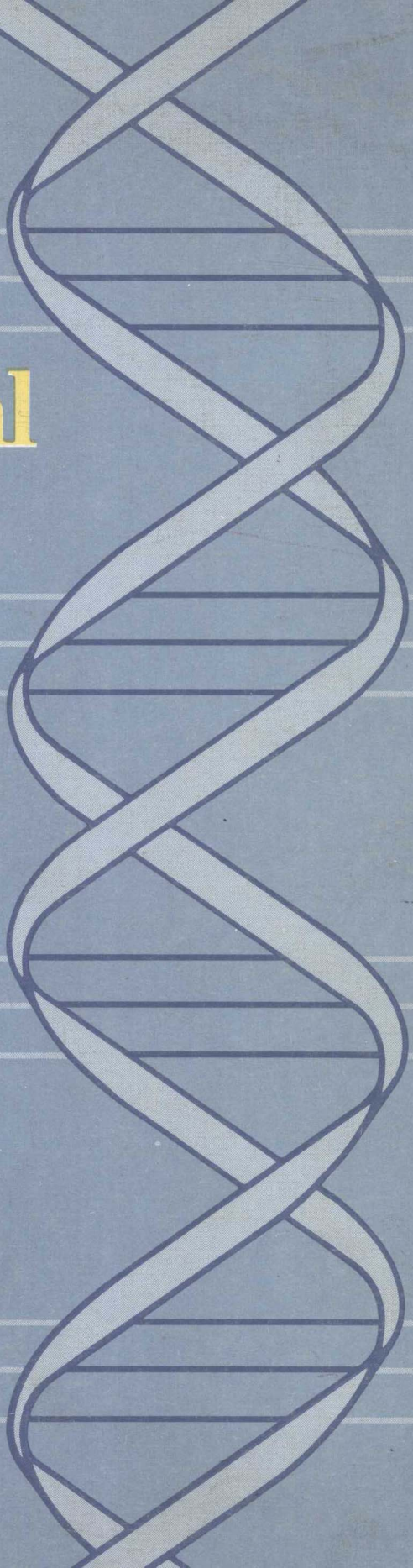
Second Edition

Pharmaceutical Biotechnology

A PROGRAMMED TEXT



Edited by

S. William Zito

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TECHNOMIC
PUBLISHING CO., INC.
LANCASTER · BASEL

Pharmaceutical Biotechnology

a **TECHNOMIC**® publication

Published in the Western Hemisphere by
Technomic Publishing Company, Inc.
851 New Holland Avenue, Box 3535
Lancaster, Pennsylvania 17604 U.S.A.

Distributed in the Rest of the World by
Technomic Publishing AG
Missionsstrasse 44
CH-4055 Basel, Switzerland

Printed in the United States of America

10 9 8 7 6 5 4 3 2

Main entry under title:
Pharmaceutical Biotechnology: A Programmed Text, Second Edition

A Technomic Publishing Company book
Bibliography: p.
Includes index p. 265

Library of Congress Catalog Card No. 97-60885
ISBN No. 1-56676-519-6

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Preface

The need for a second edition of this text can certainly be justified by the explosion of knowledge in the area of pharmaceutical biotechnology since the first edition was released in 1992. While new techniques and topics have been introduced to ensure that content is current, the format of the text has remained essentially unchanged. The text is designed to compliment existing courses in the pharmacy curriculum that may not be including biotechnology concepts in the formalized course structure. Chapters are sequenced to follow the progression of required scientific and clinical coursework found in the curricula of most pharmacy programs. The text begins with an overview of biotechnology principles and techniques. The various sections on pharmacy encompass both the basic pharmaceutical and clinical sciences. Included are chapters with focused biotechnology discussions relevant to medicinal chemistry, pharmacology, pharmaceutics, and pharmacotherapeutics. Each chapter contains specific learning objectives, as well as a pretest and posttest to allow assessment of content mastery after study.

This second edition benefits from the comments of colleagues who have utilized the first edition. As a result of their input, new authors have been added, and the chapters on Introduction to Recombinant DNA Technology, Medicinal Chemistry, Pharmacology, and Pharmacotherapeutics have been expanded. In addition, two new chapters on Pharmacokinetics of Peptide and Proteins, and Gene Therapy have been included. To our faculty colleagues, I trust you will find this text an effective way to incorporate the essential topic of biotechnology into the pharmacy curriculum. To our student readers, I hope you will enjoy this programmed method of learning about biotechnology and its importance to the practice of pharmacy.

My contributing colleagues and I wish to thank the Grant Awards to Pharmacy Schools (GAPS) program of the SmithKline Beecham Foundation for their financial support of the first edition of this text.

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CONTENTS

CHAPTER 1

The Basic Principles of Recombinant DNA Technology

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INTRODUCTION

The discovery and confirmation of DNA as the genetic material launched an era over the last fifty years that has seen an explosive growth in the fundamental understanding of how genes work and in the development of new technologies based on the manipulation of genes. These technologies have been applied to animal and plant breeding, drug development, and diagnosis and treatment of disease. Genetic engineering provides a new means to produce biologically important materials in quantities greater than could be derived from the natural sources. The selection of improved plant strains and animal breeds can be accelerated by the judicious use of DNA technology. Further, diagnosis of many genetic and viral diseases is now possible through the use of DNA probes. Understanding the pathology of many diseases has advanced as a result of the ability to probe these processes at the genetic level. Finally, the correction of genetic defects by gene replacement therapy has been initiated and strategies to combat infectious diseases and cancer are under development. This extraordinary and rapid progress launches an era of hopeful expectation and excitement about the vast knowledge yet to be discovered and elucidated.

LEARNING OBJECTIVES

(1) To review the fundamental concepts and terminology associated with DNA structure and gene expression.

(2) To describe the basic strategy for cloning a gene.

(3) To discuss the use of cDNA and genomic clones in the study of gene expression.

(4) To discuss production of pharmaceuticals using recombinant DNA.

PRE-TEST

Answer True or False

1. The two strands of DNA helix are joined by covalent bonds between the nitrogenous bases.

2. A nucleotide consists of a sugar, phosphate, and nitrogenous base.

3. Restriction enzymes randomly cut DNA.

4. The enzyme that catalyzes transcription is DNA polymerase.

5. Transfer RNA contains a codon and an amino acid binding site.

6. The genetic code is stored in the ribosomal RNA sequence.

7. Eukaryotic genomes contain more sequences than appear in the mature mRNAs that are translated.

8. Each gene can encode only one polypeptide.

9. A DNA probe will hybridize to a complementary sequence through the formation of hydrogen bonds.

10. Bacteriophages are enzymes that degrade foreign DNA.

Answers:	1. F	3. F	5. F	7. T	9. T
	2. T	4. F	6. F	8. F	10. F

NUCLEIC ACID STRUCTURE AND FUNCTION

Investigations of bacteria and later viruses, called bacteriophages, led to the fundamental discovery that DNA and not protein constituted the genetic material. DNA satisfies the two criteria for functioning as the genetic material. First, it is replicated, providing a mechanism for faithfully passing a copy of the genetic information from generation to generation. Second, it functions as an informational molecule, specifically; it directs the synthesis of cellular proteins.

DNA consists of two strands wound in a double helix. The nucleotides of each strand are linked by covalent bonds between the 3′ hydroxyl of one nucleotide and the 5′ phosphate of the next. Synthesis of DNA strands always occurs in the 5′ to 3′ direction, since the growing chain has the 3′ hydroxyl available to form a phosphodiester bond with the 5′ phosphate of the incoming nucleotide. (Figure 1-1). The nitrogenous bases, which in DNA are of four types, adenine (A), thymine (T), cytosine (C) and guanine (G), form hydrogen bonds with the bases on the opposite strand according to the complementary base pairing rule; i.e., A-T and G-C. The two strands are antiparallel in orientation; i.e., one is 5′–3′ and the complementary strand is 3′–5′.

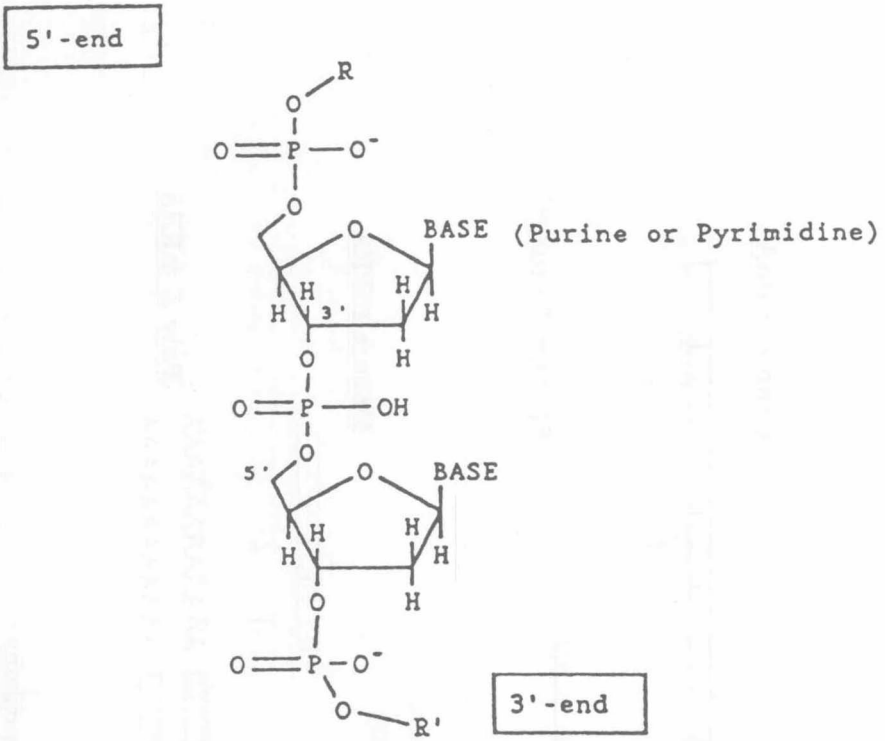

Figure 1-1. Phosphodiester linkage.

The information for protein synthesis lies in the DNA base sequence. In distinction to prokaryotes, the more complex eukaryotic genes contain coding and non-coding regions (Figure 1-2). Those regions that contain information for the *synthesis of cellular proteins* are called *exons*, while the intervening regions, called introns, contain important information for regulating transcription and processing of transcripts. In eukaryotes, the primary transcript, or heteronuclear RNA, contains both intronic and exonic sequences. Specific splicing enzymes present in the nucleus recognize sequences at the intron-exon junctions, splice out the intronic sequences, and join the exonic sequences. Interestingly, recent experiments have revealed enzymatic activity residing within some RNAs, suggesting that ribozymes may contribute to processing RNA. The mature mRNA, which is transported to the cytoplasm, acquires a polyadenylated tail. One advantage of the eukaryotic arrangement is that multiple transcripts can be derived from the same gene by alternative splicing of the primary transcript increasing the informational content. Alternately, spliced transcripts have been observed in the tissue-specific processing of proteins (Figure 1-2).

Ribonucleic acid, RNA, is involved in the reading of the genetic code in the DNA and its expression as protein molecules in a cell. RNA is a single stranded molecule containing a ribose sugar and having thymidine (T) replaced by uracil (U). The synthesis of RNA from the DNA template catalyzed by RNA polymerase is transcription (Figure 1-3). The code inscribed in the mRNA is translated into protein at the ribosome. The nucleotide sequence is read in triplets called *codons*, each codon designating a specific amino acid. Transfer RNA contains an anticodon that forms hydrogen bonds with the mRNA codon containing the complementary triplet of bases. The tRNA also contains an amino acid

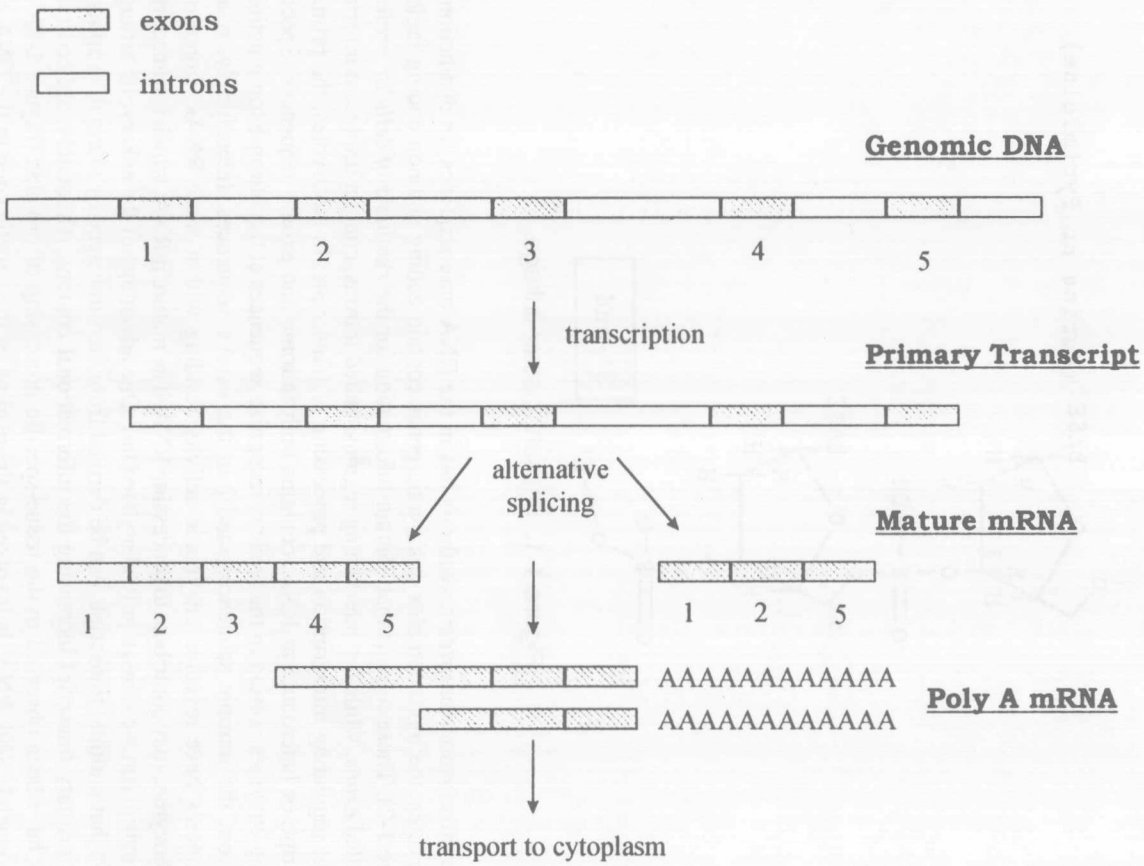
exons
introns
Genomic DNA
1
2
3
4
5
transcription
Primary Transcript
alternative
splicing
Mature mRNA
1 2 3 4 5
1 2 5
AAAAAAAAAAAA
AAAAAAAAAAAA
Poly A mRNA
transport to cytoplasm

Figure 1-2.

binding site and becomes charged with a specific amino acid in the cytoplasm. This amino acid is brought to the protein synthesis site at the ribosome and inserted into the growing polypeptide chain when the complementary codon of the mRNA sits in that site.

An understanding of these fundamental mechanisms is the basis for their application to practical problems exemplified by the emergence of genetic engineering and DNA biotechnology. Some goals of this research include correction of genetic defects by replacement of mutant genes with normal ones; accelerating selection of improved crops by introduction of useful genes into plants; improved diagnosis of viral, oncogenic, and genetic diseases; and better understanding of the pathological processes in disease and large-scale production of therapeutic proteins.

RECOMBINANT DNA

Three key discoveries provided the basis for engineering recombinant DNA molecules, that is, molecules in which a piece of DNA is inserted into a particular site in another piece of DNA. The first was the discovery of restriction enzymes, which cut the DNA at sequence specific sites. These exist in bacteria along with modification enzymes, which together provide a restriction-modification system for degrading foreign DNA while protecting the bacteria's own DNA. The bacteria tag their own DNA with methylated groups via specific modification systems and protect it from degradation by restriction enzymes. To date, hundreds of restriction enzymes have been purified from various strains of bacteria and their sequence specificities determined. Each enzyme binds to the DNA at sequence-specific sites and cleaves the helix at specific nucleotide residues. This site is called the *recognition sequence*. Recognition sites consisting of four base pairs are relatively more frequent in the genome than those of six or eight base pairs. DNA incubated with restriction enzymes under appropriate incubation conditions will yield fragments of double stranded molecules of varying lengths depending upon the frequency of sites for that enzyme within the DNA. When DNA is cut by a restriction enzyme, the fragments may possess blunt ends or single stranded tails. Sticky, or cohesive ends, result if the two strands are not cut at the corresponding bases in both strands (Figure 1-4). The sticky ends may then be annealed to other DNA molecules that contain complementary sequence. The reannealed DNA fragments are joined together by DNA ligase, an enzyme that catalyzes the formation of phosphodiester bonds between adjacent nucleotides. These provide the basic tools with which to recombine DNA fragments from different sources, i.e., generate recombinant molecules.

The isolation of sufficient amounts of material to analyze gene structure and function requires synthesizing many copies of the DNA sequence. This relies on the introduction of the sequence into a vector, which can be amplified in bacterial hosts. An important step that facilitated gene cloning was the discovery of plasmids. A plasmid is an extrachromosomal piece of circular DNA that is replicated in bacteria. These genetic elements often carry genes for sex factors and antibiotic resistance and are found in multiple copies in bacterial cells. Plasmids are transferable from bacteria to bacteria. Bacterial cultures are treated so that the cells become competent to take up DNA from the media environment by the process of transformation. Once inside the bacterial cells, the plasmids replicate. Plasmids have been designed that replicate to high copy numbers in bacterial hosts, which dramatically increases the yield of recombinant DNA that can be prepared from a bacterial culture. Plasmids differ structurally from the large chromosomal DNA and are easily