KAZAN FEDERAL UNIVERSITY
INSTITUTE OF FUNDAMENTAL MEDICINE AND BIOLOGY
DEPARTMEMNT OF BIOCHEMISTRY

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Principles of Biochemistry for Medical Students

Summaries of Lectures

Kazan – 2014

The code of a training direction (speciality): 060101.65: Medical care

Course Syllabus: "Medical care" (face study, 2014)

Discipline: "Biochemistry" (specialty, 1st and 2nd years of study)

Curriculum: 252 academic hours (including lectures - 42, laboratory classes – 108, self-preparation – 66. The forms of control: mid and final exams

Annotation: The electronic course includes the curriculum outline, slide presentations, a list of basic terms, and tests. The course is mainly intended for self-education but can also be used during classes' hours.

The goal of the course entitled "Biochemistry" is to get knowledge about basic chemical reactions underlying the life.

Working with the literature is better to begin with going through the lectures. You should read carefully with a pencil in your hand and mark of three types: what is clear, what needs to be specified, and what is totally unclear. Then you should open a textbook and find answers to your questions followed by putting down commentaries to your lectures. After that you can go to the unclear items using actively the recommended literature and consulting with a mentor.

On addition to the midterm exams, the main way to control your knowledge would be the final exam in Biochemistry.

Topics:

1. Protein Structure and Function
2. Enzymatic catalysis
3. Nucleotides, Nucleic Acids, and Nucleoproteins
4. Carbohydrates and Lipids
5. Vitamins and Hormones
6. Metabolism of Carbohydrates
7. Electron Transport and Oxidative Phosphorylation
8. Metabolism of Lipids
9. Nitrogen Metabolism
10. Flow of Genetic Information

Key words: biochemical process, macromolecule, protein, carbohydrate, vitamin, metabolism, anabolism, catabolism, metabolism of carbohydrates, metabolism of proteins and amino acids, metabolism of nucleic acids, metabolism of lipids

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URL: http://tulpar.kfu.ru/course/view.php?id=2016

Content.

Lecture 1

Protein Structure and Functions

Annotation

1. Amino acids, peptides, and polypeptides (proteins)

Proteins are large biological molecules consisting of one or more long chains of amino acid residues. Proteins differfrom one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes, and which usually results in folding of the protein into a specific three-dimensional structure that determines its activity.

2. The three-dimensional structures of proteins (major types of protein conformation) Secondary and tertiary structure: the three-dimensional (3D) structure of a protein, which results from a large number of non-covalent interactions between amino acids. Quaternary structure: non-covalent interactions that bind multiple polypeptides into a single, larger protein. E.g., hemoglobin has quaternary structure due to association of two alpha-chain and two beta-chain polypepetides.

3. Physicochemical properties of proteins and peptides

High molecular weight, high viscosity in solutions, slow diffusion, swelling capacity, amphoteric , optical activity , lowosmolality , high oncotic pressure, ability to undergo denaturation.

4. Functional diversity of proteins (e.g., hemoglobins and myoglobin, contractile proteins, collagen, fibrinogen/fibrin)

Proteins have vary diverse functions: antibodies are specialized proteins involved in defending the body from antigens, contractile proteins are responsible for movement, enzymes are proteins that facilitate biochemical reactions, etc.

5. Classification of proteins

Based on structure: Fibrous, globular and intermediate proteins. Based on composition: simple and conjugated.

6. Myoglobin and hemoglobin

Myoglobin and hemoglobin are hemeproteins whose physiological importance is related to their ability to bind molecular oxygen.

7. Electrophoresis

Electrophoresis is an analytical method used for the separation and characterization of proteins and nucleic acids.

Key words

Amino acids, peptides, and polypeptides, structure of a protein, classification of proteins, myoglobin and hemoglobin, electrophoresis.

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http://themedicalbiochemistrypage.org/

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Plan.

1. Amino acids, peptides, and polypeptides
2. The three-dimensional structures of proteins (major types of protein conformation)
3. Physicochemical properties of proteins and peptides

4. Functional diversity of proteins (e.g., hemoglobins and myoglobin, contractile proteins, collagen,fibrinogen/fibrin)
5. Classification of proteins

Lecture 1 Protein Structure and Functions

Amino acids, peptides, and polypeptides

Proteins are large biological molecules, or macromolecules, consisting of one or more long chains of amino acid residues. Proteins differ from one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes, and which usually results in folding of the protein into a specific three-dimensional structure that determines its activity.

All amino acids have a central or alpha-carbon, to which are bonded 4 groups:

- a hydrogen
- an amino group
- a carboxyl group (or acid group)
- a unique side chain, also known as a R-group

The 20 amino acid side chains confer different properties, including solubility in water. Some side chains are very non-polar; others are polar, positively or negatively charged.

Amino acids are covalently bonded together in chains by peptide bonds. If the chain length is short (say, less than 30 amino acids) it is called a peptide; longer chains are called polypeptides or proteins. Peptide bonds are formed between the carboxyl group of one amino acid and the amino group of the next amino acid. Peptide bond formation occurs in a condensation reaction involving loss of a molecule of water.

The head-to-tail arrangement of amino acids in a protein means that there is a amino group on one end (called the amino-terminus or N-terminus) and a carboxyl group on theother end (carboxyl-terminus or C-terminus). The carboxyl-terminal amino acid corresponds to the last one added to the chain during translation of the messenger RNA.

The three-dimensional structures of proteins (major types of protein conformation)

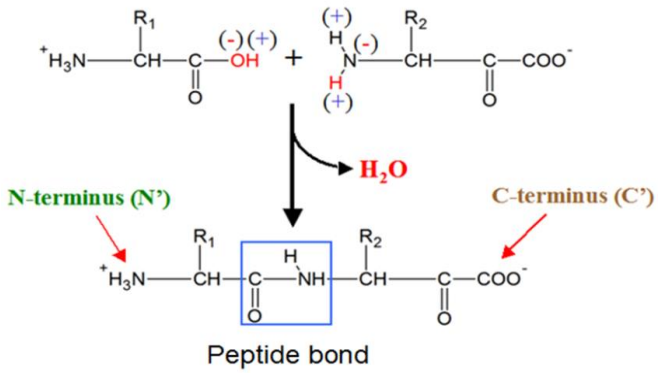
Primary structure (linear polymer of amino acids) (held together with peptide bonds)

Secondary structure (standard 3-D patterns)(a-helix, ß-sheet, held together with H-bonds between backbone atoms)

Tertiary structure (detailed 3-D conformation) (bonds between side-chain atoms)

Quaternary structure (combined polymer chains)

Primary structure describes the unique order, in which amino acids are linked together to form a protein. Proteins are constructed from a set of 20 amino acids.



Secondary structure refers to the shape of a folded protein due exclusively to hydrogen bonding between its backbone amide and carbonyl groups. Secondary structure does not include bonding between the R-groups of amino acids, hydrophobic interactions, or other interactions associated with tertiary structure.

The two most commonly encountered secondary structures of a polypeptide chain are alpha-helices and beta-pleated sheets. These structures are the first major steps in the folding of a polypeptide chain, and they establish important topological motifs that dictate subsequent tertiary structure and the ultimate function of the protein

Characteristics of the α-helix:

- 3.6 amino acids per turn
- 0.54 nm per turn
- side chains pointed out
- H-bonds parallel to axis

- dipole moment (negative at the C-end)
- no proline

β-PLEATED SHEETS:

This structure occurs when two or more segments of a polypeptide chain overlap one another and form a row of hydrogen bonds with each other.

Parallel and anti-parallel arrangement is the direct consequence of the directionality of the polypeptide chain. In anti-parallel arrangement, the C-terminus end of one segment is on the same side as the N-terminus end of the other segment. In parallel arrangement, the C-terminus end and the N-terminus end are on the same sides for both segments. The "pleat" occurs because of the alternating planes of the peptide bonds between amino acids; the aligned amino and carbonyl group of each opposite segment alternate their orientation from facing towards each other to facing opposite directions.

Tertiary structure: the three-dimensional (3D) structure of a protein, which results from a large number of non-covalent interactions between amino acids.

Tertiary structure involves the following types of bonds between and among side chains:

Hydrogen bonds (-O-H…O-)

Ionic or electrostatic bonds (attractive or repulsive)

Van der Waal's bonds (short distance attraction)

Disulfide covalent bonds between Cys residues (-CH2-S-S-CH2-)

Hydrophobic interactions (repulsive interaction of hydrophobic side chains with water solvent

Quaternary structure: non-covalent interactions that bind multiple polypeptides into a single, larger protein. E.g., hemoglobin has quaternary structure due to association of two alpha-chain and two beta-chain polypepetides

Protein structure determination

The three-dimensional shape of a protein is determined by its primary structure. The order of amino acids establishes a protein's structure and specific function. The distinct instructions for the order of amino acids are designated by the genes in a

cell. When a cell perceives a need for protein synthesis, the DNA unravels and is transcribed into an RNA copy of the genetic code followed by protein synthesis.

Physicochemical properties of proteins and peptides

Properties of proteins

High molecular weight

High viscosity in solutions

Slow diffusion

Swelling capacity

Amphoteric

Optical activity

Low osmolality

High oncotic pressure

Ability to undergo denaturation

Optical activity

Amino acids, except for glycine, have at least one “asymmetric” or chiral α-carbon atom and, hence, are optically active. All amino acids found in proteins have the same configuration on the αC-atom: they are considered L-amino acids.

Denaturation is a process, in which proteins lose their tertiary and secondary structure by application of some external compound or stress, such as a strong acid or base, a concentrated inorganic salt, an organic solvent (e.g., alcohol), or heat. If proteins are denatured, this results in disruption of their activity. Denatured proteins lose solubility and aggregate.

Functional diversity of proteins (e.g., hemoglobins and myoglobin, contractile proteins, collagen, fibrinogen/fibrin)

Type of protein	Example	Function
Enzymes	Amylase	Digestion
Transport	Hemoglobin Myoglobin Albumin Lipoprotein	Transports O2 in blood Transports O2 in muscle Transports fatty acids Transports lipids
Storage	Ovalbumin Milk Ferritin	Egg-white protein Milk Iron storage in spleen
Contractile	Myosin, actin	Muscle movement
Protection	Antibodies Fibrinogen, thrombin	Fight infection Blood clotting
Hormones	Insulin Growth hormone	Carbohydrate metabolism Growth and regeneration
Structural	Glycoproteins Collagen Elastin	Cell walls, skin Tendons, bones, cartilage Ligaments
Toxins	Clostridium botulinum Ricin Snake venom	Botulism food poisoning Castor bean toxin Snake venom

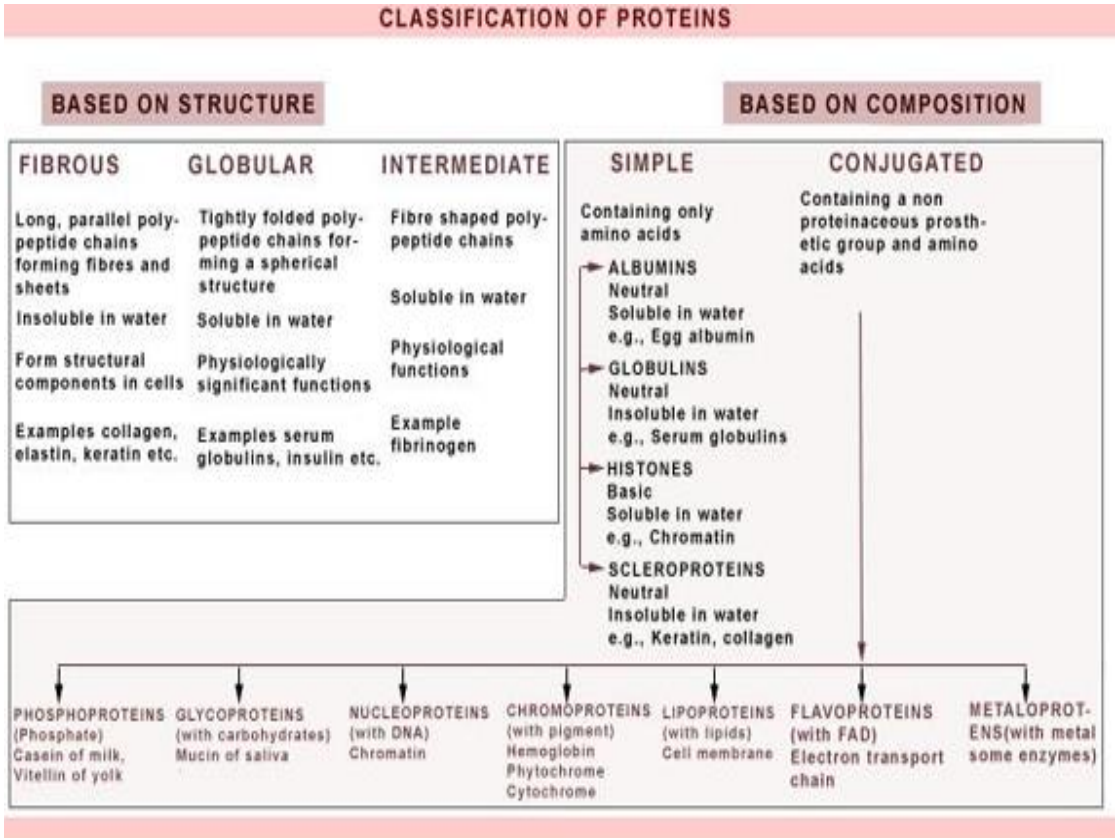
Antibodies are specialized proteins involved in defending the body from antigens. They are utilized by the immune system to identify and defend against bacteria, viruses, and other foreign bodies. Contractile proteins are responsible for movement. Examples include actin and myosin. These proteins are involved in muscle contraction and movement.

Enzymes are proteins that facilitate biochemical reactions. They are referred to as catalysts because they speed up chemical reactions. Examples include the enzymes lactase and pepsin. Lactase breaks down the sugar lactose found in milk. Pepsin is a digestive enzyme that works in the stomach to break down proteins in food.

Hormonal proteins are messenger proteins, which help to regulate certain bodily activities. Examples include insulin and somatotropin. Insulin regulates glucose metabolism by controlling the blood sugar levels. Somatotropin is a growth hormone that stimulates protein production in muscle cells. Structural proteins are fibrous and stringy and provide support. Examples include keratin, collagen, and elastin. Keratins strengthen protective coverings such as hair, quills, feathers, horns, and beaks. Collagens and elastin provide support for connective tissues such as tendons and ligaments. Storage proteins store amino acids. Examples include ovalbumin and casein. Ovalbumin is found in egg whites and casein is a milk-based protein. Transport proteins are carrier proteins which move molecules from one

place to another around the body. Examples include hemoglobin and cytochromes. Hemoglobin transports oxygen through the blood. Cytochromes operate in the electron transport chain.

Classification of proteins



Questions for self-control

1. Proteins as biopolymers. Aminoacids as monomers of proteins. Classification of proteins according to their structure, description of different classes.
2. Primary structure of proteins. Definition, description. Polymorphic and homologous proteins.
3. Secondary and tertiary structures of protein molecules. Domains as protein structural elements.
4. Quaternary structure of proteins. Hemoglobin and allosteric enzymes as an examples of oligomeric proteins. Supramolecular complexes, examples.
5. Protein-ligand interactions and functional activity of proteins. Classification of proteins according to their biological activity, examples.

6. Physico-chemical properties of proteins: ionization, solubility, optical properties of protein solutions.

7. Chemical properties of proteins: hydrolysis, phosphorylation, methylation, γ-carboxylation, glycosylation, and significance of these reactions.

Lecture 2.

Enzymatic catalysis

Annotation

1. Enzyme terminology and classification

Enzymes are biological molecules responsible for the thousands of metabolic processes that sustain life. The international Union of Biochemistry and Molecular Biology (IUBMB) developed a system of nomenclature, in which enzymes are divided into six major classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases.

2. Kinetics of enzyme-catalytic reactions

Enzyme kinetics is the study of the chemical reactions that are catalysed by enzymes. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

3. Enzyme inhibition

Types of enzyme inhibition: reversible inhibition (competitive and noncompetitive inhibition) and irreversible inhibition (Inhibitor binds covalently).

4. Allosteric regulation of enzymatic activity

Enzyme molecules may possess a site called a regulatory site distinct from the active site. The regulatory site has an affinity for certain molecules called modulators. When the modulator molecule binds to the regulatory site it effects a change in the enzyme conformation that alters the enzyme catalytic rate or affinity of the substrate or both. This type of regulation is called allosteric (allo - other; steric – shape-related).

5. Structure, vitamins, cofactors and coenzymes

A cofactor is a non-protein chemical compound that is required for the protein's biological activity. These proteins are commonly enzymes, and cofactors can be considered "helper molecules" or coenzymes that assist in biochemical

transformations. Cofactors can be classified depending on how tightly they bind to an enzyme, with loosely bound cofactors termed coenzymes and tightly bound cofactors termed prosthetic groups. An inactive enzyme without the cofactor is called an apoenzyme, while the complete enzyme with cofactor is called a holoenzyme.

6. Isoenzymes

Isoenzymes (aka isozymes or "multiple enzyme forms") are enzymes that catalyze the same chemical reaction but differ in amino acid sequence and physical properties.

Key words

Enzyme,kinetics of enzyme-catalytic reactions, reversible inhibition, competitive and noncompetitive inhibition, irreversible inhibition, allosteric regulation cofactors and coenzymes, isozymes.

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Plan.

1. Enzyme terminology and classification
2. Kinetics of enzyme-catalytic reactions
3. Enzyme inhibition
4. Allosteric regulation of enzymatic activity
5. Structure of enzymes
6. Vitamins and coenzymes

Lecture 2. Enzymatic catalysis

Enzyme terminology and classification

Enzymes are biological molecules responsible for the thousands of metabolic processes that sustain life.

Enzymes are highly selective catalysts, greatly accelerating the rate of metabolic reactions, from the digestion of food to the synthesis of DNA. Most enzymes are proteins, although some catalytic RNA molecules have been identified.

Nomenclature

Recommended name

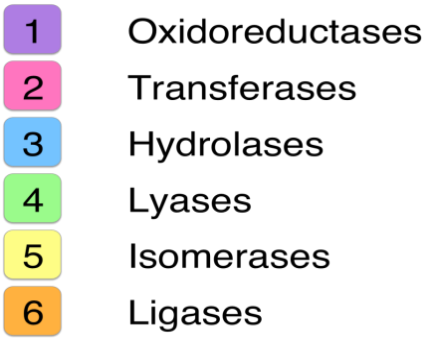
1. Most commonly used enzyme name have the "-ase" attached to substrate of the reaction Glucosidase

- or contain description of the action performed Lactatdehydrogenase

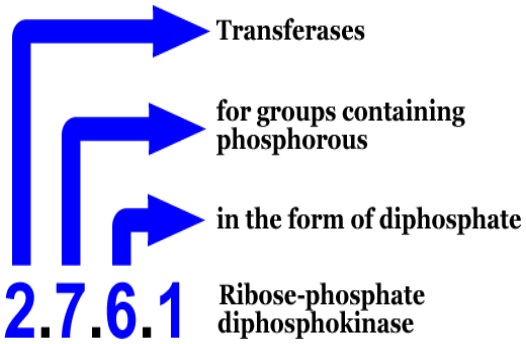
2. Some enzymes retain original trivial names Pepsin

Systematic name

The international Union of Biochemistry and Molecular Biology (IUBMB) developed a system of nomenclature, in which enzymes are divided into six major classes.



Each enzyme has a code number (EC) that characterizes the reaction type as to class (first digit), subclass (second digit), and subsubclass (third digit). The fourth digit is for the specific enzyme.



Kinetics of enzyme-catalytic reactions

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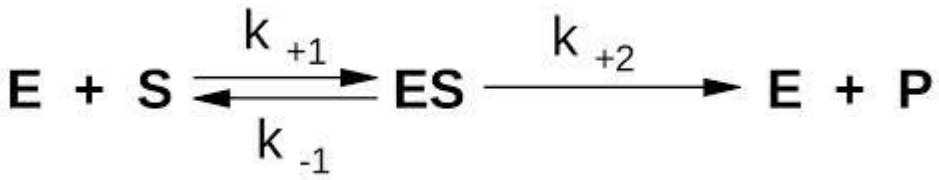
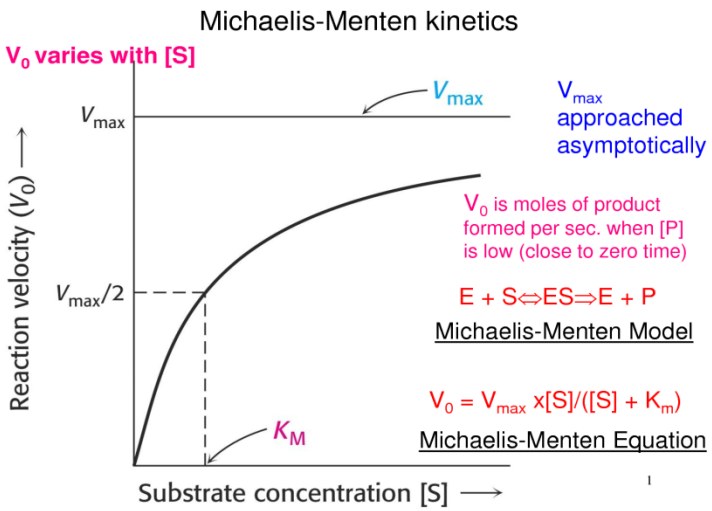


E = enzyme S = substrate

ES = enzyme-substrate complex P = product

k1,k-1 and k2 = rate constants

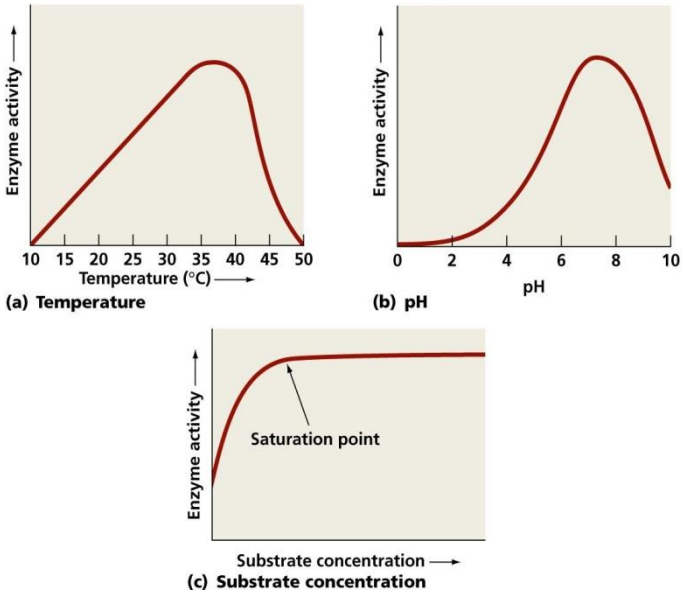


The Michaelis-Menten constant (Km) is the substrate concentration required for an enzyme to reach one half its maximum velocity. Each enzyme has a characteristic Km for a given substrate.

Increases in temperature will speed up the rate of non-enzymatic reactions, and so temperature increase speeds up enzyme mediated reactions, but only to a point. When heated too much, enzymes (since they are proteins) become denatured.

Concentration of substrate and product also control the rate of reaction, providing a feedback mechanism. Activation protects a cell from the hazards or damage the enzyme might cause ig it is always active.

Changes in pH will also affect the enzyme. Enzymes are adapted to operate at a specific pH or within a pH range.



Enzyme inhibition

- Reversible inhibition

Competitive inhibition

Noncompetitive inhibition

- Irreversible inhibition

Inhibitor binds covalently

Allosteric regulation of enzymatic activity

Enzyme activity can be regulated in several ways.

On a genetic level, enzymes are controlled by special DNA pathways called operons that can be activated or shut down due to the presence or absence of the substrate.

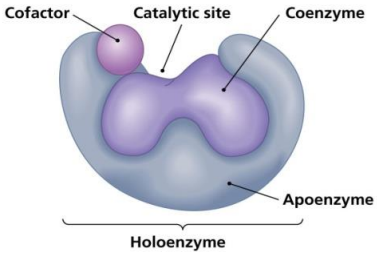
In allosteric regulation an enzyme having an allosteric site either closes the active site, preventing the formation of the enzyme-substrate complex or allows the active site to open.

Allosteric regulation

Enzyme molecules may possess a site called a regulatory site distinct from the active site. The regulatory site has an affinity for certain molecules called modulators. When the modulator molecule binds to the regulatory site it effects a change in the enzyme conformation that alters the enzyme catalytic rate or affinity of the substrate or both. This type of regulation is called allosteric (allo - other; steric – shape-related).

Modulators involved in allosteric regulation can either increase (stimulate) or decrease (inhibit) enzyme activity.

Structure of enzymes



A cofactor is a non-protein chemical compound that is required for the protein's biological activity. These proteins are commonly enzymes, and cofactors can be considered "helper molecules" or coenzymes that assist in biochemical transformations.

Cofactors can be classified depending on how tightly they bind to an enzyme, with loosely bound cofactors termed coenzymes and tightly bound cofactors termed prosthetic groups. An inactive enzyme without the cofactor is called an apoenzyme, while the complete enzyme with cofactor is called a holoenzyme

Vitamins and coenzymes.

Vitamin	Name	Active Form (co-factor)	Biochemical Function	Physiological/cellular Role
B_5	Pantothenic Acid	Coenzyme A	Acyl Transfer	• Energy production from foodstuff • Fatty acid synthesis
B_6	Pyridoxine	Pyridoxal Phosphate (PLP)	• Transamination • Racemization • Decarboxylation • β/γ-Elimination	• Amino acid breakdown • Glycogen breakdown
B_7	Biotin	Biotin	Carboxylation	• Glucose & fatty acid synthesis • Leucine synthesis
B_9	Folic Acid	Tetrahydrofolate (THF)	One-Carbon Group Transfer	Amino Acid & nucleotide synthesis
B_{12}	Cobalamin	Coenzyme B_{12}	• Intramolecular Rearrangements • Methyl transfer	• Nucleotide synthesis • Amino acid metabolism • Fatty acids breakdown • Folic acid regeneration
C	Ascorbic Acid	Ascorbic Acid	Proline Hydroxylation	Collagen synthesis
			Reduction	Antioxidation
D	Calciferol	Calcitriol	Gene expression	Bone growth

Questions for self-control

1. Enzymes: general characteristic and their biological role. International classification of enzymes. Nomenclature of enzymes, examples.
2. Chemical nature of enzymes. Functional sites (active site, regulative sites). Enzymes cofactors, their role in catalysys.
3. Mechanisms of regulation of enzymes activity, examples.

4. Properties of enzymes: catalytical activity, specificity of action, dependence of their activity from pH, temperature, substrate and enzyme concentration.

Medical enzymology. The use of enzymes in diagnostic and treatment purposes.

Lecture 3

Nucleotide, Nucleic Acids and Nucleoproteins

Annotation

1. Nicleic acids are the acids found in cell nuclei and are involved in the transmission of genetic information. They are complex biopolymers containing a carbohydrate, a phosphate ester and a heterocyclic aromatic unit, the base. DNA and RNA are polymers of nucleotides.

2. Nucleosides are formed by covalently joining the sugar to a heterocyclic base (bond formed is called a glycosidic bond.)Purine amd pyrimidine nitrogeneous bases, sugars (ribose and doxyribose).

3. Mononucleotides and Oligonucleotides

A dinucleotide (dimer) of DNA or RNA is formed by covalently linking the 5′-phosphate group of one nucleotide to the 3′-hydroxyl group of another to form a phosphodiester bond. An oligonucleotide (oligomer) is formed when several such bonds are made, and naturally-occurring nucleic acids are linear, high molecular weight molecules of this kind. At physiological pH (7.4) each phosphodiester group exists as an anion (hence the term nucleic acid).

4. DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms. Nearly every cell in a person's body has the same DNA. Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitichondrial DNA or mtDNA). The double helix is the dominant tertiary structure for biological DNA.

5. Histones

In eukaryotic cells the DNA in the nucleus is strung around a series of spool-shaped proteins known as histones. Their chief functions are to compact and control the long threads of DNA. They compact the DNA by interacting with each other to form a structure like a compact spool.

6. RNA or ribonucleic acid molecules are single stranded nucleic acids composed of nucleotides. RNA plays a major role in protein synthesis as it is involved in the transcription, decoding, and translation of the genetic code to produce proteins.

Key words

Nicleic acids, nucleosides, mononucleotides and oligonucleotides, DNA, RNA, genetic code.

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Plan.

1. History of nucleic acid research
2. Oligonucleotides
3. DNA
4. RNA
5. The "central dogma" of biology

Lecture 3 Nucleotide, Nucleic Acids and Nucleoproteins

History of nucleic acid research

1869 - Miescher – Isolated nuclein from soiled bandages

1902 - Garrod – Studied rare genetic disorder: alkaptonuria; concluded that specific gene is associated with absence of a specific enzyme

1903 - Sutton – Chromosome structure

1913- Morgan – Gene mapping

1926- Sumner – Purified urease; identified enzymes to be proteins

1928 - Griffith – Transforming principle – a chemical transferred from dead bacteria to living cells caused genetically converted strains ("transformation")

1944 - Avery, McCarty, and Macleod – Identified Griffith's "transformation principle" as DNA

1947- Chargaff – Base pairing

1950's- Franklin – X-ray crystallography of DNA

1953 - Watson and Crick – DNA double helix

Nucleic acids are the acids found in cell nuclei and are involved in the transmission of genetic information.

They are complex biopolymers containing a carbohydrate, a phosphate ester and a heterocyclic aromatic unit, the base.

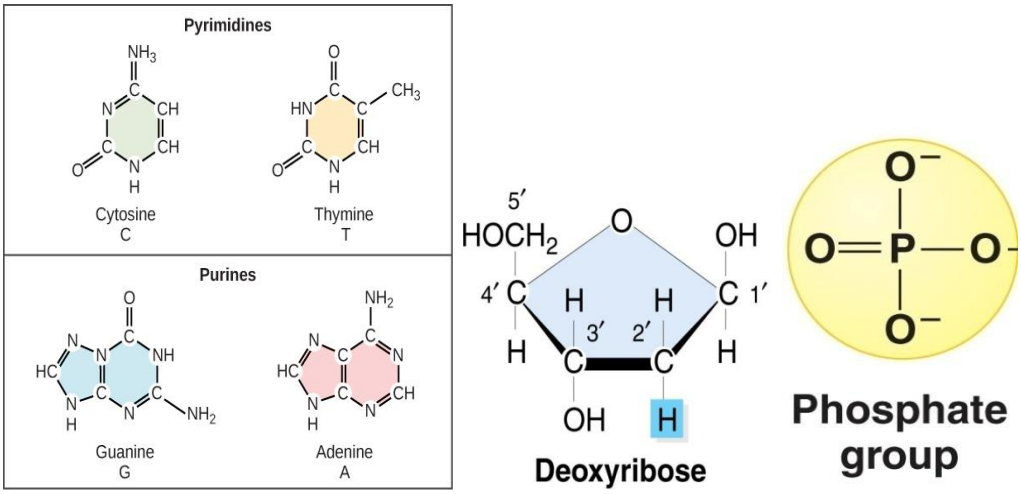
Oligonucleotides

A dinucleotide (dimer) of DNA or RNA is formed by covalently linking the 5′-phosphate group of one nucleotide to the 3′-hydroxyl group of another to form a phosphodiester bond. An oligonucleotide (oligomer) is formed when several such bonds are made, and naturally-occurring nucleic acids are linear, high molecular weight molecules of this kind. At physiological pH (7.4) each phosphodiester group exists as an anion (hence the term nucleic acid).

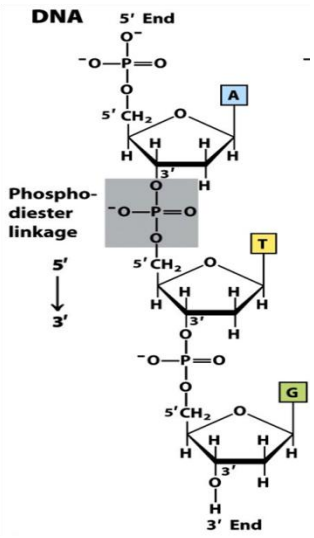
DNA

DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms. Nearly every cell in a person's body has the same DNA. Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small

amount of DNA can also be found in the mitochondria (where it is called mitichondrial DNA or mtDNA).

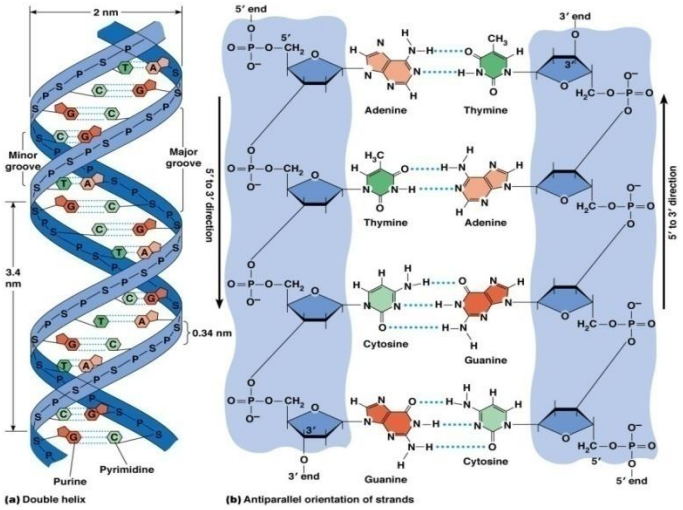


The primary structure of DNA



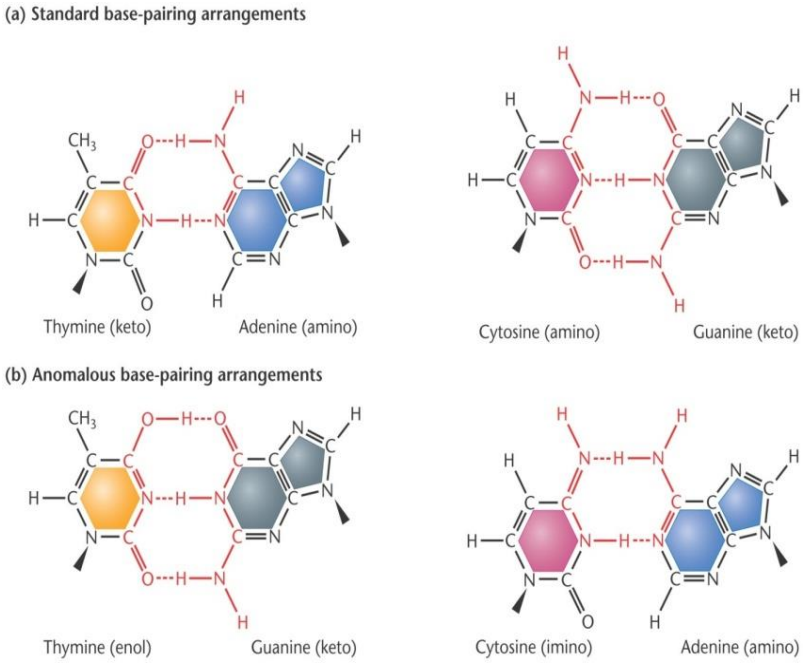
Secondary structure of DNA

The sequence of one strand of DNA precisely defines the sequence of the other; the two strands are said to be complementary, and are sometimes called reverse complements of each other. The two strands are antiparallel, with the 5′-end of one strand adjacent to the 3′-end of the other. The two strands coil around each other to form a right-handed double helix, with the hydrophobic base pairs in the centre and the sugars and negatively charged phosphates forming the external hydrophilic backbone. The term "right-handed" indicates that the backbone at the front of the molecule facing the observer slopes down from top right to bottom left.

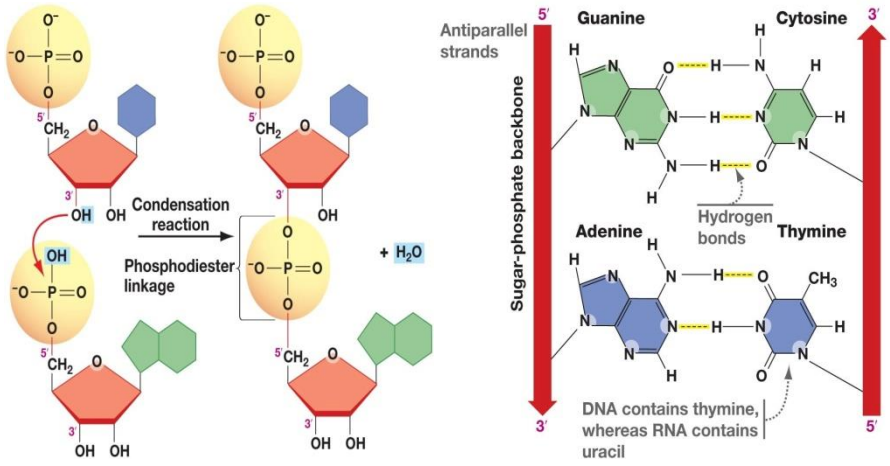


Complementary interaction of nitrogenous bases

The stability of the DNA is derived from both base stacking and hydrogen bonding.



Bonds of DNA



Tertiary structure DNA

The principal form of double helical DNA, B-DNA, has a wide major groove and a narrow minor groove running around the helix along the entire length of the molecule. Proteins interact with the DNA in these grooves (principally in the major groove) and some small drug molecules bind in the minor groove.

RNA

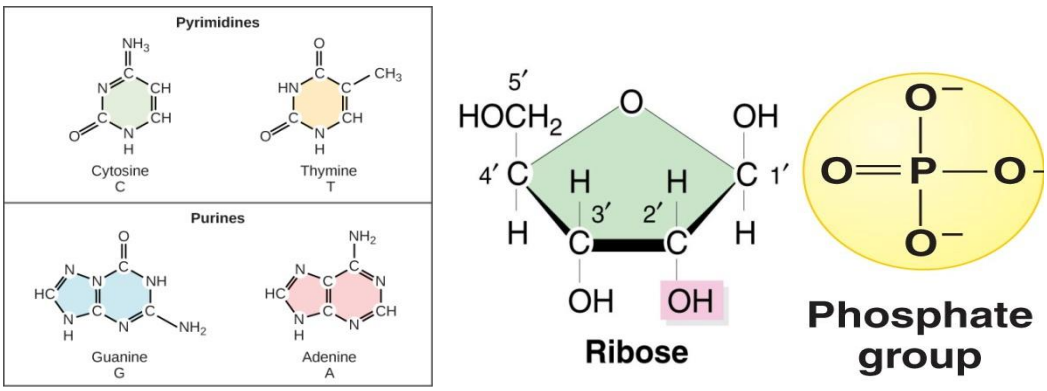
•RNA or ribonucleic acid molecules are single stranded nucleic acids composed of nucleotides. RNA plays a major role in protein synthesis as it is involved in the transcription, decoding, and translation of the genetic codeto produce proteins.

•RNA nucleotides contain three components:

Nitrogenous Base Five-Carbon Sugar Phosphate Group

•RNA nitrogenous bases include adenine (A), guanine (G), cytosine

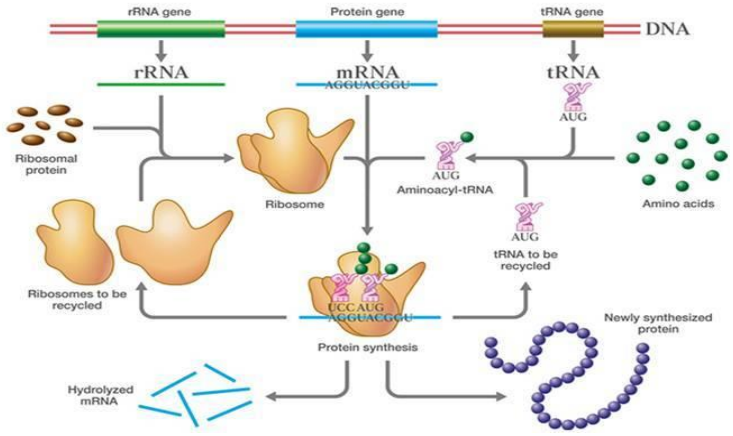
(C) and uracil (U). The five-carbon (pentose) sugar in RNA is ribose. RNA molecules are polymers of nucleotides joined to one another by covalent bonds between the phosphate of one nucleotide and the sugar of another. These linkages are called phosphodiester linkages.



Primary structure consists of a linear sequence of nucleotides that are linked together by phosphodiester bonds.

The three types of RNA are:

- rRNA (ribosomal RNA) - guides the translation of mRNA into a protein
- mRNA (messenger RNA) - carries genetic information from the nucleus to the cytoplasm
- tRNA (transfer RNA) - brings amino acids to ribosomes during protein synthesis



Ribosomal ribonucleic acid is the RNA component of the ribosome, and is essential for protein synthesis in all living organisms. It comprises the predominant material within the ribosome, which is ca. 60% rRNA and 40% protein by weight.

Messenger RNA (mRNA) is synthesized from a gene segment of DNA which ultimately contains the information on the primary sequence of amino acids in a protein to be synthesized. The genetic code as translated is for mRNA not DNA. The messenger RNA carries the code into the cytoplasm where protein synthesis occurs.

Transfer RNA (tRNA) contains about 75 nucleotides, three of which are called anticodons, and one amino acid. The tRNA reads the code and carries the amino acid to be incorporated into the developing protein.

There are at least 20 different tRNA's - one for each amino acid.

The “central dogma” of biology

DNA is transcribed to RNA; mRNA is translated to proteins.

Questions for self-control

1. Nucleic acids: definition, variants and biological functions. Primary structure of nucleic acids.
2. Secondary structure of DNA and RNA. Higher order structures of DNA, the role of proteins in its formation.

Lecture 4

Carbohydrates and Lipids

Annotation

1. Definition and structure of carbohydrates

A carbohydrate is a biomolecule consisting of carbon (C), hydrogen (H), and oxygen (O) atoms, usually with a hydrogen: oxygen atom ratio of 2:1. Carbohydrates have the empirical formula Cm(H2O)nwhere m could be different from n. Carbohydrate is a synonym of saccharide or sugar.

2. Classification of carbohydrates

The most abundant monosaccharide in nature is the six-carbon sugar D-glucose. A disaccharide is the carbohydrate formed when two monosaccharides. An oligosaccharide is a saccharide polymer containing a small number (typically 3 to 30) of monosaccharides. Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages.

3. Biological importance of carbohydrates

Energy sources (glucose, galactose, fructose, starch, and glycogen, supporting structures (components of DNA and RNA – ribose and desoxiribose), immune system, signal transduction, etc.

4. Definition and classification of lipids

The lipids (from the Greek lipos, fat) are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents (e.g. ether, chloroform, acetone & benzene) and general insolubility in water. All lipids are hydrophobic: that’s the one property they have in common. This group of molecules includes fats and oils, waxes, phospholipids, steroids (like cholesterol), and some other related compounds.

5. Fats and fatty acids

They are esters of glycerol with various fatty acids. Since the 3 hydroxyl groups of glycerol are esterified, the neutral fats are also called triglycerides or triacylglyceroles. Esterification of glycerol with one molecule of fatty acid gives

monoglyceride or monoacylglycerole, and that with 2 molecules gives diglyceride or diacylglycerole

6. Biological importance of lipids

They are more palatable and storable to unlimited amount compared to carbohydrates.They have a high-energy value(25% of body needs) and they provide more energy per gram than carbohydrates and proteins (but carbohydrates arethe preferable source of energy), etc.

Keywords

Carbohydrate, monosaccharide, polysaccharide, oligosaccharide, glucose, galactose, fructose, starch, and glycogen, lipids, oils, waxes, phospholipids, steroids, monoglyceride, triglycerides.

Recommendations: The goal of the course entitled “Biochemistry” is to get knowledge about basic chemical reactions underlying the life.

Working with the literature is better to begin with going through the lectures. You should read carefully with a pencil in your hand and mark of three types: what is clear, what needs to be specified, and what is totally unclear. Then you should open a textbook and find answers to your questions followed by putting down commentaries to your lectures. After that you can go to the unclear items using actively the recommended literature and consulting with a mentor.

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http://biochem.stanford.edu/

Plan.

1. Definition and structure of carbohydrates
2. Classification of carbohydrates
3. Biological importance of carbohydrates
4. Definition of lipids
5. Classification of lipids
6. Biological importance of lipids

Lecture 4. Carbohydrates and Lipids

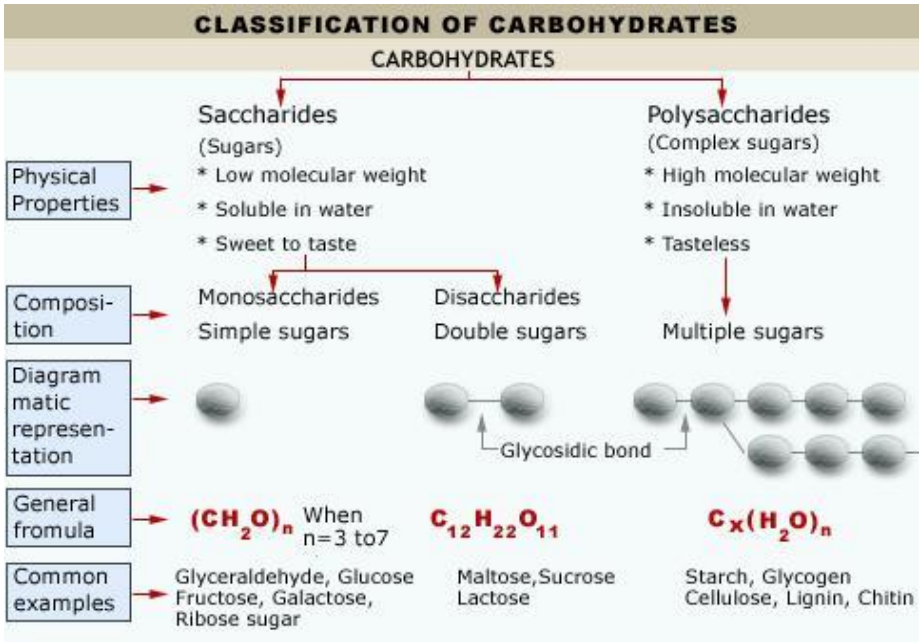
Definition and structure of carbohydrates

A carbohydrate is a biomolecule consisting of carbon (C), hydrogen (H), and oxygen (O) atoms, usually with a hydrogen:oxygen atom ratio of 2:1.

Carbohydrates have the empirical formula Cm(H2O)n where m could be different from n. Carbohydrate is a synonym of saccharide or sugar.

Classification of carbohydrates

Carbohydrates are technically hydrates of carbon; structurally it is more accurate to view them as polyhydroxy aldehydes and polyhydroxy ketones.

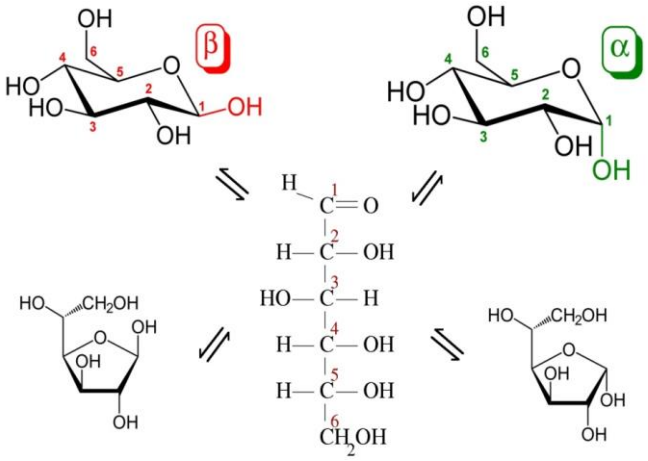


Mono- and disaccharides

Monosaccharides, or simple sugars, consist of a single (poly)hydroxy aldehyde or ketone unit.

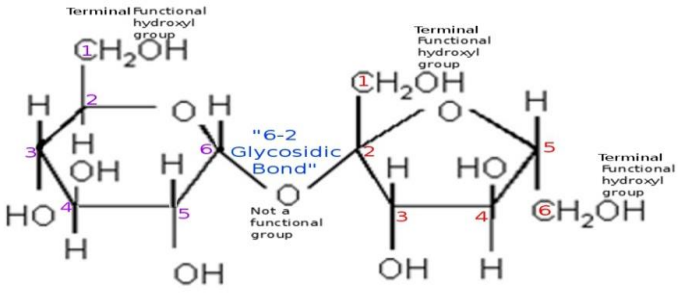
The most abundant monosaccharide in nature is the six-carbon sugar D-glucose, sometimes referred to as dextrose.

Carbohydrate isomers



Disaccharides

A disaccharide is the carbohydrate formed when two monosaccharides undergo a condensation reaction which involves the elimination of water. Like monosaccharides, disaccharides form an aqueous solution when dissolved in water.



Three common examples are sucrose, lactose and maltose.

Sucrose consists of one molecule of glucose and one of fructose; *lactose*, found in the milk of all mammals, consists of glucose and galactose; and *maltose*, a product of the breakdown of starches during digestion, consists of two molecules of glucose.

Reducing disaccharide

The disaccharides are classified as reducing and non-reducing disaccharides. The disaccharides that have hemiacetals are grouped under reducing sugars. Hemiacetals contain a free aldehyde group which can be oxidized into carboxylic acids (or diverse products).

Non-reducing disaccharide

Another type is non-reducing disaccharides. Here, the disaccharide has an acetal or ketal which cannot be oxidized readily and neither monosaccharide has a free hemiacetal unit. This is because both anomeric carbon atoms are involved in the α(1□2)glycosidic bond.

Properties of Disaccharides

Disaccharides are crystalline, water-soluble, sweet to the taste, and must be digested to monosaccharides before they can be absorbed and used for energy. Disaccharides are highly soluble compounds in water due to the presence of abundant hydroxyl groups in those molecules.

Three nutritionally important disaccharides are succrose, lactose, and maltose. Each of these has the same chemical formula (C12H22O11) and each has at least one glucose unit as part of their structure.

Oligosaccharides

An oligosaccharide (from the Greek oligos, a few, and sacchar, sugar) is a saccharide polymer containing a small number (typically 3 to 30) of monosaccharides.

Most of the oligosaccharides are not found as isolated molecules. Instead, they may be attached to other biomolecules like proteins or lipids, generally referred to as glycoconjugates. For example, the blood group serotypes (A, B, AB and 0) are the result of various oligosaccharides involved in cellular recognition.

The lipids on the surface of the erythrocytes are conjugated with various oligosaccharides.

Polysaccharides

Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages. Upon hydrolysis they give the constituent monosaccharides or oligosaccharides. They range in structure from linear to highly branched.

Starch (found in plants) is a polymer of glucose linked in a main chain through α(1,4) links with α(1,6) branches. Amylose is starch with no branches, while amylopectin has branches.

Homopolysaccharides are the polysaccharides which contain only a single type of monosaccharides. Examples are starch, amylose, amylopectin, cellulose, and glycogen.

Heteropolysaccharides are composed of different types of monosaccharides or their derivatives.

Starch granules consist of about 20% amylose and 80% amylopectin.

Glycogen is the main carbohydrate storage in animals. Muscle and liver glycogen consists of glucose residues in α(1□4) links with lots of α(1□6) branches (many more branches than in starch).

Heteropolysaccharides

Glycosaminoglycans (GAGs), unlike starch and glycogen, are linear polymers with disaccharide repeating units.

Heparin is a highly sulfated glycosaminoglycan which is widely used as an injectable medication to prevent blood clotting (an anticoagulant) and has the highest negative charge density of any known biological molecule.

Biological importance of carbohydrates

Energy sources

Glucose, galactose, fructose, starch, and glycogen.

Supporting structures

Components of nucleotides and nucleic acids (DNA and RNA) – ribose and desoxiribose.

Immune system

Olygosaccharides modify cell surface proteins to identify native versus foreign cells.

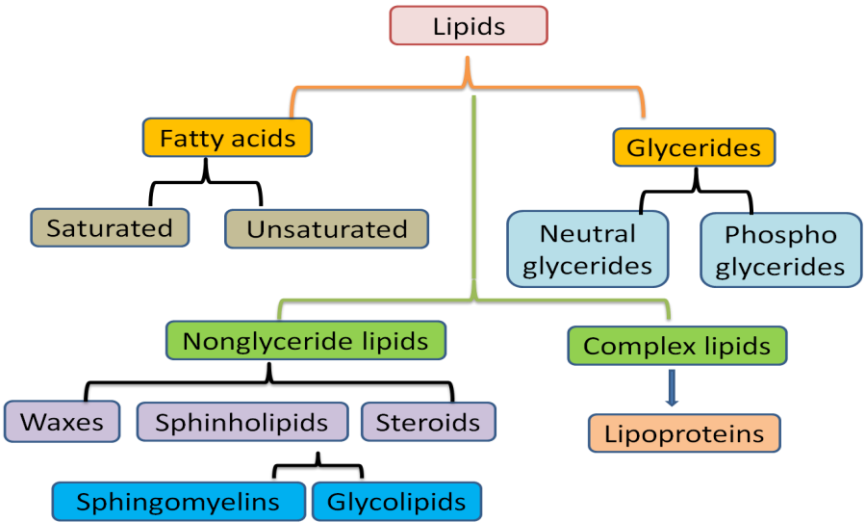
Signal transduction

Many cell surface receptors have carbohydrate side groups that are critical for function

Definition and classification of lipids

The lipids (from the Greek lipos, fat) are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents (e.g. ether, chloroform, acetone & benzene) and general insolubility in water.

All lipids are hydrophobic: that's the one property they have in common. This group of molecules includes fats and oils, waxes, phospholipids, steroids (like cholesterol), and some other related compounds.



Fats and fatty acids

They are esters of glycerol with various fatty acids. Since the 3 hydroxyl groups of glycerol are esterified, the neutral fats are also called triglycerides or triacylglyceroles.

Esterification of glycerol with one molecule of fatty acid gives monoglyceride or monoacylglycerole, and that with 2 molecules gives diglyceride or diacylglycerole.

They are called neutral lipids because they are uncharged due to absence of ionizable groups.

The neutral fats are the most abundant lipids in nature. They constitute about 98% of the lipids of adipose tissue, and 30% of plasma or liver lipids.

Triglycerides

Simple triglycerides: If the three fatty acids connected to glycerol are of the same type, the triglyceride is called simple triglyceride, e.g., tristearin.

Mixed triglycerides: If they are of different types, it is called mixed triglycerides, e.g., stearo-diolein or palmito-oleo-stearin.

Natural fats are mixtures of mixed triglycerides with a small amount of simple triglycerides.

Fatty acids

Fatty acids are aliphatic mono-carboxylic acids that are mostly obtained from the hydrolysis of natural fats and oils.

Fatty acids have the general formula R-(CH2)n-COOH and mostly have straight chain (a few exceptions have branched and heterocyclic chains). In this formula "n" is mostly an even number of carbon atoms (2-34) with a few exceptions that have an odd number.

Complex lipids

They are lipids that contain additional substances, e.g., sulfur, phosphorus, amino group, carbohydrate, or proteins besides fatty acid and alcohol.

Compound or conjugated lipids are classified into the following major types according to the nature of the additional group:

- phospholipids
- glycolipids
- lipoproteins

Most phospholipids contain a diglyceride, a phosphpate group, and a simple organic molecule such as choline.

Glycolipids are lipids with a carbohydrate attached.

A lipoprotein is a biochemical assembly that contains both proteins and lipids. The lipids or their derivatives may be covalently or non-covalently bound to the proteins.

Lipoproteins in blood plasma

Plasma lipoprotein particles are classified under chylomicrons, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and high-density (HDL) lipoproteins which enable fats to be carried in the blood stream.

Steroids and prostaglandins

Steroids are characterized by having a carbon skeleton with four fused rings. The functional groups attached to the rings distinguish the different molecules.

Prostaglandins

The prostaglandins are a group of hormone-like lipid compounds that are derived enzymatically from fatty acids and have important functions in the animal body. Every prostaglandin contains 20 carbon atoms, including a 5-carbon ring. They are derived from arachidonic acid.

Biological importance of lipids

They are more palatable and storable to unlimited amount compared to carbohydrates. They have a high-energy value (25% of body needs) and they provide more energy per gram than carbohydrates and proteins (but carbohydrates are the preferable source of energy). Supply the essential fatty acids that cannot be synthesized by the body. Supply the body with fat-soluble vitamins (A, D, E and K). Tissue fat is an essential constituent of cell membrane and nervous system.

Questions for self-control

1. Carbohydrates: definition, classification, functions in the body.
2. Lipids: definition, classification, functions in the body.

Lecture 5

Vitamins and Hormones

Annotation

1. Review of vitamins

A vitamin is an organic compound required by an organism as a vital nutrient in limited amounts. An organic chemical compound (or related set of compounds) is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained from the diet. Vitamins perform numerous essential functions.

2. Water-soluble vitamins

Water-soluble vitamins are absorbed in the duodenum and jejunum with water and enter directly into the blood stream.The water-soluble vitamins are: B1(thiamine), B2(riboflavin), B3(niacin), B5(pantothenic acid), B6(pyridoxal, pyridoxamine, and pyridoxine),B7(biotin), B9(folic acid or folate), B12(cobalamin), vitamin C (ascorbic acid).

3. Fat-soluble vitamins

Fat-soluble vitamins are absorbed in the duodenum. Vitamin A is mainly stored in the liver. Vitamins K and E are partially stored in the liver. Vitamin D is mainly stored in the fat and muscle tissue.

4. Review of hormones

Hormones are organic chemical messengers produced and secreted by endocrine cells into the bloodstream. Hormones regulate, integrate and control a wide range of physiologic functions. Hormones serve as a major form of communication between different organs and tissues. Hormones regulate a variety of physiological and behavioral activities, including digestion, metabolism, respiration, tissue function, sensory perseption, sleep, excretion, lactation, stress, growth abd development, movement, reproduction, and mood. Generally, only a small amount of hormone is required to alter cell metabolism.

5. Classification of hormones

By the site of production: hypothalamic hormones, pituitary hormones, thyroid hormones, parathyroid hormone, pancreatic hormones, adrenal hormones, gonadal hormones. Classes of hormones: water soluble and lipid soluble. By solubility: water soluble (polar): proteins, glycoproteins, polypeptides, amino acid derivatives and lipid soluble (nonpolar): steroids, amino acid derivatives, fatty acid.

Key words

Vitamins, water-soluble vitamins , fat-soluble vitamins, hormones, hypothalamic hormones, pituitary hormones, thyroid hormones, parathyroid hormone, pancreatic hormones, adrenal hormones, gonadal hormones

Recommendations: The goal of the course entitled "Biochemistry" is to get knowledge about basic chemical reactions underlying the life.

Working with the literature is better to begin with going through the lectures. You should read carefully with a pencil in your hand and mark of three types: what is clear, what needs to be specified, and what is totally unclear. Then you should open a textbook and find answers to your questions followed by putting down commentaries to your lectures. After that you can go to the unclear items using actively the recommended literature and consulting with a mentor.

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Plan.

1. Review of vitamins
2. Water-soluble vitamins

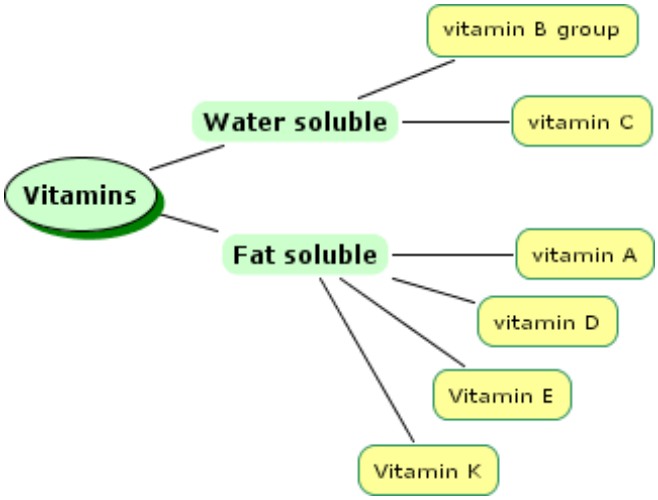
3. Fat-soluble vitamins
4. Review of hormones
5. Hypothalamic hormones
6. Pituitary hormones
7. Thyroid hormones
8. Parathyroid hormone
9. Pancreatic hormones
10. Adrenal hormones
11. Gonadal hormones

Lecture 5. Vitamins and Hormones

Review of vitamins

A vitamin is an organic compound required by an organism as a vital nutrient in limited amounts. An organic chemical compound (or related set of compounds) is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained from the diet. Thus, the term is conditional both on the circumstances and on the particular organism. For example, ascorbic acid (vitamin C) is a vitamin for humans, but not for most other animals.

Deficiencies can result in potentially serious consequences. Supplementation is important for the treatment of certain health problems but there is little evidence of benefit when used by those who are otherwise healthy



Solubility in water and fat influences a vitamin's:

1) Digestion; 2) Absorption; 3) Transportation; 4) Storage; 5) Excretion

All vitamins contain carbon, hydrogen, and oxygen.

Some vitamins contain nitrogen and sulfur.

Chemical structure of each vitamin is unique.

Each vitamin is a singular unit.

Vitamins are absorbed intact.

Vitamins perform numerous essential functions.

The many roles of vitamins

Metabolic Function	Vitamins That Play a Role
Antioxidants	Vitamin C, vitamin E
Blood clotting and red blood cell synthesis	Folate, vitamin B_6, vitamin B_{12}, vitamin K
Bone health	Vitamin A, vitamin C, vitamin D, vitamin K
Energy	Biotin, niacin (B_3), pantothenic acid, riboflavin (B_2), thiamin (B_1), vitamin B_6, vitamin B_{12}
Growth and reproduction	Vitamin A, vitamin D
Immune function	Vitamin A, vitamin C, vitamin D
Protein metabolism	Folate, vitamin B_6, vitamin B_{12}

Vitamin absorption and storage. Water-soluble vitamins are absorbed in the duodenum and jejunum. Absorbed with water and enter directly into the blood stream. Most are not stored in the body. Excess intake excreted through the urine. Important to consume adequate amounts daily. Dietary excesses can be harmful.

General properties of vitamins

	Water-Soluble Vitamins	Fat-Soluble Vitamins
Absorbed in the	Small Intestine	Small Intestine
Hydrophobic or Hydrophilic	Hydrophilic	Hydrophobic
Absorbed into the	Blood	Lymph
Stored in the body	Not Generally	Yes
Can build up and become toxic	Not Generally	Yes
Need to consume daily	Yes	No

Bioavailability of vitamins

Varies based

- on amount in food

-preparation efficiency of digestion and absorption of food

-individual nutritional status

- natural or synthetic

Fat-soluble vitamins are generally less bioavailable than water-soluble vitamins

Vitamins from animal foods are generally more bioavailable than those in plant foods

Destruction of vitamins

Water-soluble vitamins can be destroyed by:

-exposure to air

-exposure to ultraviolet light

-water

-changes in pH

-heat

-food preparation techniques

Fat-soluble vitamins tend to be more stable.

Toxicity with Overconsumption

Vitamin toxicity, aka hypervitaminosis:

-rare

-results from ingesting excess vitamins and tissue saturation

-can damage cells

Dietary Reference Intakes include tolerable upper intake limits (UL) for most vitamins to prevent excess.

Provitamine (or previtamine) is a substance that an organism can transform into a vitamin (e.g., carotene, which is converted to vitamin A in the liver).

Best Sources of Vitamins

Whole foods

- Fruits, vegetables, and whole grains

- Rich in vitamins, phytochemicals, antioxidants, and fiber

Most people do not need supplements

Vitamin	RDA/ AI		Best Sources	Functions
	Men	Women		
Thiamin (B1)	1.2mg	1.1mg	Fortified cereals and oatmeals, meats, rice and pasta, whole grains, liver	Helps the body release energy from carbohydrates during metabolism; growth and muscle tone
Riboflavin	1.3mg	1.1mg	Whole grains, green leafy vegetables, organ meats, milk, eggs	Helps the body release energy from protein, fat, and carbohydrates during metabolism
Niacin	16mg	14mg	Meat, poultry, fish, enriched cereals, peanuts, potatoes, dairy products, eggs	Involved in carbohydrate, protein, and fat metabolism
Pantothenic acid	5mg	5mg	Lean meats, whole grains, legumes	Helps release energy from fats and vegetables
Folate	400ug	400ug	Green leafy vegetables, organ meats, dried peas, beans, lentils	Aids in genetic material development; involved in red blood cell production
B6	1.3mg	1.3mg	Fish, poultry, lean meats, bananas, prunes, dried beans, whole grains, avocados	Helps build body tissue and aids in metabolism of protein
B12	2.4ug	2.4ug	Meats, milk products, seafood	Aids cell development, functioning of the nervous system, and the metabolism of protein and fat
Biotin	30ug	30ug	Cereal/grain products, yeast, legumes, liver	Involved in metabolism of protein, fats, and carbohydrates
Vitamin C	90mg	75mg	Citrus fruits, berries, and vegetables- especially peppers	Essential for structure of bones, cartilage, muscles, and blood vessels; helps maintain capillaries and gums and aids in absorption of iron.

<u>The B vitamins are:</u>

B_1 (thiamine)

B_2 (riboflavin)

B_3 (niacin)

B_5 (pantothenic acid)

B_6 (pyridoxal, pyridoxamine, and pyridoxine)

B_7 (biotin)

B_9 (folic acid or folate)

B_{12} (cobalamin)

Fat-soluble vitamins

Vitamin	RDA/ AI		Best Sources	Functions
	Men	Women		
Vitamin A (carotene)	900ug	700ug	Yellow or orange fruits and vegetables, green leafy vegetables, fortified oatmeal, liver, dairy products	Formation and maintenance of skin, hair, and mucous membranes; helps people see in dim light; bone and tooth growth
Vitamin D	5ug	5ug	Fortified milk, sunlight, fish, eggs, butter, fortified margarine	Aids in bone and tooth formation; helps maintain heart action and nervous system function
Vitamin E	15mg	15mg	Fortified and multigrain cereals, nuts, wheat germ, vegetable oils, green leafy vegetables	Protects blood cells, body tissue, and essential fatty acids from harmful destruction in the body
Vitamin K	120ug	90ug	Green leafy vegetables, fruit, dairy, grain products	Essential for blood-clotting functions

Vitamin absorption and storage

Fat-soluble vitamins are absorbed in the duodenum.

Storage

Vitamin A is mainly stored in the liver.

Vitamins K and E are partially stored in the liver.

Vitamin D is mainly stored in the fat and muscle tissue.

Can build up in body to point of toxicity.

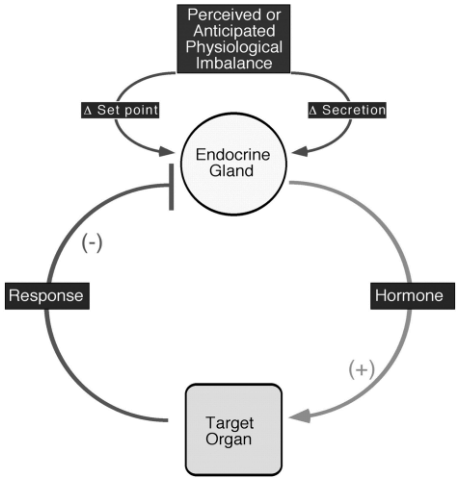
Review of hormones

Hormones are organic chemical messengers produced and secreted by endocrine cells into the bloodstream. Hormones regulate, integrate and control a wide range of physiologic functions. Hormones serve as a major form of communication between different organs and tissues. Hormones regulate a variety of physiological and behavioral activities, including digestion, metabolism, respiration, tissue function, sensory perseption, sleep, excretion, lactation, stress, growth abd development, movement, reproduction, and mood. Generally, only a small amount of hormone is required to alter cell metabolism. Endocrine glands, which are special groups of cells, make hormones. The major endocrine glands are the pituitary, pineal, thymus, thyroid, adrenal glands, and pancreas. In addition, men produce hormones in their testes and women produce them in their ovaries.

Functions of the endocrine system

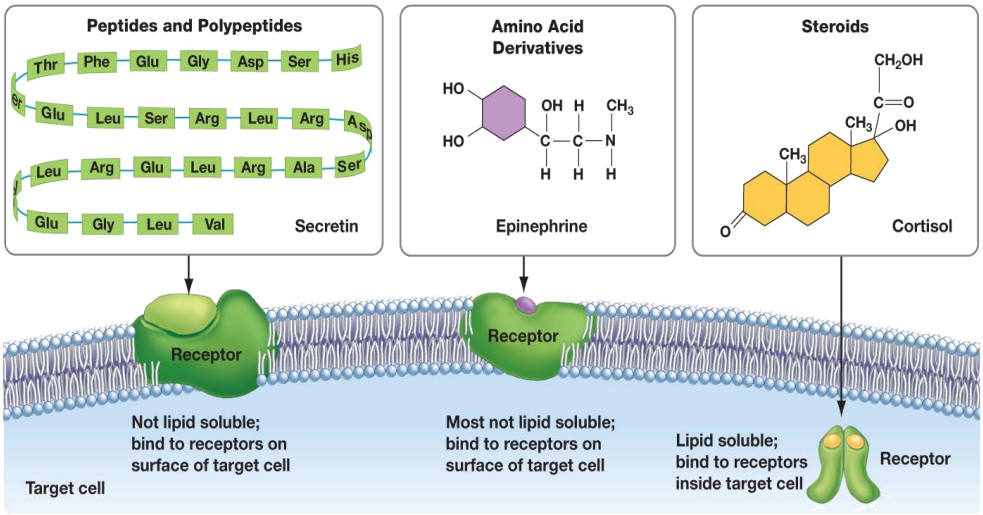
Contributes to and interacts with the control and integration functions of the nervous system. Important in the maintenance of homeostasis (set points), usually through negative feedback. Occasionally involved in processes with controlled movement away from set point (positive feedback).

Feedback loops in hormones



Endocrine glands are ductless glands comprised of endocrine cells. This means that these glands do not have ducts that lead to the outside of the body. These glands secrete hormones directly into the blood stream.

<u>Types of hormones</u>



- Classes of hormones: water soluble and lipid soluble.

- Water soluble (polar): proteins, glycoproteins, polypeptides, amino acid derivatives.
- Lipid soluble (nonpolar): steroids, amino acid derivatives, fatty acids.
- Different classes have different mechanisms of action, different modes of transport through the body, and differing stability in the circulation.

Some specific actions of hormones

Fetal development and differentiation

Cell growth and cancer

Metabolism

Cardiovascular function

Renal function

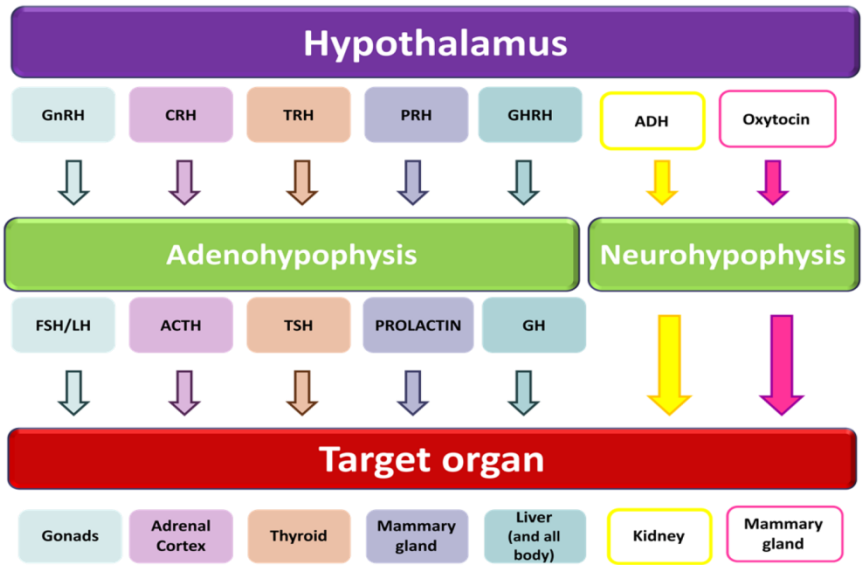
Skeletal function

Reproductive function

Immune function

Central nervous system function

Hierarchy of the endocrine system



Hypothalamic hormones

A major organ of the endocrine system, the anterior pituitary (adenohypophysis), is the anterior lobe that together with the posterior lobe, the (neurohypophysis) makes up the pituitary gland (hypophysis). Proper functioning of the anterior pituitary is regulated by hypothalamus. The hypothalamus secretes tropic hormones that affect the anterior pituitary

Gonadotropin-releasing hormone - GnRH

Controls (follicle stimulating hormone) FSH + (luteinizing hormone) LH release. Stumulates the ovaries and testes (gonads).

Thyrotropin-releasing hormone - TRH

Promotes (thyroid stimulating hormone) TSH and PRL (prolactin) secretion.

Corticotropin-releasing hormone - CRH

Promotes (adrenocorticotropic hormone) ACTH secretion

Growth Hormone-releasing hormone - GH

Promotes (growth hormone) GH secretion.

Somatostatin

Inhibits GH and TSH secretion

Antidiuretic Hormone -ADH

Increases water retention thus reducing urine volume and prevents dehydration. Also called casopressin because i can cause vasoconstriciton. Involves osmodetectors and blood osmolarity. Also functions as a neurotransmitter.

Oxytocin - OT

Surge of hormone released during sexual arousal and orgasm. Stimulate uterine contractions and propulsion of semen. Promotes feelings of sexual satisfaction and

emotional bonding between partners. Stumulates labor contractions during child brith. Stimulates flow of milk during lactation. Promotes emotional bonding between lactating mother and infanct.

Pituitary hormones

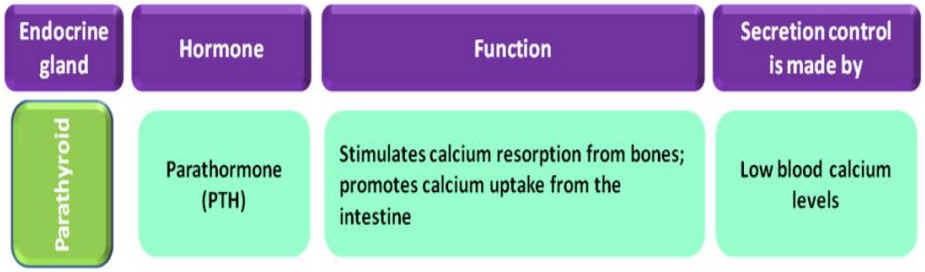
Anterior pituitary hormones

HORMONES OF THE ANTERIOR PITUITARY		
Adrenocorticotropic hormone (ACTH)	Adrenal cortex	Stimulates production of corticosteroid hormones
Follicle-stimulating hormone (FSH)	Female: Ovaries Male: Testes	Female: Stimulates growth of ovarian follicles Male: Stimulates sperm production
Luteinizing hormone (LH)	Female: Ovaries Male: Testes	Female: Stimulates ovulation, estrogen and progesterone synthesis in ovary Male: Stimulates androgen synthesis in testes
Thyroid-stimulating hormone (TSH)	Thyroid gland	Stimulates thyroid hormone synthesis and secretion
Prolactin (PRL)	Female: Mammary glands Male: Not known	Female: Stimulates milk production in mammary glands Male: May play a role in the sensitivity of the testes interstitial cells to LH
Growth hormone (GH)	Almost every cell in the body	Increased growth and metabolism in target cells; synthesis of somatomedin in the liver to stimulate growth at epiphyseal plate
Melanocyte-stimulating hormone (MSH)	Melanocytes	Stimulates synthesis of melanin and dispersion of melanin granules in epidermal cells

Thyroid hormones

Endocrine gland	Hormone	Function	Secretion control is made by
Thyroid	T3/T4	Stimulates metabolic rate for growth	TSH (Adenohypophysis)
	Calcitonin	Regulates calcium levels in the blood (decreases the resorption of calcium from bones)	Raised blood calcium levels

Parathyroid hormone



Parathyroid hormone (PTH) is one of the two major hormones modulating calcium and phosphate homeostasis, the other being calcitriol (1,25-dihydroxyvitamin D) . The minute-to-minute regulation of serum ionized calcium is exclusively regulated through PTH, maintaining the concentration of this cation within a narrow range, through stimulation of renal tubular calcium reabsorption and bone resorption.

Pancreatic hormones

Primary hormones secreted by the pancreas include:

Gastrin: This hormone aids digestion by stimulating certain cells in the stomach to produce acid.

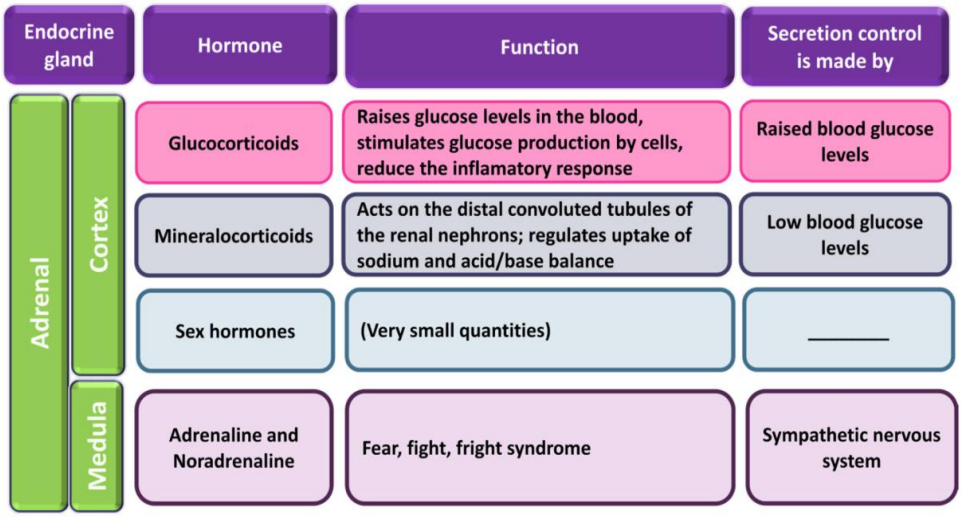
Glucagon: Glucagon helps insulin maintain normal blood glucose by working in the opposite way of insulin. It stimulates your cells to release glucose, and this raises your blood glucose levels.

Insulin: This hormone regulates blood glucose by allowing many of your body's cells to absorb and use glucose. In turn, this drops blood glucose levels.

Somatostatin: When levels of other pancreatic hormones, such as insulin and glucagon, get too high, somatostatin is secreted to maintain a balance of glucose and/or salt in the blood.

Vasoactive intestinal peptide (VIP): This hormone helps control water secretion and absorption from the intestines by stimulating the intestinal cells to release water and salts into the intestines.

Adrenal hormones



Adrenal cortex hormones

The adrenal cortex is devoted to production of corticosteroids and androgen hormones. Specific cortical cells produce particular hormones including aldosterone, cortisol, and androgens such as androstendione.

Aldosterone (mineralocorticoids)

Aldosteron's effects are on the distal tubule and collecting duct of the kidney where it causes increased reabsorption of sodium and increased excretion of both potassium and hydrogen ions. Sodium retention is also a response of the distal colon, and sweat glands to aldosterone receptor stimulation.

Cortisol (glucocorticoids)

Cortisol is the main glucocorticoid under normal conditions and its actions include mobilization of fats, proteins, and carbohydrates, but it does not increase under starvation conditions. Additionally, cortisol enhances the activity of other hormones including glucagon and catecholamines.

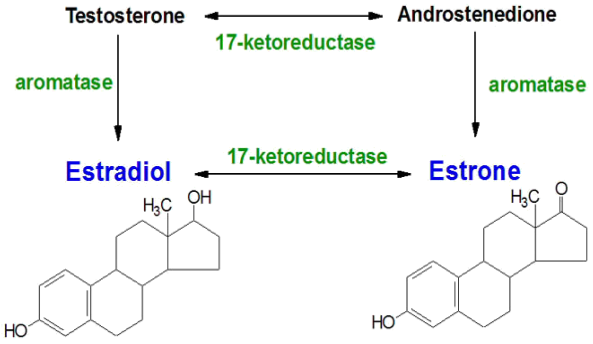
Effects of glucocorticoids

Glucocoticoids cause their effects by binding to the glucocorticoid receptor (GR). The activated GR complex, in turn, up-regulates the expression of anti-inflammatory proteins in the nucleus (a process known as transactivation) and represses the expression of proinflammatory proteins in the cytosol by preventing the translocation of other transcription factors from the cytosol into the nucleus (transrepression).

Adrenal medullary hormones

The **adrenal medulla** is part of the afrenal gland. It is located at the center of the gland, being surrounded by the afrenal cortex. It is the innermost part of the adrenal gland, consisting of cells that secrete epinephrine (adrenaline), noepinephrine (noradrenaline), and a small amount of dopamine in response to stimulation by sympathetic preganglionic neurons.

Gonadal hormones



Sex steroids, also known as gonadal steroids, are steroid hormones that interact with androgen or estrogen receptors. Their effects are mediated by slow genomic mechanisms through nuv;ear receptors as well as by fast nongenomic mechanisms through membrane-associated receptors and signaling cascades. The term sex

hormone is nearly always synonymous with sex steroid. Natural sex steroids are made by the gnads (ovaries or testes) and by adrenal cortex.

Questions for self-control

1. Definition of vitamins. Classification according to their solubility. Vitamins disbalance, its possible reasons.
2. Water-soluble vitamins: thiamin, riboflavin, pantotenic acid, niacine, pyridoxin. Their role in metabolic processes.
3. Water-soluble vitamins: biotin, folic acid, cobalamine, ascorbic acid. Their role in metabolic processes.

Fat-soluble vitamins A, D, E, K. Their role in metabolic and physiological processes.

4. Definition, biological functions and classifications of hormones.
5. The general scheme of hormonal regulation system: hypothalamus-hypophysis-peripheral glands axis.
6. Mechanism of action for the hormons which act via second messenger system.
7. Mechanism of action for the hormons which act via cytoplasmic or nuclear receptors.
8. Insulin, cortisol and thyreoid hormones: place of biosynthesis, transport in blood, mechanism of action and main metabolic effects.
9. Adrenalin, aldosteron and glucagon: place of biosynthesis, transport in blood, mechanism of action and main metabolic effects.

Lecture 6.

Metabolism of Carbohydrates

Annotation

1. Carbohydrate digestion and absorption

Dietary carbohydrates are digested with salivary amylase, pancreatic amylase, maltase, lactase and sucrose areenzymes. The absorbed monosaccharides are either hexoses or pentoses. The absorbed pentoses are excreted in urine because the body does not deal with them. The absorbed hexoses are glucose, fructose, or galactose. Fructose and galactose are converted into glucose in the liver.

2. Glycolysis

Glycolysis is the metabolic pathway that converts glucose into pyruvate. The free energy released in this process is used to form the high-energy compounds ATP and NADH. glycolysis produces 4 ATP and 2 NADH, but uses 2 ATP in the process for a net of 2 ATP and 2 NADH

3. The oxidative decarboxylation of pyruvate

Pyruvate decarboxylation is the biochemical process that uses pyruvate to form acetyl-CoA, releasing NADH, and carbon dioxide via decarboxylation.

4. Krebs cycle

The Krebs Cycle, also known as citric acid cycle or tricarboxylic acid cycle (TCA cycle), is the central metabolic pathway in all aerobic organisms. The cycle is a series of eight reactions that occur in the mitochondrion. These reactions take a two-carbon molecule (acetyl) and completely oxidize it to carbon dioxide.

5. Gluconeogenesis

Gluconeogenesis is the metabolic process by which organisms produce glucose from non-carbohydrate precursors.

6. Pentose phosphate pathway

The pentose phosphate pathway (also called the phosphogluconate pathway and the hexose monophosphate shunt) is a biochemical pathway parallel to glycolysis that generates NADPH and pentoses (5-carbon sugars).

7. Glycogenesis and glycogenolysis

Glycogenesis is the process of glycogen synthesis, in which glucose molecules are added to chains of glycogen for storage. Glycogenolysis is a process by which glycogen, the primary carbohydrate stored in the liver and muscle cells.

Key words

Carbohydrate digestion and absorption, glycolysis, pyruvate decarboxylation, Krebs cycle, gluconeogenesis, pentose phosphate pathway, glycogenesis and glycogenolysis

Recommendations: The goal of the course entitled "Biochemistry" is to get knowledge about basic chemical reactions underlying the life.

Working with the literature is better to begin with going through the lectures. You should read carefully with a pencil in your hand and mark of three types: what is clear, what needs to be specified, and what is totally unclear. Then you should open a textbook and find answers to your questions followed by putting down commentaries to your lectures. After that you can go to the unclear items using actively the recommended literature and consulting with a mentor.

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Plan.

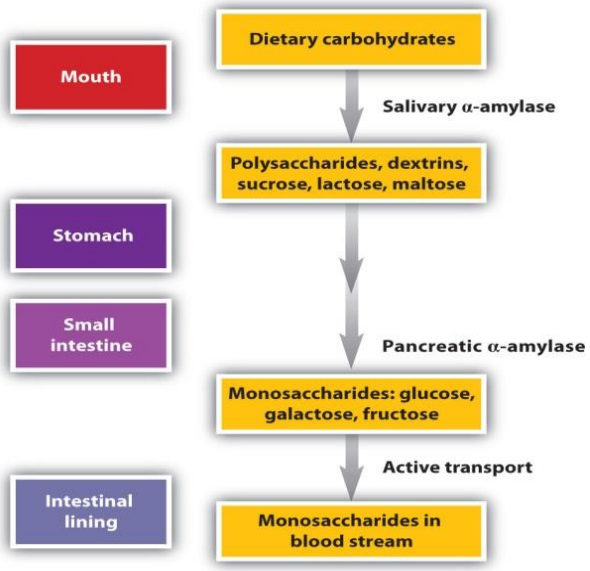
1. Carbohydrate digestion
2. Monosaccharide absorption and glucose transporters
3. Blood glucose levels and ways to use glucose

4. Glycolysis
5. The oxidative decarboxylation of pyruvate
6. Krebs cycle
7. Gluconeogenesis and the Cori cycle
8. Pentose phosphate pathway
9. Glycogenesis and glycogenolysis
10. Regulation of the blood glucose level
11. Summary of the glucose metabolism

Lecture 6. Metabolism of Carbohydrates

Carbohydrate digestion

Salivary amylase partially digests starch and glycogen to dextrin and few maltoses. Pancreatic amylase completely digests starch, glycogen, and dextrin into maltose and few glucose. Amylase is a hydrolytic enzyme responsible for splitting α-1,4-glycosidic link. Maltase, lactase and sucrose are enzymes secreted from intestinal mucosa, which hydrolyses the corresponding disaccharides to produce glucose, fructose, and galactose. HCl secreted from the stomach can partially hydrolyse the disaccharides and polysaccharides



Monosaccharide absorption and glucose transporters

- The absorbed monosaccharides are either hexoses or pentoses.
- The absorbed pentoses are excreted in urine because the body does not deal with them.
- The absorbed hexoses are glucose, fructose, or galactose.
- Fructose and galactose are converted into glucose in the liver.

Relative absorption

Glucose and galactose are absorbed very fast.

Galactose is absorbed more rapidly than glucose.

Fructose and mannose have an intermediate absorption rate.

Pentoses are absorbed slowly.

Mechanisms of absorption

Simple absorption (passive diffusion): Depends upon the concentration gradient of sugar between intestinal lumen and intestinal mucosa. This is true for all monosaccharides, especially fructose & pentoses

Facilitative diffusion by a Na+- independent glucose transporter system (GLUT5). There are mobile carrier proteins responsible for transport of fructose, glucose, and galactose along their concentration gradient.

Active transport by Na+-dependent glucose transporter system (SGLUT1). In the intestinal cell membrane there is a mobile carrier protein coupled with Na+-K+ pump. The carrier protein has 2 separate sites, one for Na+, the other for glucose. It transports sodium ions (with concentration gradient) and glucose (against its concentration gradient) to the cytoplasm of the cell. Na+ ions are expelled outside the cell by Na+-K+ pump, which needs ATP and expels 3 Na+ against 2 K+.

Exit of all sugars from mucosal cell to the blood occurs by facilitative transport through GLUT2.

Glucose transporters

GLUT1 is present mainly in red blood cells and retina.

GLUT2 is present in liver, kidneys, pancreatic B-cells, and lateral border of small intestine, for rapid uptake and release of glucose.

GLUT3 is present mainly in brain.

GLUT4 is present in heart, skeletal muscles, and adipose tissues. It is for insulin-stimulated uptake of glucose.

GLUT5 is present in small intestine and testes for glucose and fructose transport.

SGLUT1 is present in small intestine and kidneys, Na+-dependent, for active transport of glucose and galactose from lumen of small intestine and re-absorption of glucose from glomerular filtrate in proximal renal tubules.

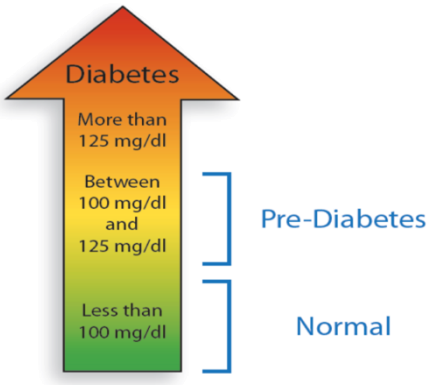
Blood glucose levels and ways to use glucose

Blood glucose comes from 3 main sources:

absorbed glucose from food

cleavage of liver glycogen (glycogenolysis)

synthesis of glucose from other substances (gluconeogenesis).



The absorbed glucose has the following pathways.

Oxidation

1. For provision of energy:

glycolysis and Krebs cycle

2. Not for energy production:

synthesis of phospho-pentoses & NADPH in the pentose phosphate pathway;

synthesis of glucuronic acid in the uronic acid pathway.

Synthesis of other carbohydrates such as:

mannose, fucose, neuraminic acid for glycoprotein formation;

galactose and lactose in mammary gland;

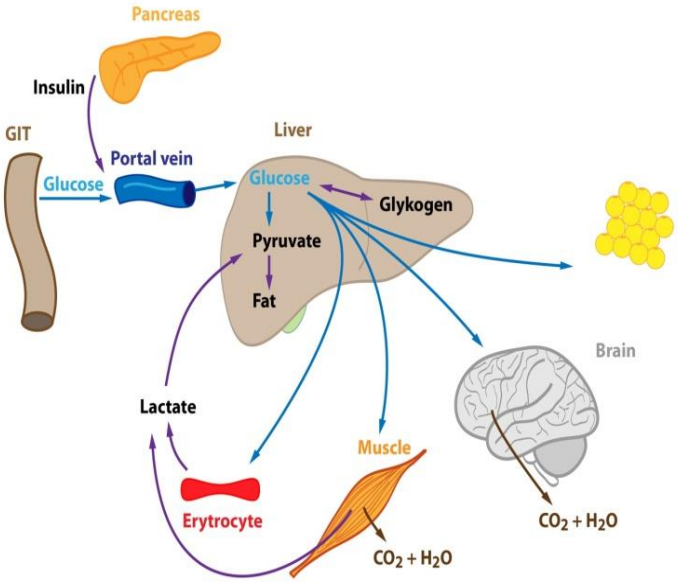
fructose in seminal vesicles;

amino sugar (glucosamine) for mucopolysaccharides and glycoprotein formation.

Synthesis of non-essential amino acids

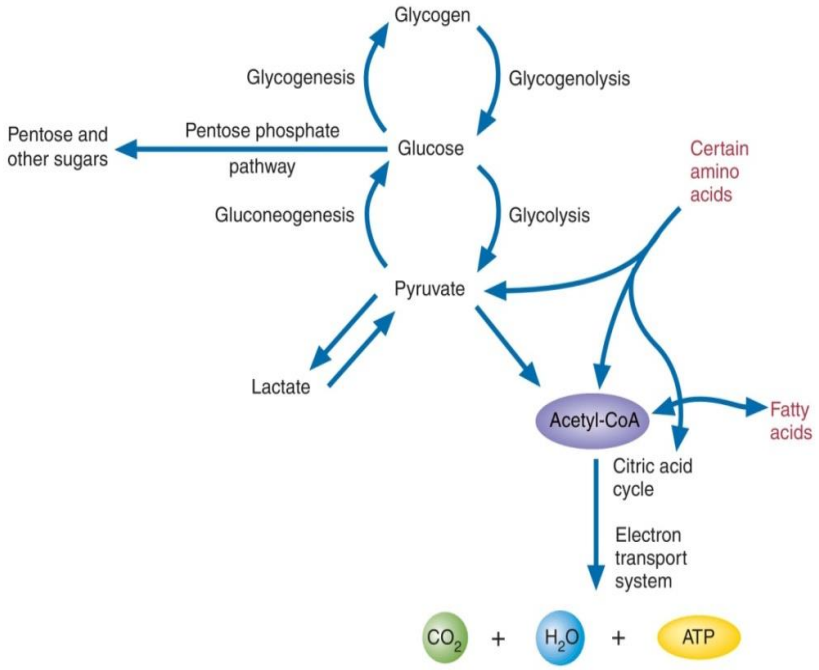
Excess glucose is stored as glycogen in liver and muscles

More excess glucose is stored as lipid in adipose tissue



Carbohydrate metabolic pathways

- Glycolysis - the oxidation metabolism of glucose to obtain ATP and pyrivate.
- Pyruvate from glycolysis enters the Krebs cycle, also known as the citric acid cycle, after moving through oxydative phosphorylation of pyrivate.
- The pentose phosphate pathway, which acts in the conversion of hexoses into pentoses and in NADPH regeneration.
- Glycogenesis - the conversion of excess glucose into glycogen as a cellular storage mechanism.
- Glycogenolysis - the breakdown of glycogen into glucose.
- Gluconeogenesis - de novo synthesis of glucose molecules from non-sugar compounds.



Glycolysis

Glycolysis is the metabolic pathway that converts glucose into pyruvate. The free energy released in this process is used to form the high-energy compounds ATP and NADH.

Glycolysis: summary

Goal: break glucose down to form two pyruvates

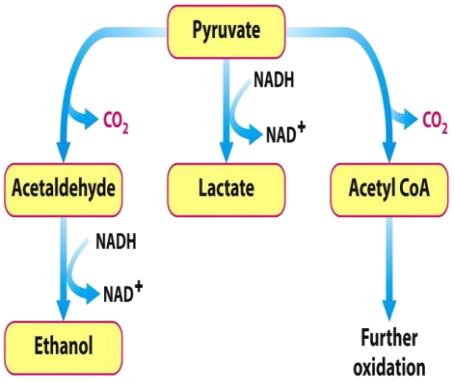
Where: the cytoplasm

Equation: C6H12O6 + 2ATP □ 2 pyruvate + 4 ATP +2 H2O

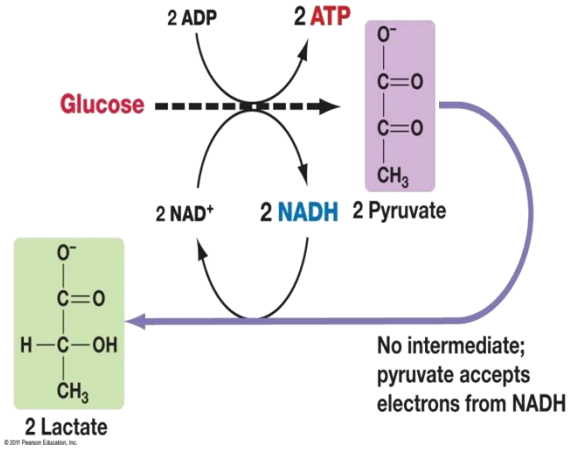
Balance: glycolysis produces 4 ATP and 2 NADH, but uses 2 ATP in the process for a net of 2 ATP and 2 NADH

Note: this process does not require O2 and does not yield much energy

The fates of pyruvate



Lactic acid fermentation



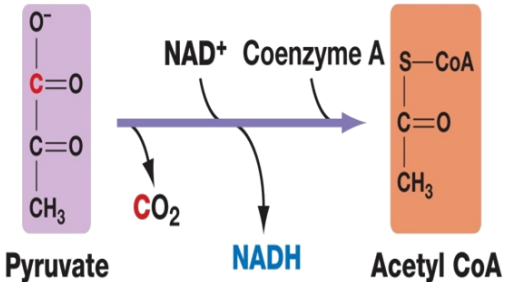
Lactic acid is formed in the ***anaerobic*** **glycolysis.** When sufficient oxygen is not present in the muscle cells for further oxidation of pyruvate, it is converted to **lactic acid** by the enzyme ***lactate dehydrogenase***.

The main goal of fermentation reactions is to convert NADH to NAD^+ (to use in glycolysis).

Only 2 ATP is gained per 1 glucose molecule in the **anaerobic glycolysis**

The oxidative decarboxylation of pyruvate

Pyruvate decarboxylation is the biochemical process that uses pyruvate to form acetyl-CoA, releasing NADH, and carbon dioxide via decarboxylation. It forms an important link between the metabolic pathways of glycolysis and the citric acid cycle.



Structure of the pyruvate dehydrogenase complex

5 cofactors:

TPP (thiamine pyrophosphate)

Lipoamide

Coenzyme A

FAD

NAD+

5 apoenzymes:

Pyruvate dehydrogenase (E1)

Dihydrolipoyl transacetylase (E2)

Dihydrolipoyl dehydrogenase (E3)

Pyruvate dehydrogenase kinase (regulatory)

Pyruvate dehydrogenase phosphatase (regulatory)

Krebs cycle

The Krebs Cycle, also known as citric acid cycle or tricarboxylic acid cycle (TCA cycle), is the central metabolic pathway in all aerobic organisms.

The cycle is a series of eight reactions that occur in the mitochondrion.

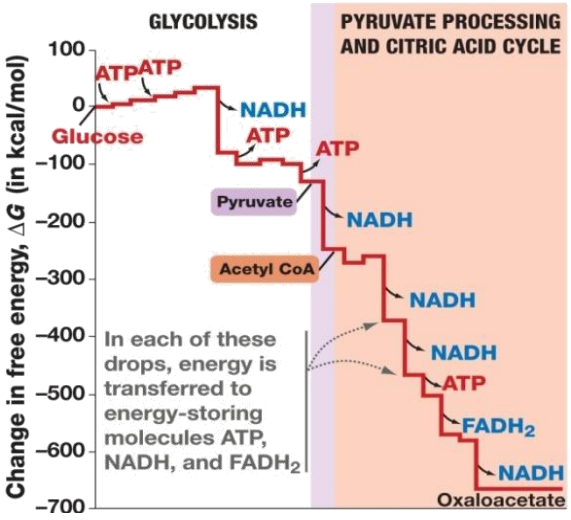
These reactions take a two-carbon molecule (acetyl) and completely oxidize it to carbon dioxide.

The cycle is summarized in the following chemical equation:

$$\text{acetyl CoA} + 3\ \text{NAD} + \text{FAD} + \text{ADP} + \text{HPO4}^{-2} \rightarrow$$

$$2\ \text{CO2} + \text{CoA} + 3\ \text{NADH}^{+} + \text{FADH}^{+} + \text{ATP}$$

Glucose catabolism: energy release



Net energy production

Glycolysis: 2 ATP

Krebs Cycle: 2 ATP

Electron Transport Phosphorylation: 32 ATP

Each NADH produced in glycolysis is worth 2 ATP (2 x 2 = 4) because the NADH is worth 3 ATP, but it costs an ATP to transport the NADH into the mitochondria, so there is a net gain of 2 ATP for each NADH produced in gylcolysis.

Each NADH produced in the conversion of pyruvate to acetyl-CoA and Krebs Cycle is worth 3 ATP (8 x 3 = 24)

Each FADH2 is worth 2 ATP (2 x 2 = 4)

4 + 24 + 4 = 32

Net Energy Production: 36 ATP per 1 glucose molecule

Gluconeogenesis and the Cori cycle

Gluconeogenesis is the metabolic process by which organisms produce glucose from non-carbohydrate precursors. Glucose is the only energy source used by the brain (with the exception of ketone bodies during times of fasting), testes, erythrocytes, and kidney medulla. In mammals this process occurs in the liver and kidneys.

In humans the main gluconeogenic precursors are lactate, glycerol, alanine, and glutamine. Altogether, they account for over 90% of the overall gluconeogenesis.

Gluconeogenesis is one of the two main mechanisms in humans and many other animals used to keep blood glucose levels from dropping too low (hypoglycemia). The other means of maintaining blood glucose levels is through the degradation of glycogen (glycogenolysis).

Synthesis of glucose from three-carbon precursors is essentially a reversal of glycolysis. Although many of the steps of gluconeogenesis are the simple reversal of steps of glycolysis, there are three important steps in glycolysis that are replaced by four different steps in gluconeogenesis.

The reactions unique to gluconeogenesis are the following:

Pyruvate + CO_2 + H_2O + ATP Oxaloacetate + ADP + Pi + $2H^+$

This reaction is catalyzed by pyrivate carboxylase. Unlike the other reactions of glycolysis and gluconeogenesis, this reaction occurs in the mitochondrial matrix.

Oxaloacetate + GTP Phosphoenolphyrivate + CO_2 + GDP)

This reaction is catalyzed by phosphoenolpyruvate carboxykinase.

Fructose-1,6-bisphosphate + H_2O Fructose-6-phosphate + Pi

This reaction is catalyzed by fructoso-1,6-bisphiosphatase.

Glucoso-6-phosphate + H_2O Glucose + Pi

This reaction is catalyzed by glucose-6-phosphatase.

In all the other reactions, gluconeogenesis proceeds simply by reversing the corresponding reaction of glycolysis.

The **Cori cycle** (also known as Lactic acid cycle) refers to the metabolic pathway in which lactate produced by anaerobic glycolysis in the muscles moves to the liver and is converted to glucose, which then returns to the muscles and is metabolized back to lactate.

Pentose phosphate pathway

The pentose phosphate pathway (also called the phosphogluconate pathway and the hexose monophosphate shunt) is a biochemical pathway parallel to glycolysis that generates NADPH and pentoses (5-carbon sugars).

There are two distinct phases in the pathway. The first is the oxidative phase, in which NADPH is generated, and the second is the non-oxidative synthesis of pentoses. In mammals the pentose phosphate pathway takes place in the cytosol.

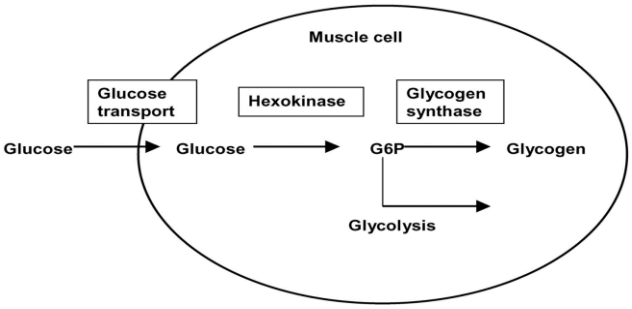
The primary results of the pathway are:

- the generation of NADPH used in reductive biosynthesis reactions within cells (e.g. fatty acid synthesis);
- production of ribose-5-phosphate, used in the synthesis of nucleotides and nucleic acids.

Glycogenesis and glycogenolysis

Glycogenesis is the process of glycogen synthesis, in which glucose molecules are added to chains of glycogen for storage.

Glycogenesis takes place when blood glucose levels are sufficiently high to allow excess glucose to be stored in liver and muscle cells.



Step 1 – Formation of UDP-glucose

Step 2 - Elongation

Step 3 – Branching

Glycogenolysis

Glycogenolysis is a process by which glycogen, the primary carbohydrate stored in the liver and muscle cells, is broken down into glucose to provide immediate energy and to maintain blood glucose levels during fasting.

Glycogenolysis occurs primarily in the liver and is stimulated by the hormones glucagon and epinephrine (adrenaline).

The overall reaction for the breakdown of glycogen to glucose-1-phosphate is:

glycogen(n residues) + Pi glycogen(n-1 residues) + glucose-1-phosphate

Glycogen breakdown is performed by phosphorylase

Phosphorylase a (active) and b (inactive)

Glycogen metabolism

Glycogen synthesis and breakdown are tightly coordinated: the same hormones activate glycogen phosphorylase and inactivate glycogen synthase and vice versa.

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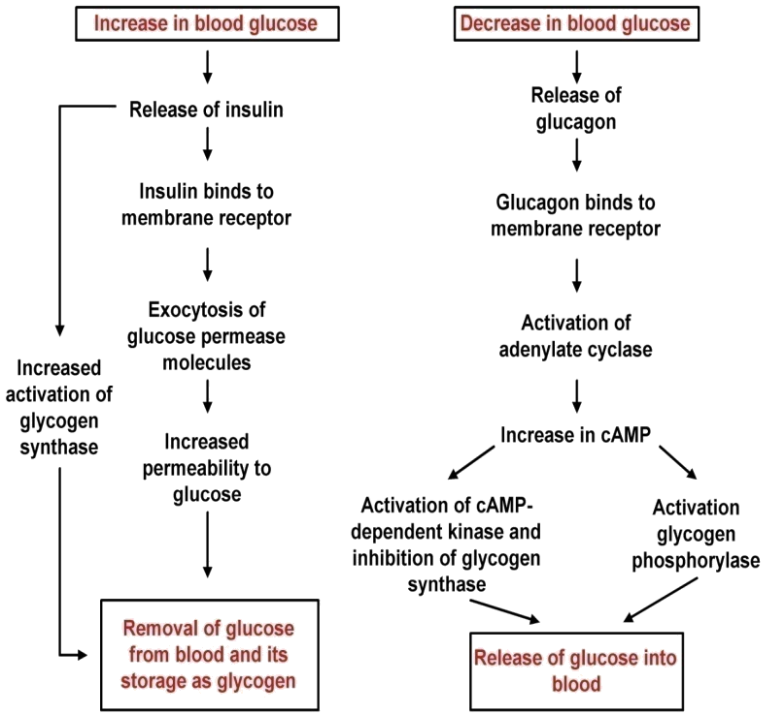
Regulation of the blood glucose level



Lecture 7.

Electron Transport Chain and Oxidative Phosphorylation

Annotation

1. Energy turnover

All processes in the body involve changes in energy. The original energy source for food energy is the sun. Energy from the sun is converted by photosynthesis into the production of glucose. Glucose is the hydrocarbon source, from which plants synthesize other organic compounds.

2. Metabolism: catabolism and anabolism

Metabolism is the sum of all of the chemical activities that cells undergo throughout their lives. Metabolism is composed of two main subdivisions: anabolism (biosynthesis) and catabolism. (the breakdown of biological molecules)

3. High-energy compound – ATP

The primary molecule used to store and deliver energy for all cell functions is adenosine triphosphate (ATP), a form of modified nucleotide composed of the nitrogenous base adenine, the five-carbon sugar ribose and three phosphate groups, the last two of which are linked by high-energy covalent bonds.

4. Substrate and oxidative phosphorylation

The reaction that involves the addition of a phosphate group (Pi) to a molecule is phosphorylation. There are two kinds of phosphorylation: substrate level phosphorylation and oxidative phosphorylation

6. Electron transport system, chemiosmotic theory

The electron transport chain (ETC) is a process in which the NADH and FADH2produced during catabolic processes are oxidized, thus releasing energy in the form of ATP. The mechanism by which ATP is formed in the ETC is called oxidative (chemiosmotic) phosphorolation. According to the chemiosmotic coupling hypothesis, proposed by Peter Mitchell, the electron transport chain and oxidative phosphorylation are coupled by a proton gradient across the inner mitochondrial membrane.

7. Mitochondrial ATP synthesis

ATP synthase is a huge molecular complex (>500 kDa) embedded in the inner membrane of mitochondria. Its function is to convert the energy of protons (H+) moving down their concentration gradient into the synthesis of ATP

Key words

Metabolism, catabolism, anabolism, ATP, substrate phosphorylation, oxidative phosphorylation, Electron transport system (ETC), chemiosmotic theory, mitochondrial ATP synthesis

Recommendations: The goal of the course entitled “Biochemistry” is to get knowledge about basic chemical reactions underlying the life.

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http://en.wikipedia.org/wiki/Biochemistry

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Plan.

1.Energy turnover

2..Metabolism: catabolism and anabolism

3. Mitochondria are a source of energy

4. Substrate and oxidative phosphorylation

5. Electron transport system, chemiosmotic theory

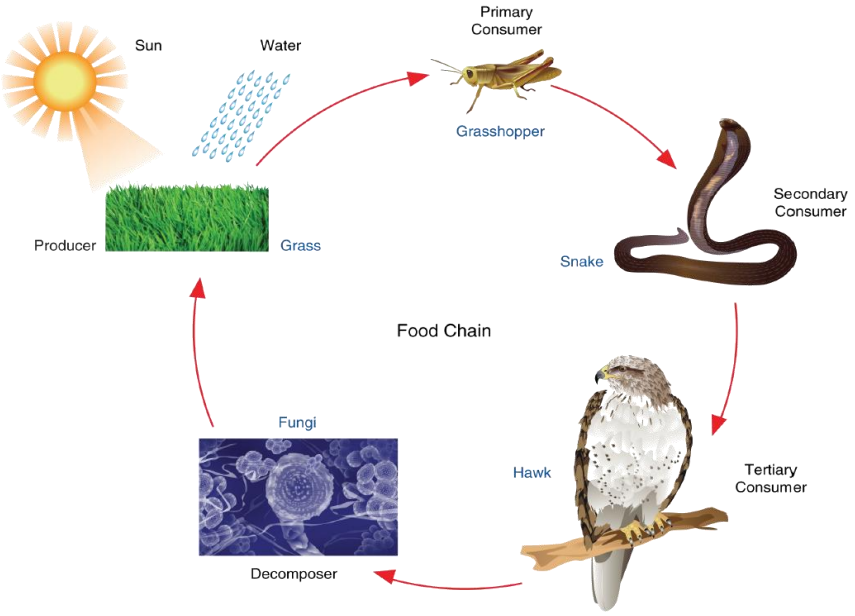
6. Mitochondrial ATP synthesis

7. Regulation of cellular respiration

Lecture 7. Electron Transport Chain and Oxidative Phosphorylation

Energy turnover

1. All processes in the body involve changes in energy.
2. The original energy source for food energy is the sun.
3. Energy from the sun is converted by photosynthesis into the production of glucose.
4. Glucose is the hydrocarbon source, from which plants synthesize other organic compounds.



Metabolism: catabolism and anabolism

Metabolism is the sum of all of the chemical activities that cells undergo throughout their lives.

Metabolism is composed of two main subdivisions:

Catabolism is the breakdown of biological molecules. Cells require nutrients containing energy stored in the carbon-to-carbon bonds of organic compounds, as

well as inorganic substances that act as key components in all metabolic activities. Catabolic reactions serve to release energy and make substances in nutrients available for use.

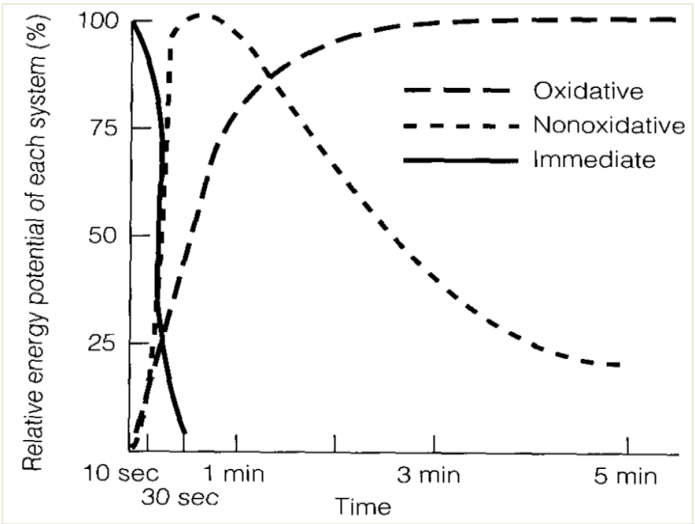
Anabolism (biosynthesis) is the building of new, novel organic compounds, utilizing the substances broken down by catabolic reactions. Anabolic pathways are necessary to synthesize metabolic and genetic materials, as well as those necessary for growth and reproduction

Amphibolic pathways

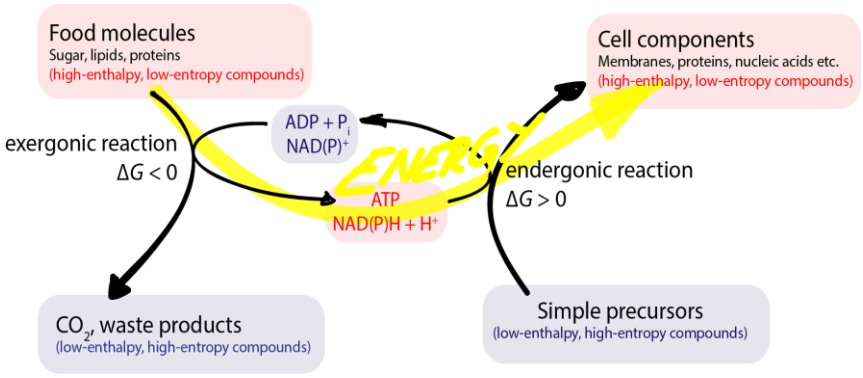
Both types of reactions, anabolic and catabolic, occur through a series of enzyme-mediated steps called metabolic pathways. Linked anabolic and catabolic processes are called amphibolic pathways.

An important example of an amphibolic pathway is the Krebs cycle, which involves both the catabolism of carbohydrates and fatty acids and the synthesis of anabolic precursors for amino-acid synthesis (e.g. α-ketogluturate and oxaloacetate).

Metabolism: energy source



Endergonic and exergonic reactions



Exergonic refers to chemical reactions that proceed spontaneously from reactants to products with the release of energy. *Endergonic* reactions require energy input to proceed. These terms are precisely defined thermodynamic concepts based on changes in an entity called Gibbs free energy (G) accompanying reactions. Reactions in which G decreases ($\Delta G<0$) are exergonic, and those in which increases ($\Delta G>0$) are endergonic.

High-energy compound – ATP

Certain compounds are encountered in the biological system which, on hydrolysis, yields energy. The term high-energy compounds or energy rich compounds are usually applied to substances which possess sufficient free energy to liberate at least 7 Cal/mol at pH 7.0. Certain other compounds which liberate less than 7 (table 1) Cal/mol (lower than ATP hydrolysis to ADP + Pi) are referred to as low- energy compounds.

There are 3 major sources of ~P taking part in energy conservation or energy capture:

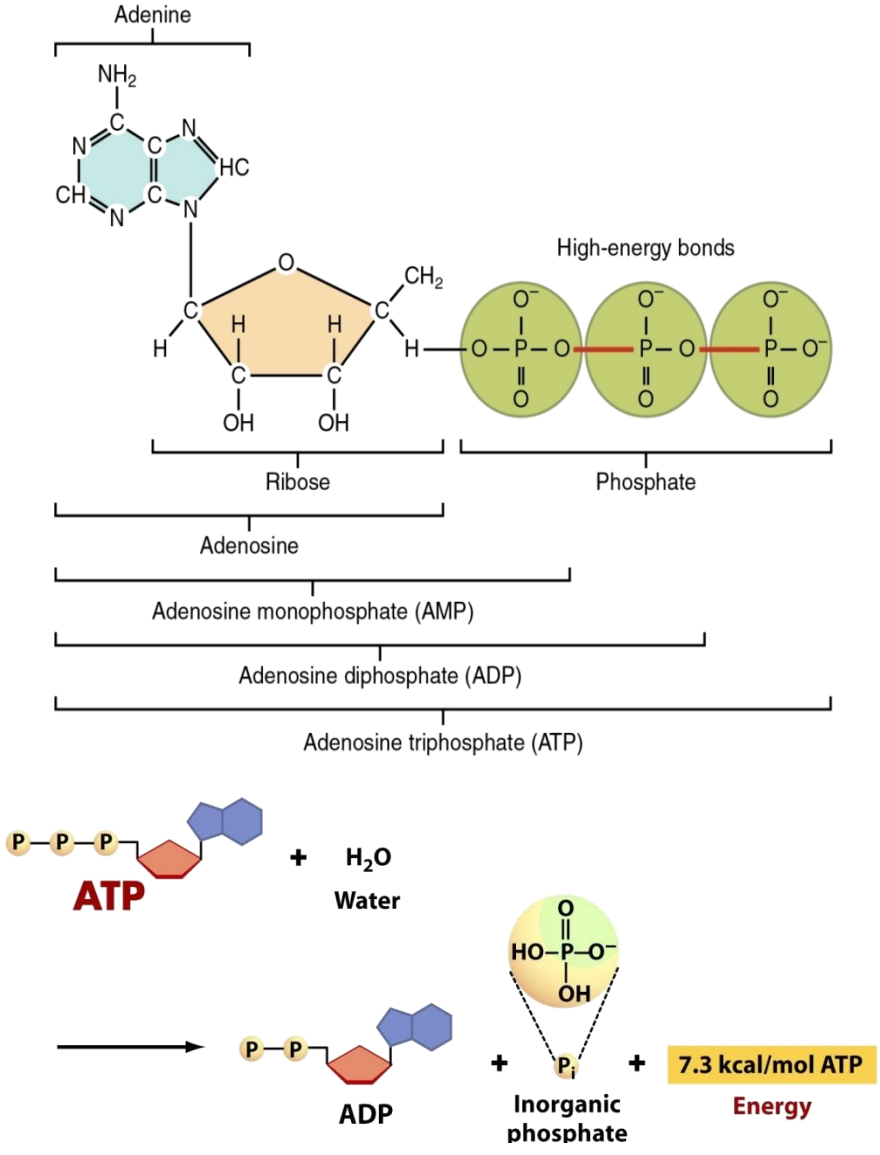
1) Oxidative phosphorylation. This is the greatest quantitative source of ~P in aerobic organisms. The free energy to drive this process comes from respiratory chain oxidation within mitochondria.

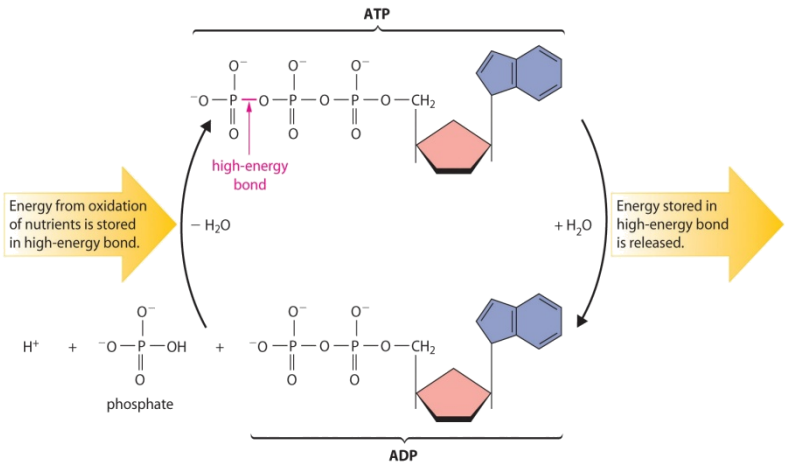
2) Glycolysis. A net formation of 2 ~P results from the formation of lactate from one molecule of glucose generated in 2 reactions catalyzed by phosphoglycerate kinase and pyruvate kinase, respectively.

3) The citric acid cycle. One ~P is generated directly in the cycle at the succinyl thiokinase step.

ATP – the most important high energy compound

Adenosine triphosphate (ATP) is a unique and the most important high energy molecule in the living cells. The ATP molecule is a purine (adenine) nucleotide in which the adenine is attached in a glycosidic linkage to D – ribose. Three phosphoryl groups esterified to the 5 position of the ribose moiety in phosphoanhydride bonds. The two terminal phosphoryl groups (i.e., β and γ) are involved in the phosphoric acid anhydride bonding and are designated as energy rich or high energy bonds.





"High energy" bonds are often represented by the "~" symbol (squiggle), with ~P representing a phosphate group with a high free energy of hydrolysis.

Potentially two "high energy" bonds can be cleaved, as two phosphates are released by hydrolysis from ATP (adenosine triphosphate), yielding ADP (adenosine diphosphate), and ultimately AMP (adenosine monophosphate).

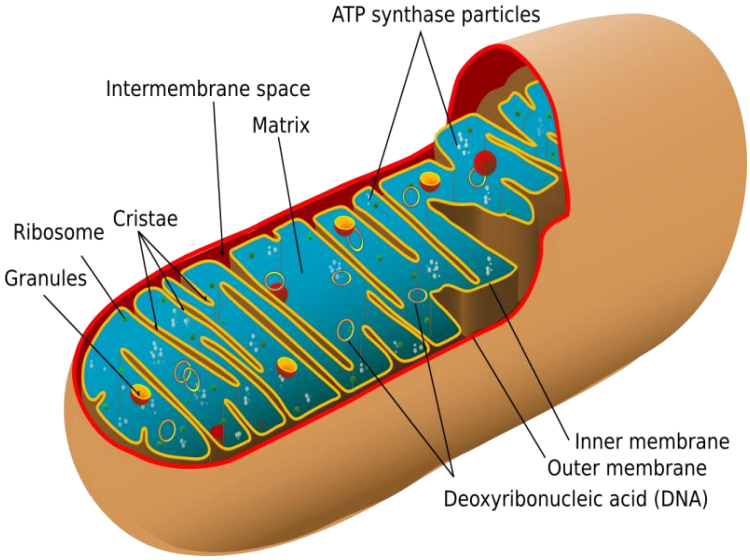
AMP~P~P → AMP~P + Pi (ATP → ADP + Pi)

AMP~P → AMP + Pi (ADP → AMP + Pi)

AMP~P~P → AMP + P~Pi (ATP → AMP + PPi)

P~P → 2 Pi

Mitochondria are a source of energy



Mitochondrial structure plays a critical role in forming and utilizing the proton gradient to synthesize ATP.

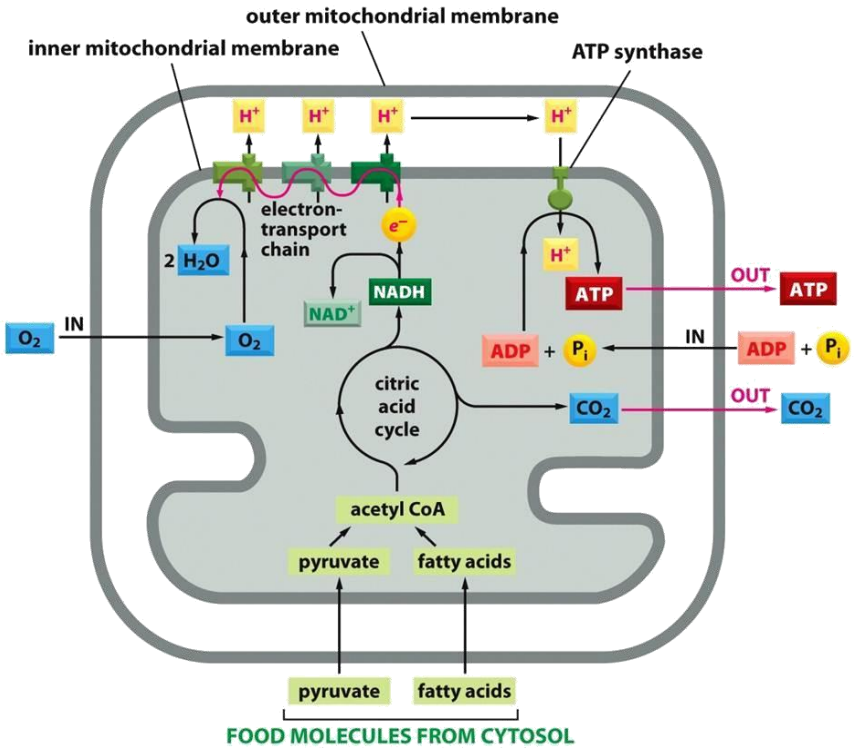
Outer membrane: It is smooth and is composed of equal amounts of phospholipids and proteins. The outer membrane is freely permeable to nutrient molecules,ions,energy molecules like the ATP and ADP molecules.

Inner membrane: The inner membrane of mitochondria is more complex in structure. It is folded into a number of folds many times and is known as the cristae. The cristae and the proteins of the inner membrane aids in the production of ATP molecules. Various chemical reactions takes place in the inner membrane of the mitochondria. Unlike the outer membrane, the inner membrane is strictly permeable, it is permeable only to oxygen, ATP and it also helps in regulating transfer of metabolites across the membrane.

Intermembrane space: It is the space between the outer and inner membrane of the mitochondria,it has the same composition as that of the cell's cytoplasm.

Matrix: The matrix of the mitochondria is a complex mixture of proteins and enzymes. These enzymes are important for the synthesis of ATP molecules, mitochondrial ribosomes, tRNAs and mitochondrial DNA.

Mitochondria are rod shaped organelles that are responsible for converting oxygen and nutrients into usable energy.



Substrate and oxidative phosphorylation

ATP synthesis via phosphorylation

Glycolysis and the citric acid cycle yield NADH and FADH2.

Both these electron carriers are energy-rich molecules because their electrons have a high transfer [redox] potentials.

Oxidative phosphorylation is the process of converting this high redox potential into energy-rich ATP molecules.

This process, together with the reactions that form the electron carriers is often called respiration.

The reaction that involves the addition of a phosphate group (Pi) to a molecule is phosphorylation.

<u>There are two kinds of phosphorylation:</u>

1. Substrate level phosphorylation

Phosphate from one molecule (substrate) is transferred to another molecule:

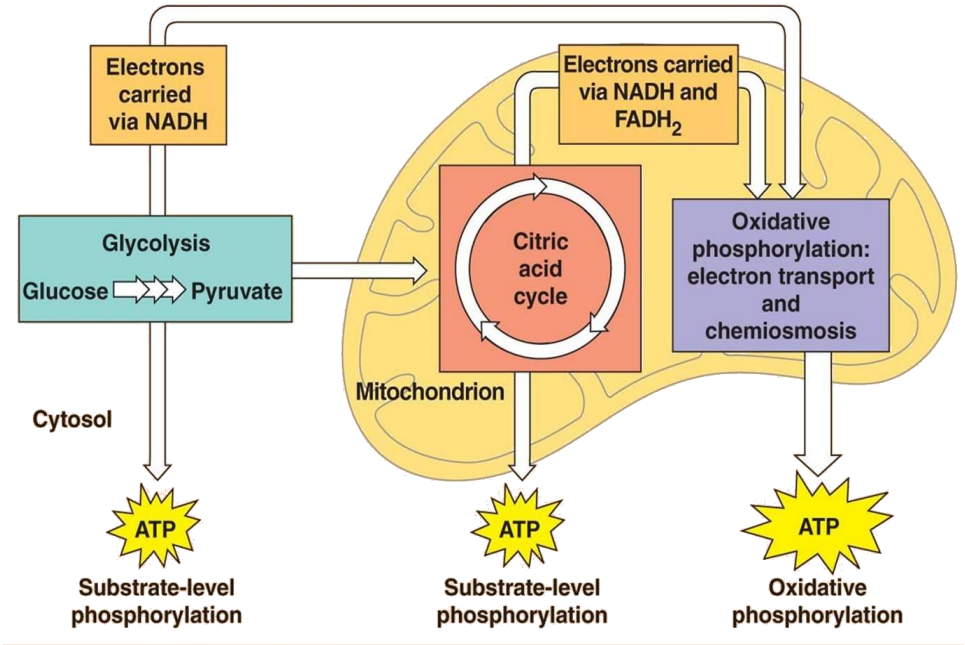
$$X\text{-}P + ADP + energy \rightarrow X + ATP$$

2. Oxidative phosphorylation

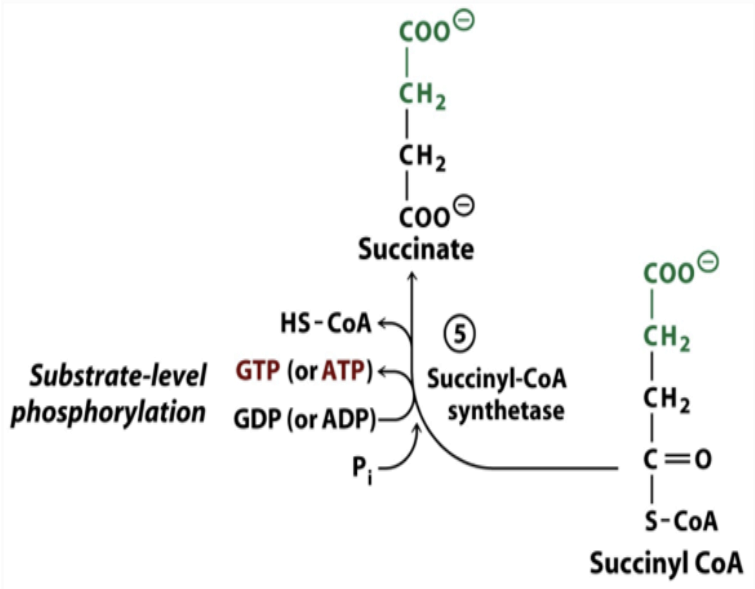
Involves the energy released when compounds (NADH + FADH2) release their electrons and the electrons are transferred along an electron transport chain. The

electrons are finally given to oxygen. ATP results from chemiosmotic coupling which captures the energy that is released as the electrons are transported and uses it to synthesize ATP by means of ATP synthase:

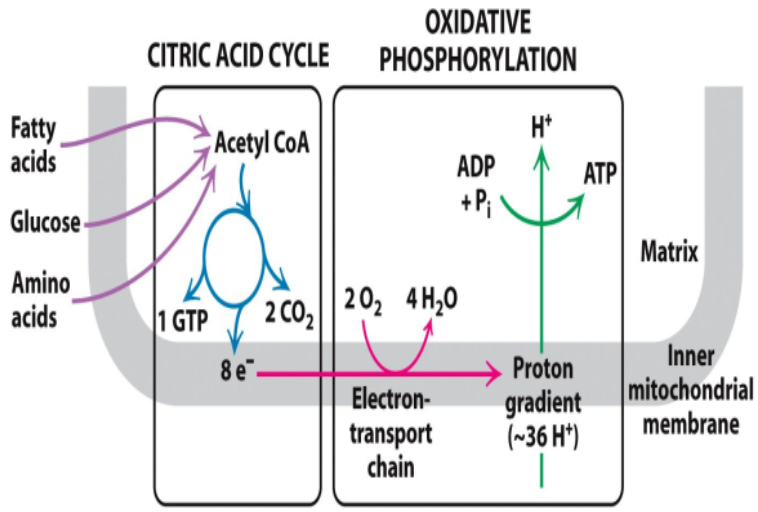
$$ADP + Pi \rightarrow ATP$$



Substrate-level phosphorylation



Oxidative phosphorylation



Importance oxidative phosphorylation

- Only 4 of 38 ATP ultimately produced by respiration of glucose are produced by substrate-level phosphorylation. Two are produced during glycolysis, and 2 are produced during the citric acid cycle.
- Energy produced in electron transport chain gives a maximum yield of 34 ATP by oxidative phosphorylation via ATP-synthase.
- Substrate-level phosphorylation and oxidative phosphorylation give a bottom line of 38 ATP.

Electron transport system, chemiosmotic theory

Chemiosmosis: a process via which oxidative phosphorylation takes place at the end of the Electron Transport Chain (ETC) to produce 90% of ATP via ATP-synthase.

Or, is the process in which ATP synthesis powered by the flow of H+ back across ATP synthase. ATP-synthase: an enzyme presents in the inner mitochondrial membrane and used in making ATP by using H+ (protons).

NAD+: Nicotinamide adenine dinucleotide, which is a co-enzyme that helps electron transfer during redox reactions in cellular respiration.

FAD: Flavin adenine dinucleotide, which is an electron acceptor that helps electron transfer during Krebs Cycle and Electron Transport Chain in cellular respiration.

Oxidative phosphorylation = ETC+ ATP synthesis

Electron transport chain (ETC)

The electron transport chain (ETC) is a process in which the NADH and FADH2 produced during catabolic processes are oxidized, thus releasing energy in the form of ATP. The mechanism by which ATP is formed in the ETC is called oxidative (chemiosmotic) phosphorolation.

The electron transport or respiratory chain gets its name from the fact electrons are transported to meet up with oxygen from respiration at the end of the chain. The overall electron chain transport reaction is:

$$2H^+ + 2e^+ + 1/2\ O_2 \longrightarrow H_2O + \text{energy}$$

2 hydrogen ions, 2 electrons, and an oxygen molecule react to form as a product water with energy released in an exothermic reaction. This relatively straightforward reaction actually requires eight or more steps. The energy released is coupled with the formation of three ATP molecules per every use of the electron transport chain.

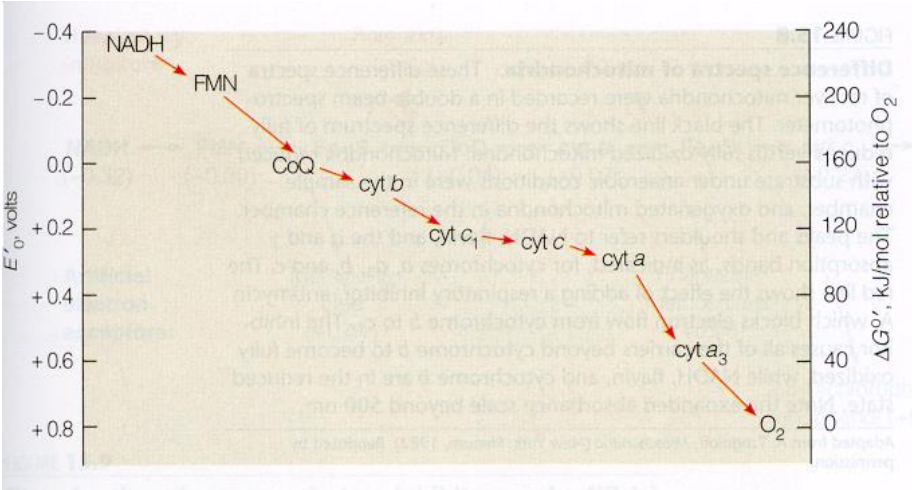
The electron transport chain generates no ATP directly. Rather, its function is to break the large free energy drop from food to oxygen into a series of smaller steps that release energy in manageable amounts.

This pathway of electron transport from NADH to H_2O through a variety of electron carriers allows the transformation of redox energy into a proton gradient.

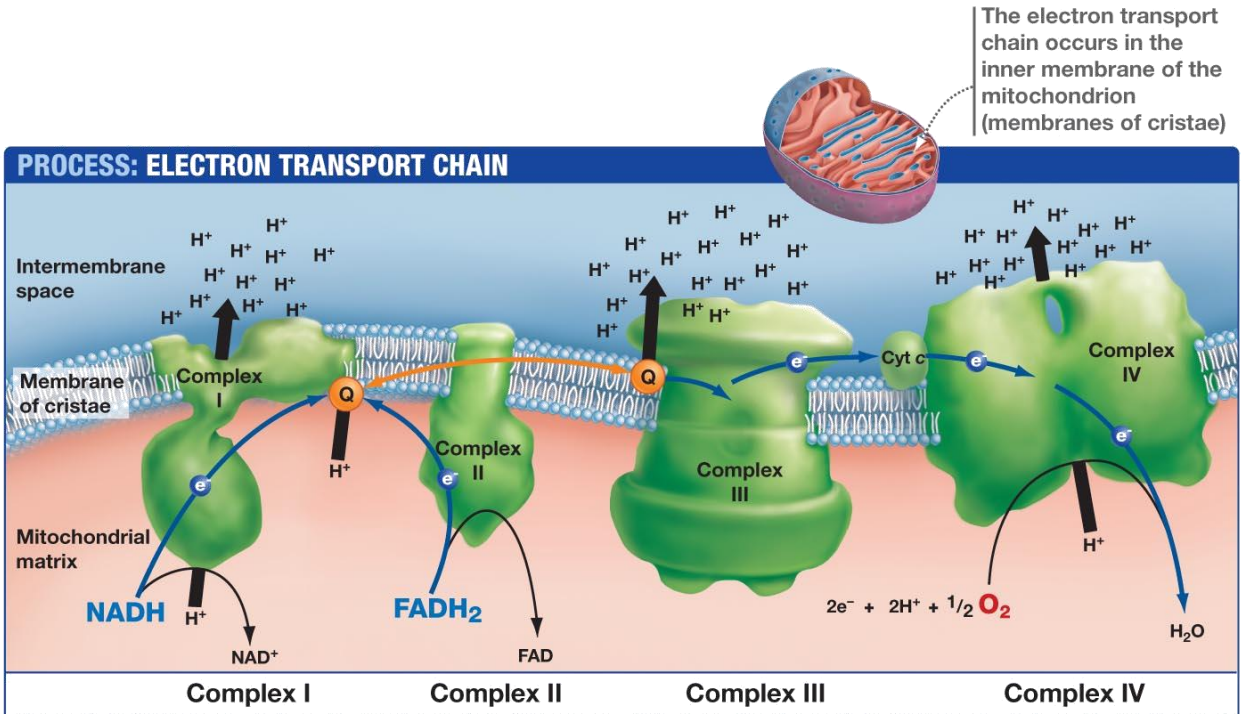
How do scientists know the order of the electron

carriers in this pathway?

The reduction potential (E'0) of each carrier has been determined over the years. Once a reduction potential for a carrier is known, it is relatively easy to place it in its correct position relative to the others since electrons move spontaneously from carriers with lower reduction potentials to carriers with higher reduction potentials.



Rather than occurring in a single step, electrons from NADH pass through groups of carriers, mostly within the mitochondrial inner membrane, eventually reaching oxygen. The most interesting of these carriers are three groups of protein complexes often identified as "Complex I, II, III, and IV."



Complex I (NADH-ubiquinone oxidioreductase): A protein that receives electrons from NADH and passes them on to ubiquinone.

Complex II (succinate-ubiquinone oxidioreductase): A protein that receives electrons from succinate (an intermediate metabolite of the Krebs cycle) to yield fumarate and FADH2.

Coenzyme Q (ubiquinone): Ubiquinone (the oxidized form of the molecule) receives electrons from several different carriers; from complexes I, II, etc. It is now the reduced form (ubiquinol) which passes its electron off to complex III.

Complex III (ubiquinol-cytochrome c oxidioreductase): A protein that receives electrons from ubiquinol which are then passed on to cytochrome c.

Complex IV (cytochrome c oxidase): A protein that that receives electrons from cytochrome c and transfers them to oxygen to produce water within the mitochondria matrix.

ATP Synthase: A protein consisting of several different subunits. This protein is directly responsible for the production of ATP via chemiosmotic phosphorylation. It uses the proton gradient created by several of the other carriers to drive a mechanical rotor. The energy from that rotor is then used to phosphorylate ADP to ATP.

Coenzymes and prosthetic groups

NAD+/NADH (Nicotinamide adenine dinucleotide)

FAD/FADH2 (Flavin adenine dinucleotide)

FMN/FMNH2 (Flavin mononucleotide)

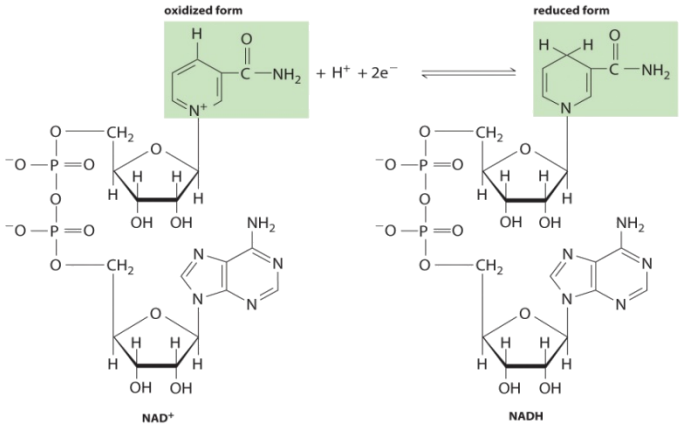
Coenzyme Q/QH2 (Ubiquinone/Ubiquinol)

Heme in cytochromes

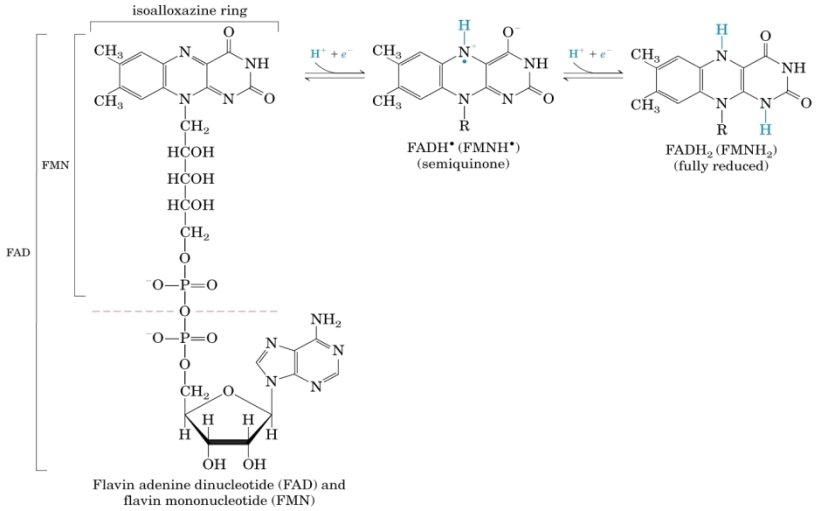
Iron-Sulfur Proteins

<u>Copper Proteins</u>

The nicotinamide ring of NAD^+, which is derived from the vitamin niacin, accepts $2e^-$ and one H^+ in going to the reduced state, as NAD+ becomes NADH.

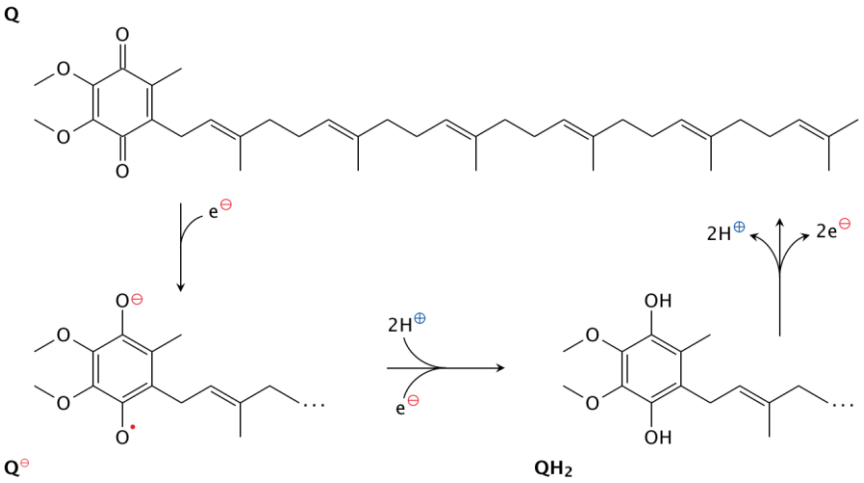


The portion of FAD or FMN that undergoes reduction is the dimethylisoalloxazine ring, derived from the vitamin riboflavin, as FAD or FMN becomes $FADH_2$ or $FMNH_2$.

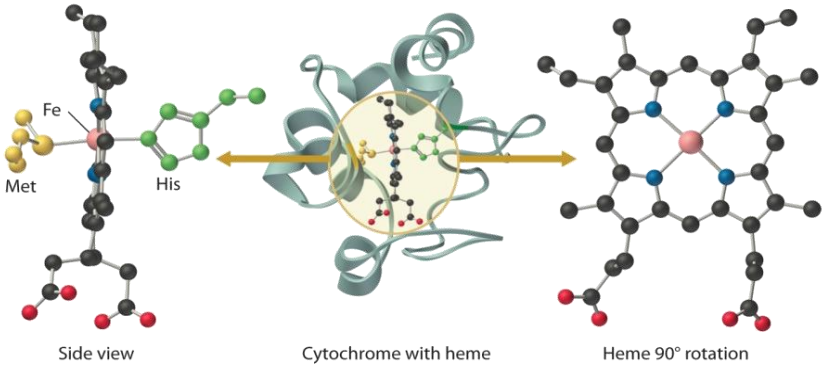


Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

Coenzyme Q/Coenzyme QH_2

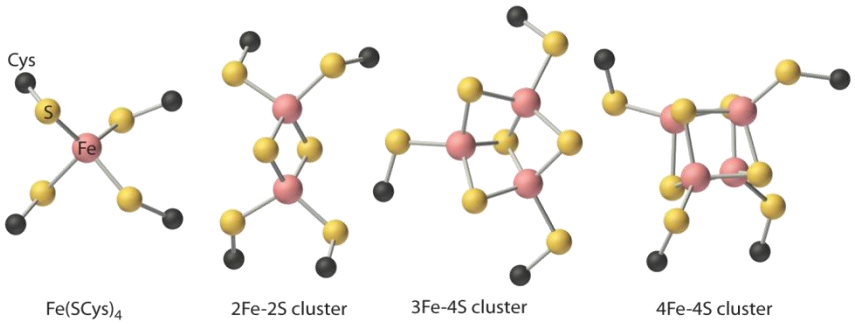


The heme iron is involved in one electron transfers involving the Fe^{2+} and Fe^{3+} oxidation states.

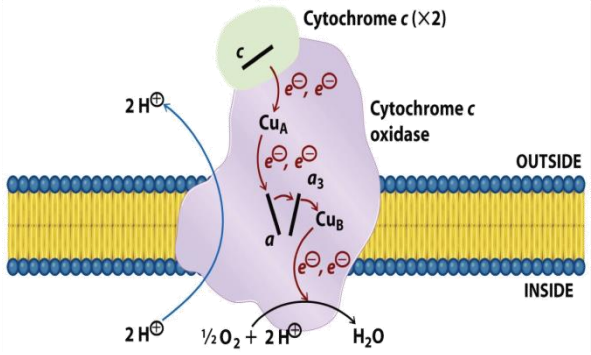


In the electron transport chain there are many iron-sulfur proteins which participate in one electron transfers involving the Fe2+ and Fe3+ oxidation states. These are non-heme iron-sulfur proteins.

The simplest iron-sulfur protein is FeS in which iron is coordinated by four cysteines. The second form is Fe2S2 which contains two irons complexed to 2 cysteine residues and two inorganic sulfides. The third form is Fe3S4 which contains 3 iron atoms coordinated to three cysteine residues and 4 inorganic sulfides. The last form is the most complicated Fe4S4 which contains 4 iron atoms coordinated to 4 cysteine residues and 4 inorganic sulfides



Copper bound proteins participate in one electron transfers involving the Cu^{+} and Cu^{2+} oxidation states.



How ETC works

Energy obtained through the transfer of electrons down the ETC is used to pump protons from the mitochondrial matrix into the intermembrane space, creating an electrochemical proton gradient across the mitochondrial inner membrane (IMM). This electrochemical proton gradient allows ATP synthase to use the flow of H+ through the enzyme back into the matrix to generate ATP from ADP and inorganic

phosphate. Complex I (NADH coenzyme Q reductase) accepts electrons from the Krebs cycle electron carrier NADH and passes them to coenzyme Q (UQ), which also receives electrons from complex II (succinate dehydrogenase). UQ passes electrons to complex III (cytochrome bc1 complex), which passes them to cyrochrome c (cyt c). Cyt c passes electrons to Complex IV (cyrochrome oxidase), which uses the electrons and hydrogen ions to reduce molecular oxygen to water.

Four membrane-bound complexes have been identified in mitochondria. Each is an extremely complex transmembrane structure that is embedded in the inner membrane. Three of them are proton pumps. The structures are electrically connected by lipid-soluble electron carriers and water-soluble electron carriers. The overall electron transport chain:

NADH → Complex I → Q → Complex III → cytochrome c → Complex IV → O2

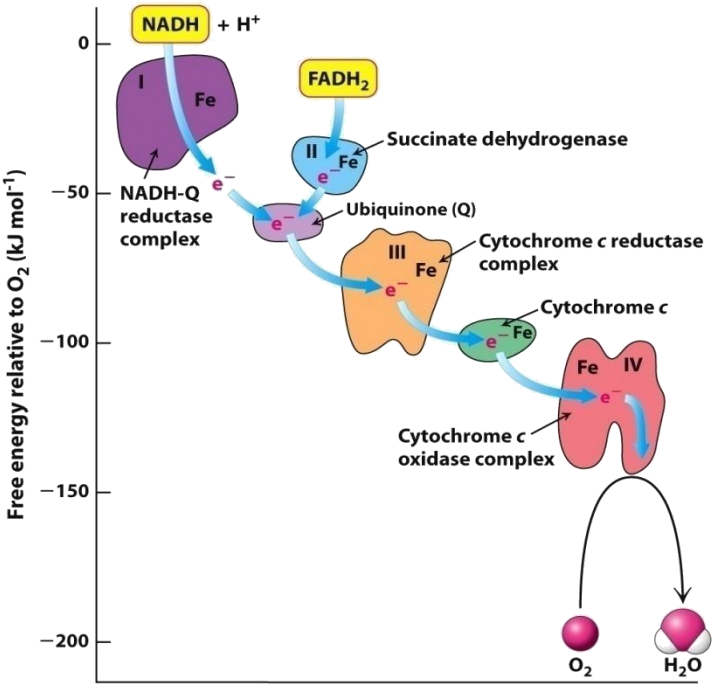
↑

Complex II

↑

Succinate

Complexes I, II, III, and IV in ETC



In *Complex I* two electrons are removed from NADH and transferred to a lipid-soluble carrier, *ubiquinone* (Q). The reduced product, QH_2, freely diffuses within the membrane, and Complex I translocates four protons (H^+) across the membrane, thus producing a proton gradient.

During their transit, electrons pass through a flavin [isoalloxazine] ring and iron-sulfur clusters.

The reduction and subsequent oxidation of the isoalloxazine requires two hydrogen atoms. Due to the unique structure of the NADH-Q oxidoreductase complex, hydrogens are drawn from the matrix during reduction and then ejected into the intermembrance space during oxidation.

Electrons are transported through the membrane from Site 1 to Complex 3 by ubiquinol (ubiquinone).

This fat-soluble carrier picks up electrons from Complex 1 (Q→QH2), then diffuses laterally through the membrane delivering electrons to Complex 3 (QH2→Q), then returns to Site 1 for more.

In *Complex II* additional electrons are delivered into the quinone pool (Q) originating from succinate and transferred (via FAD) to Q. Complex II is a parallel electron transport pathway to complex I, but unlike complex I, no protons are transported to the intermembrane space in this pathway.

FADH2 delivers electrons to the electron transport pathway by reducing ubiquinol (Q) to ubiquinone (QH2).

FADH2 is formed in the TCA cycle when succinate is oxidized to malate.

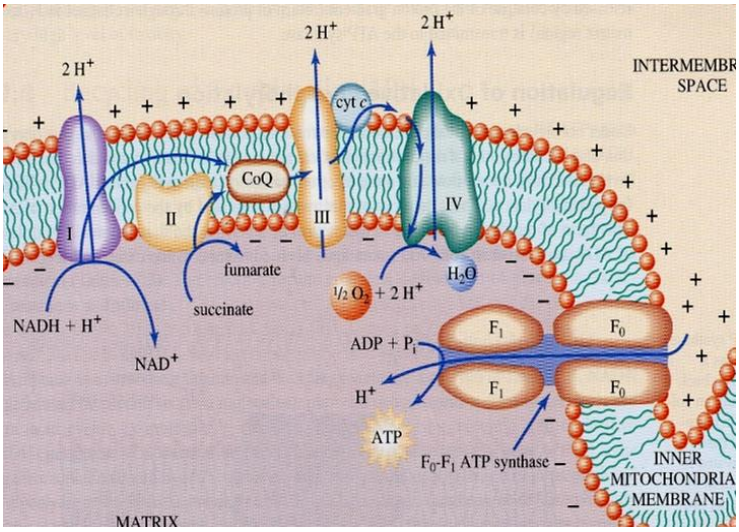
Electrons from FADH2 reduce Q to QH2 and flow through electron transport, ending up on oxygen.

Consequently, less ATP is formed from the oxidation of FADH2 than from NADH.

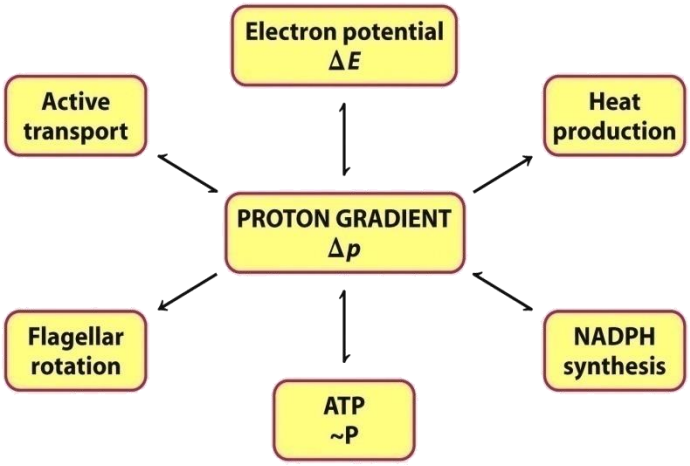
Complex III transfers the electrons from CoQH to reduce cytochrome c (which is the substrate for Complex IV) through a very unique electron transport pathway called the Q-cycle.

Proton (electrochemical) gradient

An electrochemical gradient is a gradient of electrochemical potential across a membrane. The gradient consists of two parts, the electrical potential and a difference in the chemical concentration across a membrane. The energy is stored in the form of chemical potential, which accounts for an ion's concentration gradient across a cell membrane, and electrostatic energy, which accounts for an ion's tendency to move under influence of the transmembrane potential.



Proton gradient as an energy source



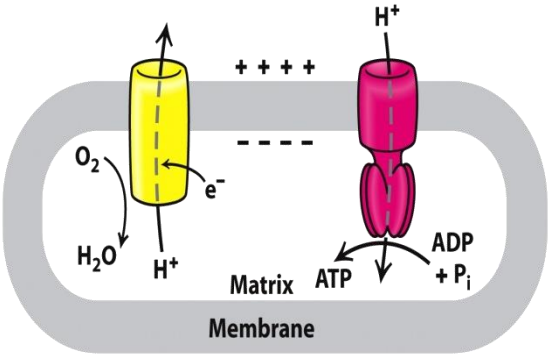
Mitochondrial ATP synthesis

Coupling of ETC with ATP synthesis

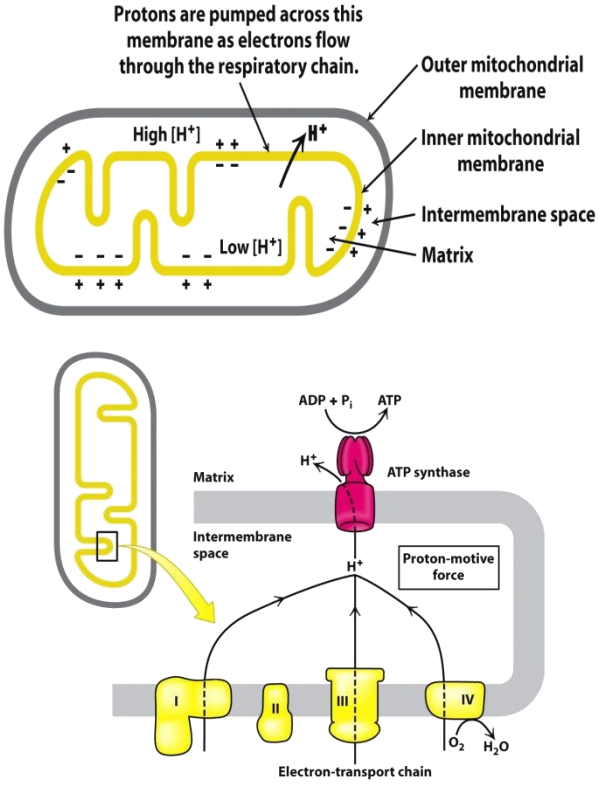
According to the chemiosmotic coupling hypothesis, proposed by Peter Mitchell, the electron transport chain and oxidative phosphorylation are coupled by a proton gradient across the inner mitochondrial membrane. The efflux of protons from the

mitochondrial matrix creates an eletrochemical gradient (proton gradient). This gradient is used by the ATP syntase complex to make ATP via oxidative phosphorylation.

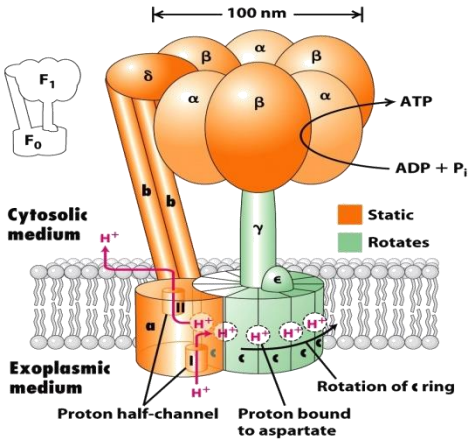
The FO component of ATP syntase acts as an ion channel that provides for a proton flux back into the mitochondrial matrix. This reflux releases free energy produced during the generation of the oxidized forms of the electron carriers (NAD+ and Q). The free energy is used to drive ATP synthesis, catalyzed by the F1 component of the complex.



Chemiosmotic hypothesis



ATP Synthase structure



ATP synthase is a huge molecular complex (>500 kDa) embedded in the inner membrane of mitochondria. Its function is to convert the energy of protons (H^+) moving down their concentration gradient into the synthesis of ATP.

How efficient the respiration is?

Only 4 of 38 ATP ultimately produced by respiration of glucose are produced by substrate-level phosphorylation. Two are produced during glycolysis, and 2 are produced during the citric acid cycle.

NADH and FADH2 account for the vast majority of the energy extracted from the food. These reduced coenzymes link glycolysis and the citric acid cycle to oxidative phosphorylation, which uses energy released by the electron transport chain to power ATP synthesis.

Complete oxidation of glucose releases 686 kcal/mol. Phosphorylation of ADP to form ATP requires at least 7.3 kcal/mol. Efficiency of respiration is 7.3 kcal/mol times 38 ATP/glucose divided by 686 kcal/mol glucose, which equals 0.4 or 40%. Approximately 60% of the energy from glucose is lost as heat. Some of that heat is used to maintain our high body temperature (37°C). Cellular respiration is remarkably efficient in energy conversion.

Regulation of cellular respiration

The dependence of oxidative phosphorylation on ADP reveals an important general feature of this process: Respiration is tightly coupled to the synthesis of ATP. This regulatory phenomenon, called respiratory control, makes biological sense, because

it ensures that substrates will not be oxidized wastefully. Instead, their utilization is controlled by the physiological need for ATP.

Questions for self-control

1.Catabolism of nutrients, its significance for the life. General description of catabolic phases

2. Mitochondrial respiratory chains. Their structure and work. Oxidative phosphorylation as the main pathway of ATP synthesis.

3.Macroergic compounds: definition, classification, biological function. The key role of ATP.

4.Biological oxidation, its functions in the body. Enzymes involved in dehydrogenation, oxygenation reactions and removal of electrons, examples.

5.Mitochondrial respiratory chains. Their structure and work. Oxidative phosphorylation as the main pathway of ATP synthesis.

Lecture 8.

Metabolism of Lipids

Annotation

1. Fat digestion and absorption

Most of the fat in the human diet is in the form of triacylglycerol (TAG), which consists of three fatty acids linked to glycerol. In the digestive tract, TAG is hydrolyzed by the enzyme lipase, to release free fatty acids and monoglycerides.

2. Lipolysis

Lipolysis is the biochemical pathway responsible for the catabolism of triacylglycerol (TAG) stored in cellular lipid droplets. The hydrolytic cleavage of TAG generates non-esterified fatty acids, which are subsequently used as energy substrates, essential precursors for lipid and membrane synthesis, or mediators in cell signaling processes.

3. Fatty acid degradation

β-Oxidation is the process by which fatty acid molecules are broken down in the mitochondria to generate acetyl-CoA, which enters the Krebs cycle, and NADH and FADH2, which are used by the electron transport chain.

4. Ketone body metabolism

Ketone bodies are three water-soluble compounds that are produced as by-products when fatty acids are broken down for energy in the liver and kidney.

5. Fatty acid synthesis

Fatty acids can be synthesized from acetyl-CoA. This is the major pathway for utilizing excess dietary carbohydrates and protein. Fatty acid synthesis occurs mainly in the fat tissue and the liver. It runs in the cytosol, which keeps it apart from mitochondrial β-oxidation. In broad outline, fatty acid synthesis is a reversal of β-oxidation.

6. Biosynthesis of cholesterol

Cholesterol is known as a "sterol" because it is made out of a alcohol and steroid. Cholesterol is present in most animal membranes with varying amounts but is absent in prokaryotes and intracellular membranes.

7. Lipoprotein metabolism, hypercholesterolemia

Chylomicrons carry triglycerides (fat) from the intestines to the liver, skeletal muscle, and to adipose tissue. Very low density lipoproteins (VLDL) carry (newly synthesised) triglycerides from the liver to adipose tissue. Low density lipoproteins (LDL) carry cholesterol from the liver to cells of the body.

Key words

Fat digestion and absorption, lipolysis, β-Oxidation, ketone body metabolism, fatty acid synthesis biosynthesis of cholesterol, hypercholesterolemia.

Recommendations: The goal of the course entitled “Biochemistry” is to get knowledge about basic chemical reactions underlying the life.

Working with the literature is better to begin with going through the lectures. You should read carefully with a pencil in your hand and mark of three types: what is clear, what needs to be specified, and what is totally unclear. Then you should open a textbook and find answers to your questions followed by putting down commentaries to your lectures. After that you can go to the unclear items using actively the recommended literature and consulting with a mentor.

Internet sources

http://www.biochemweb.org/

http://www.1lec.com/Biochemistry/

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http://biochem.stanford.edu/

Plan.

1. Fat digestion and absorption
2. Lipolysis

3. Fatty acid degradation
4. Ketone body metabolism
5. Fatty acid synthesis
6. Biosynthesis of cholesterol
7. Lipoprotein metabolism, hypercholesterolemia

Lecture 8. Metabolism of Lipids

Fat digestion and absorption

Most of the fat in the human diet is in the form of triacylglycerol (TAG), which consists of three fatty acids linked to glycerol. In the digestive tract, TAG is hydrolyzed by the enzyme lipase, to release free fatty acids and monoglycerides.

Emulsification and digestion

The digestive enzyme, lipase, is water soluble and can only work at the surface of fat globules. Digestion is greatly aided by emulsification, the breaking up of fat globules into much smaller emulsion droplets. Bile salts and phospholipids are amphipathic molecules that are present in the bile. Motility in the small intestine breaks fat globules apart into small droplets that are coated with bile salts and phospholipids, preventing the emulsion droplets from re-associating.

The emulsion droplets are where digestion occurs. Emulsification greatly increases the surface area where water-soluble lipase can work to digest TAG. Another factor that helps is colipase, an amphipathic protein that binds and anchors lipase at the surface of the emulsion droplet.

Bile acids aid in fat absorption and modulate cholesterol levels. They are produced from cholesterol in the liver and are stored in the gall bladder. Gall bladder contraction with feeding releases bile acids into the intestine. Bile acids undergo enterohepatic circulation, i.e. they are absorbed in the intestine and taken up by hepatocytes for re-excretion into bile.

<u>Function of bile acids</u>

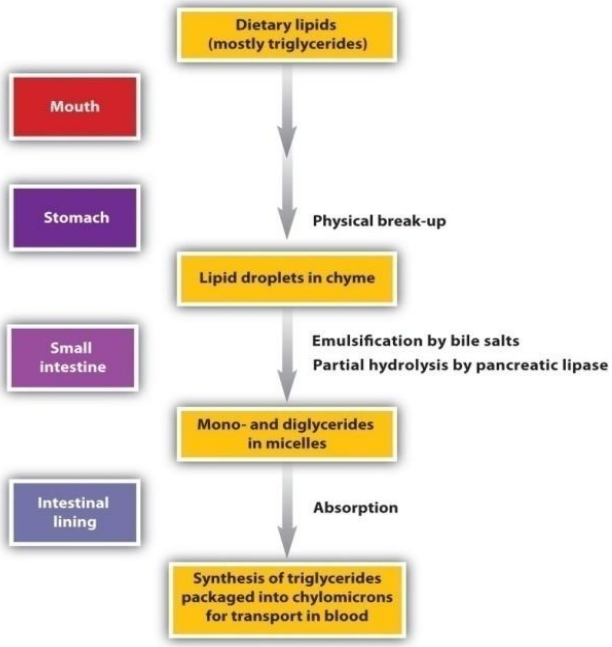
Their synthesis and subsequent excretion in the feces represent the only significant mechanism for the elimination of excess cholesterol.

Bile acids and phospholipids solubilize cholesterol in the bile, thereby preventing the precipitation of cholesterol in the gallbladder.

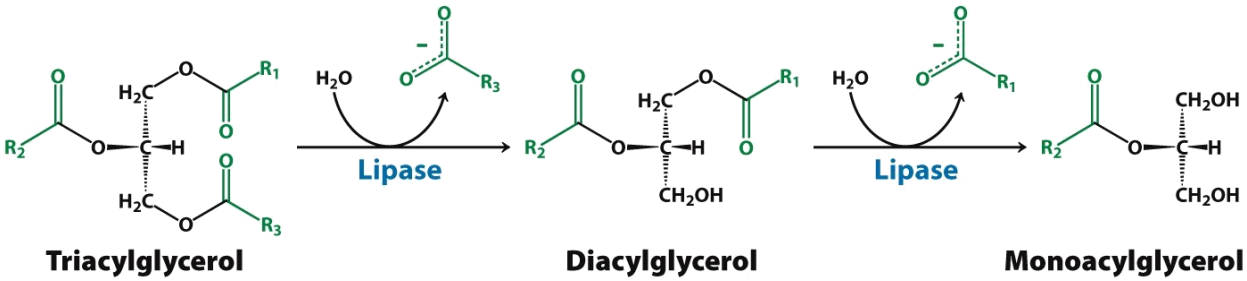
They facilitate the digestion of dietary triacylglycerols by acting as emulsifying agents that render fats accessible to pancreatic lipases.

They facilitate the intestinal absorption of fat-soluble vitamins.

Steps of lipid digestion

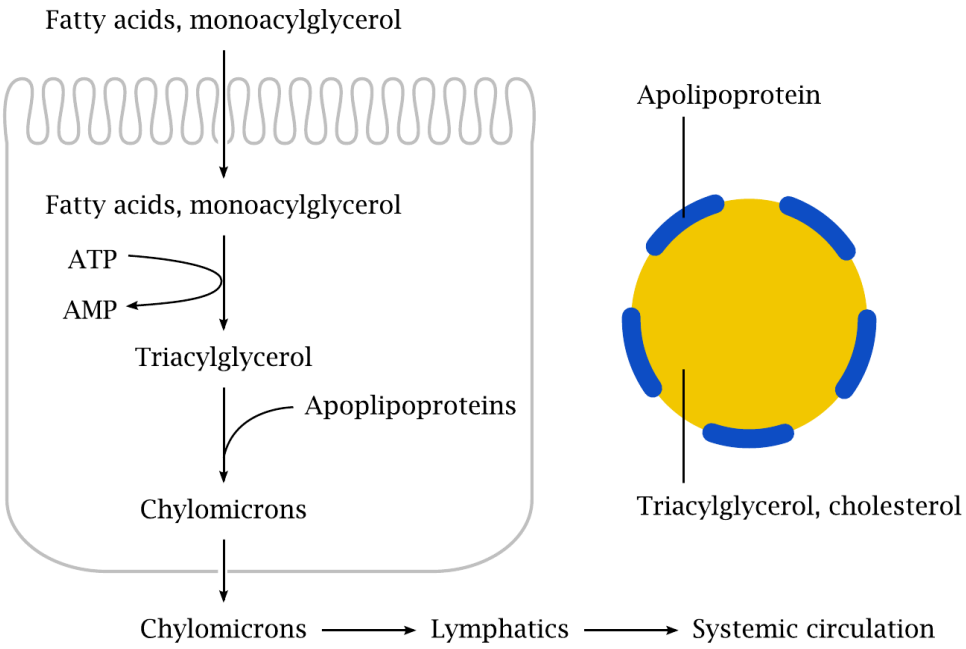


Action of pancreatic lipase

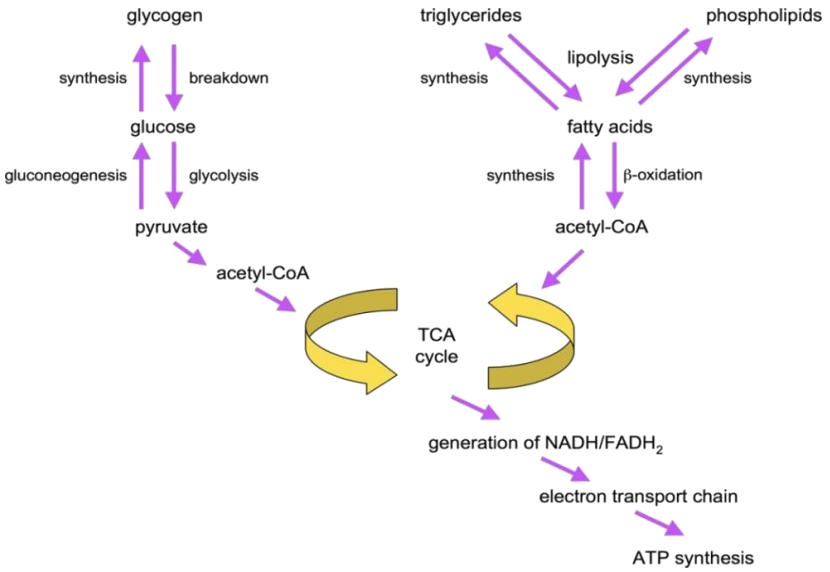


One of the primary tasks of pancreatic lipase is to break down triglycerides. This is critical since these particular lipids cannot be absorbed through the intestinal lining without first undergoing hydrolysis. The enzyme acts as a catalyst to promote the conversion of triacylglycerols into 2-monoacylglycerols and fatty acids. The successful hydrolysis of triglycerides is dependent on the adequate availability of bile salts provided by the liver.

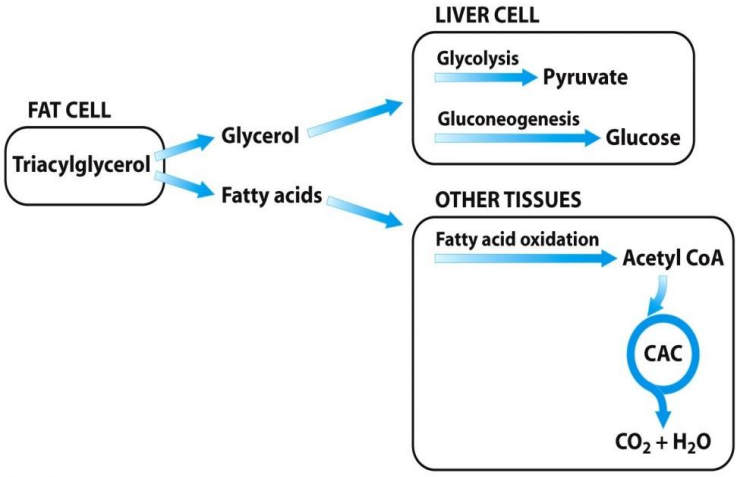
Fat digestion and absorption



Lipid catabolism



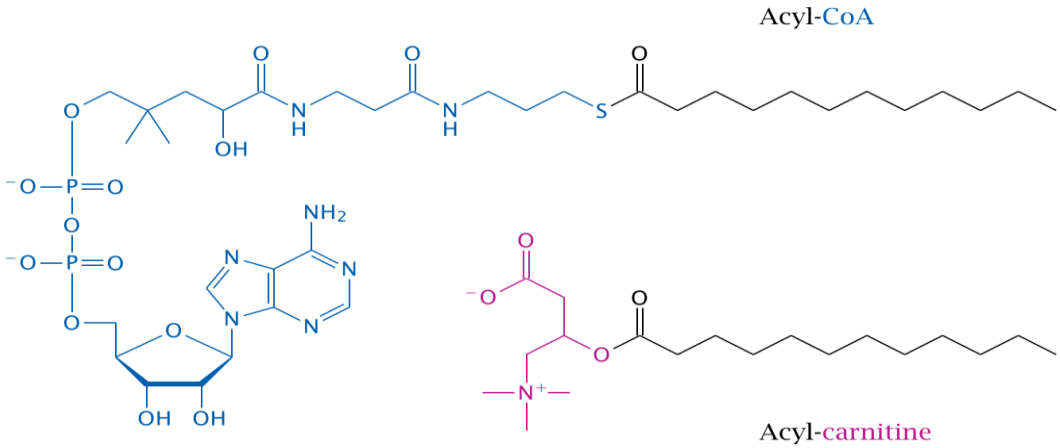
Lipolysis



Lipolysis is the biochemical pathway responsible for the catabolism of triacylglycerol (TAG) stored in cellular lipid droplets. The hydrolytic cleavage of TAG generates non-esterified fatty acids, which are subsequently used as energy substrates, essential precursors for lipid and membrane synthesis, or mediators in cell signaling processes.

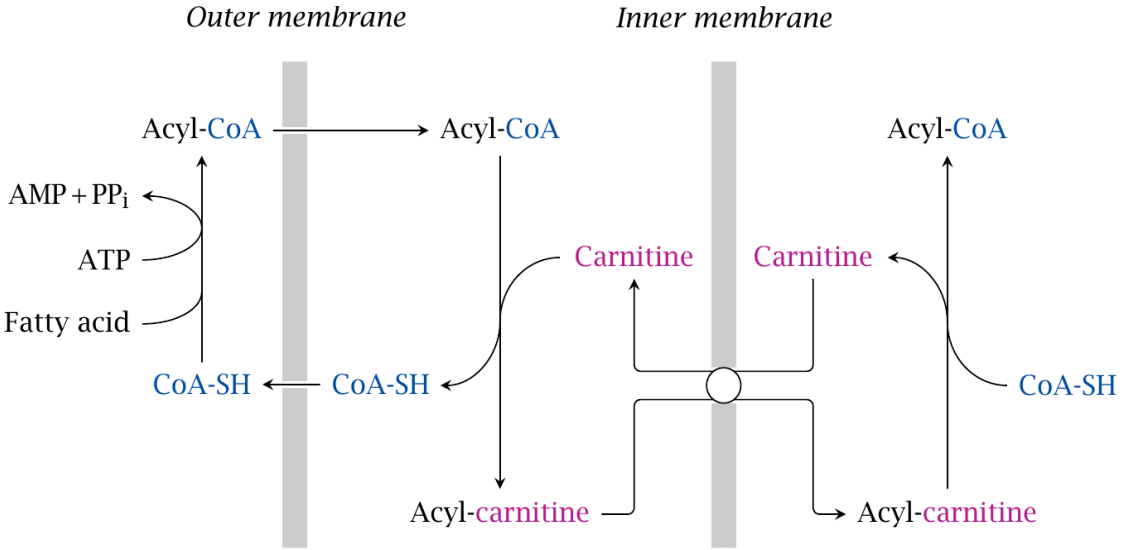
Fatty acid degradation

Activation of fatty acids



There are two activated forms of fatty acids. Fatty acids are initially activated to fatty acyl-CoA in the cytosol. This is also the form in which they enter degradation by β-oxidation. However, during transport, the CoA-moiety is transiently replaced by carnitine. This slide shows the structures of both the CoA- and the carnitine-activated forms; the entire transport process is outlined in the next one.

Transport of fatty acids to the mitochondrion

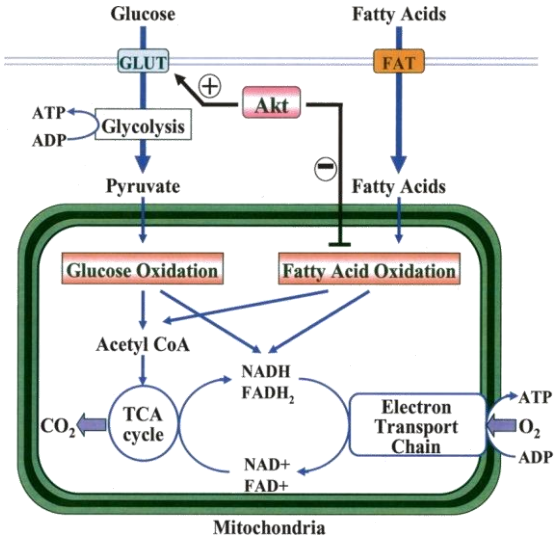


β-Oxidation is the process by which fatty acid molecules are broken down in the mitochondria to generate acetyl-CoA, which enters the Krebs cycle, and NADH and FADH2, which are used by the electron transport chain.

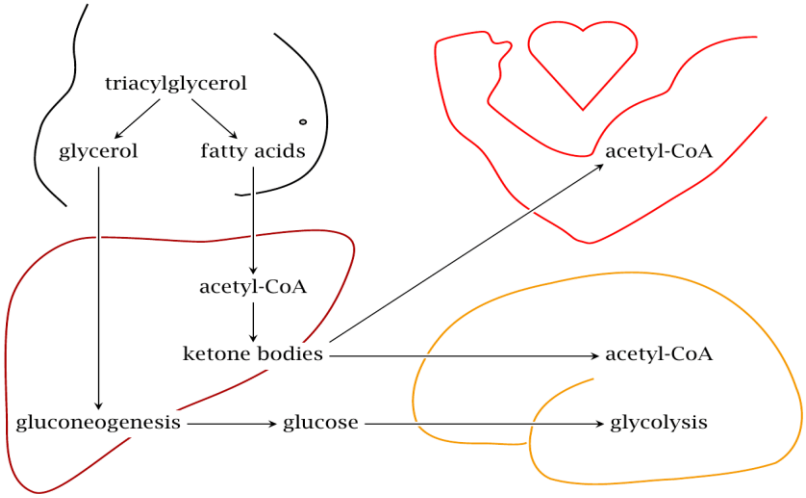
Once inside the mitochondria, the β-oxidation of fatty acids occurs via five recurring steps: activation by ATP ,oxidation by FAD, hydration, oxidation by NAD^+, thyolisis.

The final product is acetyl-KoA, the entry molecule for the Krebs cycle

Fatty acids and glucose oxidation



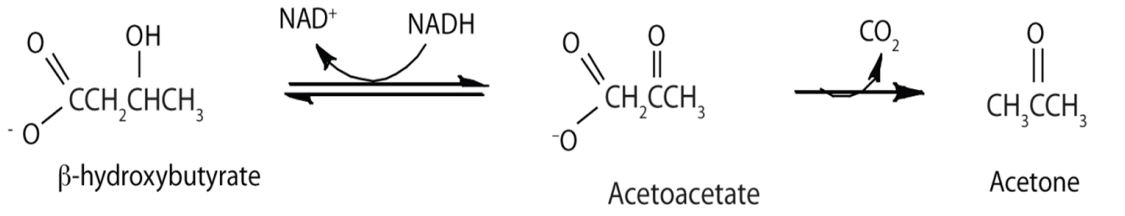
Organs in triacylglycerol utilization



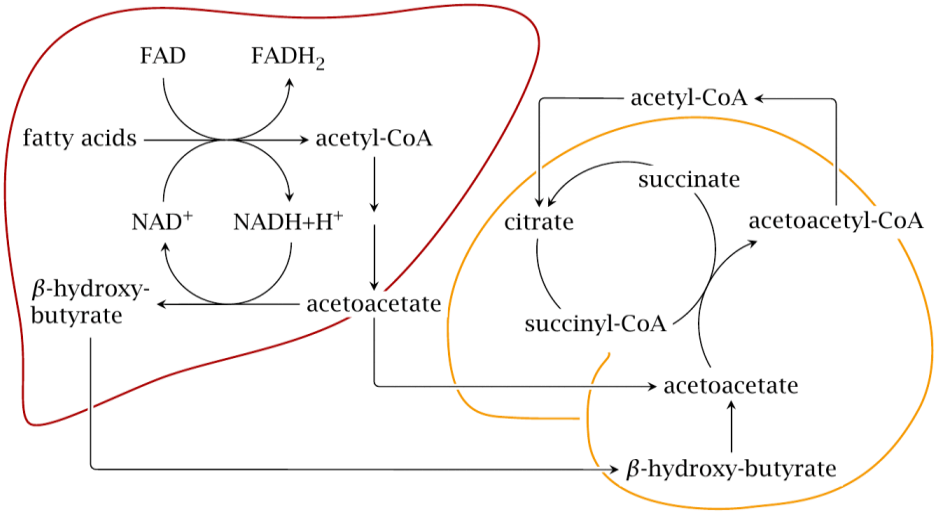
Ketone body metabolism

Ketone bodies are three water-soluble compounds that are produced as by-products when fatty acids are broken down for energy in the liver and kidney. They are used

as a source of energy in the heart and brain. In the brain, they are a vital source of energy during fasting.



Ketone body metabolism



Decarboxylation of acetoacetate

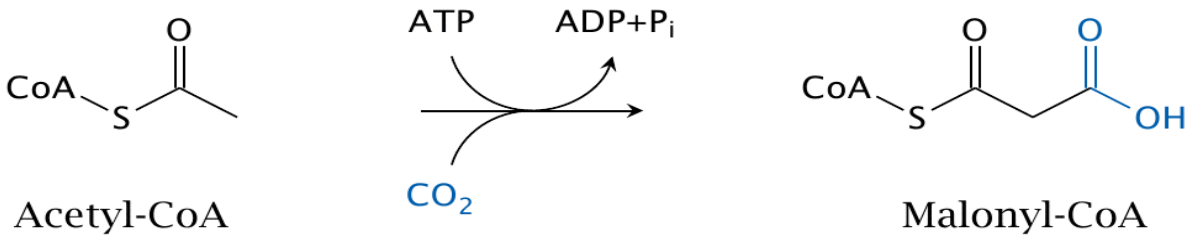
Acetone can serve as a precursor for gluconeogenesis

Fatty acid synthesis

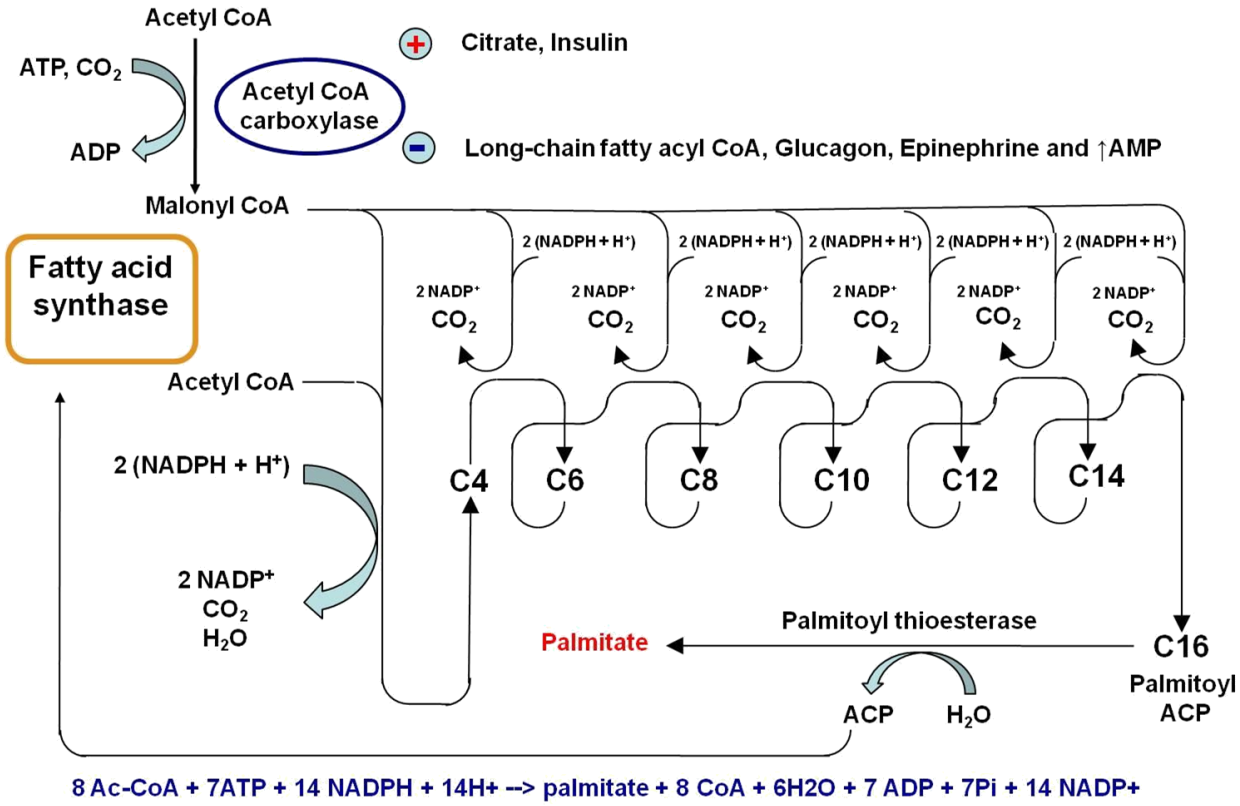
Fatty acids can be synthesized from acetyl-CoA. This is the major pathway for utilizing excess dietary carbohydrates and protein. Fatty acid synthesis occurs mainly in the fat tissue and the liver. It runs in the cytosol, which keeps it apart from mitochondrial β-oxidation. In broad outline, fatty acid synthesis is a reversal of β-oxidation: two carbon atoms at a time are added to a growing fatty acid molecule, and the new β carbon is then reduced to the alkane level. However, the mechanistic details are somewhat different.

The bulk of the work in fatty acid synthesis is accomplished by a single enzyme, fatty acid synthase, which is quite an amazing molecule: it combines six active sites

with eight distinct catalytic activities on a single polypeptide chain. Its product is palmitic acid (hexadecanoic acid). Fatty acids vary in their chain lengths and degree of bond saturation. These variants are derived from palmitate through chain elongation and desaturation, which are accomplished by separate enzymes called elongases and desaturases.

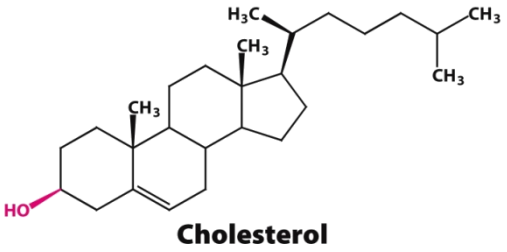


The only reaction in palmitate synthesis that is not carried out by fatty acid synthase itself is catalyzed by acetyl-CoA carboxylase



8 Ac-CoA + 7ATP + 14 NADPH + 14H+ --> palmitate + 8 CoA + 6H2O + 7 ADP + 7Pi + 14 NADP+

Biosynthesis of cholesterol

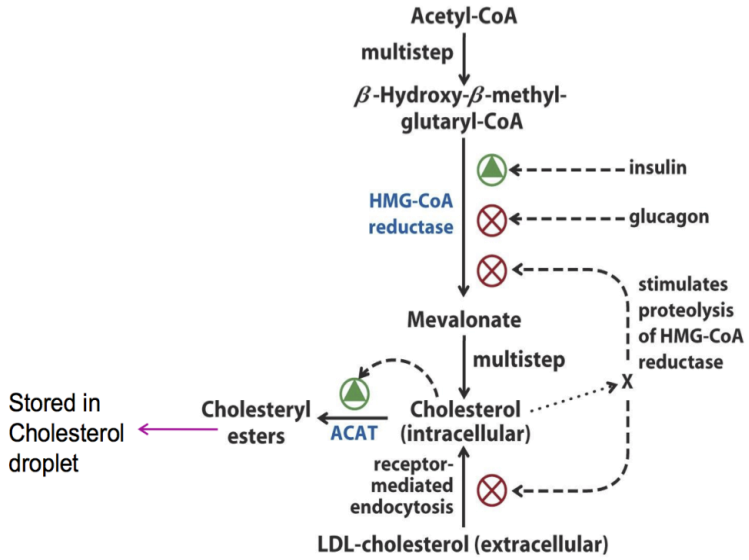


Cholesterol

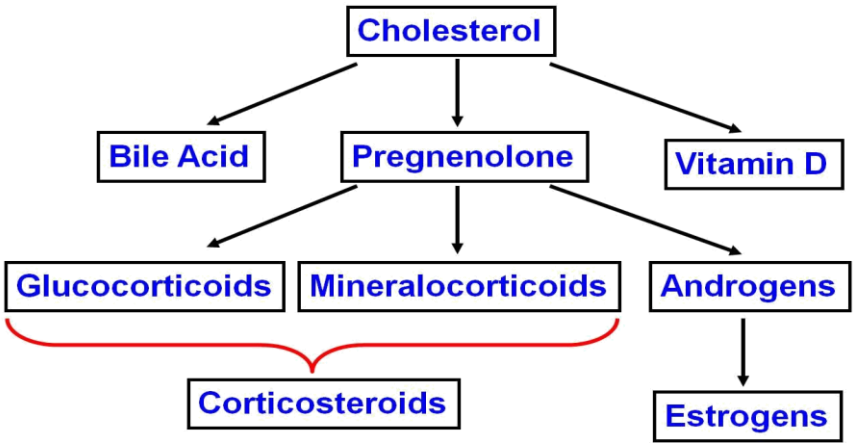
Cholesterol is known as a "sterol" because it is made out of a alcohol and steroid. Cholesterol is present in most animal membranes with varying amounts but is absent in prokaryotes and intracellular membranes. Stages of cholesterol synthesis Formation of mevalonate (6C) by the condesation of three acetate (2C) molecules. Formation of activated isoprene (5C) units (Δ3-Isopentenyl pyrophosphate and dimethylallyl pyrophosphate) from mevalonate.

Formation of squalene (30C) (6-isoprenes) by polymerisation of activated isoprene units.Formation of cholesterol by cyclization of squalene (to form the sterol nucleus) and a number of other reactions.

Regulation of cholesterol synthesis



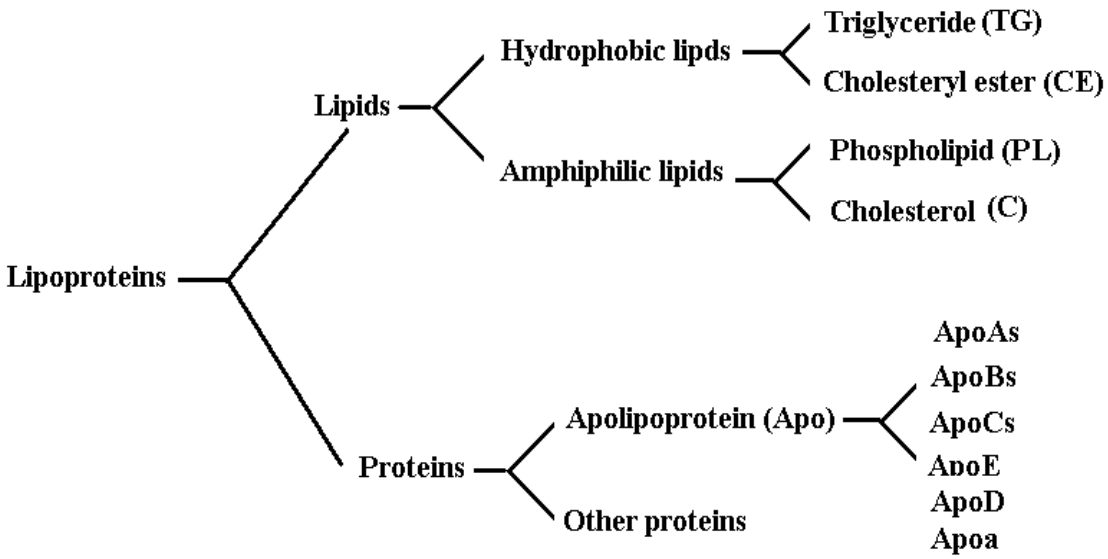
Functions of cholesterol



Lipoprotein metabolism, hypercholesterolemia

A lipoprotein is a biochemical assembly that contains both proteins and lipids. The lipids or their derivatives may be covalently or non-covalently bound to the proteins. Examples include the high density (HDL) and low density (LDL) lipoproteins which enable fats to be carried in the blood stream, the transmembrane proteins of the mitochondrion and the chloroplast, and bacterial lipoproteins function of lipoprotein particles is to transport water-insoluble lipids (fats) and cholesterol around the body in the blood.

Lipoprotein structure



Types of lipoproteins (by density)

Lipoproteins may be classified as follows, listed from larger and less dense ones to smaller and denser ones. Lipoproteins are larger and less dense, if they consist of more fat than of protein.

Chylomicrons carry triglycerides (fat) from the intestines to the liver, skeletal muscle, and to adipose tissue.

Very low density lipoproteins (VLDL) carry (newly synthesised) triglycerides from the liver to adipose tissue.

Low density lipoproteins (LDL) carry cholesterol from the liver to cells of the body. LDLs are sometimes referred to as the "bad cholesterol" liporpotein.

High density liporpoteins (HDL) collect cholesterol from the body's tissues, and bring it back to the liver. HDLs are sometimes referred to as the "good cholesterol" lipoprotein

Questions for self-control

1. Digestion of food lipids, role of bile acids in digestion and absorption. Chylomicrons as transporting form of exogenous lipids.
2. Triacylglycerol synthesis and breakdown in the adipose tissue, regulation and biological role of this processes.
3. Fatty acids biosynthesis: general description. Beta-oxidation of fatty acids: description of the process, its connections with Krebs cycle and respiratory chain.
4. Ketone bodies synthesis and oxidative breakdown, significance of their metabolism.
5. Lipid transport in blood. The structure, metabolism and biological functions of plasma lipoproteids.
6. Cholesterol biosynthesis, regulation of the process. Normal range of cholesterol level in blood plasma.
7. Hypercholesterolemia and atherosclerosis. Atherogenic coefficient

Lecture 9.

Nitrogen Metabolism

Annotation

1. Overview of nitrogen metabolism

Nitrogen is a critical chemical element in both proteins and nucleic acids, and thus every living organism must metabolize nitrogen to survive.

2. Essential and non-essential amino acids

Essential amino acids cannot be made by the body. As a result, they must come from food. "Non-essential" means thatour bodies produce an amino acid, even if we don't get it from the food we eat. Conditional amino acids are usually not essential, except in times of illness and stress.

3. Protein digestion and amino acid absorption

The digestion of protein entails breaking first into peptides and second into individual amino acids. The pepsins are enzymes secreted by the stomach in the presence of acid that breaks down proteins (proteolysis). Trypsin and chymotrypsin are pancreatic protease enzymes that are involved in protein digestion. The surface of intestinal epithelial

cells is rich in endopeptidases and aminopeptidases. The end products of cell surface digestion are free amino acids and di- and tripeptides.

4. Amino acid metabolism

Amino acids are depleted by three different routes: used to synthesize body proteins, used as a precursors of nitrogen containing small molecules, converted to glucose, glycogen, fatty acids or CO2.

5. Transamination and deamination reactions

Transamination, as the name implies, refers to the transfer of an amine group from one molecule to another. This reaction is catalyzed by a family of enzymes called transaminases or aminotransferases. Actually, the transamination reaction results in the exchange of an amine group on one acid with a ketone group on another acid.

6. Ammonia and its detoxification. Urea cycle.

The level of ammonia in the blood must be kept very low, because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system (CNS). There must, therefore, be metabolic mechanisms by which nitrogen is ultimately disposed to maintain low levels of circulating ammonia. One of them is urea synthesis in the liver (urea cycle).

Keywords

Nitrogen metabolism, essential amino acids, non-essential amino acids, proteolysis endopeptidases, aminopeptidases, transamination reactions, deamination reactions, ammonia and its detoxification, urea cycle.

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Plan.

1. Overview of nitrogen metabolism

2. Nitrogen balance
3. Essential and non-essential amino acids
4. Protein digestion and amino acid absorption
5. Amino acid metabolism
6. Transamination and deamination reactions
7. Ammonia and its detoxification
8. Urea cycle
9. Synthesis of amino acids
10. Purine and pyrimidine metabolism

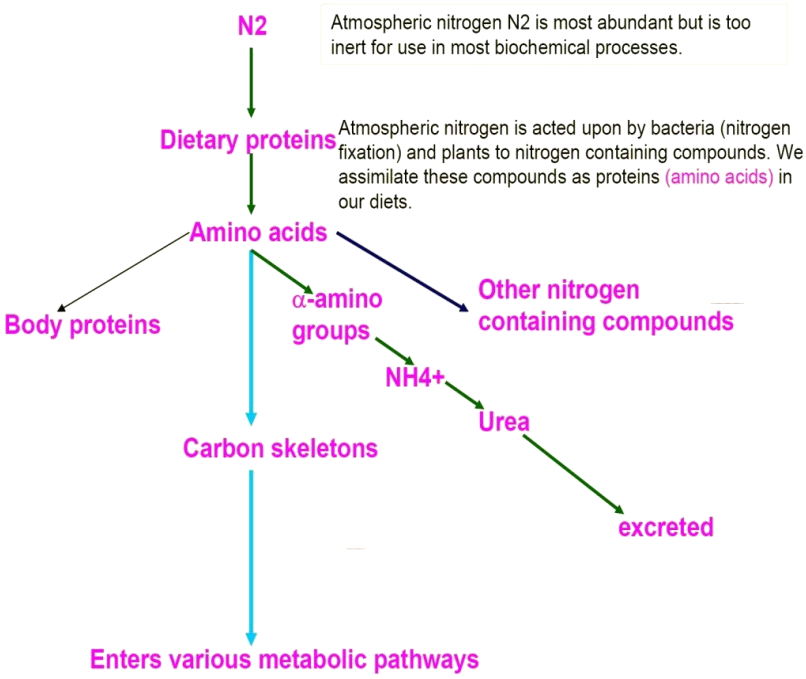
Lecture 9. Nitrogen Metabolism

Overview of nitrogen metabolism

Nitrogen is a critical chemical element in both proteins and nucleic acids, and thus every living organism must metabolize nitrogen to survive.

Humans are totally dependent on other organisms for converting atmospheric nitrogen into forms available to the body.

Nitrogen metabolism is no less important than carbohydrate and lipid metabolism. Proteins make up the structural tissue for muscles and tendons, transport oxygen or hemoglobin, catalyze all biochemical reactions as enzymes, and regulate reactions as hormones. Human bodies must be able to synthesize the many proteins, amino acids, and other non-protein nitrogen containing compounds needed for growth, replacement, and repair. Proteins in excess are used to supply energy or build reserves of glucose, glycogen, or lipids.



Nitrogen balance

In humans, reduced nitrogen enters the body through nitrogen containing foods such as proteins in our diet. Digestion of dietary protein begins in the stomach, where the proenzyme pepsinogen is converted to active pepsin and catalyzes the primary stage of proteolysis. However, the majority of proteolysis takes place in the duodenum, with the aid of pancreatic proteases. These proteases (serine protease and zinc peptidase) act as both endo- and exo-peptidases; aiding amino acid and peptide degradation. The amino acids and peptides produced are taken up by the mucosal wall enterocytes of the intestines. Amino acids are the building blocks of proteins and hence are essential to growth and repair of cells.

Nitrogen cannot be stored in the body like carbohydrates and fats. The rapid degradation of proteins means that deficiencies of just one amino acid can quickly limit essential protein synthesis. In order to maintain a healthy homeostasis of essential proteins in the body a **nitrogen balance** must be maintained – whereby nitrogen intake matches nitrogen excretion. Growth requires a positive nitrogen balance - in which the amount of nitrogen ingested exceeds that excreted. A disease can be caused by inadequate protein intake and/or deficiency of one or more essential

amino acids – termed a negative nitrogen balance. Nutritionally, the dietary proteins obtained from plants tend to be harder to digest and less concentrated than those from animal sources. Plant-sourced proteins are also more likely to be deficient in lysine, methionine and tryptophan residues.

A 70 kg person (154 lb) typically consumes 100 g protein per day.

To stay in nitrogen balance that person must excrete 100 g of N products per day.

The body makes 400 g of protein per day and 400 g are broken down.

300 g of amino acids are recycled into new protein, 100 g are degraded.

Total protein = 500 g/day, 400 g degraded, 400 resynthesized and 100 g catabolized.

Nitrogen metabolism is often referred to as protein or amino acid metabolism which denotes the various biochemical processes responsible for the synthesis and breakdown of proteins and amino acids.

Dietary proteins are first broken down to individual amino acids by various enzymes and hydrochloric acid present in the gastro-intestinal tract.

When proteins are digested or broken down, amino acids are left. The human body uses amino acids to make proteins to help the body:

- Break down food
- Grow
- Repair body tissue
- Perform many other body functions

Amino acids can also be used as a source of energy by the body.

Essential and non-essential amino acids

Amino acids are classified into three groups:

Essential amino acids

Non-essential amino acids

Conditional amino acids

Essential amino acids

Essential amino acids cannot be made by the body. As a result, they must come from food. The nine essential amino acids are: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

Non-essential amino acids

"Non-essential" means that our bodies produce an amino acid, even if we don't get it from the food we eat. They include: alanine, asparagine, aspartic acid, and glutamic acid.

Conditional amino acids

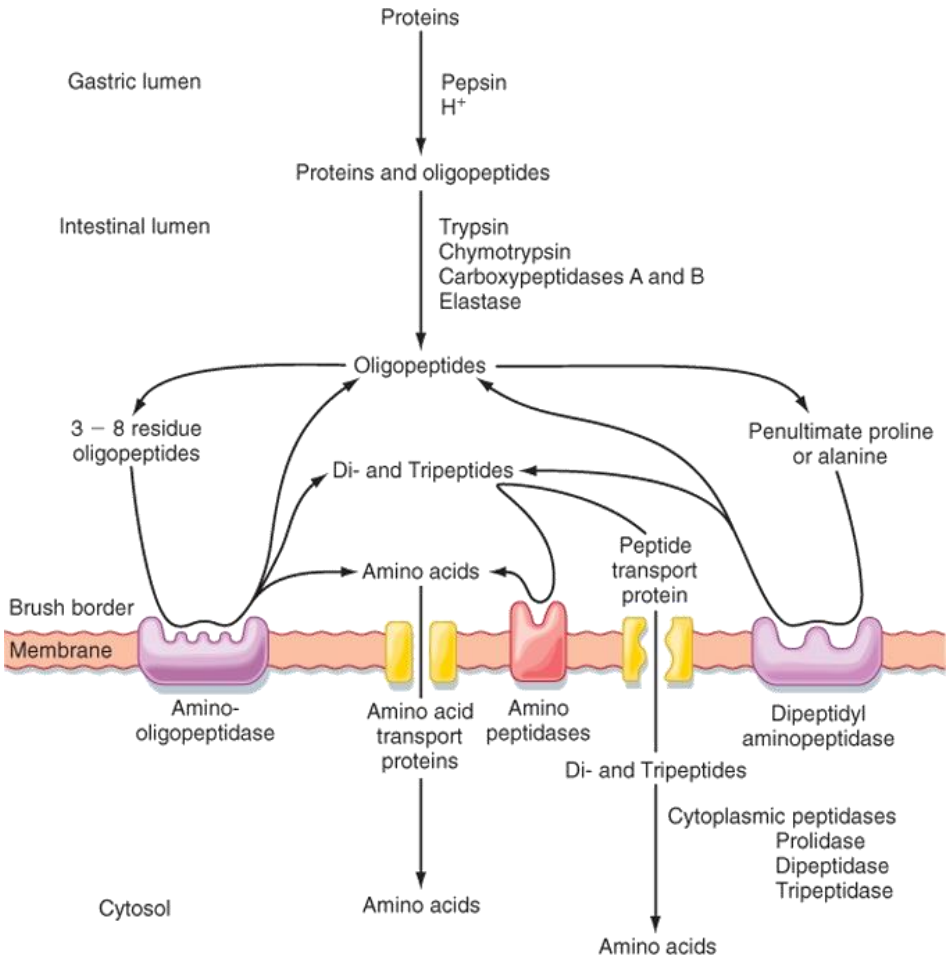
Conditional amino acids are usually not essential, except in times of illness and stress. They include: arginine, cysteine, glutamine, tyrosine, glycine, ornithine, proline, and serine.

Protein digestion and amino acid absorption

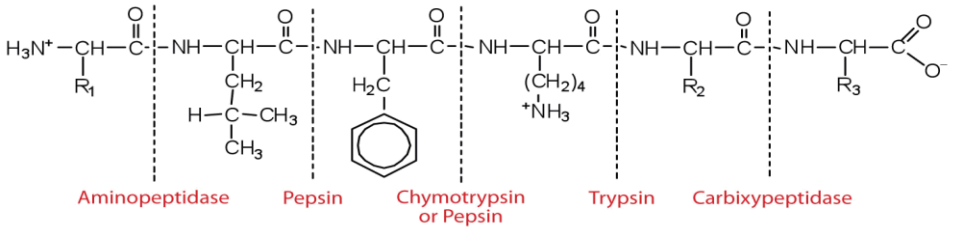
The digestion of protein entails breaking first into peptides and second into individual amino acids. The pepsins are enzymes secreted by the stomach in the presence of acid that breaks down proteins (proteolysis). The pepsins account for about 10 to 15 percent of protein digestion. Their ability to break down protein is restricted by the acidic environment with a pH between 1.8 and 3.5. The trypsins (proteolytic enzymes secreted by the pancreas) are much more powerful than pepsins, so the greater part of protein digestion occurs in the duodenum and upper jejunum. Therefore, even after total removal of the stomach, protein digestion usually is not impaired.

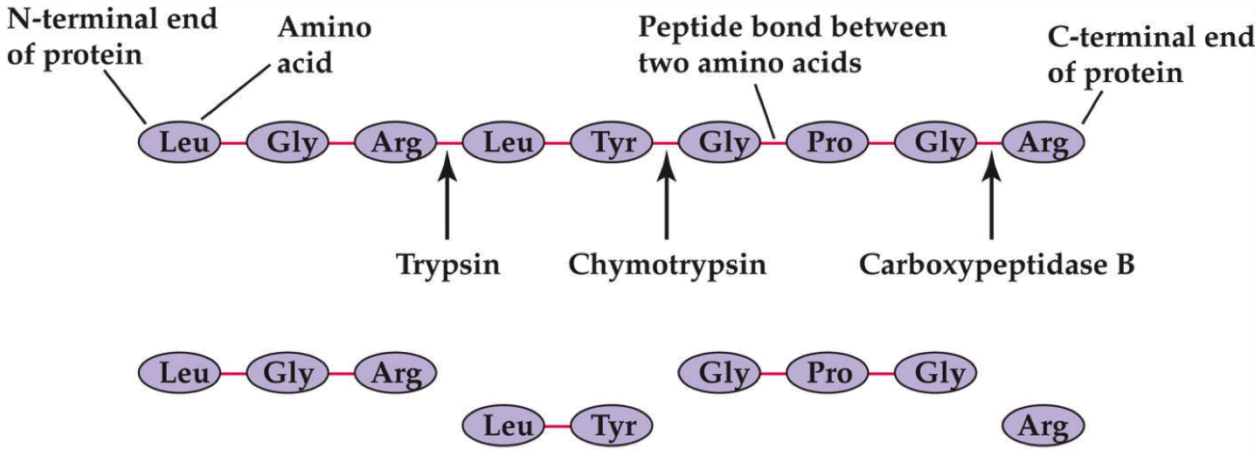
Trypsin and chymotrypsin are pancreatic protease enzymes secreted by the pancreas that are involved in protein digestion. From the stomach, protein digestion carries on in the duodenum.

As well as pepsin, trypsin continues the disintegration of proteins into amino acids. Hydrolysis involves the insertion of a water molecule between two amino acids, which forces the bond between them to break. Because amino acids have very small dimensions, they are able to penetrate the intestinal lining. Once in the bloodstream, amino acids are transported by blood plasma and red blood cells to various tissues, depending on where cell structures need to be created or repaired.

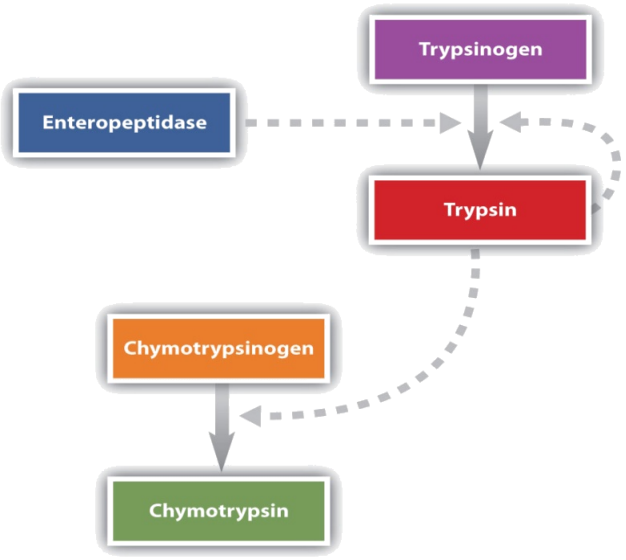


Proteolytic enzymes have different specificity for the amino acid R-group adjacent to the peptide bond.





Activation of zymogens

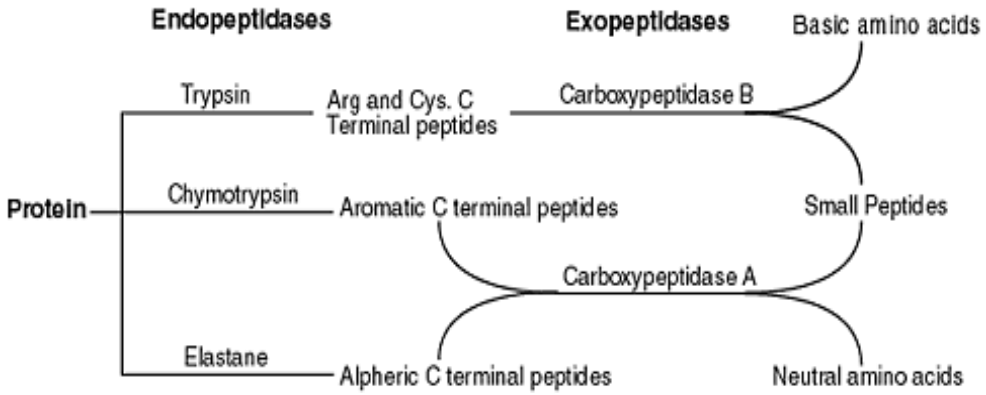


Digestion at the surface of intestinal epithelial cells

Since pancreatic juice does not contain appreciable aminopeptidase activity, final digestion of dipeptides and small peptides depends on brush border enzymes.

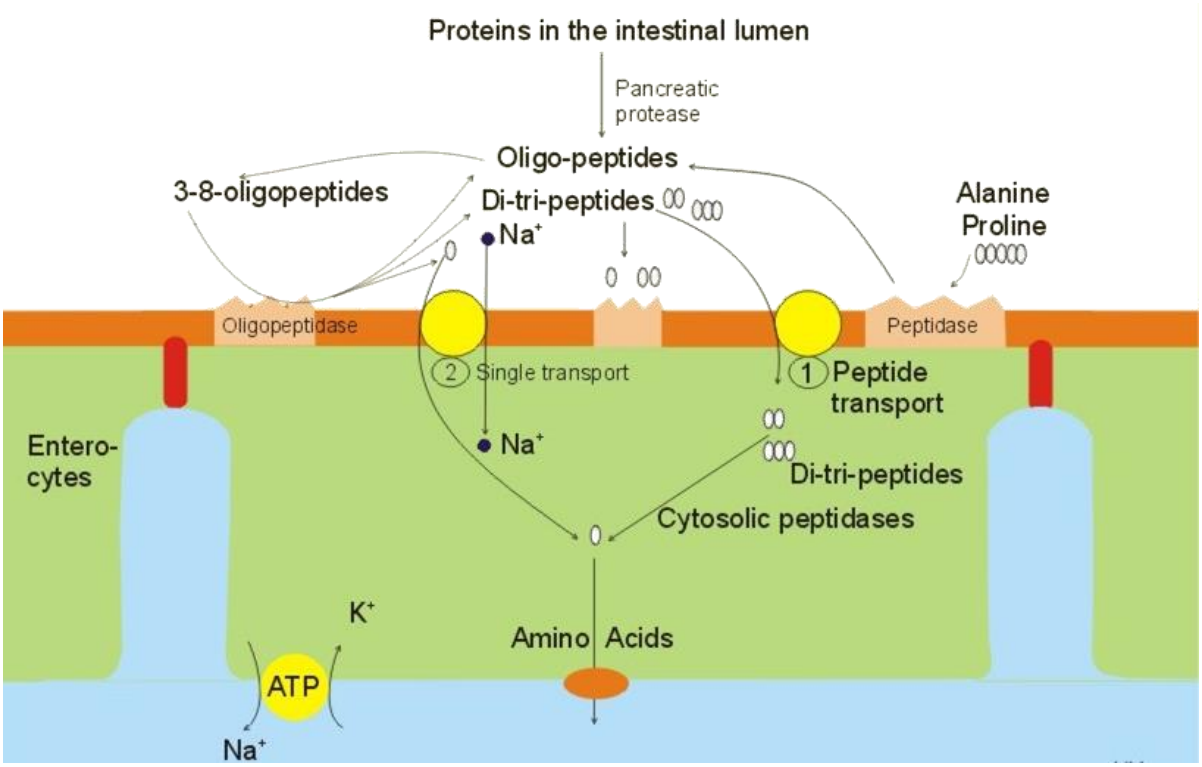
The surface of intestinal epithelial cells is rich in endopeptidases and aminopeptidases.

The end products of cell surface digestion are free amino acids and di- and tripeptides.



Absorption of amino acids

Free amino acids are taken into enterocytes by a Na+-linked secondary transport system. Di- and tripeptides are taken up by H+-linked transport system and hydrolyzed to free amino acids in cytosol and released into the portal system



Amino acid metabolism

Amino acid pool (in cells, blood, and extracellular fluids)

is supplied by three sources:

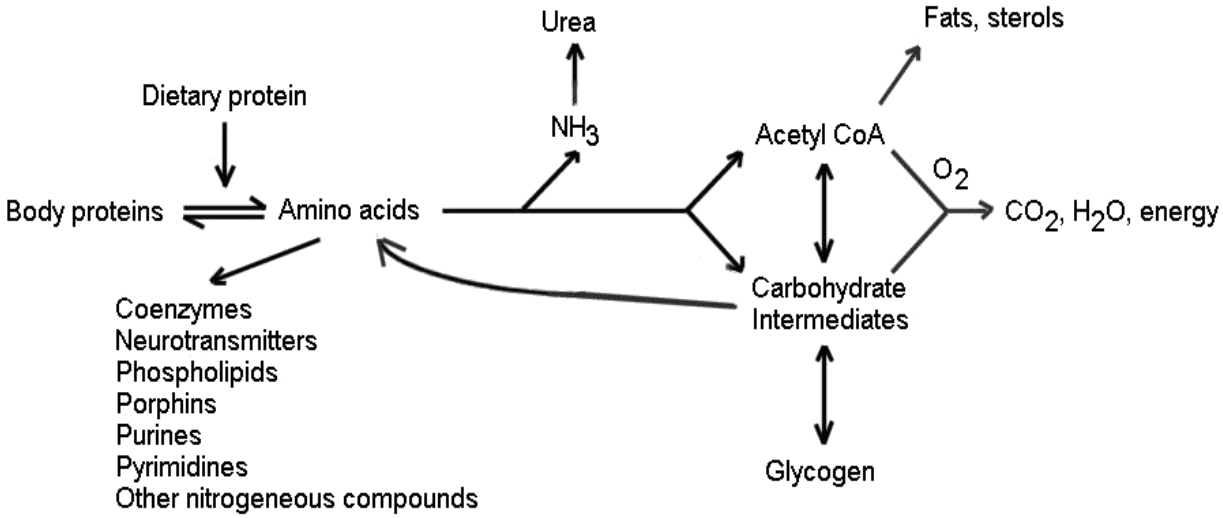
- Degradation of body proteins
- Derived from dietary proteins
- Synthesis of non-essential amino acids

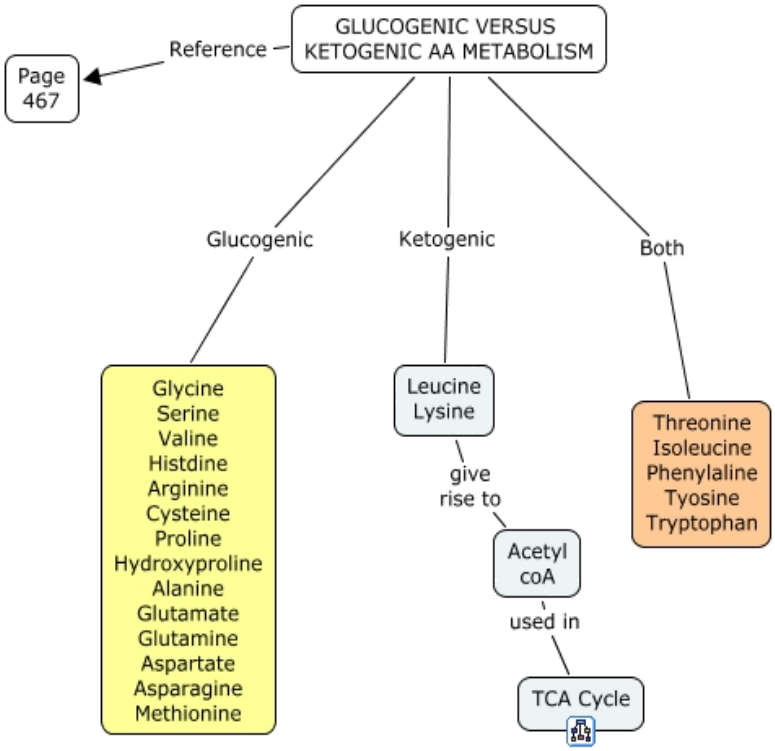
Amino acids are depleted by three different routes:

- Used to synthesize body proteins
- Used as a precursors of nitrogen containing small molecules (porphyrins, neurotransmitters, nucleotides

etc).

- Converted to glucose, glycogen, fatty acids or CO_2





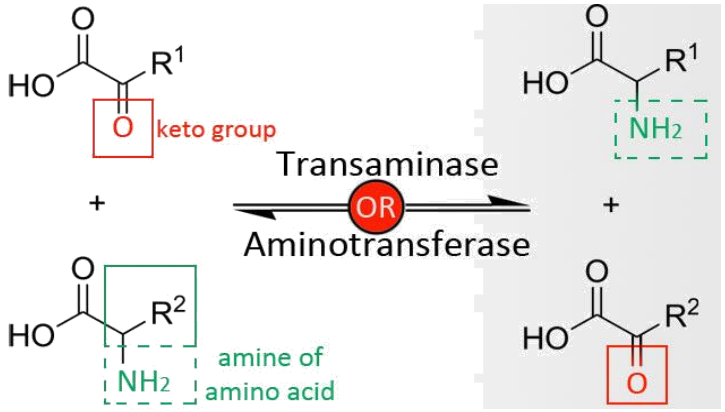
Transamination and deamination reactions

Transamination, as the name implies, refers to the transfer of an amine group from one molecule to another. This reaction is catalyzed by a family of enzymes called transaminases or aminotransferases. Actually, the transamination reaction results in the exchange of an amine group on one acid with a ketone group on another acid.

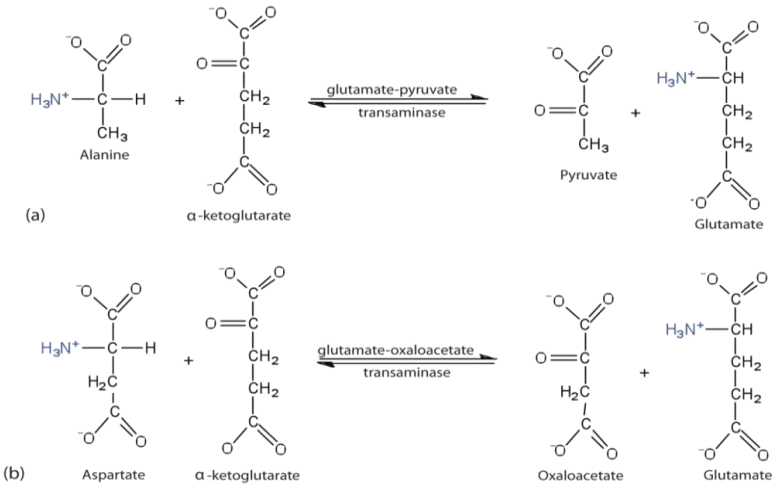
The most usual and major keto acid involved with transamination reactions is alpha-ketoglutaric acid, an intermediate in the citric acid cycle. A specific example is the transamination of alanine to make pyruvic acid and glutamic acid.

Other amino acids which can be converted after several steps through transamination into pyruvic acid include serine, cysteine, and glycine.

Transamination reactions are controlled by enzymes called aminotransferases or transaminases. These enzymes are responsible for the removal and attachment of amino groups to the α-carbons of amino acids and keto acids, with the aid of a vitamin B6-derived pyridoxal-phosphate coenzyme.



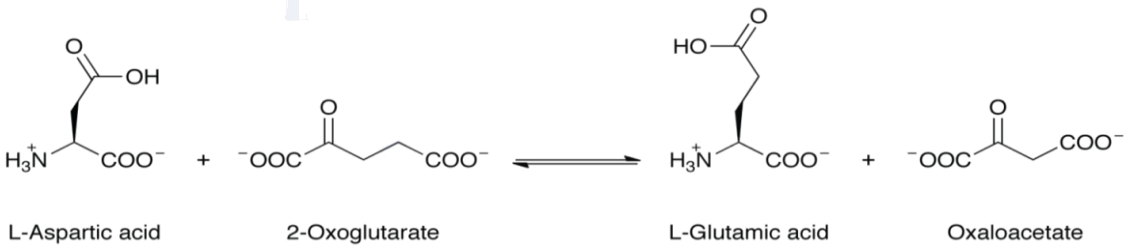
<u>Mechanism for transamination</u>

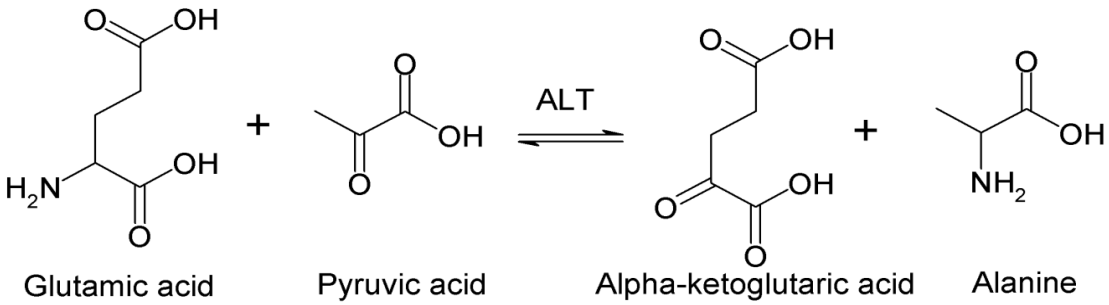


Glutamate is a major component of many aminotransferase reactions.

Aspartate aminotransferase (AST) and alanine aminotrasferase (ALT)

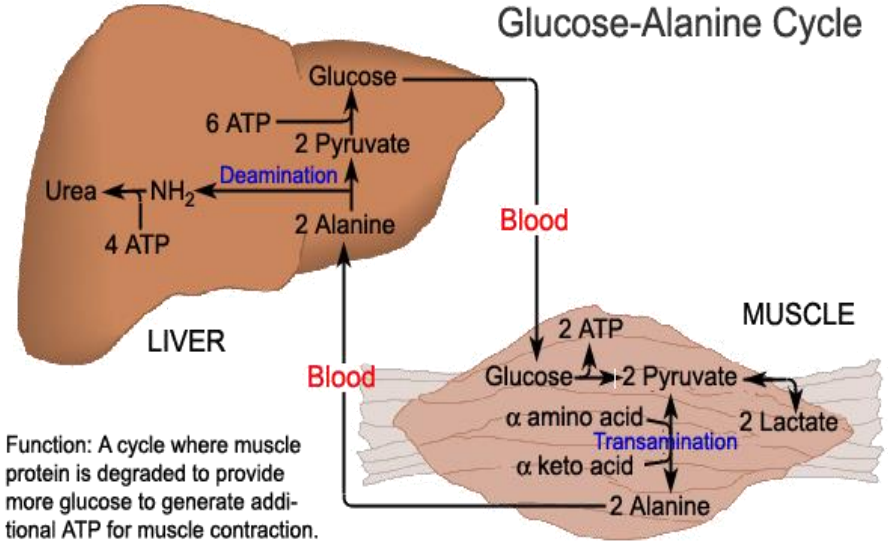
High serum levels of AST and ALT are reasonably sensitive indicators of liver damage or injury from different types of diseases or conditions.





Glucose-alanine cycle

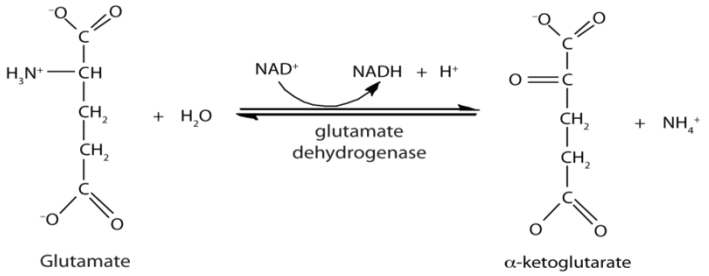
The principle role of the glucose-alanine cycle is to allow skeletal muscle to eliminate nitrogen whilst simultaneously replenishing its energy supply.



Indirect deamination via glutamate

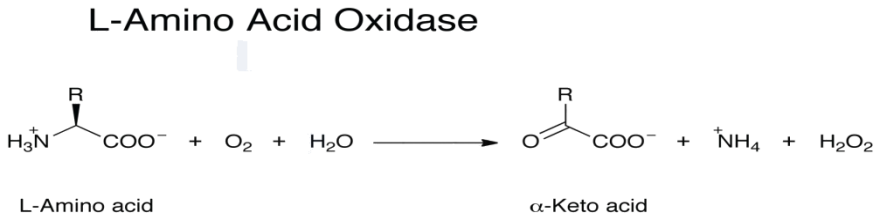
In liver, many of the amino groups are removed from α-amino acids by transamination to form glutamate.

Glutamate is transported from cytosol to mitochondria where it undergoes oxidative deamination by glutamate dehydrogenase (transdeamination of amino acids).

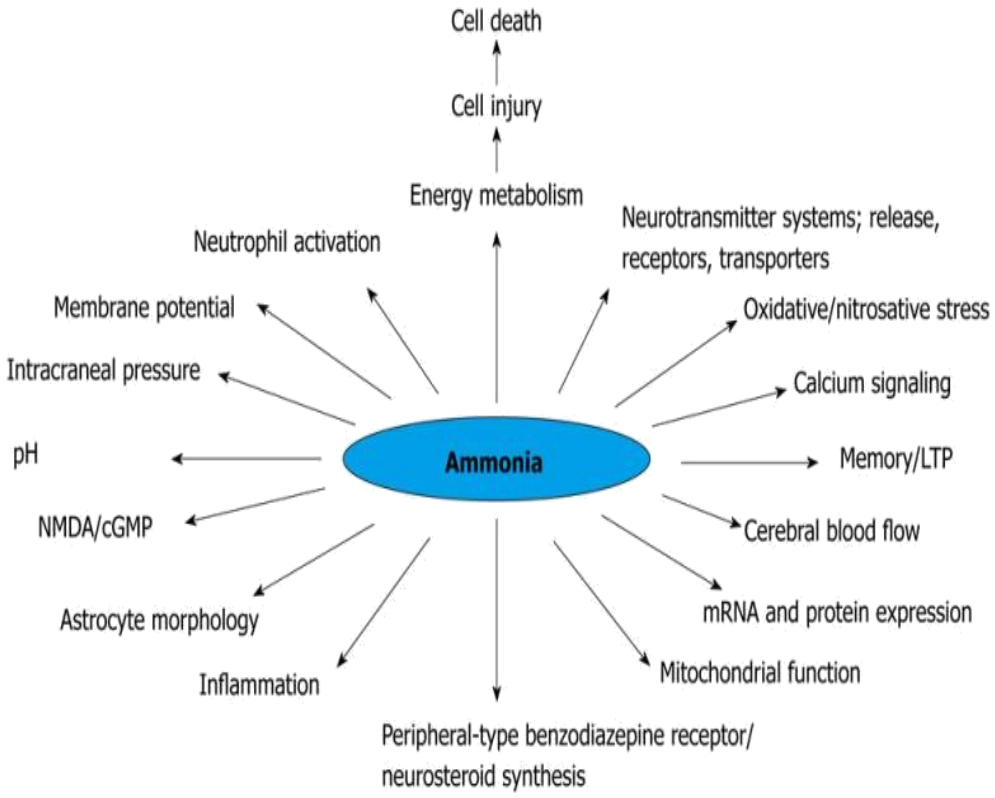


Direct oxidative deamination

Deamination is also an oxidative reaction that occurs under aerobic conditions in all tissues but especially the liver. During oxidative deamination, an amino acid is converted into the corresponding keto acid by the removal of the amine functional group as ammonia and the amine functional group is replaced by the ketone group. The ammonia eventually goes into the urea cycle.



Ammonia and its detoxification



Amino acids are quantitatively the most important source of ammonia, because most Western diets are high in protein and provide excess amino acids, which travel to the liver and undergo transdeamination— the linking of *aminotransferase* and *glutamate dehydrogenase* reactions—producing ammonia. However, substantial amounts of ammonia can be obtained from other sources.

Sources of ammonia

From amino acids.

From glutamine: Glutamine hydrolysis by glutaminase (from kidney and intestine) form ammonia. From kidney, ammonia is excreted into the urine.

From bacterial action in the intestine: Ammonia is formed from urea by the bacterial urease. This ammonia is absorbed from the intestine by the way of portal vein and then converted in to urea in liver.

From amines: amines from diet, and the monoamines that serves as hormones or neurotransmitters gives rise to ammonia by the action of amine oxidase.

From purines and pyrimidines: In the catabolism of purines and pyrimidines, amino groups attached to the rings are released as ammonia

Detoxification of ammonia

The level of ammonia in the blood must be kept very low, because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system (CNS). There must, therefore, be metabolic mechanisms by which nitrogen is ultimately disposed to maintain low levels of circulating ammonia. One of them is urea synthesis in the liver (urea cycle) and the other is glutamine synthesis in the brain

Ammonia transport

Urea: Formation of urea in the liver is the major route of disposal for ammonia.

Glutamine: It is a nontoxic storage and transport form of ammonia.

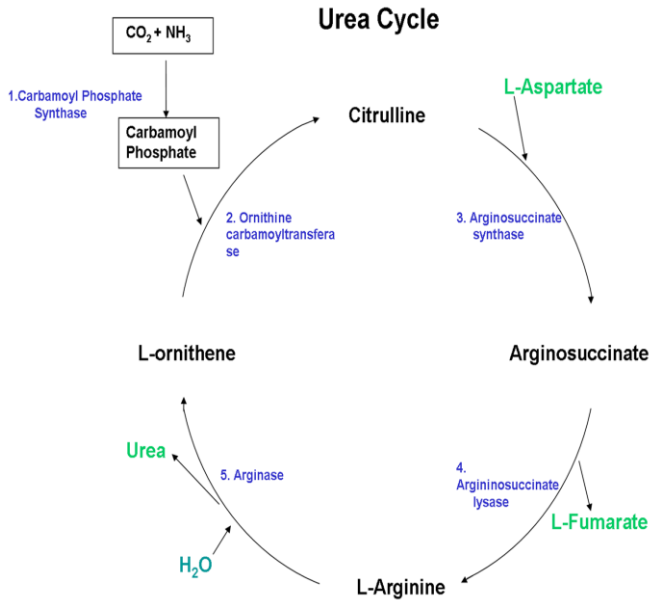
Urea cycle

The urea cycle (also known as the ornithine cycle) is a cycle of biochemical reactions occurring in many animals that produces urea from ammonia (NH_3).

The urea cycle consists of five reactions: two mitochodrial and three cytosolic. The cycle converts two amino groups, one from NH4+ and one from Asp, and a carbon atom from HCO3−, to the relatively nontoxic excretion product urea at the cost of four "high-energy" phosphate bonds (3 ATP hydrolyzed to 2 ADP and one AMP). Ornithine is the carrier of these carbon and nitrogen atoms.

One turn of the cycle:

- consumes 2 molecules of ammonia
- consumes 1 molecule of carbon dioxide
- creates 1 molecule of urea ((NH2)2CO
- regenerates a molecule of ornithine for another turn.



The urea cycle (part I)

The urea cycle runs only in the liver. It begins with the incorporation of ammonia into carbamoylphosphate by the corresponding synthetase. This reaction occurs in three successive steps. The first step uses ATP to activate bicarbonate to carbonylphosphate, which then captures free ammonia to form carbamate. Another ATP-dependent step activates that intermediate to carbamoylphosphate. The carbamoyl group will find its way into the urea that is produced by the urea cycle.

The urea cycle (part II)

The subsequent reactions in the urea cycle are as follows:

1. The carbamoyl group is transferred from carbamoylphosphate to the δ-amino group of ornithine, a non-standard amino acid homologous to lysine, by ornithine transcarbamylase. This reaction yields citrulline.

2. Citrulline and aspartate form argininosuccinate, catalyzed by argininosuccinate synthetase. This reaction again requires ATP, which is converted to AMP in the process.

3. Argininosuccinate is cleaved to fumarate and arginine by argininosuccinase.

4. Urea is released from arginine by arginase, which regenerates ornithine and closes the cycle.

Stoichiometry of the urea cycle

$$\text{Aspartate} + NH_3 + CO_2 + 3\ ATP \rightarrow$$

$$\text{Urea} + \text{Fumarate} + 2\ ADP + AMP + 2\ P_i + PP_i + 3\ H_2O$$

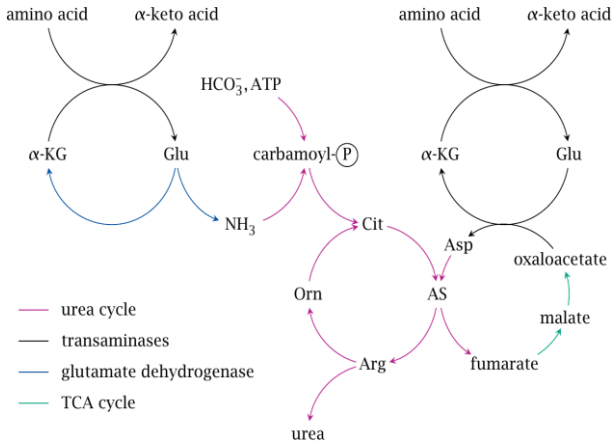
Four high energy phosphates are consumed in the synthesis of each molecule of urea.

Two ATPs are required to make carbomoyl phosphate.

One ATP is required to make argininosuccinate.

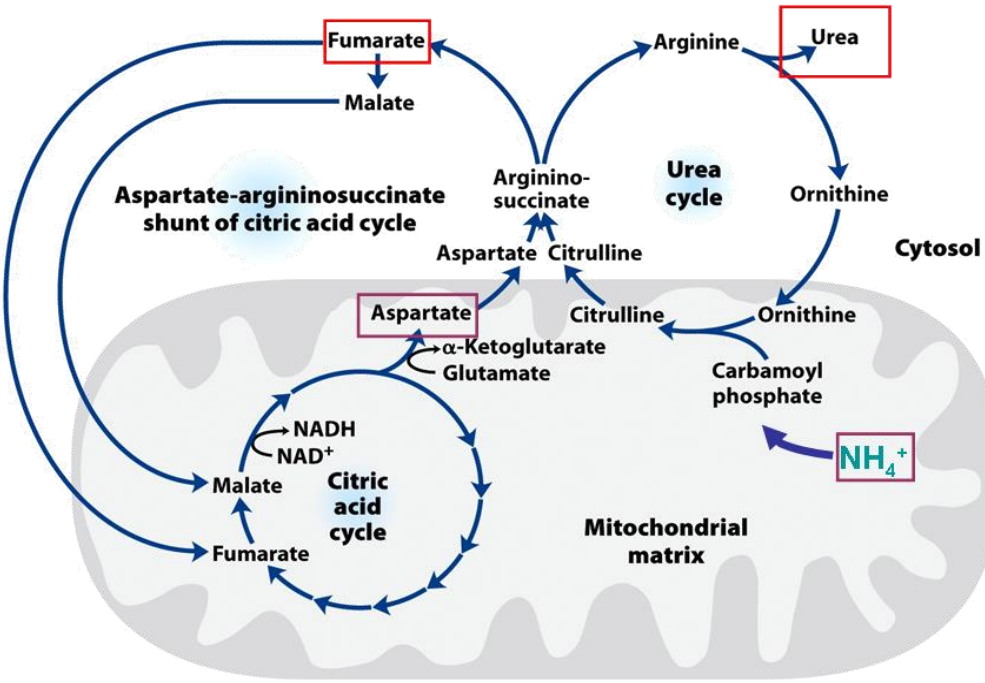
Two ATPs are required to restore 2 ADPs to two ATPs + two to restore AMP to ATP

Krebs cycle and ornithine cycle

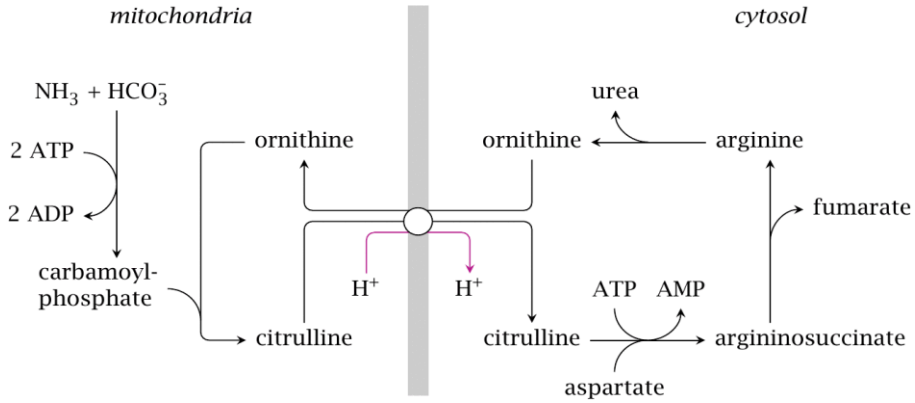


- The urea cycle and the tricarboxylic acid cycle are coupled together through fumarate and aspartate.
- Fumarate is the precursor to oxaloacetate

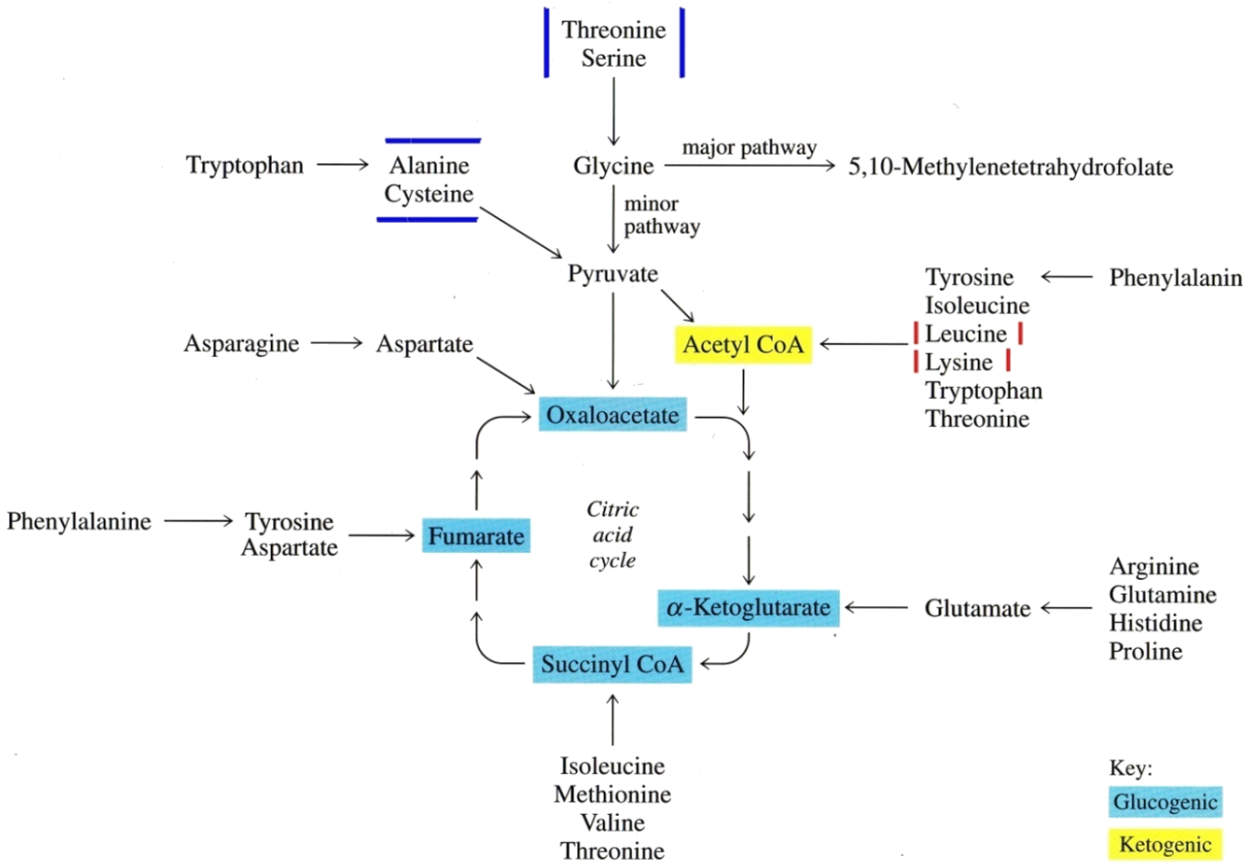
- Oxaloacetate can: be transaminated to aspartate and feed back into urea cycle condense with AcCoA and feed into citric acid cycle proceed into gluconeogenesis be converted to pyruvate.



The urea cycle spans mitochondria and cytosol



Degradation of the carbon skeleton of amino acids



Synthesis of amino acids

Synthesis of non-essential amino acids

Ignoring tyrosine (as it's immediate precursor is phenylalanine, an essential amino acid), all of the non-essential amino acids (and we will include arginine here) are synthesized from intermediates of major metabolic pathways. Furthermore, the carbon skeletons of these amino acids are traceable to their corresponding alpha-ketoacids. Therefore, it could be possible to synthesize any one of the nonessential amino acids directly by transaminating its corresponding a-ketoacid, if that ketoacid exists as a common intermediate.

Three very common alpha-ketoacids can be transaminated in one step to their corresponding amino acid:

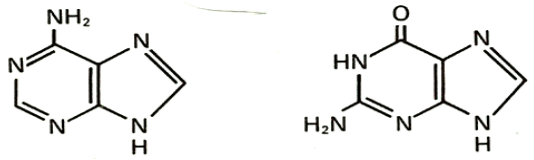
Pyruvate (glycolytic end product) --> alanine

Oxaloacetate (citric acid cycle intermediate) --> aspartate

a-ketoglutarate (citric acid cycle intermediate) --> glutamate

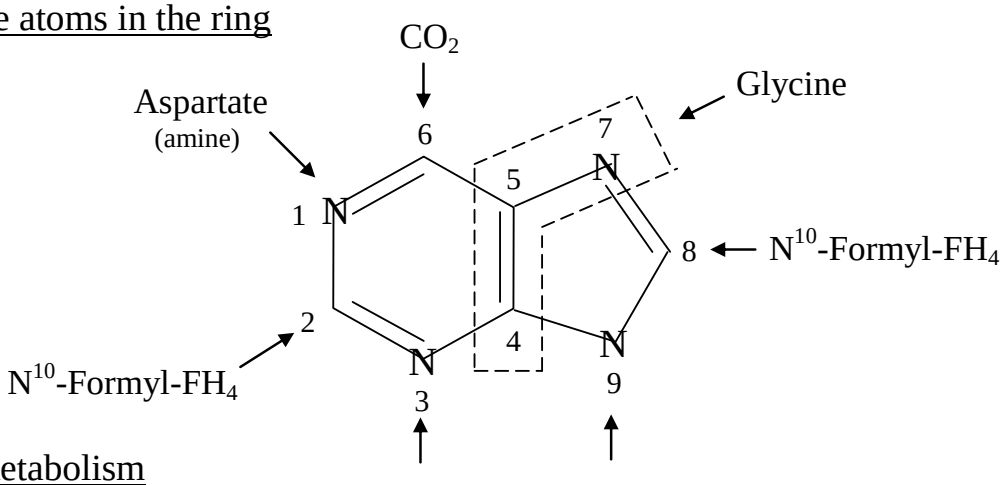
Purine and pyrimidine metabolism

Purine metabolism

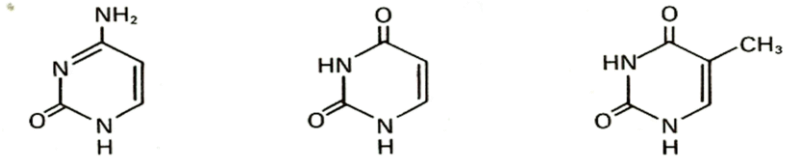


Adenine (A) Guanine (G) Major purine bases

Sources of the atoms in the ring

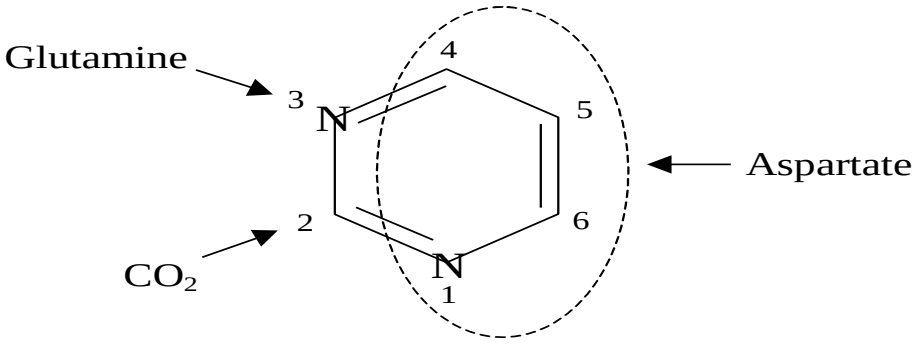


Pyrimidine metabolism

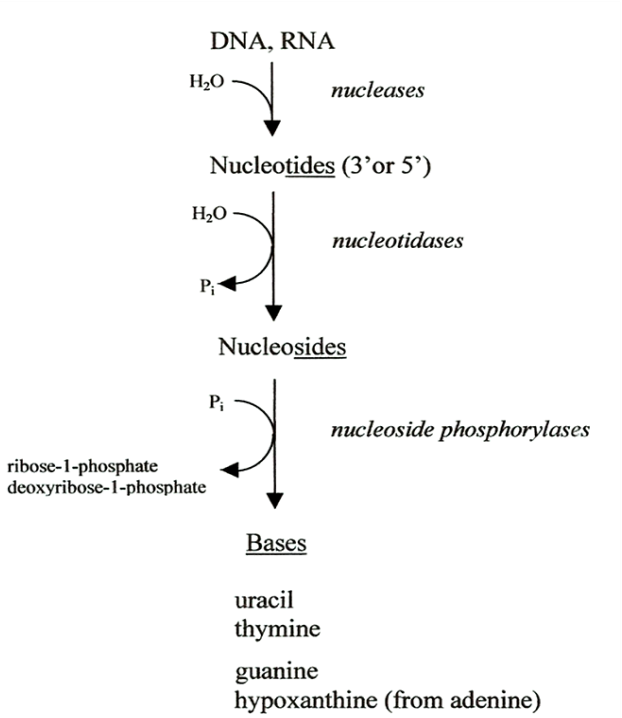


Cytosine (C) Uracil (U) Thymine (T) Major pyrimidine bases

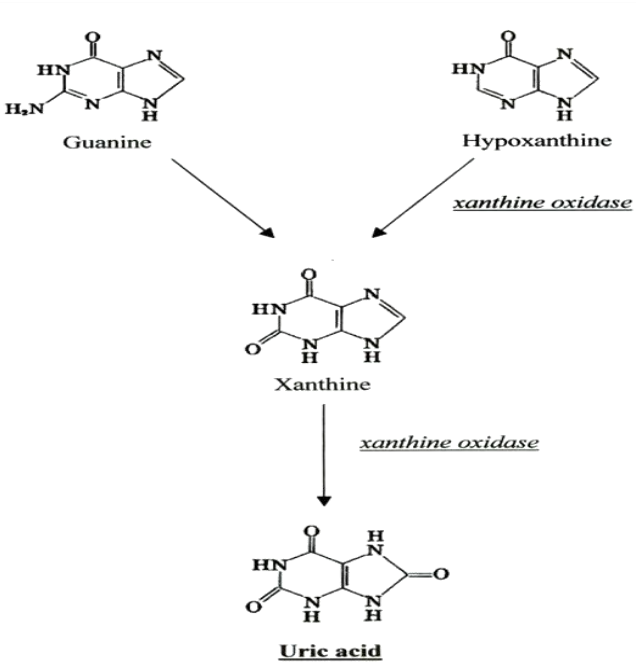
Sources of the atoms in the ring



DNA and RNA degradation



Degradation of purines



Questions for self-control

1. Deamination of aminoacids. Description of direct and indirect oxidative deamination. Transreamination, its biological function.
2. Toxicity of ammonia. Inactivation of ammonia in the body: safe transport of ammonia from the tissues to liver and kidney. The final detoxication of ammonia.
3. Decarboxilation of aminoacids. Biogenic amines, their functions. Inactivation of biogenic amines.
4. Metabolism of one-carbon units, the role of folic acid and cobalamine. The consequences of these vitamines deficiency.
5. Metabolism of aromatic aminoacids: Phe, Tyr. Phenylketonuria.

Lecture 10.

Flow of Genetic Information

Annotation

1. The central dogma of molecular biology

DNA- RNA - Protein

2. Replication of DNA

Replication is a complex process whereby the ―parent‖ strands of DNA in the double helix are separated, and each one is copied to produce a new (daughter) strand. DNA replication occurs during the S Phase of the cell cycle. It is semi-conservative, i.e. it produces two copies that each contain one of the original strands and one new strand. It takes place in a 5' to 3' direction with a leading strand and a lagging strand (which is discontinuous), and the use of an RNA primer.

3. DNA repair mechanisms

Since many mutations are deleterious, DNA repair systems are vital to the survival of all organisms.Living cells contain several DNA repair systems that can fix different type of DNA alterations.

4. Synthesis of RNA (transcription). mRNA processing.

Transcription is the first step in decoding a cell's genetic information. During transcription, enzymes called RNA polymerases build RNA molecules that are complementary to a portion of one strand of the DNA double helix. Transcription is the synthesis of RNA from a DNA template. RNA synthesis is catalyzed by RNA polymerase, which pries the DNA strands apart and hooks together the RNA nucleotides. The RNA is complementary to the DNA template
strand.

5. The codon message

Three-base segments of mRNA that specify amino acids are called codons. The three-base segments of DNA that the codons are transcribed from are called triplets. The genetic code refers to the relationship between the nucleotide base sequence of DNA (the triplets), the corresponding codons of mRNA, and the amino acids for which the codons code.

6. Translation of mRNA (initiation, elongation, termination)

After the transcription of DNA to mRNA is complete, translation — or the reading of these mRNAs to make proteins — begins. The synthesis of proteins (translation) is catalyzed by the ribosome.

7. Eukariotyc gene expression

The sequence of nucleotides in each gene contains information for assembling the string of amino acids that make up a single protein molecule. Proteins fold into complex, three-dimensional shapes to become key cell structures and regulators of cell functions.

Key words

DNA, RNA, replication, DNA repair mechanisms transcription, mRNA processing, codon message, initiation, elongation, termination, translation.

Recommendations: The goal of the course entitled “Biochemistry” is to get knowledge about basic chemical reactions underlying the life.

Working with the literature is better to begin with going through the lectures. You should read carefully with a pencil in your hand and mark of three types: what is clear, what needs to be specified, and what is totally unclear. Then you should open a textbook and find answers to your questions followed by putting down commentaries to your lectures. After that you can go to the unclear items using actively the recommended literature and consulting with a mentor.

Internet sources

http://www.biochemweb.org/

http://www.1lec.com/Biochemistry/

http://www.bioch.ox.ac.uk/

http://www.biology.arizona.edu/biochemistry/biochemistry.html

http://pubs.acs.org/journal/bichaw

http://en.wikipedia.org/wiki/Biochemistry

http://www.biochemistry.org/

http://themedicalbiochemistrypage.org/

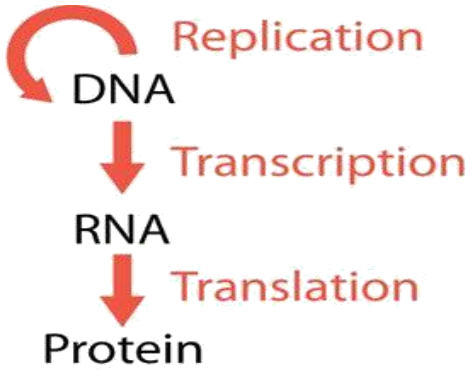
http://biochem.stanford.edu/

Plan.

1. The central dogma of molecular biology
2. Replication of DNA
3. DNA repair mechanisms
4. Synthesis of RNA (transcription)
5. mRNA processing
6. From transcription to translation
7. The codon message
8. Translation of mRNA (initiation, elongation, termination)
9. Eucariotyc gene expression

Lecture 10. Flow of Genetic Information

10. 1. The central dogma of molecular biology



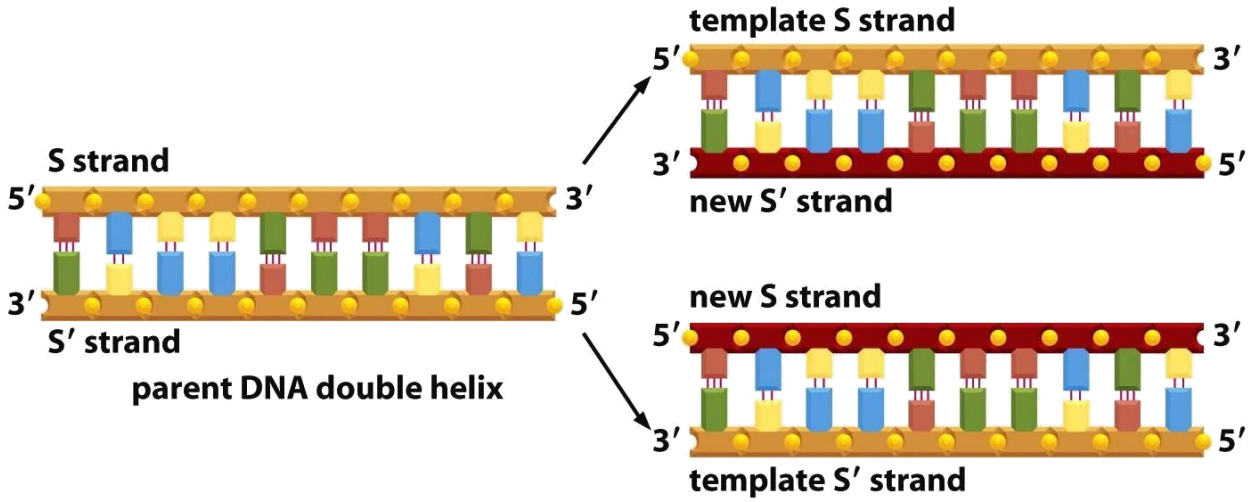
The central dogma of molecular biology, showing the flow of genetic information via the three fundamental processes of **replication, transcription, and translation**.

Replication of DNA

Before a cell can divide, it must duplicate all its DNA. In eukaryotes, this occurs during S phase of the cell cycle.

Replication is a complex process whereby the "parent" strands of DNA in the double helix are separated, and each one is copied to produce a new (daughter) strand. This process is said to be "semiconservative" because one strand from each parent is conserved and remains intact after replication has taken place.

The DNA being replicated must be in a ready state for the start of replication, and there also has to be a clear start point from which replication proceeds. As each piece of DNA must only be copied once, there also has to be an end point to replication. DNA replication must be carried out accurately, with an efficient proof reading and repair mechanism in place for any mismatches or errors. And finally, the system of replication must also be able to distinguish between the original DNA template and then newly copied DNA.



- DNA replication begins with the "unzipping" of the parent molecule as the hydrogen bonds between the base pairs are broken.
- Once exposed, the sequence of bases on each of the separated strands serves as a template to guide the insertion of a complementary set of bases on the strand being synthesized.
- The new strands are assembled from deoxynucleoside triphosphates.
- Each incoming nucleotide is covalently linked to the "free" 3' carbon atom on the pentose as the second and third phosphates are removed together as a molecule of pyrophosphate (PPi).
- The nucleotides are assembled in the order that complements the order of bases on the strand serving as the template.
- Thus each C on the template guides the insertion of a G on the new strand, each G a C, and so on.

- When the process is complete, two DNA molecules are formed identical to each other and to the parent molecule.

Replication enzymes and proteins

DNA Polymerase: Matches the correct nucleotide and then joins adjacent nucleotides together.

Primase: Provides and RNA primer to start polymerisation.

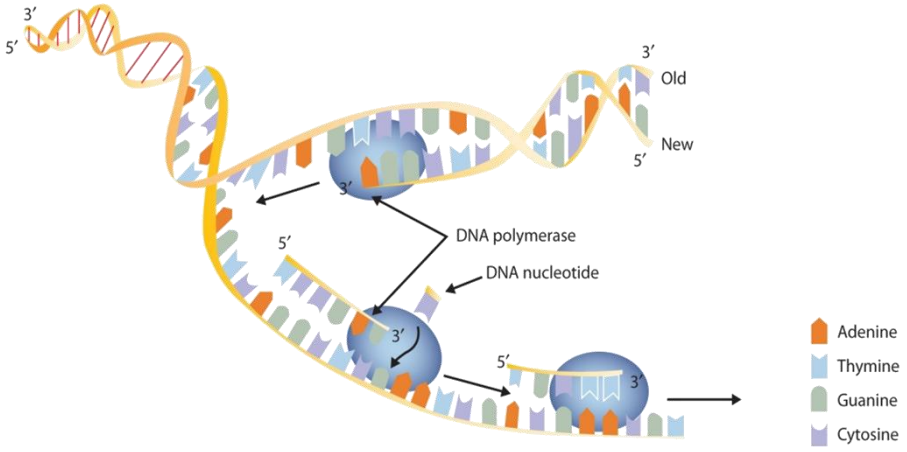
Ligase: Joins adjacent DNA strands together.

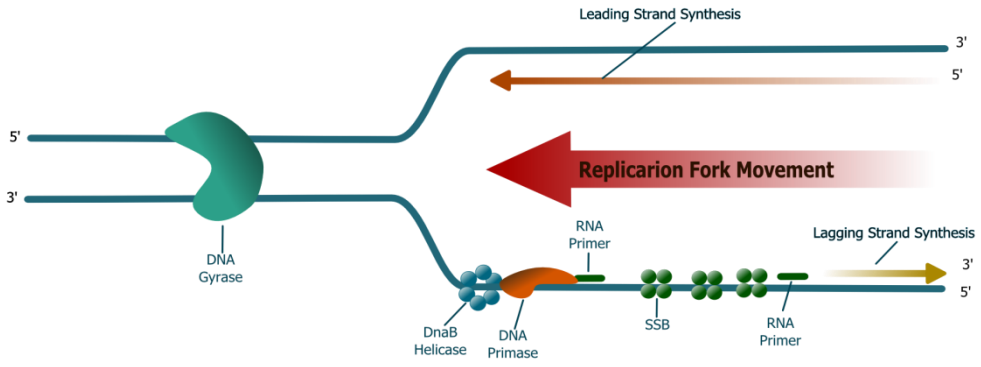
Helicase: Unwinds the DNA and melts it.

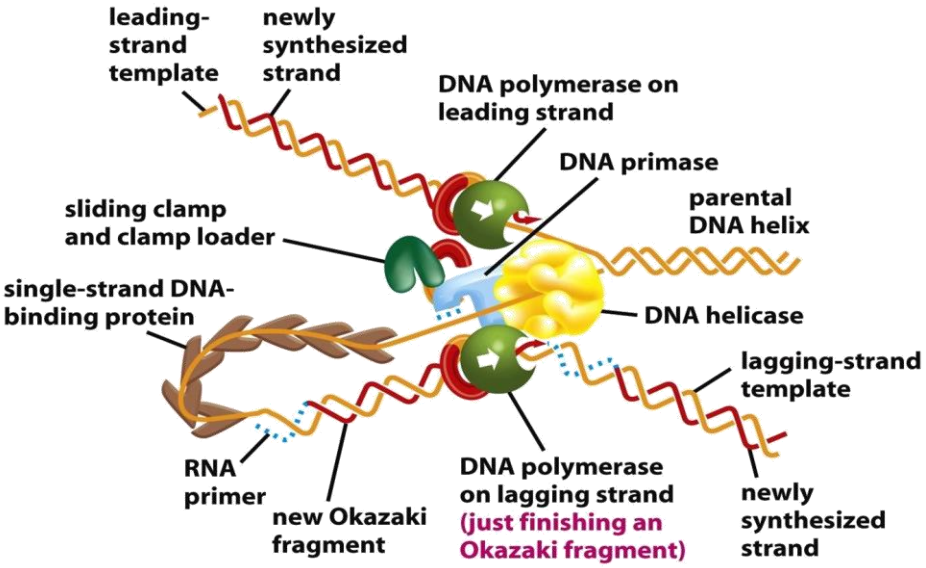
Single Strand Binding Proteins: Keep the DNA single stranded after it has been melted by helicase.

Gyrase: A topisomerase that relieves torsional strain in the DNA molecule.

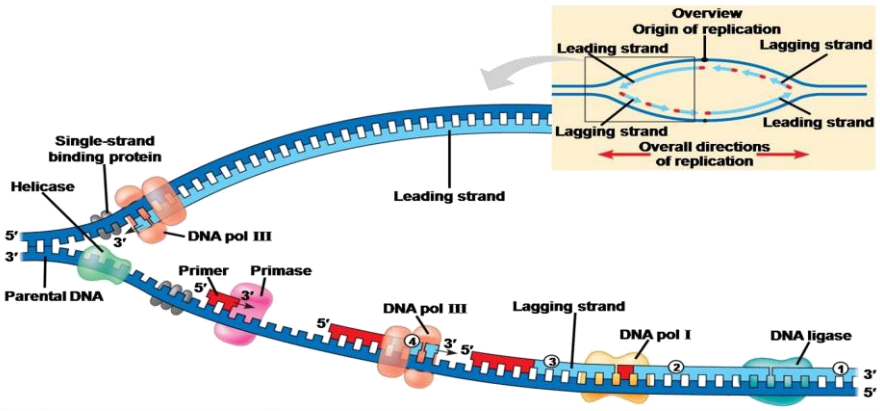
Telomerase: Finishes off the ends of the DNA strand.

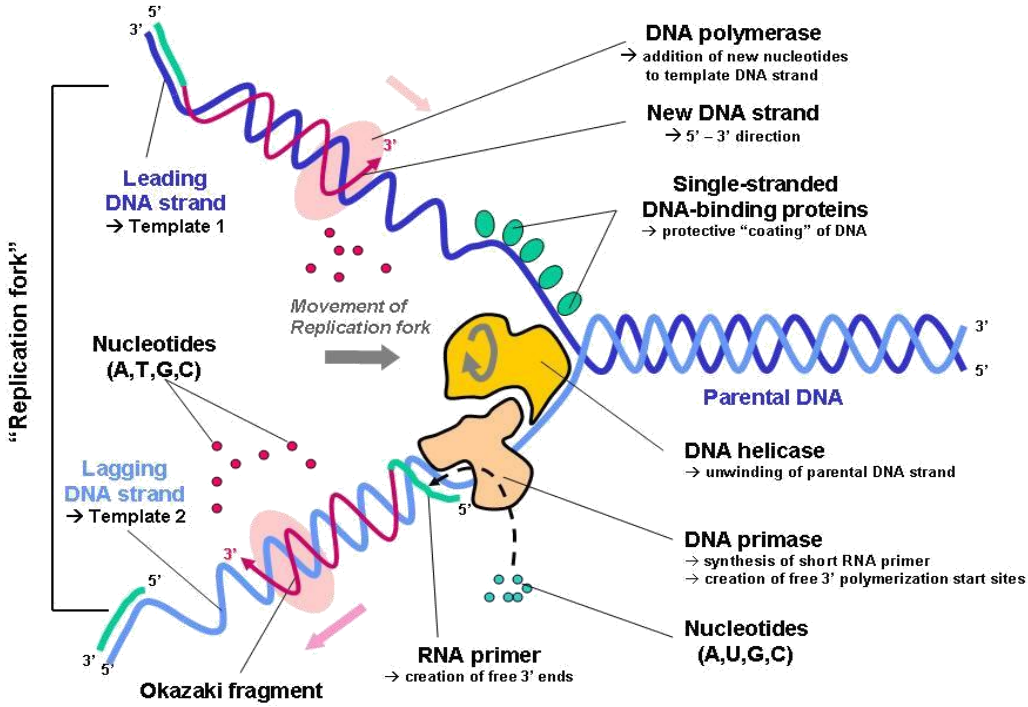






The average human chromosome contains 150 x 10^6 nucleotide pairs which are copied at about 50 base pairs per second. The process would take a month (rather than the hour it actually does) but for the fact that there are many places on the eukaryotic chromosome where replication can begin. Replication begins at some replication origins earlier in S phase than at others, but the process is completed for all by the end of S phase. As replication nears completion, "bubbles" of newly replicated DNA meet and fuse, finally forming two new molecules.





DNA replication (summary)

DNA replication occurs during the S Phase of the cell cycle.

It is semi-conservative, i.e. it produces two copies that each contain one of the original strands and one new strand.

It takes place in a 5' to 3' direction with a leading strand and a lagging strand (which is discontinuous), and the use of an RNA primer

In bacteria, there is only a single origin of replication.

In eukaryotes, there are multiple origins of replication

Replication is bi-directional.

DNA repair mechanisms

Since many mutations are deleterious, DNA repair systems are vital to the survival of all organisms.

Living cells contain several DNA repair systems that can fix different type of DNA alterations.

DNA repair mechanisms fall into 2 categories:

- repair of damaged bases,
- repair of incorrectly basepaired bases during replication.

In most cases, DNA repair is a multi-step process:

- an irregularity in DNA structure is detected,
- the abnormal DNA is removed,
- normal DNA is synthesized.

Cells possess a large number of different types of repair systems. Those repair systems can be grouped into main several broad categories.

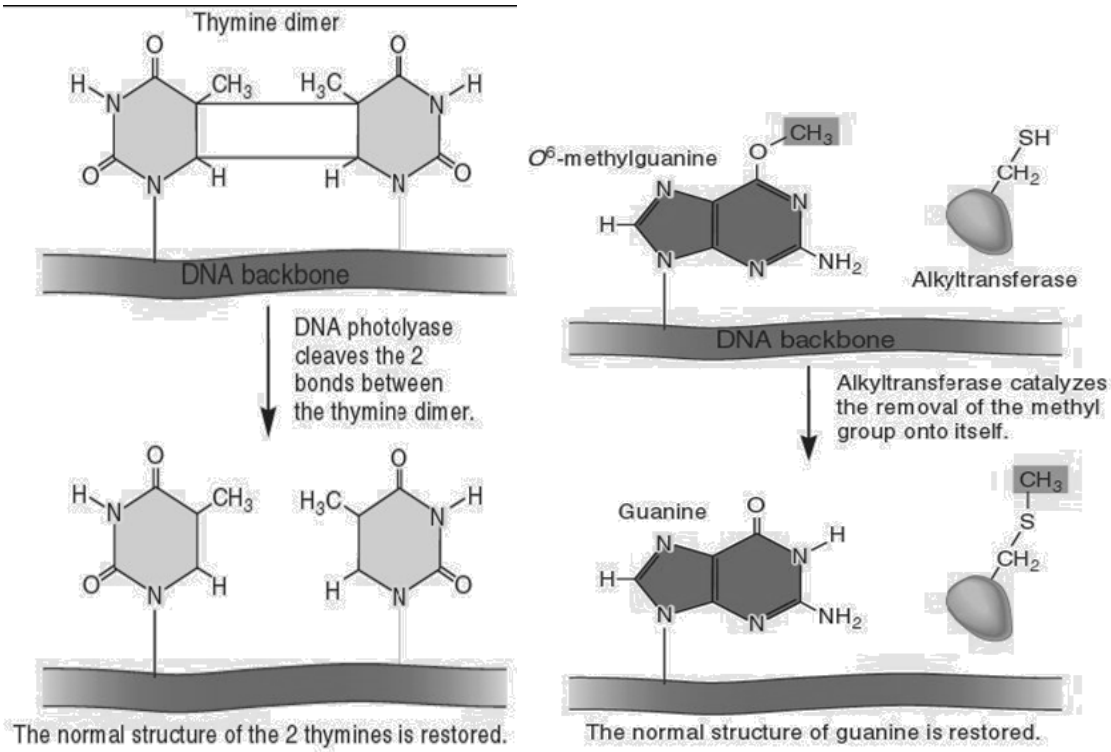
Direct reversal of damage – as the name suggests, these systems act directly on damaged nucleotides, converting each one back to its original structure.

Excision of damaged region, followed by precise replacement:

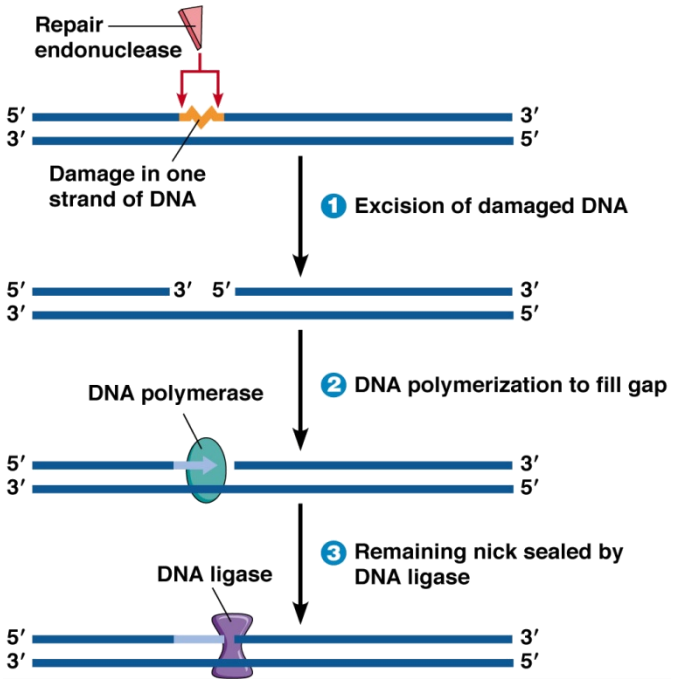
- base excision repair;
- nucleotide excision repair;
- mismatch repair.

Recombination repair is used to mend double-strand breaks.

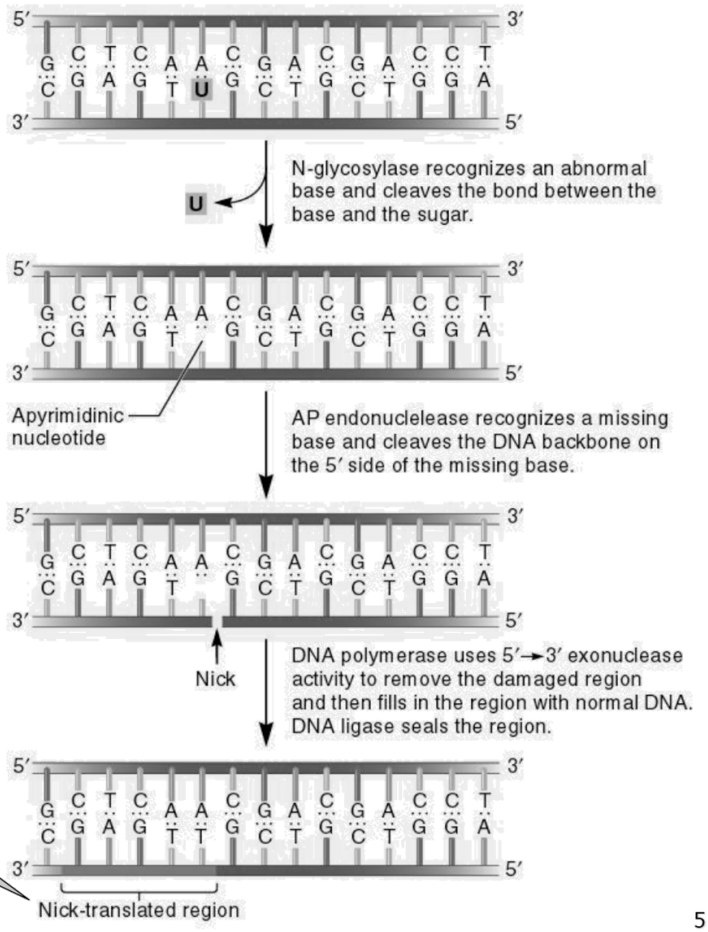
Damage tolerance - tries to minimize the effects of damage that has not been repaired.



DNA excision repair



Base Excision Repair System



Depending on whether a purine or pyrimidine is removed, this creates an apurinic and an apyrimidinic site, respectively

Nick replication would be a more accurate term

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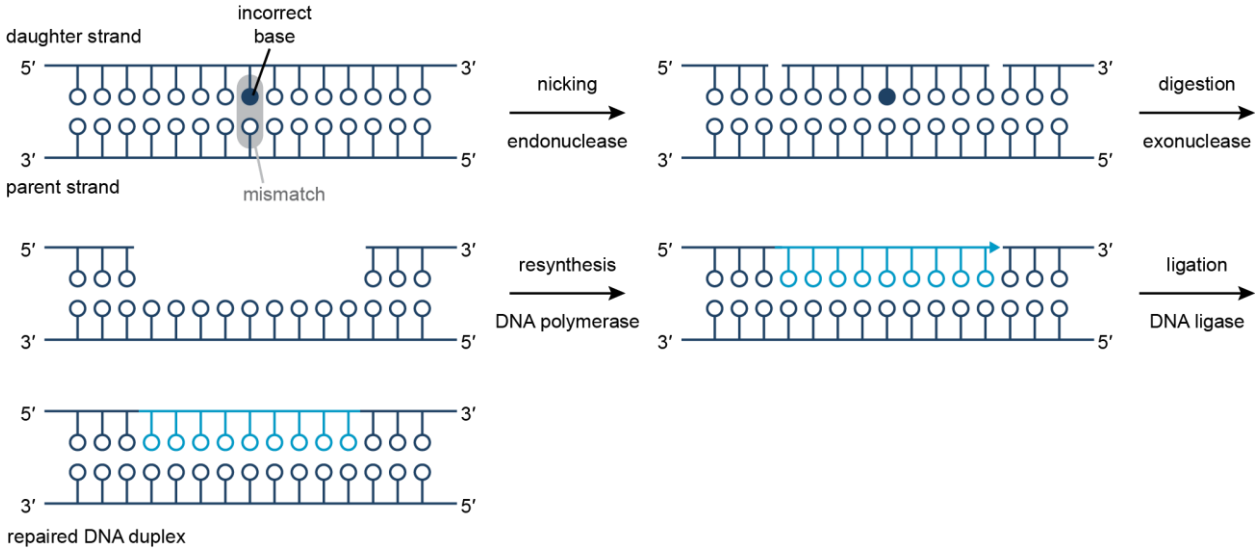


All major DNA repair mechanisms take advantage of the fact that DNA is double-stranded and strands are complementary. Therefore if damage is present in just one strand, the damage can be accurately repaired by excision and replacing it with new DNA synthesized using the complementary strand as template.

Base excision repair

Repair of small, non-bulky DNA lesions (methylated, oxidised, reduced bases).

Modified or damaged base is removed by a DNA glycosylase , creating an apurinic or apyrimidinic (AP) site.

AP-deoxyribose is then released by AP exonucleases. Missing nucleotide replaced by DNA polymerase and ligated.

No known human diseases associated with defects in base excision.

3 main steps:

1. Removal of the incorrect base by an appropriate DNA N-glycosylase to create an AP site. AP site is identical to one created by spontaneous base loss.

2. Nicking of the damaged DNA strand by AP endonuclease upstream of the AP site, thus creating a 3'-OH terminus adjacent to the AP site.

3.Extension of the 3'-OH terminus by a DNA polymerase, accompanied by excision of the AP site

Nucleotide excision repair

Nucleotide excision repair is a more flexible damage repair mechanism. It is used to correct more extreme forms of damage: closslinks, bases modified by addition of

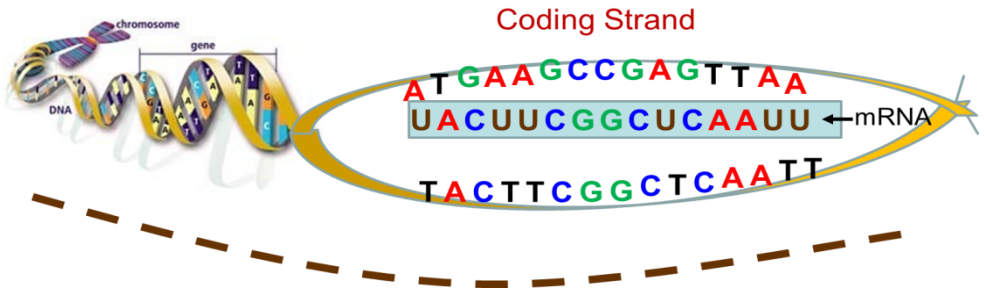
large chemical groups, etc. Generally, it recognizes damaged regions based on their abnormal structure as well as on their abnormal chemistry, then excises and replaces them.

It uses different enzymes.

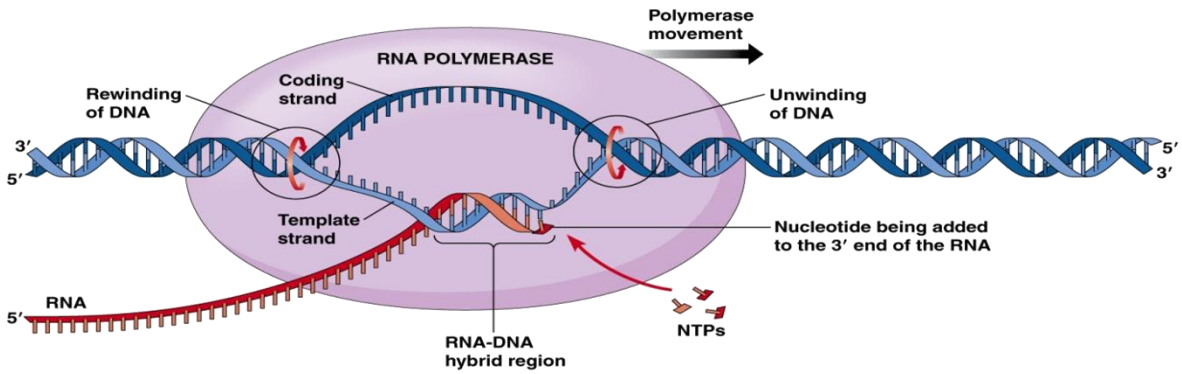
Even though there may be only a single "bad" base to correct, its nucleotide is removed along with many other adjacent nucleotides; that is, nucleotide excision repair removes a large "patch" around the damage.

Flow of info from DNA to RNA

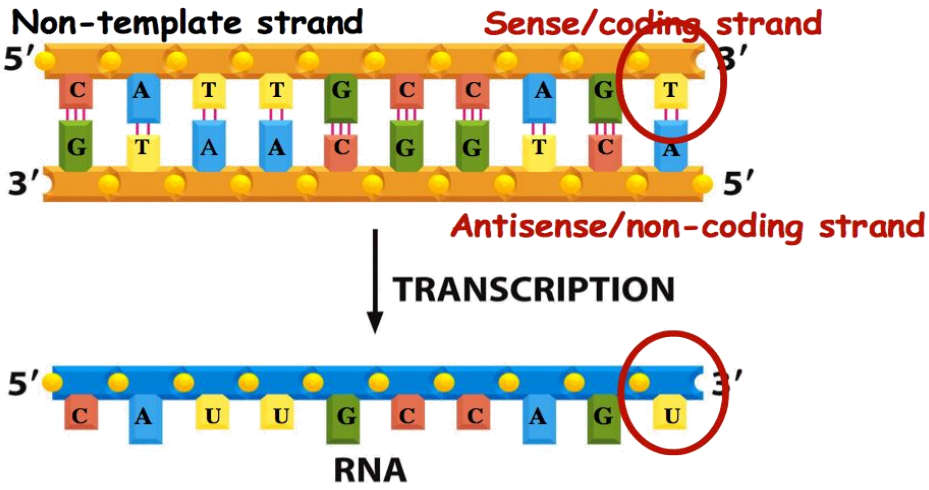
Transcription is the first step in decoding a cell's genetic information. During transcription, enzymes called RNA polymerases build RNA molecules that are complementary to a portion of one strand of the DNA double helix.



Synthesis of RNA (transcription)



- Transcription is the synthesis of RNA from a DNA template.
- RNA synthesis is catalyzed by RNA polymerase, which pries the DNA strands apart and hooks together the RNA nucleotides.
- The RNA is complementary to the DNA template strand.



- RNA synthesis follows the same complementary base-pairing rules as both sides of the DNA molecule do, except for uracil (U) substitutes for thymine (T) in RNA molecules.
- A multi-protein subunit called RNA polymerase is responsible for synthesising all three classes of RNA molecules found in prokaryotes: rRNA, mRNA, and tRNA. mRNA is the RNA you're used to - the one that codes for proteins - but rRNA, or ribosomal RNA, and tRNA are instrumental in the cell too.

Transcription has three main events.

Initiation

The template strand is transcribed while the coded strand is not transcribed

RNA polymerase: enzyme that catalyzes formation of RNA from a DNA template. Nucleotides are added to the 3' end of the growing polymer.

RNA polymerase binds to promoter region: sequence of nucleotides in DNA that indicate where the RNA polymerase complex should bind to initiate transcription.

Elongation

RNA polymerase works along DNA molecule, synthesizing strand of mRNA that's complementary to template strand of DNA.

Transcribe only one strand of DNA template (no Okazaki fragment).

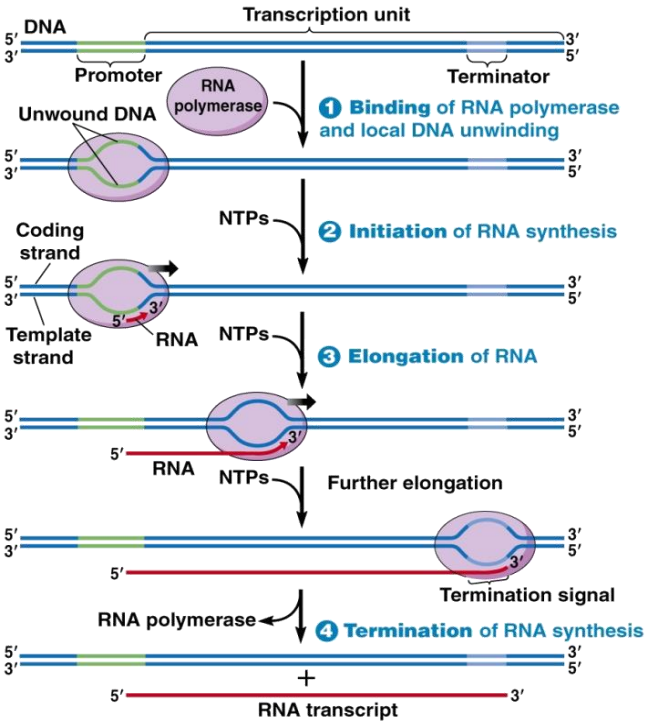
1. RNA polymerase moves along DNA.   2. RNA polymerase binds promoter region and starts synthesis of another mRNA molecule.

Termination

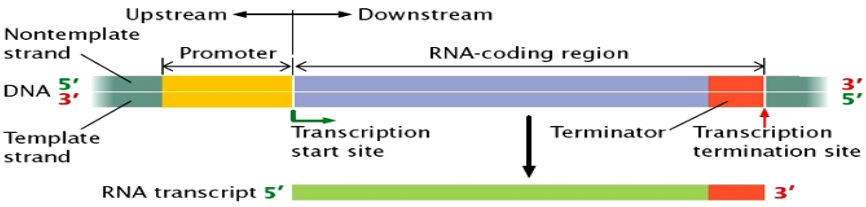
Specific nucleotide sequences in template DNA serve as signal to STOP transcription.

When RNA complex reach this signal, it detaches from the DNA strand.

mRNA strand is released from transcription assembly, and double helix reforms.

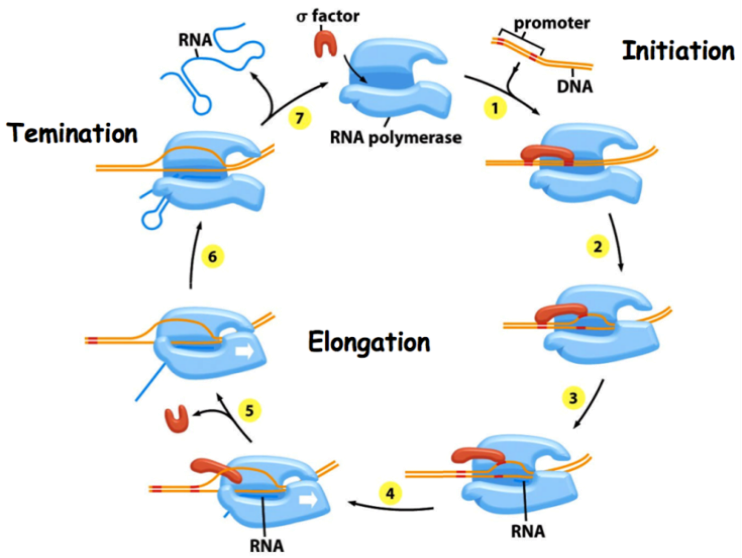


The transcription unit

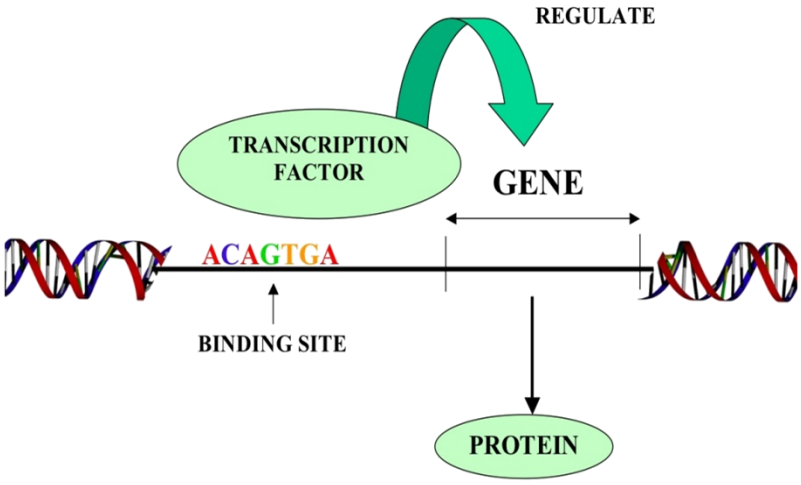


The transcription unit is the stretch of DNA that is used in transcription.

The transcription cycle



Transcription factors



A transcription factor is any protein other than RNA-polymerase that is required for transcription.

Functions of transcription factors:

bind to RNA-polymerase,

bind another transcription factor,

bind to cis-acting DNA sequences.

RNA-polymerase and the group of protein that directly interact with it are called the basal transcription apparatus. This is the apparatus that is directly responsible for transcription.

Basal transcription apparatus = RNA polymerase + general factors; both needed to initiate transcription.

Regulators of gene transcription

Promoters

Enhancers

- Upstream sequences in eukaryotes that help to control the expression of genes
- Can be thousands of nucleotides away from the protein-coding region

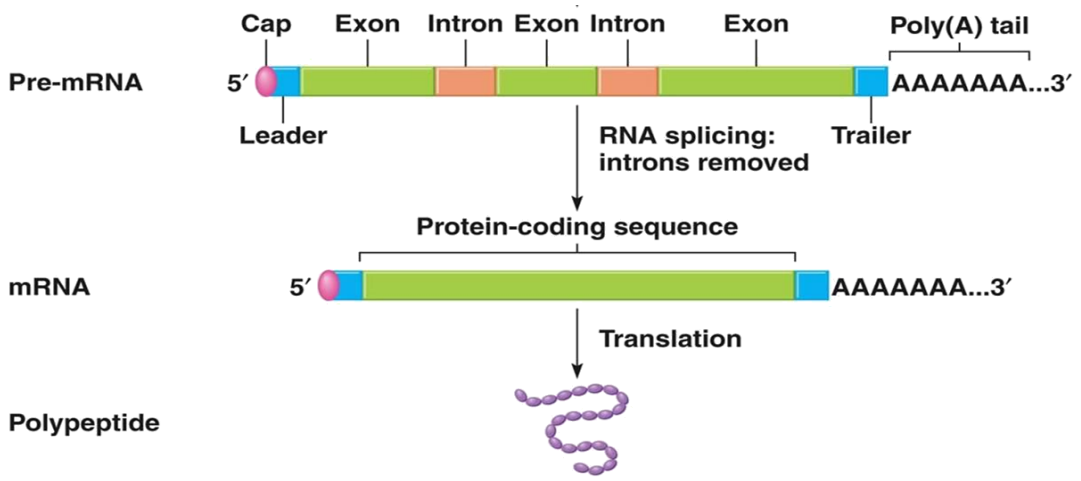
Silencers – When transcription factors bind, they prevent a gene from being transcribed

mRNA processing

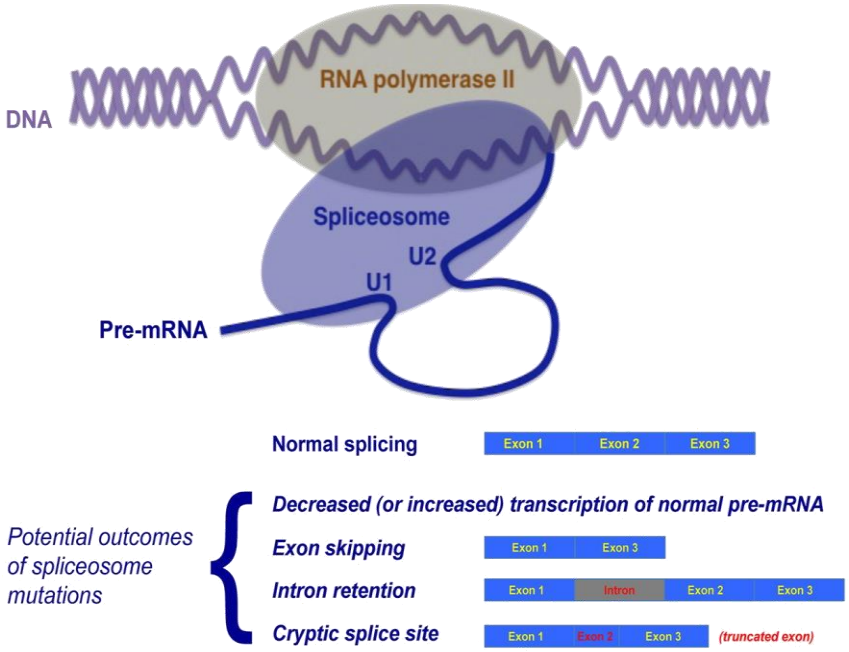
In eukaryotes, coding regions of a gene (the expressed regions, or exons) are often interrupted by noncoding regions (intervening sequences, or introns).

In the nucleus, RNA polymerase synthesizes an RNA transcript containing exons and introns.

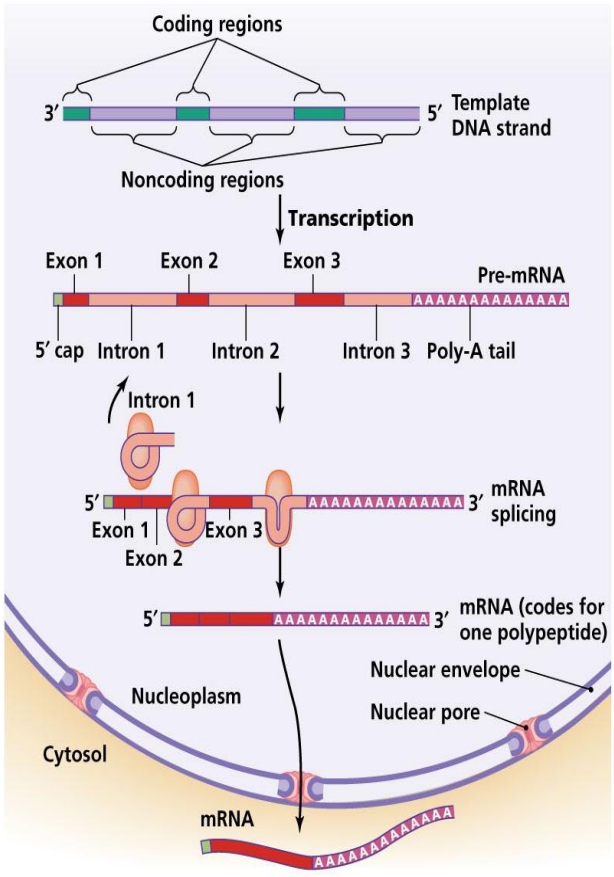
The introns must be removed from the RNA transcript before the resulting mRNA can be translated – ribozymes remove the introns and splice the exons together. The mRNA is then moved through the nuclear membrane and into the cytoplasm, where translation takes place.



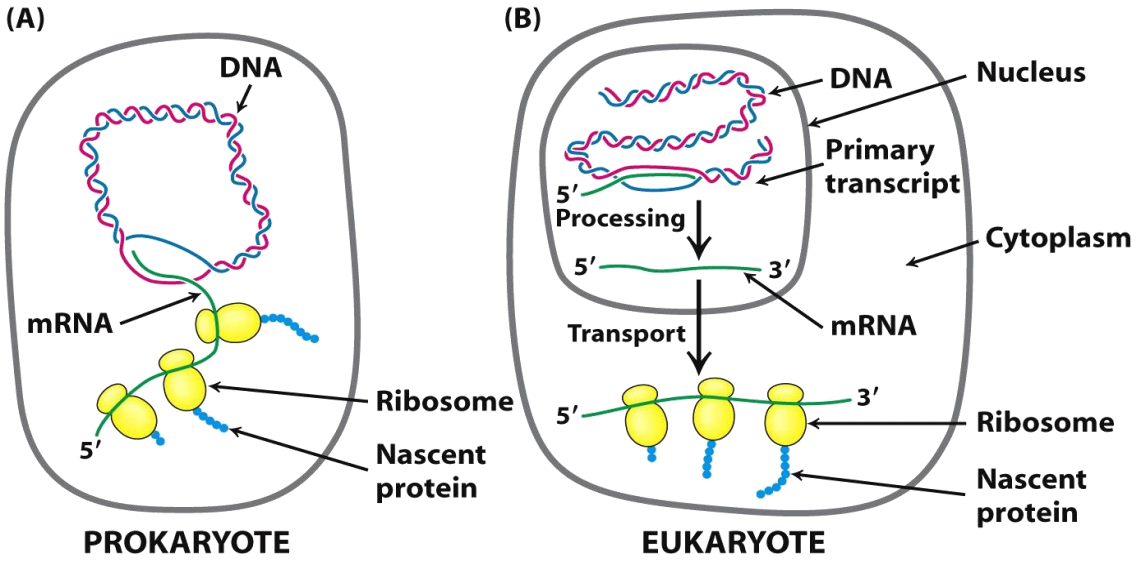
Splicing and the spliceosome

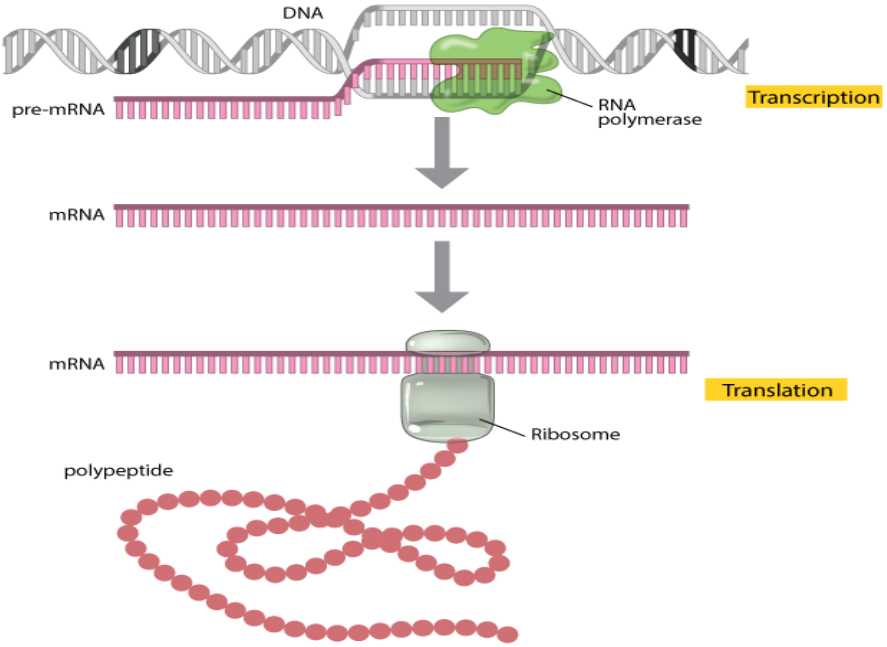


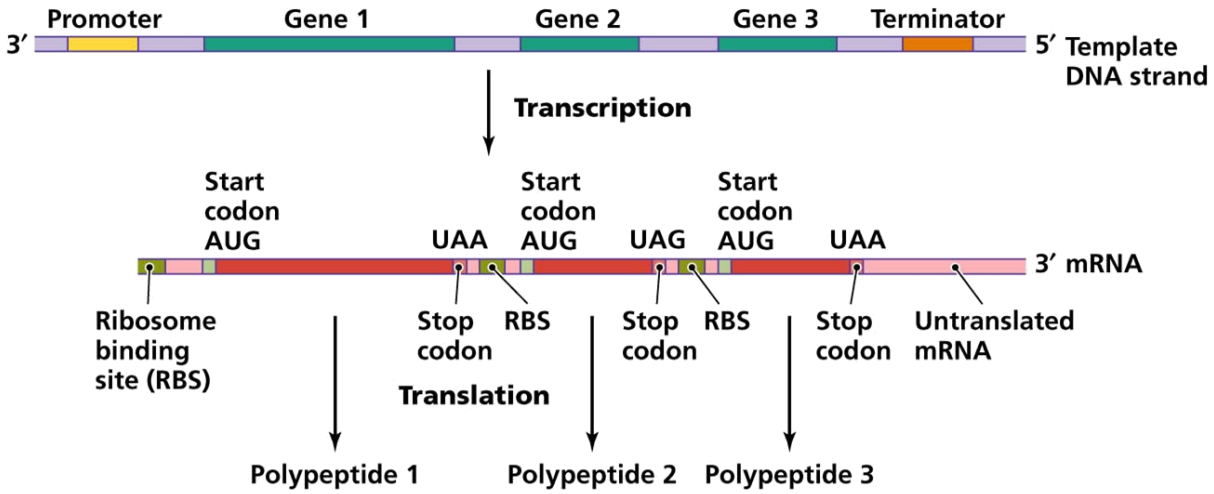
Transcription and processing



From transcription to translation







Translation is the process by which a protein is synthesized from the information contained in a molecule of messenger RNA (mRNA). During translation, an mRNA sequence is read using the genetic code, which is a set of rules that defines how an mRNA sequence is to be translated into the 20-letter code of amino acids, which are the building blocks of proteins. The genetic code is a set of three-letter combinations of nucleotides called codons, each of which corresponds with a specific amino acid or stop signal. Translation occurs in a structure called the ribosome, which is a factory for the synthesis of proteins.

The codon message

Three-base segments of mRNA that specify amino acids are called codons. The three-base segments of DNA that the codons are transcribed from are called triplets.

The genetic code refers to the relationship between the nucleotide base sequence of DNA (the triplets), the corresponding codons of mRNA, and the amino acids for which the codons code.

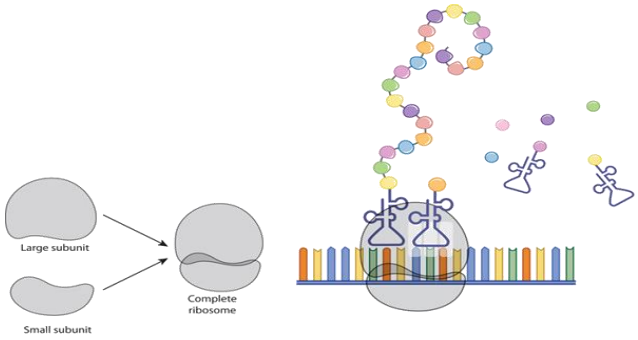
Specific amino acids are attached to molecules of tRNA. Another portion of the tRNA has a three base sequence called an anticodon.

The anticodons are complementary and antiparallel to the codons, the codons are complementary and antiparallel to the triplets.

- Of the 64 codons, 61 are sense codons (which code for amino acids), and 3 are nonsense codons (which do not code for amino acids and are stop signals for translation).

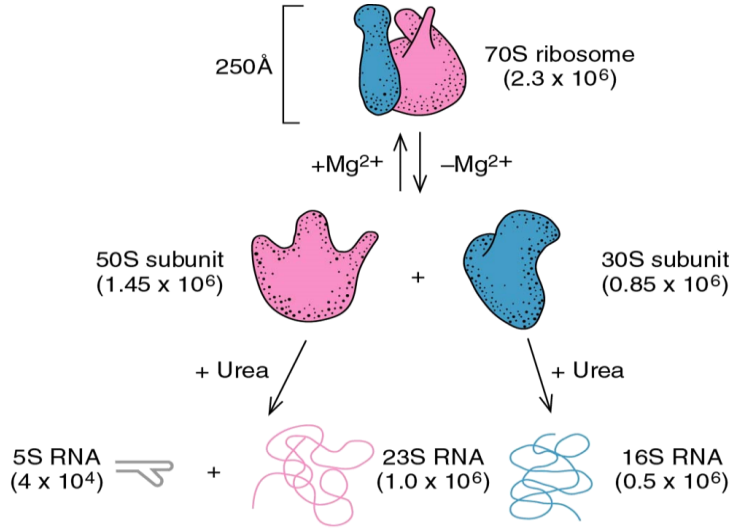
- The genetic code is redundant; that is, most amino acids are coded for by more than one codon.
- The genetic code is also degenerate, meaning in many cases that the first two nucleotides in a codon are specific and the third nucleotide may be U or C, or A or G, or any of the four (isoleucine has AU as the first two nucleotides and can have U, C, or A in the third position; AUG codes for methionine and serves as the start codon for translation).

Translation of mRNA (initiation, elongation, termination)

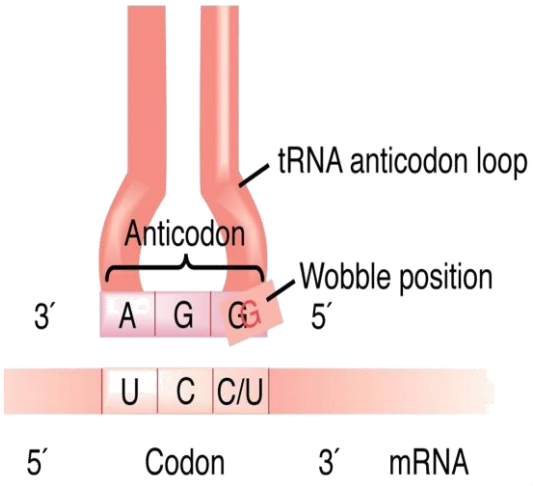


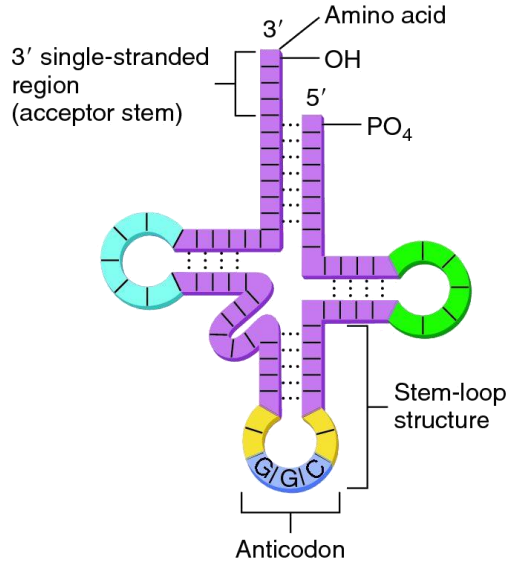
After the transcription of DNA to mRNA is complete, translation — or the reading of these mRNAs to make proteins — begins. Recall that mRNA molecules are single stranded, and the order of their bases — A, U, C, and G — is complementary to that in specific portions of the cell's DNA. Each mRNA dictates the order in which amino acids should be added to a growing protein as it is synthesized. In fact, every amino acid is represented by a three-nucleotide sequence or codon along the mRNA molecule. For example, AGC is the mRNA codon for the amino acid serine, and UAA is a signal to stop translating a protein — also called the stop codon

Translation and ribosomes



The synthesis of proteins (translation) is catalyzed by the ribosome. The ribosome is made up of a large and small subunit, and is a large enzyme comprised mostly of ribosomal RNA (rRNA), with proteins interspersed like islands in a sea of RNA. Besides the rRNA, the ribosome contains binding sites for tRNA and mRNA. The rRNA forms most of the ribosomal structure and performs the catalytic steps of peptide synthesis, the mRNA delivers the genetic message, and tRNA translates the genetic code into peptide sequence.



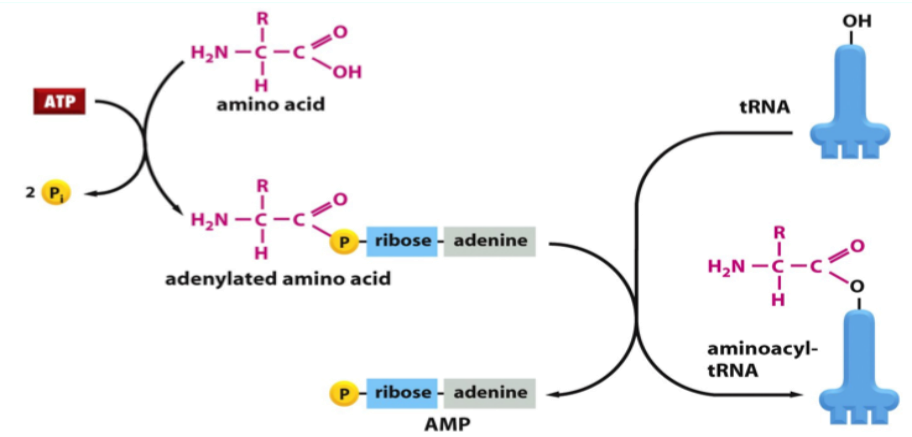


A tRNA is an adaptor molecule, typically 73 to 94 nucletides in length, that serves as the physical link between the nucleotide sequence of DNA and RNA and the amino acid sequence of proteins. It does this by carrying an amino acid to the ribosome as directed by a three-nucleotide sequence (codon) in mRNA. As such, tRNAs are a

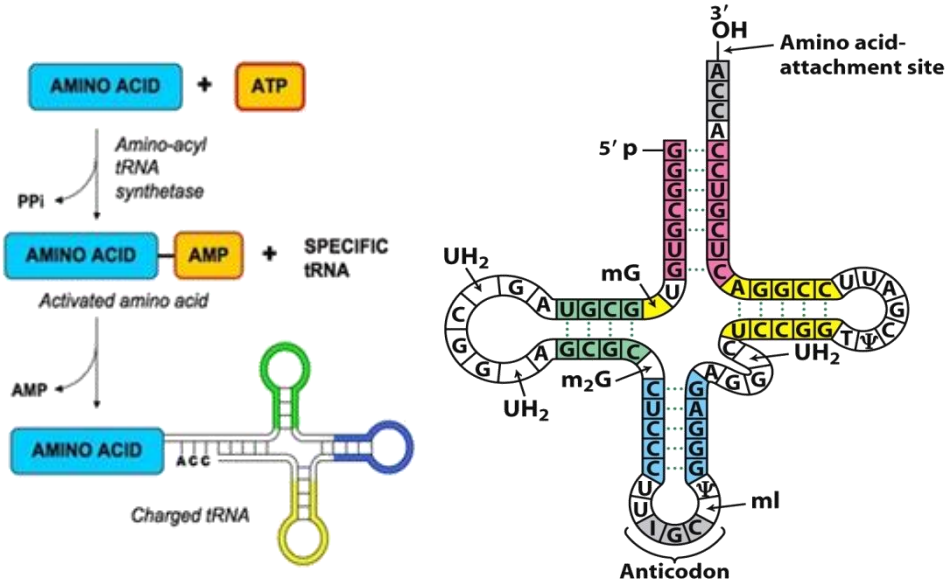
necessary component of protein translation, the biological synthesis of new proteins according to the genetic code.

The specific nucleotide sequence of an mRNA specifies which amino acids are incorporated into the protein product of the gene from which the mRNA is transcribed, and the role of tRNA is to specify which sequence from the genetic code corresponds to which amino acid. One end of the tRNA matches the genetic code in a three-nucleotide sequence called the anticodon. The anticodon forms three base pairs with a codonin mRNA during protein biosynthesis.

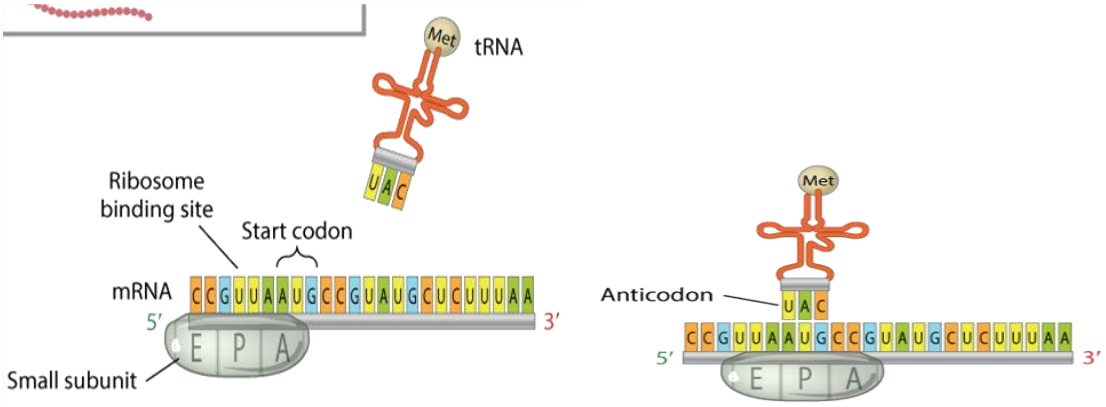
Amino acid activation



The first step of protein synthesis, whereby an amino acid reacts with adenosine triphosphate in the presence of aminoacyl tRNA synthetase to produce an amino acid adenylate, which provides the energy necessary for the attachment of the amino acid to a specific transfer RNA molecule.

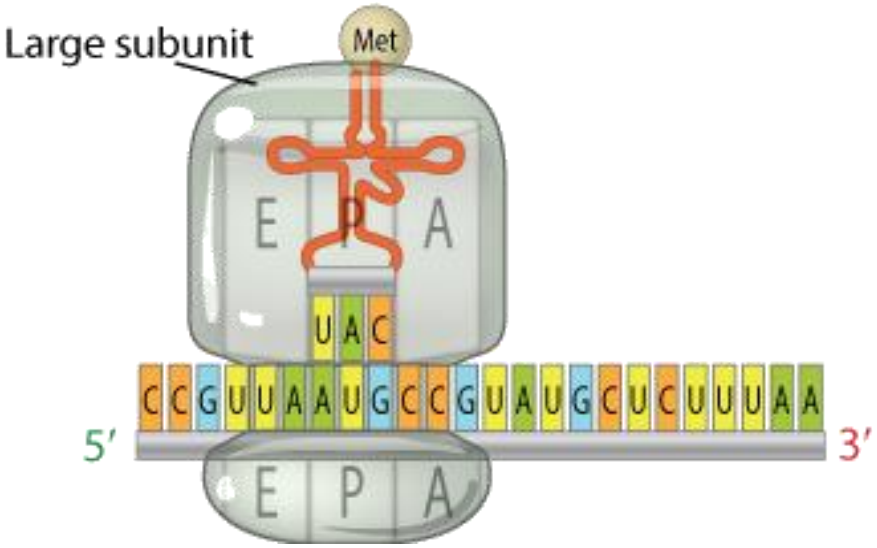


Translation initiation



When translation begins, the small subunit of the ribosome and an initiator tRNA molecule assemble on the mRNA transcript. The small subunit of the ribosome has three binding sites: an amino acid site (A), a polypeptide site (P), and an exit site (E). The initiator tRNA molecule carrying the amino acid methionine binds to the AUG start codon of the mRNA transcript at the ribosome's P site where it will become the first amino acid incorporated into the growing polypeptide chain. Here, the initiator tRNA molecule is shown binding after the small ribosomal subunit has assembled on the mRNA; the order in which this occurs is unique to prokaryotic cells. The complex then binds the mRNA transcript, so that the tRNA and the small ribosomal subunit bind the mRNA simultaneously.

The translation initiation complex



- The large ribosomal subunit binds to the small ribosomal subunit to complete the initiation complex.

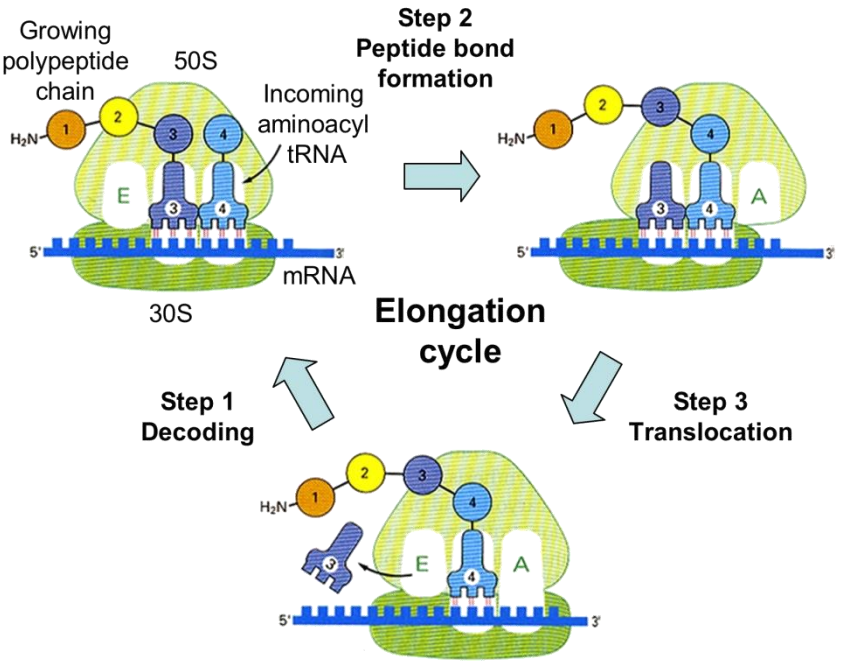
- The initiator tRNA molecule, carrying the methionine amino acid that will serve as the first amino acid of the polypeptide chain, is bound to the P site on the ribosome. The A site is aligned with the next codon, which will be bound by the anticodon of the next incoming tRNA.

The elongation phase

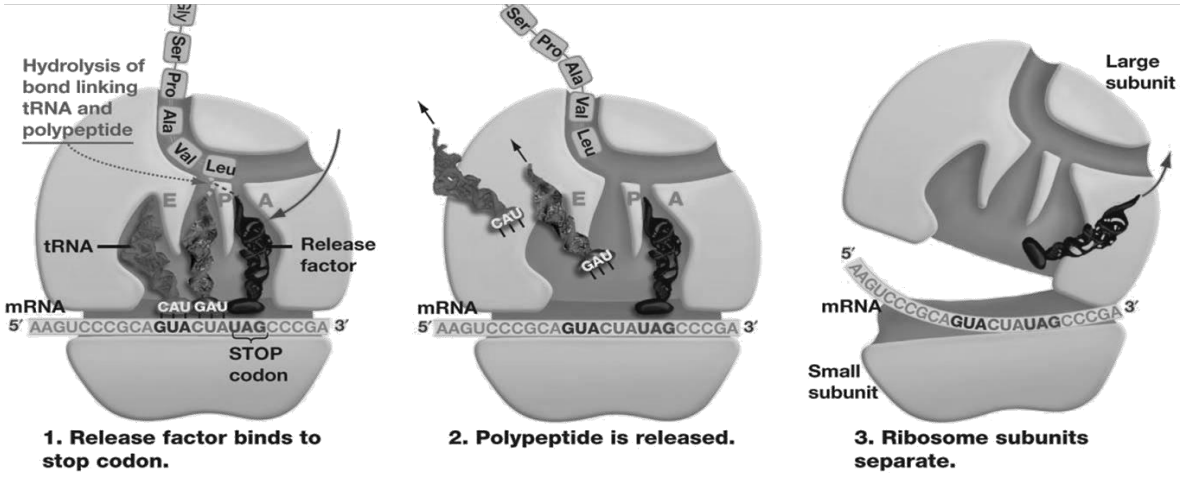
The next phase in translation is known as the elongation phase . First, the ribosome moves along the mRNA in the 5'-to-3'direction, which requires the elongation factor G, in a process called translocation. The tRNA that corresponds to the second codon can then bind to the A site, a step that requires elongation factors , as well as guanosine triphosphate (GTP) as an energy source for the process.

Next, peptide bonds between the now-adjacent first and second amino acids are formed through a peptidyl transferase activity. For many years, it was thought that an enzyme catalyzed this step, but recent evidence indicates that the transferase activity is a catalytic function of rRNA. After the peptide bond is formed, the ribosome shifts, or translocates, again, thus causing the tRNA to occupy the E site. The tRNA is then released to the cytoplasm to pick up another amino acid. In addition, the A site is now empty and ready to receive the tRNA for the next codon.

This process is repeated until all the codons in the mRNA have been read by tRNA molecules, and the amino acids attached to the tRNAs have been linked together in the growing polypeptide chain in the appropriate order. At this point, translation must be terminated, and the nascent protein must be released from the mRNA and ribosome.

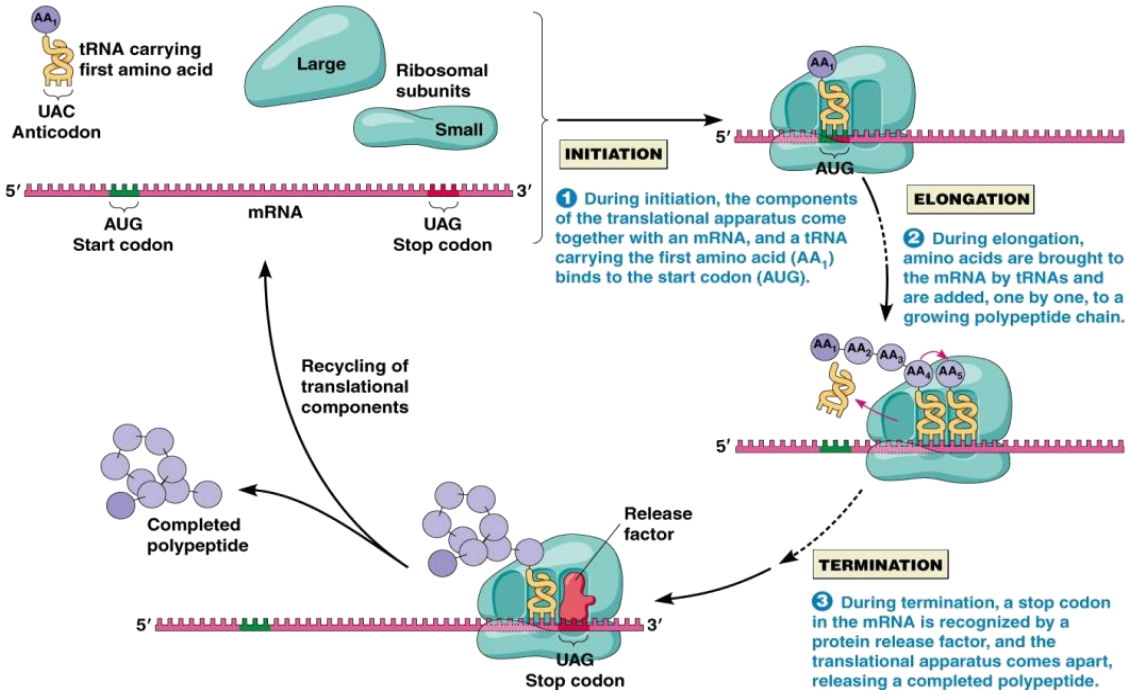


Termination of translation



There are three termination codons that are employed at the end of a protein-coding sequence in mRNA: UAA, UAG, and UGA. No tRNAs recognize these codons. Thus, in the place of these tRNAs, one of several proteins, called release factors, binds and facilitates release of the mRNA from the ribosome and subsequent dissociation of the ribosome.

Summary of translation



Eucariotyc gene expression

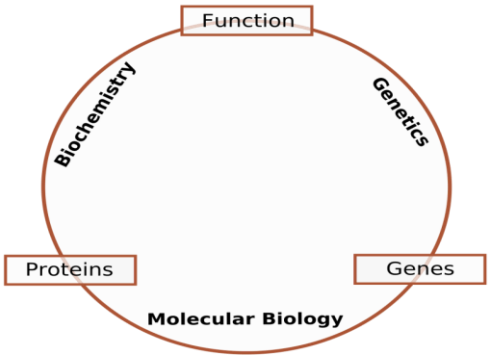
The sequence of nucleotides in DNA contain information.

This information is put to work through the production of proteins.

Proteins fold into complex, three-dimensional shapes to become key cell structures and regulators of cell functions.

The sequence of nucleotides in each gene contains information for assembling the string of amino acids that make up a single protein molecule.

Foundations of biology



Questions for self-control

1. DNA replication: general description and biological role of the process.
2. RNA synthesis: general description and biological role of the process.

3. Protein biosynthesis: general description and biological role of the process

INTERNET SOURCES

http://www.biochemweb.org/

http://www.1lec.com/Biochemistry/

http://www.bioch.ox.ac.uk/

http://www.biology.arizona.edu/biochemistry/biochemistry.html

http://pubs.acs.org/journal/bichaw

http://en.wikipedia.org/wiki/Biochemistry

http://www.biochemistry.org/

http://themedicalbiochemistrypage.org/

http://biochem.stanford.edu/

http://www.grsmu.by/page/open/review/235

Glossary

Acetyl CoA. Acetyl-coenzyme A, a high-energy ester of acetic acid that is important both in the tricarboxylic acid cycle and in fatty acid biosynthesis.

Active site. The region of an enzyme molecule that contains the substrate binding site and the catalytic site for converting the substrate(s) into product(s).

Active transport. The energy-dependent transport of a substance across a membrane.

Adenine. A purine base found in DNA or RNA.

Adenosine. A purine nucleoside found in DNA, RNA, and many cofactors.

Adenosine diphosphate (ADP). The nucleotide formed by adding a pyrophosphate group to the 5'-OH group of adenosine.

Adenosine triphosphate (ATP). The nucleotide formed by adding yet another phosphate group to the pyrophosphate group on ADP.

Adenylate cyclase. The enzyme that catalyzes the formation of cyclic 3',5' adenosine monophosphate (cAMP) from ATP.

Aldehyde. A molecule containing a doubly bonded oxygen and a hydrogen attached to the same carbon atom.

Allosteric enzyme. An enzyme whose active site can be altered by the binding of a small molecule at a nonoverlapping site.

Anomers. The sugar isomers that differ in configuration about the carbonyl carbon atom. This carbon atom is called the anomeric carbon atom of the sugar.

Antibody. A specific protein that interacts with a foreign substance (antigen) in a specific way.

Anticodon. A sequence of three bases on the transfer RNA that pair with the bases in the corresponding codon on th messenger RNA.

Antigen. A foreign substance that triggers antibody formation and is bound by the corresponding antibody.

Antiparallel b-pleated sheet (b-sheet). A hydrogen bonded secondary structure formed between two or more extended polypeptide chains.

Asymmetric carbon. A carbon that is covalently bonded to four different groups.

Beta-sheet (b-sheet). A sheetlike structure formed by the interaction between two or more extended polypeptide chains.

Beta-oxidation (b-oxidation). Oxidative degradation of fatty acids that occurs by the successive oxidation of the b-carbon atom.

Base. The adenine, guanine, cytosine or thymine group attached to a nucleotide or nucleoside. Also may be used to refer to a nucleic acid unit within a polynucleotide chain, as when a gene is said to be 2000 bases long.

Bilayer. A double layer of lipid molecules with the hydrophilic ends oriented outward, in contact with water, and the hydrophobic parts oriented inward.

Bile salts. Derivatives of cholesterol with detergent properties that aid in the solubilization of lipid molecules in the digestive tract.

Biochemical pathway. A series of enzyme-catalyzed reactions that results in the conversion of a precursor molecule into a product molecule.

Bond energy. The energy required to break a bond.

Branchpoint. An intermediate in a biochemical pathway that can follow more than one route in following steps.

Buffer. A conjugate acid-base pair that is capable of resisting changes in pH when acid or base is added to the system. This tendency will be maximal when the conjugate forms are present in equal amounts.

cAMP. 3',5' cyclic adenosine monophosphate. The cAMP molecule plays a key role in metabolic regulation.

Carbohydrate. A polyhydroxy aldehyde or ketone.

Carboxylic acid. A molecule containing a carbon atom attached to a hydroxyl group and to an oxygen atom by a double bond.

Catabolism. That part of metabolism that is concerned with degradation reactions.

Catalyst. A compound that lowers the activation energy of a reaction without itself being consumed.

Catalytic site. The site of an enzyme involved in the catalytic process.

Chemiosmotic coupling. The coupling of ATP synthesis to an electrochemical potential gradient across a membrane.

Chromatin. The nucleoprotein fibers of eukaryotic chromosomes.

Chromosome. A thread-like structure, visible in the cell nucleus during metaphase, that carries the hereditary information.

Citric acid cycle. See tricarboxylic acid (TCA) cycle.

Codon. In a messenger RNA molecule, a sequence of three bases that represents a particular amino acid.

Coenzyme. An organic molecule that associates with enzymes and affects their activity.

Cofactor. A small molecule required for enzyme activity. It could be organic in nature, like a coenzyme, or inorganic in nature, like a metallic cation.

Complementary base sequence. For a given sequence of nucleic acids, the nucleic acids that are related to them by the rules of base pairing.

Conformation. The three-dimensional arrangement adopted by a molecule, usually a complex macromolecule. Molecules with the same configuration can have more than one conformation.

Cytidine. A pyrimidine nucleoside found in DNA and RNA.

Cytochromes. Heme-containing proteins that function as electron carriers in oxidative phosphorylation and photosynthesis.

Cytoplasm. The contents enclosed by the plasma (or cytoplasmic) membrane, excluding the nucleus.

Cytosine. A pyrimidine base found in DNA and RNA.

Cytoskeleton. The filamentous skeleton, formed in the eukaryotic cytoplasm, that is largely responsible for controlling cell shape.

Cytosol. The liquid portion of the cytoplasm, including the macromolecules but not including the larger structures like subcellular organelles or cytoskeleton.

Dalton. A unit of mass equivalent to the mass of a hydrogen atom (1.66×10^{-24} g)

Deamination. The enzymatic removal of an amine group, as in the deamination of an amino acid to an alpha keto acid.

Dehydrogenase. An enzyme that catalyzes the removal of a pair of electrons (and usually one or two protons) from a substrate molecule.

Denaturation. The disruption of the native folded structure of a nucleic acid or protein molecule; may be due to heat, chemical treatment, or change in pH.

Dimer. Structure resulting from the association of two subunits.

Dissociation constant. An equilibrium constant for the dissociation of a molecule into two parts (e.g., dissociation of acetic acid into acetate anion and a proton); K_d.

Disulfide bridge. A covalent linkage formed by oxidation between two cysteine SH groups either in the same polypeptide chain or in different polypeptide chains. Reversible by adding reducing agents.

DNA. Deoxyribonucleic acid. A polydeoxyribonucleotide in which the sugar is deoxyribose; the main repository of genetic information in all cells and most viruses.

DNA polymerase. An enzyme that catalyzes the formation of 3'-5' phosphodiester bonds from deoxyribonucleotide triphosphates.

Domain. A segment of a folded protein structure showing conformational integrity. A domain could include the entire protein or just a fraction of the protein. Some proteins, such as antibodies, contain many structural domains.

Double helix. A structure in which two helically-twisted polynucleotide strands are held together by hydrogen bonding and base stacking.

Electrophoresis. The movement of particles in an electrical field. A commonly-used technique for analysis of mixtures of molecules in solution according to their electrophoretic mobilities.

Elongation factors. Protein factors uniquely required during the elongation phase of protein synthesis. Elongation factor G (EF-G) brings about the movement of the peptidyl tRNA from the A site to the P site of the ribosome.

Endergonic reaction. A reaction with a positive standard free energy change.

End-product (feedback) inhibition. The inhibition of the first enzyme in a pathway by the end product of that pathway.

Endocrine glands. Specialized tissues whose function is to synthesize and secrete hormones.

Endonuclease. An enzyme that breaks a phosphodiester linkage at some point within a polynucleotide chain.

Endopeptidase. An enzyme that breaks a polypeptide chain at an internal peptide linkage.

Endoplasmic reticulum. A system of double membranes in the cytoplasm that is involved in the synthesis of transported proteins. The rough endoplasmic reticulum has ribosomes associated with it. The smooth endoplasmic reticulum does not.

Energy charge. The fractional degree to which the AMP-ADP-ATP system is filled with high-energy phosphates (phosphoryl groups).

Entropy. The randomness of a system.

Enzyme. A moleucle, most often a protein, that contains a catalytic site for a biochemical reaction.

Eukaryote. A cell or organism that has a membrane-bound nucleus.

Excision repair. DNA repair in which a damaged region is replaced.

Exergonic reaction. A chemical reaction that takes place with a negative change in standard free energy.

Exon. A segment within a gene that carries part of the coding information for a protein.

Exonuclease. An enzyme that breaks a phosphodiester linkage at one or the other end of a polynucleotide chain so as to release single or small nucleotide residues.

Fatty acid. A long-chain hydrocarbon containing a carboxyl group at one end. Saturated fatty acids have completely saturated hydrocarbon chains. Unsaturated fatty acids have one or more carbon-carbon double bonds in their hydrocarbon chains.

Feedback inhibition. See end-product inhibition.

Fermentation. The energy-generating breakdown of glucose or related molecules by a process that does not require molecular oxygen.

Free energy. That part of the energy of a system that is available to do useful work.

Gene. A segment of the genome that codes for a functional product.

Genome. The total genetic content of a cell or a virus.

Genotype. The genetic characteristics of an organism (distinguished from its observable characteristics, or phenotype).

Globular protein. A folded protein that adopts an approximately globular shape. May also be called soluble proteins.

Gluconeogenesis. The production of sugars from nonsugar precursors such as lactate or amino acids. Applies more specifically to the production of free glucose by vertebrate livers.

Glycogen. A polymer of glucose residues in 1,4 linkage, with 1,6 linkages at branchpoints.

Glycogenic. Describing amino acids whose metabolism may lead to gluconeogenesis.

Glycolysis. The catabolic conversion of glucose to pyruvate with the production of ATP.

Glycosidic bond. The bond between a sugar and an alcohol. Also the bond that links two sugars in disaccharides, oligosaccharides, and polysaccharides.

Guanine. A purine base found in DNA or RNA.

Guanosine. A purine nucleoside found in DNA and RNA.

Half-life. The time required for the disappearance of one half of a substance.

Helix. A spiral structure with a repeating pattern.

Heme. An iron-porphyrin complex found in hemoglobin and cytochromes.
Hemiacetal. The product formed by the condensation of an aldehyde with an alcohol; it contains one oxygen linked to a central carbon in a hydroxyl fashion and one oxygen linked to the same central carbon by an ether linkage.

Heteropolymer. A polymer containing more than one type of monomeric unit.

Hexose. A sugar with a six-carbon backbone.

High-energy compound. A compound that undergoes hydrolysis with a high negative standard free energy change.

Histones. The family of basic proteins that is normally associated with DNA in most cells of eukaryotic organisms.

Holoenzyme. An intact enzyme containing all of its subunits and any necessary cofactors with full enzymatic activity.

Homopolymer. A polymer composed of only one type of monomeric building block.

Hormone. A chemical substance made in one cell and secreted so as to influence the metabolic activity of a select group of cells located at other sites in the organism.

Hormone receptor. A protein that is located on the cell membrane or inside the responsive cell and that interacts specifically with the hormone.

Hydrogen bond. A weak, noncovalent, attractive force between one electronegative atom and a hydrogen atom that is covalently linked to a second electronegative atom.

Hydrolysis. The cleavage of a molecule by the addition of water. Hydrophilic. Preferring to be in contact with water.

Hydrophobic. Preferring not to be in contact with water, as is the case with the hydrocarbon portion of a fatty acid or phospholipid chain.

Immunoglobulin. A protein made in a B plasma cell and usually secreted; it interacts specifically with a foreign agent. Synonymous with antibody. It is composed of two heavy and two light chains linked by disulfide bonds. Immunoglobulins can be divided into five classes (IgG, IgM, IgA, IgD, and IgE) based on their heavy-chain component.

In vitro. Literally, "in glass," describing whatever happens in a test tube or other receptacle, as opposed to what happens in whole cells of the whole organism (*in vivo*).

Induced fit. A change in the shape of an enzyme that results from the binding of substrate.

Initiation factors. Those protein factors that are specifically required during the initiation phase of protein synthesis.

Intron. A segment of the nascent transcript that is removed by splicing. Also refers to the corresponding region in the DNA. Synonymous with intervening sequence.

Isoelectric point or pH. The pH at which a protein has no net charge.

Isomerase. An enzyme that catalyzes an intramolecular rearrangement.

Isomerization. Rearrangement of atomic groups within the same molecule without any loss or gain of atoms.

Isozymes. Multiple forms of an enzyme that differ from one another in one or more of the properties.

Km. See Michaelis constant.

Ketogenic. Describing amino acids that are metabolized to acetoacetate and acetate.

Ketone. A functional group of an organic compound in which a carbon atom is double-bonded to an oxygen. Neither of the other substituents attached to the carbon is a hydrogen. Otherwise the group would be called an aldehyde.

Ketone bodies. Refers to acetoacetate, acetone, and b-hydroxybutyrate made from acetyl-CoA in the liver and used for energy in nonhepatic tissue.

Ketosis. A condition in which the concentration of ketone bodies in the blood or urine is unusually high.

Kilobase. One thousand bases in a DNA molecule.

Kinase. An enzyme catalyzing phosphorylation of an acceptor molecule, usually with ATP serving as the phosphate (phosphoryl) donor.

Krebs cycle. See tricarboxylic acid (TCA) cycle.

Law of mass action. The finding that the rate of a chemical reaction is a function of the product of the concentrations of the reacting species.

Ligand. A (usually small) molecule that binds to another, such as oxygen when it binds to myoglobin.

Ligase. An enzyme that catalyzes the joining of two molecules together. In DNA it joins 5'-OH to 3' phosphates.

Lipid. A biological molecule that is soluble in organic solvents. Lipids include steroids, fatty acids, prostaglandins, terpenes, and waxes.

Lipid bilayer. Model for the structure of the cell membrane based on the interaction between the hydrophobic regions of phospholipids.

Lyase. An enzyme that catalyzes the removal of a group to form a double bond, or the reverse reaction.

Lysosome. An organelle that contains hydrolytic enzymes designed to break down proteins that are targeted to that organelle.

Membrane. A sheet-like composite of protein and lipid that is the boundary of cells and organelles.

Membrane protein. A protein that is associated with a membrane, rather than found free in the cell. A membrane protein may be integral (embedded or buried) in the membrane, or peripheral (attached more loosely, by interactions with either lipid or intergral membrane proteins).

Membrane transport. The facilitated transport of a molecule across a membrane.

Messenger RNA (mRNA). The template RNA carrying the message for protein synthesis.

Metabolism. The sum total of the enzyme-catalyzed reactions that occur in a living organism.

Michaelis constant (Km). The substrate concentration at which an enzyme-catalyzed reaction proceeds at one-half of the maximum velocity.

Michaelis-Menten equation (also known as the Henri-Michaelis-Menten equation). An equation relating the reaction velocity to the substrate concentration of an enzyme.

Mismatch repair. The replacement of a base in a heteroduplex structure by one that forms a Watson-Crick base pair.

Mitochondrion. An organelle, found in eukaryotic cells, in which oxidative phosphorylation takes place. It contains its own genome and unique ribosomes to carry out protein syn thesis of only a fraction of the proteins located in this organelle.

Mitosis. The process whereby replicated chromosomes segregate equally toward opposite poles prior to cell division.

Monomer. One unit of a protein or other structure.

Mutagen. An agent that can bring about a heritable change (mutation) in an organism.

Mutagenesis. A process that leads to a change in the genetic material that is inherited in later generations.

Mutant. An organism that carries an altered gene or change in its genome.

Mutation. The genetically inheritable alteration of a gene or group of genes.

Myosin. The main protein of the thick filaments in a muscle myofibril. It is composed of two coiled subunits (M_r about 220,000) that can aggregate to form a thick filament, which is globular at each end.

Negative control. Repression of biological activity by the presence of a specific molecule.

Nitrogen cycle. The passage of nitrogen through various valence states, as the result of reactions carried out by a wide variety of different organisms.

Nitrogen fixation. Conversion of atmospheric nitrogen into a form that can be converted by biochemical reactions to an organic form. This reaction is carried out by a very limited number of microorganisms.

Nitrogenous base. An aromatic nitrogen-containing molecule with basic properties. Such bases include purines and pyrimidines.

Noncompetitive inhibitor. An inhibitor of enzyme activity whose effect is not reversed by increasing the concentration of substrate molecule.

Nuclease. An enzyme that cleaves phosphodiester bonds of nucleic acids.

Nucleic acids. Polymers of the ribonucleotides or deoxyribonucleotides.

Nucleolus. A spherical structure visible in the nucleus during interphase. The nucleolus is associated with a site on the chromosome that is involved in ribosomal RNA synthesis.

Nucleosome. A complex of DNA and an octamer of histone proteins in which a small stretch of the duplex is wrapped around a molecular bead of histone.

Nucleoside. An organic molecule containing a purine or pyrimidine base and a five-carbon sugar (ribose or deoxyribose).

Nucleotide. An organic molecule containing a purine or pyrimidine base, a five-carbon sugar (ribose or deoxyribose), and one or more phosphate groups. A phosphoester of a nucleoside.

Nucleus. In eukaryotic cells, the centrally-located organelle that encloses most of the chromosomes. Minor amounts of chromosomal substance are found in some other organelles, most notably the mitochondria and the chloroplasts.

Okazaki fragment. A short segment of single-stranded DNA that is an intermediate in DNA synthesis. In bacteria, Okazaki fragments are 1000-2000 bases in length; in eukaryotes, 100-200 bases in length.

Oligonucleotide. A polynucleotide containing a small number of nucleotides. The linkages are the same as in a polynucleotide; the only distinguishing feature is the small size.

Oligosaccharide. A molecule containing a small number of sugar residues joined in a linear or a branched structure by glycosidic bonds.

Operon. A group of contiguous genes that are coordinately regulated by two cis-acting elements, a promoter and an operator. Found only in prokaryotic cells.

Optical activity. The property of a molecule that leads to rotation of the plane of polarization of plane-polarized light when the latter is transmitted through the substance. Chirality is a necessary and sufficient property for optical activity.

Organelle. A subcellular membrane-bounded body with a well-defined function.

Osmotic pressure. The pressure generated by the mass flow of water to that side of a membrane-bounded structure that contains the higher concentration of solute

molecules. A stable osmotic pressure is seen in systems in which the membrane is not permeable to some of the solute molecules.

Oxidation. The loss of electrons from a compound.

Oxidative phosphorylation. The formation of ATP as the result of the transfer of electrons to oxygen.

Oxido-reductase. An enzyme that catalyzes oxidation-reduction reactions.

Pentose. A sugar with five carbon atoms.

Pentose phosphate pathway. The pathway involving the oxidation of glucose-6-phosphate to pentose phosphates and further reactions of pentose phosphates.

Peptide. An organic molecule in which a covalent amide bond is formed between the a-amino group of one amino acid and the a-carboxyl group of another amino acid, with the elimination of a water molecule. The resulting connection is called a peptide bond.

Peroxisomes. Subcellular organelles that contain flavin-requiring oxidases and that regenerate oxidized flavin by reaction with oxygen.

Phenotype. The observable trait(s) that result from the genotype in cooperation with the environment.

Phenylketonuria. A human disease caused by a genetic deficiency in the enzyme that converts phenylalanine to tyrosine. The immediate cause of the disease is an excess of phenylalanine, which can be alleviated by a diet low in phenylalanine.

Phosphodiester. A molecule containing two alcohols esterified to a single molecule of phosphate. For example, the backbone of nucleic acids is connected by 5'-3' phosphodiester linkages between the adjacent individual nucleotide residues.

Phosphogluconate pathway. Another name for the pentose phosphate pathway. This name derives from the fact that 6-phosphogluconate is an intermediate in the formation of pentoses from glucose.

Phospholipid. A lipid containing charged hydrophilic phosphate groups; a component of cell membranes.

Phosphorylation. The formation of a phosphate derivative of a biomolecule.

Plasma membrane. The membrane that surrounds the cytoplasm.

Plasmid. A circular DNA duplex that replicates autonomously in bacteria. Plasmids that integrate into the host genome are called episomes. Plasmids differ from viruses in that they never form infectious nucleoprotein particles.

Polar group. A hydrophilic (water-loving) group.

Polymerase. An enzyme that catalyzes the synthesis of a polymer from monomers.

Polynucleotide. A chain structure containing nucleotides linked together by phosphodiester (5'-3') bonds. The polynucleotide chain has a directional sense with a 5' and a 3' end.

Polypeptide. A linear polymer of amino acids held together by peptide linkages. The polypeptide has a directional sense, with an amino- and a carboxy-terminal end.

Polysaccharide. A linear or branched chain structure containing many sugar molecules linked by glycosidic bonds.

Positive control. A system that is turned on by the presence of a regulatory protein.

Posttranslational modification. The covalent bond changes that occur in a polypeptide chain after it leaves the ribosome and before it becomes a mature protein.

Primary structure. In a polymer, the sequence of monomers and the covalent bonds. In proteins, it refers to the amino acid sequence.

Primer. A structure that serves as a growing point for polymerization. Short primers of DNA are often used in sequencing and mutagenesis procedures.

Primosome. A multiprotein complex that catalyzes synthesis of RNA primer at various points along the DNA template.

Prokaryote. A unicellular organism that contains a single chromosome, no nucleus, no membrane-bound organelles, and has characteristic ribosomes and biochemistry.

Promoter. That region of the gene that signals RNA polymerase binding and the initiation of transcription.

Prostaglandin. An oxygenated eicosanoid that has a hormonal function. Prostaglandins are unusual hormones in that they usually have effects only in that region of the organism where they are synthesized.

Prosthetic group. Synonymous with coenzyme except that a prosthetic group is usually more firmly attached to the enzyme it serves.

Protamines. Highly basic, arginine-rich proteins found complexed to DNA in the sperm of many invertebrates and fish.

Protein subunit. One of the components or monomers of a multicomponent protein.

Proteoglycan. A protein-linked heteropolysaccharide in which the heteropolysaccharide is usually the major component.

Proton acceptor. A functional group capable of accepting a proton from a proton donor molecule.

Purine. A heterocyclic ring structure with varying functional groups. The purines adenine and guanine are found in both DNA and RNA.

Pyrimidine. A heterocyclic six-membered ring structure. Cytosine and uracil are the main pyrimidines found in RNA, and cytosine and thymine are the main pyrimidines found in DNA.

Quaternary structure. In a protein, the way in which the different folded subunits interact to form the multisubunit protein.

Redox couple. An electron donor and its corresponding oxidized form.

Redox potential (E). The relative tendency of a pair of molecules to release or accept an electron. The standard redox potential (E^0) is the redox potential of a solution containing the oxidant and reductant of the couple at standard concentrations.

Regulatory enzyme. An enzyme in which the active site is subject to regulation by factors other than the enzyme substrate. The enzyme frequently contains a nonoverlapping site for binding the regulatory factor that affects the activity of the active site.

Regulatory gene. A gene whose principal product is a protein designed to regulate the synthesis of other genes.

Renaturation. The process of returning a denatured structure to its original native structure, as when two single strands of DNA are reunited to form a regular duplex, or an unfolded polypeptide chain is returned to its normal folded three-dimensional structure.

Replication fork. The Y-shaped region of DNA at the site of DNA synthesis; also called a growth fork.

Replicon. A genetic element that behaves as an autonomous replicating unit. It can be a plasmid, phage, or bacterial chromosome.

Repressor. A regulatory protein that inhibits transcription from one or more genes. It can combine with an inducer (resulting in specific enzyme induction) or with an operator element (resulting in repression).

Reverse transcriptase. An enzyme that synthesizes DNA from an RNA template, using deoxyribonucleotide triphosphates.

Ribose. The five-carbon sugar found in RNA.

Ribosomal RNA (rRNA). The RNA parts of the ribosome.

Ribosomes. Small cellular particles made up of ribosomal RNA and protein. They are the site, together with mRNA, of protein synthesis.

RNA (ribonucleic acid). A polynucleotide in which the sugar is ribose.

RNA polymerase. An enzyme that catalyzes the formation of RNA from ribonucleotide triphosphates, using DNA as a template.

RNA splicing. The excision of a segment of RNA, followed by a rejoining of the remaining fragments.

Salting in. The increase in solubility that is displayed by typical globular proteins upon the addition of small amounts of certain salts, such as ammonium sulfate.

Salting out. The decrease in protein solubility that occurs when salts such as ammonium sulfate are present at high concentrations.

Salvage pathway. A family of reactions that permits, for instance, nucleosides as well as purine and pyrimidine bases resulting from the partial breakdown of nucleic acids to be re-utilized in nucleic acid synthesis.

Second messenger. A diffusible small molecule, such as cAMP, that is formed at the inner surface of the plasma membrane in response to a hormonal signal.

Secondary structure. In a protein or a nucleic acid, any repetitive folded pattern that results from the interaction of the corresponding polymeric chains. In proteins, the most common are b-strands (sheets) and a-helices.

Semiconservative replication. Duplication of DNA in which the daughter duplex carries one old strand and one new strand.

Soluble protein. See globular protein.

Splicing. See RNA splicing.

Stem cell. A cell from which other cells stem or arise by differentiation.

Stereoisomers. Isomers that are nonsuperimposable mirror images of each other.

Steroids. Compounds that are derivatives of a tetracyclic structure composed of a cyclopentane ring fused to a substituted phenanthrene nucleus.

Structural domain. An element of protein tertiary structure that forms an independent folding unit.

Structural gene. A gene encoding the amino acid sequence of a polypeptide chain.

Structural protein. A protein that serves a structural function.

Substrate. A molecule that is acted upon, and chemically changed, by an enzyme.

Subunit. Individual polypeptide chains in a protein.

Suppressor gene. A gene that can reverse the phenotype of a mutation in another gene.

TCA cycle. See tricarboxylic acid cycle.

Template. A polynucleotide chain that serves as a surface for the absorption of monomers of a growing polymer and thereby dictates the sequence of the monomers in the growing chain.

Termination factors. Proteins that are exclusively involved in the termination reactions of protein synthesis on the ribosome.

Tertiary structure. In a protein or nucleic acid, the final folded form of the polymer chain.

Tetramer. Structure resulting from the association of four subunits.

Thymidine. One of the four nucleosides found in DNA.

Thymine. A pyrimidine base found in DNA.

Topoisomerase. An enzyme that changes the extent of supercoiling of a DNA duplex.

Transamination. Enzymatic transfer of an amino group from an a-amino acid to an a-keto acid.

Transcription. RNA synthesis that occurs on a DNA template.

Transfection. An artificial process of infecting cells with naked viral DNA.

Transfer RNA (tRNA). Any of a family of low-molecular weight RNAs that transfer amino acids from the cytoplasm to the template for protein synthesis on the ribosome.

Transferase. An enzyme that catalyzes the transfer of a molecular group from one molecule to another.

Transformation. Genetic exchange in bacteria that is mediated via purified DNA. In somatic cell genetics the term is also used to indicate the conversion of a normal cell to one that grows like a cancer cell.

Transition state. The activated state in which a molecule is best suited to undergoing a chemical reaction.

Translation. The process of reading a messenger RNA sequence for the specified amino acid sequence it contains.

Transport protein. A protein whose primary function is to transport a substance from one part of the cell to another, from one cell to another, or from one tissue to another.

Tricarboxylic acid (TCA) cycle. The cyclical process whereby acetate is completely oxidized to CO2 and water, and electrons are transferred to NAD^+ and flavine. The TCA cycle is localized to the mitochondria in eukaryotic cells and to the plasma membrane in prokaryotic cells. Also called the Krebs or citric acid cycle.

Trypsin. A proteolytic enzyme that cleaves (cuts) peptide chains next to the basic amino acids arginine and Iysine.

Unwinding proteins. Proteins that help to unwind double-stranded DNA during DNA replication.

Urea cycle. A metabolic pathway in the liver that leads to the synthesis of urea from amino groups and CO_2. The function of the pathway is to convert the ammonia resulting from catabolism to a nontoxic form, which is then secreted.

van der Waals forces. Refers to the combined effect of two types of interactions, one attractive and one repulsive. The attractive forces are due to favorable interactions among the induced instantaneous dipole moments that arise from fluctuations in the electron charge densities of neighboring nonbonded atoms. Repulsive forces arise when noncovalently bonded atoms come too close together.

Vitamin. A trace organic substance required in the diet of some species. Many vitamins are precursors of coenzymes.

Watson-Crick base pairs. The type of hydrogen-bonded base pairs found in DNA, or comparable base pairs found in RNA. The base pairs are A-T, G-C, and A-U.

Zwitterion. A dipolar ion with spatially-separated positive and negative charges. For example, most amino acids are zwitterions, having a positive charge on the a-amino group and a negative charge on the a-carboxyl group but no net charge on the overall molecule.

Zymogen. An inactive precursor of an enzyme. For example, trypsin exists in the inactive form trypsinogen before it is converted to its active form, trypsin.

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129. Metabolism of methionine, formation of S-adenosylmethionine, its role in transmethylation reactions. Synthesis of creatine.

130. Metabolism of phenylalanine and tyrosine. Disorders of phenylalanine and tyrosine metabolism (phenylketonuria, alkaptonuria, albinism).

131. Biosynthesis of purine nucleotides: synthesis of phosphoribosylamine, origin of atoms in the purine ring. Inosinic acid as a precursor for synthesis of adenylic and guanylic acids. Regulation of synthesis of purine nucleotides.

132. Biosynthesis of pyrimidine nucleotides. Regulation of biosynthesis of pyrimidine nucleotides.

133. Degradation of nucleic acids in the gastrointestinal tract and tissues. Degradation of purine and pyrimidine nucleotides.

134. Re-utilization of nucleosides and nitrogenous bases for synthesis of nucleotides. Disorders of metabolism of nucleotides: xanthinuria, orotaciduria, gout.

135. Heme synthesis, reactions.

136. Disorders in bilirubin metabolism: jaundice, its types. Differential diagnosis for jaundices of different types.

Flow of Genetic Information

137. Biosynthesis of DNA in eukaryotic cells: scheme, enzymes, regulation.

138. Reverse transcription, biological role.

139. Biosynthesis of RNA in eukaryotic cells: steps, enzymes. Regulation of transcription. Processing of RNA.

140. The genetic code: its characteristic features.

141. Activation of amino acids. Adaptor function of tRNA. Formation and structure of aminoacyl-tRNA.

142. Structure of ribosomes, their function in protein synthesis.

143. Biosynthesis of protein in eukaryotic cells: steps, scheme. Posttranslational processing of proteins.

144. Regulation of protein synthesis. Antibiotics as inhibitors of protein synthesis.

145. Genetic engineering, cloning of DNA.