Cellular Biology

Biotechnology requires an understanding of characteristics of life and cellular components -- including biochemistry (chemistry of living organisms), cytology (study of cells), anatomy (structure) and physiology (function).

© Characteristics of LIFE – growth, reproduction, response to stimulus, breakdown food molecules (respiration), production of waste products, composed of cells (unicellular or multicellular)

Levels of ORGANIZATION – understanding how each level of biological organization works is necessary for the development and manufacture of biotechnology product.

ATOMS – smallest units of matter that are easily manipulated in cells

MOLECULES – atoms combine to form molecules. Molecules are the building blocks of cells.

ORGANELLES – specialized microscopic structures within cells that perform a specific function

CELLS – basic unit of life

TISSUES – a group of cells that function together

ORGANS – tissues that act together to form a specific function in an organisms

ОРGANISMS – a single living thing such as bacteria, protist, fungi, plants and animals

Cellular structures and Processes

There are two types of cells – prokaryotic and eukaryotic. Prokaryotic cells lack a nucleus, lack membrane-bound organelles and are unicellular. Bacteria are prokaryotes. Eukaryotic cells have a nucleus, have membrane-bound organelles and can be unicellular or multicellular. Eukaryotes include protist, fungi, plants and animals.

The cells of unicellular and multicellular organism are microscopic factories that produce thousands of molecules. Companies exploit the manufacturing capability of cells and use these cells to produce particular molecules in large amounts.

Cellular STRUCTURES –

- Plasma (cell) Membrane – outer cellular boundary that regulates the movement of materials into and out of the cell. Plasma membrane is composed of lipids and proteins. These membrane proteins can act as structural molecules that maintain cell shape. Some membrane proteins can act as identification or recognition molecules – one way that molecules recognize specific cells is by binding with a recognition or receptor protein on the membrane surface.

- For example: When an insulin molecule binds to an insulin receptor membrane protein to set a series of cellular reactions into motion that increase the cell’s glucose intake.

*Adapted from “Biotechnology: Science for the New Millennium” by Ellyn Daugherty.*
- **Cell Walls** – provide a rigid structural support around the cell membrane. Plant cell walls are composed of cellulose (polysaccharide). Bacteria, fungi and protist have cell walls. Animal cells do NOT have cell walls.

- **Nucleus** – contains the DNA on chromosomes for constructing molecules
  - This 'control center' will provide instructions for protein production which may function as enzymes, pigments, antibodies or hormones.

- **Chromosomes** – long strands of DNA intertwined with protein molecules. The actual instructions (genetic code of A, C, G, T) for molecular construction are found within the DNA.

- **Cytoplasm** – gel-like liquid of thousands of molecules suspended in water, outside the nucleus.

- **Cytoskeleton** – a protein network in the cytoplasm that gives cells structural support.

- **Lysosomes** – a membrane-bound organelle responsible for the breakdown of cellular waste.

- **Ribosomes** – responsible for protein production.

- **Chloroplast** – specialized organelle in plants responsible for photosynthesis (using light energy to generate chemical energy in the form of sugar molecules). Pigments are located in the chloroplast and absorb various wavelengths of UV light.

- **Mitochondria** – specialized organelle responsible for cellular respiration (breaking down food molecules to generate chemical energy in the form of ATP).
Cells in different organs and organisms have different structures and functions. Depending on cellular function, some cells have different numbers of some structures. For example, muscle cells have more ribosomes and mitochondria than 'typical cells' because of their increased protein and energy production. Liver cells have more lysosomes for waste removal than most cells.

The size and shape of a cell are directly related to its structure and function. For example, skin cells are flat and fit tightly together to cover and protect.

Types of CELLS used in Biotechnology

Many different types of cells are used and studied more than others in biotechnology.

- Chinese hamster ovary (CHO) cells – manipulate CHO cells to make them produce proteins different from those they normally create. Genes of interest from an assortment of organisms can be inserted and incorporated into the DNA of CHO cells, which read the DNA and begin producing the new proteins. For example: A protein that boosts RBC production used to treat anemia are produced using CHO cells.
- Vero cells (African green monkey kidney epithelial cells)
- HeLa (human epithelial cells)
- E. coli – human growth hormone (hGH) was first produced commercially using this bacteria
- Fungal cells and many bacterial cells are used in biotech labs.

CENTRAL DOGMA – states that the DNA code is rewritten into mRNA and then decoded into a protein.

DNA → mRNA → protein

TRANSCRIPTION

When cells make proteins, they transcribe (copy) a segment (gene) of DNA into a new version of the code, a messenger RNA (ribonucleic acid) – mRNA. The mRNA transcript determines the amino acid and how the amino acid will be arranged into a protein. Proteins are unique because of their amino acid sequence.

TRANSLATION

In eukaryotic cells, the mRNA which carries a protein code, moves from the nucleus to the cytoplasm. In the cytoplasm, the mRNA molecule binds to a ribosome and is translated into a protein molecule. As the ribosome reads the mRNA (A,C,G,U) code, it assembles the 20 different amino acids into a polypeptide chain. The polypeptide chain folds into a protein.

Amino acids are subunits of proteins. Each amino acid is connected by peptide bonds which form a polypeptide chain. There are several hundreds of amino acids in a typical protein chain -- the 20 different amino acids can be arranged in various orders, depending on the original DNA and mRNA sequences.

*Adapted from “Biotechnology: Science for the New Millennium” by Ellyn Daugherty.*
**MOLECULES in cells**

Macromolecules are involved in biotechnology applications – engineered molecules are the basis of many products. These **organic molecules** contain carbon and are produced only in cells. Most of the very large molecules are found in structural components of the cells, many are enzymes involved in cellular processes, and some are regulatory molecules or transport molecules.

- Macromolecules are composed of repeating units chained together. The smaller units are called monomers. Monomers link together to form larger molecules called polymers.
- Four main classes of macromolecules – **carbohydrates, lipids, proteins and nucleic acids** – give structure and function to cells.

<table>
<thead>
<tr>
<th>CARBOHYDRATES – 3 types of carbs</th>
<th>Manufactured to store or transport glucose</th>
<th>Energy-storage molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monosaccharides</strong> – are the monomer units that cells use to build polymers.</td>
<td><strong>Disaccharides</strong> – a polymer that consists of two sugar molecules</td>
<td>Structural supporting molecules</td>
</tr>
<tr>
<td>o Simple sugars – glucose, fructose, galactose</td>
<td>o Sucrose (table sugar) – composed of glucose and fructose</td>
<td>o Cellulose – in plant cell walls</td>
</tr>
<tr>
<td>Immediate energy sources – energy molecules</td>
<td>o Lactose (milk sugar) – composed of glucose and galactose</td>
<td>o Chitin – in fungal cell walls</td>
</tr>
<tr>
<td>o Glucose</td>
<td>o Maltose (malt sugar) – composed of two glucose molecules</td>
<td>Carbohydrates inside and covering plant cells present problems to biotechs trying to isolate proteins and DNA from cells. Polysaccharides become sticky compounds – which interfere with purification procedures.</td>
</tr>
<tr>
<td>o Fructose and galactose are converted to glucose and used for energy.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structural molecules found in larger molecules</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o Deoxyribose – 5 carbon sugar in DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o Ribose – 5 carbon sugar in RNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monosaccharides differ based on arrangement of atoms – have same molecular formula. C₆H₁₂O₆</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells break down glucose and transfer released energy to ADP to produce ATP during cell respiration. Cells store glucose monomers in larger polymers to be used as energy reserves.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotechs use glucose as a food source for cell cultures.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Polysaccharides – long polymers composed of many monosaccharides.</td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from “Biotechnology: Science for the New Millennium” by Ellyn Daugherty.*
**LIPIDS – 3 groups which differ chemically and functionally**
Hydrocarbons composed of carbon and hydrogen and a few oxygen molecules.
Insoluble in water – hydrophobic.

<table>
<thead>
<tr>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>o Includes animal fats and plant oils</td>
<td>o Forms a phospholipid bilayer in membranes that regulates transport of materials in and out of the cell</td>
<td>o Composed of three overlapping 6-Carbon rings bound to a single 5-Carbon ring.</td>
</tr>
<tr>
<td>o Energy storage molecules</td>
<td>o Composed of two fatty acid chains attached to a glycerol molecule; a phosphate group is attached to the end of the glycerol.</td>
<td>o Functions include – acting as hormones (testosterone &amp; estrogen), venoms and pigments.</td>
</tr>
<tr>
<td>o Composed of glycerol and 3 fatty acid chains</td>
<td></td>
<td>o Important steroid - Cholesterol is found in cell membranes of most eukaryotic cells.</td>
</tr>
</tbody>
</table>

PROTEINS

- Estimated that more than 75% of the dry mass of cell is protein.
- Proteins are often the manufactured product in biotechnology. Typical cells produce more than 2000 different proteins – quantity varies.
- Expression popular in biotech circle: “Where DNA is the flash of biotechnology, proteins are the cash!”

*Amino acids are the monomers of proteins* – when bonded together, polypeptide chains are formed. Polypeptides are not functional until they fold into a 3-dimensional shape. Folded polypeptides are called proteins. The way a polypeptide folds into a functional protein is determined by the amino acids in the chain and by their order. The amino acid sequence is ultimately determined by a cell’s DNA code.

There are 20 different amino acids. All amino acids have a basic identical structure except the ‘R’ group is unique. The ‘R’ group results in unique characteristics of each amino acid – the chemical nature of the ‘R’ group results in attractions and repulsions of certain amino acids. This unique chemical nature results in various folding patterns of the polypeptide chain. The ‘R’ groups of protein chains can interact between proteins as well – many proteins function by attracting or repelling other protein chains.

*Adapted from “Biotechnology: Science for the New Millennium” by Ellyn Daugherty.*
**Amino Acid structure:** The core consist of a central Carbon atom attached to an amino group (-NH$_2$) and a carboxyl group (-COOH). The difference in the 20 amino acids is the ‘R’ group.

<table>
<thead>
<tr>
<th>Protein Groups</th>
<th>Examples</th>
<th>Specific Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural</td>
<td>Collagen</td>
<td>Component of skin, bones, ligaments and tendons.</td>
</tr>
<tr>
<td></td>
<td>Keratin</td>
<td>Components of nails and hair.</td>
</tr>
<tr>
<td>Enzyme</td>
<td>Amylase</td>
<td><strong>Enzymes speed up the rate of chemical reactions.</strong></td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
<td>Break down starch</td>
</tr>
<tr>
<td>Transport</td>
<td>Hemoglobin</td>
<td>Carries oxygen in blood – primary function</td>
</tr>
<tr>
<td></td>
<td>Cytochrome C</td>
<td>Break down bacterial cell walls</td>
</tr>
<tr>
<td>Contractile</td>
<td>Myosin &amp; Actin</td>
<td>Involved in muscle contraction</td>
</tr>
<tr>
<td>Hormone</td>
<td>Insulin</td>
<td>Regulates blood sugar</td>
</tr>
<tr>
<td>Antibody</td>
<td>HER2 antibody</td>
<td>Recognizes a breast cancer protein</td>
</tr>
<tr>
<td></td>
<td>Gamma globulin</td>
<td>Recognizes a variety of foreign proteins</td>
</tr>
<tr>
<td>Pigment</td>
<td>Melanin</td>
<td>Modified amino acid – pigment in human cells</td>
</tr>
<tr>
<td>Recognition</td>
<td>gp120</td>
<td>Protein on HIV surface</td>
</tr>
<tr>
<td></td>
<td>MHC protein</td>
<td>Self-recognition proteins on cell surface</td>
</tr>
<tr>
<td>Toxins</td>
<td>Botox</td>
<td>Neurotoxin made by Clostridium botulin</td>
</tr>
</tbody>
</table>

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**NUCLEIC ACIDS**

- Information carrying molecules direct the synthesis of all other cellular molecules – genetic information stored in the sequence of DNA.

- Nucleic Acids are long, complex molecules composed of monomer units called nucleotides. Each nucleotide has a nitrogenous base, a 5-Carbon sugar and a phosphate group (PO₄). Nucleotides are linked together in either a single chain (RNA) or a double chain (DNA).

**Types of nucleic acids – DNA and RNA**

1. **DNA** (deoxyribonucleic acid) – located in the nucleus of eukaryotic cells and in the cytoplasm of prokaryotic cells (bacteria). DNA is a double helix containing millions of nucleotides. The arrangement of nucleotides on the DNA molecule translates into a set of instructions for the production of a cell’s proteins. This blueprint of molecular construction is the “genetic code”.

2. **RNA** (ribonucleic acid) – the RNA molecule is synthesized from a DNA template molecule. mRNA (messenger) functions to transfer the genetic information from the chromosomes to the ribosomes where proteins are made. At the ribosome, tRNA (transfer) and rRNA (ribosomal) translate the genetic code into the amino acid chain which folds to form a protein.

**Genetic Code** – determined by the arrangement of the nitrogen bases on the DNA molecule. DNA contains A (adenine), T (thymine), G (guanine) and C (cytosine). RNA contains G, C, A, and U (uracil). The DNA sequence is read three bases at a time.

- Example: DNA strand CGGATGACCATA, the mRNA strand is GCC/UAC/UGG/UAU and codes for 4 amino acids: alanine, tyrosine, tryptophan, tyrosine

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If we change, manipulate, add or delete from A, C, G, and T code – could alter the amino acid sequence of a protein. If altered, could give an organism new molecules and new characteristics or fix genetic mistakes – could improve quality of human life and correct errors in genetic code.

Genetic engineers isolate and alter the DNA codes for a particular protein or group of proteins. The goal is to manufacture large amounts of the protein to market. Sometimes the manipulated protein gives the organism a desired characteristic such as protection from the corn borer insect given to genetically engineered Syngenta Bt corn.

**GENETIC ENGINEERING**

Significant breakthrough in the manipulation of plant and animal cells occurred when scientists learned how to move pieces of DNA within and between organisms. The key was the discovery of enzymes that cut DNA into fragments containing one or more genes. These DNA pieces could be separated from each other and pasted together using other enzymes. New combinations of genetic information were formed – resulting in recombinant DNA (rDNA).

rDNA contains fragments of DNA from different organisms – they have been engineered. If the DNA fragments contain genes of interest (code for a desired product), they can be pasted into vector molecules and carried back into cells. Once in cells, they are transcribed and translated into proteins.

The 1st genetic engineering took place in 1973 when Stanley Cohen (Stanford University), Herb Boyer (University of California) and Paul Berg (Stanford University) excised a segment of amphibian DNA from the African clawed toad and pasted it into a small ring of bacterial DNA called a plasmid. The new recombinant DNA plasmid contained DNA from two species – a bacterium and an amphibian). The recombinant plasmid was inserted into a healthy E. coli cell. The E. coli read the toad DNA code, synthesized molecules encoded in the rDNA.

The 1st genetically engineered product to reach the market was human insulin for the treatment of diabetes. Using genetic engineering, scientists transferred the human insulin gene into a bacterial plasmid. The rDNA plasmid was inserted into E. coli cells (bacteria), which read the DNA and synthesized insulin molecules. The cells were grown in large volumes and the insulin protein was purified out of the cell culture. The FDA approved recombinant human insulin (rh-insulin) for marketing in 1982.

Reasons scientist might want to make recombinant human insulin:
1. diabetic patients don’t make enough insulin or their insulin doesn’t function properly.
2. there is a large market for insulin since any children are born with Type I diabetes require daily injections of insulin.

Until the 1980s, diabetic patients had to use insulin derived from cow, sheep or pig pancreases. Livestock insulin works well in many patients, but some patients have allergic reactions or the insulin doesn’t perform up to expectations. Another disadvantage of livestock insulin is the price and availability.

Robert Swanson and Dr. Herb Boyer founded the 1st biotechnology company, Genentech, Inc. with the rh-Insulin as their first product.

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**CELLULAR BIOLOGY In Biotechnology Standards:**

HS-EB-6: Compare and contrast common organisms used in biotechnology and relate the manipulation of living organisms to product and procedure development.

6.2 Distinguish between prokaryotic cells, eukaryotic cells, and non-living entities, such as viruses.

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