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DEPARTMENT OF PG MICROBIOLOGY

STUDY MATERIAL

SEMESTER-II

MBY-202: CELL BIOLOGY AND ENZYMOLOGY

MBY-202: CELL BIOLOGY AND ENZYMOLOGY

STRUCTURE AND FUNCTIONS POF CHLOROPLAST AND MITOCHONDRIA:

Chloroplasts

Structure:

1. **Outer Membrane:** Smooth and permeable to small molecules and ions.
2. **Inner Membrane:** Less permeable and contains transport proteins.
3. **Intermembrane Space:** The space between the outer and inner membranes.
4. **Stroma:** The dense fluid within the chloroplast, containing enzymes, DNA, and ribosomes.
5. **Thylakoids:** Membranous sacs stacked into grana; site of the light-dependent reactions.
6. **Granum (plural Grana):** Stacks of thylakoids.
7. **Lamellae:** Connections between grana.
8. **Chlorophyll:** The green pigment located in the thylakoid membranes.

Functions:

1. **Photosynthesis:** Conversion of light energy into chemical energy.
    - **Light-Dependent Reactions:** Occur in the thylakoid membranes; produce ATP and NADPH.
    - **Calvin Cycle (Light-Independent Reactions):** Occur in the stroma; use ATP and NADPH to synthesize glucose.
2. **Synthesis of Fatty Acids and Amino Acids:** Chloroplasts are involved in the synthesis of these essential molecules.
3. **Reduction of Nitrate to Ammonia:** A step in nitrogen assimilation in plants.

Mitochondria

Structure:

1. **Outer Membrane:** Smooth and contains proteins called porins that allow the passage of ions.
2. **Inner Membrane:** Folded into cristae to increase surface area; contains proteins for the electron transport chain.
3. **Intermembrane Space:** The space between the inner and outer membranes.
4. **Matrix:** The innermost compartment filled with enzymes, mitochondrial DNA, and ribosomes.
5. **Cristae:** Infoldings of the inner membrane to enhance ATP production efficiency.

Functions:

1. **ATP Production:** Through oxidative phosphorylation in the electron transport chain.

2. **Citric Acid Cycle (Krebs Cycle):** Occurs in the matrix; generates high-energy electron carriers (NADH and FADH2).
3. **Regulation of Metabolic Pathways:** Mitochondria play a key role in metabolism and energy production.
4. **Apoptosis (Programmed Cell Death):** Mitochondria release factors that trigger cell death.
5. **Calcium Storage:** Mitochondria help regulate intracellular calcium levels.
6. **Heat Production:** In brown adipose tissue, mitochondria generate heat through a process called non-shivering thermogenesis.

Comparative Points:

1. **Energy Conversion:**
   - Chloroplasts: Convert solar energy into chemical energy (glucose).
   - Mitochondria: Convert chemical energy from food into ATP.
2. **Genetic Material:**
   - Both chloroplasts and mitochondria contain their own DNA and ribosomes, supporting the endosymbiotic theory.
3. **Membranes:**
   - Both organelles have double membranes, with the inner membrane highly specialized to their functions.
4. **Origin:**
   - Both are believed to have originated from free-living prokaryotes that were engulfed by ancestral eukaryotic cells (endosymbiosis).

Understanding the structure and function of chloroplasts and mitochondria is essential in cell biology, as they play crucial roles in energy metabolism and cellular function.

MESOSOMES:

Mesosomes

Introduction: Mesosomes are specialized structures observed in prokaryotic cells, particularly in Gram-positive bacteria. Their existence and functional significance have been a topic of debate, with some considering them artifacts of cell preparation for electron microscopy. However, they are often described as folded invaginations in the plasma membrane of bacteria.

Structure:

1. **Invaginations:** Mesosomes appear as folded or convoluted invaginations of the bacterial plasma membrane.
2. **Types of Mesosomes:**
   - **Septal Mesosomes:** Located near the site of cell division, possibly involved in forming cross-walls (septum).

- **Lateral Mesosomes:** Found at the cell periphery, not associated with cell division.

Functions:

1. **Cell Division:**
   - Mesosomes are thought to play a role in the segregation of replicated chromosomes to daughter cells during binary fission.
   - They may help in the formation of the septum, the dividing wall between two new cells.
2. **DNA Replication:**
   - Mesosomes might anchor the bacterial chromosome to ensure proper segregation during cell division.
3. **Respiration:**
   - The folded membranes increase surface area, which may enhance the enzymatic activities associated with cellular respiration.
4. **Secretion:**
   - Mesosomes are proposed to be involved in the secretion processes, aiding in the export of extracellular enzymes and other substances.
5. **Increased Membrane Surface Area:**
   - By increasing the surface area of the plasma membrane, mesosomes could potentially support more metabolic processes such as enzyme attachment and substrate transport.

Controversy and Artifactual Nature:

- **Artifact Theory:** Some researchers argue that mesosomes are artifacts created by the chemical fixation process used during the preparation of samples for electron microscopy. This view suggests that the invaginations are not present in living cells but form due to the interaction of fixation chemicals with the bacterial membrane.
- **Evidence Supporting Artifacts:**
  - Mesosomes are not consistently observed across different studies and conditions.
  - Advanced imaging techniques and better preservation methods often do not show mesosomes, indicating they might not be natural structures.

Current Understanding:

- The consensus in modern microbiology is leaning towards mesosomes being artifacts of cell preparation rather than functional cellular structures. However, some older literature and certain studies still discuss their potential roles, highlighting the historical significance and the complexity of studying subcellular structures.

Conclusion: While mesosomes were once thought to be important bacterial organelles involved in various cellular processes, most contemporary evidence suggests they are artifacts of sample preparation for electron microscopy.

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NUTRIENT TRANSPORT:

Introduction: Nutrient transport is essential for cell survival, growth, and function. It involves the movement of substances like ions, sugars, amino acids, and other nutrients across cell membranes.

Types of Transport:

1. **Passive Transport:**
    - **Diffusion:** Movement of molecules from an area of high concentration to low concentration without energy input.
    - **Facilitated Diffusion:** Transport of substances across a membrane through a protein channel or carrier, down their concentration gradient.
    - **Osmosis:** Diffusion of water across a selectively permeable membrane.
2. **Active Transport:**
    - **Primary Active Transport:** Direct use of ATP to transport molecules against their concentration gradient (e.g., Na+/K+ pump).
    - **Secondary Active Transport:** Utilizes the energy from the electrochemical gradient created by primary active transport. Includes:
        - **Symport:** Both substances move in the same direction (e.g., glucose-Na+ symport).
        - **Antiport:** Substances move in opposite directions (e.g., Na+/Ca2+ exchanger).

Mechanisms of Transport:

1. **Simple Diffusion:**
    - Molecules move through the lipid bilayer without the aid of membrane proteins.
    - Example: Oxygen and carbon dioxide.
2. **Facilitated Diffusion:**
    - **Channel Proteins:** Form pores through the membrane (e.g., ion channels).
    - **Carrier Proteins:** Bind to molecules and change shape to shuttle them across the membrane.
    - Example: Glucose transport through GLUT proteins.
3. **Active Transport:**
    - **Na+/K+ Pump:** Pumps 3 Na+ out and 2 K+ into the cell, using ATP.
    - **Proton Pump (H+-ATPase):** Transports protons out of the cell, important in plants and fungi.
4. **Secondary Active Transport:**
    - **Symport:** Example is the SGLT (sodium-glucose linked transporter) which brings glucose into cells with Na+.
    - **Antiport:** Example is the Na+/Ca2+ exchanger, which removes Ca2+ from cells while bringing in Na+.

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Endocytosis and Exocytosis:

1. **Endocytosis:**
    - **Phagocytosis:** "Cell eating"; large particles are engulfed (e.g., white blood cells engulfing bacteria).
    - **Pinocytosis:** "Cell drinking"; small particles in extracellular fluid are engulfed.
    - **Receptor-Mediated Endocytosis:** Specific molecules bind to receptors and are then engulfed (e.g., LDL cholesterol uptake).
2. **Exocytosis:**
    - Vesicles containing substances fuse with the plasma membrane, releasing their contents outside the cell.
    - Important for secretion of hormones, neurotransmitters, and waste removal.

Transport in Plants:

1. **Xylem:** Transports water and minerals from roots to shoots.
    - Driven by transpiration, capillary action, and root pressure.
2. **Phloem:** Transports sugars and other metabolic products from sources (e.g., leaves) to sinks (e.g., roots, fruits).
    - Involves pressure-flow mechanism.

Transport in Animals:

1. **Blood Circulation:**
    - **Arteries and Veins:** Transport blood throughout the body.
    - **Capillaries:** Site of nutrient and gas exchange.
2. **Lymphatic System:**
    - Transports lymph, containing nutrients, waste products, and immune cells.

Transport Proteins:

- **Uniporters:** Transport one type of molecule.
- **Symporters:** Transport two different molecules in the same direction.
- **Antiporters:** Transport two different molecules in opposite directions.

Factors Affecting Transport:

- **Concentration Gradient:** Difference in concentration across the membrane.
- **Membrane Permeability:** How easily a substance can pass through the membrane.
- **Temperature:** Higher temperatures increase kinetic energy and transport rate.
- **Membrane Surface Area:** Larger area increases the rate of transport.
- **Presence of Transport Proteins:** Specific proteins facilitate the movement of substances.

Understanding nutrient transport mechanisms is crucial for comprehending how cells maintain homeostasis, obtain necessary nutrients, and remove waste products

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CELL CYCLES:

Introduction: The cell cycle is a series of stages that a cell goes through to grow and divide. It ensures that DNA is accurately replicated and distributed to daughter cells. The cell cycle is divided into interphase and the mitotic (M) phase.

Phases of the Cell Cycle

1. **Interphase:**
    - The period of growth and DNA replication between cell divisions.
    - Subdivided into three phases: G1, S, and G2.

    G1 Phase (Gap 1):

    - The first stage after cell division.
    - The cell grows and performs normal functions.
    - Prepares for DNA synthesis.
    - G1 checkpoint ensures the cell is ready for DNA replication.

    S Phase (Synthesis):

    - DNA replication occurs.
    - Each chromosome is duplicated, resulting in two sister chromatids.

    G2 Phase (Gap 2):

    - Further cell growth and preparation for division.
    - The cell produces proteins and organelles.
    - G2 checkpoint ensures all DNA is replicated and undamaged.
2. **Mitotic (M) Phase:**
    - Division of the cell's nucleus and cytoplasm.
    - Includes mitosis and cytokinesis.

    Mitosis:

    - Division of the nucleus into two genetically identical daughter nuclei.
    - Subdivided into five stages: prophase, prometaphase, metaphase, anaphase, and telophase.

        Prophase:

        - Chromatin condenses into visible chromosomes.
        - Mitotic spindle begins to form.
        - Nuclear envelope starts to disintegrate.

Prometaphase:

- Nuclear envelope breaks down completely.
- Spindle fibers attach to kinetochores on the chromosomes.

Metaphase:

- Chromosomes align at the cell's equatorial plate (metaphase plate).
- Metaphase checkpoint ensures all chromosomes are attached to the spindle apparatus.

Anaphase:

- Sister chromatids separate and move toward opposite poles of the cell.
- Spindle fibers shorten, pulling chromatids apart.

Telophase:

- Chromatids reach the poles and decondense back into chromatin.
- Nuclear envelopes re-form around each set of chromosomes.
- Mitotic spindle disassembles.

Cytokinesis:

- Division of the cytoplasm to form two distinct daughter cells.
- In animal cells, a cleavage furrow forms to split the cells.
- In plant cells, a cell plate forms to separate the cells.

Regulation of the Cell Cycle

- The cell cycle is tightly regulated by checkpoints and regulatory proteins to ensure proper division and function.
- **Checkpoints:**
  - **G1 Checkpoint:** Assesses cell size, nutrients, growth factors, and DNA damage.
  - **G2 Checkpoint:** Checks for DNA replication completeness and DNA damage.
  - **M (Spindle) Checkpoint:** Ensures all chromosomes are properly attached to the spindle fibers before anaphase.
- **Regulatory Proteins:**
  - **Cyclins:** Proteins that regulate the cell cycle by activating cyclin-dependent kinases (CDKs).
  - **Cyclin-Dependent Kinases (CDKs):** Enzymes that, when activated by cyclins, phosphorylate target proteins to drive the cell cycle forward.
  - **CDK Inhibitors (CKIs):** Proteins that can inhibit CDKs and halt the cell cycle in response to DNA damage or other signals.

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Cell Cycle and Cancer

- Uncontrolled cell division due to malfunctioning cell cycle regulation can lead to cancer.
- Mutations in genes that regulate the cell cycle, such as **proto-oncogenes** (promote cell division) and **tumor suppressor genes** (inhibit cell division), can contribute to cancer development.

Conclusion

Understanding the cell cycle is crucial for insights into cellular growth, division, and the basis of many diseases, including cancer. Proper regulation ensures healthy cell function and organismal development

MEIOSIS:

Introduction: Meiosis is a specialized type of cell division that reduces the chromosome number by half, producing four haploid cells from one diploid cell. It is essential for sexual reproduction, ensuring genetic diversity through recombination and independent assortment of chromosomes.

Phases of Meiosis:

Meiosis consists of two consecutive divisions: meiosis I and meiosis II. Each division has several stages.

Meiosis I (Reductional Division):

1. **Prophase I:**
    - Chromosomes condense and become visible.
    - Homologous chromosomes pair up (synapsis) forming tetrads.
    - Crossing over occurs at chiasmata, where non-sister chromatids exchange genetic material.
    - The nuclear envelope breaks down, and spindle fibers form.
2. **Metaphase I:**
    - Tetrads (homologous pairs) align at the metaphase plate.
    - Microtubules attach to the kinetochores of homologous chromosomes.
3. **Anaphase I:**
    - Homologous chromosomes are pulled apart to opposite poles.
    - Sister chromatids remain attached at their centromeres.
4. **Telophase I:**
    - Chromosomes reach the poles and the cell divides (cytokinesis).
    - Two haploid cells are formed, each with half the number of chromosomes, but each chromosome still consists of two sister chromatids.

Meiosis II (Equational Division):

1. **Prophase II:**
   - Chromosomes condense again if they had decondensed.
   - A new spindle apparatus forms in each haploid cell.
   - The nuclear envelope, if reformed, breaks down.
2. **Metaphase II:**
   - Chromosomes align at the metaphase plate in each haploid cell.
   - Microtubules attach to the kinetochores of sister chromatids.
3. **Anaphase II:**
   - Sister chromatids are finally separated and pulled to opposite poles of the cell.
4. **Telophase II:**
   - Chromatids (now individual chromosomes) reach the poles.
   - Nuclear envelopes reform around each set of chromosomes.
   - Cytokinesis occurs, resulting in four haploid daughter cells.

Key Features of Meiosis:

1. **Reduction of Chromosome Number:**
   - Meiosis reduces the chromosome number by half, creating haploid cells (n) from a diploid cell (2n).
2. **Genetic Variation:**
   - **Crossing Over:** Exchange of genetic material between non-sister chromatids during prophase I leads to new combinations of alleles.
   - **Independent Assortment:** Random orientation of homologous pairs during metaphase I results in different combinations of maternal and paternal chromosomes in the gametes.
3. **Two Rounds of Division:**
   - Meiosis involves two sequential rounds of nuclear and cytoplasmic division (meiosis I and meiosis II) without an intervening round of DNA replication.

Comparison with Mitosis:

1. **Chromosome Number:**
   - Mitosis: Maintains the same chromosome number (2n to 2n).
   - Meiosis: Reduces chromosome number by half (2n to n).
2. **Genetic Variation:**
   - Mitosis: Produces genetically identical daughter cells.
   - Meiosis: Produces genetically diverse gametes.
3. **Number of Divisions:**
   - Mitosis: One division resulting in two daughter cells.
   - Meiosis: Two divisions resulting in four haploid cells.

Importance of Meiosis:

1. **Sexual Reproduction:**

- Produces gametes (sperm and eggs) necessary for sexual reproduction.

2. **Genetic Diversity:**
   - Generates genetic variation, which is crucial for evolution and adaptation in changing environments.
3. **Maintaining Chromosome Number:**
   - Ensures that offspring have the same chromosome number as their parents by halving the chromosome number in gametes, which restores the diploid number upon fertilization.

Summary:

Meiosis is a critical process in sexually reproducing organisms that generates haploid cells and contributes to genetic diversity through crossing over and independent assortment. This genetic variation is fundamental for evolution and species adaptation. Understanding meiosis is essential for studying genetics, development, and reproductive biology.

RAS PATHWAY:

Introduction: The RAS pathway is a critical signal transduction pathway that regulates cell growth, differentiation, and survival. It is often implicated in cancer when mutated or dysregulated.

Key Components:

1. **RAS Protein:**
   - A small GTPase that acts as a molecular switch.
   - Alternates between an active (GTP-bound) and inactive (GDP-bound) state.
   - Three main isoforms: HRAS, KRAS, and NRAS.
2. **Growth Factor Receptors:**
   - Typically receptor tyrosine kinases (RTKs) such as EGFR (Epidermal Growth Factor Receptor).
   - Binding of growth factors (e.g., EGF) triggers receptor dimerization and autophosphorylation.
3. **Adaptor Proteins:**
   - Grb2 (Growth factor receptor-bound protein 2) binds to phosphorylated RTKs.
   - Sos (Son of Sevenless), a guanine nucleotide exchange factor (GEF), is recruited by Grb2 to the plasma membrane.
4. **Downstream Effectors:**
   - **RAF Kinase:** A serine/threonine kinase activated by GTP-bound RAS.
   - **MEK (MAPK/ERK Kinase):** Activated by RAF through phosphorylation.
   - **ERK (Extracellular signal-Regulated Kinase):** Activated by MEK and translocates to the nucleus to regulate gene expression.

Steps in the RAS Pathway:

1. **Activation of RTKs:**
   - Binding of a growth factor to its receptor (e.g., EGF to EGFR) leads to receptor dimerization.
   - Autophosphorylation of tyrosine residues on the intracellular domain of the receptor.
2. **Recruitment of Adaptor Proteins:**
   - Grb2 binds to the phosphorylated tyrosines on the activated receptor.
   - Sos is recruited to the membrane via Grb2.
3. **Activation of RAS:**
   - Sos acts as a GEF, facilitating the exchange of GDP for GTP on RAS, activating RAS.
4. **Activation of RAF:**
   - GTP-bound RAS recruits and activates RAF kinase (often RAF1).
5. **Activation of MEK:**
   - RAF phosphorylates and activates MEK1/2.
6. **Activation of ERK:**
   - MEK phosphorylates and activates ERK1/2.
7. **Regulation of Gene Expression:**
   - Activated ERK translocates to the nucleus.
   - ERK phosphorylates various transcription factors, leading to changes in gene expression that promote cell growth, differentiation, and survival.

Regulation of the RAS Pathway:

1. **GTPase-Activating Proteins (GAPs):**
   - Enhance the intrinsic GTPase activity of RAS, promoting the hydrolysis of GTP to GDP, thus inactivating RAS.
2. **Negative Feedback:**
   - ERK can phosphorylate SOS and other upstream components, reducing their activity and providing negative feedback.
3. **Regulation by Phosphatases:**
   - Protein tyrosine phosphatases dephosphorylate RTKs.
   - MAPK phosphatases (MKPs) dephosphorylate and inactivate ERK.

Clinical Significance:

1. **Cancer:**
   - Mutations in RAS (e.g., KRAS mutations) can lead to constitutive activation, promoting uncontrolled cell proliferation.
   - Mutations in other pathway components (e.g., BRAF mutations in melanoma) can also drive oncogenesis.
2. **Therapeutic Targets:**
   - Targeting RTKs with monoclonal antibodies (e.g., trastuzumab against HER2).

- Small molecule inhibitors against RAF (e.g., vemurafenib for BRAF-mutant melanoma), MEK, and ERK.
- Direct RAS inhibitors are in development but challenging due to the high affinity of RAS for GTP/GDP.

Summary:

The RAS pathway is crucial for transmitting signals from cell surface receptors to the nucleus, affecting cell fate decisions. Its dysregulation is a common feature in many cancers, making it a significant focus of research and therapeutic intervention. Understanding the detailed mechanisms of this pathway aids in developing targeted treatments for RAS-driven cancers.

MEMBRANE RECEPTORS:

Introduction: Membrane receptors are proteins located on the cell surface that interact with external molecules (ligands) to initiate a cellular response. They play crucial roles in cell communication, signal transduction, and cellular homeostasis.

Types of Membrane Receptors:

1. **G-Protein-Coupled Receptors (GPCRs):**
   - **Structure:** Seven transmembrane alpha-helices.
   - **Function:** Activate G-proteins in response to ligand binding.
   - **Mechanism:**
     1. Ligand binds to GPCR.
     2. GPCR undergoes a conformational change.
     3. Activated GPCR binds to a G-protein, causing the exchange of GDP for GTP on the G-protein.
     4. G-protein dissociates into α and βγ subunits, which interact with target proteins.
     5. Signal is terminated when GTP is hydrolyzed to GDP.
2. **Receptor Tyrosine Kinases (RTKs):**
   - **Structure:** Single transmembrane helix, extracellular ligand-binding domain, intracellular tyrosine kinase domain.
   - **Function:** Phosphorylate tyrosine residues on themselves and other proteins.
   - **Mechanism:**
     1. Ligand binds to RTK.
     2. RTK dimerizes and autophosphorylates tyrosine residues.
     3. Phosphorylated tyrosines serve as docking sites for intracellular signaling proteins.
     4. Downstream signaling pathways are activated (e.g., MAPK/ERK pathway).
3. **Ion Channel Receptors:**
   - **Structure:** Multi-subunit proteins forming a pore through the membrane.
   - **Function:** Allow the flow of ions across the membrane in response to ligand binding.

- **Mechanism:**
    1. Ligand binds to the receptor.
    2. Channel opens, allowing specific ions (e.g., Na+, K+, Ca2+, Cl-) to pass through.
    3. Change in ion concentration leads to cellular responses (e.g., muscle contraction, neurotransmission).

4. **Ligand-Gated Ion Channels:**
    - Subtype of ion channel receptors directly controlled by the binding of a ligand.
    - Example: Nicotinic acetylcholine receptors at neuromuscular junctions.
5. **Enzyme-Linked Receptors:**
    - **Structure:** Extracellular ligand-binding domain, single transmembrane helix, intracellular enzymatic domain.
    - **Function:** Act as enzymes or are associated with enzymes.
    - **Mechanism:**
        1. Ligand binds to the receptor.
        2. Receptor activates its intrinsic enzyme activity or associated enzyme.
        3. Enzyme catalyzes a specific reaction, leading to a cellular response.
    - Example: Guanylyl cyclase receptors that convert GTP to cGMP.
6. **Integrins:**
    - **Structure:** Heterodimers of alpha and beta subunits.
    - **Function:** Mediate cell adhesion to the extracellular matrix (ECM) and transmit signals from the ECM to the cell.
    - **Mechanism:**
        1. Ligand (e.g., fibronectin) binds to integrin.
        2. Integrin undergoes conformational changes.
        3. Interaction with intracellular proteins and cytoskeleton components.
        4. Activation of intracellular signaling pathways (e.g., FAK, Src).

Signal Transduction Pathways:

1. **GPCR Pathways:**
    - **cAMP Pathway:**
        1. GPCR activates adenylyl cyclase via G-protein.
        2. Adenylyl cyclase converts ATP to cAMP.
        3. cAMP activates protein kinase A (PKA).
        4. PKA phosphorylates target proteins, leading to cellular responses.
    - **Phosphoinositide Pathway:**
        1. GPCR activates phospholipase C (PLC) via G-protein.
        2. PLC cleaves PIP2 into IP3 and DAG.
        3. IP3 releases Ca2+ from the endoplasmic reticulum.
        4. DAG activates protein kinase C (PKC).
2. **RTK Pathways:**
    - **MAPK/ERK Pathway:**
        1. Activated RTK phosphorylates and activates RAS.
        2. RAS activates RAF.
        3. RAF phosphorylates MEK.

4. MEK phosphorylates ERK.
5. ERK translocates to the nucleus and regulates gene expression.

- **PI3K/AKT Pathway:**
  1. Activated RTK activates PI3K.
  2. PI3K converts PIP2 to PIP3.
  3. PIP3 recruits and activates AKT.
  4. AKT promotes cell survival and growth.

3. **Ion Channel Pathways:**
   - **Voltage-Gated Channels:**
     - Open in response to changes in membrane potential.
     - Crucial in action potential propagation in neurons.
   - **Ligand-Gated Channels:**
     - Open in response to specific neurotransmitter binding.
     - Example: GABA receptors allowing Cl- influx, leading to inhibitory signals in neurons.

Regulation and Termination:

1. **Desensitization:**
   - Receptors become less responsive to continuous stimulation.
   - Mechanisms include receptor phosphorylation, internalization, and degradation.
2. **Receptor Downregulation:**
   - Prolonged exposure to ligand can lead to decreased receptor numbers on the cell surface.
   - Receptors are internalized and degraded in lysosomes.
3. **Negative Feedback:**
   - Signal transduction pathways often have built-in feedback mechanisms to shut down signaling.
   - Example: Phosphatases dephosphorylate activated proteins, turning off the signal.

Clinical Relevance:

1. **Drug Targets:**
   - Many drugs target membrane receptors (e.g., beta-blockers, antihistamines).
   - Understanding receptor pathways aids in the development of new therapeutics.
2. **Disease Implications:**
   - Mutations in receptor genes can lead to diseases (e.g., cancer, diabetes, cystic fibrosis).
   - Targeted therapies can correct dysfunctional signaling pathways.

MBY-202: CELL BIOLOGY AND ENZYMOLOGY

NOMENCLEATURE AND CLASIFICATION OF ENZYMES:

Introduction: Enzymes are biological catalysts that speed up chemical reactions in cells without being consumed in the process. They are highly specific for their substrates and operate under mild conditions. Understanding enzyme nomenclature and classification is crucial for studying their functions and mechanisms.

Enzyme Nomenclature

1. **Common Names:**
    - Often based on the substrate they act on or the type of reaction they catalyze, followed by the suffix "-ase."
    - Example: Lactase (acts on lactose), DNA polymerase (polymerizes DNA).
2. **Systematic Names:**
    - More descriptive and provide detailed information about the enzyme's catalytic activity.
    - Based on the enzyme's substrate and the reaction it catalyzes.
    - Example: Alcohol dehydrogenase systematically named as alcohol

      + oxidoreductase.

Enzyme Classification

Enzymes are classified into six major classes based on the type of reaction they catalyze, as per the Enzyme Commission (EC) numbering system. Each enzyme is assigned a unique EC number, consisting of four numbers separated by periods (e.g., EC 1.1.1.1).

1. **Oxidoreductases (EC 1):**
    - Catalyze oxidation-reduction (redox) reactions where one molecule is oxidized and another is reduced.
    - Example: Alcohol dehydrogenase (EC 1.1.1.1) catalyzes the oxidation of alcohols.
2. **Transferases (EC 2):**
    - Transfer functional groups (e.g., methyl, phosphate, amino) from one molecule to another.
    - Example: Hexokinase (EC 2.7.1.1) transfers a phosphate group from ATP to glucose.
3. **Hydrolases (EC 3):**
    - Catalyze the hydrolysis of various bonds (e.g., peptide, ester, glycosidic) by adding water.
    - Example: Lipase (EC 3.1.1.3) hydrolyzes ester bonds in lipids.
4. **Lyases (EC 4):**
    - Catalyze the addition of groups to double bonds or the formation of double bonds by removing groups without hydrolysis.

- Example: Fumarase (EC 4.2.1.2) catalyzes the addition of water to fumarate to form malate.

5. **Isomerases (EC 5):**
   - Catalyze the rearrangement of atoms within a molecule, resulting in the formation of isomers.
   - Example: Phosphoglucose isomerase (EC 5.3.1.9) converts glucose-6-phosphate to fructose-6-phosphate.
6. **Ligases (EC 6):**
   - Catalyze the joining of two molecules with the concomitant hydrolysis of a diphosphate bond in ATP or another nucleotide.
   - Example: DNA ligase (EC 6.5.1.1) joins DNA strands together by forming a phosphodiester bond.

Detailed Classification Example

For a clearer understanding, let's consider an enzyme with the EC number 2.7.1.1:

- **Class 2:** Transferases
  - **Subclass 7:** Transferring phosphorus-containing groups
    - **Sub-subclass 1:** Phosphotransferases with an alcohol group as acceptor
      - **Serial number 1:** Hexokinase

Factors Affecting Enzyme Activity

1. **Substrate Concentration:**
   - As substrate concentration increases, the reaction rate increases until a maximum velocity (Vmax) is reached.
2. **pH:**
   - Each enzyme has an optimal pH range where it is most active. Deviations can denature the enzyme or affect its binding with the substrate.
3. **Temperature:**
   - Enzymatic activity increases with temperature up to an optimum point. Beyond this, enzymes can denature and lose activity.
4. **Inhibitors:**
   - **Competitive Inhibitors:** Compete with the substrate for the active site.
   - **Non-Competitive Inhibitors:** Bind to an allosteric site, changing the enzyme's shape and function.
   - **Uncompetitive Inhibitors:** Bind only to the enzyme-substrate complex.
5. **Cofactors and Coenzymes:**
   - Non-protein molecules required for enzyme activity. Cofactors are typically metal ions, while coenzymes are organic molecules (e.g., vitamins).

Enzyme Kinetics

- **Michaelis-Menten Kinetics:**

  - Describes the rate of enzymatic reactions by relating reaction rate (v) to substrate concentration ([S]).
  - Key parameters:
    - **Vmax:** Maximum rate of the reaction.
    - **Km (Michaelis constant):** Substrate concentration at which the reaction rate is half of Vmax.
- **Lineweaver-Burk Plot:**
  - A double reciprocal plot used to determine Km and Vmax.

Conclusion

Understanding enzyme nomenclature and classification provides a structured way to study and discuss enzymes. This knowledge is essential for exploring enzyme functions, mechanisms, and their applications in biotechnology, medicine, and research.

FACTORS EFFECTING ENZYME ACTIVITY:

Introduction: Enzymes are biological catalysts that accelerate chemical reactions in cells. Their activity can be influenced by several factors, which can affect the rate at which they catalyze reactions. Understanding these factors is crucial for optimizing enzymatic reactions in various biological and industrial processes.

Factors Affecting Enzyme Activity:

1. **Substrate Concentration:**
   - **Effect:** As substrate concentration increases, the rate of reaction increases until it reaches a maximum velocity (Vmax).
   - **Mechanism:** More substrate molecules increase the likelihood of enzyme-substrate complex formation. Beyond a certain concentration, all active sites of the enzyme molecules are occupied (saturation point), and the rate plateaus (Vmax).
2. **Enzyme Concentration:**
   - **Effect:** Increasing enzyme concentration increases the reaction rate, provided that substrate concentration is not limiting.
   - **Mechanism:** More enzyme molecules provide more active sites for the substrate to bind, leading to more product formation.
3. **Temperature:**
   - **Effect:** Enzyme activity increases with temperature up to an optimal point; beyond this point, activity decreases.
   - **Mechanism:** Higher temperatures increase molecular motion, enhancing substrate and enzyme interactions. However, excessive heat can denature the enzyme, causing it to lose its functional shape.
   - **Optimal Temperature:** Each enzyme has an optimal temperature range where its activity is highest. For many human enzymes, this is around 37°C (98.6°F).
4. **pH:**

- **Effect:** Each enzyme has an optimal pH range; deviations from this range reduce activity.
- **Mechanism:** pH affects the ionization of amino acids at the active site and substrate, altering binding and catalysis. Extreme pH levels can denature the enzyme.
- **Optimal pH:** Varies among enzymes; for example, pepsin in the stomach has an optimal pH of around 2, while trypsin in the small intestine works best at a pH of about 8.

5. **Inhibitors:**
   - **Types and Effects:**
     - **Competitive Inhibitors:** Compete with the substrate for the active site. Increase in substrate concentration can overcome inhibition.
     - **Non-Competitive Inhibitors:** Bind to an allosteric site, changing the enzyme's shape and reducing its activity regardless of substrate concentration.
     - **Uncompetitive Inhibitors:** Bind only to the enzyme-substrate complex, preventing the reaction from completing.
   - **Mechanism:** Inhibitors interfere with enzyme activity by altering the enzyme's ability to bind substrates or catalyze reactions.
6. **Cofactors and Coenzymes:**
   - **Effect:** Necessary for the proper functioning of many enzymes.
   - **Mechanism:** Cofactors (metal ions) and coenzymes (organic molecules) assist in enzyme catalysis by stabilizing the enzyme-substrate complex or participating in the reaction.
   - **Examples:** Magnesium ions (Mg^{2+}) for DNA polymerase, NAD^+ for dehydrogenases.
7. **Allosteric Regulation:**
   - **Effect:** Enzymes can be activated or inhibited by molecules that bind to allosteric sites.
   - **Mechanism:** Binding of allosteric regulators induces conformational changes that increase or decrease enzyme activity.
   - **Example:** ATP acts as an allosteric inhibitor for some enzymes in glycolysis, reducing their activity when cellular energy levels are high.
8. **Post-Translational Modifications:**
   - **Effect:** Modifications such as phosphorylation, methylation, and acetylation can alter enzyme activity.
   - **Mechanism:** These modifications can change the enzyme's shape, charge, or binding affinity, affecting its catalytic properties.
   - **Example:** Phosphorylation of enzymes in signal transduction pathways can rapidly activate or deactivate them.

Enzyme Kinetics:

- **Michaelis-Menten Kinetics:**
  - Describes the rate of enzymatic reactions by relating reaction rate (v) to substrate concentration ([S]).

  - **Vmax:** Maximum rate of the reaction when the enzyme is saturated with substrate.
  - **Km (Michaelis constant):** Substrate concentration at which the reaction rate is half of Vmax. Indicates the affinity of the enzyme for its substrate (lower Km means higher affinity).
- **Lineweaver-Burk Plot:**
  - A double reciprocal plot (1/v vs. 1/[S]) used to determine Km and Vmax.
  - Useful for distinguishing different types of enzyme inhibition.

Practical Applications:

1. **Industrial Enzyme Use:**
   - Optimizing conditions (temperature, pH, substrate concentration) for maximum enzyme efficiency in processes like fermentation, detergent formulation, and biocatalysis.
2. **Medical Enzyme Inhibition:**
   - Designing drugs that target specific enzymes involved in disease pathways, such as protease inhibitors for HIV treatment or COX inhibitors for pain and inflammation.
3. **Biotechnological Research:**
   - Using knowledge of enzyme activity and regulation to engineer enzymes with desired properties for research and therapeutic purposes.

Summary:

Enzyme activity is influenced by several factors, including substrate and enzyme concentration, temperature, pH, inhibitors, cofactors, and regulatory mechanisms. Understanding these factors allows for the optimization of enzymatic reactions in various fields, from industrial applications to medical therapies.

MBY-202: CELL BIOLOGY AND ENZYMOLOGY

ENZYME INHIBITORS:

Introduction: Enzyme inhibitors are molecules that bind to enzymes and decrease their activity. They play crucial roles in regulating enzymatic activity in biological systems and are also utilized in various therapeutic and research applications.

Classification of Enzyme Inhibitors:

Enzyme inhibitors can be classified into several types based on their mechanism of action and interaction with enzymes:

1. **Competitive Inhibitors:**
   - **Mechanism:** Compete with the substrate for binding to the active site of the enzyme.
   - **Effect:** Increase in substrate concentration can overcome competitive inhibition.
   - **Example:** Statins inhibit HMG-CoA reductase by competing with HMG-CoA, reducing cholesterol synthesis.
2. **Non-Competitive Inhibitors:**
   - **Mechanism:** Bind to the enzyme at a site other than the active site (allosteric site), inducing a conformational change that reduces enzyme activity.
   - **Effect:** Cannot be overcome by increasing substrate concentration.
   - **Example:** Allosteric inhibitors like ATP can inhibit phosphofructokinase, a key enzyme in glycolysis, by binding to an allosteric site.
3. **Uncompetitive Inhibitors:**
   - **Mechanism:** Bind to the enzyme-substrate complex, preventing the release of products.
   - **Effect:** Only effective when the enzyme has bound to the substrate.
   - **Example:** Methotrexate inhibits dihydrofolate reductase, an enzyme involved in DNA synthesis, by binding to the enzyme-substrate complex.
4. **Mixed Inhibitors:**
   - **Mechanism:** Bind to the enzyme at an allosteric site, affecting both the enzyme alone and the enzyme-substrate complex.
   - **Effect:** Can bind to either the free enzyme or the enzyme-substrate complex, altering enzyme activity.
   - **Example:** Sildenafil (Viagra) acts as a mixed inhibitor of phosphodiesterase-5 (PDE-5), affecting both the enzyme alone and the enzyme-substrate complex involved in regulating blood flow.
5. **Irreversible Inhibitors:**
   - **Mechanism:** Form a covalent bond with the enzyme, permanently inactivating it.
   - **Effect:** Requires the synthesis of new enzyme molecules to restore enzymatic activity.
   - **Example:** Aspirin irreversibly inhibits cyclooxygenase (COX) enzymes involved in prostaglandin synthesis, reducing inflammation and pain.

Applications of Enzyme Inhibitors:

1. **Therapeutic Uses:**
    - **Drug Development:** Designing medications to target specific enzymes involved in disease pathways, such as protease inhibitors for HIV or COX inhibitors for pain relief.
    - **Antibiotics:** Inhibiting bacterial enzymes crucial for their survival and replication.
    - **Cancer Treatment:** Targeting enzymes involved in cell division or DNA repair.
2. **Research Tools:**
    - Studying enzyme functions and pathways by selectively inhibiting specific enzymes.
    - Elucidating metabolic pathways and regulatory mechanisms.
3. **Industrial Applications:**
    - Controlling enzymatic processes in food production, fermentation, and biocatalysis.
    - Improving efficiency and yield in industrial processes by inhibiting unwanted side reactions.

Factors Influencing Inhibition:

1. **Concentration of Inhibitor:**
    - Higher inhibitor concentrations typically lead to greater inhibition until saturation is reached.
2. **Affinity of Inhibitor for Enzyme:**
    - Affinity determines how tightly an inhibitor binds to the enzyme and affects the extent of inhibition.
3. **Specificity of Inhibition:**
    - Some inhibitors may target specific enzymes or enzyme isoforms, while others may have broader effects.
4. **Reversibility of Inhibition:**
    - Reversible inhibitors bind non-covalently and can dissociate from the enzyme, allowing enzymatic activity to recover.
    - Irreversible inhibitors form strong covalent bonds and permanently inactivate the enzyme.

Summary:

Enzyme inhibitors play critical roles in regulating enzyme activity and are valuable tools in medicine, research, and industry. Understanding their classification, mechanisms of action, and applications helps in designing therapeutic agents, elucidating biochemical pathways, and optimizing biotechnological processes. Each type of inhibitor offers unique advantages and considerations depending on the desired outcome and application context.

MBY-202: CELL BIOLOGY AND ENZYMOLOGY

IMMOBILIZATION OF ENZYMES:

Introduction: Immobilization of enzymes refers to the process of fixing enzymes onto a solid support or within a matrix, thereby enhancing their stability, reusability, and functionality in various industrial, medical, and research applications. This technique has numerous advantages over free enzymes, including improved operational stability and easier separation from reaction products.

Methods of Enzyme Immobilization:

1. **Adsorption:**
    - **Process:** Enzymes are physically adsorbed onto the surface of a support material through weak interactions (e.g., hydrogen bonding, van der Waals forces).
    - **Support Materials:** Silica, activated carbon, ion-exchange resins.
    - **Advantages:** Simple process, retains enzyme activity.
    - **Disadvantages:** Weak binding can lead to enzyme leaching.
2. **Covalent Bonding:**
    - **Process:** Enzymes are covalently bound to the support material through reactive functional groups (e.g., amino, carboxyl groups).
    - **Support Materials:** Agarose, cellulose, magnetic beads.
    - **Advantages:** Strong attachment, minimal enzyme leaching, enhanced stability.
    - **Disadvantages:** Complex process, may affect enzyme structure and activity.
3. **Encapsulation:**
    - **Process:** Enzymes are entrapped within a porous matrix or microcapsule, providing a protective environment.
    - **Support Materials:** Alginate, polyvinyl alcohol (PVA), gelatin.
    - **Advantages:** Protects enzymes from harsh conditions, allows diffusion of substrates and products.
    - **Disadvantages:** Mass transfer limitations, potential for enzyme inactivation due to diffusion barriers.
4. **Cross-Linking:**
    - **Process:** Enzymes are cross-linked to form a stable enzyme-support complex.
    - **Cross-Linking Agents:** Glutaraldehyde, carbodiimides (e.g., EDC), genipin.
    - **Advantages:** Enhances stability and reusability, minimal enzyme leaching.
    - **Disadvantages:** Cross-linking agents may affect enzyme structure or activity.
5. **Entrapment within Membranes:**
    - **Process:** Enzymes are incorporated into or attached to membranes or films that allow substrate diffusion.
    - **Materials:** Polymers like polyethyleneimine (PEI), polyacrylamide.
    - **Advantages:** Provides a stable environment, facilitates continuous processes.
    - **Disadvantages:** Limited types of enzymes suitable for membrane entrapment, potential for membrane fouling.

Advantages of Immobilized Enzymes:

- **Operational Stability:** Immobilized enzymes exhibit higher stability under a range of pH, temperature, and solvent conditions compared to free enzymes.
- **Reusability:** Can be reused multiple times, reducing costs and improving efficiency in industrial processes.
- **Enhanced Productivity:** Facilitates continuous processing and higher enzyme loading, leading to improved productivity.
- **Easy Separation:** Simplifies product purification and separation from reaction mixtures, reducing downstream processing steps.

Applications of Immobilized Enzymes:

1. **Biocatalysis in Industry:**
    - Production of pharmaceuticals, fine chemicals, food products (e.g., glucose isomerase for high-fructose corn syrup).
    - Enzymatic degradation of pollutants in wastewater treatment.
2. **Biomedical Applications:**
    - Enzyme biosensors for medical diagnostics (e.g., glucose sensors).
    - Drug delivery systems utilizing immobilized enzymes for controlled release.
3. **Biofuel Production:**
    - Immobilized enzymes used in the conversion of biomass into biofuels (e.g., cellulases for ethanol production).
4. **Bioremediation:**
    - Removal of environmental contaminants using immobilized enzymes (e.g., degrading pesticides in soil).

Challenges and Considerations:

- **Enzyme Loading:** Achieving high enzyme loading without compromising activity.
- **Mass Transfer Limitations:** Ensuring efficient diffusion of substrates and products within the immobilization matrix.
- **Cost and Scalability:** Immobilization techniques should be cost-effective and scalable for industrial applications.
- **Compatibility:** Ensuring compatibility of immobilization materials with specific enzymes to maintain activity and stability.

Future Directions:

- **Nanotechnology:** Developing nanomaterials for precise control over enzyme immobilization and improved performance.
- **Multi-Enzyme Systems:** Engineering complex systems of immobilized enzymes for cascade reactions and enhanced efficiency.
- **Biocompatibility:** Exploring biocompatible materials and methods to expand biomedical applications of immobilized enzymes.