Biology 5

Cell Biology and Genetics

By Mr. Charles K. Twesigye
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African Virtual University
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I. Biology 5, Cell Biology and Genetics

By Mr. Charles K. Twesigye, Kyambogo University and Prof William J Fraser, University of Pretoria.

II. Prerequisite Course or Knowledge

You should have an understanding of mitotic cell division and the cell cycle, cell and cell structure, diploid and haploid phases of sexual life-cycles and gamatogenesis before starting on this module. These topics are covered in units on cells, the origin of life and continuity of life normally taken at advanced levels of school biology.

III. Time

120 hours
IV. Materials

This module covers Cell Biology and Genetics. Section A of the module introduces molecular and structural organization of prokaryotic and eukaryotic cells, while section B includes a detailed study of classical transmission of genetic information and provides an introduction to the principles of genetics. To achieve these outcomes, you will be given the opportunity to engage with on-line learning experiences where the specific websites have been linked to the learning content and also to access the learning content by means of CD-Rom and hard copies. We have also recommended your participation in a number of laboratory and field-related activities during the course of the module.

Section of a protein structure showing serine and alanine residues linked together by peptide bonds. Carbons are shown in white and hydrogens are omitted for clarity (Taken from http://en.wikipedia.org/wiki/Proteins on 5 November 2006).

V. Module Rationale

The purpose with this module is to give you a better understanding of cellular biology in order to prepare you for the complicated biochemical and physiological processes to follow. The module also focuses on genetics as it relates to the function and structures of cells. The learning experiences you are about to encounter in this module will also serve as a foundation for further studies in advanced molecular biology and biochemistry. The module has been designed in such a way that your personal study skills will enable you work more effectively through the learning and assessment tasks. Although you might not have direct access to a laboratory where you would be able to study cells microscopically, the module will prepare you for such encounter as well as the application of the science process skills in the science classroom. We will also suggest a number of alternative options to practical work to you in this module.supplemental to the work done in this module.

VI. Overview

Rod-shaped bacterium, E. coli (prokaryote) dividing by binary fission taken from http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookmito.html on 27th August 2006.
6.1 Outline

We have structured the module for you into two main sections namely Cell Biology and Genetics. Section A will introduce you to cells (cell theory), the molecular and structural organizations of prokaryotic and eukaryotic cells (emphasis on eukaryotic cells). Other topics to be covered under section A include cell division, nucleic acids, colloidal systems (enzyme kinetics and metabolisms) and techniques in cell biology. Section B starts with the history of genetics and moves on to genetic code and chromosomal theory (multiple alleles, sex linkages traits, crossing-over and mapping). This section also covers mutations and variations; elements of population genetics and the application of genetics in biotechnology, agriculture, medicine and industry. The section will also introduce you to principles of genetics with specific reference to the classical transmission of genetic information. It is necessary to have a better understanding of the structure and function of cells (Section A) before you can link any advanced process such as cell division and the transfer of characteristics (Section B) to cell characteristics.

The content of the module can be outlined as follows:

<table>
<thead>
<tr>
<th>Unit</th>
<th>Topic</th>
<th>Theory Time</th>
<th>Practical Time</th>
<th>Total Time</th>
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</thead>
<tbody>
<tr>
<td>5.1.1</td>
<td>Introduction to cells (cell theory and discovery)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5.1.2</td>
<td>Prokaryote and Eukaryote cells (General features)</td>
<td>15</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>5.1.3</td>
<td>Structure and functions of cell organelles (Eukaryotic cell, ER, Golgi apparatus, cell membrane, etc)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5.1.4</td>
<td>Cell division (mitosis, control of cell growth, meiosis)</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>5.1.5</td>
<td>Nucleic acids (DNA &amp; RNA) and Protein synthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.1.6</td>
<td>Colloidal systems (enzyme kinetics and metabolism)</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>5.1.7</td>
<td>Techniques in Cell Biology (microscopic and cytological techniques)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.2.1</td>
<td>History of genetics (mendelism)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.2.2</td>
<td>An overview of genetic code and chromosomal theory (multiple alleles, sex linkage traits, crossing-over and mapping)</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Mutations and variation (chromosomal aberrations, gene mutations)</td>
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<td></td>
<td></td>
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<tr>
<td>5.2.4</td>
<td>Elements of Population and quantitative genetics (Phylogenetic variations, DNA-polymorphism, random mating, Hardy-Weinberg principle, Causes of evolution)</td>
<td>15</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>5.2.5</td>
<td>Application of genetics in Biotechnology (Bio-safety issues Genetic engineering in Agriculture, Medicine, Industry etc.)</td>
<td>5</td>
<td>5</td>
<td>10</td>
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<td></td>
<td></td>
<td>60</td>
<td>60</td>
<td>120</td>
</tr>
</tbody>
</table>
6.2 Graphic Organizer
VII. General Objective(s)

When you have mastered this module, you should have achieved the following general objectives:

1. To understand the cell theory and its scientific discovery.
2. To understand the gross and the fine structures of prokaryotic and eukaryotic cells.
3. To demonstrate and describe the structure and functions of cell organelles.
4. To describe and demonstrate the process of cell division.
5. To understand the nature and structure of nucleic acids and their role in protein synthesis.
6. To describe the chemical nature of enzymes and their role in metabolism.
7. To effectively explain and demonstrate the use of the light microscopy and related techniques in the study of cells.
8. To describe Mendel’s breeding experiments and explain Mendel’s results in terms of the particulate theory of inheritance.
9. To examine the genetic code and the chromosomal theory.
10. To describe mutations and their role in causing variation in populations.
11. To explain the Hardy-Weinberg equilibrium.
12. Describe application of genetics in biotechnology and demonstrate the scientific relevance of these principles to society and our everyday lives in general.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Topic</th>
<th>Specific objective</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1.1</td>
<td>Introduction to cells (cell theory and discovery)</td>
<td>1. To understand the cell theory</td>
</tr>
<tr>
<td>5.1.2</td>
<td>Prokaryote and Eukaryote cells (General features)</td>
<td>2. When you have worked through this unit you should be able to distinguish between prokaryote and eukaryote cells.</td>
</tr>
<tr>
<td>5.1.3</td>
<td>Structure and functions of cell organelles (Eukaryotic cell, ER, Golgi apparatus, cell membrane, etc)</td>
<td>3. Understand the relationship between structure and the functions of the different cell organelles.</td>
</tr>
<tr>
<td>5.1.4</td>
<td>Cell division (mitosis, control of cell growth, meiosis)</td>
<td>1. Understand the phases of meiotic division and describe the events that occur at each phase.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. State and explain the basic differences between meiosis and mitosis.</td>
</tr>
<tr>
<td>5.1.5</td>
<td>Nucleic acids (DNA &amp; RNA) and Protein synthesis</td>
<td>1. Understand the biochemical properties of cells with specific reference to carbohydrates, proteins and lipids as they apply to the structure and functions of cells.</td>
</tr>
<tr>
<td>5.1.6</td>
<td>Colloidal systems (enzyme kinetics and metabolism)</td>
<td>2. Name major ways in which enzymes resemble or differ from other catalysts and their role in metabolism.</td>
</tr>
<tr>
<td>Unit</td>
<td>Topic</td>
<td>Specific objective</td>
</tr>
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</tr>
<tr>
<td>5.1.7</td>
<td>Techniques in Cell Biology (microscopic and cytological techniques)</td>
<td>1. To understand the important steps in the preparation of specimens for examination by means of light and electron microscopes and to state the main principles involved in each stage.</td>
</tr>
<tr>
<td>5.2.1</td>
<td>History of genetics (Mendelism)</td>
<td>1. To examine a brief overview of the modern history of genetics and to understand Mendel’s rules of inheritance.</td>
</tr>
<tr>
<td>5.2.2</td>
<td>An overview of genetic code and chromosomal theory (multiple alleles, sex linkages traits, crossing-over and mapping) DNA and RNA</td>
<td>2. Explain inheritance involving multiple alleles.</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Mutations and variation (chromosomal aberrations, gene mutations)</td>
<td>3. Describe the morphology, structure and functional significance of chromosomal and gene mutations.</td>
</tr>
<tr>
<td>5.2.4</td>
<td>Elements of Population and quantitative genetics genetics genetics (Phylogenetic variations, DNA-polymorphism, random mating, Hardy-Weinberg principle, Causes of evolution)</td>
<td>1. Define and recognize examples of continuous and discontinuous variation. 2. Explain the genetic origins of variation and its role in evolution 3. Explain the Hardy-Weinberg equilibrium and use the Hardy-Weinberg equation to determine gene and genotypic frequencies of populations.</td>
</tr>
<tr>
<td>5.2.5</td>
<td>Application of genetics in Biotechnology (Bio-safety issues Genetic engineering in Agriculture , Medicine, Industry etc.)</td>
<td>1. Outline the contribution of applied genetics in Biotechnology, Medicine and Agriculture. 2. Explain the importance of bio safety measures in Biotechnology</td>
</tr>
</tbody>
</table>
IX. Pre-Assessment One


9.1 Rationale

Cell Biology and Genetics

Rationale: Effective learning depends on what you know about a subject before you attempt to master the new learning material. You are now going to do a short test to assess your knowledge on the content dealt with in this module.

Multiple choice questions

Answer the following multiple choice questions and check your answers against the attached mark sheet.
1. What would be the major chemical component of the structure of a plant cell visible under the light microscope?
   (a) DNA
   (b) Cellulose
   (c) Lipids
   (d) Proteins

2. Which is the main disadvantage in using Drosophila for breeding experiments?
   (a) Small size of the larvae
   (b) Short life-cycle
   (c) Mating soon after emergence of flies
   (d) Large numbers of offspring produced

3. Study the following illustration of a cell division stage where the chromosomes have gathered at the equator and answer the question to follow:
In which organ of the human body did this process take place?

(a) Liver  
(b) Spleen  
(c) Ovarium  
(d) Bone marrow

4. Study the following illustration very carefully:

The illustration is a representation of a …

(a) cell wall.  
(b) root hair.  
(c) cilium.  
(d) cell membrane.

5. Which of the following is not necessary for chromosome replication?

(a) Adenosine triphosphate  
(b) Ribosomes  
(c) Nuclear enzymes  
(d) A DNA template

6. A mRNA molecule is …

(a) transcribed into DNA.  
(b) translated into protein.  
(c) transcribed from protein.  
(d) translated from DNA.

7. What is the following process called?

(a) Fertilisation  
(b) Crossing-over  
(c) Regeneration  
(d) Meiosis
8. Which one of the following arrangements represents a nucleotide in an mRNA molecule?
   (a) Guanine-deoxyribose-phosphate
   (b) Uracil-deoxyribose-phosphate
   (c) Thymine-ribose-phosphate
   (d) Adenine-ribose-phosphate

9. The sugar found in DNA molecule is …
   (a) sucrose.
   (b) ribose.
   (c) ribulose.
   (d) deoxyribose.

10. Which of the following is not a mutagen?
    (a) X-rays
    (b) Ultraviolet rays
    (c) High temperature
    (d) Low temperature

11. Among a randomly mating population, the offspring genotypic frequencies of the different types of the parental gametes and
    (a) adding them together.
    (b) diving the frequencies in half.
    (c) finding the product of their combined frequencies.
    (d) finding the possible combinations by using a Punnet square.

12. Using the Hardy-Weinberg equation to find the frequency of an allele in a population you would only need the frequency of the …
    (a) heterozygote.
    (b) dominant or recessive phenotype.
    (c) heterozygote x2.
    (d) dominant and recessive phenotypes.
13. Study the following illustration of the crossing of parents representing specific characteristics:

What would be genotype ratio of the F2 generation should one cross or use the results of the F1 generation as parents?

(a) 9:3:3:1  
(b) 1:1  
(c) 1:2:1  
(d) One pure-breeding generation

14. The use of the Hardy-Weinberg equation for a population shows that …

(a) immigration of new mating types can be accounted for.  
(b) the results of breeding over a number of generations can be predicted.  
(c) the proportion of phenotypes is 3:1.  
(d) there are twice as many dominant phenotypes.

15. Which of the following is true about enzyme competitive inhibition?

(a) The structure of the inhibitor is entirely different from the substrate.  
(b) The structure of the inhibitor molecule is similar to the substrate.  
(c) The inhibitor forms a complex at a point other than the active site on the enzyme.  
(d) The inhibitor alters the structure of the enzyme in such a way that even if the substrate gets attached, the products will not be formed.

16. Which one of the following is the best description of a prosthetic group. It is

(a) an activator.  
(b) an inhibitor.  
(c) a coenzyme.  
(d) a cofactor.
17. The active site of an enzyme is made up:
   (a) similar amino acids.
   (b) essential amino acids.
   (c) catalytic amino acids.
   (d) only acidic amino acids.

18. Most CO₂ from catabolism is released during:
   (a) glycolysis
   (b) electron transport
   (c) the Krebs cycle
   (d) oxidative phosphorylation

19. The enzyme catalysing the reaction below is characterised as Glucose + ATP
    → Glucose phosphate + ADP
   (a) isomarase
   (b) hexokinase
   (c) transferase
   (d) phospholyrase

20. A model of enzyme action where there is exact complementarity between enzyme and substrate is described as the
   (a) lock and key
   (b) potential for a substrate to bind to the enzyme recognition site
   (c) induction to the correct conformational change
   (d) induced fit model

SOMETHING TO DO ON THE WEB

You can visit the following website to test your knowledge on the functions of various cell organelles in a eukaryotic cell. This is a very basic assessment and applies mainly to learners who have just left school or Grade 12. It remains however a very preparatory assessment that will give you a very good indication of your current understanding and knowledge of cell structure and function.

The test is called ‘Cell organelles’ and is found at http://www.quia.com/jg/65947.html. Another option would also be to go to

http://www.quia.com/servlets/quia.activities.common.ActivityPlayer?
AP_rand=1039286581&AP_activityType=1&AP_urlIId=65947&gameType=list

and to follow the instructions explained at the site. It deals with matching items as well as with associations one has to make to come to the correct answers.

<table>
<thead>
<tr>
<th>Matching</th>
<th>Flashcards (Java / non-Java)</th>
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</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>Word Search</td>
</tr>
</tbody>
</table>
9.2 Pedagogical Comment For Learners

One could expect to achieve an average mark of lose to 60% and beyond for this multiple-choice test. Should your performance be below this mark, then you will have to do a lot of preliminary reading to familiarise yourself with the basics of cell biology and genetics dealt with in the module. Since effective learning and the mastery of the specific learning objectives depend on what you already know about the subject before attempting to master the new subject content, it is highly recommended that you do some preliminary reading on the subject before commencing with the learning activities.

X. Key Concepts (Glossary)

MITOSIS
During the process of mitosis the parent cell containing a given number of chromosomes (for example 2n) divides to form two daughter nuclei or cells containing the same number (for example 2n) and kinds of chromosomes as that of the parent nucleus or cell.

MEIOSIS
Meiosis refers to the type of cellular division that normally occurs as part of sexual reproduction when the new daughter cells receive that haploid (n) number of chromosomes.

PROKARYOTE
Organism (which is often also reference to a given type of cell) that lacks the membrane-bounded nucleus and organelles containing membranes that are typical of eukaryotes.

EUKARYOTE (EUKARYOTIC CELL)
Cell that contains a well-defined and membraned nucleus and membranous organelles. These structures are normally absent in prokaryotic cells or organisms.

ORGANELLE
Membranous microscopic structures found in cells having specific structures and functions for example lysosomes, mitochondria, nucleus and ribosomes.

PROTEINS
Proteins are large organic compounds made of amino acids arranged in a linear chain and joined together between the carboxyl atom of one amino acid and the amine nitrogen of another (http://en.wikipedia.org/wiki/Proteins, consulted 5 November 2006).
LIPIDS
Lipids are hydrocarbon-containing organic compounds essential in providing stored energy and organ protection within a living organism. Lipids are soluble in non-polar solvents (such as ether and chloroform) and are relatively insoluble in water. Lipid molecules have these properties because they consist of mainly carbon (http://en.wikipedia.org/wiki/Lipids, consulted 5 November 2006).

CARBOHYDRATES
Carbohydrates oxygen, hydrogen, and carbon atoms. They may also contain other elements such as sulphur or nitrogen, but these are usually minor components. They consist of monosaccharide sugars that have the general chemical formula C\(_n\)(H\(_2\)O\(_n\)) or are derivatives of such. The smallest value for \(n\) is 3 (http://en.wikipedia.org/wiki/Carbohydrates, consulted 5 November 2006).

ENZYMES
Enzymes are globular molecules with catalytic properties. They are almost invariably proteins, although a few are made of RNA (these are called ribosomes). A catalyst is a substance which alters the rate of a chemical reaction without itself undergoing permanent change. As they are not altered by the reactions they catalyze, enzymes can be used over and over again. They are therefore effective in very small amounts. Enzymes cannot cause reactions to occur, but only speed up ones which would otherwise take place extremely slowly. The word ‘enzyme’ means ‘in yeast’ and was used because they were first discovered in an extract of yeast.

METABOLISM
is the sum total of the cell’s chemical activity. One aspect of metabolism is how the cell handles small molecules such as sugars, fatty acids, nucleotides, amino acids and so on. There are two types of chemical reactions: (i) The build-up of complex compounds from simple ones by synthetic reactions collectively known as anabolism (ii) The breakdown of complex compounds from simple ones by reactions collectively known as catabolism.

GENETICS
Genetics (from the Greek 'genno' γεννω = give birth) is the science of genes, heredity, and the variation of organisms. The word "genetics" was first suggested to describe the study of inheritance and the science of variation by the prominent British scientist William Bateson in a personal letter to Adam Sedgwick, dated April 18, 1905. Bateson first used the term "genetics" publicly at the Third International Conference on Genetics (London, England) in 1906 (http://en.wikipedia.org/wiki/Genetics, consulted 27 September 2006).
PHENOTYPE
The phenotype of an individual organism is either its total physical appearance and constitution or a specific manifestation of a trait, such as size, eye colour, or behaviour that varies between individuals. Phenotype is determined to some extent by genotype, or by the identity of the alleles that an individual carries at one or more positions on the chromosomes. Many phenotypes are determined by multiple genes and influenced by environmental factors. Thus, the identity of one or a few known alleles does not always enable prediction of the phenotype (http://en.wikipedia.org/wiki/Phenotype, consulted 27 September 2006).

DOMINANCE
In genetics, the term dominant gene refers to the allele that causes a phenotype that is seen in a heterozygous genotype. Every person has two copies of every gene, one from mother and one from father. If a genetic trait is dominant, a person only needs to inherit one copy of the gene for the trait to be expressed (http://en.wikipedia.org/wiki/Dominant_gene, consulted 27 September 2006).

RECESSIVE GENE
In genetics, the term "recessive gene" refers to an allele that causes a phenotype (visible or detectable characteristic) that is only seen in a homozygous genotype (an organism that has two copies of the same allele) and never in a heterozygous genotype. Every person has two copies of every gene, one from mother and one from father. If a genetic trait is recessive, a person needs to inherit two copies of the gene for the trait to be expressed. Thus, both parents have to be carriers of a recessive trait in order for a child to express that trait. If both parents are carriers, there is a 25% chance with each child to show the recessive trait (http://en.wikipedia.org/wiki/Recessive, consulted 27 September 2006).

CHROMOSOME
A chromosome is a large macromolecule into which DNA is normally packaged in a cell. Minimally, it is a very long, continuous piece of DNA (a single DNA molecule), which contains many genes, regulatory elements and other intervening nucleotide sequences. The word chromosome comes from the Greek σωμα (‘chroma’, colour) and χρωμα (soma, body) (http://en.wikipedia.org/wiki/Chromosome, consulted 27 September 2006).

LAW OF SEGREGATION
If the two alleles differ, then one, the dominant allele, is fully expressed in the organism's appearance; the other, the recessive allele, has no noticeable effect on the organism's appearance. In other words, the dominant allele is expressed in the phenotype of the organism; however this does not always hold true. Today, we know several examples that disprove this "law", e.g. *Mirabilis jalapa*, the "Japanese wonder flower" (Fig. 3). This is called incomplete dominance. There is also co-dominance on a molecular level, e.g. people with sickle cell anaemia, when normal and sickle-shaped red blood cells mix and prevent malaria. The
two alleles for each characteristic segregate during gamete production. This is the last part of Mendel's generalization. The two alleles of the organism are separated into different gametes, ensuring variation (http://en.wikipedia.org/wiki/Law_of_segregation, consulted 27 September 2006).

**XI. Compulsory Readings**

An illustration of a fruit fly Drosophila melanogaster (SEM X60) taken from http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookgeninteract.htm where permission was given by Dennis Kunkel at www.DennisKunkel.com to use the image in the former website.

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**Reading 1**

**Complete reference**: The structure and function of Prokaryotic and Eukaryotic cells.

1. The Study Guide to the Science of Botany that is used in this section of the work is a textbook at Wikibooks shelved under Biology and intended to establish a course of study in the subject of Botany, utilizing articles provided in Wikipedia(http://www.Wikipedia.org/), with links to other relevant web sites and other Wikibooks as appropriate. In some cases, portions of the text from Wikipedia articles have been used to materially develop introductory text within

2. Cell Biology, Mark Dalton and others http://en.wikibooks.org/wiki/Cell_biology of which 30 pages covering cell structure and function have to be read prior to working through the learning activities to follow. the Guide. Focus your attention mainly to Chapter 2 of the Study Guide as it applies to the structure and function of ells only.
Abstract: The introductory section address the differentiation of cells with specific reference to cell structure and its relationship to cell division (mitosis and meiosis) and the transfer of genetic material.

Rationale: The main aim with the readings is to allow you to come to terms with the structures of prokaryotic and eukaryotic cells and also to relate the structure of the cells to function with specific reference to mitosis, meiosis and the transfer of genetic material.

Reading 2: Mitochondrion - From Wikipedia, the free encyclopaedia

Abstract: The chapter commences describing the structure of a mitochondrion, followed by energy conversion and the release of great amounts of heat. The chapter also links to a later section of the work where the task of the mitochondrion in terms of genetic transfer and population genetic studies will be highlighted. What the chapter also traces is to explain how mitochondrial inheritance occurs and what influence this could have on future generations.

Rationale: The main purpose with this section of the work is to give you the opportunity in working through an on-line text with the main intention of acquainting yourself with the basic structure and function of mitochondria. The text is vividly illustrated containing a variety of on-line links that will give you access to the discussion and description of all the minute details relating to cell mitochondria.

Reading 3: Cell organelles

Abstract: The following description applies specifically to the second ‘Wikipedia’ references listed as URL in this section. It mentions the fact that eukaryotes are the most structurally complex known cell type, and by definition are in part organized by smaller interior compartments, that are themselves enclosed by lipid membranes that resemble the outer most cell wall. The larger organelles, such as the nucleus and vacuoles, are easily visible with moderate magnification (although sometimes a clear view requires the application of chemicals that selectively stain parts of the cells); they were among the first biological discoveries made after the invention of the microscope. The article continues to explain that
not all eukaryotic cells have all of the organelles listed and occasionally, exceptional species of cells are missing organelles that might otherwise be considered universal to eukaryotic cells (such as mitochondria). There are also occasional exceptions to the number of membranes surrounding organelles.

Rationale: We have included this article to your reading resources as it could be regarded as a very comprehensive reader illustrating and explaining the structures and function of the majority of organelles contained in prokaryotic and eukaryotic cells. The different organelles are thoroughly compared with vivid and very clear descriptions relating to structure and function. It is once again a very good text to work through and to be reminded of the major differences of prokaryotic and eukaryotic cells.

Reading 4: Cell Membranes Tutorial (*optional)

Complete reference:
http://www.biology.arizona.edu/cell_bio/problem_sets/membranes/index.html

Abstract: As explained in the above-mentioned website “this exercise introduces the dynamic complexes of proteins, carbohydrates, and lipids that comprise cell membranes. You should learn that membranes are fluid, with components that move, change, and perform vital physiological roles as they allow cells to communicate with each other and their environment. We also show that membranes also are important for regulating ion and molecular traffic flow between cells and that defects in membrane components, lead to many significant diseases.”

The website suggests that you follow the following instructions:
“The following problems have multiple choice answers. Correct answers are reinforced with a brief explanation. Incorrect answers are linked to tutorials to help solve the problem.”

Rationale: The completion of a simple hands-on assessment activity will assist you in mastering the cell membranes more effectively.

Reading 5: Cell nucleus


Abstract: The following paragraph was taken from the following website: http://en.wikipedia.org/wiki/Cell_nucleus. “The article illustrates that in cell biology the nucleus is found in all eukaryotic cells and contains the nuclear genes that form most of the cell's genetic material. The section explains that nuclei have two primary functions namely to control chemical reactions within the cytoplasm and also to store information needed for cellular division. Aside from containing the cell's genome, the nucleus contains certain proteins whose interplay is thought to regulate the expression of genes. Gene expression at the nuclear level involves complex processes of transcription, pre-mRNA processing and the export of the mature mRNA to the cytoplasm.”
Rationale: The section will provide you with a very thorough briefing on the structure of the components of the cell nucleus in the first place, but will also highlight the different functions of the nucleus as it applies to cell metabolism and genetics.

**Reading 6: Introduction to genetics**

1. Complete reference: Genetics/Introduction
   From Wikibooks, the open-content textbooks collection
2. Online Biology Book
   http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookgenintro.html

**Abstract:** The short article illustrates that genetics is the study of the function and behaviour of genes and that offspring receive a mixture of genetic information from both parents. This process contributes to the great variation of traits that we see in nature, such as the colour of a flower’s petals, the markings on a butterfly’s wings, or such human behavioural traits as personality or musical talent. Geneticists also seek to understand how the information encoded in genes is used and controlled by cells and how it is transmitted from one generation to the next. They also look how tiny variations in genes can disrupt an organism’s development or cause disease. The article also explains how modern genetics involves genetic engineering, a technique used by scientists to manipulate genes.

**Rationale:** Understanding the rationale behind genetics as field of study, will help us to come to terms with the changes often experienced in genetic material, the loci where these changes are actually taking place as well as the mechanism behind the transfer of fixed characteristics from one generation to the other. The examples provided in these introductory learning activities should prepare you to comprehend the more advanced descriptions and activities to follow.

**Reading 7: Fundamental understanding of Mendel’s law of dominance**

1. Complete reference: Genetics/Mendelian Inheritance
   From Wikibooks, the open-content textbooks collection
2. Online Biology Book
   http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookTOC

**Abstract:** The short article explains that Mendel's first step in the many experiments he conducted was breeding pure breeding strains of peas. The traits (traits = characteristics) he studied included pea colour, height and whether the peas were wrinkled or smooth.
Mendel crossed the pure breeding Parental Generation (designated P). He found that the first generation (F1) was exclusively phenotypically (phenotype = externally visible characteristic such as pea colour) one of the parental types. Mendel then crossed his F1 generation with itself. He found that the F2 generation showed a surprising trait, three quarters were like the F1 generation, while the remaining quarter were like the other Parents. From this Mendel realised that there were two versions of each loci, one of which expressed dominance over the other. He called this bi-particulate (bi = two) Inheritance. If a gene was following this 3:1 pattern it was said to be segregating normally.

**Rationale:** The purpose of allowing you to work through this introductory passage on inheritance (with specific reference to the P1 and F2 generations) is to illustrate to you how the simple crossing of two individuals containing pure traits such as a pure breeding characteristic for shortness and a pure breeding characteristic for tallness would produce offspring reflecting only one of these characteristics in the first generation, called the F1 generation. The examples contained in the text are very clear and self-explanatory.

**Reading 8: Mitosis**

**Complete reference:**
http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookmito.html and http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookTOC.html

**Abstract:** The second website (consulted 5 October 2006) listed above under (2) explains the reading of this specific article as follows:

“Despite differences between prokaryotes and eukaryotes, there are several common features in their cell division processes. Replication of the DNA must occur. Segregation of the "original" and its "replica" follow. Cytokinesis ends the cell division process. Whether the cell was eukaryotic or prokaryotic, these basic events must occur.”

**Rationale:** The article has been selected as a very basic comparative study between binary fission and mitoses. The article explains the two processes very carefully and with simple examples. The article has been selected as introductory passage for the other processes (meiosis) and activities to follow.

**Reading 9: Meiosis**

**Complete reference:**
(1) http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookmeiosis.html
(2) http://en.wikipedia.org/wiki/meiosis

**Abstract:** The Wikipedia website (consulted 27 September 2006) listed above summarises meiosis as follows:
“Meiosis is the process that transforms one diploid cell into four haploid cells in eukaryotes in order to redistribute the diploid cell's genome. Meiosis forms the basis of sexual reproduction and can only occur in eukaryotes. In meiosis, the diploid cell's genome, which is composed of ordered structures of coiled DNA called chromosomes, is replicated once and separated twice, producing four sets of haploid cells each containing half of the original cell's chromosomes. These resultant haploid cells will fertilize with other haploid cells of the opposite gender to form a diploid cell again. The cyclical process of separation by meiosis and genetic recombination through fertilization is called the life cycle. The result is that the offspring produced during germination after meiosis will have a slightly different blueprint which has instructions for the cells to work, contained in the DNA. This allows sexual reproduction to occur.”

**Rationale:** Understanding meiosis will assist you in coming to a better understanding of genetic transfer and of the role chromosomes play during the transfer of characteristics.

**Reading 10: Genetic manipulation – terminator, Terminator Technology, and Genetically modified organisms**

**Complete reference:**

**Abstract:** A genetically modified organism (GMO) is an organism whose genetic material has been altered using techniques in genetics generally known as recombinant DNA technology. Recombinant DNA technology is the ability to combine DNA molecules from different sources into the one molecule in a test tube. Thus, the abilities or the phenotype of the organism, or the proteins it produces, can be altered through the modification of its genes.

The term generally does not cover organisms whose genetic makeup has been altered by conventional cross breeding or by "mutagenesis" breeding, as these methods predate the discovery of the recombinant DNA techniques. Technically speaking, however, such techniques are, by definition, genetic modification.

**Rationale:** Terminator Technology is the colloquial name given to proposed methods for restricting the use of genetically modified plants by causing second generation seeds to be sterile. The technology was under development by the U.S. Department of Agriculture and Delta and Pine Land Company in the 1990s and is not yet commercially available. Because some stakeholders expressed concerns that this technology might lead to dependence for poor smallholder farmers, Monsanto, an agricultural products company, pledged not to commercialize the technology even if and when is becomes commercially available.
Reading 11: Genetic manipulation – terminator

Complete reference:
http://www.gse.buffalo.edu/FAS/Bromley/classes/socprac/readings/Steinbrecher.htm


Abstract: According to Steinbrecher and Mooney (1998:276) in The Ecologist 28(5) “Monsanto's latest flagship technology makes a nonsense of its claim that it seeks to feed the worlds hungry. On the contrary, it threatens to undermine the very basis of traditional agriculture - that of saying seeds from year to year. What's more, this "gene cocktail" will increase the risk that new toxins and allergens will make their way into the food chain”.

Rationale: Because of the relevant importance of genetic manipulation in agriculture, we have decided to include this article dealing with the controversial manipulation of agricultural products and the responses it has evoked the last few years.
XII. Compulsory Resources

Go to the website http://www.mblab.gla.ac.uk/~julian/Dict.html, retrieved on the 7th of November 2006 that will give you access to.

**The Dictionary of Cell and Molecular Biology**  
**Third edition**

You can use the online dictionary should you require information on biological terms and concepts.

Figure: The structure of two prokaryotic cells

**Resource 1: Introduction to genetics**

**Complete reference:**  
http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookgenintro.html  

**Abstract:** According to the website listed above “Mendel studied the inheritance of seed shape first. A cross involving only one trait is referred to as a monohybrid cross. Mendel crossed pure-breeding (also referred to as true-breeding) smooth-seeded plants with a variety that had always produced wrinkled seeds (60 fertilizations on 15 plants). All resulting seeds were smooth. The following year, Mendel planted these seeds and allowed them to self-fertilize. He recovered 7324 seeds: 5474 smooth and 1850 wrinkled. To help with record keeping, generations were labelled and numbered. The parental generation is denoted as the P1 generation. The offspring of the P1 generation are the F1 generation (first filial). The self-fertilizing F1 generation produced the F2 generation (second filial).”

**Rationale:** The reading will give you a basic understanding of the principles of genetics.
Resource 2: Mitosis

Complete reference:
http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookmito.html
Consulted 26th August 2006.

Abstract: Despite differences between prokaryotes and eukaryotes, there are several common features in their cell division processes. Replication of the DNA must occur. Segregation of the "original" and its "replica" follow. Cytokinesis ends the cell division process. Whether the cell was eukaryotic or prokaryotic, these basic events must occur.

Rationale: The website, of which a number of illustrations have been incorporated into this module, explains mitosis (as well as meiosis) very clearly to the ‘visual’ learner.

Resource 3: Eukaryotic vs Prokaryotic cells

Complete references:
http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/Chap2.html#
Consulted 28th August 2006.

Abstract: The chapter refers to the Basic Principle of Life with specific reference to the structure and function of Eukaryotic and Prokaryotic Cells. The chapter also compares Eukaryotic and Prokaryotic Cells and explains the common characteristics of these cells. The structure and function of the organelles in Eukaryotic cells are also discussed in the suggested reading material.

Rationale: Working through this recommended chapter will not only prepare you in understanding the underpinning substance related to Eukaryotic and Prokaryotic cells, but the site also takes you through a number

Resource 4: Monohybrid inheritance


Abstract: The purpose of this section is to illustrate to you that monohybrid inheritance is the inheritance of a single characteristic. The different forms of the characteristic are usually controlled by different alleles of the same gene. For example, a monohybrid cross between two pure-breeding plants (homozygous for their respective traits), one with yellow seeds and one with green seeds, would be expected to produce an F1 (first) generation with only yellow seeds because the allele for yellow seeds is dominant to that of green.
Rationale: The example and illustration form the foundation of genetics as studied by Mendel and remain a valuable starting point when having to master the principles and practices of more complicated genetic manipulations and calculations.

Resource 5: Dihybrid cross


Abstract: The paragraph will illustrate to you how a dihybrid (two-hybrid) cross is a cross in which two hybrids are mated to test for dominant genes and recessive genes in two separate characteristics, and such a cross has a variety of uses of in Mendelian genetics and genetic linkage experiments. The dihybrid genotype is usually created when two different parental individuals that are true breeding (homozygous) for different alleles of two genes are sexually crossed or mated together. Their resulting progeny possesses the "dihybrid" genotype and is heterozygous for alleles of two genes. The dihybrid is also often referred to as the double heterozygote. When two dihybrids with the same genotype are mated together, the mating is referred to as a dihybrid cross.

Rationale: Dihybrid crossing illustrates what happens when two individuals with two characteristics each would cross. The estimation of the genotype and phenotype of the offspring is more complicated than in the case with monohybrid crossing. It illustrates very clearly how more than one characteristic would eventually emerge in the F2-generation.
XIII. Useful Links


Useful Link #1

**Title**: Mitochondrial division  
**URL**: http://agrippina.bcs.deakin.edu.au/beech/Mt_div.html

**Screen capture**: Downloaded from the following website on the 20th of August 2006. http://agrippina.bcs.deakin.edu.au/beech/Mt_div.html

**Description**: According to the website http://agrippina.bcs.deakin.edu.au/beech/Mt_div.html retrieved on the 4th of November 2006 "Mitochondria are descended from bacteria (specifically, the alpha-proteobacteria) that formed an endosymbiotic relationship with the ancestors of our cells probably around two billion years ago (see figure). On the otherhand, the origins of eukaryotic cells are uncertain although they appear to be a the product of the fusion between some kind of bacteria and another bacterial-like cell called an archeabacterium".

**Rationale**: The duplication of cellular inclusions is not only confined to the nucleus but can also be traced to different organelles that play important roles in the maintenance of cellular metabolism.
Useful Link # 2

Title: Genes
URL : http://en.wikipedia.org/wiki/genes

Description: This stylistic schematic diagram shows a gene in relation to the double helix structure of DNA and to a chromosome (right). Introns are regions often found in eukaryote genes which are removed in the splicing process: only the exons encode the protein. This diagram labels a region of only 40 or so bases as a gene. In reality many genes are much larger, as are introns and exons (Figure retrieved from http://en.wikipedia.org/wiki/genes on 16 September 2006).

Rationale: Working through this section of the work will allow you to understand the link between DNA, RNA, genes and chromosomes more easily. You will be dealing with mitosis and meiosis shortly in this module as well as with introductory genetics in the second part of the module. The gene ‘bridges’ the structural ‘anatomy’ of a chromosome and the molecular configuration of the DNA ‘carrying’ certain characteristics.
Useful link 3

Title: Biomedia

URL: http://ebiomed.com/teach/Teach_main.html retrieved on the 8th of November 2006.

Description: The website focuses on the use of video in the classroom. It contains discussion papers providing tips and rationale to help get more education out of classroom video. Also go to http://ebiomed.com/teach/freebies.html dealing with the use of video in the classroom as downloaded on 8 November 2006.

Rationale: According to the website "this collection of links is designed to complement a CD-ROM, Visualizing Cell Biology - An Interactive Learning Guide. These links provide enhancements and visual resources to help you learn basic cell biology concepts, as well as extend your knowledge based on the educational materials available on the web".
Useful link 4

**Title:** Free videos and Lesson Plans


According to the following website retrieved on 17 September 2006 (http://www.pubinfo.vcu.edu/secretsofthesequence/about_us.asp) the goal of the Center for Life Sciences Education at Virginia Commonwealth University in Richmond, VA, is "to promote scientific literacy at local, regional and national levels by:

- increasing public awareness of the technical and bioethical issues surrounding the life sciences discoveries of the 21st century,
- educating pre-college students about the life sciences, and
- providing information and professional development to K-12 science teachers across the nation."
The above-listed site “will take you and your students to laboratories where scientists are investigating fascinating questions. SOSq creates an avenue for students to learn from leading scientists and ethicists about the profound moral, ethical and legal impact of recent discoveries in the life sciences. Secrets of the Sequence videos engage students and introduce them to a variety of traditional and cutting edge topics”.

**Description:** All 50 videos have an accompanying classroom-tested lesson that encourages students to further explore the video topics. Each lesson includes background information, state and national science standards, discussion questions and answers, teacher notes and an activity that will ensure a hands-on, “minds-on” experience.

**Rationale:** Biology teachers depend heavily on images and illustrations to assist then in the clarification of complicated phenomena, terms, concepts and definitions. This site will provide you will valuable links where such materials related to biology could be retrieved.

**Useful Link #5**

**Title:** Lysosome structure and function  
**URL:** http://en.wikipedia.org/wiki/lysosome

**Description:** The following paragraph was taken from the following website: http://en.wikipedia.org/wiki/lysosome illustrating that lysosomes are organelles that contain digestive enzymes (acid hydrolases) to digest macromolecules. The site continues to explain that lysosomes are found in "only" animal cells, and are built in the Golgi apparatus. All these enzymes are produced in the endoplasmic reticulum, and transported and processed through the Golgi apparatus. The Golgi apparatus produces lysosomes by budding. Each acid hydrolase is then targeted to a lysosome by phosphorylation. The lysosome itself is likely safe from enzymatic action due to having proteins in the inner membrane which has a three-dimensional molecular structure that protects vulnerable bonds from enzymatic attack.

**Rationale:** We have selected this specific site for reflection mainly because of the selective and comprehensive coverage of lysomatic structure and function in the chapter. That fact that the section clearly indicates the function and activity of various enzymes should be noted while the importance of phagosytosis is also raised in the chapter. The reader is encouraged to work through the chapter very carefully and to assess the learning material in terms of her or his prior knowledge of cell organelles.
Useful Link #6

Title: Cell organelles, structure and function

URL: http://library.thinkquest.org/12413/structures.html retrieved on the 8th of November 006.

Description: The website http://library.thinkquest.org/12413/structures.html retrieved on the 8th of November explains that "within cells there is an intricate network of organelles that all have unique functions. These organelles allow the cell to function properly. The website gives you the opportunity to click on the organelle's names in a list to directly reach the feature on that structure.

Rationale: The website supplements work already done on cellular inclusions and function and will provide you with additional information of the structure and function of various organelles found in plant and animal cells.

Useful Link #7

Title: The Sourcebook for Teaching Science


Description: The website to follow reports on The Sourcebook for Teaching Science: Strategies, Activities, and Internet Resources (http://www.csun.edu/science/biology/index.html retrieved on 12 November 2006). According to this website “it provides new and experienced teachers with a wealth of useful teaching strategies, resources, lessons, activities, and ideas to enhance science teaching and learning. All ideas and activities are based upon learning theory, and are designed to stimulate student interest and involvement in the science curriculum. As students are engaged in the activities described in this resource, they acquire knowledge and understanding of key scientific concepts and the relevance of these to their everyday lives”.

**Rationale:** This module also covers a number of issues related to the teaching of biology and we thought it well to include references in the module that will provide access to teaching and learning strategies to you.

**Useful link 8**

**Title:** Biomolecular Images and Movies for Teaching


**Description:** According to the [http://www.chem.ucsb.edu/~molvisual/](http://www.chem.ucsb.edu/~molvisual/) the purpose of this site “is to facilitate visualization-based instruction by offering access to structure files, static images, movie clips, and pre-configured visualization scripts for many macromolecules that are discussed in undergraduate and graduate biochemistry courses. The images and movie clips can be readily incorporated into slideshows. The structure files and visualization scripts allow quick generation of interactive three-dimensional views of the important parts of the structure with the help of program PyMOL. Each image is accompanied by a brief annotation that documents the purpose of the image.”

**Rationale:** Finding and downloading good quality videos for teaching and studying purposes are often problematic for teachers and students. The site contains excellent visualisations and illustrations and if accessed effectively, create a better understanding of many of the topics dealt with in this module.
Useful link 9

**Title:** Best educational resources for teachers to aid the use of ICT

**URL:** http://www.bigbrownenvelope.co.uk/, downloaded 7 November 2006.

**Abstract:** According to Big Brown Envelope “teachers, and trainers, are continuously reinventing the wheel when it comes to teaching materials. This is often unnecessary since someone out there probably has already got something that meets the need. On this site we hope you will find useful materials to help with your classroom teaching.”

Useful link 10

**Title:** The Biology Corner

**URL:** http://www.biologycorner.com/, downloaded 7 November 2006.

**Abstract:** The webpage located at http://serc.carleton.edu/resources/1000.html describes ‘GeoTimes’ as “the monthly news magazine for geoscience professionals and enthusiasts published by the American Geological Institute.”
Covered are research findings, industry trends, and developments in politics, education, and technology as they relate to the earth sciences. Available online for each current or back issue is a brief synopsis of the respective cover article, highlights of the issue (From the Editor), shorter news articles (News Notes), selected up-to-date news (Web Extras), briefs on the interface between politics and the geosciences (Political Scene), and reports of recent natural occurrences of particular interest to geoscientists (GeoPhenomena).”

Useful link 11


Title: A bank of digital resources for teaching biology

The BIODIDAC Project - Description according to the following website http://biodidac.bio.uottawa.ca/Thumbnails/catquery.htm, lists their objective as follows. The following three paragraphs were taken from that site:

**Objective**: Create a bank of digital images, video, and animations that can be used and adapted for teaching Biology.

Copying the material, modifying and adapting it to meet the professor's needs, and subsequent distribution to students is permitted with the condition that this is noncommercial, that the supplier (BIODIDAC) of the material is acknowledged, and that its use is registered.

**Why?**

There is too little digital material that can be freely used for teaching. BIODIDAC aims at filling this void, at least partially.

**Contents**

BIODIDAC now contains 6153 items.
Useful link 12

Title: Teaching Strategies - Scientific Inquiry: Learning Science by Doing Science
XIV. Learning Activities

Title: The structure and function of cell organelles

Specific objectives with this learning activity:

When you have worked through this learning activity you should be able to distinguish between prokaryote and eukaryote cells with specific reference to the structure of the two cell types, as well as the functions of the different cell organelles contained within these cell types. The section will conclude with a description of the DNA and RNA structure of a cell and guidelines towards your participation in working together in coming to a better understanding of mitosis and meiosis.

Summary of the learning activity:

The Study Guide to the Science of Botany that is used in this section of the work is a textbook at Wikibooks shelved under Biology and intended to establish a course of study in the subject of Botany, utilizing articles provided in Wikipedia (http://www.Wikipedia.org/), with links to other relevant web sites and other Wikibooks as appropriate. In some cases, portions of the text from Wikipedia articles have been used to materially develop introductory text within the Guide. You will therefore often be requested to refer to this section of the work as we work through the learning activity. This specific learning activity has been structured as a cooperative learning experience where you will have to work as team members in the achievement of the objectives set for this section of the module.

Kindly consult the following sources for more information on cellular structure:

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

Cell Membranes
http://www.biology.arizona.edu/cell_bio/problem_sets/membranes/index.html

Cell nucleus
http://en.wikipedia.org/wiki/Cell_nucleus

Organelles
http://en.wikipedia.org/wiki/organelles

Lysosomes
http://en.wikipedia.org/wiki/lysosome
Chromosomes
http://en.wikipedia.org/wiki/Chromosomes

List of relevant useful links:

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

Formative evaluation

Description of the Learning activity

Please note that this is a group activity. When the task cannot be performed cooperatively, then at least attempt to do each assignment individually.

The achievement of the outcomes of this learning activity will be determined by your ability to work as members of a group and for this reason it is suggested that you join your fellow students as part of a cooperative learning group. We suggest you follow the following approach:

1. The learning task will be broken down into five different components and five team members or students clustered into five groups should therefore share their expertise and experiences when having to work through the learning materials and complete the assignments that follow.

2. Compulsory reading has been broken down into 5 sections and each group or person representing the group has to work through the compulsory reading and set out and requested in the compulsory reading section of this learning activity. See ‘Compulsory reading 1’ The structure and function of Prokaryotic and Eukaryotic cells.

3. Each group or group member addresses one of the following readings and prepare for the assessment tasks to follow:
   3.1. The structure of prokaryotic cells (inclusive of all organelles)
   3.2. The structure of eukaryotic cells (inclusive of all organelles)
   3.3. The function of cell organelles
   3.4. The processes of mitosis and meiosis
   3.5. Chromosomes as carriers of the genetic material

4. Each group or member (should the group only consist of one member) have to prepare a 40-minute PowerPoint slide-show or set of transparencies that will be presented at a seminar organised by all five groups. The groups will then compile a joint report on all five sections covered by the five groups and distribute the report among all members of the groups.
GROUP 1: The structure of prokaryotic cells

We would suggest you also use the article on organelles taken from Wikipedia as your prime source of information when having to understand the structure and function of different eukaryotic cell organelles. This article is found at http://en.wikipedia.org/wiki/organelles and is a very comprehensive and detailed comparative between prokaryotic and eukaryotic cells. Study this article in detail and attempt to answer all questions contained at the end of this Group activity.

Then go to Chapter II dealing with Eukaryotic vs. Prokaryotic cells at http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/Chap2.html#... and work through the text in an attempt to distinguish between the two different cell types. Commence by identifying what makes prokaryotic cells so special. You will be told that prokaryotic cells are very primitive and lack certain structures found mainly in eukaryotic cells. Begin by listing those structures found mainly in prokaryotic cells. How do these differ from eukaryotic cells?

You have to work through the reading material and come to an understanding of the main characteristics of the two major taxonomic groups namely the Eubacteria and the Archaea or Archaebacteria. The recommended text will explain to you why the Eubacteria are considered the more commonly known form.

The article on organelles focus specifically on the following:

(a) Specific prokaryotic organelles and their functions
(b) Plasmids and magnetosomes
(c) Flagella and nucleoids

Do the following as members of the group when working through the recommended text. Remember that the details that you will be collecting will later be used as frame of reference when comparing it to the characteristics of eukaryotic cells collected by Group 2:

(a) List the most prominent organisms that are classified as Prokaryotes or Prokaryotic cells.
(b) List their most prominent and distinguishable cellular characteristics as this group of organisms.
(c) Discuss and explain what the roles and functions of the organelles are in maintaining cellular support activities.
(d) Write one page on the economic and medical importance of these organisms.
(e) Identify at least two prominent Prokaryotes and explain why they are biologically important to humans.
GROUP 2: The structure of eukaryotic cells

The eukaryotic cells represent a very complex and integrated set of characteristics that became known as the more advanced cells. The study of cell organelles and cellular inclusions had mostly been associated with eukaryotic structure and function.

1. Cell Membranes (A tutorial)

Visit the following website where each of the separate sections are actually part of a tutorial designed as a questionnaire to be answered by you at the end of the learning experience. This section deals mainly with the cell membrane and is an excellent way to work you through good selection of questions focusing on 15 subsections associated with membrane structure, support and function.

http://www.biology.arizona.edu/cell_bio/problem_sets/membranes/index.html

To each of the following a number of questions are set that have to be answered by you.

- Membrane components
- Lipids and aqueous barriers
- Hydrophobic forces
- Osmosis
- Membrane transport
- Membrane proteins
- Diffusion
- Co-transport
- Water flow solution
- Membrane stability
- Phospholipids
- Penetrating lipid bi-layer
- Cell junctions
- Energy requirements for transport
- Oral rehydration
- Membrane flow

Figure: The structure of a mitochondrion (Reference: http://en.wikipedia.org/wiki/Mitochondria, - Wikipedia, the free encyclopedia.htm, downloaded 27th August 2006).
2. Mitochondria

Your reference for this section of the work is contained in the document ‘Mitochondrion - From Wikipedia, the free encyclopaedia’ (see http://en.wikipedia.org/wiki/Mitochondria or also http://en.wikipedia.org/w/index.php?title=Mitochondria&redirect=no).

3. Cell nucleus

4. Chromosomes

5. Lysosomes

6. Ribosomes

GROUP 3: The function of cell organelles

It will be Group 3’s function to list all organelles found in animal cells and plane cells and then to scribe the different functions of these organelles. Identify all organelles associated with and responsible for the replication and duplication of genetic material. Explain very specific the contribution of each of the following organelles to mitosis, meioses, the duplication of DNA and the transfer of genetic material:

(a) Cell nucleus
(b) Mitochondria
(c) Cytoplasm
(d) Ribosomes

GROUP 4: The processes of mitosis and meiosis

A. SOMETHING ON BINARY FISSION

DON’T CONFUSE THIS WITH MITOSIS OR MEIOSIS AS WILL BE EXPLAINED FURTHER ON IN THIS MODULE!

Go to the link supplied at the bottom of the yellow illustration of a cell and activate the animation by clicking on the red circle that represents the nucleus of the cell. This prokaryote cell division is termed binary fission. The prokaryotic chromosome is a single DNA molecule that first replicates, and then attaches each copy to a different part of the cell membrane. When the cell begins to pull apart, the replicate and original chromosomes are separated. Following cell splitting (cytokinesis), there are then two cells of identical genetic composition (except for the rare chance of a spontaneous mutation).
Go to the following website to observe and animated GIF illustration of binary fission:
http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/Chap2.html#two_bact_groups

B. MITOSIS

BACKGROUND: This section discusses mitosis – that is cell division where the given number of chromosomes is being maintained such as when body cells, for example epidermal cells, divide to replace damaged tissue.


Have a look at each of the following illustrations showing and representing one of the phases of mitosis. Notice that the cell division starts with prophase where the chromatin begins to coil with the formation of the chromatids, chromosomes and the spindle. The chromatides appear more prominent and pairs to form the chromosomes.
When the next phase, namely metaphase commences, the chromosomes arrange along the equator of the spindle where the spindle attaches to the kinetochore fibres.

**Metaphase**
Metaphase follows Prophase. The chromosomes (which at this point consist of chromatids held together by a centromere) migrate to the equator of the spindle, where the spindles attach to the kinetochore fibres.

**Anaphase**
Anaphase begins with the separation of the centromeres, and the pulling of chromosomes (we call them chromosomes after the centromeres are separated) to opposite poles of the spindle.

The following diagrammes and illustrations were retrieved from the website http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookmito.html on 27 September 2006.

**Telophase**
Telophase is when the chromosomes reach the poles of their respective spindles, the nuclear envelope reforms, chromosomes uncoil into chromatin form, and the nucleolus (which had disappeared during Prophase) reform. Where there was one cell there are now two smaller cells each with exactly the same genetic information. These cells may then develop into different adult forms via the processes of development.

**Cytokinesis**

Cytokinesis is the process of splitting the daughter cells apart. Whereas mitosis is the division of the nucleus, cytokinesis is the splitting of the cytoplasm and allocation of the Golgi apparatus, plastids and cytoplasm into each new cell.

This concludes mitosis.

We recommend you visit the following website and access one or two of the videos that deal specifically with mitosis. The animated cellular movement will really enhance the theoretical processes explained in this section of the work. The website is located at http://cellimages.ascb.org/ as retrieved on the 8th of November 2006. You could also visit specifically the following site of which the images will follow. The site was accessed on the 8th of November 2006.

http://cellimages.ascb.org/cdm4/item_viewer.php?CISOROOT=/p4041coll2&CISOPTR=38&REC=1

According to http://cellimages.ascb.org/ “the Image and Video Library of The American Society for Cell Biology (ASCB) is a collection of peer-reviewed cell images, video clips, and digitized texts that illustrate the structure, function and biology of the cell, the fundamental unit of life.”
The Image & Video Library of The American Society for Cell Biology (ASCB) is a collection of peer-reviewed cell images, video clips, and digitized texts that illustrate the structure, function, and biology of the cell, the fundamental unit of life.

### Collections

- **Founders**
- **Cytoplasm**
- **Nucleus**
- **Landmark Papers**
- **Resource of the Month**
- **Video**

#### Cytoplasm

#### Nucleus

#### Landmark Papers

#### Resource of the Month

#### Video

*Meeting in the Middle before Parting: Anaphase Lagging*

The video, part of a series, features the process of anaphase lagging, where chromosomes fail to capture the mitotic spindle, resulting in cell division abnormalities. By entering this site, you agree to the Terms and Conditions of Use.
Title: Meiosis

BACKGROUND: This section discusses meiosis – that is cell division where the given number of chromosomes are halved, for example from 2n to n, as when diploid (2n) spermatogonia (in the testes) undergo division to produce haploid (n) spermatids that will eventually develop into haploid (n) spermatozoa or sperm.

Work through page 27 of ‘Cell Biology, Edition 1, 2006 found at http://en.wikibooks.org/wiki/Cell_biology to prepare for the assignment to follow at a later stage in this tutorial. The following illustrations, diagrams and accompanying discussions were taken from http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookmito.html on 27 September 2006.

IMPORTANT NOTICE: DURING MEIOSIS THE NUCLEUS DIVIDES TWICE – THE FIRST DIVISION SEES THE NUMBER OF CHROMOSOMES BEING HALVED FROM 2N > N IN EACH NUCLEUS. THIS IS REFERED TO AS MEIOSIS ONE OR I.

DURING MEIOSIS II THE CHROMOSOMES BEHAVE JUST LIKE IN MITOSIS.

FURTHER NOTE THAT METAPHASE I WOULD THEREFORE REFER TO METAPHASE OF MEIOSIS I, WHILE METAPHASE II WOULD REFER TO METAPHASE IN THE MEIOSIS II PHASE!

Events of Prophase I (save for synapsis and crossing over) are similar to those in Prophase of mitosis: chromatin condenses into chromosomes, the nucleolus dissolves, nuclear membrane is disassembled, and the spindle apparatus forms.

**Metaphase I**
Metaphase I is when tetrads line-up along the equator of the spindle. Spindle fibres attach to the centromere region of each homologous chromosome pair. Other metaphase events as in mitosis.

**Anaphase I**
Anaphase I is when the tetrads separate, and are drawn to opposite poles by the spindle fibres/fibers. The centromeres in Anaphase I remain intact.

**Telophase I**
Telophase I is similar to Telophase of mitosis, except that only one set of (replicated) chromosomes is in each "cell". Depending on species, new nuclear envelopes may or may not form. Some animal cells may have division of the centrioles during this phase.


**Prophase II**
During Prophase II, nuclear envelopes (if they formed during Telophase I) dissolve, and spindle fibers reform. All else is as in Prophase of mitosis. Indeed Meiosis II is very similar to mitosis.


**Meiosis II**
Prophase II
The chromosomes condense again, following a brief interphase in which DNA does not replicate

**Metaphase II**
Metaphase II is similar to mitosis, with spindles moving chromosomes into equatorial area and attaching to the opposite sides of the centromeres in the kinetochore region.

**Anaphase II**
During Anaphase II, the centromeres split and the former chromatids (now chromosomes) are segregated into opposite sides of the cell.


**Telophase II**
Telophase II is identical to Telophase of mitosis. Cytokinesis separates the cells.

SOMETHING TO DO: Visit the following website http://www.biology.uc.edu/vgenetic/meiosis/ (retrieved on the 6th of November from http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookmeiosis.html) at experience an animated illustration of cells dividing. The second reference might be a better option.

**Comparison of Mitosis and Meiosis**
Mitosis maintains diploid level, while meiosis reduces it. Meiosis may be considered a reduction phase followed by a slightly altered mitosis. Meiosis occurs in a relative few cells of a multi-cellular organism, while mitosis is more common.
Meiosis

Prophase I

Parent cell (2n)

Chiasma (at least one per pair of homologs)

Homolog pairs align at the equatorial (metaphase) plate

Metaphase I

Two daughter cells

Centromeres do not divide

Sister chromatids remain together during anaphase; homologs separate

DNA does not replicate

Metaphase II

Four daughter cells

Centromeres divide

**GROUP 5: Chromosomes as carriers of the genetic material**

There is a clear link between the structure of chromosomes, their replication and duplication during meiosis and mitosis, and the ‘carrying’ of genetic material according to Mendel’s different laws.

Write an essay on the qualities and characteristics of chromosomes as carriers of genetic information and explain how the structure of a chromosome enhances the carrying capacity of the chromosome.

**Formative evaluation**

Submit the following assignments to your lecturer for marking. This will give you some indication of your understanding of the basic theoretical foundations you should have dealt with by now.

**Assignment 1:** Explain in not more than five A4-pages how the process of mitosis depends on the biochemical structure and composition of the chromosomes to take place successfully. Focus on each of the following aspects:

a. The role carbohydrates play in the composition of the chromosome material.

b. The composition of the nucleotides (components of chromosomes) and their ability to align during mitosis and meiosis.

c. The main differences between meiosis and mitosis in terms of the duplication of genetic material.

Maximum number of marks: 50

**Assignment 2:** Explain what would be the main reasons why mitosis only applies to somatic cells (that is body cells where the diploid (ploid) number of chromosomes have to be maintained). What is the main function of chromosomes and genes and what would be the impact of additional genetic material gain or loss on anatomical characteristics?

Maximum number of marks: 20
Unit 3: The biochemical properties of cells

Learning activity 1: Carbohydrates, proteins and lipids

The biochemical properties of cells, with specific reference to the structure and function of carbohydrates, proteins and lipids will not be dealt with you specifically in this module BUT you are expected to work through the documentation listed and submit the assignment applicable to this section of the work as listed. You should be able to achieve the following outcomes at the completion of this section of the work:

1. Understand the chemical composition of the carbohydrates, proteins and lipids as found in plant and animal cells and systems,
2. Be able to link the composition, location and structure of the different cell organelles to function, but then more specifically to the role of DNA and RNA in mitosis, meiosis and the transfer of genetic materials during cell division,
3. Know and understand what role proteins play in enzyme activity and how different environmental factors could impact on the performance of enzymes under specific conditions.

Access the following websites and retrieve the appropriate materials from each of them:

   2.1 Enzymes (Website reference retrieved on 6 November 2006) http://en.wikipedia.org/wiki/Enzyme (Website reference retrieved on 6 November 2006). Also visit the following website that will inform you on enzyme actions and activities:
   http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookEnzym.html

The following three illustrations were taken from the website http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookCHEM2.html retrieved on the 6th of November 2006 and illustrate molecular structure of DNA and RNA.
Assignment

The following assignment is based on the self-study section you will have to work through. This unit will not be dealt with in this module but you have to be acquainted with the content covered by the unit.

Write a ten-page assignment on the following topic and submit the assignment for assessment to your lecturer.

The carbohydrates, proteins and lipids play important roles in the synthesis of DNA and RNA as well as in the duplication of new genetic material during mitosis and meiosis.

Explain the function of carbohydrates, proteins and lipids during the above-mentioned processes with specific reference to each of the following:

a. The function and transferability of cell membranes and cell walls
b. The structure and composition of nuclear materials with specific reference to chromosomes and chromatids
c. The role of enzymes in DNA synthesis (including the function of ribosomes)
d. DNA synthesis and chromosomes
e. The location and function of genetic material (genes)
Unit 4: The structure and functions of DNA and RNA

Learning activity 1: Structure and function of DNA

A number of the following sections were retrieved from http://en.wikipedia.org/wiki/DNA on 6 November 2006. The following two illustrations are both representations of the double DNA helix. The illustration to the left is also called a so-called ball-and-stand model of DNA. Image from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com). Taken from http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookDNAMOLGEN.html on the 8th of November 2006.

Double helix illustrations of a sections of a DNA molecule

Section of DNA illustrated at the right was copied from http://en.wikipedia.org/wiki/DNA on 6 November 2006.

Introduction

The following three paragraphs were taken from http://en.wikipedia.org/wiki/DNA. DNA is responsible for the genetic propagation of most inherited traits. In humans, these traits range from hair color to disease susceptibility. The genetic information encoded by an organism's DNA is called its genome. During cell division, DNA is replicated, and during reproduction is transmitted to offspring.

In eukaryotic cells, such as those of plants, animals, fungi and protists, most of the DNA is located in the cell nucleus, and each DNA molecule is usually packed into a chromosome that are passed to daughter cells during cell division. By contrast, in simpler cells called prokaryotes, including the eubacteria and archaia, DNA is found directly in the cytoplasm (not separated by a nuclear envelope) and is circular. The cellular organelles known as chloroplasts and mitochondria also carry DNA.
In humans, the mother’s mitochondrial DNA together with 23 chromosomes from each parent combine to form the genome of a zygote, the fertilized egg. As a result, with certain exceptions such as red blood cells, most human cells contain 23 pairs of chromosomes, together with mitochondrial DNA inherited from the mother. Lineage studies can be done because mitochondrial DNA only comes from the mother, and the Y chromosome only comes from the father.

The composition of DNA

The following six paragraphs taken from http://en.wikipedia.org/wiki/DNA on 6 November 2006, explain the common structure of a DNA molecule with some reference to its comparison to RNA. Although sometimes called "the molecule of heredity", DNA macromolecules as people typically think of them are not single molecules. Rather, they are pairs of molecules, which entwine like vines, in the shape of a double helix (see the illustration above).

DNA consists of a pair of molecules, organized as strands running start-to-end and joined by hydrogen bonds along their lengths. Each strand is a chain of chemical "building blocks", called nucleotides, of which there are four types: adenine (abbreviated A), cytosine (C), guanine (G) and thymine (T). (Thymine should not be confused with thiamine, which is vitamin B1.) The DNA of some organisms, most notably of the PBS1 phage, have Uracil (U) instead of T.

Each strand of DNA is a covalently linked chain of nucleotides, with alternating sugar (deoxyribose)- phosphates forming the "backbone" for the nucleobases ("bases"). The negatively-charged phosphate groups between each deoxyribose make DNA an acid in solution and allow DNA molecules of different sizes to be separated by electrophoresis. Because DNA strands are composed of these nucleotide subunits, they are polymers. The major difference between DNA and RNA is the sugar, 2-deoxyribose in DNA and ribose in RNA.
The above-mentioned illustrations were taken from the website http://en.wikipedia.org/wiki/DNA on 7 November 2006.

**DNA replication**

The replication or duplication of DNA is discussed in the following paragraph. The information contained in the paragraph as well as the illustration to follow were retrieved from http://en.wikipedia.org/wiki/DNA on the 7th of November 2006.

The double-stranded structure of DNA provides a mechanism for DNA replication: the two strands are separated, and then each strand's complement is recreated by exposing the strand to a mixture of the four bases. An enzyme makes the complement strand by finding the correct base in the mixture and bonding it with the original strand. In this way, the base on the old strand dictates which base appears on the new strand, and the cell ends up with an extra copy of its DNA.

DNA replication or DNA synthesis is the process of copying the double-stranded DNA prior to cell division. The two resulting double strands are generally almost perfectly identical, but occasionally errors in replication or exposure to chemicals, or radiation can result in a less than perfect copy (see mutation), and each of them consists of one original and one newly synthesized strand. This is called *semiconservative replication*. 
Learning activity 2: Structure and function of RNA

The structure and functions of RNA, with specific reference to the synthesis of proteins, are dealt with in the following paragraph. The information and illustrations where appropriate were retrieved from the website http://en.wikipedia.org/wiki/RNA on 7 November 2006. You are also requested to visit the following website that will give you more information on DNA and RNA: http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookDNAMOLGEN.html. The site is active and was visited and accessed on 6 November 2006.

Introduction

Ribonucleic acid (RNA) is a nucleic acid polymer consisting of nucleotide monomers. RNA nucleotides contain ribose rings and uracil unlike deoxyribonucleic acid (DNA), which contains deoxyribose and thymine. It is transcribed from DNA by enzymes called RNA polymerases and further processed by other enzymes. RNA serves as the template for translation of genes into proteins, transferring amino acids to the ribosome to form proteins, and also translating the transcript into proteins.

RNA is primarily made up of four different bases: adenine, guanine, cytosine, and uracil. The first three are the same as those found in DNA, but in DNA thymine replaces uracil as the base complementary to adenine. This base is also a pyrimidine and is very similar to thymine. Uracil is energetically less expensive to produce than thymine, which may account for its use in RNA. In DNA, however, uracil is readily produced by chemical degradation of cytosine, so having thymine as the normal base makes detection and repair of such incipient mutations more efficient. Thus, uracil is appropriate for RNA, where quantity is important but lifespan is not, whereas thymine is appropriate for DNA where maintaining sequence with high fidelity is more critical.

There are also numerous modified bases found in RNA that serve many different roles. Pseudouridine (β) and the DNA nucleoside thymidine are found in various places (most notably in the TβC loop of every tRNA). Another notable modified base is Inosine (a deaminated Guanine base), which allows a "wobble codon" sequence in tRNA. There are nearly 100 other naturally occurring modified bases, many of which are not fully understood.

Transfer RNA (tRNA)

The structure and function of transfer RNA (tRNA) is discussed in the following paragraph and were retrieved from http://en.wikipedia.org/wiki/TRNA on the 7th of November 2006. The illustration to the left (see Transfer RNA (tRNA) below) was retrieved on the 8th of November 2006 from the website http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookPROTSYn.html.
An anticodon (sometimes called nodoc from the reversed letters of the word codon) is a unit made up of three nucleotides that correspond to the three bases of the codon on the mRNA. Each tRNA contains a specific anticodon triplet sequence that can base-pair to one or more codons for an amino acid. For example, one codon for lysine is AAA; the anticodon of a lysine tRNA might be UUU. Some anticodons can pair with more than one codon due to a phenomenon known as wobble base pairing. Frequently, the first nucleotide of the anticodon is one of two not found on mRNA: inosine and pseudouridine, which can hydrogen bond to more than one base in the corresponding codon position. In the genetic code, it is common for a single amino acid to occupy all four third-position possibilities; for example, the amino acid glycine is coded for by the codon sequences GGU, GGC, GGA, and GGG.

To provide a one-to-one correspondence between tRNA molecules and codons that specify amino acids, 61 tRNA molecules would be required per cell. However, many cells contain fewer than 61 types of tRNAs because the wobble base is capable of binding to several, though not necessarily all, of the codons that specify a particular amino acid.[1]

**Messanger RNA, ribosomes and protein synthesis**

Messanger RNA carries information from the DNA and plays an important role in the synthesis of proteins. This procedure will briefly be explained in the following paragraph. The information contained in the following paragraph was retrieved from http://en.wikipedia.org/wiki/Messenger_RNA on the 7th of November 2006.

Messenger Ribonucleic Acid (mRNA) is RNA that encodes and carries information from DNA during transcription to sites of protein synthesis to undergo translation in order to yield a gene product.
The illustration above explains the "life cycle" of an mRNA in a eukaryotic cell. RNA is transcribed in the nucleus; once completely processed, it is transported to the cytoplasm and translated by the ribosome. At the end of its life, the mRNA is degraded. Captured from http://en.wikipedia.org/wiki/Messenger_RNA on 6 November 2006.

According to http://en.wikipedia.org/wiki/Ribosome protein synthesis begins at a start codon near the 5' end of the mRNA. The small ribosomal subunit, typically bound to a tRNA containing the amino acid methionine, binds to an AUG codon on the mRNA and recruits the large ribosomal subunit. The large ribosomal subunit contains three tRNA binding sites, designated A, P, and E. The A site binds an aminoacyl-tRNA (a tRNA bound to an amino acid); the P site binds a peptidyl-tRNA (a tRNA bound to the peptide being synthesized); and the E site binds a free tRNA before it exits the ribosome.
The above mentioned figure was captured from http://en.wikipedia.org/wiki/Ribosome on 6 November 2006 and illustrates the translation of mRNA (1) by a ribosome (2) into a polypeptide chain (3). The mRNA begins with a start codon (AUG) and ends with a stop codon (UAG).

In this illustration both ribosomal subunits (small and large) assemble at the start codon (towards the 5’ end of the mRNA). The ribosome uses tRNA which matches the current codon (triplet) on the mRNA to append an amino acid to the polypeptide chain. This is done for each triplet on the mRNA, while the ribosome moves towards the 3’ end of the mRNA. Usually in bacterial cells, several ribosomes are working parallel on a single mRNA, forming what we call a polyribosome or polysome.

You can read more about protein synthesis at the following site: http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookPROTSYn.html.
UNIT 5: Colloidal systems (enzyme kinetics and metabolism)

Abstract: Enzymes are proteins that catalyze (i.e. accelerate) chemical reactions. In these reactions, the molecules at the beginning of the process are called substrates, and the enzyme converts them into different molecules, the products. Almost all processes in the cell need enzymes in order to occur at significant rates. Since enzymes are extremely selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.


5.1 Enzyme

Learning objectives

After studying this unit you should:

1. have an appreciation of the chemical environment in a cell
2. know that enzymes are biological catalysts
3. be able to describe the properties of enzymes that are typical of catalysts
4. be able to describe the properties of enzymes that are typical of proteins
5. understand the standard terminology of enzyme kinetics, including simple Michaelis-Menton kinetics
6. be able to discuss models for the mechanism of enzyme action
7. appreciate that enzymes cooperate to form biochemical pathways
8. be familiar with a range of enzyme activators and inhibitors
9. know examples to illustrate all the above points.

Figure 5.1.1: Ribbon diagram of the enzyme TIM, surrounded by the space-filling model of the protein. TIM is an extremely efficient enzyme involved in the process that converts sugars to energy in the body.
Enzymes are proteins that catalyze (i.e. accelerate) chemical reactions. In these reactions, the molecules at the beginning of the process are called substrates, and the enzyme converts them into different molecules, the products. Almost all processes in the cell need enzymes in order to occur at significant rates. Since enzymes are extremely selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.

Like all catalysts, enzymes work by lowering the activation energy ($\Phi G^\ddagger$) for a reaction, thus dramatically accelerating the rate of the reaction. Most enzyme reaction rates are millions of times faster than those of comparable uncatalyzed reactions. As with all catalysts, enzymes are not consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions. However, enzymes do differ from most other catalysts by being much more specific. Enzymes are known to catalyze about 4,000 biochemical reactions. Not all biochemical catalysts are proteins, since some RNA molecules called ribozymes also catalyze reactions.

Enzyme activity can be affected by other molecules. Inhibitors are molecules that decrease enzyme activity; activators are molecules that increase activity. Many drugs and poisons are enzyme inhibitors. Activity is also affected by temperature, pH, and the concentration of substrate. Some enzymes are used commercially, for example, in the synthesis of antibiotics. In addition, some household products use enzymes to speed up biochemical reactions (e.g., enzymes in biological washing powders break down protein or fat stains on clothes; enzymes in meat tenderizers break down proteins, making the meat easier to chew).
Etymology and history

Eduard Buchner

As early as the late 1700s and early 1800s, the digestion of meat by stomach secretions and the conversion of starch to sugars by plant extracts and saliva were known. However, the mechanism by which this occurred had not been identified.

In the 19th century, when studying the fermentation of sugar to alcohol by yeast, Louis Pasteur came to the conclusion that this fermentation was catalyzed by a vital force contained within the yeast cells called "ferments", which were thought to function only within living organisms. He wrote that "alcoholic fermentation is an act correlated with the life and organization of the yeast cells, not with the death or putrefaction of the cells."

In 1878 German physiologist Wilhelm Kühne (1837–1900) coined the term enzyme, which comes from Greek ἐνζυμον "in leaven", to describe this process. The word enzyme was used later to refer to nonliving substances such as pepsin, and the word ferment used to refer to chemical activity produced by living organisms.

Like all proteins, enzymes are made as long, linear chains of amino acids that fold to produce a three-dimensional product. Each unique amino acid sequence produces a unique structure, which has unique properties. Individual protein chains may sometimes group together to form a protein complex. Most enzymes can be denatured—that is, unfolded and inactivated—by heating, which destroys the three-dimensional structure of the protein. Depending on the enzyme, denaturation may be reversible or irreversible.

Specificity

Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity. Enzymes can also show impressive levels of stereospecificity, regioselectivity and chemoselectivity.

Some of the enzymes showing the highest specificity and accuracy are involved in the copying and expression of the genome. These enzymes have "proof-reading" mechanisms. Here, an enzyme such as DNA polymerase catalyses a reaction in a first step and then checks that the product is correct in a second step. This two-step process results in average error rates of less than 1 error in 100 million reactions in high-fidelity mammalian polymerases. Similar proofreading mechanisms are also found in RNA polymerase\textsuperscript{[14]}, aminoacyl tRNA synthetases\textsuperscript{[15]} and ribosomes.\textsuperscript{[16]}
Some enzymes that produce secondary metabolites are described as promiscuous, as they can act on a relatively broad range of different substrates. It has been suggested that this broad substrate specificity is important for the evolution of new biosynthetic pathways.\textsuperscript{[17]}

"Lock and key" model

Enzymes are very specific, and it was suggested by Emil Fischer in 1894 that this was because both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This is often referred to as "the lock and key" model. However, while this model explains enzyme specificity, it fails to explain the stabilization of the transition state that enzymes achieve.

Induced fit model

![Diagram showing induced fit model](Image)

**Figure 5.1.2** Diagrams to show the induced fit hypothesis of enzyme action.

In 1958 Daniel Koshland suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site can be reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the amino acid side chains which make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, such as glycosidases, the substrate molecule also changes shape slightly as it enters the active site.

Mechanisms

Enzymes can act in several ways, all of which lower $\Phi G^\dagger$

- Lowering the activation energy by creating an environment in which the transition state is stabilised (e.g. straining the shape of a substrate - by binding the transition-state conformation of the substrate/product molecules, the enzyme distorts the bound substrate(s) into their transition state form, thereby reducing the amount of energy required to complete the transition).
• Providing an alternative pathway (e.g. temporarily reacting with the substrate to form an intermediate which would be impossible in the absence of the enzyme).
• Reducing the reaction entropy change by bringing substrates together in the correct orientation to react. Considering $\beta H^+$ alone overlooks this effect.

Dynamics and function

Recent investigations have provided new insights into the connection between internal dynamics of enzymes and their mechanism of catalysis. An enzyme's internal dynamics are described as the movement of internal parts (e.g. amino acids, a group of amino acids, a loop region, an alpha helix, neighboring beta-sheets or even entire domain) of these biomolecules, which can occur at various time-scales ranging from femtoseconds to seconds. Networks of protein residues throughout an enzyme's structure can contribute to catalysis through dynamic motions. Protein motions are vital to many enzymes, but whether small and fast vibrations or larger and slower conformational movements are more important depends on the type of reaction involved. These new insights also have implications in understanding allosteric effects, producing designer enzymes and developing new drugs.

Allosteric modulation

Allosteric enzymes change their structure in response to binding of effectors. Modulation can be direct, where the effector binds directly to binding sites in the enzyme, or indirect, where the effector binds to other proteins or protein subunits that interact with the allosteric enzyme and thus influence catalytic activity.

Cofactors and coenzymes

Cofactors

Some enzymes do not need any additional components to show full activity. However, others require non-protein molecules to be bound for activity. Cofactors can be either inorganic (e.g., metal ions and iron-sulfur clusters) or organic compounds, (e.g., flavin and heme). Organic cofactors (coenzymes) are usually prosthetic groups, which are tightly bound to the enzymes that they assist. These tightly-bound cofactors are distinguished from other coenzymes, such as NADH, since they are not released from the active site during the reaction.

An example of an enzyme that contains a cofactor is carbonic anhydrase, and is shown in the ribbon diagram above with a zinc cofactor bound in its active site. These tightly-bound molecules are usually found in the active site and are involved in catalysis. For example, flavin and heme cofactors are often involved in redox reactions.
Enzymes that require a cofactor but do not have one bound are called apoenzymes. An apoenzyme together with its cofactor(s) is called a holoenzyme (i.e., the active form). Most cofactors are not covalently attached to an enzyme, but are very tightly bound. However, organic prosthetic groups can be covalently bound (e.g., thiamine pyrophosphate in the enzyme pyruvate dehydrogenase).

Coenzymes

Coenzymes are small molecules that transport chemical groups from one enzyme to another. Some of these chemicals such as riboflavin, thiamine and folic acid are vitamins, this is when these compounds cannot be made in the body and must be acquired from the diet. The chemical groups carried include the hydride ion (H+ + 2e-) carried by NAD or NADP+, the acetyl group carried by coenzyme A, formyl, methenyl or methyl groups carried by folic acid and the methyl group carried by S-adenosylmethionine.

Since coenzymes are chemically changed as a consequence of enzyme action, it is useful to consider coenzymes to be a special class of substrates, or second substrates, which are common to many different enzymes. For example, about 700 enzymes are known to use the coenzyme NADH.

Coenzymes are usually regenerated and their concentrations maintained at a steady level inside the cell: for example, NADPH is regenerated through the pentose phosphate pathway and S-adenosylmethionine by methionine adenosyltransferase.

Thermodynamics

Figure: 5.1.3 Diagram of a catalytic reaction, showing the energy niveau at each stage of the reaction. The substrates usually need a large amount of energy to reach the transition state, which then decays into the end product. The enzyme stabilizes the transition state, reducing the energy needed to form this species and thus reducing the energy required to form products.
As all catalysts, enzymes do not alter the position of the chemical equilibrium of the reaction. Usually, in the presence of an enzyme, the reaction runs in the same direction as it would without the enzyme, just more quickly. However, in the absence of the enzyme, other possible uncatalyzed, "spontaneous" reactions might lead to different products, because in those conditions this different product is formed faster.

Furthermore, enzymes can couple two or more reactions, so that a thermodynamically favorable reaction can be used to "drive" a thermodynamically unfavorable one. For example, the hydrolysis of ATP is often used to drive other chemical reactions.

Enzymes catalyze the forward and backward reactions equally. They do not alter the equilibrium itself, but only the speed at which it is reached. For example, carbonic anhydrase catalyzes its reaction in either direction depending on the concentration of its reactants.

\[
\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3
\]  
(in tissues; high CO2 concentration)

\[
\text{H}_2\text{CO}_3 \rightarrow \text{CO}_2 + \text{H}_2\text{O}
\]  
(in lungs; low CO2 concentration)

Nevertheless, if the equilibrium is greatly displaced in one direction, that is, in a very exergonic reaction, the reaction is effectively irreversible. Under these conditions the enzyme will, in fact, only catalyze the reaction in the thermodynamically allowed direction.

Kinetics

Mechanism for a single substrate enzyme catalyzed reaction. The enzyme (E) binds a substrate (S) and produces a product (P).

Enzyme kinetics is the investigation of how enzymes bind substrates and turn them into products. The rate data used in kinetic analyses are obtained from enzyme assays. In 1913 Leonor Michaelis and Maud Menten proposed a quantitative theory of enzyme kinetics, which is referred to as Michaelis-Menten kinetics. Their work was further developed by G. E. Briggs and J. B. S. Haldane, who derived kinetic equations that are still widely used today.
The major contribution of Michaelis and Menten was to think of enzyme reactions in two stages. In the first, the substrate binds reversibly to the enzyme, forming the enzyme-substrate complex. This is sometimes called the Michaelis-Menten complex in their honor. The enzyme then catalyzes the chemical step in the reaction and releases the product.

![Saturation curve for an enzyme reaction showing the relation between the substrate concentration (S) and rate (v).](image)

**Figure 5.1.4** Saturation curve for an enzyme reaction showing the relation between the substrate concentration (S) and rate (v).

Enzymes can catalyze up to several million reactions per second. For example, the reaction catalysed by orotidine 5′-phosphate decarboxylase will consume half of its substrate in 78 million years if no enzyme is present. However, when the decarboxylase is added, the same process takes just 25 milliseconds. Enzyme rates depend on solution conditions and substrate concentration. Conditions that denature the protein abolish enzyme activity, such as high temperatures, extremes of pH or high salt concentrations, while raising substrate concentration tends to increase activity. To find the maximum speed of an enzymatic reaction, the substrate concentration is increased until a constant rate of product formation is seen. This is shown in the saturation curve, shown on the right. Saturation happens because, as substrate concentration increases, more and more of the free enzyme is converted into the substrate-bound ES form. At the maximum velocity ($V_{\text{max}}$) of the enzyme, all enzyme active sites are saturated with substrate, and the amount of ES complex is the same as the total amount of enzyme.

However, $V_{\text{max}}$ is only one kinetic constant of enzymes. The amount of substrate needed to achieve a given rate of reaction is also important. This is given by the Michaelis-Menten constant ($K_m$), which is the substrate concentration required for an enzyme to reach one-half its maximum velocity. Each enzyme has a
characteristic $K_m$ for a given substrate, and this can show how tight the binding of the substrate is to the enzyme. Another useful constant is $k_{cat}$, which is the number of substrate molecules handled by one active site per second.

The efficiency of an enzyme can be expressed in terms of $k_{cat}/K_m$. This is also called the specificity constant and incorporates the rate constants for all steps in the reaction. Because the specificity constant reflects both affinity and catalytic ability, it is useful for comparing different enzymes against each other, or the same enzyme with different substrates. The theoretical maximum for the specificity constant is called the diffusion limit and is about $10^8$ to $10^9$ (M$^{-1}$ s$^{-1}$). At this point every collision of the enzyme with its substrate will result in catalysis, and the rate of product formation is not limited by the reaction rate but by the diffusion rate. Enzymes with this property are called catalytically perfect or kinetically perfect. Example of such enzymes are triose-phosphate isomerase, carbonic anhydrase, acetylcholinesterase, catalase, fumarase, β-lactamase, and superoxide dismutase.

Some enzymes operate with kinetics which are faster than diffusion rates, which would seem to be impossible. Several mechanisms have been invoked to explain this phenomenon. Some proteins are believed to accelerate catalysis by drawing their substrate in and pre-orienting them by using dipolar electric fields. Other models invoke a quantum-mechanical tunneling explanation, whereby a proton or an electron can tunnel through activation barriers, although for proton tunneling this model remains somewhat controversial. Quantum tunneling for protons has been observed in tryptamine. This suggests that enzyme catalysis may be more accurately characterized as "through the barrier" rather than the traditional model, which requires substrates to go "over" a lowered energy barrier.

**Inhibition**

![Figure 5.1.5](image)

Figure 5.1.5 Competitive inhibitors bind reversibly to the enzyme, preventing the binding of substrate. On the other hand, binding of substrate prevents binding of the inhibitor. Substrate and inhibitor compete for the enzyme.

Enzyme reaction rates can be decreased by various types of enzyme inhibitors.
Reversible inhibitors

Competitive inhibition

In competitive inhibition the inhibitor binds to the substrate binding site (figure right, top, thus preventing substrate from binding (EI complex). Often competitive inhibitors strongly resemble the real substrate of the enzyme. For example, methotrexate is a competitive inhibitor of the enzyme dihydrofolate reductase, which catalyzes the reduction of dihydrofolate to tetrahydrofolate. The similarity between the structures of folic acid and this drug are shown in the figure to the right bottom.

Non-competitive inhibition

Non-competitive inhibitors can bind either to the active site, or to other parts of the enzyme far away from the substrate-binding site. Moreover, non-competitive inhibitors bind to the enzyme-substrate (ES) complex and to the free enzyme. Their binding to this site changes the shape of the enzyme and stops the active site binding substrate(s). Consequently, since there is no direct competition between the substrate and inhibitor for the enzyme, the extent of inhibition depends only on the inhibitor concentration and will not be affected by the substrate concentration.

Irreversible inhibitors

Some enzyme inhibitors react with the enzyme and form a covalent adduct with the protein. The inactivation produced by this type of inhibitor cannot be reversed. A class of these compounds called suicide inhibitors includes efloornithine a drug used to treat the parasitic disease sleeping sickness.

Uses of inhibitors

Inhibitors are often used as drugs, but they can also act as poisons. However, the difference between a drug and a poison is usually only a matter of amount, since most drugs are toxic at some level, as Paracelsus wrote, “In all things there is a poison, and there is nothing without a poison.” Equally, antibiotics and other anti-infective drugs are just specific poisons that can kill a pathogen but not its host.

An example of an inhibitor being used as a drug is aspirin, which inhibits the COX-1 and COX-2 enzymes that produce the inflammation messenger prostaglandin, thus suppressing pain and inflammation. The poison cyanide is an irreversible enzyme inhibitor that combines with the copper and iron in the active site of the enzyme cytochrome c oxidase and blocks cellular respiration.
In many organisms inhibitors may act as part of a feedback mechanism. If an enzyme produces too much of one substance in the organism, that substance may act as an inhibitor for the enzyme that produces it, causing production of the substance to slow down or stop when there is sufficient amount. This is a form of negative feedback.

**Biological function**

Enzymes serve a wide variety of functions inside living organisms. They are indispensable for signal transduction and cell regulation, often via kinases and phosphatases. They also generate movement, with myosin hydrolysing ATP to generate muscle contraction and also moving cargo around the cell as part of the cytoskeleton. Other ATPases in the cell membrane are ion pumps involved in active transport. Enzymes are also involved in more exotic functions, such as luciferase generating light in fireflies.

Viruses can contain enzymes for infecting cells, such as the HIV integrase and reverse transcriptase, or for viral release from cells, like the influenza virus neuraminidase.

An important function of enzymes is in the digestive systems of animals. Enzymes such as amylases and proteases break down large molecules (starch or proteins, respectively) into smaller ones, so they can be absorbed by the intestines. Starch is inabsorbable in the intestine but enzymes hydrolyse the starch chains into smaller molecules such as maltose and eventually glucose, which can then be absorbed. Different enzymes digest different food substances. In ruminants which have a herbivorous diets, bacteria in the gut produce another enzyme, cellulase to break down the cellulose cell walls of plant fiber.

Several enzymes can work together in a specific order, creating metabolic pathways. In a metabolic pathway, one enzyme takes the product of another enzyme as a substrate. After the catalytic reaction, the product is then passed on to another enzyme. Sometimes more than one enzyme can catalyse the same reaction in parallel, this can allow more complex regulation: with for example a low constant activity being provided by one enzyme but an inducible high activity from a second enzyme.

Enzymes determine what steps occur in these pathways. Without enzymes, metabolism would neither progress through the same steps, nor be fast enough to serve the needs of the cell. Indeed, a metabolic pathway such as glycolysis could not exist independently of enzymes. Glucose, for example, can react directly with ATP to become phosphorylated at one or more of its carbons. However, if hexokinase is present, glucose-6-phosphate is the only product, as this reaction will occur most swiftly. Consequently, the network of metabolic pathways within each cell depends on the set of functional enzymes that are present.
Control of activity

There are five main ways that enzyme activity is controlled in the cell.

1. Enzyme production (transcription and translation of enzyme genes) can be enhanced or diminished by a cell in response to changes in the cell's environment. This form of gene regulation is called enzyme induction and inhibition. For example, bacteria may become resistant to antibiotics such as penicillin because enzymes called beta-lactamases are induced that hydrolyse the crucial beta-lactam ring within the penicillin molecule. Another example are enzymes in the liver called cytochrome P450 oxidases, which are important in drug metabolism. Induction or inhibition of these enzymes can cause drug interactions.

2. Enzymes can be compartmentalized, with different metabolic pathways occurring in different cellular compartments. For example, fatty acids are synthesized by one set of enzymes in the cytosol, endoplasmic reticulum and the Golgi apparatus and used by a different set of enzymes as a source of energy in the mitochondrion, through β-oxidation.

3. Enzymes can be regulated by inhibitors and activators. For example, the end product(s) of a metabolic pathway are often inhibitors for one of the first enzymes of the pathway (usually the first irreversible step, called committed step), thus regulating the amount of end product made by the pathways. Such a regulatory mechanism is called a negative feedback mechanism, because the amount of the end product produced is regulated by its own concentration. Negative feedback mechanism can effectively adjust the rate of synthesis of intermediate metabolites according to the demands of the cells. This helps allocate materials and energy economically, and prevents the manufacture of excess end products. Like other homeostatic devices, the control of enzymatic action helps to maintain a stable internal environment in living organisms.

4. Enzymes can be regulated through post-translational modification. This can include phosphorylation, myristoylation and glycosylation. For example, in the response to insulin, the phosphorylation of multiple enzymes, including glycogen synthase, helps control the synthesis or degradation of glycogen and allows the cell to respond to changes in blood sugar. Another example of post-translational modification is the cleavage of the polypeptide chain. Chymotrypsin, a digestive protease, is produced in inactive form as chymotrypsinogen in the pancreas and transported in this form to the stomach where it is activated. This stops the enzyme from digesting the pancreas or other tissues before it enters the gut. This type of inactive precursor to an enzyme is known as a zymogen.

5. Some enzymes may become activated when localized to a different environment (e.g. from a reducing (cytoplasm) to an oxidising (periplasm) environment, high pH to low pH etc). For example, hemagglutinin of the influenza virus undergoes a conformational change once it encounters the acidic environment of the host cell vesicle causing its activation.
Since the tight control of enzyme activity is essential for homeostasis, any malfunction (mutation, overproduction, underproduction or deletion) of a single critical enzyme can lead to a genetic disease. The importance of enzymes is shown by the fact that a lethal illness can be caused by the malfunction of just one type of enzyme out of the thousands of types present in our bodies.

**Naming conventions**

An enzyme's name is often derived from its substrate or the chemical reaction it catalyzes, with the word ending in -ase. Examples are lactase, alcohol dehydrogenase and DNA polymerase. This may result in different enzymes, called isoenzymes, with the same function having the same basic name. Isoenzymes have a different amino acid sequence and might be distinguished by their optimal pH, kinetic properties or immunologically. Furthermore, the normal physiological reaction an enzyme catalyzes may not be the same as under artificial conditions. This can result in the same enzyme being identified with two different names. *E.g.* Glucose isomerase, used industrially to convert glucose into the sweetener fructose, is a xylose isomerase in vivo.

The International Union of Biochemistry and Molecular Biology have developed a nomenclature for enzymes, the EC numbers; each enzyme is described by a sequence of four numbers preceded by "EC". The first number broadly classifies the enzyme based on its mechanism:

The top-level classification is

- EC 1  *Oxidoreductases*: catalyze oxidation/reduction reactions
- EC 2  *Transferases*: transfer a functional group (*e.g.* a methyl or phosphate group)
- EC 3  *Hydrolases*: catalyze the hydrolysis of various bonds
- EC 4  *Lyases*: cleave various bonds by means other than hydrolysis and oxidation
- EC 5  *Isomerases*: catalyze isomerization changes within a single molecule
- EC 6  *Ligases*: join two molecules with covalent bonds

The complete nomenclature can be browsed at [http://www.chem.qmul.ac.uk/iubmb/enzyme/](http://www.chem.qmul.ac.uk/iubmb/enzyme/).
Industrial applications

Enzymes are used in the chemical industry and other industrial applications when extremely specific catalysts are required. However, enzymes in general are limited in the number of reactions they have evolved to catalyse and also by their lack of stability in organic solvents and at high temperatures. Consequently, protein engineering is an active area of research and involves attempts to create new enzymes with novel properties, either through rational design or in vitro evolution.

Assessment questions

5.1. (a) What advantages do enzymes have over conventional catalysts?
(b) What disadvantages are there in using enzymes rather than conventional catalysts?

5.2 (a) Which type of inhibition alters the $K_m$ but not the $V_{max}$?
(b) Which type of inhibition alters the $V_{max}$ but not the $K_m$?
(c) Which type of inhibition eventually reduces the rate of the reaction to zero?

5.3 (a) List three different types of enzyme inhibition
(b) Nerve gases exist that inhibit essential enzyme-catalysed reactions in cells.
(c) What type of enzyme inhibitors have been used as nerve gases? Why is this type of inhibitor used for this purpose?

5.4. (a) When an immobilised enzyme is made, the enzyme molecules are often encased in a gel-like substance. Why must this gel-like substance be permeable to small molecules?
(b) What is the advantage of being able to reuse the enzyme?

5.5 Genetic engineering often involves transferring a gene from a eukaryotic cell, for example a human or a flowering plant, into a bacterium.
(a) Why is the bacterial transcription control sequence added to the gene before it is placed in the bacteria?
(b) Some enzymes are covalently modified after synthesis. What problems would you anticipate if you were producing such an enzyme in a bacterial cell?

5.6 A 70 kg human male contains 15kg of fat stored as triglycerides in his fatty tissue but only 0.225kg of glycogen stored in his liver and muscle. Triglycerides contain about 592 000kJ kg$^{-1}$ while glycogen contains about 3800 kg$^{-1}$.
(a) If all the energy had to be stored as glycogen how much will the man weigh? Triglycerides are insoluble in water while glycogen binds water molecules, forming a shell of water molecules around each molecule of glycogen.
(b) suggest two reasons why humans use triglycerides as long term energy stores rather than glycogen.
(c) Why is glycogen rather than triglycerides used for energy storage in muscle cells?
Stach has a similar energy content per kilogram to glycogen.
(d) Why is starch used as long term energy storage molecule in many plants when animals use triglycerides rather than glycogen, a very similar energy storage molecule?

5.7 (a) What substances are used to make ATP?
(b) What is the name of an enzyme used to make ATP?
(c) ATP can be produced in a cell by two different types of process. What are they?

5.2 Metabolism

Abstract: Metabolism is the biochemical modification of chemical compounds in living organisms and cells. It is through the process of metabolism that organisms process nutrients into the biochemical tools and structures they need to maintain a living state. Metabolism has two distinct divisions: anabolism, in which a cell uses energy and reducing power to construct complex molecules and perform other life functions such as creating cellular structure; and catabolism, in which a cell breaks down complex molecules to yield energy and reducing power. Without energy every molecule would be absolutely still and life would be impossible. Cells are packed with energy in different forms: chemical energy, potential energy and kinetic energy. Every chemical reaction organisms need a constant supply of energy to stay alive. This section deals with how energy supplied by the sun is transferred to every living cell.

Learning Objectives

After studying this unit you should:

1. understand how the energy in light is transferred to the chemical energy of carbohydrate by photosynthesis
2. understand how the energy in carbohydrates is converted to the chemical energy in ATP by anaerobic and aerobic respiration
3. have an appreciation of the relative efficiency of aerobic and anaerobic respiration
4. understand that fats and proteins can be used as respiratory substrates
5. know a range of energy storage molecules
6. appreciate that the Krebs cycle is used as a metabolic hub.
Cell metabolism involves complex sequences of controlled chemical reactions called metabolic pathways, usually a sequence of enzymatic steps. Enzymes are crucial to metabolism because they allow organisms to greatly accelerate slow favorable reactions as well as couple unfavorable reactions to available energy sources. By providing energy to metabolic processes (energy usually in the form of ATP) cells can successfully power reactions that would otherwise never occur.
The term *metabolism* is derived from the Greek Μεταβολισμός – Metabolismos for "change", or "overthrow". The total metabolism are all biochemical processes of an organism. The cell metabolism includes all chemical processes in a cell. The dynamic energy budget theory aims to quantify the metabolic rate of individual organisms.

**Anabolism**

Anabolism is a constructive metabolic process whereby energy is consumed to synthesize or combine simpler substances, such as amino acids, into more complex organic compounds, such as enzymes and proteins.

**Catabolism**

Catabolism is a type of metabolic process occurring in living cells by which complex molecules are broken down to produce energy and reducing power. The primary purpose of catabolism is to regenerate ATP, the primary energy currency of all cells. On balance, catabolic reactions are normally exothermic.

**Carbohydrate catabolism**

*Main article: Carbohydrate catabolism*

Carbohydrate catabolism is the breakdown of carbohydrates into smaller units. The empirical formula for carbohydrates, like that of their monomer counterparts, is \( C_n(H_{2m}O_l) \). Carbohydrates literally undergo combustion to retrieve the large amounts of energy in their bonds.

**Fat catabolism**

Fat catabolism, also known as lipid catabolism, is the process of lipids or phospholipids being broken down by lipases. The opposite of fat catabolism is fat anabolism, involving the storage of energy, and the building of membranes.

**Protein catabolism**

Protein catabolism is the breakdown of proteins into amino acids and simple derivative compounds, for transport into the cell through the plasma membrane and ultimately for the polymerisation into new proteins via the use of ribonucleic acids (RNA) and ribosomes. Amino acids can also be converted into glucose and used for energy, through gluconeogenesis.
UNIT 6: Techniques in Microscopy

Abstract: Microscopy is any technique for producing visible images of structures or details too small to otherwise be seen by the human eye, using a microscope or other magnification tool. It is often used more specifically as a technique of using a microscope. Microscopy has evolved with the development of the microscopes with which to work with. Hence there are three main branches of microscopy; optical, electron and scanning probe microscopy. Optical and electron microscopy involves the diffraction, reflection, or refraction of radiation incident upon the subject of study, and the subsequent collection of this scattered radiation in order to build up an image. This process may be carried out by wide field irradiation of the sample (for example standard light microscopy and transmission electron microscopy) or by scanning of a fine beam over the sample (for example confocal microscopy and scanning electron microscopy).

Learning objectives

After studying this unit you should be able to:

1. define and use, or recognise definitions and applications of key concepts
2. define resolving power of a microscope
3. outline the important steps in the preparations of specimens for examination by means of the light and electron microscopes and state the main principles involved in each stage.
4. explain the similarity between the light microscope and the transmission electron microscope, and outline possible advantages of the electron microscope over the light microscope.
5. outline some of the difficulties in interpreting microscope images.

6.1 The theory

There is a very wide diversity among living organisms, in size, shape, colour, behavior and habitat. Despite this diversity there are also similarities. The fundamental similarity is known as the cell theory. Living cells are generally small, delicate and transparent so that finding out what they are made of, what is inside them and how they work is not easy.

The small size of cells and the lack of contrast between their structural components are two particular problems that have to be overcome. Microscopes of different kinds can be used to produce a magnified image of cells. By far the most common type of microscope is the compound or, simply, light microscope. Lack of contrast between the various structures inside cells make them more or less transparent to light. For light microscopy this problem can be overcome by using dyes which impact colour to subcellar structures. Some of the dyes can be used on living cells without demaging them, but the majority are used on dead cells.
For electron microscopy the colour impacting dyes are replaced by chemicals which interfere with the passage of electrons with which the specimen is illuminated. The end results are similar to those obtained by using dyes: contrast is enhanced allowing previously invisible structures to be seen or photographed. It should be noted that preparing cells for microscopic examination may cause them to change so that the image obtained may not be an accurate guide to the structure of untreated cells.

To understand both the limitation of the light microscopy and the major advantage electron microscopy requires an understanding of a property called the resolution or resolving power of the instruments.

6.2 Preparative Techniques and Procedures

Fixation

Fixation is the rapid killing and preservation of biological material. Correct fixation is essential. The material must be fixed to preserve the three-dimensional arrangements of constituents the tissue and of the contents of the cells. It will also prevent autolysis (digestion of the cell by its own enzymes) and bacterial or fungal attack, and make the tissue resistant to any damage that might be caused by later procedures.

Fixation may be by either physical or chemical means. Physical methods involve immersing the specimen in liquid nitrogen, thereby freezing it so rapidly that ice crystals, which would disrupt and distort the issue, do not form. This method of fixation is often essential if it is necessary to preserve the tissue structure and to prevent any damage occurring to the enzymic components of the cell. Frozen tissue is of course only fixed while frozen, and if brought to room temperature, would rapidly undergo autolysis. Thus, if it is necessary to keep permanent preparations of frozen sections, they must be chemically fixed (after thawing).

Embedding

You may know from your own experiences with the light microscope that even quite small specimens may be too thick to allow enough light through them for details of structure to be seen. To obtain thin enough specimens from them, cells, tissues, organs or whole organisms have to be sliced into sections each about 5-10µm thick.

Sectioning

To produce thin sections (1-20µm for light microscopy, but 50 – 100nm for electron microscopy), an instrument called a microtome is used. All microtomes consist of a specimen holder, a sharp cutting edge and a means of regulating the thickness of the section being cut. The sharp cutting edge may be that of a steel razor for wax-embedded specimens, or of a glass or diamond knife for resin-embedded specimens. Sections of frozen specimens are cut with a microtome mounted in a freezer maintained at -20°C. When the sections have been cut, they
are supported by being mounted either on glass microscope slides for examinations with the light microscope or on a grid of fine copper strands for electron microscopy.

**Staining**

Thin sections of cells or tissues are usually transparent or nearly so. To overcome this lack of contrast it is usually necessary to stain (dye) the sections before they are examined. Many of the stains in common use are dissolved in water, so for these, sections prepared from wax-embedded material must first be treated with a wax solvent and then rehydrated (by passing them through decreasing concentrations of ethanols) before they can be stained—the reverse of the procedure used to embed the material.

For light microscopy, most of the commonly used stains are organic aromatic dyes originally produced for use in the textile industry. Some, like iodine blue, colour all tissues; others colour only particular tissues, parts of tissues or components of cells. Of these more specific stains, there are essentially two groups; basic and acidic. The specificity of these stains depends on the difference in charge of different cell components. A commonly used basic stain in haematoxylin—the colour-imparting (chromogenic) group is cationic (positively charged) and reacts primarily with negatively charged molecules, such as the nucleic acids in the nucleus, to produce a blue colour. Haematoxylin is the most frequently used in combination with a second dye, eosin. In an acidic solution, the chromogenic group of eosin is ionic (negatively charged) and it will react with basic groups in the cell, which are largely in the cytoplasm, staining them red. Haematoxylin and eosin are used together as routine stains for most animal tissues and you often find this staining technique referred to as “h and e.”

**The interpretation of images**

There is no simple way to be sure that the image down a microscope, or in a photograph taken of such an image, corresponds closely to any reality in the living cell. To appreciate this, remember what has been done in the earlier stages of preparation. The specimen will have been immersed in a fixative, dehydrated, impregnated with a mounting medium—all before it is ready for final examination with a light microscope. If an electron microscope is used, the specimen will have been fixed, dehydrated, embedded in resin, sectioned, stained, and then dehydrated in a vacuum before being bombarded with a beam of electrons. These procedures can cause shrinkage, expansion, or other distortions of the specimen or parts of it. Prolonged immersion in ethanol can extract some tissue and cell components more than others. The specimen may be compressed or torn during sectioning. Such alterations in cell structure are called artifacts. If, however, similar images are seen after using a variety of different techniques of fixation, dehydration, embedding, and staining, it reasonable to conclude that we are looking at a real structure. But the problems of interpreting the image produced by any microscope do not end even if artefacts are eliminated.
6.2 Light Microscopy

The light microscope, so called because it employs visible light to detect small objects, is probably the most well-known and well-used research tool in biology. Yet, many students and teachers are unaware of the full range of features that are available in light microscopes. Since the cost of an instrument increases with its quality and versatility, the best instruments are, unfortunately, unavailable to most academic programs. However, even the most inexpensive "student" microscopes can provide spectacular views of nature and can enable students to perform some reasonably sophisticated experiments. A beginner tends to think that the challenge of viewing small objects lies in getting enough magnification. In fact, when it comes to looking at living things the biggest challenges are, in order: 1. Obtaining sufficient contrast, 2. Finding the focal lane, 3. Obtaining good resolution, 4. Recognizing the subject when one sees it. This reading will describe types of optics that are used to obtain contrast, suggestions for finding specimens and focusing on them, and advice on using measurement devices with a light microscope. http://www.ruf.rice.edu/~bioslabs/methods/microscopy/microscopy.html

(Accessed on 11 February 2007)

6.2.1 Types of light microscopes

The bright field microscope is best known to students and is most likely to be found in a classroom. Better equipped classrooms and labs may have dark field and/or phase contrast optics. Differential interference contrast, Nomarski, Hoffman modulation contrast and variations produce considerable depth of resolution and a three dimensional effect. Fluorescence and confocal microscopes are specialized instruments, used for research, clinical, and industrial applications. Other than the compound microscope, a simpler instrument for low magnification use may also be found in the laboratory. The stereo microscope, or dissecting microscope usually has a binocular eyepiece tube, a long working distance, and a range of magnifications typically from 5x to 35 or 40x. Some instruments supply lenses for higher magnifications, but there is no improvement in resolution. Such "false magnification" is rarely worth the expense.

6.2.2 Bright Field Microscopy

With a conventional bright field microscope, light from an incandescent source is aimed toward a lens beneath the stage called the condenser, through the specimen, through an objective lens, and to the eye through a second magnifying lens, the ocular or eyepiece. We see objects in the light path because natural pigmentation or stains absorb light differentially, or because they are thick enough to absorb a significant amount of light despite being colorless. A *Paramecium* should show up fairly well in a bright field microscope, although it will not be easy to see cilia or most organelles. Living bacteria won't show up at all unless the viewer hits the focal plane by luck and distorts the image by using maximum contrast.
A good quality microscope has a built-in illuminator, adjustable condenser with aperture diaphragm (contrast) control, mechanical stage, and binocular eyepiece tube. The condenser is used to focus light on the specimen through an opening in the stage. After passing through the specimen, the light is displayed to the eye with an apparent field that is much larger than the area illuminated. The magnification of the image is simply the objective lens magnification (usually stamped on the lens body) times the ocular magnification.

Students are usually aware of the use of the coarse and fine focus knobs, used to sharpen the image of the specimen. They are frequently unaware of adjustments to the condenser that can affect resolution and contrast. Some condensers are fixed in position, others are focusable, so that the quality of light can be adjusted. Usually the best position for a focusable condenser is as close to the stage as possible. The bright field condenser usually contains an aperture diaphragm, a device that controls the diameter of the light beam coming up through the condenser, so that when the diaphragm is stopped down (nearly closed) the light comes straight up through the center of the condenser lens and contrast is high. When the diaphragm is wide open the image is brighter and contrast is low.

A disadvantage of having to rely solely on an aperture diaphragm for contrast is that beyond an optimum point the more contrast you produce the more you distort the image. With a small, unstained, unpigmented specimen, you are usually past optimum contrast when you begin to see the image.

**Using a bright field microscope**

First, think about what you want to do with the microscope. What is the maximum magnification you will need? Are you looking at a stained specimen? How much contrast/resolution do you require? Next, start setting up for viewing.

**Mount the specimen on the stage**

The cover slip must be up if there is one. High magnification objective lenses can't focus through a thick glass slide; they must be brought close to the specimen, which is why coverslips are so thin. The stage may be equipped with simple clips (less expensive microscopes), or with some type of slide holder. The slide may require manual positioning, or there may be a mechanical stage (preferred) that allows precise positioning without touching the slide.

**Optimize the lighting**

A light source should have a wide dynamic range, to provide high intensity illumination at high magnifications, and lower intensities so that the user can view comfortably at low magnifications. Better microscopes have a built-in illuminator, and the best microscopes have controls over light intensity and shape of the light beam. If your microscope requires an external light source, make sure that the light is aimed toward the middle of the condenser. Adjust illumination so that the field is bright without hurting the eyes.
Adjust the condenser

To adjust and align the microscope, start by reading the manual. If no manual is available, try using these guidelines. If the condenser is focusable, position it with the lens as close to the opening in the stage as you can get it. If the condenser has selectable options, set it to bright field. Start with the aperture diaphragm stopped down (high contrast). You should see the light that comes up through the specimen change brightness as you move the aperture diaphragm lever.

Think about what you are looking for

It is a lot harder to find something when you have no expectations as to its appearance. How big is it? Will it be moving? Is it pigmented or stained, and if so what is its color? Where do you expect to find it on a slide? For example, students typically have a lot of trouble finding stained bacteria because with the unaided eye and at low magnifications the stuff looks like dirt. It helps to know that as smears dry down they usually leave rings so that the edge of a smear usually has the densest concentration of cells.

Focus, locate, and center the specimen

Start with the lowest magnification objective lens, to home in on the specimen and/or the part of the specimen you wish to examine. It is rather easy to find and focus on sections of tissues, especially if they are fixed and stained, as with most prepared slides. However it can be very difficult to locate living, minute specimens such as bacteria or unpigmented protists. A suspension of yeast cells makes a good practice specimen for finding difficult objects.

- Use dark field mode (if available) to find unstained specimens. If not, start with high contrast (aperture diaphragm closed down).
- Start with the specimen out of focus so that the stage and objective must be brought closer together. The first surface to come into focus as you bring stage and objective together is the top of the cover slip. With smears, a cover slip is frequently not used, so the first thing you see is the smear itself.
- If you are having trouble, focus on the edge of the cover slip or an air bubble, or something that you can readily recognize. The top edge of the cover slip comes into focus first, then the bottom, which should be in the same plane as your specimen.
- Once you have found the specimen, adjust contrast and intensity of illumination, and move the slide around until you have a good area for viewing.

Adjust eyepiece separation, focus

With a single ocular, there is nothing to do with the eyepiece except to keep it clean. With a binocular microscope (preferred) you need to adjust the eyepiece separation just like you do a pair of binoculars. Binocular vision is much more sensitive to light and detail than monocular vision, so if you have a binocular microscope, take advantage of it.
One or both of the eyepieces may be a telescoping eyepiece, that is, you can focus it. Since very few people have eyes that are perfectly matched, most of us need to focus one eyepiece to match the other image. Look with the appropriate eye into the fixed eyepiece and focus with the microscope focus knob. Next, look into the adjustable eyepiece (with the other eye of course), and adjust the eyepiece, not the microscope.

**Select an objective lens for viewing**

The lowest power lens is usually 3.5 or 4x, and is used primarily for initially finding specimens. We sometimes call it the scanning lens for that reason. The most frequently used objective lens is the 10x lens, which gives a final magnification of 100x with a 10x ocular lens. For very small protists and for details in prepared slides such as cell organelles or mitotic figures, you will need a higher magnification. Typical high magnification lenses are 40x and 97x or 100x. The latter two magnifications are used exclusively with oil in order to improve resolution.

Move up in magnification by steps. Each time you go to a higher power objective, re-focus and re-center the specimen. Higher magnification lenses must be physically closer to the specimen itself, which poses the risk of jamming the objective into the specimen. Be very cautious when focusing. By the way, good quality sets of lenses are parfocal, that is, when you switch magnifications the specimen remains in focus or close to focused.

Bigger is not always better. All specimens have three dimensions, and unless a specimen is extremely thin you will be unable to focus with a high magnification objective. The higher the magnification, the harder it is to "chase" a moving specimen.

**Adjust illumination for the selected objective lens**

The apparent field of an eyepiece is constant regardless of magnification used. So it follows that when you raise magnification the area of illuminated specimen you see is smaller. Since you are looking at a smaller area, less light reaches the eye, and the image darkens. With a low power objective you may have to cut down on illumination intensity. With a high power you need all the light you can get, especially with less expensive microscopes.

**When to use bright field microscopy**

Bright field microscopy is best suited to viewing stained or naturally pigmented specimens such as stained prepared slides of tissue sections or living photosynthetic organisms. It is useless for living specimens of bacteria, and inferior for non-photosynthetic protists or metazoans, or unstained cell suspensions or tissue sections. Here is a not-so-complete list of specimens that might be observed using bright-field microscopy, and appropriate magnifications (preferred final magnifications are emphasized).
• Prepared slides, stained - bacteria (1000x), thick tissue sections (100x, 400x), thin sections with condensed chromosomes or specially stained organelles (1000x), large protists or metazoans (100x).
• Smears, stained - blood (400x, 1000x), negative stained bacteria (400x, 1000x).
• Living preparations (wet mounts, unstained) - pond water (40x, 100x, 400x), living protists or metazoans (40x, 100x, 400x occasionally), algae and other microscopic plant material (40x, 100x, 400x). Smaller specimens will be difficult to observe without distortion, especially if they have no pigmentation.

**Care of the microscope**

• EVERYTHING on a good quality microscope is unbelievably expensive, so be careful.
• Hold a microscope firmly by the stand, only. Never grab it by the eyepiece holder, for example.
• Hold the plug (not the cable) when unplugging the illuminator.
• Since bulbs are expensive, and have a limited life, turn the illuminator off when you are done.
• Always make sure the stage and lenses are clean before putting away the microscope.
• NEVER use a paper towel, your shirt, or any material other than good quality lens tissue or a cotton swab (must be 100% natural cotton) to clean an optical surface. Be gentle! You may use an appropriate lens cleaner or distilled water to help remove dried material. Organic solvents may separate or damage the lens elements or coatings.
• Cover the instrument with a dust jacket when not in use.
• Focus smoothly; don't try to speed through the focusing process or force anything. For example if you encounter increased resistance when focusing then you've probably reached a limit and you are going in the wrong direction.

### 6.3 The electron microscope


The electron microscope is a type of microscope that uses electrons to create an image of the target. It has much higher magnification or resolving power than a normal light microscope, up to two million times, allowing it to see smaller objects and details.

#### 6.3.1 Transmission Electron Microscope (TEM)

The original form of electron microscopy, Transmission electron microscopy (TEM) involves a high voltage electron beam emitted by a cathode and formed by magnetic lenses. The electron beam that has been partially transmitted through
the very thin (and so semitransparent for electrons) specimen carries information about the inner structure of the specimen. The spatial variation in this information (the "image") is then magnified by a series of magnetic lenses until it is recorded by hitting a fluorescent screen, photographic plate, or light sensitive sensor such as a CCD (charge-coupled device) camera. The image detected by the CCD may be displayed in real time on a monitor or computer.

Resolution of the high-resolution TEM (HRTEM) is limited by spherical aberration and chromatic aberration, but a new generation of aberration correctors has been able to overcome spherical aberration. Software correction of spherical aberration has allowed the production of images with sufficient resolution to show carbon atoms in diamond separated by only 0.89 ångström (89 picometers) and atoms in silicon at 0.78 ångström (78 picometers) at magnifications of 50 million times. The ability to determine the positions of atoms within materials has made the HRTEM an indispensable tool for nano-technologies research and development in many fields, including heterogeneous catalysis and the development of semiconductor devices for electronics and photonics.

6.3.2 Scanning Electron Microscope (SEM)

Unlike the TEM, where electrons are detected by beam transmission, the Scanning Electron Microscope (SEM) produces images by detecting secondary electrons which are emitted from the surface due to excitation by the primary electron beam. In the SEM, the electron beam is rastered across the sample, with detectors building up an image by mapping the detected signals with beam position.

Generally, the TEM resolution is about an order of magnitude better than the SEM resolution, however, because the SEM image relies on surface processes rather than transmission it is able to image bulk samples and has a much greater depth of view, and so can produce images that are a good representation of the 3D structure of the sample.

6.3.3 Reflection Electron Microscope (REM)

In addition there is a Reflection Electron Microscope (REM). Like TEM, this technique involves electron beams incident on a surface, but instead of using the transmission (TEM) or secondary electrons (SEM), the reflected beam is detected. This technique is typically coupled with Reflection High Energy Electron Diffraction and Reflection high-energy loss spectrum (RHELS). Another variation is Spin-Polarized Low-Energy Electron Microscopy (SPLEEM), which is used for looking at the microstructure of magnetic domains.
6.3.4 Sample Preparation

Materials to be viewed under an electron microscope may require processing to produce a suitable sample. The technique required varies depending on the specimen and the analysis required:

Cryofixation - freezing a specimen so rapidly, to liquid nitrogen or even liquid helium temperatures, that the water forms vitreous (non-crystalline) ice. This preserves the specimen in a snapshot of its solution state. An entire field called cryo-electron microscopy has branched from this technique. With the development of cryo-electron microscopy (CEMOVIS), it is now possible to observe virtually any biological specimen close to its native state.

Fixation - preserving the sample to make it more realistic. Glutaraldehyde - for hardening - and osmium tetroxide - which stains lipids black - are used.

Dehydration - replacing water with organic solvents such as ethanol or acetone.

Embedding - infiltration of the tissue with a resin such as araldite or epoxy for sectioning.

Sectioning - produces thin slices of specimen, semitransparent to electrons. These can be cut on an ultramicrotome with a diamond knife to produce very thin slices. Glass knives are also used because they can be made in the lab and are much cheaper.

Staining - uses heavy metals such as lead, uranium or tungsten to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to electrons (weak phase objects). In biology, specimens are usually stained "en bloc" before embedding and also later stained directly after sectioning by brief exposure to aqueous (or alcoholic) solutions of the heavy metal stains.

Freeze-fracture or freeze-etch - a preparation method particularly useful for examining lipid membranes and their incorporated proteins in "face on" view. The fresh tissue or cell suspension is frozen rapidly (cryofixed), then fractured by simply breaking or by using a microtome while maintained at liquid nitrogen temperature. The cold fractured surface (sometimes "etched" by increasing the temperature to about -100°C for several minutes to let some ice sublime) is then shadowed with evaporated platinum or gold at an average angle of 45° in a high vacuum evaporator. A second coat of carbon, evaporated perpendicular to the average surface plane is often performed to improve stability of the replica coating. The specimen is returned to room temperature and pressure, then the extremely fragile "pre-shadowed" metal replica of the fracture surface is released from the underlying biological material by careful chemical digestion with acids, hypochlorite solution or SDS detergent. The still-floating replica is thoroughly washed from residual chemicals, carefully fished up on EM grids, dried then viewed in the TEM.
Ion Beam Milling - thins samples until they are transparent to electrons by firing ions (typically argon) at the surface from an angle and sputtering material from the surface. A subclass of this is Focused ion beam milling, where gallium ions are used to produce an electron transparent membrane in a specific region of the sample, for example through a device within a microprocessor. Ion beam milling may also be used for cross-section polishing prior to SEM analysis of materials that are difficult to prepare using mechanical polishing.

Conductive Coating - An ultrathin coating of electrically-conducting material, deposited either by high vacuum evaporation or by low vacuum sputter coating of the sample. This is done to prevent the accumulation of static electric fields at the specimen due to the electron irradiation required during imaging. Such coatings include gold, gold/palladium, platinum, tungsten, graphite etc. and are especially important for the study of specimens with the scanning electron microscope.

Assessment questions

1. In which of the following statements is the resolving power of a microscope correctly defined or used?
   a) Resolving power means the ability to make out a fine detail.
   b) A microscope with which two images of a single object are obtained is said to have a high resolving power.
   c) The higher the magnification of a microscope, the greater is its resolving power, because at higher magnification, images can be obtained of smaller objects.
   d) The smaller the distance between two objects, the greater the resolving power of the microscope must be in order to continue to see them as two separate objects.

2. In what order are the following processes carried out when preparing a specimen for microscope examination? Embedding; dehydration; rehydration; mounting; fixation; staining; sectioning.
   a) What are the main differences in technique that can be related directly to the use of light or of electron microscopy?

3. In which of the following circumstances would it be more advantageous to use a transmission electron microscope (TEM) than to use a light microscope? Briefly explain why.
   a) Examining an anphid from a pot plant in order to identify it.
   b) Examining a kidney cell to look at its membrane.
   c) Examining a cell to estimate the number of mitochondria it contains.
   d) Examining sections of the skin to look for the pattern of capillaries.
UNIT 7 History of Genetics

Title of Learning Activity: The History of Genetics

Learning activity 1

Summary of the learning activity

After breeding experiments with the garden pea, Mendel came to the conclusion that each of the characters he investigated was under the control of two factors that we now know as alleles. Each plant contains either two similar or different alleles and they are passed on unchanged to the next generation. The alleles segregate during meiosis and come together again at random when gametes unite at fertilization. Subsequent work on inheritance in both plants and animals has confirmed the basic laws discovered by Mendel. The first of the two basic laws, the law of segregation is explained in this unit. One of the consequences of the law of segregation is that the ratio of different types in the progeny of a cross can be predicted. A 3:1 phenotypic ratio from Aa X Aa where A is dominant to a, is obtained. The ratio is 3 dominant trait: 1 recessive trait. A 1:2:1 genotypic ratio from the same cross is obtained. The ratio is 1AA: 2Aa: 1aa. A 1:1 phenotypic and genotypic ratio from Aa x aa (test cross) is obtained. The ratio is 1 dominant trait (Aa): 1 recessive trait (aa). To be able to appreciate the Law of segregation and Mendel’s contribution to the science of genetics you will have to study this module and go through the recommended reading materials and try out the suggested practical work as well as visit the relevant web-based materials and ICTs support systems.

Specific objectives for Learning Activity 2

1. To examine a brief overview of the modern history of genetics
2. Describe Mendel’s breeding experiments and his contribution to the study of genetics
3. Explain Mendel’s results in terms of the particulate theory of inheritance.
4. Describe the morphology, structure and functional significance of chromosomes
5. Explain inheritance involving multiple alleles.

It is necessary for you to read through the following sections before continuing with the learning activity.

7.1 Mendel’s experiments and conclusions

The laws of heredity were worked out by the Austrian monk, Gregor Mendel, and published in 1866. His contribution to genetics is so important that the adjective ‘Mendelian’ is now used to describe the kind of experiments he did and the principles he formulated.
Mendel was not the first to carry out breeding experiments but he was the first to analyse the results numerically and thereby discover certain consistencies that he explained in terms of 'hereditary factors'. He made a careful choice of organism with which to do his breeding experiments. The garden pea, Pisum sativum, satisfied his requirements for the following reasons.

(a) There exist many easily recognizable, distinct forms or varieties.
(b) The flowers are normally self-fertilized but it is possible to remove the stamens from a flower before they are mature and to pollinate the stigma with pollen from a different variety.
(c) The plants resulting from cross-fertilization are fully viable and fertile.
(d) The plants are easy to cultivate.
(e) The one-year life cycle is short enough to be able to collect data from several generations.

Throughout this module the terms 'character' and 'trait' will be used in the restricted sense. Mendel was quite aware that characters often had more than two traits but he deliberately restricted his investigations. He took each character in turn and cross-fertilized plants that showed the two alternative traits. For instance, he took several white flowered plants and removed the stamens from all the young flowers. Then he dusted the stigmas of the white flowers with pollen taken from purple flowers. He also did the reciprocal cross in which he removed stamens from purple flowers and pollinated them with pollen from white flowers. With all the pairs of traits he used, he found that in the generation resulting from the cross fertilizations (called the first filial or F1 generation) the plants all showed the same trait. All the offspring were like one of the parents and were not intermediate in appearance. Furthermore, the appearance of the F1 generation was the same regardless of which plant bore the seeds.

Mendel called the trait that was shown by the F1 generation the dominant trait. The other he called the recessive trait because it seems to recede out of sight in this generation. But, as later experiments demonstrated, it can reappear in subsequent generations. (Diagramme to follow retrieved from http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookgenintro.html on 4 November 2006).
Plants of the FI generation were then allowed to self-fertilize and the progeny were collected. In this second generation (called the second filial or F2 generation) some plants showed the dominant trait and some showed the recessive trait. For each character, Mendel looked at about one thousand F2 plants and counted the numbers having the dominant and the recessive trait. For the seed characters, he was able to count many more individuals because he did not have to grow the seeds in order to find out what the plants would look like.
This is a ratio of 1:2:1. It was from such results that Mendel worked out his first law of inheritance. His most important conclusion was that the units of inheritance remain as separate 'particles' when they are passed from generation to generation. They are not changed or 'diluted' and although their effects may be hidden, the particles themselves are passed on unchanged. This is called the idea of particulate inheritance.

**Particulate inheritance explained in modern terms**

We know today that genes control every characteristic. Genes can exist in alternative forms called alleles that control the alternative traits of a character. The genes are the 'particles' that are transmitted unchanged from one generation to the next. A gene is represented by a symbol say the letter 'A'. Two alleles, being different forms of the gene, are known by alternative forms of the symbol, e.g. A and a. If A represents the allele for the dominant trait and a represents the allele for the recessive trait A and a are called the dominant and recessive alleles respectively. Crosses like those described above, which taken account of only one pair of alleles, are called monohybrid crosses. Each diploid organism carries two alleles of the gene. If the two alleles are the same, the organism is said to be homozygous (Greek *homos* means 'the same'). It is homozygous for the dominant allele, it is called homozygous dominant (represented *AA*). A homozygote is pure breeding because crossed to a similar homozygote, all the offspring will be the same as the parent(s). If the organism carries two different alleles, it is said to be heterozygous (represented *Aa*). Such a plant or animal is called a heterozygote (Greek *heteros* means 'different').

The words homozygous and heterozygous describe an individual's genetic makeup, i.e. its genotype, whereas its outward appearance is called its phenotype (Greek *phainomai* (phenomenon) means 'appear'). Colour, form, physiology and behaviour are all aspects of the phenotype. The homozygous recessive genotype (*aa*) shows the recessive trait while both the homozygous dominant genotype (*AA*) and the heterozygote (*Aa*) show the dominant trait. Note that one does not speak of the 'heterozygous dominant' since there is only one kind of heterozygote.

**The monohybrid backcross or testcross**

In the example above, the grey F 2 progeny are either homozygous dominant (*GG*) or heterozygous (*Gg*). The exact genotype is not apparent from the phenotype. The genotype of the albino can only be *gg*. The way to discover an unknown genotype is by carrying out a further cross known as the testcross. A testcross always involves crossing the unknown genotype to the homozygous recessive. This is the genotype of one of the parents in the standard monohybrid cross and so it is also known as a backcross.
If the unknown genotype is $GG$, all the backcross progeny will inherit a $G$ from that parent and will show the dominant trait, grey. If the unknown genotype is $Gg$, each of its offspring has a $1 \text{ in } 2$ chance of receiving a $G$ and the same chance of receiving $g$. They will all inherit a $9$ from the albino parent. Therefore, on average, the offspring of a heterozygote and a homozygous recessive show a ratio of $1$ heterozygote: $1$ homozygous recessive. If even a single offspring in a testcross shows the recessive phenotype, we know that both of its parents must carry a recessive allele and therefore the unknown genotype must be heterozygous.

**The Physical basis of the Law of Segregation**

By 1916 it was known that genes are located in a linear sequence along Chromosomes. Diploid organisms have two complete sets of chromosomes in each cell and therefore have two copies of each gene. The position of a gene in relation to other genes on the chromosome is known as the gene locus and it is often more convenient to speak of a gene locus when it is of no consequence which particular allele occupies it. In haploid cells, each gene locus is represented only once, since there is only one set of chromosomes. Haploid organisms cannot be described as being either homozygous or heterozygous and there is no question of dominance or recessivity. This makes the genetics of haploid organisms (e.g. bacteria) quite straightforward. In polyploids, each set of chromosomes and therefore each locus is represented three, four or more times, making the study of heredity in these organisms correspondingly more complicated. This module is concerned with heredity in diploids where each chromosome is represented twice in each cell, The two copies have the same sequence of genes although the chromosomes are not necessarily identical because the alleles at each locus may be different, The two chromosomes carrying the same gene loci are called homologues; they are said to be homologous. Thus in the heterozygote, $Aa$, one homologue carries allele $A$ and the other carries allele $a$ at the same gene locus. One of the homologues in each cell is a copy of the original chromosome that was donated to the zygote by the male parent and is known as the paternal homologue. It contains a copy of one of the chromosomes that came from the female gamete and is known as the maternal homologue. Both homologues are fully functional in the cell regardless of the sex of the organism.

In order to explain his results, Mendel assumed that the 'factors' determining each trait were present in pairs in the parent plants but segregated at gamete formation such that each gamete received only one of the factors. Later observations on the behaviour of chromosomes in meiosis provided a physical parallel and indeed were taken as evidence that the segregating factors were situated on the chromosomes. It is assumed that you understand the principles of gamete formation by meiosis.
No attempt is made here to describe the events of meiosis but it might be helpful to clarify the terms 'chromosome' and 'chromatid'. Achromosome is one long molecule of DNA arranged on a framework of protein molecules. In the inter-phase prior to the first division of the nucleus, the DNA replicates (makes an exact copy of itself) and each replica also becomes associated with protein. The two replicas shorten in preparation for cell division and become visible under the microscope, lying alongside each other and joined at a constriction (represented by a circle in the diagrams) called the centromere. Each replica is called a chromatid. The chromatids are identical to the original chromosome that replicated. To draw an analogy, chromatids are like twin sisters. When they are considered in relation to each other, they are called chromatids (‘sisters’), and when each is being considered alone, it is called a chromosome (‘a girl’). A chromatid is a chromosome, just as a sister is a girl. The chromatids pull apart at anaphase and at telophase, the name chromosome is used again. It is only the name and not the structure that has changed. The analogy with twin sisters is a pertinent one because chromosome replicas are often called sister chromatids.

Key words
Allele; Backcross; Character; Chromosome; Genotype; Phenotype; Pure breeding; Out breeding; Dominant; Recessive; True breeding; Test cross; Reciprocal cross; F1 generation; F2 generation; Trait; Monohybrid cross; Monohybrid inheritance; Dihybrid inheritance; Multiple alleles; Sex determination and sex linkage; Genes and chromosomes; Population genetics; Genetic variation in populations.

Summary assignment
(1) Refer back to the objectives of section 1 and for each objective, write brief notes to show your understanding of these objectives.
(2) State the five principles established by Mendel.
(3) State Mendel’s first and second Laws
(4) Draw annotated diagrams to explain Mendel’s Laws.

Self assessment Questions
1. In pea plants, what types of offspring would you expect, in terms of genotypes and phenotypes, from crossing:
   (a) a heterozygote and a tall homozygote
   (b) a tall homozygote and a short individual?
   Explain your answer fully by means of diagrams.
2. If the factor for blue eyes is recessive to the factor for brown eyes explain, by means of diagrams, how brown-eyed parents can have a blue-eyed child.
3. In rabbits, black fur is dependent on the dominant factor B and brown fur depends on its recessive factor b. Normal length fur is dependent on the dominant factor R and short fur depends on its recessive factor r.

(a) Two rabbits with black fur are mated. Most of their offspring are black but some individuals with brown fur are produced that must be the genotypes of the parents for coat colour. Explain your answers.

(b) The two rabbits in (a) both have long fur. All their offspring had long fur. Comment on the genotypes of the parents for fur length alone.

7.2 Patterns of Inheritance

Learning Activity 2: Patterns of Inheritance

With the understanding of chromosome behaviour during cell division and the haploid nature of gametes, renewed interest was shown in the way characteristics were passed between parent and offspring. As a result, a large number of animals and plants were studied and the patterns of inheritance observed and explained in terms of chromosome behaviour. The chromosomal basis of inheritance became well established. This Unit focuses on transmission genetics that concerns the process by which genes are inherited. It is assumed that the learner has knowledge of the structure of DNA and its role in protein synthesis and also of the principles of mitosis and meiosis. These topics are normally taught before transmission genetics in advanced level courses in biology.

It is also important to be aware that of all biological topics, genetics is often regarded as one of the most difficult and some of the reasons for this have been identified (Twesigye, 1991, 1994, 2006).

For example, there is a special vocabulary associated with the subject and it also requires logical thinking, the use of symbols and some mathematics. For this to improve, learning material must be presented in an orderly sequence. I hope that this module will show that genetics is not difficult if learners are active participants in the learning process.

This module provides a logical progression through transmission genetics. The self-assessment, questions integrated in the module are graded difficulty and allow practice in dealing with new concepts introduced in each section. The questions are instructive and the data presented to demonstrate genetics principles have been taken from published research studies. Most examples and case studies have been chosen from animals and plants available locally in Africa.

Detailed answers have been provided for the self-assessment questions so that you can learn from your mistakes. The problems at the end of each module will help you to assess your ability to recognize the concepts required for their solution and also to simulate conditions that you will encounter in an examination.
To aid your understanding, these are questions within the module that are intended to make you pause and consolidate what you have covered. Do not skip these questions, as they are an essential part of the learning process in as far as promoting understanding is concerned.

Most of the problems included in this module have been based on original research studies by investigators who helped to establish the body of knowledge in genetics. You are free to select the problems you attempt but if you have covered A-level biology you should be able to solve most of the problems at the beginning of each module. The questions at the end of each section should be attempted after studying the relevant sections.

The test questions at the end of the module are mixed, both in ideas and in the level of difficulty that ranges from moderately easy to difficult. But even these can be solved after studying this module. Develop a study habit of solving problems and take an active role in the learning process. You will then enjoy genetics.

Objectives

After completing this section you should be able to do the following:

(a) Describe the relationship between characters, chromosomes and genes using the terms: loci, alleles, homologous pair, homozygous and heterozygous.
(b) Use a bead model to explain inheritance of characteristics.
(c) Explain the 3:1 monohybrid ratio in terms of probability.
(d) Explain Mendelian inheritance in terms of chromosomes, genes and meiosis.
(e) Predict situations where Mendel’s ratios will not be obtained.
(f) Explain the use of testcrosses in genetic studies.
(g) Describe breeding experiments using Drosophila.
(h) Explain why 1:2:1 phenotype ratios occur.
(i) Solve genetics problems involving sex and autosexual linkage.
(j) Explain the significance of crossing-over in the inheritance of linked characteristics.
(k) Explain inheritance involving multiple alleles.
(l) Describe the inheritance of haemophilia and blood groups.
(m) Define the terms 'super gene' and pleiotropism.
(n) Explain inheritance involving multiple genes and interaction.
7.3 Learning Activity 3: Practical: “Breeding” with beads

Materials
3 containers, 100 red beads, 100 yellow beads, plastic 500 cm³ beakers

Procedure
You will need to work in pairs for this investigation.

(a) Place 100 red beads in one container to represent the gametes of the tall parents. Place 100 yellow beads in the second container to represent the gametes of the dwarf parents.
(b) Withdraw a bead from each container. Each bead withdrawn represents a gamete containing a single gene of a pair. Place the beads together. This represents the process of fertilization, by which the paired nature of genes in the offspring is re-established. The pair of beads represents the genotype of an individual of the F₁ generation.
(c) If you continued to withdraw pairs of beads as above, what would be the genotype of all the F₁ individuals?
(d) To stimulate the gametes of F₁ generation, place 100 beads (50 red and 50 yellow) in each container. One container represents the female gametes produced by the F₁-generation; the other represents the male gametes produced by F₁ generation.
(e) Shake the containers vigorously for 30s.
(f) To produce the F₂ generation, withdraw a bead from each container (with your eyes closed) and place them together. Your partner should note the combination of genes obtained. This represents the genotype of an F₂ individual.
(g) Discard the pair of beads into the spare container.
(h) Repeat steps (f) and (g) until all the beads have been paired and their combination noted.
(i) Calculate the ratio of phenotypes of the F₂ individuals.
(j) Record the ratios obtained by other groups in your set and calculate a set average ratio.

Discussion of results
1. Why were the beads shaken in step (e) and withdrawn with closed eyes?
2. Present your results in the most appropriate way.
3. How does your ratio and the set average ratio compare with Mendel’s prediction? Explain any differences.
4. Explain how this practical acts as a model for inheritance and breeding in peas.
7.4 Learning Activity 4: Breeding with flies

Learning Objectives
1. Be able to identify by name all the equipment and supplies used.
2. Recognize mutants and wild-type flies.
3. Be able to sex Drosophila.
4. Be able to prepare media for culturing flies.

Assignments
1) Prepare four bottles of media.
2) State Stock Cultures:
   + = Wild type
   vg = vestigial wings
   e = ebony-coloured body
   w = white eyes
   L = lobed
   B = bar eyes
   cn = cinnabar
3) Practice sexing flies and identifying mutants

Mendel’s Law of Independent Assortment

Assignments
1. Review the section on "Patterns of Inheritance".
2. Check your stock cultures.
3. Bring your laboratory book and any relevant reading material.
4. Bring along your calculator!

Learning Objectives
1. Learn to sex flies rapidly and accurately.
2. Secure virgin females.
3. Design and carry out a successful experiment.
4. Collect accurate data from F1 and F2 phenotype flies.
5. Apply the Chi-Square Test to experimental data.
Principles of Probability

Learning Objectives

1. Correlate the laws of probability to genetic principles governing independent events occurring simultaneously or separately.
2. Learn to expand the binomial \( (a+b)^2 \) in order to calculate the probabilities for certain combinations of events.
3. Learn to relate the importance of probability principles to the field of genetic counselling.

Learning Objectives

1. Learn the values of and the uses for this statistical tool in genetics.
2. Learn to apply the Chi-Square Test to any experimental data and to accurately interpret results (see probability \( X^2 \) – table ??).

The reader could access the following web-page where the following three paragraphs were discussed by the authors as retrieved from the following website on 4 November 2006 (http://www.tccc.cc.nc.us/wtrotter/biology_110_assign_02.htm).

\[ \chi^2 = \frac{\text{sum of (observed frequency - expected frequency)}^2}{\text{expected frequency}} \]

Very large Chi-square results mean that the frequencies that you observed are far from what was expected (Mendel's Ratios)

Extremely small Chi-square results mean that the observed frequencies fit the ratios very closely, even more than chance would allow. (Remember: How the chromosomes line up in metaphase of meiosis, and fertilization are both random events.)

Put your Chi-square data into the table of Chi-square value at the correct number of degrees of freedom, and if the result is a probability between .80 and .10, then that is just about right.
7.4 learning to handle Drosophila and recognise their characteristics

7.4.1 Materials

- Culture of Drosophila for Genetics Experiments
- Etheriser and ether to anaesthetize flies for examination, white paper or tile, paintbrush for sorting flies, emergency etheriser, binocular microscope or magnifying glass.

NB Ether fumes are highly inflammable, and can cause dizziness and nausea.

DO NOT (a) work close to a naked flame in the laboratory
(b) leave containers of ether open,
(c) breath in the fumes

7.4.2 Procedure

(a) Remove the top of the etheriser.
(b) Add a few drops of ether to the cotton wool around the funnel
(c) Replace the top of the ether bottle and etheriser as soon as possible
(d) Tap the flies to the bottom of the culture bottle
(e) Invert the culture bottle over the funnel of the etheriser. Hold it in contact with the funnel and tap the flies into the etheriser

NB DO NOT leave flies in either too long or they will die and will no longer be usable for further crosses. Over-etherised flies may be recognized by arched wings and folded legs.

(f) Once all the flies have stopped moving (this might be after a few seconds) tip onto a white paper surface and examine with a binocular microscope or magnifying glass. The flies can be moved using the paintbrush.

(g) The flies should remain anaesthetised for about ten minutes. However, if any start moving before you have finished your investigation, you may use an emergency etheriser (see figure).

(h) Investigate the eye colour, wing length and body colour of the flies and sort them into males and females.

You can now perform your own breeding experiments with an animal that has been used extensively in genetics experiments—the fruit fly Drosophila. It is easy to keep in the laboratory, requiring simple food and little maintenance while producing many offspring in a short period of time. Familiarize yourself with the following information before you begin the practical.

(a) Life-cycle of Drosophila
(b) Distinguishing male and female flies
(c) Characteristics of fruit flies
Question: Suggest reasons why Drosophila is used so often as an experimental organism in genetics: You should think of at least five reasons.

7.4.3 Setting up a Drosophila cross

Although you will only be carrying out a single cross here, it is possible to look on this investigation as an amalgam of three crosses in one. The three crosses you can consider from this single investigation are:

A. monohybrid cross: normal body x ebony body
B. monohybrid cross: vestigial wings x normal wings
C. dihybrid cross: normal body x ebony body vestigial wings x normal wings

NB: To obtain meaningful results, female flies used in crosses must be virgins. Mating may occur within a day of emergence of the adults. Therefore, females must be removed immediately they emerge from the pupa and kept separate from males until ready for use.

7.4.4 Materials

Culture of pure-breeding vestigial-winged, normal-bodied flies, and a culture of pure-breeding normal-winged, ebony-bodied flies (males and females separated), ether and etheriser, white paper or tile, paintbrush, binocular microscope or magnifying glass, freshly prepared culture bottles, labels, incubator at 25°C.

7.4.5 Procedure

(a) Take a culture bottle and label as follows: Date; your initials; Female phenotype x male phenotype (pure breeding); NB. The female parent is always written first.
(b) Anaesthetize the flies and spread on to a white surface. Place 10 vestigial-winged females (grey-bodied) and 15 ebony-bodied males (long-winged) in the tube. Some groups should set up the reciprocal cross of 10 ebony-bodied females and 15 vestigial winged males.
(c) Leave the culture bottles on their sides until the flies recover, to prevent them falling into the medium.
(d) Place the bottles in an incubator at 25°C.
(e) One week later, remove the parent flies
(f) After a further 3-4 days, anaesthetize the F1 flies, examine carefully and record numbers and types of flies.
(g) Take a fresh bottle of culture medium. Transfer 10 female and 15 male flies from the F1 generation into this tube. Label appropriately.
(h) Repeat steps (c) and (d) as for the first cross.
(i) Collate results from the whole class. Compare your individual results with the class result. Comment upon any differences.
7.4.6 Discussion of results

1) Using appropriate symbols, discuss the inheritance of body colour and wing shape in Drosophila using your results.

2) Carry out statistical analysis of your results to find out to what extent the departure from the expected ratio is due to random variation alone or whether some other factor is having an effect.

7.5.1 Learning Activity 5: Statistical Analysis in Genetics

In this section, a simple statistical test will be used to analyse results of genetic experiments. Biologists often use statistics as a tool and may not have to go into the details underlying mathematical theory behind the tests. However, this information may be obtained from references at the end of this section.

Example:

Suppose cut-leaved tomato plants were self pollinated and the offspring comprised 160 cut-leaved plants and 40 potato leaved plants. The actual results for the ratio of cut-leaved potato leaved plants is not exactly 3:1 but rather 4:1. As a matter of fact the predicted ratios of the outcomes of genetic crosses are rarely identical with those observed experimentally. The expected ratios are based on a consideration of the probability of certain types of egg and sperm combining. Generally speaking, the larger the sample, the smaller the deviation between observed and expected results should be.

It is important to know when the deviation between the observed results and the expected results is too great to be due to chance alone. Such a large deviation usually indicates that the original hypothesis must be rejected or modified.

STEP 1

(a) First, it is necessary to calculate the expected results. The total number of offspring = 200. If the character differences showed an exact 3:1 ratio, we would expect 150 cut-leaved tomato plants and 50 potato leaved tomato plants. The 3:1 ratio represents 3 part: 1 part = 4 parts in total. Total number of individuals = 200

Number of individuals in 1 part = \( \frac{200}{4} = 50 \)

Number of individuals in 3 parts = \( 50 \times 3 = 150 \)

(b) It is now necessary to determine how the observed results differ from the expected results. The difference between the observed and expected values for each type of plant, usually termed the deviation, may be calculated by subtraction.
(c) Next, the values for the deviation of each type of plant must be incorporated in a single value. At the same time, it is necessary to make allowances for the size of the sample. Each deviation is squared, and each squared deviation is then divided by the expected number of its type. The resulting values are then added together to give a single value called the chi-square or $x^2$.

Table 1 Calculation of $x^2$ values

<table>
<thead>
<tr>
<th>Cut-leaved plants</th>
<th>Potato leaved plants</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed number</td>
<td>160</td>
<td>40</td>
</tr>
<tr>
<td>Expected number</td>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td>Deviation</td>
<td>10</td>
<td>-10</td>
</tr>
</tbody>
</table>

$$d^2 = 100 - 100$$

$$e = \frac{0.66 + 2}{2}$$

$$X = \frac{\sum d^2}{e} = 0.66 + 2 = 2.66$$

The value for $x^2$ obtained above represents a measure of the deviation of the observed results from the expected results.

**STEP 2**

In this example, only 2 types of plant are being considered – cut-leaved and potato leaved. When considering examples involving more than 2 types of plant e.g. hairy plants with cut leaves, hairy plants with potato leaves, hairless plants with cut leaves and hairless plants with potato leaves, larger values of $x^2$ are permitted before the results are considered invalid. To allow for this, a factor known as degrees of freedom (N) is introduced. The number of degrees of freedom is one less than the number of types of classes. Thus, in results involving a 3:1 ratio, there is one degree of freedom. A 1:2:1 ratio involves 2 degrees of freedom.
A 9:3:3:1 ratio involves 3 degrees of freedom. In the example of cut-leaved and potato leaved tomato plants under consideration there is one degree of freedom, i.e. \( N = 1 \).

**STEP 3**

Using the value of \( x^2 \) (2.66) and the value for degree of freedom (1), it is now possible to determine the probability of the observed deviation being due to chance by using a chi-square table.

**TABLE 2 Chi-Square Table**

<table>
<thead>
<tr>
<th>( N )</th>
<th>0.99</th>
<th>0.98</th>
<th>0.95</th>
<th>0.90</th>
<th>0.80</th>
<th>0.70</th>
<th>0.60</th>
<th>0.50</th>
<th>0.40</th>
<th>0.30</th>
<th>0.20</th>
<th>0.10</th>
<th>0.05</th>
<th>0.02</th>
<th>0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.000</td>
<td>0.001</td>
<td>0.004</td>
<td>0.016</td>
<td>0.064</td>
<td>0.148</td>
<td>0.455</td>
<td>1.074</td>
<td>1.642</td>
<td>2.706</td>
<td>3.84</td>
<td>5.412</td>
<td>6.635</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>0.40</td>
<td>0.103</td>
<td>0.211</td>
<td>0.446</td>
<td>0.713</td>
<td>1.386</td>
<td>2.408</td>
<td>3.129</td>
<td>4.605</td>
<td>5.991</td>
<td>7.824</td>
<td>9.210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.115</td>
<td>0.185</td>
<td>0.521</td>
<td>0.713</td>
<td>1.386</td>
<td>2.408</td>
<td>3.129</td>
<td>4.605</td>
<td>5.991</td>
<td>7.824</td>
<td>9.210</td>
<td>11.341</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.297</td>
<td>0.429</td>
<td>0.711</td>
<td>1.064</td>
<td>1.649</td>
<td>2.195</td>
<td>3.357</td>
<td>4.878</td>
<td>5.989</td>
<td>7.779</td>
<td>9.488</td>
<td>11.668</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.554</td>
<td>0.752</td>
<td>1.145</td>
<td>1.610</td>
<td>2.343</td>
<td>3.000</td>
<td>4.351</td>
<td>6.064</td>
<td>7.289</td>
<td>9.236</td>
<td>11.070</td>
<td>13.388</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The horizontal row of figures adjacent to the calculated value for \( N \) is selected. In this case, it will be the top line of the table. Within that row, the value, which most nearly resembles the value calculated for \( x^2 \), is then found. Finally, it is necessary to move up vertically from this value to read off the probability. In the tomato plant example being considered, \( P = 0.1-0.2 \).

**STEP 4**

The probability of getting large deviations by chance is low. Therefore, high values for \( x^2 \), and the correspondingly low values for \( P \), indicate that the hypothesis is unlikely to be true. It is necessary to decide what is the lowest value of \( P \) that is acceptably consistent with the hypothesis being true. For most scientific work, this value is accepted to be 0.05. In some cases, a much lower value is used, for instance, in trials on the effects of new drugs. In the tomato plant example, the value obtained for \( P \) was 0.1 – 0.2. This is greater than 0.05. Therefore, the deviation is accepted as being due to chance. Thus the hypothesis has been confirmed.
7.6.1 Learning activity 6: Population Genetics

Genes in populations and the Hardy-Weinberg Equation

In previous work we have seen how Mendelian genetic analysis can be used to calculate the expected proportions of different genotypes and phenotypes in the progeny of two parents whose genotypes are known. The same principles can be applied when the progeny does not come from just one pair of parents but from a large number of interbreeding individuals. The method used to calculate the expected proportions of different genotypes in a population was published in 1908 by the British mathematician G. H. Hardy and, independently, by the German physician W. Weinberg. It is now known as the Hardy-Weinberg equation.

If you cross a single pair of parents, both heterozygous Aa, the proportion of genotypes in their progeny can be calculated using a Punnett square (http://www.athro.com/evo/gen/punnett.html). In a monohybrid cross between heterozygotes, the two types of gametes occur in equal proportions.

Background Reading on ‘Population Genetics’

The following reading material were retrieved from Wikipedia, the free encyclopaedia, located at http://en.wikipedia.org/wiki/Population_genetics and accessed on the 16th September 2006.

Population genetics is the study of the allele frequency distribution and change under the influence of the four evolutionary forces: natural selection, genetic drift, mutation, and gene flow. It also takes account of population subdivision and population structure in space. As such, it attempts to explain such phenomena as adaptation and speciation. Population genetics was a vital ingredient in the modern evolutionary synthesis, its primary founders were Sewall Wright, J. B. S. Haldane and R.A. Fisher, who also laid the foundations for the related discipline of quantitative genetics.

Scope and theoretical considerations

Perhaps the most significant "formal" achievement of the modern evolutionary synthesis has been the framework of mathematical population genetics. Indeed some authors (Beatty 1986) would argue that it defines the core of the modern synthesis.

Lewontin (1974) outlined the theoretical task for population genetics. He imagined two spaces: a "genotypic space" and a "phenotypic space". The challenge of a complete theory of population genetics is to provide a set of laws that predictably map a population of genotypes ($G_1$) to a phenotype space ($P_1$), where selection takes place, and another set of laws that map the resulting population ($P_2$) back to genotype space ($G_2$) where Mendelian genetics can predict the next generation of genotypes, thus completing the cycle. Even leaving aside for the moment the non-Mendelian aspects revealed by molecular genetics, this is clearly a gargantuan task. Visualizing this transformation:
$G_1 \xrightarrow{T_1} P_1 \xrightarrow{T_2} P_2 \xrightarrow{T_3} G_2 \xrightarrow{T_4} G'_1 \xrightarrow{\ldots}$

(adapted from Lewontin 1974, p. 12).

$T_1$ represents the genetic and epigenetic laws, the aspects of functional biology, or development, that transform a genotype into phenotype. We will refer to this as the "genotype-phenotype map". $T_2$ is the transformation due to natural selection, $T_3$ are epigenetic relations that predict genotypes based on the selected phenotypes and finally $T_4$ the rules of Mendelian genetics.

In practice, there are two bodies of evolutionary theory that exist in parallel, traditional population genetics operating in the genotype space and the biometric theory used in plant and animal breeding, operating in phenotype space. The missing part is the mapping between the genotype and phenotype space. This leads to a "sleight of hand" (as Lewontin terms it) whereby variables in the equations of one domain, are considered parameters or *constants*, where, in a full-treatment they would be transformed themselves by the evolutionary process and are in reality *functions* of the state variables in the other domain. The "sleight of hand" is assuming that we know this mapping. Proceeding as if we do understand it is enough to analyze many cases of interest. For example, if the phenotype is almost one-to-one with genotype (sickle-cell disease) or the time-scale is sufficiently short, the "constants" can be treated as such; however, there are many situations where it is inaccurate.

**Population geneticists**

The three founders of population genetics were the Britons R.A. Fisher and J.B.S. Haldane and the American Sewall Wright. Fisher and Wright had some fundamental disagreements and a controversy about the relative roles of selection and drift continued for much of the century between the Americans and the British. The Frenchman Gustave Malécot was also important early in the development of the discipline. John Maynard Smith was Haldane's pupil, whilst W.D. Hamilton was heavily influenced by the writings of Fisher. The American George R. Price worked with both Hamilton and Maynard Smith. On the American side, Richard Lewontin and the Japanese Motoo Kimura were heavily influenced by Wright. Luigi Luca Cavalli-Sforza is a Stanford-based population geneticist particularly interested in human population genetics.

**References**


UNIT 8 Chromosomal theory and Application of Genetics in Biotechnology

Summary

The following section was retrieved from Wikipedia, the free encyclopaedia located at http://en.wikipedia.org/wiki/Allele on the 6th of September 2006.

In genetics, an allele is any one of a number of viable DNA codings occupying a given locus (position) on a chromosome. Usually alleles are DNA sequences that code for a gene, but sometimes the term is used to refer to a non-gene sequence. An individual's genotype for that gene is the set of alleles it happens to possess. In a diploid organism, one that has two copies of each chromosome, two alleles make up the individual's genotype.

An example is the gene for blossom colour in many species of flower — a single gene controls the colour of the petals, but there may be several different versions (or alleles) of the gene. One version might result in red petals, while another might result in white petals. The resulting colour of an individual flower will depend on which two alleles it possesses for the gene and how the two interact.

8.1 Introduction

Organisms that are diploid such as humans have paired homologous chromosomes in their somatic cells, and these contain two copies of each gene. An organism in which the two copies of the gene are identical — that is, have the same allele — is said to be homozygous for that gene. An organism that has two different alleles of the gene is said to be heterozygous. Phenotypes (the expressed characteristics) associated with a certain allele can sometimes be dominant or recessive, but often they are neither. A dominant phenotype will be expressed when at least one allele of its associated type is present, whereas a recessive phenotype will only be expressed when both alleles are of its associated type.

However, there are exceptions to the way heterozygotes express themselves in the phenotype. One exception is incomplete dominance (sometimes called blending inheritance) when alleles blend their traits in the phenotype. An example of this would be seen if, when crossing Antirrhinums — flowers with incompletely dominant "red" and "white" alleles for petal colour — the resulting offspring had pink petals. Another exception is co-dominance, where both alleles are active and both traits are expressed at the same time; for example, both red and white petals in the same bloom or red and white flowers on the same plant. Co-dominance is also apparent in human blood types. A person with one "A" blood type allele and one "B" blood type allele would have a blood type of "AB". A wild type allele is an allele which is considered to be "normal" for the organism in question, as opposed to a mutant allele which is usually a relatively new modifica-
tion. (Note that with the advent of neutral genetic markers, the term 'allele' is now often used to refer to DNA sequence variants in non-functional, or junk DNA. For example, allele frequency tables are often presented for genetic markers, such as the DYS markers.)

**Equations**

There are two simple equations for the frequency of two alleles of a given gene (see Hardy-Weinberg principle). The second is a consequence of the first, obtained by squaring both sides and applying the binomial theorem to the left-hand side:

Equation 1: \( p + q = 1 \),

Equation 2: \( p^2 + 2pq + q^2 = 1 \) where \( p \) is the frequency of one allele and \( q \) is the frequency of the other allele. \( p^2 \) is the population fraction that is homozygous for the \( p \) allele, \( 2pq \) is the frequency of heterozygotes and \( q^2 \) is the population fraction that is homozygous for the \( q \) allele. Natural selection can act on \( p \) and \( q \) in Equation 1, and obviously affect the frequency of alleles seen in Equation 2. It should be noted that the second equation can be derived from the first (or vice versa) since \( p^2 + 2pq + q^2 = 1 \) implies \((p + q)^2 = 1 \) and \( p \) and \( q \) are positive numbers.

**Genomics**

The following paragraphs were taken from Wikipedia the free encyclopaedia located at http://en.wikipedia.org/wiki/Genomics and accessed on the 16th of September 2006.

Genomics is the study of an organism's genome and the use of the genes. It deals with the systematic use of genome information, associated with other data, to provide answers in biology, medicine, and industry. Genomics has the potential of offering new therapeutic methods for the treatment of some diseases, as well as new diagnostic methods. For example, for women newly diagnosed with breast cancer, a genomic test called Onco\textit{type} DX assesses a patient’s individual risk of breast cancer recurrence and likely benefit from chemotherapy, which can help doctors make more informed and more personalized treatment decisions. Other applications are in the food and agriculture sectors.

The major tools and methods related to genomics are bioinformatics, genetic analysis, measurement of gene expression, and determination of gene function.

**History**

Genomics appeared in the 1980s and took off in the 1990s with the initiation of genome projects for several species. The related field of genetics is the study of genes and their role in inheritance. The first genome to be sequenced in its entirety was that of bacteriophage \( \Phi \)-X174; (5,368 bp) in 1980. The first free-living organism to be sequenced was that of \textit{Haemophilus influenzae} (1.8Mb) in 1995,
and since then genomes are being sequenced at a rapid pace. A rough draft of the human genome was completed by the Human Genome Project in early 2001 amid much fanfare.

The growth of the "omics"

The original use of the suffix "ome" (from the Greek for 'all', 'every' or 'complete') was "genome", which refers to the complete genetic makeup of an organism. Because of the success of large-scale quantitative biology projects such as genome sequencing, the suffix "ome" has been extended to a host of other contexts. For instance, proteome refers to the totality of proteins (expressed genes that are translated) in an organism, tissue type or cell. Proteomics is now well-established as a term for studying the proteome.

Comparative genomics

Main article: Comparative genomics

Comparison of genomes has resulted in some surprising biological discoveries. If a particular DNA sequence or pattern is present among many members of a clade, that sequence is said to have been conserved among the species. Evolutionary conservation of a DNA sequence may imply that it confers a relative selective advantage to the organisms that possess it. Conservation also suggests that sequence has functional significance. It may be a protein coding sequence or regulatory region. Experimental investigation of some of these sequences has shown that some are transcribed into small RNA molecules, although the functions of these RNAs were not immediately apparent.

The identification of similar sequences (including many genes) in two distantly related organisms, but not in other members of one of the clades, has led to the theory that these sequences were acquired by horizontal gene transfer. This phenomenon is most prominent in bacteria, although it also seems that genes were transferred from Archaea to Eubacteria. It has also been noticed that bacterial genes exist in eukaryotic nuclear genomes and that these genes generally encode mitochondrial and plastid proteins, giving support to the endosymbiotic theory of the origin of these organelles. This theory holds that the mitochondria and chloroplast organelles found in many animal and plant genomes were originally free-living bacteria that were absorbed by an ancestral eukaryote, and that subsequently became an integral part of the eukaryotic cell.

Genetic similarity

It is often stated that a particular organism shares X percent of its DNA with humans. This number indicates the percentage of base pairs that are identical between the two species. Here is a list of genetic similarity to humans, with sources, where known.
These numbers were found in various secondary sources, and were likely derived from differing methodologies (such as DNA-DNA hybridization or sequence alignment) which might give different results applied to the same pair of species. Therefore, they should be regarded only as rough approximations.

<table>
<thead>
<tr>
<th>Species</th>
<th>Similarity</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>99.9%</td>
<td>quoted by U.S.A. President Clinton, Jan 2000, State of the Union address; also Human Genome Project</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>100%</td>
<td>identical twins</td>
</tr>
<tr>
<td></td>
<td>98.4%</td>
<td>sources: Americans for Medical Progress; Jon Entine in the San Francisco Examiner</td>
</tr>
<tr>
<td>Bonobo</td>
<td>98.7%</td>
<td>Richard Mural of Celera Genomics, quoted on MSNBC</td>
</tr>
<tr>
<td>Mouse</td>
<td>98%</td>
<td>source: Americans for Medical Progress</td>
</tr>
<tr>
<td>Dog</td>
<td>85%</td>
<td>comparing all protein coding sequences, NHGRI</td>
</tr>
<tr>
<td>C. elegans</td>
<td>95%</td>
<td>Jon Entine in the San Francisco Examiner</td>
</tr>
<tr>
<td>Banana</td>
<td>50%</td>
<td>Jon Entine in the San Francisco Examiner</td>
</tr>
<tr>
<td>Daffodil</td>
<td>35%</td>
<td>Steven Rose in The Guardian 22 January 2004</td>
</tr>
</tbody>
</table>

Sources and external links

- PLoS Primer: Comparative Genomics
- The Paleobiotics Lab
- Genomics Online Database - http://wit.integratedgenomics.com/GOLD
- Joint Genome Institute
- The Institute for Genomic Research - http://www.tigr.org
- The Sanger Institute - http://www.sanger.ac.uk
• http://www.dbbm.fiocruz.br/genomics/genomics.html
• http://www.dbbm.fiocruz.br.genome/tcruzi/tcruzi.html
• Translational Genomics Research Institute
• Australian Centre for Plant Functional Genomics
• International Genomics Consortium
• Global Musa Genomics Consortium
• Functional Annotation of the Mouse database
• International Journal of Medical Sciences
• Dengueinfo.org - Dengue Virus full genome database
• http://www.dengueinfo.org/
• The National Office of Public Health Genomics

8.2 The teaching of science in the classroom

Learning activity 7:

As a science teacher one has to be acquainted with different strategies and approaches towards the teaching of biology and life sciences in the classroom. The most prominent approach currently being followed in science teaching is the application of the science processes in classroom practices. This would apply to theoretical and practical work.

The learning activity to follow will demonstrate to you how one could use DNA as theme to teach the topic according to the so-called scientific method or science process skills. The entire section was taken from Wikipedia, the free encyclopaedia on the 21 of September 2006 as located at the website http://en.wikipedia.org/wiki/Scientific_method.

Scientific method

The scientific method is a body of techniques for investigating phenomena and acquiring new knowledge, as well as for correcting and integrating previous knowledge. It is based on gathering observable, empirical, measurable evidence, subject to the principles of reasoning.

Although procedures vary from one field of inquiry to another, there are identifiable features that distinguish scientific inquiry from other methods of developing knowledge. Scientific researchers propose specific hypotheses as explanations of natural phenomena, and design experimental studies that test these predictions for accuracy. These steps are repeated in order to make increasingly dependable predictions of future results. Theories that encompass wider domains of inquiry serve to bind more specific hypotheses together in a coherent structure. This in turn aids in the formation of new hypotheses, as well as in placing groups of specific hypotheses into a broader context of understanding.
Among other facets shared by the various fields of inquiry is the conviction that the process must be objective so that the scientist does not bias the interpretation of the results or change the results outright. Another basic expectation is that of making complete documentation of data and methodology available for careful scrutiny by other scientists and researchers, thereby allowing other researchers the opportunity to verify results by attempted reproduction of them. This also allows statistical measures of the reliability of the results to be established. The scientific method also may involve attempts, if possible and appropriate, to achieve control over the factors involved in the area of inquiry, which may in turn be manipulated to test new hypotheses in order to gain further knowledge.

There are multiple ways of outlining the basic method shared by all of the fields of scientific inquiry. The following examples are typical classifications of the most important components of the method on which there is very wide agreement in the scientific community and among philosophers of science, each of which are subject only to marginal disagreements about a few very specific aspects.

The scientific method involves the following basic facets:

- **Observation.** A constant feature of scientific inquiry.
- **Measurement.** Applies to the ability to use different techniques to establish distance, space, weight (mass), speed, colour, etc.
- **Description.** Information must be reliable, i.e., replicable (repeatable) as well as valid (relevant to the inquiry).
- **Prediction.** Information must be valid for observations past, present, and future of given phenomena, i.e., purported "one shot" phenomena do not give rise to the capability to predict, nor to the ability to repeat an experiment.
- **Control.** Actively and fairly sampling the range of possible occurrences, whenever possible and proper, as opposed to the passive acceptance of opportunistic data, is the best way to control or counterbalance the risk of empirical bias.
- **Falsifiability,** or the elimination of plausible alternatives. This is a gradual process that requires repeated experiments by multiple researchers who must be able to replicate results in order to corroborate them. This requirement, one of the most frequently contended, leads to the following: All hypotheses and theories are in principle subject to disproof. Thus, there is a point at which there might be a consensus about a particular hypothesis or theory, yet it must in principle remain tentative. As a body of knowledge grows and a particular hypothesis or theory repeatedly brings predictable results, confidence in the hypothesis or theory increases.
- **Causal explanation.** Many scientists and theorists on scientific method argue that concepts of causality are not obligatory to science, but are in fact well defined only under particular, admittedly widespread conditions. Under these conditions the following requirements are generally regarded as important to scientific understanding:
• Identification of causes. Identification of the causes of a particular phenomenon to the best achievable extent.

• **Co-variation of events.** The hypothesized causes must correlate with observed effects.

• **Time-order relationship.** The hypothesized causes must precede the observed effects in time.

The following is a more specific and technical description of the hypothesis/testing method, discussion of which follows below. This general set of elements and organization of procedures will in general tend to be more characteristic of natural sciences and experimental psychology than of disciplines such as sociology and a number of other fields commonly categorized as social sciences. Among the latter, methods of verification and testing of hypotheses may involve less stringent mathematical and statistical interpretations of these elements within the respective disciplines. Nonetheless the cycle of hypothesis, verification and formulation of new hypotheses will tend to resemble the basic cycle described below.

**The essential elements of a scientific method are iterations, and orderings of the following:**

- Characterizations (Quantifications, observations, and measurements)
- Hypotheses (theoretical, hypothetical explanations of observations and measurements)
- Predictions (reasoning including logical deduction from hypotheses and theories)
- Experiments (tests of all of the above)

The element of observation includes both unconditioned observations (prior to any theory) as well as the observation of the experiment and its results. The element of experimental design must consider the elements of hypothesis development, prediction, and the effects and limits of observation because all of these elements are typically necessary for a valid experiment. Imre Lakatos and Thomas Kuhn had done extensive work on the "theory laden" character of observation. Kuhn (1961) maintained that the scientist generally has a theory in mind before designing and undertaking experiments so as to make empirical observations, and that the "route from theory to measurement can almost never be travelled backward". This perspective implies that the way in which theory is tested is dictated by the nature of the theory itself that led Kuhn (1961, p. 166) to argue "once it has been adopted by a profession ... no theory is recognized to be testable by any quantitative tests that it has not already passed". Each element of scientific method is subject to peer review for possible mistakes. These activities do not describe all that scientists do (see below) but apply mostly to experimental sciences (e.g., physics, chemistry). The elements above are often taught in the educational system.
The scientific method is not a recipe: it requires intelligence, imagination, and creativity. Further, it is an ongoing cycle, constantly developing more useful, accurate and comprehensive models and methods. For example, when Einstein developed the Special and General Theories of Relativity, he did not in any way refute or discount Newton's *Principia*. On the contrary, if one reduces out the astronomically large, the vanishingly small, and the extremely fast from Einstein's theories — all phenomena that Newton could not have observed — one is left with Newton's equations. Einstein's theories are expansions and refinements of Newton's theories, and the observations that increase our confidence in them also increase our confidence in Newton's approximations to them.

A linearized, pragmatical scheme of the four above points is sometimes offered as a guideline for proceeding:

1. Define the question
2. Gather information and resources
3. Form hypothesis
4. Perform experiment and collect data
5. Analyse data
6. Interpret data and draw conclusions that serve as a starting point for new hypotheses
7. Publish results

The iterative cycle inherent in this step-by-step methodology goes from point 3 to 6 back to 3 again.

While this schema outlines a typical hypothesis/testing method, it should also be noted that a number of philosophers, historians and sociologists of science (perhaps most notably Paul Feyerabend) claim that such descriptions of scientific method have little relation to the ways science is actually practiced.

**DNA example**

Each element of scientific method is illustrated below by an example from the discovery of the structure of DNA:

- *DNA/characterizations*
- *DNA/hypotheses*
- *DNA/predictions*
- *DNA/experiments*

The examples are continued in "Evaluations and iterations" with DNA/iterations.
The scientific method depends upon increasingly more sophisticated characterizations of subjects of the investigation. (The subjects can also be called lists of unsolved problems or the unknowns.) For example, Benjamin Franklin correctly characterized St. Elmo's fire as electrical in nature, but it has taken a long series of experiments and theory to establish this. While seeking the pertinent properties of the subjects, this careful thought may also entail some definitions and observations; the observations often demand careful measurements and/or counting.

The systematic, careful collection of measurements or counts of relevant quantities is often the critical difference between pseudo-sciences, such as alchemy, and a science, such as chemistry. Scientific measurements taken are usually tabulated, graphed, or mapped, and statistical manipulations, such as correlation and regression, performed on them. The measurements might be made in a controlled setting, such as a laboratory, or made on more or less inaccessible or non-manipulatable objects such as stars or human populations. The measurements often require specialized scientific instruments such as thermometers, spectrometers, or voltmeters, and the progress of a scientific field is usually intimately tied to their invention and development.

Measurements (one of the basic science process skills) demand the use of operational definitions of relevant quantities. That is, a scientific quantity is described or defined by how it is measured, as opposed to some more vague, inexact or "idealized" definition. For example, electrical current, measured in amperes, may be operationally defined in terms of the mass of silver deposited in a certain time on an electrode in an electrochemical device that is described in some detail. The operational definition of a thing often relies on comparisons with standards: the operational definition of "mass" ultimately relies on the use of a value such as a certain kilogram of platinum-iridium kept in a laboratory in France.

The scientific definition of a term sometimes differs substantially from their natural language usage. For example, mass and weight overlap in meaning in common discourse, but have distinct meanings in physics. Units of measure characterize scientific quantities. These can then be described in terms of conventional physical units when communicating the work.

Measurements in scientific work are also usually accompanied by estimates of their uncertainty. The uncertainty is often estimated by making repeated measurements of the desired quantity. Uncertainties may also be calculated by consideration of the uncertainties of the individual underlying quantities that are used. Counts of things, such as the number of people in a nation at a particular time, may also have an uncertainty due to limitations of the method used. Counts may only represent a sample of desired quantities, with an uncertainty that depends upon the sampling method used and the number of samples taken.
New theories sometimes arise upon realizing that certain terms had not previously been sufficiently clearly defined. For example, Albert Einstein's first paper on relativity begins by defining simultaneity and the means for determining length. These ideas were skipped over by Isaac Newton with, "I do not define time, space, place and motion, as being well known to all." Einstein's paper then demonstrates that they (viz., absolute time and length independent of motion) were approximations. Francis Crick cautions us that when characterizing a subject, however, it can be premature to define something when it remains poorly understood. In Crick's study of consciousness, he actually found it easier to study awareness in the visual system, rather than to study Free Will, for example. His cautionary example was the gene; the gene was much more poorly understood before Watson and Crick's pioneering discovery of the structure of DNA; it would have been counterproductive to spend much time on the definition of the gene, before them.

DNA/characterizations

The history of the discovery of the structure of DNA is a classic example of the elements of scientific method: in 1950 it was known that genetic inheritance had a mathematical description, starting with the studies of Gregor Mendel. But the mechanism of the gene was unclear. Researchers in Bragg's laboratory at Cambridge University made X-ray diffraction pictures of various molecules, starting with crystals of salt, and proceeding to more complicated substances. Using clues which were painstakingly assembled over the course of decades, beginning with its chemical composition, it was determined that it should be possible to characterize the physical structure of DNA, and the X-ray images would be the vehicle.

Hypothesis development

A hypothesis is a suggested explanation of a phenomenon, or alternately a reasoned proposal suggesting a possible correlation between or among a set of phenomena. Normally hypotheses have the form of a mathematical model. Sometimes, but not always, they can also be formulated as existential statements, stating that some particular instance of the phenomenon being studied has some characteristic and causal explanations, which have the general form of universal statements, stating that every instance of the phenomenon has a particular characteristic. Scientists are free to use whatever resources they have — their own creativity, ideas from other fields, induction, Bayesian inference, and so on — to imagine possible explanations for a phenomenon under study. Charles Sanders Peirce, borrowing a page from Aristotle (Prior Analytics, 2.25) described the incipient stages of inquiry, instigated by the "irritation of doubt" to venture a plausible guess, as abductive reasoning. The history of science is filled with stories of scientists claiming a "flash of inspiration", or a hunch, which then motivated them to look for evidence to support or refute their idea. Michael Polanyi made
such creativity the centrepiece of his discussion of methodology. Karl Popper, following others, notably Charles Peirce, has argued that a hypothesis must be falsifiable, and that a proposition or theory cannot be called scientific if it does not admit the possibility of being shown false. It must at least in principle be possible to make an observation that would show the proposition to be false, even if that observation had not yet been made.

William Glen observes that

the success of a hypothesis, or its service to science, lies not simply in its perceived "truth", or power to displace, subsume or reduce a predecessor idea, but perhaps more in its ability to stimulate the research that will illuminate … bald suppositions and areas of vagueness.

In general scientists tend to look for theories that are "elegant" or "beautiful". In contrast to the usual English use of these terms, they here refer to a theory in accordance with the known facts, which is nevertheless relatively simple and easy to handle. If a model is mathematically too complicated, it is hard to deduce any prediction. Different individuals and cultures perceive 'simplicity' differently.

DNA/hypotheses

Linus Pauling proposed that DNA was a triple helix. Francis Crick and James Watson learned of Pauling's hypothesis, understood from existing data that Pauling was wrong and realized that Pauling would soon realize his mistake. So the race was on to figure out the correct structure. Except that Pauling did not realize at the time that he was in a race!

Predictions from the hypotheses

Any useful hypothesis will enable predictions, by reasoning including deductive reasoning. It might predict the outcome of an experiment in a laboratory setting or the observation of a phenomenon in nature. The prediction can also be statistical and only talk about probabilities. It is essential that the outcome be currently unknown. Only in this case does the eventuation increase the probability that the hypothesis be true. If the outcome is already known, it's called a consequence and should have already been considered while formulating the hypothesis.

If the predictions are not accessible by observation or experience, the hypothesis is not yet useful for the method, and must wait for others who might come afterward, and perhaps rekindle its line of reasoning. For example, a new technology or theory might make the necessary experiments feasible.
When Watson and Crick hypothesized that DNA was a double helix, Francis Crick predicted that an X-ray diffraction image of DNA would show an X-shape. Also in their first paper they predicted that the double helix structure that they discovered would prove important in biology writing "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material".

Once predictions are made, they can be tested by experiments. If test results contradict predictions, then the hypotheses are called into question and explanations may be sought. Sometimes experiments are conducted incorrectly and are at fault. If the results confirm the predictions, then the hypotheses are considered likely to be correct but might still be wrong and are subject to further testing. Depending on the predictions, the experiments can have different shapes. It could be a classical experiment in a laboratory setting, a double-blind study or an archaeological excavation. Even taking a plane from New York to Paris is an experiment that tests the aerodynamical hypotheses used for constructing the plane. Scientists assume an attitude of openness and accountability on the part of those conducting an experiment. Detailed record keeping is essential, to aid in recording and reporting on the experimental results, and providing evidence of the effectiveness and integrity of the procedure. They will also assist in reproducing the experimental results. This tradition can be seen in the work of Hipparchus (190 BCE - 120 BCE), when determining a value for the precession of the Earth over 2100 years ago, and 1000 years before Al-Batani.

Before proposing their model Watson and Crick had previously seen x-ray diffraction images by Rosalind Franklin, Maurice Wilkins, and Raymond Gosling. However, they later reported that Franklin initially rebuffed their suggestion that DNA might be a double helix. Franklin had immediately spotted flaws in the initial hypotheses about the structure of DNA by Watson and Crick. The X-shape in X-ray images helped confirm the helical structure of DNA.
Evaluation and iteration

Testing and improvement

The scientific process is iterative. At any stage it is possible that some consideration will lead the scientist to repeat an earlier part of the process. Failure to develop an interesting hypothesis may lead a scientist to re-define the subject they are considering. Failure of a hypothesis to produce interesting and testable predictions may lead to reconsideration of the hypothesis or of the definition of the subject. Failure of the experiment to produce interesting results may lead the scientist to reconsidering the experimental method, the hypothesis or the definition of the subject. Other scientists may start their own research and enter the process at any stage. They might adopt the characterization and formulate their own hypothesis, or they might adopt the hypothesis and deduce their own predictions. The experiment is often not done by the person who predicted the outcome. The characterization often is based on experiments done by someone else. Published results of experiments can also serve as a hypothesis predicting their own reproducibility.

DNA/iterations

After considerable fruitless experimentation, being discouraged by their superior from continuing, and numerous false starts, Watson and Crick were able to infer the essential structure of DNA by concrete modelling of the physical shapes of the nucleotides which comprise it. They were guided by the bond lengths that had been deduced by Linus Pauling and the X-ray diffraction images of Rosalind Franklin.

Confirmation

Science is a social enterprise, and scientific work tends to be accepted by the community when it has been confirmed. Crucially, experimental and theoretical results must be reproduced by others within the science community. Researchers have given their lives for this vision; Georg Wilhelm Richmann was killed by lightning (1753) when attempting to replicate the 1752 kite experiment of Benjamin Franklin.[7]
Models of scientific inquiry

Classical model

The classical model of scientific inquiry derives from Aristotle, who distinguished the forms of approximate and exact reasoning, set out the threefold scheme of abductive, deductive, and inductive inference, and also treated the compound forms such as reasoning by analogy.

Pragmatic model

Charles Peirce considered scientific inquiry to be a species of the genus inquiry, which he defined as any means of fixing belief, that is, any means of arriving at a settled opinion on a matter in question. He observed that inquiry in general begins with a state of uncertainty and moves toward a state of certainty, sufficient at least to terminate the inquiry for the time being. He graded the prevalent forms of inquiry according to their evident success in achieving their common objective, scoring scientific inquiry at the high end of this scale. At the low end he placed what he called the method of tenacity, a die-hard attempt to deny uncertainty and fixate on a favoured belief. Next in line he placed the method of authority, a determined attempt to conform to a chosen source of ready-made beliefs. After that he placed what might be called the method of congruity, also called the a priori, the dilettante, or the what is agreeable to reason method. Peirce observed the fact of human nature that almost everybody uses almost all of these methods at one time or another, and that even scientists, being human, use the method of authority far more than they like to admit. But what recommends the specifically scientific method of inquiry above all others is the fact that it is deliberately designed to arrive at the ultimately most secure beliefs, upon which the most successful actions can be based.

Philosophy and sociology of science

While the philosophy of science has limited direct impact on day-to-day scientific practice, it plays a vital role in justifying and defending the scientific approach. Philosophy of science looks at the underpinning logic of the scientific method, at what separates science from non-science, and the ethic that is implicit in science.

We find ourselves in a world that is not directly understandable. We find that we sometimes disagree with others as to the facts of the things we see in the world around us, and we find that there are things in the world that are at odds with our present understanding. The scientific method attempts to provide a way in which we can reach agreement and understanding. A "perfect" scientific method might work in such a way that rational application of the method would always result in agreement and understanding; a perfect method would arguably be algorithmic, and so not leave any room for rational agents to disagree. As with all philosophical
topics, the search has been neither straightforward nor simple. Logical Positivist, empiricist, falsificationist, and other theories have claimed to give a definitive account of the logic of science, but each has in turn been criticised.

Thomas Samuel Kuhn examined the history of science in his *The Structure of Scientific Revolutions*, and found that the actual method used by scientists differed dramatically from the then-espoused method. Paul Feyerabend similarly examined the history of science, and was led to deny that science is genuinely a methodological process. In his book *Against Method* he argues that scientific progress is not the result of applying any particular method. In essence, he says that "anything goes", by which he meant that for any specific methodology or norm of science, successful science has been done in violation of it. The strong programme seeks to apply sociological methods to all of science.
Notes and references


Further reading

1. Bacon, Francis. *Novum Organum* (The New Organon), 1620. Bacon's work described many of the accepted principles, underscoring the importance of Theory, empirical results, data gathering, experiment, and independent corroboration.


Formative evaluation

The purpose of this assignment is to determine whether you would be able to design a lesson plan (learning task) using the science process skills. Now do the following:

Design a lesson plan (learning task) with a clear focus on the development of learners’ research capacities and skills when conducting a simple investigation. The activities (outcomes or objectives) of the learning tasks (lesson plan) should be to allow your learners to conduct any simple investigation where they have to apply the science process skills in a laboratory investigation. Your lesson plan (learning task(s)) should contain elements of the following:

(a) Tasks and activities focusing the learners’ attention on given observations.
(b) Applying measurement skills through the use of measuring instruments. These could include rulers, tapes, scales, balances, rubrics, checklists, worksheets, etc.
(c) Developing a hypothesis.
(d) Setting up an experiment to test the hypothesis.
(e) Conducting an investigation to test the hypothesis.
(f) Synthesising your data.
(g) Assessing your findings

Total score: 50
8.3. Pedagogical Learning Activity 8: Planning and Preparing a Lesson Plan

Of all biological topics, genetics is often regarded as one of the most difficult and some of the reasons for this can be identified (Twesigye, 1991, 1994, 2006). There is a special vocabulary associated with the subject and it also requires logical thinking, the use of symbols and some mathematics.

With the above observations in mind, make a one hour lesson plan involving demonstration of use of symbols, logical thinking and calculation of phenotypic and genotypic ratios. Your lesson plan should include the following components:

(a) materials
(b) procedure
(c) class activity
(d) discussion of results by the class
(e) evaluate the success of the lesson and give feedback to learners.

Pedagogical Comment

To help you understand the material covered in this module, we have made every effort to provide pedagogical guidance by way of learning aids. These aids have been designed and presented in a concise and clear way so as to make them understandable to you.

Study Objectives - Each unit begins with a brief introduction and study objectives. These objectives preview the unit and highlight the most important concepts.

Study Outline - The unit topics are provided as an outline that consist of words or phrases that clearly define what the various sections of the unit contain.

Summary - Each unit summary recaps the study objectives at the beginning of each unit to help you determine if you have gained an understanding of the material presented in the study objectives.

Exercises and Problems - At the end of each unit are numerous problems to test your understanding of the material. This section includes critical thinking questions designed to help you develop an ability to evaluate and solve problems.
List of relevant useful links:


XV. Synthesis Of The Module

The completion of this module taught you how to demonstrate practical knowledge and understanding of cell biology and genetics. You should now be able to apply the knowledge and understanding gained through this course to your courses in your major area of study. The ability to integrate knowledge from this course to your other areas of study underscores our major educational goal of educating the whole person, with balanced emphasis placed on the development of mind, spirit, and body. Desired outcomes from this course include skills in problem solving, data analysis, knowledge synthesis, evaluation and the design of scientific investigations. Practical work in cell biology and genetics offers you an opportunity for applying biological know in every day life. Experiments are designed to demonstrate and complement the information being taught in the lecture portion.

The science of genetics includes the rules of inheritance in cells, individuals, and populations and the molecular mechanisms by which genes control the growth, development, and appearance of an organism. The understanding of genetics also plays a crucial role in promoting understanding others areas in biology and other life sciences because genes not only control cellular processes, they also determine the course of evolution. Genetic concepts provide the framework for the study of modern biology.

This module provided you with a balanced treatment of the major areas in cell biology and genetics in order to prepare you for advanced course in biology and other life sciences and also for your role as a biology teacher. Genetics is commonly divided into three areas: classical, molecular and population, although molecular advancements have blurred these distinctions. We feel that a historical approach provides a sound introduction to the field and a thorough grounding in Mendelian genetics is necessary for an understanding of molecular and population genetics.

A comprehensive glossary has been provided to help maintain continuity in the case of changing the order in which activities have been arranged. We have made it clear that an understanding of genetics is crucial to advancements in medicine, agriculture, and many industries. In this module we have stressed the importance of critical thinking, an approach that puts emphasize understanding over memorization, problem solving over passive reading, and active participation in the learning process. The module included a reference section with a reading list of review articles, magazines and journals that contain non-technical summaries as well as material at the cutting edge of cell biology and genetics. The World Wide Web also should provide valuable learning resources. You will begin with a search engine such ‘Google’ or ‘Altavista’ and type in a key word or a phrase such as “genetic variation”.
In this module we have presented genetics as the study of inheritance in all of its forms. It was our purpose in this module to introduce and describe the processes and patterns of inheritance and at the same time present a historical overview of the advancements that have contributed to our current understanding of genetics. In this regard the module starts with events beginning with the discovery of cells and microscopes that is also the beginning of the modern history of genetics. The modern history of genetics has been divided into four periods: before 1860, 1860–1900, 1900–1944, and 1944 to the present. The period from 1944 to the present is the era of molecular genetics, beginning with the demonstration that DNA is the genetic material and culminating with our current explosion of knowledge due to recombinant DNA technology also known as genetic engineering.

In this module we have attempted to present a balanced view of the various topics that make up cell biology and genetics by bringing together this information from a historical perspective. Historically, geneticists have worked in three different areas, each with its own particular problems, terminology, tools, and organisms. These areas are classical genetics, molecular genetics, and evolutionary genetics. In classical genetics, we are concerned with the chromosomal theory of inheritance that is the concept that genes are located in a linear fashion on chromosomes and that the relative positions of genes can be determined by their frequency in offspring.

Molecular genetics is the study of the genetic material: its structure, replication, and expression, as well as the information revolution emanating from the discoveries of recombination and expression, as well as the information revolution coming from the genetic engineering, including the Human Genome Project. Evolutionary genetics is the study of the mechanisms of evolutionary changes in populations. To day these three areas have become less clearly defined because of advances made in molecular genetics. Information generated from molecular genetics allows us to understand better the structure and functioning of chromosomes on one hand and the mechanism of natural selection on the other.
XV. Summative Evaluation

Summative Evaluation will include tests, a project, course assignments, oral presentation, and final examination at the end of the module. Learners will submit answers to instructors by email, speed delivery and any other suitable mode of delivering information. Instructors will use email to give feedback to learners. As learners you are required to give feedback to instructors on the suitability of this module. Your objective evaluation of this module will help us to improve its quality and this in turn will lead to improvement in the overall quality of learning through this Open Distance Learning mode.

Samples of Assessment Questions

1. True breeding, purple-stem tomato plants were crossed green-stem plants and all the F1 plants had purple stems. When the later were crossed the F2 generation comprised 3,087 purple-stem and 1,096 green-stem plants. How would you explain these results? Does your hypothesis fit the observed results satisfactorily?

2. When a true breeding purple-stem, potato leaf strain was crossed with a true breeding green-stem, cut-leaf strain, the F2 generation was made up of the following:

<table>
<thead>
<tr>
<th></th>
<th>purple-cut</th>
<th>purple potato</th>
<th>green-cut</th>
<th>green potato</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>250</td>
<td>88</td>
<td>79</td>
<td>31</td>
</tr>
</tbody>
</table>

Explain these results using diagrams to illustrate genotypes and phenotypes of each step. Test your explanation using the x² method.

3. In guinea pigs, rough coat R is dominant over smooth coat r and black coat is dominant over white coat b. If smooth black animal is back-crossed a smooth white animal, the offspring comprise 6 smooth black animals and 7 smooth white animals. What are the genotypes of the parents? Diagram the crosses showing all pertinent genotypes, phenotypes and gametes. Test the validity of your explanation by the x² method.

4. Suppose the F₂ offspring produced as a result of a Drosophila cross were as follows:

<table>
<thead>
<tr>
<th></th>
<th>410</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type wings and eyes</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>110</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type wings and scarlet eyes</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>truncated wings and wild type eye</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>truncated wings and scarlet eyes</td>
<td></td>
</tr>
</tbody>
</table>

Would these results fit the typical Mendelian pattern expected?
5. In Andalusian fowls, the heterozygous condition of the alleles for black plumage (B) and white (b) is blue. Blue fowls were crossed and the following offspring were produced:

<table>
<thead>
<tr>
<th>black</th>
<th>blue</th>
<th>white</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>85</td>
<td>37</td>
</tr>
</tbody>
</table>

Explain these results by means of diagrams. Determine the values for \( \chi^2 \). Can the deviation observed be accepted as sampling error?

**Answers to pre-assessment questions**

1. b
2. c
3. c
4. d
5. b
6. b
7. b
8. d
9. d
10. d
11. d
12. b
13. c
14. b
15. b
16. d
17. c
18. c
19. b
20. a
Answers to assessment questions on the section on enzymes:

Answers to assessment questions

5.1. (a) Very specific
(b) Can be denatured

5.2. (a) Competitive inhibition
(b) Non-competitive inhibition.
(c) Irreversible inhibition.

5.3. (a) Competitive, non-competitive, irreversible.
(b) Irreversible (ensure death)
(c) Competitive (binds to active site)
(d) Non-cometive (binds away from the active site)

5.4. (a) So the substrate can reach the active site.
(b) Cheaper.

5.5. (a) So that the bacteria can transcribe the gene.
(b) Bacteria lack the enzymes that make the modifications: protein may not function.

5.6. (a) $2337 \text{ kg of glycogen} + (70-15) = 2392 \text{ kg}.$
(b) Less mass, does not diffuse in tissue fluid as insoluble in water.
(c) Quickly converted to glucose for use as energy
(d) Mass does not matter to plant, as not mobile; starch less soluble in water than glycogen.

5.7 (a) ADP, Pi
(b) ATPase
(c) Enzyme-catalysed reactions, using energy stored in a H+ concentration gradient.

Answers to questions on Cell Microscopy

1. Resolving power is used correctly in (a) and (d); (b) is wrong-if this happened, it would be very inconvenient; in (c), magnification and resolving power are confused.

2. (a) The correct order is: fixation; dehydration; embedding; sectioning; rehydration; staining; mounting.
(b) The main differences in embedding, staining and specimen mounting:
For embedding, the medium is related to thinness of the section- much thinner for the electron microscope than for the light microscope. Resin is normally used for electron microscopy and parafin wax for light microscopy. For staining: for the light microscope, stains are dyes that absorb different wavelengths of transmitted light; but for the electron microscope, stains are metal compounds that impart differences in electron density when taken up by cell components.
For specimen mounting: for the light microscope, the stained specimen is mounted in a transparent medium on a transparent (glass) slide; for the electron microscope the stained specimen is mounted on a fine mesh metal grid (usually copper) which supports it.

For fixation, dehydration and sectioning: no differences in principle, but differences in substances used.

3. For circumstances (b) and (c) you would choose a TEM because a high resolving power is needed and the preparative processes will not destroy the part you want to see.

For circumstances (a) and (d), you do not need high resolving power and as you need to scan fairly objects, you would choose a light microscope with appropriate magnification.
XVII. REFERENCES


XVIII. Main Author of the Module

Charles K. Twesigye is a Senior Lecturer in the Department of Biological Sciences, Faculty of Science, Kyambogo University, Kampala, Uganda. He graduated in 1982 from Makerere University with a BSc.Dip.Ed (Botany and Zoology) before joining Namilyango College as a graduate teacher for Biology and Agriculture. From 1984 to 2005, Twesigye served as a senior examiner for the Uganda National Examinations’ Board and as Chief Examiner in Advanced Biology from 1999 to 2005. He returned to Makerere University in 1990 for graduate studies in Science Education and obtained an M.Ed(Sc.) in 1991 before joining Kyambogo University in 1992. He returned to Makerere University in 1997 to pursue a Master of Science in Genetics which he completed in 1999 and continued teaching genetics, cell biology and evolutionary biology at Kyambogo University. His research interests focus on Ecological and Conservation Genetics of African fishes, mammals and a wide range of environmental issues in the Great Lakes Region of Africa.

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