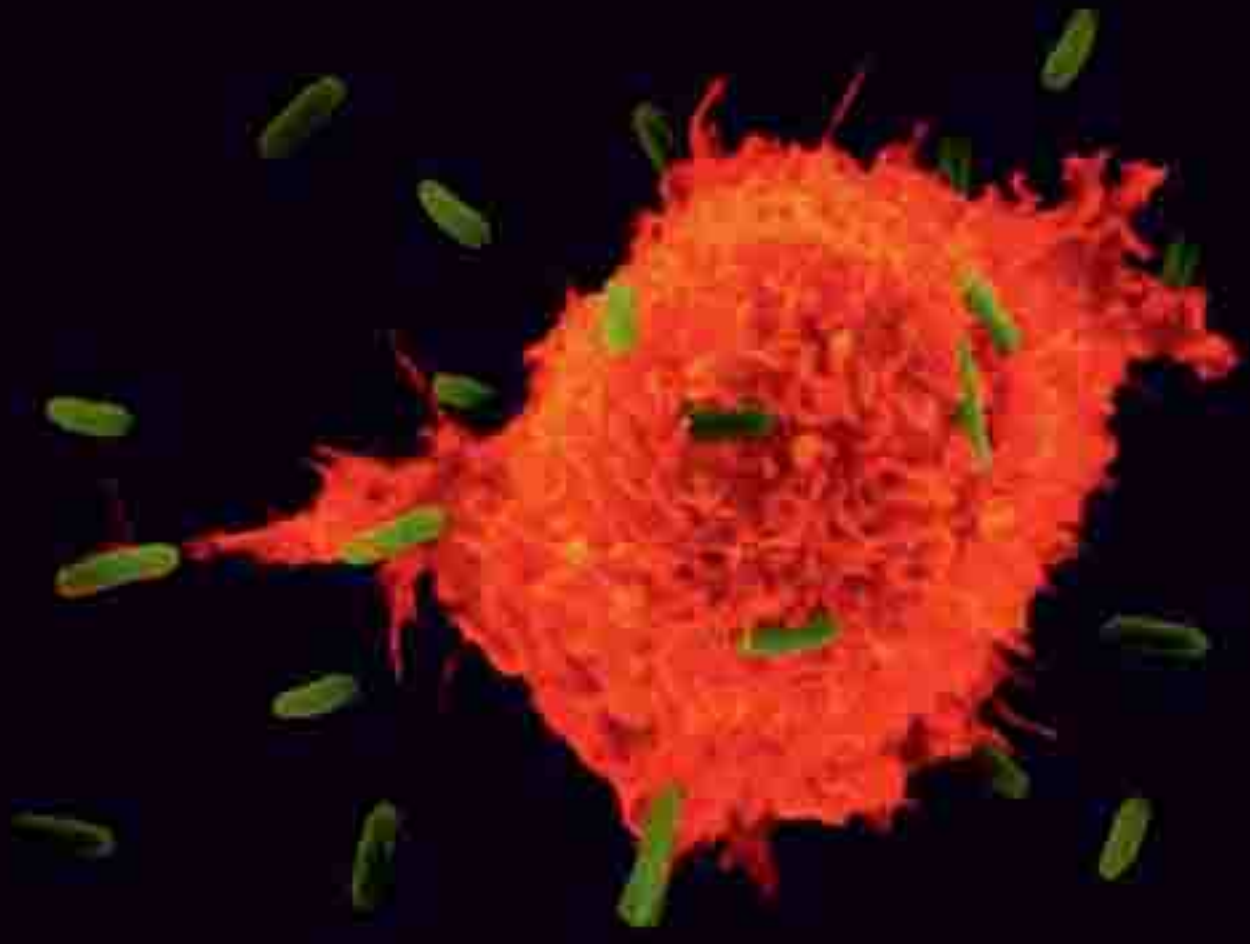
Life Sciences

Fundamentals and Practice - II

Fifth edition



Pranav Kumar | Usha Mina

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Part – II

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Preface

Life Sciences have always been a fundamental area of science. The exponential increase in the quantity of scientific information and the rate, at which new discoveries are made, require very elaborate, interdisciplinary and up-to-date information and their understanding. This fourth edition of Life sciences, Fundamentals and practice includes extensive revisions of the previous edition. We have attempted to provide an extraordinarily large amount of information from the enormous and ever-growing field in an easily retrievable form. It is written in clear and concise language to enhance self-motivation and strategic learning skill of the students and empowering them with a mechanism to measure and analyze their abilities and the confidence of winning. We have given equal importance to text and illustrations. The fourth edition has a number of new figures to enhance understanding. At the same time, we avoid excess details, which can obscure the main point of the figure. We have retained the design elements that have evolved through the previous editions to make the book easier to read. Sincere efforts have been made to support textual clarifications and explanations with the help of flow charts, figures and tables to make learning easy and convincing. The chapters have been supplemented with self-tests and questions so as to check one's own level of understanding. We hope you will find this book interesting, relevant and challenging.

Acknowledgements

Our students were the original inspiration for the first edition of this book, and we remain continually grateful to all of them, because we learn from them how to think about the life sciences and how to communicate knowledge in most meaningful way. We thank, Dr. Diwakar Kumar Singh, Harleen Kaur and Mr. Ajay Kumar, reviewers of this book, whose comment and suggestions were invaluable in improving the text. Any book of this kind requires meticulous and painstaking efforts by all its contributors. Several diligent and hardworking minds have come together to bring out this book in this complete form. We are much beholden to each of them and especially to Dr. Neeraj Tiwari. This book is a team effort, and producing it would be impossible without the outstanding people of Pathfinder Publication. It was a pleasure to work with many other dedicated and creative people of Pathfinder Publication during the production of this book, especially Pradeep Verma.

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Chapter 01

Genetics

All living organisms reproduce. Reproduction results in the formation of offspring of the same kind. However, the resulting offspring need not and, most often, does not totally resemble the parent. Several characteristics may differ between individuals belonging to the same species. These differences are termed *variations.* The mechanism of transmission of characters, resemblances as well as differences, from the parental generation to the offspring, is called *heredity.* The scientific study of heredity, variations and the environmental factors responsible for these, is known as *genetics* (from the Greek word *genno* = give birth). The word *genetics* was first suggested to describe the study of inheritance and the science of variation by prominent British scientist William Bateson.

Genetics can be divided into three areas: classical genetics, molecular genetics and evolutionary genetics. In *classical genetics,* we are concerned with Mendel's principles, sex determination, sex linkage and cytogenetics. *Molecular genetics* is the study of the genetic material: its structure, replication and expression, as well as the information revolution emanating from the discoveries of recombinant DNA techniques. *Evolutionary genetics* is the study of the mechanisms of evolutionary change or changes in gene frequencies in populations (population genetics).

Classical genetics

1.1 Mendel's principles

Gregor Johann Mendel (1822–1884), known as the *Father of Genetics,* was an Austrian monk. In 1856, he published the results of *hybridization experiments* titled *Experiments on Plant Hybrids* in a journal "The proceeding of the Brunn society of natural history" and postulated the principles of inheritance which are popularly known as **Mendel's laws**. But his work was largely ignored by scientists at that time. In 1900, the work was independently rediscovered by three biologists - Hugo de Vries of Holland, Carl Correns of Germany and Erich Tschermak of Austria. Mendel did a statistical study (he had a mathematical background). He discovered that individual traits are inherited as discrete *factors* which retain their physical identity in a hybrid. Later, these factors came to be known as *genes*. The term was coined by Danish botanist Wilhelm Johannsen in 1909. A gene is defined as a *unit of heredity* that may influence the outcome of an organism's traits.

Mendel's experiment

Mendel chose the garden pea, *Pisum sativum*, for his experiments since it had the following advantages.

1. Well-defined discrete characters
2. Bisexual flowers
3. Predominant self fertilization
4. Easy hybridization
5. Easy to cultivate and relatively short life cycle

Characters studied by Mendel

The characteristics of an organism are described as *characters* or *traits*. Traits studied by Mendel were clear cut and discrete. Such clear-cut, discrete characteristics are known as *Mendelian characters.* Mendel studied seven characters/ traits (all having two variants) and these are:

		Dominant	*Recessive*
1.	Stem length	Tall	Dwarf
2.	Flower position	Axial	Terminal
3.	Flower colour	Violet	White
	Seed coat colour	Grey	White
4.	Pod shape	Inflated	Constricted
5.	Pod colour	Green	Yellow
6.	Cotyledon colour	Yellow	Green
7.	Seed form	Round	Wrinkled

Flower colour is positively correlated with seed coat colours. Seeds with white seed coats were produced by plants that had white flowers and those with gray seed coats came from plants that had violet flower.

Allele

Each gene may exist in alternative forms known as *alleles*, which code for different versions of a particular inherited character. We may also define alleles as genes occupying corresponding positions on homologous chromosomes and controlling the same characteristic (e.g. height of plant) but producing different effects (tall or short). The term *homologous* refers to chromosomes that carry the same set of genes in the same sequence, although they may not necessarily carry identical alleles of each gene.

Wild-type versus Mutant alleles

Prevalent alleles in a population are called *wild-type alleles*. These alleles typically encode proteins that are made in the right amount and function normally. Alleles that are present at less than 1% in the population and have been altered by mutation are called *mutant alleles.* Such alleles *usually* result in a reduction in the amount or function of the wild-type protein and are most often inherited in a recessive fashion.

Dominant and Recessive alleles

A *dominant allele* masks or hides expression of a recessive allele and it is represented by an *uppercase letter.* A *recessive allele* is an allele that exerts its effect only in the *homozygous state* and in heterozygous condition its expression is masked by a dominant allele. It is represented by a *lowercase letter*.

Homozygous and Heterozygous

Each parent (diploid) has two alleles for a trait — they may be:

1. **Homozygous**, indicating they possess two identical alleles for a trait.
   a. *Homozygous dominant* genotypes possess two dominant alleles for a trait (TT).
   b. *Homozygous recessive* genotypes possess two recessive alleles for a trait (tt).
2. **Heterozygous** genotypes possess one of each allele for a particular trait (Tt).

1.1.4 Lethal alleles

Certain genes are absolutely essential for survival. The alleles created by mutations in these genes are called *lethal alleles*. The phenotypic manifestation of these alleles is the death of the organism. Lethal alleles may be *recessive* or *dominant*. Recessive lethal alleles are lethal when present in homozygous conditions whereas dominant lethal alleles show lethal effects even in heterozygous conditions. Dominant lethal alleles are very rare. Lethal alleles fall into four categories:

- *Early onset* : Lethal alleles which result in early death of an organism, during embryogenesis.
- *Late onset* : Lethal genes which have delayed effect so that the organism can live for some time but eventually succumb to the disease.
- *Conditional* : Lethal alleles which kill organism under certain environmental conditions only. For example, a temperature sensitive lethal allele may kill organism at high temperature, but not at low temperature.
- *Semilethal* : Lethal alleles which kill only some individuals in the population but not all.

1.1.5 Penetrance and expressivity

The percentage of individuals that shows a particular phenotype among those capable of showing it, is known as *penetrance*. Let us take an example of polydactyly in human, which is produced by a dominant gene. Homozygous recessive genotype does not cause polydactyly. However, some heterozygous individuals are not polydactylous. If suppose 20% of heterozygous individuals do not show polydactyly, this means that the gene has a penetrance of 80%. Degree of expression of a trait is controlled by a gene. A particular gene may produce different degrees of expression in different individuals. This is known as *expressivity*. Different degrees of expression in different individuals may be due to variation in the allelic constitution of the rest of the genome or to environmental factors. Thus, the terms penetrance and expressivity quantify the modification of gene expression by varying environment and genetic background; they measure respectively the percentage of cases in which the gene is expressed and the level of expression.

Phenocopy

A phenotype that is not genetically controlled but looks like a genetically controlled one is called *phenocopy*. It is an environmentally induced phenotype that resembles the phenotype determined by the genotype. An example of a phenocopy is Vitamin-D-resistant rickets. A dietary deficiency of vitamin D, for example, produces rickets that is virtually indistinguishable from genetically caused rickets.

1.1.6 Probability

The chance that an event will occur in the future is called the event's *probability*. For example, if you flip a coin, the probability is 0.50, or 50%, that the head side will be showing when it lands. The probability depends on the number of possible outcomes. In this case, there are two possible outcomes (head and tail), which are equally likely. This allows us to predict that there is a 50% chance that a coin flip will produce head. The general formula for the probability is:

$$\text{Probability} = \frac{\text{Number of times an event occurs}}{\text{Total number of events}}$$

$$P_{head} = 1 \text{ head}/(1 \text{ head} + 1 \text{ tail}) = 1/2 = 50\%$$

A probability calculation allows us to predict the likelihood that an event will occur in the future. The accuracy of this prediction, however, depends to a great extent on the size of the sample.

In genetic problems, we are often interested in the probability that a particular type of offspring will be produced. For example, when two heterozygous tall pea plants (Tt) are crossed, the phenotypic ratio of the offspring is 3 tall : 1 dwarf. This information can be used to calculate the probability for either type of offspring:

$$\text{Probability} = \frac{\text{Number of individuals with a given phenotype}}{\text{Total number of individuals}}$$

P_{tall} = 3 tall/(3 tall + 1 dwarf) = 3/4 = 0.75 = 75% and P_{dwarf} = 1 dwarf/(3 tall + 1 dwarf) = 1/4 = 0.25 = 25%

The probability of obtaining a tall plant is 75% and a dwarf plant 25%. When we add together the probabilities of all the possible outcomes (tall and dwarf), we should get a sum of 100% (here, 75% + 25% = 100%).

There are two basic laws of probability that are used for genetic analysis. The first law, the *multiplicative law* (*product rule*) *of probability,* states that the chance of two or more *independent events* occurring together is the product of the probability of the events occurring separately. Independent events are events whose outcomes do not influence one another. This is also known as the **and rule**. *The product rule can be used to predict the probability of independent events that occur in a particular order.*

Example 1,

A Mendelian cross has been made between pea plants that are heterozygous for plant height (Tt). What is the probability that the offspring will be homozygous recessive (tt)?

We can find the answer by applying the *product rule*. First, the probability that an egg will receive a 't' allele = 1/2 and a sperm will receive a 't' allele = 1/2. The overall probability that two recessive alleles will unite, one from the egg ***and*** one from the sperm, simultaneously, at fertilization is: 1/2 × 1/2 = 1/4.

Example 2,

A cross has been made between two plants of genotypes AabbCcDd and AaBbCcdd. What is the probability that the offspring will be of genotype *aabbccdd*?

If we assume that all the gene pairs assort independently, then we can do this calculation easily by using the *product rule*. The four different gene pairs are considered individually, as if four separate crosses, and then the appropriate probabilities are multiplied together to arrive at the answer. From Aa × Aa, one-fourth of the progeny will be aa; from bb × Bb, one-half of the progeny will be bb; from Cc × Cc, one-fourth of the progeny will be cc; and from Dd × dd, one-half of the progeny will be dd. Therefore, the overall probability of progeny of genotype aabbccdd will be 1/4 × 1/2 × 1/4 × 1/2 = 1/64.

The second law is the **additive law** (*sum rule*) *of probability.* It states that the probability that one of two or more mutually exclusive events will occur is equal to the sum of the individual probabilities of the events. This is also known as the *either or rule. The sum rule can be used to predict the occurrence of mutually exclusive events.* Mutually exclusive events are events in which the occurrence of one possibility excludes the occurrence of the other possibilities.

Example 1,

In a Mendelian cross between pea plants that are heterozygous for flower colour (Rr), what is the probability of the offspring being a heterozygote?

There are two ways in which a heterozygote may be produced: the dominant allele (R) may be in the egg and the recessive allele (r) in the sperm or the dominant allele may be in the sperm and the recessive in the egg. Consequently, the probability that the offspring will be heterozygous is the sum of the probabilities of those two possible ways:

Probability that the dominant allele will be in the egg with the recessive in the sperm is 1/2 × 1/2 = 1/4.

Probability that the dominant allele will be in the sperm and the recessive in the egg is 1/2 × 1/2 = 1/4.

Therefore, the probability that a heterozygous offspring will be produced is 1/4 + 1/4 = 1/2.

Example 2,

A heterozygous pea plant that is tall with yellow seeds, TtYy, is allowed to self-fertilize. What is the probability that an offspring will be either tall with yellow seeds, tall with green seeds, or dwarf with yellow seeds?

The problem involves three mutually exclusive events, we can use the *sum rule* to solve it.

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1.2 Chromosomal basis of inheritance

In 1902, *Walter S. Sutton* and *T. Boveri* proposed the *chromosomal theory of heredity*. The theory provides a way to explain how the cellular transmission or chromosomes passes genetic determinant (i.e. genes) from parent to offspring. According to this view:

1. Chromosome contains the genetic material (genes) that is transmitted from parent to offspring.
2. Chromosomes are replicated and passed along generation after generation from parent to offspring.
3. The nuclei of most eukaryotic cells contain chromosomes that are found in homologous pairs (i.e. they are diploid). One member of each pair is inherited from the mother, the other from the father. At meiosis, one of the two members of each pair segregates into one daughter nucleus and the other segregates into different daughter nucleus. Therefore, gametes contain one set of chromosomes (i.e. they are haploid) as shown in figure 1.6.
4. During gamete formation, different types of chromosomes segregate independently of each other.
5. Each parent contributes one set of chromosomes to its offspring.

   Hence, the chromosome theory of inheritance describes the relationship between Mendel's Law and chromosomal transmission.

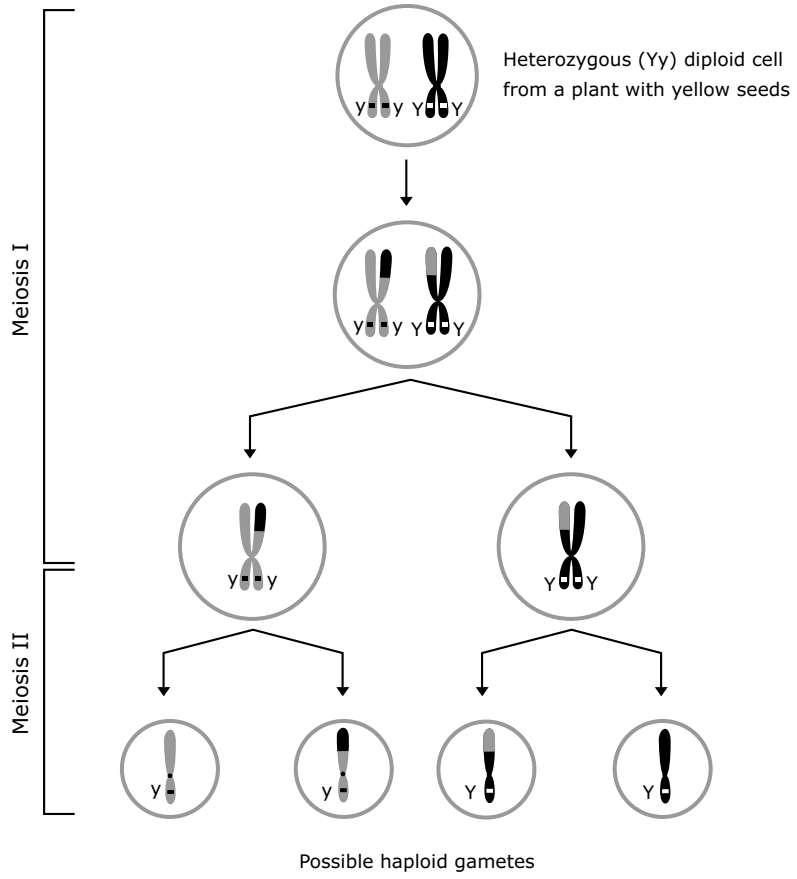


Figure 1.6 Segregation of homologous chromosome during meiosis explains Mendel's law of segregation.

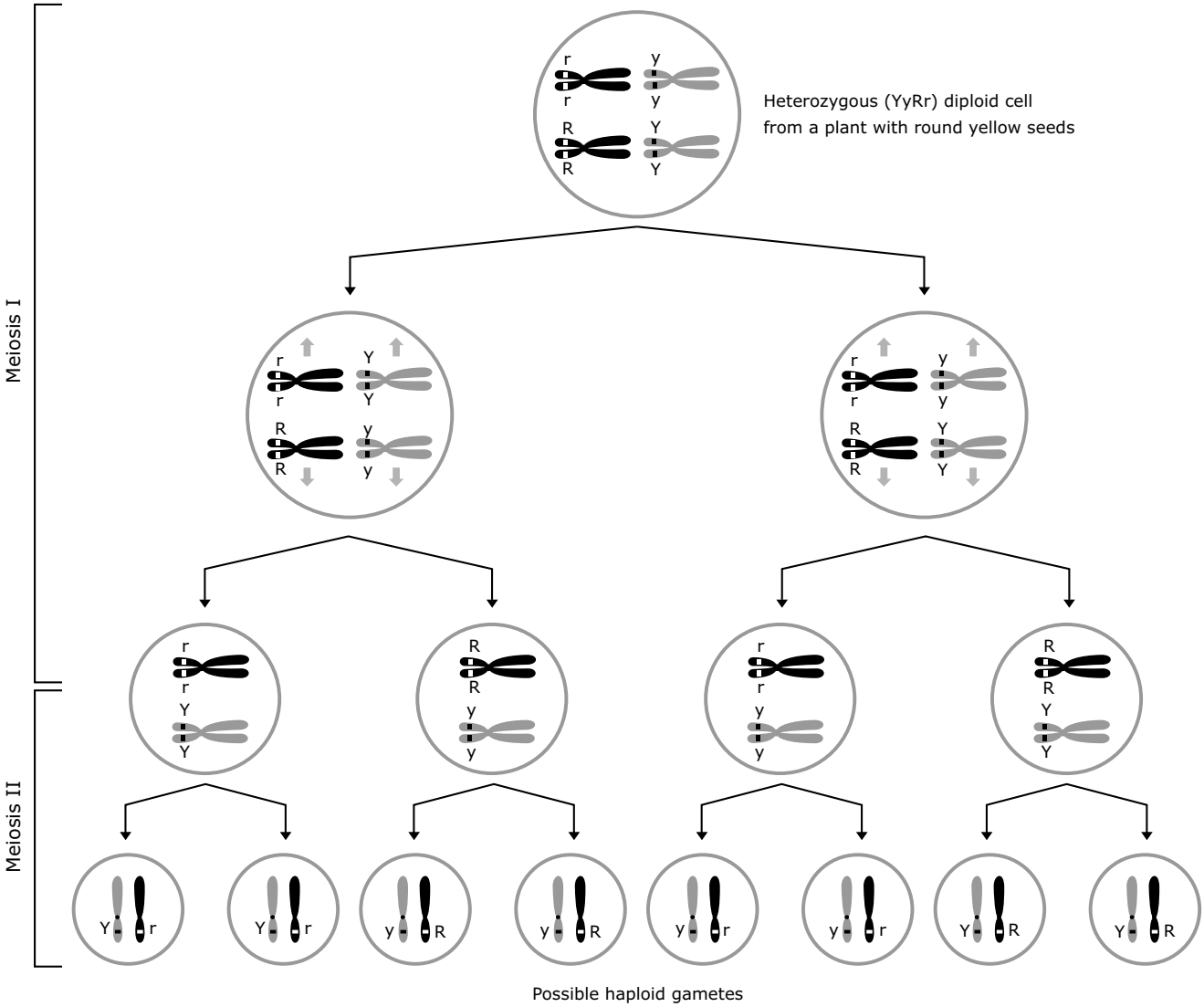


Figure 1.7 Random alignment of bivalents during prophase of meiosis I explains Mendel's law of independent assortment.

1.3 Gene interaction

According to Mendel, genes are functioning independently of each other i.e. each of seven traits considered was controlled by a single gene. But many traits of an organism are determined by the complex contribution of many different genes. When two or more different genes (non-allelic) influence the outcome of single trait, this is known as a *gene interaction*.

The first case of two different genes interacting to affect a single trait was discovered by William Bateson and Reginald Punnett in 1906. They discovered an unexpected gene interaction when they studied crosses involving the sweet pea, *Lathyrus odoratus*. When they crossed true breeding purple flowered plant to a true breeding white flowered plant, the F1 generation was all purple flowered plants and the F2 generation (produced by self fertilization of the F1 generation) contained purple and white flowered plants in a 3 : 1 ratio. But when they crossed two different varieties of white flowered plants then all F1 generation plants had purple flowers. When these purple flower plants were allowed to self fertilized, the F2 generation contained purple and white flowers in a ratio of 9 purple : 7 white. How can this unexpected result be explained? This surprising result was explained by Bateson

1.5 Tetrad analysis

Certain species of lower eukaryotes, particularly unicellular algae and fungi, which spend the greatest part of their life cycle in the haploid state, have also been used in mapping studies. The sac fungi (ascomycetes) have been particularly useful to geneticists because of their unique style of sexual reproduction.

Fungi may be unicellular or multicellular. Fungal cells are typically haploid (1n) and can reproduce asexually. In addition, fungi can also reproduce sexually by the fusion of two haploid cells to create a diploid zygote (2n). The diploid zygote can then proceed through meiosis to produce four haploid cells, which are called *spores.* This group of four spores is known as a **tetrad**. In some species, meiosis is followed by a mitotic division to produce eight cells, known as an **octad**. The cells of a tetrad or octad are contained within a sac known as an *ascus* (plural, asci).

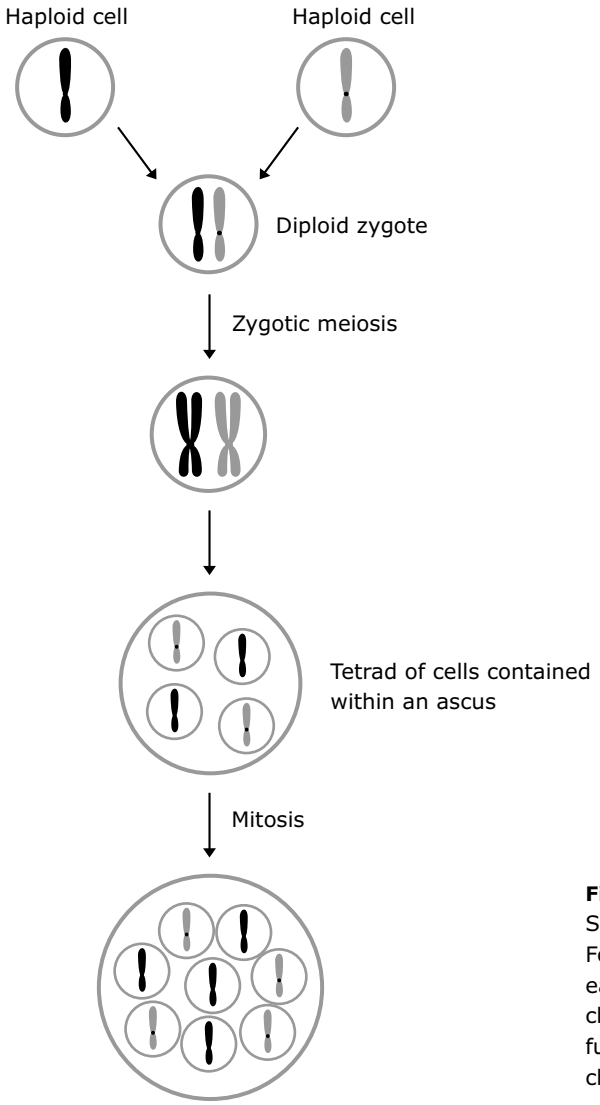


Figure 1.15
Sexual reproduction in ascomycetes. For simplicity, this diagram shows each haploid cell as having only one chromosome per haploid set. However, fungal species actually contain several chromosomes per haploid set.

Ordered *or* unordered tetrad/octad

The arrangement of spores within an ascus varies from species to species. In some cases, the ascus provides enough space for the tetrads or octads of spores to randomly mix together. This is known as an *unordered tetrad* or *octad*. These occur in fungal species such as *S. cerevisiae*. By comparison, other species of fungi produce a very tight ascus that prevents spores from randomly moving around. This can create a *linear tetrad or octad* found in *N.crassa.*

Saccharomyces cerevisiae *Neurospora crassa*

Figure 1.16 Different arrangements of fungal spores.

A key feature of linear tetrads or octads is that the position and order of spores within the ascus reflects their relationship to each other as they were produced by meiosis and mitosis. This idea is schematically shown in figure 1.17. After the original diploid cell has undergone chromosome replication, the first meiotic division produces two cells that are arranged next to each other within the sac. The second meiotic division then produces four cells that are also arranged in a straight row. Due to the tight enclosure of the sac around the cells, each pair of daughter cells is forced to lie next to each other in a linear fashion. Likewise, when each of these four cells divides by mitosis, each of the daughter cells is located next to each other.

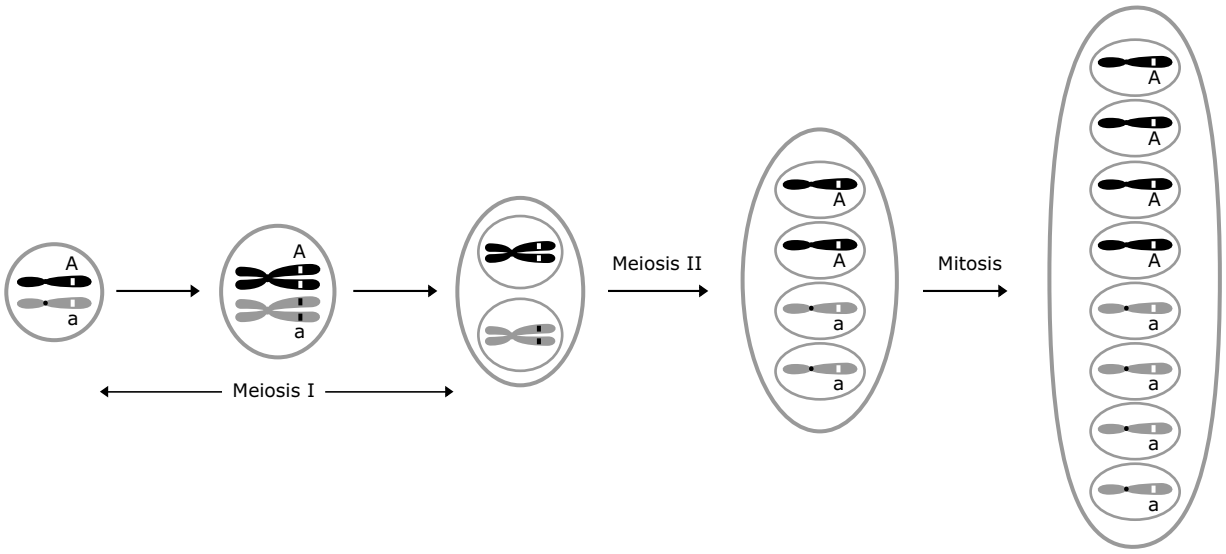


Figure 1.17 Formation of a linear octad in *N. crassa*.

1.5.1 Analysis of ordered tetrad

Linear tetrad analysis can be used to map the distance between a gene and the centromere. This approach has been extensively exploited in *N. crassa.* In *N. crassa,* the products of meiosis are contained in an ordered array of spores. Each mature ascus contains eight ascospores in four pairs, each pair representing one of the products of meiosis. The ordered arrangement of meiotic product makes it possible to map each gene with respect to its centromere; i.e. to determine the recombination frequency between a gene and its centromere. Two cases are possible depending on whether or not there is a crossover between the locus and its centromere.

First case

In the absence of crossing over between a gene and its centromere, the alleles of the gene (for example A and a) must separate in the first meiotic division, this separation is called **First Division Segregation** (FDS). Octad contains a linear arrangement of four haploid cells carrying the A allele, which are adjacent to four haploid cells that contain an allele i.e. 4:4 arrangement of spores within the ascus (figure 1.18).

1.7 Quantitative inheritance

The classical Mendelian traits are qualitative in nature; i.e. traits that are easily classified into distinct phenotypic categories. These discrete phenotypes are under the genetic control of only one or a very few genes with little or no environmental modification to obscure the gene effects.

In contrast to this, the variability exhibited by many traits fails to fit into separate phenotypic classes (*discontinuous variability*), but forms a spectrum of phenotypes that blend imperceptively from one type to another (*continuous variability*). Traits that exhibit continuous variation can usually be quantified by measuring, weighing, counting and so on. Traits such as body weight gains, mature plant heights, egg, or milk production records, and yield of grain per acre are **quantitative** or **complex traits** (historically referred to as *metric traits*) with continuous variability.

Let us compare the discontinuous and continuous variation by considering the example – height of pea plants and kernel colour of wheat. Height of pea plant is a Mendelian trait. So, in terms of height, pea plants can be either tall or dwarf; there is no overlap. All of the F1 generation plants are tall and when these plants are self-fertilized, the ratio of tall and dwarf plant in the F2 is 3 : 1 respectively. This is inheritance involving one locus with two alleles.

Kernel colour of wheat varies continuously from one phenotypic extreme i.e. dark red to the other i.e. white with no clear-cut breaks in between. When a particular strain of wheat having dark red kernels is crossed with another strain having white kernels, all the F1 plants have kernels that are intermediate in colour. When these plants are self-fertilized, the ratio of kernels in the F2 is 1 red : 2 intermediate : 1 white.

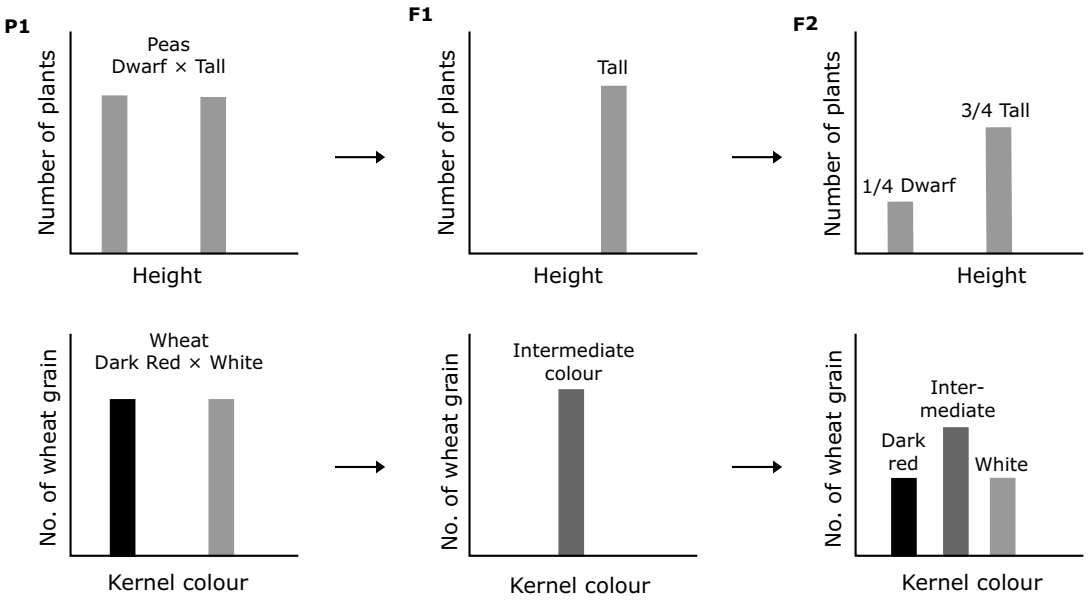


Figure 1.29 Comparison of discontinuous variation in qualitative trait (height in pea plant) with continuous variation in quantitative trait (kernel colour in wheat).

The basic differences between qualitative and quantitative traits involve the number of genes contributing to the phenotypic variability and the degree to which the phenotype can be modified by environmental factors. Quantitative traits are governed by many genes at different loci and each contributes such a small amount to the phenotype that their individual effects cannot be detected by Mendelian methods. Each gene exerts a small additive effect i.e. effects of the genes are cumulative. As more than one gene determines the expression of a given trait, quantitative traits are also referred to as **polygenic traits**. In quantitative traits, no allelic pairs exhibit dominance and there is no genetic interaction between alleles of different loci and no linkage between the loci is involved. Each gene locus may be occupied by either a *contributing allele*, which contributes a constant amount to the phenotype, or by a non-contributing allele, which does not contribute quantitatively to the phenotype.

in the subsequent generations? If the genotypes we assigned to the parents are correct, then the genotype of F1 individuals from each cross are Dd (from DD×dd and dd×DD). If the female genotype does control the phenotype of its offspring, then we would predict that all the F2 snails would have right coils. This is the exact result that is seen. But what would the genotypes of the F2 snails be? If we intermate snails with the genotype Dd, the genotypic ratio should be 3 D_ to 1 dd. These genotypes would not be expressed as a phenotype until the F3 generation. These are the results that were obtained. A general conclusion from all traits that express a *maternal effect* is that the normal Mendelian ratios are expressed one generation than expected. Cytological analysis of developing eggs has provided the explanation of above mentioned result: the genotype of the mother determines the orientation of the mitotic spindle during the second cleavage (mitotic) division in the zygote, and this, in turn, controls the direction of shell coiling of the offspring.

1.9 Cytogenetics

A chromosome is an organized structure of DNA and protein that is found in the nucleus of a eukaryotic cell. The study of the structure, function and abnormalities of chromosome is called *cytogenetics*, a discipline that combines cytology with genetics.

1.9.1 Human karyotype

The number, sizes and shapes of the metaphase chromosomes constitute the *karyotype* or *karyogram,* which is distinctive for each species. The useful karyotypic characteristics are: chromosome size, chromosome number, sex chromosomes, centromere position, nucleolar organizer position, heterochromatin pattern, secondary constriction and banding patterns. Karyotype consisting of a photograph or diagram of all the metaphasic chromosomes arranged in homologous pairs according to decreasing length and position of centromere is described as **idiogram**.

Table 1.6 Symbol used in describing a karyotype

Symbol	*Meaning*
p (*petit*)	Short arm
q (*queue*)	Long arm
13p	Short arm of chromosome 13
13q	Long arm of chromosome 13
del	Deletion
del(2)	Deletion in chromosome 2
dup	Duplication
dup(1)	Duplication in chromosome 1
inv	Inversion
inv(4)	Inversion in chromosome 4
t	Translocation
t(2;5)	Reciprocal translocation between a chromosome 2 and a chromosome 5
tel	Telomere
cen	Centromere
+ or –	Indicate gain or loss of part of chromosome
2q–	Deletion of the long arm of chromosome 2

Tijo and *Levan* (1956) of Sweden found that human cells have 23 pairs or 46 chromosomes. Of the 23 pairs, 22 are perfectly matched in both males and females, and are called *autosomes*. The remaining pair, the sex chromosomes, consists of two similar chromosomes in females and two dissimilar chromosomes in males. In human, females are designated XX and males XY.

Denver system

According to 'Denver system' of classification, the 22 pairs of human chromosomes are placed in seven groups as;

	Group	*Position of centromere*	*Idiogram number*
I	(A)	Metacentric or submetacentric	1, 2, 3
II	(B)	Submetacentric	4, 5
III	(C)	Submetacentric	6, 7, 8, 9, 10, 11, 12 and X
IV	(D)	Acrocentric	13, 14 and 15
V	(E)	Metacentric or submetacentric	16, 17 and 18
VI	(F)	Metacentric	19 and 20
VII	(G)	Metacentric	21, 22 and Y

1.9.2 Chromosome banding

Chromosome banding is a cytological procedure of differential staining of mitotic chromosome along the longitudinal axis. The differential staining reactions reflect the heterogeneity and complexity of the chromosome along its length. The molecular mechanisms involved in producing the various banding patterns are not precisely defined. Chromosome *painting* is different from *banding.* It refers to the hybridization of fluorescently labeled chromosome-specific, composite probe pools to chromosome.

The most common methods of dye-based chromosome banding are G- (Giemsa), R- (reverse), C- (centromere) and Q- (quinacrine) banding. Bands that show strong staining are referred to as *positive bands*; weakly staining bands are *negative bands*. Features of commonly used banding techniques are described in the table 1.7.

Table 1.7 Chromosome banding techniques

Technique	*Procedure*	*Banding pattern*
G-banding	Mild proteolysis with trypsin followed by staining with Giemsa (G stand for Giemsa).	Dark bands are AT-rich (gene poor) Pale bands are GC-rich (gene rich)
R-banding	Heat denature followed by staining with Giemsa. Reverse of G-banding and R stand for Reverse.	Dark bands are GC-rich Pale bands are AT-rich
Q-banding	Stain with Quinacrine mustard (a fluorescent stain). Q stands for Quinacrine.	Dark bands are AT-rich Pale bands are GC-rich
C-banding	Denature with barium hydroxide and then stain with Giemsa. C stands for Constitutive heterochromatin.	Dark bands contain constitutive heterochromatin

Regions and bands

A *region* is an area that lies between two landmarks. Regions are divided into bands. A *band* is that part of a chromosome that is distinctly different from the adjacent area by virtue of being lighter or darker in staining intensity. Each band is approximately 5 to 10 megabase pairs of DNA that may include hundreds of genes. The bands and the regions to which they belong are identified by numbers, with the centromere serving as the point of reference for the numbering scheme. In designating a particular band, four items are required: the chromosome number, the arm symbol, the region number and the band number within that region. A band within a region is numbered in sequence with band 1 being nearest to the centromere.

For example,

13q14 Region 1, band 4 of the long arm of chromosome 13.

Band can further be divided into *sub-bands*. By convention, a decimal point is placed before any sub-band number. Sub-bands are numbered sequentially from centromere outward.

13q14.2 Sub-band 2 of 13q14

Ring chromosomes and Isochromosomes

A *ring chromosome* (denoted by the symbol, r) is formed when a break occurs on each arm of a chromosome leaving two sticky ends on the central portion that reunite as a ring.

An *isochromosome* is an abnormal chromosome that has lost one of its arms and replaced it with an exact copy of the other arm. The most probable explanation for the formation of an isochromosome is that the centomere has divided transversely rather than longitudinally.

1.9.5 Position effect

The change in the phenotypic expression of one or more genes as a result of a change in position in the genome is called *position effect.* Position effect may be exhibited if a gene located in euchromatin is brought near heterochromatin. These effects are either *stable*, as in the Bar eye of *Drosophila* or *variegated*, as with *Drosophila* eye colour.

The white gene controls eye pigment production in *Drosophila*. The locus for white gene is near the tip of the X-chromosome. Wild type flies with a normal white gene ($white^+$) have normal pigment production, which gives them red eyes, but if the white gene is mutated and inactivated, the mutant flies ($white^-$) make no pigment and have white eyes. In case of *position effect variegation,* the eyes are mottled, with both red and white patches. The white patches represent cells in which $white^+$ gene is inactive, whereas red patches represent cells with active $white^+$ gene. Inactivation of $white^+$ gene in cells of white patches is due to change in the position of $white^+$ gene from euchromatin region to adjacent heterochromatin region. This difference in gene expression is an example of position effects because the activity of a gene depends on its position along a chromosome.

There is also a position effect in the *Bar system*. Both a homozygous Bar and a heterozygous double Bar have four copies of the 16A regions. It would therefore be reasonable to expect that both genotypes would produce the same phenotype. However, the homozygous Bar has about 70 facets in each eye, whereas the heterozygote has about 45.

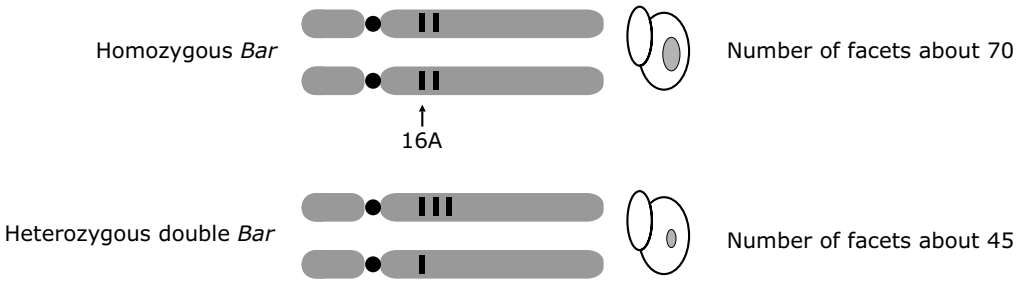


Figure 1.41 Position effect and Bar eye in *Drosophila*. In homozygous Bar females, there are four copies of 16A loci, two on each homolog; these flies have about 70 facets in their reduced eyes. In females heterozygous for double-Bar, there are also four copies of 16A loci, three on the double-Bar chromosome and one on the normal chromosome; even though the number of loci is the same, these flies have smaller eyes, with about 45 facets.

1.10 Population genetics

Population genetics is the study of changes in the frequencies of alleles and genotypes within a population (an interbreeding group of individuals of a particular species at a particular place). Populations are usually subdivided into partially isolated breeding groups called *demes*. The complete set of genetic information contained within the members in a population is called the *gene pool*. The gene pool includes all alleles present in the population.

1.11 Genome

Genome is the sum total of all genetic material of an organism which store biological information. The nature of the genome may be either DNA or RNA. All eukaryotes and prokaryotes always have a DNA genome, but viruses may either have a DNA genome or RNA genome. The eukaryotic genome consists of two distinct parts: Nuclear genome and organelles (mitochondrial and chloroplast) genome. The nuclear genome consists of linear dsDNA. In a few lower eukaryotes, double-stranded circular plasmid DNA (for example, 2-micron circle in yeast) is also present within the nucleus.

The amount of DNA present in the genome of a species is called a **C-value**, which is characteristic of each species. The value ranges from $<10^6$ bps as in smallest prokaryote, *Mycoplasma* to more than 10^{11} bps for eukaryotes such as amphibians. The genomes of higher eukaryotes contain a large amount of DNA.

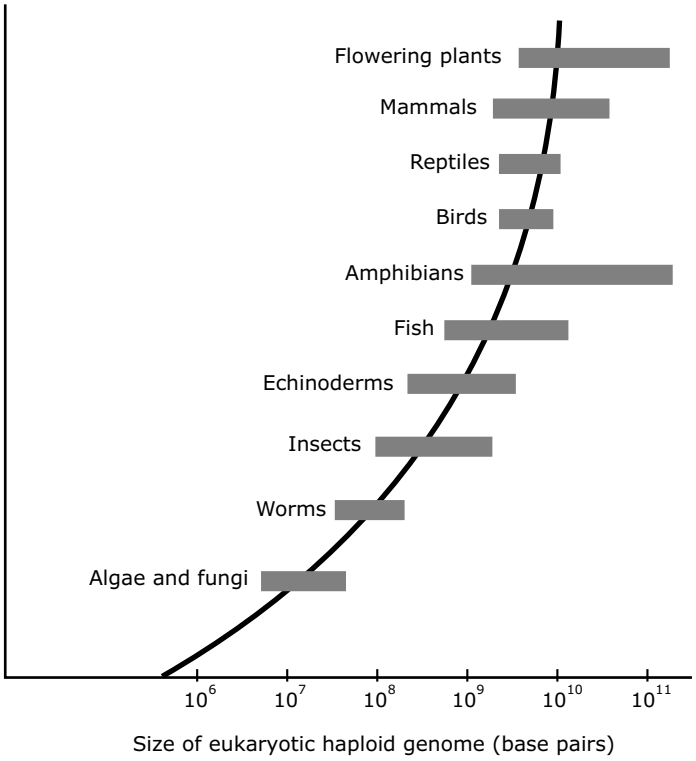


Figure 1.43 The DNA content of the haploid genome of a range of phyla. The range of values within a phylum is indicated by the shaded area.

The DNA content of the organism's genome is related to the morphological complexity of lower eukaryotes, but varies extensively among the higher eukaryotes. In lower eukaryotic organisms like yeast, amount of DNA increases with increasing complexity of organisms. However, in higher eukaryotes there is no correlation between increased genome size and complexity. This lack of correlation between genome size and genetic complexity refers to **C-value paradox**. For example, a man is more complex than amphibians in terms of genetic development, but some amphibian cells contain 30 times more DNA than human cells. Moreover, the genomes of different species of amphibians can vary 100-fold in their DNA contents.

Table 1.9 Genome size in some eukaryotes

Organism	**Genome size** (Mb)
S. cerevisiae (yeast)	12
A. thaliana (mustard plant)	120
D. melanogaster (fruit fly)	170
H. sapiens (human)	3,300
H. vulgare (barley)	5,300

1.11.1 Genome complexity

Genome complexity is the total length of different sequences of DNA. It can be measured through the *renaturation kinetics* of denatured DNA. Renaturation of DNA occurs through complementary base pairing. Renaturation of DNA depends on the random collision of the complementary strands, and follows second-order kinetics. A DNA renaturation (reassociation) reaction is described by the $Cot_{1/2}$. If large DNA is sheared into uniform fragments and allowed to renature, then the rate of renaturation of denatured DNA is expressed as

$$\frac{dC}{dt} = -kC^2$$

where k is the second-order rate constant. C is the concentration of single-stranded DNA at time t and the second order rate equation for two complementary strands coming together is given by the rate of decrease in C.

Starting with a concentration, C_0, of completely denatured DNA at $t = 0$, the amount of single-stranded DNA remaining at some time t is

$$\frac{C}{C_0} = \frac{1}{(1 + k.C_0.t)}$$

The time for half of the DNA to renature (when $C/C_0 = 0.5$) is defined as $t = t_{1/2}$. Then,

$$0.5 = \frac{1}{(1 + k.C_0.t_{1/2})} \text{ and thus } 1 + k.C_0.t_{1/2} = 2, \text{ yielding}$$

$$C_0.t_{1/2} = \frac{1}{k}$$

The product of $C_0 \times t_{1/2}$ is called the $Cot_{1/2}$. It is inversely proportional to the rate constant. Since the $Cot_{1/2}$ is the product of the concentration and time required to proceed halfway, a greater $Cot_{1/2}$ implies a slower reaction. The renaturation of DNA usually is followed in the form of a Cot curve. A graph of the fraction of single-stranded DNA reannealed $(1 - C/C_0)$ as a function of Cot on a semilogarithmic plot is referred to as a **Cot curve**.

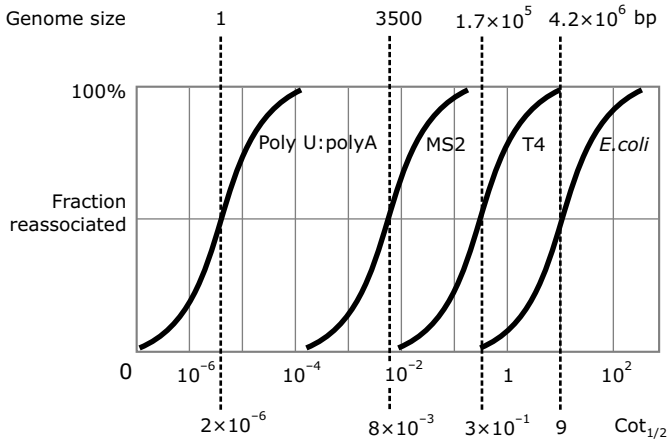


Figure 1.44
Cot curve of dsDNA from the indicated source.

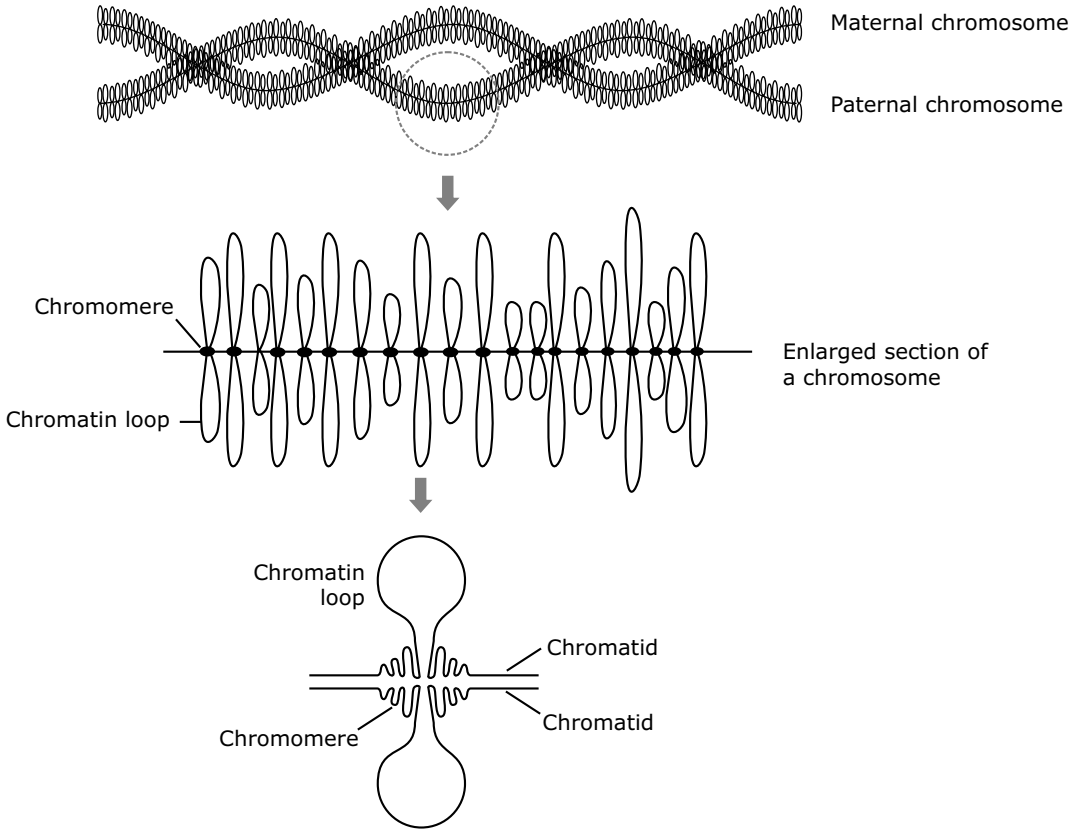


Figure 1.71 Lampbrush chromosome structure. Most of the DNA in each chromosome remains highly condensed in the chromomeres. Each of the two chromosomes shown consists of two closely apposed sister chromatids. This four stranded structure is characteristic of diplotene stage of meiosis.

1.12.6 B–chromosomes

The B-chromosomes (also referred to as *supernumerary* or *accessory chromosomes*) are additional (extra) chromosomes that are present in some individuals in some species. In eukaryotic cells normal chromosomes are termed as A-chromosomes. Most B-chromosomes are mainly or entirely heterochromatic and genetically inert. They are thought to be selfish genetic elements with no defined functions. The evolutionary origin of B-chromosomes is not clear, but presumably they must have been derived from heterochromatic segments of normal A-chromosomes.

1.13 DNA replication

Transmission of chromosomal DNA from generation to generation is crucial to cell propagation. This can only be achieved when chromosomal DNA is accurately replicated, providing two copies of the entire genome for faithful distribution into each daughter cell.

1.13.1 Semiconservative replication

It is crucial that the genetic material is reproduced accurately. When Watson and Crick worked out the double-helix structure of DNA in 1953, they recognized that the complementary nature of the two strands-A paired with T and G paired with C-might play an important role in its replication. Because the two polynucleotide strands are joined only

by hydrogen bonds, they are able to separate without requiring breakage of covalent bonds. If the two strands of a parental double helix of DNA are separated, the base sequence of each parental strand could serve as a template for the synthesis of a new complementary strand, producing two identical progeny double helices. This process is called **semiconservative** replication because the parental double helix is half conserved, each parental single strand remaining intact. The alternative methods are *conservative* and *dispersive.* In **conservative** replication, the whole original double helix acts as a template for a new one, one daughter molecule would consist of the original parental DNA, and the other daughter would be totally new DNA. In **dispersive** replication, some parts of the original double helix are conserved, and some parts are not. In this model, the parental double helix is broken into double-stranded DNA segments and just like conservative mode of replication acts as templates for the synthesis of new double-stranded DNA segments. The segments then reassemble into complete DNA double helices, each with parental and progeny DNA segments interspersed.

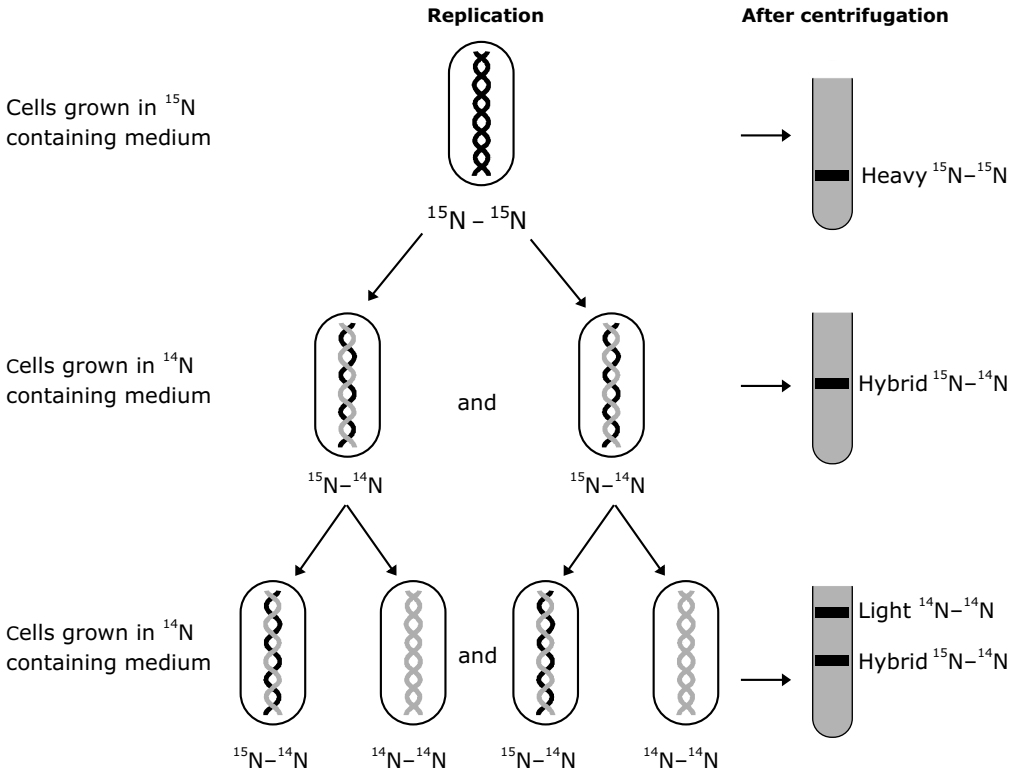


Figure 1.72 The Meselson-Stahl experiment showed that DNA replicates by a semiconservative mechanism. In this experiment, *E. coli* cells initially were grown in a medium containing ammonium salts prepared with heavy nitrogen (^{15}N) until all the cellular DNA was labeled. After the cells were transferred to a medium containing the normal light isotope (^{14}N), samples were removed periodically from the cultures and the DNA in each sample was analyzed by equilibrium density-gradient centrifugation. This technique can separate heavy-heavy (^{15}N-^{15}N), light-light (^{14}N-^{14}N), and heavy-light (^{15}N-^{14}N) duplexes into distinct bands. After one generation of growth, all the extracted DNA had the density of ^{15}N-^{14}N DNA. After two generations, approximately half the DNA had the density of ^{15}N-^{14}N DNA; the other half had the density of ^{15}N-^{14}N DNA. With additional generations, a large fraction of the extracted DNA consisted of ^{14}N-^{14}N duplexes; ^{15}N-^{15}N duplexes never appeared. These results match the predicted pattern for the semiconservative replication mechanism.

Meselson and Stahl experimentally demonstrated the semiconservative replication of DNA in *E. coli* in 1958. The figure 1.72 illustrates a prediction of this model. If the parental DNA carries a *heavy* density label because the organism has been grown in medium containing a suitable isotope (such as ^{15}N), its strands can be distinguished from those that are synthesized when the organism is transferred to a medium containing normal *light* isotopes.

1.13.6 Replication of mitochondrial DNA

Small and mostly circular mitochondrial and chloroplast DNA use a slightly different process of replication. Replication of circular double stranded mitochondrial DNA starts at a specific origin. But duplex DNA uses different origin sequences to initiate replication of each DNA strand. Initially, only one of the two parental strands is used as a template for synthesis of a new strand. Synthesis proceeds for only a short distance, displacing the original complementary strand, which remains single-stranded. This pattern of replication generates a **displacement** or **D loop** (hence, termed as *displacement replication*). A single D loop is found as an opening of 500–600 bases in mammalian mitochondria. Some mitochondrial DNAs possess several D loops which reflects the presence of multiple origins. Replication of the complementary strand is initiated when its origin is exposed by the movement of the first replication fork. The similar mechanism is employed in chloroplast DNA. Mammalian mitochondrial DNA is replicated by the DNA polymerase γ. The replisome machinery is formed by DNA polymerase, TWINKLE and mitochondrial SSB proteins. TWINKLE is a helicase, which unwinds short stretches of dsDNA in the 5′ to 3′ direction.

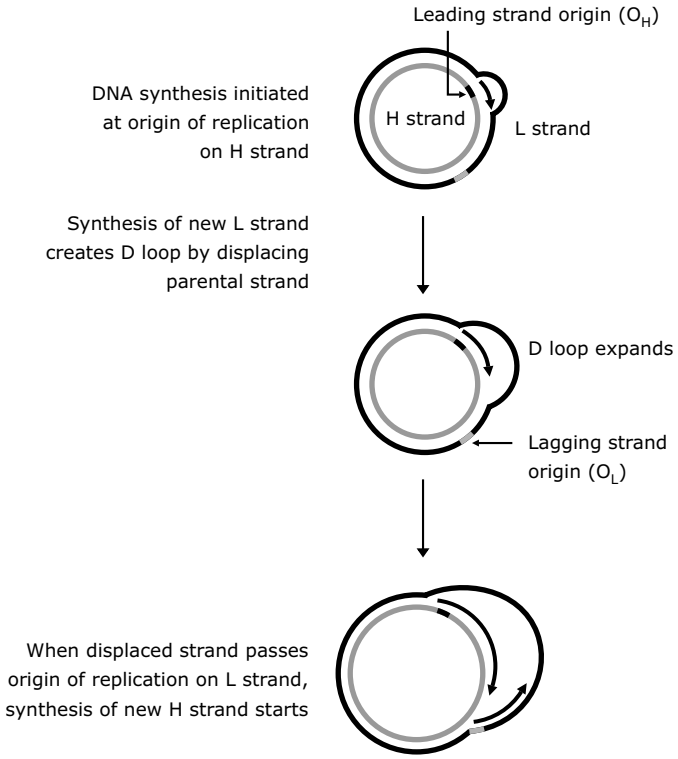


Figure 1.86 Replication of mammalian mitochondrial DNA. Replication starts at a specific origin in the circular duplex DNA. Initially only one of the two parental strands (the H strand in mammalian mitochondrial DNA) is used as a template for synthesis of a new strand. Synthesis proceeds for only a short distance, displacing the original partner (L) strand, which remains single-stranded. There is separate origins for L and H strand.

1.14 Recombination

Genomes are dynamic entities that change as a result of mutations and recombinations. Recombination is a large-scale rearrangement of a DNA molecule that involves the breakage and reunion of DNA. It was first recognized as the process responsible for crossing-over during meiosis of eukaryotic cells, and was subsequently implicated in the integration of the transferred DNA into bacterial genomes after conjugation, transduction or transformation. Genetic recombination events fall into two general classes:

Homologous recombination

Recombination which involves the exchange of homologous segments between any two homologous DNA molecules (or segments of the same molecule) that share an extended homology.

Site-specific recombination

Recombination between two dsDNA molecules that have only short regions of nucleotide sequence similarity.

A different type of event called **transposition**, which is related to the processes of recombination, allows one DNA sequence to be inserted into another without relying on sequence homology. It provides a means by which certain elements move from one chromosomal location to another.

1.14.1 Homologous recombination

Homologous recombination (also termed as *general recombination*) is the most important version of recombination in nature, being responsible for meiotic crossing-over in eukaryotes and the integration of acquired DNA by the process of conjugation, transduction and transformation into bacterial genomes. It involves a reciprocal exchange of sequences of DNA.

Holliday model for homologous recombination

An appealing scheme for homologous recombination was proposed by Robin Holliday in 1964. The Holliday model (also known as *heteroduplex model*) describes recombination between two homologous double-stranded molecules, those with identical or nearly identical sequences. But, it is equally applicable to two different molecules that share a limited region of homology, or a single molecule that recombines with itself because it contains two separate regions that are homologous with one another.

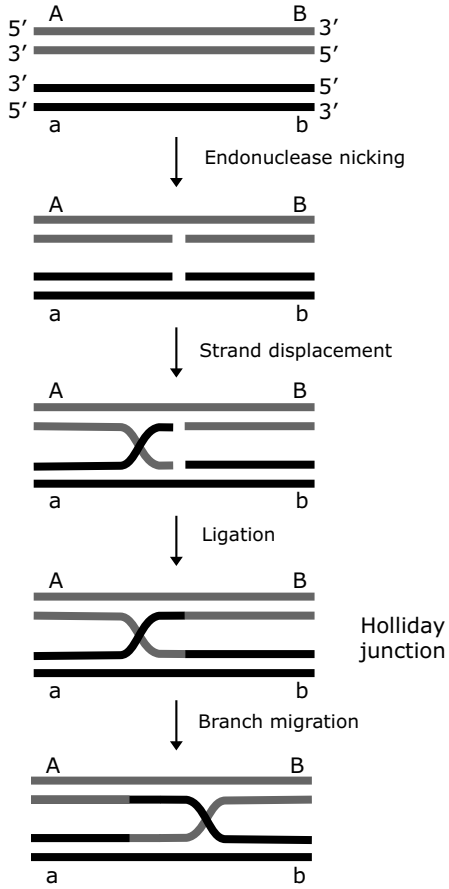


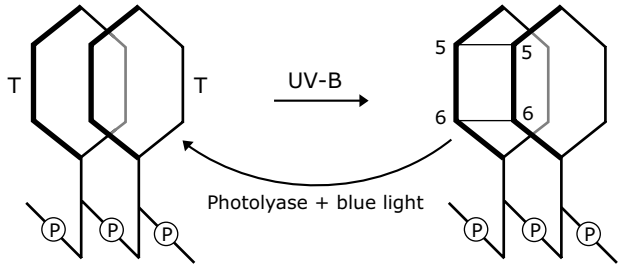
Figure 1.87
The Holliday model for homologous recombination. Single-strand nicks are introduced at the same position on both parental molecules. The nicked strands then exchange by complementary base pairing, and ligation produces a crossed-strand intermediate called a Holliday junction.

1.15 DNA repair

Although the genetic variation is important for evolution, the survival of the individual demands genetic stability also. Maintaining genetic stability requires not only an extremely accurate mechanism for replicating DNA, but also mechanisms for repairing the many accidental lesions that occur continually in DNA. Most such spontaneous changes in DNA are temporary because they are immediately corrected by a set of processes that are collectively called as *DNA repair*. Without repair systems, a genome would not be able to maintain its essential cellular functions. Most cells possess four different categories of DNA repair system: Direct repair, Excision repair, Mismatch repair and Recombination repair.

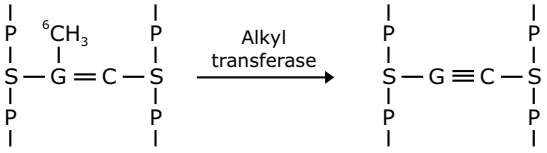
1.15.1 Direct repair

Direct repair systems act directly on damaged nucleotides, converting each one back to its original structure. But only a few types of damaged nucleotide can be repaired directly. One very common type of UV radiation mediated damages, *pyrimidine dimers,* are repaired by a light-dependent direct system called **photoreactivation**. In *E. coli*, the process involves the enzyme called **DNA photolyase**. When stimulated by light with a wavelength between 300 and 500 nm, the enzyme binds to pyrimidine dimers and converts them back to the original monomeric nucleotides. Photoreactivation is a widespread but not universal type of repair.



Adjacent thymines Thymine dimer

Another example is the repair of O^6-methylguanine, which forms in the presence of alkylating agents and is a common and highly mutagenic lesion. It tends to pair with thymine rather than cytosine during replication. Direct repair of O^6-methylguanine is carried out by O^6-methylguanine DNA methyltransferase (an alkyl transferase), which catalyzes the transfer of the methyl group of O^6-methylguanine to a specific *Cys* residue in the same protein.



1.15.2 Excision repair

Excision repair involves the excision of a segment of the polynucleotide containing a damaged site, followed by resynthesis of the correct nucleotide sequence by a DNA polymerase. These pathways fall into two categories:

Base-excision repair

Base excision repair involves removal of a damaged nucleotide base, excision of a short piece of the polynucleotide and resynthesis with a DNA polymerase. It is used to repair many minor damage like alkylation and deamination resulting from exposure to mutagenic agents. Enzyme **DNA glycosylase** initiates the repair process. A DNA

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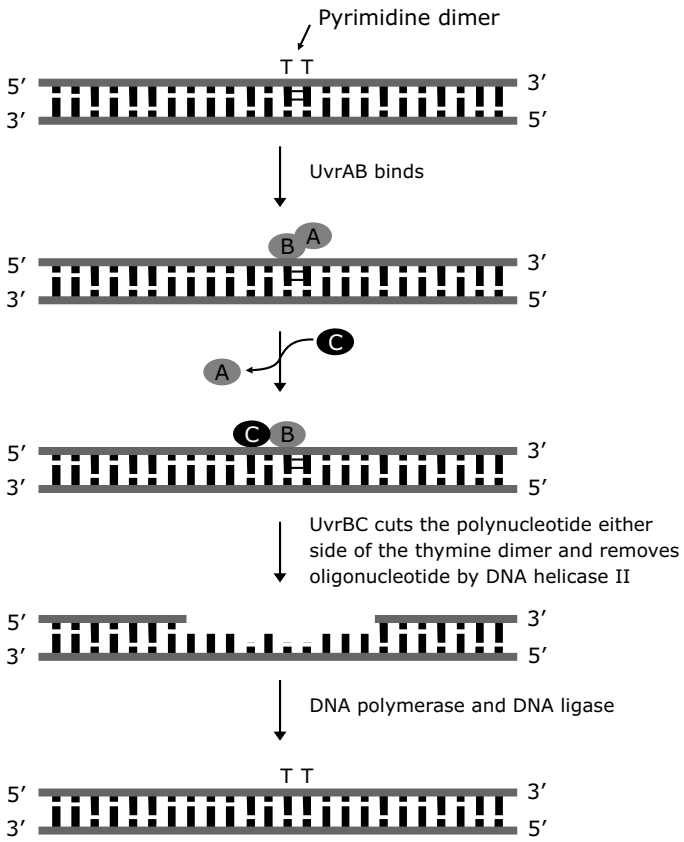

Figure 1.94 Nucleotide excision repair (A-UvrA, B-UvrB, C-UvrC).

1.15.3 Mismatch repair

The mismatch repair system can detect mismatches that occur in DNA replication. Enzyme systems involved in mismatch repair are as follows:

1. Recognize mismatched base pairs.
2. Determine which base in the mismatch is the incorrect one.
3. Excise the incorrect base and carry out repair synthesis.

The repair must be made in the daughter polynucleotide because it is in this newly synthesized strand that the error has occurred; the parent polynucleotide has the correct sequence. How does the repair process know which strand is which? When mismatch errors occur during replication in *E. coli*, it is possible to distinguish the original strand of DNA. Immediately after replication of methylated DNA, only the original parental strand carries the methyl groups.

During the period while the newly synthesized strand awaits the introduction of methyl groups, the two strands can be distinguished. In *E. coli*, the answer is that the daughter strand is, at this stage, undermethylated and can be distinguished from the parent polynucleotide, which has a full complement of methyl groups. *E. coli* DNA is methylated because of the activities of the *DNA adenine methylase* (**Dam**), which converts adenines to 6-methyladenines in the sequence 5′-GATC-3′, and the *DNA cytosine methylase* (**Dcm**), which converts internal cytosines to 5-methylcytosines in 5′-CCAGG-3′ and 5′-CCTGG-3′. These methylations are not mutagenic, the modified nucleotides having the same base-pairing properties as the unmodified versions. There is a delay between DNA replication and methylation of the daughter strand, and it is during this window of opportunity that the repair system scans the DNA for mismatches and makes the required corrections in the undermethylated, daughter strand.

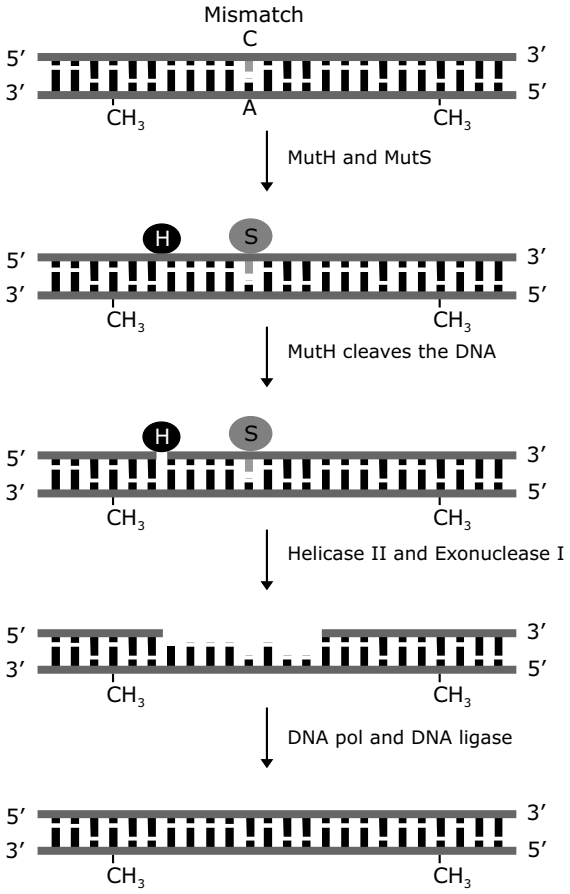

Figure 1.95 Long patch mismatch repair in *E. coli.* H-MutH; S-MutS.

There are three mismatch repair systems in *E. coli*; on the basis of the relative lengths of the unmethylated strand excised and repaired by components of the repair system. These are categorized as *long patch, short patch* and *very short patch*. In *E. coli* the long patch system involves three Mut proteins, coded by the mut genes. These mut proteins are MutH, MutL and MutS. Recognition of the sequence GATC and of the mismatch are specialized functions of the MutH and MutS proteins, respectively. The role of MutL is not clear. The MutH protein cleaves the unmethylated strand on the 5' side of the G in the GATC sequence. The combined action of DNA helicase II and exonuclease I then removes a segment of the new strand between the cleavage site and a point just beyond the mismatch. The resulting gap is filled in by DNA polymerase and the nick is sealed by DNA ligase.

1.15.4 Recombinational repair

Recombinational repair is a process of filling a gap in one strand of dsDNA by retrieving a homologous single strand from another dsDNA. It is a *post-replication repair* method because it occurs after replication. An example is illustrated in figure 1.96 by considering a damage, such as a pyrimidine dimer, on one strand of a double helix. When the DNA is replicated, the dimer prevents the damaged site from acting as a template. Replication is forced to bypass it. This results in a gap in the newly synthesized strand. Whereas the other parental strand forms a normal complementary strand. The gap opposite the damaged site in the one double strand is filled by taking the homologous single strand of DNA from the normal duplex. Following this single-strand exchange, the recipient duplex has a parental (damaged) strand facing a normal strand. The donor duplex has a normal parental strand facing a gap; that can be filled by repair synthesis in the usual way, generating a normal duplex.

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A more serious problem occurs if one of the parent strand being replicated contains a single-strand nick. If a break or nick in the phosphodiester backbone of DNA strand is not repaired before a replication fork passes, the replication process leads to double strand break in one of the daughter double helix and the replication fork is lost. This is called *replication fork collapse*. The break can be repaired by a form of homologous recombination between the broken end and the second, undamaged DNA helix. End of the broken DNA is processed by RecBCD to create a 3′ single stranded overhang. RecA mediates the invasion of 3′ overhang into donor homologous duplex. The donor DNA duplex acts as a template for DNA synthesis, resulting in the formation of Holliday junction. Cleavage of the resulting Holliday junction restores the replication fork.

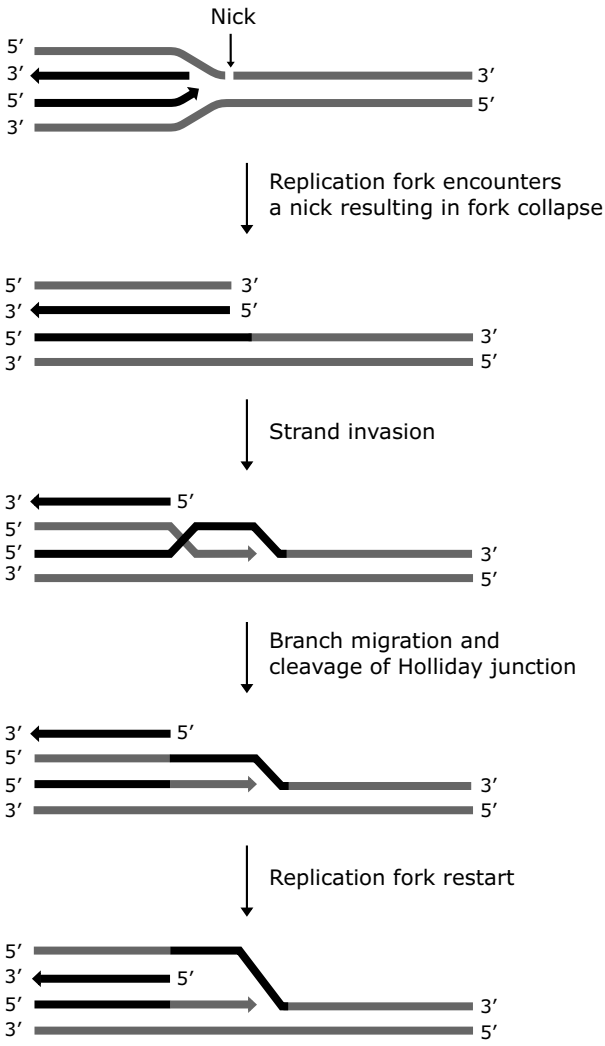

Figure 1.98
Recombinational repair of a collapsed replication fork. Replication fork collapse when the advancing fork encounters a nick on the template strand. The broken arm is processed by RecBCD to create 3′ ssDNA overhang. The 3′ overhang invades the donor DNA and forms a D-loop. Invasion is followed by formation of Holliday junction. Resolution of the Holliday junction restores the replication fork.

1.15.5 Repair of double strand DNA break

Ionizing radiation, oxidizing agents and replication errors may cause double-strand break in DNA. If these lesions were left unrepaired, they would lead to the breakdown of chromosomes into smaller fragments.

Two distinct mechanisms have evolved to repair these damages – *Non-homologous* and *homologous end joining*. The choice of cells to use either non-homologous or homologous end joining is largely dependent on the phases of the cell cycle. Non-homologous end joining is present throughout the cell cycle but is particularly common in the G0 and G1 phases whereas homologous end joining predominates in the S and G2 phases.

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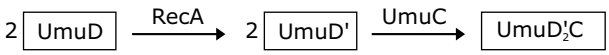
as a co-protease and stimulates protease activity. It also promotes base-pairing between a single-strand of DNA and its complement in a dsDNA. The LexA repressor regulates the transcription of all of the SOS genes. LexA represses SOS response genes by binding to a 20 bp stretch of DNA called an *SOS box*.

When the genome is subjected to heavy damage through exposure to UV light or a DNA-damaging reagent, DNA repair becomes significantly less accurate and a high mutation rate is observed. Higher levels of DNA damage effectively bring normal DNA replication to a halt in *E. coli* because normal DNA replication with DNA polymerase III cannot proceed past many types of DNA lesions. In this situation, DNA polymerase V (or UmuD'$_2$C) performs the DNA replication. Protein RecA triggers the activation of DNA polymerase V. The UmuD (umu for *U*V *mu*tagenesis) protein is cleaved to UmuD' when RecA is activated; the cleavage event activates UmuD.



DNA polymerase V (a Y-family DNA polymerase) lacks 3'→5' exonuclease proofreading activity. Hence it is also known as *error-prone DNA polymerase.* Because proper base pairing is often impossible at the site of a lesion, this **translesion DNA synthesis** (TLS) is an *error-prone replication*.

Problem

For each DNA repair process in column I, list all characteristics from column II that correctly describe that process.

Column I	*Column II*
a. Nucleotide excision repair	1. RecA protein participates.
b. Photo reactivation	2. Damaged nucleotides are removed by breaking phosphodiester bonds.
c. Base excision repair	3. A free radical mechanism is involved.
d. SOS repair	4. The repair enzyme functions only once.
e. Alkyl transferase repair	5. The key enzyme contains a bound folate cofactor.
f. Mismatch repair	6. No bases or nucleotides are removed from the DNA.
	7. Deficiency of this enzyme in humans increases the risk of skin cancer.
	8. This system is responsible for error prone replication.
	9. This process begins up to 1 kbp away from the site to be repaired.
	10. DNA ligase catalyzes the final reaction.

Solution

a. 2, 7, 10
b. 3, 5, 6
c. 2, 10
d. 1, 8, 10
e. 4, 6
f. 9, 10

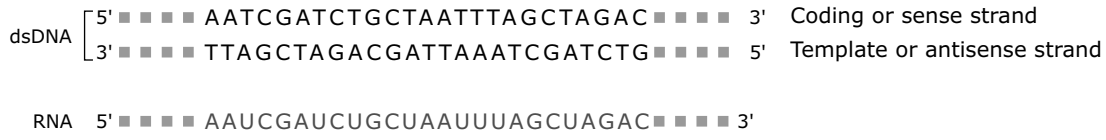
1.16 Transcription

Transcription is a process of formation of transcript (RNA). It takes place by the usual process of complementary base pairing, catalyzed and scrutinized by the enzyme *RNA polymerase*. It occurs unidirectionally in which RNA chain (transcript) is synthesized from the 5' to 3' direction.

1.16.1 Transcription unit

Transcription is a selective process. Each transcribed segment of DNA is called a *transcription unit.* In eukaryotes, a transcription unit typically carries the information of just one gene and it is termed as *monocistronic* transcription unit. In prokaryotes, a set of adjacent genes is often transcribed as a unit termed *polycistronic* transcription unit. The immediate product of transcription is called the **primary transcript**. The eukaryotic transcription unit may be *simple* or *complex*. The primary transcript produced from a *simple* transcription unit is processed to yield a single type of mRNA, encoding a single protein. In the case of *complex* transcription units, which are quite common in multicellular organisms, the primary RNA transcript can be processed in more than one way, leading to formation of more than one type of mRNAs, encoding more than one type of polypeptides. Transcription starts from the first base pair that is called the **start point**. From this point, RNA polymerase moves along the template, synthesizing RNA, until it reaches a terminator sequence. Sequences prior to the startpoint are described as *upstream* of it; those after the startpoint (within the transcribed sequence) are *downstream* of it.

During transcription, only one strand of the transcription unit is transcribed. Therefore, the transcript is identical in sequence with one strand of the DNA, which is called the **coding strand** and complementary to the other strand, called **template strand**. The coding strand is also known as the *sense* (+) strand while the template strand is the *antisense* (–) strand. In principle, any region of the DNA double helix could be copied into two different RNA molecules - one from each of the two DNA strands. In reality, only one DNA strand is used as a template in each region.



1.16.2 Prokaryotic transcription

RNA polymerase

DNA dependent RNA synthesis is catalyzed by the enzyme DNA dependent RNA polymerase (simply called RNA polymerase). It was discovered by Samuel B. Weiss and Jerard Hurwitz in 1960. In prokaryotes, single type of RNA polymerase appears to be responsible for the synthesis of all different types of RNA such as mRNA, rRNA and tRNA.

Eubacterial RNA pol is a multisubunit enzyme made up of five different polypeptides – α, β, β', ω, σ. The holoenzyme ($\alpha_2\beta\beta'\omega\sigma$) can be separated into two components, the core enzyme ($\alpha_2\beta\beta'\omega$) and the sigma factor (the σ polypeptide). The complete enzyme or holoenzyme in *E. coli* has a molecular mass of ~465 kDa. The α subunit is required for assembly of the core enzyme and plays a role in promoter recognition. The α subunit also plays a role in the interaction of RNA polymerase with some regulatory factors. The β and β' subunits together make up the catalytic center. β subunit involves in chain elongation. The σ subunit is concerned specifically with promoter recognition. The ω subunit facilitates assembly of RNA polymerase and stabilizes assembled RNA polymerase. The catalytic activity of RNA pol is provided by core complex composed of β and β' subunits, ω subunit and two copies of α subunit.

Table 1.22 RNA polymerase subunits and their functions

Subunits	*Gene*	*Function*
α	*rpoA*	assembly of the core enzyme and promoter recognition
β	*rpoB*	catalytic center
β'	*rpoC*	catalytic center
ω	*rpoZ*	assembly of RNA polymerase
σ	*rpoD*	promoter recognition and transcription initiation

1.17 RNA processing

In eukaryotes, transcription and translation take place in different cellular compartments: transcription takes place in the nucleus, whereas translation takes place in the cytoplasm. In prokaryotes, transcription of mRNA and translation occur simultaneously. Thus, mRNA molecules undergo little or no modification after synthesis by RNA polymerase in prokaryotes. In contrast, pre-tRNA and pre-rRNA undergo processing like cleavage, addition of nucleotides and chemical modification after synthesis. Although both prokaryotes and eukaryotes modify pre-tRNA and pre-rRNA, eukaryotes very extensively process pre mRNA destined to become mRNA. The primary transcript of an RNA polymerase is referred to as pre-RNA. Processing of eukaryotic pre-mRNA involves 5′ capping, 3′ cleavage/polyadenylation, splicing and RNA editing before being transported to the cytoplasm, where they are translated by ribosomes.

1.17.1 Processing of eukaryotic pre–mRNA

5′-capping

Eukaryotic mRNA has a peculiar enzymatically appended cap structure consisting of 7-methylguanosine residue joined via a 5′-5′ triphosphate bridge. During transcription, 7-methylguanosine is added to the 5′ end of nascent mRNA. The initial steps in RNA capping are catalyzed by a dimeric capping enzyme, which associates with the phosphorylated carboxyl-terminal tail domain (CTD) of RNA polymerase II.

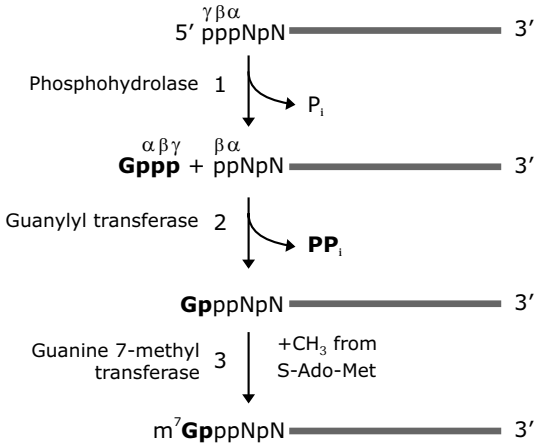


Figure 1.117 The reactions that cap the 5′ end of each RNA molecule synthesized by RNA polymerase II. The final cap contains a novel 5′-to-5′ linkage between the positively charged 7-methyl G residue and the 5′ end of the RNA transcript. The letter N represents any one of the four ribonucleotides, although the nucleotide that starts an RNA chain is usually a purine.

One subunit of the capping enzyme removes the γ-phosphate from the 5′ end of the nascent RNA emerging from the surface of an RNA polymerase II. The other subunit transfers the GMP moiety from GTP to the 5′-diphosphate of the nascent transcript, creating the guanosine 5′-5′-triphosphate structure. In the final steps, separate enzymes transfer methyl groups from *S*-adenosylmethionine to the N^7 position of the guanine at the 5′ end of the nascent RNA. If mRNA has a methyl group on N^7 position of guanine at the 5′ end, then it is called **cap 0**. This is the first methylation step and occurs in all eukaryotes. In some higher eukaryotes, methyl group addition also occurs at second base. But this happens only when the position is occupied by adenine; the reaction involves addition at the N^6 position.

1.22 RNA interference

RNA interference (abbreviated RNAi) is an evolutionarily conserved mechanism of gene regulation that is induced by *small silencing RNA* in a sequence-specific manner. In 1998, Fire and Mello first established this in *C. elegans*. Historically, RNA interference was known by other names, including *post transcriptional gene silencing* (PTGS), *transgene silencing* and *quelling*. RNAi has been observed in all eukaryotes, from yeast to mammals. RNA interference has an important role in post-transcriptional gene regulation, transposon regulation and defending cells against viruses. Two types of small silencing RNA molecules – small interfering RNA (siRNA) and microRNA (miRNA) – are central to RNA interference.

siRNAs mediated RNAi

In the siRNAs mediated RNAi pathway, the dsRNAs are processed into siRNAs duplexes comprised of two ~21 nucleotides long strands with two nucleotides overhangs at the 3' ends by an enzyme called Dicer. **Dicer** is a ~200 kDa multidomain, an RNase III family enzyme that functions in processing dsRNA to siRNA. The Dicer includes an ATPase/RNA helicase domain, catalytic RNase III domains, and dsRNA binding domain. Dicer and a dsRNA binding protein (together form the RISC loading complex) then load the RNA duplex into RISC. The siRNA is thought to provide target specificity to RISC through base pairing of the guide strand with the target mRNA. Only one of the two strands, which is known as the *guide strand*, directs the gene silencing. The other *anti-guide strand* or *passenger strand* is degraded during RISC activation. The active components of an RNA-induced silencing complex (RISC) are endonucleases called **argonaute** proteins, which cleave the target mRNA strand complementary to their bound siRNA.

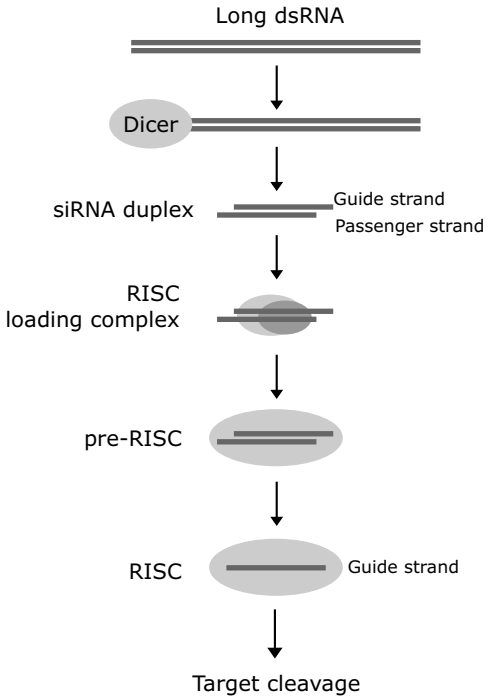


Figure 1.153 dsRNA precursors are processed by Dicer to generate siRNA duplexes containing guide and passenger strands. RISC-loading complex loads the duplex into RISC. The passenger strand is later destroyed and the guide strand directs RISC to the target RNA.

miRNAs mediated RNAi

miRNAs (microRNAs) are small, non-coding RNA molecules encoded in the genomes of plants, animals and their viruses. These highly conserved, 20–25 mer RNAs appear to regulate gene expression post-transcriptionally by

1.23 Genetic code

General features of genetic code

- The genetic code is a triplet code called a *codon*.

  How many nucleotides in DNA are needed to specify each amino acid in a protein? We know that the information in DNA must reside in the sequence of the four nucleotides that constitute the DNA: A, T, G and C. A doublet code involving two adjacent nucleotides would not be adequate, as four kinds of nucleotides taken two at a time can generate only $4^2 = 16$ different combinations. But with three nucleotides per word, the number of different words that can be produced with an alphabet of just four letters is $4^3=64$. This number is more than sufficient to code for 20 different amino acids. Such mathematical arguments led biologists to suspect the existence of a triplet code. Later Francis Crick, Sydney Brenner, and their colleagues provided genetic evidence for the triplet nature of the code by studying the mutagenic effects of the chemical proflavin on bacteriophage T4.
- Certain codons contain *start* and *stop* signals to *initiate* and *terminate* translation. The initiation codon is usually AUG, which specifies methionine. In few mRNA, GUG or UUG also acts as initiation codon. Out of 64 codons, three do not code for any amino acids and called a *stop* or *termination* codons (UAA, UAG, and UGA).
- The code is **unambiguous**, meaning that each triplet specifies only a single amino acid.
- No internal punctuation (commas) is used in the code. Thus, the code is said to be commaless. Once the translation of mRNA begins, the codons are read one after the other with no breaks between them.
- The code is **degenerate**, meaning that a given amino acid can be specified by more than one triplet codon. This is the case for 18 of the 22 amino acids. The different codons for a given amino acid are said to be *synonymous.* For example, UUU and UUC are synonyms for phenylalanine, whereas serine is encoded by the synonyms UCU, UCC, UCA, UCG, AGU and AGC.

Table 1.32 Amino acids and their synonymous codons

Amino acids	*Number of synonymous codon*
Leu, Ser, Arg	6
Gly, Pro, Ala, Val, Thr	4
Ile	3
Phe, Tyr, Cys, His, Gln, Glu, Asn, Asp Lys	2
Met, Trp	1

- The code is **nonoverlapping**. After translation commences, any single ribonucleotide at a specific location within the mRNA is part of only one triplet.
- It is usual to describe the genetic code as a **universal code**, meaning that the same code is used throughout all life forms. This is not strictly true. There is a few example of *context dependent codons* also. For example, selenocysteine is coded by UGA and pyrrolysine by UAG. These codons, therefore, have a dual meaning because they are mainly used as stop codons. Similarly, some differences in the genetic code have been found, especially in the mitochondria, chloroplast, some protozoans and others as mentioned in table 1.33. In this context the code is *nearly universal*. With only minor exceptions, a single coding dictionary is used by almost all viruses, prokaryotes, archaea and eukaryotes.

Table 1.33 Some differences between the *universal* code and mitochondrial genetic codes.

Codon	*Universal code*	*Unusual code*	*Occurrence*
UGA	Stop	*Trp*	*Mycoplasma, Spiroplasma,* mitochondria of many species
CUG	Leu	*Thr*	Mitochondria in yeasts
UAA, UAG	Stop	*Gln*	*Acetabularia, Tetrahymena,* Paramecium, etc.
UGA	Stop	*Cys*	*Euplotes*

Activation of ubiquitin: Ubiquitin is activated by an E1; *ubiquitin-activating enzyme.* E1 becomes covalently linked to free ubiquitin through the free C-terminal residue of ubiquitin, in an energy-dependent manner.

Transfer of ubiquitin from E1 to E2: The activated ubiquitin is subsequently transferred to a cysteine residue present on an E2; *ubiquitin-conjugating enzyme*.

Ligation of ubiquitin to target protein: Finally, E3; *ubiquitin ligases* (~500 in humans) transfer the activated ubiquitin from E2 to a Lys amino acid residue of its target protein, forming an isopeptide bond. A ubiquitinated protein is proteolytically degraded to short peptides in an ATP-dependent process mediated by proteasome.

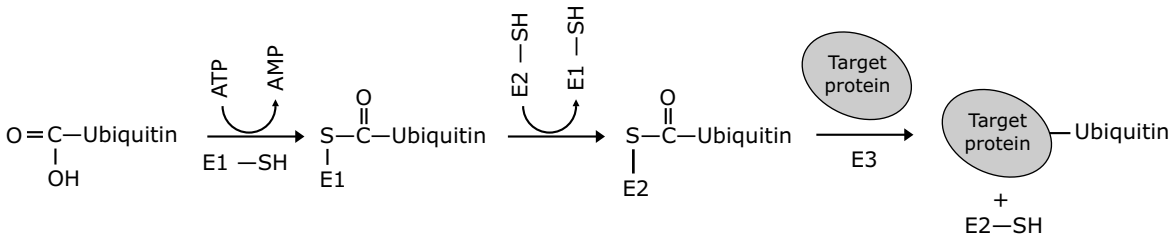


Figure 1.174 The reactions involved in the attachment of ubiquitin to a protein. In the first part of the process, ubiquitin's terminal carboxyl group is joined, via a thioester linkage, to E1 in a reaction driven by ATP hydrolysis. The activated ubiquitin is subsequently transferred to a sulfhydryl group of E2 and then in a reaction catalyzed by E3, to the amino group of a lysine residue on a target protein.

1.25 Mutation

Genome is not a static entity. It is subject to different types of heritable changes. A sudden and heritable change in the sequence of an organism's genome that gives rise to alternate forms of any gene is called **mutation**. It can simply be put as an abrupt change in the genotype of an organism that is not the result of recombination. The process by which mutations is produced is called **mutagenesis**. An organism exhibiting a novel phenotype as a result of the presence of a mutation is referred to as a **mutant**. In a broad sense, the term mutations include all types of heritable genetic change of an organism not explainable by recombination of preexisting genetic variability. Such genetic changes include ploidy, chromosomal aberrations and changes in individual genes. Generally, the change in individual gene is known as *gene mutation*.

General characteristics of mutation

- Mutations are generally recessive, but dominant mutations also occur.
- Mutations are generally harmful to the organisms.
- Mutations are random, occur at any time and in any cell of an organism.
- Mutations are recurrent i.e. the same mutation may occur again and again.

Role of mutation

- Ultimate source of all genetic variation and it provides the raw material for evolution.
- Mutation results into the formation of alleles. Without mutation, all genes would exist in only one form.
- Organisms would able to evolve and adapt to environmental change.

Molecular basis of gene mutation

Mutations arise in two ways : Some mutations are *spontaneous* that occur without treatment of the organism with an exogenous mutagen. *Mutagen* is an agent that leads to an increase in the frequency of occurrence of mutations. Spontaneous mutations account for the 'background rate' of mutation and are presumably the ultimate source of natural genetic variation that is seen in populations. Spontaneous mutations can occur because of replication errors, spontaneous lesions and transposition of transposable elements during the normal growth of the cell. Other mutations called *induced* mutations arise because a mutagen has reacted with the parent DNA, causing a structural change that affects the base-pairing capability of the altered nucleotide.

Concept of cistron and genetic complementation

The word *cistron* was coined in 1956 by Seymour Benzer. He used the term to identify a segment of a genome that is responsible for a single genetic 'function', as determined by the cis-trans complementation test. The underlying idea of the test is that two mutations might produce similar phenotypic effects in several different ways. First, the two mutations might alter the same gene and, consequently, affect the same enzymatically catalysed step in a biochemical pathway. Alternatively, the two mutations might affect genes that encode enzymes for different steps in a single biochemical pathway. A third possibility is that two mutations might block steps in two different biochemical pathways that converge.

It is important to realize that a gene is not a point on a chromosome, but a segment of a chromosome. Mutations within a single gene may occupy different sites (that is, different DNA bases) within the gene. Any two mutations within a single gene are said to be 'alleles' in the sense that they affect the same genetic function. Mutations at exactly the same site are called homoalleles; mutations at different sites within a gene are heteroalleles.

Problem

Seven arginine requiring mutants of *E. coli* were independently isolated. All pairwise matings were done to determine the number of complementation groups involved. If a (+) in the following table indicates growth and a (–) no growth on minimal medium, how many complementation groups are involved?

1	2	3	4	5	6	7	
–	+	+	+	+	–	–	1
	–	+	+	–	+	+	2
		–	–	+	+	+	3
			–	+	+	+	4
				–	+	+	5
					–	–	6
						–	7

Solution

A group of mutants which do not complement each other belongs to single complementation group. Thus, we conclude that there are three complementation groups present: 1, 6 and 7 are mutually non-complementing, as are 2 and 5 and 3 and 4.

1.26 Developmental genetics

A multicellular animal or plant arises from a single cell – a fertilized egg. During development, the cell divides repeatedly to produce many different cells. The genetically identical cells come to differ from one another by expressing distinct sets of genes during development. The differential gene expression controls cell proliferation, cell specialization, cell interactions and cell movement. The strategies used to instruct genetically-identical cells to express distinct sets of genes and thereby differentiate into diverse cell types are mRNA localization, cell-to-cell contact and signaling through the diffusion of a secreted signaling molecule. In this section, we will encounter these strategies during embryonic development in some model organisms.

1.26.1 Genetic control of embryonic development in *Drosophila*

Embryonic development in *Drosophila* is an orderly sequence of change and is controlled by the differential expression of genes. *Drosophila* displays a **holometabolous** method of development, meaning that they have three distinct stages of their post-embryonic life cycle, each with radically different body plans: *larva*, *pupa* and finally, *adult* (imago).

Imago consists of a *head* followed by three *thoracic* segments (T1 to T3) and eight or nine abdominal segments (A1 to A9). Segment T1 carries a pair of legs, T2 carries a pair of legs plus a pair of wings and T3 carries a pair of legs plus a pair of halteres.

In *Drosophila,* after fertilization, the diploid nuclei undergo a series of nuclear divisions and forms a *syncytium*— a group of nuclei without cell membranes. Most of nuclei then migrate from the middle of the egg toward the surface, where they form a monolayer called the *syncytial blastoderm*. Later, plasma membrane encloses each nucleus and thereby converting the syncytial blastoderm into a *cellular blastoderm.* A small subset of nuclei present in the extreme posterior end of the egg are segregated into cells; these *pole cells* are the primordial germ cells that will give rise to eggs or sperm.

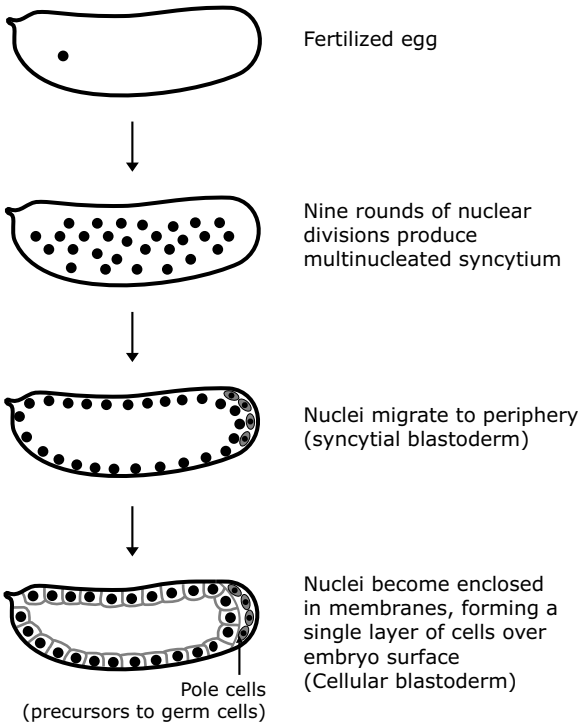


Figure 1.185 Early stages of embryonic development in *Drosophila*.

Three important classes of pattern-regulating genes specify the basic features and functions during embryonic development: *Maternal effect genes, Segmentation genes and Homeotic genes.*

Maternal effect genes

Maternal effect genes are expressed during oogenesis by the mother (expressed prior to fertilization) and develop the anterior-posterior and dorsal-ventral polarity of the egg. The anterior end of the egg becomes the head; the posterior end becomes the tail. The dorsal side is on top; the ventral side is underneath. The products of maternal effect genes*,* called *maternal mRNAs,* are produced by *nurse cells* and *follicle cells* and deposited in the egg cell (oocyte). At the start of development, gradients of maternal mRNA and their products are established in the oocyte along the anterior-posterior and dorsal-ventral axes.

About 30 maternal effect genes involved in pattern formation have been identified. In particular, products of four maternal effect genes are critical to the formation of the anterior-posterior axis. The product of two maternal effect genes, *bicoid* and *hunchback*, regulates the formation of anterior structures, while another pair of maternal effect genes, *nanos* and *caudal*, specifies proteins that regulate the formation of the posterior parts of the embryo.

Chapter 02

Recombinant DNA technology

Recombinant DNA technology (also known as genetic engineering) is the set of techniques that enable the DNA from different sources to be identified, isolated and recombined so that new characteristics can be introduced into an organism. The invention of recombinant DNA technology—the way in which genetic material from one organism is artificially introduced into the genome of another organism and then replicated and expressed by that other organism—was largely the work of Paul Berg, Herbert W. Boyer, and Stanley N. Cohen, although many other scientists made important contributions to the new technology as well. Paul Berg developed the first recombinant DNA molecules that combined DNA from SV40 virus and lambda phage. Later in 1973, Herbert Boyer and Stanley Cohen develop recombinant DNA technology, showing that genetically engineered DNA molecules may be cloned in foreign cells.

One important aspect in recombinant DNA technology is *DNA cloning.* It is a set of techniques that are used to assemble recombinant DNA molecules and to direct their replication within host organisms. The use of the word cloning refers to the fact that the method involves the replication of a single DNA molecule starting from a single living cell to generate a large population of cells containing identical DNA molecules.

2.1 DNA cloning

DNA cloning is the production of a large number of identical DNA molecules from a single ancestral DNA molecule. The essential characteristic of DNA cloning is that the desired DNA fragments must be *selectively amplified* resulting in a large increase in copy number of selected DNA sequences. In practice, this involves multiple rounds of DNA replication catalyzed by a DNA polymerase acting on one or more types of template DNA molecule. Essentially two different DNA cloning approaches are used: *Cell-based* and *cell-free DNA cloning.*

Cell-based DNA cloning

This was the first form of DNA cloning to be developed, and is an *in vivo* cloning method. The first step in this approach involves attaching foreign DNA fragments *in vitro* to DNA sequences which are capable of independent replication. The recombinant DNA fragments are then transferred into suitable host cells where they can be propagated selectively.

The essence of cell-based DNA cloning involves following steps:

Construction of recombinant DNA molecules

Recombinants are hybrid DNA molecules consisting of autonomously replicating DNA segment plus inserted elements. Such hybrid molecules are also called *chimera*. Recombinant DNA molecules are constructed by *in vitro* covalent attachment (ligation) of the desired DNA fragments (target DNA) to a replicon (any sequence capable of independent DNA replication). This step is facilitated by cutting the target DNA and replicon molecules with specific restriction endonucleases before joining the different DNA fragments using the enzyme DNA ligase.

Transformation

The recombinant DNA molecules are transferred into host cells (often bacterial or yeast cells) in which the chosen replicon can undergo DNA replication independently of the host cell chromosome(s).

Selective propagation of cell clones

Selective propagation of cell clones involves two stages. Initially the transformed cells are plated out by spreading on an agar surface in order to encourage the growth of *well-separated* cell colonies. These are cell clones (populations of identical cells all descended from a single cell). Subsequently, *individual* colonies can be picked from the plate and the cells can be further expanded in liquid culture.

Isolation of recombinant DNA clones

Isolation of recombinant DNA clones by harvesting expanded cell cultures and selectively isolating the recombinant DNA.

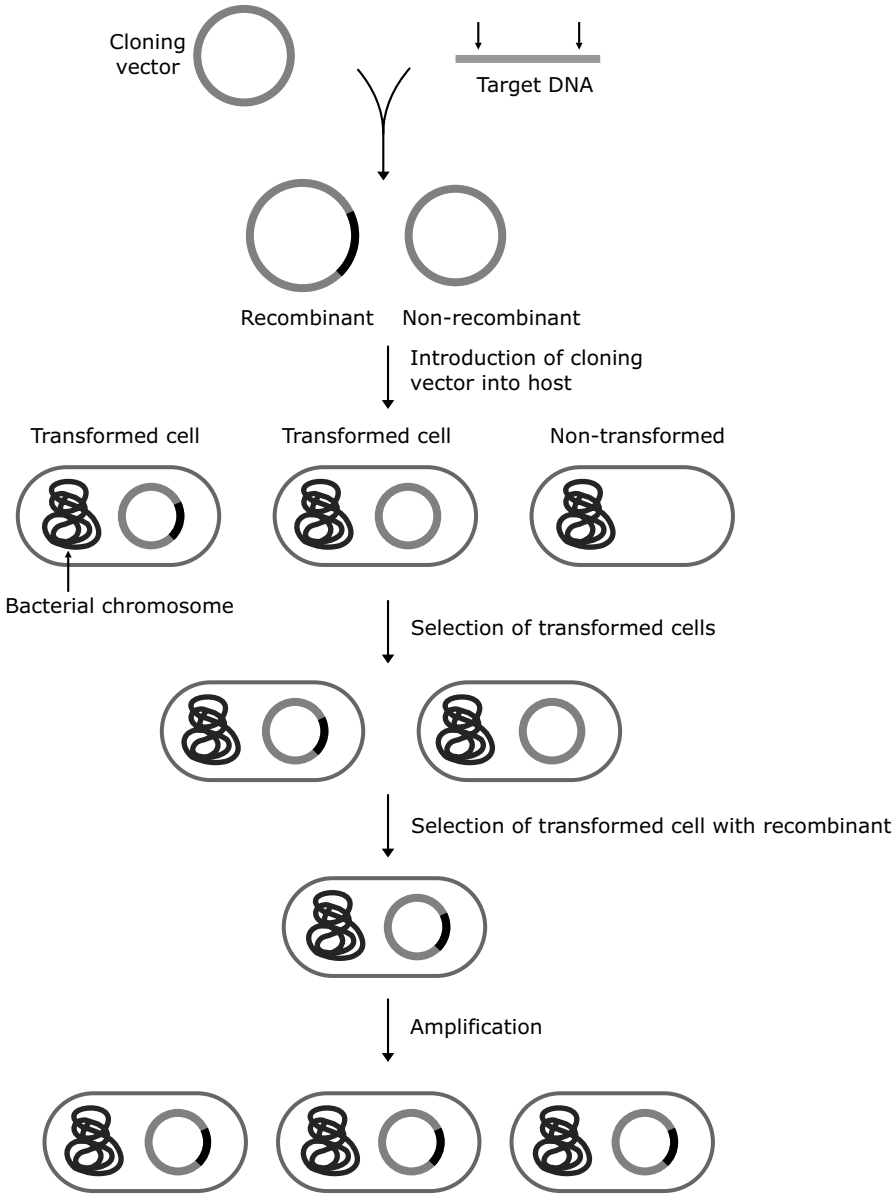


Figure 2.1 An overview of DNA cloning in bacteria using a plasmid vector.

Cell-free DNA cloning

The polymerase chain reaction (PCR) is a newer form of DNA cloning which is enzyme mediated and is conducted entirely *in vitro*. PCR (developed in 1983 by Kary Mullis) is a revolutionary technique used for selective amplification of specific target sequence of nucleic acid by using short primers. It is a rapid, inexpensive and simple method of copying specific DNA sequence.

2.2 Enzymes for DNA manipulation

The enzymes used in the recombinant DNA technology fall into four broad categories:

2.2.1 Template–dependent DNA polymerase

DNA polymerase enzymes that synthesize new polynucleotides complementary to an existing DNA or RNA template are included in this category. Different types of DNA polymerase are used in gene manipulation.

DNA polymerase I (Kornberg enzyme) has both the 3′-5′ and 5′-3′ exonuclease activities and 5′-3′ polymerase activity.

Reverse transcriptase, also known as RNA-directed DNA polymerase, synthesizes DNA from RNA.

Reverse transcriptase was discovered by *Howard Temin* at the University of Wisconsin, and independently by *David Baltimore* at about the same time. The two shared the 1975 Nobel Prize in Physiology or Medicine.

Taq DNA polymerase is a DNA polymerase derived from a thermostable bacterium, *Thermus aquaticus*. It operates at 72°C and is reasonably stable above 90°C and used in PCR. It has a 5′ to 3′ polymerase activity and a 5′ to 3′ exonuclease activity, but it lacks a 3′ to 5′ exonuclease (proofreading) activity.

2.2.2 Nucleases

Nucleases are enzymes that degrade nucleic acids by breaking the phosphodiester bonds that link one nucleotide to the next. Ribonucleases (RNases) attack RNA and deoxyribonucleases (DNases) attack DNA. Some nucleases will only attack single stranded nucleic acids, others will only attack double-stranded nucleic acids and a few will attack either kind. Nuclease are of two different kinds – exonucleases and endonucleases. *Exonucleases* remove nucleotides one at a time from the end of a nucleic acid whereas e*ndonucleases* are able to break internal phosphodiester bonds within a nucleic acid. Any particular exonuclease attacks either the 3′-end or the 5′-end but not both.

Mung bean nuclease

The mung bean nuclease is an endonuclease specific for ssDNA and RNA. It is purified from mung bean sprouts. It digests single-stranded nucleic acids, but will leave intact any region which is double stranded. It requires Zn^{2+} for catalytic activity.

S1 nuclease

The S1 nuclease is an endonuclease purified from *Aspergillus oryzae*. This enzyme degrades RNA or single stranded DNA, but does not degrade dsDNA or RNA-DNA hybrids in native conformation. Thus, its activity is similar to mung bean nuclease, however, the enzyme will also cleave a strand opposite a nick on the complementary strand.

RNase A

RNase A is an endonuclease, which digests ssRNA at the 3′ end of pyrimidine residues.

RNase H

It is an endonuclease which digests the RNA strand of an RNA-DNA heteroduplex. The enzyme does not digest ss or dsDNA.

thermodynamically less stable than DNA because of the 2′ hydroxyl group on the ribose ring that promotes hydrophilic attack on the 5′-3′ phosphodiester bond to form a 2′-3′ cyclic phosphate. Therefore, even if all RNases are eliminated or inhibited during RNA purification, RNA spontaneously degrades while in solution. To circumvent this biological decay of RNA, purified samples are stored at –20°C as ethanol precipitates.

The purification of mRNA involves two basic steps: 1. Biochemical separation of total cellular RNA from DNA and protein using a strong protein denaturant to inhibit cellular RNases, and 2. Isolation of poly A tail mRNA using an oligo dT affinity matrix. A common method used to isolate mRNA from tissue culture cells is outlined in the following figure 2.4. Guanidinium thiocyanate is a protein denaturant that lyses the cells and inhibits cellular RNases.

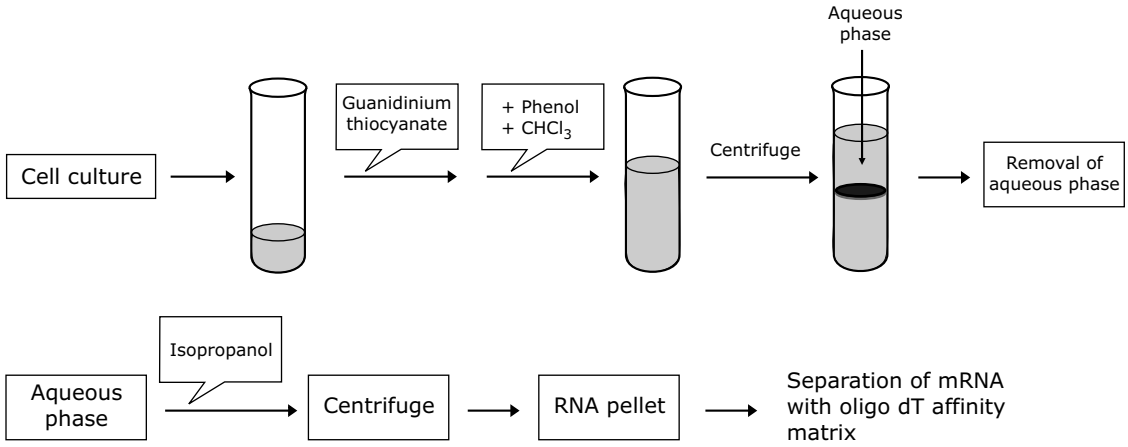


Figure 2.4 Isolation and purification of mRNA from tissue culture cells using guanidinium thiocyanate and oligo dT cellulose. This purification method is based on the finding that RNA preferentially partitions to the aqueous phase in a solution containing guanidinium thiocyanate at pH 4 in the presence of phenol and chloroform. Under these conditions, proteins partition to the organic phase and most of the large DNA fragments trapped in the interphase. Poly A+ mRNA is purified from total cellular RNA using polyadenylated and therefore, oligo dT affinity ligand.

2.4 Vectors

The term *vector* refers to the DNA molecules that act as transporting vehicle which carries foreign DNA from the test tube to the host cell for the purpose of cloning and expression. **Cloning vectors** are used to clone foreign DNA whereas **expression vectors** are engineered so that any foreign DNA can be transcribed in RNA and translated into protein. A viral DNA or plasmid is generally used as a vector. The important features of a *cloning vector* are as follows:

1. Ability to replicate in host cells.

   All cloning vectors have origin of replication for autonomous replication within the host cell. The origin of replication is a specific sequence in DNA from where replication starts. When foreign DNA is linked to vector containing origin of replication then along with vector replication, foreign (desirable) DNA also starts replicating within the host cell.

2. Unique restriction enzyme sites for insertional cloning.

   All cloning vectors have features that allow a foreign DNA to be conveniently inserted into the vector. This may be a *multiple cloning site* (also called polylinker site) which contains many unique restriction sites. The restriction sites in the polylinker site are first cleaved by restriction enzymes, and a target gene is then ligated into the vectors using DNA ligase.

3. Genetic marker to select for host cells containing the vector.

   Genetic marker is a gene that allow the selection of transformed from nontransformed cell and recombinant containing transformed cell from non-recombinant containing transformed cell. Marker genes belong to two

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It is small (only 4361 base pairs) and maintained in the host in relatively high copy number, 20–30 copies per cell. It has genes conferring ampicillin resistance and tetracycline resistance on its host and has single cleavage sites for PstI, EcoRI, HindIII, BamI, SalI and ClaI. pBR322 is genetically engineered from DNA derived from three different naturally occurring plasmids (R1, R6-5 and pMB1). Antibiotic resistance genes amp^R and tet^R are derived from plasmid R1 and R6-5, respectively. The origin of replication is derived from pMB1. The valuable features of pBR322 have been enhanced by the construction of a series of plasmids termed pUC (produced at the University of California). The plasmid vector pUC19 (2,686 bp long) contains a polylinker with unique cloning sites for multiple restriction nucleases and an ampicillin resistance gene to permit identification of transformed cells. In addition, a selection of recombinants is achieved by insertional inactivation of a component of the β-galactosidase gene, a complementary portion of this gene being provided by using a specially modified *E. coli* host cell.

Bacterial Artificial Chromosome (BAC)

BAC cloning vectors were developed by Mel Simon and his colleagues. BAC vectors are maintained in *E. coli* as large single copy plasmids and contain an insert of 50–300 kb. BAC vectors contain the F-plasmid origin of replication, F-plasmid genes control plasmid replication, plasmid copy number and the bacterial chloramphenicol acetyltransferase gene for plasmid selection.

2. Cloning vectors based on viral DNA

Viral vectors are those in which the gene or genes of interest are incorporated into the genome of a virus. Because viruses infect cells with high efficiency, the cloned gene can be introduced into cells at a significantly higher frequency than by simple transformation. Some viral vectors are specialized for producing high levels of proteins encoded by the cloned genes and other viral vectors, such as the bacterial M13-based vectors, are designed to facilitate sequencing and the generation of mutations in cloned genes.

Cloning vector based on λ phage

Lambda phage, a temperate phage, infects bacteria *E. coli* and replicates by a *lytic* or *lysogenic* pathway. Its genome consists of single linear dsDNA of ~48 kb. However, at either end of the molecule is a short 12 nucleotides stretch, in which the DNA is single-stranded described as cohesive ends or sticky ends. The lambda cohesive ends are called the ***cos* ends** and they play two distinct roles during the lambda infection cycle. First, they allow the linear DNA molecule that is injected into the cell to be circularized. The second role of the *cos* sites is rather different and comes into play during the lytic cycle. During lytic cycle, a large number of new lambda DNA molecules are produced by the rolling circle mechanism of replication. The result is a catenate consisting of a series of linear λ genomes joined together at the *cos* sites. The role of the *cos* sites is now to act as recognition sequences for an endonuclease that cleaves the catenate at the *cos* sites, producing individual lambda genomes.

In the *lytic pathway*, viral functions are fully expressed: viral DNA and proteins are quickly produced and packaged into virus particles, leading to the lysis of the host cell and release of virus particles, or *virions.* In the *lysogenic pathway*, the phage DNA becomes integrated into the host-cell genome and can be replicated together with host-cell DNA for many generations, remaining inactive. Certain environmental changes can trigger the expression of this dormant viral DNA, which leads to the formation of progeny virus and lysis of the host.

There is one problem in using λ phage DNA as cloning vector. This is *size limitation*. Normal λ phage genome size is about 49 kb, whereas the capacity of capsid to incorporate maximum genome size is about 53 kb. Thus a λ phage DNA molecule that can be increased in size by the addition of only 3 kb of new DNA. However, this problem has overcome by deletion of non-essential DNA of about 15 kb. This means that as much as 18 kb of new DNA can now be added. The non-essential region, in fact, contains the genes involved in lysogenic pathway. A deleted λ genome is, therefore, non-lysogenic and can follow only the lytic cycle.

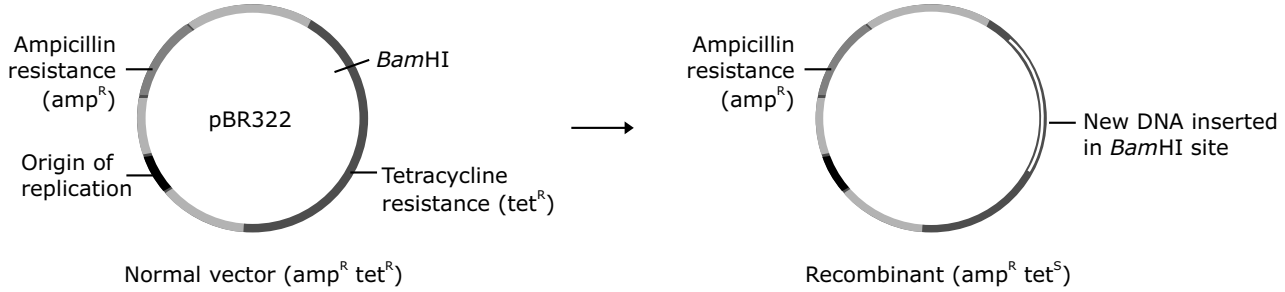
Two basic types of lambda vectors have been developed:

Insertion λ vectors

Insertion vectors are the simplest form of lambda cloning vectors. In the development of this vector, a large segment of the nonessential region of λ phage genome has been deleted and the two fragments ligated together. It

2.6 Recombinant screening

A selective medium enables transformants to be distinguished from non-transformants. The next problem is to determine which of the transformed colonies comprise cells that contain recombinant DNA molecules, and which contain self-ligated vector molecules. With most cloning vectors insertion of a DNA fragment into the plasmid destroys the integrity of one of the genes present on the molecule. Recombinants can, therefore, be identified because the characteristic coded by the inactivated gene is no longer displayed by the host cells (called **insertional inactivation**). Most commonly recombinant selection is carried out by insertional inactivation of antibiotic resistance gene. In this case the insertion of new DNA fragments (insert) occurs at the site within the gene that confers resistance towards a particular antibiotic.



Insertional inactivation does not always involve antibiotic resistance genes. For example in pUC8, gene LacZ′, which codes for part of enzyme β-galactosidase is used for insertional inactivation. Recombinant pUC8 involves insertional inactivation of the lac Z′ gene, can be identified because of their inability to synthesize β-galactosidase. β-galactosidase, coded by lacZ gene, causes the breakdown of lactose to glucose plus galactose. lacZ′, a modified lacZ gene, codes for the α peptide portion of β-galactosidase.

2.7 Introduction of DNA into the host cells

2.7.1 In bacterial cells

The process of transferring exogenous DNA into bacterial cells is called **transformation**. There are basically two general methods for transforming bacteria.

The first is a *chemical transformation method* utilizing $CaCl_2$ and heat shock to promote DNA entry into cells. The chemical method uses bacteria that are incubated with DNA on ice cold salt solution containing $CaCl_2$ followed by a brief heat shock at 42°C. Exactly how this treatment works is not understood. Possibly $CaCl_2$ causes the DNA to precipitate onto the surface of the cells, or perhaps the salt is responsible for some kind of change in the cell wall that improves DNA binding.

A second method is called *electroporation*. It uses a short pulse of electric charge to facilitate DNA uptake. Electroporation induces formation of microscopic pores within a biological membrane. These pores, called *electropores*, allow molecules, ions and water to pass from one side of the membrane to the other.

2.7.2 In plant cells

Gene transfer to plant cells is achieved using three different methods:

First, the natural ability of certain bacteria of the genus *Agrobacterium* to naturally transfer DNA to the genomes of infected plant cells. These bacteria are also known as *natural genetic engineers* of plants since these bacteria have ability to transfer T-DNA of their plasmid into plant genome upon infection of cells at the wound site. Ti and Ri plasmids can be used as gene vectors for delivering useful foreign genes into target plant cells and tissues. The foreign gene is cloned in the T-DNA region of Ti plasmid by replacing unwanted sequences.

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In transfected cells, *transgene* present in two different states. In a large proportion of transfected cells, the *transgenes* do not integrate into the genome and are maintained in the nucleus in an extrachromosomal state. If it does not contain an origin of replication, it persists for just a short time before it is degraded. This is known as *transient transfection.* In second types of transfection called *stable transfection*, foreign DNA must be maintained permanently in the cell. If the exogenous DNA is non-replicative, stable transfection must occur by integration of the DNA into the genome. Alternatively, the foreign DNA may be carried in an episomal vector, whose moderate replication rate does not cause cell death. The stable transfection is required for the long-term production of foreign proteins, for gene silencing by antisense RNA synthesis and for the generation of transgenic animals. It is also highly desirable in many gene therapy applications.

Transfection strategies : Two basic DNA transfection strategies have been developed to deliver DNA to cells. These termed as stealth and attack strategies of DNA transfection. The *stealth strategy* is based on the use of positively charged carrier molecules that are mixed with the experimental DNA *in vitro* and then applied directly to the cell culture media. These carrier-DNA complexes attach to cell membranes and stimulate the uptake of the *stealth* DNA molecules. The three most commonly used stealth transfection methods are calcium phosphate precipitation, DEAE dextran-mediated gene transfer and liposome-mediated gene transfer. Each of these techniques is sensitive to cell type differences owing to the requirement for selective interactions between the carrier molecule and the cell membrane. The *attack strategy* uses physical methods to force DNA into cells. Two attack DNA transfection methods have been developed: biolistics and microinjection.

Chemical transfection techniques : In chemical transfection techniques, cells take up DNA complexed with another molecule by endocytosis. The chemical transfection is carried out by precipitating DNA in the presence of the cells. This can be achieved by washing cultured cells in a phosphate buffer, adding the DNA, and then adding calcium chloride to the mixture. Under these circumstances, it is thought that the precipitate settles on the surface of cells and is then internalized through endocytosis.

An alternate chemical transfection method utilizes diethylaminoethyl dextran (DEAE-dextran), a soluble polycationic carbohydrate that promotes interactions between DNA and the cell and thus their internalization.

Liposomes and Lipofection : *Liposomes* are unilaminar phospholipid vesicles into which DNA can be packaged. When mixed with cells in culture, the vesicles fuse with the cell membrane and deliver DNA directly into the cytoplasm. The efficiency of liposome-mediated gene transfer can be enhanced by incorporating viral proteins that facilitate the active fusion between viral envelopes and cell membranes. Such fusogenic particles, have been termed *virosomes.*

Lipofection involves cationic/neutral lipid mixtures, which spontaneously associate with negatively charged DNA to form complexes. Unlike liposome-mediated transfection, where the DNA is encapsulated within a lipid vesicle, lipofection involved in the formation of a DNA lipid complex (*lipoplex*) which is taken up efficiently by endocytosis.

Cell or protoplast fusion : Certain chemicals, such as polyethylene glycol (PEG), act as fusogens, agents that cause cell membranes *to* fuse together. This can be exploited to transfect animal cells by mixing them with other cells containing large amounts of plasmid DNA. Schaffner first successfully used bacterial protoplasts to transfect mammalian cells in culture by treating bacterial cells with chloramphenicol to amplify the plasmid contents and lysozyme to remove the cell wall. The protoplasts were then induced to fuse with mammalian cells.

Microinjection : The direct microinjection of DNA into the cytoplasm or nuclei of cultured cells is sometimes used as a transfection method. Although highly efficient on an individual cell basis, the procedure is time consuming, and only a small number of cells can be treated. One major advantage of this technique is that direct nuclear delivery avoids exposing the foreign DNA to any cytoplasmic organelles; so it is delivered intact. Microinjected DNA, therefore, suffers a less mutation than DNA delivered by most chemical transfection methods. The most significant use of microinjection is the introduction of DNA into the oocytes and eggs of animals, either for transient expression analysis (e.g. in *Xenopus)* or to generate transgenic animals.

Particle bombardment : Particle bombardment (also known as *microballistic* or microprojectile transfection) is a relatively recent transfection technique. The procedure involves coating micrometer-sized gold or tungsten particles with DNA and then accelerating the particles into cells using blast of high pressure He gas or an electrical discharge.

Receptor-mediated transfection : Receptor-mediated transfection involves the delivery of DNA to particular cells by conjugation to a specific ligand. The ligand interacts with receptors on the cell surface, allowing both and the attached DNA to be internalized. One problem associated with this technique is that the ligand-DNA complexes are internalized via endocytotic vesicles that generally fuse with lysosomes, resulting in degradation of the DNA and consequent failure of gene expression. Some of the DNA escapes this fate and finds its way to the nucleus to be expressed, but the mechanism by which this occurs is not understood. Receptor-mediated transfection is highly efficient in cell culture, resulting in the transfection of up to 90% of cells carrying the appropriate receptor. Less success has been observed for *in vivo* gene transfer, partly because the ligand-DNA complexes are degraded in serum, and partly because the size of the particles appears to be a critical parameter for the transfection of different cell types.

Table 2.4 Examples of the major categories of transfection method

Category	*Examples*
Chemical	Calcium phosphate, DEAE-dextran, lipofection
Poration	Electroporation
Fusion	Liposome/virosome delivery, protoplast fusion
Physical	Particle bombardment, microinjection
Receptor mediated	Conjugation to various ligands

Transduction : It describes virus-mediated gene transfer. Certain animal viruses naturally infect human and mammalian cells. They include both DNA viruses (e.g. SV40, adenovirus) and RNA viruses (e.g. HIV and other retroviruses). Modified forms of these viruses can be used as vectors to transfer exogenous genes into suitable target cells at high efficiency. Development of viral vectors for DNA transfer to animal cells utilizes several advantageous characteristics of animal viruses:

1. They have evolved efficient mechanisms to adsorb to the surface and gain entry into cells without damaging them.
2. They deliver their nucleic acid intact because it is initially packaged in a proteinaceous capsid.
3. Viral genomes contain strong regulatory elements that can be exploited to drive high-level foreign gene expression.
4. Many animal viruses have a broad host range and can thus replicate in diverse cell types.
5. Many viruses can stably transform cells by integration or latent episomal replication.

2.8 Polymerase chain reaction

PCR is a rapid and versatile *in vitro* method for amplifying defined target DNA sequences present within the source of DNA. This technique was formulated in 1985 by *Kerry Mullis*. Usually, the method is designed to permit *selective amplification* of a specific target DNA sequence(s) within a heterogeneous collection of DNA sequences (e.g. total genomic DNA or a complex cDNA population). To permit such selective amplification, some prior DNA sequence information from the target sequences is required. This information is used to design two oligonucleotide primers (amplimers) which are specific for the target sequence and which are often about 15–25 nucleotides long. After the primers are added to denatured template DNA, they bind specifically to complementary DNA sequences at the target site. In the presence of a suitably heat-stable DNA polymerase and DNA precursors (the four deoxynucleoside triphosphates, dATP, dCTP, dGTP and dTTP), primer initiates the synthesis of new DNA strands which are complementary to the individual DNA strands of the target DNA segment, and which will overlap each other.

Primer design

To permit selective amplification, some prior DNA sequence information from the target DNA is required. The information is used to design two primers (amplimers), which are specific for sequences flanking the target DNA sequence.

Problems due to the prokaryotic host, E. coli

Processing of proteins

Prokaryotes do not carry out the same kind of post-translational modifications such as glycosylation and phosphorylation as eukaryotes do. This affects a protein's activity or stability, or at least its response to antibodies.

Folding

In prokaryotic expression systems, most protein products of cloned eukaryotic genes become insoluble aggregates called *inclusion bodies* due to incorrect folding and are very difficult to recover as functional proteins. Target proteins expressed in *E. coli* may be mis-folded for a variety of reasons, including the exposure of hydrophobic residues that are normally in the core of the protein, the lack of its normal interaction partners and inappropriate or missing post-translational modifications.

Eukaryotic expression systems

Problems associated with obtaining active recombinant proteins from genes cloned in prokaryotic host can be mitigated by using eukaryotic expression system. Eukaryotic systems for the expression of protein include: yeast, mammalian cells and baculovirus cells (insect). Advantages of eukaryotic protein expression systems include very high levels of expression. The proteins are easy to purify using special tags which are included into the vectors including His, Myc and other tags. The disadvantages of the systems include the fact that eukaryotic cells do grow slower than prokaryotic cells.

2.10.3 Fusion protein

Proteins may be expressed by expression vectors as native polypeptide or fusion proteins; the latter often to facilitate protein purification or analysis. *Fusion proteins* (also called hybrid protein, chimeric protein) are the products of two or more coding sequences from different genes that have been cloned together and that, after translation, form a single polypeptide sequence. Fusion protein protects the cloned gene product from attack by host cell proteases. In a number of cases, cloned gene proteins have been found to be resistant to degradation when they are part of a fusion protein, whereas, when expressed as separate intact proteins, they are susceptible to degradation by proteolytic enzymes (proteolysis).

Fusion proteins are constructed at the DNA level by ligating together a portion of the coding regions of two or more genes. In its simplest form, a fusion vector system entails the insertion of a target gene or gene segment into the coding region of a cloned host gene. Knowledge of the nucleotide sequences of the various coding segments that are joined at the DNA level is necessary to ensure that ligation produces the correct reading frame.

2.11 DNA sequencing

The term *DNA sequencing* encompasses biochemical methods for determining the order of the nucleotide bases, adenine, guanine, cytosine and thymine, in a DNA oligonucleotide.

The methodologies for DNA sequencing:

The *chain termination method* (by Sanger), in which the sequence of a ssDNA molecule is determined by enzymatic synthesis of complementary polynucleotide chains, these chains terminating at specific nucleotide positions.

The *chemical degradation method* (by Maxam and Gilbert), in which the sequence of a dsDNA molecule is determined by treatment with chemicals that cut the molecule at specific nucleotide positions.

The *pyrosequencing method,* in which the addition of a deoxynucleotide to the end of the growing strand is detectable because it is accompanied by the release of a flash of light.

Chain termination method

Chain termination method relies on the use of dideoxyribonucleoside triphosphates, derivatives of the normal deoxyribonucleoside triphosphates that lack the 3' hydroxyl group.

2.12 Genome mapping

A map of the locations of identifiable landmarks on chromosomes is known as *genome map*. There are two kinds of maps - genetic and physical map. The **genetic map** gives the relative position of genetic markers (gene loci) according to the frequency of recombination, expressed in term of centimorgans (cM). Genetic maps illustrate the order of genetic markers on a chromosome and the relative distances between those markers. Genetic mapping uses classical genetic techniques to determine distances between markers. **Physical maps**, by contrast, always give the physical, DNA-base-pair distances from one genetic marker to another.

There is a difference between a genome map and a genome sequence. A genome sequence spells out the order of every DNA base in the genome, while a map simply identifies a series of landmarks in the genome. A genome map is less detailed than a genome sequence.

2.12.1 Genetic marker

A gene or DNA sequence having a known location on a chromosome and associated with a particular trait or gene is used as a *genetic marker*. Genes were the first markers to be used to prepare the first genetic maps of fruit fly. There are three major types of genetic markers:

1. Morphological (also classical or visible) markers which are based on phenotypic traits or characters;
2. Biochemical markers, which are based on gene products; and
3. DNA (or molecular) markers, which reveal sites of variation in DNA.

Morphological markers are usually visually characterized phenotypic characters such as flower colour, seed shape, growth habits or pigmentation. Biochemical markers are differences in gene products that are detected by electrophoresis and specific staining. The major disadvantages of morphological and biochemical markers are that they may be limited in number and are influenced by environmental factors or the developmental stages. A *molecular* or *DNA marker* is defined as a particular segment of DNA that is representative of the differences at the genome level. Molecular markers should not be considered as normal genes, as they usually do not have any biological effect, and instead can be thought of as constant landmarks in the genome. They are identifiable DNA sequences, found at specific locations of the genome, and transmitted by the standard laws of inheritance from one generation to the next. An ideal molecular marker should have the following criteria:

1. be polymorphic and evenly distributed throughout the genome,
2. provide adequate resolution of genetic differences,
3. have linkage to distinct phenotypes.

2.12.2 Types of DNA markers

Various types of DNA markers have been described in the literature. They can be broadly divided into two classes based on the method of their detection: Hybridization-based (such as RFLP) and PCR based (such as RAPD, AFLP, SSLP). PCR-based techniques can further be subdivided into two subcategories: arbitrarily primed PCR-based techniques or sequence nonspecific techniques (such as RAPD, AFLP) and sequence targeted PCR-based techniques (such as SSLP, SNP).

DNA markers may be described as *codominant* or *dominant*. This description is based on whether markers can discriminate between homozygotes and heterozygotes. Codominant markers indicate differences in size whereas dominant markers are either present or absent.

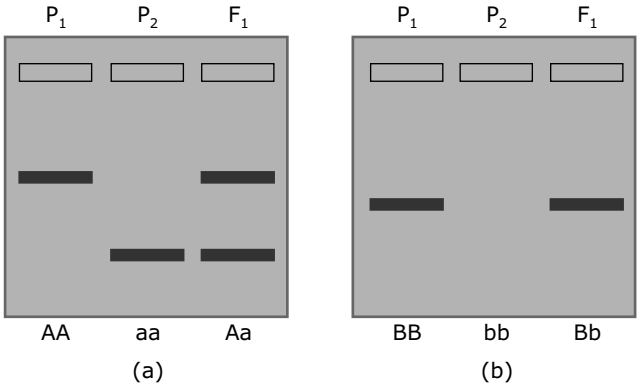


Figure 2.22 Comparison between (a) codominant and (b) dominant markers. Codominant markers can clearly discriminate between homozygotes and heterozygotes whereas dominant markers do not. Genotypes at two marker loci (A and B) are indicated below the gel diagrams.

RFLPs

RFLP (Restriction Fragment Length Polymorphisms) is the most widely used hybridization-based molecular marker. RFLP markers were first used in 1975 to identify DNA sequence polymorphisms for genetic mapping. RFLPs arise because mutations can create or destroy the sites recognized by specific restriction enzymes, leading to variations between individuals in the length of restriction fragments produced from identical regions of the genome. Although two individuals of the same species have almost identical genomes, they will always differ at a few nucleotides due to point mutation and insertion/deletion. Some of the differences in DNA sequences at the restriction sites can result in the gain, loss or relocation of a restriction site.

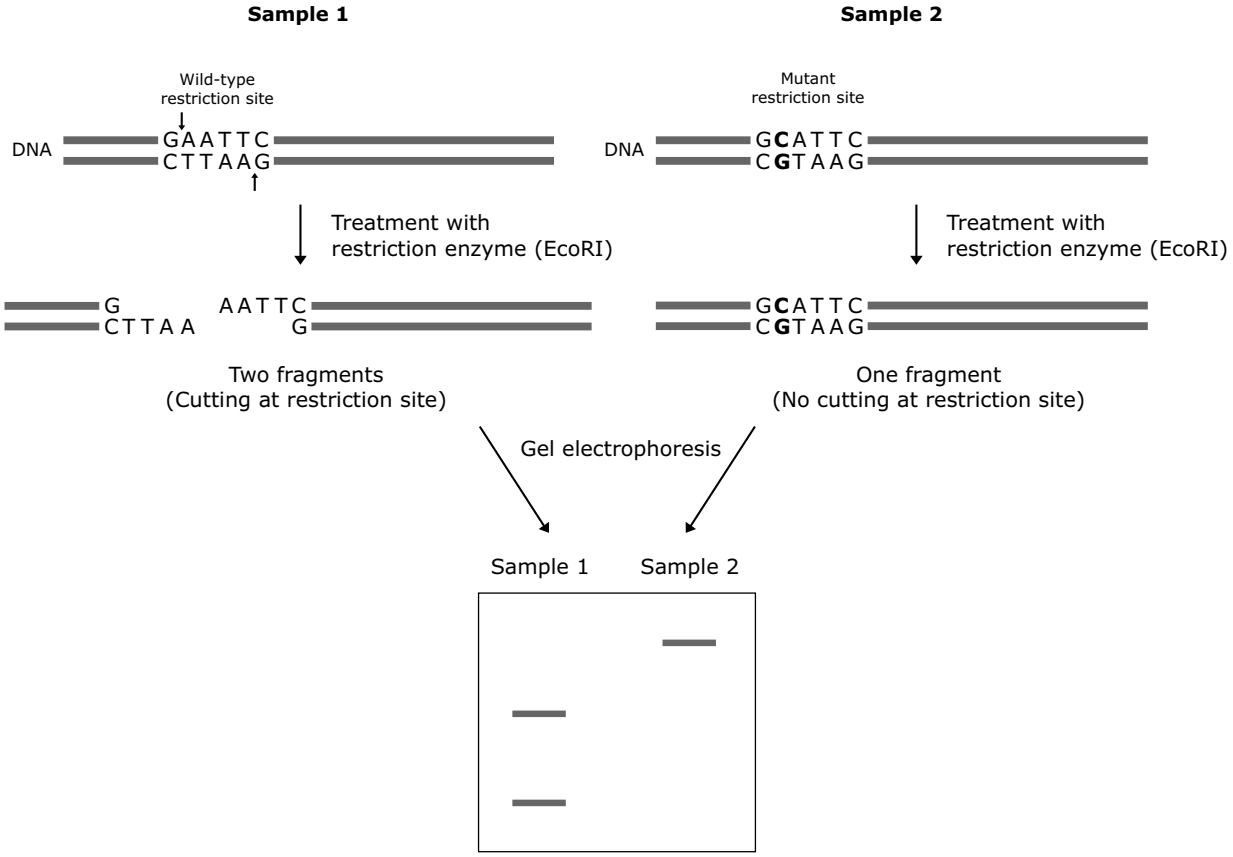


Figure 2.23 Restriction-fragment length polymorphisms.

2.13 DNA profiling

DNA profiling or *DNA typing* is a technique used to distinguish between individuals of the same species using only samples of their DNA. Although the term *DNA fingerprinting* is sometimes used, it is not really appropriate. The DNA profiling technique was first reported in 1984 by Sir Alec Jeffreys at the University of Leicester in England. The chemical structure of everyone's DNA is the same. The only difference between persons is the order of the base pairs. Hence, every person could be identified solely by the sequence of their base pairs. Although two individuals will have the vast majority of their DNA sequence in common, DNA profiling exploits highly variable repeat sequences called VNTRs (Variable Number Tandem Repeats). VNTR is a general term which includes microsatellite VNTR and minisatellite VNTRs, but is often used loosely to mean the minisatellite class. Within a species, the nucleotide sequences of the repeat units composing VNTR are highly conserved among individuals. However, differences in the number of repeats, and thus in the length of simple-sequence tandem arrays containing the same repeat unit, are quite common among individuals. These DNA polymorphisms form the basis of *DNA fingerprinting.*

The high variability in minisatellites makes them especially useful for genomic mapping, because there is a high probability that individuals will vary in their alleles at such a locus. Scientists can use these regions to generate a DNA profile of an individual, using samples from blood, bone, hair and other body tissues and products. In human chromosomes, many VNTR regions are bordered by restriction endonuclease sites on either side. Alleles of a given VNTR differ from each other in the size of the segment cut by the restriction endonuclease, giving rise to nearly unique RFLP patterns. This is the basis of the technique known as DNA fingerprinting. With this technique, forensic scientists have been able to find missing persons, determine whether or not an individual is the parent of a child, and establish beyond reasonable doubt that a person is guilty of a crime for which he or she has been accused.

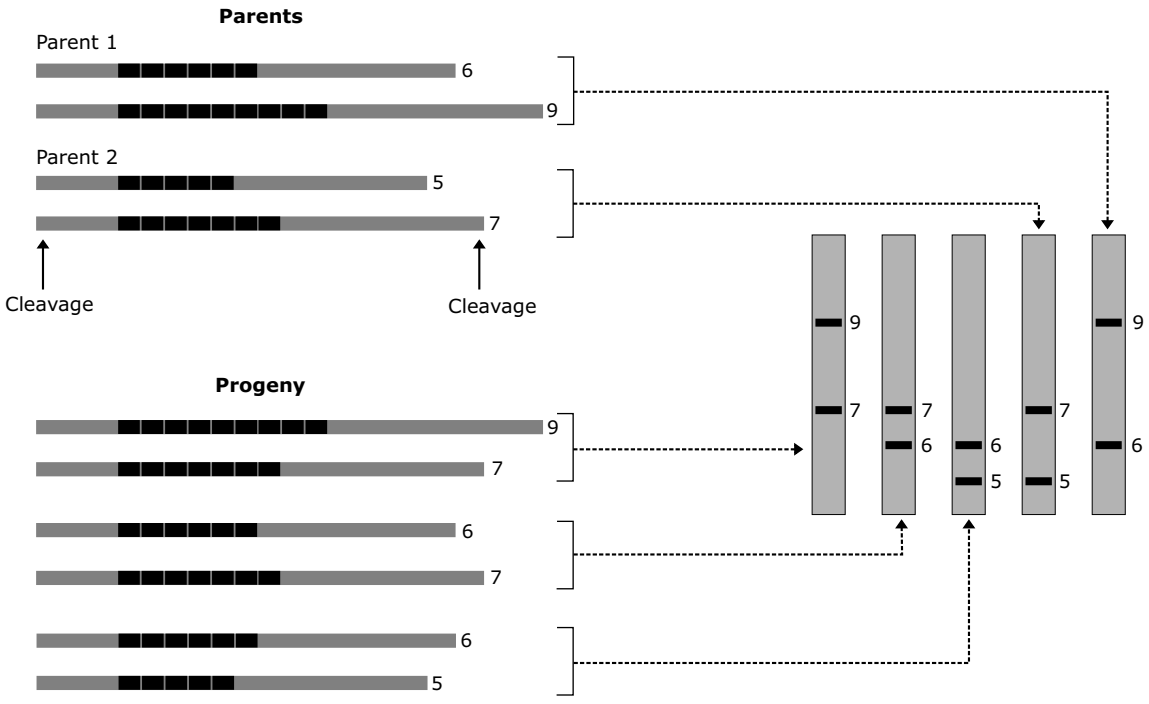


Figure 2.27 Alleles may differ in the number of repeats at a VNTR locus, so that cleavage on either side generates, restriction fragments that differ in length. By using a minisatellite with alleles that differ between parents, the pattern of inheritance can be followed.

An example of mapping by minisatellites is illustrated in figure 2.27. In this case two individuals are heterozygous at a minisatellite locus. All progeny gain one allele from each parent in the usual way, and it is possible unambiguously to determine the source of every allele in the progeny. DNA fingerprinting analyzes the differences between the fragments generated by using restriction enzymes. Because these are unique to every individual, the presence of a particular subset in any two individuals can be used to define their common inheritance (e.g. a parent-child relationship).

2.14 Genetic manipulation of animal cells

2.14.1 Transgenesis and transgenic animals

Transgenesis may be defined as the introduction of exogenous (foreign) DNA into the genome, such that it is stably maintained in a heritable manner. Animals that have been permanently engineered by gene insertion are called *transgenic animals*, and any foreign genes that are added are called *transgenes*.

Genetically modified, *genetically engineered* and *transgenic* organisms are often used interchangeably; yet, they do not mean the same thing. A g*enetically modified* organism is one that has had its genetic material altered through any method, including conventional breeding. A *genetically engineered* organism is one that has been genetically modified using recombinant DNA technology. A transgenic organism has been genetically engineered using a foreign gene, usually belonging to a different species.

Production of transgenic animals

The first transgenic animals were produced in 1981 by Ralph Brinster and Richard Palmiter. They succeeded in introducing a gene for rat growth hormone (GH) into the fertilized eggs of mice. The injected DNA was constructed so as to contain the coding portion of the rat GH gene in a position just downstream from the promoter region of the mouse metallothionein gene. A variety of methods have been developed for the production of transgenic animals. All are based on the introduction of the DNA into a single cell that contributes to the development of the animal. In mammals, the vast majority of transgenic experiments have been performed using mice. Methods for producing transgenic mice involve the removal of fertilized eggs or early embryos from donor mothers, *in vitro* transfer of transgene and then their return to foster-mothers, where development continues. For mouse transgenesis, DNA can be introduced into the mice by

1. Pronuclear microinjection (microinjection of DNA into the male pronucleus of one-cell embryos).
2. Introduction of genetically engineered embryonic stem cells into an early stage developing embryo.
3. Introduction of retroviral vectors in the cells of an early stage embryo.

Pronuclear microinjection

In pronuclear microinjection, transgene is injected into the male pronucleus just after fertilization. The male pronucleus is relatively larger than the female nucleus and closer to the oocyte surface. Microinjection is done using a microneedle. The microinjected transgene randomly integrates into chromosomal DNA, usually at a single site, and usually as multiple copies. It is more common for the DNA to integrate after one or two cell divisions, in such cases the resulting mouse is a **mosaic** containing both transfected and non-transfected cells.

If the DNA integrates prior to the first division of the zygote, every cell in the resulting animal will contain the same transgene. The surviving intact eggs after microinjection are implanted into a host female mouse. Three weeks after the birth of the pups, genomic DNA from mouse are analyzed. Mice that score positive by test are then bred to establish founder transgenic lines. If the DNA integrates after the first division of the zygote, only some of the cells in the embryo will incorporate the transgene. However, if those cells contribute to the germ line of the embryo, transgenic gametes will be produced and the subsequent generation of animals will be transgenic.

2.17.3 Molecular farming

It is an application of genetic engineering in which genes, primarily of human or animal origin are introduced into plants or farm animals for cost effective production of therapeutic products such as antibodies, blood products, cytokines, growth factors, hormones, recombinant enzymes and human and veterinary vaccines. Therapeutic compounds so produced are also known as biopharmaceuticals (pharmaceuticals from biological organisms).

The organisms in which genes coding for the target therapeutically active compound introduced are often referred to as expression system. Expression system studied so far include bacteria, yeast, plant viruses, animal cell culture, transgenic plants and transgenic animals. Initially bacteria were the most widely used expression systems but due to the complexity of the most therapeutic proteins to be produced and simplicity of the bacterial system, new expression systems were explored. As of now the plants are the preferred and most widely used expression system in comparison to other systems. The first recombinant pharmaceutical protein produced in the plant was human serum albumin, first produced in 1990 in transgenic tobacco and potato plants.

Table 2.10 Examples of some pharmaceutical recombinant human proteins expressed in plant systems

Tobacco, sunflower (plants)	Growth hormone
Tobacco, potato (plants)	Serum albumin
Tobacco (plants)	Epidermal growth factor
Rice (plants)	Alpha-interferon
Tobacco (cell culture)	Erythropoietin
Tobacco (plants)	Haemoglobin
Tobacco (cell culture)	Interleukins-2 and 4
Tobacco (root culture)	Placental alkaline phosphatase

2.18 Plant tissue culture

The field of plant tissue culture is based on the fact that plants can be separated into their component parts (organs, tissues or cells), which can be manipulated *in vitro* and then grown back into complete plants. Plant cells or tissues will continue to grow if supplied with the appropriate nutrients and conditions. The culture of plant cells, tissues and organs such as roots, shoot tips and leaves in artificial nutrient media aseptically is referred to as *plant tissue culture*.

Plant cells - *unique features*

A plant cell is a eukaryotic cell and shares similar features with the typical eukaryote cell. However some features are uniquely present in plant cells. Their distinctive features include:

- A cell wall outside the cell membrane which is composed of cellulose, hemicellulose, pectin and in many cases lignin.
- A large central vacuole enclosed by a membrane known as the *tonoplast which* maintains the cell's turgor, controls movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.
- Specialized cell-cell communication through plasmodesmata, pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.
- Plastids such as chloroplasts which contain chlorophyll for photosynthesis, amyloplasts for starch storage, elaioplasts for fat storage and chromoplasts for the synthesis and storage of pigments.
- A specialized peroxisome called glyoxysome for the operation of glyoxylate cycle.
- Cytokinesis by formation of a phragmoplast and cell plates.
- Absence of centrioles in MTOC that are present in animal cells.

2.18.1 Cellular totipotency

Totipotency is the ability of a single cell to divide and produce all the differentiated cells in an organism. In a multicellular organism, a cell after regulated division undergoes for cell differentiation. It is a process of specializing cells' functions. Isolated cells from differentiated tissues are generally non-dividing and quiescent; to express totipotency the differentiation process has to be reversed (called *de-differentiation*) and repeated again (called *re-differentiation*). A differentiated cell reverting to an undifferentiated state is termed *dedifferentiation*, whereas the ability of a dedifferentiated cell to form a whole organism or organs is termed *redifferentiation*. Theoretically, all living cells can revert to an undifferential status through this process. However, the more differentiated a cell has been, the more difficult it will be to induce its de-differentiation. In plants, even highly mature or differentiated cells have the ability to regress to a meristematic state as long as they are viable and express totipotency. This phenomenon of totipotency is an amazing developmental plasticity that sets plant cells apart from most of their animal counterparts. In animals the differentiation is irreversible.

2.18.2 Tissue culture media

The plant material will only grow *in vitro* when provided with specialized media. Growth and morphogenesis of plant *in vitro* are largely governed by the composition of the culture media. Media compositions are formulated considering the requirements of a particular culture system. Culture media used for the *in vitro* cultivation of plant cells are composed of four basic components:

1. Essential elements, or mineral ions, supplied as a complex mixture of salts;
2. An organic supplement supplying vitamins and/or amino acids;
3. A source of fixed carbon; usually supplied as the sugar sucrose and
4. A gelling agent.

The principal components of most plant tissue culture media are inorganic nutrients (*macronutrients* like nitrogen, phosphorus, potassium, calcium, magnesium and sulphur and *micronutrients* like iron, manganese, zinc, boron, copper and molybdenum), *carbon source* (the most preferred carbon source in plant tissue culture is sucrose), *organic supplements* such as yeast extract, *growth regulators*, *antibiotics* such as kanamycin and a *gelling agent* (agar). The concentration of inorganic and organic constituents in the plant tissue culture media is expressed in mass values (mg/*l* or ppm).

Plant material can be cultured either in a liquid medium or on a medium that has been partially solidified with a gelling agent. The method employed will depend on the type of culture and its objective. Gelling or solidifying agents are commonly used for preparing semisolid or solid tissue culture media. Agar, a polysaccharide obtained from seaweeds, has several advantages over other gelling agents. First, agar gels do not react with media constituents. Secondly, they are not digested by plant enzymes and remain stable at all feasible incubation temperatures. Liquid media are essential for suspension cultures. They are also used in some micropropagation work. Very small organs (e.g. anthers) are often floated on the top of liquid medium and plant cells or protoplasts can be cultured in very shallow layers of static liquid, providing there is sufficient gaseous diffusion.

Growth regulators

Plant growth regulators are compounds, which, at very low concentration, are capable of modifying growth or plant morphogenesis. There are seven main classes of plant growth regulator used in plant cell culture, namely: 1. auxins; 2. cytokinins; 3. gibberellins; 4. abscisic acid; 5. ethylene; 6. polyamines and 7. jasmonic acid

Auxins: *Main effects in tissue culture system*

1. Adventitious root formation (at high concentration)
2. Adventitious shoot formation (at low concentration)
3. Induction of somatic embryos
4. Cell division

2.19 Animal cell culture

Cells in animals exist in an organized tissue matrix which require for their controlled growth and differentiation. These cells from intact organisms may be isolated, maintained and grown *in vitro* in culture media aseptically containing a suitable mixture of nutrients and growth factors. This process is called *animal cell culture.*

2.19.1 Primary and secondary cultures

A *primary cell culture* is prepared by inoculating cells directly from tissues of an organism into culture media (that is, without cell proliferation *in vitro*). With the exception of some cells derived from tumors, most primary cell cultures have a limited lifespan. After a certain number of divisions, cells undergo the process of senescence and stop dividing. In these cells, the limited proliferation capacity reflects a progressive shortening of the cell's telomeres, the repetitive DNA sequences and associated proteins that cap the ends of each chromosome.

The primary cell culture is of two types depending on the kind of cells in culture – attachment culture and suspension culture. *Attachment culture* involves the adherent or anchorage dependent cells. To survive and grow, most cells require a surface to which they can attach, thus they are anchorage dependent. Without the surface attachment, these cells cannot survive. These adherent cells are usually derived from tissues of organs such as kidney, where they are immobile and embedded in connective tissue. *Suspension culture* involves non-adherent or *anchorage independent* cells which do not require attachment for growth or do not attach to the surface of the culture vessels. Lymphocytes are anchorage independent cells commonly grown in culture.

A *secondary culture* is prepared by subculturing a primary culture. *Subculture* (or *passage*) refers to the transfer of cells from one culture vessel to another. In most cases, cells in primary cultures can be removed from the culture dish and made to proliferate to form a large number of secondary cultures.

2.19.2 Cell line

When a primary cell culture is subcultured, it becomes a *cell line*. The cell lines may be *finite cell line* or *infinite cell line*. A *finite cell line* (or normal cell line) is a line of cells that will undergo only a finite number of divisions in cell culture and eventually undergoes senescence. It has a limited number of possible subcultures or passages. Normal mammalian cells generally have a finite life span in culture; that is, after a number of divisions characteristic of the species and cell type, the cells stop dividing. These cell lines exhibit the property of contact inhibition, density limitation and anchorage dependence.

A cell line that has the potential to be subcultured indefinitely is termed *infinite* (immortal or continuous) cell line. Tumor cells or normal cells that have undergone transformation induced by chemical carcinogens or viruses can be propagated indefinitely in tissue culture; thus, have unlimited number of possible subcultures.

Infinite cell lines are also known as *transformed* cell lines due to altered growth properties of immortalized cells. Transformed cells do not necessarily mean cancer or tumor cells. Transformed cell lines do *not* exhibit the property of contact inhibition, density-dependent inhibition of proliferation and anchorage dependence. They have a reduced requirement for serum or growth factors for optimal growth. A transformed cell line often has an abnormal chromosome number (aneuploid) and overproduces different proteins. Cancer cells are naturally immortal. Thus all cancerous cell lines are transformed, although it is not clear whether all transformed cell lines are cancerous.

The first cell line—the mouse fibroblast L cell—was derived from cultured mouse subcutaneous connective tissue by exposing the cultured cells to a chemical carcinogen. Another important cell line, the HeLa cell, was derived from a 31-year-old black woman named Henrietta Lacks, who died of cervical cancer in 1951. Since these early cell lines, hundreds of cell lines have been established.

Chapter 03

Plant Physiology

3.1 Plant–water relationship

Water is essential for life. The most abundant substance of the living cell is water. It accounts for about 70% of a cell's weight. It is essential for all physiological activities of the plant. It provides the medium in which most substances remain dissolved. Water (H_2O) is made up of two hydrogen atoms and one oxygen atom, with a total atomic mass of 18 daltons. It is a *polar* molecule. Although water is electrically neutral, it has a partial positive charge on each hydrogen and a partial negative charge on oxygen.

Water acts as an *excellent solvent*. It dissolves more substances than any other liquid. This is because it has very high value of *dielectric constant*, which is a measure of the capacity to neutralize the attraction between electrical charges. Because of this property, water is an especially powerful solvent for electrolytes and polar molecules such as sugars.

Water has a *high specific heat* (the amount of energy required to raise the temperature of a unit mass of a substance by 1°C is called its specific heat). The high specific heat of liquid water is caused by the arrangement of its molecules, which allows the hydrogen and oxygen atoms to vibrate freely, almost as if they were free ions. Thus, they can absorb large quantities of energy without much temperature increase. That's why plants can resist large fluctuations in temperature.

Water has a *high heat of vaporization* (the energy necessary to go from a liquid to a gas). 586 cal are required to convert 1 g of water at 20°C to 1 g of water vapour at 20°C. Thus, evaporation from leaves cools the plant.

The extensive hydrogen bonding in water gives rise to the property known as *cohesion*. Cohesion gives water a high tensile strength which is the ability to resist stretching (tension) without breaking. Cohesion among water molecules also accounts for *surface tension*.

3.1.1 Diffusion and osmosis

Diffusion is the random movement of molecules along the concentration gradient (from an area of higher concentration to an area of lower concentration) by their own kinetic energy. It is a spontaneous and passive process. The rate of diffusion depends on several factors such as concentration difference, size of molecules and temperature. The rate of diffusion of molecules down a concentration gradient is given by the *Fick's law:*

$$J = -D\left(\frac{\Delta C}{\Delta x}\right)$$

Where J is the flux per unit area, D is the *diffusion coefficient* (usually expressed as cm^2/sec) and ΔC is the difference in concentration between two regions separated by a distance Δx. The negative sign accounts for the fact that diffusion is toward the lower concentration.

Osmosis is a specialized case of diffusion that involves the passive transport of water (i.e. solvent). In osmosis, water moves through a *semipermeable membrane* from a region of its higher concentration to a region of its lower

concentration. Semipermeable membrane selectively allows the passage of a solvent while restricting the movement of solutes. Plasma membranes of plant cells are *selectively permeable* not semipermeable membrane because they allow the movement of solvent (water) as well as solutes. The selective permeability is due to the presence of the discriminating barrier of the lipid bilayer and the specific transport proteins.

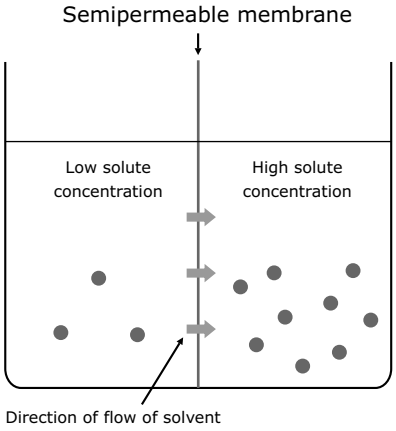


Figure 3.1 Osmosis is the diffusion of water (solvent) across a semipermeable membrane.

In osmosis, selective diffusion of solvent is driven by the internal energy of the solvent molecules. It is convenient to express the available energy per unit volume in terms of *osmotic pressure*. It is defined as the pressure required to completely stop the entry of water into an osmotically active solution across a semipermeable membrane. It is also defined as the minimum pressure needed to stop osmosis. It is measured in atmospheres or bars (1 bar equal to 100 kilopascals). The osmotic pressure is directly proportional to the difference in the concentration of the total number of solute molecules on each side of the membrane.

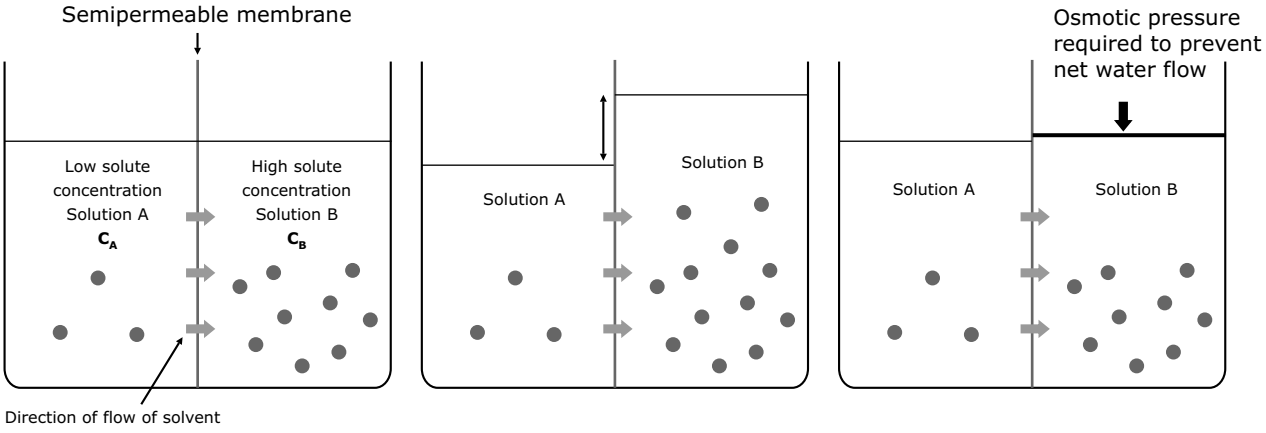


Figure 3.2 Osmotic pressure. Solutions A and B are separated by a semipermeable membrane. If the total concentration of solutes in solution B is greater than solution A, water will tend to flow across the membrane from solution A to solution B. The osmotic pressure between the solutions is the minimum pressure that would have to be applied to solution B to stop osmosis. From the van't Hoff equation, osmotic pressure is given by $\pi = RT(C_B - C_A)$, where R is the gas constant and T is the absolute temperature.

Tonicity

Tonicity is the measure of the osmotic pressure gradient of two solutions separated by a semipermeable membrane. There are three types of tonicity that one solution can have relative to another: hypertonic, hypotonic

3.3 Ascent of sap

Water and minerals from the soil enter the plant through the epidermis of roots, radially cross the root cortex, and pass into the xylem. From there the xylem sap, the water and dissolved minerals in the xylem, moves upward. It is vitally important for a plant to transport water and minerals from the soil to its uppermost leaves. The upward movement of minerals and water against gravitational force from root to aerial parts of the plant through xylem is called as *ascent of sap*.

3.3.1 Xylem anatomy

Xylem is a complex tissue consisting of living and non-living cells. The conducting cells in the xylem are typically non-living and include two types of tracheary elements – **tracheids** and **vessel elements**. Both of these cell types have thick, lignified secondary cell walls and are dead at maturity. Vessel elements are present only in angiosperm and small group of gymnosperms. In addition to tracheary elements, the xylem tissue also contains parenchyma cells (storage function) and fibers (mechanical function).

Tracheids are elongated and spindle-shaped cells. The walls of these cells are heavily lignified with openings in the walls called *pits*. Water flows between tracheids by means of numerous pits in their lateral walls. Pits are microscopic regions where the secondary wall is absent and the primary wall is thin. Pits of one tracheid are typically located opposite to the pits of an adjoining tracheid forming *pit pairs.* The porous layer between pit pairs, consisting of two primary walls and a middle lamella, is called the *pit membrane. Vessel elements* are shorter and wider than tracheids and have perforated end walls that form a *perforation plate* at each end of the cell. Like tracheids, vessel elements have pits on their lateral walls. The perforated end walls allow vessel members to be stacked end to end to form a larger conduit called a *vessel*. Because of their open end walls, vessels provide a very efficient low-resistance pathway for water movement. In rooted plants, transport of water and minerals through xylem is essentially *unidirectional*, from roots to the stems.

3.3.2 Mechanism of ascent of sap

Unlike animals, plants do not have a heart or a circulatory system to move water from the soil to the leaves. Despite this, the upward flow of water through the xylem occurs at fairly high rates. How is this movement accomplished? A longstanding question is whether water is *pushed* or *pulled* through the plant.

As we mentioned previously some roots develop root pressure, a positive hydrostatic pressure in their xylem. However, root pressure is typically less than 0.1 MPa and disappears when the transpiration rate is high, so it is clearly inadequate to move water up a tall tree. It is also not a universal phenomenon. Instead, water at the top of a tree develops a large tension (a negative hydrostatic pressure), which *pulls* water through the xylem.

Most researchers agree that water is mainly pulled through the plant, and that the driving force for this process is *transpiration* from the leaves. Transpiration generates a negative pressure in the xylem of leaves, which pulls the water upward. The most accepted theory for upward movement of water in the xylem is the **cohesion-tension theory** (also known as *transpiration pull*). This theory was proposed by *Dixon* and *Jolly*. Some physical properties of water support the formation of water column in xylem vessel and their upward movement are described below:

1. *Cohesion* : it is mutual attraction between water molecules.
2. *Adhesion* : it is attraction of molecules to the hydrophilic walls of tracheary elements.
3. *Surface tension* : water molecules are attracted to each other in the liquid phase more than to water in the gas phase.

These properties give water high *tensile strength* (i.e. an ability to resist a pulling force) and high *capillarity* (i.e. the ability to rise in a thin tube). In plants, capillarity is aided by the small diameter of the tracheary elements - the tracheids and vessel elements.

Due to the fact that transpiration 'pulls' the sap from the soil to the leaves, water in the xylem is in a state of tension. In this state, negative pressures in the xylem water may cause *cavitation* (embolisms) in the xylem.

Cavitation is the appearance of a gas bubble within the liquid phase. It is more common in wide vessels than in tracheids and can occur during drought stress or when xylem sap freezes in winter. Such cavitation can block water transport and lead to severe water deficits in the leaf. However, the impact of xylem cavitation on the plant is minimized by several means. The principal mechanism for minimizing the effect of cavitation is a structural one. The end walls of vessels and the pores prevent the bubble from spreading from tube to tube.

3.4 Transpiration

Transpiration is the loss of water from aerial parts of plants (especially leaves) in the form of water vapour. The driving force for transpiration is the difference in water vapour pressure between the leaf air spaces and the external air. Higher vapour pressure difference between leaf air spaces and the external air increase the transpiration. Transpiration may occur through the stomata, cuticle or lenticels of plants. Accordingly it is termed as stomatal, cuticular or lenticular transpiration.

1. Cuticular transpiration

   Cuticle is a layer of wax-like covering on the epidermis of leaves and herbaceous stems. It is mainly composed of wax and cutin. *Cutin* is a principal constituent of the cuticle. It is a polymer consists of long-chain hydroxy or epoxy fatty acids that are attached to each other by ester linkages. Cuticle is meant to check transpiration. However, some water may be lost through it. The loss of water in the form of water vapour through the cuticle is known as cuticular transpiration, which accounts for 5 to 10% of the total transpiration by plants.

2. Lenticular transpiration

   A *lenticel* is an opening in the bark of stems and roots that allows gases to be exchanged between atmosphere and the inner living cells of a plant. Loss of water in the form of water vapour through the lenticels is called lenticular transpiration. It accounts for only 1-5% of the total water loss by the plant.

3. Stomatal transpiration

   Stomata (sing. stoma) are specialized epidermal structures that are responsible for modulating the exchange of gases between the plant and the environment. They act as a *turgor-operated valves*. The primary function of stomata is to allow gas exchange between the plant's internal tissues and the atmosphere. Loss of water in the form of water vapour through the stomata is called stomatal transpiration. It accounts for ~90% of the total transpiration by the plant.

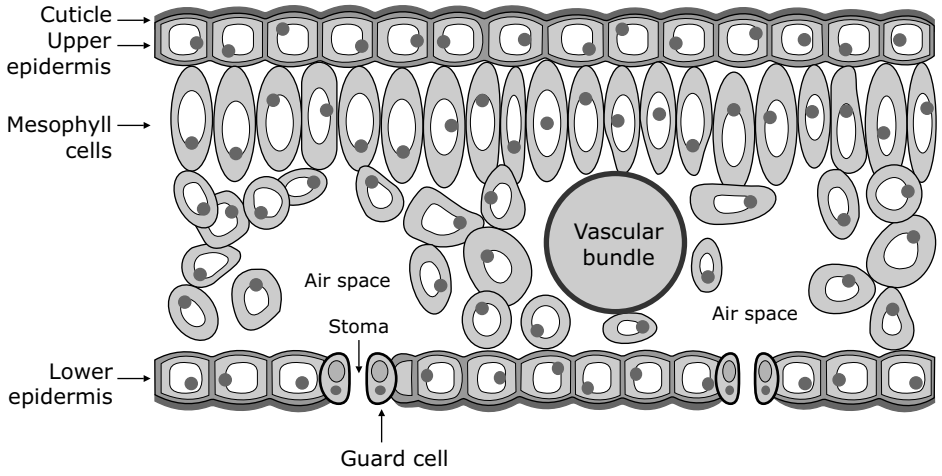


Figure 3.6 Diagrammatic representation of a typical dorsiventral leaf shown in cross-section.

The xylem tracheary elements are dead cells. To enter the tracheary elements, the ions must exit the symplast by crossing a plasma membrane. The process where ions exit the symplast and enter the conducting cells of xylem is called *xylem loading.*

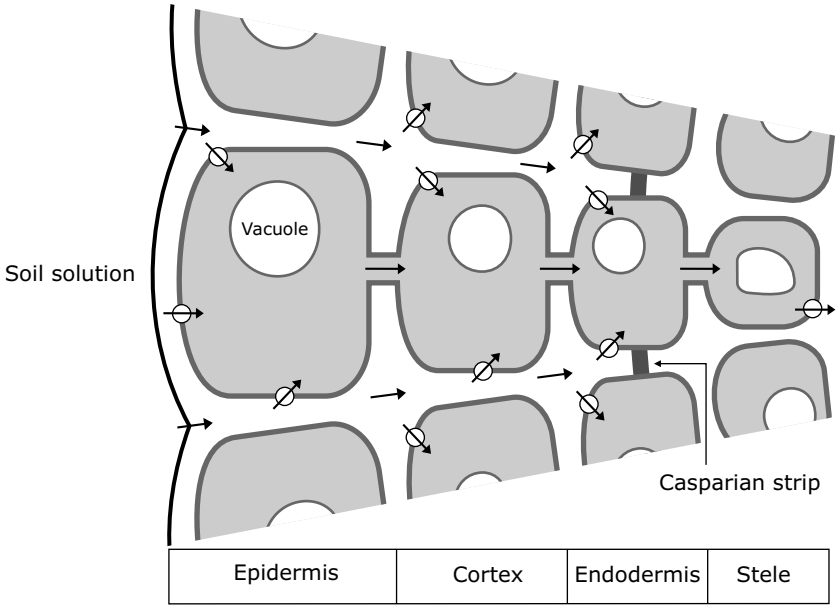


Figure 3.9 The radial path of ion movement through a root. Arrows indicate the alternate paths that may be taken by nutrient ions as they move from the soil solution into the vascular elements in the stele. Arrows with circles indicate carrier/channel mediated transport of ions across plasma membranes.

3.6 Mineral nutrition

Plants are autotrophs. Unlike heterotrophic organisms, plants depend on inorganic nutrients for synthesis of complex organic compounds. Plants derrive these nutrients from air, water and soil. Inorganic nutrients acquired in the form of inorganic ions from soil are called *mineral nutrients.* The study of the uptake of mineral nutrients and their use by plants is called *mineral nutrition*. Most plants require a relatively small number of nutrient elements in order to complete their growth and life cycle. However, more than sixty elements are reported in different angiospermic plants but only seventeen elements are considered as essential (till now investigated) for proper growth and development. An *essential element* is defined as one whose absence prevents a plant from completing its life cycle or one that has a specific physiological role.

Criteria for essentiality

Any element which is present in a plant cannot be considered as an essential element. For example, some plant species accumulate elements such as selenium, gold and strontium but they are not essential. The criteria for essentiality of elements was proposed by Arnon and Stout (1939). According to them, three criteria must be met for an element to be considered essential. These criteria are:

1. A plant must be unable to complete its life cycle in the absence of the essential element.
2. The function of the essential element must *not* be replaceable by another element i.e. deficiency of any one element cannot be met by supplying some other element.
3. The essential element must be directly involved in plant metabolism.

3.7.1 Allocation and partitioning of photoassimilates

Allocation refers to fate of fixed carbon either newly assimilated in the source or delivered to a sink. Allocation of carbon in source includes the storage, utilization and export to other parts of the plant. In sinks, transported sugars are allocated to growth processes or to storage.

Partitioning is the differential distribution of photoassimilates within the plant. Partitioning mechanisms determine the quantities of fixed carbon delivered to different sink tissues. In general, sinks are competitive and photoassimilate is partitioned to all active sinks. If the number of sinks is less, a higher proportion of the photoassimilate is directed to each of the sinks. Partitioning of assimilate between competing sinks depends primarily on three factors: the nature of vascular connections between source and sinks, the proximity of the sink to the source and sink strength. The sink strength is a measure of the capacity of a sink to accumulate metabolites. It is given as the product of sink size and sink activity. Sink size is the total mass of the sink and sink activity is the rate of uptake of photoassimilates per unit weight of sink tissue.

3.8 Plant hormones

Plant growth and development involves the integration of many environmental and endogenous signals that, together with the intrinsic genetic program, determine plant form. Fundamental to this process are several growth regulators collectively called the *plant hormones* or *phytohormones*. According to a standard definition, plant hormones are small organic compounds, synthesized by specific plant cells/tissues, active in low concentration and promote or inhibit growth and developmental processes. The definition of a hormone used in animal physiology does not apply to plant hormones. Although like animal hormones, plant hormones are naturally occurring organic substances that profoundly influence physiological processes at low concentration. The site of synthesis and mode of transport for plant hormones, however, is not always so clearly localized. Although some tissues may be characterized by higher hormones level than others, synthesis of plant hormones appears to be much more diffuse and not always localized to discrete organ or tissue. Plant hormones are able to exert their action locally or at a distance (e.g., some are transported from one organ to another organ to produce their physiological effect, and some others bring about changes in the same tissue, or within the same cell where they are synthesized). These characteristics have led to consider that transport is not an essential property of a plant hormone.

Growth in plants is defined as *an irreversible increase in size or volume*. This is mainly driven by turgor pressure. During this process, cells increase in volume and become highly vacuolate. Growth also can be measured in terms of change in living biomass over a particular period of time. However, the living biomass of plants fluctuates in response to changes in water status, so this criterion may be a poor indicator of actual growth. In these situations, measurements of dry weight are often more appropriate. The cell number is commonly used to measure the growth of unicellular organisms such as the green alga *Chlamydomonas.* In multicellular plants, however, cell number can be a misleading growth measurement because cells can divide without increasing in volume.

Types of plant hormones

The concept of plant hormones originates from a classical experiment on phototropism, the bending of plants toward light, carried out by Charles Darwin and his son Francis in 1880. The Darwins were able to demonstrate that when oat seedlings were exposed to a lateral light source, a transported signal originating from the plant shoot apex promoted differential cell elongation in the lower parts of the seedling that resulted in it bending toward the light source. This signal was subsequently shown to be IAA, the first known plant hormone. A small number of plant hormones have been shown to influence the plant growth and development. Tremendous progress has been made in the identification of plant hormones, discovery of their effects on plants and elucidation of their chemical structures. For some of the plant hormones considerable knowledge about their biosynthetic pathways has been obtained.

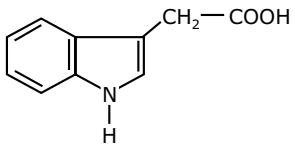
Based on function or chemical structure, there are five major groups of plant hormones. These groups are: auxins, gibberellins, cytokinins, abscisic acid and ethylene. In addition, there is a variety of other plant hormones including the brassinosteroids, polyamines, jasmonic acid, salicylic acid and others.

3.8.1 Auxin

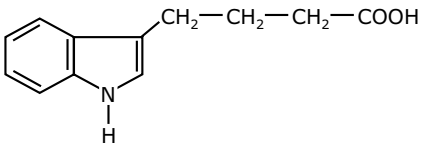
Auxin, the first plant hormone was discovered by Frits Went as a growth promoting chemical in the tip of oat (*Avena sativa*) coleoptiles. Because the chemical isolated by Went promoted the elongation of the coleoptile, it was eventually named auxin (derived from the Greek word *auxein*, meaning *to increase*).

Biosynthesis and transport

Various naturally occurring auxins are known, namely IAA (indole 3-acetic acid), IBA (indole 3-butyric acid) and PAA (phenyl acetic acid). Indole-3-acetic acid is the principal naturally occurring auxin in higher plants. There are more than 200 auxin analogs with different chemical structures known to have the common auxin activity. The only common features shared by these compounds are an unsaturated planar aromatic ring structure and a carboxyl side chain.



Indole-3-acetic acid
(IAA)



Indole-3-butyric acid
(IBA)

Major (primary) sites for IAA synthesis are the shoot apical meristem, young leaves and developing fruits and seeds. Intracellularly IAA is found in the chloroplast as well as cytosol. In plants, IAA occurs in both conjugated and free forms. It has been found to be conjugated to both high (such as glycoproteins) and low-molecular-weight compounds (such as glucose). IAA conjugates are located exclusively in the cytosol. There are multiple pathways for the biosynthesis of IAA. However, two major routes for the production of IAA can be:

Tryptophan-dependent pathways

The similarity of chemical structure of IAA and tryptophan suggested a connection between these. Considerable research has shown that tryptophan, one of the protein amino acids, is a precursor of auxin biosynthesis. The *indole-3-pyruvic acid pathway* is the most common tryptophan dependent pathway. Overall, the conversion of tryptophan to IAA involves:

1. Deamination of tryptophan, (catalyzed by trp transaminase).
2. Decarboxylation of indole-3-pyruvic acid (catalyzed by indole-3-pyruvic acid decarboxylase).
3. Oxidation of indole-3-acetaldehyde (catalyzed by indole-3-acetaldehyde dehydrogenase).

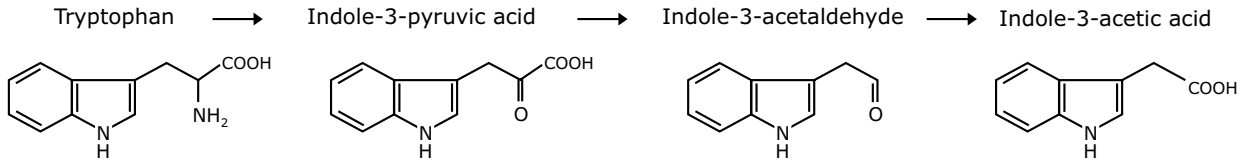


Figure 3.16 Tryptophan-dependent pathways of IAA biosynthesis.

Tryptophan-independent pathway

In addition to the tryptophan-dependent pathways, recent genetic studies have provided evidence that plants can synthesize IAA via one or more tryptophan-independent pathways. This route doesn't involve tryptophan directly as a precursor to the formation of auxin. The precise pathway for tryptophan-independent IAA synthesis is not known.

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around the cell plasma membrane. A $2H^+$–IAA^- symporter-mediated secondary active uptake mechanism is responsible for auxin accumulation. Molecular genetic studies in *Arabidopsis thaliana* have identified a permease type IAA influx carrier termed AUX1.

Auxin efflux is mediated by auxin efflux carriers PIN-FORMED (PIN) proteins. The PIN proteins (named after the pin-shaped inflorescences formed by the *pin1* mutant of *Arabidopsis*) are a plant-specific family of transmembrane proteins that transport the auxin. They are asymmetrically localized on the plasma membrane of cells and their asymmetrical distribution determines the directionality of intercellular auxin flow. Until now, eight members of the PIN protein family have been isolated in *Arabidopsis* and are commonly referred to as PIN1 to PIN8. The PIN1, PIN2, PIN3, PIN4 and PIN7 proteins are localized at the plasma membrane, where they act as auxin efflux carriers.

Auxin is also transported non-polarly in phloem. Most of auxins that are synthesized in mature leaves appears to be transported to the rest of plant non-polarly. Non-polar transport of auxin in the phloem is passive in nature. It is rapid, long-distance source-to-sink transport of auxins.

In root, on the other hand, there appear to be two transport streams. An acropetal stream, arriving from the shoot, flows through xylem parenchyma cells in the central cylinder of the root and directs auxin toward the root tip. A basipetal stream then reverses the direction of flow, moving auxin away from the root tip, or basipetally, through the outer epidermal and cortical cell files.

Physiological effects

- Cell elongation : Auxins stimulate cell elongation/expansion in stems and coleoptiles. According to the *acid growth hypothesis*, auxin promotes elongation growth by increasing cell wall extensibility. Auxin stimulates proton pumping by activating (directly or indirectly) proton pump, H^+-ATPase, present on the plasma membrane. As a result, the pH of the cell wall falls as low as 4.5. The low pH activates a class of cell wall protein, termed *expansins,* that disrupts the hydrogen bonding between cellulose microfibrils, causing the laminate structure of the cell wall to loosen. With the rigidity of the wall reduced, the cell can elongate. The expansin-triggered loosening of the wall is reversed when the pH is raised back to normal, showing that expansin does not break covalent bonds in cellulose. Like auxin, a fungal compound *fusicoccin* induces rapid cell elongation and triggers proton pumping out of sensitive cells, with accompanying wall loosening. The action of fusicoccin or auxin can be blocked by permeating the cell wall with buffers that prevent the extracellular pH from being lowered.
- Cell differentiation : The differentiation of relative amounts of xylem and phloem are regulated by auxin concentration. In general, high auxin concentrations induce the differentiation of xylem and low auxin concentrations induce phloem differentiation.
- Rooting : Auxins inhibit elongation of the primary root, but stimulates root initiation on stem cuttings (*adventitious roots*) and lateral root development. Lateral roots are commonly found above the root hair zone and originate from cells present in the pericycle. Auxin stimulates these pericycle cells to divide. The dividing cells gradually give rise to the lateral root.
- Apical dominance : In most plants, the growing apical bud inhibits the growth of lateral buds—a phenomenon called *apical dominance*. Removal of the shoot apex usually results in the growth of lateral buds. In the presence of apical bud, the outgrowth of the lateral bud is inhibited by auxin that is transported basipetally from the apical bud.
- Fruit set : *Fruit set* is defined as the transition of a quiescent ovary to a rapidly growing young fruit. Auxin promotes fruit development. After fertilization, fruit growth may depend on auxin produced in developing seeds. Auxin is produced in the endosperm and the embryo of developing seeds. In some plant species, seedless fruits may be induced by treatment of the unpollinated flowers with auxin. The production of such seedless fruits is called *parthenocarpy*.
- Tropic response : Auxin induces differential growth in plant organs under the influence of directional stimuli (i.e. light, gravity). This is termed as tropic response. According to the Cholodny–Went theory, which states that tropic stimuli induce the lateral redistribution of auxin, resulting in unequal accumulation between opposing regions of a responding organ and thus differential growth.

3.8.2 Gibberellins

Gibberellins are a group of *tetracyclic diterpenoid* compounds made up of four isoprene units with the *ent*-gibberellane ring skeleton that function as plant growth regulators influencing a range of developmental processes in higher plants including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction and leaf and fruit senescence. Gibberellins were discovered by Kurosawa in Japan in the course of his studies on fungal diseases of rice. In particular, he was investigating the mechanism by which the fungal pathogen *Gibberella fujikuroi* led to foolish seedling (bakanae) disease in rice plants infected with this pathogen. Kurosawa found that the abnormal growth promoted by the fungi was due to a compound produced by *G. fujikuroi*. This compound was first isolated in 1935 by Yabuta and Sumuki, from fungal strains, *Gibberella fujikuroi*. Yabuta named the isolate as gibberellin. All gibberellins are derived from the *ent*-gibberellane skeleton. Unlike the classification of auxins which are classified on the basis of function, gibberellins are classified on the basis of structure as well as function. There are currently 136 gibberellins identified from plants, fungi and bacteria. The gibberellins are named $GA_1...GA_n$ in order of discovery. They all have either 19 carbon atoms (C_{19}-GAs) or 20 carbon atoms (C_{20}-GAs). C_{19}-GAs are more biologically active than C_{20}-GAs. There are certain structural requirements for gibberellin activity. A carboxyl group at carbon-7 is a feature of all biologically active gibberellins.

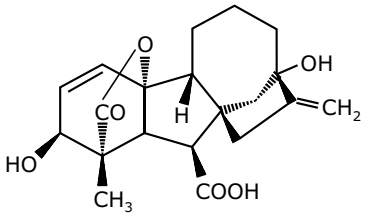

Figure 3.18 Structure of gibberellin GA1.

Biosynthesis and transport

Gibberellins are synthesized primarily in the apical tissues and young leaves. It is uncertain whether root tissues also produce gibberellins. The highest levels of gibberellins are found in immature seeds and developing fruits. Gibberellins are synthesized by the condensation of four isoprenoids subunits. The basic biological isoprene unit is isopentenyl pyrophosphate (IPP). IPPs condense to produce 20 carbons geranylgeranyl pyrophosphate (GGPP). GGPP acts as a biosynthetic precursor for gibberellins. Gibberellins biosynthetic pathway can be divided into three stages, each completes in a different cellular compartment.

Stage 1 : Geranylgeranyl pyrophosphate (a 20-carbon linear molecule) is converted to *ent*–kaurene *in plastids*.

Stage 2 : Kaurene is transported from the plastid to the endoplasmic reticulum (ER). In the ER, *ent*-kaurene is oxidized to GA_{12}, the first gibberellin in the biosynthetic pathway in all plants and the precursor of all other gibberellins. The hydroxylation of carbon–13 of GA_{12} gives GA_{53}.

Stage 3 : *In the cytosol*, G_{12} or G_{53}, each of which has 20 carbon atoms is converted to other gibberellins. G_{19}-GAs arise by oxidative elimination of carbon-20 in the form of CO_2. Enzyme GA 20-oxidase catalyzes the oxidation steps including the removal of carbon-20 as CO_2.

Certain commercial chemicals block the synthesis of gibberellins. Some of these chemicals are Phosphon D, AMO-1618, Cycocel (CCC), ancymidol, and paclobutrazol. These chemicals inhibit the first stage of gibberellin biosynthesis.

Gibberellins are translocated *via* the phloem and xylem. The gibberellins that are synthesized in the shoot are transported to the rest of the plant via the phloem. The presence of gibberellins in root exudates and root extracts supports the synthesis of gibberellins in roots. The gibberellins that are synthesized in the root are transported to the shoot via the xylem. The movements of gibberellins in plants do not exhibit polarity like auxins. Gibberellins are capable of moving both up and down the stem.

pathway emanating from it. In its unbound, active state, the receptor activates Raf-like serine/threonine kinase, CTR1 (Constitutive Triple Response1). The function of CTR1 in ethylene signaling depends on its serine/threonine kinase activity and the association of its N-terminal domain with the ethylene receptor. By an unknown signaling mechanism, active CTR1 stimulates the ubiquitylation and degradation of a nuclear gene regulatory protein called EIN3, which is required for the transcription of ethylene-responsive genes. The EIN protein gets its name from the finding that plants with inactivating mutations in the gene that encodes it are ethylene insensitive. Ethylene binding inactivates the receptors, altering their conformation so that they no longer bind to CTR1. As a result, CTR1 is inactivated, and the downstream signaling pathway emanating from it is blocked; the EIN3 protein is no longer ubiquitylated and degraded and can now activate the transcription of the large number of ethylene-responsive genes.

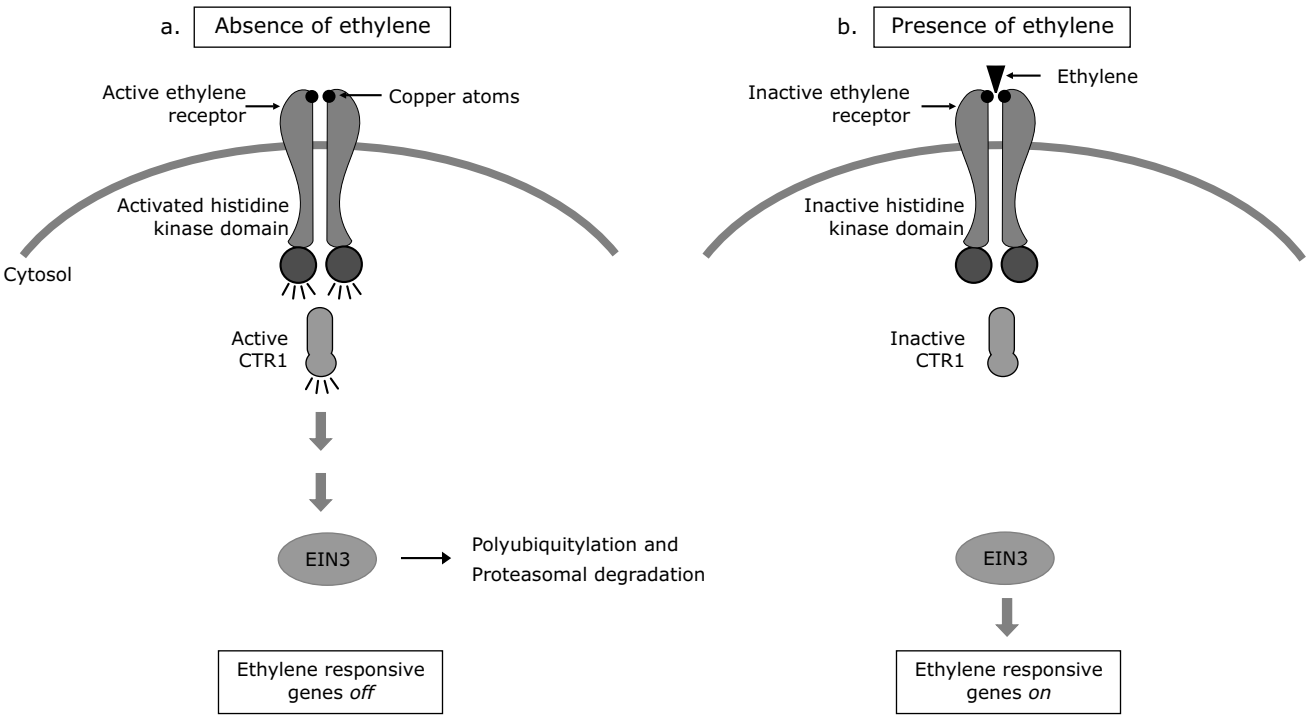

Figure 3.24 A current view of the ethylene signaling pathway. (a) In the absence of ethylene, both the receptors and CTR1 are active, causing the ubiquitylation and destruction of the EIN3 protein, the gene regulatory protein in the nucleus that is responsible for the transcription of ethylene-responsive genes. (b) The binding of ethylene inactivates the receptors and disrupts the interaction between the receptors and CTR1. The EIN3 protein is not degraded and can therefore activate the transcription of ethylene responsive genes.

3.9 Photomorphogenesis

Light has profound effects on the growth and development of plants. Light is vital for photosynthesis, but is also necessary for plant growth and development. It can be observed in seedlings grown in the dark. Seedlings grown in the dark have a pale, unusually tall and spindly appearance known as etiolated growth. The light-mediated changes in plant growth and development, independent of photosynthesis, are called *photomorphogenesis* (from the Latin word meaning *light from begins*). Light acts as a signal to initiate and regulate photomorphogenesis. There are two light-sensing systems involved in these responses, the *blue light sensitive system* (cryptochrome, phototropin) and the *red light sensitive system* (phytochrome).

Pre-chilling: Low temperature can release fully hydrated seeds from dormancy. Some seeds can be induced to germinate by a period of pre-chilling (*stratification*). It requires a period of low temperature (0–10°C) in order to germinate.

Seed germination

By definition, *seed germination* incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis. It is the resumption of growth of the embryo of the mature seed. Seed germination depends on both internal and external conditions. The most important external factors include temperature, water, oxygen and sometimes light or darkness. Germination of many seeds is influenced by light. Seeds that are stimulated to germinate by light are described as *positively photoblastic*; seeds whose germination is inhibited by light are said to be *negatively photoblastic*. The germination of positively photoblastic seeds (such as lettuce, *Lactuca sativa,* seed) is regulated by phytochrome. Red light irradiation induces the germination of lettuce seeds, and far-red irradiation given after red light cancels the effect of red light. Phytochrome regulates lettuce seed germination via the control of the endogenous level of gibberellin.

Based on the fate of the cotyledons, two kinds of seed germination occur – *epigeal* and *hypogeal germination.*

Epigeal germination is characteristic of bean and pine seeds and is considered evolutionarily more primitive than hypogeal germination. During germination, the cotyledons are *raised above the ground* where they continue to provide nutritive support to the growing points.

Hypogeal germination is characteristic of pea seeds and all grasses. During germination, the cotyledons or comparable storage organs *remain beneath the soil* while the plumule pushes upward and emerges above the ground. In hypogeal germination, the epicotyl is the rapidly elongating structure.

3.13 Plant development

Angiosperms (flowering plants) are the most advanced terrestrial plants. The development of a flowering plant, like that of an animal, begins with the division of a fertilized egg to form an embryo with a polarized organization: the apical part of the embryo will form the shoot, the basal part, the root, and the middle part, the stem.

Sexual life cycle of angiosperms

In flowering plants, the reproductive organs are in the flower. Meiosis and fertilization are two essential processes in the sexual cycle of flowering plants. Sexual reproduction consists of two generations — sporophytic and gametophytic. Gametophyte is characterized by nutritionally independent extremely short haploid phase. The sporophytic generation begins when an egg nucleus unites with a sperm nucleus, producing an embryo, and the second sperm nucleus fuses with the polar nuclei (secondary nucleus) producing triploid endosperm and continues with the development of seed, seedling, mature plant and flowers. Sporophytic tissues contain a diploid chromosome number. The flower contains spore-forming organs called anthers and ovaries. Anthers and the ovaries produce haploid (*n*) microspores and megaspores, respectively. Thus, alternation of sporophytic and gametophytic generations occurs during the life cycle of flowering plants and this form of life cycle is termed as *haplodiplontic* life cycle.

Sporophyte generation

Sporophyte bears spore-producing organs and produces spores by the process of *sporogenesis* (micro-and megasporogenesis).

Microsporogenesis is the formation of the *microspores*. The microspores give rise to the male gametophyte. The anther of the microsporophyll or stamen bears the microsporangia or pollen sacs, the function of which is to produce the microspores or pollen grains.

Anther is the fertile portion of the stamen. A typical anther is tetrasporangiate type. Each anther lobe has two microsporangia (pollen sac). In each lobe, some hypodermal cells become more prominent and constitute the *archesporium*. The archesporial cells divide in a plane parallel to the outer wall of the anther lobe (periclinal division)

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Gametophyte generation

Gametophyte body forms gametes. The *male gametophyte* forms male gametes. Before the pollen is shed, a mitotic division into vegetative *tube cell* and *generative* cell gives rise to the two-celled gametophyte. The tube cell guides the pollen germination and the growth of the pollen tube after the pollen lands on the stigma of carpel. The generative cell may immediately divide mitotically into two *sperms*, or male gametes, or the second mitotic division may occur after the pollen grain germinates. Among the angiosperms the pollen is shed in a two-celled state as well as in a three-celled state. The mitotic division resulting in the formation of the generative nucleus is followed by cytokinesis resembling that in somatic cells.

Megaspores give rise to the **embryo sac**. Embryo sac is also known as *female gametophyte*. There are numerous variations in angiosperm embryo sac formation. The most common type of embryo sac formation is polygonum type. The *Polygonum* type of embryo sac is called *monosporic* because it is derived from one of four megaspores resulting from meiosis. In monosporic condition, the nuclei of megaspore undergo three successive mitotic divisions and gives an eight-nucleated embryo sac. The eight-nucleate cell is organized into the seven-celled embryo sac. The three cells at the micropylar end constitute the *egg apparatus* which is composed of the *egg* and two *synergids*. At the opposite end of the embryo sac are three *antipodal cells*. Between the two groups of cells is the large *central cell* containing the two *polar nuclei* derived one from each of the two groups of four nuclei.

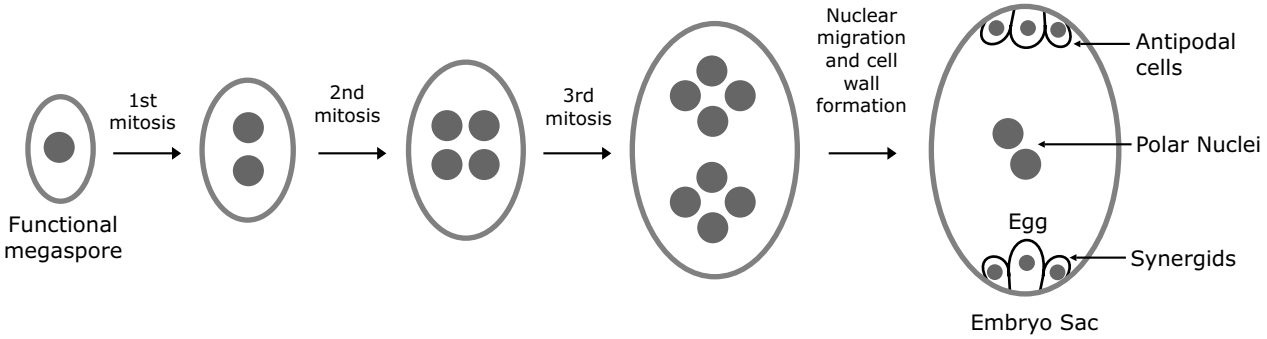

Figure 3.32 Development of embryo sac from megaspore (nucellus and integuments not shown).

The two polar nuclei eventually come together and fuse to form a diploid **secondary nucleus**. Thus, the mature embryo sac is a *seven-celled* structure. All the cells of the embryo sac are haploid, except the central cell which is diploid (due to the fusion of two polar nuclei).

Role of synergids, antipodal cells and egg

Synergids direct the pollen tube growth by secreting some chemotropically active substances. Pollen tube discharges the content at one of the synergids. It also acts as potential egg cell in case the egg is non-functional. *Antipodal cells* are haustorial in function. They do not take part in fertilization. After fertilization, antipodal cells degenerate. The *egg* takes part is fertilization. In fertilization one male gamete fuses with the egg nucleus to form a zygote. Zygote divides and redivides to form a multicellular structure called an *embryo*.

Fertilization

The pollen normally germinates on the stigma but most of the information on pollen tube growth is derived from studies of pollen germinated in cultures. In most of the plants, a pollen produces only one tube (**monosiphonous**) but in the members of Malvaceae, Cucurbitaceae etc., several pollen tubes come from a single pollen grain (**polysiphonous**). The cytoplasm of the growing pollen tube commonly accumulates at the tip, with some differentiation between the apical and subapical regions. The vegetative nucleus, the sperms (or the generative nucleus), the cytoplasmic organelles and small vesicles, all transferred from the pollen grain, occur in the subapical region. The apical end is devoid of organelles, but contains numerous vesicles.

3.14 Plant secondary metabolites

Plants are capable of synthesizing diverse types of organic molecules, which may be divided into two major groups: primary and secondary metabolites. *Primary metabolites* are metabolic intermediates or products found in all living systems, essential to growth and life, and biosynthesized by a limited number of biochemical pathways. These metabolites are involved in a primary metabolic process such as respiration and photosynthesis. Secondary metabolites are metabolic intermediates or products which are not essential to growth and life of the producing plants but rather required for the interaction of plants with their environment and produced in response to stress. Plant secondary metabolites can be divided into four major classes: terpenes, phenolics, glycosides and alkaloids.

3.14.1 Terpenes

Terpenes constitute a large class of natural products built up from isoprene units. There is a difference in terpenes and terpenoids. Terpenes are technically only hydrocarbons, while terpenoids are oxygenated hydrocarbons. The basic molecular formulae of terpenes are multiples of $(C_5H_8)_n$ where 'n' is the number of linked isoprene units (*isoprene rule*). Thus, terpenes are also termed as isoprenoid compounds. One isoprene unit is termed as hemiterpene, C_5H_8.

Isoprene Isoprene unit

Classification of terpenes:

The classification of terpenes is based on the number of isoprene units present in their structure.

Number of isoprene units	*Name*	*Carbon atoms*
2 unit	Monoterpenes	C_{10}
3 unit	Sesquiterpenes	C_{15}
4 unit	Diterpenes	C_{20}
6 unit	Triterpene	C_{30}
8 unit	Tetraterpene	C_{40}
More than 8	Polyterpenes	

Biosynthesis

There are two biosynthetic pathways for terpenes– MVA (mevalonic acid) pathway and MEP (methylerythritol phosphate) pathway. In a MVA pathway, acetyl-coenzyme A acts as precursor. Three molecules of acetyl-CoA are joined together to form mevalonic acid. This key six-carbon molecule is then pyrophosphorylated, decarboxylated and dehydrated to yield isopentenyl pyrophosphate (IPP). IPP isomerizes to *dimethylallyl pyrophosphate* (DMAPP). DMAPP acts as the prenyl donor to a molecule of IPP producing *geranylpyrophosphate* (GPP) by a head-to-tail condensation reaction. GPP can then link to another molecule of IPP to give the 15-carbon compound farnesyl pyrophosphate (FPP), the precursor of nearly all the sesquiterpenes.

The addition of yet another molecule of IPP gives the 20-carbon compound geranylgeranyl pyrophosphate (GGPP), the precursor of the diterpenes. FPP and GGPP can also dimerize in a head-to-head fashion to form the precursors of the C30 and the C40 terpenes respectively. The C10–C20 pyrophosphates undergo a wide range of cyclizations and rearrangements to produce the parent carbon skeletons of each terpene class. Finally as a result of variety of oxidations, reductions, isomerizations, conjugations and other transformations, the parent skeletons of each terpene class are converted to thousands of distinct terpene metabolites.

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Diterpenes	Anti-cancer drug	Taxol
	Phytoalexin	Casbene
	Phytohormone	Gibberellin
	Antioxidant	Dihydroleucodine
Triterpenes	Membrane component	Sitosterol
	Therapeutic use	Cardenolides
Tetraterpenes	Plant pigment	β-carotene
Polyterpenes	Photosynthesis	Chlorophyll
	Electron transport	Plastoquinone
	Industrial raw material	Rubber

3.14.2 Phenolics

A *phenolic* is a chemical compound, which is characterized by the presence of aromatic ring structure bearing one or more hydroxyl groups. Phenolics are the most abundant secondary metabolites of plants (more than 8000 phenolic structures are known) ranging from simple molecules such as phenolic acid to highly polymerized substances such as tannins.

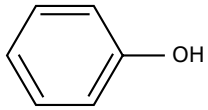

Figure 3.39 Structure of phenol.

All phenolic compounds exhibit absorption in the UV region of the electromagnetic spectrum and those that are coloured absorb strongly in the visible region as well. Each class of phenolic compounds has distinctive absorption characteristics. Plant phenolics are chemically heterogeneous compounds. Due to chemical diversity, phenolics play a variety of roles in the plant. Plant phenolics are generally involved in defense against ultraviolet radiation and serve as defense compounds against herbivores and pathogens and provide mechanical support. They are ubiquitous in all plant organs and are, therefore, an integral part of the human diet. Phenolics are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, *etc.*) and beverages (tea, coffee, beer, wine, *etc.*). Phenolics also help in attracting pollinators and fruit dispersers, or in reducing the growth of nearby competing plants. Several classes of phenolics have been categorized on the basis of their basic skeleton:

Table 3.6 Important classes of phenolic compounds in plants

No. of C-atoms	*Basic skeleton*	*Class*
6	C_6	Simple phenols, benzoquinones
7	$C_6 - C_1$	Phenolic acids
8	$C_6 - C_2$	Acetophenone, phenylacetic acid
9	$C_6 - C_3$	Phenylpropanoids, hydroxycinnamic acid, coumarins
10	$C_6 - C_4$	Naphthoquinone
13	$C_6 - C_1 - C_6$	Xanthone
14	$C_6 - C_2 - C_6$	Stilbene, anthraquinone
15	$C_6 - C_3 - C_6$	Flavonoids, isoflavonoids
18	$(C_6 - C_3)_2$	Lignans, neolignans
30	$(C_6 - C_3 - C_6)_2$	Biflavonoids

Chapter 04

Human Physiology

Like all multicellular animals, human body is composed of different cell types. Groups of cells similar in structure and function are organized into *tissues*. Different tissues grouped together into a structural and functional unit called *organs*. An *organ system* is a group of organs that function together to carry out the principal activities of the body.

4.1 Tissue level organization

A *tissue* is a group of similar cells that usually have a common embryonic origin and functions together to carry out specialized activities.

4.1.1 Type of tissues

Body tissues can be classified into four basic types according to function and structure:

1. *Epithelial tissue* consists of cells that form membranes, which cover and line the body surfaces and of glands, which are derived from these membranes.
2. *Connective tissue* protects and supports the body and its organs. Various types of connective tissues bind organs together, store energy reserves as fat, and provide immunity to disease-causing organisms.
3. *Nervous tissue* detects changes in a variety of conditions inside and outside the body and responds by generating action potentials (nerve impulses) that help maintain homeostasis.
4. *Muscular tissue* generates the physical force needed to make body structures move.

1. Epithelial tissue

An *epithelial tissue* consists of cells arranged in continuous sheets, in either single or multiple layers. Because the cells are closely packed and are held tightly together by many cell junctions, there is little intercellular space between adjacent plasma membranes. Epithelial tissue may be divided into two types:

A. *Covering and lining epithelium* forms the outer covering of the skin and some internal organs. It also forms the inner lining of blood vessels, ducts and body cavities, and the interior of the respiratory, digestive, urinary and reproductive systems.

B. *Glandular epithelium* makes up the secreting portion of glands such as the thyroid gland, adrenal glands and sweat glands.

A. Covering and lining epithelium

The covering and lining epithelial tissue is further classified according to the two characteristics like the arrangement of cells into layers and the shapes of the cells.

According to the arrangement of cells into layers

Simple epithelium is a single layer of cells that functions in diffusion, osmosis, filtration, secretion and absorption. It may be:

a. *Simple squamous epithelium*

Description: Single layer of flat cells; centrally located nucleus.

Location: Lines heart, blood vessels, lymphatic vessels, air sacs of lungs, glomerular (Bowman's) capsule of kidneys and inner surface of the tympanic membrane (eardrum); forms epithelial layer of serous membranes, such as the peritoneum.

Function: Filtration, diffusion, osmosis and secretion in serous membranes.

b. *Simple cuboidal epithelium*

Description: Single layer of cube-shaped cells; centrally located nucleus.

Location: Covers the surface of ovary, lines anterior surface of the capsule of the lens of the eye, forms the pigmented epithelium at the posterior surface of the eye, lines kidney tubules and smaller ducts of many glands, and makes up the secreting portion of some glands such as the thyroid gland and the ducts of some glands such as the pancreas.

Function: Secretion and absorption.

c. *Simple columnar epithelium* (nonciliated and ciliated)

Nonciliated

Description: Single layer of nonciliated column-like cells with nuclei near base of cells; contains goblet cells and cells with microvilli in some locations.

Location: Lines the gastrointestinal tract (from the stomach to the anus), ducts of many glands and gallbladder.

Function: Secretion and absorption.

Ciliated

Description: Single layer of ciliated column-like cells with nuclei near base; contains goblet cells in some locations.

Location: Lines a few portions of upper respiratory tract, uterine (fallopian) tubes, uterus, some paranasal sinuses, central canal of spinal cord and ventricles of the brain.

Function: Moves mucus and other substances by ciliary action.

d. *Pseudostratified columnar epithelium* (nonciliated and ciliated)

Description: Not a true stratified tissue; nuclei of the cells are at different levels; all cells attached to the basement membrane, but not all reach the apical surface.

Location: Pseudostratified ciliated columnar epithelium lines the airways of upper respiratory tract.

Function: Secretion and movement of mucus by ciliary action.

Stratified epithelium consists of two or more layers of cells that protect underlying tissues in locations where is considerable wear and tear. It may be:

a. *Stratified squamous epithelium*

Description: Several layers of cells; cuboidal to columnar shape in deep layers; squamous cells form the apical layer and several layers deep to it; cells from the basal layer replace surface cells as they are lost.

Location: Keratinized variety forms superficial layer of skin; nonkeratinized variety lines wet surface, such as the lining of the mouth, esophagus, part of epiglottis, part of pharynx and vagina, and covers the tongue.

Function: Protection.

4.2.9 Peripheral nervous system

The CNS communicates with the body by means of nerves that exit from the brain (cranial nerves) and spinal cord (spinal nerves). These nerves, together with aggregations of cell bodies located outside the CNS, constitute the peripheral nervous system (PNS). The PNS composed of the afferent and efferent fibers. The *efferent division* of the PNS is the communication link by which the CNS controls the effector organs. Cardiac muscle, smooth muscle, most exocrine glands, some endocrine glands, and adipose tissue are innervated by the **autonomic nervous system**, the involuntary branch of the peripheral efferent division. Skeletal muscle is innervated by the **somatic nervous system**, the branch of the efferent division subject to voluntary control.

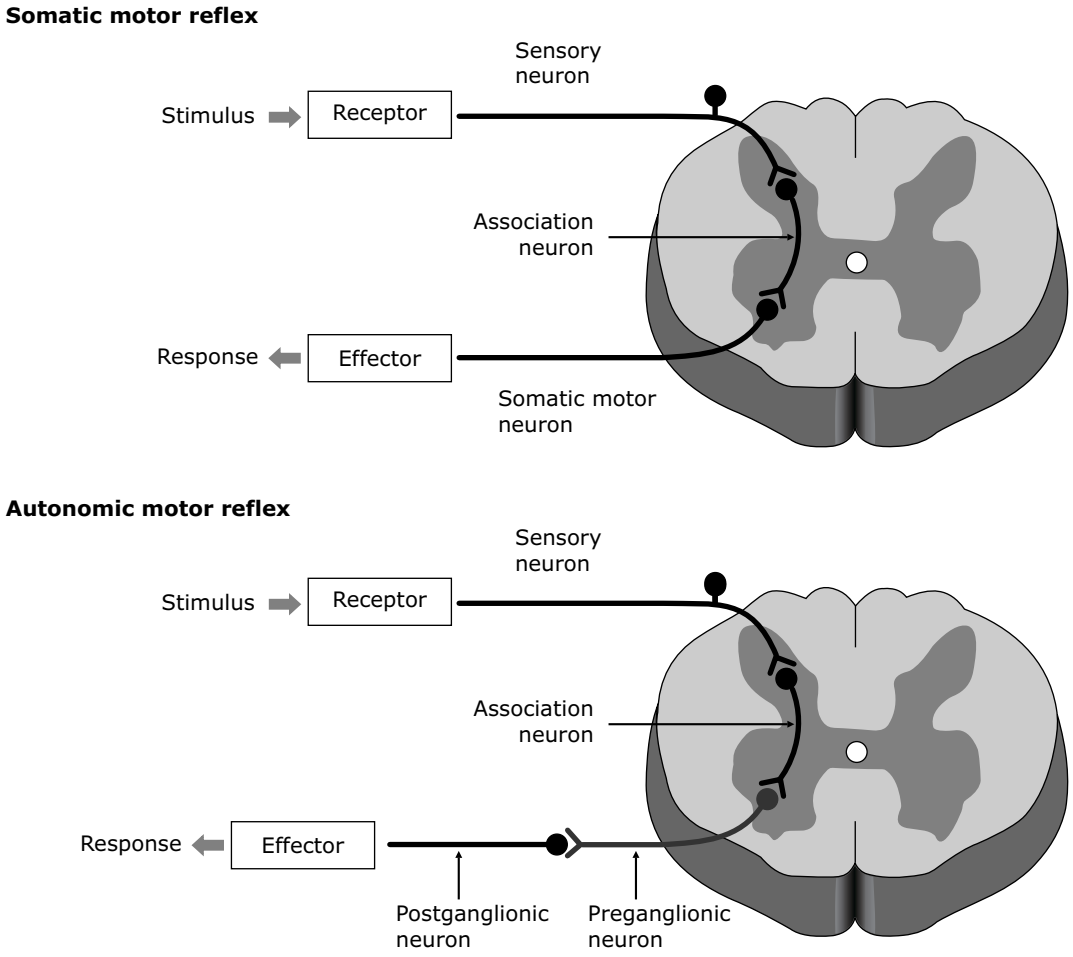

Figure 4.9 Comparison of a somatic motor reflex and an autonomic motor reflex. In a skeletal muscle reflex, a single somatic motor neuron passes from the CNS to the skeletal muscle. In an autonomic reflex, a preganglionic neuron passes from the CNS to an autonomic ganglion, where it synapses with a second autonomic neuron. It is that second, or postganglionic, neuron that innervates the smooth muscle, cardiac muscle or gland.

4.2.10 Autonomic nervous system

The *autonomic nervous system* regulates the activities of involuntary smooth muscle, cardiac muscles and glands without consulting the will. The system consists of preganglionic fibres from CNS, ganglia and post ganglionic fibres. It is also called *visceral* nervous *system* because the autonomic neurons innervates the viscera.

Unlike somatic motor neurons of somatic nervous system, which conduct impulses along a single axon from the spinal cord to the neuromuscular junction, autonomic motor control involves two neurons in the efferent pathway. The first of these neurons has its cell body in the gray matter of the brain or spinal cord. The axon of this neuron does not directly innervate the effector organ but instead synapses with a second neuron within an *autonomic ganglion* (a ganglion is a collection of cell bodies outside the CNS). The first neuron is thus called a **preganglionic neuron**. The second neuron in this pathway, called a **postganglionic neuron**, has an axon that extends from the autonomic ganglion to an effector organ, where it synapses with its target tissue.

Preganglionic autonomic fibers originate in the midbrain and hindbrain and in the upper thoracic to the fourth sacral levels of the spinal cord. The origin of the preganglionic fibers and the location of the autonomic ganglia help to distinguish the *sympathetic* and *parasympathetic divisions* of the autonomic system.

Sympathetic and parasympathetic divisions

The autonomic nervous system has two subdivisions - the *sympathetic* and the *parasympathetic nervous systems*. The sympathetic and parasympathetic divisions of the autonomic system have some structural features in common. Both consist of preganglionic neurons that originate in the CNS and postganglionic neurons that originate outside of the CNS in ganglia. However, the specific origin of the preganglionic fibers and the location of the ganglia differ in the two divisions of the autonomic system.

The **sympathetic division** is also called the *thoracolumbar division* of the autonomic system because its preganglionic fibers exit the spinal cord from the first thoracic (T1) to the second lumbar (L2) levels. Most sympathetic nerve fibers, however, separate from the somatic motor fibers and synapse with postganglionic neurons within a double row of sympathetic ganglia, called *paravertebral ganglia*, located on either side of the spinal cord. Ganglia within each row are interconnected, forming a *sympathetic chain of ganglia* that parallels the spinal cord on each lateral side.

The **parasympathetic division** is also known as the *craniosacral division* of the autonomic system. This is because its preganglionic fibers originate in the brain (specifically, in the midbrain, medulla oblongata, and pons) and in the second through fourth sacral levels of the spinal column. These preganglionic parasympathetic fibers synapse in ganglia that are located next to—or actually within—the organs innervated. These parasympathetic ganglia, called *terminal ganglia,* supply the postganglionic fibers that synapse with the effector cells.

The sympathetic division of the autonomic system activates the body to 'fight or flight,' largely through the release of norepinephrine from postganglionic fibers and the secretion of epinephrine from the adrenal medulla. The parasympathetic division often produces antagonistic effects through the release of acetylcholine from its postganglionic fibers. The actions of the two divisions must be balanced in order to maintain homeostasis.

Table 4.3 Effect of the sympathetic and parasympathetic divisions of the autonomic nervous system

Target organ/system	*Parasympathetic effects*	*Sympathetic effects*
Digestive system	Increases peristalsis and amount of secretion by digestive glands; relaxes sphincters	Decreases activity of digestive system and constricts digestive system sphincters (For example, anal sphincter)
Liver	No effect	Causes glucose to be released to blood
Lungs	Constricts bronchioles	Dilates bronchioles
Urinary bladder/Urethra	Relaxes sphincter (allows voiding)	Constricts sphincters (prevents voiding)
Kidneys	No effect	Decreases urine output
Heart	Decreases rate	Increases rate and force of heartbeat
Blood vessels	No effect on most blood vessels	Constricts blood vessels in viscera (dilates those in skeletal muscle and heart); increases blood pressure
Glands-salivary lacrimal	Stimulates; increases production of saliva and tears	Inhibits; result in dry mouth and dry eyes

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Table 4.4 Comparison of the somatic system and the autonomic system

Feature	*Somatic system*	*Autonomic system*
Effector organs	Skeletal muscles	Cardiac muscle, smooth muscle and glands
Presence of ganglia	No ganglia	Ganglia present
Number of neurons from CNS to effector	One	Two
Effect of nerve impulse on muscle	Excitatory only	Either excitatory or inhibitory
Type of nerve fibers	Fast-conducting, thick, myelinated	Slow-conducting; preganglionic fibers and lightly myelinated but thin; postganglionic fibers unmyelinated and very thin.

4.3 Sensory organs

Sensory organs at the periphery of the body are the *windows of the brain* because they keep the brain aware of what is going on in the external world. When stimulated, sensory receptors generate nerve impulses that travel to the central nervous system (CNS). Nerve impulses arriving at the cerebral cortex of the brain result in sensation. We see, hear, taste and smell with our brain, not with our sensory organs.

4.3.1 Eye

The eyes are complex sense organs. Each eye has a layer of receptors, a lens system that focuses light on these receptors, and a system of nerves that conducts impulse from the receptors. The eye may be compared to a video camera, which collects images, converts them into electrical pulses, records them on magnetic tape and allows their visualization by decoding the taped information. The eye focuses on an image by projecting that image onto the retina. A series of events begins, the first of which is photochemical, followed by biochemical events that amplify the signal and finally electrical impulses are sent to the brain, where the image is reconstructed in *the mind's eye*.

Anatomy of the eyeball

The human eye can also be divided into two main segments: *the anterior segment* and *the posterior segment*. The anterior segment of the eye includes the structures - cornea, iris, ciliary body and lens. **Aqueous humor** fills the space within the anterior segment. Anterior segment has two chambers - *anterior chamber* (between cornea and iris) and *posterior chamber* (between iris, zonule fibers and lens). The aqueous humor brings nutrients to the cornea and to the lens, and it removes the products of metabolism from them. *Posterior segment* includes vitreous chamber (between the lens and the retina). The **vitreous humor** fills the vitreous chamber and it is a gelatinous mass that helps maintain the shape of the eye while allowing it to remain somewhat pliable. The *lens* is bathed on one side by aqueous humor and supported on the other side by vitreous humor. It has no blood supply, but is metabolically active. It obtains nutrients from the aqueous humor. The lens is mostly water and proteins. The majority of vertebrate lens proteins are the α-, β and γ crystallins.

Anatomically, the wall of the eyeball consists of three layers: *fibrous tunic*, *vascular tunic* and *retina*. The **fibrous tunic** is the superficial coat of the eyeball and consists of the anterior cornea and posterior sclera. The **cornea** is a transparent coat that covers the coloured iris. Because it is curved, the cornea helps focus light onto the retina. Its outer surface consists of non-keratinized stratified squamous epithelium. The middle coat of the cornea consists of collagen fibers and fibroblasts, and the inner surface is simple squamous epithelium. The **sclera** the *white* of the eye, is a layer of dense connective tissue made up mostly of collagen fibers and fibroblasts. The sclera covers the entire eyeball except the cornea.

The **vascular tunic** (or *uvea*) is the middle layer of the eyeball. It is composed of three parts: *choroid*, *ciliary body* and *iris*. The *choroid* is a highly vascularized structure which is the posterior portion of the vascular tunic. It is deeply pigmented with melanin. It provides blood supply and absorbs scattered light. The choroid coat forms the iris in the front of the eye.

Hyperparathyroidism, an elevation in the level of parathyroid hormone, is often caused due to tumor in one of the parathyroid gland. This causes excessive resorption of bone matrix, raising the levels of calcium and phosphate ions in blood causing bones to become soft and easily fractured. High blood calcium levels also promote the formation of kidney stones.

Adrenal gland disorders

Hypersecretion of cortisol causes *Cushing's syndrome*. The prominent characteristics of this syndrome are related to the exaggerated effects of glucocorticoid, with the main symptoms caused by excessive gluconeogenesis. When too many amino acids are converted into glucose, the body suffers from combined glucose excess (high blood glucose) and protein shortage.

Addison's disease is caused by hyposecretion of both glucocorticoids and mineralocorticoids, which results in hypoglycemia, sodium and potassium imbalance, dehydration, hypotension, rapid weight loss and generalized weakness.

Table 4.9 A partial listing of the endocrine glands

Endocrine gland	*Major hormones*	*Primary target organs*
Adrenal cortex	Glucocorticoids	Liver and muscles
	Aldosterone	Kidneys
Adrenal medulla	Epinephrine	Heart, bronchioles and blood vessels
Hypothalamus	Releasing and inhibiting hormones	Anterior pituitary
Small intestine	Secretin and cholecystokinin	Stomach, liver and pancreas
Islets of Langerhans	Insulin	Many organs
	Glucagon	Liver and adipose tissue
Kidneys	Erythropoietin	Bone marrow
Liver	Somatomedins	Cartilage
Ovaries	Estradiol and progesterone	Female reproductive tract and mammary glands
Parathyroid glands	Parathyroid hormone	Bone, small intestine and kidneys
Pineal gland	Melatonin	Hypothalamus and anterior pituitary
Pituitary, anterior	Trophic hormones	Endocrine glands and other organs
Pituitary, posterior	Antidiuretic hormone	Kidneys and blood vessels
	Oxytocin	Uterus and mammary glands
Stomach	Gastrin	Stomach
Testes	Testosterone	Prostate, seminal vesicles and other organs
Thymus	Thymopoietin	Lymph nodes
Thyroid gland	T_4 and T_3, calcitonin	Most organs

4.5 Respiratory system

The respiratory system consists of the nose, pharynx (throat), larynx, trachea, bronchi and lungs. The respiratory system has the following functions:

1. Helps in gaseous exchange – intake of oxygen for delivery to body cells and elimination of carbon dioxide produced by cells.
2. Helps to regulate blood pH.
3. Contains receptors for the sense of smell, filters inspired air, produces vocal sounds (phonation), and excretes small amounts of water and heat.

4.5.1 Respiration

The process of gas exchange in the body, called respiration, has three basic steps:

1. *Pulmonary ventilation* or breathing is the inhalation (*inspiration*) and exhalation (*expiration*) of air between the atmosphere and the alveoli of the lungs. Air flows between the atmosphere and the alveoli of the lungs because of alternating pressure differences created by contraction and relaxation of the respiratory muscles.
2. *External* (pulmonary) *respiration* is the exchange of gases between the alveoli of the lungs and the blood in pulmonary capillaries across the respiratory membrane. In this process, pulmonary capillary blood gains oxygen and loses carbon dioxide.
3. *Internal* (tissue) *respiration* is the exchange of gases between blood in systemic capillaries and tissue cells. In this step, the blood loses oxygen and gains carbon dioxide. Within cells, the metabolic reactions that consume oxygen and give off carbon dioxide during the production of ATP are termed *cellular respiration*.

4.5.2 Mechanics and breathing

The respiratory system includes the *respiratory airways*, the *lungs* and the *respiratory muscles* of the chest and abdomen. The respiratory airways are tubes that carry air between the atmosphere and the air sacs. The airways begin with the nostrils, which are lined with hairs that filter out dust and other particles. As the air passes through the nasal cavity, extensive array of cilia on its epithelial lining further cleans the air and moistens it. The nasal passages open into the **pharynx** (throat), which serves as a common pathway for both the respiratory and the digestive systems. Two tubes lead from the pharynx - the **trachea** (windpipe), through which air enters into the lungs and the esophagus, the tube through which food passes to the stomach. Air normally enters the pharynx through the nose, but it can enter by the mouth as well. Because the pharynx serves as a common passageway for food and air, reflex mechanisms close off the trachea during swallowing so that food enters the esophagus and not the airways. The esophagus stays closed except during swallowing to keep air from entering the stomach during breathing. The **larynx** or *voice box* is located at the entrance of the trachea. Beyond the larynx, the trachea divides into two main branches, the right and left **bronchi**, which enter the right and left lungs, respectively. Within each lung, the bronchus continues to branch into progressively narrower, shorter, and more numerous airways, much like the branching of a tree. The smaller branches are known as **bronchioles**. At the ends of the terminal bronchioles are the **alveoli**, the tiny air sacs where gases are exchanged between air and blood.

Humans possess a pair of lungs located in the chest or thoracic cavity. The two lungs hang free within the cavity, being connected to the rest of the body only at one position where the lung's blood vessels and bronchus enter. The thoracic cavity is bounded on its sides by ribs, which are capable of flexing and the *diaphragm,* which forms the floor of the thoracic cavity, is a large, dome-shaped sheet of skeletal muscle that separates the thoracic cavity from the abdominal cavity. Each lung is covered by a very thin, smooth membrane called the *pleural membrane*. A second pleural membrane marks the interior boundary of the thoracic cavity, dividing it into two halves; each lung is thus suspended in its own cavity. Within the cavity, the weight of the lungs is supported by the intrapleural fluid. Breathing or *pulmonary ventilation* consists of two phases: *inspiration* and *expiration.* Inspiration or inhalation and expiration or exhalation are accomplished by alternately increasing and decreasing the volumes of the thorax and lungs.

Inspiration

The **inspiratory muscles** – *diaphragm* and *external intercostal muscles* – contract to accomplish an inspiration during quiet breathing. Before the beginning of inspiration, all respiratory muscles are relaxed. At the onset of inspiration, the inspiratory muscles are stimulated to contract, enlarging the thoracic cavity. The major inspiratory muscle is the diaphragm. The relaxed diaphragm has a dome shape. When the diaphragm contracts, it descends downward, enlarging the volume of the thoracic cavity by increasing its vertical dimension. Seventy-five percent of the enlargement of the thoracic cavity during quiet inspiration is accomplished by contraction of the diaphragm. Two sets of intercostal muscles lie between the ribs. The *external intercostal muscles* lie on top of the *internal intercostal muscles.* Contraction of the external intercostal muscles enlarges the thoracic cavity in both the lateral and the anteroposterior dimensions. When the external intercostals contract, they elevate the ribs and subsequently the sternum upward and outward.

Bicarbonate ions

The greatest percentage of carbon dioxide (about 70%) is transported in blood plasma as bicarbonate ions. As carbon dioxide diffuses into systemic capillaries and enters red blood cells, it reacts with water in the presence of the enzyme *carbonic anhydrase* (CA) to form carbonic acids, which dissociates into H^+ and HCO_3^-. Thus, as blood picks up carbon dioxide, bicarbonate accumulates inside RBCs. Some bicarbonate ions move out into the blood plasma, down its concentration gradient. In exchange, chloride ions move from plasma into the RBCs. The exchange of negative ions which maintains the electrical balance between blood plasma and RBC cytosol, is known as the *chloride shift.* The amount of carbon dioxide that can be transported in the blood is influenced by the percent saturation of hemoglobin with oxygen. The lower the amount of oxy-hemoglobin, the higher the carbon dioxide carrying capacity of the blood, a relationship known as the **Haldane effect**. Two characteristics of deoxyhemoglobin give rise to the Haldane effect:

a. Deoxyhemoglobin binds to and thus transports more carbon dioxide than does $Hb–O_2$.
b. Deoxyhemoglobin also buffers more H^+ than does $Hb–O_2$. Thereby removing H^+ from solution and promoting the conversion of carbon dioxide to carbonic acid via the reaction catalyzed by carbonic anhydrase.

Table 4.10 Important respiratory states

Apnea	Transient cessation of breathing.
Asphyxia	Lack of oxygen in tissues.
Cyanosis	Blueness of the skin resulting from insufficient oxygenated blood in the arteries.
Eupnea	Normal breathing.
Hypercapnia	Excess CO_2 in the arterial blood.
Hyperpnea	Increased pulmonary ventilation that matches increased metabolic demands, as in exercise.
Hypocapnia	Below-normal CO_2 in the arterial blood.
Hypoxia	Insufficient O_2 at the cellular level.

4.6 Cardiovascular system

The **cardiovascular system** consists of three interrelated components: *blood*, the *heart* and *blood vessels*. The branch of science concerned with the study of blood, blood-forming tissues and the disorders associated with them is *hematology*. Human has closed blood vascular system. In this system, blood is pumped through a closed system of heart, arteries, veins and capillaries without coming in direct contact of body tissues or body cavity. Blood flows at high pressure.

4.6.1 Physical characteristics of blood

Blood is slightly alkaline (pH ranging from 7.35 to 7.45). Blood constitutes about 20% of extracellular fluid, amounting to 8% of the total body mass. The blood volume is 5 to 6 liters in an average–sized adult male and 4 to 5 liters in an average–sized adult female.

Functions of blood

1. Transportation of oxygen, carbon dioxide, nutrients, hormones, heat and wastes.
2. Regulation of pH, body temperature and water content of cells.
3. Protection against blood loss through clotting and against disease through phagocytic activity with blood cells and antibodies.

Components of blood

Blood is a connective tissue composed of a liquid extracellular matrix called *blood plasma* that dissolves and suspends various cells and cell fragments. So, blood has two components:

1. *Blood plasma*, a watery liquid extracellular matrix that contains dissolved substances and
2. *Formed elements*, which are cells and cell fragments.

4.7 Digestive system

The digestive system includes the gastrointestinal (GI) tract and the accessory digestive organs. The **GI tract** or *alimentary canal* is a continuous tube that extends from the mouth to the anus. The organs of the GI tract include the *oral cavity, pharynx, esophagus, stomach, small intestine* and *large intestine.* The *accessory digestive organs* include the teeth, tongue, salivary glands, liver, gallbladder and pancreas. The GI tract from the esophagus to the anal canal is composed of four layers, or *tunics.* Each tunic contains a dominant tissue type that performs specific functions in the digestive process. The four tunics of the GI tract, from the inside out, are the *mucosa* (mucous membrane layer)*, submucosa* (submucosal layer)*, muscularis* (smooth muscle layer) and *serosa* (serous membrane layer). The mucosa, which lines the lumen of the GI tract, is the absorptive and major secretory layer.

Functions of digestive system

1. Ingestion: taking food into the mouth.
2. Secretion: release of water, acid, buffers and enzymes into the lumen of the GI tract.
3. Mixing and propulsion: churning and propulsion of food through the GI tract.
4. Digestion: mechanical and chemical breakdown of food.
5. Absorption: passage and assimilation of digested products from the GI tract into the blood and lymph.
6. Defecation: the elimination of feces from the GI tract.

4.7.1 Digestive activities in the GI

Mouth

The mouth is formed by the cheeks, hard and soft palates, lips and tongue. Digestion starts in mouth with breakdown of large pieces of food into smaller particles. *Saliva,* secreted by three pairs of salivary glands. Saliva, which contains mucus, moistens and lubricates the food particles. The most important salivary proteins are *amylase, mucus* and *lysozyme.* Saliva begins digestion of carbohydrate in the mouth through action of *salivary amylase*, an enzyme that breaks polysaccharides down into maltose, a disaccharide consisting of two glucose molecules

Function

- Cut, tear, and pulverize food by the activity of teeth.
- Secrete saliva from the salivary glands that softens, moistens and dissolves food and cleanses mouth and teeth. Salivary amylase present in saliva splits starch into smaller fragments.
- Secrete lingual lipase from lingual glands.

Pharynx and esophagus

Pharynx receives a bolus from the oral cavity and passes it into the esophagus. Swallowing occurs in the pharynx. It is a reflex action performed automatically, without conscious thought. During swallowing, food normally enters the esophagus because the air passages are blocked.

The esophagus is the portion of the GI tract that connects the pharynx to the stomach. It is a muscular tube approximately 25 cm long. Skeletal muscles surround the esophagus just below the pharynx and form the *upper esophageal sphincter***,** whereas the smooth muscles in the last portion of the esophagus form the *lower esophageal sphincter.* The esophagus plays no role in the chemical digestion of food. Its sole purpose is to conduct the food bolus from the mouth to the stomach. The *peristaltic wave* pushes the bolus from the beginning to the end of the esophagus. The term **peristalsis** refers to ringlike contractions of the circular smooth muscle that move progressively forward, pushing the bolus into a relaxed area ahead of the contraction. The peristaltic wave takes about 5 to 9 seconds to reach the lower end of the esophagus.

Stomach

The stomach is a sac like organ, located between the esophagus and the small intestine. Its functions are to store, dissolve and partially digest the food particles and to regulate the rate at which the stomach's contents empty into

The countercurrent multiplier mechanism has certain special features which are as follows:

1. The descending limb of Henle's loop is permeable to water, whereas the ascending limb has poor water permeability.
2. The ascending limb of the nephron is the site for active transport of sodium.
3. The regulatory influence of the ADH is exerted on the permeability of the distal tubules and the collecting ducts. High hormone levels increase water permeability and its absence reduces it to a low level.

From the structure of the urinary tubules, it is seen that the descending, ascending and collecting tubule are very close to each other. The flow in the ascending tubule runs counter to that in the descending and the collecting tubule. Sodium and other ions are transported out of the filtrate in the proximal tubule where water also moves out. By the time urine reaches the loop of Henle, about 80 percent of total filtrate is out of the tubule. The loop of Henle absorbs lot of sodium and making the urine concentrated or hyperosmolar.

In the ascending tubule and the loop of Henle, there is no movement of water consequent upon the active transport of sodium, making the filtrate less concentrated. The fluid returned to the distal tubule is hypotonic, but this segment is able to reabsorb water and the fluid reaching the collecting tubules is isotonic. There is further transport of sodium from the collecting duct, but as the fluid passes through the medulla, water diffuses out of the filtrate and the fluid reaching the pelvis of the kidney is the concentrated form of urine.

The countercurrent mechanism is also maintained by the vasa rectae. In vasa recta, the flow of blood run counter to the flow of urine. The flow of blood in the medullary tissue is downward and in the cortex it is upward. As the blood flows through the medulla, water diffuses out and sodium diffuses in, whereas reverse movement takes place in the cortex, leaving most of the sodium to remain in the medullary interstitial fluid circulation.

4.9 Reproductive system

The reproductive system of sexually reproducing animal consists of:

- Primary sex organs (called gonads) which produce gametes and hormones.
- Secondary sex organs which participate in reproduction but not form gametes.
- Accessory sex organs cause differences in the appearance of two sexes.

The primary reproductive organs, or gonads, consist of the ovaries and testes. These organs are responsible for producing the egg and sperm cells and hormones. These hormones function in the maturation of the reproductive system, the development of sexual characteristics, and have important roles in regulating the normal physiology of the reproductive system. All other organs, ducts, and glands in the reproductive system are considered secondary, or accessory, reproductive organs.

4.9.1 Male reproductive system

The male reproductive system consists of glands with their ducts and supporting structures

1. The glands include a pair of testes, a pair of seminal vesicles, a pair of bulbourethral (Cowper's) glands, and one prostate gland.
2. Ducts of testes include a pair of epididymis, a pair of vas deferens, a pair of ejaculatory ducts, and one urethra.
3. Supporting structures are divided into: *Internal* – a pair of spermatic cords and *External* - scrotum and penis.

Testes

The male gonads, testes, begin their development high in the abdominal cavity, near the kidneys. During the last two months before birth, or shortly after birth, they descend through the inguinal canal into the **scrotum**, a pouch that extends below the abdomen, posterior to the penis. Although this location of the testes, outside the abdominal cavity, may seem to make them vulnerable to injury, it provides a temperature about 3°C below normal body

4.10 Embryonic development

Embryogenesis is a developmental process that usually begins once the egg has been fertilized. It involves multiplication of cells by mitosis and their subsequent growth, movement, and differentiation into all the tissues and organs of a living baby.

4.10.1 Fertilization

Fertilization is a process whereby two gametes fuse together to form a zygote. The **male gamete**, the sperm cell, is a small cell with a greatly reduced cytoplasm and a haploid nucleus. Sperm usually consist of two morphologically and functionally distinct regions enclosed by a single plasma membrane: the *tail* and the *head.* The head contains a nucleus and an acrosomal vesicle. The DNA in the nucleus is highly condensed and transcriptionally inactive because the normal histones are replaced by a special class of packaging proteins known as *protamines.* The acrosomal vesicle (or acrosome) is a specialized secretory vesicle present in most animal sperm. It is derived from the Golgi apparatus and contains hydrolytic enzymes needed to digest extracellular coats surrounding the egg. It can be considered as a modified secretory vesicle.

The motile tail of a sperm is a long flagellum. The active bending of the flagellum is caused by the sliding of adjacent microtubule doublets past one another, driven by dynein motor proteins, which use the energy from ATP hydrolysis to slide the microtubules. The ATP is generated by a large number of highly specialized mitochondria that are concentrated in the anterior part of the sperm tail (called the *midpiece*). The neck (region between head and midpiece) contains the two centrioles (proximal and distal). Axonemal structure of flagella grows out of distal centriole.

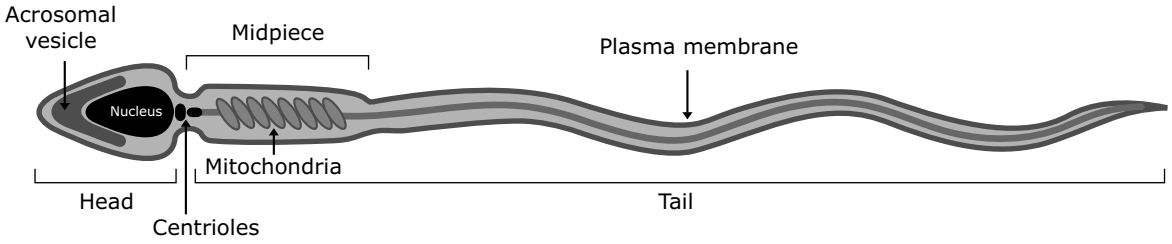

Figure 4.33 A longitudinal section of human sperm.

The **female gamete** (egg) of most animals is giant single cell. It contains all the materials needed for initial development of the embryo. There are three main ways to provide nutrition to the developing embryo: 1. supply the embryo with yolk; 2. form a larval feeding stage between the embryo and the adult; or 3. create a placenta between the mother and the embryo.

A placental mammalian egg is not as large as the egg of a frog or bird. Because the embryo can start to grow early by taking up nutrients from the mother via the placenta. The egg cytoplasm usually contains nutritional reserves in the form of **yolk**, which is rich in lipids, proteins and polysaccharides and is often contained within discrete structures called *yolk granules.* In some species, a membrane encloses each yolk granule. Yolk is usually synthesized outside the ovary and imported into the oocyte. In birds, amphibians and insects, yolk proteins are made by liver cells (or their equivalents), which secrete these proteins into the blood. Within the ovaries, oocytes use receptor-mediated endocytosis to take up the yolk proteins from the extracellular fluid. Yolk is often concentrated toward one pole of the egg, called the *vegetal pole*; the yolk concentration decreases significantly toward the opposite pole, the *animal pole*. The animal pole is also the site where the polar bodies of oogenesis bud from the cell. In eggs that develop into large animals outside the mother's body, yolk can account for more than 95% of the volume of the cell. In mammals, whose embryos are largely nourished by their mothers via the placenta, there is little, if any, yolk. The **egg coat** is another peculiarity of eggs. It is a specialized form of extracellular matrix consisting largely of glycoproteins, present as a layer immediately surrounding the egg plasma membrane. In non-mammalian eggs,

Chapter 05

Ecology

5.1 What is Ecology?

Ecology is the study of relationships between living organisms and their environments, the interaction of organisms with each other and the pattern and cause of the abundance and distribution of organisms in nature. Thus, ecology is the science that attempts to answer questions about how the nature works. The term ecology was coined by German biologist Ernst Haeckel combining two Greek words, *oikos* (meaning 'house' or 'dwelling place') and *logos* (meaning the study of) to denote such relationship between the organisms and their environment.

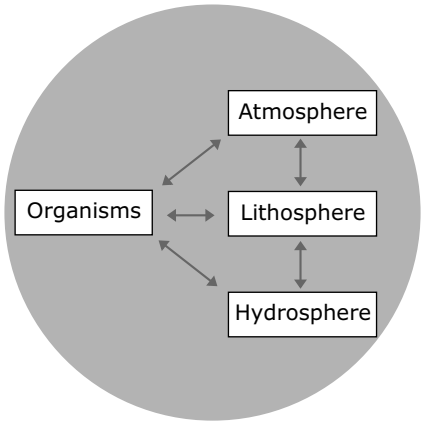

Figure 5.1 Organisms interact with physical environment comprised of atmosphere, hydrosphere and lithosphere.

Level of organization

Ecological patterns and processes vary as a function of the scale at which they operate. The basic level of ecological organization starts with the **individual** (a single plant, insect or bird). The next level of organization is the **population**. Populations are a collection of individuals of the same species within an area or region. The next, more complex, level of organization is the **community**. Communities are made up of different populations of interacting plants, animals and microorganisms within some defined geographical area. The next level of organization is the **ecosystem**. An ecosystem consists of different communities of organisms associated within a physically defined space. Terrestrial ecosystems can be grouped into units of similar nature, termed **biomes** (such as a deciduous forest, grassland, coniferous forest, etc.), or into a geographic unit, termed **landscapes**, containing several different types of ecosystems.

Based on the level of organization ecology is classified into autecology and synecology. **Autecology** is the study of interaction between organisms and their environments at the level of an individual, a population or an entire species. **Synecology** is the study of a biotic community. It is also called *community ecology*. It is the synecology which describes the biotic community as a whole, especially the links between organisms.

5.2 Environment

Organisms and their environments are dynamic and interdependent. The term environment etymologically means surroundings. Thus, the environment includes everything (biotic as well as abiotic) that surrounds an organism and all the abiotic components (like air, water, soil, radiations) affecting the biotic components described as *environmental factors* or *ecological factors*.

Soil

Soil is the uppermost weathered layer of the earth's crust. It is a mixture of weathered mineral rock particles, organic matter (i.e. both living and dead), water and air. Soil is a biologically active matrix and home for plant roots, seeds, animals, bacteria, fungi, algae and viruses. The study of soil is called *pedology*.

Soil composition

Soils are composed of *mineral particles*, *organic matter*, *air* and *water*. Soil mineral particles include sand (0.05-2.0 mm), silt (0.002-0.05 mm) and clay (<0.002 mm). The relative proportions of sand, silt, and clay in a soil are referred as **soil texture**. Soils are also composed of organic matter, which include *living biomass*, *detritus* (dead tissue) and *humus* (non-living, non tissue). **Humus** is an amorphous and a colloidal mixture of complex organic substances. It is made up of *humic* substances (comprise about 60 to 80% of the soil organic matter and characterized by dark coloured amorphous substance) and *non-humic* substances (refers to the group of identifiable biomolecules that are mainly produced by microbial action and less resistant to breakdown). Soil air is the mixture of gases that are present in soil pores that are not filled with water. Oxygen and carbon dioxide are important constituents, and their concentration in the soil affects many processes (e.g. nitrification and denitrification). Soil water can contribute up to 30% of soil volume, and is essential for the activity and physiological functioning of organisms in the soil.

Soil profile

The mineral and organic components of soil are differentiated into horizons or strata of variable depth. Each horizon differs in morphology, physical structure, and chemical and biological characteristics. These horizons are evident when a vertical cut is made through the soil, revealing the *soil profile*. The widely accepted structure of the soil profile is as follows:

- O Organic litter of loose leaves and debris.
- A_1 Rich in humus and dark in colour.
- A_2 Zone of maximum leaching of minerals; readily available minerals to plant roots present in this layer.
- B Little organic material and chemical composition is largely that of the underlying rock; also referred to as the *zone of accumulation* since minerals from above and below tend to concentrate here.
- C Parent rock, which is weakly weathered.
- D Unweathered bedrock.

The soil profile and the relative thickness of the horizons are generally characteristic for different climatic regions and different topographical situations. For example, in grassland soil humification is rapid, but mineralization is slow. In forest soil litter and root decay slowly. Hence humus layer is narrow, but mineralization is rapid so B horizon is broad.

5.7 The ecosystem concept

An ecosystem (A.G. Tansley, 1935 proposed the term *ecosystem*) is a functional unit comprising all the organisms in a particular place interacting with one another and with their physical environment, and interconnected by an ongoing flow of energy and a cycling of materials. An ecosystem can be visualized as a functional unit of nature. All ecosystems are 'open' systems in the sense that energy and matter are transferred in and out. An ecosystem could be of any size depending on the communities to be studied and its boundaries can be either real (natural) or arbitrary.

The ecosystem is the first unit, in the hierarchical organization of the living system from molecule to biosphere, which is complete i.e., it has all components: biological and physical, necessary for survival. Accordingly, it is the basic unit around which theories and experiments of ecology are organized.

Organism ➡ Population ➡ Community ➡ **Ecosystem** ➡ Landscape ➡ Biome ➡ Biosphere

5.7.1 Ecosystem components

Ecosystem consists of various non-living, **abiotic**, and living, **biotic** components. The abiotic components of an ecosystem include various physical and chemical factors. The *physical factors* having the greatest effect on the ecosystem are: light, temperature, precipitation, air, soil, fire and water. However, the *chemical factors* are: pH, salinity, nutrient contents, moisture contents (for terrestrial ecosystem), amount of toxic substances and level of dissolved oxygen (in aquatic ecosystems). Organisms that make up the **biotic** component of an ecosystem are usually classified as *autotrophs* and *heterotrophs*, based on how they get their food or organic nutrients they need to survive.

Autotrophs

Autotrophs are organisms that can manufacture the organic compounds they need as nutrients from simple inorganic compounds obtained from their environment. They are the ultimate sources of organic compounds for all nonautotrophic organisms, and for this reason, biologists refer to autotrophs as the *producers* of the biosphere. There are two kinds of autotrophs - photoautotrophs and chemoautotrophs. Most autotrophs are photoautotrophs and make their organic nutrients they need through *photosynthesis*. In most terrestrial ecosystems, green plants are the primary producers. In aquatic ecosystems, most of the primary producers are phytoplankton, consisting of various species of floating and drifting bacteria and protist. Some autotrophs, mostly specialized bacteria, can extract inorganic compounds from their environment and convert them into organic nutrient compounds without using sunlight. These autotrophs are called *chemotrophs*.

Heterotrophs

Heterotrophs are organisms which cannot synthesize the organic nutrients they need and get them by feeding on the tissues of producers or other consumers. Fungi, animals and most bacteria are heterotrophs. Heterotrophs can be classified into *consumer* and *decomposer.*

Consumers are organisms, such as animals, that feed on producers and other consumers. There are several classes of consumers, depending on their food sources: *herbivores* (feed directly on plants), carnivores (feed on animals), omnivores (eat both plants and animals) and *detritivores* (eat detritus). *Detritus* are the dead bodies and waste product of living and once living organisms or simply, decomposing organic material. Earthworms are a well-known example of *detritivores* (also known as *detrivores* or *detritus feeders*), eating rotting plant leaves and other debris. Some detritus feeders, such as dung beetles, eat feces. Consumers can also be classified into primary, secondary and tertiary consumers based on feeding level or trophic level. Primary consumers feed on producers and belong to the second trophic level. The secondary consumers feed only on primary consumers, and they belong to the third trophic level. Producers belong to the first trophic level.

5.7.8 Ecological pyramid

The different trophic levels of an ecosystem are related to one another and can be summarized in the form of ecological pyramids. The base of each pyramid represents the producers or the first trophic level while the apex represents tertiary or high-level consumers; other consumer trophic levels are in between. There are three kinds of ecological pyramids possible which are discussed below.

Pyramid of numbers

A graphic representation of the total number of individuals of different species belonging to each trophic level in an ecosystem is known as *pyramid of number*. It consists of a number of horizontal bars depicting specific trophic levels which are arranged sequentially from primary producer level through herbivore, carnivore onwards. The length of each bar represents the total number of individuals at each trophic level in an ecosystem.

For most ecosystems, like grassland ecosystem, pyramids of numbers are upright because numbering of organisms decreases at successively higher trophic levels. However, there are some ecological systems for which pyramid of numbers may be inverted. For example, if we depict the situation of a single tree along with its dependent insect population, we would get an inverted pyramid. Since the tree is a primary producer, it would represent the base of the pyramid and the dependent phytophagous insect population will represent the second trophic level.

Pyramid of biomass

Pyramid of biomass represents the total dry weight of living beings of different species at each trophic level at a particular time. It is usually determined by collecting all the organisms occupying each trophic level separately and measuring their dry weight.

This eliminates the size difference problem because all kinds of organisms at a trophic level are weighed. Generally, the biomass of producers is much greater than the biomass of herbivores and the biomass of herbivores is greater than that of carnivores and so on. In other words, we find that biomass decreases at each trophic level if we move from producer to top carnivore. Therefore, diagrammatic representation of biomass of individuals belonging to different trophic levels invariably assumes the shape of an upright pyramid. This, however, is not always the case. In some aquatic ecosystems, like large lakes and oceans, the pyramid of biomass, sometimes assumes an inverted form. Since microscopic phytoplanktonic algae are the primary producers in the aquatic system, being single-celled organisms, they do not accumulate much biomass and consequently, at a given time, the total weight or the standing crop of phytoplankton is less as compared to herbivores or other consumers. This is the reason for the base of the pyramid in aquatic ecosystem being smaller than the upper structure.

Pyramid of energy

An energy pyramid more accurately reflects the laws of thermodynamics, with loss of energy being depicted at each transfer to another trophic level, hence the *pyramid is always upright.*

Energy pyramids in the case of aquatic ecosystem are also upright, even where the pyramid of biomass is inverted. In energy pyramids, a given trophic level, always has a smaller energy content than the trophic level immediately below it. This is due to the fact that some energy is always lost as heat is going from one trophic level to the next. Each bar in the pyramid indicates the amount of energy utilized at each trophic level in a given time annually per unit area.

Figure 5.8 An upright pyramid of numbers

Figure 5.9 An inverted pyramid of Biomass

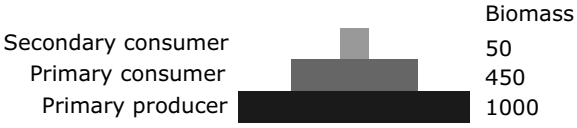

Figure 5.10 An upright pyramid of Biomass

Figure 5.11 An upright pyramid of Energy

5.7.9 Transfer efficiencies

In ecosystems, living organisms are linked together by feeding relationships. Producers (or autotrophs) have the ability to fix carbon through photosynthesis *via* green chloroplasts in their leaves. Herbivores are the primary consumers of organic molecules fixed by the producers. Carnivores are secondary consumers, living on the organic molecules of the herbivores. There may be several levels of carnivores in any one ecosystem; in such cases the ultimate level will be occupied by the top carnivore. The final groups of organisms in an ecosystem are decomposers or detritivores, small animals, bacteria and fungi which can break down the complex organic chemicals of dead materials and waste products.

The proportions of net primary production that flow along each of the possible energy pathways depend on transfer efficiencies in the way energy is used and passed from one step to the next. A knowledge of the values of just three categories of transfer efficiency is all that is required to predict the pattern of energy flow. These are *consumption efficiency* (CE), *assimilation efficiency* (AE) and *production efficiency* (PE).

Consumption efficiency is the percentage of total productivity available at one trophic level (P_{n-1}) that is actually consumed (ingested) by a trophic compartment one level up (I_n).

$$\text{Consumption efficiency (CE)} = \frac{I_n}{P_{n-1}} \times 100$$

In the case of secondary consumers, it is the percentage of herbivore productivity eaten by carnivores. Consumption efficiencies of herbivores are very low, reflecting either the difficulty of utilizing plant material or the low herbivore densities.

Assimilation efficiency is the percentage of food energy taken into the guts of consumers in a trophic compartment (I_n) that is assimilated across the gut wall (A_n) and becomes available for incorporation into growth or used to do work. The remainder is lost as feces and enters the base of the decomposer system.

$$\text{Assimilation efficiency (AE)} = \frac{A_n}{I_n} \times 100$$

For herbivores and detritivores, assimilation efficiencies are low (20–50%) while high values are achieved for carnivores (80–90%).

Production efficiency is the percentage of assimilated energy (A_n) that is incorporated into new biomass (P_n). The remainder is entirely lost to the community as respiratory heat.

$$\text{Production efficiency (PE)} = \frac{P_n}{A_n} \times 100$$

Production efficiency varies mainly according to the taxonomic class of the organisms concerned. Invertebrates in general have high efficiencies (30–40%), losing little energy in respiratory heat. Among the vertebrates, ectotherms have intermediate values for PE (around 10%), whilst endotherms, with high energy expenditure associated with maintaining a constant temperature, convert only 1–2% of assimilated energy into production. Similarly, herbivores tend to have higher production efficiency, but lower assimilation efficiency than carnivores.

5.9 Population ecology

A *population* is a collection of individuals of the same species that live together in a region. *Population ecology* is the study of populations (especially population abundance) and how they change over time. It studies the spatial and temporal patterns in the abundance and distribution of organisms and of the mechanisms that produce those patterns. The aims of population ecology are threefold: 1. to elucidate general principles explaining these dynamic patterns; 2. to integrate these principles with mechanistic models and evolutionary interpretations of individual life-history tactics, physiology, and behaviour as well as with theories of community and ecosystem dynamics; and 3. to apply these principles to the management and conservation of natural populations.

5.9.1 Population characteristics

A population has several characteristics or attributes which is a function of the whole group and not of the individual. Different populations can be compared by measuring these attributes. These attributes are population density, natality, mortality, growth forms, etc. The study of the group characteristics or parameters of the population, their changes over time and prediction of future changes is known as **demography**.

Population density

The size of the population is represented by its fundamental property called *density*. Density is expressed as the total number of individuals per unit area or volume at a given time. Two types of densities are described - **crude density** (it is the density per unit total space) and **specific** (ecological) **density** (it is the density per unit of habitat space i.e. available area or volume that can actually be colonized by the population).

Natality

Natality is the ability of individuals of a population to produce new individuals. The natality rate is equivalent to the birth rate in the terminology of human population study (demography). Maximum (sometimes called *absolute* or *physiological*) natality is the theoretical maximum production of new individuals under ideal environmental conditions (i.e. no ecological limiting factors) and is a constant for a given population. *Ecological* or *realized* natality refers to population increase under an actual or specific environmental condition. It is not a constant for a population but may vary with the size and age composition of the population and the physical environmental conditions. Natality is generally expressed as a rate determined by dividing the number of new individuals produced by time (*absolute* or *crude natality rate*) or as the number of new individuals per unit of time and per unit of population (the *specific natality rate*).

Mortality

Mortality refers to the death of individuals in the population. It is equivalent to the death rate in human demography. Like natality, mortality may be expressed as the number of individuals dying in a given period.

Ecological or *realized* mortality is the loss of individuals, under a given environmental condition. Like ecological natality, it is not a constant but varies with population and environmental conditions. A theoretical *minimum mortality*, a constant for a population, represents the loss under ideal or non-limiting conditions. Information of the death and survivor of a population with respect to age is represented in the form of table known as **life table**.

What is really vital for the population is not which members die, but which member survives? Consequently specific mortality rate of a population is expressed by survivorship curve. **Survivorship curves** plot the number of surviving individuals to a particular age. Survivorship curves are of three general types:

A *highly convex curve* (type I) is characteristic of the species in which the population mortality rate is low until near the end of the life span. Many species of large animals such as deer, mountain sheep and man, show such curves.

A *highly concave curve* (type III) is characteristic of those species where the mortality rate is high during the young stages. Oysters or shell fish show this type of curve. In oysters mortality is extremely high during free

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The proportion of individuals in each age group is called the *age structure* of that population. A group of individuals which are all roughly of same age is called a *cohort*. Age distribution influences both natality and mortality of the population. The ratio of various age groups in a population determines the current reproductive status of the population.

The age structure of any population can be classified into three categories, i.e. *Pre-reproductive*, Reproductive and Post-reproductive ages. The relative duration of these age groups in proportion to the life span varies greatly with different organisms. In man, the three ages are relatively equal in length.

Age pyramids

The easiest and most convenient way to represent the age distribution in a population is to arrange the data in the form of age pyramid. An *age pyramid* is a vertical bar graph which represents the number or proportion of individuals in various age groups at any given time i.e. age pyramid is the model representing geometrically the proportions of different age groups in the populations of any organisms. There are three types of hypothetical age pyramids:

Expanding population

In a rapidly growing population birth rate is high and population growth may be exponential, as in the case of the housefly, yeast and alga. Under these conditions each successive generation will be more numerous than the preceding one; as a result, a *pyramid-shaped* age structure results.

Stable population

As the rate of growth decreases and stabilizes, the pre-reproductive and reproductive age groups become more or less equal in size; the post-reproductive groups, remains as the smallest. The graphic representation of this stabilized population is *bell-shaped*.

Diminishing population

If the birth rate is drastically reduced, the pre-reproductive group dwindles in proportion to the reproductive and post-reproductive groups, resulting in an *urn-shaped* age structure. This is representative of a population that is dying off.

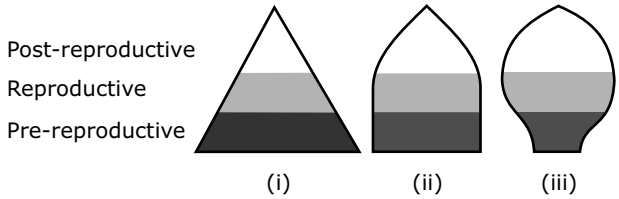

Figure 5.23 Age structure of different types of populations: (i) expanding population (indicating a high percentage of young individuals in a population) (ii) stable population (indicating moderate proportion of young to old individuals) (iii) diminishing population (indicating a low number of young individuals).

Population dispersal

A population is inherently dynamic in nature since individuals are always leaving or entering the populations. But such changes normally do not affect the size of a population. Population dispersal is the movement of individuals into or out of the population or the population area. It occurs in three following ways–

Emigration – one way outward movement of individuals from an area.

Immigration – one way inward movement of individuals into an area.

Migration – periodic departure and return of individuals to the same area.

5.9.2 Population growth

A population is a changing entity. The term population growth refers to the change in the number of individuals in a population with time. Change in population is determined by four factors: birth, death, emigration and immigration. Population growth can be exponential growth or logistic growth. In the simplest case of population growth there is no limitation on growth. In such situations two things occur: the population displays its intrinsic rate of increase and experiences exponential growth.

Exponential growth

A population shows *exponential growth* if all members have abundant resource availability and are free to reproduce at their physiological capacity. This is also known as *geometric growth*. This form of growth may be represented by the simple model based on the exponential equation:

$$\frac{dN}{dt} = rN \text{ with a definite limit on N}$$

Where N = population size,

r = *intrinsic rate of natural increase*. It is described as the maximum potential of reproduction in an individual in a particular set of conditions.

In geometric growth, rate of increase is expressed as a constant fraction or an exponent by which a particular population is multiplied (like 2, 4, 8, 16...). By contrast, a pattern of growth that increases at a constant amount per unit of time (i.e. 1, 2, 3, 4 or 1, 3, 5, 7...) is called *arithmetic growth*.

The integral form of the exponential growth equation is:

$$N_t = N_0 e^{rt}$$

Where, N_t = Population density after time t

N_0 = Population density at time zero

r = Intrinsic rate of natural increase

e = The base of natural logarithms.

The population size that increases exponentially at a constant rate, results in a *J-shaped* growth curve when population size is plotted over time. The J-shaped curve of exponential growth is characteristic of some population that is introduced into a new or unfilled environment.

Logistic growth

The exponential growth assumes resources that are unlimited, but it is never the case in the real situation. As population density increases, each individual has access to fewer resources. It means a particular environment can only support a maximum population size. The number of individuals of a particular species that a particular environment can support indefinitely is defined as *carrying capacity* (represented by the letter K). Carrying capacity is not fixed, but varies over space and time with the abundance of limiting resources. It is determined by various factors including predation, competition and climatic conditions. All factors which limit a population growth are collectively known as the *environmental resistance* to population growth. Since such factors are many and varied, it is clear that the carrying capacity of any area for a population may vary over a period of time. So, the growth of a population eventually slows as the population reaches the carrying capacity for environment. This result in an S-shaped curve of population growth known as *Verhulst-Pearl logistic growth.*

5.11 Succession

Succession is a universal process of directional change in community composition, on an ecological time scale. Succession is a unidirectional progressive series of changes which leads to the establishment of a relatively stable **climax community**. Climax community marks the end point of succession. An intermediate stage found in the successional process advancing towards its climax community is called *seral community* (or sere) and the temporary stage is called a *seral stage.* In most cases more than one seral stage evolves until climax community are attained. Each seral stage is a snapshot of a continuum that is changing over time, but each has its characteristic species composition. Eventually succession slows as the community reaches a steady equilibrium with the environment. Successional changes include structural (changes in species composition and species diversity) and functional (like energy content) changes. In the table 5.7, trends of changes during the early and late stages of succession are described.

Table 5.7 Trends of changes in early and late successional stages.

Attribute	**Early**	**Late**
Community structure		
Total organic matter	Small	Large
Inorganic nutrients	Extrabiotic	Intrabiotic
Species diversity (Richness)	Low	High
Species diversity (Evenness)	Low	High
Biochemical diversity	Low	High
Stratification and spatial heterogeneity	Poorly organized	Well-organized
Life history		
Niche specialization	Broad	Narrow
Size of organism	Small	Large
Life cycles	Short, simple	Long, complex
Nutrient cycling		
Mineral cycles	Open	Closed
Nutrient exchange rate, between organisms and environment	Rapid	Slow
Role of detritus in nutrient regeneration	Unimportant	Important
Selection pressure		
Growth form	r-selection	K-selection
Production	Quantity	Quality
Overall homeostasis		
Internal symbiosis	Undeveloped	Developed
Nutrient conservation	Poor	Good
Stability (resistance to external perturbations)	Poor	Good
Entropy	High	Low
Information	Low	High
Community energetics		
Gross production/community respiration (P/R ratio)	Greater or less than 1	Approaches 1
Gross production/standing crop biomass (P/B ratio)	High	Low
Biomass supported/unit energy flow (B/E ratio)	Low	High
Net community production (yield)	High	Low
Food chains	Linear, predominantly grazing	Weblike, predominantly detritus

Source: The strategy of ecosystem development. Science 164: 262–270, 1969

5.11.4 Model of succession

There are three models to explain the ecological processes of community change during succession. These models are *facilitation model, tolerance model* and *inhibition model.*

Facilitation model

The classical model that explains the mechanisms of succession is the *facilitation model.* According to this model, certain pioneer species with qualities ideal for early succession can colonize the newly exposed landforms after an ecological disturbance. These initial species modify the site, making it more suitable for invasion by other species, for example, by carrying out the earliest stages of soil development. Once established, the later-successional species eliminate the pioneers through competition. This ecological dynamic proceeds through a progression of stages in which earlier species are eliminated by later species, until the climax stage is reached. This model seems to be most appropriate in explaining changes in many primary successions, but less so for secondary successions.

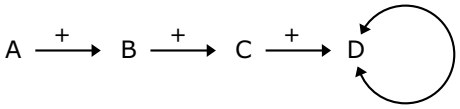

Figure 5.33 Facilitation model. It is based on the assumption that species of a previous stage are replaced by the succeeding stage. And at each stage the species modify their own environment to make it progressively less suitable for themselves and increasingly more suitable for succeeding species. ('+' indicates facilitation and circular arrow indicates that the species replaces itself).

Tolerance model

According to tolerance model, new pioneer species neither inhibit nor facilitate the growth and success of other species. All species in the succession are capable of establishing on a newly disturbed site, although with varying successes in terms of the rapid attainment of a large population size and biomass. In contrast with the facilitation model, the early occupants of the site do not change environmental conditions in ways that favour the subsequent invasion of later-successional species. Rather, with increasing time, the various species sort themselves out through their differing tolerances of the successionally increasing intensity of biological stresses associated with competition. In the tolerance model, competition-intolerant species are relatively successful in early successional stages when site conditions are characterized by a free availability of resources. However, these species are eliminated later on because they are not as competitive as later species, which eventually develop a climax community.

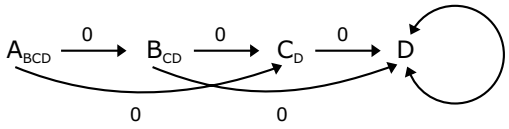

Figure 5.34 Tolerance model. In this model, the presence of early successional species is not essential, that is, any species can start succession. Some species are competitively superior and they eventually predominate in the climax community. Species that are more tolerant of limited resources, replace the other species. Succession proceeds either by the invasion of later species or by a thinning out of the initial colonists, depending on the starting conditions. In the figure, the capital letters A-D represent dominant species and subscript indicate that species are present as minor components. '0' indicates no effect and circular arrow indicates that the species replaces itself.

Inhibition model

A third suggested mechanism of succession is the *inhibition model*. As with the tolerance model, both early and later-successional species can establish populations soon after disturbance. However, some early species make the site less suitable for the development of other species. For example, some plants are known to secrete toxic biochemical into the soil (these are called allelochemicals), which inhibits the establishment and growth of other species.

5.12 Biodiversity

Biodiversity, short for **biological diversity**, refers to all of the population, species and communities in a defined area. In contrast to the more specific term *species diversity* the term *biodiversity* was coined to emphasize the many complex kinds of variations that exist within and among organisms at different levels of organization. The term biodiversity refers to the totality of genes, species and ecosystems of a region.

5.12.1 Levels of biodiversity

Biological diversity includes three hierarchical levels: *Genetic, species* and *ecosystem* diversity

Genetic diversity

Genetic diversity refers to the variation of genes within a species. The differences could be in alleles, in entire genes or in chromosomal structures. The genetic diversity enables the population to adapt to its environment and respond to natural selection. The amount of genetic variation is the basis of speciation. It has a key role in the maintenance of diversity at the species and community levels. Genetic diversity within a species often increases with environmental variability.

Species diversity

Species are distinct units of diversity, each playing a specific role in an ecosystem. Species diversity refers to the variety of species within a region. The simplest measure of species diversity is species richness, i.e. the number of species per unit area. Generally, the greater the species richness, the greater is the species diversity. Number of individuals among the species may also vary, resulting into differences in evenness, or equitability and consequently in diversity.

Species richness and evenness

Species richness is only one aspect of diversity. Not all species exist in equal numbers: some are rare, some are common but not numerous, and others are very abundant. Imagine two forest stands, both of which contain a total of 100 individuals belonging to 5 different species. In one forest stand, there are 20 individuals of each species. In the other, one species has 60 individuals, while each of the other four species has 10 individuals. These two samples differ in a property called *evenness.* The first, in which the species are represented by the same number of individuals, is more even, and thus has one of the essential elements of being more diverse than the second. The species diversity of a community depends on both its richness and its evenness: higher species numbers, with the individuals more evenly distributed among them, contribute to higher community diversity.

Ecosystem diversity

Ecosystems include all the species, plus all the abiotic factors characteristic of a region. For example, a desert ecosystem has soil, temperature, rainfall patterns, and solar radiation that affect not only what species occur there, but also the morphology, behaviour and the interactions among those species. Ecosystem diversity describes the number of niches, trophic levels and various ecological processes that sustain energy flow, food webs and the recycling of nutrients.

5.12.2 Components and gradients of biodiversity

Whittaker (1972) described three terms for measuring biodiversity over spatial scales: alpha, beta and gamma diversity. *Alpha diversity* refers to the diversity within a particular habitat or ecosystem. It is expressed as a number of species per unit area. If we examine the change in species diversity between two ecosystems then we are measuring the beta diversity. We are counting the total number of species that are unique to each of the ecosystems being compared. *Beta diversity* allows us to compare diversity between ecosystems. *Gamma diversity* is a measure of the overall diversity for the different ecosystems within a region.

Imprinting

Behaviours may be **innate**, developmentally fixed and need no prior learning experience, or **learned**, improving over time based on specific experiences through trial and error. Complex innate behaviours are called *instincts which* may involve many behavioural components, as demonstrated in the courtship patterns of most animals and "dancing patterns used by honeybees to communicate the direction and distance of food sources. An individual can modify its behaviour to suit different environmental or social circumstances. Learned behaviour is more flexible and often more complex than innate behavior. Learned behavior derives largely from practice, allowing individuals to modify their behaviour on the basis of their own or others' past experiences.

A type of behaviour that includes both learned and innate components is **imprinting**. A widely applicable definition of imprinting is that it is a learning process that restricts preferences to a specific class of objects. It implies some sensitive period when imprinting can occur. Imprinting is distinguished from other types of learning by having a sensitive period, also called a *critical period*, a limited developmental phase when certain behaviours can be learned. During the sensitive period, the young imprint on their parent and learn the basic behaviours of their species, while the parent learns to recognize its offspring.

Two kinds of imprinting have been extensively studied.

Filial imprinting concerns the development of a social preference of a young animal for its parent(s). The typical example is the young of ducks and geese, which instantly follow their mother almost the first day after they were hatched. This is often called the *following response*. The chicks of these birds imprint on any individual present at hatching time, including a human, and will follow this individual as a mother figure. In species with parental care, the benefits of filial imprinting are obvious because it helps an offspring to attach to its parent.

Sexual imprinting is the process by which young animals learn the characteristics of future mates. The effects of this early learning process become manifest in adult mate choice. The social parents usually serve as templates for the young in the establishment of mate preference. There is also some evidence that siblings may influence the development of mate recognition.

5.13 Environmental pollution

Pollution is the undesirable change in the physical, chemical, or biological characteristics of the air, water, or land that can harmfully affect the health, survival, or activities of human or other living organisms. Any substance introduced into the environment that adversely affects the physical, chemical or biological properties of the environment that have a harmful effect on the ecosystem as a whole is termed *pollutant*. There are three major types of environmental pollution: air pollution, water pollution and soil pollution.

5.13.1 Air pollution

Air pollution may be defined as any atmospheric condition in which *substances* are present at concentrations above their normal ambient levels to produce a *measurable effect* on man, animals, vegetation, or materials. Substances mean any natural or anthropogenic (man-made) chemical compounds capable of being airborne. They may exist in the atmosphere as gases, liquid drops or solid particles.

Types of air pollutants

Air pollutants can be classified as either *primary* or *secondary*.

Primary air pollutants are substances which are directly emitted into the atmosphere from natural and anthropogenic sources, such as ash from a volcanic eruption and the carbon monoxide gas from motor vehicles.

Secondary pollutants are not emitted directly. Rather, they form in the air when primary pollutants react or interact in the atmosphere. Example of a secondary pollutant includes ozone, which is formed when hydrocarbons and nitrogen oxides (NOx) combine in the presence of sunlight.

Criteria pollutants

Criteria pollutants are those air pollutants for which ambient air standards have been defined to protect human health and welfare. Criteria air pollutants are six major pollutants defined by EPA (Environmental Protection Agency). These are carbon monoxide, lead, nitrogen dioxide, ozone, particulate matter and sulfur dioxide.

Carbon monoxide : CO is a colourless, odourless and tasteless gas and the most abundant of the criteria pollutants. It is produced as a result of incomplete combustion of carbonaceous fuels. Incomplete combustion yields CO instead of CO_2. Vehicular emissions are the major source of CO emission. It comprises ~70% of total CO emissions. CO has greater affinity for hemoglobin than does O_2. Hence, it interferes with the blood's ability to carry O_2 to the cells of the body. It readily binds with hemoglobin to form *carboxyhemoglobin*.

Ozone : Tropospheric ozone (O_3) is a secondary air pollutant. Sources of ozone in the troposphere are both natural and anthropogenic. Natural sources are responsible for an average background surface ozone concentration of 20–30 ppb. Ozone is produced during the NO-catalyzed oxidation of hydrocarbons in the presence of sunlight. NOx and hydrocarbons initiate a complex set of reactions that produce a number of secondary pollutants known as *photochemical oxidants*. Ozone is the most abundant photochemical oxidant and a major constituent of **photochemical smog**. Smog is a term that was derived from smoke and fog. Photochemical smog is also known as *Los Angeles smog*. It requires neither smoke nor fog. This type of smog has its origin in the nitrogen oxides and hydrocarbon emitted by automobiles and other sources, which then undergo photochemical reactions in the lower atmosphere. The highly toxic gas ozone arises from the reaction of nitrogen oxides with hydrocarbon in the presence of sunlight, and some nitrogen dioxide is produced from the reaction of nitrogen oxide with sunlight. Other major components of photochemical smog are formaldehyde, acrolein, peroxyacetyl nitrate (PAN) and peroxybenzoyl nitrate. The resulting smog causes a light brownish colouration of the atmosphere, reduced visibility, plant damage, irritation of the eyes, and respiratory distress. Photochemical smog has a high concentration of oxidizing agents and is, therefore, called as *oxidizing smog*.

The second type of smog is *sulfurous smog,* which is also called *London smog*. It results from a high concentration of sulfur oxides in the air and is caused by the use of sulfur-bearing fossil fuels, particularly coal. Chemically, it is a reducing mixture and so it is also called as *reducing smog*.

Oxides of nitrogen : Two important oxides of nitrogen (NOx) considered as air pollutants are nitric oxide (NO) and nitrogen dioxide (NO_2). Almost all NOx emissions occur in the form of NO. NO can oxidise to NO_2. NO_2 may react with hydrocarbons in the presence of sunlight to form *photochemical smog*. NO_2 also reacts with OH radical to form nitric acid (HNO_3).

Particulate matter : Atmospheric particulate matter is defined as solid or liquid dispersed matter which is smaller than about 500μm. Particulate matters which are less than 10 microns in diameter are known as PM10. Solid particles are termed *dusts* if they are generated by grinding or crushing operations. Solid particles are called *fumes* if they are formed when vapours condense whereas liquid particles may be called *mist*. Smoke and soot are terms used to describe particles composed primarily of carbon that results from incomplete combustion.

Sulfur dioxide : Major source of sulfur dioxide emissions are the fossil fuel combustions. Sulfur dioxide, once released, can be converted to SO_3. SO_3 reacts very quickly with water to form sulfuric acid, which is the principal cause of acid rain.

Lead : Lead is emitted to the atmosphere primarily in the form of inorganic particulates. Most lead emission occurred in the past from motor vehicles as a result of burning of gasoline containing the antiknock additive, tetraethyl lead.

Air quality standards

EPA has established two levels of national ambient air quality standards: primary and secondary. *Primary standards* are required to be set at levels that will protect public health including the most sensitive individuals. *Secondary standards* are established to protect public welfare (e.g. crops, animals, structures). Secondary standards are meant to be more stringent than primary standards.

5.13.2 Greenhouse effect

Various gases in the atmosphere absorb incoming short wave solar radiation and long wave or thermal radiation radiated by the Earth. The ability of gases to absorb radiation varies with the wavelength. All of the incoming solar radiation with wavelengths less than 0.3 μM is absorbed by oxygen and ozone. This absorption occurs in the stratosphere. Most of the long wavelength radiation (greater than 4 μM) radiated by the Earth is absorbed by atmospheric gases, most importantly water vapour, CO_2, N_2O and CH_4. Outgoing radiation between wavelength 7 and 12 μM easily passes through the atmosphere. This region is referred to as the *atmospheric radiative window*.

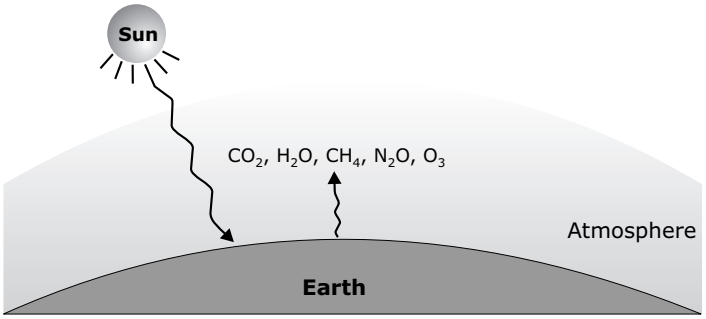

Figure 5.38 Greenhouse gases trap long wavelength energy from the earth's surface, heating the atmosphere, which, in turn, heats the earth.

Radiatively active gases that absorb wavelengths longer than 4 μM are called *greenhouse gases*. These gases absorb most of the outgoing thermal radiation attempting to leave the Earth's surface. This absorption heats the atmosphere, which in turn, radiates energy back to the Earth. The green house gases act as a thermal blanket around the Earth, raising the Earth's temperature. This effect is known as **greenhouse effect**. The greenhouse effect is a natural phenomenon. It is responsible for Earth having an average near surface air temperature 33°C warmer than it would have if it did not have greenhouse gases in the troposphere.

Global warming

Global warming is defined as the increase in the average temperature of the Earth. Over the last 100 years, the average temperature of the air near the Earth´s surface has risen a little less than 1° Celsius (0.74 ± 0.18°C). The rate of warming over the last 50 years is almost double that for the period 1906-2005 as a whole. Increasing greenhouse gas concentrations resulting from human activity such as fossil fuel burning and deforestation is mainly responsible for the observed temperature increase.

An increase in global temperature will cause sea levels to rise and will change the amount and pattern of precipitation, probably including expansion of subtropical deserts. The continuing retreat of glaciers, permafrost and sea ice are expected with warming being strongest in the Arctic. Other likely effects include increases in the intensity of extreme weather events, species extinctions and changes in agricultural yields.

Chapter 06

Evolution

6.1 Origin of Life

Abiogenesis

Abiogenesis is the generation of life from non-living matter. Abiogenesis is now more precisely known as *spontaneous generation*. This theory states that complex living organisms are generated from decaying organic substances e.g. organism like mice spontaneously appears in stored grain or maggots spontaneously appear in meat.

Francesco Redi, an Italian physician, was the first who disproved the *Theory of Spontaneous Generation* by performing a controlled experiment. In 1668, Redi performed an experiment to check whether maggots really came from decaying meat. He did this by placing meat in a number of jars and covering half of them with fine gauze while leaving the others uncovered. Maggots developed only on the meat in the uncovered jars. From this, Redi concluded that the maggots did not come from the meat, but from tiny eggs that flies had laid on the meat. Since flies could not land on the meat in the covered jars, they could not lay eggs on that meat, and no maggots formed. Therefore, decaying meat could not produce maggots.

Later, *Lazzaro Spallanzani* (an Italian naturalist) performed a similar experiment with broth. He put broth into two glass flasks and sterilized them by boiling the flask containing broth. One of the flasks was left open to the air. The other flask was sealed up to keep out any organisms that might be floating in the air. Microorganisms developed only in the uncovered flask. From this, Spallanzani concluded that the microorganisms did not come from the broth, but were in the air that entered the flask.

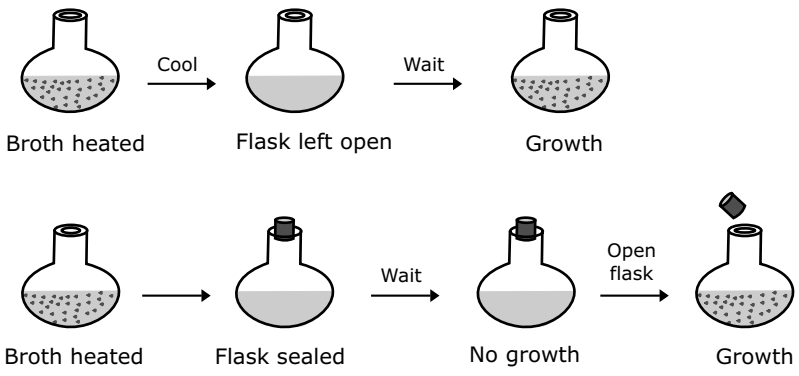

Figure 6.1 Sterilized broth in open flask developed microbes whereas in sealed flask microbes not developed.

Unfortunately, many scientists were not convinced by his experiment. *Louis Pasteur,* a French chemist, finally disproved the *Theory of Spontaneous Generation* in the mid 1800's. He performed the same type of experiment as Spallanzani. Louis Pasteur, however, allowed air to enter into the flask of sterile broth.

He performed experiments with two flasks – one with a straight neck and other with S-shaped neck. Flask with a straight neck allowed both air and microorganisms to enter whereas the other flask with S-shaped neck allowed only air to enter but not microorganisms. The broth in the straight neck flask became contaminated with microorganisms but the broth in the flask with an S-shaped neck did not become contaminated. Therefore, Louis Pasteur showed that even though air could get in the flask, the broth did not produce microorganisms.

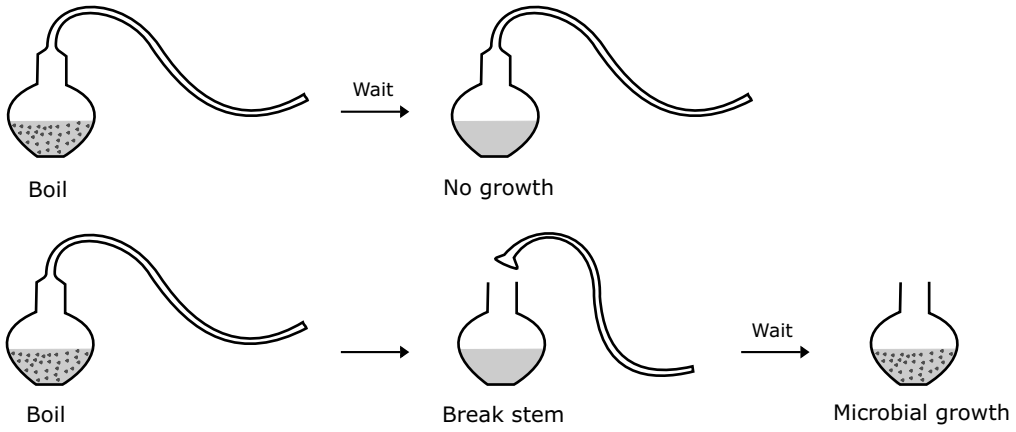

Figure 6.2 No microbial growth occurs in flask with an S-shaped neck whereas microbes growth occurs in flask with broken stem.

Scientists finally were convinced that living things, no matter how small, do not come from nonliving things. The present theory of where living things come from is called **biogenesis**. This theory states that living things come only from other living things. For example, mice come only from mice, and microorganisms such as bacteria can only come from other bacteria. Since spontaneous generation was now proved incorrect, many scientists began to wonder *how life started on the Earth*. Oparin and Haldane attempted to answer this question. They proposed that life had arisen from simpler molecules on the lifeless earth under much different atmospheric conditions than exist today. However, instead of life arising suddenly, as previous spontaneous generation theories proposed, Oparin and Haldane believed that it occurred over a very long period of time.

Chemical evolution

Our current understanding of conditions on prebiotic Earth and the idea of a gradual chemical evolution toward life were first proposed independently by A.I. Oparin and J.B.S. Haldane. The Oparin-Haldane model suggested that under the strong reducing conditions theorized to have been present in the atmosphere of the early Earth (between 4.0–3.5 billion years ago), inorganic molecules would spontaneously form organic molecules (simple sugars and amino acids). Oparin argued that a *primeval soup* (or Primordial soup) of organic molecules could be created in an oxygenless atmosphere through the action of sunlight. These would combine in evermore complex ways until they formed coacervate droplets. These droplets would *grow* by fusion with other droplets, and *reproduce* through fission into daughter droplets.

Around the same time in the year 1929, *J.B.S. Haldane* published a paper in which he proposed that the Earth's prebiotic oceans would have formed a *hot dilute soup* in which organic compounds could have formed.

In 1953, *Stanley Miller*, along with his graduate advisor *Harold Urey*, tested this hypothesis by constructing an apparatus that simulated the Oparin-Haldane *early earth*.

6.3 Evidences of evolution

Evidences supporting the evolution model come from multiple, distinct areas of biology and geology. These pieces of evidence can be grouped into four categories:

- Direct observation of evolutionary change
- Homology and development
- Vestigial traits
- Fossil record and biogeography

Direct observation of evolutionary change

Small-scale evolution can be observed in nature or generated experimentally in the laboratory. The classic story of the *peppered moth* in Britain during the industrial revolution is an example of microevolution. *Grant* and *Grant* have recorded evolutionary change in beak shape within and among populations of Darwin's finches, over periods as short as two years.

We can also drive genetic change in laboratory populations of *Drosophila* and other model organisms in the laboratory. By selecting certain phenotypic traits, we can generate directional change in characteristics such as abdominal bristle number, lifespan and avoidance to certain chemicals. Artificial selection experiments are also the foundation of agricultural improvement over the past 10,000 years.

Homology and development

One of the most compelling lines of evidence supporting the common ancestry of species is that of similar structural elements across functionally diverse forms. It becomes evident from *comparative anatomy.* We find the same bones in many different types of animals, but these bones are often modified to do different things. The hopping legs of the frog contain the same bones as our own legs, but the frog's legs are highly modified to fulfill a different function (hopping). The wing of a bird and the forelimb of a bat contain exactly the same bones as the arm of a human, but the size, shape, and even internal structure of these bones are all adapted to play a different role in each animal.

We call structures like the wings of a bird and the forelimbs of a bat **homologous structures**. Homologous structures are structures that are derived from a common ancestor. Even if they are superficially different, they are developmentally related. Homology does not mean that these structures must share the same function. A character shared between two species but not present in their common ancestor is called **homoplasy**.

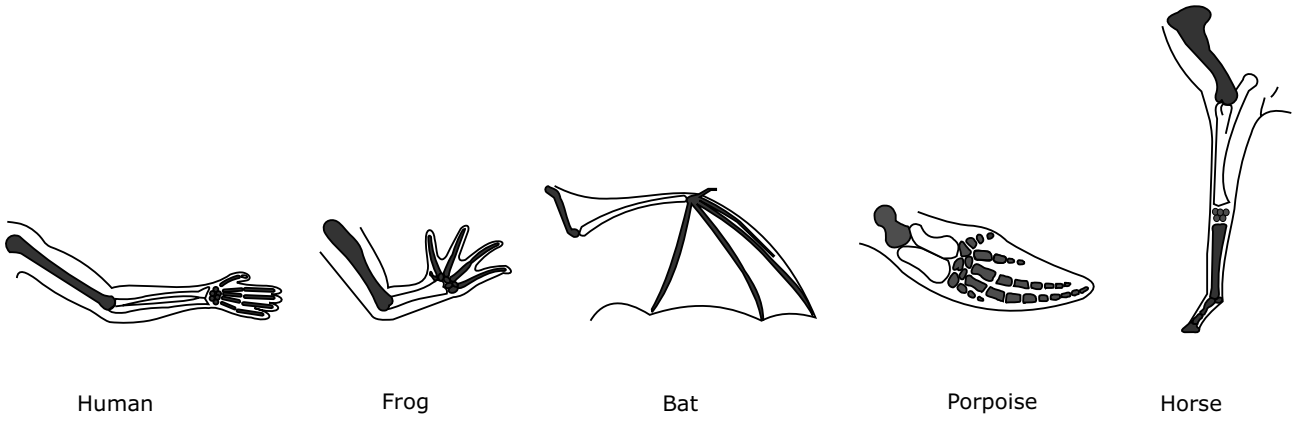

Figure 6.8 Homology between the forelimbs of four mammals and a frog, showing the ways in which the proportions of the bones have changed in relation to the particular way of life of the organism.

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Problem

Whether the limbs modified into wings of bats and the wings of birds is an example of evolutionary analogy or homology? What about whale fins compared to fish fins?

Solution

Bat and bird wings have the same function and the same origin (they are modified limbs) so they are analogous and homologous organs. Whale fins are a modification of the posterior limbs while fish fins although having the same function, do not come from modified limbs; so they are analogous but not homologous structures.

6.4 Natural selection

Selection is a composite of all the forces that cause differential survival and differential reproduction among genetic variants. When the selective agencies are primarily those of human choice, the process is called *artificial selection*. When the selective agencies are not those of human choice, it is called *natural selection.* Natural selection is the nonrandom process by which biological traits become more or less common in a population as a function of the differential reproduction of their bearers or differences in the rate of survival. Natural selection can act on any heritable phenotypic trait and operate among any entities that reproduce, show inheritance of their characteristics from one generation to the next, and vary in fitness.

Natural selection is the machine that drives evolution. Some evidence of natural selection has been seen in nature, but not to an extent that would change a species in any meaningful way.

Evidences of natural selection

Industrial melanism

Industrial melanism is a phenomenon used to describe the evolutionary process in which initially light coloured organism's population become dark as a result of natural selection. Industrial melanism affected over 70 species of moths in England. It has been best studied in the peppered moth, *Biston betularia*. Prior to 1800, the typical moth species had a light colour pattern. Dark coloured or melanic moths were rare. During the industrial revolution, soot and other industrial wastes darkened tree trunks and killed off lichens. Because light coloured peppered moths rely on camouflage to avoid predation, this sudden change in their environment made them highly vulnerable to predators. In course of time, the light-coloured moth became rare and the dark coloured moth became abundant. The cause of this change was thought to be selective predation by birds, which favoured camouflage colouration in the moth. By 1886, dark coloured (melanic) were far more common — illustrating rapid evolutionary change.

Evolution of drug resistant HIV

The evolution of drug resistance in HIV also provides an example of natural selection. Researchers have developed numerous drugs to combat this pathogen. Drug 3TC (lamivudine) is one of them. It is a nucleoside inhibitor and acts as a molecular analog of cytidine. When reverse transcriptase places a 3TC molecule, instead of a C, in a replicating DNA chain, chain elongation is stopped and the reproduction of HIV is also stopped. The 3TC resistant varieties of HIV carry slightly different versions of reverse transcriptase that are able to discriminate between the drug and the normal C nucleotide. The viruses that carry these genes have no advantage in the absence of 3TC; in fact, they replicate slowly than those that carry the most common form. But once 3TC is added to their environment it becomes a powerful selecting force, favouring reproduction of resistant individuals. Here drug does not create resistant HIV; it selected resistant HIV that was already present in the population. The increase in the frequency of drug-resistant HIV is almost certainly driven by natural selection.

Punctuated equilibria and phyletic gradualism

According to the theory of *punctuated equilibrium* (evolution by jerks), evolution proceeds relatively rapidly during speciation: between speciation events, the population remains relatively constant in a condition called *stasis*. Stasis is broken by rare events of large net change, characterized by rapid events of branching speciation called cladogenesis. It considers that most sexually reproducing species will show little change for most of their geological history. When phenotypic evolution occurs, it is localized in rare events of branching speciation and occurs relatively quickly compared to the species full and stable duration on earth.

Theory of punctuated equilibrium is commonly contrasted against the hypothesis of *phyletic gradualism*, which states that most evolution occurs uniformly and by the steady and gradual transformation of whole lineages (anagenesis). According to phyletic gradualism:

- Evolution occurs at a constant rate.
- New species arise by the gradual transformation of ancestral species.

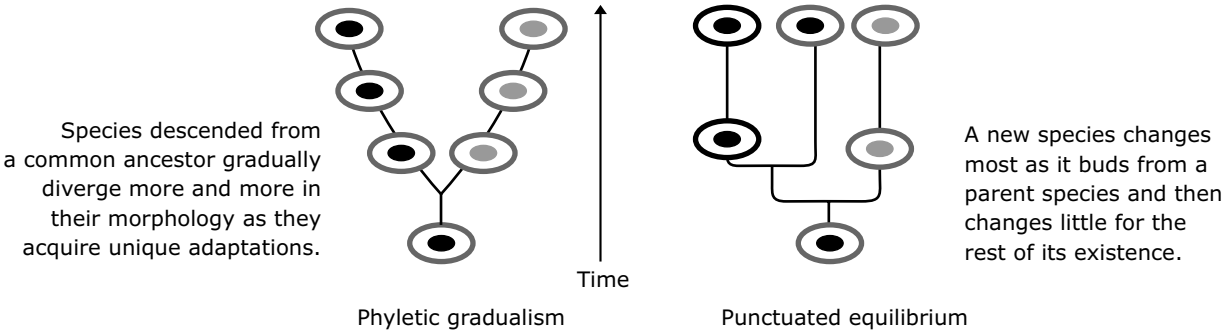


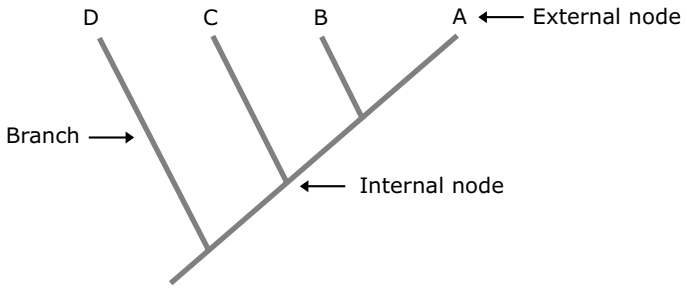
Figure 6.12 Phyletic gradualism and punctuated equilibrium.

6.10 Molecular phylogeny

Phylogenetics is the science of estimating and analyzing evolutionary relatedness among various group of organisms. *Molecular phylogenetics* is the use of the structure of molecules to gain information on an organism's evolutionary relationships. Nucleic acids (DNA and RNA) and proteins are 'information molecules' in that they retain information of an organism's evolutionary history. The approach is to compare nucleic acid or protein sequences from different organisms using computer programmes and estimate the evolutionary relationships based on the degree of homology between the sequences. Nucleic acids and proteins are linear molecules made of smaller units called nucleotides and amino acids, respectively. The nucleotide differences within a gene or amino acid differences within a protein reflect the evolutionary distance between two organisms. In other words, closely related organisms will exhibit fewer sequence differences than distantly related organisms.

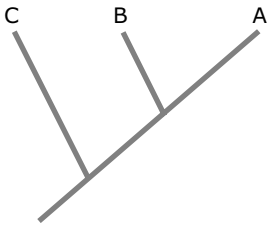
Phylogenetic tree

In phylogenetic studies, the most convenient way of visually presenting evolutionary relationships among a group of organisms is through illustrations called *phylogenetic trees*. Phylogenetic tree is represented by *lines* and *nodes*. Nodes can be internal or external (terminal). The different sequences of DNA/proteins, which are compared, are located at *external nodes* but are connected via branches to *interior nodes,* which represent ancestral forms for two or more sequences. *Branch* defines the relationship between the sequence of DNA/proteins in terms of descent and ancestry. The length of the branches indicates the degree of difference between the sequence represented by the nodes. The branch lengths are proportional to the predicted evolutionary time between organisms or sequences. The branching pattern of the tree is termed as *topology*.

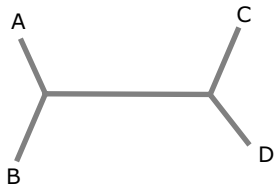


An evolutionary tree

A phylogenetic tree may be *rooted* or *unrooted.* A *rooted tree* infers the existence of a common ancestor and indicates the direction on the evolutionary process. A rooted tree in which every node has two descendants is called a *binary tree*. An *unrooted tree* does not infer a common ancestor and shows only the evolutionary relationships between the organisms.



Rooted tree

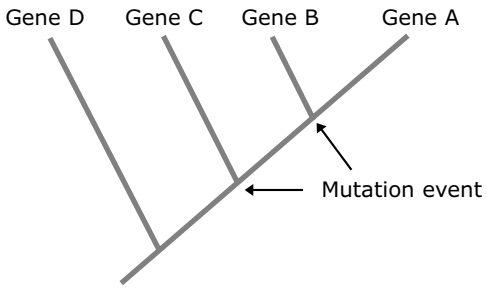


Unrooted tree

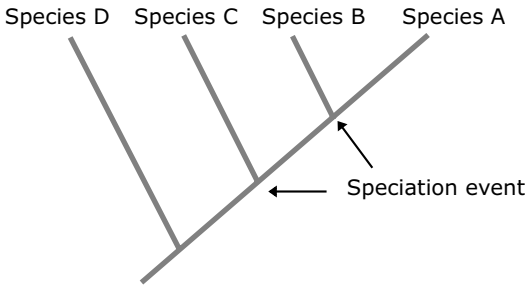
Gene trees versus Species trees

A *gene tree* is a model of how a gene evolves through duplication, loss and nucleotide substitution. As a gene at a locus in the genome replicates and its copies are passed on to more than one offspring, branching points are generated in the gene tree. A *species tree* depicts the pattern of branching of species lineages via the process of speciation. Speciation occurs by the population of the ancestral species splitting into two groups that are unable to interbreed.

An internal node in a gene tree indicates the divergence of an ancestral gene into two genes with different DNA sequences, usually resulting from a mutation of one sort or another. An internal node in a species tree represents what is called a *speciation event*, whereby the population of the ancestral species splits into two groups are no longer able to interbreed. These two events, mutation and speciation, do not always occur at the same time.



An evolutionary gene tree



An evolutionary species tree

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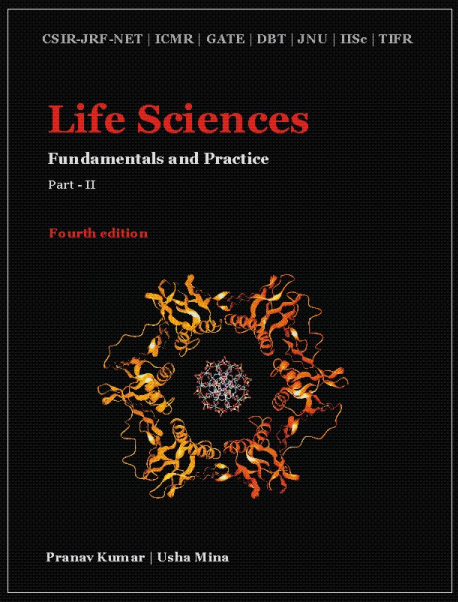

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