

Table of Contents

Module 1	6
Microscopy	6
Biochemistry: Important Advance.....	7
Genetic: Information Flow and inheritance	7
Carbon-containing molecules are stable	7
Bond Polarity	7
Water is cohesive: network of hydrogen-bonded molecules, hydrogen bond is weak compared to covalent bonds.	7
Selectively Permeable Membranes	8
Cellular membrane is a hydrophobic barrier	8
Membrane lipids Are Amphipathic.....	8
Membranes are Selectively Permeable	8
Module 2- Macromolecules of the cell	9
Proteins	9
Cell processes.....	9
Small molecules	9
Levels of organization in Protein structure	9
4 Protein major classes	10
The monomers are amino acids.....	10
The structure of 20 amino acids	10
The polymers are Polypeptides and Proteins	10
Peptide bond formation.....	11
Polypeptide and Proteins.....	11
Primary structure	11
Secondary structure.....	11
Categories of Proteins.....	11
Tertiary Structure.....	12
Quaternary structure	12
Disulfide Bond formation in Insulin	12
The Primary structure of Insulin	12
Structure of Hair	12
The roles of DNA and RNA in Protein synthesis	12
Nucleic Acids.....	12
Transcription and Translation	12
Nucleic Acids	12
The Monomers are Nucleotides	13
Polymers: DNA and RNA	13
A DNA molecule is double-stranded Helix	13
RNA Structure	14
The Phosphorylated Forms of Adenosine	14

Hydrogen Bonding in Nucleic Acid Structure	14
Polysaccharides	14
Polysaccharides.....	14
The Monomers are Monosaccharides	14
Polysaccharides.....	14
Polysaccharide Polymers are Storage and Structural	14
Glycogen (storage)	14
Starch (storage-plants).....	15
Cellulose (structural polysaccharide).....	15
Lipids	15
Fatty Acids Are the Building Blocks of several classes of Lipids	16
Triacylglycerol Are Storage Lipids	16
Phospholipids Are Important in Membrane Structure	16
Steroids Are Lipids with a Variety of Functions.....	16
Terpenes are Formed from Isoprene	16
Module 3 – Introduction to Cells and Organelles.....	17
Types of Cells and Their Properties	17
There Are Three Limitations on cell size	17
Eukaryote Cells use Organelles to compartmentalize Cellular Function	17
Chromosome.....	18
Genetic Information.....	18
The Eukaryotic Cell – Plasma Membrane, Organelles, And the Endosymbiont Theory	18
Mitochondrion	18
The Chloroplast.....	18
The nucleus is the information center of the Eukaryotic cell	18
The Endoplasmic Reticulum	19
The Golgi Complex	19
Cell Vesicles, Structural Components, and Examples of Cellular Invaders	19
Ribosome	20
Cytoplasm	20
Cytoskeleton	20
Microtubules.....	21
Microfilaments.....	21
ECM and Cell Wall Are “Outside” the cell	21
The ECM	21
Vacuoles.....	21
Secretory Vesicles	21
Lysosome	22
The Phagolysosome	22
Peroxisome	22
Hydrogen Peroxide	22
Viruses.....	22
Bacteriophage	22
Module 4 -Enzymes.....	22
Enzyme Structure.....	22
Factors Affecting enzymes	23

Module 5 - Membranes and the Endomembrane System 23

Membrane Characteristics and Composition..... 23

The functions of the membrane	23
Membranes are sites of specific proteins and functions	23
Regulation of Transport across the cell is a main function of membrane protein	24
Membrane Proteins Mediate Cell Adhesion and Cell-to-Cell Communication	24
Membrane proteins play role in other cell functions	24
Membrane Structure: Fluid Mosaic Model.....	24
Three classes of membrane proteins.....	24
Main class of membrane lipids: phospholipids, glycolipids and sterols.....	24
Phospholipids.....	24
Glycolipids.....	24
Cerebrosides	24
Gangliosides.....	24
Sterols	25
Membrane Asymmetry: most lipids are distributed unequally between two monolayers	25
Lipids move freely within their monolayer	25
Membrane fluidity is measured using fluorescence recovery after photobleaching (FRAP).....	25
Membrane functions properly only in the fluid state	25

Membranes Functions and the Endomembrane System..... 25

Effects of Sterol on Membrane Fluidity	25
Lipid rafts localized regions involved in cell signaling.....	26
Function of the lipid rafts.....	26
Receptors in lipid rafts	26
The membrane consists of a mosaic of proteins: evidence from Freeze-Fracture Microscopy	26
Membrane Proteins are Oriented Asymmetrically Across the Lipid Bilayer	26
DNA Sequencing.....	26
The Endomembrane System	27
Membrane biosynthesis.....	27
Variations in amount of Rough and Smooth ER	27
The Golgi Complex	27
Roles of the ER and Golgi Complex in Protein Trafficking.....	27
Protein and Lipid Tags.....	27
Exocytosis and Endocytosis: Transporting Material Across the Plasma Membrane.....	27
Phagocytosis.....	27
Receptor-Mediated Endocytosis.....	28
Process of receptor-mediated endocytosis	28

Module 6 – Membrane Transport 28

Transport Across Membranes: Overcoming the Permeability Barrier. 28

Cell Transport.....	28
Simple diffusion: unassisted movement down the gradient.	28
Osmosis of water across membrane.....	28
Second Law of Thermodynamics	29
Solute size	29
Solute Polarity.....	29
Ion permeability.....	29
Rate of simple diffusion directly proportional to concentration gradient.....	29
Facilitated diffusion: protein-mediated movement down the gradient	30
Carrier and channel proteins facilitate transport by different mechanisms.....	30
Glucose transporter: a uniport carrier	30

Active transport: protein-mediated movement up the gradient.....	30
Pumps	30
Na ⁺ /K ⁺ pump.....	31
Module 8 - DNA, Chromosomes, the Nucleus	31
Information and DNA.....	31
DNA	31
DNA in the genome.....	32
Restriction Enzymes.....	32
The Nucleus	32
Module 9 – Signal Transduction	32
Receptor-Ligand Interactions	32
Signal Transduction Mechanisms.....	32
Different Types of Chemical Signals Can Be Received by Cells	32
Receptor Binding Involves Specific Interactions Between Ligands and Their Receptor	33
Receptor-ligand interactions.....	33
Receptor Affinity.....	33
Coreceptors.....	33
Receptor Down-regulation.....	33
Agonist and Antagonists	33
Receptor Binding activates a sequence of signal transduction events within the cell	33
Signal Amplification	34
GPCRs And Second Messengers	34
Categories of Receptors	34
G Protein-Linked Receptors	34
The structure and regulation of G protein-linked receptors.....	34
The structure, activation, and inactivation of G proteins	34
Regulation of G protein-linked receptors	34
G protein Inactivation	35
Cyclic AMP is a second messenger whose production is regulated by some G proteins.....	35
G proteins are active for only a short period of time.....	35
Many G Proteins use inositol trisphosphate (IP3) and Diacylglycerol (DAG) as second messenger	35
Calcium in signaling.....	35
Protein Kinase-associated receptors	35
Protein Kinase-associated receptors.....	35
Growth factors often bind protein kinase-associated receptors	35
Receptor tyrosine kinases aggregate and undergo autophosphorylation.....	36
The activation of receptor tyrosine kinases.....	36
Dominant negative mutant receptors are important tools for studying receptor function	36
Constitutive mutations	36
Disruptions of growth factor signaling can lead to cancer.....	36
Hormone signaling	37
Hormones can be classified by the distance they travel and by their chemical properties.....	37
Chemical classification of endocrine hormones	37
Control of Glucose metabolism is a good example of endocrine regulation	37
Intracellular effects of adrenergic hormonal control of glycogen degradation.....	37
Insulin signaling acts through PI 3-kinase to regulate resting glucose levels.....	37

Diabetes	38
Steroid hormone receptors act primarily in the nucleus, not the cell surface	38
Module 10 – Recombinant Technology	38
DNA technology.....	38
The PCR Revolution.....	38
PCR.....	38
DNA Extraction.....	38
Copying DNA	39
Technologies And Utilities.....	39
Historical Perspective.....	39
Basic Principles.....	39
Major technologies	39
Principal uses of chips.....	39
DNA chips.....	39
DNA chip design	40
cDNA (complementary DNA) fragments arrays on nylon and glass.....	40
Scanning the arrays.....	40
The Affymetrix approach	40
Module 11 – Cell Cycle, Mitosis and DNA Replication	41
Mitosis and the Cell Cycle	41
The Cell Cycle, DNA Replication and Mitosis	41
Overview of the cell cycle	41
Mitosis is a relatively short part of the cell cycle	41
Chromosomes in Mitosis	41
Mitosis is subdivided into Prophase, Prometaphase, Metaphase, Anaphase and Telophase	41
Prophase	42
Centromere	42
Kinetochores	42
Metaphase	42
Anaphase	42
Telophase.....	43
The mitotic spindle is responsible for chromosome movements during mitosis	43
Spindle Assembly and Chromosome Attachment.....	43
Spindle Assembly	43
So what Drives Chromosomes Movement.....	43
Cytokinesis Divides the Cytoplasm	43
Myosin and cleavage.....	43
Cell Division is Sometimes Asymmetric	44
DNA Replication.....	44
DNA Replication	44
DNA Replication is usually bidirectional	44
Eukaryotic DNA Replication involves multiple replicons	44
Replication licensing ensures that DNA molecules are duplicated only once prior to each cell division	45
DNA polymerase catalyze the elongation of DNA chains	45
Biotechnology functions of DNA polymerases.....	45
DNA is synthesized as discontinuous segments that are joined together by DNA ligases.....	45
Proofreading is performed by the 3'→5' Exonuclease activity of DNA polymerase	45
DNA synthesis requires RNA primers.....	45
The process of DNA synthesis.....	45

Unwinding the DNA double helix requires DNA helicases, Topoisomerases, and single-strand DNA binding proteins	46
Summary: DNA Replication	46
Telomeres solve the DNA end-replication problem.....	46
Telomeres and telomerase	46
Most cells have a limited life span	46
DNA damage and repair.....	46
DNA damage can occur spontaneously or in response to mutagens	47
Translesion synthesis and excision repair correct mutations involving abnormal nucleotides	47
Progression through the cell cycle is controlled at several key transition points.....	47
Checkpoint pathways monitor chromosome-to-spindle attachments, completion of DNA replication, and DNA damage	48
Checkpoints.....	48
Apoptosis	49
Necrosis.....	49
Apoptosis is triggered by death signals or withdrawal of survival factors.....	49
Damaged cells can trigger their own apoptosis	49
Module 12 – Regulation of Gene Expression	50
The Regulation of Gene Expression	50
Gene regulation	50
Catabolic and anabolic pathways utilize different strategies for adaptive enzyme synthesis.....	50
Catabolic pathways and substrate induction.....	50
Anabolic pathways and end product repression	50
Anabolic pathways and end product repression	50
Lac repressor is an allosteric protein whose binding to DNA is controlled by lactose.....	51
Genetic repression	51
Effector Molecules	51
Genes involved in lactose catabolism are organized into an inducible operon.....	51
The lac operon	51
Repressor protein	52
Reversible interaction of the lac repressor.....	52
The genes involved in tryptophan synthesis are organized into a repressible operon.....	53
Eukaryotic gene expression is regulated at multiple levels	53

Module 1

Microscopy

- Light microscope: resolution: 1 μm .
- Smaller limit of resolution of a microscope, the greater its **resolving power**.
- Phase contrast/differential interference contrast microscopy exploits differences in the phase of light passing through a structure with a refractive index different than the surrounding medium, shows images in 3-D.
- **Fluorescent microscopy**: detects fluorescent dyes to how location of substances in the cell.
- **Confocal scanning**: uses a laser beam to illuminate a single plane of a fluorescently labeled specimen (3-D reconstruction).
- **The electron microscope**: uses an electron beam rather than light – limit of resolution: 0.1-0.2nm- magnification 100,000X than light microscope.

- **TEM:** transmission electron microscopy. Electrons are transmitted through the specimen.
- **SEM:** scanning electron microscopy. The surface of a specimen is scanned by a beam of electrons deflected from specimen 'surface'.

Biochemistry: Important Advance

- **Chromatography:** techniques used for sample preparation which let separate molecules by size, charge or binding affinity.
- **Electrophoresis:** the cell is loaded into a gel and then an electric field is applied to the gel. This Electric field moves the molecules through the gel differentially
- Since DNA molecules are negatively charged, when the electric field is applied to the gel, the DNA molecules moved towards the positive charges. But larger molecules move slowly and run through the argos matrix and run next to a sample of known molecular weight called the DNA ladder. Uses an electrical field to move proteins, DNA or RNA molecules through a medium based on size/charge.
- **Mass spectrometry or MassSpec:** determine size and composition of protein by measuring mass to charge ratio of ions in a sample.

Genetic: Information Flow and inheritance

- Humans have 23 pairs of chromosomes.
- Uses **ultracentrifugation** and **electrophoresis** to separate DNA and RNA molecule.
- **Recombinant DNA technology**, restriction enzymes cut DNA at specific places to create recombinant DNA molecules with DNA from different sources.
- **DNA sequencing:** methods to determine base sequences of DNA molecules.
- Possible to sequence entire genome (entire DNA content of a cell).
- **Cell membrane:** a barrier which maintains physical integrity.
- **Covalent bonds:** sharing of a pair of electrons between 2 atoms.

Carbon-containing molecules are stable

- Valence: Carbon (4), Nitrogen (3), Oxygen (2) and Hydrogen (1).
- Stability is expressed as bond energy.
- Bond energy is expressed as cal/mol, amount of energy required to break one mole (6×10^{23}) of bonds.
- A calorie is the required energy to increase the T of 1g of water by 1°C .

Bond Polarity

Polar bond result from a high electronegativity (affinity for electrons) of O2 and sulfur compared to carbon and hydrogen.

Water molecules are polar: electrons drawn by oxygen, partial negative charge at the end of O2, a partial positive charge around hydrogen molecules, bent shape.

Water is cohesive: network of hydrogen-bonded molecules, hydrogen bond is weak compared to covalent bonds.

- Combined effect of many hydrogen bonds accounts for water's high
 - **Surface tension:** vast number of hydrogen bonds.
 - **Specific heat** gives water its T stabilizing capacity.
Specific heat: amount of heat a substance must absorb to raise its T by 1°C .
Specific heat of water: 1 cal per gram.

- **Boiling point.**
- **Heat of vaporization.**
- Water changes temperature very slowly.
- Water is an excellent solvent: due to its polarity. Most of the molecule in cells are also polar, and so can form hydrogen or ionic bonds with water.
- Takes part in many chemical reactions.

Selectively Permeable Membranes

A barrier such

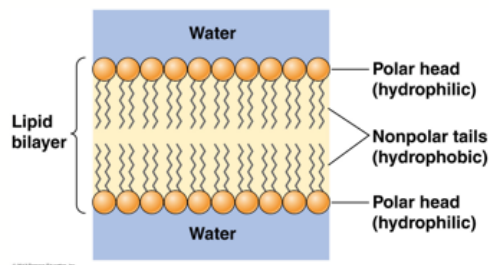
- Impermeable to much of the cell contents.
- Insoluble in water.
- Permeable to water.

Cellular membrane is a hydrophobic barrier

- Consists of: **phospholipids, glycolipids, membrane proteins, and sterols:** cholesterol(animal), ergosterols (fungi) and phytosterol (plants).

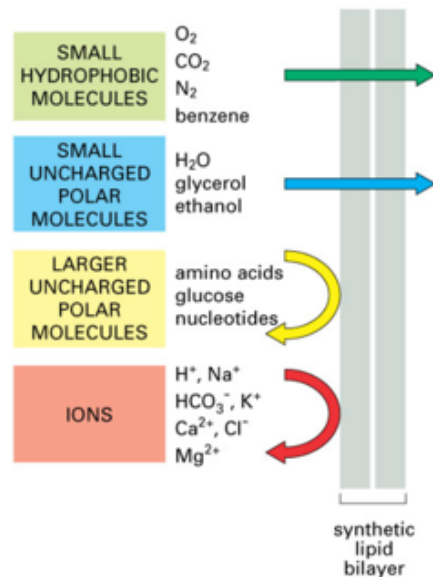
Membrane lipids Are Amphipathic

- **Phospholipid** have a polar head, due to negatively charged phosphate group linked to a positively charged group (serine, choline, ethanolamine), and two non-polar hydrocarbon tails.
- A Membrane is a lipid bilayer with Proteins embedded within it
- Polar heads of membrane phospholipids face outward toward aqueous environment.
- Hydrophilic tails are oriented inward.



Membranes are Selectively Permeable

- Cellular constituents are mostly polar or charged and are prevented from entering or leaving the cell.
- Because of the hydrophobic interior, impermeable to most polar molecules and very impermeable to ions.
- Non-polar and very small molecules diffuse.
- The rate at which a molecule diffuse across lipid bilayer depends on its size and solubility.



Module 2- Macromolecules of the cell

4 major classes of macromolecules:

- Nucleic Acids
- Proteins
- Polysaccharides
- Lipids

Proteins

Cell processes

Transcription takes DNA and makes RNA out of it. Nature makes an RNA copy of DNA and by post-transcription makes mRNA out of it. **Translation** is the process where small molecules are added to the mRNA to build up first **polypeptides**, amino-acids in small repeat units. Then there is a process to convert polypeptides into a protein: post-translation. And when it takes a 3D shape it takes protein activity to effect particular functions like an effector molecule and then activate more functions.

Small molecules

- **Amino acid**: monomeric components of proteins.
- **Aromatic bases** (purines and pyrimidines): components of nucleic acids: DNA and RNA.
- **Sugars** (monosaccharides):
 - **Ribose**: components of nucleic acids
 - **Glucose**: used in metabolism to make energy
- **Lipids**: components of phospholipids.

Levels of organization in Protein structure

- **Primary**: Amino acid sequence based on covalent peptide bonds.
- **Secondary**: fold alpha-helix, beta-sheet or random coil based on hydrogen bonds.

- **Tertiary:** 3D folding of a single polypeptide chain based on hydrogen bonds, disulfide bonds, electrostatic interactions and hydrophobic effect.
- **Quaternary** (macromolecule): association of two or more polypeptides with same interactions seen in tertiary structure.

4 Protein major classes

- enzymes, (**catalysts**) that greatly increase rates of chemical reactions in cells.
- **Structural proteins:** provide support and shape to cells and organelles, giving cells their characteristic appearances.
- **Motility proteins:** play key roles in the contraction and movement of cells and intracellular structures.
- **Regulatory proteins:** are responsible for control and coordination of cellular functions, ensuring that cellular activities are regulated to meet cellular needs.
- **Mono-functional proteins:** have a single function: catalytic, structural, motility, or regulatory.
- **Bi-functional proteins** plays two different roles.

The monomers are amino acids

- Proteins are linear polymers of amino acids.
- 60 different kinds of amino acids, but only 20 are used in protein synthesis.
- **Every amino acid has the basic structure with a carbonyl group, an amino group, a hydrogen atom, and a R-group all attached to a single carbon atom.**
- The R group determines the characteristics (size, polarity and pH) for each type of amino acid.
- Amino acids such as **valine, methionine, and alanine** are nonpolar (hydrophobic), while amino acids such as **serine, threonine, and cysteine** are polar (hydrophilic). The R groups of lysine and arginine are positively charged so these amino acids are also known as basic (high pH) amino acids. Proline is an exception to the standard structure of an amino acid because its R group is linked to the amino group, forming a ring-like structure.
- Except for glycine, for which the R group is a hydrogen atom, all amino acids have at least one asymmetric carbon atom. Therefore, most amino acids exist in two isomeric forms, L and D-amino acids.

The structure of 20 amino acids

- **Group A:** hydrophobic and nonpolar R groups.
- **Group B:** hydrophilic and polar R group, uncharged.
- **Group C:** hydrophilic, polar R group, and protonated or ionized at cellular pH.

The polymers are Polypeptides and Proteins

- Stepwise addition of new amino acid to a growing chain of amino acids by a **dehydration (condensation) reaction:** formation of polymers and water molecule.
- The reaction could be reversed by adding back water molecules; used by cells to excrete water waste.
- -H and -OR groups are removed as water comes out and the covalent bond between the carboxyl group and an amino group is called **peptide bond**.

Peptide bond formation

Always an N-terminus at one end and C-terminus at the other end.

Polypeptide and Proteins

- Product of amino acid polymerization is a polypeptide (polymers of peptides).
- Protein is a polypeptide or polypeptides that have folded properly, combined with other additional components needed for proper functioning, and is now functional.
- Protein is a polypeptide chain that have attained a unique stable, 3-D shape and it is biologically stable.
- Monomeric protein consists in a single polypeptide vs. multimeric proteins, two polypeptides: a dimer, 3 polypeptides: a trimer.
- **Ribonuclease** is a monomeric protein.
- Hemoglobin is a multimeric protein. It contains 4 polypeptides, (2 alpha-subunit and 2 beta-subunits), alpha-chains and beta-chains.
- Each subunit contains a **heme group** with an iron atom. Each heme iron can bind a single oxygen molecule.
- **Homomeric**, and **heteromeric**: protein made up at least of two different polypeptides chains.
- Protein structure is determined by its amino acid sequence which drives the folding and intramolecular bonding of the linear amino acid chain, which ultimately determines the protein's unique three-dimensional shape.

Primary structure

- Primary structure is the amino acid sequence of the constituent polypeptides.
- Amino acids are always written from **the N-terminal** to the **C-terminal**, direction in which the polypeptide is synthesized.
- Once incorporated into a polypeptide chain, individual amino acids are called **amino acid residues**.
- **Disulfide bond**: very stable bond between two sulfur atoms of 2 cysteine amino acid residues.

Secondary structure

Because of the folding groups of amino acids are close to each other.

The group interactions result in two structural patterns: the alpha-helix and beta sheet conformations.

Categories of Proteins

- **Fibrous proteins**: have extensive secondary structure (either a helix or beta sheet) giving them a highly ordered and repetitive structure.
- **Globular proteins**: most of the proteins.
 - The polypeptide chain is folded in a compact structure. It is folded locally into alpha-helical or Beta-sheet structures. These regions are folded on one another to give the protein its compact, globular shape.
 - The folding is possible because the interspersed random coils allowing the polypeptide to loop and fold.
 - Have unique tertiary structures

- They consist of a number of segments called domains. A **domain** is a discrete, locally folded unit of tertiary structure. A domain typically contains 50-350 amino acids, and usually has a specific function.

Tertiary Structure

3-D folding of a single polypeptide chain.

Quaternary structure

- Level of organization concerned with subunit interactions and assembly.
- Association of 2 or more polypeptides to form a multimeric protein.
- Applied only to multimeric proteins.
- The bonds and forces that maintain quaternary structure are the same as those responsible for tertiary structure: **hydrogen bonds, electrostatic interactions, hydrophobic interactions, and covalent disulfide bonds.**

Disulfide Bond formation in Insulin

It could be reversed.

The Primary structure of Insulin

Insulin consists of two polypeptides, A and B chains. The two chains are covalently linked by two inter-chain disulfide bonds.

Structure of Hair

Alpha keratin protein: 3 helices of alpha-keratin wrap into protofibrils which then bond together to form microfibrils. Microfibril= 9 + 2 protofibril based structure. Microfibrils aggregate to form macrofibrils.

The roles of DNA and RNA in Protein synthesis

mRNA: directs amino acids sequence of polypeptides.

tRNA: binds to amino acids and directs them to proper locations within the growing polypeptide chain.

rRNA: components of the ribosomes that serve as the site of protein synthesis.

Nucleic Acids

Transcription and Translation

- **Transcription:** DNA molecule is transcribed into an RNA molecule.
- **Translation:** takes RNA and converts into protein.

Nucleic Acids

- Nucleic acids are macromolecules critical in the storage, transmission and expression of genetic information.
- Are linear polymers of nucleotides, strung together in a genetically determined order.
- Two major types are DNA and RNA.
- DNA contains the sugar deoxyribose, RNA contains 5-carbon sugar ribose in each of its nucleotides.
- DNA plays as the repository of genetic information, whereas RNA molecules play several different roles in the expression of that information during protein synthesis.
- **mRNA:** directs amino acids sequence of polypeptides that is during polypeptide synthesis.
- **tRNA:** binds to amino acids and directs them to proper locations within the growing polypeptide chain.

- **rRNA:** components of the ribosomes that serve as the site of protein synthesis.

The Monomers are Nucleotides

- Nucleic acids are informational macromolecules that contain non-identical monomeric units in a specified sequence.
- The monomeric units of nucleic acids are called **nucleotides**.
- DNA and RNA each contain only four different kinds of nucleotides.
- Each nucleotide consists of **a five-carbon sugar, a phosphate group, and a nitrogen-containing aromatic base. The sugar is either D-ribose (for RNA) or D-deoxyribose (for DNA).**
- **The phosphate is joined by a phosphoester bond to the 5' carbon of the sugar (-P-O-C-), and the base is attached at the 1' carbon. The base may be either a purine or a pyrimidine.**
- DNA contains the **purines: adenine (A) and guanine (G)** and the **pyrimidines: cytosine (C) and thymine (T)**. RNA also has **adenine, guanine, and cytosine** but contains the pyrimidine **uracil (U)** in place of thymine.
- ATP is the energy-rich compound used to drive a variety of reactions in the cell, including the activation of monomers for polymer formation.
- Nucleotides play two roles in the cell:
 - Monomeric units of nucleic acids
 - Serve as intermediates in various energy transferring reactions.

Polymers: DNA and RNA

- Nucleic acids are linear polymers formed by linking each nucleotide to the next through a phosphate group.
- The result of a condensation reaction with the -H and -OH groups coming off from the sugar and the phosphate group respectively is a **3',5' phosphodiester bond**.
- Incoming nucleotides must be added in a specific, genetically determined sequence. The **template to specify nucleotide order is DNA for both DNA and RNA synthesis.**
- **Purines: Adenine, Guanine**
- **Pyrimidines: Thymine, Uracil, Cytosine**
- Purine and pyrimidine bases have carbonyl groups and nitrogen atoms capable of hydrogen bond formation under appropriate conditions.
- Pairing of A with T (or U) and G with C

A DNA molecule is double-stranded Helix

- The double helix consists of two complementary chains of DNA twisted together around a common axis to form a right-handed helical structure.
- The two chains are oriented in opposite directions along the helix, one in 5'3' direction and the other in 3'-5' direction.
- The sugar phosphate backbones of the two strands could be envisioned as the sides of a circular staircase where each step corresponds to a pair of bases held in place by hydrogen bonding.
- The right-handed helix is an idealized version of the B-DNA, the main form of DNA.
- Z-DNA is a left-handed double helix, with a longer, thinner sugar phosphate backbone.

RNA Structure

- Secondary and tertiary structures are well understood only for tRNA molecules.
- **A nucleotide is composed of three components, namely a nitrogenous base, phosphate group, and sugar. A nucleoside is composed of two components, namely a nitrogenous base and sugar.** This is the basic difference between a nucleotide and a nucleoside.

The Phosphorylated Forms of Adenosine

- Adenosine occurs as the free nucleoside, the monophosphate (AMP), the diphosphate (ADP), and the triphosphate (ATP).
- The bond that links the first phosphate to the ribose of adenosine is a low-energy **phosphoester** bond, whereas the bonds that link the second and third phosphate groups to the molecule are higher-energy **phosphoanhydride** bonds.

Hydrogen Bonding in Nucleic Acid Structure

A-T pair held together by two hydrogen bonds, whereas the C-G pair has three hydrogen bonds.

Polysaccharides

Polysaccharides

- No known informational role in the cell.
- They are the storage polysaccharides **starch** and **glycogen** and the structural polysaccharide **cellulose**.
- Each of these polymers contains the 6-carbon sugar, **glucose** and its single repeat unit.

The Monomers are Monosaccharides

- The repeats are single sugar called monosaccharides.
- A sugar can be an aldehyde or ketone that has two or more hydroxyl groups.
- 2 categories of sugars: **aldosugars**, with a terminal carbonyl group and the **ketosugars**, with an internal carbonyl group.
- Sugars are classified as triose (3 carbons), a tetrose (4), a pentose (5), a hexose (6), or a heptose (7).
- Most common: aldohexose D-glucose, $C_6H_{12}O_6$.

Polysaccharides

Glucose also occurs in disaccharides consisting of 2 monosaccharide units linked covalently.

- **Maltose**: 2 glucose units linked together.
- **Lactose**: glucose linked to a galactose.
- **Sucrose**: glucose linked to a fructose.

Polysaccharide Polymers are Storage and Structural

- Polysaccharides perform either storage or structural functions in cells. The most familiar storage polysaccharides are the starch of plant cells and the glycogen of animal cells. Both of these polymers consist of alpha-d-glucose units linked together by a **glycosidic** bond.
- Glycosidic bond: covalent bond between a carbohydrate (sugar) with its hydroxyl group (OH: either in alpha or beta configuration).

Glycogen (storage)

- Glycogen is highly branched, with linkages occurring every 8 to 10 glucose units along the backbone and giving rise to short side chains of about 8 to 12 glucose units.

- Glycogen is stored mainly in the liver and in muscle tissue.
In the liver it is used as a source of glucose to maintain blood sugar levels, whereas in muscle it serves as a fuel source to generate the ATP needed for muscle contraction.

Starch (storage-plants)

- Starch occurs both as un-branched amylose and as branched amylopectin.
- Like glycogen, **amylopectin** has a (1 → 6) branches along the backbone and give rise to longer chains.
- Starch deposits are about 10-30% amylose and 70-90% amylopectin.

Cellulose (structural polysaccharide)

- Cellulose is an important polymer quantitatively; more than half of the carbon in higher plants is present in cellulose.
- Like starch and glycogen, cellulose is also a polymer of glucose, but the repeating monomer is **beta-d-glucose** and the linkage is therefore beta (1 → 4).
- Cellulose forms rigid, linear rods. These aggregate into **microfibrils**.
- Plant and fungal cell walls consist of these rigid microfibrils of cellulose embedded in a **non-cellulosic matrix**.
- Mammals do not possess an enzyme that utilize cellulose as food (cannot cleave glycosidic bonds).

Polysaccharides are also important macromolecules in cell structure and function. How are they similar to proteins and nucleic acids, and how do they differ?

- Made of repeating monomers: amino acids for proteins, nucleotides for nucleic acids, monosaccharides for sugars.
- A monomer joins with another monomer with the release of water molecules, leading to the formation of a covalent bond: hydrogen of one monomer combines with hydroxyl group of another monomer (dehydration synthesis), it requires energy. The reverse reaction is hydrolysis. Each macromolecule is broken down by a specific enzyme:
 - Proteins by enzymes pepsin and peptidase, and by hydrochloric acid
 - Lipids by lipase
 - Carbohydrates by amylase, sucrase, lactase or maltase.
- Storage or structural role based on type of glycosidic bonds.

Lipids

- The distinguished feature of lipids is their **hydrophobic nature**.
- They resemble one another more in their soluble properties than in their chemical structures.
- **Not the result of stepwise polymerization found for proteins, nucleic acids, and polysaccharides.**
- Rich in nonpolar hydrocarbon regions and have relatively few polar groups.
- Some lipids are amphipathic having both a polar and a nonpolar region.
- They play at least 3 main roles in the cell:
 - Energy storage
 - Membrane structure

- Transmission of chemical signals into and within cells.
- The six main classes of lipids are **fatty acids, triacylglycerols, phospholipids, glycolipids, steroids, and terpenes.**

Fatty Acids Are the Building Blocks of several classes of Lipids

- A fatty acid is a long, unbranched hydrocarbon chain with a carboxyl group at one end.
- It is amphipathic; the carboxyl group renders one end (“head”) polar whereas the hydrocarbon, “tail” is nonpolar.
- Fatty acid yields a great deal of energy upon oxidation.
- **Fatty acids without double bonds are saturated fatty acids: every carbon atom in the chain has the maximum number of hydrogen atoms attached to it.**
- **Unsaturated fatty acids contain one or a few double bonds.**
- General formula: n carbon atoms is $C_nH_{2n}O_2$.

Triacylglycerol Are Storage Lipids

- Triacylglycerols (triglycerides) consist of a glycerol molecule with 3 fatty acids linked to it.
- Glycerol is 3-carbon alcohol with a hydroxyl group on each carbon.
- Fatty acids are linked to glycerol by ester bonds, formed by the removal of water.
- Triglycerides are synthesized stepwise, with one fatty acid added at a time.
- Monoglycerides contain a single esterified fatty acid, diglycerides have 2, triglycerides have 3.
- Triglycerides are usually solid or semi-solid at room temperature and are called fats.
- In plants, most triglycerides are liquid at room temperature – vegetable oils.

Phospholipids Are Important in Membrane Structure

- Critical to the bilayer structure found in all membranes.
- Phospholipids are phosphoglycerides or sphingolipids.

Steroids Are Lipids with a Variety of Functions

- Are derivatives of a 4-membered ring compound called phenanthrenes which makes them structurally distinct from other lipids.
- Only property that links to other classes of lipids: relatively nonpolar and therefore hydrophobic.
- **Cholesterol** is an amphipathic molecule, with a polar head group and a nonpolar hydrocarbon body and tail.
- **Cholesterol** found primarily in membranes.
- **Cholesterol** is the starting point for the synthesis of all the steroid hormones, which include the male and female sex hormones, the glucocorticoids and mineralocorticoids.

Terpenes are Formed from Isoprene

Terpenes, synthesized from 5-carbon compound isoprene, also called isoprenoids.

Isoprene and its derivatives are joined together in various combinations to produce vitamin A1, carotenoid pigments.

Lipids, our final class of macromolecules, are quite different from the other three classes, yet have some similarities. Explain.

- **Fatty acids**

- **Phospholipids:** phosphoglycerides and sphingolipids
- **Glycolipids**

Considered as macromolecules due to their high molecular weight and their frequent association with macromolecules, particularly proteins. Lipids vary substantially in chemical structure but are grouped together because they share the common property of being hydrophobic and thus are nearly insoluble in water.

- Lipids are not polymers.
- Fatty acids are lipids consisting of a long hydrocarbon chain of 12–20 carbon atoms with a carboxylic acid group at one end.
- Phosphoglycerides and sphingolipids are types of phospholipids that make up the lipid bilayer of biological membranes. They are amphipathic molecules with two hydrophobic fatty acid chains and a polar phosphate-containing head group.
- **Glycolipids** are similar to phospholipids but contain a polar carbohydrate group instead of phosphate. They are often found on the outer surface of membranes, where they play a role in cell recognition.
- Other important cellular lipids are the steroids (including cholesterol and steroid hormones) and the terpenes (including vitamin A and some important coenzymes).

Module 3 – Introduction to Cells and Organelles

Types of Cells and Their Properties

- The main distinction between two cell types (Prokaryotes vs. Eukaryotes) is the membrane bound nucleus of eukaryotic cells.
- Prokaryotic cells can be divided into **bacteria** and **archaea**.
- Most bacteria and archaea surrounded by an extra-cellular structure: **cell wall**.
- Bacterial cell walls consist of **peptidoglycan**.
- Phylogenetic Tree of Life: ancestral cell -> bacteria, Archaea, Eukarya.
- Gram's stain: staining to distinguish bacteria:
 - **Gram-positive** microorganisms have higher peptidoglycan content, whereas gram-negative organisms have higher lipid content.

There Are Three Limitations on cell size

- *Need to maintain adequate surface area to volume ratio*
Cells that are specialized for absorption have characteristics to maximize surface area/volume.
- *Diffusion rates of Molecules*
Eukaryotic cells avoid the problem of slow diffusion rates by using carrier proteins or vesicles.
- *The Need for adequate local concentration of essential substances*
The larger the cell is, the more difficult to maintain these higher concentrations required for different type of reactions to occur.

Eukaryote Cells use Organelles to compartmentalize Cellular Function

- As cell size increases, the number of molecules increase proportionately with volume.

The challenges of diffusing macro-molecules across the cell or accumulating higher concentrations in certain regions of cells can be mitigated by eukaryote cell development of organelles.

Chromosome

DNA is tightly packed into gene and chromosome and contained in the nucleus.

Genetic Information

- Eukaryotic cells replicate DNA and then distribute their chromosomes into daughter cells by **mitosis and meiosis, followed by cytokinesis, division of the cytoplasm**
- Bacterial and archaeal cells replicate their DNA and divide by binary fission.
- In the process of binary fission, an organism duplicates its genetic material, or deoxyribonucleic acid (DNA), and then divides into two parts (cytokinesis), with each new organism receiving one copy of DNA.

<https://biologydictionary.net/difference-binary-fission-mitosis/>

The Eukaryotic Cell – Plasma Membrane, Organelles, And the Endosymbiont Theory

- A typical eukaryotic cell has: a **plasma membrane**, a **nucleus**, a membrane bounded **organelle**, and the **cytosol** supported by a **cytoskeleton**.
- The Plasma Membrane defines cell boundaries and retains content.
- Membrane proteins are also amphipathic, with oligosaccharides attached to them: **glycoproteins**.
- Plasma membrane is selectively permeable membrane: only certain compounds can move across this membrane, tight control of transport across in either direction for the cell.

Mitochondrion

- Site of aerobic respiration.
- Oxidation of sugars and other fuel molecules extract energy from food and stores it as ATP.
- Most molecules for mitochondrial functions, are localized on the **cristae** or the matrix.
- Tissues with high demand for ATP have many mitochondria, located within the cell at the site of greatest energy needs (e.g., sperm and muscle cells).
- We inherit mitochondrial DNA (mtDNA) only from our mothers.

The Chloroplast

- Site of photosynthesis in plants and algae.
- Large and quite be numerous.
- Like mitochondrion, contain own ribosomes, and a small circular DNA molecule.

The nucleus is the information center of the Eukaryotic cell

- The nuclear envelope has numerous pores controlled by various proteins constituting a **nuclear pore complex**.
- The nucleus includes the **nucleolus**, the site of ribosomal RNA synthesis and ribosome assembly.
- The **endosymbiont theory**: mitochondria and chloroplasts and bacteria are similar; it suggests that mitochondria and chloroplasts originated from prokaryotes.
- Similarities:
 - Circular DNA molecules without associated histone.
 - rRNA sequences

- ribosome size
- sensitivities to inhibitors of RNA and protein synthesis.
- Type of proteins used in protein synthesis.
- Surrounded by double membranes
- Inner membrane has bacterial-type lipids

The Endoplasmic Reticulum

- **Cisternae:** is the lumen; tubular membranes and flattened sacs.
- **Lumen:** internal space of ER.
- **Rough ER:**
 - Rough ER studded with ribosomes on cytoplasmic side.
 - free ribosomes not associated with rough ER.
 - Secretory and membrane proteins are made by ribosomes on rough ER.
 - Proteins intended to be used within the cytosol or for import into organelles are made on free ribosomes.
- **Smooth ER:**
 - No role in protein synthesis.
 - Involved in synthesis of lipids and steroids (cholesterol and its derivatives).
 - Responsible for inactivating and detoxifying substances.
 - Carbohydrate metabolism
 - Calcium storage
- **Sarcoplasmic reticulum** has critical functions in contraction.

The Golgi Complex

- Consists in small number of flattened cisternae.
- At the **cis face**, transition vesicles from the ER fuse with the cis-Golgi network (CGN). At the **trans face**, transport vesicles bud from the trans-Golgi network (TGN) and carry lipids and proteins to other components of the endomembrane system.
- Two models for movement through the Golgi: **stationary cisternae model**: shuttle vesicles carry material from ER through successive Golgi compartments, **cisternae maturation model**: cisternae gradually change in composition as themselves move forward. In both models, enzymes and lipids needed in earlier compartments move backward in retrograde movement.
- Packaging station or the *post office*; processing and packaging secretory proteins and involved in complex polysaccharide synthesis.
- Role of sending off proteins to distant locations within the cells through vesicles.
- Accepts vesicles that bud off of the ER.
- The contents of vesicles from the ER are modified and processed in the Golgi complex.
- Secretory and membrane proteins are mainly **glycosylated** (addition of short-chain carbohydrates).

Cell Vesicles, Structural Components, and Examples of Cellular Invaders

Endomembrane system: ER, Golgi, secretory vesicles and lysosomes.

The cytoplasm contains the cytosol and cytoskeleton

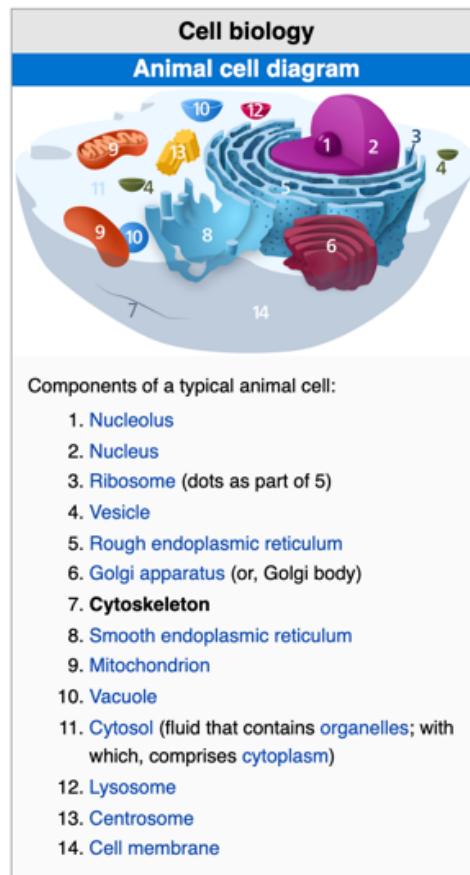
Ribosome

- Ribosomes read or translate mRNA to link amino acids together and form proteins.
- Very small, can only be seen under microscope electronic.
- Small sedimentation coefficients.
- Sedimentation coefficient: is a measure of how rapidly the particle sediments when subjected to centrifugation.

Cytoplasm

- Interior of the cell not occupied by the nucleus.
- Cytosol is the semifluid substance in which organelles are suspended.
- Cytosol is permeated by the cytoskeleton.
- Synthesis of fats and proteins and initial step of releasing energy from sugars take place in the cytosol.

Cytoskeleton



- The **cytoskeleton** is a 3-D array of interconnected **microfilaments**, **microtubules**, and **intermediate filaments**.
- Gives cell its definitive shape and internal organization.
- Plays also a role in cell movement and cell division.

- Serves as a framework for positioning and moving organelles, and macromolecules within the cell.

Microtubules

- Are critical to mitosis.
- They form the **mitotic spindle fibers** that separate chromosomes prior to cell division.
- Play a role in the organization of the cytoplasm: overall shape, organelle organization, movement of macromolecules, distribution of microfilaments.
- Cylinders of longitudinal arrays of **protofilaments** with a hollow center called a **lumen**.
- Each protofilament is a linear polymer of **tubulin** with polarity.
- **Tubulin** is a dimeric protein (alpha-tubulin and beta-tubulin).

Microfilaments

- Smallest components of the cytoskeleton.
- Form connections with plasma membrane to give structure and affect movement.
- But also help to move cell in specific way during cell division.
- Are polymers of the protein **actin**.
- Actin is synthesized as a monomer called **G-actin**.
- Subunits are polymerized into **F-actin**.
- Have a polarity.

ECM and Cell Wall Are “Outside” the cell

- For plant and fungal cells, cells walls consist in cellulose microfibrils.
- Bacterial cell walls are composed of **peptidoglycans**, long chains of GlcNAc and MurNAc
- These are held together by peptide bonds between a small number of amino acids, forming a netlike structure.

The ECM

- Support role
- In animal cells: a network of **proteoglycans** surrounds **collagen** fibers.
- Regulate processes:
 - Cell motility and migration.
 - Cell division.
 - Cell recognition and adhesion.
 - Cell differentiation during embryonic development.

Vacuoles

- Membrane containers for temporary storage and movement of compound.
- Plant vacuole large to keep the plant upright.

Secretory Vesicles

- After being processed by the Golgi complex, materials are exported from the cell into secretory vesicles.
- They move to the plasma membrane and fuse with it, releasing their content outside the cell.
- **Endomembrane system of the cell: ER, Golgi, secretory vesicles and lysosomes.**

Lysosome

- Single membrane organelles that store **hydrolases**, enzymes that can digest any biological molecules.
- A special carbohydrate coating on the inner lysosome membrane protects it from digestion.
- All of the lysosomal enzymes are **acid hydrolases**, are active at low pH, but not at higher pH (7.2), pH of the inner cell.

The Phagolysosome

- **Phagocytosis**: ingestion of bacteria by phagocytes.
- **Phagolysosome**: merge of phagosomes with lysosomes to destroy bacterial pathogens.

Peroxisome

- Similar to lysosome but contains peroxide.
- Helps to break down fatty acids.

Hydrogen Peroxide

- H_2O_2 highly toxic to cells but can be formed into water and oxygen by the enzyme **catalase**.
- These reactions are confined to peroxisomes that contain **catalase**, so that cells are protected from the harmful effects of peroxide.
- Peroxide production is increased during cellular stress (infection, disease, UV exposure) and can serve as a useful biomarker for early infections.

Viruses

- Small.
- Invade and infect cells, using synthetic machinery to produce more viruses' particles.
- No cytoplasm, organelles, or ribosomes and consist of only a few different molecules of nucleic acid and protein.
- Consist of a coat (capsid) or protein surrounding a core, containing DNA or RNA.
- Viruses that infect bacteria are **bacteriophages** or **phages**.
Each virus has a characteristic shape, defined by its protein capsid.
- Some viral capsids consist of a single type of protein, while more complex viruses have capsids with a number of different proteins
- Some viruses are surrounded by a membrane, and are called **enveloped viruses**: HIV.

Bacteriophage

- In theory, bacteriophage exist for every type of bacterium.
- Present in every ecosystem.
- Can be highly specific for their hosts.

Module 4 -Enzymes

Enzyme Structure

- Catalysts lower the temperature which a chemical reaction occurs and make it easier to happen.
- All reactions occur at 98.6 degrees F.
- An extremophile is an organism that thrives in extreme environments.
- Activation Energy = Transition state energy – Reactant state energy
- With enzyme, we drop the activation energy.

- In the reaction sequence with catalyst, number of molecules increases for same amount of time and energy.
- The substrate of an enzyme are the **reactants** that are activated by the enzyme.
- Enzymes are specific to their substrates.
- Specificity is determined by the active site.
- The **shape** and the chemical environment inside the **active site** permit a chemical reaction to proceed more easily. Active site is where the reaction happens converting reactants to products.
- Substrate molecules coming, are bound to the enzyme surface in just the right orientation and shape, electronic substrate bonding happens with the chemical environment, pulls the molecules apart to form the product.

Factors Affecting enzymes

- **Substrate concentration:** more enzymes, more reactions.
- **pH:** most of enzymes react at cellular pH.
- **Temperature:** specific T at which enzyme works.
- **Inhibitor:** can slow down the rate of the chemical reaction, even can stop the enzyme to function.
- Denature of the enzyme: pull off water molecules from the enzyme.
- Enzyme substrate complex: when the substrate locks into the enzyme.
- At the end the enzyme can go back at the reactance side of the reaction coordinate diagram: **reversibility**.
- More material present, the more reaction takes place as long there is enough reactant present.
- Reaction velocity does not increase beyond **V_{max}**.

Module 5 - Membranes and the Endomembrane System

Membrane Characteristics and Composition

The functions of the membrane

- 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.

Membranes are sites of specific proteins and functions

- Different functions associated with membrane proteins: act as enzymes, integral proteins, signaling molecules
- **Differential centrifugation:** purify or tagging proteins based on localization and molecular weight
- **Immunostaining:** tag a specific protein with an antibiotic of an epitope of a protein. Allows different, assessment of different regions within the cell by microscopy.

Regulation of Transport across the cell is a main function of membrane protein

- Receptors are specific proteins which by binding trigger changes in cell function allowing signals to be transmitted from the outer of the cell to its interior.
- Chemical signal molecules usually bind to membrane proteins, receptors, on the outer surface of the plasma membrane.
- Growth factor stimulates the cell to continue to replicating.

Membrane Proteins Mediate Cell Adhesion and Cell-to-Cell Communication

Cadherins (bind Ca^{2+}) promote adhesion between similar types of cells in a tissue.

Adhesive junctions, Tight junctions, Gap junction.

Membrane proteins play role in other cell functions

- Uptake from the cell: **endocytosis** and secreting of substances: **exocytosis**.
- Take part in targeting, sorting and modification of proteins in the ER and Golgi complex.
- Autophagy: self-recycling.

Membrane Structure: Fluid Mosaic Model

Model has 2 key features:

- A fluid lipid bilayer.
- A mosaic of proteins attached to or embedded in the bilayer.
- Not homogenous
- Ordered thru dynamic microdomains: **lipid rafts**.

Three classes of membrane proteins

- **Integral** membrane proteins: hydrophobic segments embedded within membrane interior and hydrophilic regions that extend outward into the aqueous phase on one or both sides of the membrane (transmembrane proteins).
- **Peripheral** proteins (hydrophilic and located on surface of the bilayer).
- **Lipid-anchored** proteins attached to the bilayer by covalent attachments to lipid molecules embedded in the lipid bilayer.

Main class of membrane lipids: phospholipids, glycolipids and sterols

Phospholipids

- Includes glycerol-based **phosphoglycerides** and sphingosine-based **sphingolipids**.
- **Amphipathic**: can easily form lipid bilayers.
- Small polar head group and lipid backbone.

Glycolipids

- Glycerol-based and sphingosine-based **glycosphingolipids**.
- Formed by addition of carbohydrates to lipids.
- Most common of glycosphingolipids are **cerebrosides** and **gangliosides**.
- Main function: maintain membrane stability and facilitate cell-cell communication.
- Especially prominent in brain and nerve cells.

Cerebrosides

- Single uncharged sugar as its head group: **galactose**.

Gangliosides

- Expressed on the surface of the plasma membrane, can be involved in immune reactions (ex. ABO blood cells).

Sterols

- **cholesterol**: main sterol in animal cell membrane, it stabilizes and maintains membranes, adds firmness and integrity to the plasma membrane and prevents it from becoming overly fluid.
- Plant cell membrane: **phytosterol**, fungi cell membrane: **ergosterol**.

Membrane Asymmetry: most lipids are distributed unequally between two monolayers

- Refers to the difference in the kind of lipids and degree of saturation of fatty acids in the phospholipids.
- Once established membrane asymmetry does not change much.
- Movement of lipids from one monolayer to another requires their hydrophilic heads to move through the hydrophobic interior of the bilayer.

Lipids move freely within their monolayer

- Rotation, lateral diffusion, transverse diffusion
- Movements are rapid and random.

Membrane fluidity is measured using fluorescence recovery after photobleaching (FRAP).

Tags by covalently linking membrane molecules with fluorescent dye or for proteins genetically tagging using GFP, high-intensity laser beam, at first dark tiny spots which, after a while, become fluorescent and then undistinguishable.

Membrane functions properly only in the fluid state

- Membrane has an optimal temperature: more fluid with increase of T and vice versa.
- When temperature increases, membrane fluidity increases.
- When temperature decreases, membrane fluidity decreases.
- Long-chain and saturated fatty acids decrease fluidity.
- Short-chain and unsaturated fatty acids increase fluidity.
- Below T_m , any functions that rely on membrane fluidity will be disrupted.
- Long-chain of acid chains and saturated fatty have higher T_m (saturated with H_2 and no double bonds); they pack together well in the membrane.
- In plants, most triacylglycerols are liquid at room temperature, as the term vegetable oil suggests. Because the fatty acids of oils are predominantly unsaturated, their hydrocarbon chains have kinks that prevent an orderly packing of the molecules. As a result, vegetable oils have lower melting temperatures than most animal fats do.

Membranes Functions and the Endomembrane System

Effects of Sterol on Membrane Fluidity

- Most membrane fatty acids vary in chain length and degree of saturation: helps those membranes to be fluid at physiological temperatures.
- Cholesterol molecules are rigid and can act as spacers within the hydrocarbon chain of phospholipids to prevent a tightly packed layer and helps to reduce the tendency of the membrane to gel.
- **Fluidity buffer**: help to maintain the correct amount to fluidity.
- **Sterols** decrease the permeability of membranes to ions and small polar molecules (block routes through membrane).

Lipid rafts localized regions involved in cell signaling

- Regions with concentrated lipids: lipid microdomains.
- Dynamic and changing composition.
- Lipid rafts in outer monolayer of animal cells have elevated levels of cholesterol and glycosphingolipids, and are less fluid than the rest of the membrane.

Function of the lipid rafts

- Thought to have roles in detecting and responding to extracellular signals.
 - transport of nutrients and ions across membranes
 - binding of activated immune system cells to their microbial targets
 - transport of cholera toxin into intestinal cells

Receptors in lipid rafts

- Can precipitate the downstream cellular signaling cascade that organized with lipid rafts interior to the membrane., for ex. in **phosphorylation** (addition of a phosphate/phosphoryl group PO_3^-) event.
- The addition of phosphoryl groups is called **phosphorylation** and occurs most commonly by transfer of the phosphoryl group from ATP to the hydroxyl group of a serine, threonine, or tyrosine residue in the protein. Enzymes that catalyze the phosphorylation of other enzymes (or of other proteins) are called **protein kinases**. The reversal of this process is called **dephosphorylation**, which removes a phosphoryl group from a phosphorylated protein, and is catalyzed by enzymes called **protein phosphatases**. Depending on the enzyme, phosphorylation may activate or inhibit the enzyme.
- When a receptor molecule on the outer surface of the plasma, binds its ligands, it can move into lipid rafts located in the outer membrane. These lipid rafts are connected to lipid rafts on the inner monolayer. Some lipid rafts contain *kinases* (enzymes) that generate second messengers in a cell via phosphorylation of target molecules.
- Cholera toxin binds to a receptor which is associated with lipid microdomains.

The membrane consists of a mosaic of proteins: evidence from Freeze-Fracture Microscopy

- Bilayer is frozen and then hit with diamond knife. Resulting fracture follows plane between two mono layers of lipid membrane.

Membrane Proteins are Oriented Asymmetrically Across the Lipid Bilayer

- Once in place proteins cannot move across the membrane

DNA Sequencing

- Gives an idea of different protein regions and an idea of which portions of the protein are likely transmembrane regions: identification of likely structure and orientation of protein in membrane.
- Reveals understanding of structural and functional relationship between proteins.
- Allows the determination of amino acid sequences of a protein without need to isolate it in purified form.
- Allows specific mutation in the protein sequence to determine effects on a protein function.

The Endomembrane System

- Membranes define cellular borders and organelles but also involved in **transport, signaling and adhesion**: this system is called **endomembrane**.
- **ER, Golgi, secretory vesicle and lysosomes**: make up the **endomembrane system**.

Membrane biosynthesis

- Fatty acids for membrane phospholipids are synthesized in the cytoplasm and incorporated into the ER membrane on cytosolic side.
- Transferred to other cellular membranes using vesicles from the ER membrane which fuse with other organelles of the endomembrane system.

Variations in amount of Rough and Smooth ER

- Cells involved in synthesis of secretory proteins have prominent rough ER networks: e.g., fibroblasts in skin secrete collagen.
- Cells producing steroid hormones tend to have extensive network of smooth ER. (e.g., cells of adrenal glands).

The Golgi Complex

- In ER, **glycoproteins** are sorted and packaged for transport via the **trans-Golgi** network or TGN.
- Many of the proteins with hydrophilic regions exposed on the external side of the plasma membrane have carbohydrate side chains known as oligosaccharides attached to them and are therefore called **glycoproteins**.
- Materials to be exported from the cell are packaged into secretory vesicles.
- These move to the plasma membrane and fuse into it releasing their contents outside.
- Many of the proteins trafficked through the membrane go through a process called **glycosylation**: addition of carbohydrates side chains to proteins (to a hydroxyl or other function group). It helps in proper folding of proteins, stability and in cell-to-cell adhesion.
- This forms **glycoproteins**.
- Initial glycosylation occurs in the ER.
- All carbohydrate side chains have a common **core oligosaccharide**.

Roles of the ER and Golgi Complex in Protein Trafficking

- Proteins synthesized in the rough ER must be directed to a variety of locations.
- Each protein contains a chemical tag, targeting to a specific transport vesicle.

Protein and Lipid Tags

- A tag could be an amino acid sequence, **a hydrophobic domain, or oligosaccharide side chain or some other feature (depending on the protein and destination)**.
- Membrane lipids can also be tagged to help vesicle to reach their destinations.
- Lipid tags can be one or **more phosphate group**.

Exocytosis and Endocytosis: Transporting Material Across the Plasma Membrane

Endocytic vesicle.

Phagocytosis

- Ingestion of large particles up to and including whole cells or microorganisms.
- “Professional phagocytes”: neutrophils, macrophages, and dendritic cells.

Receptor-Mediated Endocytosis

- A receptor-mediated drives endocytosis (or **clathrin-dependent endocytosis**), to ingest growth factors, hormones, serum proteins, enzymes, cholesterol, antibodies, iron, viruses, bacterial toxins.

Process of receptor-mediated endocytosis

1. The receptor-ligand complexes diffuse laterally into coated pits.
2. Additional proteins on the cytosolic surface of the membrane: adaptor proteins: **clathrin**, **dynamin**, induce curvature and invagination of the pit.
3. Eventually the pit pinches off forming a coated vesicle.
4. The **clathrin** coat is released leaving an uncoated vesicle
5. Coat proteins and dynamin are recycled to the plasma membrane and the uncoated vesicle fuses with an endosome.

Module 6 – Membrane Transport

Transport Across Membranes: Overcoming the Permeability Barrier.

Cell Transport

- Cell transport is the ability of the cell to move ions and organic molecules across membranes selectively.
- Most substances that move across the membranes are not macromolecules or fluids but dissolved ions and small organic molecules-solutes.
- Common ions transported: **sodium, potassium, calcium, chloride**, and **hydrogen**.
- Most of the molecules are metabolites-substrates, intermediates and products in the various metabolic pathways: **sugars, amino acids**, and **nucleotides**.
- More than 2/3 of the energy your body expends in the resting state is used to maintain gradients of ions such as H⁺, K⁺, Na⁺, and Ca²⁺.
- **Electrochemical gradient**: concentration gradient + membrane potential (charge gradient of all the ions across the membrane).
- Stored energy gradient used to drive uptake of other solutes, including synthesis of ATP.
- In nerve cells, gradients of K⁺ and Na⁺ responsible for transmission of nerve impulses.

Simple diffusion: unassisted movement down the gradient.

- Because of the hydrophobic interior of the membrane, simple diffusion relevant only for small, nonpolar molecules.
- **Facilitated diffusion mediated by carrier protein: GLUT1** – glucose transporter. **Anion** exchange protein Cl⁻ in (chloride), HCO₃⁻ out (bicarbonate).
- **Facilitated diffusion mediated by channel protein. Aquaporin channel proteins.**
- **Active transport** – Na⁺/K⁺ pump: 3 Na⁺ out, 2 K⁺ in, driven by hydrolysis of ATP, electrochemical potential across membrane.

Osmosis of water across membrane

- Water molecules not charged; concentration similar on opposite sides of membrane.
- Water tends to move from regions of lower solute concentration (higher free energy) to regions of higher solute concentration (lower free energy).
- Diffusion always moves solutes toward an equilibrium.

- For most cells, water will move inward because the concentration of solutes is almost always higher inside a cell than outside.
- **Osmosis:** movement of water in response to differences in solute concentration.

Second Law of Thermodynamics

- Diffusion always proceeds from regions of higher energy to lower free energy: molecules flow down their concentration gradient, and ions flow down their electrochemical gradient.
- 3 main factors affecting diffusion: size, polarity and charge.

Solute size

- Size rule holds up to about the size of glucose (ethanol and glycerol are able to diffuse, glucose not).
- Water, O₂ and CO₂ can diffuse across a bilayer by simple diffusion.

Solute Polarity

- The more hydrophobic or nonpolar, a substance is, the more readily and rapidly it can move across the membrane.

Ion permeability

- Lipid bilayer very impermeable to ions.
- Impermeability very important: cells must maintain an ion gradient across its plasma membrane in order to function: either a gradient of sodium ions (animal cells) or protons (mitochondria, chloroplasts).
- Proteins that facilitate ion transport provide hydrophilic channels.

Table 8-1 Comparison of Simple Diffusion, Facilitated Diffusion, and Active Transport

Properties		Simple Diffusion	Facilitated Diffusion	Active Transport
Solutes transported	Examples			
Small nonpolar	Oxygen	Yes	No	No
Large nonpolar	Fatty acids	No	Yes	No
Small polar	Water, glycerol	Yes	Yes	No
Large polar	Glucose	No	Yes	Yes
Ions	Na ⁺ , K ⁺ , Ca ²⁺	No	Yes	Yes
Thermodynamic properties				
Direction relative to electrochemical gradient		Down	Down	Up
Effect on entropy		Increased	Increased	Decreased
Metabolic energy required		No	No	Yes
Intrinsic directionality		No	No	Yes
Kinetic properties				
Membrane protein mediated		No	Yes	Yes
Saturation kinetics		No	Yes	Yes
Competitive inhibition		No	Yes	Yes



Rate of simple diffusion directly proportional to concentration gradient

- Simple diffusion thermodynamically always an **exergonic process** (no energy required)
- Simple diffusion is a **linear relationship** between the inward flux of the solute across the membrane and the concentration gradient of the solute, with **no saturation at high concentrations**.

Facilitated diffusion: protein-mediated movement down the gradient

- **Facilitated diffusion is subject to saturation and follows Michaelis-Menten kinetics.**
- **Facilitated diffusion or passive transport** does not require energy, **process is exergonic**:
- Example movement of glucose across the plasma membrane of an erythrocyte. Concentration of glucose is higher in blood plasma than in erythrocytes, so transport of glucose across the plasma membrane is passive.

Carrier and channel proteins facilitate transport by different mechanisms

- Channel proteins form **hydrophilic channels** through the membrane.
 - **Pores**: large and nonspecific channels. Formed by transmembrane proteins called **porins**, allow molecules weight up to about 600Da to diffuse across the membrane.
 - Most channels are small and highly selective: **ion channels** – **more rapid** no need for a protein to change its shape and capture a solute.
- Carrier proteins are called **permeases**.
 - Like enzymes very specific.
 - Carrier proteins differ in number of salutes transported (**uniport**), and the direction in which they move.
 - Glucose carrier protein is a **uniporter**.
- When two solutes are transported simultaneously and their transport coupled: **co-transport**.
- Same direction: **symport** – opposite direction: **antiport**.

Glucose transporter: a uniport carrier

- The erythrocyte is capable of glucose uptake by facilitated diffusion, in erythrocyte: GluT1 (glucose transporter).
- GluT1 provides a hydrophilic channel for n-glucose molecules alternating between T1 and T2 conformations.

Active transport: protein-mediated movement up the gradient.

- Always moves solutes away from thermodynamic equilibrium (up a concentration or electrochemical gradient), therefore always requires energy (ATP → ADP).
- Process **endergonic**, occurs only when coupled to an exergonic process.
- Performs 3 major functions:
 1. Makes possible the uptake of essential nutrients from environment or surroundings of the cell.
 2. Allowed secretory products and waste materials to be removed from the cell or organelles.
 3. Enables the cell to maintain constant, non-equilibrium intracellular concentration of specific ions: K⁺, Na⁺, Ca²⁺, H⁺.

Pumps

- Membrane proteins involved in active transport.
- Passive transport: inherently nondirectional w.r.t membrane. Active transport has **directionality: unidirectional or vectorial process**.
- **Direct or primary active transport**: coupled to an exergonic chemical reaction, most commonly hydrolysis of ATP.

- **Indirect or secondary active transport:** driven by the co-transport of cations-protons down the electrochemical gradient: exergonic inward movement of protons provides energy to move the solute against its concentration gradient.

Na⁺/K⁺ pump

- Uses ATP for energy, example of a transport ATPase.
- Directional: 2 K⁺ in, 3 Na⁺ out.
- E1, E2 conformational changes.

Module 8 - DNA, Chromosomes, the Nucleus

Information and DNA

- **Replication:** 2 DNA copies are distributed to daughter cells when cell divides.
- **DNA replication:** DNA synthesis.
- **Mitosis:** cell division.
- **Transcription:** involves the use of selected segments of DNA as templates for the synthesis of mRNA and other RNA molecules.
- **Translation:** amino acids are joined in a sequence dictated by the sequence of nucleotides in mRNA. protein synthesis (cytoplasm).
- **Expression of genetic information:** transcription and translation.

DNA

- Helix is right-handed.
- Contains 10 nucleotide pairs per turn and advances 0.34nm per nucleotide pair.
- Each complete turn of the helix adds 3.4nm to the length of the molecule.
- Diameter of helix: 2nm.
- Pyrimidine-Purine pairing.
- Two chains of DNA complementary to each other.
- Two chains create a **major groove** and **minor groove** which play significant roles in the interactions of variety of molecules.
- **Antiparallel orientation** of the two DNA strands.
- Nucleotides linked by **phosphodiester bonds**.
- **5'-3' orientation- 3'-5' in opposite strand:** 5' carbon linked to 3' carbon.
- **Supercoiled DNA:** DNA double helix twisted upon itself.
- **Circular DNA** molecules are **negatively supercoiled**.
- Raise temperature to denature DNA: **DNA melting temperature** T_M.
- G-C: 3 H₂ bonds, A-T: 2 H₂ bonds.
- **Strand separation** can be readily achieved because the two DNA strands are bound together by relatively **weak, non-covalent bonds**.
- Strand separation is integral part of DNA replication and RNA synthesis, transcription.
- By raising slowly, the temperature: **DNA denaturation happens** (two strands separated), when lowering the temperature, the reverse happens and **is DNA renaturation**.
- Maximum absorption of UV lights **around 260nm**. As strands separate, absorbance of solution increases rapidly.

- Ability to renature nucleic acids forms the basis of **nucleic acid hybridization**.

DNA in the genome

- Genome of an organism or virus: complete copy of all the genetic information.
- For many viruses or prokaryotes, genome resides in on DNA molecule.
- Eukaryotic cells have a nuclear genome, a mitochondrial genome.
- Nuclear genome consists of multiple DNA molecules.
- Genome size increases with complexity of the organism with exceptions.
- Genome size is less important than the number and identity of functional genes.
- DNA must be efficiently packaged into cells and yet remain accessible to cellular machinery for DNA replication and transcription.

Restriction Enzymes

- Cut DNA molecules in places where it encounters a specific recognition sequence, called a **restriction site**.
- Cleave DNA into fragments ranging from a few hundred to a few thousand base pairs
- More amenable to manipulation.
- Gel electrophoresis is the technique used to separate the fragments to each other, their number and lengths.

The Nucleus

- Site where the chromosomes are localized and replicated and where DNA is transcribed.
- Both the repository of most cell's genetic information and control center for expression of the information.

There are two types of cell division: **mitosis** and **meiosis**. Most of the time when people refer to "cell division," they mean mitosis, the process of making new body cells. Meiosis is the type of cell division that creates egg and sperm cells.

Module 9 – Signal Transduction

Receptor-Ligand Interactions

Signal Transduction Mechanisms

- Receptors are located on receiving cells that can be quite distant from the secreting cell.
- Multicellular organisms can control the activities of specialized cells through the release of chemical messengers.
- The ability of the cell to respond to ligand-receptor binding by altering its behavior or gene expression is signal transduction.

Different Types of Chemical Signals Can Be Received by Cells

- **Endocrine signals:** produced far from the target tissues, which they reach via the circulatory system.
- **Paracrine signals:** are diffusible and act over short range.
- **Juxtacrine signals:** require physical contact between sending and receiving cells.
- **Autocrine signals:** act on same cell that produces them.

Receptor Binding Involves Specific Interactions Between Ligands and Their Receptor

- Messengers bind to receptors in a highly specific way.
- The binding site (or binding pocket) on the receptor fits the messenger very closely.
- Necessary amino acid side chain, positioned form chemical bonds with the messenger.

Receptor-ligand interactions

- Binding of a receptor and a ligand resembles binding of an enzyme and its substrate.
- A receptor specific for a certain ligand: **cognate receptor**.
- A receptor bound to its ligand is **occupied**.

Receptor Affinity

- **Receptor affinity**: relationship between the ligand in solution and the number of receptors occupied.
- **High ligand affinity**: almost all the receptors are occupied at a low concentration of free ligand.
- **Dissociation constant K_d**, [free ligand] needed to produce a state in which half of receptors occupied.
- High ligand affinity \Leftrightarrow low K_d.

Coreceptors

- Receptor-ligand interactions can be affected by coreceptors on the cell surface.
- They help to facilitate receptor-ligand interaction via physical interaction with receptor.
- Sometimes localized in regions like lipid rafts or lipid microdomains.
- **Toll-like receptors**: ways in which a cell recognizes pathogen.
- **HSPGs**: Heparan Sulfate Proteoglycans.

Receptor Down-regulation

- Cells sense ligand concentration changes rather than fixed concentrations.
- When receptors occupied for prolonged periods, the cells adapt to no longer respond to ligand.
- **Receptor down-regulation**:
 - Cells reduce density of receptors on their cell surface via **receptor-mediated endocytosis**.
 - Cells can adapt to signals by **desensitization** (common method: **phosphorylation**, add. phosphate group to a molecule), alterations to lower receptor affinity for the ligand.

Agonist and Antagonists

- **Agonists**: drugs that activate the receptor they are bound to.
- **Antagonists**: bind receptors without triggering a change, prevent naturally occurring messenger from activating the receptor.
- It is possible to make synthetic ligands that bind even more tightly or selectively than the real ligand.

Receptor Binding activates a sequence of signal transduction events within the cell

- When a ligand binds to its cognate receptor it either induces a change in receptor conformation or causes receptors to cluster (coreceptors).
- Once this takes place, a **preprogrammed** sequence of events is initiated inside the cell.

- Cells can be exposed to a multitude of signals at any given moment.
- Cells must **integrate** these signals to produce appropriate responses (i.e., **signal integration**) without under or over responding.
- A single receptor can **activate multiple pathways, or multiple pathways can converge onto the same molecules**.
- MAP Kinase pathway: ligands that signal the cell to grow and divide (**mitogen**).
- MAPKs phosphorylate transcription factors that enter the nucleus to alter gene expression.

Signal Amplification

- Very small quantities of ligand often sufficient to elicit responses from target cell.
- At each step in resulting cascade of event, a signaling intermediate stimulates the production of many molecules for next step.
- **Signal amplification**: multiplication of the effects of the signal.
- **Epinephrine**: part of light-fly response system.

GPCRs And Second Messengers

Categories of Receptors

Can be classified

- Ligand-gated channel (ion channels that allows neuro-transmitter to pass through).
- Plasma membrane receptors: linked to G proteins or protein kinases.

G Protein-Linked Receptors

- **G protein-linked receptor** family: ligand binding causes a change in receptor conformation that activates a G protein (guanine-nucleotide binding protein: **GDP and GTP**).
- The G protein then binds a target protein, such as an enzyme or channel protein, altering target's activity.
- Many Seven-Transmembrane receptors act via G proteins.
- **Opioid receptors**: G-protein-coupled receptor (GPCRs).

The structure and regulation of G protein-linked receptors

- The receptor forms 7 transmembrane alpha helices connected by alternating cytosolic or extracellular loops.
- Extracellular portion of each receptor has a unique messenger-binding site.

The structure, activation, and inactivation of G proteins

- G Proteins act like molecular switches: on when bound to guanosine triphosphate (**GTP**) and off bound to guanosine diphosphate (**GDP**).
- Large **heterotrimeric G proteins** (3 subunits) and **small monomeric G proteins**.
- Heterotrimeric G proteins mediate signal transduction through G protein-linked receptors and have **G α , G β and G γ subunits**.

Regulation of G protein-linked receptors

- Important for cells to be able to stop G protein-linked receptors to activate G proteins by:
 - Phosphorylation of amino acids in the cytosolic domain by G protein-linked receptor kinases (**GRKs**) which act on activated receptors.
 - **Desensitization**, or adaption to a persistent stimulus: e.g., **β -arrestin** binding to the receptor, blocks further G protein-mediated signaling and targets receptor for internalization.

G protein Inactivation

- G proteins remain active as long the G_{α} subunit is bound to GTP and separate from the $G_{\beta\gamma}$ subunit.
- Once the G_{α} subunit has hydrolyzed GTP to GDP, it is re-associates with $G_{\beta\gamma}$.
- Some G_{α} subunits are not very efficient at GTP hydrolysis (i.e., turning off) so G protein activity (for GTP hydrolysis) is greatly enhanced by regulators of G-protein-signaling (RGS) proteins.
- Most important G protein function: release or formation of second messenger.

Cyclic AMP is a second messenger whose production is regulated by some G proteins.

- **Cyclic AMP (cAMP):** formed from cytosolic ATP by adenylyl cyclase, an enzyme anchored in the plasma membrane.
- Enzyme is inactive until bound to activated $G_{s\alpha}$ (by receptor-ligand stimulated acquisition of GTP and release from $G_{s\beta\gamma}$).

G proteins are active for only a short period of time

- Because G proteins remain active for a very short time, they can respond quickly to changing conditions:
- Once a G protein becomes inactive, adenylyl cyclase stops making new cAMP.
- The cAMP that remains is degraded.
- cAMP very important in many cellular events: main target is protein kinase A (**PKA**).
- PKA 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 site slows.**

Many G Proteins use inositol trisphosphate (IP3) and Diacylglycerol (DAG) as second messenger
Calcium in signaling

- Ca^{2+} : 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.
- Ca^{2+} fluorescent dyes.

Protein Kinase-associated receptors

Protein Kinase-associated receptors

- Important not only as receptors, but also function as kinases (**kinases phosphorylate proteins by transferring phosphate groups to them**).
- Function in many important cellular processes.
- Ligand binding stimulate their kinase activities.
- Signaling of **these receptor protein kinases**: transmitted through **protein Kinase-associated receptors**.

Growth factors often bind protein kinase-associated receptors

For cells to divide they need enough nutrients for growth and signals to stimulate cell growth.

- Cultured cells in vitro will not grow, even with enough nutrients, unless blood serum is provided.
- Messengers in the serum that stimulate growth are called **growth factors**.
- Several growth factors stimulate receptor tyrosine kinases:
 - **Insulin**
 - **Insulin-like growth factor-1**
 - **Fibroblast growth factor**
 - **Epidermal growth factor**
 - **Nerve growth factor**

Receptor tyrosine kinases aggregate and undergo autophosphorylation

- Many **receptor tyrosine kinases (RTKs)** trigger a chain of events in the cell that culminate in cell growth, proliferation, or specialization.
- **RTKs**: often consist in a single polypeptide chain with one transmembrane segment.
- **Extracellular part of the receptor: ligand-binding domain.**
- **On cytosolic side: tyrosine kinase domain.**

The activation of receptor tyrosine kinases

- Signal transduction initiated upon ligand binding that causes aggregation of receptor tyrosine kinases.
- In some cases, receptors dimerize upon ligand binding, and phosphorylate each other: **autophosphorylation**.
- 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.

Dominant negative mutant receptors are important tools for studying receptor function

- One approach: introduce mutations into the receptor to determine the effect: e.g., **fibroblast growth factors (FGFs) and their receptor tyrosine kinases (FGFRs)**.
- **Normal FGFs** undergo autophosphorylation in response to ligand binding.
- Some types of mutant FGFRs can bind ligands but cannot undergo autophosphorylation.
- These mutant receptors interfere with normal receptor function because they can dimerize w/ normal receptors.
- **Dominant negative mutation**: a mutant that overrides normal function.

Constitutive mutations

- Some mutations make FGFRs active in signaling, even when not bound to ligand.
- **Mutation constitutively active**: cause receptor to stay switched “on” all the time.

Disruptions of growth factor signaling can lead to cancer

Some cancers can result from the loss of regulation of growth factor signaling: e.g., **mutation in Ras often associated with cancer**.

Mutations in **EGFR** can result in **breast cancer, glioblastoma (brain cancer) and fibrosarcoma (bone cancer)**.

Growth factor receptor pathways share common themes

- Ligand binding often results in activation and/or clustering of receptors, followed by a cascade of events, often phosphorylation (addition of a phosphate group).
- **Phosphorylation may be catalyzed by the receptor, or by Janus activated kinase, when activated by a receptor.**

Hormone signaling

Plants and animals use secreted chemical signals called hormones to coordinate function of cells and tissues over long distances.

Hormones can be classified by the distance they travel and by their chemical properties

- **Endocrine hormones:** travel from sending to receiving cells via circulatory system.
- Synthesized by: **endocrine tissues** and secreted directly into bloodstream, with a lifespan ranging from few seconds to many hours.
- As they circulate, they encounter their receptors in target tissues.

The cells in **target tissues have hormone-specific receptors** embedded in their plasma membranes (or, in the case of steroid hormones, in their nucleus or cytosol).

Chemical classification of endocrine hormones

Endocrine hormones fall into four categories

- **Amino acid derivatives (e.g., epinephrine),**
- **Peptides (e.g., vasopressin).**
- **Proteins (e.g., insulin).**
- **Lipid-like hormones such as steroids (e.g., testosterone).**

Control of Glucose metabolism is a good example of endocrine regulation

- **Adrenergic hormones: epinephrine and norepinephrine,** function to put the body on hold and redirect resources to the heart and skeletal muscles in dangerous or stressful situations.
- They bind to a family of G protein-linked receptors, adrenergic receptors, **classified as α - and β -adrenergic receptors.**
- These receptors stimulate different pathways (and have different effects) because they are linked to different G proteins.

Intracellular effects of adrenergic hormonal control of glycogen degradation

- One effect of adrenergic hormones **is to stimulate the breakdown of glycogen to provide muscle cells with an adequate supply of glucose (energy).**
- The breakdown of glycogen is facilitated by the **enzyme glycogen phosphorylase**, resulting in release of a **glucose-1-phosphate**.
- Glycogen degradation begins when an epinephrine molecule binds to a **β -adrenergic receptor** on a liver or muscle cell.

Insulin signaling acts through PI 3-kinase to regulate resting glucose levels

Specialized cells in pancreas, the **islets of Langerhans** secrete two peptide hormones that regulate glucose levels.

- **Glucagon:** acts to increase blood glucose through glycogen breakdown.
- **Insulin:** reduces blood glucose levels by stimulating uptake into muscle and adipose cells, and stimulating glycogen synthesis.

Diabetes

- **Insulin:** is a peptide hormone that has rapid and longer-lasting effects on a variety of cells (compared to adrenergic hormones)
- **Type I diabetes:** is an **autoimmune disorder resulting in loss of insulin** - producing cells in the islets of Langerhans (only 5% of people diagnosed with diabetes have this form). It can be somewhat successfully treated with insulin.
- **Type II diabetes:** appears to result from resistance to insulin and so is not as effectively treated with insulin.

Steroid hormone receptors act primarily in the nucleus, not the cell surface

- Steroid receptor proteins mediate actions of steroid hormones such as **progesterone, estrogen, testosterone, and glucocorticoids**.
- Steroid hormones **are lipid signaling molecules**.
- The hormone enters the target cell and binds its receptor, triggering a cascade of events that activate (sometimes inhibit) transcription of a set of target genes.
- Can diffuse through the plasma membrane.

Module 10 – Recombinant Technology

DNA technology

The PCR Revolution

- DNA fingerprinting analysis can be used to identify and characterize particular sequences contained in as little as 1 microgram of DNA, the amount in a small drop of blood
- But sometimes even that amount of DNA may not be available. In such cases another method, called the polymerase chain reaction (PCR), can come to the rescue. With PCR, it is possible to rapidly replicate, or amplify, selected DNA segments that are initially present in extremely small amounts.

PCR

- The keys to the simplicity of PCR are an unusual DNA polymerase and the fact that synthetic primers can set up a chain reaction that produces an exponentially growing population of specific DNA molecules.
- To carry out PCR, it is usually necessary to know part of the base sequence of the DNA segment that one wishes to amplify. Based on this information, short single-stranded DNA primers are chemically synthesized; these primers are 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.
- DNA polymerase is then added to catalyze the synthesis of complementary DNA strands using the two primers as starting points.
- *Thermus aquaticus*, an inhabitant of thermal hot springs where the waters are normally 70-80°C. The optimal temperature for this enzyme, called Taq polymerase, is 72°C, and it is stable at even higher temperatures - a property that made possible the automation of PCR.

DNA Extraction

- Chemical treatments cause cells and nuclei to burst

- DNA spooling: DNA is inherently sticky, and can be pulled out of the mixture.

Copying DNA

- PCR: Polymerase Chain Reaction.
- A method of making many copies of a piece of DNA.

Technologies And Utilities

Historical Perspective

DNA hybridization (1960s)

Detection of hybrids (methods)

- hydroxyapatite (bone material)
- radio active labelling
- enzyme-linked detection
- fluorescent labelling

Fixing sample on solid support

- Southern blots(1970s)
- Northern blots
- Dot blots

Basic Principles

Main novelty DNA chips is scale

- hundreds or thousands of probes rather than tens
- it cannot be achieved manually

Probes are attached to solid supports

Robotics are used extensively

Informatics is a central component at all stages

Major technologies

- cDNA probes usually produced by PCR, attached to either nylon (cheaper, hold less samples) or glass supports
- **Oligonucleotides** attached to glass support
- **Oligonucleotides** synthesized in situ on silica wafers
- (Affymetrix method)
- Probes attached to tagged beads

Principal uses of chips

Genome-scale gene expression analysis

- **Differentiation**
- Responses to environmental factors
- Disease processes
- Effects of drugs

Detection of sequence variation

- **Genetic typing**
- Detection of somatic mutations (e.g., in **oncogenes**)
- **Direct sequencing**

DNA chips

- Probes are DNA fragments, usually amplified by PCR.

- Probes are deposited on a solid support, either positively charged.
- nylon or glass slide.
- Samples are labeled using fluorescent dyes.
- At least two samples are hybridized to chip.
- Fluorescence at different wavelengths measured by a scanner.

DNA chip design

Probe selection

- Non-redundant set of probes
- Includes genes of interest to project
- Corresponds to physically available clones

Chip layout

- Grouping of probes by function
- Correspondence between wells in micro-titre plates and spots on the chip

cDNA (complementary DNA) fragments arrays on nylon and glass

Nylon arrays

- Up to about 1000 probes per filter
- Use radiolabeled cDNA target
- Can use phosphor imager or X-ray film

Glass arrays

- Up to about 40,000 probes per slide, or 10,000 per 2cm² area (limited by arrayer's capabilities)
- Use fluorescent targets
- Require specialized scanner

Scanning the arrays

Laser scanners

- Excellent spatial resolution
- Good sensitivity, but can bleach fluorochromes
- Still rather slow

CCD scanners

- Spatial resolution can be a problem
- Sensitivity easily adjustable (exposure time)
- Faster and cheaper than lasers

In all cases, raw data are images showing fluorescence on surface of chip.

The Affymetrix approach

- Probes are oligos synthesized in situ using a photolithographic approach
- There are at least 5 oligos per cDNA, plus an equal number of negative controls
- The apparatus requires a fluidics station for hybridization and a special scanner
- Only a single fluorochrome is used per hybridization
- It is a very expensive approach!

Module 11 – Cell Cycle, Mitosis and DNA Replication

Mitosis and the Cell Cycle

The Cell Cycle, DNA Replication and Mitosis

- Cell growth is generally accompanied by cell division, whereby one cell gives rise to 2 new daughter cells.
- All the genetic information in the nucleus must be accurately duplicated and carefully distributed to the daughter cells.
- In doing this a cell passes through a series of stages known as the **cell cycle**.

Overview of the cell cycle

- Begins when two new cells are formed by division of a parent cell and ends when one of these cells divides again.
- Two copies of each chromosome made during S phase are distributed into daughter cells during M phase.
- **M (Mitosis) phase:** the cells actually divide; nucleus first, followed by cytoplasm.
- **Mitosis: nuclear division; cytokinesis: cytoplasm division.**

Mitosis is a relatively short part of the cell cycle

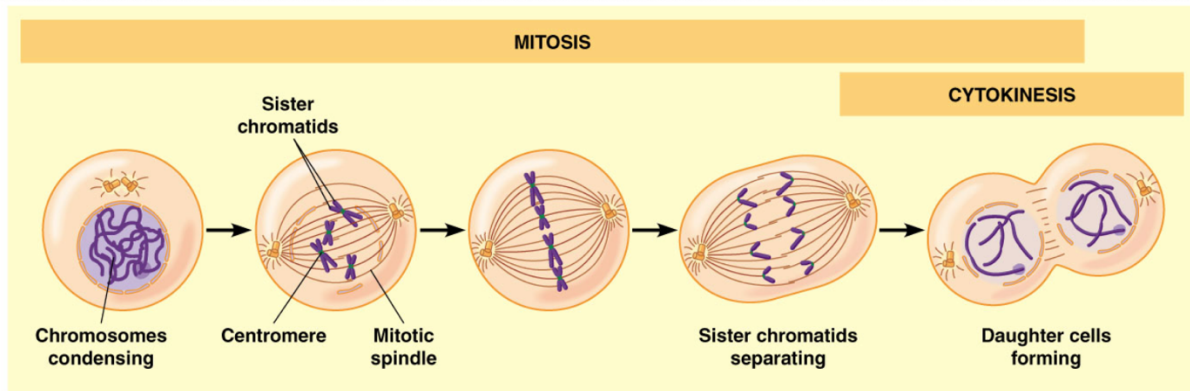
- Cells spend very little time in M phase.
- Most of the time spent in interphase, composed of **G1 phase, S phase (when DNA is replicated), and G2 phase.**
- Overall length of the cell cycle **called generation time**; in cultured mammalian cells about 18-24 hours.

Chromosomes in Mitosis

- 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.

Mitosis is subdivided into Prophase, Prometaphase, Metaphase, Anaphase and Telophase

Mitosis is divided into 5 stages based on changing appearance and behavior of chromosomes. Events during each stage are directed toward the correct distribution of one copy of each chromosome into daughter nuclei.



(a) The M (mitotic) phase

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.
- 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.

Centromere

- 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.

Kinetochores

- 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 chromosomes to be identified. 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.

The mitotic spindle is responsible for chromosome movements during mitosis

- **Mitotic spindle:** The microtubule-containing apparatus responsible for separation of chromatids into daughter cells

Spindle Assembly and Chromosome Attachment

Microtubules have an inherent polarity (the two ends have different chemical properties).

- (-) end: is at the initiating centrosome: end where MT is initiated (the centrosome in the case of the spindle)
- (+) end: points away from the centrosome: where most growth occurs.
- Tubulin subunits are added and removed during mitosis.

Spindle Assembly

- During late prophase, MT growth speeds up dramatically and initiation of new MTs at centrosomes increases.
- When the nuclear envelope disintegrates, kinetochores and MTs can come into contact.
- When (+) end of MTs and kinetochore bind, the MT becomes **kinetochore MT**.

So what Drives Chromosomes Movement

- Several motor proteins play active roles in mitosis:
 - Kinesins
 - Dynein
 - Myosin
- The use energy from ATP to change shape and exert force that causes movement of attached proteins.
- Motor proteins play at least 3 distinct roles in movement oof anaphase chromosomes.
 - Move chromosome apart
 - Attach centrosome to plasma membrane
 - Causes spindle to attach

Cytokinesis Divides the Cytoplasm

- After the chromosomes have separated, cytokinesis divides the cytoplasm in two.
- This usually starts in late anaphase or early telophase.

Myosin and cleavage

- Contraction of the ring generated by interactions between **actin** and motor protein: **myosin**.

- **Members of Rho-GTP binding proteins** regulate assembly and activation of the contractile ring.
- **RhoA** is recruited to the cleavage furrow to activate proteins needed for actin polymerization and stimulate activation of myosin.

Cell Division is Sometimes Asymmetric

- Cytokinesis is not always symmetric; sometimes the spindle forms in asymmetric fashion.
- Can result in one large and one small cell.
- Occurs frequently during embryonic development; sometimes cells formed this way have differing developmental potentials.

DNA Replication

DNA Replication

Mechanism depends on double-helical structure of DNA.

Semiconservative replication: one strand of every new DNA molecule is derived from the parent molecule and the other is new.

Two parental DNA strands unwind and each specifies a new daughter strand by base-pairing rules.

DNA Replication is usually bidirectional

- DNA replication is especially well understood in *Escherichia coli*.
- Replication very similar in prokaryotes and eukaryotes.
- Early biologists studied replication in *E. coli*; They grew cells in a medium containing ^3H -thymidine.
- They visualized the circular chromosomes by autoradiography and observed **replication forks**.
- These are formed where replication begins and then proceeds in *bidirectional fashion* away from the origin

Eukaryotic DNA Replication involves multiple replicons

- **Replicons:** in eukaryotes replication of linear chromosomes is initiated at multiple sites, creating replication units called replicons.
- **Origin of replication:** at the center of each replicon is a DNA sequence called an origin of replication, where synthesis is initiated by several groups of initiator proteins (a single eukaryotic chromosome may contain several thousand replicons).
- First, a multi-subunit protein complex the **origin recognition complex (ORC)** binds the replication origin.
- Next: the **minichromosome maintenance (MCM)** proteins bind the origin.
- The MCM proteins include **several DNA helicases** that unwind the double helix.
- **Pre-replication complex:** all the DNA-bound proteins bound to this point; the DNA is “licensed” for replication.
- After replication begins, two replication forks synthesize DNA in opposite directions, forming a **replication bubble** that grows as replication proceeds.

Replication licensing ensures that DNA molecules are duplicated only once prior to each cell division

- Licensing provided by binding of MCM proteins to the origin, which requires both ORC and helicase loaders.
- Ensures that after DNA is replicated at each origin, the DNA cannot be licensed for replication again until after mitosis.
- After replication begins, the MCM proteins are removed from the origins and cannot bind again (**due to Cdk and geminin binding**).

DNA polymerase catalyze the elongation of DNA chains

- **DNA polymerase**: an enzyme that can copy DNA molecules.
- Incoming nucleotides are added to the 3' hydroxyl end of the growing DNA chain, so **elongation occurs in the 5' to 3' direction**.
- 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 deoxynucleoside triphosphate (5' to 3')

Biotechnology functions of DNA polymerases

- DNA polymerases have practical applications in biotechnology
- The polymerase chain reaction is a technique in which a DNA polymerase is used to amplify tiny samples of DNA

DNA is synthesized as discontinuous segments that are joined together by DNA ligases

- 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.

Proofreading is performed by the 3'→5' Exonuclease activity of DNA polymerase

- About 1 of every 100,000 nucleotides incorporated during DNA replication is correct.
- Such mistakes fixed by a **proofreading mechanism**.
- Almost all DNA polymerase have a 3' → 5' **exonuclease activity**.
- **Exonucleases** degrade nucleic acids from the ends of the molecules. Exonuclease activity of DNA polymerase allows it to remove incorrectly base-paired nucleotides and incorporate the correct base.
- **Endonucleases** make internal cuts (vital for repair).

DNA synthesis requires RNA primers

- Natural DNA synthesis (not PCR) is initiated by formation of **short RNA primers**.
- These are synthesized by **primase** using a single DNA strand as the template.

The process of DNA synthesis

- Once the RNA primer is made, a **DNA polymerase III** adds deoxynucleotides (A's, C's, T's, or G's) to the 3' end of the primer.

- **For the leading strand, just one primer is needed, but the lagging strand needs a series of primers to initiate each Okazaki fragment.**
- When the DNA chain reaches the next Okazaki fragment the RNA is degraded and replaced with DNA; adjacent fragments are joined together by DNA ligase.

Unwinding the DNA double helix requires DNA helicases, Topoisomerases, and single-strand DNA binding proteins

- During DNA replication the two strands of the double helix must unwind at each replication fork.
- 3 classes of proteins facilitate the unwinding:
 - **DNA helicases:** responsible for unwinding the DNA using energy from ATP hydrolysis.
 - **Topoisomerases:** create swivel points in the DNA molecule by making and then quickly sealing double-strand or single-stranded breaks (e.g., **gyrase**).
 - **SSB:** keep the DNA unwound and accessible to the replication machinery.

Summary: DNA Replication

- Starting at the origin of replication, the machinery at the replication fork adds proteins required for synthesizing DNA.
- These are **DNA helicase, DNA gyrase, SSB, primase, DNA polymerase, and DNA ligase.**
- Several other proteins are used to improve the efficiency, e.g., a ring-shaped sliding clamp keeps DNA polymerase firmly attached to DNA.

Telomeres solve the DNA end-replication problem

- Linear DNA molecules have a problem completing DNA replication on the lagging strand, because primers are required.
- Each round of replication would end with the loss of some nucleotides from the ends of each linear molecule.
- Eukaryotes solve this problem with telomeres, highly repeated sequences at the ends of chromosomes.

Telomeres and telomerase

- 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 is restricted to germ cells and few other types of actively proliferating cells.

Most cells have a limited life span

- 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.

DNA damage and repair

- DNA must be accurately passed on to daughter cells.

- In addition to ensuring that replication is faithful, this also means that DNA alterations must be repaired.
- DNA alterations, or mutations, can arise spontaneously, or through exposure to environmental agents.

DNA damage can occur spontaneously or in response to mutagens

- During DNA replication, some types of mutations occur through spontaneous hydrolysis reactions:
 - **Depurination:** loss of a purine base (A or G)
 - **Deamination:** removal of a base's amino group, changing its base-pairing properties.
- DNA damage can be caused by mutation-causing agents: **mutagens:**
 - Environmental mutagens fall into two categories: chemicals and radiation.
 - Mutagenic chemicals alter DNA structure through a variety of mechanisms.

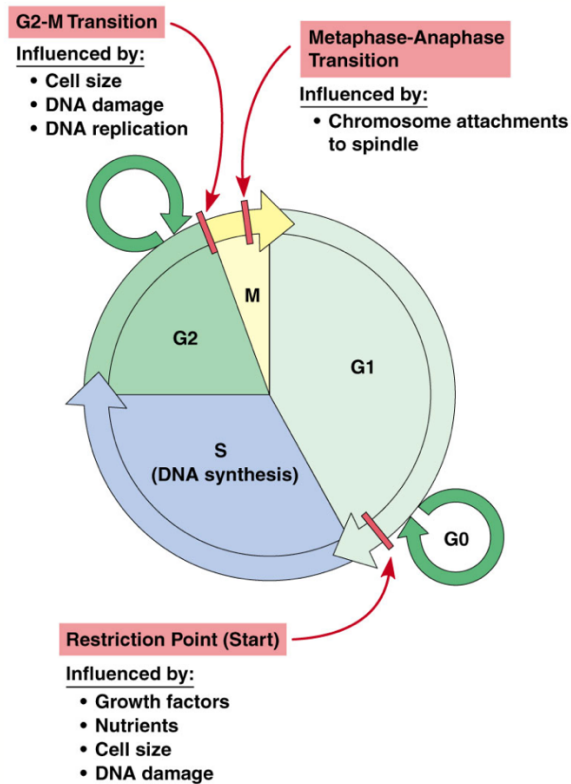
Translesion synthesis and excision repair correct mutations involving abnormal nucleotides

- A variety of mechanisms are used for DNA repair:
- **Translesion synthesis:** repairs during replication. Involves specialized DNA polymerase.
- Repair endonucleases are recruited to DNA by proteins that recognize damage.
- Excision repair: repairs during DNA replication. Abnormal nucleotides are removed and replaced:
 - *E. coli* has nearly 100 genes that code for proteins involved in this process.
 - Excision repair works by a basic 3-step process:
 - Excision of damaged DNA.
 - DNA polymerization to fill gap.
 - Remaining nick sealed by DNA ligase.

Progression through the cell cycle is controlled at several key transition points

Control of the cell cycle must:

- Ensure that events of each phase are carried out in correct order and at appropriate time.
- Ensure that each phase is completed before next one begins.
- Respond to external conditions.



Checkpoint pathways monitor chromosome-to-spindle attachments, completion of DNA replication, and DNA damage

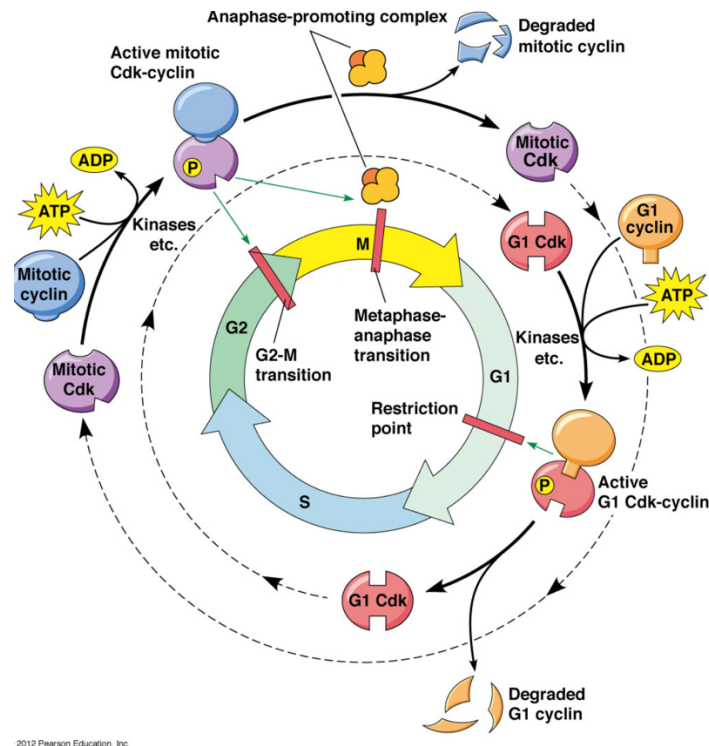
- If cells proceeded from one phase of the cell cycle to the next without completing each step, daughter cells might be abnormal: e.g., **aneuploidy** (incorrect number of chromosomes) could result.
- Cells use a series of checkpoints that ensure each phase is completed properly before next one begins.

Checkpoints

- The **mitotic spindle checkpoint** prevents anaphase from beginning before the chromosomes are all attached to the spindle.
- The **DNA replication checkpoint** ensures that DNA synthesis is complete before the cell begins mitosis.

A multiple series of **DNA damage checkpoints** monitor DNA for damage and halt the cell cycle at various points:

- **p53 protein, the “guardian of the genome,”** plays a central role in these checkpoint pathways:
 - Regulator of cell cycle, and therefore tumor suppressor.
 - Classified as a “tumor suppressor gene” by Bert Vogelstein at JHMI in 1989.
 - p53 stimulates production of enzymes involved in DNA repair.
 - But if the damage cannot be repaired, p53 activates genes needed to trigger cell death by apoptosis.



Apoptosis

- 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**.

Necrosis

- Cell death called necrosis sometimes follows tissue injury
- Necrosis involves swelling and rupture of injured cells, whereas apoptosis involves a specific series of events that lead to dismantling of the cell contents

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.

Module 12 – Regulation of Gene Expression

The Regulation of Gene Expression

Gene regulation

- Gene regulation is an important part of almost every process in nature.
- Most genes are not expressed all the time.
- Selective gene expression enables cells to synthesize only those gene products that are of immediate use.
- In prokaryotes, mechanisms of gene regulation operate mainly at the level of **transcription**.

Catabolic and anabolic pathways utilize different strategies for adaptive enzyme synthesis

Bacteria use 2 main strategies for regulating enzyme synthesis:

- **Catabolic** (degradative)
- **Anabolic** (synthetic) pathway
- Enzymes that catalyze such pathways are often **regulated coordinately**.

Catabolic pathways and substrate induction

- **Catabolic enzymes degrade** specific substrates, often to obtain **energy**,
- Catabolic pathway that degrades disaccharide lactose into simple sugars that can be metabolized by glycolysis.
- Hydrolysis of lactose into monosaccharides glucose and galactose is catalyzed by **enzyme β -galactosidase**.
- However, before lactose can be hydrolyzed, it must first be transported into the cell.
- **Galactoside permease** is responsible for this transport, and its synthesis is regulated coordinately with enzyme β -galactosidase.

Anabolic pathways and end product repression

- Regulation of **anabolic** pathways is the opposite of catabolic pathways.
- Anabolic pathway for synthesizing the amino acid **tryptophan** from starting compound **chorismate**.
- **Chorismate** is an important biochemical intermediate in plants and microorganisms.
- Enzymes that catalyze the 6 steps of this pathway are regulated coordinately at the genetic level.
- For anabolic pathways, the amount of enzyme produced by a cell usually correlates with intracellular concentration of the end-product of the pathway.

Anabolic pathways and end product repression

- As the concentration of **tryptophan** rises, it is advantageous for the cell to economize on its metabolic resources by reducing its production of the enzymes involved in synthesizing tryptophan.
- It is equally important that the cell be able to turn the production of these enzymes back on when the level of tryptophan decreases again.
- This control is made possible by **the ability of the end-product of an anabolic pathway. Tryptophan represses (reduces or stops) further production of the enzymes involved in its formation**
- The reduction in the expression of the enzyme-coding genes is **called end-product repression**.

- Most biosynthetic pathways in bacterial cells are regulated in this way.

Lac repressor is an allosteric protein whose binding to DNA is controlled by lactose

- A key feature of the operon model is that genes with metabolically related functions are clustered together so their transcription can be regulated as a single unit.
- For induction to occur, an additional gene must be present, a regulatory gene that they named **lacI** (for inducibility).
- Whereas normal bacteria produce, β -galactosidase, galactoside permease and transacetylase only when an inducer is present, deletion of the lacI gene yielded cells that always produce these proteins, even when inducer is absent.
- **The lacI gene codes for a product that normally inhibits, and thereby regulates, expression of the lacZ, lacY, and lacA genes.**
- The regulatory gene product that inhibits expression of other genes is called a **repressor**.

Genetic repression

- **True genetic repression** always has an effect on protein synthesis.
The end products of biosynthetic pathways often have an inhibitory effect on enzyme activity.
Feedback inhibition differs from repression in both mechanism and result:
- **feedback inhibition**, molecules of enzyme are still present, but their catalytic activity is inhibited.
- **end product repression**, the enzyme molecules are never made.

Effector Molecules

- One feature common to both induction and repression of enzyme synthesis is **that control is exerted at the gene level in both cases**.
- Control is triggered by small organic molecules called **effectors** present within the cell or the cell's surroundings.
- **Effectors induce shape changes in allosteric proteins that control gene expression.**
- **For catabolic pathways, effectors are almost always substrates** (lactose example) – they function as inducers of gene expression and enzyme synthesis.
- **For anabolic pathways, effectors are usually end products** (example – tryptophan) - they usually lead to the repression of gene expression and repression of enzyme synthesis.

Genes involved in lactose catabolism are organized into an inducible operon

- Classic example of an inducible enzyme system occurs in the bacterium Escherichia coli.
- It involves a group of enzymes involved in lactose catabolism - the enzymes that catalyze the steps shown in Figure 23-3.
- Much of what we know about the regulation of gene expression in bacteria, including the vocabulary used to express that knowledge is based on the pioneering studies of this system carried out by French molecular geneticists Francois Jacob and Jacques Monod.

The lac operon

- The lac operon consists of 3 structural genes (**lacZ, lacY, and lacA**) **preceded by a promoter (Plac) and a special nucleotide sequence called the operator (O), which actually overlaps the promoter.**

- **Transcription of the lac operon begins at the promoter**, which is the site of RNA polymerase attachment, and then proceeds through the operator and all the structural genes until finally ending at a terminator sequence.
- The result is a single molecule of mRNA coding for the polypeptide products of all three structural genes. Such mRNA molecules, which code for more than one polypeptide, are called polygenic mRNAs; they occur only in prokaryotic cells.
- Clustering related genes into an operon for transcription into a single polygenic mRNA allows the synthesis of several polypeptides to be controlled in a single step.
- The crucial step in this control is the interaction between an operator site in the DNA and a repressor protein.
- The repressor protein, called **the lac repressor**, is encoded by the **lacI regulatory gene**, which is located outside the operon (although it happens to be located adjacent to the lac operon it regulates).
- **The lac repressor is a DNA-binding protein that specifically recognizes and binds to the operator site of the lac operon.** When the repressor is bound to the operator (Figure 23-4a), RNA polymerase cannot bind to the promoter and thus transcription of the structural genes is not possible.
- Binding of the repressor to the operator inactivates the operon and keeps its structural genes turned off.

Repressor protein

- If binding of the repressor to the operator blocks transcription, **how do cells turn on transcription of the lac operon, as occurs in the presence of inducers such as lactose?**
- **Answer - inducer molecules bind to the lac repressor, thereby altering its conformation so that the repressor can no longer bind to the lac operator site in the DNA.**
- **Without repressor bound to it, the operator site is unoccupied and RNA polymerase can bind to the promoter and proceed down the operon, transcribing the lacZ, lacY, and lacA genes into a single polygenic mRNA molecule (Figure 23-4b).**
- A crucial feature of a repressor protein, therefore, is its ability to exist in two forms, only one of which binds to the operator.
- **Repressor: allosteric protein.** It can exist in either of **2 conformational states**, depending whether or not an effector molecule is present: in one state the protein is active; in the other state it is nearly inactive.
- When the effector molecule binds to the protein, it induces a change in the conformational state of the protein and therefore in its activity.
- **The binding is readily reversible, however**, departure of the effector results in the protein's rapid return to the alternative form.

Reversible interaction of the lac repressor

- Figure 23-5 shows the reversible interaction of the lac repressor with its effector, called **allolactose, an isomer of lactose produced after lactose enters the cell.**
- The conformational form assumed by the repressor protein in the absence of allolactose recognizes and binds to the operator (**thereby inhibiting transcription**), whereas the form with allolactose attached to it does not.

- The result is that the **repressor protein inhibits transcription of the lac operon in the absence of allolactose, when there is no need to produce the catabolic enzymes encoded by the lac operon.**
- **In the presence of allolactose, the repressor converts to its inactive form, which does not recognize the operator and hence does not prevent transcription of the lac structural genes by RNA polymerase.**
- Lactose triggers the induction of the enzymes encoded by the lac operon. **Because the lac operon is turned off unless induced, it is said to be an inducible operon.**

The genes involved in tryptophan synthesis are organized into a repressible operon

- Although much of the work leading to the initial formulation of the operon concept involved the lac operon of *E. coli*, a number of other bacterial regulatory systems are now known to follow the same general pattern, that is, **genes coding for enzymes of a given metabolic pathway are clustered together in a group that serves as a unit of both transcription and regulation.**
- One or more operators, promoters, and regulatory genes are usually involved, although there is **sufficient variation from one operon to another to preclude many generalizations.**
- **Operons coding for enzymes involved in catabolic pathways generally resemble the lac operon in being inducible; that is, they are turned on by a specific allosteric effector, usually the substrate for the pathway involved.**
- **In contrast, 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. The tryptophan (trp) operon is a good example of a repressible operon.**
- **The trp operon** contains the structural genes coding for the enzymes that catalyze the reactions involved in tryptophan biosynthesis, as well as the DNA sequences necessary to regulate the production of these enzymes.
- **The effector molecule in this case is the end-product of the biosynthetic pathway, the amino acid tryptophan.**

Expression of the enzymes produced by the trp operon is repressed in the presence of tryptophan (**Figure 23-6a**) and de-repressed in its absence (**Figure 23-6b**).

Thus, unlike the lac system, the regulatory gene for this operon, called **trpR**, codes for an allosteric repressor protein that is active (binds to operator DNA) when the effector is attached to it and that is inactive in its free form.

- The effector in such systems (in this case, tryptophan) is sometimes referred to as a **co-repressor because it is required, along with the repressor protein, to shut off transcription of the operon.**

Eukaryotic gene expression is regulated at multiple levels

Gene expression in eukaryotic cells acts at several different 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.

