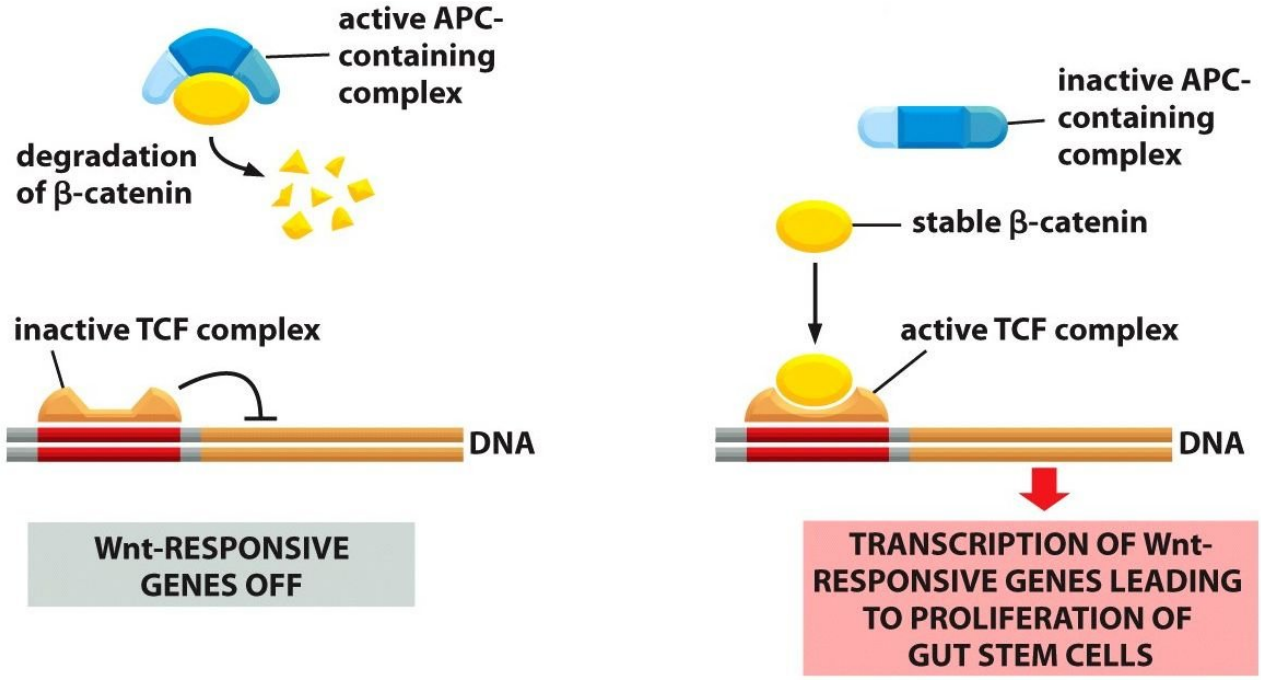


Figure 20-51 Essential Cell Biology 3/e (© Garland Science 2010)

APC / TCF complex (simplified)



Summary

- Multiple cell junctions will keep cells together
- Collagen and proteoglycan extracellular matrix unites connective tissues
- Stem cells are specialized on the new cell production
- Cancer is often the result of oncogene activation or deactivation of tumor suppressor gene

Short anonymous absolutely voluntary survey

1. What do you **like** most in advanced cell course?
2. What do you **dislike** most in advanced cell course?
3. **Which lab** do you remember most of all?
4. Please grade (1—bad, 5—excellent):
   (a) Lectures
   (b) Labs
   (c) Final questions
   (d) Exams

For Further Reading

References

[1] A. Shipunov. *Advanced Cell Biology* [Electronic resource]. 2011—onwards. Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

[2] B. Alberts et al. *Essential Cell Biology*. 3rd edition. Garland Science, 2009. *Chapter 20*.