

Ursula Goodenough

Genetics

Third Edition

Q3
G553
(3)



Ursula Goodenough

Washington University



61950

SAUNDERS COLLEGE PUBLISHING

Philadelphia New York Chicago
San Francisco Montreal Toronto
London Sydney Tokyo Mexico City
Rio de Janeiro Madrid

HOLT-SAUNDERS JAPAN



SBY63/03



Preface

Do DNA sequences belong in a genetics textbook? This was the most important question I faced as I undertook writing a third edition of this text in the midst of the cloning/sequencing "revolution." The DNA sequences prevailed, as a glance through the text will indicate. My reasoning can be summarized as follows.

Genetics seems to have been embroiled in an identity crisis since 1953, when genes began to be actively analyzed as molecular units as well as heritable units of function. Scientists who studied the molecular properties of genes came to call themselves molecular biologists, whereas those who continued to study genes as heritable units of function called themselves geneticists. Pedagogical distinctions soon followed: molecular biology textbooks were devoid of mapping functions and epistasis, whereas genetics textbooks rarely mentioned histone phosphorylation or C_0t plots. A dichotomy also developed in teaching styles: whereas undergraduate courses in molecular biology were full of the "latest news," with relatively little attention paid to the development of the field, courses in genetics were usually taught in an historical fashion, with much emphasis placed on the intellectual approaches taken during and since Mendel's original experiments.

I have written this edition with the conviction that these distinctions are already obsolete in practice and should be made obsolete in the classroom. Molecular biologists, now able to clone genes at will and sequence them in weeks, have come knocking on the doors of geneticists to obtain strains with interesting genetic properties. Geneticists, meanwhile, have come to realize that gene fine structure will never again be productively tackled by the heroic genetic approaches of Benzer, Sherman, or Judd, and they are increasingly receptive to the knocks on the door from their molecular colleagues. Population geneticists are using restriction enzymes to detect polymorphisms; evolutionists are discovering that the genomes of modern organisms contain a rich fossil record of their genetic ancestry;

molecular biologists attend conferences on hybrid dysgenesis in *Drosophila* and the dissociator-activator system in maize. With the old distinctions rapidly blurring in the laboratory, why should they continue to be perpetrated on undergraduates?

I usually hear three answers to this question. One is the notion that the molecular material is "too hard," that students won't understand it. This is certainly true for non-science majors, for whom this text is definitely not written, but biology majors take required chemistry courses that provide ample background for understanding the principles of molecular genetics when they are carefully presented. Indeed, it is my experience that most students are able to understand the implications of a DNA sequence far more readily than a coefficient of coincidence, but perhaps other instructors have a different impression. Then there is the intellectual argument. Once students grasp the construction of a brilliant genetic proof, are they not better trained in science? I strongly agree that they are, and the text includes detailed presentations of many "strictly genetic" experiments. But if these experiments are presented in a molecular vacuum, then the training is incomplete: the student emerges steeped in genetic logic but has not been allowed to combine that logic with a knowledge of how genes are constructed and how they recombine, mutate, and express themselves.

Finally, it is argued that students will learn "that stuff" in another course. I agree that such subjects as DNA replication, renaturation kinetics, and sequencing are rightly considered in considerable depth in biochemistry and molecular biology courses. They are presented quite superficially in this text. It hardly follows, however, that genes should not be the focal subject of a genetics course.

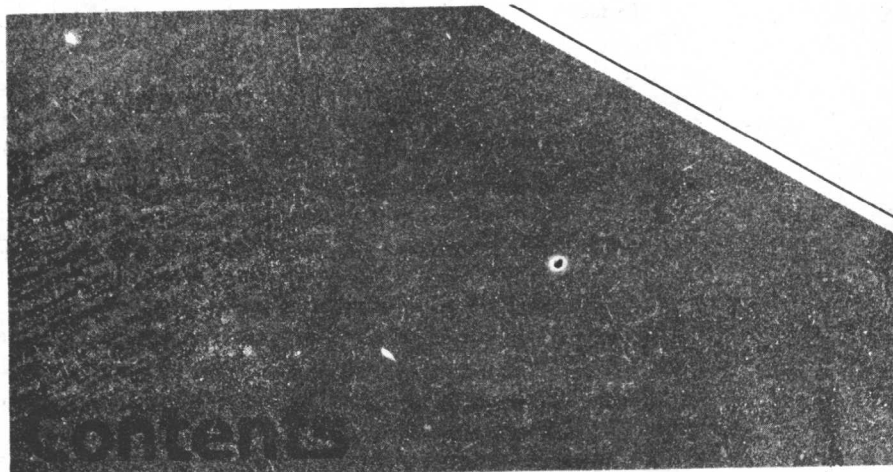
In a field moving as rapidly as genetics, it proves impossible to simply "update" a previous edition. Therefore, this is once again an almost entirely new book. Major changes from the second edition include the following:

1. New chapters have been added on somatic cell genetics, transposable elements, and immunogenetics, three of the most rapidly advancing fields in the past five years.
2. Such topics as mutation, recombination, chromosome structure, and gene structure are each presented in two chapters, the first giving a general overview and the second a more detailed molecular view (see Table of Contents). This should allow instructors more flexibility in determining the "molecular depth" of their assignments.
3. Population and quantitative genetics have been expanded considerably.
4. By popular request, Mendel's experiments are included *per se*.

I have been fortunate to obtain excellent critiques on the previous and current editions. Particular thanks go to Drs. Julian Adams (University of Michigan), Fred Allendorf (University of Montana), Alan Atherly (Iowa

State), C. William Birky (Ohio State), Barbara Brownstein (Temple University), Darrel Falk (Syracuse University), Peter Kuempel (University of Colorado), Gustavo Maroni (University of North Carolina), H. James Price (Texas A. & M University), W. Stuart Riggsby (University of Tennessee), Raymond Rodriguez (University of California, Davis), Carol Sibley (University of Washington), Richard Siegal (U.C.L.A.), Edward Simon (Purdue University), Millard Susman (University of Wisconsin), Eric Weinberg (University of Pennsylvania), and Herbert Wiesmeyer (Vanderbilt University) for their overall reviews and to Drs. Douglas Berg (Washington University), Richard Borowsky (New York University), Nam-Hai Chua (Rockefeller University), Jeffrey Davidson (Eleanor Roosevelt Institute, Denver), W. J. Dickinson (University of Utah), John Drake (National Institute of Environmental Health Sciences), Sarah Elgin (Washington University), Nancy Martin (University of Texas, Dallas), Janice Pero and Vicki Sato (Harvard University), and Christopher Woodcock (University of Massachusetts, Amherst) for their individual chapter reviews. Michael Brown, Biology Editor at Saunders, provided important help at many stages in the production process.

This edition was written almost entirely in the serenity of Chilmark, Massachusetts, in the loving presence of my four children—Jason, Mathea, Jessica, and Thomas—and my husband, John Heuser. The book is the product of the peace of mind, and hence the clarity of thought, that they all bring to me.



The 20 chapters displayed in black present the "core material" of present-day genetics; the 8 chapters displayed in gray offer detailed presentations of topics in molecular genetics.

Preface iii

1 DNA (and RNA) as the Genetic Material in Chromosomes 1

Introduction	1
The Requirements to Be Met by Genetic Material	2
The Structure of DNA and RNA	4
Relating DNA Structure to Its Genetic Requirements	14
Experiments Indicating DNA and RNA as the Genetic Material	17
Questions and Problems	23

2 Cell Cycles, Chromosome Duplication, and Mitosis 25

Introduction	25
The Bacterial Cell Cycle and Chromosome Replication	26
The Eukaryotic Cell and Cell Cycle	34
The Karyotype	48
Atypical Eukaryotic Cell Cycles	57
Questions and Problems	60

vi

3 Molecular Organization of Chromosomes 62

Introduction	62
Protein Structure	63
Chromosomal Proteins	73
The 10 nm Nucleosome Filament of Chromatin	74
Higher Orders of Chromatin Organization	81
<i>Questions and Problems</i>	92

4 Molecular Analysis of Chromosomal DNA and Genetic Engineering 93

Introduction	93
Overall Composition of Genomic DNA	94
Reassociation Kinetics (C_0T Plots) of Genomic DNA	98
The Kinetic Classes of Eukaryotic DNA	102
Restricting, Sequencing, and Cloning DNA	108
<i>Questions and Problems</i>	122

5 The Meiotic Transmission of Chromosomes 124

Introduction	124
Meiosis	125
Life Cycles of Sexually Reproducing Organisms: Mitosis-Meiosis Alternations	137
Meiotic Errors	145
<i>Questions and Problems</i>	153

6 Mendelian Inheritance of Genes Carried by Autosomes and Sex Chromosomes 153

Introduction	154
Mendel's Experiments	154
Segregation of Alleles	159
Independent Assortment	170
Sex-Linked Inheritance	182
<i>Questions and Problems</i>	196

7 Mutation: Induction and Detection of Mutant Organisms and Chromosomes 201

Introduction	201
Screening Procedures	204
Characterizing Mutant Karyotypes	212
Mutagens, Clastogens, and Carcinogens	220
Questions and Problems	227

8 DNA Replication and Repair Mechanisms and Their Contribution to Mutagenesis 230

Introduction	230
DNA Replication Mechanisms	231
Direct Mutagenesis Mechanisms	237
Repair and Misrepair Mechanisms	245
Questions and Problems	255

9 Genes and Gene Transcripts: General Features 258

Introduction	258
General Features of Transcription	259
Anatomy of Structural Genes	267
"Split Genes" in Eukaryotes	272
Visualizing Structural Gene Transcription	277
Questions and Problems	282

10 Genes and Gene Transcripts: Specific Genes 285

Introduction	285
Transfer RNA Genes	286
Ribosomal RNA Genes	294
Structural Eukaryotic Genes	300
Questions and Problems	306

11 Structural Gene Expression and the Genetic Code 308

- Introduction 308
- Protein Synthesis 309
- "Cracking" the Genetic Code 317
- Nonsense Mutations and Chain Termination 330
- Suppressor Mutations 332
- Questions and Problems 336

12 Mapping Viral Chromosomes 340

- Introduction 341
- Viral Infection Cycles 341
- Complementation Analysis 348
- Recombination-Frequency Mapping 352
- Deletion Mapping 367
- Mapping without Recombination 375
- Approaches to Solving Mapping and Complementation Problems 385
- Questions and Problems 389

13 Mapping Bacterial Chromosomes and Plasmids 399

- Introduction 400
- Molecular Overview of Bacterial Conjugation 400
- Mapping by Bacterial Conjugation 405
- Bacterial Transformation 413
- Generalized Transduction 417
- Specialized Transduction 421
- Plasmids 425
- Approaches to Solving Mapping Problems 432
- Questions and Problems 433

14 Mapping Eukaryotic Chromosomes in Sexual Crosses 437

- Introduction 438
- Classical Studies on Linkage and Recombination 438
- Mapping *Drosophila* in Sexual Crosses 444
- Cytological Mapping 450
- Linkage Groups and Chromosomes 456

Mapping by Tetrad Analysis	459
Approaches to Solving Mapping Problems in Eukaryotes	472
Questions and Problems	475

15 Somatic Cell Genetics 482

Introduction	482
The Parasexual Cycle of <i>Aspergillus</i>	483
Genetic Analysis of Cultured Somatic Cells	490
Gene Transfer or Eukaryotic Transformation	498
The Human Chromosome Map	503
Questions and Problems	507

16 Extranuclear Genetic Systems 511

Introduction	512
Molecular Studies of Mitochondrial Genetic Functions	512
Genetic Analysis of the Yeast Mitochondrial Genome	515
Mitochondrial Genomes of Higher Eukaryotes	523
Chloroplast Genomes	527
Endosymbiosis and the Origins of Organelle Genetic Systems	531
Inheritance of Preformed Structures	533
Questions and Problems	535

17 General Recombination Mechanisms 538

Introduction	539
Models of General Recombination	539
Enzymes that Mediate General Recombination	547
Formation and Segregations of Physical and Genetic DNA Hybrids during General Recombination	550
Mismatch Repair of Heteroduplex DNA during General Recombination	554
Questions and Problems	565

18 Transposition and Mutagenesis by Temperate Viruses and Transposable Elements 569

Introduction	569
Integration and Excision by Temperate Bacteriophages	570
Integration and Excision by Transposable Elements	577
Questions and Problems	589

98-302

B2

19 Related Genes: Alleles, Isoloci, and Gene Families 591

- Introduction 591
- Traits Controlled by a Single Gene Locus 592
- Isozymes Specified by Isoloci 604
- Gene Families 606
- Distinguishing Alleles, Isoloci, and Gene Families in Genetic Crosses 613
- Questions and Problems* 615

20 Immunogenetics 618

- Introduction 619
- Properties of the Immunoglobins 619
- Construction and Expression of Light-Chain Genes 625
- Construction and Expression of Heavy-Chain Genes 631
- Somatic Mutation of Antibody Genes, and a Summary of Antibody Diversity Mechanisms 637
- Questions and Problems* 640

21 Genes that Cooperate to Produce Complex Phenotypes and Quantitative Traits 642

- Introduction 642
- Clustered Genes Specifying One Trait 643
- Dispersed Genes Specifying One Trait 652
- Biochemical Genetics 654
- Polygenes and Continuous Variation 664
- Questions and Problems* 675

22 Control of Gene Expression in Bacteria 681

- Introduction 682
- General Features of Gene Regulation 682
- Regulation of Lactose Utilization 687
- Regulation of Tryptophan Biosynthesis 697
- Translational Control 704
- Questions and Problems* 705

23 Control of Gene Expression in Bacteriophages and Eukaryotic Viruses 709

- Introduction 709
Regulation of Gene Expression by Lytic Bacteriophages 710
Regulation of Gene Expression during Phage λ Infection 713
Gene Regulation during SV40 Infection 723
Questions and Problems 726

24 Control of Gene Expression in Eukaryotes: Short-Term Regulation 728

- Introduction 728
Short-Term Regulation in Fungi 729
Short-Term Regulation in Higher Eukaryotes 733
Mechanisms of Short-Term Regulation in Eukaryotes 739
Questions and Problems 742

25 Control of Gene Expression in Eukaryotes: Long-Term Regulation 745

- Introduction 746
General Features of Long-Term Differentiation 746
The Differentiation of the Egg and Maternal Influences on Development 753
Development Genetics of *Drosophila* 760
Developmental Genetics of Vertebrates 774
Differential Expression of Hemoglobin Genes 783
Questions and Problems 788

26 Population Genetics I: General Principles and Mendelian Populations 792

- Introduction 792
General Principles of Population Genetics 793
Genetic Variability in Populations 797
Mendelian Populations 802
Questions and Problems 814

27 Population Genetics II: Evolutionary Agents 819

Introduction	820
Fitness	820
Selection	823
Migration	842
Random Drift in Small Populations	846
The Contributions of Selection and Drift to Polymorphism	851
<i>Questions and Problems</i>	855

28 Populations Genetics III: Speciation and Molecular Evolution 858

Introduction	858
Speciation	859
Molecular Evolution	864
<i>Questions and Problems</i>	875

Boxed Reference Material

CsCl Gradient Centrifugation	30
Autoradiography	32
Antibodies and Antibody Testing	43
Staining and Banding Chromosomes	53
Chromatography	67
Gel Electrophoresis	69
Properties of Enzymes Relevant to Molecular Genetics	79
DNA Sequencing	114
Sucrose Gradient Centrifugation	273
Southern Blot Hybridization	381

Index 879

1

DNA (and RNA) as the Genetic Material in Chromosomes

- A. Introduction
- B. The Requirements to Be Met By Genetic Material
- C. The Structure of DNA and RNA
 - 1.1 The Polynucleotide
 - 1.2 The Double Helix
- D. Relating DNA Structure to Its Genetic Requirements
 - 1.3 DNA as a Coded Molecule
- E. Experiments Indicating DNA and RNA as the Genetic Material
 - 1.4 DNA Replication
 - 1.5 DNA Expression
 - 1.6 DNA Variation
 - 1.7 Transformation Experiments
 - 1.8 Hershey-Chase Experiments
 - 1.9 Experiments with RNA Viruses
- F. Questions and Problems

Introduction

Today, when the terms **gene** or **genetics** are mentioned, most biologists immediately think of **DNA**. DNA, or **deoxyribonucleic acid**, is well known as the chemical bearer of genetic information; **RNA** (**ribonucleic acid**) serves this function in certain viruses.

In the history of genetics as a science, DNA became the center of attention only relatively recently. Focus first centered on **heredity**, on the **patterns of inheritance** of a given trait (blue eyes, red flower color, short tail) from parent to offspring. It was postulated that these inherited traits were somehow dictated by genes and that genes were linearly arranged along the chromosomes of higher animals and plants. "Maps" of gene order on chromosomes were constructed, and many of the details of gene transmission from generation to generation were worked out well before much was known about what a gene is and how it acts.

As the science of genetics developed, increased attention was given to how genes function, and more experimental use was made of microorganisms, notably bacteria and bacterial viruses. During this period it was proposed, with good evidence, that the function of most genes is to specify the formation of proteins. When it was eventually established that most genes are borne within molecules of DNA, primary attention was given to the chemical nature of the gene itself.

In beginning our text with DNA and RNA and in developing a molecular picture of genes and gene function at the same time as we establish patterns of heredity, we are, in one sense, violating the sequence set by scientific history. In another sense, of course, we are more closely following evolutionary history, since genes almost certainly developed their fundamental properties well before the hereditary patterns exhibited by modern organisms were established.

The Requirements to be Met by Genetic Material

Certain requirements must be met by any molecules if they are to qualify as the substances that transmit genetic information. These requirements extend directly from what is known about the continuity of species and the process of evolutionary change.

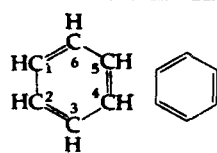
1. **Genetic material must contain biologically useful information that is maintained in a stable form.**
2. **Genetic information must be reproduced and transmitted faithfully** from cell to cell or from generation to generation.
3. **Genetic material must be able to express itself** so that other biological molecules, and ultimately cells and organisms, will be produced and maintained. Implicit in this requirement is that some mechanism be available for decoding, or translating, the information contained in the genetic material into its "productive" form. A narrow, but important, distinction is thus made between a molecule that can generate only its own kind and a molecule that can also generate new kinds of molecules. A salt crystal can "seed" a salt solution so that new salt crystals are formed, but this is the extent of its influence over its surroundings.
4. **Genetic material must be capable of variation.** This requirement is somewhat contradictory to the first requirement, which demanded stability of the genetic material. There is, in fact, no *a priori* reason why genetic material should have built-in provisions for change; one could certainly design a hypothetical genetic system in which information would be rigidly conserved from one generation to another. The dominant theme in the history of life is, however, organic evolution, and

this demands that genetic material be capable of change, if only infrequently.

Two sources of change have been recognized in present-day genetic systems: **mutations** and **recombination**. A mutation changes the nature of the information transmitted from parent to offspring and thus represents a relatively drastic way of bringing about variation. If the change is deleterious (and it usually is), the offspring may be greatly handicapped and may die soon after conception, or else it may introduce a deleterious gene into the population. Recombination is a more moderate way of producing variation. It occurs during the course of some sort of sexual process, and it involves the precise shuffling of parental genetic information such that new combinations of genes are produced. These are then inherited by the offspring.

With these four requirements in mind, we can study the physical and chemical properties of DNA and RNA, putting the molecular facts into a genetic context. Table 1-1 reviews some key definitions from organic chemistry that are relevant to the next few sections of this chapter.

TABLE 1-1 Definitions from Organic Chemistry

	<p>Benzene ring, with = indicating double bonds where two carbon atoms share four electrons between them. The common abbreviated version of a benzene ring is shown at right.</p>
—CH ₃	Methyl group
—OH	Hydroxyl group
=O	Keto group
$\begin{array}{c} \text{H} \\ \\ -\text{C}=\text{O} \end{array}$	Aldehyde group
$\begin{array}{c} \text{O} \\ \\ -\text{C}-\text{OH} \end{array}$	Carboxyl group, characteristically acidic (—COO [−])
—NH ₂	Amino group, characteristically basic (—NH ₃ ⁺)
Covalent bond	A strong bond formed when two atoms share a pair of electrons between them.
Hydrogen bond	A weak attractive force between an electronegative (electron-seeking) atom (usually N or O) and a hydrogen atom covalently linked to a second electronegative atom (usually O—H or N—H).
Hydrolysis	Breaking a large molecule into two or more smaller molecules by adding water.

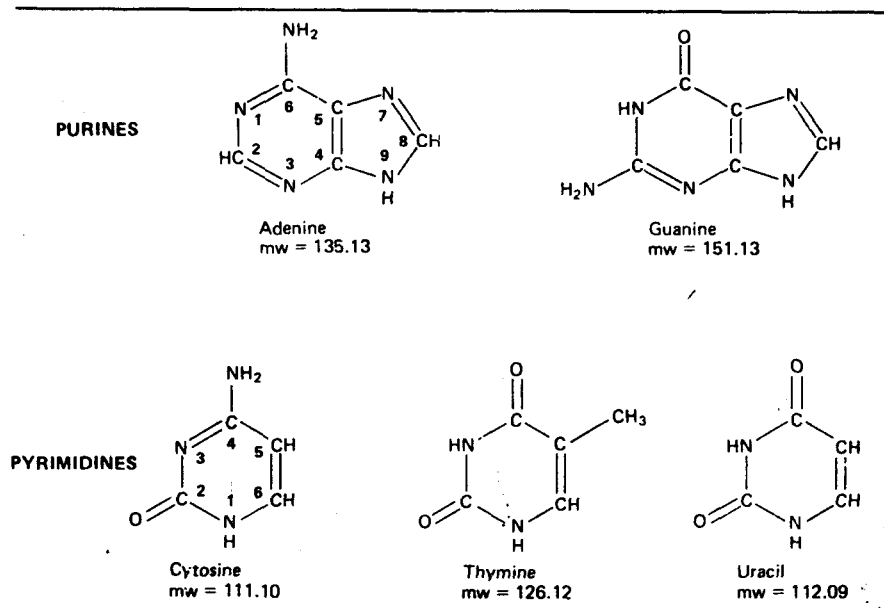
The Structure of DNA and RNA

1.1 The Polynucleotide

DNA and RNA are composed of two different classes of nitrogen-containing bases, the **purines** and the **pyrimidines**. The two most commonly occurring purines in DNA are **adenine** and **guanine**, and the common pyrimidines are **cytosine** and **thymine**. Their structures are shown in Figure 1-1. Thymine is not found in most species of RNA; instead, one finds the pyrimidine **uracil**, which is also shown in Figure 1-1. Modified forms of these bases (5-methyl cytosine, for example) are occasionally found as well, particularly in certain specialized forms of RNA described in Chapter 10.

The purines and the pyrimidines can be seen in Figure 1-1 to contain several conjugated double bonds. Molecules containing such bonds are potentially able to exist in a number of different chemical forms, for their hydrogen atoms have a certain freedom. A hydrogen atom can, for example, move away from an amino group ($-\text{NH}_2$), leaving an imino group ($-\text{NH}$) and a net negative charge that is absorbed by the conjugated ring system of the molecule. Such chemical fluctuations are called **tautomeric shifts**, and the different molecular structures that result are called **tautomers**. It turns out that under physiological conditions, the purines and pyrimidines exist almost invariably in the forms that have been drawn in Figure 1-1; the other tautomeric forms of these bases rarely occur. In other

FIGURE 1-1 Purine and pyrimidine bases. Molecular weights (mw) are given in dalton units (see Table 1-2).



03010