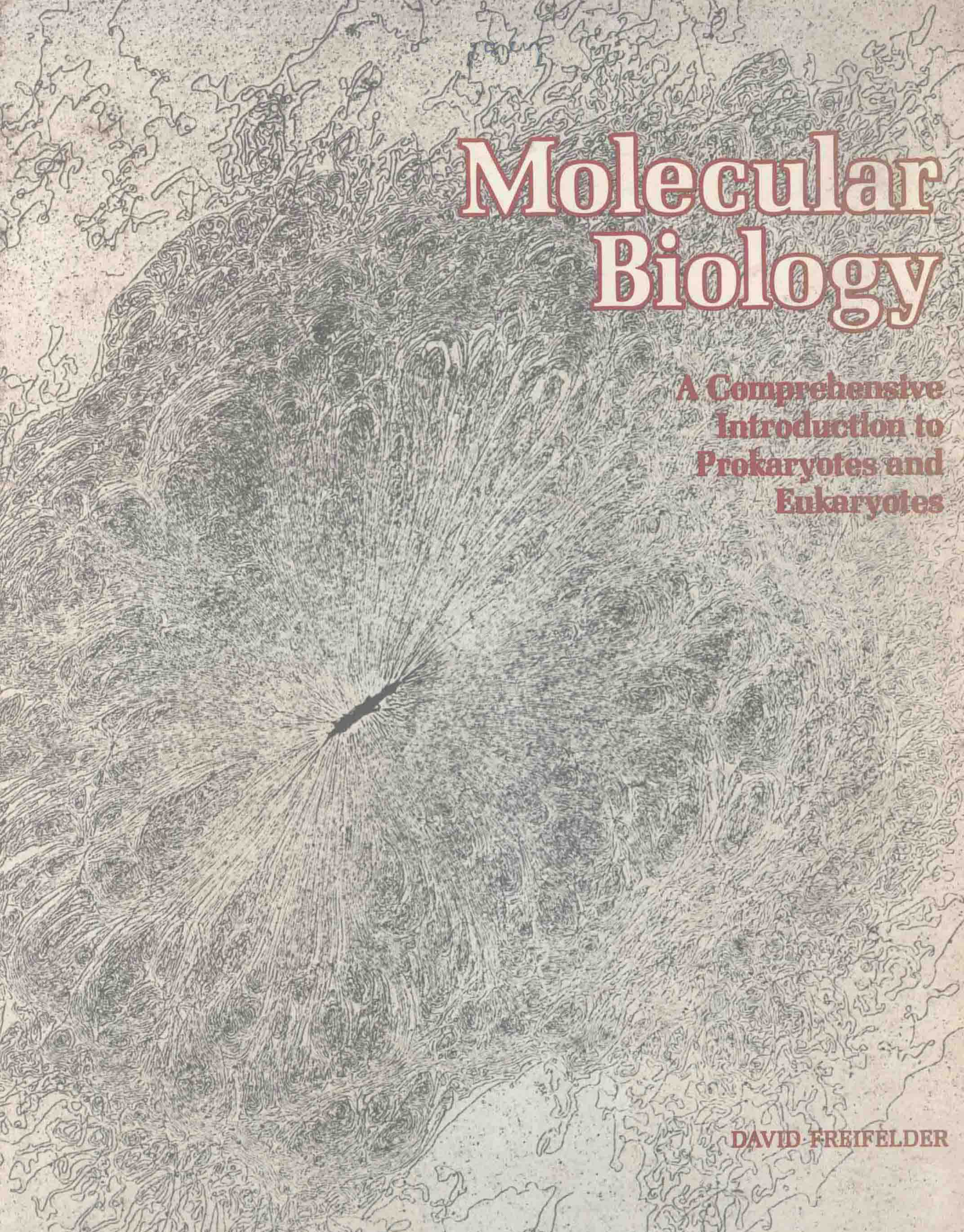

Molecular Biology

A Comprehensive Introduction to Prokaryotes and Eukaryotes

DAVID FREIFELDER

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To my parents,
MORRIS *and* FLORENCE,

and to my children,
RACHEL *and* JOSHUA

rather lengthy chapters on the chemical and physical properties of DNA and on nucleic acid synthesis has been used in a one-semester course in nucleic acids that I taught several years ago. Classical genetics, microbial genetics, and general features of genetic recombination are likewise treated in detail; my experience has been that when supplemented with sets of problems these topics can serve as a core for a molecular genetics course. (A separate problems book is available to make this application possible.) I expect that the book will be useful in advanced genetics courses, also. Finally, reviewers of the text who teach in medical schools have pointed out to me that the text can be used as a component of medical biochemistry and cell biology courses, in keeping with the current trend of emphasizing molecular processes in such courses. Before writing my text, I made a survey to determine the college year in which molecular biology and molecular genetics are usually taught. This was found to range from the sophomore year to the first year of graduate school. Thus, in an effort to accommodate various curricula, I have included in each chapter of my book basic information (sometimes in the form of a review or an overview), advanced topics, and discussion of many specific systems.

Molecular Biology differs in organization from other molecular biology texts: it follows my philosophy that molecular biology must emphasize both molecules and biology, and that to be molecular, it must also be chemical and physical—quite a tall order for a single book. With this goal in mind, I chose to present a biological introduction to the subject first (Chapter 1) before getting into the complexity of molecular detail, so that the student will know the phenomena that molecular biologists would at present like to explain and will be familiar with the biological systems used in research. A second introductory discussion, Chapter 2, reviews those aspects of organic chemistry needed by the reader and provides some basic biochemical concepts. The first major discussion following the introductory material is concerned with physical biochemistry; hence, there is extensive early coverage of the properties of macromolecules; this is a notable feature of the organization of this book. Early presentation of this material is based on my teaching experience, in which I have concluded that students have an easier time understanding the great variety of phenomena in which macromolecular interactions play a role if they have first acquired a thorough understanding of macromolecular structure. For example, the student should have a clear understanding of the significance of molecular shape and the interactions that give rise to the shape of a particular molecule; how, otherwise, can the concepts of templates, active sites, specificity of binding, and mutation be understood?

Following the treatment of macromolecules, the text turns to what is traditionally thought of as molecular biology—namely, DNA, RNA, and protein synthesis. In each case, both prokaryotic and eukaryotic systems are described in the same chapter; the prokaryotic systems are given in greater detail, as more is understood about these systems, and the similarities and differences between prokaryotes and eukaryotes are pointed out.

The next major unit of the book is concerned with how the basic synthetic processes are integrated to enable a prokaryotic cell or particle to function efficiently and to respond to a changing environment; here, the text examines in some detail metabolic regulation in bacteria and phages, stressing the notion of efficiency.

Features of genetic exchange—natural systems such as plasmids, transposons, and homologous recombination, and the artificial process of genetic engineering—form the next unit; parts of each chapter are presented as a survey, for a beginning course, but discussions of particular models are provided for use in courses of greater depth.

The final unit of the book is concerned exclusively with eukaryotic systems. It is divided into two parts, the first on animal and plant viruses—their basic biology and how they are regulated—the second on regulation in numerous eukaryotic systems, in both unicells and animal tissue.

A problems book (*Problems for Molecular Biology*, Science Books International/Van Nostrand Reinhold Company, 1983), containing more than 800 problems with complete and detailed solutions, is also available as a supplement to this text and is matched to the text chapter by chapter. Drill questions to test a student's mastery of facts are given for each major topic and many problems of a more substantive and sometimes difficult nature are also included. These problems have all been used in prior courses and have been extensively checked for ambiguities and correctness of solutions by undergraduate students at Brandeis University and graduate students and faculty at the University of California, Berkeley.

Inasmuch as *Molecular Biology* can be used in its entirety in either a condensed one-semester course, such as I have taught, or a more relaxed full-year course, instructors will have to be selective of the material for one-semester courses. To aid in this selection, summaries of the chapters—and especially of the points of view taken in the chapters—are presented in the following paragraphs.

Since the *modus operandi* of molecular biologists requires knowledge of phage biology, bacteriology, cell biology, and classical and microbial genetics, these topics are presented in Chapter 1 in a phenomenological way. Commonly used genetic tools such as recombinational mapping and complementation are reviewed. A brief discussion is also given of the logical approach taken in molecular biology that distinguishes it from the biology of the earlier part of the century. The basic life cycles of particular biological systems used by molecular biologists—namely, *E. coli*, phage, viruses, yeasts, and animal cells—

and the means for handling these systems—liquid culture, growth on agar, colony and plaque counting, and eukaryotic cell culture—are described in some detail. Chapter 2 provides a parallel review of elementary organic chemistry (e.g., functional groups) and introduces the molecules commonly found in biological systems. Since in many schools the study of molecular biology precedes that of biochemistry and cell biology, such biochemical topics as carbohydrate metabolism, the role of ATP in biochemical reactions, and the concept of the metabolic pathway are also included. A discussion of growth media for various organisms is also provided. To some students, little of the introductory material will be new, but my experience as a teacher has demonstrated to me that most college students have forgotten a great deal of what they learned in previous courses and profit from such a review. Instructors for advanced courses may choose to omit Chapters 1 and 2, but I advise having the student read these chapters quickly and reflect on possibly forgotten topics.

The text then turns to a study of macromolecules. Chapter 3 presents the general properties of macromolecules and some of the more common techniques used in their study. Chapters 4 and 5 treat nucleic acids and proteins, respectively, in considerable detail, with respect to both their chemistry and physical properties; structure is related to function whenever possible. A goal in the treatment of nucleic acids is to provide the student with the information needed to understand the mechanisms of replication and transcription, which are examined in later chapters. Furthermore, the reader will examine in some detail the properties of nucleic acids that researchers utilize when designing experiments investigating fundamental biochemical mechanisms. Chapter 5 has three goals—to give the student an appreciation of the variety of possible protein structures, to relate protein structure to biological function, and to indicate how a single amino acid change in a mutant can have a profound effect on protein function. In recent years knowledge of macromolecular structure has reached the point where examination of complex systems of macromolecules—for example, antibodies, chromatin, membranes, nuclear bodies, cell walls, and so forth—is profitable; these and other topics are included in Chapter 6. Some of these topics may be omitted from a short course, but the sections on immunoglobulins and chromatin should be included, for knowledge of these structures will be required in later chapters of the book.

To keep their science courses contemporary, most instructors cannot afford to spend much time on the story of how molecular biology evolved as a science; indeed, I believe many students find the topic less interesting nowadays than instructors do. (An instructor's interest is often derived from acquaintance with the personages involved, but increasingly the material must be considered history.) Accordingly, very little history is given in this book; however, I have included a brief presentation of the discovery of the genetic nature of DNA, for it still seems worth while to me to give beginning students some idea of the foundation of the field of study on which they are embarking. This historical interlude is presented in Chapter 7 but is given as part of a more general discussion of those properties that enable nucleic acids to serve as the repository of genetic information.

The treatment of what is traditionally thought of as molecular biology begins in Chapter 8 with DNA replication—an uncommon starting point. I have chosen this organization for five reasons: (1) the essential concepts of a template and of chemical polarity are easily introduced with this approach; (2) DNA replication examplifies, in a simple way, the kinds of geometric problems faced by many biochemical systems; (3) this topic shows how complicated a "simple" process can be; (4) the concepts of both variability of genetic material (for example, mutational changes) and protection of existing information (for example, repair processes) are more obvious here than in other topics; (5) the notion of regulation (that is, when DNA synthesis begins in a cell) can be introduced. The chapter on DNA replication is very lengthy because I have chosen to present examples of almost every known mode of replication in both prokaryotes and eukaryotes. The instructor will probably have to be selective and may choose to delay certain topics—for example, phage DNA replication—to later chapters.

Chapter 9, the subject of which is DNA repair, and Chapter 10, which is concerned with mutations, follow logically from a treatment of DNA replication, for in these chapters I have discussed the imperfections of biological systems and how cells deal with the problem of damage to their DNA by many environmental agents and reagents. Most of the material in these chapters consists of well-understood subjects; the main exception is SOS repair, a subject still actively being investigated.

By the time the reader has completed Chapter 10, he or she will have become familiar with the structure of the important intracellular molecules and will have examined systems for synthesis of one type of macromolecule. In so doing, the foundation will have been laid for understanding how a cell manages to do what it has to do at the appropriate time and how it responds to environmental factors. Thus, the reader is ready to be presented with the mechanisms of RNA synthesis and protein synthesis in prokaryotes and eukaryotes, which is done in Chapters 11–13. Chapter 11 deals with transcription; as in Chapter 8, the basic phenomenon is described first; then this is elaborated on in considerable detail. Special attention has been paid to essential features (such as recognition sites) of the macromolecules required for transcription, and the value of understanding the complete nucleotide sequence is made evident. Chapters 12 and 13 are concerned with protein synthesis. This immense topic has been divided into two parts—namely, the information problem, which is presented in Chapter 12, and the chemistry, which is given in Chapter 13. The structures of the relevant molecules (tRNA and the aminoacyl synthetases) and particles (ribosomes) are described twice—first in a simple way and then in considerable detail. The chemistry of protein synthesis is presented fairly completely and, in some cases, as somewhat advanced material, but chapter sections have been arranged in such a way that more detailed sections may be omitted, if desired. For example, a student can learn about protein synthesis by reading an overview consisting of a few pages, but if detail is wanted, a sequence of sections consisting of nearly twenty pages can be used.

Having completed all units on macromolecular synthesis, the reader is then ready to study how such synthesis is regulated. Chapter 14 is concerned with regulation in prokaryotes and presents the operon concept. (Regulation in eukaryotic cells is delayed to Chapter 22 because of its complexity, but it may be covered after Chapter 14.) The *lac* operon serves as the basis for the chapter because most of the features and concepts of regulation were developed by investigating this system. Discussion of the *lac* operon is rather lengthy, though, and some instructors may wish to omit certain subsections. In the early days of molecular biological research, when the *lac* operon was first being studied, it appeared that a general mechanism of regulation—namely, via operons—had been uncovered and that the *lac* system was the prototype. However, work from numerous laboratories has shown that though operons are indeed a general phenomenon in prokaryotes, a great many different regulatory programs exist for different metabolic systems; furthermore, the *lac* system has been found to be considerably more complex than was earlier thought. In Chapter 14 the reader is exposed to a wide variety of systems—the negatively regulated, the positively regulated, those with one promoter or with more than one promoter, degradative systems and biosynthetic systems, and so forth. The chapter is rather long and I assume that instructors will pick and choose among the systems. (Although a large number of operons are presented, selectivity has nonetheless been necessary and no doubt some instructors will find that a favorite system has been omitted.)

Chapters 15 and 16 treat the bacteriophages, elegant systems that exemplify the principles of regulation and of macromolecular synthesis. The study of phage biology is not simple because, though the life cycles of most phages follow the same basic pattern, details of the modes of nucleic acid replication and regulation often differ from one phage species to the next. Also, the reader will be introduced in this chapter to the first exception to the rule that DNA is the genetic material—namely, the RNA-containing phages. Many phage species are examined in these chapters—each one selected to demonstrate some feature of phage biology that can be easily observed with that phage. For example, T4 illustrates the mechanisms of taking over a host cell and of cleaving DNA prior to packaging, λ is more useful for examining the regulation of transcription and for studying a system with alternate life styles, φX174 illustrates the problems faced by a single-stranded DNA molecule, and the RNA-containing phages show that a genetic system can function without utilizing DNA, though special problems are encountered. Chapter 15 deals exclusively with the lytic life cycle; in Chapter 16, lysogeny, primarily of phage λ, and the related phenomenon, specialized transduction, are examined.

At this point in the book, the student will have seen how prokaryotic cells grow and maintain themselves in a changing and often hostile environment, and how continuous existence from one generation to the next is achieved. However, one of the essential features of life is that living organisms change in time. Organisms generally evolve slowly by accumulating mutations, but discontinuities in genetic composition can also occur by exchange of genetic material. Such exchange, called genetic recombination, occurs in a variety of ways, which are treated in some detail in Chapters 17–19. These three chapters are concerned respectively with plasmids and their transmissibility, the mechanism of homologous recombination, and transposition. Again, basic phenomena are described simply early in each chapter and details are given in later sections. Inclusion of all of these chapters may be impossible in a short course; however, if genetic engineering is to be covered later in the course, Chapter 17, on plasmids, should be included. The relatively new subject of transposition—treated in Chapter 19—is also important if regulation in eukaryotic systems is to be part of the course; this chapter can, however, be covered briefly by omitting the models for transposition.

Genetic engineering, the basis of the new biotechnology, is presented in Chapter 20. I have chosen to place this important topic late in the book because it is difficult to appreciate the applications and problems of this technique before learning about macromolecular synthesis and regulation. However, many instructors will prefer to discuss this topic early in their courses, so the use of probes can be included in the study of transcription and regulation. If this is to be the case, I would recommend that this chapter be preceded by Chapter 17 (plasmids) and parts of Chapter 11 (transcription).

In all earlier chapters except for Chapter 14 (prokaryotic regulation), both prokaryotic and eukaryotic systems are described and compared. The remainder of the book deals exclusively with eukaryotes. The animal viruses, which are given a lengthy treatment in Chapter 21, exhibit great variety in structure and in life cycles. Here the reader can see that just as the study of phage has given important insight into macromolecular processes and regulation in bacteria, study of viruses gives information about regulation in eukaryotes. Of course, animal viruses possess a great deal of intrinsic interest also, both as highly regulated systems and as agents of disease. A great many viruses are examined in Chapter 21; each one exemplifies a particular mode of transcription, replication, or production of viral proteins, the mode varying with the particular genetic material possessed by that virus. Two points emphasized in this chapter are that different virus types use rather different strategies for generating mRNA and that viruses use a variety of mechanisms to deal with the problem of making a sufficient number of protein types from a rather limited amount of nucleic acid. In a full-year course students will profit by studying this chapter completely; in a short course there surely will not be enough time, but I urge the instructor to find a place at least for poliovirus or vesicular stomatitis virus in order to demonstrate the role of polyproteins in eukaryotic systems.

The final chapter in this book—Chapter 22—is concerned with regulation in eukaryotes. Both unicellular organisms, such as yeast, and differentiated animal cells are included. The unusual organization of eukaryotic DNA, and gene families, as well as various modes of DNA deletion and amplification and recombination are all important topics. The role of hormones as regulators is examined in some detail. Several topics—such as synthesis of antibodies and of vitellogenin—are given rather lengthy treatments. This chapter will necessarily be frustrating to some readers because more questions are raised than answered. Because the field of eukaryotic regulation is advancing so rapidly, the instructor will find it necessary to supplement the text with the latest information, for no textbook can be up to date in this rapidly progressing field. I have chosen to present systems that examplify general principles and are reasonably well understood. This chapter should certainly be included in any course in molecular biology except one devoted exclusively to prokaryotes, because this subject is the molecular biology of the future.

An introductory textbook must necessarily contain a large number of terms not previously encountered by the reader. To aid in recognizing these terms and in finding the definitions at a later time, new terms are printed in **boldface** type where they first appear in the text.

Molecular Biology is the result of the effort of a large number of people. Many scientists served as consultants, reviewers, and contributors of illustrations. My consultants examined a proposed seven-page table of contents, answered a questionnaire concerned with the content of various courses and the background of students taking these courses, and let me know what they felt should be in a molecular biology book. My reviewers read preliminary and revised drafts of every chapter, corrected my mistakes, and gave me the latest unpublished information from their and other laboratories. These reviewers are listed following this preface. Many people contributed photographs and electron micrographs. I owe special thanks to Robley Williams (Professor of Molecular

Biology at the University of California, Berkeley) for allowing me to look through his immense collection of electron micrographs and use whatever I needed. The contributions of three people were indispensable in completing the manuscript. Mary Jane Voll (Professor of Microbiology at the University of Maryland) and Peter Gray (Professor of Biochemistry at the University of Oklahoma) read the entire second draft, then still in need of a great deal of revision, and gave me valuable scientific and pedagogical advice; Cedric Davern (Vice-President of the University of Utah) read the entire final draft before manuscript editing was begun. I was exceedingly fortunate to have the help of Peter Geiduschek (Professor of Biology at the University of California, San Diego), who read the page proofs of the entire book and examined all of the completed figures; he found errors I had missed and provided last-minute updating.

Arthur Bartlett, my friend and publisher of this and my earlier books, supported me more than amply in all phases of the work. Hal Lockwood (of Bookman Productions in San Rafael, California) designed and was responsible for the production of the book. I was most fortunate to have a great scientific illustrator, Donna Salmon, already known for her magnificent drawings in Lubert Stryer's *Biochemistry,* consent to illustrate the book. Not only is Donna a skilled illustrator, designer, and technician, but, through her unusual understanding of the scientific message of each figure, frequently she was able to add something for which each student and reader must be grateful. I was also fortunate to have the advice and editorial criticism of Kirk Sargent, the manuscript editor of this and two of my earlier books, who established a standard of clarity of writing that I could not have achieved myself and who made many valuable suggestions about increasing the information content of the illustrations. My long-time secretary and friend, Mildred Kravitz, typed most of the rough draft, and Elizabeth Lindheim, Judith Day, and Thomas Armstrong entered the final copy into my word processor. My daughter Rachel helped me in proofreading; it was my computer-expert son Joshua who helped me to master the capabilities of the word processor. Indexing was accomplished by "Indexor," a new computer program (Compress, Inc., Wentworth, New Hampshire) written by Jeremy Sagan and provided to me before it was commercially available. To all of these people I owe my thanks.

Now, after two and a half years of the most difficult work my students have ever suggested that I undertake, this book is ready for them and for other readers. I hope that the effort invested in it will have, as its result, that instructors and students of biology everywhere will find the book a new and valuable tool. If so, my great love of teaching will be satisfied and I will be content.

February 1983 David Freifelder
San Diego, California

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