PREFACE

One of my most exciting educational experiences was my introductory molecular biology course in graduate school. My professor used no textbook, but assigned us readings directly from the scientific literature. It was challenging, but I found it immensely satisfying to meet the challenge and understand, not only the conclusions, but how the evidence supported those conclusions.

When I started teaching my own molecular biology course, I adopted this same approach, but tried to reduce the challenge to a level more appropriate for undergraduate students. I did this by narrowing the focus to the most important experiments in each article, and explaining those carefully in class. I used hand-drawn cartoons and photocopies of the figures as illustrations.

This approach worked well, and the students enjoyed it, 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 bacteria. 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 bacterial 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 and silencers can interact with coactivators and corepressors to modify histones, and thereby 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 four kinds of posttranscriptional control of gene expression: (1) RNA interference; (2) modulating mRNA stability (using the transferrin receptor mRNA as the prime example); (3) control by microRNAs, and (4) control of transposons in germ cells by Piwi-interacting RNAs (piRNAs).

Chapters 17–19 describe the translation process in both bacteria 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 bacteria. 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 bacteria 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.

Chapters 24 and 25 present concepts of genomics, proteomics, and bioinformatics. Chapter 24 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). Chapter 25 deals with functional genomics (transcriptomics), proteomics, and bioinformatics.

New to the Fifth Edition

The most obvious change in the fifth edition is the splitting of old Chapter 24 (Genomics, Proteomics, and Bioinformatics) in two. This chapter was already the longest in the book, and the field it represents is growing explosively, so a split was inevitable. The new Chapter 24 deals with classical genomics: the sequencing and comparison of genomes. New material in Chapter 24 includes an analysis of the similarity between the human and chimpanzee genomes, and a look at the even closer similarity between the human and Neanderthal genomes, including recent evidence for interbreeding between humans and Neanderthals. It also includes an update on the new field of synthetic biology, made possible by genomic work on microorganisms, and contains a report of the recent success by Craig Venter and colleagues in creating a living *Mycoplasma* cell with a synthetic genome.

Chapter 25 deals with fields allied with Genomics: Functional Genomics, Proteomics, and Bioinformatics. New material in Chapter 25 includes new applications of the ChIP-chip and ChIP-seq techniques—the latter using next-generation DNA sequencing; collision-induced dissociation mass spectrometry, which can be used to sequence proteins; and the use of isotope-coded affinity tags (ICATs) and stable isotope labeling by amino acids (SILAC) to make mass spectrometry (MS) quantitative. Quantitative MS in turn enables comparative proteomics, in which the concentrations of large numbers of proteins can be compared between species.

All but the introductory chapters of this fifth edition have been updated. Here are a few highlights:

- Chapter 5: Introduces high-throughput (next generation) DNA sequencing techniques. These have revolutionized the field of genomics. Chromatin immunoprecipitation (ChIP) and the yeast two-hybrid assay have been moved to Chapter 5, in light of their broad applicabilities. A treatment of the energies of the β-electrons from ^{3}H, ^{14}C, ^{35}S, and ^{32}P has been added, and the fluorography technique, which captures information from the lower-energy emissions, is discussed.
- Chapter 6: Adds a discussion of FRET-ALEX (FRET with alternating laser excitation), along with a description of how this technique has been used to support (1) the stochastic release model of the ϕ-cycle and (2) the scrunching hypothesis to explain abortive transcription. This chapter also updates the structure of the bacterial elongation complex, including a discussion of a two-state model for nucleotide addition.
- Chapter 7: Introduces the riboswitch in the mRNA from the *glmS* gene of *B. subtilis,* in which the end product of the gene turns expression of the gene off by stimulating the mRNA to destroy itself. This chapter also introduces a hammerhead ribozyme as a possible mammalian riboswitch that may operate by a similar mechanism.
- Chapter 8: Introduces the concepts of anti-sigma factors, and anti-anti-sigma factors as controllers of transcription during sporulation in *B. subtilis*.
- Chapter 9: Emphasizes the dynamic nature of protein structure, and points out that a given crystal structure represents just one of a range of different protein conformations.
- Chapter 10: Presents a new study by Roger Kornberg's group that identifies the RNA polymerase II trigger loop as a key determinant in transcription specificity, along with a discussion of how the enzyme distinguishes between ribonuncleotides and deoxyribonucleotides.
- This chapter also introduces the concepts of core promoter and proximal promoter, where the core promoter contains any combination of TFIIB recognition element, TATA box, initiator, downstream promoter element, downstream core element, and motif ten element, and the proximal promoter contains upstream promoter elements.
- Chapter 11: Introduces the concept of core TAFs—those associated with class II preinitiation complexes from a wide variety of eukaryotes, and introduces the new nomenclature (TAF1–TAF13), which replaces the old, confusing nomenclature that was

based on molecular masses (e.g., $TAF_{II}250$). This chapter also describes an experiment that shows the importance of TFIIB in setting the start site of transcription. It also shows that a similar mechanism applies in the archaea, which use a TFIIB homolog known as transcription factor B.

- Chapter 12: Introduces the technique of chromosome conformation capture (3C) and shows how it can be used to detect DNA looping between an enhancer and a promoter. This chapter also introduces the concept of imprinting during gametogenesis, and explains the role of methylation in imprinting, particularly methylation of the imprinting control region of the mouse *Igf2/H19* locus. It also introduces the concept of transcription factories, where transcription of multiple genes occurs. Finally, this chapter refines and corrects the concept of the enhanceosome.
- Chapter 13: Presents a new table showing all the ways histones can be modified in vivo; brings back the solenoid, alongside the two-start helix, as a candidate for the 30-nm fiber structure; and presents evidence that chromatin adopts one or the other structure, depending on its nucleosome repeat length. This chapter also introduces the concept of specific histone methylations as markers for transcription initiation and elongation, and shows how this information can be used to infer that RNA polymerase II is poised between initiation and elongation on many human protein-encoding genes. It also emphasizes the importance of histone modifications in affecting not only histone-DNA interactions, but also nucleosome-nucleosome interactions and recruitment of histone-modifying and chromatin-remodeling proteins. Finally, this chapter shows how PARP1 (poly[ADP-ribose] polymerase-1) can facilitate nucleosome loss from chromatin by poly(ADP-ribosyl)ating itself.
- Chapter 14: Introduces the exon junction complex (EJC), which is added to mRNAs during splicing in the nucleus, and shows how the EJC can stimulate transcription by facilitating the association of mRNAs with ribosomes. This chapter also introduces exon and intron definition modes of splicing and shows how they can be distinguished experimentally. This test has revealed that higher eukaryotes primarily use exon definition and lower eukaryotes primarily use intron definition.
- Chapter 15: Demonstrates that a subunit of CPSF (CPSF-73) is responsible for cutting a pre-mRNA at a polyadenylation signal. It also shows that serine 7, in addition to serines 2 and 5 in the repeating heptad in the CTD of the largest RNA polymerase subunit, can be phosphorylated, and shows that this serine 7 phosphorylation controls the expression of certain genes (e.g., the U2 snRNA gene) by controlling the 3′-end processing of their mRNAs.
- Chapter 16: Identifies a single enzyme, tRNA 3′ processing endoribonuclease, as the agent that cleaves excess nucleotides from the 3′-end of a eukaryotic tRNA precursor; points out the overwhelming prevalence of trans-splicing in *C. elegans.;* presents a new model for removal of the passenger strand of a double-stranded siRNA—cleavage of the passenger strand by Ago2; introduces Piwi-interacting RNAs (piRNAs) and presents the ping-pong model by which they are assumed to amplify themselves and inactivate transposons in germ cells; introduces plant RNA polymerases IV and V, and describes their roles in gene silencing. This chapter also greatly expands the coverage of miRNAs, and points out that hundreds of miRNAs control thousands of plant and animal genes, and that mutations in miRNA genes typically have very deleterious effects. Chapter 16 also updates the biogenesis of miRNAs, introducing two pathways to miRNA production: the Drosha and mirtron pathways. Finally, this chapter introduces P-bodies, which are involved in mRNA decay and translational repression.
- Chapter 17: Updates the section on eukaryotic viral internal ribosome entry sequences (IRESs). Some viruses cleave eIF4G, leaving a remnant called p100. Poliovirus IRESs bind to p100 and thereby gain access to ribosomes, but hepatitis C virus IRESs bind directly to eIF3, while hepatitis A virus IRESs bind even more directly to ribosomes. This chapter also refines the model describing how the cleavage of eIF4G affects mammalian host mRNA translation. Different cell types respond differently to this cleavage. Finally, this chapter introduces the concept of the pioneer round of translation, and points out that different initiation factors are used in the pioneering round than in all subsequent rounds.
- Chapter 18: Introduces the concept of superwobble, which holds that a single tRNA with a U in its wobble position can recognize codons ending in any of the four bases, and presents evidence that superwobble works. This chapter also introduces the hybrid P/I state as the initial ribosomal binding state for fMet-$tRNA^{Met}f$. In this state, the anticodon is in the P site, but the fMet and acceptor stem are in an "initiator" site between the P site and the E site. This chapter also describes no-go decay, which degrades mRNA containing a stalled ribosome, and introduces the concept of codon bias to explain inefficiency of translation. Finally, this chapter explains how the slowing of translation by rare codons can influence protein folding both negatively and positively.

- Chapter 19: Includes a new section based on recent crystal structures of the ribosome in complex with various elongation factors. One of these structures involves aminoacyl-tRNA and EF-Tu, and has shown that the tRNA is bent by about 30 degrees in forming an A/T complex. This bend is important in fidelity of translation, and also facilitates the GTP hydrolysis that permits EF-Tu to leave the ribosome. Another crystal structure involves EF-G–GDP and shows the ribosome in the post-translocation E/E, P/P state, as opposed to the spontaneously achieved pre-translocation P/E, A/P hybrid state. This chapter also provides links to two excellent new movies describing the elongation process and an overview of translation initiation, elongation, and termination. Finally, this chapter describes crystal structures that illustrate the functions of two critical parts of RF1 and RF2 in stop codon recognition and cleavage of polypeptides from their tRNAs.
- Chapter 20: Introduces the controversial proposal, with evidence, that DNA replication in *E. coli* is discontinuous on both strands. This chapter also introduces ACL1, a chromatin remodeler recruited via its macrodomain to sites of double-strand breaks by poly(ADP-ribose) formed at these sites by poly(ADP-ribose) polymerase 1 (PARP-1).
- Chapter 21: Presents a co-crystal structure of a β dimer bound to a primed DNA template, showing that the β clamp really does encircle the DNA, but that the DNA runs through the circle at an angle of 20 degrees with respect to the horizontal. This chapter also includes a corrected and updated Figure 21.17 (model of the polIII* subassembly) to show a single **-subunit and the two **-subunits joined to the core polymerases through their flexible C-terminal domains. This section also clarifies that the **- and **-subunits are products of the same gene, but the former lacks the C-terminal domain of the latter. This chapter also introduces the complex of telomere-binding proteins known as shelterin, and focuses on the six shelterin proteins of mammals and their roles in protecting telomeres, and in preventing inappropriate repair and cell cycle arrest in response to normal chromosome ends.
- Chapter 22: Adds a new figure (Figure 22.3) to show how different nicking patterns to resolve the Holliday junction in the RecBCD pathway lead to different recombination products (crossover or noncrossover recombinants).
- Chapter 23: Reports that piRNAs targeting P element transposons are likely to be the transposition suppressors in the P-M system. Similarly, piRNAs appear to play the suppressor role in the I-R transposon system.

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