GENES & GENOMES

A CHANGING PERSPECTIVE

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The cover photograph shows an electron micrograph of eukaryotic chromatin in which both ends of a DNA loop appear to emanate from adjacent points in the protein matrix located at the bottom of the micrograph. (Photograph supplied by U. K. Laemmli.)

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Preface

This book is about the molecular structures and mechanisms that underlie the utilization of genetic information by complex organisms. We hope, in the presentation, to capture the sense of discovery, understanding, and anticipation that has followed from the recombinant DNA breakthrough.

To introduce these developments, Perspective I and Chapters 1 through 3 emphasize the classical genetic and biochemical studies from the beginning of this century to circa 1972. These studies, carried out largely with microorganisms, defined our knowledge of the structure and function of genes and genomes. The emergence of the recombinant DNA methods in the early 1970s and the associated advance in rapid DNA sequencing technology soon thereafter provided the means for a molecular approach to the analysis of more complex genomes. Thus, the core of this book deals with research covering less than two decades and reveals the extraordinary depth and breadth of opportunity that these methods provide to biology.

The book has been a long time in the works. The idea for it originated with a series of public lectures one of us (P.B.) gave in 1979 at the University of Pittsburgh. The efforts to prepare the lectures for ublication were frustrating. In speaking to an audience of nonbiologists, many interesting and exciting details of the science were omitted. A book that lacked such detail would surely be unsatisfactory. As the concept of the book expanded, the need to share the effort became apparent. Thus, the two of us set to work. With only two authors, both working over all the material, we hoped to obtain a unified, coherent approach and style. But we also recognized that it would be foolhardy to attempt the comprehensive coverage attained by larger groups of authors in several excellent recent texts. We also faced the continuing task of updating previously written sections in order to have the final manuscript reasonably

current. To avoid the risk of being overwhelmed by the explosive pace at which new information emerged, we limited our goals. Thus, we have focused on selected specific areas that have already been studied in some depth and that illustrate the progress that is being made.

Our overall plan was to emphasize by depth and experimental approach the science that has come to be called molecular genetics, as it is applied to eukaryotes. To accomplish this, and yet have a text that can be used by readers with only limited prior knowledge of biochemistry, cell biology, and genetics, we have provided background materials in two ways. First, Chapters 1, 2, and 3, respectively, summarize the essential information on the structures of DNA, RNA, and proteins; the various cellular transactions involving DNA (replication, repair, and recombination); and the fundamental mechanisms of transcription, translation, and control of gene expression. Readers who are well versed in this material may want to skip the first three chapters. Second, the essays denoted Perspectives I, II, and III provide a historical introduction and overview of the concepts described in the chapters comprising Parts I, II, and III of the book, respectively. These essays do not say much about how these concepts were discovered and verified. Rather, they attempt to convey how the intellectual framework of modern biology was constructed from diverse studies involving chemistry, genetics, microbiology, cell biology, and evolutionary biology. Thus, Perspective I, which introduces Chapters 1, 2, and 3, traces the historical path to our present views on inheritance. It introduces, along the way, the concepts of a gene, the transmission and segregation of genes, the rationale for the early mapping of genetic determinants to specific chromosomal locations, the identification of genes as segments of nucleic acid, and the emergence of the informational relationships between DNA, RNA, and proteins.

The four chapters in Part II describe tools and methods for constructing, cloning, selecting, and characterizing recombinant DNA molecules as well as other experimental methods in molecular genetics. The emphasis is on the logic of the methods; the chapters do not constitute a laboratory manual. Readers interested in detailed procedures are directed, by reference, to the several excellent manuals published during the last decade. The Perspective II essay that precedes these chapters stresses the historical development of the tools and methods from a large and disparate body of fundamental research in enzymology, bacterial and bacteriophage genetics, and nucleic acid chemistry.

The principal aim of the book is realized in Part III, which describes the major concepts that define current understanding of the genetic information systems of eukaryotes. The Perspective III essay includes an introduction to the distinctive properties of eukaryotic genomes, including introns and the complex signals that regulate transcription, as well as the abundance of sequence repetition in the genome, and the related question of the role of reverse transcription in the origin of eukaryotic DNA segments. The essay ends with a description of the unifying concepts pro-

vided by thinking about biological evolution as a process that fundamentally involves the reshaping of nucleic acid and, consequently, protein structure.

The first of the three chapters in Part III (Chapter 8) examines the structure of eukaryotic genes and our present understanding of their expression, including (1) the complex signals for regulating transcription and (2) the origins, locations, and structures of introns and the mechanisms by which introns are removed from primary transcripts by splicing. Fundamental to these descriptions is the use of reverse genetics—the design and construction of specifically mutated DNA segments—for the analysis of structure-function relationships in eukaryotic genes. Chapter 9 focuses on the way genetic information is organized in complex genomes. The emphasis is on the distribution of genes and other DNA sequences, including their relation to chromosome morphology. The concept of the genome as a record of evolutionary history threads throughout. The chapter concludes with a description of the genomes of the intracellular organelles—mitochondria and chloroplasts. The diverse mechanisms involved in random and nonrandom rearrangements of genomic DNA are described in Chapter 10. These include amplifications, deletions, and transpositions, both those that are unprogrammed and lead to mutagenesis and those such as yeast mating type switches and immunoglobulin gene construction, which are programmed and regulate gene expression in precise and essential ways.

This volume closes with the Perspective IV essay, which illustrates briefly how the general concepts presented earlier apply to specific and complex biological systems. Perspective IV provides an introductory overview to Part IV of the book, which will appear in a separate volume. Part IV emphasizes that genes operate in complex, multicomponent, interacting systems. In each system, the general operating principles are applied in different ways, leading to the great diversity of living things. In the past, much of this diversity was described in phenomenologic terms. Now the phenomenology can be reinterpreted with molecular descriptions of the regulation, in time and place, of gene expression. Part IV also describes how, as a consequence of the recombinant DNA methods, biology has changed from a descriptive to a manipulative science. The genotypes, and thus the phenotypes of individual proteins and of whole cells and organisms, can be altered, providing future opportunities to investigate fundamental biological processes as well as to address critical problems facing our species and the planet we inhabit. Many of the opportunities will be realized only after we acquire greater understanding of the structure of genomes. This includes locating the positions of genes and extensive sequence information. Part IV introduces the concepts fundamental to current efforts to map and sequence the genomes of several species, including humans.

In 1980, when we first began thinking about this book, we, and many other biologists, were just emerging from a period when

public policy issues vied with science for daily attention. The exciting research stemming from the recombinant DNA methodology was only one aspect of what has been called a "revolution in biology." Besides initiating a new era in our ability to understand living things, the revolution initiated unprecedented social concern over the impact of biological research.

The earliest public concerns reflected questions raised by the community of biological scientists and centered on the safety of recombinant DNA experiments. Laboratory safety has always concerned people working with pathogenic microorganisms—viruses and bacteria. These concerns were renewed about 1971 as more work was carried out worldwide on viruses that cause tumors in experimental animals. The distinctive life cycle of these viruses compared to the more familiar viruses (for example, those that cause measles and polio) and the possibility that some of them might cause human cancer prompted a serious look at the possible hazards to scientists and their students. It was in this context that the recombinant DNA methods emerged. The general atmosphere of concern was also influenced by the then pervasive sense of social responsibility in the United States, a sense that grew out of the prior decade's turmoils over civil rights and the war in Vietnam.

The earliest prototypes for recombinant DNA experiments involved the joining of DNA from a virus causing tumors in small laboratory rodents to parts of the DNA of a well-studied bacterial virus. Plans to introduce the new DNA molecule into bacterial cells aroused serious reservations, but these were stilled by the decision to defer such attempts. The next major step involved constructing recombinant DNA with a gene that makes bacterial cells resistant to antibiotics. The ability to transform living cells genetically by introducing such novel DNA molecules into bacteria raised questions about whether such bacteria might cause tumors or acquire natural resistance to medically important antibiotics. The issues were raised at scientific meetings and in private conversations. At one such meeting in June, 1973, the participating scientists called attention to this promising line of research and asked the National Academy of Sciences to undertake a focused study of the possible risks of recombinant DNA experimentation. To publicize this request, their letter was published in *Science*.

The Academy responded (as academies typically do) by forming a committee, this one of scientists actively engaged in recombinant DNA and related work. This committee met in April, 1974, and proposed two major steps that became, to their surprise, front page news. First, they recommended a worldwide moratorium on those recombinant DNA experiments that involved tumor virus DNAs or introduced genes for potent toxins or antibiotic resistance into bacteria that normally did not have such genes. Second, they called for an international, broad-gauged discussion of the issues, to be held at a meeting the following winter.

Although there were rumblings of discontent and even accusations that U.S. scientists were trying to slow up everyone else's work so they could win a race to the major discoveries promised by

the new methods, the moratorium was, as far as anyone knows, universally honored. Molecular biologists, virologists, microbiologists, and biochemists from the United States and abroad, as well as science administrators, journalists, and even lawyers, readily accepted invitations to the proposed conference.

That gathering took place in February, 1975, at Asilomar, a state of California meeting center on the edge of the Pacific, a few miles south of Stanford University's Hopkins Marine Station in Pacific Grove, where these words are being written. The Organizing Committee had arranged for prior working group sessions and working paper preparation by experts in several different areas for presentation. There was a good deal of discussion, some heated, about the reality of the dangers being discussed. There was broad agreement but not unanimity that if risks of constructing hazardous organisms existed, their likelihood was very low. But consensus on a future course of action seemed remote until the lawyers took their turn. They underscored the personal legal responsibility of scientists, even for highly unlikely events. They also reminded us that the public can be very restrictive about remote risks if fear of consequences, however unlikely, is great. The message was clear. Continuing a responsible stance was the only sensible course. On the last day, the Organizing Committee's proposed report was debated vigorously but finally accepted. The Asilomar Conference's recommendations were widely reported in the press and later published in several scientific journals. Similar conclusions had been reached by a British government committee under Lord Ashby a few weeks earlier.

The recommendations made at Asilomar provided the framework and starting point for official U.S. action, which began the day after the conference closed. A committee organized by the National Institutes of Health (NIH) began work on guidelines to govern all recombinant DNA experiments carried out in institutions with their funding. The original guidelines, published in June, 1986, were intentionally strict, with the expectation that, as experience and knowledge accumulated, they could be revised. To this day, no untoward events to laboratory personnel or the public are known to have originated from the tens of thousands of recombinant DNA experiments that have been conducted. The containment requirements for most routine recombinant DNA experimentation have been eliminated or relaxed. The only experiments that still require strict containment within the NIH guidelines (or those in most other countries) involve recombinants that include extensive DNA regions from highly pathogenic organisms. Interestingly, recombinant DNA techniques have made the study of some important but very dangerous infectious agents of humans and animals feasible and safe.

Soon after Asilomar, and with varying intensity until the present, public interest and concern have continued over recombinant DNA experiments and their extension to the genetic engineering of whole organisms—bacteria, plants, and animals. At first, the focus was the same as that of the majority of the scientific community—

the potential of the experiments to create disease-producing agents. Local and state governments passed laws and ordinances mandating compliance with the NIH guidelines or somewhat more restrictive rules. Bills proposing to make the NIH guidelines law and mandating punishments for noncompliance were introduced and debated in Congress, but none ever passed. These numerous independent debates, in various locales and at many levels, ultimately validated the NIH approach to the problem—both the scientific risk analysis and the administrative organization.

Later, and to this day, other kinds of issues dominate the public debate. A few scientists raised questions about the possible evolutionary consequences of passing DNA across species boundaries, for example, inserting human DNA into bacteria. These questions attracted attention from many nonscientists, but the issue is now viewed as inconsequential. There are myriad opportunities in nature for such exchanges of DNA, and they have probably occurred many times. Recombinant DNA experiments do not significantly increase the opportunities. Moreover, most organisms harboring recombinant DNA molecules are unlikely to thrive except under very special laboratory conditions. The latter argument is also pertinent to the recent discussions regarding government regulation of the deliberate release of genetically engineered organisms into the environment for various agricultural purposes. Although careful consideration of the potential problems associated with such releases is essential, excessive requirements, including protective garb like astronaut suits, are, from a scientific standpoint, unwarranted.

The possible application of recombinant DNA techniques to the development of biological warfare agents is raised continually; the United States is party to a 1972 international convention prohibiting such work. The Defense Department continues to support work aimed at defense against biological weapons that others might develop. This too has been debated because the differences between research intended for offensive and defensive purposes are not easily distinguished. This debate, properly, attracts scientists and nonscientists alike because it is fundamentally a political and social question, not a scientific one, as are discussions by the public of other questions arising out of the new biology. What are the advantages and disadvantages of gene therapy for humans should it ever become feasible? How should society, employers, and individuals deal with the growing amount of information about people's genes that will be acquired as a result of the new technologies? Are there valid ethical concerns associated with introducing human genes into animals? Should genetically engineered animals and plants be patentable?

The early public fears about the biological revolution engendered many negative attitudes about the research. Biologists then feared the worst: highly restrictive laws or regulations that would seriously hinder further experimentation and its promise of new knowledge and beneficial applications to medicine, agriculture, and industry. Many scientists regretted the initial open discussion of

the issues in the face of successful demagoguery by critics and the tendency of newspapers to build hype rather than carefully explore difficult issues. Yet it has turned out well. The science described in this book attests to that, as do the growing number of important products being produced by the young and energetic biotechnology industry. Perhaps the moratorium and initially restrictive guidelines held things up, but only briefly. The early caution in the face of ignorance was prudent, even though hindsight suggests that the risk scenarios were far less likely than we had supposed.

MAXINE SINGER AND PAUL BERG

Acknowledgments

Contrary to the widely held image, science is a very social activity, and most scientific work depends on a good deal of collaboration and consultation. This book is no exception. We are grateful for the cooperation and constructive, patient help of our colleagues who read and criticized sections or chapters of the text: David Finnegan, Claude Klee, Arthur Kornberg, I. R. Lehman, Howard Nash, Bruce Paterson, Carl Schmid, and Robert Tjian. We also thank those who provided unpublished information and manuscripts.

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Molecular geneticists think about genes and genomes visually. We "see" DNA and schemes describing how DNA functions in our minds' eyes. Thus, the diagrams in scientific papers and textbooks like this one are critical to our understanding and memory. The wonderful pictures and diagrams in this book were prepared by Charlene Kornberg and her colleagues at Stanford University Medical School. They are an integral part of the narrative, and in many instances, they clarify and amplify the text. Our own naivete

about the difficult process of preparing the drawings and our insistence on an abundance of pictures made the job burdensome and long. We thank all those who worked so diligently under Charlene Kornberg's direction: Meryle Colten, Butch Colyear, Mike Maystead, Eunice Ockerman, Lois Schoen, Kelly Solis-Navarro, Karen Sullivan, and Linda Toda. But it is we, not they, who are responsible for any errors that may remain.

Among the splendid advantages available to modern biologists are the libraries wisely established by earlier generations. During our work, we have spent time reading and writing at three such places, all conveniently remote from telephones and daily responsibilities. The librarians and other staff members of the Marine Biological Laboratory at Woods Hole, Massachusetts, of The Jackson Laboratory at Bar Harbor, Maine, and of the Hopkins Marine Station of Stanford University at Pacific Grove, California, were all hospitable and helpful.

Harry Woolf, then director of the Institute for Advanced Studies at Princeton, New Jersey, graciously provided opportunities to work at that quiet place. His kind interest, hospitality, and friendship, and the fine food offered by the Institute's eclectic chef, Franz Moehn, made those days memorable. Curiously, our ability to work uninterrupted at Princeton was assured by the almost universal disinterest of the institute's physicists and mathematicians in talking to biologists.

Quiet, uninterrupted days for work were also assured during Paul Berg's tenure as a fellow of Clare Hall, Cambridge. The lively intellectual community there, presided over by Michael Stoker, was congenial and stimulating.

Over the years, the science we hoped to capture here progressed at an extraordinary and unprecedented pace. Continual revisions put heavy loads on the good humored and expert people who typed and retyped innumerable drafts. Without Eleanor Olson and Dot Potter at Stanford and May Liu and Gail Gray in Bethesda, nothing would have been accomplished.

Millie Berg and Dan Singer have been understandably impatient at times, but their support was always clear and always welcomed. So, too, the younger generations of the Berg and Singer families, though none is a biochemist or molecular biologist, have bolstered us with their curiosity, enthusiasm, pride, and love.

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