CHAPTER 1

Historical Perspectives and Milestones

CHAPTER OUTLINE

INTRODUCTION

The term *biotechnology* represents a fusion or an alliance between biology and technology. It is a newly discovered discipline for old-age practices. Biotechnology, as an integral component of biosciences, has derived its strength from different disciplines of science and technology. It is an interdisciplinary pursuit with multidisciplinary applications.

DISCOVERY OF CELL AND CHROMOSOME THEORY OF INHERITANCE

Robert Hooke is credited with the discovery of cell. An important discovery made by Robert Brown (1831) was the presence of a small sphere within the cells of orchid roots. In 1839, Hugo von Mohl and J. Purkinje named the jelly-like substance as *protoplast*. In 1885, Virchow explained that cells are derived from pre-existing cells.

In 1902, cytologists Walter Sutton and Theodor Boveri independently came to the conclusion that the behaviour of chromosomes at meiosis can serve as the cellular basis of both segregation and independent assortment. The theory of heredity of chromosomes was further expanded by Thomas H. Morgan on fruit fly drosophila. The term *factor* as the basic unit of inheritance was replaced with the term *gene* by Johannsen in 1909. The term *mutation* was coined in 1901 by Hugo de Vries to explain the variation he observed in the plant *Oenothera lamarckiana* (primrose).

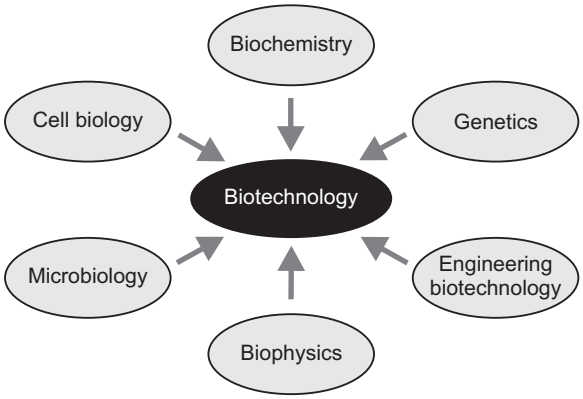


Figure 1.1 Basic strength for advancement of biotechnology.

The origin of biotechnology dates back to 6000 BC, the same year when the yeast was first used to produce beer and wine. The history of biotechnology begins with zymotechnology, which commenced with a focus on brewing techniques for beer. By World War I, zymotechnology expanded to cover larger industrial issues, and the potential of industrial fermentation gave rise to biotechnology. Yoghurt was produced from bacteria. Louis Pasteur, also known as the *father of biotechnology,* identified the role of microorganisms in fermentation. The biotechnological revolution began in the late 1970s and early 1980s when scientists understood the genetic constitution of living organisms. Biotechnology, as an applied bioscience, has been effectively utilized for better industrial therapeutic production. As a matter of fact, every discipline of science has contributed either directly or indirectly to the growth of biotechnology (Fig. 1.1).

MAJOR BRANCHES OF BIOTECHNOLOGY

Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents—microorganisms, plants and animal cells. Biotechnology can be represented as a mixture of various biological sciences (biosciences) for better service in the field of pharmaceuticals (Fig. 1.2).

Agricultural Biotechnology

It deals with a group of scientific techniques that are used to create, improve or modify plants and animals. It encompasses the knowledge of biosciences concerned with plants and agriculture, such as plant tissue culture, production of haploid plants, somaclonal variation, micropropagation cryopreservation methodology in the genetic engineering of plants, application of transgenic plants, etc.

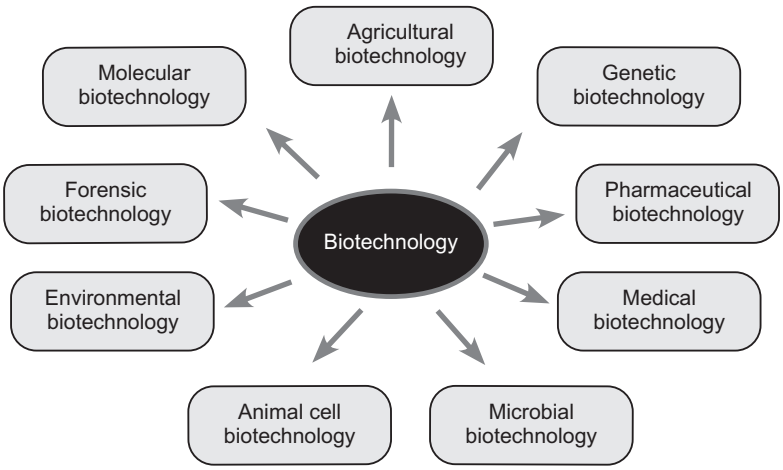


Figure 1.2 Branches of biotechnology.

Forensic Biotechnology

It is the method for detecting unique DNA pattern of organism, and it includes DNA fingerprinting.

Medical Biotechnology

It deals with the utilization diagnostic kits for the detection of different diseases. It has wide applications in human health care, and it includes information related to gene therapy, DNA in disease diagnosis, fingerprinting, specialized products of DNA biotechnology, etc.

Pharmaceutical Biotechnology

It is a major branch of biotechnology, which includes production of therapeutic proteins and hormones, fermentation products such as antibiotics, vaccines and drugs, etc.

Environmental Biotechnology

It encompasses the information on biotechnology with references to environment such as environment pollution, treatment modalities, etc.

Microbial Biotechnology

The information pertaining to fermentation technology, downstream processing, enzyme technology, microbial production of organic solvents, organic acids, antibiotics, amino acids,

vitamins, foods and beverages, polysaccharides, biomass–bioenergy, etc., is covered under microbial biotechnology.

Biotechnology has several other branches also, with varied applications, such as molecular biotechnology, aquatic biotechnology, animal biotechnology, environmental biotechnology, leather biotechnology, mining and metal biotechnology, textile biotechnology, genetic engineering, etc.

ADVANCES IN BIOTECHNOLOGY

With the advent of information technology, modern technologies give rise to genomics, proteomics and cellomics that promise to develop into the technology with applications in medicine, food, agriculture, etc. The present focus of biotechnology is centred basically on genomics and postgenomic. Since 2001, different methods have been developed enabling rapid sequencing of more than 50 microbial, plant, animal and human genomes.

Newer frontiers in biotechnology include development of nucleic acid probes, production of monoclonal antibodies and molecular markers. Development of microbial inoculants covering biopesticides, biofertilizers and genetic modifications of microbes through DNA recombination are other important applications. Novel approaches such as proteomics and structure biology are contributing to the understanding of chemistry of life and disease.

Different techniques of population genetics and biometric analysis are complemented with modern methods in reproductive biology and gene diagnosis. Artificial insemination, in vitro fertilization and embryo transfer are important applications of biotechnology in animal breeding.

SIGNIFICANT MILESTONES IN BIOTECHNOLOGICAL RESEARCH

During the 20th century, the pharmaceutical industry witnessed a series of developments in science and technology, which had generated new opportunities for biotechnological discoveries. The important historical developments in biotechnology, recent milestones in biotechnology and chronological sequences of important biotechnological products (drugs, vaccines and other therapeutics) are listed in Tables 1.1, 1.2 and 1.3, respectively.

HISTORICAL CONTRIBUTIONS OF SOME LEADING SCIENTISTS

1. Antony van Leeuwenhoek (1632–1723) is the greatest of all microscopists. He was the first to observe and accurately describe the shape of human red blood cells. He observed and

Table 1.1 Important historical developments in biotechnological research

Year	Scientist	Discovery
1928	Alexander Fleming	Discovery of penicillin from common moulds
1944	Avery, MacLeod and McCarty	Identification of DNA as the genetic material
1953	Watson and Crick	Determination of DNA structure
1958	Meselson and Stahl	Semiconservative application of DNA
1961	Jacob and Monod	Lac operon model for gene regulation
1972	Khurana, et al.	Synthesis of *tRNA* gene
1975	Kohler and Milstein	Production of monoclonal antibodies
1976	Sanger and Gilbert	Techniques to develop DNA sequence
1978	Joshua Lederberg	Production of insulin in *E. coli*
1983	Kary Mullis	First artificial chromosome synthesized
1989	UC Davis and researchers	Recombinant vaccine against deadly rinderpest virus
1998	Thomson and Gearhart	Technique for culturing embryonic stem cells
2010	Craig Venter	Creation of first self-replicating synthetic bacterial cell

Table 1.2 Recent milestones in biotechnology

Year	Discovery
1977	First genome sequence
1983	Use of Ti plasmids to genetically transform plants
1987	Gene transfer by biolistic transformation
1988	Development of polymerase chain reaction (PCR)
1990	Official launching of human genome project
1994–95	Genetic and physical maps of human chromosome elucidated
1996	First eukaryotic organism sequence
1997	First mammalian, Dolly (a sheep), developed by nuclear cloning
2000	First plant genome sequence (*Arabidopsis thaliana*)
2001	Human genome, the first mammalian genome sequence
2002	First crop plant genome sequenced
2003	Mouse genome, the experimental model to men, sequenced
2005	Artificial cell for use in nanotechnology, nanobiotechnology, blood substitutes, regenerative medicine and gene therapy
2006	Artificial cell encapsulated bone marrow stem cells regenerated liver resulting in recovery

Table 1.3 Chronological sequence of important biotechnological products (drugs, vaccines and other therapeutics)

Year of discovery	*Product*	*Company name*
1982	Humulin	Eli Lilly and Company
1986	Digibind	Burroughs Wellcome
	Roferon-A	Hoffmann-La-Roche
	Intron-A	Schering-Plough
	Recombivax HB	Merck
1989	Engerix-B	SmithKline Beecham
1990	Actimmune	Genetech
1991	Leukin	Immune
1995	Follitropin alpha	Ares Serono
	Betaferon	Schering AG
1996	Humalog	Eli Lilly
1997	Com hep A and B vaccines	GlaxoSmithkline Biologicals
1998	Simulect	Novartis Pharmaceuticals
1999	Procomvax	Sanofi Pasteur MSD
	Intron A	SP Europe
2000	ViraferonPeg	SP Europe
	Insulin glargine	Aventis Pharma Deutschland GmbH
	Hexavac	Pasteur Merieux MSD
	Luveris	Merck Serono Limited
	Fasturtec	Sanofi-Synthelabo
	Metalyse	Boehringer Ingelheim International GmbH
2001	Tenecteplase	Boehringer Ingelheim International GmbH
	Nespo	Dompe Biotec S.p.A.
	Aranesp	Amgen Europe B.V.
	Nonfact	Sanquin
	Replag	TKT Europe-5S AB
2002	Anakinra	Amgen Europe B.V.
	Neulasta	Amgen Europe B.V.
	Pegfilgrastim	Novatech Biopharmaceutical Co Ltd
	Neupopeg	Dompe Biotec S.p.A.
	Ambirix	GlaxoSmithKline
	Ultratard	Novo Nordisk
	Actraphane	Novo Nordisk
	Forsteo	Eli Lilly and Company
	Teriparatide	Eli Lilly and Company
	Somavert	Pfizer Limited

(Continued)

Year of discovery	Product	Company name
2003	Zevalin	Schering AG
	Advate	Baxter AG
	Humira	Abbott Laboratories
2004	Trudexa	Abbott Laboratories
2005	Fendrix	GlaxoSmithKline
	Comb vaccine	GlaxoSmithKline Biologicals
2010	Coagulation factor VIIa	EMD Biosciences

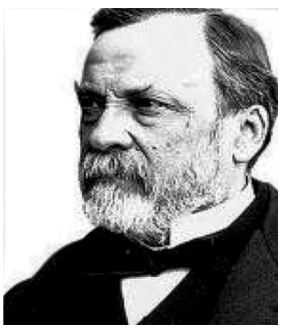
measured a large number of minute living organisms, including bacteria and protozoa and communicated them to the Royal Society of London (1684).

2. John Needham (1713–81) was the greatest supporter of the theory of spontaneous generation. Spontaneous generation is the hypothesis that some vital force contained in or given to organic matter can create living organisms from inanimate objects.
3. Louis Pasteur (1822–95) first demonstrated that air contains microscopically observable organized structures. He passed large quantities of air through a tube that contained a plug of guncotton to serve as filter. The guncotton was then removed and dissolved in a mixture of alcohol and ether, and the sediment was examined under a microscope. He found that this sediment contains not only organic matter, but also large number of small microorganisms.

   Louis Pasteur opened the field of sterilization by stating that boiling rendered fluid sterile. He introduced the method of sterilizing glassware by dry heat at 170°C. Louis Pasteur in 1880 isolated the bacterium responsible for chicken cholera and grew it in pure culture. He invented the vaccines for anthrax and rabies. He knew that the causative agent of rabies attacks the brain and spinal cord.

Antony van Leeuwenhoek

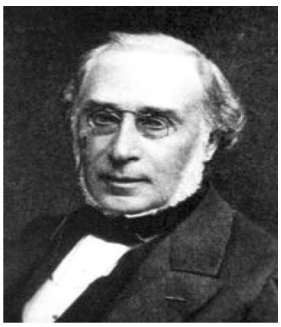
John Needham



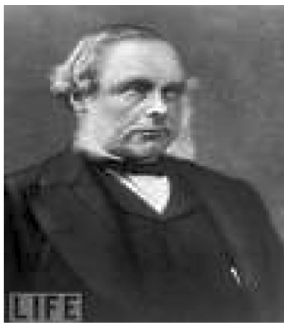
Louis Pasteur

4. Augustino Bassi (1773–1856) presented convincing evidence that living organism was the cause of disease. He demonstrated that a fungus that caused a disease in silkworm could be transmitted from one silkworm to another.

5. Lord Joseph (1827–1912), a famous English surgeon, first introduced antiseptic for the prevention and cure of wound healing.
6. Robert Koch (1843–1910) was a German physician who isolated *Bacillus anthracis*, the causative agent of anthrax. He was one of the first scientists to demonstrate the role of bacteria in causing diseases.



Augustino Bassi



Lord Joseph

Robert Koch

7. Richard Petri (1852–1921) designed a special plate to hold solid culture media. This plate has great significance in microbiology and is referred to as *Petri plate*.
8. Paul Ehrlich (1904) found that the dye trypan red was active against trypanosomes that cause African sleeping sickness. This dye with antimicrobial activity was referred to as a *magic bullet*.
9. Sahachiro Hata (1909) introduced the drug salvarsan for the treatment of syphilis caused by *Treponema pallidum.*

Richard Petri

Paul Ehrlich

Sahachiro Hata

The period from 1857 to 1914 is considered as the *Golden Age of Microbiology,* because significant advances made during this period led to the establishment of microbiology as an important discipline of science.

The discovery of microorganisms responsible for wide-spectrum ailments in human beings had been a significant contribution of microbiologists across the globe. It paved way for pinpointed

Table 1.4 Milestones in discovery of causative agents

Year	Causative agent	Discoverer	Disease
1874	*Mycobacterium leprae*	Hansen	Leprosy
1877	*Actinomyces bovis*	Bollinger	Actinomycosis
1879	*Neisseria gonorrhoeae*	Albert Neisser	Gonorrhoea
1880	*Salmonella typhi*	Eberth	Typhoid fever
1880	*Plasmodium* spp.	Laveran	Malaria
1882	*Mycobacterium tuberculosis*	Robert Koch	Tuberculosis
1883	*Vibrio cholerae*	Robert Koch	Cholera
1885	*Clostridium tetani*	Arthur Nicolaier	Tetanus
1894	*Yersinia pestis*	Alexander Yersin	Plague
1900	Avian influenza virus	WHO	Bird flu
1906	*Bordetella pertussis*	Border and Gangou	Whooping cough
1983	Human immunodeficiency virus	Luc Montagnier	AIDS
1983	*Helicobacter pylori*	R. Warren and B. Marshall	Gastritis
1984	Human T-cell virus	Robert, et al.	Leukaemia
2003	SARS coronavirus	Carlo Urbani	Respiratory disease

Abbreviations: SARS, severe acute respiratory syndrome; WHO, World Health Organization.

Table 1.5 Discovery of antibiotics

Year	Source	Discoverer	Antibiotics
1929	*Penicillium notatum*	Alexander Fleming	Penicillin
1944	*Streptomyces griseus*	Walksman, et al.	Streptomycin
1947	*Streptomyces venezuelae*	P.R. Burkholder	Chloramphenicol
1949	*Streptomyces fradiae*	Waksman and Lechevalier	Neomycin
1950	*Streptomyces noursei*	Hazen and Brown	Nystatin
1956	*Streptomyces nodosus*	Gold, et al.	Amphotericin A
2000	*Micromonospora* spp.	Fernandez-Chimeno, et al.	Cytotoxic macrolide, IB-96212

research, which ultimately resulted in the discovery of wide range of antibiotics. The chronologically ordered milestones in discovery of causative agents and discovery of antibiotics are given in Tables 1.4 and 1.5, respectively.

Gerhardt Domagk found that prontosil—an azo dye from *para*-aminobenzene-sulphonamide—was active against specific bacteria. The first success in treating streptococcal infection was reported in 1935. Sir Alexander Fleming accidentally discovered a substance produced by *Penicillium notatum*. He extracted a compound from the fungus and named it *penicillin,* which could destroy several pathogenic bacteria. The commercial production of penicillin was taken up by US firms in 1941 during World War II. S.A. Waksman (1944) reported production of streptomycin from two different strains of actinomycetes.

GENETIC EVALUATION

Historical Perspective

Gregor Johann Mendel (1900) is known as the *father of genetics*. W. Bateson and R.C. Punnet (1906) reported the first case of linkage in sweet pea and proposed the presence or absence theory. The British physician, Sir Archibald Garrod (1908) first proposed one gene–one product hypothesis. W. Johnson (1909) coined the term *gene* that acts as hereditary unit. T.H. Morgan (1926) discarded all the previous adjusting theories and put forward the particulate gene theory. In 1933, Morgan was awarded Nobel Prize for his research in explaining gene theory.

In 1940, Beadle and Tatum proposed one gene–one protein hypothesis, which explained that one gene encodes one protein and is known as *overlapping gene* (genes within genes). In 1955, Benzer found that cultures of T4 bacteriophage formed plaques on other plates of *E. coli.* G.W. Beadle and E.L. Tatum (1958) with Lederberg received a Nobel Prize for their contribution to physiological genetics.

Discovery of genetic code was possible with the significant contributions made by Francis Crick, Severo Ochoa, M.W. Nirenberg and Har Gobind Khorana early in 1960. For this work, Khorana shared Nobel Prize with Nirenberg and Holley in 1968. Jacob and Monod (1961), explained regulation of gene activity. In 1962, Benzer coined the term *mutant* to denote the smallest unit of chromosome that undergoes mutational changes. Shapiro and coworkers (1969) published the first picture of isolated genes. Thomas Cech in 1986 discovered that pre-rRNA isolated from ciliated protozoa *Tetrahymena thermophila* is self-splicing.

The International Human Genome Project began in 1990 with the following objectives:

1. Developing ways of mapping the human genome at increasing fine level of precision
2. Storing the information in databases and developing tools for data analysis
3. Addressing the ethical, legal and social issues that may arise from execution of this project

Robert and Sharp in 1993 independently hybridized the mRNA of adenovirus with their progeny or DNA segments of virus.

Gene Cloning

Gene cloning is a technological tool used for identifying, isolating and copying a gene coding for a valuable polypeptide with an objective of making available gene for analysis or for production of protein. In 1972, the first recombinant molecule was reported to generate DNA fragments by an enzyme *lipase*. Recombinant technology has resulted into large-scale production of vaccines, hormones and blood clotting factors. A new era was started with the discovery of hybridoma technology, a method for producing pure, identical antibodies against specific antigens. George Milstein and Cesar Kohler (1975) created hybridomas by the fusion of cancer cell with antibodies producing lymphocytes from immunized animal, which resulted in the production of monoclonal antibodies.

Gene Therapy

Discovery of DNA structure is the most important historical achievement in the field of biotechnology. Watson and Crick in 1953 proposed the structure for DNA, for which they were awarded Nobel Prize. According to these two scientists, the DNA molecule consists of deoxyribose sugar, nitrogen bases and phosphoric acid. Erwin Chargaff (1948) used the technique of paper chromatography for revealing the basic composition of DNA. He discovered that in the DNA of different types of organisms, the total amount of purines is equal to the total amount of pyrimidines.

Many attempts in human gene therapy made as early as in 1979 were not successful. The gene therapy involving genetic manipulations may provide new approaches for treating a disorder caused by pathogen that is resistant to the conventional drug.

Protein Biosynthesis

It is the process through which cells build proteins. The term is also used to refer only to protein translation. But more often it refers to a multistep process, beginning with amino acid synthesis and transcription of nuclear DNA into messenger RNA, which is then used as input to translation. The proteins can be synthesized directly from genes by translating mRNA. When a protein needs to be available on short notice or in large quantities, a protein precursor is produced. A *pro-protein* is an inactive protein containing one or more inhibitory peptides that may be activated when the inhibitory sequence is removed by proteolysis during posttranslational modification. It contains a signal sequence (*N*-terminal signal peptide) that specifies its insertion into or through membranes (Fig. 1.3).

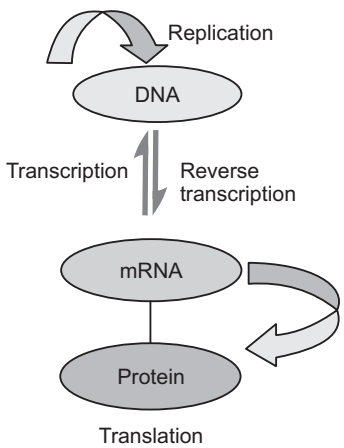


Figure 1.3 Protein biosynthesis.

DNA Replication

It is the basis for biological inheritance. It reflects a fundamental process occurring in all living organisms to copy their DNA. The process is known as *replication* as each strand of the original double-stranded DNA molecule serves as template for reproduction of the complementary strand. As a result of this, two identical DNA molecules are produced from a single double-stranded DNA molecule. Cellular proofreading and error toe-checking mechanisms ensure near-perfect fidelity for DNA replication. The DNA replication in a cell is initiated at specific locations in the genome, known as *origin.* Unwinding of DNA at the origin and synthesis of new strands form a replication

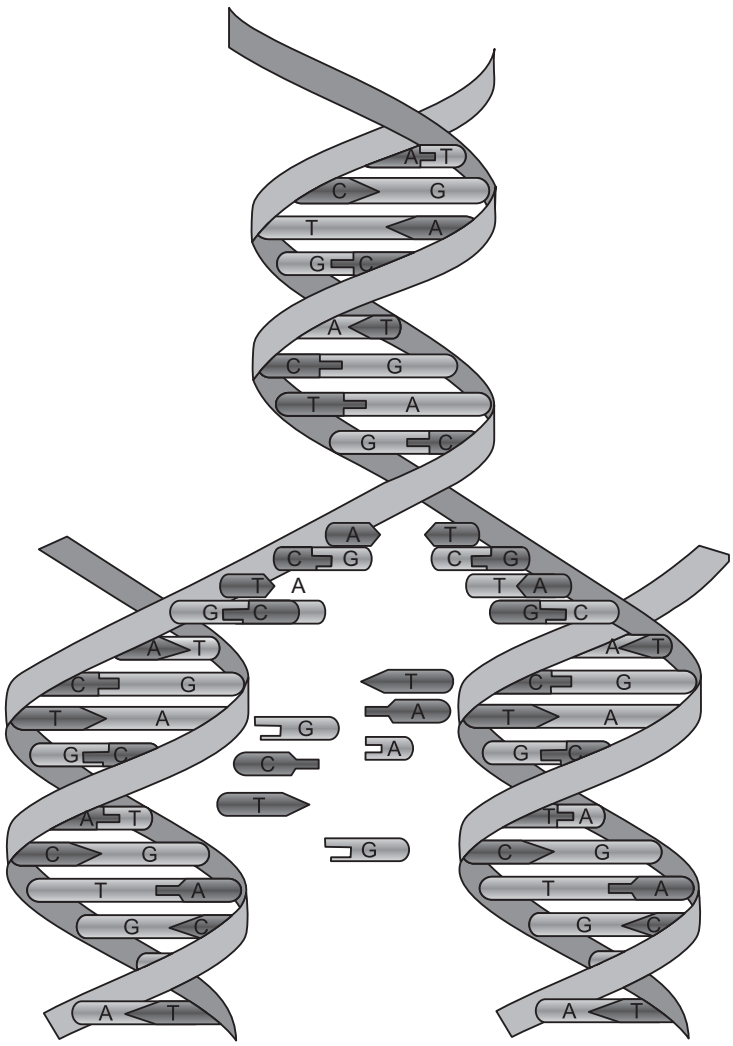


Figure 1.4 DNA replication.

fork. *DNA polymerase* is the enzyme that synthesizes the new DNA by adding nucleotides matching to the template strand. In addition to this, a number of other proteins are associated with the fork and assist in the initiation and continuation of DNA synthesis. DNA replication can also be achieved in vitro (outside a cell). DNA polymerases isolated from cells and artificial DNA primers may be used to initiate the process of DNA synthesis at known sequences in a template molecule. The PCR is a common laboratory technique that employs such artificial synthetic process in a cyclic manner to amplify a specific target DNA fragment from a pool of DNA (Fig. 1.4).

The DNA double helix is stabilized by hydrogen bonds between the bases attached to the strands. The four bases found in DNA are adenine (A), cytosine (C), guanine (G) and thymine (T). These four bases are attached to the sugar/phosphate groups to form the complete nucleotide.

Cytosine and thymine are six-membered rings and are known as *pyrimidines*, whereas adenine and guanine are fused five- and six-membered heterocyclic compounds (*purines*). A fifth pyrimidine base known as *uracil* (U) normally takes the place of thymine in RNA and differs from thymine by lacking a $–CH_3$ group on its ring.

HISTORICAL PERSPECTIVES OF PLANT TISSUE CULTURE

The cell therapy advanced by Schleiden and Schwann (1838) revealed the principle of tissue culture. According to biologist Gautheret (1985), the discovery of tissue culture could be traced to the pioneering experiment conducted by Hennery–Louis on wound healing in plant showing spontaneous callus formation on the decorticated region of the Elm plant.

According to Haberlandt's hypothesis (1902), a cell is capable of autonomy and has potential of totipotency. The term *totipotency* was first coined by Morgan. Hannig started his research work by taking embryogenic tissue instead of single cells. He excised nearly matured embryos of some crucifers such as *Raphanus sativus*, *Raphanus landra* and successfully cultivated (in vitro) them on artificial medium consisting of mineral salts and sugar.

Symun (1908) established the basis for callus culture and to some extent also for micropropagation Kotte and Robbins (1922) simultaneously put forward a new approach to tissue culture and reported that a true in vitro culture could be developed using meristematic cells. White (1934–39) carried out in vitro techniques for tissue culture by changing the nature of nutrient medium. Michael (1939) demonstrated the role of sodium nitrate in protoplast fusion. In the same year, Steward et al. successfully raised a large number of plantlets from carrot root suspension cultures. Overbeek et al. (1941) used coconut milk for embryo development and callus formation in datura, and it proved to be a very important turning point in the development of embryo culture. Later on, this was helpful in the development of several hybrids. Muir and associates (1954) reported that pieces of callus of *Tagetes erecta* and *Nicotiana tabacum* could be cultured in the form of cell suspensions. Skoog and Miller (1957) proposed the concept of hormone-controlled organ formation. In 1960, Cocking introduced protoplasmic plant tissue cultures.

QUESTIONS

1. Define biotechnology. Enlist different branches of biotechnology.
2. Mention in brief the contributions of some leading scientists in biotechnology.
3. Discuss the significant milestones in biotechnological research.
4. Give a brief account of discovery of cell and chromosome theory of inheritance.