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# Module 2- Macromolecules of the cell

## For each of the six biological polymers listed, indicate which of the properties apply. Each polymer has multiple properties, and a given property may be used more than once.

Polymers:

(a)  Cellulose

(b)  Messenger RNA

(c)  Globular protein

(d)  Amylopectin

(e)  DNA

(f)  Fibrous protein

Properties  
1. Branched-chain polymer  
2. Extracellular location  
3. Glycosidic bonds  
4. Informational macromolecule

5. Peptide bond  
6. beta linkage  
7. Phosphodiester bridge  
8. Nucleoside triphosphates  
9. Helical structure possible

10. Synthesis requires a template.

A: 2-3-6

B: 4-7-9-10

C: 4-5-9-10

D: 1-3-9

E: 4-7-9-10

F: 4-5-9-10

## Protein Bonds

|  |  |  |
| --- | --- | --- |
| **Bond** | **Amino Acids** | **Levels of Structure** |
| Peptide | All | Primary |
| Hydrogen | All | Secondary |
| Disulfide (covalent) | Cysteine | Tertiary |
| Hydrogen | All | Secondary |
| Hydrophobic | Leucine | Tertiary, Quaternary |
| Ionic | Glutamate | Tertiary, Quaternary |

****

**Protein Structure:**

* Carboxyl group (CO2)
* Amino group
* R group
* Alpha carbon
* Hydrogen atom

## Features of Nucleic Acids

For each of the following features of nucleic acids, indicate whether it is true of DNA only (D), of RNA only (R), of both DNA and RNA (DR), or of neither (N).

(a)  Contains the base uracil. **R**

(b)  Contains the nucleotide deoxythymidine monophosphate**. N**

(c)  Is usually double-stranded. **D**

(d)  Is a polymer. **DR**

(e)  Contains a phosphate group. **DR**

(f)  Is an inherently directional molecule, with an N-terminus on one end and a C-terminus on the other end. **N**

**Like proteins, nucleotides are important informational macromolecules. How are they similar to proteins and how do they differ in terms of monomer types and assembly, polymer structure, and cellular functions?**

Like the proteins, nucleic acids DNA and RNA are composed of monomers, nucleotides for the nucleic acids which are linked together by phosphodiester bridges. As in proteins, the order of monomers carries information, which is genetic information.

## Wrong Again. For each of the following false statements, change the statement to make it true, and explain why it is false as written:

(a) Nucleic acids are polymers consisting of chemically ~~identical~~ repeating nucleotide monomers.

(b)  A protein may have an alpha helical secondary structure. An alpha helix is spiral in shape and stabilized by covalent bonds between the NH group and the CO group in the polypeptide backbone.

(c)  Whereas a protein can be denatured by high-temperature treatment, extremes of pH both of which disrupt ~~generally have no effect on~~ tertiary structure.

(d)  Nucleic acids are synthesized from monomers that contain a high energy phosphodiester bond. They are already activated and do not require carrier molecule.

~~are activated by linking them to a carrier molecule in an energy-requiring reaction.~~

(e)  The disaccharide sucrose comprises two monosaccharide ~~glucose~~ monomers covalently linked together.

(f)  A beta-pleated sheet is an extended sheet-like conformation with the R groups of successive amino acids jutting out on the alternating ~~same~~ side of the sheet.

(g)  It is not easy to predict the final folded structure of a protein from its amino acid sequence using today’s powerful supercomputers.

## Telling Them Apart. For each of the following pairs of molecules, specify a property that would distinguish between them, and indicate two different tests that could be used to make that distinction:

(a)  The protein insulin and the DNA in the gene that encodes insulin

*Phosphodiester bonds in DNA but not in protein.*

(b)  The DNA that encodes insulin and the messenger RNA for insulin

*Presence of purine thymine or pentose deoxyribose in DNA but not in RNA.*

(c)  Starch and cellulose

*Starch repeating unit: alpha-D glucose, cellulose repeating unit: beta-D glucose.*

*Use the enzyme amylase that can digest alpha (1-4) but not beta (1-4).*

(d)  Amylose and amylopectin

*Starch occurs in branched amylose alpha (1-6) glycosidic bonds or unbranched amylopectin alpha(1-4) glycosidic bonds.*

(e)  The monomeric protein myoglobin and the tetrameric protein hemoglobin

*Presence of 4 subunits in hemoglobin but not in myoglobin.*

(f)  A triacylglycerol and a phospholipid with a very similar fatty acid content

*Presence of glycerol but absence of phosphorus in triacylglycerol.*

(g)  A glycolipid and a sphingolipid

*Carbohydrate group (glycolipid) instead of phosphate group (sphingolipid).*

**Examples of proteins**

* *Structural proteins*: collagen, keratin.
* *Motility proteins*: Actin (microfilaments), tubulin (microtubules).
* *Regulatory proteins*: transcription factor bind to DNA sequences to turn genes on.
* *Signaling proteins*: GLUT1. Glucose transporter, found in cells that import glucose, K+ channels.
* *Receptor proteins*: insulin receptor binds to insulin to initiate glucose utilization, found in cell, Ach.
* *Defensive proteins*: antibodies.
* *Storage proteins*: Ferritin stores iron.

# Module 3 – Introduction to Cells and Organelles

**Describe and similarities and differences between archaea, bacteria and eukaryotes**

* They came from the same ancestor cell.
* Eukaryote cell has a plasma membrane, a nucleus, membrane bounded organelles and cytosol supported by the cytoskeleton.
* Main distinction between prokaryote (bacteria and archaea) and eukaryote cell (plant, animal, fungi, algae and protozoa) types is the membrane-bound nucleus of eukaryotic cells.
* **Eukaryotic DNA is organized into linear molecules complexed with large amounts of histones.**
* **Bacterial DNA is present as a circular molecule associated with few proteins.**
* **Archaea DNA is circular and complexes with proteins similar to eukaryotic histone proteins.**



**Discuss the 3 main limitations on cell size**

1. Need to maintain adequate surface area to volume ratio

*Larger cells have proportionally smaller surface areas. Beyond a certain threshold of this ratio, large cells do not have enough surface area to accommodate the need for nutrients and release of enough wastes. Cells like cells lining the small intestine have characteristics like fingerlike projections that increase the surface area.*

1. Rate of diffusion of proteins decreases as the size of molecules increases

*Eukaryotic cells avoid the problem by using carrier proteins or vesicles.*

1. Need for adequate local concentrations and essential substances

*To maintain the necessary concentration of a specific molecule, number of molecules must increase with cell volume. An effective solution to the concentration problem is the compartmentalization of activities within organelles.*

**Discuss the role of plasma membrane**

**The main role: ensures that cell contents are retained.**

* Serves as a permeability barrier between the cell and outside environment.
* Localizes and organizes different functions within the cell.
* Facilitates transport of different molecules within the cell between organelles and also its outside environment: nutrients, ions or water, and wastes.
* Helps the cell to perceive its external environment and respond appropriately thru receptor mediated signal transduction, transmission of signals from outer surface to cell interior.
* Mediate interactions with other cells.

**List several eukaryotic organelles and their basic functions**

* **Mitochondrion**

Site of aerobic respiration

Provide energy to cell by oxidation of sugars and other fuel molecules.

* **Rough ER**

Has ribosomes either on the side of the membrane facing the cytosol or free in the cytosol which synthesize proteins; some of them to be transported out of the cell.

* **Smooth ER**

Involved in the synthesis of lipids and steroids such as cholesterol and steroid hormones derived from it.

* **Golgi Complex**

The post office: involved in processing and packaging secretory vesicles which are then passed to other components of the cell, and in polysaccharide synthesis. Glycoproteins and membrane lipids from the ER undergo further process: sorted and are packaged for transport (via the trans-Golgi network or TGN).

* **Lysosome**

Storage for hydrolase enzymes capable of digesting any biological molecules.

*Cells involved in synthesis of secretory proteins have prominent rough ER networks (fibroblasts in skin secrete collagen). Cell producing steroid hormones have extensive networks of smooth ER (e.g., cells of adrenal gland).*

**Describe the Endosymbiont Theory**

Suggests that mitochondria and chloroplast evolved from the same ancestor bacteria. This is based on similarities in size, membrane lipid composition, rRNA sequences, presence of circular DNA molecules, and bacterial type ribosomes, and ability to reproduce autonomously.

**Describe the eukaryotic cytoskeleton and its structural components**

* Eukaryotic **cytoskeleton** is an array of fibers giving structure to the cytoplasm giving the cell its shape. In addition, it plays a role in cell movement and cell division.
* A 3-D array of interconnected **microfilaments, microtubules, and intermediate filaments.**
* A **microtubule** is a cylinder of **protofilaments** with a hollow center (lumen). Each protofilament is a linear polymer of **tubulin** with polarity. **Tubulin** consists of two proteins: **alpha-tubulin and beta-tubulin.**
* **Microfilaments** are polymers of F**-actin** strands twisted in a helical structure. F-actin polymers are made of **G-actin.** Microfilaments have a polarity.

**Explain key characteristics of prions, viruses, and bacteriophages**

* **Viruses** are small and consists of a coat of protein surrounding a core, containing DNA or RNA. They have no cytoplasm, organelles or ribosome and infect cells, using their machinery to produce more viruses. When they infect bacteria, they are called **bacteriophages** or **phages**. They are responsible for many diseases, also important tools as research tools.
* **Prions** are infective particles which induce existing, properly folded proteins to convert into disease-associated prion form, and they induce amyloid plaques.
* A **bacteriophage** exists in theory for every type of bacterium, can be highly specific for their hosts.

**Wrong Again. For each of the following false statements, change the statement to make it true.**

(a)  The mitochondria of bacterial cells and human cells are quite identical.

(b)  Ribosomes are enclosed by a membrane in bacterial cells.

*Ribosomes are not membrane bound.*

(c)  Instead of a cell wall, ***some*** eukaryotic cells have an extracellular matrix for structural support.

(d)  All the ribosomes found in a typical human muscle cell are identical.

*Cytoplasmic ribosomes are the eukaryotic types, mitochondrial ribosomes are the prokaryotic type.*

(e)  DNA is found only in the nucleus of a cell.

*DNA is found in the nucleus of a eukaryotic cell but also in the mitochondria and in the chloroplasts.*

(f)  Because bacterial cells have no organelles, they cannot carry out either ATP synthesis or photosynthesis.

*Carry out ATP synthesis using the plasma membrane.*

(g)  A large amount of the DNA in eukaryotic cells has no function and is called “junk DNA.”

*Some of this non-coding DNA is used to produce non-coding RNA: tRNA, regulatory and ribosomal RNA.*

**Toward an Artificial Cell. Scientists have recently constructed an artificial ribosome in vitro from purified ribosomal proteins and rRNAs. (Some of the following questions may require sleuthing in earlier chapters to answer.)**

1. What types of intermolecular forces do you think are holding the individual proteins and rRNAs together in this macromolecular complex?

*Ionic bonds, hydrogen bonds, hydrophobic bonds between nonpolar groups, van der Waals interactions.*

1. Describe how high temperature, high salt, or low pH would disrupt its structure, causing the ribosome to fall apart.

*High temperature will break the weak hydrogen bonds, and denature the protein. High salt will interfere with ionic bonding, extremes of pH can change the charge on acidic and basic residues of the proteins, interfering w/ both ionic and hydrogen bonding.*

1. If you were asked to determine which organism the ribosomal components were purified from, how could you do this?

*You could sequence the rRNA to determine the source organism. For the ribosomal proteins, you could sequence the proteins themselves or the genes that encode them.*

1. What other molecules would you have to add to the test tube for the ribosomes to make polypeptides?

*Need to add amino acids, mRNA to translate tRNAs, aminoacyl-tRNA synthetases, and a source of ATP.*

Sentence Completion. Complete each of the following statements about cellular structure in ten words or less.

(a) Unlike animal cells, plant cells have . . . *a rigid cell wall, plastids and large vacuole.*

(b) When placed in a glass of water, a dried date . . .

(c) A cellular structure that is visible with an electron microscope but not with a light microscope is . . . *a ribosome, virus, microtubule, microfilaments etc.…*

(d) Several environments in which you are more likely to find archaea than bacteria are … *salt water, hot spring, acidic environments and sulfur-containing environments.*

One reason that it might be difficult to separate lysosomes from peroxisomes by centrifugation techniques is that . . . *they are very similar in size.*

(f)  The nucleic acid of a virus is composed of… *DNA or RNA but not both.*

Telling Them Apart. Suggest a way to distinguish between the

two elements in each of the following pairs.

(a)  Plant peroxisomes; thylakoids

(b)  Rough ER; smooth ER ribosome on cytoplasmic side of the cell.

(c)  Animal peroxisomes; leaf peroxisomes

(d)  Smooth ER; mitochondria

(e)  Vacuole; nucleus

(f)  Polio virus; herpes simplex virus

(g)  Eukaryotic ribosomes; bacterial ribosomes

Protein Synthesis and Secretion. Order events 1–7 so that they represent the correct sequence corresponding to steps a–g, tracing a typical secretory protein from the initial transcription (readout) of the relevant genetic information in the nucleus to the eventual secretion of the protein from the cell by exocytosis.

Transcription > (a) > (b) > (c) > (d) > (e) > (f) > (g) > Secretion

1. The RNA transcript is transported from the nucleus to the cytoplasm.
2. The RNA message associates with a ribosome and begins synthesis of the desired protein on the surface of the rough ER.
3. As the protein is synthesized, it passes across the ER membrane into the lumen of the rough ER, and from there via a vesicle to the Golgi apparatus.
4. The protein is partially glycosylated within the lumen of the rough ER.
5. Final sugar groups are added to the protein in the Golgi apparatus.
6. The protein is packaged into a secretory vesicle and released from the Golgi apparatus.
7. The secretory vesicle arrives at and fuses with the plasma membrane.

**Are They Alive? Biologists sometimes debate whether viruses should be considered alive. Let’s join in the debate.**

1. What are some ways in which viruses resemble cells?

*They contain nucleic acid (DNA or RNA) and proteins; they are composed primarily of carbon, hydrogen, and oxygen; they are too small … they sometimes have a membrane covering;*

1. What are some ways in which viruses differ from cells?

*They are much smaller than most cells; they have DNA or RNA but not both; they cannot replicate on their own; they do not make their own membrane; they have, at most a few enzymes; they do not have cytoplasm or nucleus.*

1. Choose either of the two following positions and defend it: (1) Viruses are alive. (2) Viruses are not alive.

*Do not satisfy: metabolism, irritability and ability to reproduce.*

(d)  Why do you suppose that viral illnesses are more difficult to treat than bacterial illnesses?

(e)  Design a strategy to cure a viral disease without harming the patient.

# Module 4 – Enzymes

**Describe the basic properties of the enzymes**

<https://infinitabiotech.com/blog/properties-of-enzymes/>

* Act as biological catalyst by increasing the rate of reactions without increasing the temperature.
* Are globular proteins.
* A complex 3-D structure.
* They are not depleted and remain unchanged at the end of a reaction.
* Specificity.

**Explain why enzymes are good biological catalysts**

* They increase the rate of a reaction by lowering the activation energy requirements, without increasing the temperature.
* They change the rate at which equilibrium is achieved without changing its position.
* Most of the enzyme catalyzed reactions are reversible.

**Explain why enzymes only work on a single substrate**

Because of the precise chemical fit between the active site of the enzyme and its reactants, enzymes are very specific.

Two models to explain this specificity: *lock-and-key* and *induce-fit* (conformational change of the enzyme).

**Explain that enzymes function by lowering the activation energy for biochemical reactions**

Before a chemical reaction happens, there is an activation energy, which is the minimal amount of energy the reactants must contain before collisions between them, will be successful in giving rise to products. Enzymes by lowering the activation energy, ensure that a higher proportion of molecules, possess enough energy to undergo reaction without increasing the temperature.

**The Need for Enzymes. You should now be in a position to appreciate the difference between the thermodynamic feasibility of a reaction and the likelihood that it will actually proceed.**

1. Define the terms activation energy and transition state.

**Activation energ**y: *minimum amount of energy*, reactants must contain before a chemical reaction happens.

**Transition state**: *chemical state* which separates the state in which molecules exist as reactants and the state in which they exist as product.

1. Describe the effect of heat on enzyme activity and explain why using heat to alter enzyme activity is problematic in cells.

Reaction rate is the highest at the optimal temperature (370c for human enzymes). Above this optimal temperature, enzyme activity decreases sharply until the enzyme is denatured (inactive).

1. An alternative solution is to lower the activation energy barrier. What does it mean in molecular terms to say that a catalyst lowers the activation energy barrier of a reaction?

A catalyst by lowering the activation energy requirements, allows a higher proportion of the molecules to possess sufficient energy to undergo reaction without elevation of temperature.

1. Organic chemists often use inorganic catalysts such as nickel, platinum, or cations in their reactions, whereas cells use proteins called enzymes. What advantages can you see to the use of enzymes? Can you think of any disadvantages?

**Advantages**: specificity and more exact control.

**Disadvantages**: more susceptible to inactivation by heat, pH, substrate concentration and; also, more energy needed to be expanded to synthesize the enzyme molecules.

**Temperature and pH Effects. Figure 6-4 illustrates enzyme activities as functions of temperature and pH. In general, the activity of a specific enzyme is highest at the temperature and pH that are characteristic of the environment in which the enzyme normally functions.**



1. **Explain the shapes of the curves in Figure 6-4 in terms of the major chemical or physical factors that affect enzyme activity.**

*Figure 6-4a*: The velocity of the reaction increases as the temperature is increased consistent with the effect of temperature in general on chemical reaction, which usually double in reaction velocity for every 100C increase. As the T is raised above the optimum, sharp decline in activity as the enzyme undergoes denaturation.

*Figure 6-4b*: pH optimum corresponds to the ionizable groups on both the enzyme and the substrate molecules, are in the most favorable form for chemical reactivity. pH away from optimum, results in loss of enzyme activity due to *titration* of the ionizable groups on the enzyme or substrate.

1. **For each enzyme in Figure 6-4, suggest the adaptive advantage of having the enzyme activity profile shown in the figure.**

*Figure 6-4a* shows that both enzymes are maximally active at or near the temperature of the milieu in which they are found.

*Figure 6-4b* shows the differences in pH optima for the two enzymes reflects the different environments in which the two enzymes are active.

**(c)- Some enzymes have a very flat pH profile—that is, they have essentially the same activity over a broad pH range. How might you explain this observation?**

They have no amino acids at its active site that undergo ionization or protonation, and probably catalyzes a reaction in which neither substrates nor the products can be ionized or protonated.

# Module 5 – Membrane and the Endomembrane systems

**Describe 5 important function of membranes and give examples**

1. **Boundary and permeability barrier**

The plasma membrane surrounds the cell and regulates passage of molecules both into and out of the cells. Also, intracellular membranes compartmentalize functions in eukaryotic cells.

1. **Organization and localization of function**

Adequate of local concentration of essential substances.

Mitochondrial membranes are critical for respiration.

1. **Cell-to-cell interactions**

Cell-cell interaction allow cells to communicate with each other to changes in their microenvironment and is essential for the survival of the cell.

*Cadherin* is a membrane protein which has extracellular sequences of amino acids that binds Ca2+, and promote adhesion between similar types of cells in tissue.

1. **Signal transduction**

Chemical signal molecules bind to membrane protein receptors, on the outer surface of plasma membrane; which are transmitted to the interior of the cell: e.g., muscle and liver cell membrane contain insulin receptors and can respond to this hormone, which helps cells take in glucose.

1. **Transport processes**

Membranes are sites of specific proteins which carry out and regulate the transport of substances across the membrane: e.g., **aquaporin** which is an integral membrane protein that transports water.

**Differential centrifugation**: used to separate organelles by size and density differences.

**Immunostaining**: technique in which antibodies are labeled with a fluorescent dye to enable them to be identified and localized microscopically based on their fluorescence.

**Explain the Fluid Mosaic**

* The fluid part is that the plasma membrane is as lipid bilayer – main classes of lipids: phospholipids, glycolipids and sterols.
* The mosaic part includes proteins attached or embedded in the bilayer membrane, and lipid rafts and other lipid domains.

**Describe the 3 classes of membrane proteins**

* **Integral**: has one or more hydrophobic amino acid segments that anchor the protein to the membrane.
* **Peripheral**: hydrophilic and remain on the membrane surface. Typically attached to the polar head groups of phospholipids by ionic and hydrogen bonding.
* **Lipid-anchored**: hydrophilic proteins attached to the bilayer by covalent attachments to lipid molecules embedded in the lipid bilayer.

**Explain what is meant by membrane asymmetry**

Refers to the difference in both the kinds of lipids present and the degree of unsaturation of the fatty acids in the phospholipid molecule; e.g., most of the glycolipids present in plasma membrane are restricted to the outer monolayer (carbohydrate groups protrude from outer membrane surface). Once established, asymmetry mostly maintained because movement of lipids from one monolayer to the other requires the passage of hydrophilic head groups through the hydrophobic interior of the membrane**, flip-flop** or **transverse diffusion**.

**Explain laboratory techniques that can be used to study membranes and membrane-associated molecules**

**Thin-Layer Chromatography**: useful to separate membrane lipids according to their degree of polarity. The sample is spotted on a glass TLC plate. Components of the sample are carried upward by the solvent on the plate.

**FRAP** (Fluorescent recovery after photobleaching): molecules in a living cell are tagged with a fluorescent protein (e.g., GFP). A high-density laser beam is used to bleach the dye in a tiny spot on the cell surface, and is seen with a fluorescence microscope as a dark spot. Eventually fluorescent proteins diffuse in and the pot is indistinguishable from the rest of the cell surface.

**Differential scanning calorimetry**: the membrane is placed in a sealed chamber, the calorimeter, and its uptake of heat is measured as the temperature is slowly increased.

**Freeze-fracturing**: A lipid bilayer or a membrane is frozen and then hit sharply with a diamond knife. The resulting fracture often follows the plane between the two layers of membrane lipid: split between its inner and outer monolayers, revealing the inner surface of each.

**Electrophoresis**: several techniques which use electric field to separate molecules according to size.

**X-ray crystallography** – determine 3-D structure of proteins.

**DNA sequencing** - Amino acid and nucleotide sequences can be deduced from DNA thus it reveals:

* Functionally important amino acids.
* Families of homologous proteins.
* Structure and orientation of proteins in membrane.
* Functional relationships between proteins.

Also, it, allows specific mutation in the protein sequence to allow determination effects on function.

**Describe glycosylation**

Initial steps of **N-glycosylation** (addition of short-chain of carbohydrates to oligosaccharides) starts on cytosolic surface of the ER membrane; later steps take place in the lumen of the rough ER. The process is usually completed within the Golgi complex. It consists in adding carbohydrate chains to specific amino acid residues of proteins to form **glycoproteins**. Enzymes catalyzes this reaction.

**N-linked glycosylation (or N-glycosylation):** involves the addition of a specific **oligosaccharide** unit to the *nitrogen atom on the terminal amino group of certain asparagine residues*.

**O-linked glycosylation:** involves addition of an **oligosaccharide** to the oxygen atom on the hydroxyl group of certain serine or threonine residues. Each step of glycosylation is strictly dependent on preceding modifications.

**Describe the theory of lipid rafts and give examples of where they have important functions**

Lipid rafts or lipid microdomains are involved *in cell signaling*. In the outer membrane layer of animal cells, they are characterized by elevated concentrations of cholesterol and glycosphingolipids. Moreover, the phospholipids and glycosphingolipids in lipid rafts, are more saturated, and the rigidity and hydrophobic nature of the cholesterol, and the hydrocarbon tails of the glycosphingolipids and the phospholipids, allow tight packing, making lipid rafts thicker and less fluid than the rest of the membrane.

Lipid rafts have roles in:

* Detection and response to extracellular signals.

Lipid rafts containing receptors are coupled to lipid rafts on the inner mono layer. Receptor-mediate endocytosis (or clathrin-dependent cytosis) starts when a specific molecule (ligands) binds to their receptor molecules on the outer surface of the plasma. Receptor-ligand complexes accumulate in coated pits where invagination is facilitated by adaptor proteins: **clathrin and dynamin**. The coated vesicle that loses its clathrin, now fuses with an early endosome. Coat proteins and dynamin are recycled to the plasma membrane. It can also, move into lipid rafts located in the outer monolayer. Some lipid rafts contain **kinases**, enzymes that generate second messengers in a cell phosphorylation (addition of a phosphate group) of target molecules.

* Transport of nutrients and ions across membranes.
* Binding of activated immune system cells to microbial target.
* Transport of cholera toxin into intestinal cells.

**Explain how DNA sequencing is used to study membrane proteins**

**DNA sequencing** - Amino acid and nucleotide sequences can be deduced from DNA thus it reveals:

* Functionally important amino acids.
* Families of homologous proteins.
* Structure and orientation of proteins in membrane.
* Functional relationships between proteins.

Also, it, allows specific mutation in the protein sequence to allow determination effects on function.

**List the organelles that make up the endomembrane system and describe how molecules are trafficked through this system**

* ER (rough and smooth)
* Golgi complex
* Vacuoles
* Lysosome

Proteins synthesized in the rough ER must be directed to various destinations within the cell and outside. Sorting of proteins begins in the ER and early compartments of the Golgi (**vesicular transport model and cisternae maturation model**). The final sorting that will leave the Golgi complex occurs in the TGN. Once a protein reached its destination, it must be prevented from leaving. Each protein contains *a tag targeting to a transport vesicle* that will take it to the correct destination. Some tags can also be used to exclude materials from certain vesicles. Tags may be **an amino acid sequence, a hydrophobic domain**, **oligosaccharide side chain, membrane lipids, or lipid phosphate groups.**

**Describe endocytosis, exocytosis and phagocytosis**

**Endocytosis**: taking in of matter by a cell by invagination of its membrane to form a vacuole. A small segment of the plasma membrane folds inward. Then it pinches off to form an endocytic vesicle containing ingesting substances or particles.

**Phagocytosis**: a specific form of endocytosis, is the ingestion of large particle up to and including whole cell or microorganisms. For complex organisms, it is usually restricted to specialized cells called **phagocytes (neutrophils, macrophages, and dendritic cells).**

**Exocytosis**: process by which the content of a cell vacuole is released to the outside of the cell through fusion of the vacuole with cell membrane.

**Functions of Membranes. For each of the following statements, specify which one of the five general membrane functions (permeability barrier, localization of function, regulation of transport, detection of signals, or intercellular communication) the statement illustrates.**

(a)  Intracellular organelles that are engaged in degradative chemical reactions are limited by membranes. *Localization of function.*

(b)  On their outer surface, cells of multicellular organisms carry specific glycoproteins that are responsible for cell-cell adhesion. *Intercellular communication*

(c)  The interior of a membrane consists primarily of the hydrophobic portions of phospholipids and amphipathic proteins *Regulation of transport*

(d)  Cellular membranes have a two-layered structure with hydrophobic tails facing each other.

*Localization of function.*

(e)  All of the acid phosphatase in a mammalian cell is found within the lysosomes. *Localization of function.*

(f)  The membrane of a plant root cell has an ion pump that exchanges phosphate inward for bicarbonate outward. *Regulation of transport.*

(g)  Ions and large polar molecules cannot cross the membrane without the aid of a transport protein. *Permeability barrier.*

(h)  Insulin does not enter a target cell but instead binds to a specific membrane receptor on the external surface of the membrane, thereby activating the enzyme adenylyl cyclase on the inner membrane surface. *Detection of signals.*

(i) Adjacent plant cells frequently exchange cytoplasmic components through membrane-lined channels called *plasmodesmata*. *Intercellular communication*

**Wrong Again. For each of the following false statements, change the statement to make it true and explain your reasoning.**

(a)  Because membranes have a hydrophobic interior, polar and charged molecules cannot pass through membranes. *Can pass through the membrane only with the help of a transport protein embedded in the membrane.*

(b)  Different cellular organelles have membranes with an identical chemical composition.

(c)  Glycoproteins are proteins containing oligosaccharide chains which protrude from the ~~inner~~ outer membrane.

(d)  Membrane fluidity is affected by temperature. When temperature decreases, membrane fluidity ~~increases~~ decreases, and the temperature at which this occurs is known as the transition temperature (Tm).

(e)  You would expect membrane lipids from tropical plants such as palm and coconut to have ~~short-chain~~ long-chain fatty acids ~~with~~ without multiple C=C double bonds (saturated).

**Imagine that a new type of cell was discovered on Mars in an organism growing in benzene, a nonpolar liquid. The cell has a lipid bilayer made of phospholipids, but its structure is very different from that of our cell membranes.**





1. Draw what might be a possible structure for this new type of membrane. What might be characteristic features of the phospholipid head groups?

*Membrane will be likely reverse than ours: Two nonpolar groups will be facing the nonpolar solvent and it would have a hydrophilic interior.*

1. What properties would you expect to find in membrane proteins embedded in this membrane?

*Proteins embedded in the membrane, would likely have hydrophilic regions spanning the membrane with hydrophobic groups protruding from both sides.*

(c)  How might you isolate and visualize these unusual membranes?

**Temperature and Membrane Composition. Which of the following responses are likely to be seen when a bacterial culture growing at 37°C is transferred to a culture room maintained at 20°C? Explain your reasoning.**

1. No change in membrane fluidity

(b) A gradual increase in the proportion of saturated fatty acids in membrane lipids  
(c) Increased mobility of membrane proteins

(d) Increase in the activity of the desaturase enzyme

(e) Increase in the synthesis of saturated fatty acids

*a: unlikely because membrane fluidity is temperature dependent.*

*b: unlikely long-chain and saturated fatty acids increase fluidity.*

*c and d: are likely, short-chain and unsaturated fatty acids will increase membrane mobility.*

*e: unlikely because bacteria do not contain cholesterol.*

**Membrane Fluidity and Temperature. The effects of temperature and lipid composition on membrane fluidity are often studied by using artificial membranes containing only one or a few kinds of lipids and no proteins. Assume that you and your lab partner have made the following artificial membranes:**

**Membrane 1:** Made entirely from phosphatidylcholine with saturated 16-carbon fatty acids.

**Membrane 2:** Same as membrane 1, except that each of the 16-carbon fatty acids has a single cis double bond.

**Membrane 3:** Same as membrane 1, except that each of the saturated fatty acids has only 14 carbon atoms.

After determining the transition temperatures of samples representing each of the membranes, you discover that your lab partner failed to record which membranes the samples correspond to. The three values you determined are –36°C, 23°C, and 41°C. Assign each of these transition temperatures to the correct artificial membrane, and explain your reasoning.

*Membrane 1: saturated, 16C*

*Membrane2: less saturated, 16C*

*Membrane 3: saturated, 14C*

*T2 < T3 < T1*

*Double bonds are very disruptive of phospholipid packing in the membrane, so M2 has the lowest Tm.*

**The Little Bacterium That Can’t. *Acholeplasma laidlawii* is a small bacterium that cannot synthesize its own fatty acids and must therefore construct its plasma membrane from whatever fatty acids are available in the environment. As a result, the *Acholeplasma* membrane takes on the physical characteristics of the fatty acids available at the time.**

1. If you give *Acholeplasma* cells access to a mixture of saturated and unsaturated fatty acids, they will thrive at room temperature. Can you explain why?

*Under these conditions, Acholeplasma cells can incorporate an appropriate combination of saturated and unsaturated fatty acids into their membrane to obtain optimum level of membrane fluidity.*

1. If you transfer the bacteria of part (a) to a medium containing only saturated fatty acids but make no other changes in culture conditions, they will stop growing shortly after the change in medium. Explain why.

*Saturated fatty acids make a membrane less fluid. If only saturated acids are available, the Tm increases until the Tm = ambient temperature and the membrane will gel.*

1. What is one way you could get the bacteria of part (b) growing again without changing the medium? Explain your reasoning.

*Temperature could be raised to preserve membrane fluidity.*

1. If you were to maintain the *Acholeplasma* culture of part (b) under the conditions described there for an extended period of time, what do you predict will happen to the bacterial cells? Explain your reasoning.   
   *When a membrane gels, all cell functions that depends on the mobility of membrane proteins or lipids, will be impaired or disrupted. Without the ability of transport solutes, detection and transmission of signals, and to carry out other membrane dependent processes, the cell will die.*
2. What result would you predict if you were to transfer the bacteria of part (a) to a medium containing only unsaturated fatty acids without making any other changes in the culture conditions? Explain your reasoning.

*Unsaturated fatty acids increase membrane fluidity, thus increasing the permeability of the membrane to ions and other solutes, and making it impossible to maintain critical concentration gradients.*

**How do differences in the structure and arrangement of rough versus smooth ER reflect their different functions?**

*Both types of ER make phospholipids, but membrane and secretory proteins are all produced by the ribosomes on the rough ER. Other than the lipid synthesis, smooth ERs are involved in the metabolism of carbohydrates and steroids.*

<https://pediaa.com/difference-between-smooth-and-rough-er/>

**Why is it necessary for material flowing through the Golgi to move in both the anterograde and the retrograde directions?**

*Every time a secretory vesicle fuses with the plasma membrane by exocytosis, a bit of membrane that originated in the ER becomes part of the plasma membrane. To balance the flow of lipids towards the plasma membrane, and to ensure a supply of components for forming new vesicles, the vesicles from the Golgi cisternae are sent back towards the ER.*

**What features of membrane lipids and proteins contribute to their proper trafficking and targeting in cells?**

*Each protein contains a specific tag targeting a protein to a vesicle to reach its destination.*

*Membrane lipid could be also tagged to help vesicles to reach them.*

**What problems would a cell have if it could not produce lysosomes?**

*The accumulation of specific substances within the cell due to the absence of lysosomes, severely impairs the cell or destroy it.*

**Why is it important for the biochemical reactions occurring in peroxisomes to be isolated from the cytoplasm in a separate organelle?**

*The generation and degradation of hydrogen peroxide (H2O2) occurs within the peroxisome protecting other parts of the cell from exposure to this harmful compound.*

**Endoplasmic Reticulum. For each of the following statements, indicate if the statement is true of the rough ER only (R), of the smooth ER only (S), or of both rough and smooth ER (RS).**

(*a) Contains less cholesterol than does the plasma membrane RS*

*(b) Does not contain free ribosomes R  
(c) Is involved in steroid biosynthesis S  
(d) Is involved in the breakdown of polycyclic aryl hydrocarbons S*

*(e) Is the site for biosynthesis of secretory proteins R*

*(f) Is the site for the folding of membrane-bound proteins R*

*(g) Tends to form tubular structures S  
(h) Usually consists of flattened sacs R  
(i) Visible only by electron microscopy RS*

**Biosynthesis of Integral Membrane Proteins. In addition to their role in cellular secretion, the rough ER and the Golgi apparatus are also responsible for the biosynthesis of integral mem- brane proteins. More specifically, these organelles are the source of glycoproteins commonly found in the outer phospholipid monolayer of the plasma membrane.**

1. In a series of diagrams, depict the synthesis and glycosylation of glycoproteins of the plasma membrane.

*Starts in the cytosol side of the ER and ends in the ER lumen: addition of carbohydrate to integral protein.*

1. Explain why the carbohydrate groups of membrane glycoproteins are always found on the outer surface of the plasma membrane.

*Outer monolayer originally faced the rough ER and Golgi, where the enzymes involved in glycosylation are located.*

(c)  What assumptions did you make about biological membranes in order to draw the diagrams in part a and answer the question in part b?

**Cellular Digestion. For each of the following statements, indicate the specific digestion process or processes for which the statement is true: phagocytosis (P), receptor-mediated endocytosis (R), autophagy (A), or extracellular digestion (E). Each statement is true of one or more of these processes.**

(a) Can involve endocytosis only *P,R,A,E*(b) Can direct contents of newly formed vesicles to lysosomes *A*

(c) Highly efficient uptake of extracellular nutrients *P,R,E*(d) Digested material is sourced from another species *A*(e) Involved in the progression of rheumatoid arthritis  
(f) Important for certain developmental processes *P,A,E*(g) Involves acid hydrolases *P,R,A,E*  
(h) Involves fusion of endocytic vesicles with an early endosome *P,R*

(i) Involves fusion of lysosomes with the plasma membrane *E*  
(j) Occurs within lysosomes *P, R, A*(k) Serves as a source of nutrients within the cell *P,R,A*

**Lysosomal Storage Diseases. Despite a bewildering variety of symptoms, lysosomal storage diseases have several properties in common. For each of the following statements, indicate whether you would expect the property to be common to most lysosomal storage diseases (M), to be true of a specific lysosomal storage disease (S), or not to be true of any lysosomal storage diseases (N).**

(a) Impaired metabolism of glycolipids that causes mental deterioration

(b) Leads to accumulation of degradation products in the lysosome

(c) Leads to accumulation of excessive amounts of glycogen Sin the lysosome

(d) Results from an inability to regulate the synthesis of glycosaminoglycans

(e) Results from an absence of functional acid hydrolases  
(f) Results in accumulation of lysosomes in the cell  
(g) Symptoms include Muscle weakness and mental retardation.

(h) Triggers proliferation of organelles containing catalase

# Module 6 – Membrane Transport

Hydrophobic <=> non-polar

Polar <=> hydrophilic with exceptions (sugar)

**Explain how hydrophobic molecules cross cell membranes**

Small hydrophobic molecules, including, uncharged and no polar molecules (oils, steroids), can cross the bilayer membrane by simple diffusion using the concentration gradient existing between the inside of the cell and its outside. They pass though the gaps in the membrane

which are due to a mixture of unsaturated or saturated fatty acids tails in both of the monolayers of the bilayer. Large non polar molecules need to use facilitated diffusion to cross the lipid bilayer.

https://www.quora.com/How-do-hydrophobic-non-polar-molecules-cross-the-plasma-membrane-when-they-have-to-pass-through-the-polar-phosphate-group-first

<https://www-ncbi-nlm-nih-gov.proxy1.library.jhu.edu/books/NBK9847/>

**Distinguish between channel proteins and carrier proteins**

* **Channel proteins** form hydrophilic channels through the membrane that allow passage of solutes without major change in the conformation of the molecule, this process is thus quicker compared to carrier protein transport. Most of the channel proteins are small and very specific, and are referred **to ion channels**. Some of these channels, such as the **pores** found in the outer membrane of bacteria, mitochondria and chloroplasts, are relatively large and nonspecific. These pores are formed by transmembrane proteins called **porins**, and allow selected hydrophilic solutes with MW up to about 600 Da to diffuse across the membrane.
* **Carrier proteins** (also called *transporters or permeases*) bind one or more solute molecule on one side of the membrane and then undergo a conformational change that transfers the solute to the other side of the membrane, shielding the polar or charged groups of the solute from the nonpolar interior of the membrane. The carrier proteins are analogous to enzymes in their specificity and kinetics. They can specific to one compound, or a small group of closely related compounds or even to a specific stereoisomer (GLUT1 recognizes only glucose and few closely related monosaccharides, such as galactose, and it accepts the D- but not L-isomer of these sugars. Like enzymes, carrier-facilitated proteins exhibit saturation kinetics (upper limit velocity Vm, and constant Km corresp. to the concentration of solute needed to achieve ½ of Vm).

**Define diffusion. Explain why diffusion is a passive and spontaneous process**

Diffusion is the result of second law of thermodynamic which states that “chemical reactions and physical processes proceed in the direction of decreasing free energy”, for the cell, the free energy is minimized as molecules flow down their concentration gradient (meaning from higher to lower concentration regions) and as ions flow down their electrochemical gradient. As a result, whenever there exists a difference of concentration of a specific substance, a concentration gradient, diffusion happens, the substance is transported to regions of lower concentration and this process does not require any metabolic energy (exergonic).

**Explain why a concentration gradient of a substance across a membrane represents a potential energy**

A concentration gradient of a substance across membrane corresponds to an energy which is proportional to the energy released by moving the substance down its concentration gradient which is used, in indirect active transport, to move a transported solute against its concentration or electrochemical potential.

**Explain how transport protein facilitate diffusion**

A transport protein is a specialized transmembrane protein that serves either as a hydrophilic channel through an otherwise hydrophobic membrane or as a carrier that binds a specific solute on one side of the membrane and then undergoes a conformational change to move the solute across the membrane.

**Distinguish between osmosis, facilitated diffusion, and active transport**

* **Osmosis** is the diffusion of water across a selectively permeable membrane. Because most solutes cannot cross cell membranes by diffusion, water will diffuse from the side of the membrane with the lower solute concentration (more water) to the side with higher solute concentration (less water). At equilibrium, the overall solute concentration is the same.
* **Facilitated diffusion** allows large nonpolar or polar and charged solutes cross the membrane using transport proteins, it is a passive transport as the solute diffuses down the concentration or electrochemical gradient, and does not require metabolic energy (exergonic process): ex.., movement of glucose across the plasma membrane of erythrocyte or any cell. Passive transport is nondirectional.
* **Active transport** moves a solute up its concentration or electrochemical gradient, away from its thermodynamic equilibrium therefore requires energy (endergonic). It is unidirectional. It occurs only when coupled to an exergonic chemical reaction (**direct**) or exergonic inward movement of ions-protons (**indirect**).

<https://physics.stackexchange.com/questions/271228/does-there-exist-a-membrane-that-has-unbalanced-concentration-as-equilibrium>

**Describe the two forces that combine to produce an electrochemical gradient**

The movement of an ion is determined by its electrochemical gradient which is the sum of its concentration gradient of that ion and the net difference in charge for that ion across the membrane.

**Describe the process of co-transport**

Carrier proteins transport one solute (uniporter, e.g., glucose) or two solutes. When two solutes are transported and their transport is coupled such that transport of either stops if the other is absent is called **co-transport** (*coupled transport*). If the two solutes are moved in same direction: the co-transport is **symport** otherwise it is **antiport**.

**Explain how large molecules can be transported across cell membranes**

Large non polar molecules are transported by facilitated diffusion, large polar molecules are transported by facilitated diffusion or active transport.

**Define facilitated diffusion and why it is important in membrane transport**

Lipid bilayers are readily permeable to small molecules, and relatively permeable to nonpolar molecules and less permeable to polar molecules. Lipid bilayers are very impermeable to ions. Polar, large polar/non polar molecules and ions need proteins or pumps to be able to cross the lipid bilayer. Facilitated diffusion allows to move a solute down its concentration gradient or electrochemical gradient without requiring an input of metabolic energy, it also speeds up the movement of substances which could cross the plasma membrane but to a slower rate.

**Discuss in details how carrier proteins assist in moving substances up a concentration gradient**

Cells also require transport/carrier proteins that actively pump certain solutes across the membrane against their concentration or electrochemical gradients. This process is known as active transport. The pumping activity is directional because it is tightly coupled to a source of metabolic energy, such as ATP hydrolysis or an ion gradient.

The active transport can be:

* **Direct** as it involves a transport coupled to an exergonic chemical reaction mostly ATP hydrolysis.
* **Indirect** when it is driven by the co-transport of cations-protons; the exergonic inward movements of protons provide the energy to move the transported solute against its concentration gradient or electrochemical potential. Concentration gradient of one molecule provides the energy for the transport of the second molecule against its concentration gradient.

**Draw a diagram depicting Na-K pump**

1. Initial binding of 3 Na+ to E1 on inner side of the membrane
2. Na+ binding triggers autophosphorylation of the alpha subunit using ATP and ADP is released, causing E1 to E2.
3. A conformational change to E2 expels 3 Na+ to the outside of the cell.
4. 2 K+ from outside the cell bind to E2.
5. K+ binding triggers dephosphorylation causing conformational change back to E1.
6. During this process, 2 K+ expelled to the inside as ATP binds.

**For which of the three types of transport mechanisms is the magnitude of the concentration gradient relevant? For which is the electrochemical gradient important?**

*Magnitude of concentration gradient relevant: simple diffusion*

*Magnitude electrochemical gradient: indirect active transport.*

**How is osmosis different from the simple diffusion of molecular oxygen (O2) across a membrane? How are they similar?**

*Simple diffusion is the movement of solutes across a membrane permeable to the solute from the region of high concentration to low solute concentration; osmosis is the movement of water across a selectively permeable membrane, a membrane not permeable to the dissolved solute from the region where solute concentration is lower to the region with higher solute concentration, or from a more dilute solution to a region of more concentrated solution. At equilibrium the solute concentration is equal on both sides of the membrane.*

*They are both transport molecules, and do not require any input of metabolic energy to occur, and they both reach equilibrium when solute concentration is same on both sides of the membrane (follow 2nd law of thermodynamic: equilibrium: free energy of system is minimized).*

**How would you determine whether a specific integral membrane transporter is operating by facilitated diffusion or active transport?**

*Active transport is unidirectional compared to facilitated diffusion. In facilitated diffusion, the solutes are transported by the membrane transporter down their concentration or electrochemical gradients. Active transport has a directionality, an active transport system that transports a solute across the membrane in one direction will not transport that solute in the other direction. So, by changing the concentration of the solute across the membrane, a membrane transporter operating by facilitated diffusion will still operate, and if the transporter system stops functioning, the transporter is operating by active transport.*

**Both the Na+/glucose symporter and the Na+/K+ pump move sodium ions across a membrane. How is the movement different for the two types of transporters?**

*Na+/glucose symporter and the Na+/K+ pump uses as ATP as source of energy thus are active transport.*

* *Na+/glucose is a symporter uses secondary active transport and which transports the glucose and 2 Na+ ions inward the cell, in the same direction. The glucose is transported against its concentration gradient and is driven simultaneously by the inward transport of the sodium ions down their electrochemical gradient maintained by the Na+/K+ pump.*
* *Na+/K+ pump uses direct active transport, it moves 3 Na+ ions out of the cell allowing two K+ ions to move in the cell, in both cases the ions are moved against their electrochemical concentration gradients.*

**True or False? Indicate whether each of the following statements about membrane transport is true (T) or false (F). If false, reword the statement to make it true.**

*(a)  Facilitated diffusion of glucose occurs rapidly because the concentration gradient is maintained by ~~packaging intracellular glucose into vesicles~~ transporting glucose across the membrane with the glucose transporter GLUT1.*

*(b)  The exergonic movement of an ion coupled with the movement of a solute ~~down~~ up a concentration gradient is an example of secondary active transport.*

*(c)  The Keq value for the diffusion of polar molecules out of the cell is less than one because membranes are essentially impermeable to such molecules.*

*(d)  Aquaporins facilitate the rapid movement of water molecules into or out of cells. T*

*(e)  Oxygen can move freely across the plasma membrane by simple diffusion. T*

*(f)  In simple diffusion, the net rate of transport for a specific substance is ~~indirectly~~ directly proportional to the concentration difference for that substance across the membrane. T*

*(g)  ABC transporters are of medical interest because they are known to be involved in drug resistance.*

*(h) Transport channel proteins have a high level of specificity for a solute. T*

**Telling Them Apart. From the following list of properties, indicate which one(s) can be used to distinguish between each of the following pairs of transport mechanisms.**

**Transport Mechanisms**

(a)  Simple diffusion; facilitated diffusion 3,7

(b)  Facilitated diffusion; active transport 2,4,6

(c)  Simple diffusion; active transport 2,3,4,6,7

(d)  Direct active transport; indirect active transport 4,5

(e)  Symport; antiport 1

(f)  Uniport; coupled transport 5

(g)  P-type ATPase; V-type ATPase 8

**Properties**

1. Directions in which two transported solutes move

2. Direction the solute moves relative to its concentration gradient or its electrochemical potential

3. Kinetics of solute transport

4. Requirement for metabolic energy

5. Requirement for simultaneous transport of two solutes

6. Intrinsic directionality

7. Competitive inhibition

8. Sensitivity to the inhibitor vanadate

**Mechanisms of Transport. For each of the following statements, answer with a D if the statement is true of simple diffusion, with an F if it is true of facilitated diffusion, and with an A if it is true of active transport. Any, all, or none (N) of the choices may be appropriate for a given statement.**

**(a)**Requires the presence of an integral membrane protein A,F

**(b)**Solutes move down their free energy gradient in the direction of thermodynamic equilibrium. D

**(c)**Is not subject to saturation D

**(d)**Requires the hydrolysis of ATP A

**(e)**Is a way of establishing a difference in the concentration gradient of solutes across a membrane A

**(f)**Applies only to small, nonpolar solutes D

**(g)**Applies only to ions N

**(h)**Transport can occur in either direction across the membrane, depending on the prevailing concentration gradient D,F

**(i)**Has a positive ∆*G*

**(j)**Usually has intrinsic directionality A

Discounting the Transverse Carrier Model. At one time, membrane biologists thought that transport proteins might act by binding a solute molecule or ion on one side of the membrane and then diffusing across the membrane to release the solute molecule on the other side. We now know that this transverse carrier model is almost certainly wrong. Suggest two reasons that argue against such a model. One of your reasons should be based on our current under- standing of membrane structure and the other on thermodynamic considerations.

1. *Integral membrane proteins are embedded in the membrane and protrude from one or both sides base on their hydrophobic or hydrophilic regions.*
2. *To traverse the membrane, a protein will have to have its hydrophilic region(s) to move through the hydrophobic interior of the membrane which will require a lot of energy and hence thermodynamically impossible.*

**Carboxyl group: CO2**

**Hydroxyl group: OH**

**Phosphoryl group: PO3-**

**Amino group: H-N-H**



**Phosphester bond:** bond between phosphate group and sugar.

**Phosphodiester bond:** phosphate group linked to two adjacent nucleotides.

**Glycosidic bond**: bond between a carboxyl and hydroxyl group.

**Phosphorylation**: adding a phosphate group to a substrate.

**Carboxylation**: adding a carboxyl group to a substrate.

**Hydrogenation**: adding a hydrogen group to a substrate.

**Stereoisomers**: geometrical positioning of atoms are mirror images.

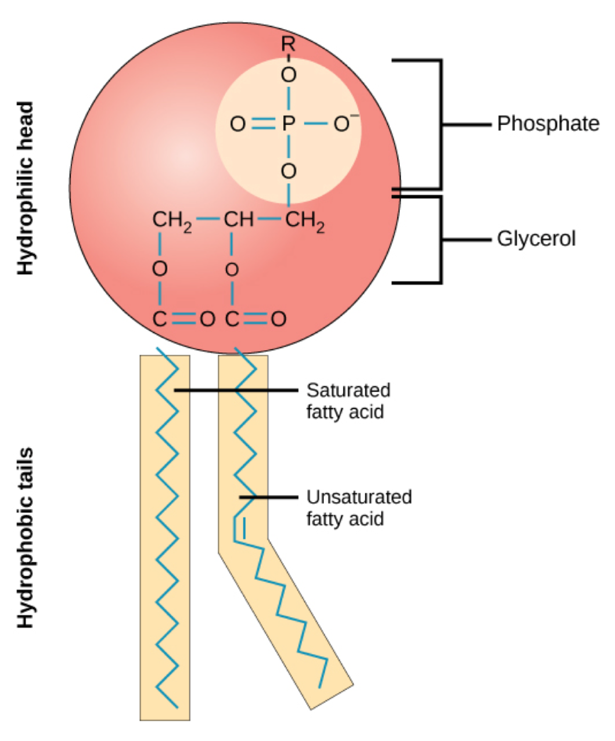
**Oligosaccharides**: saccharides with 30-50 sugars.

**Polysaccharides**: saccharides with over 50 sugars.

**Proteoglycans**: structural function.

**Glycolipids**: lipids with a carbohydrate attached, component of cell membrane, cellular recognition.

**Phospholipids**, also known as phosphatides, are a class of lipids whose molecule has a hydrophilic "head" containing a phosphate group and two hydrophobic "tails" derived from fatty acids, joined by an alcohol residue (usually a glycerol molecule).



**Glycoprotein**: proteins + oligosaccharides.

**Extracellular protein**: collagen.

# Module 8 – DNA, Chromosomes, the Nucleus

**Discuss the history of the discovery of DNA from Miescher’s discovery of DNA**

Miescher when studying fish sperm believed that DNA was involved in the transmission of hereditary information.

**Explain in details Chargaff’s rules**

* A **dsDNA** molecule globally has percentage base pair equals: %A = %T, %G=%C.
* A **sDNA** also has percentage base pair equals: %A = %T, %G=%C.
* For the same species, for all DNA samples, count of A = count of T and count of G = count of C.
* Or A + T = C + G.
* DNA base composition varies from species to species: nucleotides ratios (A/T, C/G, (A+T)/(C+G)) varies.

**Explain how genome size varies generally increases with an organism’s complexity**

Genome size increases by duplication, insertion or polyploidization (multiplication of complete chromosome). More complex organisms often have larger genomes, but there are some very complicated organisms with very small genomes and likewise some simple organisms with very large genomes (e.g., trillium).

**Discuss how tiny differences in genome sequence distinguish people from one another**

The human genome differs in all people by about. 0.001 percent. Small variation in the nucleotide sequences like a different pairs account for different gene expression and differences in people appearance and health. People who are closely related, have more similar DNA.

**Explain and contrast how bacteria package DNA in chromosomes and plasmids while eukaryotes package DNA in chromatin and chromosomes.**

* Bacteria organize the genome as chromosome which is a long either circular or linear DNA molecule; the most common arrangement being circular. In addition, bacteria cell may contain one or more **plasmids**; usually small circular molecules of DNA that carry genes both for their own replication and, often, for one or more cellular functions.
* By comparison, eukaryotic chromosome contains a single, linear DNA molecule of enormous size. When bound to proteins like **histones**, DNA is known as **chromatin**. When chromatin fibers condense and fold into much larger, compact structure they become **chromosomes**.

# Module 9 – Signal Transduction

**Describe what is meant by “signal transduction” and give examples of different types of chemical signals that can be received by cells.**

The ability of a cell to sense and respond to its environments through chemical signals using ligand-receptor binding by altering its behavior or gene expression is called signal transduction:

* MAPK/ERK pathway: a pathway that links intracellular responses to the binding of growth factors to cell surface receptors. **Epidermal growth factor signaling pathway (EGF):** growth factors bind to their receptors. Activated receptors trigger a series of events: activation of kinase Raf, active Raf phosphorylation activates MEK, which phosphorylates and activates ERKs, which phosphorylate and activate a variety of target molecules (**c-Myc**, cytoplasmic targets) which promote cell growth and division.
* Many G proteins use Inositol Trisphosphate (IP3) and Diacylglycerol (DAG) as second messengers.
* Calcium signaling:
  + Ca2+: essential role in regulating a variety of cellular functions.
  + Maintained at low concentration levels through **calcium ATPases** in the plasma membrane and ER; these transport calcium ions out of the cytosol.
  + Calcium concentrations can be released by opening calcium channels in plasma membrane as in neuronal signaling.
  + Calcium can also be released from storage in ER through IP3 receptor channel.
  + Ca2+ fluorescent dyes.
  + Ca2+ signaling in the β-cells of the pancreas leads to the release of insulin, Ca2+ signaling in muscle cells leads to muscle contraction.
* **cAMP**: another second messenger used in many different cell types is cyclic adenosine monophosphate (cyclic AMP or cAMP), a small molecule made from ATP. In response to signals, an enzyme called adenylyl cyclase converts ATP into cAMP. The enzyme is inactive until bound to activated GSα (by receptor-ligand stimulated acquisition of GTP and release form. GSβδ). Once G proteins becomes inactive, adenylyl cyclase stops making cAMP. Once generated, cAMP can activate an enzyme called protein kinase A (PKA), enabling it to phosphorylate its targets and pass along the signal. Protein kinase A phosphorylates a variety of proteins, using ATP as source of phosphate. When cAMP levels are reduced, PKA activation is also reduced; phosphorylation of cytosolic GPCR sites slows.

**Explain receptor-ligand interactions and receptor affinity**

The binding of a receptor and ligand is similar to the binding between an enzyme and its substrate. Relationship between the ligand and the number of receptors occupied is the receptor affinity. The dissociation constant, K diss the free ligand needed to produce a state in which half the receptors are occupied. Receptors with high affinity have low Kd (and vice versa).

**Describe signal amplification and give an example of this phenomenon**

Very small quantities of ligand can trigger a cascade of events, and each intermediate step of this cascade stimulates the production of many more molecules than the previous step for the next step. Multiplication of the effect of the initial signal is signal amplification.

Binding of epinephrine to G protein-linked receptor (1 molecule) can trigger the production of 108 glucose-1-phosphate.

**Describe G-protein coupled receptors (GPCR), including their structure and regulation**

Ligand biding to a GPCR causes a change in its conformational changes in the α-subunit of a G protein: α-subunit causing the GDP to dissociated; GTP can readily bind in place of GDP. GTP binding causes further conformational change; the activated α complex dissociates from the activated β-γ-complex or not. The α-subunit can now regulate the activity of the target protein. The activated target protein now relates the signal to other components in the signaling cascade. Eventually the α-subunit hydrolyses GTP to GDP which inactivates the subunit. This step is often accelerated by the binding of regulators of G-protein-signaling proteins (RGS). The inactivated α-subunit reforms an inactive G-protein with the β-γ-complex; turning off other downstream events. For long stimulation, the receptor eventually inactivated even if the activated ligand remains bound (desensitization or adaption to a persistent stimulus). G protein-linked receptor kinases (GRKs) act on activated receptors by phosphorylation of the cytosolic portion of the activated GPCR. Once the receptor has been phosphorylated, it binds with high affinity to β-arrestin which deactivates the receptor by preventing its interaction with G proteins. The receptor forms 7 transmembrane alpha helices connected by alternating cytosolic or extracellular loops.

**Provide an example where disruption of GPCR signaling relates to disease**

When *V.cholerae* bacterium secretes cholera toxin in the guts; it is the internalized via endocytosis in the intestinal cells. The toxin, in conjunction with a protein in the intestinal cells, can chemically modify Gs (by adding ADP-ribose to it) so that it can no longer hydrolyzed GTP to GDP. As a result, Gs cannot be shut off, and cAMP levels remain high. This causes prolonged activation of the cystic fibrosis chloride transporter (CFTR). The intestine now secretes large amounts of chloride ions and sodium which leads to severe dehydration and death.

The **pertussis toxin** secreted by *Bordetella pertussis* (which causes whooping cough) acts in a similar manner but on the inhibitory G protein, Gi. This protein normally shuts off adenylyl cyclase. When inactivated by pertussis toxin, Gi no longer inhibits adenylyl cyclase. The resulting fluid accumulation in the lungs leads to the characteristic cough.

**Describe protein kinase-associated receptors, including their structure and regulation**

Receptor Tyrosine Kinases (RTKs) exist ass monomers (single polypeptide chain) with one transmembrane segment. Each receptor has an extra cellular which contains the ligand-binding domain and on the cytosolic side the tyrosine kinase domain. When growth factor is present, it binds and induces dimerization of receptor monomers resulting in receptor activation. The activated receptor then autophosphorylates tyrosine in its intracellular domain. The phosphotyrosine are binding sites for intracellular signaling proteins which in turn can activate downstream proteins. An amplification signa occurs for each growth factor molecule.

* Once autophosphorylation of the receptors occurs, the receptors recruit cytosolic proteins like **Ras and MAP kinases.**
* Receptor tyrosine kinases can also activate **phospholipase C,** leading to **production of IP3 and DAG.**
* Signaling components such as those in the Ras pathway are sometimes assembled into large multiprotein complexes that make cascades more efficient.

Protein kinases and phosphatases are regulated by protein-protein interactions, binding of ligands, and reversible or irreversible covalent modifications such as **phosphorylation** and limited proteolysis.

**Explain the importance of hormone signaling as it related to normal organismal function vs. disease**

Insulin is a peptide hormone that has rapid and longer-lasting effects on a variety of cells. It reduces blood glucose levels by stimulating uptake into muscle and adipose cells, and stimulating glycogen synthesis. Breakdown of glycogen is facilitated by the enzyme glycogen phosphorylase, resulting in release of a glucose-1-phosphate. Long-term effects of insuling, such as production of enzymes involved in glycogen synthesis, require higher levels of insulin sustained over many hours. To exert its effects, insulin binds to receptor tyrosine kinases. Type I diabetes is an autoimmune disorder resulting in loss of insulin-producing cells in the islets of Langerhans. It can be somewhat treated with insulin. Type II diabetes appears to result from resistance to insulin and is not effectively treated with insulin.

# Module 10 – Recombinant DNA Technology

**Explain the underlying mechanisms of gene cloning**

Gene cloning involves the production in vitro of new DNA molecules which contain novel combinations of genes or oligonucleotides and the propagation of such recombinant DNA molecules by the exploitation in vivo of the replicative mechanisms of bacteria and other organisms.

**Discuss the practical aspects of applying recombinant DNA technology**

Gene cloning is the isolation and amplification of a specific DNA fragment. Most of these fragments are created either by digesting and existing piece of DNA with restriction enzymes or by targeting it via PCR. After successful isolation, the DNA of interest is ligated into a vector plasmid a double-stranded circular piece of DNA that can be propagated in E.coli. Vectors used in the laboratory represent a shorter version of naturally occurring plasmids: for PCR they are DNA primers, generally 15-20 nucleotides long and consist of sequences that are complementary to sequences located at the two ends of the DNA segment being amplified. For PCR DNA polymerase is then added to catalyze the synthesis of complementary DNA strands using the two primers as starting points.

Describe recombinant gene expression systems

Describe the different techniques used in recombinant DNA technology

[Tools for rDNA](https://byjus.com/biology/recombinant-dna-technology/#tools)

The **enzymes** which include the restriction enzymes help to cut, the polymerases- help to synthesize and the ligases- help to bind. The restriction enzymes used in recombinant DNA technology play a major role in determining the location at which the desired gene is inserted into the vector genome. They are two types, **namely Endonucleases and Exonucleases**.

The Endonucleases cut within the DNA strand whereas the Exonucleases remove the nucleotides from the ends of the strands. The restriction endonucleases are sequence-specific which are usually palindrome sequences and cut the DNA at specific points. They scrutinize the length of DNA and make the cut at the specific site called the restriction site. This gives rise to sticky ends in the sequence. The desired genes and the vectors are cut by the same restriction enzymes to obtain the complementary sticky notes, thus making the work of the ligases easy to bind the desired gene to the vector.

The **vectors** – help in carrying and integrating the desired gene. These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism. **Plasmids** and **bacteriophages** are the most common vectors in recombinant DNA technology that are used as they have a very high copy number. The vectors are made up of an origin of replication- This is a sequence of nucleotide from where the replication starts, a selectable marker – constitute genes which show resistance to certain antibiotics like ampicillin; and cloning sites – the sites recognized by the restriction enzymes where desired DNAs are inserted.

**Host organism** – into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA with the help of the enzymes.

There are a number of ways in which these recombinant DNAs are inserted into the host, namely – microinjection, biolistics or gene gun, alternate cooling and heating, use of calcium ions, etc.

Describe the advances made possible by recombinant DNA technology

Application of rDNA, PCR or CRISPR are numerous and cross-domains: applications are related to protein production, vaccines, growth hormones, antibodies, anticancer drugs, also:

* In agriculture and food: for production of important enzymes, production of products with less toxicity, increased yield, and nutritional values, production of products with specific savor, with increased resistance to weather vagaries, and resistance to parasite.
* In gene therapy: for treatment of genetic human, or cardiovascular diseases.
* In environment: genetic engineering has been used in various challenging environmental
* situations like in bioremediation.
* Sequencing methods overall: have been critical in understanding the mechanisms of cellular physiology, in analysis of gene expression, detection of infectious diseases by amplifying limited availability of biological samples in which the presence of the pathogen is not always detectable with other techniques, in genetic diseases and diagnostic, lastly in genetic engineering for directed mutagenesis. Sanger sequencing, another cloning technique, was a critical tool for the human genome project.
* Gene Therapy – It is used as an attempt to correct the gene defects which give rise to heredity diseases.
* Clinical diagnosis – ELISA is an example where the application of recombinant
* Recombinant DNA technology is widely used in Agriculture to produce genetically-modified organisms such as savory tomatoes, golden rice rich in proteins, Bt-cotton to protect the plant against ball worms and lot more.
* In the field of medicines, recombinant DNA technology is used for the production of Insulin.

# Module 11 – Cell cycle Mitosis and DNA replication

**Describe the 4 main parts of the cell cycle and the role of checkpoints**

The 4 phases of the cell cycle are: G1, followed by S when DNA is replicated, then G2 and Mitosis when the cells actually divide; the nucleus first, followed by the cytoplasm. The two copies of each chromosome made during S phase are distributed into daughter cells during M phase. Most of the time is spent in interphase: G1, S, and G2. Cells spend very little time in M phase. The overall length of the cell cycle; called the generation time in cultured mammalian cells is about 18-24 hours. Cells that become arrested in G1, awaiting a signal that will trigger reentry into the cell cycle are in G0. Cells use a series of checkpoints that ensure each phase is completed properly before the next one begins, if not daughter cells might be abnormal (e.g., aneuploidy (incorrect number of chromosomes) could result). Checkpoints are:

* Mitotic spindle checkpoint prevents anaphase from beginning before the chromosomes are all attached to the spindle.
* DNA replication checkpoint ensures that DNA synthesis is complete before the cell begins mitosis.
* Series of DNA damage checkpoints monitor DNA for damage and halt the cell cycle: e.g., p53 protein.

List the stages of mitosis and the role of chromosomes

* **Prophase**: After DNA replication, cells exit S phase and enter G2 phase, where final preparations are made for entry into mitosis.

Toward the end of G2, chromosomes begin to condense into more compact, folded structures.

The G2 => **prophase** transition is not sharply defined but cells are in prophase when individual chromosomes become visible.

At the beginning of mitosis, chromatin folds and condenses to produce visible chromosomes.

At this part of the cycle, DNA has replicated, so each chromosome is composed of two sister chromatids.

**The mitotic spindle microtubules** distribute the chromatids to opposite ends of the cell.

Centrosomes function as microtubule-organizing centers (**MTOCs**) where microtubules are assembled and anchored.

At the beginning of prophase, the two centrosomes separate from each other and move toward opposite sides of the nucleus.

Centrosomes complete their movement to opposite sides of the nucleus and the spindle MTs contact the condensed chromosomes.

MTs attach to chromosomes in the **centromere** region.

DNA in centromeres consists of simple tandemly **repeated CEN sequences**, with considerable variation among species.

**CENP-A** recruits additional proteins to the centromere to form the **kinetochore**, to which MTs attach.

Kinetochore proteins begin to assemble on centromeres shortly after S phase.

During prometaphase spindle MTs bind the kinetochores associated with each chromatid.

Forces exerted by these kinetochore microtubules gradually move chromosomes toward the center of the cells.

* **Metaphase**: A cell is in **metaphase** when the fully condensed chromosomes are aligned at the **metaphase plate** (a plane equidistant between the two poles of the spindle). Agents that interfere with spindle function (e.g., **colchicine**) are used to arrest cells at metaphase. Examining metaphase cells allows chromosome to be id. Generating a **karyotype**.
* **Anaphase**: Shortest phase of mitosis. Two sister chromatids of each chromosome abruptly separate and move toward opposite poles.
  + In **anaphase A,** the chromosomes are pulled toward spindle poles as kinetochore MTs get shorter.
  + **In anaphase B,** the spindle poles themselves move away from each other as polar MTs lengthen.
* **Telophase**: At the beginning of telophase, daughter chromosomes arrive at the poles of the spindle. Chromosomes uncoil into **interphase chromatin**.
  + Nucleoli reappear and nuclear envelopes reform.
  + During this period, cytokinesis also take place.

**Explain the purpose of karyotyping**

Karyotyping is the process of pairing and ordering all the chromosomes of an organism, thus providing a genome-wide snapshot of an individual’s chromosomes. Clinical cytogeneticists analyze human karyotyping to detect gross genetic changes, anomalies involving several megabases or more of DNA.

Karyotypes can reveal changes in chromosome number associated with **aneuploid** conditions, such as trisomy 21 (Down syndrome). Careful analysis of karyotypes can also reveal more subtle structural changes, such as chromosomal deletions, duplications, translocations, or inversions. In fact, as medical genetics becomes increasingly integrated with clinical medicine, karyotypes are becoming a source of diagnostic information for specific birth defects, genetic disorders, and even cancers.

**Describe the importance of motor proteins during mitosis**

Motor proteins are: kinesins, dynein and myosin.

They use energy from ATP to change shape and exert force that causes movement of attached structures. Motor proteins play at least 3 roles in the movement of anaphase chromosomes:

* Kinesin proteins can bind to the end of a microtubule and induce it to depolymerize. One of these kinesins is located at the (+) end of the kinetochore microtubules, and the other at the (-) end. The kinesin at the (+) end is embedded in the kinetochore, where it induces microtubule depolymerization and moves the chromosome toward the spindle pole. At the same time, the kinesin located at the (-) end is embedded in the spindle pole, where it induces microtubule depolymerization and “reels in” the microtubules and their attached chromosomes.
* During anaphase B, bipolar kinesin motors bind to overlapping polar microtubules causing them to slide apart, thereby forcing the spindle poles away from each other. As the MT slide apart, they are strengthened by the addition of tubulin subunits to their (+) end.
* Astral MT cytoplasmic dynein link the (+) ends of astral MTs to the cell cortex and exert a pull on the spindle poles toward the cortex by inducing astral MT depolymerization at their (+) ends.

**Explain the process of DNA replication and how the process differs between prokaryotes and eukaryotes**

DNA replication is semiconservative, the two strands of the double helix separate during DNA replication, and. Each strand serves as a template from which the new complementary strand is copied. After replication, each double-stranded DNA includes one parental strand and one daughter strand.

* **Prokaryotic DNA replication** begins at a single origin of replication (*oriC*), and proceeds in a bidirectional manner around the circular chromosome until replication is complete. The bidirectional nature of the replication creates 2 replication forks that are actively mediating the replication process.

The open regions of DNA that are actively undergoing replication are called ***replication forks***. All the proteins involved in DNA replication aggregate at the ***replication forks*** to form a replication complex called a ***replisome*.**

At the origin of replication, topoisomerase II relaxes the supercoiled chromosome. Two replication forks are formed by the opening of the double-stranded DNA at the origin, and helicase separates the DNA strands, which are coated by single-stranded binding proteins to keep the strands separated. DNA replication occurs in both directions. An RNA primer complementary to the parental strand is synthesized by **RNA primase** and is elongated by **DNA polymerase III** through the addition of nucleotides to the 3′-OH end. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches called **Okazaki fragments.** RNA primers within the lagging strand are removed by the exonuclease activity of **DNA polymerase I, and the Okazaki fragments are joined by DNA ligase.**

Cells (prokaryotes or eukaryotes) contain an enzyme called primase that synthesizes RNA fragments about ten bases long using DNA as a template. In E. coli, primase is relatively inactive unless it is accompanied by six other proteins, forming a complex called a **primosome**.

In E. coli, at least two different DNA helicases are involved in DNA replication; one attaches to the lagging strand template and moves in a 5′ S 3′ direction, and the other attaches to the leading strand template and moves 3′ S 5′. Both are part of the primosome, but the 5′ S 3′ helicase is more important for unwinding DNA at the replication fork.

The key enzyme for DNA replication **is DNA gyrase, a type II topoisomerase** (an enzyme that cuts both DNA strands). Using energy derived from ATP, DNA gyrase introduces negative supercoils and thereby relaxes positive ones. DNA gyrase serves as the main swivel that prevents overwinding (positive supercoiling) of the DNA ahead of the replication fork (Figure 17-14c). In addition, this enzyme has a role in both initiating and completing DNA replication in E. coli—in opening up the double helix at the origin of replication and in separating the linked circles of daughter DNA at the end. Similar topoisomerases of both types have been isolated in eukaryotes.

* **Eukaryotic chromosomes** are typically linear, and each contains multiple origin of replication, where DNA synthesis is initiated by several groups of initiator proteins. Replication units are called **replicons**.

After DNA synthesis has been initiated at an origin of replication, 2 replication forks begin to synthesize DNA in opposite directions away from the origin, creating a *“replication bubble”* that grows in size as replication proceeds in both directions.

Origins of replication recruit proteins:

1. A multi-subunit protein **“origin recognition complex” (ORC)** binds to a replication origin.
2. The next components to bind are **the mini-chromosome maintenance (MCM)** proteins, which include several **DNA helicases** that facilitate DNA replication by unwinding the double helix.

During DNA replication the two strands of the double helix must unwind at each replication fork, 3 classes of proteins facilitate the unwinding:

* 1. **DNA helicases:** responsible for unwinding the DNA using energy form ATP hydrolysis.
  2. **Topoisomerases:** create swivel points in the DNA molecule by making and then quickly sealing double-strand or single-stranded breaks (e.g., **gyrase**).
  3. **SSB**: keep the DNA unwound and accessible to the replication machinery

1. The recruitment of MCM proteins to the replication origin requires the participation of **helicase loaders proteins**, which mediate binding of the MCM proteins to the ORC. At this point, the complete group of DNA-bound proteins is called a **pre-replication complex.** However, replication does not begin until several more proteins, including the enzymes that catalyze DNA synthesis, are added.

DNA polymerase, an enzyme that can copy DNA molecules, catalyzes the elongation of DNA chains.

Incoming nucleotides are added to the 3’ hydroxyl end of the growing DNA chain, so elongation occurs in the 5’ to 3’ direction: each successive nucleotide is linked to the growing chain by a phosphoester bond between 5’ carbon and the hydroxyl group on the 3’ carbon of the nucleotide added in the previous step.

n the phosphate group on its 5

Several forms of DNA polymerase have been identified; the original is now called **DNA polymerase I.**

The Directionality of DNA Synthesis: DNA polymerase catalyzes the addition of deoxynucleotide triphosphate (5’ to 3’). In the DNA polymerase, incoming nucleotides (dNTPS or deoxynucleotide triphosphate: A, T, G, and C) are covalently bonded to the 3’-hydroxyl ends of the growing DNA chain. Each success

DNA is synthesized in the 5’ to 3’ direction, but the two strands of the double helix are oriented in opposite directions.

**The leading strand** is synthesized in a continuous chain.

**The lagging strand** is synthesized in discontinuous fragments called **Okasaki fragments**. These are then joined by DNA ligase to form a continuous new 3’ to 5’ DNA strand.

Natural DNA synthesis is initiated by the formation of short RNA primer. These are synthesized by primase using a single DNA strand as template. Once the RNA primer is made, a DNA polymerase III adds deoxynucleotides (A, C, T or G) to the 3’ end of the primer.

For the leading strand just one primer is needed but the lagging strand needs a series of primer to initiate each **Osaki fragment**. Once the DNA chain reaches the next Osaki fragment, the RNA is degraded and replaced with DNA (DNA polymerase I); adjacent fragments are joined together by DNA ligase.

For example, like prokaryotes, a DNA sliding clamp protein acts along with DNA polymerase during DNA synthesis. One such eukaryotic clamp protein, **proliferating nuclear cell antigen** (PCNA), was originally identified as an antigen that is expressed in the nuclei of dividing cells during S phase. PCNA is a clamp protein for DNA polymerase d. Unlike bacteria, however, eukaryotes do not rely on the ribonuclease activity of DNA polymerase I to remove RNA primers. Instead, an RNA endonuclease, **RNAse H, nicks the backbone of the RNA-DNA hybrids, and an RNA exonuclease, FEN1, removes the RNA “flap” thus created.**

**Describe some ways in which knowledge of the DNA replication process has led to genetic engineering techniques in molecular biology**

In PCR, DNA polymerases (Taq and Pfu) are used to synthesize using a DNA template and primers new strands of DNA complementary to the target sequence. The polymerase begins synthesizing new DNA from the end of the primer.

**Explain the function of telomeres**

On linear DNA molecules, when a growing lagging strand reaches the end of the DNA molecules and the last RNA primer is removed by a 5’->3’ exonuclease, the final gap cannot be filled because there is no 3’ OH end that deoxynucleotide can be added to. Each round of. Replication generates shorter and shorter DNA molecules.

Eukaryotes have solved this problem with telomers, highly repeated sequences at the ends of chromosomes. Human telomeres have 100 to 1500 copies of TTAGGG at the end of chromosomes.

These noncoding sequences ensure that the cell will not lose important genetic information if DNA molecules shorten during replication.

**Telomerase:** a polymerase that can catalyze the addition of repeats to chromosome ends (to lengthen the telomere).

In multicellular organisms, telomerase function restricted to germ cells and few other types of actively proliferating cells.

Telomere shortening occurs with each cell division in most cells.

As a result, telomere length is a sort of counting device for how many times a cell has divided; if a cell divides too many times, telomeres could be lost.

Cells at risk of loss of telomeres undergo **apoptosis**, programmed cell death.

**Discuss mechanisms of DNA damage and repair**

DNA alterations, or mutations, can arise spontaneously, or through exposure to environmental agents.

Mutations can occur spontaneously during replication:

* **DNA tautomers** (alternate resonance structures of nitrogenous bases)
* **Trinucleotide repeats** (DNA polymerase replicates a short stretch of DNA twice)
* **Depurination and deamination**
  + **Depurination**: loss of a purine base (A or G)
  + **Deamination**: removal of a base’s amino group, changing its base-pairing properties.
* Mutations caused by mutation-inducing agents or **mutagens**:
  + Environmental mutagens fall into two categories: chemicals and radiation.
  + Mutagenic chemicals alter DNA structure through a variety of mechanisms:

**Base analogues** resemble nitrogenous bases in structure and are incorporated into DNA

**Base-modifying agents** react chemically with DNA bases and alter their structure.

**Intercalating** agents insert themselves between adjacent bases of the double helix, thereby distorting DNA structure and increasing the chance that a base will be deleted or inserted during DNA replication.

DNA mutations caused by **radiation: pyrimidine dimer formation** causing kinks in DNA helix structure.

A variety. Of mechanisms have evolved for repairing damaged DNA:

* **Light-dependent repair**:

Pyrimidine dimers can be directly repaired in a light-dependent process known as photoactive repair. Photoactive repair is best understood in bacteria, but appears to occur in some eukaryotes (but not humans). It depends on the enzyme photolyase, which catalyzes the breakage of bonds between thymine dimers. The energy needed for photolyase to repair DNA is provided by visible light, which is why the enzyme has the name it does.

* **Base excision repair**: DNA glycosylase removes a modified base to create an apurinic (AP) nucleotide. AP endonuclease then removes the remainder of the nucleotid, and DNA polymerase and ligase repair the gap.
* **Nucleotide excision repair**: for removing pyrimidine dimers and other bulky lesions in DNA. **NER endonuclease** (or **excinuclease**) makes two cuts in the DNA backbone, one on either side of the distortion. Then a DNA helicase binds to the stretch of DNA between the nicks (12 nucleotides long in E. coli, 29 in humans) and unwinds it, freeing it from the rest of the DNA. Finally, the resulting gap is filled in by DNA polymerase and sealed by DNA ligase.
* **Mismatch repair**: detection by unmethylated DNA strand.

**Error-prone repair: Translesion synthesis** (TLS) is one of the pathways to overcome stalled replication in which specific polymerases (TLS polymerase) perform bypass synthesis across DNA damage. In eukaryotes, specialized bypass polymerases carry out a process similar to the SOS response known as translesion synthesis.

* **Double-strand break repair:** in **nonhomologous end-joining (NHEJ),** a set of proteins binds to the ends of the two broken DNA fragments and joins them together; it is dangerous for a cell.
  + **Synthesis-dependent strand annealing (SDSA):** SDSA repairs a double-strand break using strand invasion and D loop formation by a single strand from the DNA that needs repair. DNA synthesis causes the D loop to migrate, followed by strand disengagement and DNA synthesis and ligation to fill in gaps.
  + **Double Holliday junction formation:** a double-strand break leads to strand invasion from both strands, DNA synthesis, Holliday junction formation, and resolution of the Holliday junction in one of two ways.

**Explain apoptosis and necrosis**

* Apoptosis is described as an active, programmed process of autonomous cellular dismantling that avoids eliciting inflammation.

Damaged or diseased cells need to be eliminated. In such cases, the process must not damage surrounding cells.

Multicellular organisms accomplish this through a programmed cell death – **apoptosis**.

Apoptosis proceeds through the activation of a series of enzymes called **caspases**.

Apoptosis is triggered by death signals or withdrawal of survival factors

Two main routes by which cells can activate caspases and enter apoptosis:

* + Activation can occur directly: e.g., when human cells are infected by viruses, cytotoxic T lymphocytes are activated and induce apoptosis.
  + When survival factors are withdrawn

Site of action is the mitochondrion

Healthy cells have several **anti-apoptotic proteins in outer mitochondrial membrane**.

Damaged cells can trigger their own apoptosis

* + If a cell suffers such damage that it can’t repair itself, it may trigger its own demise.
  + It can enter apoptosis through the activity of p53.
* Cell death called necrosis sometimes follows tissue injury. Necrosis involves swelling and rupture of injured cells. Necrosis has been characterized as passive, accidental cell death resulting from environmental perturbations with uncontrolled release of inflammatory cellular contents.

# Module 12 – Regulation of gene expression

**Explain the concept of an operon and the function of the operator, repressor and co-repressor**

An **operon** is a group of genes with related functions that are clustered together with DNA sequences that allow the genes to be turned on and off simultaneously. The operon includes a regulatory gene product that inhibits the expression of other genes to be expressed and is called a **repressor protein**. The effector controls the operon activation by binding to the repressor, inducing a change in its conformational state. The repressor is an allosteric protein which has only two conformational states, depending on whether or not the appropriate effector binds to it. Transcription of the operon begins at the promoter, which is the site of RNA polymerase attachment, and then proceeds through the operator and the genes to be turned on until finally ending at a terminator sequence. The end result is an mRNA molecule encoding the polypeptide products of all the genes which were activated. Such mRNA molecules, which encode more than one polypeptide, are called **polycistronic mRNAs**.

The operator is where the active form of the repressor binds.

* Some operons are negatively regulated by the repressor: in the absence of the effector, the repressor is active and remains bound to the operator, the operon is repressed. In the presence of the effector, the repressor is converted to its inactive form, which does not bind to the operator.
* Unlike the *lac* system, when the effector is present (tryptophan in tryptophan synthesis), the repressor produced by the *trpR* gene binds to the *trp* operator, operon is repressed. The effector is in this case is called a **co-repressor**.

**Explain how repressible and inducible operons differ and how these differences reflect differences in the pathways the control**

Control of induction and repression of enzyme synthesis is triggered by small organic molecules present within the cell or in the cell’s surroundings.

* **For catabolic pathway**s, these molecules are almost always substrates and they function as inducers of gene expression and thus, of enzyme synthesis. The operon in this case is turned off unless induced (inducible operon); it is under **negative regulation**. Operons encoding enzymes involved in catabolic pathways are turned *on* by a specific allosteric effector.
* **For anabolic pathways,** the relevant molecules are usually end-products, and they usually lead to the repression of gene expression and thus the repression of enzyme synthesis. Operons that regulate enzymes involved in anabolic (biosynthetic) pathways are **repressible operons**: they are turned off allosterically, usually by an effector that is the end-product of the pathway.

**Describe how the *lac* operon functions**

The lactose (*lac*) operon of E. coli consists of a segment of DNA that includes 3 contiguous genes: *lacZ* gene (which encodes β-galactosidase; enzyme that hydrolyzes lactose), *lacY* gene (which encodes galactoside permease, the plasma membrane protein that transports lactose into the cell) and *lacA* gene (which encodes a transacetylase that adds acetyl group to lactose).

These 3 genes are transcribed and regulated coordinately. The *lacI* gene encodes the lac repressor protein **R**. In absence of effector allolactose, the repressor binds to the lac operator and inhibits transcription of the lac operon (negative regulation). The binding of allolactose to the repressor converts the repressor to a conformational form that can no longer bind to the lac operator which inhibits transcription. This way, the allolactose triggers the induction of the enzymes encodes by the lac operon (inducible operon).

**Distinguish between positive and negative controls**

If, in binding to the DNA, the regulatory protein (repressor) prevents or turns off transcription, then it is part of a negative control mechanism. If on the other hand, its binding to DNA results in the activation of transcription then the regulatory protein is part of a positive control mechanism. The lac operon is subject to both positive and negative control:

* Transcription of the lac operon is regulated by the lac repressor ( R ) which is, when active, bound to DNA preventing RNA polymerase from transcribing lac operon.
* When complexed with cAMP, the catabolite activator protein (CAP) attaches to one of its recognition sites in DNA, the binding of RNA polymerase to the promoter is greatly enhanced, thereby stimulating the initiation of transcription.

**Explain the role of promoters, enhancers, activators, and repressors in transcriptional control**

Different cell types transcribe different sets of genes to produce proteins needed for carrying out that cell’s specialized functions. Transcription factors are essential for the transcription of all the genes transcribed by a given type of RNA polymerase. For genes transcribed by RNA polymerase II, the transcription factors assemble with RNA polymerase at the core promoter, a DNA region located in the immediate vicinity of the transcriptional start site. Sequences of DNA upstream of the promoter are referred to proximal control elements: CAAT box, GC box and octamer. Transcription factors that selectively bind to one of these, or to other control sequences located outside the core promoter, are regulatory transcription factors.

* A second class of DNA control sequences are located either upstream or downstream from the genes they regulate and often lie far away from the promoter. If they stimulate gene transcription these type of control regions are **enhancers** or **silencers** if they inhibit transcription. Regions that restrict the influence of enhancers or silencers, are **insulators**. The regulatory transcription factors that bind to enhancers are **transcriptional activators** because they are involved in activating transcription. The regulatory transcription factors that bind to silencers to reduce gene transcription are or **transcriptional repressors**.

**Explain how gene expression may be controlled at the translational and post- translational level**

Eukaryotic gene expression is regulated at multiple levels:

5 main levels of control: 1) the genome 2) transcription 3) RNA processing and export from nucleus to cytoplasm 4) translation and 5) post-translational events.

* Regulatory mechanisms in the last 3 categories are all examples of post-transcriptional control which encompasses a wide variety of different processes, included in this category are reversible structural alterations that influence protein function, such as protein phosphorylation and dephosphorylation, as well as permanent alterations such as proteolytic cleavage.
* Other posttranslational events subject to regulation include the guiding of protein folding by chaperone proteins, the targeting of proteins to intracellular or extracellular locations, and the interaction of proteins with regulatory molecules or ions, such as cAMP or Ca2+.

# Module 13 – Gene expression

**Generally, describe the mechanisms of new gene editing tools such as CRISPR/Cas9**

**List the key molecules involved in transcription and translation**

### Transcription:

* RNA polymerase synthesizes RNA using DNA as a template.
* In eukaryotes, RNA polymerases require transcription factors.
* **4 distinct stages: binding, initiation, elongation, and termination.** The process of transcription begins with 1) the binding of RNA polymerase to a DNA promoter sequence, which triggers local unwinding of the DNA double helix. Using one of the two DNA strands as a template, RNA polymerase then 2) initiates the synthesis of an RNA chain. After initiation has taken place, the RNA polymerase molecule moves along the DNA template, unwinding the double helix and 3) elongating the RNA chain as it goes. The unwound region of DNA is known as a transcription bubble. During this process, the enzyme catalyzes the polymerization of nucleotides in an order determined by their base pairing with the DNA template strand.

### Translation: key players

* **Ribosomes**: carry out the process of polypeptide synthesis.
* **tRNA** molecules align the amino acids in the correct order.
* **Aminoacyl-tRNA synthetases** attach amino acids to their appropriate tRNA molecules.
* **mRNA** molecules encode the amino acid sequence information (A, C, G, U).
* **Protein factors**: facilitate some of the steps of translation.

**Explain how messenger RNAs are translated into proteins**

### Eukaryotic initiation

* **The initiation factors bind the tRNA** (these are called **eIFs**; there are about a dozen of these) and the tRNA then binds the small ribosomal subunit.
* The resulting complex then binds to the 5’ end of the mRNA, recognizing the **5’ cap.**
* After binding the mRNA, the small ribosomal subunit (including the initiator tRNA) scans along the transcript and begins translation at the first AUG (st**art codon**).
* After the initiator tRNA is base-paired with the start codon the large subunit joins the complex, facilitated by GTP hydrolysis.

### Initiation of translation in prokaryotes

Initiation of translation in bacteria can be divided into three steps:

1. Three **initiation factors** (*IF1*, *IF2*, and *IF3*) bind to the small ribosomal subunit, with GTP bound to IF2 (Step 1)
2. mRNA and the tRNA carrying the first amino acid bind to the small subunit (Step 2)
3. Once the IF3 has been released, the 30S complex can bind a free 50S subunit, generating the 70S initiation complex (Step 3)

### Chain elongation involves sequential cycles of aminoacyl tRNA binding, peptide bond formation, and translocation

* Once initiation has been completed a polypeptide chain is synthesized.
* Amino acids are added in sequence to the growing chain (**elongation**).
* Elongation involves a repetitive cycle of 3 steps:

1. **Binding of aminoacyl tRNA**

* Elongation begins as a tRNA with an anticodon complementary to the second codon binds the A site (1).
* Elongation factors don’t recognize particular anticodons, so all types (except initiator tRNAs) are brought to the A site.
* Only those with an anticodon complementary to the codon stay at the A site long enough for GTP hydrolysis to take place.
* Mechanisms for selecting against incorrect aminoacyl tRNA synthetases + proofreading result in a final error rate in translation of at most 1/10,000.

**Polypeptide chain elongation in bacteria**

1. An aminoacyl tRNA binds to the A site, escorted by EF-Tu bound to GTP. During tRNA binding, the GTP is hydrolyzed and EF-Tu is released. EF-Ts helps recycle the EF-Tu.
2. A peptide bond is formed between the carboxyl group of fMet (or, in later cycles, of the terminal amino acid) at the P site and the amino group of the newly arrived amino acid at the A site.
3. The mRNA advances by three nucleotides, the peptidyl tRNA moves from the A site to the P site, and the empty tRNA moves from the P site to the E site, accompanied by the hydrolysis of GTP bound to EF-G.
4. **Peptide bond formation**

* Once the aminoacyl tRNA is bound to the A site, a peptide bond forms between the amino group of the amino acid at the A site and the carboxyl group of the amino acid at the P site.
* The growing peptide chain is transferred to the tRNA at the A site (2).
* No ATP or GTP hydrolysis is required for this step.
* This step is catalyzed by rRNA.

1. **Translocation**

* After the peptide bond forms, the mRNA advances to bring the next codon into the proper position.
* **During this translocation, the peptidyl tRNA moves from the A to the P site, and the empty tRNA moves to the E site.**
* Once the next mRNA codon reaches the A site, the ribosome is now set to receive the next aminoacyl tRNA.
* The elongation cycle repeats and the amino terminal of the growing polypeptide passes out of the ribosome through an **exit tunnel in the 50S subunit.**
* Here **molecular chaperones** assist its folding.
* Termination of polypeptide synthesis is triggered by **release factors** that recognize mRNA stop codons.

1. Messenger RNA brings polypeptide coding information to the ribosome

* The sequence of codons in mRNA directs the order of amino acids in the polypeptide.
* mRNA is exported from the nucleus to the cytoplasm via binding to proteins that contain nuclear export signals (NES); these proteins target the mRNA through nuclear pores.
* An untranslated sequence at the 5’ end of the message precedes the start codon, the first to be translated (usually AUG).
* There is also an untranslated region at the 3’ end of the mRNA that follows **the stop codon,** which signals the end of translation. Stop codon may be UAG, UAA, or UGA.
* 5’ and 3’ untranslated regions vary in length and are essential for mRNA function.
* mRNAs have a 5’ cap and 3’ poly(A) tail within the untranslated region. The 5’ cap is important for initiating translation in eukaryotes.

**Explain how polypeptides become functional proteins**

**Explain what is meant by posttranslational processing**

After polypeptide chains have been synthesized, they often must be chemically modified before they can perform their normal functions. Such modifications are known collectively as **posttranslational modifications.**

* **In bacteria, the N-formyl methionine at the N-terminus is removed.**
* **In eukaryotes, the methionine at the N-terminus is released.**
* Sometimes whole blocks of amino acids are removed from the polypeptide, for instance certain enzymes synthesized as inactive precursors.
* These are activated by removal of sequences from one end of the protein.
* **Transport of proteins across membranes may require removal of a signal sequenc**e and some have internal amino acids that must be removed (e.g., insulin).
* **Other common processing events include chemical modifications of individual amino acid groups—by methylation, phosphorylation, or acetylation reactions, multiprotein complexes.**
* In addition to the preceding posttranslational events, some proteins undergo a relatively unusual type of processing called **protein splicing,**
* Most polypeptides synthesis (~90%) takes place in the cytosol with transcripts leaving the nucleus through nuclear pores and associating with free ribosomes.
* After polypeptides are released in the ER lumen, they fold into their final shape.
* Protein folding is often accompanied by formation of disulfide bonds.
* Most proteins synthesized on rough ER are **glycoproteins**.
* The initial glycosylation takes place in the ER as the polypeptide is being synthesized.
* In the Golgi complex, further glycosylation and processing of carbohydrate side chains occurs, and the proteins are sorted and distributed to other locations.

Describe the role of molecular chaperones