SECOND
EDITION

Microbial Genetics

Stanley R. Maloy
John E. Cronan, Jr.
David Freifelder

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Second Edition

Stanley R. Maloy
Department of Microbiology
University of Illinois, Urbana

John E. Cronan, Jr.
Departments of Microbiology and Biochemistry
University of Illinois, Urbana

David Freifelder
University of California, San Diego

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We dedicate this book to our late friend and colleague William D. Nunn

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Cover: The figure on the cover represents a physical map of the *Escherichia coli* chromosome. The outer circle shows regions of the chromosome where the DNA sequence has been determined (compiled in 1992). The eight inner circles show the positions of recognition sites of eight restriction endonucleases: *Bam*HI, *Hin*dIII, *Eco*RI, *Eco*RV, *Bgl*I, *Kpn*I, *Pst*I, and *Pvu*II. This figure was kindly provided by Kenneth E. Rudd from the National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD. The physical map is described in detail in: K. Rudd. 1993. Maps, genes, sequences, and computers: an *Escherichia coli* case study. ASM News 59, 335.

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U	Phe	Ser	Tyr	Cys	U
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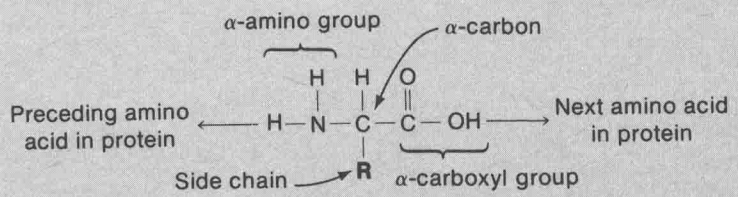
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gin	Arg	A
	Leu	Pro	Gin	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	[Met]	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	[Val]	Ala	Glu	Gly	G

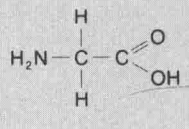
NOTE: The boxed codons are used for initiation.

BASIC STRUCTURE OF AN α-AMINO ACID

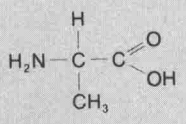


CHEMICAL STRUCTURES OF THE AMINO ACIDS

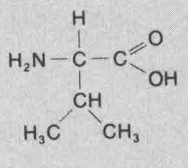
Neutral, Nonpolar



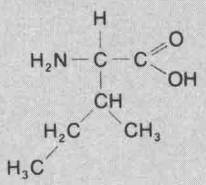
Glycine



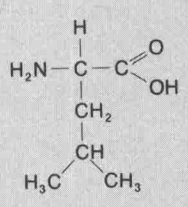
Alanine



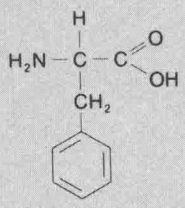
Valine



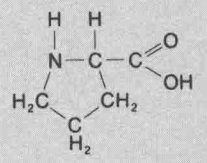
Isoleucine



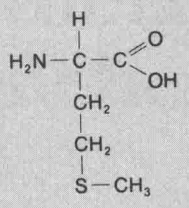
Leucine



Phenylalanine

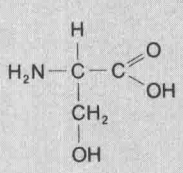


Proline



Methionine

Neutral, Polar



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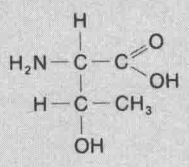
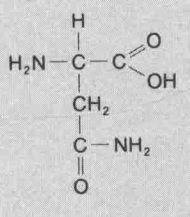
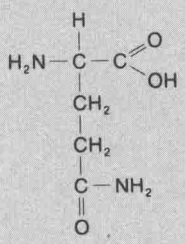


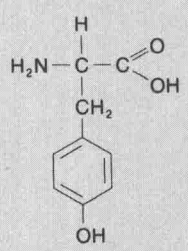
Threonine



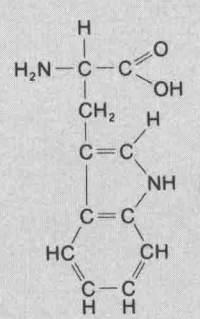
Asparagine



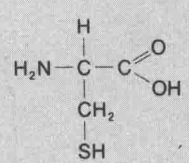
Glutamine



Tyrosine

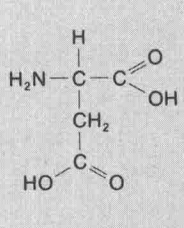


Tryptophan

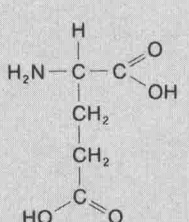


Cysteine

Acidic

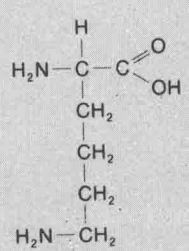


Aspartic acid

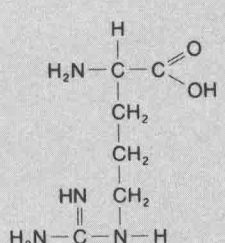


Glutamic acid

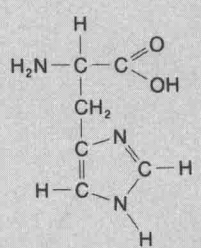
Basic



Lysine

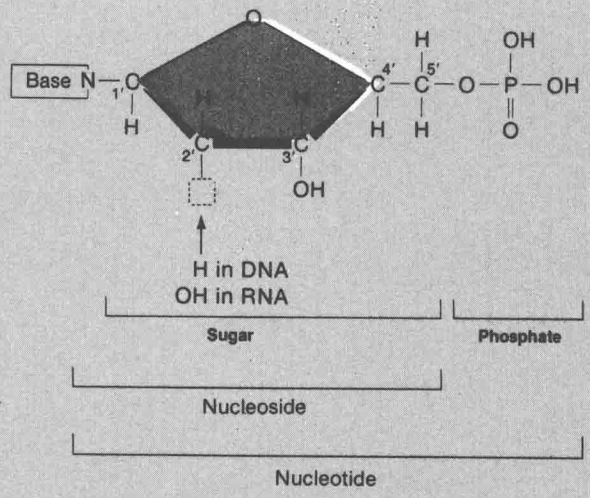


Arginine

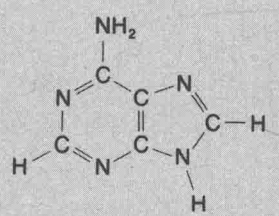


Histidine

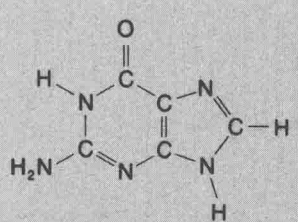
A TYPICAL NUCLEOTIDE



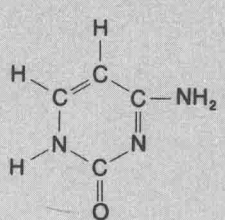
THE BASES FOUND IN NUCLEIC ACIDS



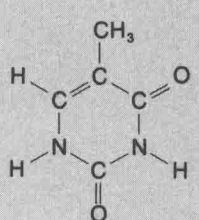
Adenine



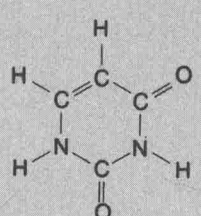
Guanine



Cytosine

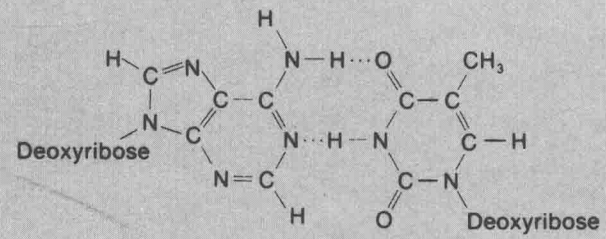


Thymine

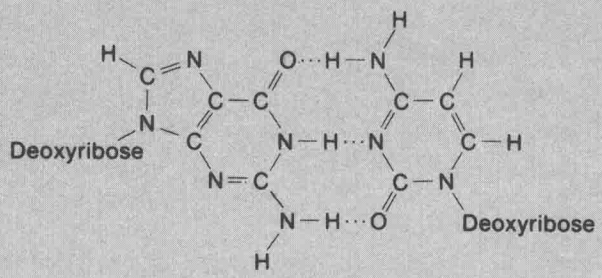


Uracil

THE TWO COMMON BASE PAIRS OF DNA



Adenine **Thymine**



Guanine **Cytosine**

TYPES OF MUTAGENS

Mutagen	Mode of action	Example	Consequence
Base analog	Substitutes for a standard base during replication and causes a new base pair to appear in daughter cells in a later generation	5-Bromouracil 2-Aminopurine	A • T → G • C, and G • C → A • T A • T → G • C
Chemical mutagen	Chemically alters a base so that a new base pair appears in daughter cells in a later generation	Nitrous acid Hydroxylamine Ethyl methane sulfonate (EMS) Ultraviolet light	G • C → A • T, and A • T → G • C G • C → A • T G • C → A • T, *G • C → C • G*, and *G • C → T • A* All single base-pair changes are possible.
Intercalating agents	Addition or deletion of one or more base pairs	Acridines	Frameshifts
Mutator genes	Excessive insertion of incorrect bases or lack of repair of incorrectly inserted bases	_____	All single base-pair changes are possible.
None	Spontaneous deamination of 5-methylcytosine (MeC)	_____	G • MeC → A • T

Note: Italicized changes in base pairs are transversions; those that are not italicized are transitions.

Microbial Genetics

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Preface

Although many general genetics and molecular biology textbooks are available, few recent textbooks focus on microbial genetics. Furthermore, although many of the general genetics and molecular biology textbooks have sections on microbial genetics, they do not cover a wide variety of specialized aspects of microbial genetics. The first edition of David Freifelder's *Microbial Genetics* sought to fill this niche.

Since the first edition was published, there have been numerous advances in microbial genetics. The development of new techniques such as polymerase chain reaction and electroporation made it possible to do molecular genetics in a variety of interesting and important bacteria that were previously genetically intractable. Genetic approaches have been essential for dissecting important microbiological problems, such as the mechanisms of virulence of bacterial pathogens and the emergence of antibiotic-resistant pathogens. Genetic analysis of Archaea (previously called Archaebacteria) has contributed to our understanding of evolution of the major groups of living organisms and the diversity of life. Each of these advances required a solid knowledge of classic microbial genetics and modern molecular biology. Because David Freifelder is now deceased, we undertook the task of rewriting and updating the second edition of *Microbial Genetics*. (It might be argued that the book would be more appropriately titled *Bacterial and Phage Genetics* because it does not deal with eukaryotic microbes. We tried to include Archaea when relevant, however, and we hope these sections will be expanded in the future. Thus, because the content is not strictly limited to bacteria, we retained the original title.) Some sections are extensively rewritten, and other sections are only slightly changed, but we retained the basic format, organization, and style of the first edition.

We were fortunate to have scientific mentors who are not only outstanding bacterial geneticists, but also have a strong passion for genetics. Dan Wulff (SUNY at Albany), John Roth (University of Utah), and Tom Silhavy (Princeton University) left a profound influence on our style of thinking about genetics.

We greatly appreciate the suggestions of several reviewers, especially Jeffrey Gardner (University of Illinois, Urbana), Michelle Igo (University of California, Davis), and Ken Noll (University of Connecticut, Storrs). In addition, we appreciate the help of a number of graduate students and undergraduates at the University of Illinois who read the manuscript and checked the problems: Scott Allen, Karen Colletta, Matt Lawes, Min-Ken Liao, Aileen Rubio, and Paula Ostrovsky

de Spicer. Art Bartlett (Jones and Bartlett Publishers) gave us the encouragement and gentle prodding needed to keep us from getting terminally sidetracked. Finally, we would like to thank our families for their support and patience with our constant preoccupation with science.

Stanley Maloy
John Cronan, Jr.

Preface to the First Edition

Experiments in microbial genetics carried out in the 1940s and 1950s gave birth to what ultimately became known as molecular biology. The elucidation of the structure of DNA by James Watson and Francis Crick, neither of whom were geneticists, had enormous impact on all of genetics and biology. Many physicists, fascinated by the new discoveries and attracted by the quantitative aspects of microbial genetics, joined classic microbiologists, geneticists, and biochemists, and what has been called the Golden Age of Biology began. During this period, genetic analysis took on new importance in that it provided the insights for developing detailed theories of gene action. Genetic results obtained with bacteria and bacteriophages led to hypotheses about gene organization, chromosome structure, regulation of gene action, genetic recombination, and many other phenomena. Although genetic results could not rigorously prove a molecular mechanism, they placed limits on hypotheses and were enormously suggestive about mechanisms. In fact, hypotheses based solely on genetic results so often proved to be correct that it became commonplace among molecular biologists to assume the validity of a mechanism before biochemical proof was obtained; certainly mechanisms that were inconsistent with genetic results were not thought worthy of consideration. A great deal of the early work in microbial genetics was compiled in an excellent and influential text—*The Genetics of Bacteria and Viruses* by William Hayes—the most recent edition of which was published in 1968. Since that time, few texts on microbial genetics have appeared, the most recent one being more than 5 years old. *Microbial Genetics* represents my attempt to bring this still-useful subject up to date.

The application of microbial genetics led to the accumulation of a huge body of knowledge and a continually greater understanding of the nature of the gene. In the early 1970s, microbial genetics itself underwent a revolution, with the development of the recombinant DNA technology. This collection of remarkable but straightforward techniques, usually called genetic engineering, allows manipulation and exchange of fragments of DNA outside of the cell and reintroduction of recombinant DNA into a new cell. In this way, novel organisms can be created, with characteristics drawn from distant species and genera. Even human genes can be transferred to a bacterium, and genes of microorganisms can be placed in animal cells. In fact, the glitter of this new technology has led to the conversion of hundreds of research laboratories to gene-cloning factories and to the development of a new industry, known as bioengineering. This new technology is the topic of the later chapters in this book.

Microbial Genetics is the result of a good deal of prodding by many

colleagues who urged me to write such a book. Although I am a "modern" molecular biologist (though I have never cloned a gene), I did acquire my research experience during the first Golden Age and worked intensively in various aspects of microbial genetics. Furthermore, since I have had the experience of writing several books, I was told that the task was clearly mine. Thus, several months ago I started to re-read ancient books and papers. This brought back many pleasant memories of lectures and meetings that were thoroughly exciting because in retrospect we seem to have known, comparatively speaking, very little about the details of genetic processes. These recollections, as well as thoughts about many former colleagues, made the process of book-writing more of a pleasure than a task.

Microbial Genetics begins with five review chapters. These include basic material about classic genetics, DNA and protein structure, and the basic biology of bacteria and bacteriophages. Students who have had a course in biochemistry can surely omit Chapters 2 and 3, and those having studied classic genetics can undoubtedly skip Chapters 1, 4, and 5. Because these chapters are meant as a review, the material is presented fairly succinctly. Furthermore, only topics needed in later discussions have been included.

Every chapter in this book ends with a substantial set of questions and problems. Most of the beginning questions simply test the memory of the student; they are designed to ensure that the reader has learned the definitions and understood the most elementary concepts. Later questions are more difficult. Complete answers to all questions and problems, with explanations, are given in the Answers section at the back of the book.

David Freifelder

Brief Contents

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