Genetic Engineering and Biotechnology

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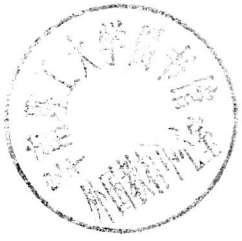


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GENETIC ENGINEERING AND BIOTECHNOLOGY

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Preface

Biotechnology, a contraction of biological technology, can be defined as application of biological organism, system or process to manufacturing and service industries. However, many scientists consider only the techniques of recombinant DNA (r-DNA) that find applications as biotechnology. Genetic Engineering is an umbrella term for a range of techniques for isolation, analyzing and manipulating DNA in vitro that result in predetermined alteration in genotype of an organism. The genetic engineers have been endowed with immense power of producing genetically modified organisms. The r-DNA technology has given a boost to production of novel variety of crop plants, medical research, environmental protection and developments in almost every facet of life that one can think of. The literature has become so vast and deep that no one can follow all the developments in the field. It is in this scenario that technology of genetic engineering as applied to biotechnological processes has been attempted in this text book.

The book deals with the principles of genetic engineering, the strategic developments in the discipline approaches adopted for modification of genetic information content of the organisms, applicability of control systems in metabolic processes and the future prospects of application of these technologies in microbes, plants and animals for benefit of humankind. In such a text repetition of the technology is imperative. However attempt has been made to avoid such repetitions as far as possible but whereever necessary it has been retained. Further an attempt has also been made to incorporate in brief the details of experimental processes to explain the strategies developed and solutions sought.

The book is expected to serve undergraduate and postgraduate students of Biotechnology (B.Sc. and M.Sc.), Engineering, Agricultural and Medical Sciences of various Indian universities. A humble attempt has been made to present the subject in its totality, in a comprehensive lucid manner and all humanly possible care has been taken to avoid mistakes. Still any mistake found in the book is my sole responsibility and for which suggestions of the reader for improvement of the text are most welcome.

I with great indebtedness acknowledge the contributions of scores of scientists who have been dedicated to Biotechnology. I am also indebted to my colleagues and students at Meerut for their unflagging support, intellectual stimulation that they have provided all these many years. I am also thankful to my family members and publishers for their constant support during the preparation of the book.

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September, 2005

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DNA : The Basic Molecule of 'Gene'

1.1. INTRODUCTION

Major molecules that constitute the living cells (biomolecules) are classified according to their physical and chemical properties. Among these nucleotides are most versatile. In comparison to other classes of molecules—amino acids, carbohydrates, lipids, etc., nucleotides are notable for their involvement in the reactions which are very essential for maintenance and propagation of life. The nucleotides are known to participate in the process of energy transfer. When in polymeric forms these constitute nucleic acids which have main role in storing and encoding the genetic informations. These molecules (nucleotides and nucleic acids both) have been now, for about two decades, known to play catalytic roles in the cells. There are varied and essential functions of life which nucleic acid performs in the cell and no other molecule is known to perform such varied functions.

The nucleotides show considerable diversity in their structure. There are eight common varieties of nucleotides which are composed of nitrogenous base linked to a sugar (Base + sugar = nucleoside) to which at least one phosphate group is also attached (Base + sugar + phosphate = nucleotide). The bases of the nucleotides are planer, aromatic and heterocyclic molecules which are structural derivatives of either purine or pyrimidine although *in vivo* these are never synthesized from either of these organic molecules.

DNA molecule stores the genetic information and it is interesting to

note that this information is stored in DNA molecules as a sequence of nucleotides. The **genetic engineering** deals with *in vitro* manipulation of these sequences through recombinant DNA technology. It is, therefore, very important to brush up our understanding of structure and organization of nucleotides in DNA molecules.

Most common purines are adenine (A) and guanine (G) and the major pyrimidines are cytosine (C), thymine (T) and uracil (U). The purines bind to 5-C sugar (Pentose via their Na atoms while the pyrimidines bind to 5-C sugar via N-1 atoms.

The ribonucleotides constitute (as polymer) the RNA wherein pentose is ribose, the deoxyribonucleotides constitute DNA wherein pentose is 2-deoxyribose (i.e. carbon position 2 lacks a hydroxyl group). The primed numbers always indicate carbon position in the sugars. The carbon position in the bases is represented as unprimed number. The usual structures of ribonucleotides and deoxyribonucleotides are represented in Fig. 1.1.

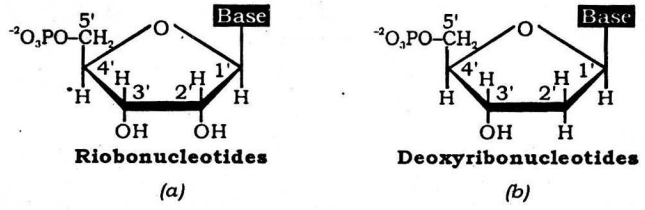
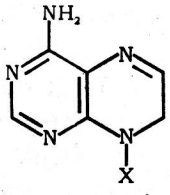
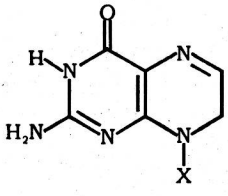
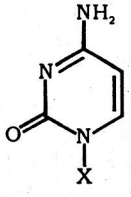
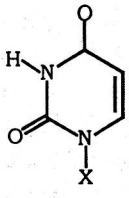
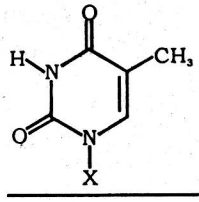


Fig. 1.1: Chemical structure of (a) ribonucleotides and (b) deoxyribonucleotides. The purine or pyrimidine base is linked to C-1' of the pentose and to the last one phosphate is attached.

Whereas adenine, guanine and cytosine are common to both RNA and DNA, the thymine is present only in DNA and instead of thymine uracil is present in RNA. The bulk of the nucleotides in the cell are found in a polymeric forms as either DNA or RNA whose primary functions are storage or transfer of information. However free nucleotides or their derivatives may also be present in the cells and may perform variety of metabolic functions which may not be related to the management of genetic information. One of the best known example of such derivatives is adenosine triphosphate (ATP - the energy currency of the cell). Some other such molecules are adenosine diphosphate (ADP), flavin adenine dinucleotide (FAD) adenosine linked to riboflavin (Vit. B_2) via two phosphate groups, nicotinamide adenine dinucleotide (NAD^+) or its related compound, nicotinamide adenine dinucleotide phosphate ($NADP^+$) and coenzyme-A (CoA). It is very well established now that these moleules have remained as key players in the metabolic field (even during prebiotic-evolutionary succession) while other structures and processes involving nucleotides have

grown up around them (Table 1.1).

TABLE 1.1: Names and Abbreviations of Nucleic Acid Bases, Nucleosides and Nucleotides

Base Formula	*Base (X = H)*	*Nucleoside (X = ribose[a])*	*Nucleotide (X = ribose phosphate[a])*
	Adenine Ade A	Adenosine Ado A	Adenylic acid Adenosine monophosphate AMP
	Guanine Gua G	Guanosine Guo G	Guanylic acid Guanosine monophosphate GMP
	Cytosine Cyt C	Cytidine Cyd C	Cytidylic acid Cytidine monophosphate CMP
	Uracil Ura U	Uridine Urd U	Uridylic acid Uridine monophosphate UMP
	Thymine Thy T	Deoxythymidine dThd dT	Deoxythymidylic acid Deoxythymidine monophosphate dTMP

1.2. STRUCTURE OF NUCLEIC ACIDS

As we have known the nucleic acids are polymers of nucleotides in the form of either DNA or RNA. In the polymers the neighbouring units of

sugars (ribose or deoxyribose) are bridged together in between position 3' (3 prime) and 5' (5 prime). As phosphate of these polynucleotides are acidic, so at physiological pH, nucleic acids are poly-anions.

The bond between two sugar units is known as phosphate diester bond as the phosphate is esterified by two sugar units. Each nucleotide that enters into the polynucleotide is known as nucleotide residue. In a polymeric molecule, the terminal sugar residue either at C5' or C3' is not linked to another nucleotide. The terminal residue whose C5' end is not linked to another nucleotide is called 5' end of the strand while the terminal residue whose C3' is not linked to another nucleotide is termed as 3' end of the strand. By property of polymerised units it may be **monomer** (single nucleotide) **dimer, trimer, tetramer** (2, 3, 4 units polymerised respectively) and so on through oligomer (a few units polymerised). As the size of the polymer increases the physical properties such as charge on the molecules, solubility and several other characteristics may change. When a polymer has similar repeating units, it is called homopolymer but when molecule has a series of non-identical units/residues it is called heteropolymer and has a property of containing some specific information in the form of its sequence of residues.

1.3. BASE COMPOSITION OF DNA : CHARGAFF'S RULE

In the late 1940s Erwin Chargaff devised the first reliable quantitative method for compositional analysis of DNA. It was demonstrated that DNA molecules have equal numbers of adenine and thymine. Similarly guanine and cytosine are present in equal numbers. It was also shown later that DNA base composition varies widely among different organisms. In bacteria for example it ranges from about 25% to 75% G+C, however, it is more or less constant among related species. In mammals G+C ranges from 39-46%. The significance of Chargaff's rule was not appreciated well at that time but with the establishment of double stranded nature of DNA, this rule has strengthened the concept and structural basis of the molecule. It is important here to remember that RNA being single stranded structure does not obey this rule.

1.3.1. Structure of DNA

After the initial experiments by Griffith (1920) on process of transformation in strains of *Diplococeus pneumoniae* (= *Streptococcus pneumoniae*) Avery, McLeod and McCarty indicated that the nature of the transforming factors was DNA. Later Harshey and Chase (1952) conclusively established that DNA was the genetic material through which the genetic

information is passed on from one generation to the other generation.

These discoveries exerted lot of pressure on scientists to establish the structure of DNA and lot of competition was set on them to become first to discover its chemical and physical structure. People at this time also knew that nucleotides were the building blocks of DNA and that these were linked by dehydration synthesis to form DNA but the exact structure/arrangement was unknown.

In the early 1950s Rosalind Franklin carried out experiments which involved bouncing X-rays off the crystals of various substances (a process which is called X-ray crystallography) including DNA, then exposing photographic film to bouncing X-rays. The scatter pattern of X-rays bouncing off the crystals of DNA is presented as a landmark photograph and is depicted below in Fig. 1.2.

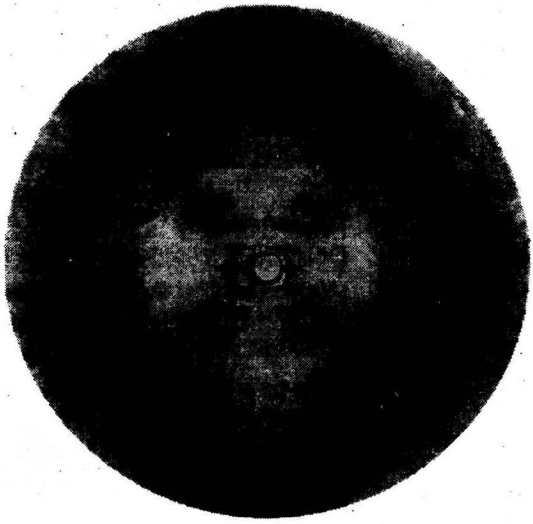


Fig. 1.2: X-ray differaction pattern of DNA crystals as obtained by Frankin and Wilkins.

Other people like Linus Pauling were also attempting to figure out the structure of DNA. James Watson while working with Francis Crick were shown Franklin's photographs of DNA X-ray crystallography. From her pictures, and basing their imagination on Chargaff's data on DNA composition Watson and Crick published a paper in 1953 in which they proposed and described a hypothetical structure for DNA. They suggested that DNA was organised into a double spiral or double helix. Some of the major characteristics of DNA as suggested by them are

1. DNA is a double helix. Two Strands of the helix wound around each other to form a right handed helix.