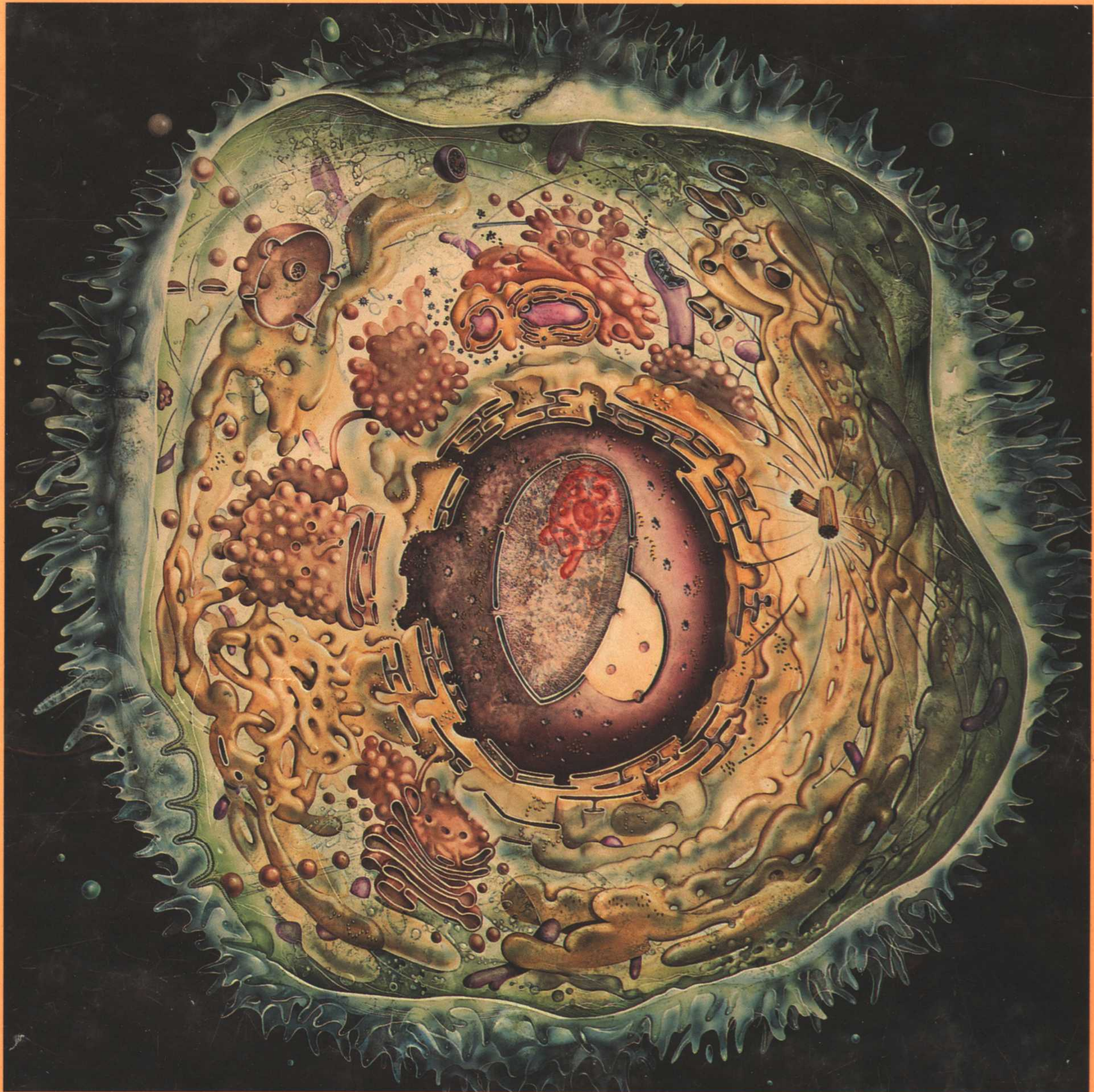
Cell and Molecular Biology

Concepts and Experiments



Gerald Karp

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PREFACE

Before I began work on this text, I drew up a number of basic guidelines regarding the type of book I planned to write. Having taught a course in cell and molecular biology for a number of years, and having written a previous text in the field, I had a clearly defined picture of the subject matter to be covered. There were more questions about the depth of coverage of topics and the way to treat the experimental nature of the material. I considered the following:

- Most introductory courses in cell and molecular biology run either a single semester or 1–2 quarters and are taken in the sophomore or junior year. I decided to draft a text that is best suited to this type of course; one whose length would not exceed about 800 pages and would not overwhelm or discourage students at this level.
- I decided to make a determined effort to elaborate on fundamental concepts, such as the relationship between molecular structure and function, the dynamic character of cellular organelles, the use of chemical energy in running cellular activities and insuring accurate macromolecular biosynthesis, and the nature of cellular regulation. I have tried to limit the amount of factual detail but, at the same time, include relatively detailed discussions of the mechanisms responsible for certain important cellular activities so that the reader could visualize how these activities might take place. I have had to be selective in choosing which mechanisms to explore in greatest depth. Examples include the mechanism by which cytochrome oxidase reduces water during aerobic respiration (page 196); the mechanism by which light absorption induces a separation of charge across the thylakoid membrane of a photosystem (page 227); the mechanism by which noncoding regions are removed from pre-mRNAs by the splicing machinery (page 478); and the mechanism by which signals are transmitted from the extracellular space to the cell interior via heterotrimeric G proteins (page 658).
- Possibly the single most important question that an author of a cell and molecular biology text must consider is how much experimental background to present. Like most biologists, I believe it is essential that students gain some knowledge about how we know what we know. There was a time not long past when a detailed discussion of a few handfuls of key experiments could serve to provide an introductory student with a strong appreciation for the experimental approach *and* a solid foundation in cell and molecular biology. Cell and molecular biology has matured and it is very difficult (impossible?) to identify individual studies that have such broad impact on the field. Experimental techniques have become more diverse and complex and it is easy to overwhelm students who have a limited background in methodologies.

  With this in mind, I decided to approach the experimental nature of the subject in two ways. As I wrote each chapter, I included enough experimental evidence to justify many of the conclusions that were being made. In addition, the salient features of key experimental techniques are described and the reader is referred to a more detailed discussion that appears in the last chapter on methodologies. Chapter 8 and 9, for example, contain introductory sections on the types of techniques that have proven most important in the analysis of cytomembranes and the cytoskeleton, respectively. Brief discussions of selected experiments have also been included in the body of the chapters to reinforce the experimental basis of our knowledge.

  A more indepth treatment of the importance of experimentation is located at the end of each chapter as a feature entitled "Experimental Pathways." Each of these narratives describes some of the key experimental findings that have led to our current understanding of a particular subject that is relevant to the chapter at hand. Because the scope of the narrative is limited, the design of the experiments can be considered in some detail. The figures and tables provided in these sections are often those that appeared in the original research article, which provides the reader with an opportunity to examine original data and to realize that its analysis is not beyond their means. The Experimental Pathways also illustrate the stepwise nature of scientific discovery, showing how the result of one study raises questions that provide the basis for subsequent studies.

- I have tried my best to make this text interesting and readable. To make the text more relevant to undergraduate readers, particularly premedical students, I have included a feature called "The Human Perspective." These sections illustrate that virtually all human disorders can be traced to disruption of activities at the cellular and molecular level. Furthermore, they reveal the importance of basic research as the pathway to understanding and eventually treating most disorders. In Chapter 11, for example, the Human Perspective describes how ribozymes may prove to be an important new tool in the treatment of cancer and viral diseases, including AIDS. In this same chapter, the reader will learn how ribozymes were first discovered in studies on the processing of ribosomal RNA in a protozoan. It becomes evident that one can never predict the practical importance of basic research in cell and molecular biology. Other examples of subjects considered in The Human Perspectives are the status of an AIDS vaccine, the prospects for gene therapy, and the cellular and molecular basis of aging.

Pedagogy

This text is divided into a relatively small number of chapters (17) for two primary reasons.

- Rather than breaking a general subject into separate chapters, its various aspects are dealt with together so that their interrelationships can be appreciated. For example, the interrelated subjects of the cytoskeleton and cell motility are covered in a single chapter, as are the subjects of membrane structure and function, membrane transport, and nerve cell excitability. Each chapter is divided into several major subdivisions which are assigned separate section numbers to facilitate reading assignments.
- Subjects such as immunity, developmental biology, and nerve and muscle physiology are treated as subdivisions of more general subjects. For example, antibody structure is discussed in the chapter describing the structure of proteins; the induction and propagation of a nerve impulse is covered in the chapter on membrane structure and function; and the DNA rearrangements that occur in antibody-producing cells are described in the chapter on the control of gene expression.

Many of the illustrations that depict complex processes have been "stepped out" (see Figure 4.50 as an example) so that information can be more easily broken down into manageable parts. Events occurring at each step are described either in the figure legend or in the corresponding text. The text includes approximately 500 photographs, which allow students to see actual representations of most subjects being discussed. Included among the photographs are a large number of full-color fluorescence micrographs that illustrate either the dynamic properties of cells (as in Figure 9.3) or provide a means to localize a specific protein (e.g., Figure 14.26) or a DNA or RNA sequence (e.g., Figure 12.25). Many of the figures include coordinated micrographs and line art drawings (such as those depicting the three-dimensional structure of tight junctions and gap junctions in Figures 7.29 and 7.31). In a few cases (see Figures 6.24 and 6.25), the line art is superimposed over the photograph to better illustrate where in the cell particular chemical reactions of a pathway occur.

End-of-chapter material includes a synopsis, review questions, analytic questions, and a listing of recent books and reviews for further reading. The synopsis is designed to identify key concepts (in bold face type) followed by a supporting discussion to help students review the material and test their comprehension. The synopsis is page referenced back to the chapter text to help in easily locating the relevant material. The references consist primarily of recent reviews that can serve as starting points for searching the literature in a particular area.

Supplements

Problems Book and Study Guide, by Nancy Pruitt, Colgate University (ISBN: 0471-14287-5). Designed to help students focus on concepts by providing key learning objectives and an outline for each chapter. Extensive review and analytical problems (with worked-out solutions) are also included. In addition, students are asked to answer questions about a key figure from each chapter of the text.

Instructor's Manual, by Joel Piperberg, Millersville University of Pennsylvania (ISBN: 0471-13591-7). Includes chapter outlines, teaching hints, analogy sections, and test questions.

CD-ROM. Includes key images from the text for use in lecture presentations.

Transparencies (ISBN: 0471-13592-5). Full-color acetates of key illustrations are included. The images have been enlarged and the fonts bolded for better projection capabilities.

Laboratory Investigations in Cell and Molecular Biology, by Allyn Bregman, SUNY-New Paltz (ISBN: 0471-51155-2). Contains 21 investigations of major topics in cell and molecular biology.

To the Student

When I began college, the thought of becoming a biology major was one of the last things on my mind. I enrolled in a physical anthropology course to fulfill the life science requirement by the easiest possible route. During that course, I learned for the first time about chromosomes, mitosis, and genetic recombination, and I became fascinated by the intricate activities that could take place in such a small volume of cellular space. The next semester, I took Introductory Biology and began to seriously consider becoming a cell biologist. I am burdening you with this personal trivia so you will understand why I wrote this book and my goals in doing so.

Even though many years have passed, I still find cell biology the most fascinating subject to explore and I still love spending the day reading about the latest findings by colleagues in the field. Thus, for me, writing a text in cell biology provides a reason and an opportunity to keep abreast of what is going on throughout the field. My primary goal in writing this text is to help generate an appreciation in students for the activities in which the giant molecules and minuscule structures that inhabit the cellular world of life are engaged. Another goal is to provide the reader with an insight into the types of questions that cell and molecular biologists ask and the experimental approaches they use to seek answers. As you read the text, think like a researcher; consider the evidence that is presented, think of alternate explanations, plan experiments that could lead to new hypotheses.

You might begin this approach by looking at one of the many electron micrographs that fill the pages of this text. To take this photograph, you would be sitting in a small, pitch-black room in front of a large metallic instrument whose column rises several meters above your head. You are looking through a pair of binoculars at a vivid, bright green screen. The parts of the cell you are examining appear dark and colorless against the bright green background. They are dark because they have been stained with heavy metal atoms that deflect a fraction of the electrons within a beam that is being focused on the viewing screen by large electromagnetic lenses in the wall of the column. The electrons that strike the screen are accelerated through the evacuated space of the column by a force of tens of thousands of volts. One of your hands may be gripping a knob that controls the magnifying power of the lenses. A simple turn of this knob can switch the image in front of your eyes from that of a whole field of cells to a tiny part of a cell, such as a few ribosomes or a small portion of a single membrane. Your other hand may be gripping another knob, which you can turn back and forth so as to bring the image of the specimen into the very sharpest focus. Once you have satisfied yourself that the image is focused, you can turn a handle that lifts the screen out of view, allowing the electron beam to strike a piece of film and produce a photographic image of the specimen.

Since the study of cell function generally requires the use of considerable instrumentation, the investigator is quite far removed from the subject being studied. To a large degree, cells are like tiny black boxes. We have developed many ways to probe the boxes, but we are always groping in an area that cannot be fully illuminated. A discovery is made or a new technique is developed and a new thin beam of light penetrates the box. With further work, our understanding of the nature of the structure or process is broadened, but we are always left with additional questions. We generate more complete and sophisticated constructions, but we can never be sure how closely our views approach the reality we assume is there. In this regard, the study of cell and molecular biology can be compared to the study of an elephant as conducted by six blind men in an old Indian fable. The six travel to a nearby palace to learn about the nature of elephants. When they arrive, each approaches the elephant and begins to touch it. The first blind man touches the side of the elephant and concludes that an elephant is smooth like a wall. The second touches the trunk and decides that an elephant is round like a snake. The other members of the group touch the tusk, leg, ear, and tail of the elephant, and each forms his impression of the animal based on his own limited experiences. Cell biologists are limited in a similar manner to what they can learn by using a particular technique or experimental approach. While each new piece of information adds to the preexisting body of knowledge to provide a better concept of the activity being studied, the total picture remains uncertain.

Before closing these introductory comments, let me take the liberty of offering the reader some advice: Don't accept everything you read as being true. There are several reasons for urging such skepticism. Undoubtedly, there are errors in this text that reflect the author's ignorance of or misinterpretation of some aspect of the scientific literature. But, more importantly, there is reason to consider the nature of biological research. Biology is an empirical science; nothing is ever *proved.* We merely compile data concerning a particular cell organelle, metabolic reaction, intracellular movement, etc., and draw some type of conclusion. Even if there is a consensus of agreement concerning the "facts" regarding a particular phenomenon, there are often several possible interpretations of the data. Hypotheses are put forth and generally stimulate further research, thereby leading to a reevaluation of the original proposal. A theory is constructed in terms of the concepts and prevailing perspectives of the time. As new techniques and information become available, new insights are made. Most hypotheses that remain valid undergo a sort of evolution and, when presented in the text, should not be considered wholly correct or incorrect. Remain skeptical.

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Several people have played particularly important roles in the development of this book and I would like to give them special thanks. I would first like to thank Sally Cheney who was the biology editor at John Wiley & Sons through 1994. In addition to being a dear friend, Sally is one of the most generous, insightful, and brightest people I have ever known. Thank you for all the confidence you have shown in me and this project. Fortunately, Sally was succeeded by a very capable individual. Since coming onto the project, David Harris has played a major role in directing the art program, providing guidance in organizing the book's contents, and assembling a support team to put all the pieces together. Among the production team at John Wiley, I want to give special thanks to Katharine Rubin and Hilary Newman who have worked so hard and effectively on this project. To Katharine, I owe my sanity. She was the central nervous system for this project, coordinating the information arriving from compositors, copyeditors, proofreaders, illustrators, photo editors, designers, and dummiers, as well as a constant barrage of text changes ordered by the author. Always calm, organized, and meticulous, she made sure everything was done correctly. Hilary is responsible for the superb photo program in the text. In addition to obtaining photos that I requested, she scoured the agencies making sure that we got the very best photos possible. She never gave up on any photo I really wanted, regardless of the number of faxes or calls that it took. Those who suffered from the "bite of the bulldog" will curse her name forever. All of the original art, which includes all but a few pieces picked up from other Wiley books, was drawn by computer by Anthony Poole. It has been a real pleasure to work with such a talented artist and a gracious person. Any success that this book might achieve will be due in large part to its fine illustration program. A special thanks is owed Cathy Lundmark, a biologist who served as copyeditor for the manuscript and who improved its quality immeasurably. I am also indebted to Dr. Mary Lee S. Ledbetter of the College of the Holy Cross, a wonderful reviewer who made valuable comments on nearly every page of the manuscript. Thanks to Dr. Elizabeth Coolidge-Stolz and Denise Lello for writing the glossary; and to Gloria Hamilton, who prepared the index. I would also like to thank Edward Starr, illustration coordinator; Ishaya Monokoff, illustration director; Pam Kennedy, production director; Laura Nicholls, designer; Rebecca Herschler, marketing manager; Marian Provenzano, acting editor; and Cathy Donovan, editorial program assistant, for their important contributions.

I am especially grateful to the many biologists who have contributed micrographs for use in this book; more than any other element, it is these photographs that bring the study of cell biology to life on the printed page. Finally, I would like to apologize, in advance, for any errors that may occur in the text, and express my heartfelt embarrassment. Any comments or criticisms from readers would be greatly appreciated. They can be directed to: Biology Editor, John Wiley & Sons, 605 Third Avenue, New York, NY 10158.

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