UNVERSITY OF ZAMBIA

SCHOOL OF NATURAL SCIENCES
DEPARTMENT OF BIOLOGICAL SCIENCES

BIO 1412 MOLECULAR BIOLOGY AND GENETICS

LECTURE MODULE

PROF. CYPRIAN KATONGO

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BIO 1412: MOLECULAR BIOLOGY AND GENETICS MODULE

Rationale

Genetic characters are inherited by offspring from their parents through the activties of nucleic acids. Therefore it is important to understand the molecular basis of inheritance of genetic characters.

Introduction

Modern genetics is based on the central dogma. The genetic code is said to be universal, i.e. it is the same for all living organisms. There are four types of nucleotides found in DNA, 20 amino acids found in proteins and there are 3 nucleotides coding for each amino acid. There is a total of 4^3 (64) possible codons for the 20 amino acids found in proteins. Therefore, there is more than one codon for the same amino acid (the third position is said to be degenerate). The 44 excess codons do not code for amino acids but are either degenerate or have other functions such as giving the stop signal. The genetic code is comma-less and non-overlapping.

Genetics is the study of heredity and variation. It is concerned with form, function and change. Genetics attempts to explain how inter-specific variation is maintained and at the same time, how intra-specific variation is generated and inherited. Modern genetics includes the study of nucleic acids and proteins. Heredity is the process of transmission of characters from one generation to another, bringing about the similarity between parents and their offspring. Differences between individuals of a family, or of a species, are referred to as variation.

The term 'Genetics' was coined by William Bateson in 1907 based on Gregor Mendel's work. William Bateson initially believed that characters were passed from parents to offspring in form of waves. He did not believe in continuous variation. After carefully studying Mendel's work, he introduced the terms, Genetics, allelomorphs, heterozygous and homozygous. Bateson also discovered linkage among genes.

1. NUCLEIC ACIDS

Objectives: At the end of this topic you should be able to:

1. Understand that ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) are composed of mononucleotides
2. Recall the basic structure of a mononucleotide; thymine, uracil and cytosine as pyrimidines; adenine and guanine ad purines
3. Understand that condensation reactions are involved in the formation of mononucleotides and polynucleotides (RNA and DNA)
4. Recall the structure and understand the roles of messenger and transfer RNA
5. Recall the structure of DNA; understand base pairing
6. Understand that a gene is a sequence of bases on the DNA molecule which codes for a sequence of amino acids in a polypeptide chain
7. Understand that DNA stores and transmits genetic information; understand that genetic information is passed on from parents to offspring through RNA; compare and contrast the structure and function of DNA with that of RNA.

INTRODUCTION

Nucleic acids are large biomolecules that store, transmit and express the genetic information of a cell. There are two types of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In most cells, DNA is the store of genetic information which is transmitted from one generation to the next. RNA is normally used as an intermediate in the transfer of information from DNA to proteins. In some viruses RNA is the store of genetic information. Nucleic acids are essential for life because they constitute the genetic material of all living organisms. The elucidation of the structure of DNA represents one of the outstanding milestones in biology because it finally solved the problem of how living cells accurately replicate themselves and encode the information needed to control their activities. Nucleic acids are made up of units called nucleotides which are arranged in extremely long molecules known as polynucleotides.
An extra-chromosomal DNA molecule is a molecule separate from the chromosomal DNA. Examples include mitochondrial, chloroplast and plasmid DNAs.
An episome is a piece of DNA which is able to move from one position to another either within the same DNA molecule or from one DNA molecule to the other. Examples include viruses and transposable elements.

1. 1. Nucleotides

A nucleotide is a monomer of a nucleic acid. It has three components: a 5- carbon sugar (pentose), a nitrogenous base and phosphoric acid. The pentose sugar is either a ribose (found in RNA) or a deoxyribose (found in DNA). There are two types of nitrogenous bases (i) pyrimidines which have one ring and purines which have two rings in their structure. There are two purines; adenine (A) and guanine (G) and three pyrimidines; cytosine (C), thymine (T) and uracil (U). RNA contains uracil while DNA contains thymine in the corresponding position.

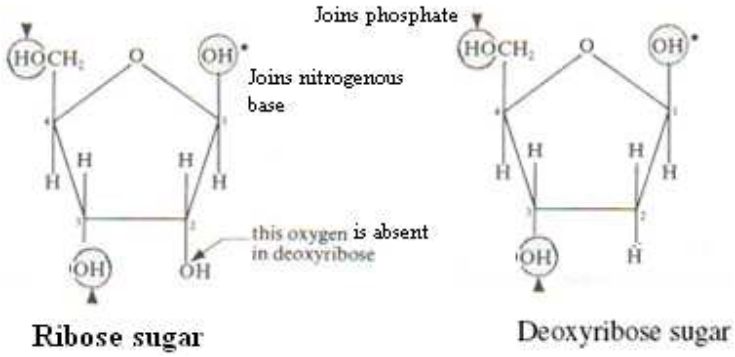


Figure 1.1. Ribose and deoxyribose sugars.

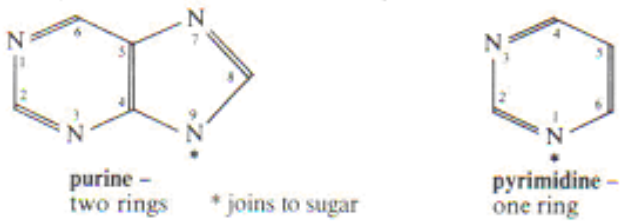


Figure 1.2. The two types of nitrogenous bases found in DNA and RNA

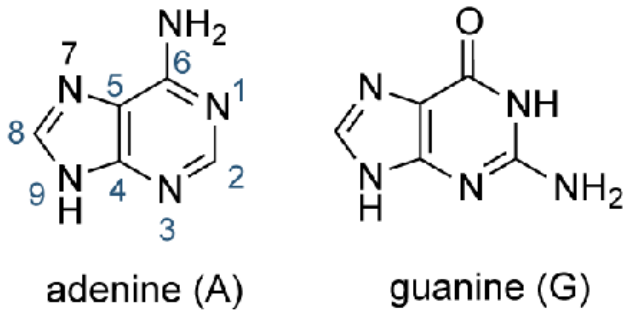


Figure 1.3 The two purine bases found in DNA and RNA

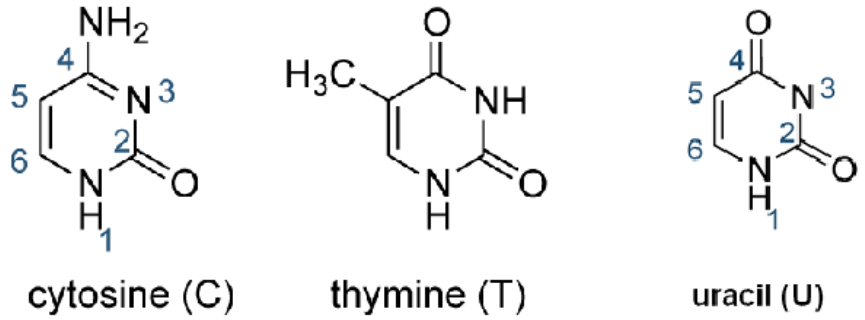


Figure 1.4 The three pyrimidine bases found in DNA (C and T) and RNA (C and U)

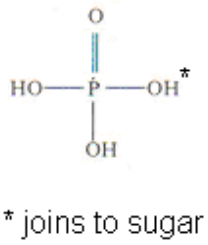
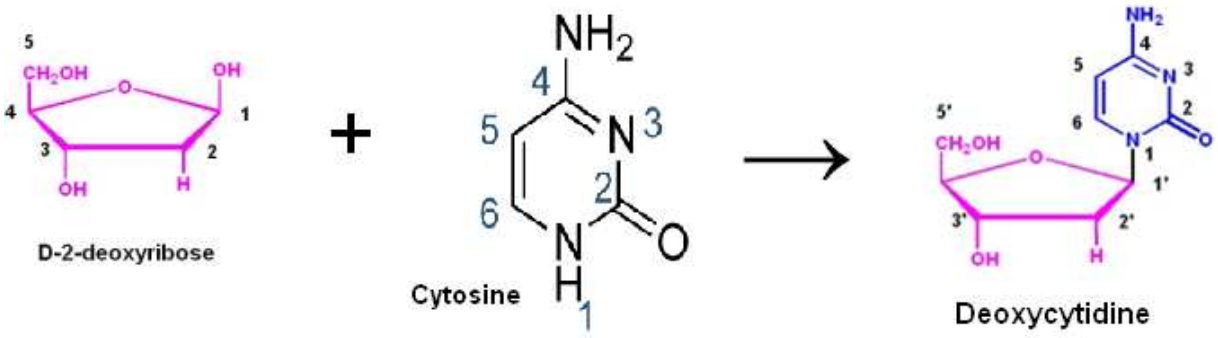
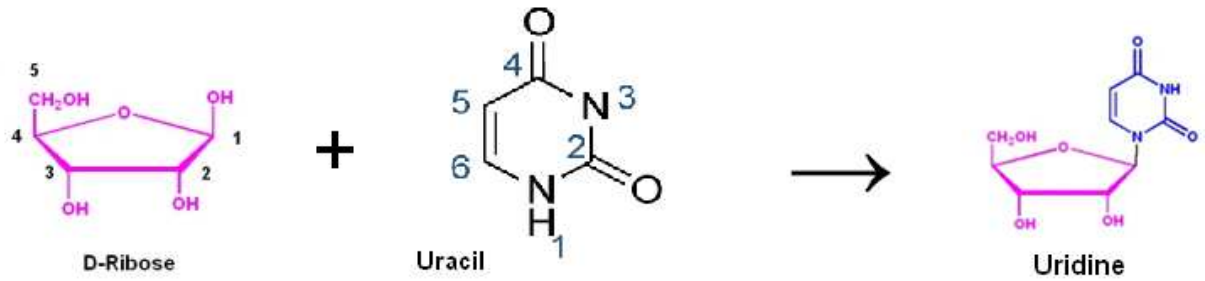


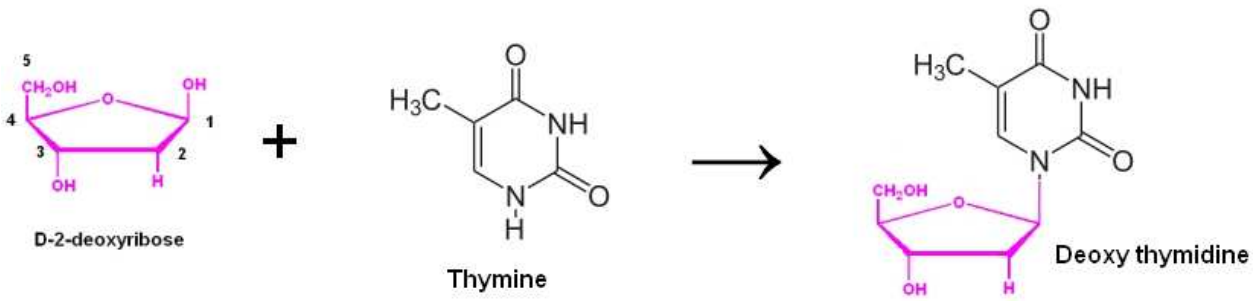
Figure 1.5 Phosphoric acid gives the nucleic acids their acidic character.

1.2 Formation of a nucleoside

A nucleoside is a molecule which is formed when a pentose sugar forms a beta glycosidic (covalent) bond with a nitrogenous base. The OH group of carbon 1 of the pentose sugar combines with the H at position 1 of a pyrimidine base to form a pyrimidine nucleoside or the H at position 9 of a purine to form a purine nucleoside. This is a condensation reaction.







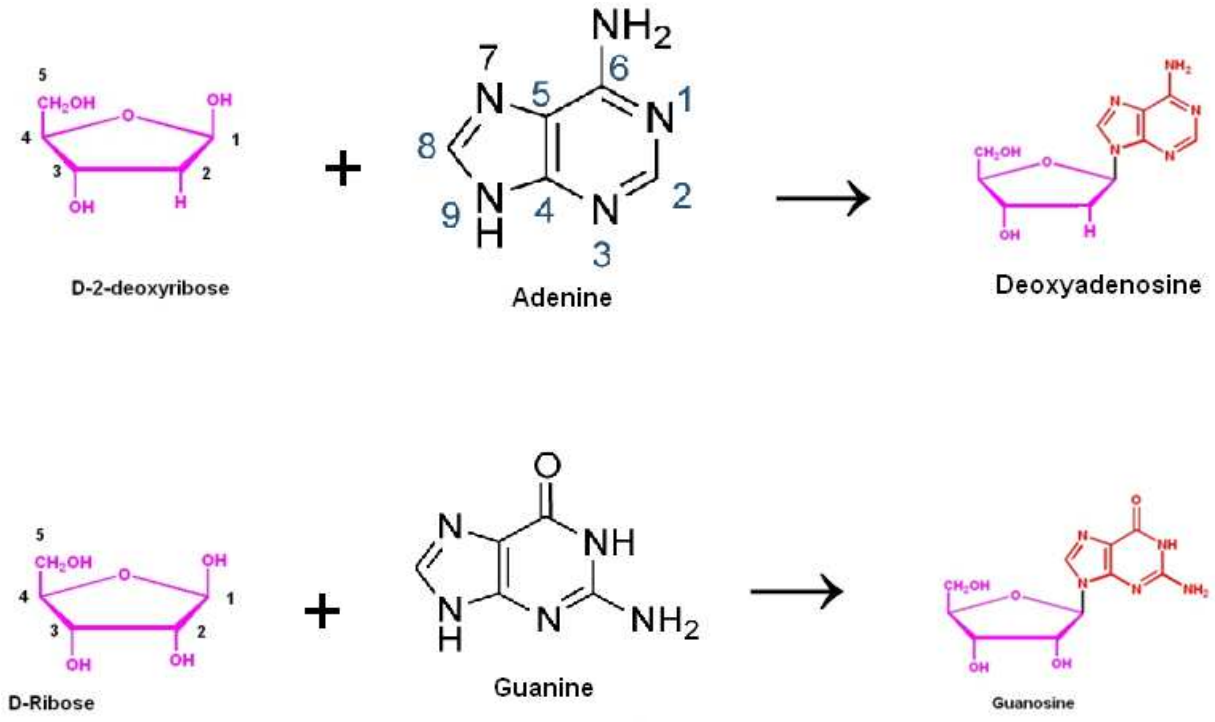


Figure 1.6 Formation of a different nucleosides by condensation.

1.3 Formation of a nucleotide

A nucleotide is formed when a nucleoside forms a phosphoester bond with a phosphate (or phosphoric acid). The OH group of carbon 5 of the pentose sugar of the nucleoside combines with H of the phosphate in a condensation reaction.

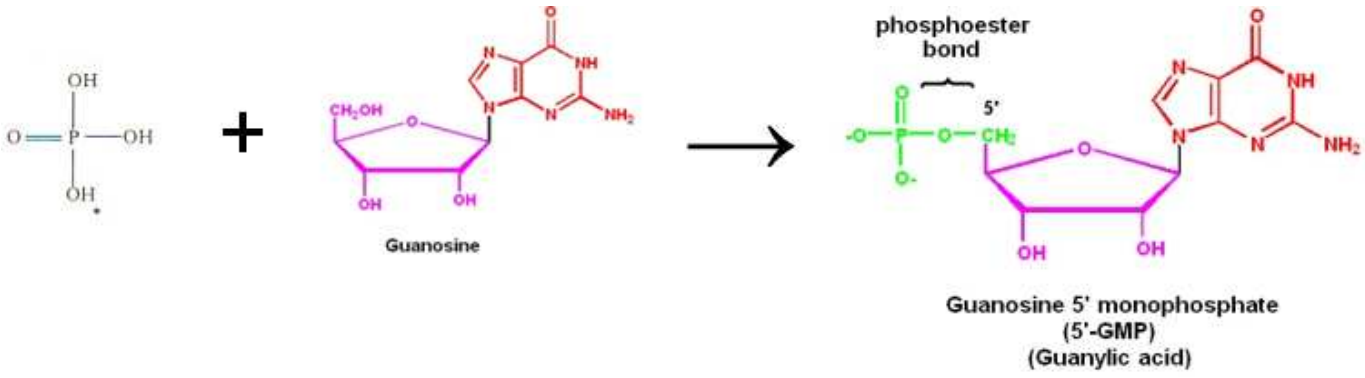


Figure 1.7 Formation of a nucleotide (guanosine monophosphate) by condensation.

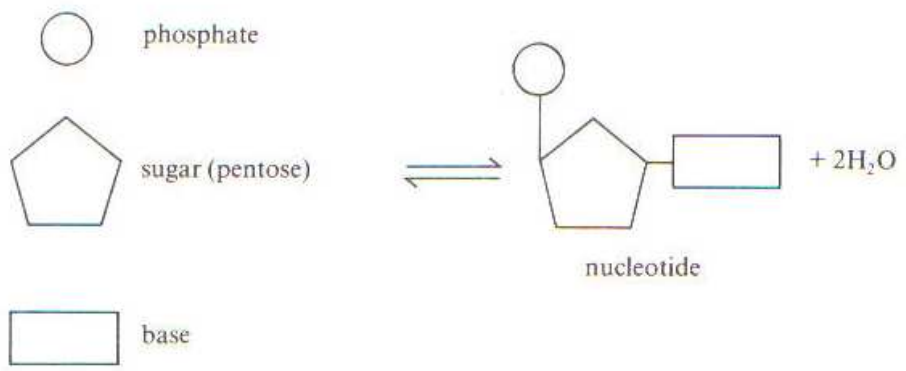


Figure 1.8 Diagrammatic representation of nucleotide formation

Table 1.1 Summary of the formation of the nucleotides of DNA and RNA

Base	**Nucleoside**	**Nucleotide**	**RNA**	**DNA**	**Symbol**
Adenine	Adenosine	Adenosine monophosphate	AMP	dAMP	A
Guanine	Guanosine	Guanosine monophosphate	GMP	dGMP	G
Cytosine	Cytidine	Cytidine monophosphate	CMP	dCMP	C
Thymine	Thymidine	Thymidine monophosphate	TMP	dTMP	T
Uracil	Uridine	Uridine monophosphate	UMP	dUMP	U

Formation of a dinulceotide

A dinclucleotide is a moleule with two nucleotides. A second monocleotide is added at carbon 3 of the pentose sugar of the first nucleotide. This is a condensation reaction between the OH of carbon 3 of the first nulceotide and H of the phosphate on carbon 5 of the second nucleotide. The bond formed between carbon 3 of the first sugar and carbon 5 of the second sugar is called a phosphodiester bridge.

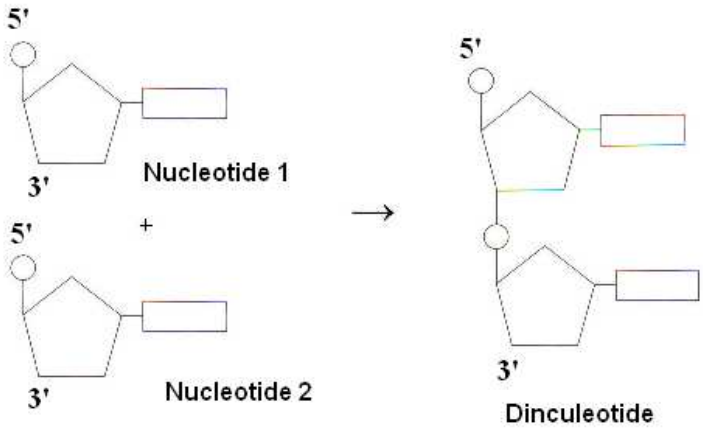


Figure 1.9 Diagrammatic representation of dinucleotide formation

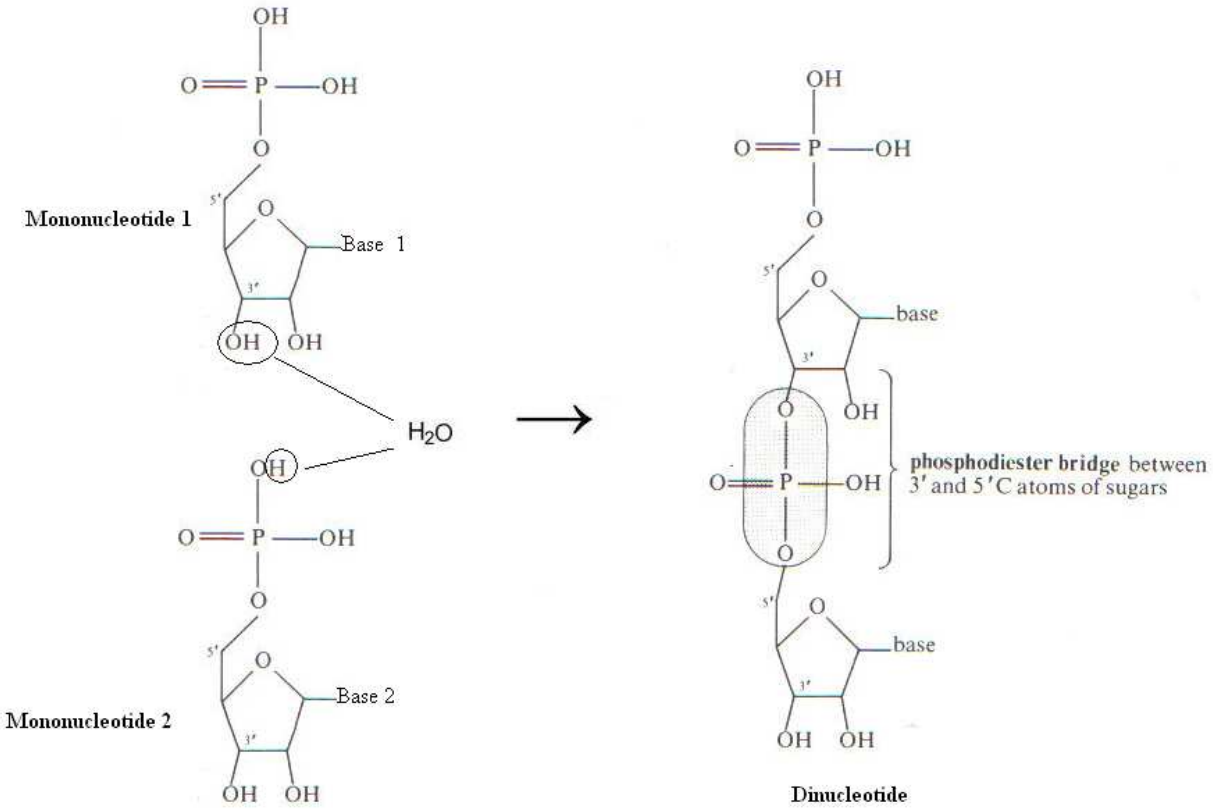


Figure 1.10 Chemical reaction during the formation of a dinucleotide of RNA

Formation of DNA and RNA polynucleotides: The dinucleotide is converted to a trinucleotide by addition of a nucleotide to the OH on carbon 3 of the sugar of the second nucleotide. The addition of more nucleotides continues at the 3′ end of the growing chain until the required length of a DNA or RNA strand is produced.

1.3 DNA Structure: The DNA molecule is made of two strands and is therefore said to be double stranded. Each strand is a polymer of the four types of nucleotide bases.

Physical properties of DNA: Maurice Wilkins and Rosalind Franklin passed X-rays through DNA fibres (X-ray crystallography) and found that (i) DNA had a regularly twisted (helical) structure (ii) there was a regular (repeating) spacing of 3.4nm between the components of DNA polymer and that the diameter of each DNA polymer was 20nm (iii) DNA was made of two strands.

Chemical properties of DNA: DNA, like RNA is acidic in nature because it's rich in phosphates. Erwin Chargaff (1949) did some chemical analysis of DNA and came up with findings known as Chargaff's rules which state that: (1) the total number of purine bases (A+G) = the total number of pyrimidine bases (C+T); (2) The number of adenine bases = the number of thymine bases (i.e. the ratio A: T = 1); (3) The number of guanine bases = the number of cytosine bases (i.e. the ratio G: C = 1).

The Watson - Crick Model of DNA structure: Watson and Crick (1953) used X-ray crystallography results from Wilkins and Franklin as well as Chargaff's rules to work out their model of DNA. Using pieces of wire and flat metal shapes, they concluded that DNA was in form of a double helix composed of two polynucleotide chains held together by hydrogen bonding between pairs of bases. They thought of DNA as similar to a ladder in which the base pairs are the steps and the sugar-phosphate backbones are the two sides. The backbone is then twisted into a double helix in which there are ten nucleotide bases per turn. They proposed that the sequence of nucleotides in one strand determines the sequence in the other meaning that the

two strands are complementary and anti-parallel. In 1962 Watson and Crick together with Wilkins were awarded the Nobel Prize for Medicine for elucidating the structure of DNA.

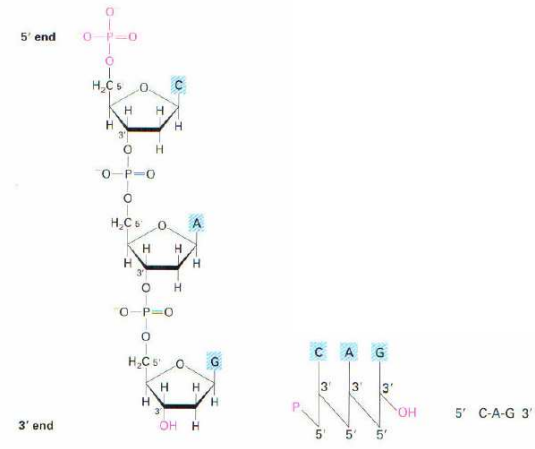


Figure 1.11 DNA single strand.

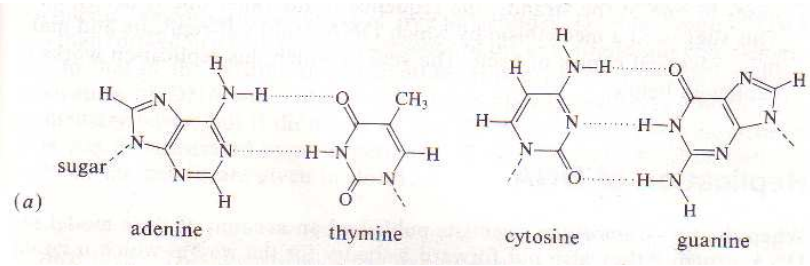


Figure 1.12 (a) The two polynucleotide strands are held together by hydrogen bonds which form between specific pairs of bases. The A-T pair has two H-bonds while the C-G pair has three H-bonds (b) Pyrimidine-pyrimidine pairs would be too narrow while purine-purine pairs would be too wide for the diameter of DNA.

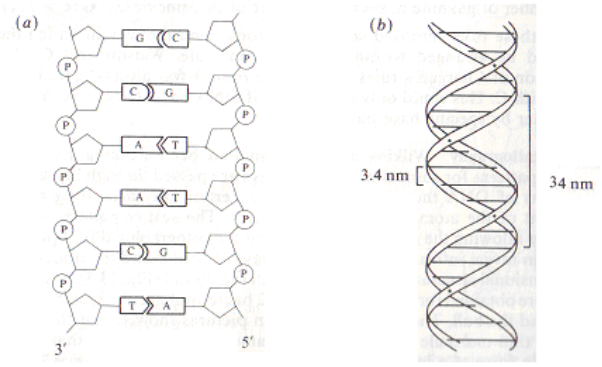


Figure 1.13 The DNA double helix (a) and double helix (b). Adenine (A) is paired with thymine (T) while guanine (G) is paired with cytosine(C). The presence of thousands of hydrogen bonds in a DNA molecule contributes greatly to the stability of the double helix.

1.4 Ribonucleic acid (RNA) Structure: RNA is a single polynucleotide which is synthesised based on the information carried by DNA. The three types of RNA are messenger, transfer and ribosomal RNAs. RNA contains adenine, guanine, cytosine and uracil but not thymine.

1.4.1 Messenger RNA: Messenger RNA (mRNA) is a single-stranded molecule formed on a single strand of DNA by transcription. The mRNA is formed according to the rules of complementary base-paring under the influence of RNA polymerase. Thus the base sequence of RNA is a template copy of the template DNA strand and varies in length according to the length of the polypeptide that it codes for. The mRNA carries the genetic code (codon) according to the template DNA.

1.4.2 Ribosomal RNA: Ribosomal RNA (rRNA) is a single-stranded nucleic acid which makes up nearly 80% of the total RNA of the cell. It is synthesised (transcribed) from the DNA located in the nucleolar organiser of several chromosomes. It combines with protein to form ribosomes which are the site of proteinsynthesis. Ribosomes are usually found in clusters (polyribosomes) linked together by a strand of mRNA.

1.4.3 Transfer RNA

Transfer RNA (tRNA) is a small single-stranded molecule with about 80 nucleotides per molecule. It is an adaptor molecule which is involved in the transfer of amino acids from the cytoplasm to the ribosomes during the process of proteinsynthesis. The tRNA has four arms (i) the acceptor arm (ii) the variable arm (iii) the D arm and (iv) the anticodon arm. Each amino acid has its own tRNA which carries a triplet anticodon for the particular amino acid. About 60 different tRNA have been identified and all have the same basic structure. The 5´ end of tRNA always ends in the base sequence of CCA. The structure of tRNA is described by the clover leaf model.

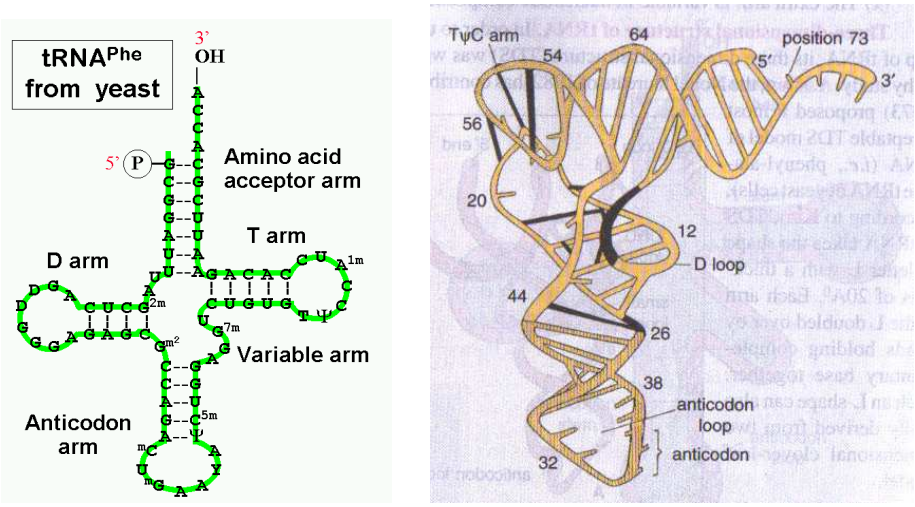


Figure 1.14 Transfer RNA (tRNA). (a) Schematic diagram of tRNA for phenylalanine.

1.2. Similarities between DNA and RNA structure and function

	DNA and RNA
1	Present in all prokaryotes and eukaryotes
2	Carry genetic information
3	Made up of nucleotides
4	Polymerization results in strand formation
5	Cleaved by nucleases

1.3 Differences between DNA and RNA structure and function

	DNA	**RNA**
1	Stores and transmits genetic information	Mainly transmits genetic information
2	Mainly double stranded	Mainly single stranded
3	Chemically stable	Chemically unstable
4	Has thymine in the place of uracil	Has uracil in the place of thymine
5	Huge molecule	Relatively smaller in size
6	Usually one type	Mainly three types (rRNA, tRNA and mRNA)

1.8 Mitochondrial DNA (**mtDNA**): This is the DNA located in organelles called mitochondria. Nuclear and mitochondrial DNA are thought to be of separate evolutionary origin, with the mtDNA being derived from the circular genomes of the bacteria that were engulfed by the early ancestors of today's eukaryotic cells. Mitochondrial DNA carries genes that code for the enzymes and other substances involved in aerobic respiration (Krebs cycle and the electron chain transport). It is able to replicate itself independently of nuclear DNA. It is inherited from the mother (maternally inherited) because the mitochondria in the sperm are usually destroyed by the egg cell after fertilization. Mutations in mtDNA cause maternally inherited diseases and are thought to be a major contributor to aging and age-associated pathology. MtDNA has been used to track the ancestry of many species back hundreds of generations through females (matrilineage). The concept of the Mitochondrial Eve is based on this type of analysis.

1.9 Chloroplast DNA (cpDNA): This is the DNA located in organelles called chloroplasts which are found in photosynthesizing cells. It is believed to have arisen when photosynthesizing bacteria where engulfed by eukaryotic cells. Chloroplast DNA is able to replicate itself independently of nuclear DNA. It carries genes that code for the enzymes and other substances involved in the process of photosynthesis. The chloroplast genome (cpDNA) of plants has been a focus of research in plant molecular evolution and systematics.

1.10 Plasmids: A plasmid is an extra-chromosomal DNA molecule separate from the chromosomal DNA which is capable of replicating independently of the chromosomal DNA. In many cases, it is circular and double-stranded. Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms (e.g., the 2-micrometre-ring in *Saccharomyces cerevisiae*). The plasmid carries all the information necessary for its independent replication. Plasmids can be advantageous to a bacterium. For example, they can carry genes that give them resistance to antibiotics or toxic metals, genes that allow the bacterium to degrade compounds that it otherwise could not use as food, and genes that allow the bacterium to infect an animal or plant cell.

1.11 Episomes: An episome is a portion of genetic material that can exist independent of the genome (the chromosomes) at some times, while at other times is able to integrate into the chromosome. An episome is distinguished from other pieces of DNA that are independent of the chromosome by their large size. Examples of episomes include transposable genes (transposons) and some viruses.

Transposons are also called mobile genetic elements because they are able to move from one position to another position on the chromosome and from one cell to another. Transposons can carry with them some drug resistance genes and can therefore spread drug resistance from one cell to another.

Viruses that integrate their genetic material into the host chromosome enable the viral nucleic acid to be produced along with the host genetic material in a non-destructive manner (lysogeny). However, the viral episome living outside the cell will destroy the host cell as it commandeers the host's replication machinery to make new copies of itself (lysis)

1.12 The gene: In biochemical terms, a gene is a sequence of nucleotide pairs along a DNA molecule which codes for an RNA or polypeptide product. Polypeptide coding genes are either (1) structural genes which code for functional proteins (enzymes, hormones, components of cell structure, antibodies, storage proteins, etc.) or (2) regulatory genes which serve to control the function of other genes. The mRNA, rRNA and tRNA are coded for by genes and are made directly by transcription from DNA. The genes which code for rRNAs are present in multiple copies and in eukaryotes they are localised at a special region in the chromosome called the secondary constriction or nucleolus organiser. Most eukaryotic genes are split i.e. contain both coding (exons) and non-coding DNA (introns, spacer DNA, microsatellites, etc.). It also transpires that large regions of the chromosomes do not appear to contain any genes at all: they are composed of stretches of repetitive (or redundant) DNA consisting of small sequences of bases which are present as millions of tandemly repeated copies of unknown function.

REVIEW QUESTIONS 1: NUCLEIC ACIDS

1. State the name given to the building blocks (monomers) of nucleic acids.
2. State the components of a nucleic acid.
3. Describe one similarity and one difference between a purine and a pyrimidine.
4. Explain the meaning of the fact that DNA strands are antiparallel.
5. Describe two structural similarities and two structural differences between DNA and RNA.
6. Describe two functional similarities and two functional differences between DNA and RNA.

2. DNA REPLICATION

Introduction: DNA is a biomolecule which is capable of reproducing itself in a process called replication. Watson and Crick proposed that the two strands were capable of separating and acting as templates to which a complementary set of nucleotides would attach by base pairing to form new DNA strands.

2.1 Semi-conservative mechanism of DNA replication: The replication of DNA is semi-conservative in which the original double helix molecule is duplicated into two identical copies. Each DNA copy contains one old strand and one new strand. Evidence of this mechanism of DNA replication came from the experiment by Meselson and Stahl who used *E.coli* cells.

The cells were initially grown in a nutrient medium containing 'heavy' nitrogen isotope (^{15}N) for many generations until all the nitrogen in their DNA contained ^{15}N. The cells were then transferred to a medium containing the 'light' nitrogen isotope (^{14}N) and different generations of bacterial cells were sampled from the medium. The DNA in each sample was analysed by density-gradient equilibrium centrifugation using caesium chloride (CsCl). In this method different concentrations of CsCl were added to a centrifuge tube. The most concentrated solution was added first followed by less concentrated solutions and the most dilute solution was added last. This CsCl solution gradient is able to separate heavy-heavy (HH), light-light (LL) and heavy-light (HL) DNA double helices into separate bands. The bands which were observed in the experiment proved that DNA undergoes the semi-conservative replication and not the conservative replication. The semi-conservative type of DNA replication has also been conformed in eukaryotic cells

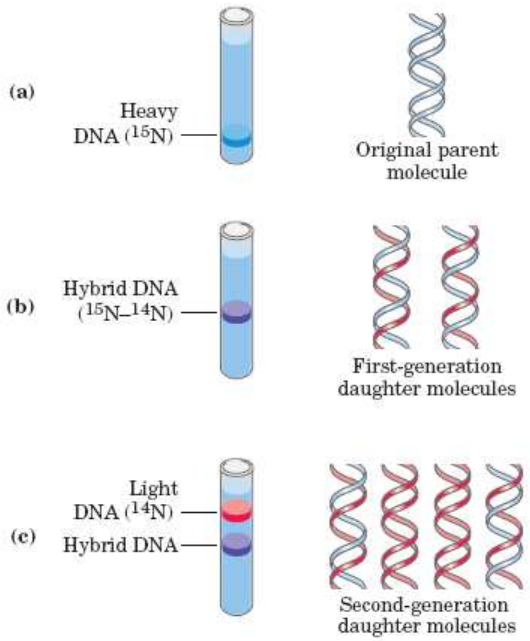


Figure 2.1a. The Meselson-Stahl experiment. (a) Cells were grown for many generations in a medium containing only heavy nitrogen, ^{15}N, so that all the nitrogen in their DNA was ^{15}N as shown by a single band (blue) when centrifuged in a CsCl density gradient. (b) Once the cells had been transferred to a medium containing only light nitrogen, ^{14}N, cellular DNA isolated after one generation equilibrated at a higher position in the density gradient (purple band). (c) Continuation of replication for a second generation yielded two hybrid DNAs and two light DNAs (red), confirming semi conservative replication.

2.2 DNA replication process in prokaryotes

DNA replication in prokaryote is well represented by *E.coli.* The process involves the following major steps: (i) Replication initiation, (ii) DNA denaturation – melting –unzipping, (iii) Priming, (iv) Elongation - DNA synthesis at growing fork (v) Separation of circular daughter molecules, (vi) Proof reading.

2.2.1 Initiation of replication in *E.coli*

The replication of DNA requires the removal of the supercoils and the attachment of enzymes and other proteins to the origin of replication on the DNA molecule. The supercoils are removed by the enzyme called topoisomerase followed by the attachment of a protein called DnaA. This results in the formation of the initiation complex.

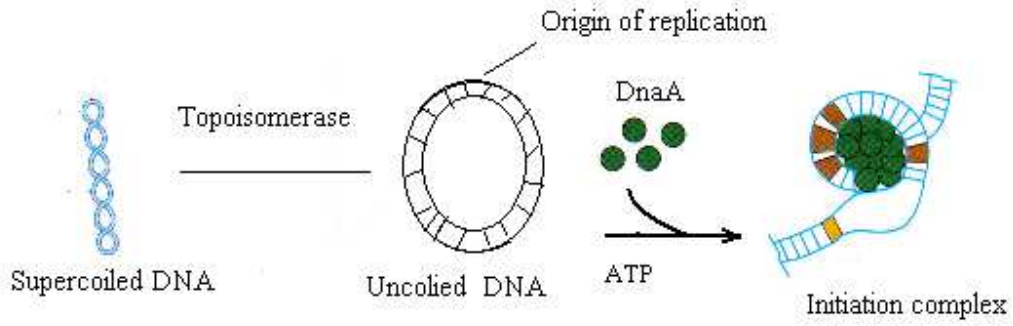


Figure 3.1b. Model of initiation of DNA replication at *E.coli* origin of replication

2.2.2 Double stranded DNA denaturation

This process is also called DNA melting or unzipping. When DnaA binds to the double helix, the first separation of the two strands takes place. This process requires ATP. This is followed by the binding of the enzyme DNA helicase which leads to the separation of more base pairs. The helicase moves along the DNA double helix using the energy from ATP hydrolysis to separate the two strands. The denaturation of double stranded DNA is in the $5' \rightarrow 3'$ direction. The single strand binding protein prevents the double helix from rejoining.

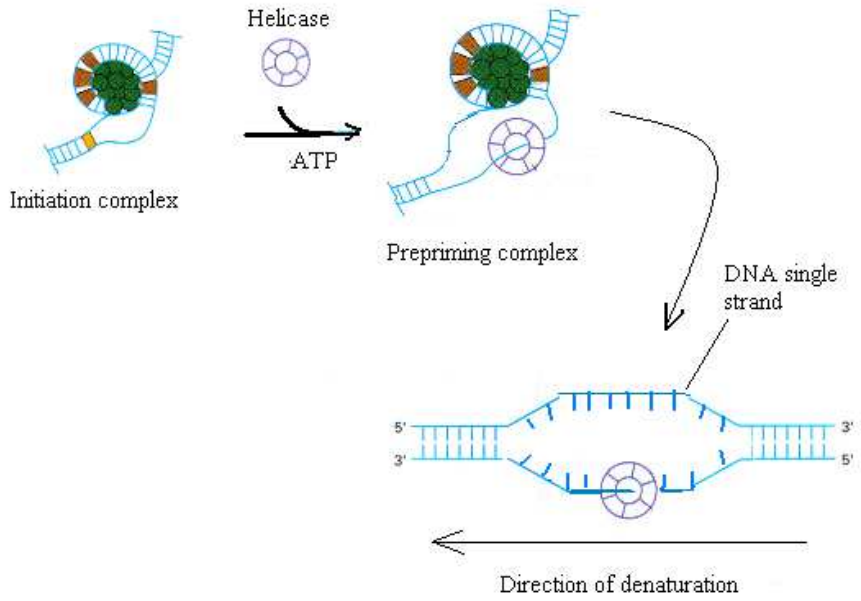
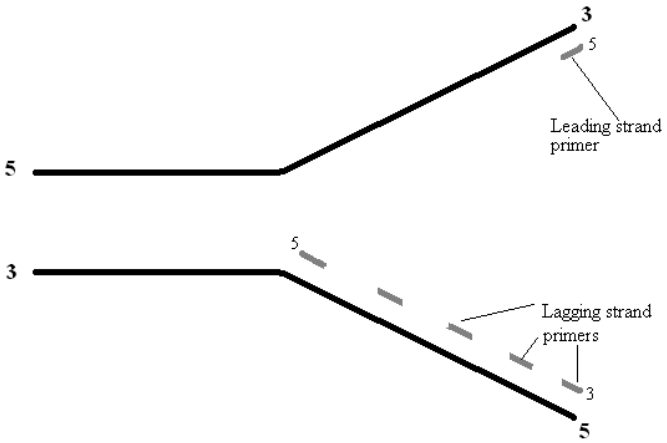


Figure 2.1e. DNA denaturation by the action of helicase in *E.coli*.

2.2.3 Formation of RNA primers in *E.coli*

This process is called priming. It is well known that DNA polymerase cannot initiate a new DNA chain but can only elongate a pre-existing DNA or RNA strand. Therefore, before the parental DNA is replicated, a short RNA molecule, complementary the DNA strand, is synthesised by RNA polymerase. This RNA molecule is called a primer and it attaches itself to the initiation complex. A primer is synthesised on each DNA strand in the 5′→3′ direction.



2.2.4 DNA synthesis - addition of nucleotides at the growing fork by *E. coli* DNA

This is a polymerization reaction catalysed by DNA polymerase. Although the two strands of DNA double helix are anti-parallel, DNA polymerase catalyses nucleotide addition at the 3'-hydroxyl end of each growing chain. Therefore each strand is elongated only in the 5′→3′ direction. Synthesis of a new DNA strand involves the addition of deoxyribonucleotide triphosphates (dNTPs) which include: dATP, dGTP, dTTP, and dCTP.

Leading strand synthesis: At each growing fork, one DNA strand, called the leading strand, is synthesized continuously from a single primer on the leading strand template. The leading strand grows in the 5'→3' direction, like the growing fork.

Lagging strand synthesis: Synthesis of the lagging strand is more complicated, because DNA polymerases can add nucleotides only to the 3' end of a primer or growing DNA strand. Movement of the growing fork exposes the lagging-strand template on which short RNA primers are copied. Each of these primers is then elongated by addition of dNTPs to its 3′ end. . In *E.coli*, this reaction is catalysed by DNA polymerase III (poly III). The resulting short fragments containing RNA covalently linked to DNA are called Okazaki fragments. In bacteria and bacteriophage, Okazaki fragments contain 1000-2000 nucleotides. The overall direction of growth of the lagging strand is from its 3′→5′ end, complementary to the polarity of its template but opposite to the direction of nucleotide addition by DNA polymerases. DNA polymerase I is primarily involved in removing RNA primers from Okazaki fragments (exonuclease activity) and filling the resultant gaps using its 5′→3′ polymerizing activity. DNA ligase joins adjacent completed Okazaki fragments.

DNA polymerase II functions in the inducible SOS response and also fills gaps and appears to facilitate DNA synthesis directed by damaged templates. DNA polymerase II resembles DNA polymerase I in its activity, but is a DNA repair enzyme, bringing about the growth in 5′→3′ direction using free 3′- OH groups.

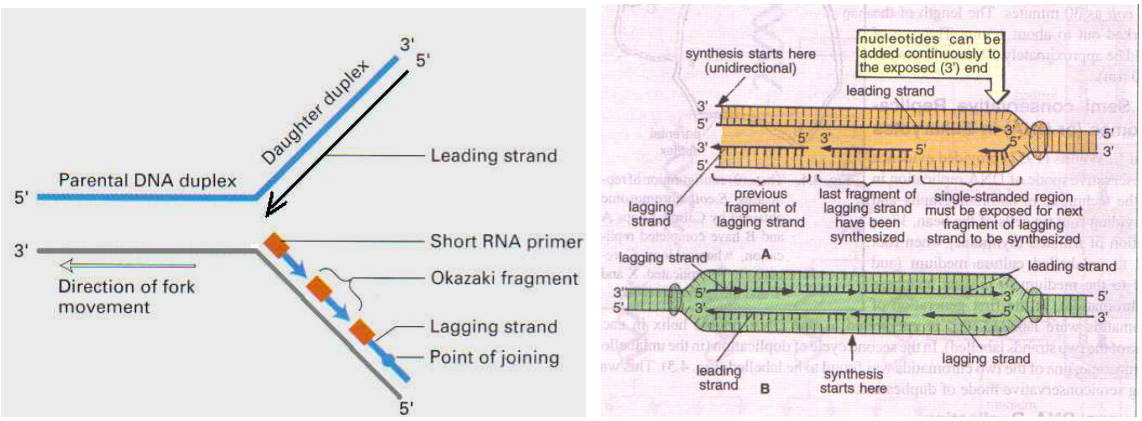


Figure 3.1g. DNA replication at a growing fork.

Separation of circular daughter molecules

During DNA replication the parental strands remain intact and retain their superhelicity. This poses stearic and topological constraints to the completion of the replication of a circular DNA molecule as the two growing forks approach each other. In *E.coli*, decatenation of the two daughter cells is catalyzed by Topo II (DNA gyrase) and topoisomerase IV (Topo IV). This helps separate the daughter DNA molecules from each other

Proof reading by DNA polymerase

Occasionally, a wrong base is inserted during DNA synthesis. This is corrected by the proofreading function of all bacterial polymerases.

Enzymes involved in prokaryotic DNA replication

#	Enzyme	Function
1	DnaA protein	
2	Topoisomerase	Removes coils and supercoils from DNA
3	DNA polymerase I	Removes primers from the new DNA strand
4	DNA polymerase II	Repairs mistakes during DNA polymerization and also fills in the gaps left after the removal of primers from lagging strand
5	DNA polymerase III	DNA polymerization
6	Helicase	DNA denaturation
7	Primase	Synthesis of short RNA primers on template DNA
8	Ligase	Joins DNA nucleotides through covalent bonds
9	Single strand binding protein	Prevents single stranded DNA from forming double strands

REVIEW QUESTIONS 2: DNA REPLICATION

1. Outline the central dogma of molecular biology.
2. Explain the experiments which provided evidence in support of the mechanism of DNA replication.
3. Differentiate between conservative and semi-conservative mechanisms of DNA replication.
4. Describe the various stages of prokaryotic DNA replication.
5. List the enzymes and non-enzyme proteins involved in prokaryotic DNA synthesis and explain the role of each of them.
6. Differentiate between leading and lagging strand synthesis of prokaryotic DNA.

3. TRANSCRIPTION

Objective: At the end of this topic you should be able to:

1. Understand mRNA, tRNA, and rRNA transcription
2. To describe the phases of initiation, elongation and termination
3. To explain post transcription modification during eukaryotic transcription

3.1 Introduction

The transfer of information from DNA to RNA is called transcription. The process involves initiation, elongation and termination. The stretch of DNA that is transcribed into an RNA molecule is called a *transcription unit*. The section of DNA that holds the information for one polypeptide is called a gene.

Only one of the DNA strands is used as a template for the synthesis of RNA. This is called the sense DNA strand. The other strand which is not transcribed is called the anti-sense and it has the same code as the RNA transcript except for T in place of U.

3.2 Mechanism of transcription

Transcription involves the following stages: (i) initiation (ii) elongation (iii) termination and (iv) the processing of primary transcript products into mature RNA molecules.

3.2.1 Initiation

Before transcription can take place, the double helix in the gene to be transcribed has to unwind and the two DNA polynucleotide chains have to separate (unzip) in order to expose the nucleotide bases. This is done with the help of the enzyme helicase and other proteins. This is followed by the binding of RNA polymerase to the unzipped part of DNA at a region called promoter. During the initiation phase, the first two nucleotides of the RNA (usually A and C) are joined together.

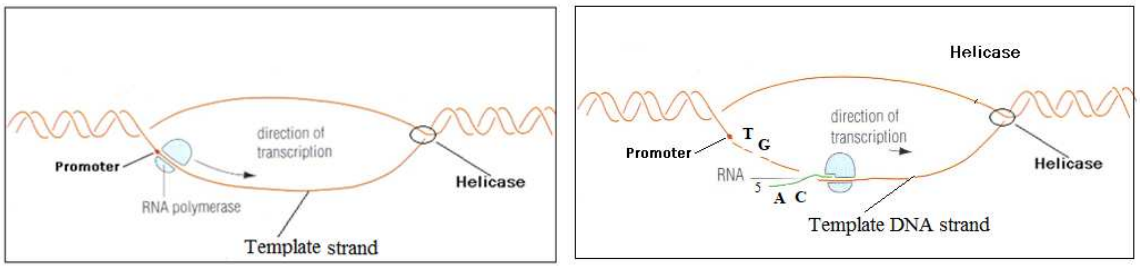


Figure 3.1. Initiation of RNA transcription

3.2.2 Elongation

The elongation phase begins when the RNA polymerase moves along the sense DNA strand binding one nucleotide at a time as it moves. RNA polymerase adds nucleotides to the 3′- end, building the new RNA in the 5′ → 3′ direction and opening the DNA chain as it moves along.

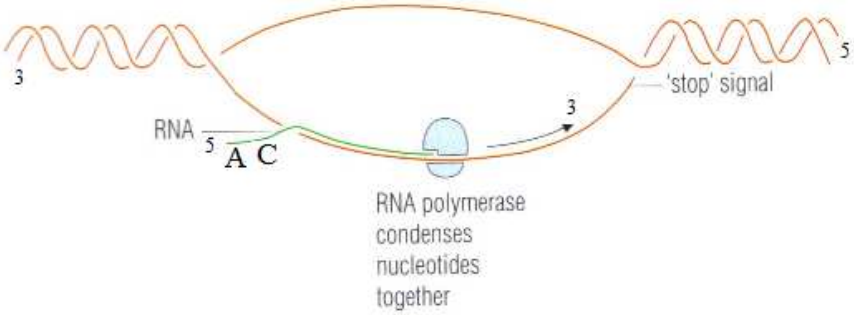


Figure 3.2. Elongation during RNA synthesis/transcription

3.2.4 Termination of transcription

There are two types of transcription termination mechanisms.

(i) The first mechanism which is referred to as rho (ρ)-independent termination of transcription, occurs at specific base sequences called palindromic sequences in the RNA molecule (AAAGGCUCC-UUUU-GGAGCCUUU). When the palindromic sequence is transcribed, it forms a hair-pin loop which disturbs the RNA polymerase and results in termination of transcription;

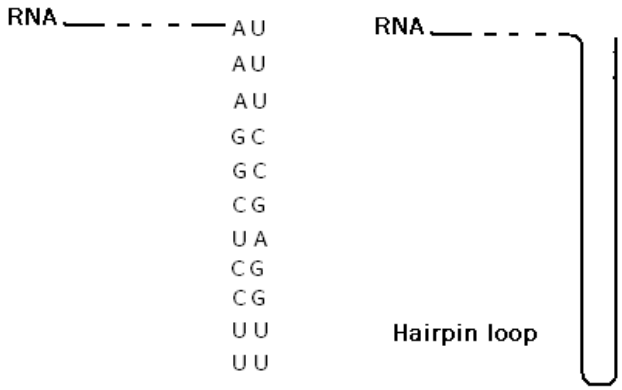


Figure 3.3 Rho-independent termination of transcription

(ii) the second mechanism is the (rho) ρ-dependent termination which uses the ρ factor to stop RNA synthesis at specific sites. When the RNA polymerase reaches certain sites of DNA the rho factor approaches the new mRNA and hooks it up thereby disturbing the RNA polymerase and causing the termination of the transcription process.

3.2.5 Modification of newly synthesized RNAs

The newly synthesized RNAs (mRNA, tRNA and rRNA) are called precursor molecules and must be modified before they become mature and functional. Modification involves removal of some nucleotides and transformation of other nucleotides.

3.2.5.1 Messenger RNA posttranscriptional modification

Precursor mRNA undergoes capping, polyadenylation and splicing before it becomes mature mRNA.

Capping is the addition of methylated guanosine head on the 5′ end: The cap protects mRNA from degradation and helps in nuclear export and ribosome binding.

Polyadenylation is the addition of poly (A) tail on the 3′ end: The poly-A tail protects mRNA from degradation and aid in the export of mRNA from the nucleus; also aids in transcription termination and in translation.

Splicing is the removal of introns and joining of exons together.

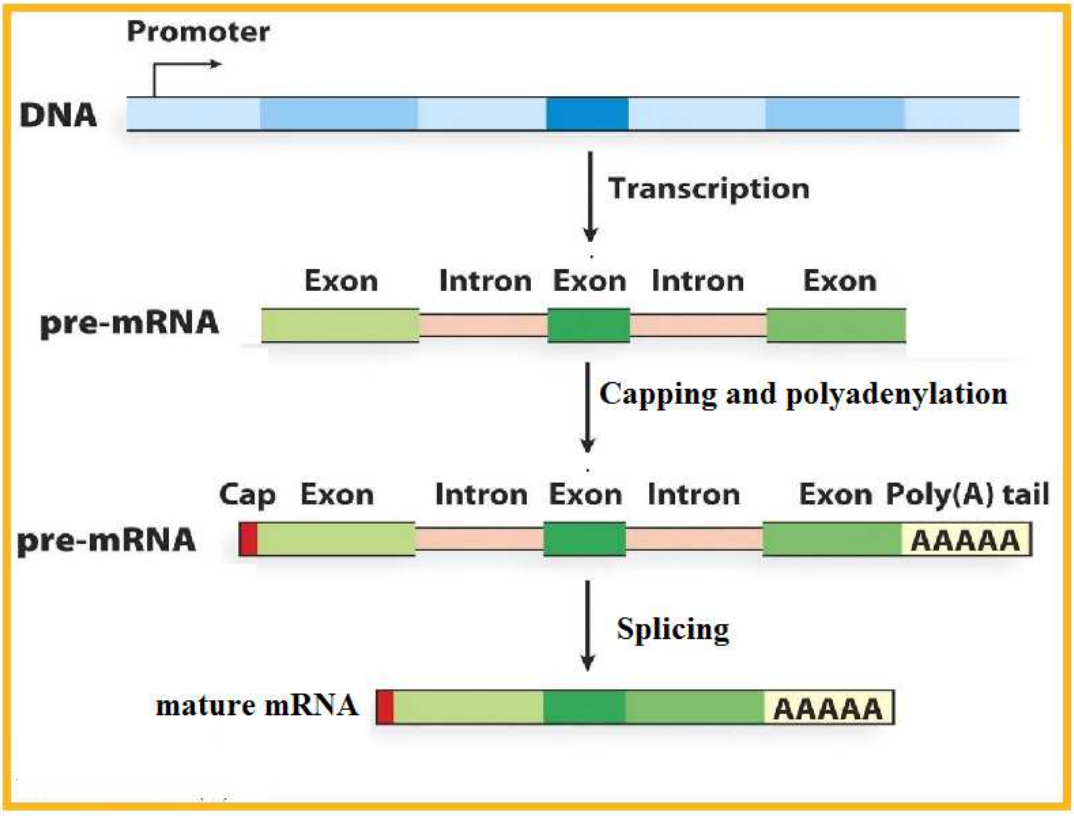


Figure 3.4 Messenger RNA post-transcription modification

3.2.5.2. Ribosomal RNA posttranscriptional modification

Ribosomal RNA (rRNA) is synthesised as precursor rRNA. Precursor rRNA undergoes methylation and shortening to form mature rRNA. Mature rRNA combines with protein to produce ribosome. A complete ribosome has two subunits. The prokaryotic ribosome is 70s (30s + 50s) while the eukaryotic ribosome is 80s (60s +40s).

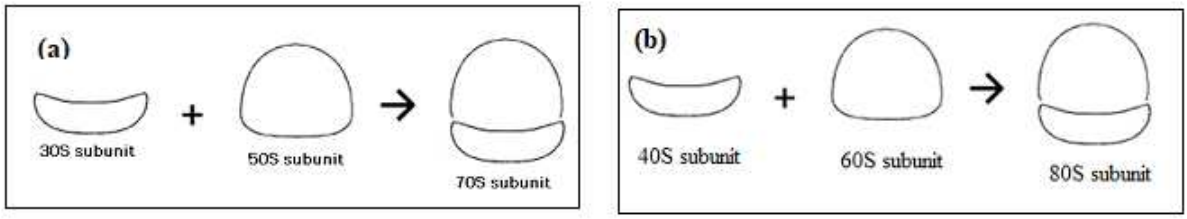


Figure 3.4 Formation of prokaryotic (a) and eukaryotic (b) ribosomes.

3.2.5.3. Transfer RNA posttranscriptional modification

Precursor tRNA is converted into cloverleaf shaped mature tRNA which has the following features: (i) D arm, (ii) T arm, (iii) anticodon and (iv) amino acid acceptor arm.

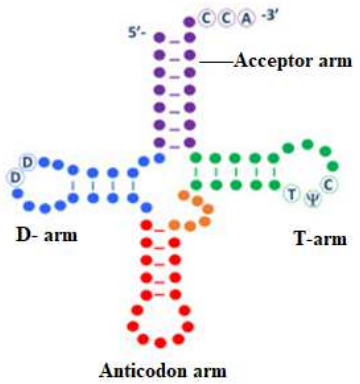


Figure 3.4 Mature of tRNA

3.2.6 Transcription inhibiting drugs

Transcription inhibitors can be used as antibiotics against, for example, pathogenic bacteria (antibacterial drugs) and fungi (antifungals). Rifampicin is an antibacterial drug which inhibits prokaryotic DNA transcription while 8-Hydroxyquinoline is an antifungal drug which is a transcription inhibitor.

REVIEW QUESTIONS 3: TRANSCRIPTION

1. Explain differences between DNA replication and transcription.
2. Explain why the DNA molecule is referred to as the blue print for protein synthesis.
3. Explain the role of the promoter site during transcription.
4. Discuss posttranscriptional modification of RNA.
5. Differentiate between introns and exons and mention the type of RNA in which they are found.
6. Describe the structure of each mature and functional prokaryotic RNA.
7. Explain the role of each RNA.

4. TRANSLATION

Objectives: At the end of this topic you should be able to

(i) Explain the functions of RNAs
(ii) Explain the role of the genetic code.
(iii) Describe the process of protein synthesis

4.1 Introduction

Translation also called proteinsynthesis is a process by which proteins are synthesised from amino acids using genetic information from DNA through RNA. During this process, messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA) are all involved. The triplet base sequences (codons) of mRNA molecules are converted into a specific sequence of amino acids in a polypeptide chain. This process occurs on ribosomes.

4.2 Role of Messenger RNA

The mRNA carries the genetic code (triplet codon) derived from the template DNA. Messenger RNA is a linear molecule which is a copy of the template DNA strand and varies in length according to the length of the polypeptide that it codes for.

4.2.1 The genetic code

The genetic code is the whole sequence of nucleotides on the mRNA which is derived from the DNA.

The characteristics of the genetic code are; (i) It has codons in which the nucleotides of mRNA are arranged as a linear sequence of successive triplets, (ii) it is non-overlapping meaning that during translation, the codons do not overlap but are read sequentially, three at a time, (iii) it is comaless meaning that all the nucleotides are used to code for amino acids with none of them used for punctuation, (v) it is universal meaning that it is the same for all organisms ranging from virus to humans, (v) it has polarity meaning that it is always read in the 5' $\rightarrow$ 3' direction and (vi) it is degenerate meaning that more than one codon may specify the same amino acid. Except tryptophan and methionine which have a single codon each, all the other 18 amino acids have more than one codon (vii) there are three codons that are not recognized by any tRNA: UAA, UAG, and UGA. These are termed nonsense codons or stop codons, because they signal protein synthesis to stop at that point.

Wobble hypothesis

The interaction between the codon in the mRNA and the anticodon in the tRNA needs to be exact in first two nucleotide positions, but does not have to be so in the third position. Non-standard base-pairing might occur between the third bases of the anticodon and of the codon. The degenerate base (third base) is said to be in the wobble position.

Table 4.1 Table of the genetic code

First Letter	Second Letter: U	Second Letter: C	Second Letter: A	Second Letter: G	Third Letter
U	UUU, UUC: Phe; UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr; UAA, UAG: Nonsense	UGU, UGC: Cys; UGA: Nonsense; UGG: Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His; CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile; AUG: Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn; AAA, AAG: Lys	AGU, AGC: Ser; AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp; GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

4.3 Role of Ribosomal RNA

Ribosomal RNA (rRNA) makes up nearly 80% of the total RNA of the cell. It combines with protein to form organelles called ribosomes. Ribosomes are the site of proteinsynthesis. Each ribosome has two subunits; a small and a large subunit. The function of the ribosome is to hold in position the mRNA, tRNA and the associated enzymes involved in protein synthesis until a peptide bond is formed between the adjacent amino acids. The ribosome has the A site (aminoacyl site) and the P site (peptidyl site).

4. Role of transfer tRNA

Transfer RNA (tRNA) acts as an intermediate molecule between the triplet code of mRNA and the amino acid sequence of the polypeptide chain. It is a small molecule with about 80 nucleotides. Each amino acid has its own tRNA which carries a triplet anticodon for the particular amino acid. The anticodon is complementary to a specific codon on mRNA. The 3´ end of the tRNA carries the sequence 5´ -CCA-3´ which is used to attach a specific amino acid.

4.3 The process of translation

The main steps involved in translation may be summarised under the following headings:

(i) Amino acid activation (ii) Binding of tRNA to ribosome (iii) polypeptide chain initiation (iv) chain elongation (v) chain termination (vi) posttranslational modification.

4.3.1 Binding of mRNA to ribosome

The small subunit and the large subunit of the ribosomes unite to make a complete ribosome. The messenger RNA binds to the small subunit of the ribosome. Ribosomes are usually found in clusters linked together by a strand of mRNA. This complex known as a polyribosome or polysome enables several copies of the same polypeptide to be produced simultaneously.

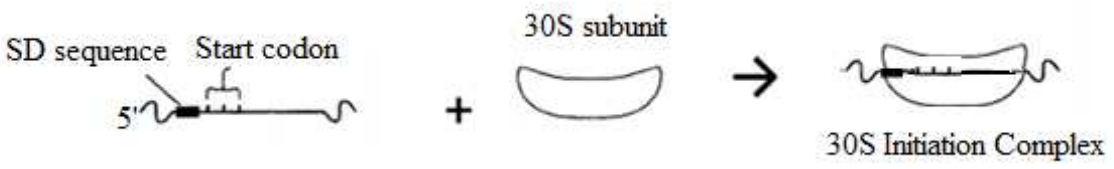


Figure 4.1a. Binding of mRNA to prokaryotic ribosome

4.3.2 Amino acid activation and attachment to tRNA

Each target amino acid is activated by ATP using the enzyme aminoacyl-tRNA synthetase. There is one enzyme for each amino acid. The activated amino acid is then attached to its specific tRNA at the CCA end of the tRNA. After this attachment, the tRNA complex is ready to transport the amino acid to the ribosome.

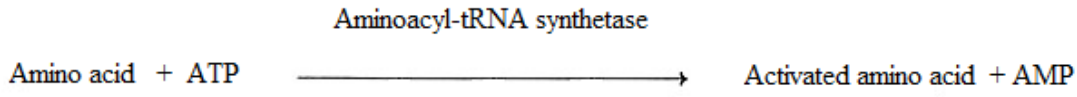


Figure 4.2. Amino acid activation

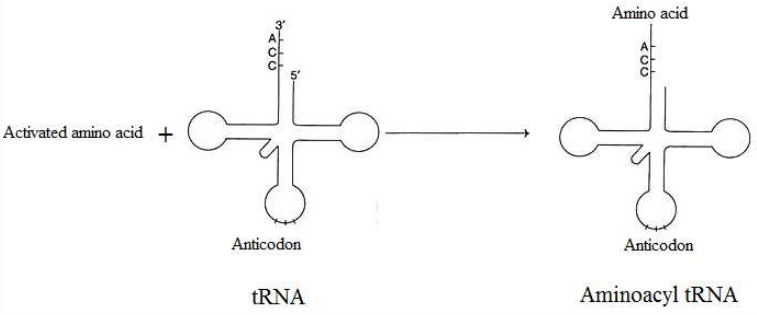
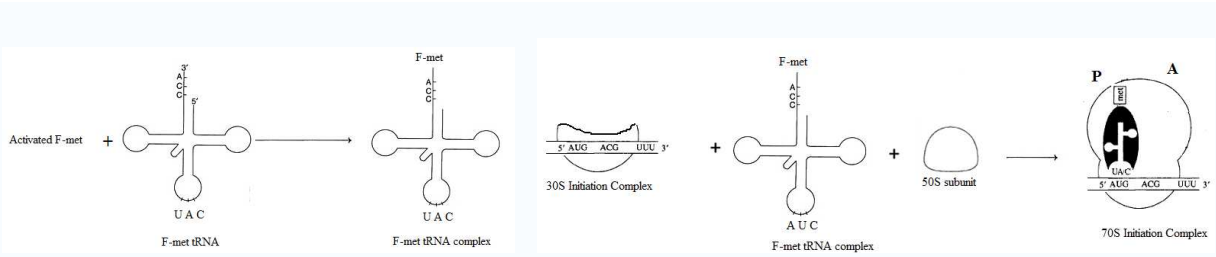


Figure 4.3. Attachment of activated amino acid to its tRNA

4.3.3 Polypeptide chain initiation

The tRNA with the anti-codon UAC for the codon AUG carries formyl methionine (fMet) to the P site of the ribosome/mRNA complex to initiate the polypeptide chain. The AUG is the mRNA start codon and the anticodon on the fmet-tRNA is UAC. Proteins called initiation factors are involved in chain initiation.



(a) (b)

Figure 4.4.(a) attachment of fmet to tRNA, (b) binding of fmet-tRNA complex to the P site

4.3.4 Chain elongation

The second amino acid is brought to the A site by its tRNA. The ribosome then translocates to the right by one codon, releasing the unloaded f-met. Meanwhile the second amino acid forms a peptide bond with fmet and the resulting dipeptide is carried by the second tRNA which is now located in the P site. Then the third amino acid is brought to the A site by its specific tRNA. The amino acids are added one at a time to the growing polypeptide chain as the ribosome moves

down the mRNA strand in the 5' → 3' direction of mRNA. Proteins called elongation factors are involved in chain elongation.

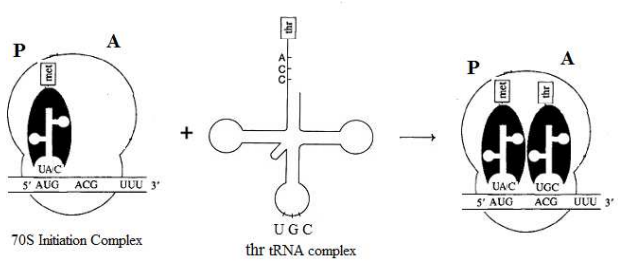


Figure 4.4. Binding of the second amino acid to the A site of a ribosome

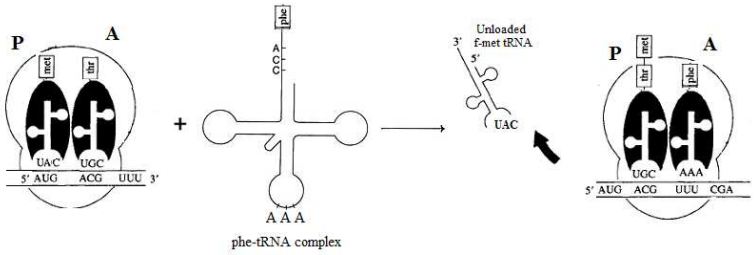


Figure 4.5 Binding of the third amino acid to the A site and translocation of ribosome

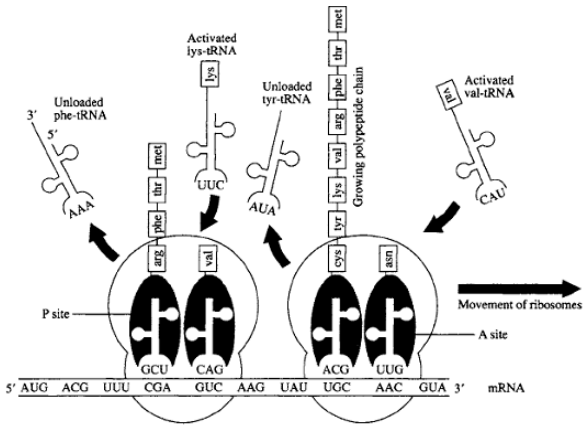


Figure 4.5. Binding of the more amino acids to the growing peptide chain

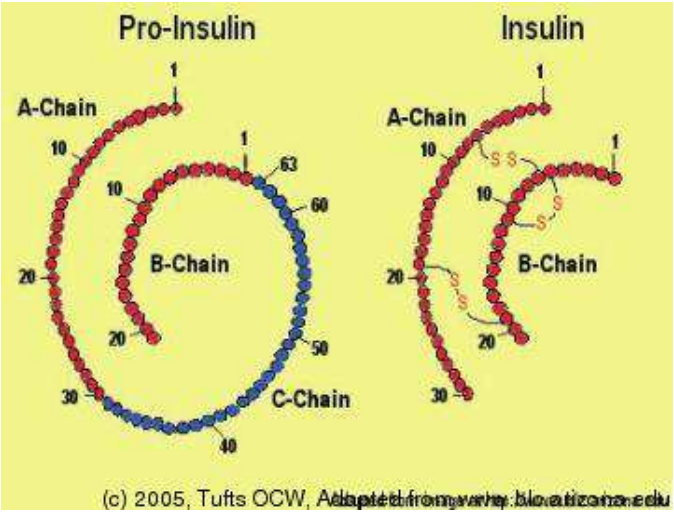
4.3.5 Chain termination

When any of the stop codons (UAA or UAG or UGA) reaches the A site, no tRNA attaches. Instead, proteins called release factors attach to the A-site. The synthesis of the polypeptide chain stops and at this point the polypeptide chain is released. The ribosome is detached from the mRNA and split in to its subunits.

4.3.6 Post-translation modification

In order to achieve its biologically active form, the new polypeptide must fold into its proper three-dimensional conformation. Before or after folding, the new polypeptide may undergo enzymatic processing, including (i) removal of amino acids; (ii) addition of functional groups to certain amino acid residues; (iii) proteolytic cleavage; (iv) and attachment of oligosaccharides.

Insulin is an example of a polypeptide which undergoes modification. It is synthesised as pro-insulin and must be modified for it to become mature insulin.



Another example of a polypeptide which undergoes post-transcriptional modification is trypsin. It is synthesised as an inactive substance called trypsinogen is modified into trypsin which is the active protein digesting enzyme.

5. REGULATION OF GENE EXPRESSION

Objectives: At the end of this topic you should be able to understand

1. The importance of regulation of gene expression
2. Use end product inhibition and the Lac operon system as examples.

5.1 Introduction

Regulation of gene expression (or gene regulation) refers to the mechanisms which the cells use to switch the processes of transcription and translation on and off. Gene regulation is important as it allows an organism to express certain proteins only when they are needed in order to avoid waste of materials and energy. The classical example of a gene regulation system is the lactose operon (lac operon), discovered by Jacob and Monod, in which enzymes involved in lactose metabolism are expressed by *E.coli* only in the presence of lactose and absence of glucose.

An operon is a group of genes which work together under the control of a single promoter. These genes may be expressed (i.e. transcribed and translated) together and their protein products may have related functions.

Gene expression in eukaryotes is more complex than in prokaryotes and its regulation takes place at different levels some of which are:

(i) Transcription of DNA into RNA

(ii) Post transcriptional modification of RNA primary transcripts

(iii)Translation of mRNA into a polypeptide chain

(iv) Post translational modification of the polypeptide chain

(v) Cell differentiation

(vi) Growth and development

Each of these steps represents a potential point at which the expression of eukaryotic genes may be turned on or off.

Up-regulation is a process in which an internal or external signal results in increased expression of one or more genes resulting in increased protein synthesis. Down-regulation is a process resulting in decreased gene and corresponding protein expression.

5.2 Inducible and repressible systems

An inducible system is normally 'switched off' except in the presence of an inducer molecule that switches the system on and allows gene expression to take place. A repressible system is normally 'switched on' except in the presence of a co-repressor molecule that suppresses gene expression.

5.3 The *Lac* operon

The *lac* operon consists of three structural genes which is controlled by a regulator gene. It also works in conjunction with a promoter sequence, an operator sequence and a terminator sequence. The three structural genes of the lac operon are: (i) *lacZ* gene which encodes the enzyme β-galactosidase (that breaks the disaccharide lactose into the monosaccharides glucose and galactose), (ii) *lacY* gene which encodes the membrane-bound protein β-galactoside permease (that pumps lactose into the cell) and (iii) *lacA* gene which encodes the enzyme β-galactoside transacetylase (that modifies lactose).

The *lac* operon is required for the efficient transport and metabolism of lactose in *Escherichia coli* and some related bacteria. In the absence of glucose, the cell can use lactose as an energy source, but it must first produce the enzymes that are needed to mobilise and digest the lactose (also called β-galactoside).

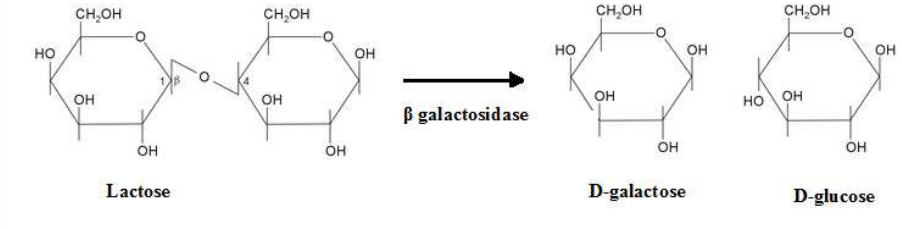


Figure 5.1 Breakdown of lactose into galactose and glucose.

The *lac* operon ensures that the cell uses energy in producing the three enzymes only when necessary. It achieves this by using the repressor molecule, which stops the production of the three enzymes in the absence of lactose. The repressor molecule has two active sites to which either an inducer molecule (lactose) may be attached to 'switch the operator gene on' or a co-repressor (glucose) molecule may be attached to 'switch the operator gene off'. When the operator gene is switched on, the structural genes carry out transcription of mRNA which is then translated into polypeptides. When the operator gene is switched off, no mRNA is transcribed and no polypeptides are synthesized.

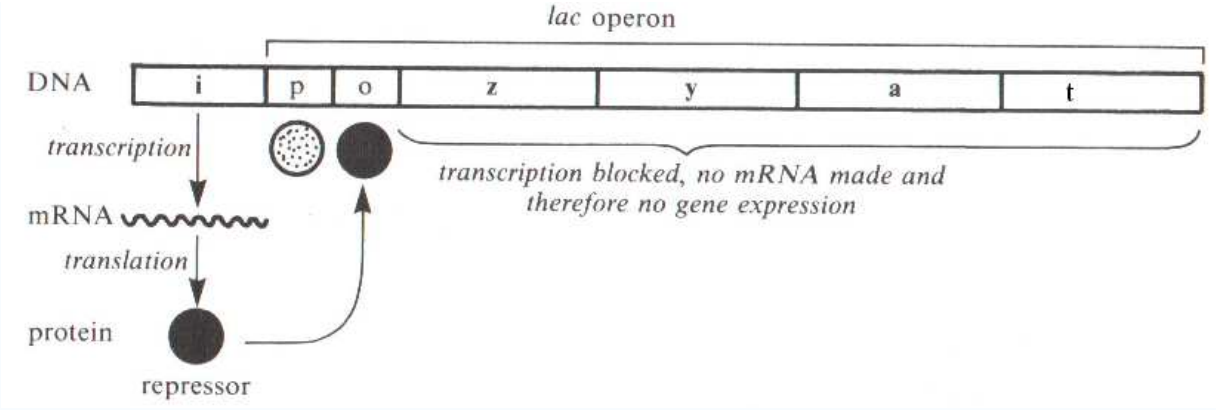


Figure 5.4. The lac Operon in the repressed state. The repressor protein binds to the operator site and prevents transcription. The genes z, y and a are therefore switched off. The symbols are: i= regulator gene, p = promoter site, o =operator site, z, y and a = structural genes, t = terminator sequence (Jones and Karp 1994:239).

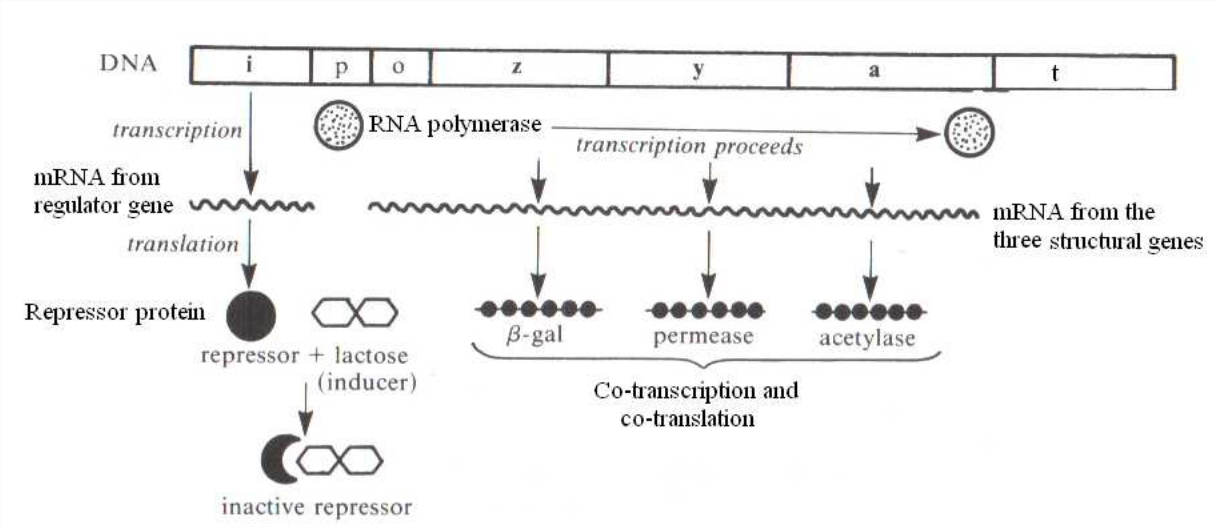


Figure 5.5. The lac Operon in the induced state. When lactose (the inducer) is present, it alters the shape of the repressor which can then no longer bind to the operator. The genes z, y and a are therefore transcribed. . The symbols are: i= regulator gene, p = promoter site, o =operator site, z, y and a = structural genes, t = terminator sequence (Jones and Karp 1994:239).

The lac Operon in the repressed state: The repressor protein binds to the operator site and prevents transcription. The genes z, y and a are therefore switched off and hence are not transcribed.

The lac Operon in the induced state: When lactose (the inducer) is present, it alters the shape of the repressor which can then no longer bind to the operator. The genes z, y and a are therefore transcribed.

5.4 End-production inhibition

When *E. coli* is grown on a glucose medium, the regulator gene produces a repressor molecule which binds the glucose (co-repressor). This makes the repressor molecule active and causes it to bind with the operator gene and switches the whole lac operon off. This is a case of end-product inhibition or repression. It also referred to as a negative feedback control mechanism in which the enzymes for glucose production (from lactose breakdown) are not synthesized because the glucose (end-product) is already there.

6. MUTAGENS

Objectives: At the end of this topic you should be able to understand

(i) The causes of gene mutations
(ii) Give examples of chemical and physical mutagens.
(iii) That viruses are implicated in mutagenesis

Causes of gene mutations

Mutations are either spontaneous or induced. Spontaneous mutations occur naturally and randomly while Induced mutations are caused by factors called mutagens. The three common types of mutagens are (i) physical, (ii) chemical and (iii) biological. Mutagens which cause cancer are also known as carcinogens.

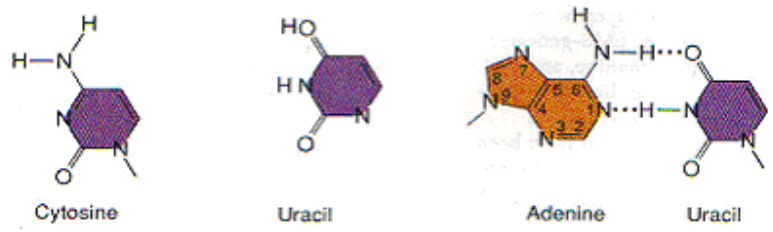
Chemical mutagens

Chemical mutagens include:

(i) Deaminating agents

These mutagens remove amino groups from nitrogenous bases. For example, they may remove the amino group from cytosine thus converting it to uracil.

This may cause change in the base paring from CG through UA to AT as the affected DNA undergoes replication. The overall change is: CG $\rightarrow$ UA $\rightarrow$ TA



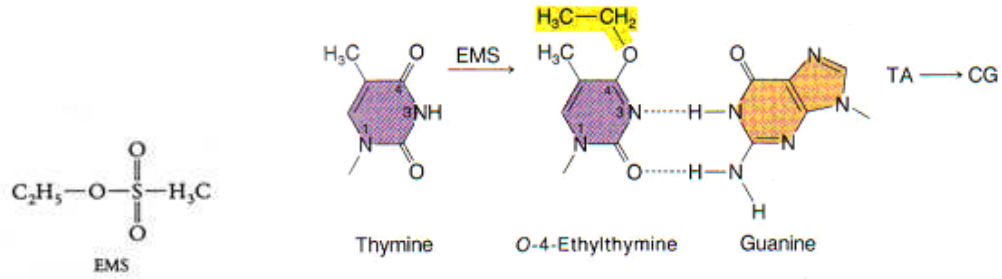
Figures 6.1. Deamination: Nitrous acid (NA) deaminates cytosine to form uracil, which bonds (pairs with adenine) like thymine (Griffiths et al. 1994: 544).

Examples include nitrosamine, nitrous acid and sodium nitrite. Meat reddening is linked to the use of sodium nitrite.

Deaminating agents are linked to gastric cancer.

(ii) **Alkylating agents**

These mutagens add alkyl groups (e.g. CH_3, CH_2CH_3) to nitrogenous bases. This may lead to deletion of such bases especially the purines (A and G) or to mispairing of bases. Examples of alkylating agents include dimethylsulphate, diethylsulphate and ethylmethane sulphate (EMS).



Figures 6.2. Alkylation: Ethylmethane sulphate (EMS) generates ethylation at the O-4 position of thymine. This causes the modified thymine to mispair with guanine leading to TA → GC transition (Griffiths et al. 1994: 543).

Ethylmethylsulphate (EMS) adds an ethyl group to the O-4 position of thymine. This causes the modified thymine to O-4 ethylthymine which pairs with guanine. As the DNA replicate this pairing leads to the change: TA → O-4Eth/G → GC.

Alkylating agents are linked to bladder, bronchial and blood cancer.

(iii) Base analogues

These mutagens are chemicals that look like normal bases and as such confuse the DNA replication system. Examples are 5-bromouracil (5-BU), an analogue of thymine and 2-amino purine (2-AP), an analogue of adenine.

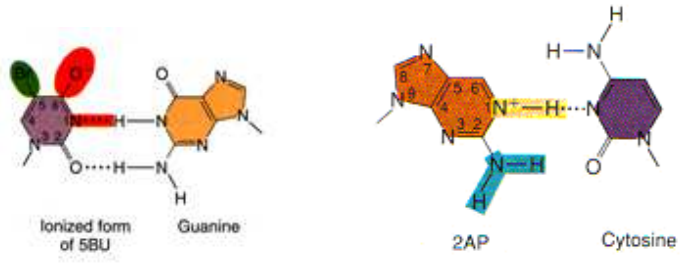


Figure 6.3. Base analogues: 5 bromouracil (5BU) an analogue of thymine pairing with adenine and 2-amino purine (2AP) an analogue of adenine pairing with cytosine (Griffiths et al. 1994: 541-2).

These mutagens cause changes in base pairing. For example 5-BU may pair with guanine and when the affected DNA replicates, it produces the change: TA → 5BU/G → to GC. Similarly 2AP may pair with cytosine leading to the change: AT → 2AP/C → GC.

(iv) Bulky addition products

These are huge molecules which are able to covalently bind to purines in DNA leading to the deletion of such purines from the affected DNA. Aflatoxin B_1 (AFB_1) is a bulk addition mutagen which is also a carcinogen found in stored seed crops such as groundnuts and maize. It is linked to liver cancer and is correlated to Hepatitis B virus occurrence.

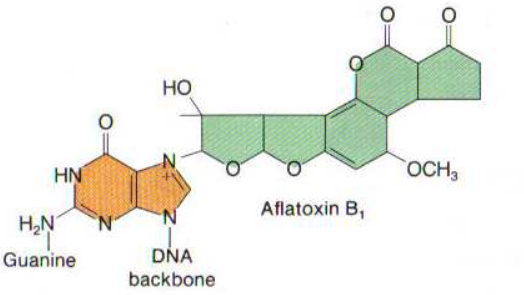


Figure 6.4. Bulky addition: The binding of Aflatoxin B_1 to guanine (Griffiths *et al*. 1994:546).

For example the attachment of AFB1 to guanine will cause the deletion of the affected guanine from the DNA leaving an apurinic site. The repair system usually inserts adenine in the site of deletion. This leads to the change: CG → C*_ → CA → TA

(v) Intercalating agents

These molecules have three rings and resemble and mimic base pairs. They are able to insert themselves (intercalate) between base pairs in the affected DNA double helix, thereby causing frame shift mutations. Examples of intercalating agents include proflavin (which is used as a

topical antibacterial and urinary antiseptic), acridine orange (which is a dye used in the laboratory detection of *Mycobacterium*) and ethidium bromide (which is used as a fluorescent dye in agarose gel electrophoresis in PCR).

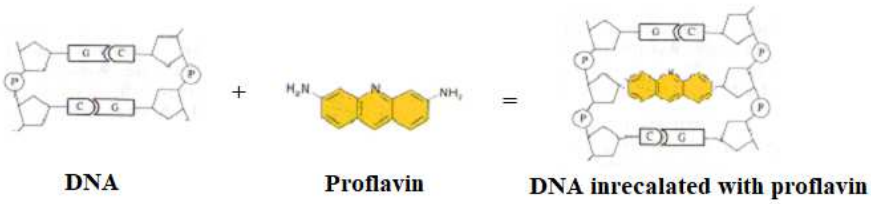


Figure 6.5. Intercalating agent: The structure of proflavin and the intercalation of proflavin between the nitrogenous bases stacked at the centre of the DNA molecule (Griffiths *et al*. 1994: 544).

Physical mutagens

The most important physical mutagens are U.V. and ionization radiations. UV radiation generates pyrimidine (thymine) dimers which cause mutations and cancer (melanomas and keratosis).

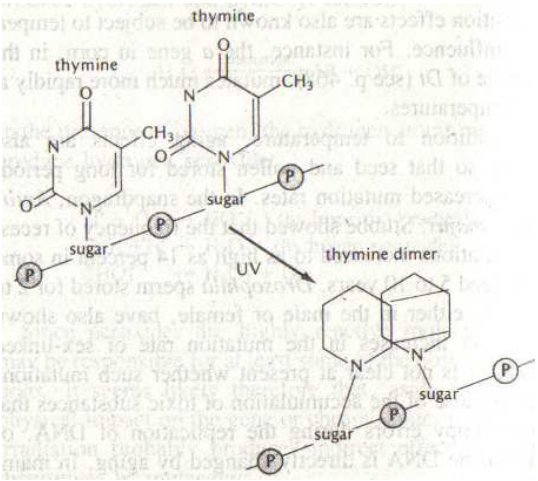


Figure 6. 6. Formation of a thymine dimer due to UV radiation (Strickberger 1985: 491).

Ionization radiations include X-rays, gamma rays, neutrons and alpha rays. These radiations lead to the generation of free radicals which cause breaks in the DNA. These breaks may result into deletions and other types of mutations in the affected DNA.

Biological mutagens

These include viruses, transposable elements and some bacteria.

Mutagenic viruses

Viruses are also potential mutagens. In instances of some acquired viral infections, the virus attaches to the cell, transfer its genetic material into the cell thus altering the original gene, causing mutation. Hepatitis B virus is an example of a DNA viral mutagen while Hepatitis C virus is an example of an RNA virus. These viruses cause cancer in liver. Other examples of viral mutagens are Human herpes virus, SV40 virus and Human Papilloma virus.

Transposable elements

A transposable element is a segment of DNA which is able to move from one position to another in the same chromosome or from one chromososme to another. When it is inserted into a new position on chromosomal DNA, it disrupt the function of the genes.

Mutagenic bacteria

Some bacteria such as *Helicobacter pylori* cause inflammation during which oxidative species are produced, causing DNA damage by reducing efficiency of DNA repair systems thereby increasing mutation.

7. GENE MUTATIONS

Objectives: At the end of this topic you should be able to

1. Understand that mutations may be spontaneous or may be induced (caused by mutagens).
2. Understand the various types of point mutations which include deletions, insertions and substitutions.
3. Use sickle cell anaemia as an example of a point mutation.

Mutation

A mutation is a change in the amount, arrangement or structure of the DNA of an organism. This change usually affects the phenotype and may be inherited by daughter cells from the mutant mother cell.

Gene or point mutation is a mutation due to a change in one a gene. It is usually a change in a single nucleotide base but may involve two or more bases. In this type of mutation new alleles of a gene are produced and this change is then transcribed into RNA and finally translated into a protein. Gene mutations may be spontaneous (natural) or may be induced (caused by external agents called mutagens). The various types of gene mutations include; (i) addition or insertion, (ii) deletion and (iii) substitution.

Insertions and deletions (Indels)

These are mutations which involve the insertion (addition) and deletion of bases in the DNA sequence. Insertion or deletion of a single nucleotide or several nucleotides which are not multiples of three will result into a frame shift in the genetic code. Frame shifts can lead to missense or nonsense mutations and most of them have negative effects on the affected individual.

Substitution

Substitution involves the replacement of one base by another base in the sequence. There are two types of substitution, i.e. (i) transitional substitution and (ii) transverse substitution.

Transitional substitution

In transitional substitution, a purine is replaced by another purine or a pyrimidine replaced by another pyrimidine. Transitional substitutions have very little or no effect on the type of protein synthesized.

Transverse substitution

In transverse substitution, a purine replaces a pyrimidine or vice-versa. Such a change will have significant negative effects on the type of protein synthesized and in many cases the protein is non-functional or inactive.

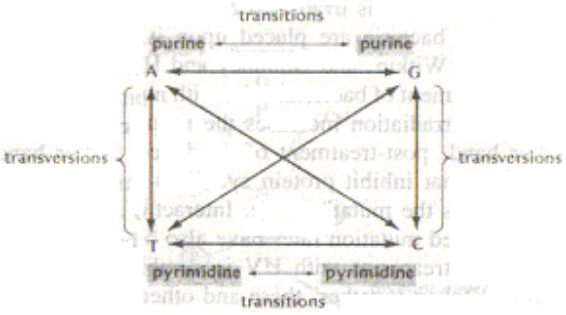


Figure 7.1. Types of possible substitutions showing the model designating purine and pyrimidine changes (Strickberger 1985).

Sickle cell anaemia: One common example of transverse substitution in humans is sickle cell haemoglobin (Hb^S) which causes a disease called sickle cell anaemia. The normal haemoglobin (Hb^A) has glutamate at position 6 of the beta-heamoglobin chain while Hb^S has valine at the same position. This difference is due to a change from the codon GAA (for glutamate) to the codon GUA (for valine). Glutamate is hydrophilic and is able to stick out into the aqueous medium of the blood allowing the red blood cells to have their normal shape. Valine on the other hand is hydrophobic and tends to stick away from the aqueous medium of the blood leading to the sickle shape in the folding of the red blood cells.

Table 7.1. Illustration of point mutations

Type of mutation	**Triplet code**						
Wild type	ACC Thr	CAC His	UCU ser	GGA gly	AUU ile	GAA glu	GCA Ala
Same silent/null mutation	ACC Thr	CAU His	UCU ser	GGA gly	AUU ile	GAA glu	GCA Ala
Missense mutation	ACC Thr	CAC His	UCU ser	GGA gly	AUU ile	GUA val	GCA Ala
Neutral mutation	ACC	CAC	UCU	GGA	GUU	GAA	GCA

	Thr	his	ser	gly	val	glu	Ala
Nonsense mutation	ACC Thr	CAC his	UCU ser	UGA stop	AUU ---	GAA ---	GCA ---
Negative (-1) Frame shift mutation Deletion of C at position 4	ACC Thr	ACU thr	CUG leu	GAA glu	UUG	AAG	CA
Positive (+1) Frame shift mutation Insertion of A at position 6	ACC Thr	CAA gln	CUC leu	UGG Trp	AAU Ile	UGA stop	AGC ---

(i) Silent or null mutation is when the same amino acid is coded for; e.g. if CAC is changed to CAU, histidine will still be translated.

(ii) A neutral mutation is where the protein will not change much because the amino acids coded for are functionally the same. If AUU (hydrophobic isoleucine) is replaced by GUU (hydrophobic valine).

(iii) A missense mutation causes the substitution of one amino acid for another functionally different amino acid; e.g. when GAA (hydrophilic glutamate) changes to GUA (hydrophobic valine).

(iv) Nonsense mutations occur when a codon mutates into UGA, UAA or UAG stop codons.

(v) Frame shift mutations occur when the reading frame on the mRNA is disturbed. They are caused by the deletion (negative frame shift) or insertion (positive frame shift) of bases in the DNA.

8. CHROMOSOMAL THEORY OF INHERITANCE

Objectives: At the end of this topic you should be able to:

1. Outline the series of events which led to the discovery of chromosomes
2. Link Chromosome bahaviour to inheritance of genetic characteristics
3. Describe the molecular structure of chromosomes

8.1 Discovery of chromosomes

The study of chromosomes and their behaviour is called cytogenetics.

Gregor Mendel an Austrian Priest and researcher proposed two laws of heredity.

Charles Darwin an English researcher believed that ‘gemmules’ traveled from every body part to the sexual organs, where they were stored.

Walther Flemming a German biologist discovered the presence of threadlike structures in the cell nucleus which were able to absorb basic dyes and became coloured. These were called chromatin or chromosomes (coloured body). He also studied the behaviour of the chromosomes in cells undergoing cell division (mitosis).

Theodor Boveri, a German embryologist discovered that the number of chromosome was reduced in the gametes during meiosis.

Walter Sutton, an American scientist observed that the behavior of chromosome undergoing meiosis during gamete formation was consistent (in agreement) with Mendel's second law of heredity. "I may finally call attention to the probability that the association of paternal and maternal chromosomes in pairs and their subsequent separation during the reducing division as indicated above may constitute the physical basis of the Mendelian law of heredity." Sutton, 2002.

Thomas Morgan, an American scientist together with his colleagues (who included his wife) provided physical evidence to link behaviour of chromosomes to an inherited characteristic. Their experiments showed very clearly that the rare white color of the eyes in *Drosophila* was due to a mutation of a gene responsible for eye color which is located on the X chromosome of *Drosophila*.

Summary

The chromosome theory of inheritance was the collaborative result of multiple researchers working over many years. The first ideas were started 1860s, when Gregor Mendel and Charles Darwin each proposed possible systems of heredity. Later on Walther Flemming discovered chromosomes and described their behavior during mitosis. The idea of a connection between chromosomes and heredity was strengthened by research done by Theodor Boveri and Walter Sutton, but direct evidence in support of chromosome theory came from Thomas Hunt Morgan's experiments with fruit flies (*Drosophila*). Therefore, after nearly 50 years of speculation, scientists were finally able to confirm that chromosomes were indeed the physical carriers of hereditary information.

8.2 Chromosome structure

In prokaryotes, genes are located in the cytoplasm either on genomic (chromosomal) DNA or on extra chromosomal (plasmid) DNA.

In Eukaryotes genes are either located in the nucleus on chromosomal DNA or in the cytoplasm on mitochondrial and/or chloroplast DNA.

Relationship between chromosome and DNA: Chromosomes are the carriers of the genes and represent the material basis of inheritance. Chemical analysis shows that chromosomes carry DNA which is the genetic material.

DNA stores and transmits genetic information from one generation to another. It remains constant in a given species and is a large, stable macromolecule which is not metabolised.

Prokaryotic (bacterial) chromosomes are made up of naked double-stranded DNA located in their cytoplasm.

In most eukaryotes the nucleus of each cell contains several pairs of homologous chromosomes which contain DNA tightly bound to proteins. Eukaryotic chromosomes are made up of equal amounts of DNA and proteins (basic histones and acidic non-histones). There are many kinds of acidic proteins but only five basic histones (H_1, H_{2A}, H_{2B}, H_3 and H_4) are common to most species.

The ‘beads’ seen when chromosomes are observed under a microscope during interphase are the nucleosomes which consist of an octamer (group of 8 molecules) comprising two molecules each of histones H_{2A}, H_{2B}, H_3 and H_4 around which DNA is wrapped ($1^1/_3$ turns). Histone 1 is involved in the packaging of nucleosomes into the solenoid fibre (**Figure 8.1**).

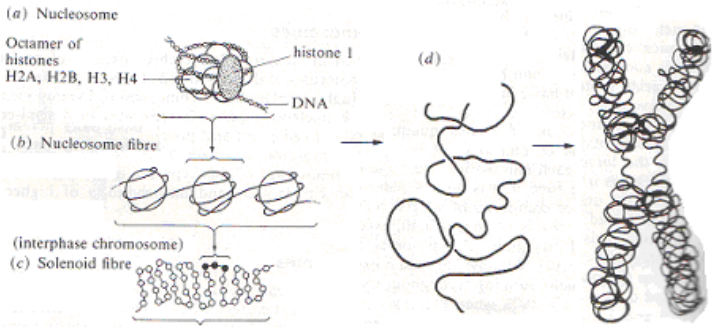


Figure 8.1. The arrangement of DNA in the chromosome (Jones and Karp, 1994:181-183).

The large amount of DNA in eukaryotic chromosomes creates a problem of packaging and organization. The chromosomes must allow for gene activity and must be able to replicate during cell division (mitosis and meiosis).

The chromosomes are in the extended position during the resting phase and DNA replication but are tightly packed and shortened during cell division.

When the chromosome is loosely coiled, during interphase, the turns that the DNA makes about the nucleosome (octamer) give a packing ratio of 10:1 and further coiling into the solenoid fibre gives a ratio of 100:1 is achieved.

When the chromatin is further condensed by super coiling and folding at metaphase, the packing ratio increases up to 10 000:1.

Genes and alleles

The gene: A gene is a sequence of nucleotide pairs along a DNA molecule, which codes for a ribonucleic acid (RNA) or a polypeptide product. Genes which code for polypeptides are divided into two main classes. These are: (1) structural genes, which code for functional proteins such as enzymes, hormones, membrane proteins, antibodies, storage proteins, etc., and (2) regulatory genes, which serve to control or regulate the activity of other genes

The allele: In diploid organisms the chromosomes exist in homologous pairs with one set coming from the mother and the other from the father. Each member of a homologous pair carries one of the alleles of each character (gene) at corresponding positions called loci (sing. locus) along its length.

An allele is a particular form of a gene occupying a fixed locus (place) on a chromosome.

Most genes have two alleles (allelic forms).

9. CELL CYCLE

OBJECTIVES: At the end of this topic you should be able to:

(i) Explain the importance of cell division

(ii) Describe the various stages of cell cycle

INTRODUCTION

The cell cycle is the series of events that take place in a cell leading to its division and duplication (replication). It is the period from the beginning of one cell division to the beginning of the next cell division. The cycle may be presented as a circle (Figure 8.1).

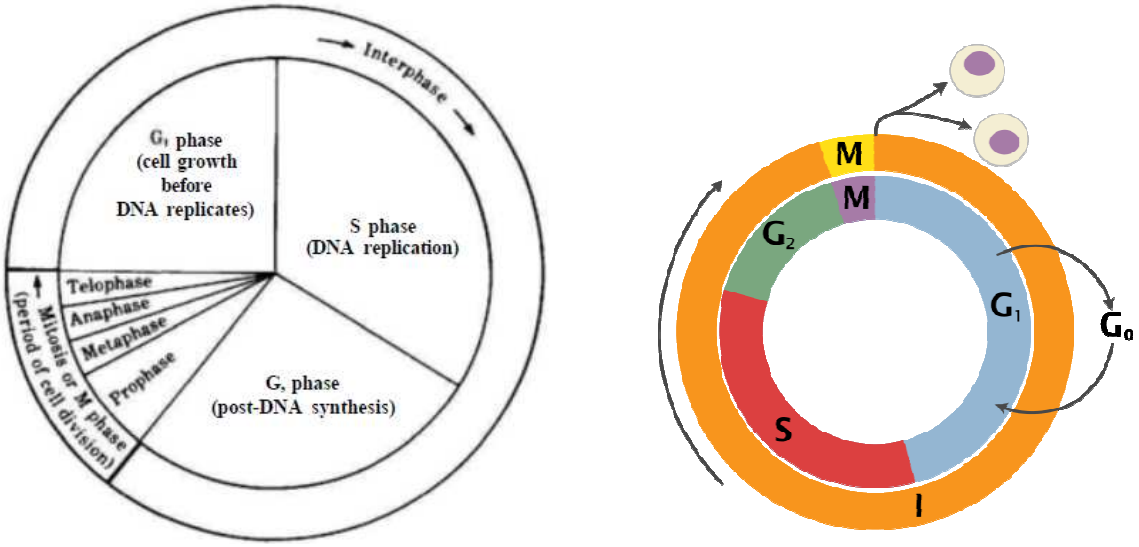


Figure 9.1 Diagram of a typical cell reproductive cycle

The time it takes to complete one cycle is called the generation time and it varies according to the type of cell involved and according to environment of the cells.

In eukaryotes, the cell cycle can be divided in two periods: (i) interphase during which the cell grows by synthesizing molecules needed for duplicating its DNA, (ii) the mitotic (M) phase during which the cell splits into two separate "daughter cells".

Cell division involves two major processes; karyokinesis (division of the nucleus) and cytokinesis (division of the cytoplasm). In some cases a cell may only undergo nuclear division without cytokinesis resulting in a cell with two or more nuclei as seen in muscle cells. The lengths of these phases differ from one cell type to another. Normal mammalian cells growing in tissue culture, for example, usually require 18-24 hours at 37°C to complete their cell cycle.

8.1 INTERPHASE

Interphase, also known as the preparatory phase, takes place before mitosis and cytokinesis. Before a cell can enter cell division, it needs to synthesize different molecules. All of the preparations are done during the interphase which is in three stages; (i) G_1, (ii) S, and (iii) G_2. During interphase, the nucleus and cytoplasm do not divide but the cell merely prepares for division.

Gap 1 phase- G_1

G_1 is the growth phase and is the first part of interphase, from the end of the previous M phase until the beginning of DNA synthesis. During this phase the biosynthetic activities of the cell take place at a high rate. This phase uses the 20 amino acids to form millions of enzymes and other proteins that are required in the S phase for DNA replication. The duration of G_1 is highly variable but is relatively long.

Synthesis phase - S

The S phase starts with DNA replication and when it is complete, all of the chromosomes have been replicated. This means each chromosome will have two sister chromatids. Thus, during this phase, the amount of DNA in the cell is doubled. This phase is completed very quickly to avoid damage to the exposed base nucleotides which are sensitive to mutagens. DNA synthesis starts at several positions on each chromosome thereby reducing the time required to replicate the whole chromosome.

Gap 2 phase - G_2

This is also called the post- DNA synthesis phase. It is the gap between DNA synthesis and mitosis during which the cell will continue to grow. The cell makes sure that everything is ready for it to enter the M (mitosis) phase.

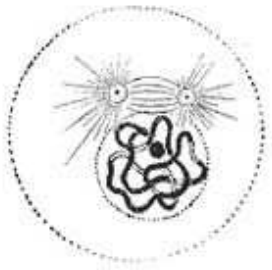
8.2 MITOSIS

The period of the cell cycle when the cell is undergoing division is called the mitotic phase (M phase). It is the process by which the chromosomes of the cell nucleus a cell separate into two identical sets and end up in two separate nuclei. It is a form of karyokinesis (nuclear division) which is followed by cytokinesis (cell division) in which the nucleus and cytoplasm, organelles, and cell membrane are divided into roughly equal amounts. The process of mitosis is fast and quite complex. It is divided into (i) prophase, (ii) metaphase, (iii) anaphase and (iv) telophase. The times spent in each of these phases are quite different but prophase usually requires longer durations than the other phases and metaphase is the shortest.

Mitosis is important for the maintenance of the chromosomal set; each daughter cell receives chromosomes that are identical in composition and equal in number to the chromosomes of the mother cell.

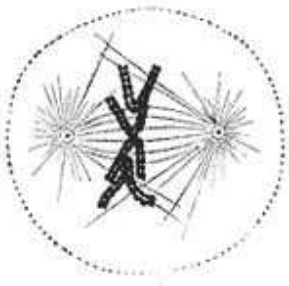
Mitosis is important for the following functions:

(i) Growth and development
(ii) Cell replacement and tissue repair e.g. in the skin and digestive and red blood cells
(iii) Tissue and organ regeneration: e.g , starfish regenerate lost arms through mitosis
(iv) Asexual reproduction e.g budding in yeast and hydra and vegetative propagation in plants.



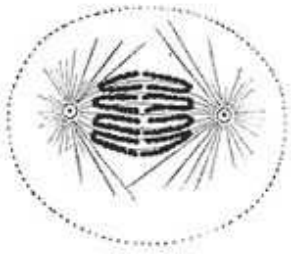
Prophase

The chromatin is condensing into chromosomes.



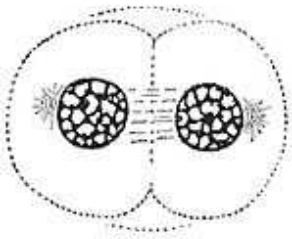
Metaphase

The chromatids align at the metaphase plate.



Anaphase

The chromosomes split and the kinetochore microtubules shorten



Telophase

The decondensing chromosomes are surrounded by nuclear membranes. Cytokinesis has already begun; the pinched area is known as the *cleavage furrow*.

Figure 8.2. Diagrams of mitosis in a hypothetical cell.

Prophase: Chromosomes become condensed by coiling and thickening. The chromosomes have replicated into chromatids but are still attached at the centromeres. The nuclear membrane disappears.

Metaphase: Chromatids align at the metaphase plate (equatorial region) and are still attached at the centromeres.

Anaphase: Chromatids split into separate chromosomes and the two sets migrate to the opposite poles of the cell.

Telophase: Chromosomes uncoil and become thin. The nuclear membrane reappears and each set of chromosomes is enclosed by a separate nuclear membrane. Cytokinesis has already begun.

Cytokinesis: In animals, the cell separates into two through a pinched area known as the *cleavage furrow* while in plants the cells separates into two by the cell plate. At the end of mitosis two daughter cells are produced and each of them contains the same number of chromosomes as the mother cell.

Role of centrioles and microtubules (spindle fibres): Microtubules (spindle fibres) which found in both plant and animal cells hold the chromosomes in place and also help with the separation of the chromatids into chromosomes. Centrioles which are present in animal cells but not in plant cells help to organize the spindle fibres during cell division.

Rapidly dividing cells: In humans, examples of rapidly dividing cells are those in the epithelium of the digestive track, in the skin and the stem cells that are used to produce blood cells. The rate at which cells divide is genetically controlled to prevent abnormal division, apoptosis and cancerous tumours.

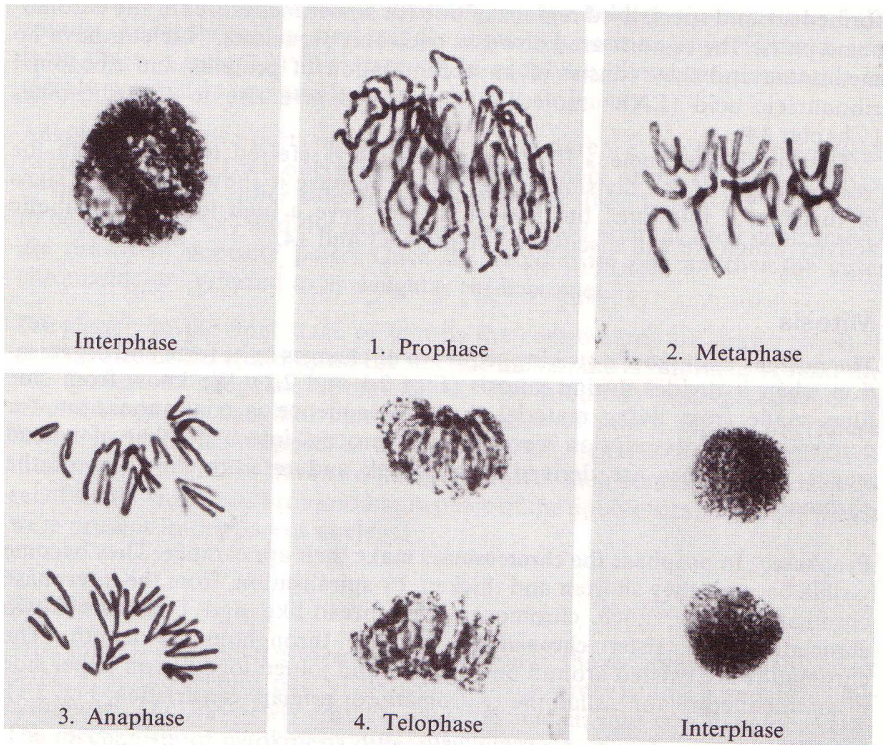


Figure 8.3. Photographs of mitosis in root meristems cells of the onion (*Allium cepa*, 2n = 2x = 16) in its somatic cell nuclei. Interphase nuclei, as they appear just before and just after mitosis are also shown.

9. Meiosis

OBJECTIVES: At the end of this topic you should be able to:

(i) Describe the various stages of Meiosis I and II

(ii) Explain the significance of meiosis

(iii) Compare and contrast mitosis and meiosis

INTRODUCTION

Meiosis is a type of cell division which is involved in the production of gametes that are involved in sexual reproduction. The male gametes are generally called sperm and the female gametes are generally called eggs. Meiosis is not a cycle like mitosis because the end products (gametes) must first undergo fertilization to produce a zygote which develops into an individual organism. This individual will then produce gametes which will undergo the same fate.

Meiosis occurs only in the specialized cells (germ line) of the reproductive organs (gonads). In animals, the testes are male gonads and the ovaries are female gonads. Gametes contain the haploid number (n) of chromosomes, but originate from diploid (2n) cells of the germ line. Meiosis ensures that the number of chromosomes is reduced by half during gamete formation in order to maintain the chromosome number of the species after fertilization.

Meiosis involves a single DNA/chromosome replication and two divisions of the cytoplasm (meiosis I and meiosis II). The DNA/chromosome replication takes place during interphase before meiosis I.

8.2.1 MEIOSIS I

The first meiotic division is a reductional division that produces two haploid cells from a single diploid cell. Meiosis I consists of four major phases (i) prophase I, (ii) metaphase I, (iii) anaphase I and (iv) telophase I.

Prophase I

Prophase I differs from the prophase of mitosis in that homologous chromosomes come to lie side by side in a pairing process called synapsis. Each pair of chromosomes is called a bivalent (2 chromosomes) with each chromosome consisting of two identical sister chromatids. A bivalent may also be called a tetrad (4 chromatids). During synapsis, non-sister chromatids (one from each of the paired chromosomes) of a tetrad may cross over and exchange portions. The point of exchange is called a chiasma (plural = chiasmata). Therefore, at a given chiasma, only two of the four chromatids cross over in a random manner. Generally, the longer the chromosome, the higher the number of crossovers.

Metaphase I

During metaphase I, the bivalents align themselves at random on the equatorial plane. This random orientation promotes independent assortment of the chromosomes and their genes.

Anaphase I

During anaphase I, the centromeres do not divide, but continue to hold sister chromatids together. Because of crossovers, sister chromatids may no longer be genetically identical. Homologous chromosomes (each consisting of 2 sister chromatids) separate and move to opposite poles. This movement reduces the chromosome number from the diploid (2n) condition to the haploid (n) condition.

Telophase I

The first meiotic division effectively ends when the chromosomes arrive at the poles. Each daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids. The nuclear membrane reappears and surrounds the haploid set of chromosomes. The chromosomes uncoil back into chromatin.

Cytokinesis in telophase I divides the diploid mother cell into 2 haploid daughter cells. This involves the formation of a cleavage furrow in animal cells or the formation of the cell plate in plant cells, occurs. Sister chromatids remain attached during telophase I.

INTERKINESIS

The period between the first and second meiotic divisions is called interkinesis or interphase II. The DNA does *not* replicate during interkinesis.

8.2.3 MEIOSIS II

The second meiotic division (meiosis II) is an equational division (mitosis-like), in which sister chromatids of the haploid cells are separated). This process is similar to mitosis, though the cells produced have half the number of chromosomes. Meiosis II also consists of four major phases (prophase II. metaphase II, anaphase II, and telophase II).

Prophase II

In prophase II, the nuclear membrane disappears and the spindle fibres reappear. The chromatids shorten and thicken in readiness for the second meiotic division.

Metaphase II

At metaphase II, the individual chromosomes line up on the equatorial plane. The new equatorial metaphase plate is rotated by 90 degrees compared to meiosis I plate and is therefore perpendicular to the metaphase I plate.

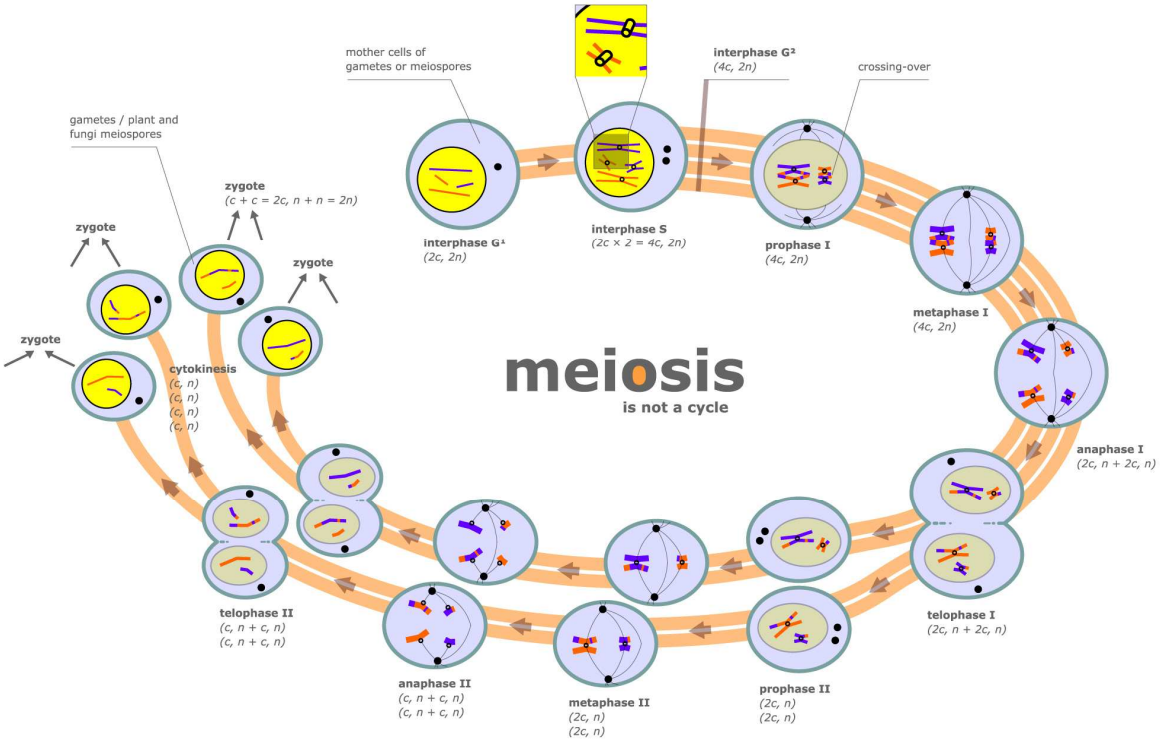
Anaphase II

During anaphase II, the centromeres of each chromosome divide, allowing the sister chromatids to be pulled apart in an equal division (mitosis-like) by the spindle fibres. The sister chromatids which are now called chromosomes move toward opposing poles.

Telophase II

During **telophase II**, which is similar to telophase I, the chromosomes uncoil and lengthen. The spindle fibres disappear and the nuclear membrane reforms and there is formation of either a cleavage furrow (in animal cells) or cell plate (in plants) producing a total of four daughter cells, each with a haploid set (half the number) of chromosomes.

In the end, one diploid mother cell divides into four haploid daughter cells as a consequence of meiosis I and meiosis II.



A diagram of the meiotic phases

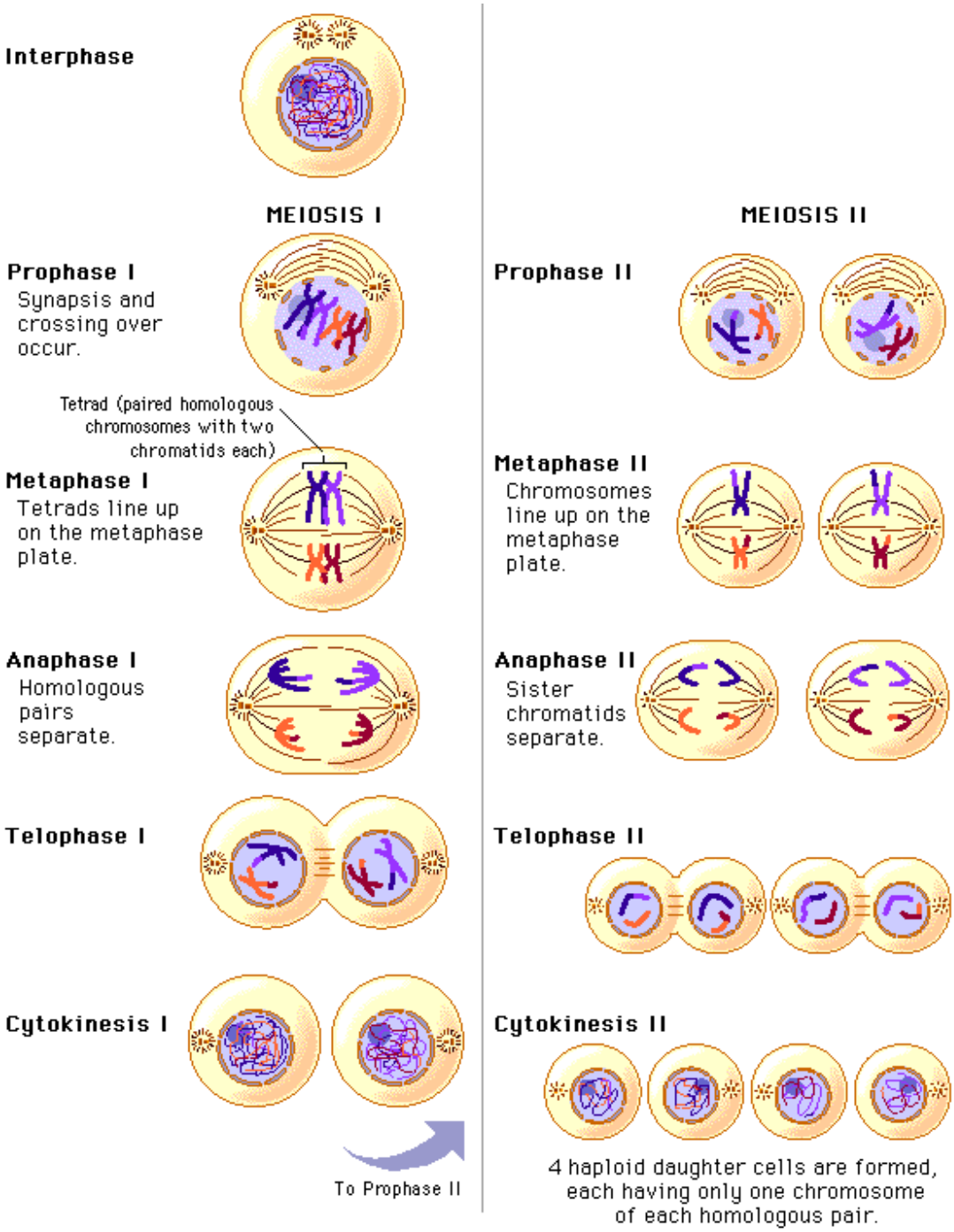

Interphase
MEIOSIS I
MEIOSIS II
Prophase I
Synapsis and crossing over occur.
Prophase II
Tetrad (paired homologous chromosomes with two chromatids each)
Metaphase I
Tetrads line up on the metaphase plate.
Metaphase II
Chromosomes line up on the metaphase plate.
Anaphase I
Homologous pairs separate.
Anaphase II
Sister chromatids separate.
Telophase I
Telophase II
Cytokinesis I
Cytokinesis II
To Prophase II
4 haploid daughter cells are formed, each having only one chromosome of each homologous pair.

Table 8.1. Characteristics of Mitosis and Meiosis

Mitosis	Meiosis
). An equational division that separates sister chromatids	1. The first stage is a reductional division which separates homologous chromosomes at first anaphasc; sister chromatids separate in an equational division at second anaphasc
2. One division per cycle, i.e., one cytoplasmic division (cytokinesis) per equational chromosomal division	2. Two divisions per cycle, i.e., two cytoplasmic divisions, one following reductional chromosomal division and one following equational chromosomal division
3. Chromosomes fail co synapse; no chiasmata form: genetic exchange between homologous chromosomes does not occur	3. Chromosomes synapse and form chiasmata; genetic exchange occurs between homologucs
4. Two products (daughtercells) produced percycle	4. Four cellular products (gametes or spores) produced per cycle
5. Genetic content of mitotic products are identical	5. Genetic content of mciotic products are different; centromeres may be replicas of either maternal or paternal centromeres in varying combinations
6. Chromosome number of daughter cells is the same as that of the mother cell	6. Chromosome number of meiotic products is half that of the mother cell
7. Mitoiic products are usually capable of undergoing additional mitotic divisions	7. Meiotic products cannot undergo another mciotic division although they may undergo mitotic division
8. Normally occurs in most all somatic cells	8. Occurs only in specialized cells of the germ line
9. Begins at the zygote state and continues through the life of the organism	9. Occurs only after a higher organism has begun to mature; occurs in the zygote of many algae and fungi

10. INTRODUCTION TO GENETICS

Objectives: At the end of this topic you should be able to

1. Define genetics and explain its significance
2. Understand the concept of gene expression and the effect of the environment on gene expression.

Genetics is defined as the study of variation and heredity. It is concerned with the generation of variation and the maintenance of this variation in a population.

Applications of genetics: Genetics can be applied in medicine, pharmaceutical industry, forensics, conservation, etc.

The genotype: This is the genetic constitution (make-up) of an individual. It shows the types of alleles present in the individual. E.g. TT is the genotype for homozygous tall pea plants, Tt = heterozygous tall, tt = homozygous short.

The phenotype: This is the physical appearance or function of an organism as a result of its genotype and its environment. The phenotype results from the expression of the genetic information through the protein (polypeptide) product. E.g. the two phenotypes for height in pea plants are tall and short.

Dominant allele

A dominant allele is an allele that masks (hides) the expression of another allele of the same gene. A dominant allele produces a dominant phenotypic character. E.g. the allele (T) for tall height in pea plants is dominant to the allele (t) for short height (T >t). TT and Tt plants will both have the tall phenotype.

Codominant alleles

Codominant alleles are contrasting alleles which are both expressed in a heterozygote (F_1). The heterozygote with such alleles exhibits the relevant characteristics of both parents. E.g. Coat colour of cattle is determined by two alleles R (red) and W (white). When red (RR) and white (WW) cattle are crossed, they produce roan (RW) offspring, which possess both red and white hairs on their skin;

Incompletely dominant alleles

A heterozygote with incompletely dominant alleles will exhibit an intermediate between the two homozygous parental phenotypic characters. E.g. (i) The flower colour in the four o' clock plant (*Mirabilis jalapa*) in which the F_2 generation has three genotypes; RR (red), RW (pink) and WW (white);

Recessive alleles

A recessive allele is one whose effect is masked (hidden) by the presence of a dominant allele of the same gene. A recessive character is only expressed by the recessive alleles in their homozygote state. E.g. (i) The allele (t) for short height in the pea plant is recessive to the allele for tall height. Plants with genotype tt will be short, TT and Tt plants will be tall (T > t).

Lethal genes

Lethal genes are genes that cause death of the individual possessing them either in the prenatal or postnatal period prior to maturity. Lethal genes usually cause death when they are in the homozygous state. E.g. (ii) The Achondroplastic dwarfism allele (A) in man is a dominant lethal allele in which AA individuals are aborted, Aa individuals are dwarf and aa individuals are normal.

Environmental effects on gene expression

The expression of certain genes is influenced by the environment in which they are expressed. The environmental may enhance or inhibit the expression of certain genes. For example height in humans is influenced by the diet and general health of the individual. Studies using identical twins have shown that although the twins carry the same gene, their phenotypes can be different if they are raised in different environments.

11. CLASSICAL MENDELIAN GENETICS I

Expected learning outcomes

At the end of this topic you should be able to understand Mendel's garden pea experiments and statistics: Punnet square; Monohybrid crosses; phenotype, genotype, homozygote, heterozygote, dominance, incomplete dominance, codominance, recessive gene; P, F_1 and F_2 generations; test cross and back cross. Mendel's First Law.

Introduction

Gregor Mendel is called the father of genetics because he was the pioneer of this field of biology. He observed that certain characters of the garden pea plant had **two alternative forms**. He saw that some plants bred true for one character while the other plants bred true for the alternative character. Mendel used the seven characters of garden peas as shown in Table 10.1.

Table 11.1 Characters and their alternative forms in the garden peas used by Mendel

#	**Character**	**Alternative Forms**
1	Length of stem	Tall or dwarf
2	Shape of seed	Round or wrinkled
3	Colour of cotyledons	Yellow or green
4	Colour of seed coat	Grey or white
5	Shape of pods	Inflated or constricted
6	Colour of pods	Green or Yellow
7	Position of flower	Axial or terminal

Suitability of the pea plant for study of inheritance

The seven characters chosen are constant and easily recognizable. For example, the flowers are clearly visible and the parts are easily distinguishable. The characters have contrasting traits (forms) e.g. smooth vs wrinkled seeds. The hybrids are fertile and both cross and self-fertilization are possible. Self-pollination is naturally favoured because the reproductive parts of the flowers are covered by the keel (two petals) and only opens after pollination has been completed (cleistogamy). Cross-pollination is possible by emasculation which involves the opening of the young bud of the female parent plant,, removal of the keel and stamen using forceps and dusting the stigma with pollen from a specific male parent plant. The pea seeds are cheap, readily available, take up little space, have a short generation time and produce many offspring.

Mendel's experiments

To study the inheritance of stem height, Mendel crossed tall plants with short plants and got hybrid plants which were all tall. He then self-pollinated the hybrid tall plants among themselves and noticed that they produced a mixture of tall and short plants. He also used the other characters and got similar results. In other experiments he studied the simultaneous inheritance of two characters (such as seed shape and seed colour) and observed that the two forms of each character are inherited independently of each other. From this work, Mendel was able to understand the nature of inheritance and came up with his two principles or laws of inheritance i.e. (1) The Law of Segregation and (2) The Law of Independent Assortment.

Mendel's First Law - The Law of Segregation

This Law states that during gamete formation, contrasting forms of a gene separate in equal numbers. This law is best illustrated by a monohybrid cross.

Monohybrid cross: This is a cross between homozygous parents that differ only in one pair of alleles of one gene, controlling one character. For example stem height in garden peas is controlled by one gene in which a pure breeding tall plant has genotype TT and a pure breeding short plant has a genotype tt. Mendel carried out separate monohybrid crosses for each of the seven characters in his study of their inheritance and he got similar results.

F_1 hybrids

The progeny or offspring of a monohybrid cross is called the first filial generation or F_1 hybrid. Usually reciprocal crosses are made in a monohybrid cross. For example, pollen from tall plants can be used to fertilise the ovules of the short plants in one cross while pollen from short plants can be used to fertilise the ovules of tall plant in the other cross.

♂ tall X short ♀ → Tall offspring } Reciprocal cross

♂ short X tall ♀ → Tall offspring

Figure 11.1 A reciprocal cross

Mendel observed that when seeds from the F_1 plants were grown, they were all identical: they all resembled the tall plants with no short or intermediate forms. Mendel reasoned that both the tall and short forms of the character were present in the hybrid but only the tall form was expressed. He called the tall form **dominant** and the short form which did not appear, he called **recessive**. He noted that tall was dominant in both cases of the reciprocal cross (Figure 10.1).

The F_2 generation

The second filial or F_2 generation is raised by allowing the F_1 hybrids to self pollinate. When Mendel examined the F_2 plants, the recessive (short) character reappeared and was seen together with the dominant (tall character). The numbers of the plants were in the ratio of 3 tall: 1 short.

The phenotypes of parental, F_1 and F_2 generations are summarised in Figure 1.2 below.

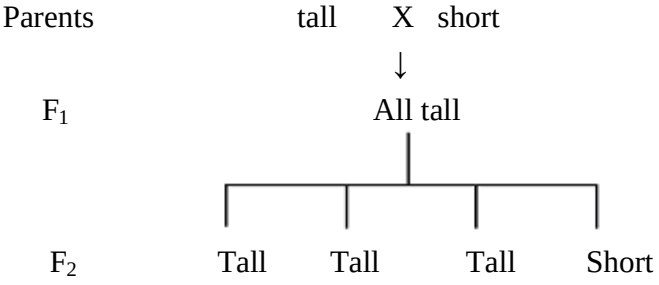


Figure 11.2. Parental**,** F_1 and F_2 generations

Mendel interpreted that the 3:1 phenotypic ratio represented a 1: 2: 1 genotypic ratio representing the binomial expression: (T + t) (T + t) = 1TT + 2Tt + 1tt

This ratio gave Mendel the idea that the character that is passed on during reproduction existed in two alternative forms (T and t) which are able to combine together randomly in pairs.

The formation of gametes, segregation of the forms (alleles) and production of F_1 and F_2 generations are illustrated in Figure 10.3.

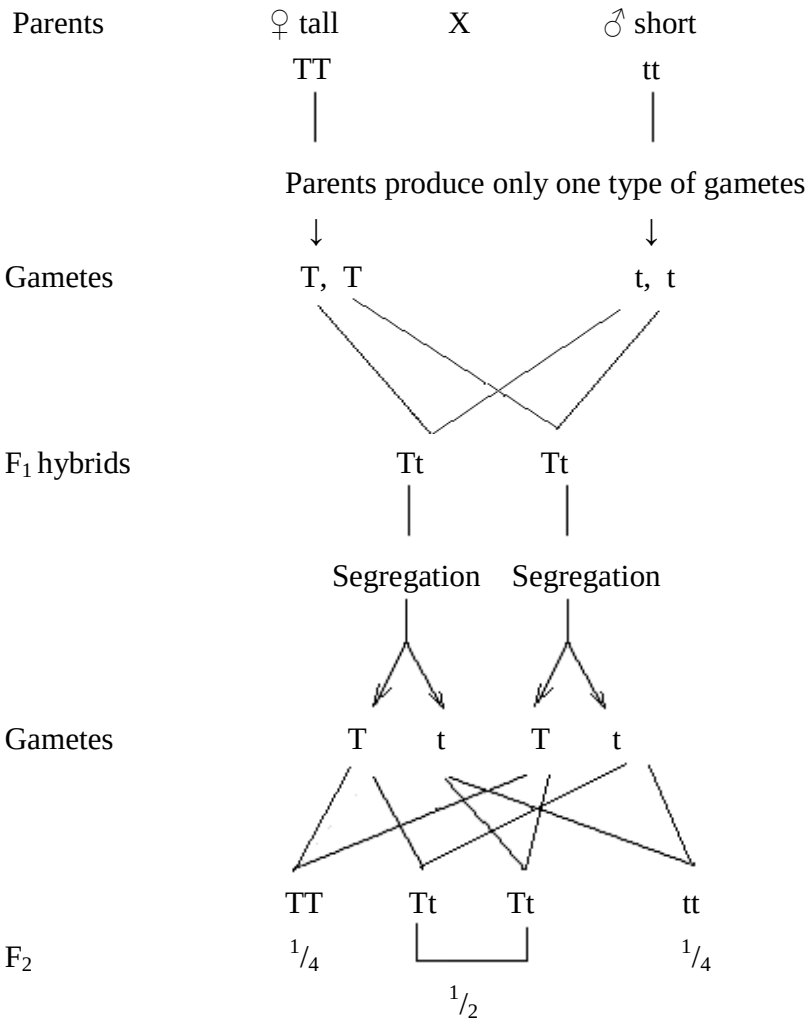


Figure 11.3. Segregation of alleles

The Punnet square: **R.C. Punnet** devised the **Punnet Square** method which clearly shows how the alternative forms of a character combine to produce the F_2 progeny.

F_1 x F_1 Cross Tt X Tt

F_1 Gametes T t T t (segregation)

F_2 progeny as depicted by the 2x2 Punnet square of a monohybrid cross

♀ / ♂	T	t
T	TT	Tt
t	Tt	tt

Figure 11.4. The 2 x 2 Punnet Square. Phenotypic ratio = 3T_ : 1tt and Genotypic ratio = 1TT: 2Tt: 1tt

Testcross

This is a breeding test, in which dominant phenotype (F_1 hybrid) is crossed with the recessive homozygote in order to verify whether it is an F1 hybrid (heterozygote) or a homozygous dominant genotype. The results of a **testcross** confirm that there are two kinds of alleles in the hybrid. A testcross in which an individual is crossed with one of its own parents is referred to as a **backcross**.

Testcross results (i)

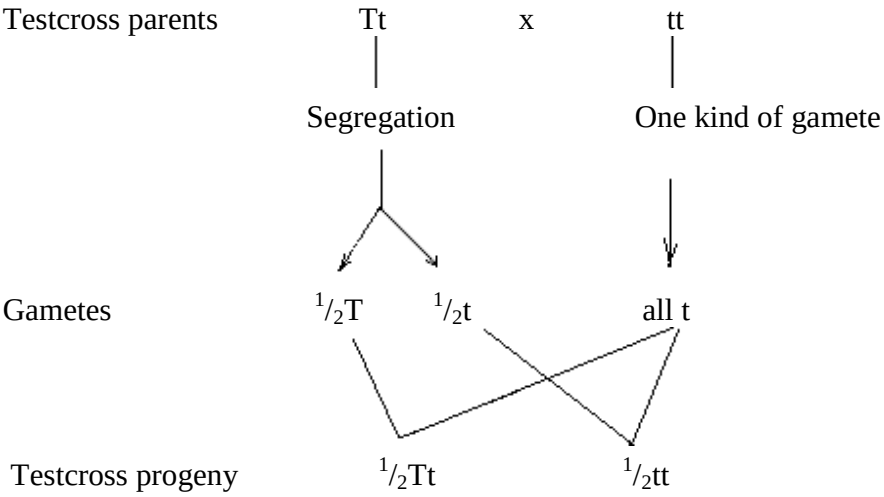


Figure 11.5. Test cross (i)

Testcross results (ii)

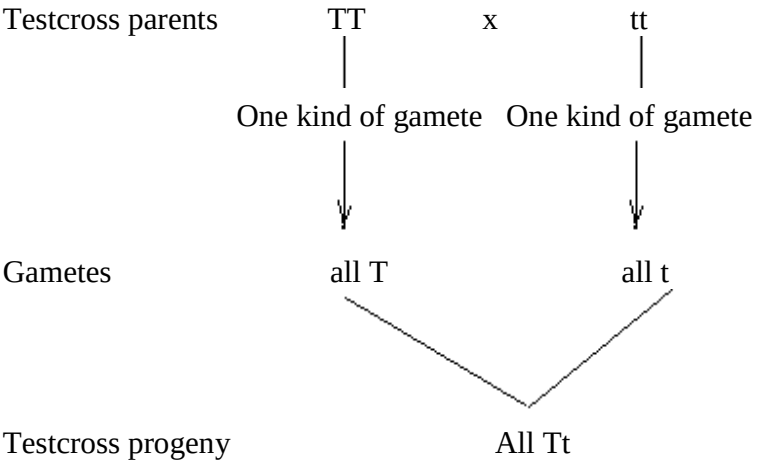


Figure 11.6. Test cross (ii)

When ½ of the progeny of the testcross are tall and ½ are short, then the dominant genotype is confirmed to be heterozygous (F_1 hybrid).

When all the progeny of the test cross are tall then the dominant genotype is confirmed to be homozygous.

12. CLASSICAL MENDELIAN GENETICS II – DIHYBRID CROSS

Expected learning outcomes: At the end of this topic you should be able to understand dihybrid crosses and Mendel's Second Law.

Mendel's 2nd Law – The Law of Independent Assortment

This law states that 'When two or more unlinked pairs genes are brought together in a cross, their alleles assort (segregate) independently of each other as a result of meiosis'. This law is best illustrated by a dihybrid cross.

Dihybrid cross

A dihybrid cross is carried out to study what happens when two pairs of contrasting characters (genes) are combined together in the same hybrid. In one such dihybrid cross, Mendel chose two characters; seed colour (yellow or green) and seed shape (round or wrinkled). This cross is summarised below:

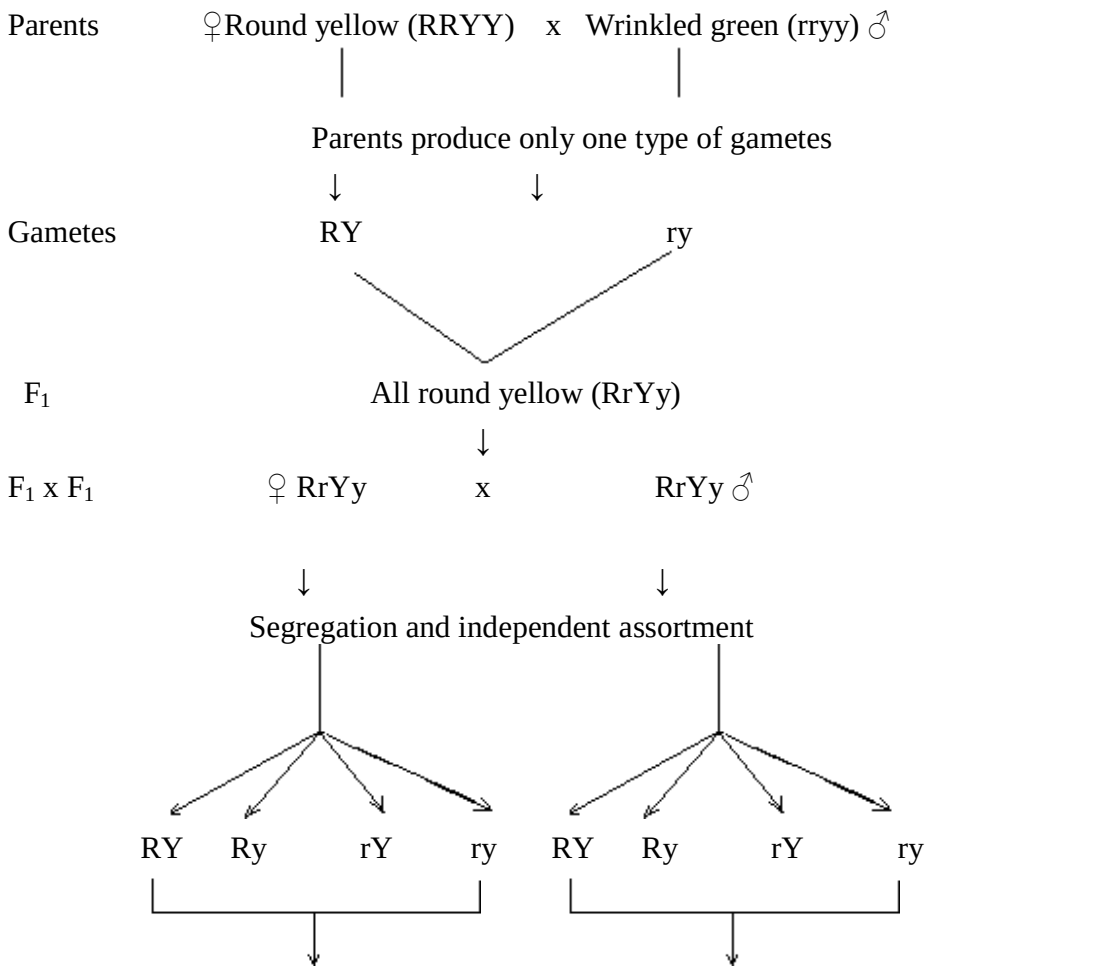


Figure 12.1. A dihybrid cross showing independent segregation (assortment).

The formation of the F_2 generation can be presented in a Punnet square as in Table 11.1.

Table 12.1. The F_2 progeny of a dihybrid cross of round yellow x wrinkled green pea plants

	¼ RY	**¼ Ry**	**¼ rY**	**¼ ry**
¼ RY	$^1/_{16}$RRYY	$^1/_{16}$RRYy	$^1/_{16}$RrYY	$^1/_{16}$RrYy
¼ Ry	$^1/_{16}$RRYy	$^1/_{16}$RRyy	$^1/_{16}$RrYy	$^1/_{16}$Rryy
¼ Ry	$^1/_{16}$RrYY	$^1/_{16}$RrYy	$^1/_{16}$rrYY	$^1/_{16}$rrYy
¼ ry	$^1/_{16}$RrYy	$^1/_{16}$Rryy	$^1/_{16}$rrYy	$^1/_{16}$Rryy

The genotypes of the F_2 of a dihybrid cross do not show any pattern while the phenotypes show a regular pattern.

There are four possible phenotypes in the F_2 i.e. (i) Round yellow - Parental (ii) round green – Parental (iii) Wrinkled yellow – Recombinant and (iv) wrinkled green – Recombinant.

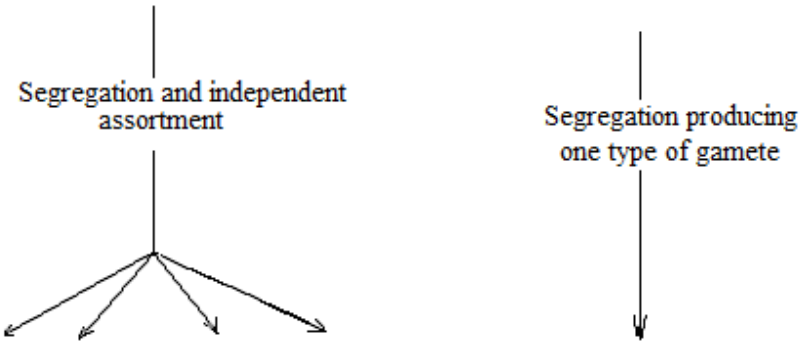
The phenotypic ratio is:

$^{9}/_{16}$ round yellow: $^{3}/_{16}$ round green: $^{3}/_{16}$ wrinkled yellow: $^{3}/_{16}$ wrinkled green = 9:3:3.1

$^{9}/_{16}$ R_Y_ : $^{3}/_{16}$ R_yy : $^{3}/_{16}$ rrY_ : $^{1}/_{16}$ rryy

Dihybrid inheritance test cross: This is a cross between a double dominant phenotype and a double recessive phenotype which confirms whether the double dominant phenotype is homozygous or heterozygous. A reciprocal cross is recommended.

Testcross Round yellow RrYy (F1 hybrid) x Wrinkled green rryy testcross parent



Gametes RY Ry rY ry x ry

F_2 $^{1}/_{4}$RrYy: $^{1}/_{4}$Rryy: $^{1}/_{4}$rrYy: $^{1}/_{4}$rryy

The expected phenotypic ratio is 1: 1: 1:1
The expected genotypic ratio is also 1: 1: 1:1

Figure 12.2. A dihybrid test cross

Autosomal linkage

Genes which are located on the same chromosome are said to be linked and they show linked inheritance. Such cannot behave independently of one another in their inheritance. Alleles that are far apart will have higher chances of crossing over than those which are close together. This is because for the alleles to crossover, there is need for the formation of a chiasma between them.

(i) Unlinked genes located on different chromosomes assort quite freely and segregate their alleles into gametes in all possible random combinations. This is a result of segregation and independent assortment

(ii) Linked genes assort much less freely. The linking together of certain genes on the same chromosome gives some control over recombination meaning that certain combinations of characters can be kept together. This is a result of crossing over in which a chiasma is formed between homologous chromosomes during meiosis.

Genetic recombination and variation

The F_1 offspring from two individuals with different (pure) genotypes but of the same species show genotypic and phenotypic variability which results from the reshuffling of alleles in the gametes of their parents. The variation that comes about by recombination of alleles is the main function of meiosis and sexual reproduction. This variation is important to the long term survival and evolution of the species.

The F_2 individuals with new combinations (which are absent in the parental types) are called recombinants and are a result of crossing over and due to independent assortment during meiosis in the F_1 gamete formation.

In general, the following genotypes and phenotypes are observed:

(i) The F_1 of a monohybrid cross (n =1) will comprise three genotypes (3^n) and two phenotypes (2^n). With independent segregation the phenotypes will be in the ratio 3:1.

(ii) The F_2 of a dihybrid cross (n =2) will comprise nine genotypes (3^n) and four phenotypes (2^n). With independent segregation the phenotypes will be in the ratio 9:3:3:1.

Question: Predict the number of genotypes and phenotypes of the F_2 of a trihybrid cross.

Solution: The F_2 of a trihybrid cross (n =3) will have 27 (3^n) genotypes and eight (2^n) phenotypes.

13. CHI-SQUARED TEST (χ^2 TEST)

Expected learning outcomes

At the end of this topic you should be able to understand the principle behind the Chi-squared test (χ^2 Test) and its application in solving genetics problems.

The Chi-squared test (χ^2 Test) is a statistical test used to confirm whether the differences between the observed values and the expected values are significant or not. It tests whether

deviations in the observed ratios from the expected Mendelian ratios of a given cross are due to chance or due to real departure (difference). The number of observations used in the chi-squared test must be based on 30 individuals or more in order to get reliable results.

Mendel had to work with very large numbers of plants to get reliable results because there were no statistical methods for assessing the reliability of his data available during his time.

In deciding whether the results obtained are within the expected ratio, the calculated χ^2 value is compared with the tabulated χ^2 value. If the calculated value is greater than the tabulated (critical value), then the departure from the expected ratio is significant, i.e. there is real departure from the expected ration; and vice-versa.

The formula for calculating the chi-squared is:

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Where Σ = sum of; O = observed values; E = expected values.

The tabulated χ^2 value is obtained from the χ^2 statistical table at 5% probability and at the appropriate degrees of freedom (df), where df = number of classes – 1.

Example

Consider the progeny from a cross between two red-flowered F_1 plants as follows;

Rr x Rr

R is the dominant allele which determines red flowers in Rr and RR plants

r is the recessive allele which gives white flowers only in rr plants.

Two possible results from 200 F_2 plants are given below;

(i) 152 red flowered plants and 48 white flowered plants

(ii) 165 red flowered plants and 35 white flowered plants

Use the chi-squared test to determine whether the two results are in the expected Mendelian ratio of an F_2 progeny of a monohybrid cross or not.

Solution

Under perfect conditions, we expect a 3 red: 1white phenotypic ratio in the F_2 progeny i.e. 150 red and 50 white flowered plants for the sample of 200. In real situations the conditions are not perfect and therefore a chi-squared test should be used.

Chi-squared test of results (i) of 152 red and 48 white

Character	O	E	O-E (deviation, d)	$\mathbf{d^2}$	$\mathbf{d^2/E}$
Red	148	150	-2	4	0.02667
White	52	50	2	4	0.08
Total (Σ)	200	200	0		**0.10667**

The calculated χ^2 value is 0.11

There are two classes (red and white) hence one degree of freedom

(df) = number of classes – 1 = 2 – 1 = 1.

From the χ^2 table, the value for 1 degree of freedom at 5% probability = 3.841 (highlighted in the table below).

		Probability							
		0.01	**0.05**	**0.10**	**0.20**	**etc**			
Degrees of freedom	**1**	6.6	3.8	2.7	1.6				
	2								
	3								
	etc								

In conclusion the calculated χ^2 value of 0.11 is less than the table χ^2 value of 3.841. Therefore the calculated χ^2 value is **not significant** and the observed values **agree** with the expected values meaning that the results are according to the expected Mendelian phenotypic ratio of 3:1.

Chi-squared test of results (ii) of 165 red and 35 white

Character	O	E	O-E (deviation, d)	$\mathbf{d^2}$	$\mathbf{d^2/E}$
Red	165	150	+15	225	1.5
White	35	50	-15	225	4.5
Total (Σ)	200	200	0		χ^2 = **6.0**

The calculated χ^2 value is 6.0.

There are two classes (red and white) hence one degree of freedom

(df) = number of classes – 1 = 2 – 1 = 1.

From the χ^2 table, the value for 1 degree of freedom at 5% probability = 3.841 (highlighted in the table below).

		Probability							
		0.01	**0.05**	**0.10**	**0.20**	**etc**			
Degrees of freedom	**1**	6.6	3.8	2.7	1.6				
	2								
	3								
	etc								

In conclusion the calculated χ^2 value of 6.0 is greater than the table χ^2 value of 3.841. Therefore the calculated χ^2 value is **significant** and the observed values **do not agree** with the expectation. This means that the results are not according to the expected Mendelian ratio. The results suggesting that there is an effect on the expected results.

Expected ratios for monohybrid and dihybrid crosses

Cross	**Progeny**	**Expected phenotypic ratio**	**No. of classes (n)**	**df**
Monohybrid cross	F_2	3:1	2	1
Monohybrid test cross	Test cross	1:1	2	1
Dihybrid cross	F_2	9:3:3:1	4	3
Dihybrid test cross	Test cross	1:1:1:1	4	3

14. EXTENSION OF MENDELIAN GENETICS - 1

Expected learning outcomes

At the end of this topic you should be able to understand

1. Autosomal linkage and recombinants,
2. Multiple alleles, illustrated by the ABO blood system and Rhesus factor in man
3. Quantitative trait inheritance

14.1 AUTOSOMAL LINKAGE

Genes which are located on the same chromosome are said to be linked and they show linked inheritance. Such cannot behave independently of one another in their inheritance. Alleles that are far apart will have higher chances of crossing over than those which are close together. This is because for the alleles to crossover, there is need for the formation of a chiasma between them.

(i) Unlinked genes located on different chromosomes assort quite freely and segregate their alleles into gametes in all possible random combinations. This is a result of segregation and independent assortment

(ii) Linked genes assort much less freely. The linking together of certain genes on the same chromosome gives some control over recombination meaning that certain combinations of characters can be kept together. This is a result of crossing over in which a chiasma is formed between homologous chromosomes during meiosis.

14.2 MULTIPLE ALLELES

It has been observed that some characters do not follow the Mendelian type of inheritance. Some of these non-Mendelian characters are controlled by one gene with more than two alleles.

Three or more kinds of alleles controlling one character (one gene) at one locus in an individual's chromosomes are referred to as multiple alleles. The multiple allelic gene will still

be represented only twice on the chromosome pair concerned, but will have more than three genotypes when the alleles are combined two at a time.

THE ABO BLOOD GROUP SYSTEM: The ABO blood group system is one of several different blood group systems in man and is a good example of multiple allelic inheritance. Blood group phenotypes are due to proteins, bound to the surface of the red blood cells which act as antigens. In the ABO system the blood groups are controlled by a single gene, designated as I, which has three alleles I^A, I^B and I^O. I^A is co-dominant to I^B ; I^A dominant to I^O; I^B dominant to I^O ($I^A = I^B > I^O$). There are six possible pair-combinations of the alleles, giving six genotypes as shown in the table:

Blood group	**Genotypes**
A	I^AI^A
	I^AI^O
B	I^BI^B
	I^BI^O
AB	I^AI^B
O	I^OI^O

Blood groups are inherited in the Mendelian fashion but each individual will only carry two out of three alleles.

Examples of crosses involving the ABO blood groups:

(i) Parents ♀Group O x ♂Group O

Genotypes ♀ I^OI^O x ♂I^OI^O

All children will be blood group O

(ii) Parents ♀Group AB x ♂Group O

Genotypes ♀ I^AI^B x I^OI^O ♂

Gametes ½ I^A, ½ I^B All I^O

Children ½ I^AI^O ½ I^BI^O

½ Group A ½ Group B

Genotypic ratio: ½ I^AI^O: ½I^BI^O

Phenotypic ratio : ½ A: ½ B

(iii) Parents ♀ Group AB x Group AB ♂

Genotypes ♀ I^AI^B x I^AI^B ♂

Gametes ½ I^A, ½ I^B ½ I^A, ½ I^B

Offpspring

♀/♂	½ I^A	½ I^B
½ I^A	¼ I^A I^A	¼ I^AI^B
½ I^B	¼ I^AI^B	¼ I^BI^B

Genotypic ratio: ¼ I^A I^A: ½ I^AI^B: ¼ I^BI^B

Phenotypic ratio : ¼ A: ½ AB: ¼ B

Red blood cell antigens and serum antibodies

The surfaces of red blood cells contain antigens while the blood serum contains natural antibodies against foreign RBC antigens. The antigen-antibody combinations for all the possible blood groups are shown in the table below:

Blood group (phenotypes)	**Genotypes**	**RBC antigens**	**Serum antibodies**
A	I^AI^A	A	Anti-B
	I^AI^O	A	
B	I^BI^B	B	Anti-A
	I^BI^O	B	
AB	I^AI^B	AB	No anti-A, no Anti-B
O	I^OI^O	No antigens	Anti-A and anti-B

When a blood group carrying a given antigen is mixed with a blood group carrying an antibody for that particular antigen, the blood cells in the mixture agglutinate (clump together).

Blood typing

The reactions are summarised in the table below:

Serum type	**Reaction to serum according to blood group**			
	O	A	B	AB
Anti-A serum	-	+	-	+
Anti-B serum	-	-	+	+

A positive result represents agglutination; a negative result represents no agglutination

Blood transfusion

When small quantities of blood are transfused some groups can be safely mixed with others as shown in table below:

Donor	**Recipient**			
	O Anti-A + Anti-B antibodies	A Anti-B antibody	B Anti-A antibody	AB No antibody
O (No antigen)	-	-	-	-
A (A antigen)	+	-	+	-
B (B antigen)	+	+	-	-
AB(A+B antigens)	+	+	+	-

The + sign represents agglutination meaning transfusion is not safe; the – sign represents no agglutination meaning transfusion is safe.

Group O can donate to any of the other groups because O has no A or B antigens on its red blood cells and cannot be agglutinated by antibodies in the recipient's serum. For this reason people of group O are referred to as universal donors.

AB blood has no anti-A or anti-B antibodies and can receive blood from any of the other groups because it cannot agglutinate their red cells. Persons of group AB are therefore known as universal recipients.

14.3 POLYGENIC INHERITANCE

A study of the phenotypic differences in any large population shows that two forms of variation occur namely (i) discontinuous variation and (ii) continuous variation.

Discontinuous variation

In this kind of variation individuals show clear-cut differences with no intermediates between them. Examples of discontinuous variation include blood groups and albinism in humans, wing lengths in *Drosophila*, melanic (dark) and light body colour in the moth *Biston betularia*, and sex in animals and plants. Characteristics showing discontinuous variation are usually controlled by one or two major genes which may have two or more allelic forms and their phenotypic expression is relatively unaffected by environmental conditions. This form of variation is also known as qualitative variation.

Continuous variation -Polygenic inheritance

This is a type of inheritance in which one character is controlled by many genes (polygenes) working together to bring about the phenotype. Each gene has two or more alleles. It involves characters that show a range measuring from one extreme to the other within a population. Characters exhibiting continuous variation are controlled by the combined effects of many genes and the environment. The frequency distribution for a character showing continuous variation is a normal curve. Most of the organisms in the population fall in the middle of the range with approximately equal numbers showing the two extreme forms of the characteristic. Individually each of these genes has little effect on the phenotype but their combined effect is significant. Polygenic characters are also called quantitative traits or continuously varying traits.

Although polygenic traits are inherited in the Mendelian fashion, they are studied by use of statistical methods dealing with populations.

In humans, height is controlled by polygenic inheritance. An individual may possess the genotype for being very tall height, but if he or she does not eat nutritious foods during the early years of growth, he/she will not be able to grow as tall as the genotype would permit.
In plants, yield is a polygenic trait which is affected by many genes controlling factors such as rate of germination, rate of photosynthesis, amount of root, drought tolerance, etc.

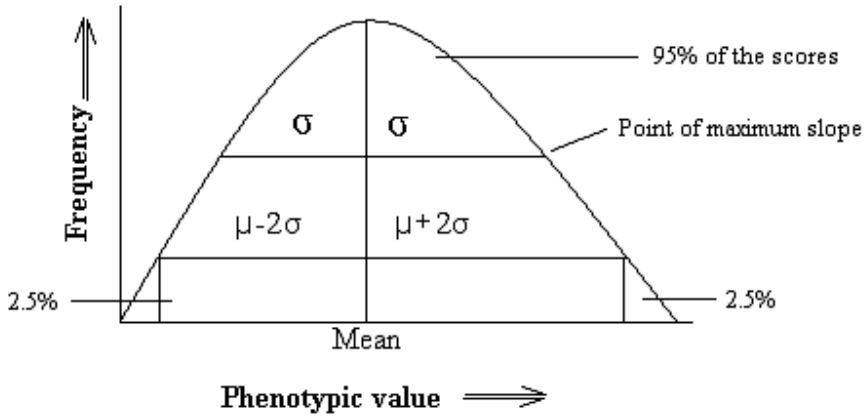


Figure 14.3.1. Normal distribution of a polygenic trait

Table 14.3.1. Comparison between qualitative and quantitative traits

	Qualitative traits	**Quantitative traits**
1	Characters are of a kind	Characters are of a degree
2	Show discontinuous variation	Show continuous variation
3	Characters controlled by single genes in which each gene has a major effect on the character	Polygenic control in which each gene has a small effect
4	Changing one gene for another at a locus causes a major difference in the phenotype	Changing one gene for another one at a locus causes relatively little difference in the phenotype
5	Based on individual mating events between couples and their progeny	Based on populations in which mating events take place at random and many different sets of offspring are produced
5	Analysed by making counts and simple ratios	Analysed by use of statistics using the chi-squared test and other tests
6	The environment has a small effect on them	The environment has a major effect on them
7	Examples; i) sweat pea flowers are either white or pink, (ii) the sex of a person is either male or female, (iii) the blood group of a person is A or B or O or AB.	Examples (i) height, intelligence, skin colour, beauty are examples of human quantitative traits (ii) crop yield (productivity) is a plant quantitative trait

15. GENE INTERACTIONS

Expected learning outcomes: At the end of this topic you should be able to understand gene interaction in terms of epistasis, hypostatis; modifications of dihybrid phenotypic ratios; recessive epistasis, dominant epistasis and duplicate recessive epistasis. You should be able to explain the biochemical interpretation of epistasis and pleiotropy (non-epistatic interaction).

Introduction

In many cases genes at different loci interact with each other leading to unexpected phenotypes. These non-allelic gene interactions include epistasis, hypostasis, complementary action and pleiotropy.

Epistasis: Epistasis means ‘standing upon’. This is the interactions between two different genes where one gene hides the expression of another gene. The epistatic gene interferes with the phenotypic expression of another (hypostatic) gene. Epistasis can be dominant or recessive.

Recessive epistasis

One form of coat colour in mice provides an example of recessive epitasis. The hair colour of the wildtype mice is described as agouti in which there is a yellow band around an otherwise black hair. The agouti allele (A) is dominant to black allele (a) and therefore individuals with the genotype AA or Aa are agouti whilst individuals with the genotype aa have black hair. Another independently inherited gene is required for the synthesis of the hair pigment. This gene has two alleles i.e. dominant allele C which is responsible for colour expression and recessive allele c which is responsible for non-expression of colour. Individuals of genotypes CC and Cc are coloured while cc individuals are albino (no colour). It has been observed that the individual with the genotype AAcc will be albino because cc is epistatic to the colour gene. The F2 generation of mice with these two gene is give in the table below:

Table 15.1. Recessive epistasis in coat colour in mice (AaCc x AaCc)

	AC	**Ac**	**aC**	**ac**
AC	AACC Agouti	AACc Agouti	AaCC Agouti	AaCc Agouti
Ac	AACc Agouti	AAcc albino	AaCc Agouti	Aacc albino
aC	AaCC Agouti	AaCc Agouti	aaCC Black	aaCc Black
Ac	AaCc Agouti	Aacc albino	aaCc Black	aacc albino

Phenotypic ratio = 9 agouti: 3 black: 4 albino (Modified 9:3:3:1 ratio)

Dominant epistasis: Dominant epistasis is seen in the inheritance of plumage (feather) colour in chickens.

Pure-breeding White Leghorns are homozygous dominant for the gene I which causes inhibition of colour in the feathers. They are also homozygous for the gene C which brings about coloured feathers. The IICC chickens are white because allele I is epistatic over C.

Pure-breeding White Wyandotte chickens are homozygous for carry the recessive i for colour inhibition and also homozygous for recessive allele c for lack of colour in feathers. The iicc chicken are white because genotype ii inhibits colour in feathers and the genotype cc is responsible for lack of colour .

When White Leghorns are crossed with White Wyandottes (I ICC x iicc) the F1 are all white (IiCc). Interbreeding among the F_1 produces F_2 which consists of white and coloured chickens in the ratio 13: 3. The genotypes containing II or Ii are white and the double recessive iicc is also white. Only the iiCC and iiCc are coloured. Here the colour inhibiting gene I is epistatic to the colour producing gene C. Individuals that carry the dominant allele I will have white feathers even if they are carrying the dominant allele C for colour production. For example an individual

with the genotype IiCC will be white and individuals which are homozygous recessive for the colour gene (cc) will also be white.

Table 15.2. Dominant epistasis in plumage colour in chickens (IiCc x IiCc)

	IC	Ic	iC	ic
IC	IICC white	IICc white	IiCC white	IiCc white
Ic	IICc white	IIcc white	IiCc white	Iicc white
iC	IiCC white	IiCc white	iiCC coloured	iiCc coloured
Ic	IiCc white	Iicc white	iiCc coloured	iicc white

Phenotypic ratio = 13 white : 3 coloured (modified from the 9:3:3:1 ratio)

Hypostasis: Hypostasis is a situation in which a (hypostatic) gene is hidden by another (epistatic) gene.

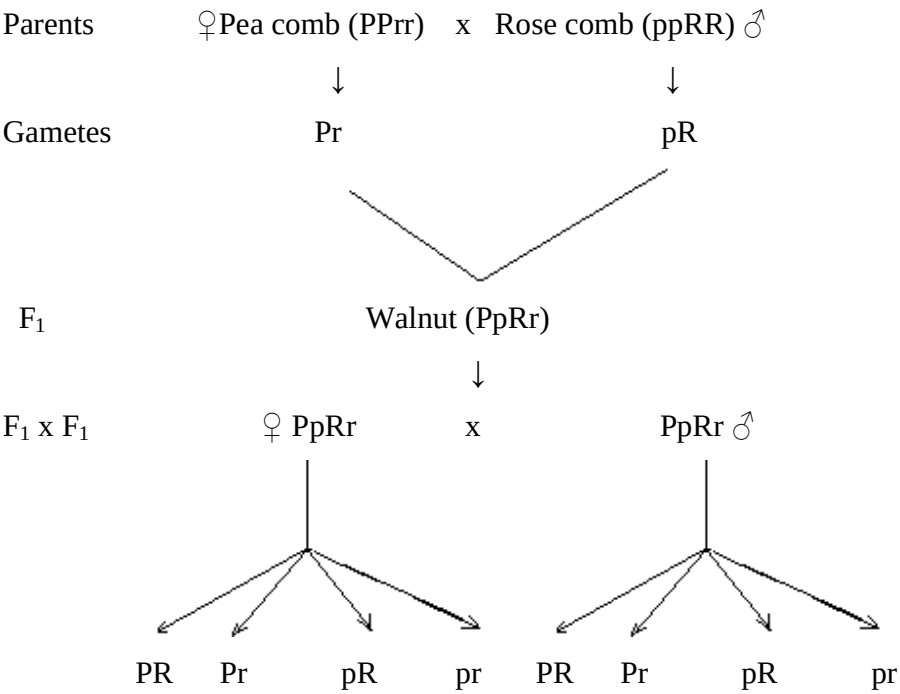
Pleiotropy: non-epistatic interaction: Pleiotropy explains the multiple phenotypic effects of a single gene. Genes that affect a fundamental process will affect many other processes in a complex organism.

There are two ways in which a single gene may affect several different characters. The first is that the gene produces a product that involved in a branched biochemical pathway, and a mutation in the gene therefore affects different branches. The second cause of pleiotropy is when the gene determines an enzyme, which is common to a number of different metabolic pathways. Mutation in the gene will then cause a block in these otherwise unrelated pathways.

For example sickle cell anaemia is due to a mutation in one gene which leads to the production of abnormal haemoglobin. The effects of the abnormal haemoglobin are many and complex and include the sickle-shape of the red blood cells and their tendency to clump together and clog blood vessels in various organs of the body. As a result, heart, kidney, spleen and brain damage are common elements to the syndrome. The defective red blood cells are also readily destroyed in the body, causing severe anaemia.

Another example of Pleiotropy**:** is albinism.

Complementary genes: Complementary genes interact together to produce an effect which is different from that given by either of them separately. One unusual gene interaction was observed by Bateson and Punnett in the character comb type in chickens. There are a number of different forms of the comb, two of which are called 'pea' and 'rose'. Both of these types breed true, but when crossed together the resultant F_1 hybrid has an entirely different form called 'walnut'. When walnut birds interbreed, the F_2 generation has four comb types: walnut, pea, rose and a new type known as single. The four phenotypes occur in the ratio 9:3:3:1.



	$^1/_4$PR	**$^1/_4$Pr**	**$^1/_4$pR**	**$^1/_4$pr**
$^1/_4$PR	PPRR walnut	PPRr walnut	PpRR walnut	PpRr walnut
$^1/_4$Pr	PPRr walnut	PPrr pea	PpRr Walnut	Pprr pea
$^1/_4$Pr	PpRR walnut	PpRr walnut	ppRR rose	ppRr rose
$^1/_4$pr	PpRr walnut	Pprr pea	ppRr rose	pprr single

Phenotypic ratio is 9 walnut: 3 pea: 3 rose: 1 single

Bateson and Punnett explained these results on the basis of the interaction of two independent genes which were called P (for 'pea') and R (for 'rose'). Pea and rose are both dominant over single. A genotype which has at least one dominant allele of the gene P has a pea comb, and one with at least one dominant allele of the gene R has a rose comb. A genotype without any dominant alleles has a single comb, which results from the interaction between two homozygous recessive pairs of alleles. When the dominant alleles of both P and R are present together, they interact to give the walnut phenotype. Pure breeding parents with pea combs are therefore of genotype PPrr and those with rose combs are ppRR. The F_1 are walnut because they have the dominant alleles of both genes (PpRr). The important clue in this cross is the way in which the different phenotypic classes are seen to be in multiples of $^1/_{16}$ths. This confirms that the F_2 must have come about by the crossing of F_1s which were heterozygous for two pairs of independently-segregating genes.

Although the phenotypic ratio is 9:3:3:1, it differs from the normal dihybrid cross because in this case there is only one character involved which is comb form.

16. MODIFIED MENDELIAN RATIOS

Lethal genes, gene linkage and gene interactions lead to modification in Mendelian ratios.

16.1 Modification due to lethal genes: Lethal genes are essential genes which have alleles that cause an organism to die only when present in the homozygous condition. There are a number of classes of lethal genes. (i) The completely fully dominant lethal allele kills the carrier in both homozygous and heterozygous conditions at the zygote stage, embryonic stage or even after birth or hatching. No individual attains the age of reproduction. (ii) The recessive lethal allele will cause death in the the homozygote for the allele. Most lethal genes are recessive (iii) Sub lethal or semi lethal genes become operative at the time the individuals become sexually mature. Such lethal genes handicap but do not destroy their possessor.

Lethal genes will eliminate one or more phenotypic classes from the adult progeny, thus modifying the phenotypic ratio. For example, the 3:1 phenotypic ratio resulting from a monohybrid cross will become 2:1 if the recessive allele is lethal.

i) Yellow fur allele (Y) in mice is a dominant lethal allele which causes death in homozygotes. It has been observed that when two yellow mice are crossed, the offspring have a phenotypic ratio of 2 yellow : 1 grey instead of the Mendelian 3:1 ratio.

F_2 ¼ YY (aborted) ½ Yy (yellow) ¼ yy (grey)

(ii) Achondroplastic dwarfism allele (A) in man is a dominant lethal allele.

F_2 ¼ AA (aborted) ½ Aa (dwarf) ¼ aa (normal)

The phenotypic ratio observed in the F_2 is 2 dwarf: 1 normal (2:1)

(iii) Chlorosis allele (C) in maize which results in plants lacking chlorophyll (chlorotic

plants).

F_2 ¼ CC (die early) ½ Cc (chlorotic plants) ½ cc (normal plants)

The phenotypic ratio observed in the F_2 is 2 chlorotic: 1 normal (2:1)

16.2 Modifications due to gene interactions: Gene interactions change the normal dihybrid phenotypic ratio (9:3:3:1), because a certain genotype will not produce the expected phenotype, but one of the other phenotypes. Thus one or more phenotypic classes disappear and another class increases in (relative) numbers. The normal dihybrid phenotypic ratio may change to 9:7 or 12:3 or 9: 6: 1 etc but the sum of the ratio numbers remains 16.

Table 16.2.1. A summary of the modified F_2 dihybrid Mendelian ratios produced by the various types of gene interactions

Type of gene interaction	**9**	**3**	**3**	**1**	**Phenotypic ratio**
	A_B_	A_bb	aaB_	aabb	
None (four distinct phenotypes)	9	3	3	1	9:3:3:1
Complementary gene action	9	7			9:7
Dominant suppression by A of B	12		3	1	13: 3
Recessive epistasis by aa of alleles B and b	9	3	4		9:3:4
Dominant epistasis by A of allele B and b	12		3	1	12:3:1

For **incomplete penetrance** and **variable expressivity**, the modified ration cannot be simply derived from the normal ratio by eliminating or joining classes as is the case with lethal or interacting genes.

16.3 Modifications due to gene linkage: When two genes are located on the same chromosome, they are said to be linked. Such linked genes do not have independent assortment and their F2 phenotypic ratio is not 9:3:3:1. Instead there are more individuals with the two parental phenotypes compared to the number of individuals with the recombinant phenotypes.

To test whether two genes are linked or not, a dihybrid test cross is carried out (on the F_1 generation). The expected ratio of this dihybrid cross is 1:1:1:1. Using the chi-squared test the phenotypic ratio of the testcross progeny is tested and if the difference between observed and expected ratio is significant, then the two genes are linked. If on the other hand, the difference between observed and expected ratio is not significant, then the two genes are not linked.

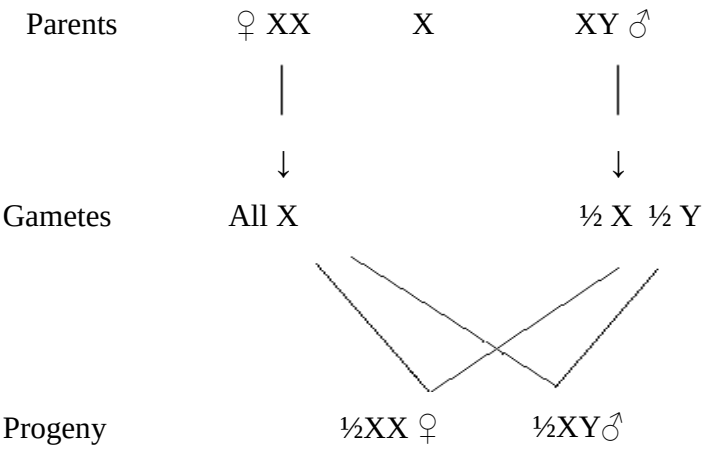
17. SEX DETERMINATION AND SEX LINKAGE

Sexual differentiation of a species into separate male and female forms is usually genetically determined. The inheritance of sex can be interpreted in Mendelian terms as a monohybrid test cross. Mating between males and females always results in two sexes among the offspring in approximately equal numbers.

Sex chromosomes are those that carry the sex-determining genes. All the other chromosomes which are not involved in sex determination are known as autosomal chromosomes or autosomes.

The XX/XY system: In human and many other animals, sex is determined by the presence of different sets of sex chromosomes designated X and Y. Females have two X chromosomes; males have an X and a Y chromosome. In most species, the Y chromosome only contains the genes responsible for the development of the male organs producing the male gametes, the testes. It is therefore considered to be genetically empty. In most cases the Y chromosome is physically smaller than the X chromosome. The female is therefore homogametic (XX) producing eggs carrying a single X while the male is heterogametic (XY) with half the gametes carrying the X chromosome and the other half carrying the Y chromosome.

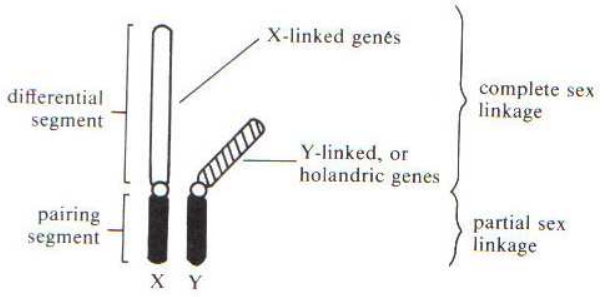
In the XX/XY system therefore, the sex of the offspring is determined by the male parent.



Sex (X-chromosome) linkage

Sex chromosomes also carry genes which are not directly involved with the determination of sex. These so called sex-linked genes are carried on the X chromosome and are present with only one allele in the male sex. In females the sex-linked genes are present with two alleles (one on each X-chromosome) like what happens with all genes located on autosomes. As a result, recessive, sex-linked alleles will always be expressed in the male's phenotype, because he will never have the dominant counterpart to mask its effect. That is the reason why sex-linked diseases caused by a recessive allele occur more frequently in men than in women.

In the XX/XY system, there are genes in the differential part of the X chromosome which have no allelic partners in the Y and vice versa. These are the sex-linked genes.



Examples of sex-linked genes include those for (i) haemophilia in humans and (ii) colour blindness in humans

Two indications of sex-linkage:

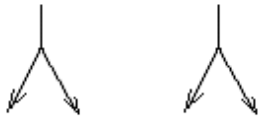
(1) When a recessive allele appears more frequently in males than in females in a pedigree.

(2) When a reciprocal cross yields a different phenotypic ratio.

Haemophilia in humans: Haemophilia is a human disease in which the main feature is a reduced ability of the blood to clot. Haemophiliacs can therefore bleed excessively even from very small wounds. The commonest form of haemophilia is caused by a recessive allele h of a single gene 'H' located on the X chromosome. 'Normal' parents can have haemophiliac children as illustrate below:

Parents Queen Victoria Prince Albert

♀ X^HX^h X^HY♂



Gametes ½X^H ½ X^h ½X^H ½Y

Children X^HX^H X^HY X^HX^h X^hY

Normal Normal Carrier Haemphiliac

♀ ♂ ♀ ♂

Although Queen Victoria was phenotypically normal, she carried the allele for haemophilia which she passes on to her children of which only her sons could be haemophiliac.

Colour blindness in humans

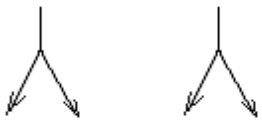
Colour blindness is the inability to distinguish between green and red. This condition results from a recessive allele c of gene C carried on the X chromosome. This condition is more frequent in males than females for obvious reasons.

The inheritance of colour blindness can be illustrated as given below:

Parents Mother Father

♀ X^CX^c X^CY♂

normal normal



Gametes ½X^C ½X^c ½X^C ½Y

Children ¼ X^CX^C ¼ X^CY ¼ X^CX^c ¼ X^hY

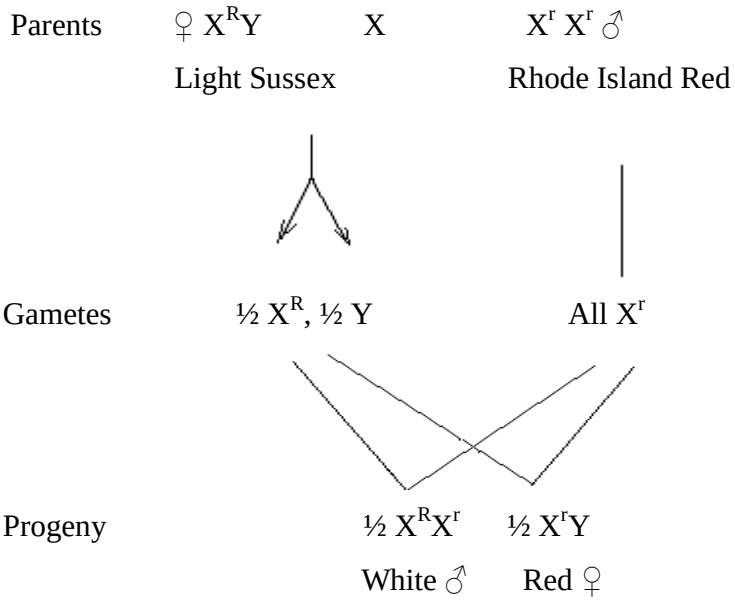
Normal Normal Carrier Colour blind

♀ ♂ ♀ ♂

Feather colour in birds

In birds, the male is heterogametic while the female is homogametic. The same principle of inheritance applies as the one for sex linkage in humans but the pattern of inheritance is reversed in the sexes.

In one example, Light Sussex chickens have mostly white plumage and the Rhode Island Red breed have mostly red. The allele R for white feathers is dominant to the allele r for red feathers. A cross between Rhode Island cockerels and Light Sussex hens produces white male offspring and red female offspring as shown below:



Holandric genes or Y-linked genes

These are genes located on the Y-chromosome whose effects only pass to male offspring from their father e.g. the pattern baldness allele and the hairy ear (hypertrichosis) allele.

Hormonal effects on sex expression

The gender of an animal can also influence or limit the effects or expression of certain sex-influenced genes. These genes are not necessarily located on the X chromosome, most of the times they are even on the autosomes. The cause of these gender-mediated differences in gene expression is the difference in level of male and female sex hormones between genders.

sex-influenced (sex-affiliated) genes: When the same genotype leads to different phenotypes in male and female, the gene involved is said to be **sex-influenced (sex-affiliated).** For example the allele responsible for pattern baldness in humans is expressed in males and not in females. This means that heterozygous dominant men are bald, while homozygous recessive women are not.

Table 17.1. Inheritance of pattern baldness

Genotype	**Male**	**Female**
BB	Bald	Bald
Bb	Bald	Nonbald
Bb	Nonbald	Nonbald

The allele B in both the homozygous and heterozygous states exerts its dominance only in the male sex while in the female only the homozygous BB exerts dominance.

Sex-limited genes: Usually sex-limited characters are expressed only in one sex even though both sexes may carry genes for such characters. The expression of these genes is determined by the presence or absence of sex hormones e.g. beards in man and milk production in females. Both men and women posses the genes for producing milk, but only in men these genes are never expressed. Sex-limited genes may be carried on autosomes and are strongly affected by the level and types of sex hormones present in the body.

18. CHROMOSOME MUTATIONS

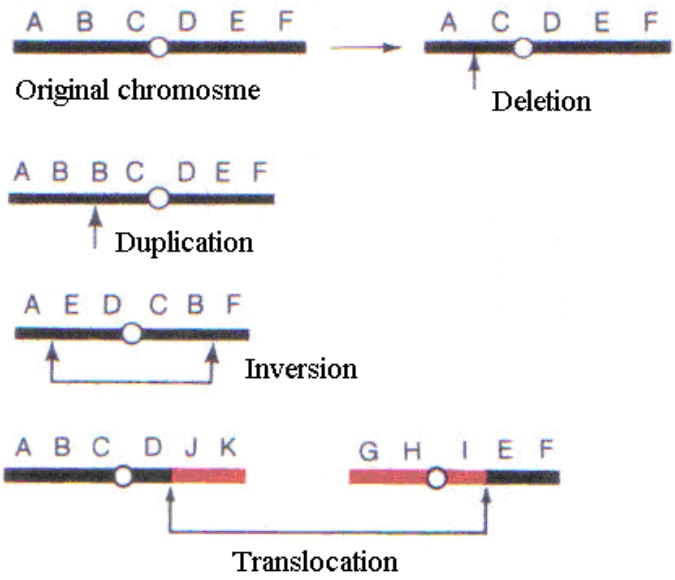
Chromosome mutation is the name given to the processes of change leading to rearrangement of chromosomes parts leading to abnormal numbers of individual chromosomes.

Two types of chromosome mutation

Chromosome mutations are of two types i.e. (1) changes in chromosome structure and (2) changes in chromosome numbers

Changes in chromosome structure

The chromosome set mutates spontaneously to produce chromosome rearrangements. The four rearrangements of chromosome structure are: deletions, duplications, inversions and translocations.



Translocations

A translocation moves a chromosome segment to another position in the genome. New gene linkages can be produced by translocations. Translocation heterozygotes may have 50% reduced fertility.

Translocations are an important cause of ill health in human populations**.** For example, translocation between chromosomes 5 and 11 leads to a duplication of a segment of chromosome 11 and a deletion of a segment of chromosome 5. Children born with this translocation show symptoms of cri du chat associated with deletion in chromosome 5.

Changes in chromosome numbers

There are two types of changes in chromosome numbers. These called euploidy and aneuploidy.

Aneuploidy

Aneuploidy is the change in number of individual chromosome. Chromosomes may be added to or subtracted from normal sets. One or more whole chromosomes may be absent from or may be added to the chromosome set (complement).

The cause of aneuploidy may be due to a process called non-disjunction. Non-disjunction is a mistake in which both chromosomes (or chromatids) pass to the same pole of a cell instead of opposite poles at anaphase during meiosis.

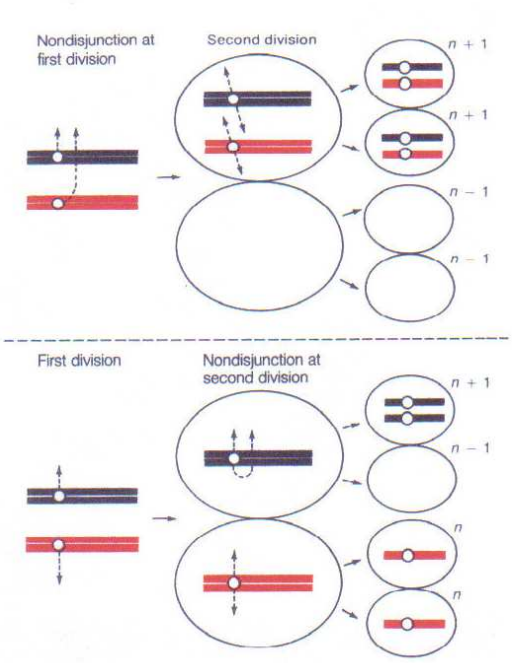


Figure 18.1. The origin of aneuploid gametes by nondisjunction at the first or second meiotic division

Aneuploid conditions are well studied in humans. Down syndrome (Trisomy 21), Klinefelter syndrome (XXY), and Turner syndrome (XO) are well documented. The spontaneous level of aneuploidy in humans is quite high and produces a major proportion of genetic diseases in human populations.

Trisomics (2n +1) - polysomy

There are several examples of viable trisomics in humans such as the Klinefelter syndrome (XXY) in which the affected persons are sterile males with effeminate tendencies; and the XYY syndrome in which the males affected are sterile and are physically normal but socially deviant. The most common is Down syndrome (Trisomy 21)

Down syndrome (Trisomy 21)

The most common type of human aneuploidy is the Down syndrome, an autosomal trisomic caused by non-disjunction of chromosome 21. There is a maternal-age effect for Down syndrome; older mothers show a greater risk of having Down syndrome children.

Facial features include eyelids which apparently slant upwards due to a fold of skin over the inner corner of the eye. The face is flat and rounded. Other symptoms include: metal retardation; short stature and relatively small skull due to poor skeletal development; heart defects occur in about one-quarter of Down's children; increased risk of respiratory and ear infections; high risk of leukaemia; coarse, straight hair.

Euploidy

Euploidy is variation in the number of whole sets (complements) of chromosomes. Diploids with two sets of chromosomes in their nuclei are regarded as being the normal form in eukaryotes.

Polyploids

Organisms with three or more complement sets of chromosomes are known as polyploids. These include triploids (2n=3x), tetraploids (2n=4x), pentaploids (2n=5x) and hexaploids (2n=6x). Polyploids are common in plants e.g. bread wheat, *Triticum aestivum*, (2n=6x=42). Another example of plant polyploidy is the potato, *Solanum tuberosum* (2n = 4x = 48). Polyploids are rare in animals where they usually fail to develop and are aborted.

Polyploids can arise due to non-disjunction in which either: (i) two gametes with unreduced chromosomes fuse to form a tetraploid zygote or (ii) a gamete with unreduced chromosomes fuses with a normal gamete to form a triploid zygote.

The unreduced gametes are formed when there is failure to form spindles at anaphase during meiosis resulting in diploid gametes.

19. HUMAN CHROMOSOMAL ABERRATIONS

Expected learning outcomes

At the end of this topic you should be able to understand chromosomal variations with an emphasis on variation in numbers.

There are two types of human chromosomal aberrations, namely (i) variation in chromosome numbers (ii) variations in chromosome structure

Variation in chromosome number

Each species has a characteristic number of chromosomes. Humans and higher organisms are diploid (2n), with two sets of homologous chromosomes: one set donated by the father, the other set by the mother. Variation in the number of sets of chromosomes (ploidy) is also commonly encountered in nature. About one-third of the flowering plants (angiosperms), for example, have more than two sets of chromosomes.

There are two types of variation in chromosome number namely (1) Euploidy and (2) Aneuploidy

Euploidy: This is a variation in the number of whole sets of chromosomes. Diploids with two sets of chromosomes in their nuclei are regarded as being the normal form in eukaryotes including humans.

Monoploids: These have only one set that develops from unfertilised eggs and are very rare. Male bees, wasps and ants are monoploids which develop parthenogenetically from unfertilized eggs.

Polyploids: These are organisms with three or more complement sets of chromosomes. They are common in plants and include triploids (2n = 2x), tetraploids (2n =3x), pentaploids (2n =5x) hexaploids (2n = 6x), etc.

Aneuploidy: Aneuploidy is the variation in number of single chromosomes. Chromosomes may be added to or subtracted from normal sets. One or more chromosomes may be absent from or may be added to the diploid or polyploid complement.

Aneuploids include:

Monosomics (2n-1): These are diploid organisms which are missing one chromosome of a single pair. Monosomics have reduced fertility and high mortality. Humans with only one sex chromosome are examples of monosomics. These are either (i) AAX0 (Turner syndrome) who are infertile females with underdeveloped external genitalia, have webbed skin in the neck, short in stature and have low intelligence or AAY0 (Rare males) who usually die when still young.

Trisomics (2n +1): These are diploid organisms with one extra chromosome. Monosomics and Trisomics arise due to nondisjunction of one of the autosome pairs resulting in 2n+1 genome in the offspring. In humans, there a number of trisomic conditions of which the most common is the trisomic for the autosomal chromosome 21 causes a genetic disease called Down's syndrome (Mongoloid idiocy). The affected individuals are mentally retarded, short, have stubby fingers and swollen tongue, exhibit abnormalities of prints on their hands and feet and have eye folds resembling those of the Mongolian race

Non-disjunction of sex chromosomes

Failure of sex chromosomes in either sex to separate during meiosis resulting in the production of both normal and rare gametes.

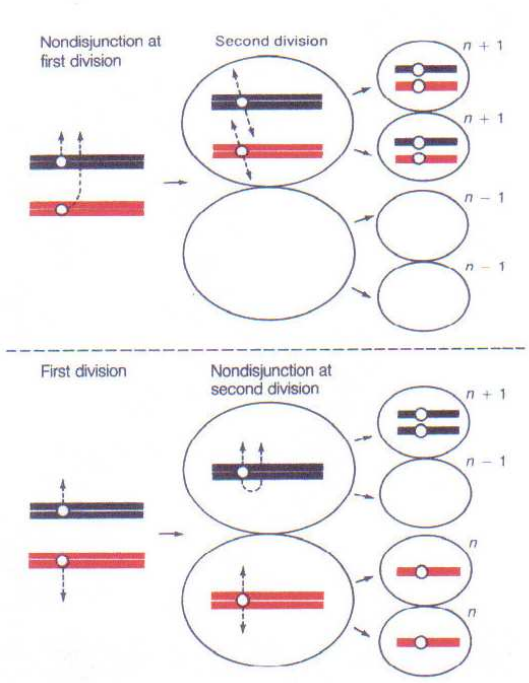


Figure 19.1. The origin of aneuploid gametes by nondisjunction at the first or second meiotic division. If the n+1 gamete fuses with a normal n gamete, the zygote will be 2n+1 while a fusion of the n-1 gamete with a normal gamete will result in a 2n-1 zygote.

Fertilization of the rare gametes results in various sex chromosomal anomalies and associated abnormal phenotypes in the offspring as shown below:

Non-disjunction in the male Non-disjunction in the female

P: 46, AA XY ♂ P: 46, AA XX ♀

Gametes AX, AY, A0, AXY, AXX, AYY AX, AXX, A0

Normal With nondisjunction Normal With nondisjunction

Table 19.1. The F_1 resulting from different types of nondisjunction of the human sex chromosomes

Genotype	**Characteristic**
46, AAXX Diploid	Normal female
46, AAXY Diploid	Normal male
47, AAXXX Trisomic	Superfemale (metafemale) range phenotypically from normal females to nearly like those with Turner's syndrome. Have a high incidence of mental retardation
47, AAXXY Trisomic	Klinefelter syndrome males: sterile, with long limbs, feminine breast development (gynecomastia) and mentally retarded
47, AAXYY Trisomic	Tall-aggressive syndrome: males. Fertile or infertile, usually very tall. Most of the very vicious murders in the world are committed by them
45, AAX0 Monosomic	Turner's syndrome: infertile females with underdeveloped external genitalia. Has a webbing of neck skin, short stature, shield like chest and low intelligence
45, AAY0 Monosomic	Rare males, usually die.

TOPIC 20 HUMAN CHROMOSOMAL ABNORMALITIES II

Expected learning outcomes

At the end of this topic you should be able to understand chromosomal variations with an emphasis on variation in structure.

Variation in chromosome structure

This variation is due to deletion (deficiency), duplication, translocation or inversion. Structural changes in chromosomes lead to formation of loops during meiosis.

Deletion (deficiency): Represent a loss in chromosomal material, which may carry one or more genes.Consider chromosome abcdefg below which loses a part fg through deletion.

Figure 20.1 . Deletion of fg from chromosome abcdefg.

A deletion heterozygote can lead to pseudo-dominance in which an allele is deleted from one chromosome making the alternative recessive allele on the intact chromosome express itself as if it were dominant.

Cri-du Chat syndrome.

In humans, a deletion of a large part of the short arm of chromosome 5 causes a genetic disease called **'Cri-du Chat syndrome'**. The affected babies have a cat-like cry, are also mentally retarded, have a moon face, low birth weight, saddle nose, small mandible and malformed low-set ears.

Partial deletions of chromosomes 4, 13 and 18 are also known to cause specific syndromes.

Duplication (addition): The presence of a section of a chromosome in excess of the normal amount is known as duplication. The repeated section of chromosomal material may be present in one pair of homologous chromosomes or may have been transposed to a nonhomologue. Generally speaking, duplications are not as deleterious as deletions. In fact duplications usually act as new sources of genetic variation. The diagram below illustrates duplication of segment ab.

Figure 20.2. Duplication of ab in chromosome abcdefg

Translocation: A translocation moves a chromosome segment to another position in the genome. Chromosomes sometimes undergo spontaneous rupture, or can be induced to rupture due to high frequency of ionisation radiation. The broken ends of such chromosomes may rejoin into a non-homologous position.

Simple translocation: Involves a single break in the chromosome and the transfer of a broken piece directly onto the end of another.

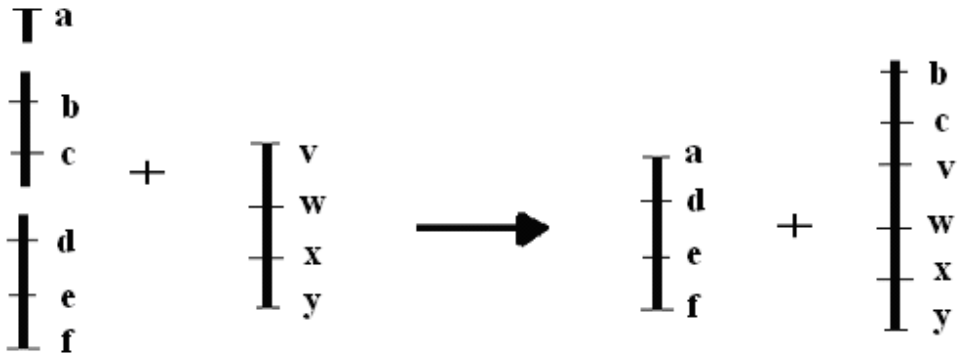


Figure 20.3. Simple translocation of bd from abcdefg to one end of vwxy

Shift (intercalary) translocation: Involves three breaks, so that a middle piece from one chromosome is inserted within the break in a nonhomologous chromosome.

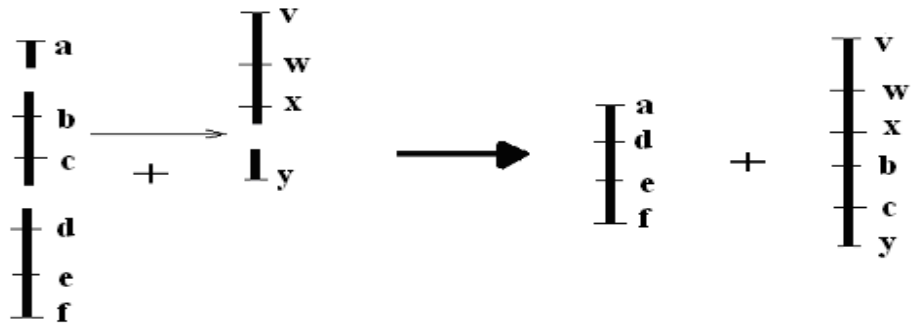


Figure 20.4. Shift translocation of bc in between x and y involving nonhomologues.

Reciprocal translocation (interchanges): These occur when single breaks in two nonhomologous chromosomes produce an exchange of chromosome segments.

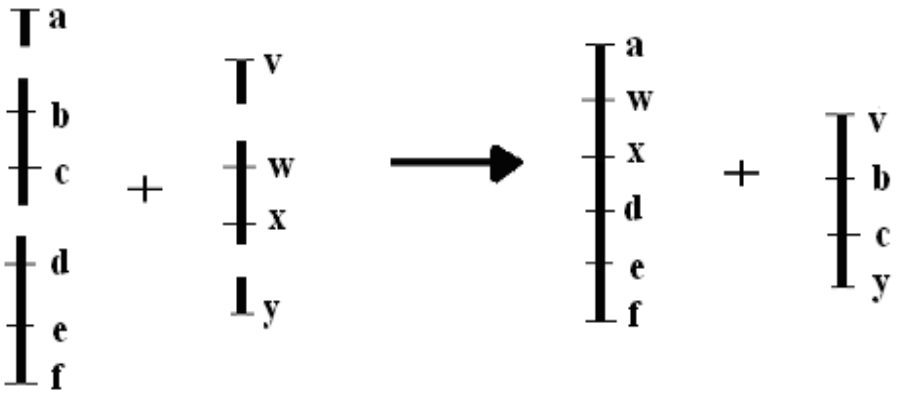


Figure 20.5. Reciprocal translocation of segments bc and wx between two homologues

The Philadelphia chromosome is caused by the reciprocal translocation between chromosome 9 and 22. It is found in about 90% of patients with chronic myelocytic leukaemia (a blood cancer). t(9;22)(q34;q11) - Philadelphia chromosome, Chronic myeloid leukaemia (CML), ALL

Reciprocal translocation between chromosomes 8 and 14 causes most cases of Burkitt's lymphoma, a cancer of the B lymphocytes. t(8;14) - Burkitt's lymphoma (c-myc)

Inversion: An inversion involves a break of segment in a chromosome and the rejoining of the segment but after a 180° turn in the segment.

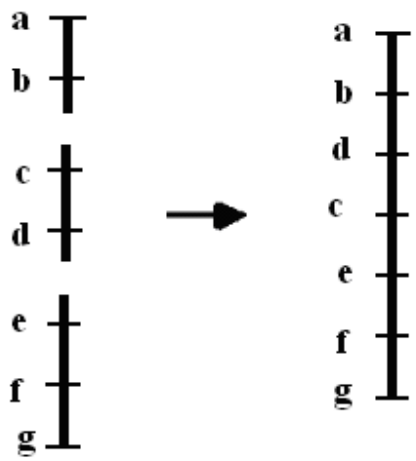


Figure 20.6. Inversion of the segment cd.

Pericentric inversions span the centromere while paracentric inversions do not span the centromeres.

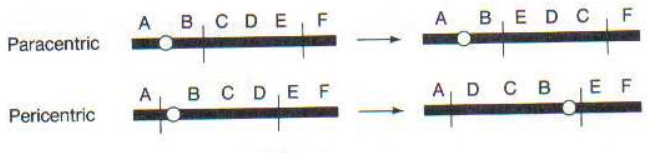


Figure 20.7. Paracentric and pericentric inversions.

An inversion can disrupt a gene if it occurs in the middle of a gene and generally speaking paracentric inversions are less deleterious than pericentric inversions.

21. DETECTION AND PEDIGREE ANALYSIS OF HUMAN GENETIC DISEASES

Expected learning outcomes

At the end of this topic you should be able to understand the methods of detection of human genetic diseases and the methods of pedigree analysis of human genetic diseases.

Detection of human genetic diseases

The first step towards the pedigree analysis of human diseases is the detection of the diseases themselves. There are a number of techniques used to diagnose genetic diseases. Prenatal diagnostic methods, for example, are used to detect disorders in unborn children. These techniques include; amniocentesis, chorionic villi sampling and ultra sound.

(i) Amniocentesis

In this method a sample of the amniotic fluid is taken using a syringe and a hypodermic needle around the sixteenth week of pregnancy. It is separated into fluid and cells. The fluid is used to detect (a) neural tube disorders and (b) biochemical (metabolic) diseases while the cells are cultured and used to study (a) foetal sex, (b) chromosomal disorders and (c) metabolic diseases.

(ii) Chorionic villi sampling

In this technique a sample of finger like projections called chorionic villi is taken from the chorion, which is the outermost membrane surrounding the foetus. The sample is taken using either a transabdominal needle guided by an ultrasound probe or through a transcervical catheter also guided by an ultrasound probe. This technique is used to study foetal sex, metabolic disorders and chromosomal disorders. Although this method produces faster results compared to amniocentesis, it cannot detect neural tube defects.

Ultra sound

Ultra sound is used together with amniocentesis and chorionic villi sampling in locating the placenta, establishing an accurate gestational stage and excluding multiple pregnancy and foetal death.

Pedigree analysis of human genetic diseases

A human pedigree is a chart of an individual's ancestors used in human genetics to analyze Mendelian inheritance of certain traits, especially of inherited diseases.

The use of pedigree analysis involves collection of information about a particular family and their ancestors. This information is then used to construct a pedigree chart (family tree). This chart contains names of people and also information about their phenotypes. Using this chart it is possible to predict the risks of a particular couple having a child who might suffer from a particular genetic disorder.

The symbols used in pedigree analysis are summarised in Figure 6.1. Consanguinity is the marriage of related individuals, for example cousins. The propositus is the individual who first draws the geneticist's attention to a particular family.

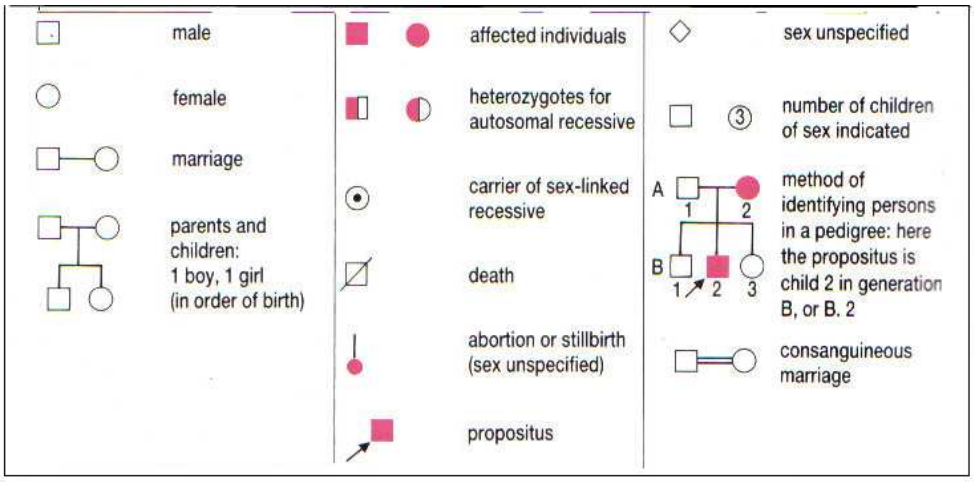


Figure 21.1. The symbols used in pedigree analysis.

Autosomal dominant traits

Autosomal traits are carried on the autosomal chromosomes (not on the sex chromosomes). Inheritance of autosomal traits is best-studied using dominant traits. Some clues used to show that a trait is not sex-linked (X-linked):

(i) Equal numbers of male and female offspring are affected

(ii) If the affected male passes the trait to his sons, it means the gene cannot be on the X-chromosome since the man only passes the Y chromosome to his sons.

(iii) If the affected male has affected daughters as well as unaffected daughters-because if the gene were X-linked dominant then all his daughters would be affected, since he transmits his X chromosome to each of them.

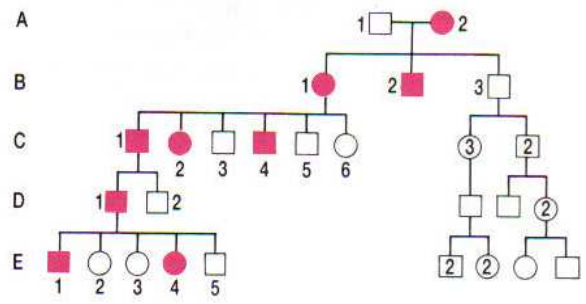


Figure 21.2. A pedigree showing a pattern of inheritance consistent with a trait inherited as an autosomal dominant.

Autosomal recessive inheritance

An autosomal recessive is characterised by; (1) rarity of the trait (2) skipping of generations and (3) consanguineous marriages. Figure 6.3 illustrates this.

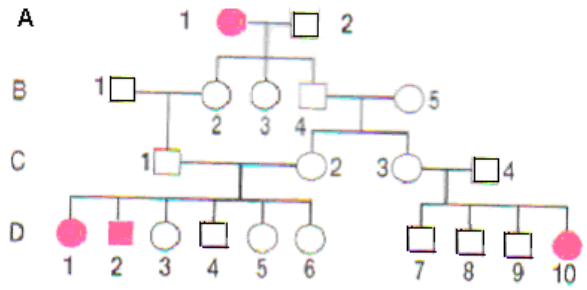


Figure 21.3. A pedigree showing a pattern of inheritance consistent with a trait inherited as an autosomal recessive.

Sex-linked traits

Sex-linked traits are carried by sex chromosomes (X or Y). Sex-linked (X-linked) are characterised by: (1) Many more males than females being affected (2) If caused by a very rare recessive gene, almost all observed cases would be males. (3) Usually none of the offspring of an affected male will be affected (if his wife is unaffected) but all his daughters will carry the allele, so half their sons, on average should be affected. Figure 6.4 illustrates this.

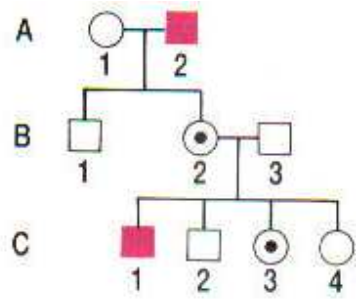


Figure 21.4. A pedigree showing the inheritance of an X-linked recessive trait.

Table 21.1 Some genetic diseases

Disease	Type of inheritance	Type of allele
Cystic fibrosis	Autosomal	Recessive
PKU	Autosomal	Recessive
Sickle cell anaemia	Autosomal	Recessive
Colour blindness	X-linked	Recessive
Haemophilia	X-linked	Recessive
Huntington's disease	Autosomal	Dominant
Muscular dystrophy	X-linked	Recessive

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