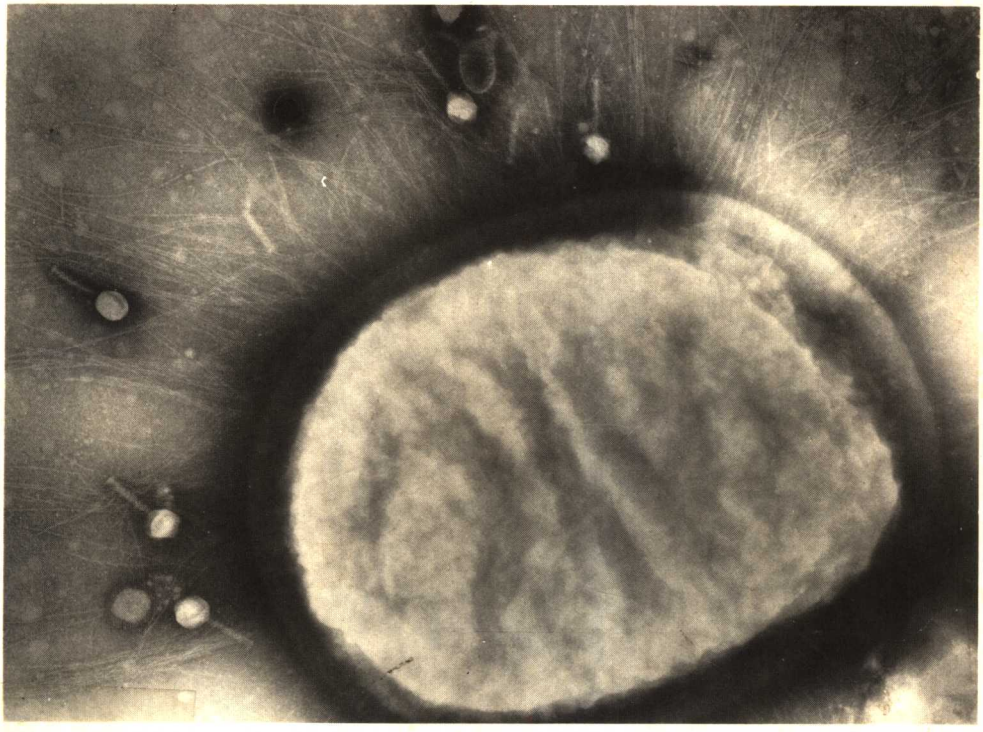
Bacteria, Plasmids, and Phages

An Introduction to Molecular Biology



E.C.C. Lin,
Richard Goldstein,
and
Michael Syvanen

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Preface

THIS BOOK GREW out of a set of lectures on bacteria and their viruses designed to provide first-year students at Harvard Medical School with the background to pursue the study of human pathogens. Thus, throughout the work we point out connections with topics of clinical interest where appropriate and describe the general features of bacterial structure, physiology, and metabolism, as well as the behavior of viruses.

A major aim of this volume, however, is to give a compact and balanced account of the molecular genetics of bacteria and their associated plasmids and phages. *Escherichia coli*, phage λ, and the F plasmid are featured because these models have been most extensively studied until recently. We hope to give the student some insight into how knowledge of the molecular biology of microorganisms is gained and how it is being applied in the emerging technology of genetic engineering.

Though we assume a modicum of exposure to general biochemical pathways, enzymatic catalysis, and the basic properties of macromolecules, we believe that this volume can be useful as a text for upper-level college students who wish to be initiated into microbiology at the molecular level. A set of questions in each chapter reviews the material presented and challenges the reader to more detailed inquiries, often in the context of laboratory experiments. A complete set of answers is provided at the back of the book.

We wish to acknowledge the generosity of our colleagues D. R. Fraenkel and B. D. Davis, who permitted us to draw from their lecture notes, and J. R. Beckwith, who made a number of helpful comments. Daniel R. Kuritzkes and Alison De Long read all the chapters from the point of view of a medical and graduate student respectively and offered useful suggestions. Yu-Mei Chen and Eric Johnson assisted with the proofreading. We are grateful to Sarah Monosson for the many hours she devoted to the preparation of this work. Finally, we have had the good fortune of being able to rely on Susan Wallace as a discerning and patient editor. We alone are responsible for any inaccuracy or inadequacy in this work, despite the competent and abundant help we have received.

E. C. C. L.
R. G.
M. S.

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Bacteriology: An Overview

THE MOST widely distributed and metabolically diverse group of organisms known is bacteria. Some species live in close association with eukaryotic organisms: the relationship can be symbiotic, as in the nitrogen fixation process, or destructive, as in processes of infectious disease. Many bacteria are ecologically important, helping to maintain the geochemical cycle. Because of their relative simplicity and amenability to experimental manipulation, bacteria and their associated genetic elements have provided some of the most fertile and elegant systems used in basic research. In many cases principles established in research with bacteria have had applications of profound importance, as in the development of life-saving vaccines and antibiotics. Research in more complex organisms, too, has often depended on ideas and techniques acquired in bacterial research.

Medical Microbiology

Infectious diseases, including those of bacterial etiology, have presented a tremendous burden to humanity. Until this century they were not only the leading cause of death but also the cause of epidemics that disabled and terrorized communities, sometimes determining the fates of armies and of nations.

The spread of certain diseases from one person to another long ago suggested the existence of invisible, transmissible agents of infection. Microscopic organisms (microbes) were not discovered, however,

until Anton van Leeuwenhoek (1632–1723) made one-lens microscopes with sufficient magnification. Among the previously invisible particles that he observed in many natural materials, some could be recognized as alive because their motility was not random. Nevertheless, these observations in natural history, though subsequently expanded by the development of the compound microscope, did not lead to an experimental science of microbiology until nearly two centuries later, when a methodology was developed for culturing various microbes in ways that avoided contamination.

Louis Pasteur (1822–1895) played a seminal role in this development. He demonstrated that certain microbes are able to live without air, and that different kinds of microbes are associated with different kinds of fermentation (suggesting biochemical specificity also in infectious diseases). Working on this assumption, Pasteur went on to develop important vaccines, such as the one against rabies. Finally, in decisively refuting the theory of the continual spontaneous generation of life (on an experimental time scale) by excluding contamination from ubiquitous organisms floating in the air, he played a role in the nineteenth-century debate over evolution. Pasteur's famous remark "dans les champs de l'observation le hasard ne favorise que les esprits préparés" ("chance favors only the prepared mind") has become a universal admonition for experimentalists. His work was crucial in allowing later investigators to isolate a great variety of microbes and to study their biochemical and pathogenic activities.

Robert Koch (1843–1910) systematized medical microbiology by perfecting the use of solid media, on which individual cells give rise to separate colonies and hence to pure cultures. He also formalized the criteria known as Koch's postulates for distinguishing a pathogenic agent from the adventitious organisms also recovered from lesions. These technical and theoretical advances led immediately to great scientific progress and are still important to clinical and experimental microbiology. Between 1879 and 1889 bacteriologists in Germany discovered the tubercle bacillus, cholera vibrio, typhoid bacillus, diphtheria bacillus, pneumococcus, staphylococcus, streptococcus, meningococcus, gonococcus, and tetanus bacillus. Even today new bacterial pathogens are being discovered—for example, the Legionnaires' disease bacillus in 1977, and virulent strains in species previously considered harmless, such as enteropathogenic strains of *Escherichia coli*.

The small size of bacteria, near the limit of resolution of the

microscope, initially impeded analysis of their internal structure and function. Progress was therefore limited to identifying species and describing their growth requirements, excretion products (including toxins), and interactions with their environment. Studies of viruses were made difficult by their requirement of an intracellular environment for multiplication. The basic cycle of viral multiplication was first deciphered in bacteria, while animal virology was long restricted to descriptive studies in the living host. Today several bacterial viruses (bacteriophages) are understood in minute detail; they not only provide valuable background for the understanding of animal viruses but also serve as model systems for genetic and biochemical research.

Despite the importance of microbiology in medicine, it should be noted that the identification of the major pathogens, however exciting, led to the development of antisera or vaccines for only a small number of those organisms. Indeed, even before the offending organisms were identified, the development of widespread sanitation early in the nineteenth century, separating sewage from the water supply, probably played a larger role than vaccines in reducing the frequency of serious infectious disease.

Sanitation, in conjunction with the introduction of antibiotics in the 1940s, had a dramatic impact on the pattern of disease prevalence, shifting it toward degenerative and neoplastic diseases and toward infections due to viruses or antibiotic-resistent bacteria.

Classification of Bacteria

The purpose of biological classification (taxonomy) is twofold: to identify species, the basic units of classification; and to arrange these in a hierarchical family tree. The arrangement has been based traditionally on degree of shared and unshared phenotypic characteristics. With higher organisms multiple kinds of evidence are used to conclude that a given hierarchy is a "natural" phylogenetic tree, reflecting lines of evolutionary descent. Moreover, with organisms that reproduce sexually, the species can generally be sharply delineated: it is a group of organisms that interbreed in nature. Thus, in the course of evolutionary divergence within a species (usually owing to geographic separation) the accumulation of mutational differences leads first to different races (subspecies), which remain

interfertile, and then to reproductively incompatible species; only rarely are intergrades observed. The evolution of reproductive barriers between species was essential in producing the wide variety of higher organisms that are found in nature; otherwise interbreeding between variants would constantly tend to homogenize the whole population.

With bacteria, however, the groupings in the conventional branching tree serve primarily to provide a determinative key, by which one can conveniently identify the species of any new specimen; phylogenetic relations are inferred but are less certain, since various characteristics (morphology, biochemical activities, ecology, and so on) do not necessarily diverge in parallel. Because these organisms multiply vegetatively rather than sexually, the interbreeding test cannot define a species. Indeed, the ability of individual genes to mutate and occasionally to be transferred independently suggests the existence of a continuous range of variation, with no basis for defining species. However, in practice, comparison of bacteria with respect to a large number of characters shows that they form clusters of biotypes, each with many common features but also with a range of variation. This pattern implies that random combinations of bacterial genes do not have equal evolutionary survival value: there must be strong selection for a balanced and coherent genome, though we cannot yet identify its detailed features.

The classification of bacteria into species thus has a rationale but also a large element of arbitrariness; taxonomists will differ in the weight that they attach to different characters, and in their tendency either to "split" (favor a large number of small groupings) or to "lump" (favor a small number of large groupings). Nevertheless, with most new isolates there is no difficulty in agreeing on a species assignment, measured against a standard type culture. The differences between strains in a species can have clinical significance. As techniques have evolved to allow recognition of subtle molecular differences, it has become increasingly clear that these are often correlated with differences in pattern or degree of pathogenicity.

With the advent of molecular genetics, comparisons between the DNA of two organisms has provided a much more direct index of evolutionary relation than those between phenotypes. The simplest measurement is the percentage of guanine and cytosine in the DNA composition: genomes of bacteria vary much more widely than those of higher organisms, ranging from 30% to 75% (vertebrate genomes

range from 36% to 43%), although within a closely related group, such as the Enterobacteriaceae, the range is narrow. A much more sensitive test is the degree to which the DNA of one organism will hybridize with that of another, after melting and then annealing under standard conditions. However, bacteria have diverged so much that DNA hybridization occurs only between organisms already known to be very close (whereas the whole range of vertebrate DNA will hybridize). Ribosomal RNA is much more conserved in evolution than are most genes, and hybridization of this RNA with homologous or heterologous DNA measures evolutionary distance over a broader range. So far, however, DNA comparison is a research tool and not a method used in diagnostic laboratories.

In comparing bacteria we should note that the components of the cytoplasm tend to be highly similar or even identical in their monomeric units (though usually not in sequence), whereas the macromolecules of the outer surface (wall, outer membrane, capsule) vary widely in their composition, including many novel sugars and modified amino acids. These external variations give rise to different serological types within a species and evidently have great evolutionary value for the pathogen, since a host who has developed protective antibodies against one type can subsequently be infected by another type in the same species.

Bacteria and the Geochemical Cycle

Pathogens of plants and animals constitute only a small fraction of recognized bacterial species, and an infinitesimal fraction of the total mass of microbes on earth. The vast majority of bacteria are beneficial or even necessary to the ecosystem, playing an essential role in the geochemical cycle by which CO_2 and N_2 are converted to organic matter and then reconverted to mineral form. The ability to fix CO_2 probably evolved first in bacteria, but the process has been largely taken over by algae in waters and higher plants on land. The chloroplasts in algae and plants are believed to be derived originally from photosynthetic bacteria that infected evolving eukaryotic cells and became established as endosymbionts. In contrast, the fixation of N_2 by reduction to ammonia and the subsequent conversion to nitrate (which will not evaporate from soil) remains an attribute only of bacteria.

In the other half of the cycle, which degrades all dead plant and animal matter, bacteria have evolved an astonishing variety of metabolic patterns and choices of nutrients. Although a given species can utilize only a limited range of substrates, the microbial kingdom taken as a whole can convert *all* natural carbon- and nitrogen-containing compounds to CO_2 and N_2. They cannot, however, attack many synthetic compounds, such as fluorocarbons and polystyrene.

The activities of bacteria in the soil have given rise to the applied field of agricultural microbiology, while the variety of fermentation patterns has led to the development of numerous cultivated strains for the production of foods and industrial chemicals. Bacteria provide numerous examples of specialized patterns of metabolism. For instance, *Propionibacter* ferments lactic acid to propionate and CO_2, which accounts for the holes in Swiss cheese. *Bdellovibrio* burrows beneath the wall of another bacterium and grows at the expense of its cytoplasmic content. Sulfur bacteria, acquiring energy by oxidizing H_2S or S, can produce up to 5% H_2SO_4 in the medium. Magnetotactic bacteria use intracellular magnetite (Fe_2O_3) crystals to orient their swimming along the earth's magnetic lines of force. Presumably by this device the cells move down into the bottom mud, where they find food.

The extreme ecological niches to which bacteria have become adapted in evolution is extraordinarily wide ranging. Many of the highly specialized species belong to the subkingdom Archaebacteria, regarded as relics of a primitive group of prokaryotes that were also ancestors of the subkingdom Eubacteria, to which most known species of bacteria belong. (Archaebacteria have cell membranes of unusual lipid composition and lack peptidoglycan walls, a hallmark of Eubacteria.) Representative of these "primitive" bacteria include the methanogens that reduce CO_2 with H_2 to give CH_4 and water; halophiles that thrive in the Dead Sea and require a salt concentration of over 10%; and thermophiles that thrive in hot springs at 90°C. There are also bacteria, the baryophiles, that live in the depths of the ocean and require a pressure of several thousand atmospheres. Even more astounding is the recent discovery that around sulfide chimneys on the deep ocean floor, some bacteria live at temperatures approaching 300°C. (At 265 times atmospheric pressure seawater remains liquid at temperatures of about 460°C.) At the opposite extreme there are the psychrophiles that can live at temperatures down to −5°C and cannot tolerate temperatures above 20°C.

Despite the great diversity among bacteria in their chemical abilities to exploit different compounds for growth and in their physical tolerance of specialized environments, the basic mechanisms by which genetic information is replicated and expressed and the basic pathways in central metabolism are highly similar and not fundamentally different from those of all other organisms. For instance, the glycolytic pathway in lactic acid bacteria is identical to that in mammalian muscle. Thus, the *diversity* of special biochemical adaptations and the *unity* of the core mechanisms of growth and reproduction constitute the two sides of evolution: divergence from a common ancestry.

Bacteria and Molecular Genetics

Bacteria were long considered essentially bags of enzymes, and their adaptive variation was vaguely thought to be an environmentally directed response of a plastic, primitive kind of genetic makeup. The existence of discrete, mutable genes was first recognized in the early 1940s, when one-step spontaneous mutations were demonstrated. However, classical genetics depended not only on recognizing heritable differences between individuals but also on carrying out crosses that yielded recombinants between two different parents. Because bacteria multiply vegetatively rather than by sexual reproduction, bacterial genetics progressed little until, in 1944, O. T. Avery's studies of pneumococcal transformation showed that genes can be transferred between bacteria and that the "transforming principle" is DNA. Soon thereafter the discovery of conjugation by Joshua Lederberg further showed that in bacteria, as in higher organisms, the genes are linked in a chromosome.

With the growing recognition of the fundamental unity of core metabolic pathways and hereditary mechanisms in bacteria and higher organisms it became possible to use bacteria as model cells for studying universal properties. Among the advantages of a bacterial system, the greatest is the ease with which one can cultivate billions of individuals and then select from these huge populations rare mutants and rare genetic recombinants. This is also true for molecular genetic studies in bacteriophage, in which the numbers of individuals per unit volume of culture can be even larger.

Avery's discovery provided an essential ingredient for developing

a rich science of bacterial genetics; but even more, by identifying the genetic material as DNA, it laid the groundwork for the emergence of molecular genetics in 1953, with James Watson and Francis Crick's model of DNA structure. Intensive studies since then have revealed many aspects of DNA that contribute to its genetic and evolutionary functions: replication, expression, mutation, recombination, repair, and rearrangement. Moreover, viruses—and also plasmids (autonomous blocks of DNA in bacteria)—can integrate into the host cell chromosome and exchange genes with it. This mode of transfer provided powerful tools for studying and manipulating genes. The possibilities are now greatly expanded by molecular recombination in vitro, using enzymes from bacteria to insert DNA of any origin into viral or plasmid DNA, and amplifying the transplanted DNA (and often their products) by having the genetic vectors multiply in bacteria or higher cells.

Similar mechanisms for interspecific recombination are active in nature, where they play an important evolutionary role in microbes and almost certainly also in higher organisms (for example, the transfer of genes from a bacterial plasmid into plant cell chromosomes causes crown gall tumors). Indeed, this kind of gene transfer across species barriers provides more direct evidence than the fossil record for the evolutionary continuity of the living world.

In probing into the mysteries of the gene, molecular geneticists have not revealed any novel chemical or physical forces, as some people expected. However, the study of molecular genetics *has* revealed a pattern of organization that involves these forces in a way that is unique to living matter: **molecular information transfer.** Three kinds can now be recognized. First, DNA can store information, pass it on to future generations by replication of the sequence of bases in the DNA, and express the information by providing a template for complementary RNA sequences. Second, translation of the one-dimensional molecular information of DNA into the three-dimensional shapes required for phenotypic expression occurs simply by spontaneous folding and aggregation of RNA and polypeptide chains as dictated by the mutual affinity of their parts. Finally, allosteric proteins also carry out information transfer: they sense a chemical concentration and transfer this information to sites where it regulates the activity of a gene, an enzyme, or a protein involved in a sensory response.

Knowledge of genetic phenomena first revealed in bacteria has

been extended to advance the understanding of human diseases. For example:

Single-gene defects There are over 2,000 known human genetic disorders. Growing out of studies of biochemical defects in microbes, research on humans has now defined several hundred genetic disorders in terms of an altered or absent protein. Many of these disorders can be diagnosed prenatally by amniocentesis, followed by culturing the recovered fetal cells and identifying a defective protein. Moreover, DNA sequencing has recently been used to reveal alterations in genes coding for hemoglobin, for though the cells from amniotic fluid do not make the product, they have the gene.

Regulatory diseases Gout is analogous to a class of bacterial mutant phenotypes caused by a genetically determined failure of feedback regulation of an enzyme, leading to overproduction of purines. More subtle variations in regulation of the amount or the activity of a specific enzyme or other protein (such as cell receptors) are being found in many other diseases.

Cell differentiation The rearrangement of DNA segments during the maturation of lymphoid cells is responsible for the generation of innumerable kinds of antibodies. Mobile genetic elements in bacteria, which are inserted or recombine with high frequency at certain DNA sequences by special site-specific enzymatic mechanisms, provide a model, and such rearrangements seem likely to occur more broadly in cell differentiation.

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