Cell Biology Lecture Notes

The Chemistry of the Cell: Cellular Chemistry

Why Chemistry?

Biology in general and cell biology in particular depend heavily on both chemistry and physics. Simply, cells and organisms follow all the laws of the physical universe, and biology is really just the study of chemistry in systems that happen to be alive. In fact, everything cells are and do has a molecular and chemical basis. Therefore, we can truly understand and appreciate cellular structure and function only when we can describe that structure in molecular terms and express that function in terms of chemical reactions and events.

5 themes in the chemistry of the cell

1. Carbon: biology deals with carbon containing molecules
Valence of four and covalent bond
Carbon containing molecules are stable
Carbon-containing molecules are diverse
Carbon-containing molecules can form isomers
2. Water: Cellular world is an aqueous world
Water molecules are polar
Water molecules are cohesive
Water is an excellent solvent
Hydrophilic and hydrophobic molecules
3. Selectively permeable membrane: Separation of two water environments
Amphipathic molecules
Membrane bilayer
Movement across the membrane
4. Polymerization: Addition of molecular building units
Monomers and polymers
Biological polymers: proteins, nucleic acids, polysaccharides and lipids(fat)
Condensation reaction
Directionality
5. Self-assembly: spontaneous assembly of the parts
Characteristics
Driving forces
Protein assembly

Reading Assignments:

Text pages 41-78.

Questions:

1. Which of the following statements is **false**?

   A. The molecules of liquid water are extensively hydrogen-bonded to one another
   B. When exposed to an aqueous environment, amphipathic molecules undergo hydrophobic interactions
   C. The water molecule is polar because it has an asymmetric charge distribution
   D. The carbon-carbon double bonds are less stable than the single bonds and therefore result in a bend or kink in the unsaturated fatty acid
   E. None of above (all are true)

2. Hydrogen bond is a covalent bond. True___ False____

3. Why are the carbon containing molecules are stable?

4. What is the currency of the biological energy?

5. Why is the polarity of water the most important chemcial property?

6. Hydrophobic interaction is _____

7. Amphiphatic molecules are _____

8. Condensation is _____

9. Self-assembly is _____

Carbohydrates and Polysaccharides

Polysaccharides: they usually consist of a single kind of repeating unit, or sometime a strictly alternating pattern of two kinds.

Monomers :Monosaccharides

1. Either consists of aldehyde or ketone functional group
2. 2 or more -OH' groups
3. Formula: $C_nH_{2n}O_n$, where n= 3 to 7
   Triose, n=3
   - glyceraldehyde
   - dihydroxyacetone

   Pentose, n=5
   - ribose
   - deoxyribose

   Hexose, n=6
   - glucose
   - fructose
   - galatose

4. Ring form and chair form
5. α and β configuration
6. Sugar derivatives

Oligosaccharides: consist of 2 to 20 monosaccharides covalently linked together

1. Glycosidic bond: covalent bond
   - α and β linkages
2. Disaccharides
   - maltose
   - lactose
   - sucrose
3. Complex oligosaccharides
   - glycoproteins
   - glycolipids

Polysaccharides

1. Storage polysaccharides
   - starch: storage polysaccharides in the plant cells
     - amylose
     - amylopectin
   - glycogen : storage polysaccharides in animal cells
2. Structural polysaccharides
   - cellulose: structural polysaccharides found in the plant cells chitin

Secondary structure of polysaccharides

1. Determining factors
    - linkage configuration
    - branching degree
2. Types
    - Loose helices
    - Rigid, liner rods

Glycosaminoglycan chains and proeoglycans in the extracellular matrix of animals

Glycosaminoglycan (GAG)
Protroglycans

Lipids: any discussion of cellular structure and chemical components would be incomplete without reference to this important group of molecules. Especially, they are frequently associated with the macromolecules, i. e. proteins.

1. Hyprophobic nature
2. Amphipathic

Triglycerides are storage lipids

1. Ester bonds
2. Fatty acids
3. Fats
4. Vegetable oils

Phospholipids are important in membrane structure

1. Phosphatidic acid
2. Phosphoester bonds

Sphingolipids are also found in membranes

1. In animal membranes
2. Sphingosine
3. Amide bonds

Steroids are lipids with a variety of functions

1. Ring structures
2. Steroids play in a variety of roles in the cells of higher organisms but not present in bacteria
3. Some mammalian hormones are steroids
    - Adrenocortical hormones
    - Sex hormones
4. Bile acids
5. Cholesterol

Proteins and Polypeptides

Monomers

- amino acids
  - α carbon
    - Families of amino acids
      - Hydrophilic amino acids
        - Non-polar amino acids
      - Hydrophobic amino acids
        - Basic amino acids
        - Acidic amino acids
        - Non-charged polar amino acids

Primary sequence

- Peptide bonds
- Primary sequences determine their higher organization

Driving forces for the higher organization of proteins (polypeptides)

- Non-covalent bonds
  - Hydrogen bonding
  - Ionic interactions
  - Hydrophobic interaction
  - van der Waals interaction
- Covalent bonds
  - Disulfide bonds

Secondary structure

- Driving force: hydrogen bonds
- α helix
- β pleated sheets

Tertiary structure

- Driving forces
  - Non-covalent bonds
    - Hydrogen bonding
    - Ionic interactions
    - Hydrophobic interaction
    - van der Waals interaction
  - Covalent bonds
    - Disulfide bonds
- The chemistry of amino acid side chain (R groups) is the determining factor

Quaternary structure

- Driving forces
  - Non-covalent bonds
    - Hydrogen bonding
    - Ionic interactions
    - Hydrophobic interaction
    - van der Waals interaction
  - Covalent bonds
    - Disulfide bonds
- Multimeric protein structure

Protein modification: post-translational modification

- Phosphorylation
- Tyrosination
- Acetylation

Classifications of proteins

- Fibrous proteins versus globular proteins
- Membrane proteins versus cytosol proteins
- Structural proteins
- Glycoproteins
- Proteoglycans

Reading Assignments:

Text pages 56-57; 111-128

Questions:

1. Which amino acid is always found on the outside of protien molecules? cluster together inside of protein molecule? within plasma membrane?

2. The shape of a protein molecule is determinedby its amino acid sequence.
   True____ False____

3. What is a peptide bond?

4. What is a difulfide bond? Which amino acid is involved?

5. What is α -carbon in an amino acid?

6. List 3 globular proteins and 3 fibrous proteins.

7. What is the tertiary of a protein? What is the quarternary structure of a protein?

Nucleic Acids

Nucleic acids play the roles in the storage, transmission and expression of genetic information.

- DNA
- RNA
  - mRNA
  - tRNA
  - rRNA

Monomers

- Nucleotides (4 different basic nucleotides for DNA and RNA, respectively)
  - 3 chemical groups
    - a pentose
      - DNA: β -D-deoxyribose
      - RNA: β -D-ribose
    - a phosphate group
    - a nitrogen containing base (purine and pyrimidine)
      - DNA: A, G, C, T
      - RNA: A, G, C, U
- Other functional roles of nucleotides
  - energy providers
  - enzyme cofactors
  - signaling molecules in intracellular signal transduction

Polynucleotide formation: 3', 5'-phosphodiester bonds

- Condensation reaction
- Sugar-phosphate is the backbone
- Intrinsic directionality (5' 3')
- Require energy and information

Hydrogen bonding between bases and complementary base pairing

- A=T(U)
- G=C

Double helix of nucleic acids

- DNA
  - 2 complementary chains of DNA twisted with each other
  - They are in opposite direction
  - Backbone: sugar and phosphate unit
  - Bases are pairing inward
  - Right handed double helix with ~ 10 nucleotide pair per turn
- RNA
  - Only local region of short complementary base pairing

What does the DNA helix tell us?

- Quantitative biochemistry
  - [A]=[T] and [G]=[C]
- Explain heredity
  - DNA replication process is semiconservative

RNA serves as an informational carrier intermediate between DNA and protein

- Prokaryotes
- Eukaryotes

Enzymes: Biological Catalysts

The law of thermodynamic spontaneity

All reactions that occur spontaneously result in a decrease in the free energy content of the system

In the cells:

1) Some reactions are thermodynamic feasible but do not occur at appreciable rates
2) The only reactions that occur at appreciable rates are those from which an enzyme is present
3) All reactions are mediated by the biological catalysts called enzymes

Activation energy

How to overcome the activation energy barrier
1) Heat
2) Lower the activation energy: catalysts

Properties of catalysts

1) Increase rates of reaction by lowering activation energy to allow more molecules to react without use of heat
2) Form transient complexes with substrates in a fashion that facilitates reaction
3) Only change rate at which reaction equilibrium is achieved, has no effect on the position of the equilibrium

Enzyme Structure

- Proteins
  - Tertiary or quaternary proteins
  - Active sites
  - Prosthelic groups
- RNAs
  - Ribozyme

Enzyme Specificity

Enzyme mechanisms

1).Random collisions
2) Driving forces
3) Induced fit
4) Form temporary covalent bonds

Enzyme sensitivity to environment

- Temperature
- pH

Enzyme kinetics

- Michaelis-Menten kinetics
- Vmax and Km

Enzyme Regulations

- Allosteric regulation
  - Negative regulation
    - Feedback inhibition
  - Positive regulation
    - Subtract activation
- Enzyme inhibitors
  - Reversible inhibitors
  - Irreversible inhibitors

Definitions

Allosteric effector
Small molecule that cause a change in the conformation of an allosteric protein (or enzyme) by binding to a site other than the active site.

Allosteric protein (allosteric enzyme)
Regulatory protein that has two alternative conformations, each with a different biological property; interconversion of the two conformations is mediated by the reversible binding of a specific small molecule to the effector site.

Allosteric regulation
Control of a reaction pathway by the effector-mediated reversible interconversion of the two conformations of an allosteric enzymes in the pathway.

How Cells are Studied I

Optic techniques for cellular and subcellular architecture

The Light Microscopy
- Limit of resolution
- Scale of cell biology
  - μ m, nm, and A
- Compound microscopy

Types of light microscopy
- **Brightfield microscopy**
  - basic form
  - inexpensive and easy
  - for color and fixed specimen and not for living species
- **Phase-contrast microscopy**
  - phase plate
  - good for living, unstained specimen
- **Dark field microscopy**
- **Fluorescence microscopy**
  - fluorescent compounds
  - exciter filter
  - barrier filter
- **Differential -interference -contrast microscopy (DIC)** (Nomarski)
  - polarizer
  - analyzer
  - Wollaston prism
  - to produce 3-D image
- **Confocal microscopy**
  - to produce 3-D image from a collection of optic sections

Sample preparation techniques in light microscopy
- Fixation
- Cryoprotection
- Embedding and sectioning
- Staining
- Labeling
  - radioisotope
  - immunolabeling

The Electron Microscopy

Use a beam of electron to produce an image

Two major types of electron microscopy

Transmission electron microscopy (TEM)

Vacuum system
Electron gun
Electromagnetic lenses and image formation
Photographic system

Sample preparation techniques in TEM microscopy

Fixation
Embedding, Sectioning, and poststaining
Electron microscopic autoradiography
Negative staining
Shadowing
Freeze-fracturing
Freeze-etching

Scanning electron microscopy (SEM): 3 D images

Second electrons

Sample preparation techniques in SEM microscopy

Fixation
Postfixation
Dehydration
Poststaining
Mounting
Coating

with a layer gold or a mixture of gold and palladium.

How Cells are Studied II

Biochemical Techniques for Cellular and Subcelllular Functions

Isolation of cells

- Source for the best yield
  - fetal or neonatal tissue
- Disrupting the extracellular matrix and intercellular junctions
  - Proteolytic enzymes
  - Chelating agents
- Approaches to separate cell types
  - Centrifugation
  - Cell sorter: fluorescence-activated cell sorter
- What to do with a uniform population of cells
  - For biochemical analysis
  - For cell culture

Fractionation of organelles and macromolecules

- Cell disruption: homogenate
- Centrifugation
  - Separation by size
  - Separation by size and shape
  - Separation by buoyant density
- Cell-free system
  - Isolation
  - Reconstitution
- Chromatography
  - Partition chromatography
  - Column chromatography
    - Ion-exchange chromatography
    - Gel-filtration chromatography
    - Affinity chromatography
    - HPLC
- Electrophoresis
    - Proteins usually have a net positive or negative charge that reflects the mixture of charged amino acids they contain. If an electric field is applied to a solution containing a protein molecules, the protein will migrate at a rate that depends onits net charge and on its size and shape
    - SDS-PAGE
      - SDS
      - β -mercaptoethanol
      - Coomassie blue
      - Silver stain
      - Western blotting
- 2-D gel electrophoresis
    - First dimension: isoelectrical focusing
    - Second dimension: SDS-PAGE

Analysis of polypeptides

Peptide mapping

Amino acid sequena

Membranes: Their Structure and Function

Generalization of membranes

They are assembly of lipids and proteins held together by noncovalent interactions. They are dynamic fluid structure. Depending on the source, membranes vary in thickness, in lipid composition and in their ratio of lipid and protein.

Functional roles of membranes

- Define and compartmentalize the cell
- Serve as the locus of specific functions
- Control movement of substances into and out of the cell and its compartments
- Play a role in cell-to-cell communication and detection of external signals

Biochemical models of membranes

- Fluid mosaic model
- Transmembrane protein structure

Three main constituents of membranes

Membrane lipids

- Approximately 50% of mass
- Lipid bilayers: amphipathic molecules
- Typical membrane lipids
  - phospholipids
  - glycolipids
  - sphingolipids
  - cholesterol
- Analysis of membrane lipids

Membrane proteins

- Association with lipids
- Peripheral membrane proteins and integral membrane proteins
- Classification of membrane proteins by function
- Studies of membrane proteins
  - Solubilization, isolation and reconstitution
  - Studies of red blood cell ghosts*

Membrane carbohydrates

- Approximately 2-10 % of mass
- Confined mainly to the non-cytosolic surface
  - On the extracellular surface of the cells
  - Inward toward the lumen of the compartment
- Covalent linkage to proteins and lipids
  - Glycoproteins and proteoglycans
  - Glycolipids
- Analysis of carbohydrate moiety of membranes
  - Lectins
- Functions of membrane carbohydrates

Membrane asymmetry

- Asymmetric distribution of lipids, proteins and carbohydrates
- Diffusion in the membranes
  - Transverse diffusion
  - Lateral diffusion

Membrane fluidity

- Lipid bilayer is a two-dimensional fluid
- Membrane fluidity depends upon its composition
  - Length of hydrocarbon chain and saturation
  - Cholesterol
  - Regulation of membrane fluidity
- Mobility of membrane proteins
- Cell fusion experiment

Transport Across Membranes

Categories of membrane transport

Cellular transport

It concerns the exchange of materials between the cells and its environment Intracellular transport It evolves movement of substances across membranes of organelles inside the cell

Transcellular transport

It involves the movement of a substance in on one side and out on the other side

Mechanisms of membrane transport for small molecules

Passive Transport:

It does not require energy; it occurs because of the tendency for dissolved molecules to move from higher to lower concentrations.

1.) Simple diffusion

Factors governing diffusion across lipid bilayers

size
polarity
ionization

Kinetics for simple diffusion

$V=k_D$ [X] outside-[X] inside

2.) Facilitated transport

Involvement of a membrane transport protein

carrier protein
channel protein

Kinetics for facilitated transport

follow Michaelis-Menten kinetics

Specificity of transport proteins

Examples

3.) Ionophores:

They are small hydrophobic molecules that dissolve in lipid bilayers and increase their ion permeability

Classes of ionophores

mobile ion carriers
channel formers

Active Transport

It requires energy; it takes place against the electrochemical gradient

1.) 3 major functions
- uptakes of fuel molecules and nutrients
- removal of waste materials, secretory products and sodium ions
- maintenance of a constant, optimal internal environment of inorganic ions

2.) Directionality

3.) Kinetics
for uncharged molecules
for charged molecules

4.) Involvement of membrane potential

5.) Simple versed coupled transport

6.) Energy source

7.) Examples

Cellular transports: exocytosis and endocytosis

Both involve the sequential formation and fusion of membrane-bounded vesicles

Exocytosis:

1.) Steps
Packing secretory vesicles
Response to extracellular signals
Fusion with membrane: recognition sites and Ca^{++}
Discharge the contents

2.) Membranes asymmetry is maintained through secretion

3.) Two pathways of exocytosis
Constitutive exocytosis
continuous secretion in all eukaryotic cells
Regulated exocytosis
extracellular triggers control the secretion in secretory cells: hormones, neurotransmitters or digestive enzymes

Endocytosis:

1.) Steps: a complementary process of exocytosis
2.) Two types of endocytosis
    - Pinocytosis: cellular drinking
        - ingestion of fluid and solutes via small vesicles in many cell types
    - Phagocytosis: cellular eating
        - ingestion of macromolecules in specified phagocytic cells
3.) Steps with pinocytosis:
    - Begins at clathrin coated pits
    - Form coated vesicles
    - Shed the coats
    - Fused with endosome
    - Lysosome
4.) Receptor-mediated endocytosis
    - Ligands and cell-surface receptors are involved
    - Example: uptake of cholesterol
5.) Transcytosis

Intracellular Transport and Compartments

- Road maps of biosynthetic protein traffic (Figure 12-7)
- Three fundamental mechanisms
  - via gated transporters
    - i.e. transport from cytosol to nucleus
  - via translocators (membrane bound translocators)
    - i.e. transport from cytosol to mitochondria (plastids), ER and peroxisome
  - via transport vesicles
    - i.e. transport from ER to Golgi etc
- Sorting signals
  - Types of sorting signals (Figure 12-8)
    - signal peptides (Table 12-3)
    - signal patches
- Ubiquitin- and ATP-dependent protease (Figure 5-39)
  - The fate of protein without sorting signals
  - Ubiquitin-enzyme complex
  - Chain of ubiquitins
  - Proteosome (large protein complex) as a trash can in the cell
- Transport between cytosol and nucleus
  - Nuclear pore complex
    - mechanism of transport
      - simple diffusion and active transport
    - more active in transcription, more number of nuclear pore
  - Nuclear localization signals
    - rich in positive charge amino acids and have proline
    - signals are not cut off after the transport
  - Export of RNA via specific receptor proteins
- Transport into mitochondria
  - Matrix target signals
    - 20-80 amino acid residues
    - at amino end
    - signals are removed after transport by protease
  - 2 stages transport
  - Chaperonins in the cytosol and mitochondria hsp70 and hsp60

- Transport into ER
  - Types of protein into ER
    - Transmembrane proteins
    - Water soluble proteins
  - Cotranslational mechanism
  - Signal hypothesis
    - ER signal peptide
    - Signal recognition particle (SRP)
    - Specific receptors on ER
    - Translocator protein (hydrophilic pore)
  - Start transfer signal and stop transfer signal.

Cytoskeleton I

A complex network of interconnected filaments and tubules called **cytoskeleton** extends **throughout the cytoplasm**, from the nucleus to the inner surface of the plasma membrane. This elaborate array of filaments and tubules forms **a highly structured yet very dynamic matrix** that helps to establish the shape of the cell and plays important roles in cell movement and cell division.

Major structural elements

Microtubules: Mts
Microfilaments: Mf
Intermediate filaments: IF

Unique to Eukaryotic cells

Microtubules

Two groups of Mts

Axonemal Mts

The highly organized, stable Mts found in specific subcellular structures associated with cellular movement, including cilia, flagella and the basal bodiesto which these appendages are attached .

Cytoplasmic Mts

Mts radiate out as lacelike threads toward the periphery of the cell from a Microtubule-organizing center (MTOC) near the nucleus, i.e. centrosome (cell center)

Monomers

α -tubulin and β -tubulin

Heterogeneity

genetic aspects
post-translational modification

Assembly of Mts

Nucleation
Tubulin monomers
Tubulin dimers
Rings
Sheet of protofilaments
Closed Mts
Elongation

Structure:

Hollow tube with a wall consisting of 13 protofilaments

Diameter:

outer: 25 nm; inner: 15 nm

Polarity: plus end and minus end

Microtubule motor proteins
- Cell motility
- Disposition and movement of orgenelles
- Determination of cell shape
- Maintenance of cell shape

Cytoskeleton II

Microfilaments (Mfs)

- Monomers: G-actin
  - actin is single most abundant protein in most cells
  - muscle cell: α -actin
  - nonmuscle cells: β -actin and γ -actin
  - actin gene is highly conserved
- Diameter: 8 nm
- Assembly of Mfs
  - spontaneous assembly of G-actin monomers into F-actins
  - possible addition of actin monomers to bith ends of the growing filament
  - accompanied with hydrolysis of ATP but not ATP energy required
- Structure
  - Two intertwined chains of F-actins
  - Treadmilling model
- Actin-binding proteins
  - length-regulating proteins
  - depolymerizing proteins
  - cross-linking and bounding proteins
    - Spectrin-ankyrin-actin network
- Myosin and actin
  - muscle striation
  - muscle contraction
- Functions
  - muscle contraction
  - amoeboid movement
  - cell locomotion
  - cytoplasmic streaming
  - cell division
  - cell shape

Intermediate filaments (Ifs)

- Monomers
  - Three distinctive domains
  - tissue specific IFs proteins
    - epithelial cells: keratins
    - mesenchymal: vimentin
    - muscle: desmin
    - glial: glial fibrillary acidic protein
    - neurons: neurofilamanet protein
  - nuclear lamina of all cells: nuclear lamains A, B, and C
    - located on the inside surface of the nuclear envelop
    - common to most animal cells
  - They are coded by a single family of related genes
    - Type I
    - Type II
    - Type III
    - Type IV
    - Type V
  - Intermediate filament typing
    - to identify the origin of tissues
  - Assembly of Ifs: Ifs are fibrous proteins
    - two IF polypeptides
    - a coiled coil dimer of two intertwined polypeptides
    - a tetrameric protofilament consisting of two aligned coile-coil dimers
    - staggered association of protofilaments into a long rope-like filament
    - final structure of intermediate filament with width of 8 protofilaments (16coiled-coil dimers; 32 monomers) in staggered overlaps
    - Regulation
      - phosphorylation of serine residue and mitosis
  - Functions
    - structure support
    - maintenance of cell shape
    - formation of nuclear lamin and scaffolding
    - strengthening of nerve axon

Energy Conversion I

Mitochondria structure

- Size
- Shape
- Matrix
- Outer membrane
- Inner membrane
- Intermembrane space

5 Stages of respiratory metabolism

1) Glycolysis
2) TCA cycle
3) Electron transport chain
4) Pumping of proton
5) Oxidative phosphorylation

The Tricarboxylic Acid Cycle: TCA cycle

- It occurs in mitochondria matrix
- Substrate: acetyl CoA
- Products: carbon dioxide and reduced coenzymes, NADH and FADH
- Reaction involved with TCA cycle
  - Conversion of pyruvate to acetyl coenzyme A
    - decarboxylation and oxidative reaction coenzyme A
  - Entry of acetate into the TCA cycle
  - The oxidative decarboxylation steps of the cycle
  - The ATP generating step of the cycle via the formation of GTP
  - Regeneration of oxaloacetate
- Regulation of TCA cycle activity
  1. NAD^+/ NADH ratio
  2. ATP/ADP ratio
  3. Pyruvate dehydrogenase
  4. Phosphofructokinase
- Summary of TCA cycle
  1. Acetate to citrate
  2. Decarboxylation
  3. Oxidation
  4. ATP generation
  5. Regeneration of oxaloacetate

Electron Transport Chain

- Outcome of TCA cycle: reduction of coenzymes
  - electrons are transferred to NAD^+ an FAD
- Definition of electron transport
  - the process of coenzymes reoxidation by transfer of electron to oxygen
  - this process is NOT directly
  - it is through a multiple process and involves a series of reversibly oxidizable electron acceptors: electron transport chain
- Reduction Potentials
  - Standard reduction potential E: a convention used to quantify the electron transfer potential of oxidation-reduction chain
- Electron Carriers of the Transport Chain
  - Flavoproteins
    - NADH dehydrogenase
  - Coenzyme A
  - Iron-sulfur proteins
    - NADH dehydrogenase
  - Cytochromes
    - heme and heme A
    - cytochrome b, c, c1, a1, and a3
  - Organization of Electron Transport Chain
    - NADH dehydrogenase
    - Coenzyme Q-cytochrome c reductase
    - Cytochrome c oxidase

Oxidative Phosphorylation

- ATP production depends upon phosphorylation events that are coupled to oxygen-dependent electron transport
  - Coupling of ATP synthesis to electron transport
    - 2 points:
    - 1) ATP generation depend on electron flow
    - 2) electron flow is possible only when ATP is synthesized
- Uncoupler: 2,4-dinitrophenol (DNP)
- ADP is the respiratory control
- Sites of synthesis
  - 1) between NADH and coenzyme Q
  - 2) between coenzyme Q and cytochrome c
  - 3) between cytochrome c and oxygen

- Chemiosmotic coupling model
  - Each of three sites of coupling along the transport chain involves electron transfer event that is accomplanied by the unidirectional
    pumping of protons across the membrane where the transport chain is localized
  - Electrochemical proton Gradient
    - Proton motive force (pmf)
  - ATP synthetase and the proton translocator
    - F_1
    - F°
  - Summary of respiratory metabolism
    - ATP yield of respiratory metabolism

Energy Conversion II

Review of chloroplast structure
- size
- shape
- inner membrane
- outer membrane
- stroma
- thylakoids, grana and stroma lamellae
- intermembrane space

Phototrophs
- photoheterotrophs
- photoautotrophs

Photosynthesis: 2 unique reactions

Light dependent reactions
- photosynthetic electron transfer reactions
- light reactions
- light driven production of ATP and NADPH

Light independent reactions
- carbon fixation reactions
- dark reactions
- conversion of carbon dioxide to carbohydrate

Oxygenic phototrophs: use water as an electron donor
- It needs energy and it comes from sunlight (photon)

Light dependent reactions to produce ATP and NADPH
- Chlorophyll
- It is the only pigment (light-absorbing compound) that can donate photoenergized electrons to organic compounds
- Chlorophyll a: common to all oxygenic phototrophs
- Chlorophyll b, c and d: a second kind of chlorophyll

Accessory pigments
- Carotenoids and phycobilins
- 2 functional roles:
  - 1.) broad absorption spectrum
  - 2) good agreement between absorption spectrum and action spectrum

Reaction centers
- P680
- P700

Photosystem I and generation of NADPH
- Photosystem I: the cluster responsible for the reduction of NADPH
- Photoreduction
- Chlorophyll and Chlorophyll*

Photosystem II and the oxidation of water

Water is not a good electron donor ($E° = + 0.86$)

Photosystem I: to reach ferredoxin

Photosystem II: to reach water

Summary of the transfer of electron from water to $NADP^+$

1.) Photosystem II: receive electron from water

2.) Photosystem II: accept electrons from plastocyanin

3.) Electron carriers link electron acceptor for photosystem II and electron donor for photosystem I

4.) Electron carriers link the electron acceptor for photosystem I with the ultimate acceptor NADP+

ATP synthesis

Electron flow downhill results in the proton pumpled across the membrane from the stroma into the intrathylakoid space. Therefore, an electrochemical proton gradient is generated.

CF_1

$CF°$

PMF in the chloroplast is due to the pH gradient

Photosynthetic carbon metabolism: The Calvin Cycle

Carbon fixation

Ribulose bisphosphate carboxylase

Reduction of 3-phosphoglycerate

Carbohydrate synthesis

glucose

sucrose

starch

Regeneration of ribulose-1,5-bisphophate

Summary: 3 ATP and 2 NADPH are used to fix 1 CO_2

The C_4 plants

Mesophyll cells

Bundle sheath cells

The Hatch-slack cycle: feeder system

Flow of Information I

The flow of genetic information between generations
The expression of genetic information

Expression of Genetic Information

Protein synthesis: translation
RNA synthesis: transcription
DNA synthesis: replication

DNA replication

- Chemistry and structure of DNA
  - Hydrogen bonds between G-C and A-T
  - Double-helix
    - B-DNA (Watson-Crick Model)
      - right-handed helix
    - Z-DNA
      - left-handed configuration
    - A-DNA
      - A right-handed helix induced by dehydration of B-DNA
  - Major and minor grooves
  - Polarity
  - Supercoiled DNA
    - Topological isomers
      - The molecules that differ only in their state of supercoiling
    - Enzymes: Topoisomerases
      - Type I
      - Type II : DNA gyrase is a Type II topoisomerase
    - Model of replication of circular DNA
      - Origin of replication
      - Replication is bidirection
      - Theta replication
      - Multiple origins of replication for Eukaryotic DNA

DNA polymerase
Multiple DNA polymerizes
In E Coli: 3 polymerases
DNA polemerase I
DNA polymerase III
In Eucayrotes
Polymerase a
Polymerase ß
Polymerase ?
Leading and lagging strands
Okazaki fragments
DNA ligase
RNA primer
Primase
Primosome
Replication forks
Unwinding the DNA
Helicase (unwinding protein)
Gyrase
Single strand binding protein
(Helix destabilizing protein)
Summary

DNA repair

RNA synthesis and processing

RNA polymerases

E coli: a single kind of polymerase consisting of a core enzyme complex as $a_2ßß'$ and a dissociate factor s (sigma)

Eukaryotes: 5 polymerases different in location, products and sensitivity to a-amanotin

RNA polymerase I
RNA polymerase II
RNA polymerase III
Mitochondrial polymerase
Chloroplast polymerase

The Steps of transcription

Binding: binding of polymerase to a promoter

Promoters

E coli:

recognition of promotors
about 40 nucleotide pairs
start site, 6-8 hexanucleotide sequence

Eukaryotes:

each of the polymerases has its
own promotors i.e. TATA box in the
promotors for polymerase II

Initiation

Unwinding of one turn of the DNA
doulbe helix
As soon as the first two rNTP
(N=a, U, G, C) in place, polymerase
joint the phosphodiester bond

Elongation

Polymerase moves up in 3' to 5' direction
RNA strand grows in 5' to 3' direction
A short DNA-RNA hybrid form
DNA return to its double helix form
(thermodynamic stability)

Termination

Termination signal (stop signal)

E coli: it is a sequence that fige rise in the RNA
product to a hairpin helix followed by
a string of U's (the hairpin structure is the factor)

? factor in other region

Processing of RNA

Ribosomal RNA

rRNA is the most abundant and most stable form of RNA

In eukaryotes

Processing of 45S to 18S, 28S and 5.8S

5S is a separate product

Transfer RNA

At 5' end, a short leader sequence is removed

At 3' end, the two terminal nucleotide (UU) is replaced with CCA which is a distinguishing characteristic of functional tRNA

Methylation

Splicing

Messenger RNA

E coli: transcription and translation are coupled processes

Eukaryotes: the compartmentization is associated with the need of mRNA processing (splicing)

Transcription unit for mRNA is monocistronic

hnRNA (heterogeneous RNA): precusor of mRNA

Introns and Exons

Splicing

Caps and Tails

Protein Synthesis

Reading Assignments:

Text pages: 223-273

Questions:

1. Z-DNA co-exists with B-DNA in the same DNA
   True_____False_____

2. DNA ligase is a Type II topoisomerase.
   True_____False_____

3. Primase is accompanied by a large complex of protein called primosome.
   True_____False_____

4. In most vertebrate cells, the clusters of genes encoding 28 s rRNA are transcribed independently
   True_____False_____

5. Transcription unit is a segment of DNA that is transcribed as a single, continues RNA with a promoter on one end and a termination signal on the other end
   True_____False_____

6. Which of the following is **false** about hnRNA (heteronuclear RNA)?

A. Contains introns
B. Lacks cap and tail
C. Can be polycistronic
D. Contains exons
E. None of the above

Recombinant DNA Technology

Restriction Enzymes

Endonucleases, are present in most bacterial cells

- Protect the bacterial cell from foreign DNA molecule, particularly those of bacteriophages
- Part of a restriction/methylation system
  - Foreign DNA is degraded by restriction enzymes, and the bacterial genome is protected by methylation
- i. e. **Ecor RI** from **E. col**i strain **R**
  HaeIII from **H**emophilus **ae**gyptius

Recognition sequences

- Specificity
- 4 or 6 nucleotide pairs
- Palindromes; twofold rotational symmetry of the sequence
- The recognition sequence has the same order of nucleotides on both strands but is read in opposite directions on the strands because of their antiparallel orientation

Restriction fragments

- With blunt ends
- With cohesive (sticky) ends

Gel electrophoresis of DNA

- Polyacryamide
- Agarose
- Because of the negative charge of their phosphate groups, DNA fragments migrate down the gel toward the anode; the technique separate DNA based on their size
- Detection of DNA
  - Ethidium bromide
  - Autoradiography

Restriction Maps

- Restriction maps indicate the location of restriction enzyme cleave sites in relation to one another

Recombinant DNA molecules

DNA cloning

1) Insertion of DNA into a cloning vector
   - bacteriaphage
   - plasmid
   - antibiotic resistance genes: selectable markers
   - DNA ligase
2) Amplification of recombinant vector molecules in bacterial cells
   - Transduction or transfection
3) Selection of bacterial cells containing recombinant DNA
4) Identification of bacterial colonies containing the DNA of interest
   - Screening
     - Colony hybridization
       - nucleic acid probe
     - Antibody approach
       - expression vectors

Genomic and cDNA libraries

Genomic library

cDNA library
- reverse transcription of mRNA
- a cDNA library will contain only those DNA sequences that are transcribed into RNA, presumably the active genes in the tissue from which the mRNA was prepared.

PCR (Polymerase Chain Reaction)

Amplification of selected DNA sequences

In the test tube

Need DNA oligonucleotide primers

Heat stable enzyme:The DNA polymerase was first isolated from bacteria able to grow in thermal hot springs (70- 80oC)

Procedures

1) reverse transcriptase synthesizes cDNA from mRNA
2) Alkali digestion of mRNA
3) DNA polymerase synthesize double strain DNA
4) Terminal transferase
5) Mix with a cloning vector with a complementary fragment

Genetic Engineering

Application of recombinant DNA technology to the practical problems

In medicine

insulin

human growth hormone and hypopituitarism

human gene therapy

Transgenic animals and plants

Regulation of Gene Expression in Eukaryotes

Differences between Prokaryotes and Eukaryotes

- Genome Size and Complexity
  - Large genome for eukaryotes
  - Uncoding sequence in eukaryotic genome
- Genomic Compartmentalization
  - Nuclear envelope serves to screen antibody
  - Transcripts
- Structural Organization of Genome
  - Highly ordered in packing in eukaryotes
  - Binding of regulatory protein to desired region
  - Regulatory elements
- Stability of mRNA
  - Greater longevity for eukaryotic mRNA
  - Environmental constancy is not assured for prokaryotes
- Protein Turnover: What to do with defective and unwanted proteins
  - Proteolytic enzymes
  - Cease cell division
  - Cease synthesis

Multiple Levels of Gene Control in Eukaryotes

- Genomic Control
  - Totipotency of differentiated cells
    1) nuclear transplantation in animals
    2) tissue culture study in plants
- Gene amplification
  - Some interesting examples take place, but it does not seem to be a critical control mechanism for most genes.
- Transcriptional Control
  - Evidence
    1) differential transcription of genes
    2) nuclear run-on transcription assays
- Two-Stage Process
    1) decondensation of coiled chromatin
    2) regulated transcription of uncoiled region
- Binding of Transcriptional Factors Regulates Transcription
    - Regulatory proteins
    - Consensus binding sites
    - Combinatorial model for gene regulation
- Cis-Acting Elements: Eukaryotic Promoters and Enhancers
    - Promoters
    - Upstream promoter region
    - Enhancers
    - Deletion mutant technique

- Trans-Acting Factors: Regulatory Proteins Bind to Promoters and Enhancers
  - 2 Structural Domains
    1) DNA binding domain
    2) transcription activation domain
  - 3 Common Structural Motifs
    1) Helix-turn-helix
    2) Zinc finger
    3) Leucine zipper
- Mechanisms of Action of Enhancers and Transcriptional Factors
  - Long range chromatin effect
  - Gateway for liner diffusion
  - Looping/interaction
- Possible Role of DNA Methylation in Regulating DNA Availability
  - Methylation of cytosine
  - DNA of inactive gene tends to have more methylation.
  - Methylation
- Posttranscriptional Control
  - RNA Processing and Translocation
    - Alternative splicing
    - Translational control
      1) Selective utilization of specific mRNA
      2) Variation in rates of mRNA degradation
      3) Availability of tRNA and tRNA synthetase
      4) Prosthetic group availability
         example: regulation of transcription by Hemin in red blood cells
- Posttranslational Control
  - Permanent Modification
    - Glycosylation
    - Proteolytic actions
    - Reversible structural modification
      - phosphorylation
- Responses to Intracellular Elements
  - Ca^{++}, cAMP, IP_3

Cell Signaling

Cell-cell communication in animal cells
- Via secreted molecules
  - paracrine signaling
  - endocrine signaling
  - synaptic signaling
- Via plasma-membrane-bound-molecules
  - Cell adhesion, cell junction and extracellular matrix

Receptors and hydrophobicity of signaling molecules
- Cell surface receptors and hydrophilic signaling molecules
- Intracellular receptors and hydrophophobic molecules

Intracellular receptors
- Diffusion into the cells
- Binding to the intracellular receptors
- Inducing the conformational change of receptor
- The activated receptor comples enters into the nucleus
- Binding to the response element (i.e. hormone response element)

Cell surface receptors
- Types of cell surface receptors
- First messenger
- Second messenger
  - Cyclic AMP (cAMP) as a second messenger
    - G proteins and cAMP synthesis
    - Regulation of G proteins
    - cAMP and glycogen degradation
  - Ca^{++} as a second messenger
    - Calcium binding protein
      - Calmodulin
  - Inositol Triphosphate (IP_3) and Diacylglycerol (DAG) as second messengers
  - Third messengers
    - Protein and protein phosphatase
  - Fourth messengers
    - Transcriptional factors (messengers in nucleus)

Signaling amplification
- Cascade of intracellular events and amplification of extracellular signals
  - Rapid turnover of intracellular mediators
  - "All or none" effect of chemical signals
    - Cooperativity
    - Activation of one enzyme and inhibition of another one with opposite reaction

Target cell adaptation

- Mechanisms
  - Down-regulation of receptors
    - Receptor sequestration
    - Receptor degradation
      - Receptor mediated endocytosis
  - Inaction of receptors
  - Inaction of none-receptor prot

Cell Junction, Cell Adhesion and Extracellular Matrix

Cell Junctions

- Three functional types
  - Occluding junctions
  - Anchoring junctions
  - Comunicating junctions
- **Tight junctions: occluding function**
  - Function
  - Features
  - Intermembrane space
  - Associated structures
  - Molecular structure
- **Anchoring junctions**
  - Function
  - Forms
    - Adherens junction
    - Desmosomes
    - Hemidesmosomes
  - Associated structures
  - Intermembrane space
  - Features
- **Gap junctions**
  - Function
  - Features
- Intermembrane spaces
- Associated structure

Cell Adhesion

- Mechanisms
  - Homophilic binding
  - Heterophilic binding
- Through an extracellular linker molecule
  - Neural Cell Adhesion Molecules (N-CAM)
  - Cardherins

Extracellular Matrix (ECM)

- Connective tissues
  - Fibroblasts
    - Chondroblasts
    - Osteoblasts
  - Components of ECM
    - Glycosaminoglycans (GAGs)
    - Fibrous proteins
      - Collagen
        - It is the major protein of ECM
          - It is also the most abundant protein in the animal cells
          - At least 10 types of collagen have been determined, 4 will be studied
            - Type I
            - Type II
            - Type III
            - Type IV
      - Elastin
        - It is a hydrophobic protein
        - It is not a glycoprotein
        - Forms a network of elastic fibers in ECM
      - Adhesive components
        - Fibronectin
          - It is a glycoprotein
          - It helps to mediate cell-matrix adhesion
          - Alternative RNA splicing produces the multiple forms of fibronectin
        - Laminin
          - One of the components of basal lamina
            - Basal laminae are continuous thin mats of specialized ECM that underlie all epithelial cell sheets and tubes and also surround the other cells
      - ECM receptors Matrix receptors
        - Low affinity binding and high concentration presence
        - Fibronectin receptor
          - Integrins

The Nervous System

CNS and PNS
The Cells
- **Neurons**
  - Cellular structures
    - Cell body
    - Axon
    - Dendrite
  - Different types of neurons
- **Glial cells**
  - Central nervous system
    - Oligodendrocyte
    - Microglia
    - Ependymal cells
    - Astrocytes
  - Peripheral nervous system
    - Schwann cells
- Blood-brain barrier

Transport Mechanisms
- Fast transport and slow transport
- Anterograde transport and retrograde transport

Synaptic Transmission
- **Synapses**
  - Electrical synapses
  - Chemical synapses
- **Chemical Synapse**
  - Neurotransmitters
    - Criteria to be a neurotransmitter
      - It must elicit the appropriate response upon microinjection into the synaptic cleft
      - It must be found to occur naturally in the presynaptic axon
      - It must be released at the right time when the presynaptic membrane is stimulated
    - Neurotransmitters are released by exocytosis
    - Neurotransmitter release is quantal and probabilistic
    - Excitatory effects and inhibitory effects
      - Excitation
      - Inhibition
    - Structure of chemical synapse
      - Synaptic cleft
      - presynaptic membrane
        - Synaptic vesicles

- Postsynaptic membrane
- Mode of action of acetylcholine
  - Acetylcholine is an excitatory neurotransmitter
  - Structure synthesis and hydrolysis of acetylcholine
  - The acetylcholine receptor
- Other neurotransmitters
  - GABA and glycine are inhibitory neurotransmitter
    - GABA: γ -aminobutyric acid
    - GABA receptors
      - Tranquilizers act on GABA receptors
        - Benzodiazepines
  - Catecholamines and aderenergic synapses
    - Catecholamines are derivatives of tyrosine
      - Dopamine
      - Norepinephrine
      - Epinephrine
    - Monoamine oxidase inactivates catecholamine
  - Neurotoxins
    - Strychnine
    - Curare

Cellular Aspects of the Immune Response

Innate immunity and adaptive immune system
The immune response
- Antigen and antigenic determinants
- Characteristics of the immune response
- Types of immune responses
  - Cell-mediated immune responses
  - Humoral immune responses

Cellular basis of the immune response
- Lymphocytes
  - T cells and B cells
- Development of lymphocytes
- Clonal selection
  - Antigen receptors
  - Formation of clones and their selection by antibodies
    - Antigen-independent differentiation
    - Antigen-dependent differentiation
- Immunological memory
  - Primary and secondary responses
  - Effector cells and memory cells
    - Production of memory cells
- Differentiation markers
  - B cells: immunoglobulins
  - T cells: CD3 complex
    - T_h cells
    - T_c cells
- Lymphocyte activation pathways
  - Graft rejection
  - The **m**ajor **h**istocompatibility **c**omplex
    - The MHC gene expression
    - Classes of MHC antigens
      - Class I antigens
      - Class II antigens
  - Pathways of the Immune response
    - Antigen processing and presentation
    - T_h cell activation
    - T_c cell activation
    - B cell activation

The structure and function and antibodies

- The antibody molecule
  - Variable domains and constant domains
  - Antigen binding sites and effector sites
- Classes of immunoglobulins in mammals
  - IgG
  - IgM
  - IgA
  - IgD
  - IgE
- Antibody valence

Monoclonal antibody

Cellular Aspects of Cancer

Cancer: lost of normal growth and positional regulation

- **Neoplastic transformation**
  - **Classification of neoplasm (tumor)**
  - **Causes of neoplastic transformation**
    - Chromosomal alteration
      - Chronic myelogenous leukemia (CML) and Philadelphia chromosome
    - Oncogenic Viruses:
      - RNA tumor viruses in the retrovirus family
        - replication cycle of retrovirus
      - How to demonstrate a viral etiology for a specific tumor
      - Patterns of infection
        - Horizontal transmission
        - Vertical transmission
    - Environmental carcinogens
      - Physical factors
      - Chemical carcinogens
        - Metabolic conversion of the procarcinogen to ultimate carcinogen
          - Mixed-function oxidase or aryl hydroxylase
      - Chemical carcinogens act by producing genetic mutations
        - Ames test is a mutagenesis assay
    - The genetic basis of neoplasia
      - Oncogene
        - Definition
        - Proto-oncogene
        - Alternation of proto-oncogene to oncogene
          - Dosage effects
          - Gene mutation
    - Tumor-suppresser genes
      - Dominant character of the oncogene
      - Recessive character of spontaneous tumors
      - rbl human gene:
        - Inactivation of rbl gene is associated with the inherited tumor bilateral retinoblastoma

Tumor Dissemination

- Tumor invasion
  - Dissemination to nearby tissue
  - Process contribute to tumor invasion
    - release of degradative enzymes
    - loss of contact paralysis
- Metastasis
  - Dissemination to distant organs
  - It can occur in 4 systems
    - peritoneal cavity
    - neural canal
    - lymphatic system
    - vascular system
  - Vascular metastasis
    - Establishment of a vascular supply