Molecular Biology

Third Edition

Robert F. Weaver

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Cover image: © *Prof. Ada Yonath, Dr. Anat Bashan, and Dr. Maggie Kessler, The Ribosome Group at The Weizmann Institute of Science, Rehovot Israel*

Cover Image
The ribosomal site for peptide bond formation. The crystal structure of the large ribosomal subunit from *Deinococcus radiodurans* (Harms et al., Cell 107, 679-688, 2001) reveals RNA exclusiveness at the peptide bond formation site. This site is located at the bottom of the peptidyl transferase center (PTC), represented here by the backbone of its components of the 23S RNA (H89-magenta; H93-pink; H68-jade; H71-orange; H69-gold) and protein L16 (green). The positions of an oligonucleotide (red) mimicking the aminoacylated tRNA acceptor stem (Bashan et al., Mol. Cell, 11, 91-102, 2003) as well as the docked (from Yusupov et al., Science 292, 883-896, 2001) A- (cyan) and P- (olive-green) sites tRNAs, indicate that remote interactions with the PTC upper rims govern their precise placement and accurate alignment, mandatory for efficient peptide bond formation.

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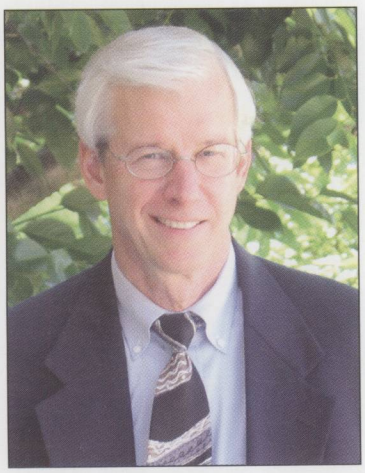
ABOUT THE AUTHOR



(*Source:* Ashvini C. Ganesh)

Rob Weaver was born in Topeka, Kansas, and grew up in Arlington, Virginia. He received his bachelor's degree in chemistry from the College of Wooster in Wooster, Ohio, in 1964. He earned his Ph.D. in biochemistry at Duke University in 1969, then spent two years doing postdoctoral research at the University of California, San Francisco, where he studied the structure of eukaryotic RNA polymerases with William J. Rutter.

He joined the faculty of the University of Kansas as an assistant professor of biochemistry in 1971, was promoted to associate professor, and then to full professor in 1981. In 1984, he became chair of the Department of Biochemistry, and served in that capacity until he was named Associate Dean of the College of Liberal Arts and Sciences in 1995.

Prof. Weaver is the divisional dean for the science and mathematic departments within the College, which includes supervising 14 different departments and programs. As a professor of molecular biosciences, he teaches courses in introductory molecular biology and the molecular biology of cancer. He directs a research laboratory in which undergraduates and graduate students participate in research on the molecular biology of a baculovirus that infects caterpillars.

Prof. Weaver is the author of many scientific papers resulting from research funded by the National Institutes of Health, the National Science Foundation, and the American Cancer Society. He has also coauthored two genetics textbooks and has written two articles on molecular biology in the *National Geographic Magazine*. He has spent two years performing research in European Laboratories as an American Cancer Society Research Scholar, one year in Zurich, Switzerland, and one year in Oxford, England.

To my parents

PREFACE

This textbook is designed for an introductory course in molecular biology. But what is molecular biology? The definition of this elusive term depends on who is doing the defining. In this book, I consider molecular biology to be the study of genes and their activities at the molecular level.

When I was a student in college and graduate school I found that I became most excited about science, and learned best, when the instructor emphasized the experimental strategy and the data that led to the conclusions, rather than just the conclusions themselves. Thus, when I began teaching an introductory molecular biology course in 1972, I adopted that teaching strategy and have used it ever since. I have found that my students react as positively as I did.

One problem with this approach, however, was that no textbook placed as great an emphasis on experimental data as I would have liked. So I tried assigning reading from the literature in lieu of a textbook. Although this method was entirely appropriate for an advanced course, it was a relatively inefficient process and not practical for a first course in molecular biology. To streamline the process, I augmented the literature readings with hand-drawn cartoons of the data I wanted to present. Later, when technology became available, I made transparencies of figures from the journal articles. But I really wanted a textbook that presented the concepts of molecular biology, along with experiments that led to those concepts. I wanted clear explanations that showed students the relationship between the experiments and the concepts. So, I finally decided that the best way to get such a book would be to write it myself. I had already coauthored a successful introductory genetics text in which I took an experimental approach—as much as possible with a book at that level. That gave me the courage to try writing an entire book by myself and to treat the subject as an adventure in discovery.

Organization

The book begins with a four-chapter sequence that should be a review for most students. Chapter 1 is a brief history of genetics. Chapter 2 discusses the structure and chemical properties of DNA. Chapter 3 is an overview of gene expression, and Chapter 4 deals with the nuts and bolts of gene cloning. All these are topics that the great majority of molecular biology students have already learned in an introductory genetics course. Still, students of molecular biology need to have a grasp of these concepts and may need to refresh their understanding of them. I do not deal specifically with these chapters in class; instead, I suggest students consult them if they need more work on these topics. These chapters are written at a more basic level than the rest of the book.

Chapter 5 describes a number of common techniques used by molecular biologists. It would not have been possible to include all the techniques described in this book in one chapter, so I tried to include the most common or, in a few cases, valuable techniques that are not mentioned elsewhere in the book. When I teach this course, I do not present Chapter 5 as such. Instead, I refer students to it when we first encounter a technique in a later chapter. I do it that way to avoid boring my students with technique after technique. I also realize that the concepts behind some of these techniques are rather sophisticated, and the students' appreciation of them is much deeper after they've acquired more experience in molecular biology.

Chapters 6–9 describe transcription in prokaryotes. Chapter 6 introduces the basic transcription apparatus, including promoters, terminators, and RNA polymerase, and shows how transcripts are initiated, elongated, and terminated. Chapter 7 describes the control of transcription in three different operons, then Chapter 8 shows how bacteria and their phages control transcription of many genes at a time, often by providing alternative sigma factors. Chapter 9 discusses the interaction between prokaryotic DNA-binding proteins, mostly helix-turn-helix proteins, and their DNA targets.

Chapters 10–13 present control of transcription in eukaryotes. Chapter 10 deals with the three eukaryotic RNA polymerases and the promoters they recognize. Chapter 11 introduces the general transcription factors that collaborate with the three RNA polymerases and points out the unifying theme of the TATA-box-binding protein, which participates in transcription by all three polymerases. Chapter 12 explains the functions of gene-specific transcription factors, or activators. This chapter also illustrates the structures of several representative activators and shows how they interact with their DNA targets. Chapter 13 describes the structure of eukaryotic chromatin and shows how activators can interact with histones to activate or repress transcription.

Chapters 14–16 introduce some of the posttranscriptional events that occur in eukaryotes. Chapter 14 deals

with RNA splicing. Chapter 15 describes capping and polyadenylation, and Chapter 16 introduces a collection of fascinating "other posttranscriptional events," including rRNA and tRNA processing, *trans*-splicing, and RNA editing. This chapter also discusses two kinds of posttranscriptional control of gene expression: (1) RNA interference; and (2) modulating mRNA stability (using the transferrin receptor gene as the prime example).

Chapters 17–19 describe the translation process in both prokaryotes and eukaryotes. Chapter 17 deals with initiation of translation, including the control of translation at the initiation step. Chapter 18 shows how polypeptides are elongated, with the emphasis on elongation in prokaryotes. Chapter 19 provides details on the structure and function of two of the key players in translation: ribosomes and tRNA.

Chapters 20–23 describe the mechanisms of DNA replication, recombination, and translocation. Chapter 20 introduces the basic mechanisms of DNA replication and repair, and some of the proteins (including the DNA polymerases) involved in replication. Chapter 21 provides details of the initiation, elongation, and termination steps in DNA replication in prokaryotes and eukaryotes. Chapters 22 and 23 describe DNA rearrangements that occur naturally in cells. Chapter 22 discusses homologous recombination and Chapter 23 deals with translocation.

Chapter 24 presents concepts of genomics and proteomics. The chapter begins with an old-fashioned positional cloning story involving the Huntington disease gene and contrasts this lengthy and heroic quest with the relative ease of performing positional cloning with the human genome (and other genomes) in hand.

New to the Third Edition

One of the most obvious changes has been the addition of Analytical Questions to each chapter (except Chapter 1). I have always intended the Review Questions to check students' retention of the material in each chapter, and the answers are readily available in the text and figures. But many users of the book have asked me for questions that require a bit more thought and extrapolation beyond the presented material. That is the purpose of the new Analytical Questions. I thank Marie Pizzorno for her contribution to this new set of questions and welcome further contributions to expand these questions in future editions.

Most of the chapters of this third edition have been updated and include new information. Here are a few highlights:

Chapter 6: A considerable amount of new structural information has been added on prokaryotic RNA polymerase, including new x-ray crystal structures of the prokaryotic RNA polymerase holoenzyme and of the holoenzyme bound to DNA.

Chapter 7: The x-ray crystal structure of the complex of *lac* DNA, CAP-cyclic AMP, and the α-CTD of RNA polymerase shows exactly what part of the CAP protein contacts the α-CTD.

Chapter 8: This chapter shows a new insight into how transcription is controlled in bacterial cells infected with λ phage, including new evidence that shows how NusA facilitates transcription termination by facilitating the formation of a hairpin at the terminator, and how the λ N protein overrides termination by inhibiting hairpin formation. Also, we know how the heat shock σ-factor appears so rapidly after heat shock in *E. coli:* Elevated temperature melts inhibitory secondary structure in the mRNA, rendering it more accessible to ribosomes.

Chapter 10: New structural information on RNA polymerase II and its mechanism is presented in Chapter 10. For example: The structure of yeast polymerase II at atomic resolution reveals a deep cleft that can accept a linear DNA template from one end to the other. The catalytic center, containing a Mg^{2+} ion, lies at the bottom of the cleft. A highly mobile clamp appears to swing open to allow the DNA template to enter the cleft.

Chapter 11: Chapter 11 examines a new class II transcription elongation factor: Sometimes, phosphorylation on serine 2 of the RNA polymerase II CTD is also lost during elongation and that can cause pausing of the polymerase. For elongation to begin again, rephosphorylation of serine 2 of the CTD must occur.

Chapter 12: This chapter presents new information on insulators and insulator regulation, and new insights into how transcription can be controlled by covalent modifications, including ubiquitination and sumoylation of transcription factors.

Chapter 13: A new concept of a histone code is introducted in Chapter 13, with the interferon-β (INF-β) gene as an example. In principle, each particular combination of methylations, acetylations, phosphorylations, and ubiquitinations can send a different message to the cell about activation or repression of transcription.

Chapter 14: A minor class of introns with 5'-splice sites and branchpoints can be spliced with the help of a variant class of snRNAs, including U11, U12, U4atac, and U6atac.

Chapter 15: The CTD of the largest subunit of RNA polymerase II serves as a platform for assembly of factors that carry out capping, polyadenylation, and

splicing. These factors come and go as needed, and the phosphorylation state of the CTD can change as transcription progresses. Also, new information on the coupling of polyadenylation and transcription termination is presented.

Chapter 16: We introduce a more widespread form of RNA editing: Some adenosines in mRNAs of higher eukaryotes, including fruit flies and mammals, must be deaminated to inosine posttranscriptionally for the mRNAs to code for the proper proteins. Enzymes known as adenosine deaminases active on RNAs (ADARs) carry out this kind of RNA editing.

Chapter 17: We introduce a new eukaryotic translation initiation factor: This factor, eIF5B, is homologous to the prokaryotic factor IF2. It resembles IF2 in binding GTP and stimulating association of the two ribosomal subunits.

Chapter 19: We examine another role for IF1 in prokaryotic translation initiation: preventing aminoacyl tRNAs from binding to the ribosomal A site until the initiation phase is over.

Chapter 24: This chapter has seen the greatest change, as befits such a rapidly evolving subdiscipline. The proteomics part of the chapter has been expanded, including new techniques to probe protein–protein interactions. To relfect this expansion, the chapter has been renamed Genomics and Proteomics. We have also designed a short tutorial on the use of the NCBI website including: querying the database for a sequence match; finding information on a gene of interest; and viewing the structure of a protein of interest in three dimensions by rotating the structure on the computer screen.

The genomics part of the chapter has also been extensively revised. For example, the positional cloning of the Huntington disease (HD) gene has been moved to the beginning of the chapter as an introduction to genomics to illustrate how laborious such searches were before the genomics era. We also present a hypothesis to explain why expansion of the polyglutamine tract in huntingtin leads to the deterioration of the central nervous system that characterizes HD.

We also show that is is possible to define the essential gene set of a simple organism by mutating one gene at a time to see which genes are required for life. In principle, it is also possible to define the minimal genome—the set of genes that is the minimum required for life.

Supplements

- A presentation CD-ROM contains digital files for all of the line art, tables, and photographs in the text in an easy-to-use format. This format is compatible with either PC or Macintosh.
- Text-Specific Website
  The following website, specific to this text, provides access to digital image files, updates, and web links for both students and instructors. Separate message boards for both instructor and student discussion are also available:

  www.mhhe.com/weaver3

ACKNOWLEDGMENTS

In writing this book, I have been aided immeasurably by the advice of many editors and reviewers. They have contributed greatly to the accuracy and readability of the book, but they cannot be held accountable for any remaining errors or ambiguities. For those, I take full responsibility. I would like to thank the following people for their help.

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GUIDE TO EXPERIMENTAL TECHNIQUES IN MOLECULAR BIOLOGY

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