ESSENTIALS OF BIOTECHNOLOGY

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PREFACE

The outstanding development of biotechnology during the past decade has been a result of science's ability to begin to decipher the codes of life, reprogram the cells and tune molecular switches adopting hosts capable of growing in large quantities. Biotechnology provides the environment on which new bodies of knowledge can expand and act as catalyst for many disciplines.

New technology, equipment and processes will be born in medicine, biochemistry, food, fuels environmental control as a result of the biological revolution of the last generation. Many of these technologies are complementary and this book views the essentials of biotechnology from a technological viewpoint. Presented is the interaction of several disciplines since all biotechnology is multidisciplinary in nature, challenging the technologist and layman alike.

The reader will find this book useful and informative. Chapters include extensive bibliographies on subject matter presented for further reference and research which will serve as a guide to the more recent literature on biotechnology. Overall this volume should prove useful to both the practitioner as well as the student.

ROBERT P. OUELLETTE
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What is Biotechnology?

INTRODUCTION

Biotechnology is exploding like a Nova, bursting on the everyday scene, challenging the technologist and the layman alike. The field is ancient and new. It is as old as thinking man; and as new as the latest discovery.

It is not possible to review, with scientific rigor, all the findings, advances and discoveries of recent years. We will limit ourselves to a broad sketch on a canvas. We hope that major lines will emerge, guiding our review of what biotechnology has achieved and what it promises to accomplish.

There are no limits to scientific inquiry, still nagging ethical questions persist. We will stay far from such crags and concentrate on the easier topic of science and technology. We display in the following pages the logic of life, the confusing terminology in use today, the prerequisite tools for biotechnological achievements, and some of the emerging concepts.

THE LOGIC OF LIFE

All life is reducible, at the molecular level, to the sequences in DNA (deoxyribonucleic acid). A precise mathematical logic insures that the DNA message is translated into RNA (ribonucleic acid) and that this last code is eventually transcribed into proteins. Proteins serve many functions but none is more important than their role as enzymes catalyzing the entire chemistry of life (Figure 1-1).

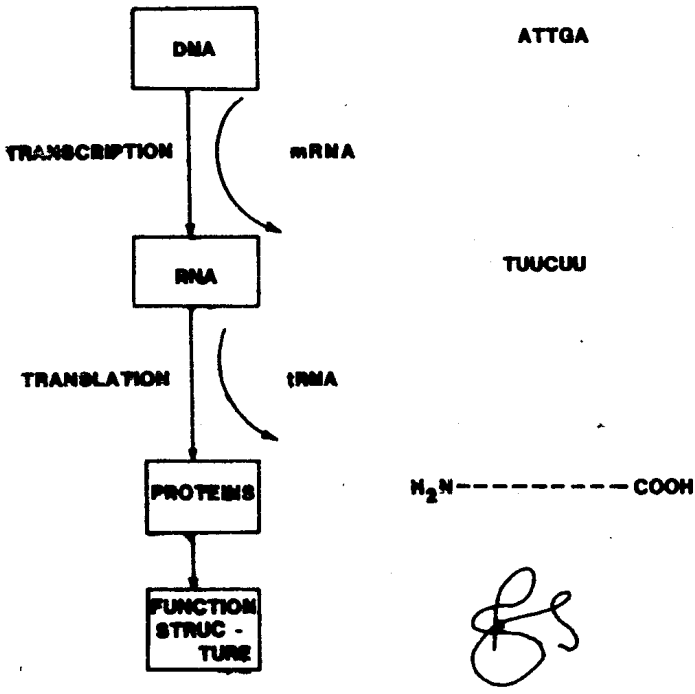

Figure 1-1. The logic of life.

The Central Dogma

A central dogma on the sense and direction of the cellular machinery from DNA to RNA to protein has been proposed. Like all dogma, it sins in its absolute character.

The central dogma of molecular biology states that information transfer never proceeds from protein to nucleic acid. This part of the dogma has never been refuted. The DNA to RNA pathway part of the original formulation had to be revised with the observation that retroviruses transcribe RNA to DNA. The common use of reverse transcriptase in genetic engineering experiments is ample confirmation of the reality of the reverse pathway.

A more modern version of the dogma would be as follows:

Indicating that RNA to DNA information transfers while rare, can occur.

The Building Blocks

Today's engineers might not assemble DNA the way nature did it, but given the basic building blocks, the logic of the assembly is easily recognized. The basic blocks are three: the bases, the sugar moitie, and the phosphate groups.

The Bases

The bases are of five kinds: 2 purines and 3 pyrimidines (Figure 1-2). The two purines, adenine and guanine, are used both in RNA and DNA; while the pyrimidine thymine is found only in DNA, and uracil appears in RNA. These bases are linked together to form a polynucleotide. The phosphate group of one nucleotide is linked to the deoxyribose group of another, and so forth.

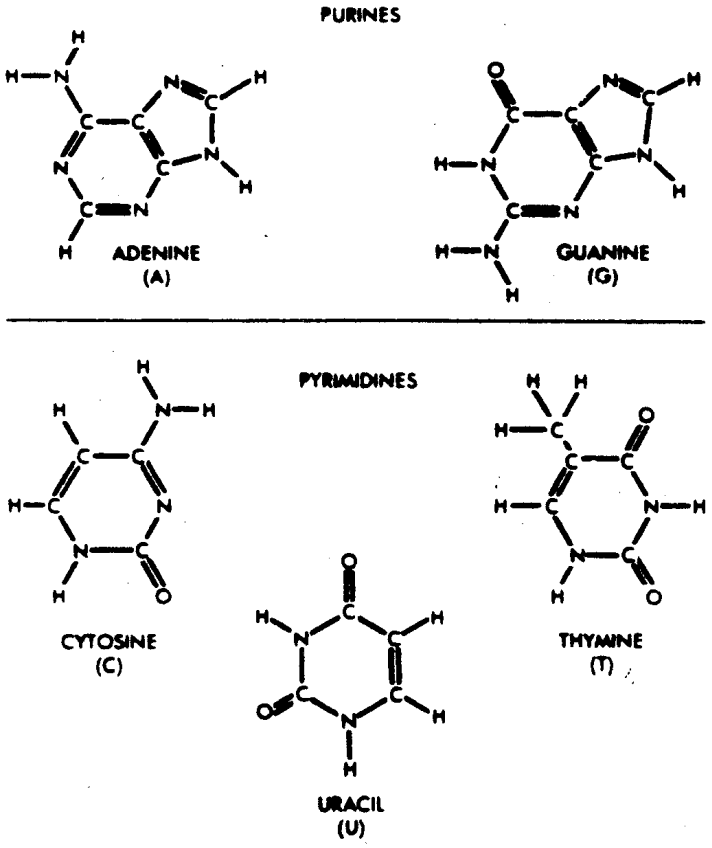

Figure 1-2. The five bases.

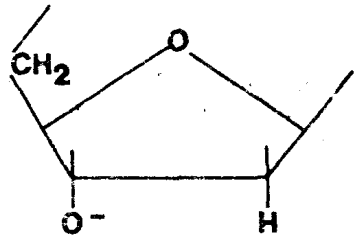

Figure 1-3. The deoxyribose.

The Sugar

The sugar backbone is a deoxyribose (Figure 1-3).

The Phosphodiester Bond

The phosphate group serves as a linkage (Figure 1-4). The linking is from the 5′ carbon of a deoxyribose molecule to the 3′ carbon of a nucleotide (Figure 1-5).

The Double Helix

The double helix of DNA is held together by hydrogen bonds between purines and pyrimidines according to an exact pairing system where A pairs with T and G with C (Figure 1-6).

The double helix structure of DNA was demonstrated in 1953 by Watson and Crick, building on the work of earlier crystallographers [1].

The double helix can now be seen as made up of sugar-phosphate backbones linking the bases. The two strands of the helix are connected by hydrogen bonds (Figure 1-7). The two strands of the DNA molecule have complementary shapes. The strands are twisted in a right-handed double helix. The DNA helix is supercoiled in a compact form. Supercoiling is controlled by enzymes: topoisomerases and gyrases.

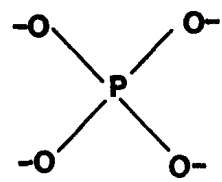

Figure 1-4. The phosphate group.

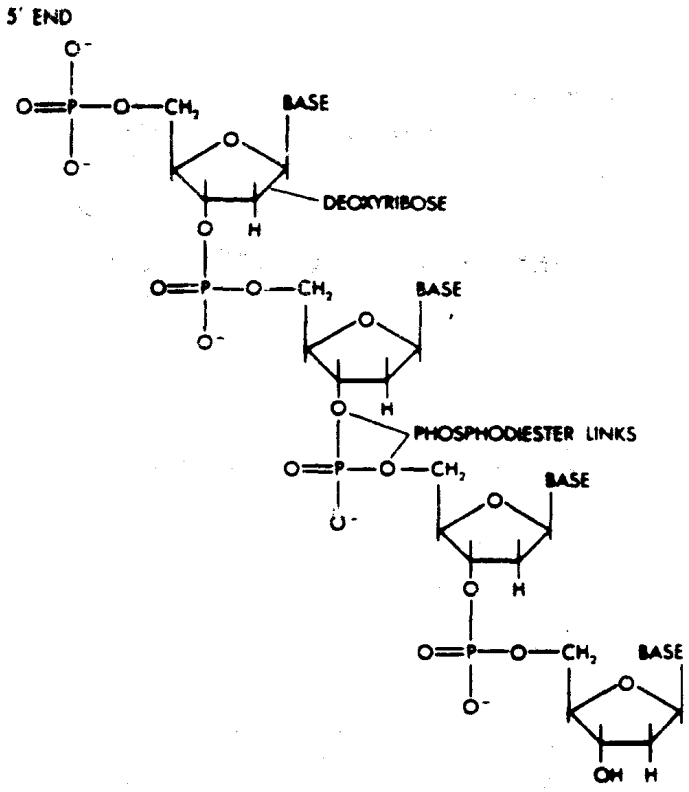

Figure 1-5. Phosphodiester bonds.

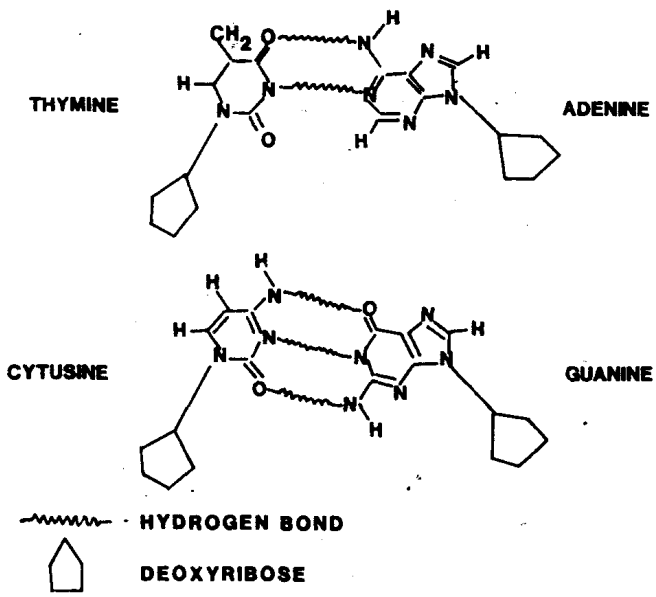

Figure 1-6. Base pairing with hydrogen bonds.

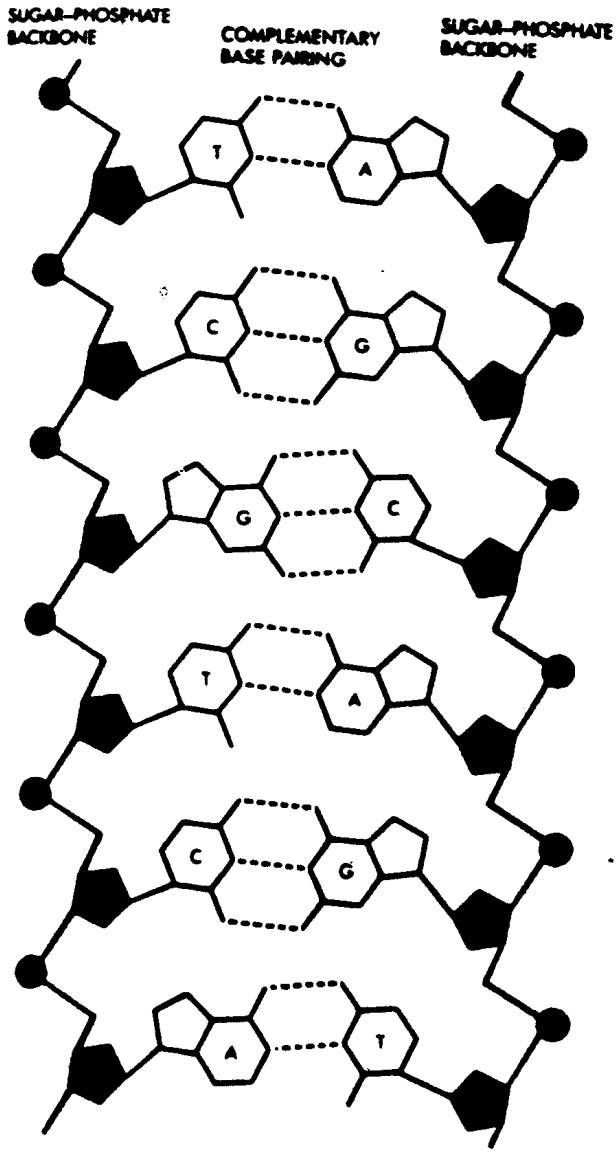

Figure 1-7. The DNA chain.

The DNA Code

We now know how the basic information is stored, transmitted from generation to generation, modified by external agents and noise, and decoded. We know how cells use this information to build structures and condition the functioning of cells, tissues, organs, and organisms.

The basic message is made up of a sequence of four bases: Adenine

(A), Thymine (T) or Uracil (U), Guanine (G), Cytocine (C). The bases are complementary, insuring geometry into the message.

A C
| |
T(U) G

The genetic code is a language based on four nucleic acid bases, through which the 64 triplets specify the 20 amino acids and operators. The DNA code is a coma free, generally non-overlapping degenerate triplet code (Table 1-1).

The Process

The conversion of the DNA code into a protein molecule is a two-step process, involving numerous enzymes.

DNA Duplication

DNA can direct its own replication. The first step in DNA replication is the separation of the DNA double helix. Each strand then serves as a

TABLE 1-1. The DNA Code.

First Position (5′ end)	Second Position				Third Position (3′ end)
	U	C	A	G	
U	PHE	SER	TYR	CYS	U
	PHE	SER	TYR	CYS	C
	LEU	SER	Stop	Stop	A
	LEU	SER	Stop	TRP	G
C	LEU	PRO	HIS	ARG	U
	LEU	PRO	HIS	ARG	C
	LEU	PRO	GLN	ARG	A
	LEU	PRO	GLN	ARG	G
A	ILE	THR	ASN	SER	U
	ILE	THR	ASN	SER	C
	ILE	THR	LYS	ARG	A
	MET	THR	LYS	ARG	G
G	VAL	ALA	ASP	GLY	U
	VAL	ALA	ASP	GLY	C
	VAL	ALA	GLU	GLY	A
	VAL	ALA	GLU	GLY	G

template for the production of two daughters DNA molecules. The separation-replication process goes hand-in-hand.

Transcription

The conversion of DNA to RNA is accomplished by an RNA polymerase. This enzyme binds to the DNA at a correct starting point, then moves rapidly down the DNA chain, adding the correct nucleotides to the RNA chain until the task is completed.

It takes several steps to transform the primary DNA into mature messenger RNA. Certain nucleotides in the chain are modified and others are excerpted by a splicing mechanism, yielding a mRNA chain shorter than the original primary DNA [2].

Translation

The synthesis of protein is accomplished by intracellular particles called ribosomes. The ribosome is a complex apparatus made up of 52 different proteins and three different RNA molecules. It is a quite efficient machine, adding up some 15 amino acids, to the growing chain, per second [3].

The instructions for coding protein are delivered to the ribosome by the messenger RNA (mRNA). Each amino acid is transported to the ribosome synthesis sites by transfer RNA's (tRNA). The tRNA carries an anticodon which matches, in a complementary fashion, the codon of the messenger RNA.

The ribosome has two binding sites, one for positioning the arriving tRNA carrying the selected amino acid, and the other for holding the growing polypeptide chain.

The process we have described above has been compared to a language, to a computer and more generally, to a finite state machine.

The skeleton of a finite state machine can be uncovered by reducing most modern devices for their bare bone essential elements. A mousetrap, a household appliance, a computer, is a finite state machine [4].

At the molecular level, the ribosome and the various transfer RNA's operate as a finite state machine. The input are the four RNA bases (A,U,G,C); the output is a protein made up of infinite rearrangement and ordering of the basic 20 amino acids. The machine must be able to recognize a start signal (AUG) and a stop signal (UAA, UAG, UGA). The machine reads three bases at a time and can perform either of three functions: start the processing, halt, or translate the input into an output (Figure 1-8).

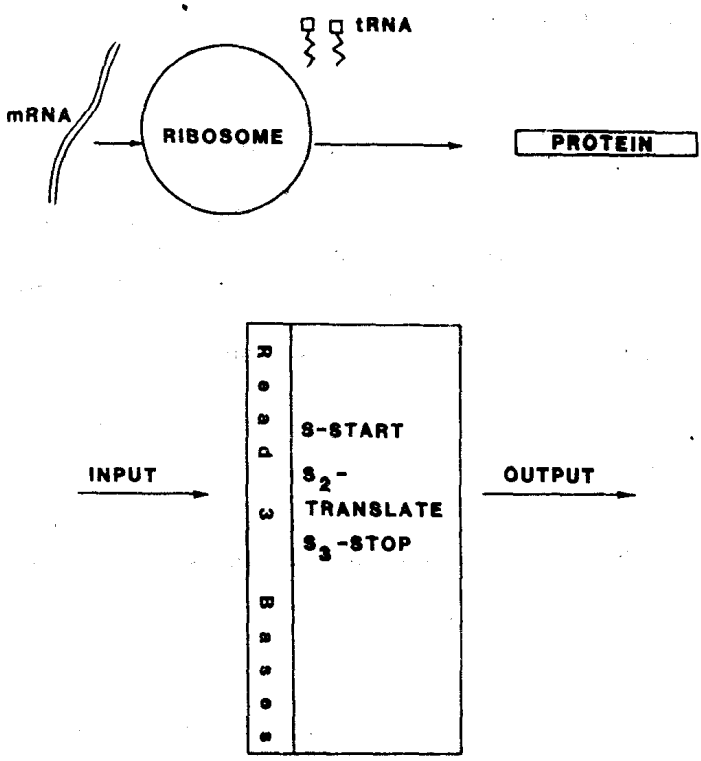

Figure 1-8.

The Protein

Twenty amino acids are commonly occurring in protein.

Glycine	GLY	Lysine	LYS
Alanine	ALA	Arginine	ARG
Valine	VAL	Asparagine	ASN
Isoleucine	ILE	Glutamine	GLN
Leucine	LEU	Cysteine	CYS
Serine	SER	Methionine	MET
Threonine	THR	Tryptophan	TRP
Proline	PRO	Phenylalanine	PHE
Aspartic acid	ASP	Tyrosine	TYR
Glutamic acid	GLU	Histidine	HIS

Proteins are the basis of all living phenomena as structural support, catalyst of equisite selectivity, storage media, transport systems, hormone messengers, etc.