CELL BIOLOGY SUMMARY
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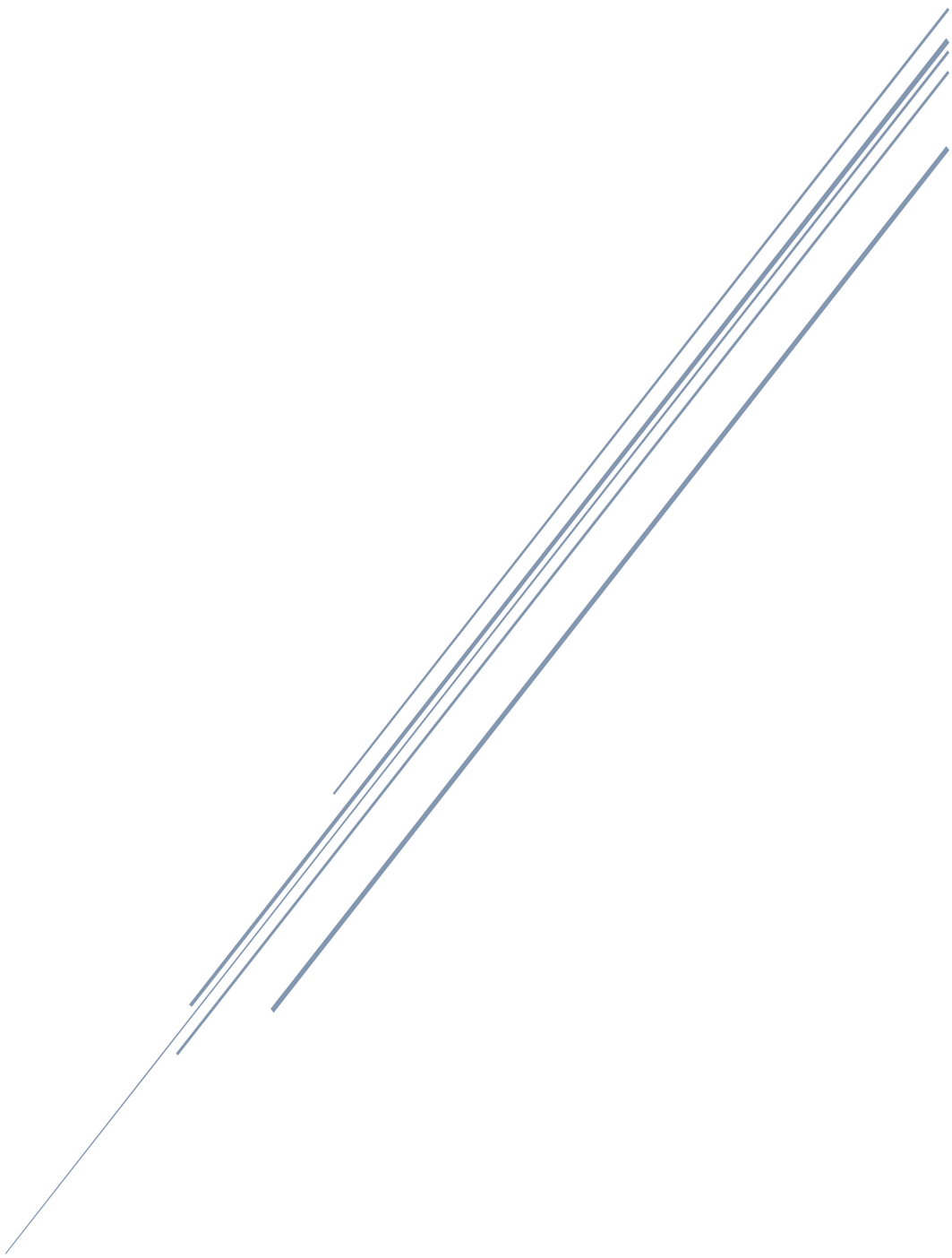

La Sapienza

By Julian Politsch

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1. Introduction to cells

1.1 Unity and diversity of cells

Cells are the fundamental units of life; all living things are made of cells. The present-day cells are believed to have evolved from an ancestral cell that excited more than 3 billion years age. Cells vary enormous in appearance and function, however all living cells have a similar basic chemistry.

With the invention of the microscope, it became clear that plants and animals are assemblies of cells, that cells can also exist as independent organisms, and that cells individually are living in the sense that they can grow, reproduce, convert energy from one form into another, respond to their environment, and so on. Although cells are varied when viewed from the outside, all living things are fundamentally similar inside. And in all living things, genetic instructions, called genes, are stored in DNA molecules. In every cell, the instructions in the DNA are read out, or transcribed, into a chemically related set of molecules made of RNA. The messages carried by the RNA molecules are in turn translated into yet another chemical form: they are used to direct the synthesis of a huge variety of large protein molecules that dominate the behavior of the cell. In sum, the reproduction process exists of replication (DNA synthesis), transcription (RNA synthesis) and translation (protein synthesis). Unfortunately, the copying of DNA is not always perfect, and the instructions are occasionally corrupted. Later is this summary we will discuss this further.

Cells are enclosed by a plasma membrane that separates the inside of the cell from the environment. And all cells contain DNA as a store of genetic information and use it to guide the synthesis of proteins. Cells in a multicellular organism, though the all contain the same DNA, can be very different. They use their genetic information to direct their biochemical activities according to cues they receive from their environment.

1.2 Cells under the microscope

Cells of animal and plant tissues are typically 5-20 micrometer in diameter and can be seen with a light microscope, which also reveals some of their internal components (organelles). The electron microscope permits the smaller organelles and even individual molecules to be seen, but specimens require elaborate preparation and cannot be viewed alive. So, the invention of the light microscope led to the discovery of cells

The presence or absence of a nucleus is used as the basis for a simple but fundamental classification of all living things. Organisms whose cells have a nucleus are called eukaryotes. Organisms whose cells do not have a nucleus are called prokaryotes. Bacteria, the simplest of present-day living cells, are prokaryotes. Different species of prokaryotes are diverse in their chemical capabilities and inhabit an amazingly wide range of habitats. Prokaryotes are divided into two groups: eubacteria and archaea. As mentioned above eukaryotic cells possess a nucleus. They probably evolved in a series of stages from cells more similar to bacteria. An important step appears to have been the acquisition of mitochondria, origination as engulfed bacteria living in symbiosis with larger anaerobic cells.

There are a lot of organelles found in eukaryotic cells: the nucleus is the most prominent organelle in most plant and animal cells. It contains the genetic information of the organism, stored in DNA molecules. The rest of the cell's contents, apart from the nucleus, constitute the cytoplasm. Chloroplasts are green organelles found only in the cells of plants and algae, not in the cells of animals or fungi. They perform photosynthesis and in the process they release oxygen as a molecular by-product. Other organelles are the mitochondria, which are generators of chemical energy for the cell. Mitochondria contain their own DNA and reproduce by dividing in two. Furthermore, they take the energy from the oxidation of food molecules to produce adenosine triphosphate (ATP). The endoplasmic reticulum (ER) is the site at which most cell membrane components, as well as materials destined for export from the cell, are made. The Golgi apparatus often modifies chemically the molecules made in the ER and directs them to various locations of the cell. Lysosomes are organelles in which intracellular digestion occurs and peroxisomes generate a dangerously reactive chemical, hydrogen peroxide. Finally, the cytoskeleton is responsible for directed cell movements.

1.3 Model organisms

Free-living single-celled eukaryotic microorganisms include some of the most complex eukaryotic cells known, and they are able to swim, mate, hunt and devour food. Other types of eukaryotic cells, derived from a fertilized egg, cooperate to form large, complex multicellular organisms composed of thousands of billions of cells.

Biologists have chosen a small number of organisms as a focus for intense investigation. These include the bacterium E. coli, brewer's yeast, a nematode worm, a fly, a small plant, a mouse and the human species itself. Although the minimum number of genes needed for a viable cell is probably less than 400, most cells contain significantly more. Yet even such a complex organism as a human has only about 30.000 genes – twice as many as a fly, seven times many as E. coli.

2. Chemical components of cells

2.1 Chemical bonds

The cell is the structural and functional unit of all known living organisms, but the smallest particle of an element that still retains its distinctive chemical properties is an atom. Each atom has as center a positively charged nucleus, which is surrounded by a cloud of negatively charged electrons. The nucleus consists of two kinds of particles:

- positively charged protons
- neutrons, which are electrically neutral

The number of protons present in an atomic nucleus determines its atomic number. Because the whole atom is electrically neutral, the number of negatively charged electron surrounding the nucleus is equal to the number of positively charged protons that the nucleus contains. Isotopes of an element have nuclei with the same number of protons (the same atomic number) but different numbers of neutrons.

The atomic weight of an atom, or the molecular weight of a molecule, is its mass relative to that of a hydrogen atom. The mass of an atom or a molecule is often specified in Daltons. If a substance has a molecular weight of M, a mass of M grams of the substance will contain 6 x 10^23 molecules. This quantity is called one mole of the substance. The concept of mole is used widely in chemistry as a way to represent the number of molecules that are available to participate in chemical reactions. There are 92 naturally occurring elements, each differing from the others in the number of protons and electrons in its atoms. Living organisms are made of only a small selection of these elements H, N, O, C.

The outermost electrons determine how atoms interact. The number and arrangement of its electrons determine the chemical properties of an atom. An atom is most stable when all of its electrons are at their lowest possible energy level and when each electron shell is completely filled. The number of electrons an atom must acquire of lose to attain a filled outer shell is known as its valence. Chemical bonds form between atoms as electrons move to reach a more stable arrangement. Clusters of two or more atoms held together by covalent bonds are known as molecules. There are two ways to create chemical bonds:

- An ionic bond is formed when electrons are donated by one atom to another.
- A covalent bond is formed when two atoms share a pair of electrons. If two pairs of electrons are shared, a double bond is formed. Double bonds are shorter and stronger than single bonds.

Also, covalent and noncovalent chemical bonds have different strengths and lengths. Noncovalent bonds as a rule are much weaker.

Another noncovalent bond is the hydrogen bond, by which water is held together. These bonds are much weaker than covalent bonds. Molecules carrying positive or negative charges (ions) dissolve readily in water and are called hydrophilic, meaning that they are ‘water-loving’. Hydrophobic (water fearing) molecules on the other hand, are uncharged and form few or no hydrogen bonds, and so do not dissolve in water.

Substances that release protons when they dissolve in water and thus forming H3O+, are termed acids. The higher the concentration of H3O+, the more acidic the solution. The opposite of an acid is a base; any molecule capable of accepting a proton is called a base or alkaline. The concentration of H3O+ is expressed using the pH scale.

2.2 Molecules in Cells

Living organisms contain a distinctive and restricted set of small carbon-based molecules that are essentially the same for every living species. The main categories are:

- Sugars: a primary source of chemical energy for cells and can be incorporated into polysaccharides for energy storage
- Fatty acids: also important for energy storage, but their most essential functions are in the formation of cell membranes. There are two kinds of fatty acids saturated and non-saturated. The first has no double bounds between its carbon atoms and contains the maximum possible numbers of hydrogens. The non-saturated fatty acids have tails with one or more double bounds. These double bounds create kinks in the molecules, interfering with their ability to pack together in a solid mass. How tightly the fatty acids, found in cell membranes, pack affects the fluidity of the membrane.
- Amino acids: the subunits of proteins. The covalent linkage between two adjacent amino acids in a protein chain is called a peptide bound, the chain of amino acids is also known as a polypeptide.
- Nucleotides: the subunits of DNA and RNA

These four families of small organic molecules, together with the macromolecules made by linking them into long chains, account for a large fraction of a cell’s mass.

The vast majority of the dry mass of al cell consists of macromolecules, formed as polymers of sugars, amino acids, or nucleotides. Macromolecules are intermediated both in size and complexity between small molecules and cell organelles. They have many remarkable properties that are not easily deduced from the subunits from which they are made. Their remarkable diversity arises from the fact that each macromolecule has a unique sequence of subunits.

Noncovalent bounds specify the precise shape of a macromolecule: weak noncovalent bonds form between different regions of a macromolecule. Two types of noncovalent bounds are discussed earlier: ionic bounds and hydrogen bounds, but there is a third type of weak bound that result from 'van der Waals attractions. These attractions are a form of electrical attraction caused by fluctuating electric charges that whenever two atoms come within a very short distance of each other. These weak noncovalent bounds can cause the macromolecule to fold into a unique three-dimensional shape with a special chemistry, as seen in proteins.

3. Energy, catalysis and biosynthesis

Living organisms are able to exist because of a continual input of energy. Part of this energy is used to carry out essential functions, like reactions that support cellular metabolism, growth and reproduction, and the remainder is lost in the form of heat.

3.1 Catalysis and the use of energy

All animals live on energy stored in the chemical bonds of organic molecules made by other organisms, which they take as in food. Animals obtain food by eating plants or by eating animals that feed on plants. But ultimately, the primary source of energy for most living organisms is the sun.

Plants and photosynthetic bacteria use solar energy to produce organic molecules from carbon dioxide. They use the energy they derive from sunlight to form chemical bonds between atoms, linking them into small chemical building blocks such as sugar, amino acids, nucleotides and fatty acids. These small molecules in turn are converted into the macromolecules that form the plant.
The reactions of photosynthesis take place in two stages:

1. In the light-dependent stage energy for the sunlight is captured and transiently stored as chemical bond energy in specialized small molecules that carry energy in their reactive chemical groups. Oxygen is released as a by-product of the first stage.
2. In the second stage the molecules that serve as energy carriers are used to help drive a carbon-fixation process in which sugars are manufactured from carbon dioxide gas and water. By producing sugars, these light-independent reactions generate a critical source of stored chemical bond energy and materials.

The net result of the entire process of photosynthesis is:

Light energy + CO_2 + H_2O ® sugars + O_2 + energy

To use to energy to live, grow and reproduce, organisms must extract it in a usable form. In both plants and animals, energy is extracted from food molecules by a process of oxidation, or controlled burning. Next to oxidation there is a process called cellular respiration. Photosynthesis and cellular respiration are complementary processes; cellular respiration uses the O_2 to form CO_2 from the same carbon atoms that had been taken up as CO_2 and converted into sugars by photosynthesis. In this process, the organisms obtain the chemical bond energy that they need to survive.

Oxidation refers to the removal of electrons and reduction (the converse of oxidation) refers to the addition of electrons. Because the number of electrons is conserved in a chemical reaction – there is no net loss or gain – oxidation and reduction always occur simultaneously.

Cells use enzymes to catalyze the oxidation of organic molecules in small steps, through a sequence of reactions that allows useful energy to be harvested.

3.2 Enzymes lower the barriers that block chemical reactions

Each of the many hundreds of chemical reactions that occur in a cell is specifically catalyzed by an enzyme. Large numbers of different enzymes work in sequence to form chains of reactions, called metabolic pathways, each performing a particular set of functions in the cell.

Catabolic reactions break down food molecules through oxidative pathways and release energy. Anabolic reactions generate the many complex molecules needed by the cell, and they require an energy input. In animal cells, both the building blocks and the energy required for the anabolic reactions are obtained by catabolism.

Enzymes catalyze reactions by binding to particular substrate molecules in a way that lowers the activation energy required for making and breaking specific covalent bonds. The rate at which an enzyme catalyzes a reaction depends on how rapidly it finds its substrate and how quickly the product forms and then diffuses away. These rates vary widely from one enzyme to another, and they can be measured after mixing purified enzymes and substrates together under a set of defined conditions. In general, the stronger the binding of the enzyme and substrate, the slower their rate of dissociation.

If a reaction leads to a release of free energy, this energy can be harnessed to do work or drive chemical reactions. Chemical reactions proceed only in the direction that leads to a loss of free energy; in other words, the spontaneous direction for any reaction is the direction that goes 'downhill'. This kind of reaction is often said to be energetically favorable. But even energetically favorable reactions require activation energy to get them started!

As mentioned above, the push over the energy barrier is greatly aided by enzymes. A substance that can lower the energy barrier, and hence the activation energy of a reaction is termed a catalyst. Like all other catalysts, enzyme molecules themselves remain unchanged after participating in a reaction and therefore can function over and over again.

If the concentration of the substrate is increased progressively from a very low value, the concentration of the enzyme-substrate complex, and therefore the rate at which product is formed, initially increases in a linear fashion in direct proportion to substrate concentration. But at a very high concentration of substrate it reaches a maximum value, termed Vmax. At this point, the active sites of all enzyme molecules in the sample are fully occupied with substrate, and the rate of product formation depends only on how rapidly the substrate molecule can be processed; also called the turnover number.

The concentration of substrate needed to make the enzyme work efficiently is often measured by a different parameter, the Michaelis' constant (Km). An enzyme's Km is the concentration of substrate at which the enzyme works at half its maximum speed (0.5 Vmax). A low value of Km indicated that a substrate binds very tightly to the enzyme, and a large value corresponds to weak binding. But enzymes cannot change the equilibrium point for reactions!

3.3 The free-energy change for a reaction determines whether it can occur

Although enzymes speed up reactions, they cannot by themselves force energetically unfavorable reactions to occur. But this can be done through enzymes that directly couple energetically favorable reactions, which release energy and produce heat, to energetically unfavorable reactions, which use this energy. But (according to the second law of thermodynamics) a chemical reaction can proceed only if it results in a net increase of the disorder in the universe. The criterion for an increase of disorder can be expressed most conveniently in term of free energy (G) of a system. The free-energy change for a reaction, ΔG, measures the disorder, and it must be less than zero for a reaction to proceed.

Energetically favorable reactions are those that create disorder by decreasing the free energy of a system to which the belong; in other words, they have a negative ΔG. Conversely, energetically unfavorable reactions, with a positive ΔG, create order in the universe. Because energetically unfavorable reactions require energy, they can take place only if they are coupled to a second reaction with a negative ΔG so large that the net ΔG of the entire process is negative.

In concluding, by creating a reaction pathway that couples an energetically favorable reaction to an energetically unfavorable one, enzymes cause otherwise impossible chemical transformations to occur.

The free-energy change for a chemical reaction, ΔG, depends on the concentration of the reacting molecules, and it may be calculated from these concentrations if the equilibrium constant (K) of the reaction (of the standard free-energy change ΔGº for the reactants) is known. Because the equilibrium constant of a reaction is related directly to the standard free energy change (ΔGº), it is often employed as a measure of the binding strength between molecules. This value is very useful as it indicates the specificity of the interactions between molecules.

Equilibrium constants govern all of the associations (and dissociations) that occur between macromolecules and small molecules in the cell. The equilibrium constant becomes larger as the binding energy between the two molecules increases, and the more likely that these molecules will be paired.

3.4 Activated carrier molecules and biosynthesis

In living systems energy capture is achieved by means of a couple reactions, in which an energetically favorable reaction is used to drive and energetically unfavorable one that produces an activated carrier molecule. Coupling

mechanisms require enzymes, and they are fundamental to all of the energy transactions in the cell. The most important of the activated carrier molecules are ATP, NADH and NADPH. ATP carries high-energy phosphate groups, whereas NADH an NADPH carry high-energy electrons.

1) ATP

The most important of the activated carrier molecules in cells is ATP (adenosine 5'-triphosphate). ATP is synthesized in an energetically unfavorable phosphorylation reaction in which a phosphate group is added to ADP (adenosine 5'-diphosphate). When required, ATP gives up this energy packet in an energetically favorable hydrolysis to ADP and inorganic phosphate. The regenerated ADP is then available to be used for another round of the phosphorylation reaction that forms ATP, creating an ATP cycle in the cell. Therefore, an energetically unfavorable biosynthetic reaction can be driven by ATP hydrolysis. For example, the synthesis of a polynucleotide likes RNA and DNA.

2) NADH an NADPH

NAD+ and NADP+ each pick up a 'packet of energy' in the form of two high-energy electrons plus a proton (H+), becoming NADH and NADPH, respectively. Like ATP, NADPH is an activated carrier that participates in many important biosynthetic reactions that would otherwise be energetically unfavorable. NADH, by contrast, has a special role as an intermediate in the catabolic system of reactions that generate ATP through the oxidation of food molecules.

Food molecules provide the carbon skeletons for the formation of larger molecules. The covalent bonds of these larger molecules are typically produced in reactions that are coupled to energetically favorable bond changes in activated carrier molecules such as ATP and NADPH.

4. Protein structure and function

Proteins are by far the most structurally complex and functionally sophisticated molecules known. Proteins are assembled from a set of 20 different amino acids, each with different chemical properties. A protein molecule is made from a long chain of these amino acids, each linked to its neighbor through a covalent peptide bond. Proteins, therefore, are also called polypeptides. Each polypeptide chain consists of a backbone that supports the different amino acid side chain. The polypeptide backbone if formed from the repeating sequence of atoms along the polypeptide chain. Attached to this repetitive chain are any of the 20 different amino acid side chains. These side chains give each amino acid its unique properties, for example hydrophobic, nonpolar or positively charged.

4.1 Shape and structure of proteins

Each type of protein has a unique amino acid sequence that determines both its three-dimensional shape and its biological activity. Long peptides are very flexible and therefore proteins can fold in enormous number of ways. The folded structure of a protein is stabilized by noncovalent interactions between different parts of the polypeptide chain.

The final folded structure, of conformation, is the one in which the free energy (G) is minimized. A protein can be unfolded, or denatured, by treatment with certain solvents that disrupt the noncovalent interactions holding the folded chain together. When the denaturing solvent is removed, the protein often refolds spontaneously, or renatures, into its original conformation.

When proteins fold improperly, they can form aggregates that can damage cells and even whole tissue. Aggregated proteins underlie a number of neurodegenerative disorders, including Alzheimer's disease and Huntington's disease. Although a protein chain can fold into its correct conformation without outside help, protein folding in a living cell in generally assisted by special proteins called molecular chaperones. These proteins bind to partly folded chains and help them to fold along the most energetically favorable pathway. However, the final three-dimensional shape of the protein is still specified by its amino acid sequence: chaperones merely make the folding process more efficient and reliable.

Although the overall conformation of each protein is unique, two regular folding patterns are often found in parts of them. Hydrogen bonds between neighboring regions of the polypeptide backbone can give rise to regular folding patterns, known as a helices and b sheets. In a helix the N-H of every peptide bond is hydrogen-bonded to the C=O of a neighboring peptide bond located four peptide bonds away in the same chain. In the case of the b sheet the individual polypeptide chains in the sheet are held together by hydrogen-bonding between peptide bonds in different strands, and the amino acid side chains in each strand project alternately above and below the plane of the sheet.

A a helix is generated when a single polypeptide chain turns around itself to form a structurally rigid cylinder. Sometimes a pair of a helices will wrap around one another to form a particularly stable structure, known as coiled-coil. This structure forms when the two a helices have most of their nonpolar (hydrophobic) side chains on one side, so that they can twist around each other with these side chains facing inwards. b Sheets are made when hydrogen

bonds form between segments of polypeptide chains lying side by side. When the structure consists of neighboring polypeptide chains that run in the same orientation, it is considered a parallel b sheet; when it forms from a polypeptide chain that fold back and forth upon itself the structure is an antiparallel b sheet. b Sheets provide an ideal ice-binding surface in an antifreeze protein.

4.2 Levels of organization

The structure of many proteins can be subdivided into smaller globular regions of compact three-dimensional structure, known as protein domain. So, a protein's structure begins with its amino acid sequence, which is thus considered its primary structure. The next level of organization includes the a helices and b sheets that form within certain segments of polypeptide chain; these folds are elements of the protein's secondary structure. The full, three-dimensional conformation formed by an entire polypeptide chain is referred as the tertiary structure. Finally, if a particular protein molecule is formed as a complex of more than one polypeptide chain, than the complete structure is designed its quaternary structure.

The same weak noncovalent bonds that enable a polypeptide chain to fold into a specific conformation also allows proteins to bind to each other to produce larger structures in the cell. Any region on a protein's surface that interacts with another molecule through sets of noncovalent bonds is termed a binding site. If a binding site recognizes the surface of a second protein, the tight binding of two folded polypeptide chains at this site will create a larger protein molecule with a precisely defined geometry. Each polypeptide chain in such a protein is called a subunit. Each of these protein subunits may contain more than one domain.

There are different types of proteins:

1. Globular proteins: in which the polypeptide chain folds up into a compact shape like a ball with an irregular surface. Enzymes tend to be globular proteins.
2. Fibrous proteins: these have a relatively simple, elongated three-dimensional structure. These proteins are especially abundant outside the cell, where they form the gel-like extracellular matrix that helps cells bind together to form tissues. These proteins are secreted by cells into their surface roundings, where they often assemble into sheets of long fibrils. Collagen is the most abundant of these fibrous proteins in animal tissues, and another example is elastin.

To help maintain their structures, the polypeptide chains in such proteins are often stabilizes by covalent cross-linkages. The most common cross-links in proteins are covalent sulfur-sulfur bonds. These disulfide bonds (also called S-S bonds) form as proteins are being exported from cells. Their formation is catalyzed in the endoplasmatic reticulum by a special enzyme that links together two –SH groups from cysteine side chains that are adjacent in the folded protein.

4.3 How proteins work

The biological function of a protein depends on the detailed chemical properties of its surface and how it binds to other molecules, called ligands. The ability of a protein to bind selectively and with high affinity to a ligand is due to the formation of a set of weak, noncovalent bonds and favorable hydrophobic interactions. Each individual bond is weak, so that an effective interaction requires that many weak bonds be formed simultaneously. The region of a protein that associates with a ligand, known as its binding sites, usually consists of a cavity in the protein surface formed by a particular arrangement of amino acids. These amino acids belong to widely separated regions of the polypeptide chain that are brought together when the proteins fold.

All proteins must bind to particular ligands to carry out their various functions. But this binding capacity seems to have been most highly developed for proteins in the antibody family. Antibodies, or immunoglobulins, are proteins produced by the immune system in response to foreign molecules. Each antibody binds to a particular target molecule, either inactivating that target directly or marking it for destruction. An antibody is Y-shaped and has two identical binding sites for its antigen, one on each arm of the Y.

For many proteins, binding to another molecule is their only function, but there are some proteins for which ligand binding is simply a necessary first step in their functions. This class of proteins is called enzymes. Enzymes are proteins that first bind tightly to specific molecules, called substrates, and then catalyze the formation or breakage of covalent bonds in these molecules. At the active site of an enzyme, the amino acid side chains of the folded protein are precisely positioned so that they favor the formation of the high-energy transition states that the substrates must pass through to be converted to product.
The three-dimensional structure of many proteins has evolved so that the binding of a small ligand can induce a significant change in protein shape.

Although the order of amino acids in proteins gives molecules their shape and the versatility to perform different functions, sometimes the amino acids by themselves are not enough. So, proteins often employ small nonprotein molecules to perform functions that would be difficult or impossible using amino acids alone. Examples of these proteins are retinal (the light-sensitive molecule attached to rhodopsin in our eyes) and heme (gives hemoglobin and blood its red color and enables hemoglobin to pick up oxygen in the lungs and release it in the tissues).

4.4 How proteins are controlled

Inside the cell most proteins and enzymes do not work continuously or at full speed. Instead, their activity is regulated so that the cell can maintain itself in a state of equilibrium, generating only those molecules it requires to thrive under the current conditions. To achieve this balans, the activities of cellular proteins are controlled in an integrated fashion, with consideration of what reactions are occurring in other parts of the cell. By coordinating when and how proteins function, the cell ensures that it does not deplete its energy reserves by accumulating molecules it does not require.

Regulation of enzyme activity occurs at many levels. At one level, the cell controls how many molecules of each enzyme it makes by regulating the expression of the gene that encodes that protein. At another level, the cell controls enzymatic activities by confining sets of enzymes to particular subcellular compartments, enclosed by distinct membranes (both mechanisms are discussed later in this summary). But the most rapid and general process used to adjust reactions rates operated at the level of the enzyme itself. In this case, an enzyme's activity changes in response to other specific molecules that it encounters.

- Feedback inhibition (negative feedback): it prevents an enzyme from acting
- Positive regulation (positive feedback): the enzyme's activity is stimulated

The interaction between sites that are located on separate regions of a protein molecule is known to depend on a conformational change in the protein: binding at one of the sites causes a shift in the protein's structure from one folded shape to a slightly different folded shape. Feedback inhibition, for example, triggers a conformational change. Many, if not most, protein molecules are allosteric: they can adopt two or more slightly different conformations that differ in catalytic activity, and by a shift from one to another, their activity can be regulated. This is true not only for enzymes but for many other proteins like receptors, structural proteins, and motor proteins. The enzyme can be turned on or off by ligands that bind to a distinct regulatory site to stabilize either the active or the inactive conformation.

Phosphorylation can control protein activity by triggering a conformational change

Enzymes are not only regulated by the binding of small molecules. A second method commonly used by eukaryotic cells to regulate protein activity involves attaching a phosphate group covalently to one of its amino acids side chains. Removal of the phosphate group by a second enzyme returns the protein to its original conformation and restores its initial activity. This reversible protein phosphorylation controls the activity of many different types of proteins in eukaryotic cells.

Protein phosphorylation involves the enzyme-catalyzed transfer of the terminal phosphate group of ATP to the hydroxyl group on a serine, threonine, of tyrosine said chain of the protein. This reaction is catalyzed by a protein kinase. The reverse reaction – removal of the phosphate group, or dephosphorylation – is catalyzed by a protein phosphatase. Cells contain hundreds of different protein kinases, each responsible for phosphorylating a different protein or set of proteins. Cells also contain many different protein phosphatases.

For many proteins, a phosphate group is added to a particular side chain and then removed in a continuous cycle. Phosphorylation cycles of this kind allow proteins to switch rapidly from one state to another. The energy required to drive this cycle is derived from the free energy of hydrolysis of ATP.

GTP-Binding proteins are also regulated by cyclic gain and loss of a phosphate group

Eukaryotic cells have another way to regulate protein activity by phosphate addition and removal. Instead of being enzymatically transferred from ATP to the protein, the phosphate is part of a guanine nucleotide (either GTP or GDP) that is bound tightly to the protein. Such GTP-binding proteins are in their active conformations with GTP bound; the protein itself then hydrolyses this GTP to GDP, by releasing a phosphate, and flips to an inactive conformation. As with protein phosphorylation, this process is reversible.

The GTP-binding proteins often bind to other proteins to control enzyme activities, and their crucial role in intracellular pathways will be discussed later in this summary.

In concluding, many thousands of proteins in a typical eukaryotic cell are regulated either by cycles of phosphorylation and dephosphorylation, or by the binding and hydrolysis of GTP by a GTP-binding protein.

4.5 Nucleotide hydrolysis

As mentioned above, conformational changes in proteins play a central part in enzyme regulation and cell signaling. But conformational changes also play another important role in the operation of the cell: they enable proteins whose major function is to move other molecules, the motor proteins, to generate the forces responsible for muscle contraction and the movements of the cell. The hydrolysis of ATP to ADP by motor proteins produces directed movements in the cell.

4.6 Proteins often form large complexes that function as protein machines

Highly efficient proteins machines are formed by assemblies of allosteric proteins. In most protein machines the hydrolysis of bound nucleoside triphosphates (ATP or GTP) drives an ordered series of conformational changes in some of the individual protein subunits, enabling the ensemble of proteins to move coordinately. In this way, the appropriate enzymes can be moved directly into the positions where they are needed to carry out successive reactions in a series as, for example, protein synthesis or DNA replication.

5. DNA and chromosomes

5.1 The structure and function of DNA

Life depends on stable and compact storage of genetic information. Genetic information is carried by very long deoxyribonucleic acid (DNA) molecules and encoded in the linear sequence of nucleotides A, T, G and C.
A DNA molecule consists of two long polynucleotide chains known as DNA chains, or DNA strands. Each of these chains is composed of four types of nucleotide subunits, and the two chains are held together by hydrogen bonds between the base portions of the nucleotides, nucleotides are composed of a five-carbon sugar to which are attached one or more phosphate groups and a nitrogen-containing base. In the case of the nucleotides in DNA, the sugar is deoxyribose attached to a single phosphate group, and the base may by adenine (A), cytosine (C), guanine (G), or thymine (T). The nucleotides are covalently linked together in a chain through the sugars and phosphates, which thus form a 'backbone' of alternating sugar-phosphate-sugar-phosphate.

The two polynucleotide chains in the DNA double helix are held together by hydrogen-bonding between the bases on the different strands; G-C and A-T. All the bases are therefore on the inside of the helix, with the sugar-phosphate backbones on the outside.
Each strand of DNA has a chemical polarity due to the linkage of alternating sugars and phosphates in its backbone The members of each base pair can fit together within the double helix only if the two strands of the helix are antiparallel, that is, only if the polarity of one strand is oriented opposite to that of the other strand. A consequence of these base-pairing requirements is that each strand of a DNA molecule contains a sequence of nucleotides that is exactly complementary to the nucleotide sequence of its partner strand. This is crucial importance for the copying of DNA, as we will see later in the summary.

5.2 The structure of eukaryotic chromosomes

Large amounts of DNA are required to encode all the information needed to make just a single-celled bacterium, and far more DNA is needed to encode the instructions for the development of multicellular organisms like ourselves.

In eukaryotic cells, enormously long double-stranded DNA molecules are package into chromosomes can be easily apportioned between the two daughter cells at each cell division. In eukaryotes the DNA in the nucleus is distributed among a set of different chromosomes. Each chromosome consists of a single, enormously long, linear DNA molecule associated with proteins that fold and pack the fine thread of DNA into a more compact structure. The complex of DNA and protein is called chromatin.
With the exception of the germ cells (sperm and eggs) and highly specialized cells that lack DNA entirely, human cells each contain two copies of each chromosome, one inherited from the mother and one from the father; the maternal and paternal chromosomes of a pair are called homologous chromosomes (homologs). The only nonhomologous chromosome pairs are the sex chromosomes in males, where a Y chromosome is inherited from the father and an X chromosome from the mother.

A display of the full set of 46 chromosomes is called the human karyotype. Cytogeneticists use alterations in banding patterns to detect chromosomal abnormalities that are associated with some inherited defects and with certain types of cancer.

The genetic material of a eukaryotic cell is contained within one or more chromosomes, each formed from a single, enormously long DNA molecule that contains many genes. In general, the more complex an organism is, the larger its genome. Furthermore, how the DNA is apportioned over chromosomes also differ from one species to another. Humans have 46 chromosomes, but a species of small deer has only 6 chromosomes. Thus, although gene number is roughly correlated with species complexity, there is no simple relationship between gene number, chromosome number and total genome size.

5.3 'Life-cycle of chromosomes'

To form a functional chromosome, a DNA molecule must be able to replicate, and the replicated copies must be separated and partitioned reliably into daughter cells at each cell division. These processes occur through an ordered series of stages, known collectively as the cell cycle.

Two stages are important:

1. the interphase, when chromosomes are duplicated;
2. and mitosis, when they are distributed to the two daughter nuclei.

During interphase, the cell is actively expressing its genes, and during this stage the chromosomes are extended as long, thin, tangled threads of DNA in the nucleus. Still during the interphase and before cell division, the DNA is replicated, and the chromosomes are duplicated. Once DNA replication is complete, the cell can enter M phase, when mitosis occurs. Mitosis is the division of the nucleus. During this stage, the chromosomes condense, gene expression largely ceases, the nuclear envelope breaks down, and the mitotic spindle forms from microtubules and other proteins. The condensed chromosomes are captured by the mitotic spindle, and one complete set of chromosomes is pulled to each end of the cell. A nuclear envelope form around each chromosome set, and in the final step of M phase, the cell divides to produces two daughter cells.

Three DNA sequence elements are needed to produce a eukaryotic chromosome that can be replicated and then segregated at mitosis. These sequences ensure that the chromosome can be replicated efficiently and passed on to daughter cells.

1. Telomere: contain repeated nucleotide sequences that enable the ends of chromosomes to be replicated. They also protect the end of the chromosome from being mistaken by the cell as a broken DNA molecule in need of repair. But the function of telomeres is discussed later in this summary.
2. Replication origin
3. Centromere: this allows one copy of each duplicated chromosome to be apportioned to each daughter cell.

The nucleus is delimited by a nuclear envelope formed by two concentric membranes. The nuclear envelope is supported by two networks of protein filaments: one, the nuclear lamina, forms a thin layer underlying and supporting the inner nuclear membrane; while the other, less regularly organized, surrounds the outer nuclear membrane. The two membranes are punctured at intervals by nuclear pores, which actively transport selected molecules to and from the cytosol.

The most obvious example of chromosome organization in the interphase nucleus is the nucleolus. This is a region where the parts of different chromosomes carrying genes for ribosomal RNA cluster together. Here, ribosomal RNAs are synthesized and combined with proteins to form ribosomes, the cell's protein-synthesizing machines.

5.4 Chromosomes and DNA

Chromosomes in eukaryotic cells consist of DNA tightly bound to a roughly equal mass of specialized proteins. These proteins fold the DNA into a more compact form so that it can fit into a cell nucleus.

The proteins that bind to the DNA to form eukaryotic chromosomes are traditionally divided into two general classes: the histones and the nonhistone chromosomal proteins. The complex of DNA and both classes of protein in chromosomes is called chromatin. Histones are responsible for the first and most fundamental level of chromatin packing: they pack DNA into a repeating array of DNA-protein particles called nucleosomes.

An individual nucleosome core particle consists of a complex of eight histone proteins (two molecules each of the histone H2A, H2B, H3 and H4) and a double stranded DNA of about 146 nucleotide pairs that winds around this histone octamer. Each nucleosome core particle is separated from the next by a region of linker DNA.

All four of the histones that make up the nucleosome core are relatively small proteins with a high proportion of positively charged amino acids. The positive charges help the histones bind tightly to the negatively charged sugar-phosphate backbone of DNA. Each of the core histones also has a long N-terminal amino acid 'tail', which extends out from the DNA histone core. These histone tails are subject to several types of covalent modification that control many aspects of chromatin structure.

Nucleosomes are further packed upon one another to generate a more compact structure, the 30-nm fiber. This happens with the aid of histone H1 molecules, which is thought to pull the nucleosomes together into a regular repeating array. This fiber can be further coiled and folded, producing more compact chromatin structures. But, some forms of chromatin are so highly compacted that the packaged genes cannot be expressed into protein.

As daughter cells complete their separation following mitosis, the mitotic chromosomes unfold into a more extended form: the interphase chromosomes. However, the chromatin in an interphase chromosome is not in the same packing state throughout the chromosome. In general, regions of the chromosome that contain genes that are being expressed are more extended, while those that contain quiescent genes are more compact. The most highly condensed form of interphase chromatin is called heterochromatin. Heterochromatin typically makes up about 10% of an interphase chromosome, and in mammalian chromosomes, it is typically concentrated around the centromere region and in the telomeres at the ends of the chromosomes. Most DNA that is folded into heterochromatin does not contain genes. However, genes that do become packaged into heterochromatin usually become resistant to

being expressed because heterochromatin is unusually compact. The rest of the interphase chromatin, which is in a variety of more extended states, is called euchromatin.

5.5 Changes in nucleosome structure allow access to DNA

Eukaryotic cells have several ways to rapidly adjust the local structure of their chromatin. One approach takes advantage of chromatin remodeling complexes, protein machines that use the energy of ATP hydrolysis to change the structure of nucleosomes.

In concluding, chromatin structure is dynamic: by temporarily altering its structure (using chromatin remodeling complexes and enzymes that modify histone tails) the cell can ensure that proteins involved in gene expression, replication, and repair have rapid, localized access to the necessary DNA sequences.

6. DNA replication, repair and recombination

The ability of a cell to maintain order in a chaotic environment depends on the accurate duplication of the vast quantity of genetic information carried in its DNA. This duplication process, called DNA replication, must occur before a cell can produce two genetically identical daughter cells.

Despite systems for protecting the genetic instructions form copying errors and accidental damage, permanent changes, or mutations, sometimes do occur. Mutations in the DNA often affect the information it encodes. Occasionally, this can benefit the organism in which a mutation occurs. However, mutations are often detrimental: they are responsible for thousands of inherited diseases and many types of cancer. Without the cellular systems that are continually monitoring and repairing damage to DNA, it is questionable whether life could exist at all.

6.1 DNA replication

As mentioned earlier, each strand of the DNA double helix contains a sequence of nucleotides that is exactly complementary to the nucleotide sequence of its partner strand. Each strand can therefore act as a template for the synthesis of a new complementary strand.
The ability of each strand of a DNA molecule to act as a template for producing a complementary strand enables a cell to copy, or replicate, its genes before passing them on to its descendants. This replication is performed by a cluster of proteins that together form a ‘replication machine’. In each round of replication, each of the two strands of DNA is used as a template for the formation of a complementary DNA strand. The original strands, therefore, remain intact through many cell generations. DNA replication is called ‘semiconservative’ because each daughter DNA double helix is composed of one conserved strand and one newly synthesized strand.

DNA synthesis

1) DNA synthesis begins at replication origins

The DNA double helix is normally very stable, because the two DNA strands are locked together by the large numbers of hydrogen bonds between the bases on both strands. The process of DNA replication begins by initiator proteins that bind to the DNA and pulled the two strands apart, by breaking the hydrogen bonds between the bases. Although the hydrogen bonds collectively make the DNA helix very stable, individually each hydrogen bond is weak. Separating a short length of DNA does not therefore require a large energy input. The positions at which the DNA is first opened are called replication origins, and they are usually marked by a particular sequence of nucleotides. An A-T base pair is held together by fewer hydrogen bonds than is a G-C base pair, therefore DNA rich in A-T base pairs is relatively easy to pull apart, and A-T-rich stretches of DNA are typically found at replication origins.
Once an initiator protein binds to DNA at the replication origin and locally opens up the double helix, it attracts a group a proteins that carry out DNA replication. This group operates as a protein machine, with each member carrying out a specific function.

2) New DNA synthesis occurs at replication forks

As a DNA molecule replicates, its two strands are pulled apart to form one or more Y-shaped replication forks. At these forks, the replication machine is moving along the DNA, opening up the two strands of the double helix and using each strand as a template to make a new daughter strand. The enzyme DNA polymerase, situated in the fork, catalyzes the addition of nucleotides to the 3’ end of a growing DNA strand by forming a phosphodiester bond between this end and the 5’-phosphate group of the incoming nucleotide.

In concluding, DNA polymerase can catalyze the growth of the DNA chain in only one direction: the 5’-to-3’ direction. This problem is solved by the use of a ‘backstitching’ maneuver. The DNA strand whose 5’ end must grow is made discontinuously, in successive separate small pieces, with the DNA polymerase working backward from the replication fork in the 5’-to-3’ direction for each new piece. These pieces, called Okazaki fragments, are later ‘stitched’ together (by the enzyme ligase) to form a continuous new strand. The DNA strand that is synthesized

discontinuously in this way is called the lagging strand; the strand that is synthesized continuously is called the leading strand. Because both of the new strands are synthesized in the 5'-to-3' direction, the DNA replication forks are asymmetrical.

DNA polymerase

DNA polymerase replicated a DNA template with remarkable fidelity, making less than one error in every 10^7 bases read. This is possible because the enzyme removes its own polymerization errors as it moves along the DNA (proofreading). Before the enzyme adds a nucleotide to a growing DNA chain, it checks whether the previous nucleotide added is correctly base-paired to the template strand. If so, the polymerase adds the next nucleotide; if not, the polymerase removes the mis paired nucleotide by cutting the phosphodiester bond it has just made, releases the nucleotide, and tries it again. Thus, DNA polymerase possess both a 5'-to-3' polymerization activity and a 3'-to-5' exonuclease (nucleic acid-degrading) activity.

This proofreading mechanism explains why DNA polymerase synthesize DNA only in the 5'-to-3' direction. If DNA is synthesized in the 3'-to-5' direction is would be unable to proofread: because if it removed an incorrectly paired nucleotide, the polymerase would create a chain end that is dead and unable to elongate.

Because the polymerase can join a nucleotide only to a base-paired nucleotide in a DNA double helix, it cannot start a completely new DNA strand. A different enzyme is needed, and it is called primase. This enzyme makes short lengths of RNA, called primers, which are subsequently erased and replaced with DNA.

For the leading strand, an RNA primer is needed only to start replication at a replication origin; once a replication fork has been established, the DNA polymerase is continuously present with a base-paired 3' end as it tracks along the template strand. But on the lagging strand, where DNA synthesis is discontinuous, new primers are needed continually.

To produce a continuous new DNA strand from the many separate pieces of RNA and DNA made on the lagging strand, three additional enzymes are needed. These act quickly to remove the RNA primer (nuclease), replace it with DNA (repair polymerase), and join the DNA fragments together (ligase).

Proteins at a replication fork

DNA replication requires the cooperation of many proteins: at the head of the replication machine is a helicase, a protein that uses the energy of ATP hydrolysis to speed along DNA, unzipping the double helix as it moves. Another component of the replication machine, the single-strand binding proteins clings to the single-stranded DNA exposed by the helicase and transiently prevents is from re-forming base pairs. Yet another protein, called a sliding clamp, keeps the DNA polymerase firmly attached to the DNA template; on the lagging strand, the sliding clamp releases the polymerase from the DNA each time an Okazaki fragment is completed. This clamp proteins forms a ring around the DNA helix and binds polymerase, allowing it to slide along a template strand as its synthesis new DNA.

Most of the proteins involved in DNA replication are thought to be held together in a large multienzyme complex that moves as a unit along the DNA, enabling DNA to be synthesized on both strands in a coordinated manner.

Genetic information can be stored stably in DNA sequences only because a variety of DNA repair enzymes continuously scan the DNA and correct replication mistakes and replace damaged nucleotides. DNA can be repaired easily because one strand can be corrected using the other strand as a template.

Telomerase replicates the ends of eukaryotic chromosomes

In eukaryotes, a special enzyme called telomerase replicated the DNA at the ends of the chromosomes. Telomerase adds multiple copies of the same telomere DNA sequence to the ends of the chromosomes, thereby producing a template that allows replication of the lagging strand to be completed.

6.2 DNA repair

To survive and reproduce, individuals must be genetically stable. This stability is achieved not only through the extremely accurate mechanism for replicating DNA, but also through mechanisms for correcting the rare copying mistakes made by the replication machinery and for repairing the accidental damage that continually occurs to the DNA. Most of these changes in DNA are only temporary because they are immediately corrected by processes collectively called DNA repair.

Only rarely do the cell's DNA replication and repair processes fail and allow a permanent change in the DNA. Such a permanent change is called a mutation. For example, a single nucleotide change causes the inherited disease sickle-cell anemia. It is very important to protect reproductive cells (germ cells) against mutation, because a mutation in one of these cells will be passed on to all the cells in the body of the multicellular organism that develops from it, including the germ cells for production of the next generation. However, the other cells (somatic cells) must also be

protected from genetic change to safeguard the health and well-being of the individual. Therefore, cells have acquired an elegant set of mechanisms to reduce the number of mutations that occur in their DNA.

DNA mismatch repair system

The rare copying mistakes that slip through the DNA replication machinery are dealt with by the mismatch repair proteins, which monitor newly replicated DNA and repair copying mistakes. The overall accuracy of DNA replication, including mismatch repair, is one mistake per 10^9 nucleotides copied.

A complex of mismatch repair proteins recognizes the DNA mismatches, removes (excises) one of the two strands of DNA involved in the mismatch, and resynthesizes the missing strand. To be effective in correcting replication mistakes, this mismatch repair system must always excise only the newly synthesized DNA, and thus, eliminating the mutation by using the original (old) template strand as the template. The importance of mismatch repair in humans was recognized when it was discovered that an inherited predisposition to certain cancers (especially some types of colon cancer) is caused by a mutation in the gene responsible for producing one of the mismatch repair proteins.

DNA is continually suffering damage in cells. There are many ways in which the DNA can be damaged, and these require other mechanism, than mismatch repair proteins, for their repair. Depurination and deamination are the most frequent chemical reactions known to create serious DNA damage in the cell. Furthermore, the ultraviolet radiation in sunlight causes also DNA damage.
The basic mechanism of DNA repair involved three steps:

- Excision
- Resynthesizes
- Ligation

In step one (excision), the damage is recognized and removed by one of a variety of different nuclease, which cleave the covalent bond that join the damaged nucleotides to the rest of the DNA molecule, leaving a small gap on one of the DNA double helix in this region. In step two (resynthesize), a repair DNA polymerase binds to the 3'-hydroxyl ends of the cut DNA strand. In then fills the gap by making a complimentary copy of the information stored in the undamaged strand. In step three (ligation), DNA ligase seals the nick left in the sugar-phosphate backbone of the repaired strand. Nick sealing requires energy from ATP hydrolysis, remakes the broken phosphodiester bond between the adjacent nucleotides.

steps two and three are nearly the same for most types of DNA repair, including mismatch repair. However, step one uses a series of different enzymes, each specialized for removing different types of DNA damage.

6.3 DNA recombination

Homologous recombination is the process by which two double-stranded DNA molecules of similar nucleotide sequence can cross over to create DNA molecules of novel sequence. Homologous recombination begins with a double-strand break in a chromosome, creating a complete break in the DNA molecule. The 5' ends at the break are then chewed back by a DNA-digesting enzyme, creating protruding single-stranded 3' ends. Each of these single strands then searches for a homologous, complementary DNA helix with which to pair, leading to the formation of a 'joint-molecule' between the two chromosomes. The nicks in the DNA strands are then sealed and this is known as a cross-strand exchange or 'Holliday junction'. To regenerate two separate DNA molecules, the two crossing strands must be cut. The structure undergoes a series of rotational movements so that the two original noncrossing strands become crossing and vice versa.

Homologous recombination provides many advantages to cells and organisms:

- The process allows an organism to repair DNA that is damaged on both strands of the double helix
- It can fix other genetic accidents that occur during nearly every round of DNA replication
- It is essential for the accurate chromosome segregation that occurs during meiosis in fungi, plants and animals.

In homologous recombination, DNA rearrangements occur between DNA segments that are very similar in sequence. A second type of recombination, called site-specific recombination, allows DNA exchange to occur between DNA double helices that are not similar in nucleotide sequences. Mobile genetic elements are thought to play a crucial role in this process. Mobile genetic elements are DNA sequences that can move from place to place in the genomes of their host. This movement creates change in the host genome provides a source of genetic variation.

More than 50% of the human genome consists of DNA that is repeated many times in the genome. Approximately two-thirds of this repeated DNA (about 34% of the total genome) consists of two classes of transposons that have multiplied to especially high copy numbers in the genome. Retrotransposons move via an RNA intermediate, instead of a DNA intermediate. One type of retrotransposon, the L1 element (or LINE-1), is a highly repeated sequence that constitutes about 15% of the total mass of the human genome.

Viruses are mobile genetic elements that can escape from cells
Viruses are little more than genes packaged in protective protein coats. They require host cells in order to reproduces themselves. Viral genomes can be made of DNA or RNA and can be single-stranded or double-stranded. One group of RNA viruses – the retrovirus – must copy their RNA genomes into DNA in order to replicate. The human immunodeficiency virus (HIV), which is the cause of AIDS, is a retrovirus.

7. From DNA to protein

When a particular protein is needed by the cell, the nucleotide sequences of the appropriate portion of the immensely long DNA molecule in a chromosome is first copies into another type of nucleic acid – RNA (ribonucleic acid). It is these RNA copies of short segments of the DNA that are used as templates to direct the synthesis of the protein.

The flow of genetic information in all living cells is therefore from DNA ® RNA ® protein. The conversation of the genetic instructions in DNA into RNAs and proteins is termed gene expression.

7.1 From DNA to RNA

To express the genetic information carried in DNA, the nucleotide sequence of a gene is first transcribed into RNA. Like DNA, RNA is a linear polymer made of four different types of nucleotide subunits linked together by phosphodiester bonds. It differs from DNA chemically in two ways:

1. The nucleotides in RNA are ribonucleotides; they contain the sugar ribose rather than deoxyribose
2. Although RNA and DNA both contain the bases adenine (A), guanine (G), and cytosine (C), RNA contains uracil (U) instead of thymine (T) found in DNA.

Next to the chemical differences, there are also important differences in overall structure. Whereas DNA always occurs in cells as a double-stranded helix, RNA is single-stranded. This difference has important functional consequences; because RNA is single-stranded it can fold up into a variety of three-dimensional shapes. As we will see later in this summary, this ability allows RNA to carry out different functions, like for example catalytic functions.

All of the RNA in a cell is made by transcription. The enzymes that carry out transcription are called RNA polymerase. RNA polymerases catalyze the formation of phosphodiester bonds that link the nucleotides together and form the sugar-phosphate backbone of the RNA chain. The RNA polymerase moves stepwise along the DNA, unwinding the DNA helix to expose a new region of the template strand for complementary base-pairing. Nucleotide sequences in the DNA molecule indicate to the RNA polymerase where to start and stop transcribing. A promotor contains a sequence of nucleotides indicating the starting point for RNA synthesis. A subunit of bacterial polymerase, called sigma (s) factor, is primarily responsible for recognizing the promotor sequence on DNA.

RNA polymerase makes about one mistake for every 10^4 nucleotides copied into RNA, compared with an error rate for DNA polymerase of about one in 10^7 nucleotides. Although RNA polymerase catalyzes essentially the same chemical reactions as DNA polymerase, there are some important differences between the two enzymes:

- RNA polymerase catalyzes the linkage of ribonucleotides, not deoxyribonucleotides
- Unlike the DNA polymerase involved in DNA replication, RNA polymerase can start an RNA chain without a primer

Several types of RNA are produces in cells

Cells make several different functional types of RNA, including messenger RNA (mRNA), which carries the instructions for making proteins, ribosomal RNA (rRNA), which is a component of ribosome's; and transfer RNA (tRNA), which acts as an adaptor molecule in proteins synthesis.

Although the templating principle by which DNA is transcribed into RNA is the same in all organisms, the way in which the RNA transcripts are handled before the can be used by the cell differs a great deal between bacteria and eukaryotes. Bacterial DNA lies directly exposed to the cytoplasm, which contains the ribosomes on which protein synthesis takes places. In eukaryotic cells, by contrast, DNA is enclosed within the nucleus. Transcription takes place in the nucleus, but protein synthesis takes place on ribosomes in cytoplasm. So, before mRNA can be translated, it must be transported out of the nucleus. In addition, before RNA exists in the nucleus, it must go through several different RNA processing steps. Two processing steps that occur only on transcripts destines to become mRNA molecules are:

- RNA capping
- Polyadenylation

These two modifications are thought to increase the stability of the eukaryotic mRNA molecule, to aid its export from the nucleus to the cytoplasm, and to generally identify the RNA molecule as an mRNA. They are also used by

the protein-synthesis machinery as an indication that both ends of the mRNA are present and that the message is therefore completed.

Genes are interrupted by noncoding sequences

In eukaryotic DNA most genes are composed of a number of smaller coding regions (exons) interspersed with noncoding regions (introns). When a eukaryotic gene is transcribed from DNA into RNA, both exons and introns are copied. After capping, as the RNA polymerase continues to transcribe the gene, the process of RNA splicing begins, in which the intron sequences are removed from the newly synthesized RNA and the exons are stitched together. RNA splicing is performed by RNA molecules that recognize intron-exon boundaries and participate in the chemistry of splicing. These RNA molecules, called small nuclear RNAs (snRNAs), bind with additional proteins to form small nuclear ribonucleoprotein particles (snRNPs). These snRNPs form the core of the spliceosome, the largely assembly of RNA and protein molecules that performs RNA splicing in the cell. To splice an RNA, a group of snRNPs assemble at an intron-exon boundary, cut out the intron, and rejoin the RNA chain.

In concluding, eukaryotic mRNAs go through several additional RNA processing steps before the leave the nucleus, including RNA capping and polyadenylation. These reactions, along with splicing, are tightly coupled to transcription and take place as the RNA is being transcribed. The mature mRNA then moves to the cytoplasm. Furthermore, RNA splicing enables eukaryotes to increase the already enormous coding potential of their genomes.

7.2 From RNA to protein

Translation is the 'transfer of the information' in RNA into proteins. Because there are only 4 different nucleotides in mRNA and 20 different types of amino acids in a protein, this translation cannot be accounted for by a direct one-to-one correspondence between a nucleotide in RNA and an amino acid in protein. The rules by which the nucleotide sequence of a gene, through the medium of mRNA, is translated into the amino acid sequence of a protein are known as the genetic code.

Translation of the nucleotide sequence of mRNA into a protein takes place in the cytoplasm on large ribonucleoprotein assemblies called ribosomes. These attach to the mRNA and move stepwise along the mRNA chain, translating the message into protein. The nucleotide sequence in mRNA is read in sets of three nucleotides (codons), each codon corresponding to one amino acid. The correspondence between amino acids and codons is specified by the genetic code. The possible combinations of the 4 different nucleotides in RNA give 64 (4x4x4) different codons in the genetic code. Most amino acids are specified by more than one codon.

Transfer RNAs

The codons in an mRNA do not directly recognize the amino acids they specify. Rather, the translation of mRNA into proteins depends on adaptor molecules that can recognize and bind both to the codon and to the amino acid. These adaptors consist of a set of small RNA molecules known as transfer RNAs (tRNAs).

tRNA acts as an adaptor molecule in protein synthesis and enzymes called aminoacyl-tRNA synthetases link amino acids to their appropriate tRNAs. Each tRNA contains a sequence of three nucleotides, the anticodon, which matches a codon in mRNA by complementary base-pairing between codon and anticodon.

Recognition and attachment of the correct amino acid depends on enzymes called aminoacyl-tRNA synthetases, which covalently couple each amino acid to its appropriate set of tRNA molecules. There is a different synthetase enzyme for each amino acid. The synthetase-catalyzed reaction that attaches the amino acid to the 3' end of the tRNA is one of the many cellular reactions coupled to the energy-releasing hydrolysis of ATP. And it produces a high-energy bond between the charged tRNA and the amino acid. The energy of this bond is used at a later stage in protein synthesis to covalently link the amino acid to the growing polypeptide chain.

Decoding of the RNA message

Each ribosome has a binding site for mRNA and three binding sites for tRNA. The tRNA sites are designated the A-, P-, and E-sites. Protein synthesis begins when a ribosome assembles at an initiation codon (AUG) in mRNA, a process that is regulated by proteins called translation initiation factors. Of all the charged tRNAs in the cell, only the charged initiator tRNA is capable of tightly binding to the P-site of the small ribosome subunit. The completed protein chain is released from the ribosome when a stop codon (UUA, UAG, of UGA) is reached. Proteins known as release factors bind to any stop codon that reaches the A-site on the ribosome, and this binding alters the activity of the peptidyl transferase in the ribosome, finally causing the release of the protein into the cytoplasm.
The stepwise linking of amino acids into a polypeptide chain is catalyzes by an rRNA molecule in the large ribosomal subunit. Thus, the ribosome is an example of a ribozyme, an RNA molecule that can catalyze a chemical reaction.

Protein breakdown

The degradation of proteins in the cell is carefully controlled. Cells possess specializes pathways to enzymatically break proteins down into their constituent amino acids – a process called proteolysis. The enzymes that degrade

proteins are known collectively as proteases. Proteases act by hydrolyzing the peptide bonds between amino acids. One function of the proteolytic pathways is to rapidly degrade those proteins whose lifetimes must be short. Another is to recognize and eliminate proteins that are damaged or misfolded. Eliminating improperly folded proteins is critical for an organism, because neurodegenerative disorders such as Huntington's and Alzheimer's are caused by the aggregation of misfolded proteins. Some proteins are degraded in the cytosol by large protein complexes called proteasomes. Proteasomes act primarily on proteins that have been marked for destruction by the covalent attachment of a small protein called ubiquitin.

7.3 RNA and the origins of life

From our knowledge of present-day organisms and their molecules, it seems likely that living systems began with the evolution of RNA molecules that could catalyze their own replication. We have seen that a protein is able to catalyze a biochemical reaction because it has a special surface on which a given substrate can react. In the same way, RNA molecules, with their unique folded three-dimensional shapes, can serve as enzymes. Although the fact that they are constructed of only four different subunits limits their catalytic efficiency and the range of chemical reactions they can catalyze compared with proteins. So, most catalytic functions in present-day cells have been taken over by proteins.

It has been proposed that, as cells evolved, the DNA double helix replaced RNA as a more stable molecule for storing increased amounts of genetic information, and proteins replaced RNAs as major catalytic and structural components.

The flow of information in present-day living cells is DNA ® RNA ® protein, with RNA serving primarily as a go-between. Some important reactions, however, are still catalyzed by RNA; these are thought to provide a glimpse into the ancient, RNA-based world.

11. Membrane structure

Cell membranes enable a cell to create barriers that confine particular molecules to specific compartments. The simplest bacteria have only a single membrane, the plasma membrane. Eukaryotic cells, however, contain in addition a profusion of internal membranes that enclose intracellular compartments. All cell membranes are composed of lipids and proteins and share a common general structure. The lipid component consists of many millions of lipid molecules forming a lipid bilayer. This lipid bilayer gives the membrane its basic structure and serves as a permeability barrier.

11.1 The lipid bilayer

The lipids in cell membranes combine two very different properties in a single molecule: each lipid has a hydrophilic ('water-loving') has and one or two hydrophobic ('water-hating') hydrocarbon tails. There are three major classes of membrane lipid molecules:

1. Phospholipids
2. Sterols
3. Glycolipids

The most abundant lipids in cell membranes are phospholipids, and the most common type of phospholipid in most cell membranes is phosphatidylcholine. Molecules with both hydrophilic and hydrophobic properties are termed amphipathic. This chemical property plays a crucial part in driving these lipid molecules to assemble into bilayers. They assemble spontaneously into bilayers when placed in water, forming closed compartments that reseals of torn.

Amphipathic molecules re subject to two conflicting forces: the hydrophilic head is attracted to water, while the hydrophobic tail shuns water and seeks to aggregate with other hydrophobic molecules. This conflict is resolved by the formation of a lipid bilayer, because the hydrophilic heads face the water at each of the two surfaces of the sheet of molecules and the hydrophobic tails are all shielded from the water and lie next to one another in the interior of this 'sandwich'. Finally, the phospholipid bilayers spontaneously close in on themselves to form sealed compartments.

The fluidity of a lipid bilayer

The lipid bilayer is fluid, and individual lipid molecules are able to diffuse within their own monolayer; they do not, however, spontaneously flip from one monolayer to the other. The two layers of the lipid bilayer have different lipid compositions, reflecting the different functions of the two faces of a cell membrane.

The fluidity of a cell membrane (the ease with which its lipid molecules move within the plane of the bilayer) is important for membrane function and has to be maintained within certain limits. The fluidity of a bilayer depends on its phospholipid composition and on the nature of the hydrocarbon tails. The closer and more regular the

packing of the tails, the more viscous and less fluid the bilayer will be. The two properties length and degree of unsaturation determine how tightly packed the bilayer is.

- A shorter chain length reduces the tendency of the hydrocarbon tails to interact with one another and therefore increases the fluidity of the bilayer.
- Each double bond in an unsaturated tail (saturated tails have no double bonds) creates a small kink in the hydrocarbon tail that makes it more difficult for the tails to pack against one another. For this reason, lipid bilayers that contain a large proportion of unsaturated hydrocarbon tails are more fluid than those with lower proportions.

In animals, membrane fluidity is modulated by the inclusion of the sterol cholesterol. Cholesterol molecules fill the spaces between neighboring phospholipid molecules left by the kinks in the unsaturated hydrocarbon tails. In this way cholesterol tends to stiffen the bilayer, making it more rigid and less permeable.

Membrane fluidity is important for many reasons:

1. It enables membrane proteins to diffuse rapidly in the plane of the bilayer and to interact with one another
2. It permits membrane lipids and proteins to diffuse from sites where they are inserted into the bilayer after their synthesis to other regions of the cell
3. It allows membranes to fuse with one another and mix their molecules
4. It ensures that membrane molecules are distributed evenly between daughter cells when a cell divides

Cells adjust their membrane fluidity by modifying the lipid composition of their membranes. Flippases play a role in synthesizing the lipid bilayer.

11.2 Membrane proteins

Although the lipid bilayer provides the basic structure of all cell membranes and serves as a permeability barrier to the molecules on either side of it, most membrane functions are carried out by membrane proteins. Plasma membrane proteins have a variety of functions: first they take care of transportation of nutrients, metabolites, and ions across the lipid bilayer. Some anchor the membrane to macromolecules on either side. Others function as receptors that detect chemical signals in the cell's environment and relay them to the cell's interior; and still others work as enzymes to catalyze specific reactions.

Proteins can be associated with the lipid bilayer of a cell membrane in several ways:

1. Transmembrane: transmembrane proteins extend across the lipid bilayer, usually as one or more a helices but sometimes as a b sheet curved into the form of a barrel
2. Membrane-associated: some membrane proteins are anchored to the cytosolic surface by an amphipathic a helix
3. Lipid-linked: membrane proteins that are attached to either side of the bilayer solely by a covalent attachment to a lipid molecule
4. Protein-attached: many proteins are attached to the membrane only by relatively weak, noncovalent interactions with other membrane proteins

Proteins that are directly attached to membranes can be removed only by disrupting the lipid bilayer detergents. Such proteins are known as integral membrane proteins. The remaining membrane proteins are known as peripheral membrane proteins; they can be released from the membrane by extraction procedures that interface with protein-protein interactions but leave the lipid bilayer intact.

Before an individual protein can be studied in detail, it must be separated from all the other cellular proteins. For most membrane proteins, the first step in this separation process involves solubilizing the membrane with agents that destroy the lipid bilayer by disrupting hydrophobic associations. The most widely used disruptive agents are detergents. Sodium dodecyl sulfate (SDS) and Trition X-100 are two commonly used detergents. The detergent disrupts the lipid bilayer and brings the protein into solution as protein-detergent complexes.

The cell surfaces

A cell membrane by itself is enormously thin and fragile. Most cell membranes are therefore strengthened and supported by a framework of proteins, attached to the membrane via transmembrane proteins. The shape of the cell and the mechanical properties of the plasma membrane are determined by a meshwork of fibrous proteins, called the cell cortex, which is attached to the cytosolic surface of the membrane.

Many of the proteins and some of the lipids exposed on the surface of cells have attached chains of sugars which help protect and lubricate the cell surface and are involved in cell-cell recognition. Some proteins, called lectins, are specialized to recognize particular oligosaccharide side chains and bind to them.

Although many membrane proteins can diffuse rapidly in the plane of the membrane, cells have ways of confining proteins to specific membrane domains and of immobilizing particular proteins by attaching them to intracellular or extracellular macromolecules.

12. Membrane transport

The lipid bilayer of cell membranes is permeable to small nonpolar molecules such as oxygen and carbon dioxide and to very small polar molecules such as water. It is highly impermeable to most large, water-soluble molecules and all ions. Transfer of nutrients, metabolites, and ions across the plasma membrane and internal cell membranes is carried out by membrane transport proteins. Cell membranes contain a variety of transport proteins, each of which is responsible for transferring a particular type of solute across the membrane. There are two classes of membrane transport proteins:

1. Carrier proteins
2. Channel proteins; most let through inorganic ions and are therefore called ion channels

The basic difference between carrier proteins and channel proteins is the way they discriminate between solutes. A channel protein discriminates mainly on the basis of size and electric charge; if the channel is open, molecules small enough and carrying the appropriate charge can slip through. A carrier protein, on the other hand, allows passage only to solute molecules that fit into a binding site on the protein; it then transfers these molecules across the membrane one at a time by changing its own conformation.

12.1 Principles of membrane transport

Living cells maintain an internal ion composition that is very different from the ion composition in the fluid around them. Na+ is the most plentiful positively charged ion (cation) outside the cell, while K+ is the most plentiful of all the solutes in a cell's environment. The high concentration of Na+ outside the cell is balanced by extracellular Cl-. The high concentration of K+ inside is balanced by a variety of negatively charged intracellular ions (anions). This differential distribution of ions inside and outside the cell is controlled in part by the activity of membrane transport proteins, and in part by the permeability characteristics of the lipid bilayer itself.

The electrochemical gradient represents the net driving force on an ion due to its concentration gradient, and a charged solute (an ion) moves spontaneously down its electrochemical gradient. Such movements are called passive transport (or facilitated diffusion), because they need no other driving forces. All channel proteins and many carrier proteins can act as a conduit for such passive transport. In active transport an uncharged solute or an ion is transported against its concentration or electrochemical gradient in an energy-requiring process. This is carried out only by special types of carrier proteins that can harness some energy source to the transport process.

Carrier proteins and their function

Although the detailed molecular mechanisms that underlie transport are known for only a few carrier proteins, the general principles are well understood. Solutes can cross the membrane by passive and active transport, and carrier proteins are capable of facilitating both types of movement. Carrier proteins bind specific solutes (inorganic ions, small organic molecules, or both) and transfer them across the lipid bilayer by undergoing conformational changes that expose the solute-binding site first on one side of the membrane and then on the other.

Cells carry out active transport in three main ways:

1. Coupled transport; they couple the uphill transport of one solute across the membrane to the downhill transport of another
2. ATP-driven pumps; they couple uphill transport to the hydrolysis of ATP
3. Light-driven pumps; they are found mainly in bacterial cells and couple uphill transport to an input of energy from light

The Na+-K+ pump in the plasma membrane of animal cells is an ATPase that actively transports Na+ out of the cell and K+ in, maintaining the steep Na+ gradient across the plasma membrane that is used to drive other active transport processes and to convey electrical signals. The downhill movement of the first soluble down its gradient provides the energy to drive the uphill transport of the second. The carrier proteins that do this are called coupled transporters. If the transporters move both solutes in the same direction across the membrane, it is called a symport. If it moves them in opposite directions, it is called an antiport. A carrier protein that ferries only one type of solute across the membrane (and is therefore not a coupled transporter) is called a uniport.

In animal cells an especially important role is played by symports that use they inward flow of Na+ down its steep electrochemical gradient to drive the import of other solutes into the cell. The epithelial cells that line the gut, for example, transfer glucose from the gut across the gut epithelium. In this way animals use the Na+ gradient to take up nutrients.

Furthermore, the Na+-K+ pump helps to maintain the osmotic balance of animal cells. Movement of water from a region of low solute concentration (high water concentration) to a region of high solute concentration (low water concentration) is called osmosis. The driving forces for the water movement is equivalent to a difference in water pressure and is called the osmotic pressure. In the absence of any counter-acting pressure, the osmotic movement of water into a cell will cause it to swell. Such effects are a severe problem for animal cells, which have no rigid external wall to prevent them from swelling. In animal cells the osmotic balance is regulated by the Na-K+- pump, which pumps out the Na+ that leaks in. At the same time, by helping to maintain a membrane potential, the Na+-K+-pump also tends to prevent the entry of Cl-, which is negatively charged.

Other cells cope with their osmotic problems in different ways. Plant cells are prevented from swelling and bursting by their tough cell wall and so can tolerate a large osmotic difference across their plasma membrane. The protozoan avoids swelling by periodically ejecting the water that moves into the cell.

Ion channels and the membrane potential

Channel proteins form aqueous pores across the lipid bilayer through which solutes can diffuse. Whereas transport by carrier proteins can be active or passive, transport by channel proteins is always passive. Most channel proteins are selective ion channels that allow inorganic ions of appropriate size and charge to cross the membrane down their electrochemical gradients. Transport through ion channels is at least 1000 times faster than transport through any known carrier protein.

There are more than a hundred types of ion channels and they mainly differ from one another with respect to their ion selectivity (the type of ions they allow to pass) and gating (the conditions that influence their opening and closing). For a voltage-gated channel, the probability of being open is controlled by the membrane potential. For a ligand-gated channel, it is controlled by the binding of some molecules (the ligand) to the channel protein. For a stress-activated channel, opening is controlled by a mechanical force applied to the channel. Even when opened by their specific stimulus, ion channels do not remain continuously open: they flicker randomly between open and closed conformations. An activating stimulus increases the proportion of time the channel spends in the open state.

All cells have an electrical potential difference, or membrane potential, across their plasma membrane. The membrane potential is determined by the unequal distribution of electric charge on the two sides of the plasma membrane and is altered when ions flow through open channels. In most animal cells, K+-selective leak channels hold the resting membrane potential at a negative value, close to the value where the driving force for movement of K+ across the membrane is almost zero.

A simple formula called the Nernst equation expresses the equilibrium quantitatively and makes it possible to calculate the theoretical resting membrane potential if the ratio of internal to external ion concentrations is known.

Ion channels and signaling in nerve cells

The fundamental task of a nerve cell (neuron) is to receive, conduct, and transmit signals. No matter what the meaning of the signal a neuron carries the form of the signal is always the same: it consists of changes in the electrical potential across the neuron's plasma membrane. Neurons are often extremely elongated, and the action potentials therefore have to travel long distances along an axon without weakening.

Every neuron consists of a cell body (containing the nucleus); a long axon, which conducts signals away from the cell body toward distant target cells; and dendrites, which extend from the cell body like antennae and provide an enlarged surface area to receive signals from the axons of other neurons. The axon divides at its far end into many branches, each of which ends in a nerve terminal; so that the neuron's message can be passed simultaneously to many target cells.

Action potentials are usually mediated by voltage-gate Na+ channels that open in response to depolarization of the plasma membrane (a shift in the membrane potential to a less negatively value). Such a depolarization is caused by the action of signaling molecules, called neurotransmitters, released by other neurons. When the voltage-gate Na+ channels are open Na+ enters the cell, depolarizes the membrane further. The membrane potential swings past zero and reaches +40mV before it returns to its resting negatively value, as the action potential terminates. The membrane is helped to return to its resting value by inactivation of the Na+ channels, opening of voltage-gated K+ channels, and through the K+ leak channels.

When the action potential reaches the ends of the axon (the nerve terminals) the signal must be relayed to the target cells that the nerve terminals contact. The signal is transmitted at specialized junctions known as synapses. At most synapses the presynaptic and postsynaptic plasma membranes are separated from each other by a synaptic cleft, which the electrical signal cannot cross. For the message to be transmitted the electrical signal is converted into a chemical signal, known as a neurotransmitter. Neurotransmitters are stored in the nerve terminals, packaged in synaptic vesicles. When the action potential reaches the terminal, voltage-gated Ca2+ channels in nerve terminals couple electrical signals to transmitter release at synapses. Transmitter-gated ion channels convert these chemical signals back into electrical signals in the postsynaptic target cell.

The response produced by a neurotransmitter at a synapse can be either excitatory or inhibitory. Excitatory neurotransmitters, mainly acetylcholine and glutamate, open transmitter-gated channels that are permeable to Na+ and thereby depolarize the postsynaptic cell membrane toward the threshold potential for firing an action potential. Inhibitory neurotransmitters, mainly GABA and glycine, open transmitter-gated Cl- channels and thereby suppress firing by keeping the postsynaptic cell membrane polarized.

Transmitter-gated ion channels are major targets for psychoactive drugs.

13. How cells obtain energy from food

As mentioned earlier, cells require a constant supply of energy to generate and maintain the biological order that keeps them alive. Perhaps the most important fuel molecules are the sugars. Plants make their own sugars by photosynthesis, whereas animals obtain sugars by eating another organism. Nevertheless, the process whereby these sugars are oxidized to generate energy is very similar in both animals and plants. Glucose and other food molecules are broken down by controlled stepwise oxidation to provide useful chemical energy in the form of the activated carriers ATP and NADH.

13.1 The breakdown of sugars and fats

The proteins, lipids and polysaccharides that make up most of the food we eat must be broken down into smaller molecules before our cells can use them; this happens in three stages:

Stage 1

In stage 1 the enzymatic breakdown of food molecules (digestion) occurs either outside the cells in our intestine or in a specialized organelle within cells called lysosome. In either case, digestive enzymes reduce the large polymeric molecules in food into their monomeric subunits (proteins into amino acids and polysaccharides into sugar). After digestion, the small organic molecules derived from the food enter the cytosol of a cell, where their gradual oxidation begins

Stage 2

The most important step of stage 2 is the degradation of glucose, a process called glycolysis. Glycolysis produces ATP without the involvement of molecular oxygen (O2 gas). It occurs in the cytosol of most cells, including many anaerobic microorganisms.

Glycolysis converts each molecule of glucose into two smaller molecules of pyruvate. During this formation two types of activated carrier molecules are produced: ATP and NADH. At the end of glycolysis, there is a net gain of two molecules ATP (4 produced, but 2 consumed during the process) and two molecules of NADH for each glucose molecule broken down. Next the pyruvate passes from the cytosol into mitochondria.

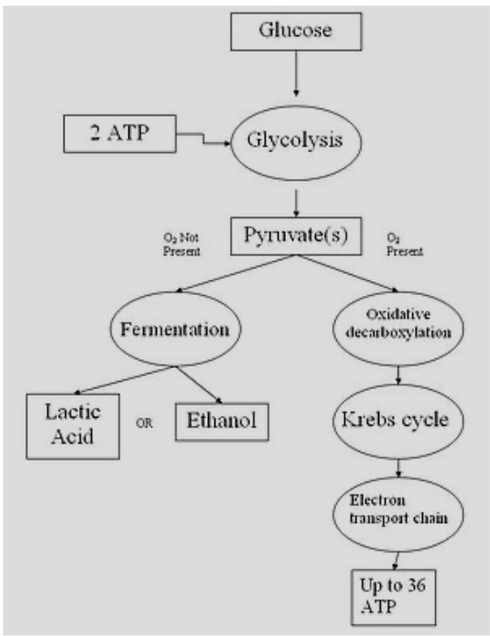
Stage 3

Stage 3 takes place in mitochondria. Here the pyruvate is converted into CO2 plus acetyl coenzyme A (CoA). The

acetyl group enters a series of reactions called the citric acid cycle. The citric acid cycle accounts for about two-thirds of the total oxidation of carbon compounds in most cells, and its major products are CO2 and NADH. The CO2 is released as a waste product, while the high-energy electrons from NADH are passed to a membrane-bound electron-transport chain, eventually combining with O2 to produce H2O. Each turn of the citric acid cycle produces one molecule of GTP and one molecule of FADH2. The energy that is stored in the high-electrons of NADH and FADH2 will be used to drive a process that produces ATP and consumes molecular oxygen (O2). It is in these final steps that most of the energy released by oxidation is harnessed to produce most of the cell's ATP. Because the energy for it ultimately derives from the oxidative breakdown of food molecules, the phosphorylation of ADP to form ATP in the mitochondrial inner membrane is known as oxidative phosphorylation.

As mentioned above, sugars are broken down by distinct sets of reactions: glycolysis (which occurs in the cytosol), the citric acid cycle (in the mitochondrial matrix), and oxidative phosphorylation (in the inner mitochondrial membrane). In total, the complete oxidation of a molecule of glucose to H20 and CO2 produces about 30 molecules of ATP (in contrast to glycolysis alone, which produces 2 molecules of ATP). Besides sugars, another major energy source in foods is fat. The fatty acids produced from fats are imported into mitochondria and converted to acetyl CoA molecules. These acetyl CoA molecules are then further oxidized through the citric acid cycle, producing NADH and FADH2, just like the acetyl CoA derived from pyruvate.

13.2 Anaerobic conditions



For most animal and plant cells, glycolysis is just a prelude to the final stage of the breakdown of food molecules. But for many anaerobic organisms, which do not use molecular oxygen and can grow and dived in its absence, glycolysis is the principle source of the cell's ATP. In these anaerobic conditions, the pyruvate and the NADH electrons stay in the cytosol. The pyruvate is converted into products that are excreted from the cell, like for example lactate and ethanol. The NADH is converted back into NAD+; this is required to maintain the reactions of glycolysis.

Storing and utilizing food

Cells store food molecules in special reservoirs. Glucose subunits are stored as glycogen in animals and as starch in plants; both animals and plants store food as fats. The food reservoirs produced by plants are the major sources of food for animals, including humans.
Molecules ingested as food are used not only as sources of metabolic energy but also as raw material for biosynthesis. Thus, many intermediates of glycolysis and the citric acid cycle are starting points for pathways that lead to the synthesis of proteins, nucleic acids, and the many other specialized molecules of the cell.

The thousands of different reactions carried out simultaneously by a cell are closely coordinated, enabling the cell to adapt and continue to function under a wide range of external conditions.

14. Energy generation in mitochondria and chloroplasts

The main energy currency in cells is ATP. In eukaryotic cells, small amounts of ATP are generated during glycolysis in the cytosol, but most ATP is produced by membrane-based processes in the mitochondria, using energy derived from oxidation of sugars and fatty acids. Similar processes also occur in the cell membrane of many bacteria. The mechanism for making ATP consists of two linked stages, both of which are carried out by protein complexes embedded in a membrane:

1. Stage 1: electrons derived from the oxidation of food molecules are transferred along an electron-transport chain, which is embedded in the membrane. These electron transfers release energy that is used to pump protons (H+) across the membrane and thus generate an electron chemical proton gradient. This gradient is a form of stored energy that can be used when the ions flow back down their electrochemical gradient.
2. Stage 2: H+ flows back down its electrochemical gradient through a protein complex called ATP synthase, which catalyzes the energy-requiring synthesis of ATP from ADP and inorganic phosphate (Pi).

The linkage of electron transport, proton pumping, and ATP synthesis is called chemiosmotic coupling. Chemiosmotic coupling first evolved in bacteria. Therefore, it is perhaps not surprising that aerobic eukaryotic cells appear to have adopted the bacterial chemiosmotic mechanisms by engulfing aerobic bacteria to form mitochondria and cyanobacteria to form chloroplasts.

14.1 Mitochondria and oxidative phosphorylation

Mitochondria are present in nearly all eukaryotic cells and contain their own DNA and RNA, and a complete transcription and translation system including ribosomes, which allows them to synthesize some of their own proteins. Defects in mitochondrial function can have serious repercussions for an organism. An inherited disorder called myoclonic epilepsy and ragged red fiber disease (MERRF) caused by a mutation in one of the mitochondrial transfer RNA genes, is characterized by a decrease in synthesis of the mitochondrial proteins required for electron transport and ATP production. As a result, patients with this disorder typically experience muscle weakness or hart problems (from the effect on cardiac muscle) and epilepsy or dementia (from the effects on nerve cells). Muscle and nervous tissue suffer most when mitochondria are defective because they need particularly large amounts of ATP to function optimally.

Composition of mitochondria

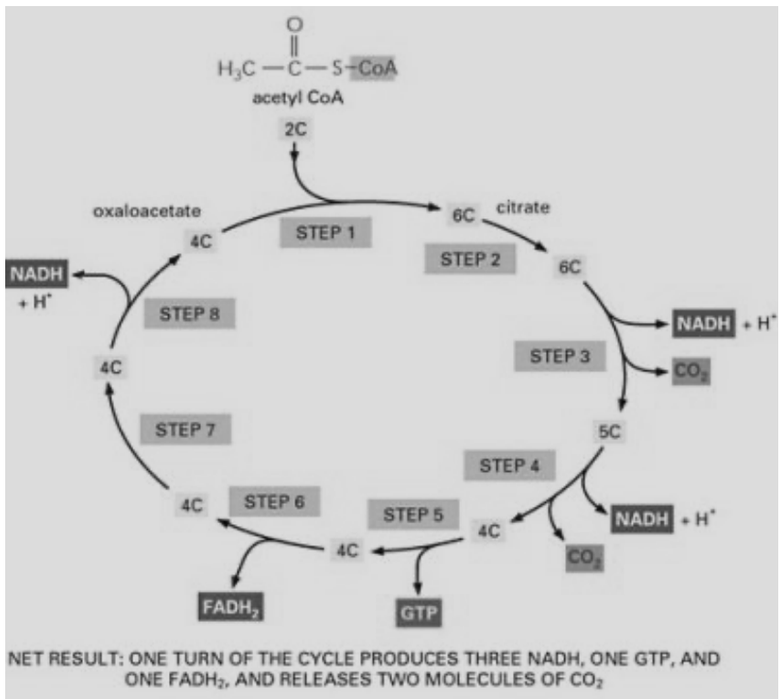
Mitochondria are enclosed by two concentric membranes. The outer and inner mitochondrial membranes create two mitochondrial compartments: a large internal space called the matrix and the intermembrane space. The matrix is a large internal space that contain a mixture of hundreds of enzymes, and it contains several identical copies of the mitochondrial DNA genome. The inner mitochondrial membrane is folded into numerous cristae, which greatly increase its total surface area. An electrochemical gradient of H+, which drives the ATP synthase, is established across this membrane. The inner membrane contains proteins with three types of function:

1. Those that carry out the oxidation reactions of the electron-transport chain
2. The ATP synthase that makes ATP in the matrix
3. Transport proteins that allow the passage of metabolites into and out the matrix.

The outer membrane contains many molecules of a transport protein called porin, which forms wide aqueous channels through the lipid's bilayer. As a result, the outer membrane is permeable to all molecules of 5000 Dalton or less. Other proteins in this membrane include enzymes involved in mitochondrial lipid synthesis and enzymes that convert lipid substrates into forms that are subsequently metabolized in the matrix.

Citric acid cycle

Mitochondria can use both pyruvate and fatty acids as fuel. Pyruvate comes mainly from glucose and other sugars, and fatty acids come from fats. Pyruvate and fatty acids enter the mitochondrion and are converted into acetyl CoA. Then they are further metabolized by the citric acid cycle, which reduces NAD+ to NADH, and FAD to FADH2. In the process of oxidative phosphorylation, high-energy electrons from NADH and FADH2 are then passed along the electron-transport chain (the respiratory chain) in the inner membrane to oxygen. Much of the energy released by electron transfers along the respiratory chain is harnessed to pump H+ out of the matrix, thereby creating a transmembrane electrochemical proton (H+) gradient. The proton pumping is carried out by three large respiratory enzyme complexes embedded in the membrane. Each complex includes transmembrane proteins that hold the entire protein complex firmly in the inner mitochondrial membrane. The three respiratory enzyme complexes are:



- NADH dehydrogenase complex
- Cytochrome b-c1 complex
- Cytochrome oxidase complex

The resulting electrochemical proton gradient across the inner mitochondrial membrane is used to drive the ATP synthesis in the process of oxidative phosphorylation. The device that makes this possible is a large membrane-bound enzyme called ATP synthase. This enzyme creates a hydrophilic pathway across the inner mitochondrial membrane that allows protons to flow down their electrochemical gradient. ATP synthase is a reversible coupling device. It can either harness the flow of protons down their electrochemical gradient to make ATP or use the energy of ATP hydrolysis to pump protons across a membrane, like H+ pumps. Whether the ATP synthase primarily makes or consumes ATP depends on the magnitude of the electrochemical proton gradient across the membrane in which it sits.
The synthesis of ATP is not the only process driven by the electrochemical proton gradient. The electrochemical gradient also drives the active transport of metabolites into and out the mitochondrion or coupled transport processes.

14.2 Chloroplasts and photosynthesis

In plants, photosynthesis is carried out in a specialized intracellular organelle, the chloroplast. Chloroplasts perform photosynthesis during daylight hours and thereby produce ATP and NADPH, which in turn are used to convert CO2 into sugars inside the chloroplast. The sugars are then used to make ATP and as starting materials for many of the other organic molecules that the plant cell needs. Chloroplasts have a highly permeable outer membrane, a much less permeable inner membrane (in which membrane transport proteins are embedded) and a narrow intermembrane space in between. Together these membranes form the chloroplast envelope. The inner membrane surrounds a large space called the stroma, which is analogous to the mitochondrial matrix and contains many metabolic enzymes. Like the mitochondrion, the chloroplast evolved from an engulfed bacterium, and it still contains its own genome and genetic system. There is, however, an important difference between the organization of mitochondria and that of chloroplasts. Compared with mitochondria, chloroplasts are larger and have an extra compartment. A chloroplast contains, in addition to an inner and outer membrane, a thylakoid membrane enclosing a thylakoid space. The thylakoid membrane contains the light-capturing systems, the electron-transport chains and ATP synthase.

The many reactions that occur during photosynthesis in plants can be grouped into two broad categories:

1. Photosynthetic electron-transfer reactions (also called the 'light-reactions'): in this reactions are water oxidized and oxygen released
2. Carbon-fixation reactions (also called the 'dark reactions'): in this reactions, which begin in the chloroplast stroma and continue in the cytosol, carbon dioxide is assimilated (fixed) to produce sugars and a variety of other organic molecules

In photosynthesis high-energy electrons are generated when sunlight is absorbed by chlorophyll; this energy is captured by protein complexes known as photosystems, which are located in the thylakoid membranes of chloroplasts.

Electron-transport chains associated with photosystems transfer electrons from water to NADP+ to form NADPH, with the concomitant production of an electrochemical proton gradient across the thylakoid membrane. Molecular oxygen is generated as a by-product. As is mitochondria, the proton gradient across the thylakoid membrane is used by an ATP synthase embedded in the membrane to generate ATP. The ATP and the NADPH made by photosynthesis are used within the chloroplast to drive the carbon-fixation cycle in the chloroplast stroma, thereby producing carbohydrate from CO2. Carbohydrate is exported to the cell cytosol, where it is metabolized to provide organic carbon, ATP (mostly via mitochondria), and reducing power for the rest of the cell.

The origins of chloroplasts and mitochondria

Both mitochondria and chloroplasts are thought to have evolved from bacteria that were endocytosed by primitive eukaryotic cells. Each retains its own genome and divides by processes that resemble a bacterial cell division. Because mitochondria and chloroplasts have component proteins that are encoded by two separate genetic systems, their growth and proliferation is complicated.

Chemiosmotic coupling mechanisms are widespread and of ancient origin. Modern microorganisms that live in environments similar to those thought to have been present on the early Earth also use chemiosmotic coupling to produce ATP.

Exam Questions:

Semester 1

- What is posttranslational control / Gene Expression?
- Explain the process of Glycolysis
- Why and how is the poly-a tale the mRNA? Why is mRNA not stable in the cytoplasm?
- How what is the process of ATP synthesis (Complete)
- Reactions in ATP synthase
- How are Nucleotides in RNA/DNA linked together
- Protein structures and what happens with them in Acidic / Basic environments or change in temperature
- Structure of Amino-Acids (Chemistry)
- Interactions of Side Chains of different Amino Groups
- What kind of amino acids are found inside alpha-helices
- How are errors in DNA replications avoided
- Why is there no high risk of change in a protein when a mutation occurred
- Explain why a single wrong amino acid can change the whole conformation of a protein
- Process of transcription? How does the RNA polymerase know where to start, why and when does it start (explanation for Eukaryotes and Prokaryotes)
- What is the spliceosome and how does it work?
- How does the spliceosome recognize where there is a start or an end of an intron?
- What do introns have in common? Are they in some kind similar?
- DNA Replication? What direction is the DNA polymerase working? Thereby which one is lagging / leading strand
- What is a ribosome, how is it built and what is the size?
- Which is the amino acid for AUG (start)
- Explain Carboxy-Terminal and Amino Terminal and which one is connected to what

Semester 1 + 2 mix

- Give an example of a cell cycle (for example of a liver cell)
- How does the cell know to start the steps?
- Asked to draw the cycle and the checkpoints
- What makes the cell to progress or stop?
    - Why and How → what could be reasons for not continuing in the cycle
- On the molecular point of view what are the checkpoint molecules that sense/send a signal / explain the signal transduction pathway that activates the cell-cycle
- How does cyclin change its concentration / How does it appear/disappear?
- Explain the S-Phase

- How is transcription regulated?
- How does the machinery of transcription know where to start?
- Explain the localization of proteins, how are mitochondrial proteins getting to where they belong
- What is ubiquitin
- Explain the process of degradation of proteins
- Tell about the process of glycosylation
- What happens in the Golgi apparatus?

- Tell about Protein synthesis and localization
- How does the cell-cycle work?
- How is transcription regulated
- How are the necessary proteins binding to DNA?
- What is a domain of a protein?
- How are external signals affecting the regulation of gene expression?
- Tell about apoptosis? What is p53 and what is it doing
- What are the mechanisms of repairing DNA? How do they work?
- What is a phagosome
- Tell about meiosis (Full)
- What does Retinoblastoma do?
- Tell me about the Golgi Apparatus
- Important covalent modifications made in the golgi and ER
-