Edward A. Birge

Bacterial and Bacteriophage Genetics

An Introduction

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With 111 Figures

Springer-Verlag New York Heidelberg Berlin

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Library of Congress Cataloging in Publication Data

Birge, Edward A. (Edward Asahel)
Bacterial and bacteriophage genetics.

(Springer series in microbiology)
Includes bibliographies and index.
1. Bacterial genetics. 2. Bacteriophage—
Genetics. I. Title. II. Series.
[DNLM: 1. Bacteria—Genetics. 2 Bacteriophages—Genetics. QW 51 B617b]
QH434.B57 589.9'015 81-151 AACR2

Printed in the United States of America

9 8 7 6 5 4 3 2 1

ISBN 0-387-90504-9 Springer-Verlag New York Heidelberg Berlin
ISBN 3-540-90504-9 Springer-Verlag Berlin Heidelberg New York

Preface

This book is intended for the student who is taking a first course in bacterial and bacteriophage genetics, rather than as a reference tool for the specialist. It presumes a knowledge of basic biology as well as familiarity with general genetics. Extensive knowledge of microbiology, although helpful, is not essential for a good understanding of the material presented herein.

In order to develop the basic concepts of bacterial and bacteriophage genetics in a volume of reasonable size, I have endeavored to avoid the strictly molecular approach as well as the thoroughly comprehensive treatment characteristic of review articles. For simplification and continuity, therefore, I have dealt primarily with *Escherichia coli* and its phages, except where other bacteria can better illustrate a particular point. This should not, however, be construed to imply that only *E. coli* is worthy of study. Rather, it is my hope that students will be able to generalize from the principles presented in this book to the specific bacterial systems which may be of more direct interest to them.

This book is not intended to cover the individual aspects of bacterial genetics in great depth. To do so would require such an excess of detail as to make the book unwieldy. Rather, it is my hope that the student who has carefully read this book will be prepared to read, with insight and comprehension, the current literature in whatever area of specific interest. Toward this end, I have tried to present bacterial genetics as a logical development of concepts. In so doing, it has occasionally been necessary to omit or defer until later chapters certain topics for which the theoretical framework has not been prepared. Cross references to such topics within the text are made by a series of numbers and letters indicating chapter, section, and part, respectively (e.g., 1.IV.A refers to Chapter 1, Section IV, Part A).

At the end of each chapter, references are provided which will assist the serious student in finding more information on individual topics. These are grouped into two classes: those articles which can be used as general background, and those which have been selected as examples of research articles which amplify the specific topics covered in the chapter. The selection of a limited number of references must of necessity be highly arbitrary, and it should be understood that there are many excellent research scientists whose works simply could not be cited because of space limitations. Indexes such as the *Science Citation Index* will assist the reader to enlarge upon the lists of references provided.

Two books may be noted as being particularly useful sources of detailed information. One is the classic book in the field, William Hayes's *Genetics of Bacteria and Their Viruses*. Although somewhat dated now, its breadth of coverage has never been equaled. The other book which may prove to be a useful descriptive reference for certain types of procedures used in bacterial genetics is David Freifelder's *Physical Biochemistry*.

If the reader of this book develops some of the same fascination which I have long found in bacterial genetics, the task of writing it will have been well worthwhile.

Tempe, Arizona, 1980 EDWARD A. BIRGE

Acknowledgments

A book such as this would not be possible without the cooperation of many people. It is a pleasure to acknowledge the support and encouragement of Dr. Mortimer P. Starr, for without his stimulus this book would never have been written. The style of writing and the presentation of the subject matter have benefited greatly from the efforts of Drs. Elizabeth M. Haines and Lynn E. Birge. The early chapters were facilitated by the congenial habitat provided by Bernard and Ruth Weber and by the comments of Drs. William F. Burke and Winifred Doane. Many of the figures in the text are taken from the research literature, and the cooperation of the authors in supplying these figures has been greatly appreciated. Preparation of the manuscript has been made easier by the efforts of Regina Derose, who typed much of the rough draft; by Valley Autographics, who prepared the finished product; and by Dr. Mark Licker, Ute Bujard, Roger Kasunic, and Marie Donovan of Springer-Verlag, who patiently dealt with the inevitable problems. However, despite all of these contributors, the ultimate responsibility for any errors of commission or omission must be mine.

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Chapter 1

Unique Features of Prokaryotes and Their Genetics

In beginning the study of the genetics of bacteria and bacteriophages, it is important to have clearly in mind the ways in which these prokaryotes and their viruses organize their genetic processes and the ways in which these processes differ from those utilized by eukaryotic organisms. This chapter provides a brief review of important cell functions, emphasizes their unique features, and then provides an overview of the basic genetic processes to be discussed later in this book.

I. The Problems Associated with Haploidy

The key feature which distinguishes prokaryotic organisms from eukaryotic organisms is the lack of an organized nucleus in the prokaryotes. Additionally, there are no organized chromosomes, mitosis, or meiosis. Moreover, prokaryotic cells do not possess any of the membrane-bound organelles, such as mitochondria or chloroplasts, characteristic of eukaryotes. Because of these differences, it has been necessary to add several new terms to those already in common genetic use. For example, the term **chromosome**, as used cytologically in connection with plant and animal cells, refers to a precisely organized structure composed of DNA and certain basic proteins, called histones, which possess regularly spaced globular regions, called chromomeres. Each chromomere consists of an octamer of two copies each of four different histones, usually denoted as H2a, H2b, H3, and H4. The spacer region is covered by a different protein, H1. In the "lower" eukaryotes, such as algae or fungi, a similar situation has been shown to prevail in many, but not all, systems. Where chromomeres have been observed, they frequently have shorter spacing between them and do not necessarily

Although bacteria do appear to have proteins associated with their DNA, they do not have the resultant highly ordered structure to which cytologists apply the term chromosome. Therefore, a new term, **genophore**, has been coined by Ris and Chandler to refer to the intertwined mass of bacterial DNA, RNA, and protein as it exists in vivo. In order to emphasize the uniqueness of the bacterial genetic arrangement, the term genophore will be used throughout this book. It should be realized, however, that in current scientific literature the terms genophore and bacterial chromosome are being used interchangeably. In some cases the term **nucleoid** (see Section II) is used synonymously with genophore.

In eukaryotic cells, the process of mitosis serves to ensure that, after cell division, each daughter cell has the appropriate chromosome complement. In the case of a diploid cell, this means two copies of each type of chromosome, one from either parent. Since bacterial cells lack the ability to undergo mitosis, they must, of necessity, be haploid. In the strict genetic sense, the term **haploid** means that there is only one copy of each piece of genetic information per cell. Later in this chapter it will be shown that the DNA in a bacterial cell is basically in the form of one continuous circle. Therefore, a haploid bacterial cell would be expected to have one circular molecule of DNA, except when it is preparing to divide. In the case of a bacterium which reproduces by binary fission, it would be expected to have two circular DNA molecules per cell just prior to septum formation and fission.

This description of the haploid state is complicated, however, by the fact that many bacteria are capable of growing at a rate such that the **generation time**, the average time interval between cell divisions, is shorter than the length of time required to replicate the entire DNA molecule in the cell (one round of DNA replication). The cell obviates this problem by beginning a second round of DNA replication prior to the completion of the first. As the generation time decreases, the time interval between the initiation of new rounds of replication also decreases. The net result of these processes is that a rapidly growing bacterial cell actually has multiple copies of most genetic information. Moreover, the genetic information located near the origin of replication is present in proportionately greater amounts than that located near the termination site (Figure 1-1).

Strictly speaking, then, it is not possible to talk about the number of sets of information per cell (**genomes**), since most of them are incompletely replicated. The term **genome equivalent**, which refers to the number of nucleotide base pairs contained in one complete bacterial genome, is generally used instead. Note that a cell containing several genome equivalents of DNA is, nonetheless, haploid, since all the DNA is of necessity identical, because it is all descended from the same original molecule. An example of this can be seen in Figure 1-1C, in which there are four copies of A but only one of J. This is similar to the case of a diploid eukaryotic cell which, just prior to cell division, actually contains four of each kind of chromosome instead of two but is still considered as diploid. Figure 1-2

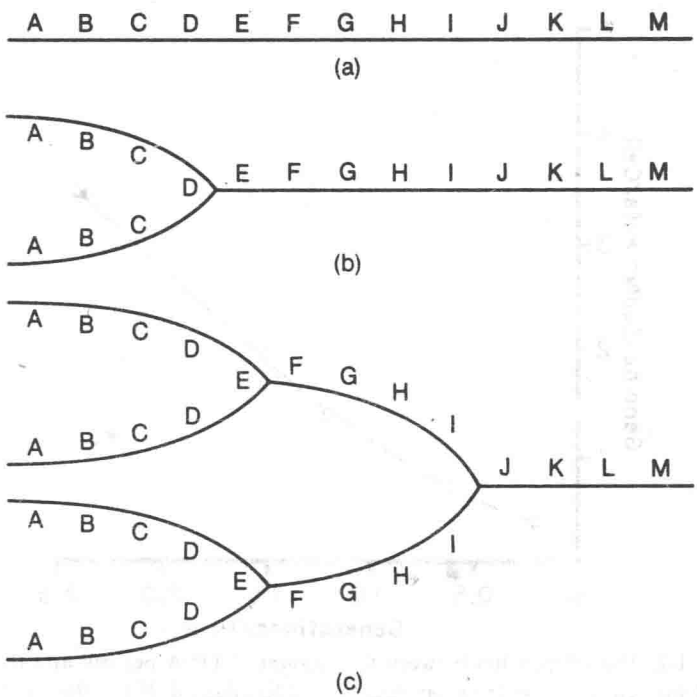

Figure 1-1. The effect of replication on gene dosage. (a) A nonreplicating DNA duplex. (b) The first round of replication has begun, initiated at the left-hand end of the duplex. (c) The second round of replication has begun before the first round of replication has finished. Once again the initiation occurred at the left-hand end, giving rise to two new replication forks. The same effect would be seen in a cell with a circular genophore except that the DNA duplex would be longer and would be looped back on itself.

demonstrates the relationship between the number of genome equivalents and the growth rate for *Escherichia coli*.

In the case of a bacterial cell which has received a new piece of DNA via some type of genetic process (see Section IV), it is possible to have two distinctly different sets of genetic information in the same cytoplasm. Such a cell is effectively diploid for that information. However, since most DNA transfer processes move only a fraction of the total genome, the resulting cell is only a partial diploid, or **merodiploid**. If the new piece of DNA is capable of self-replication, the merodiploid state may persist indefinitely. If it is not, only one of the daughter cells will be a merodiploid at each cell division, and the lone merodiploid cell will soon be lost amidst a large number of haploid cells, unless the extra DNA confers some selective advantage.

The absence of any sort of mitotic apparatus in prokaryotic cells raises the issue of how such cells manage to move their DNA molecules around so that each daughter cell receives an appropriate share. The generally accepted theory, formulated by Jacob and co-workers, is that the replicat-

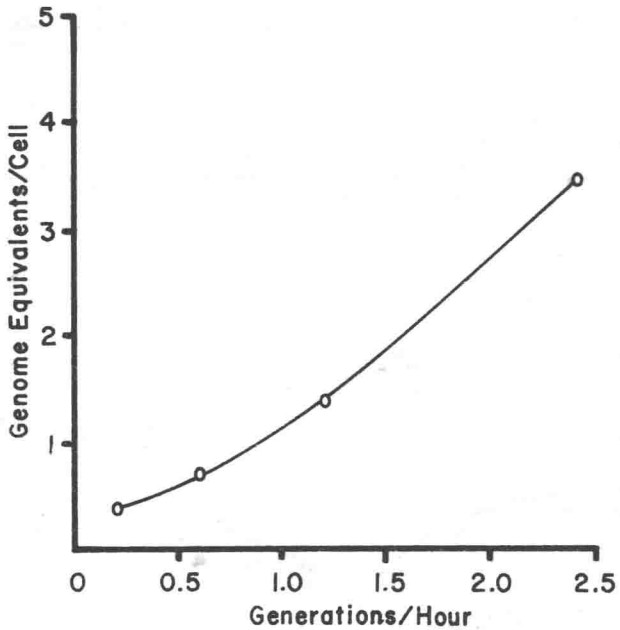

Figure 1-2. The relationship between the amount of DNA per cell and the growth rate of the cell. Data are from Maaløe, O. and Kjeldgaard, N.O. 1966. The Control of Macromolecular Synthesis. Reading, Massachusetts: Addison-Wesley.

ing DNA molecules are attached to the plasma membrane. As each new round of replication begins, a new attachment site is formed on the membrane. The plasma membrane of a bacterial cell appears to grow primarily at the region along which the new septum will form. The insertion of new membrane material into this preexisting structure implies that two points lying astride the center of the membrane, which are initially close together, will gradually separate as the membrane grows. Electron micrographic evidence indicates that the points of attachment of the replicating DNA molecules do lie on the plane of the future cell cleavage, and this mechanism apparently does shift the DNA molecules sufficiently to ensure proper segregation at the time of binary fission (Figure 1-3).

II. Maintenance and Utilization of Genetic Information

In descriptions of genetic processes there is frequently an implication that the bacterial cell is in a quiescent state, whereas, in fact, the converse is usually true. Most genetic experiments are performed with cells either "mid" or "late" in the exponential (log) phase of growth; i.e., such cells are synthesizing DNA, RNA, and protein at a rate near the maximum possible

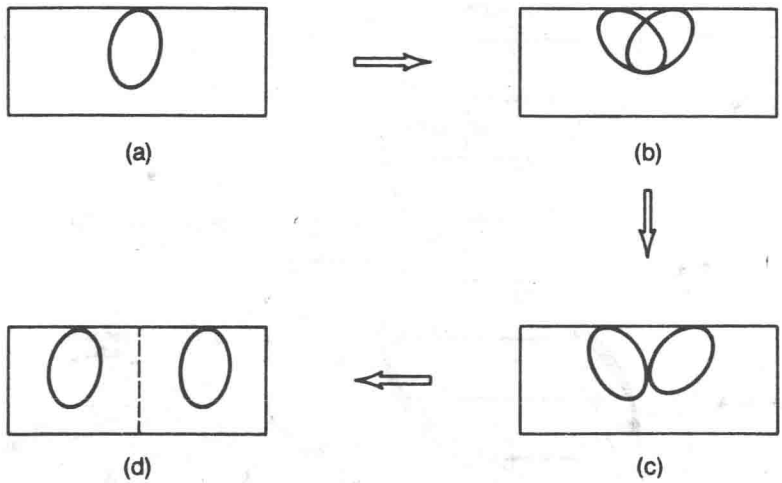

Figure 1-3. Segregation of replicating DNA. (a) A single DNA molecule is attached to the cell membrane. No DNA replication is occurring. (b) Replication has begun, and the origin region of the DNA (including the membrane attachment site) has been duplicated. The insertion of new membrane material has caused the two origin regions to become physically separated on the surface of the cell. (c) Replication of the DNA is almost complete. The membrane attachment sites have continued to separate until the physical connection between the DNA duplexes lies along the presumptive plane of cell division. (d) DNA replication has stopped, and a new round of replication has not yet been initiated. The cell will soon divide along the plane indicated by the dotted line.

for that particular growth medium. It is important, therefore, to realize that the DNA and its immediate vicinity are in a state of considerable metabolic flux and to have some basic knowledge of the biochemistry of the syntheses involved.

The DNA molecules are thought to exist in some variant of the double helical structure proposed by Watson and Crick. In this structure, there is a built-in chemical polarity due to the position of various substituents on the deoxyribose moiety (Figure 1-4). It is customary to refer to the 5′ or 3′ end of a nucleic acid, depending on the point of attachment of the last substituent (phosphate or hydroxyl group) to the pentose ring of the last nucleotide in the chain. In Figure 1-4, for example, the arrow heads are always at the 3′-hydroxyl end of the chain. Replication of the DNA is in a semiconservative mode, in which one new strand of DNA is paired to one old strand. During replication, a Y-shaped structure is produced, and the junction point of the arms and leg of the Y is called the replication fork.

However, nothing in the Watson and Crick model explicitly explained the biochemistry of replication, and as a result, the model has been greatly enlarged and altered by recent discoveries. The first complication arose when Cairns carefully extracted DNA from *E. coli* and demonstrated that it was normally in the form of a circle which formed a structure during

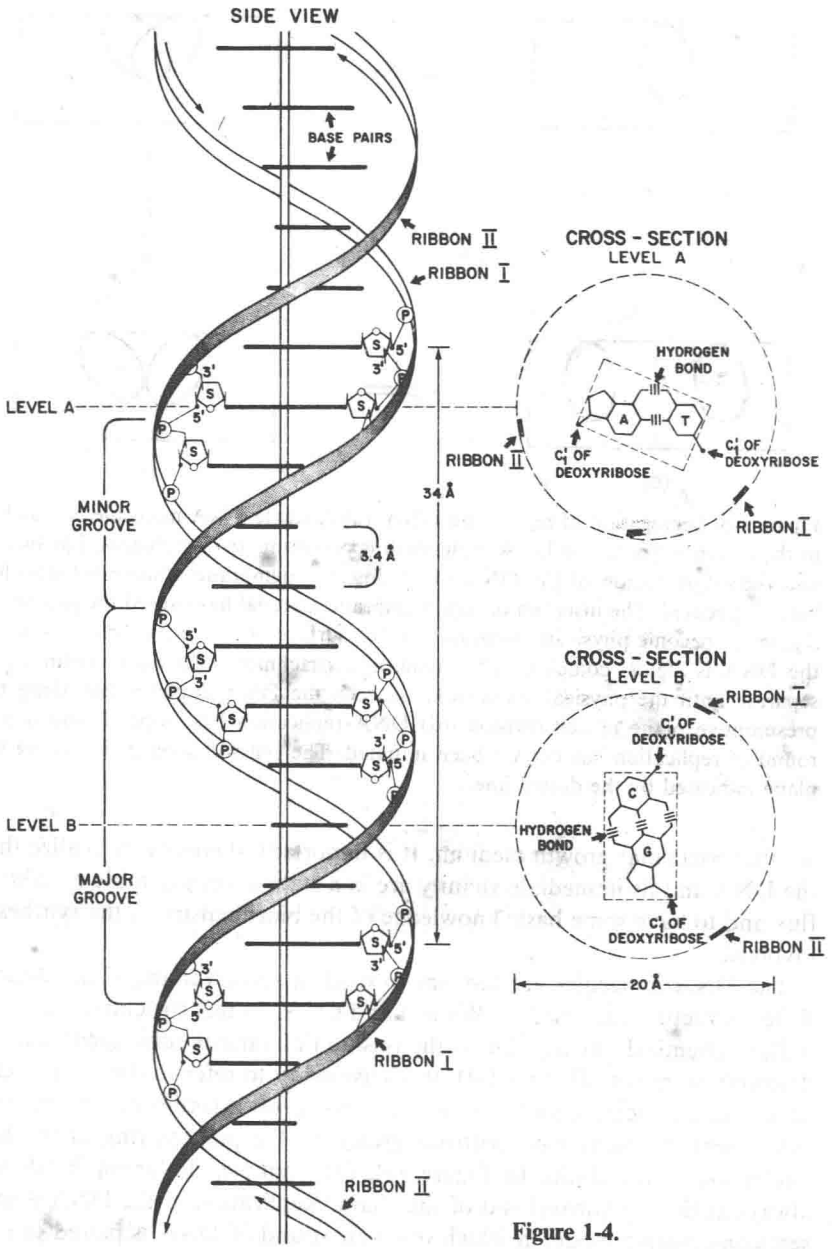

Figure 1-4.

replication rather like the Greek letter theta (Figure 1-5). In fact, this type of replication is sometimes called theta replication. Results obtained from a variety of other bacteria indicated that they, too, have circular DNA molecules and, more importantly, that in all of the bacteria examined the replication is bidirectional. That is to say, both bifurcations in Figure 1-5 represent actively replicating forks.

An even more remarkable discovery, made by Stonington and Pettijohn,

Figure 1-4. A diagrammatic representation of double-helical DNA (B form). At the left the molecule is drawn in side view with the fiber axis indicated by the vertical rod. The backbone of the molecule consists of two polynucleotide chains, which form right-handed helices. These chains are coiled together in a plectonemic (i.e., intertwined and not freely separable) manner to form a double helix having two grooves, one shallow (minor) and one deep (major), and an overall diameter of 2 nm.

Each chain is composed of D-2′-deoxyribose sugar moieties (S) linked by phosphate groups (P), thus forming 3′,5′-phosphodiester bridges and producing a long unbranched polymer. The individual bases are attached to the sugar molecules through β-*N*-glycosyl linkages. The two chains are antiparallel with the 5′-to-3′ direction proceeding upward for one chain but downward for the other. This 5′-to-3′ direction is illustrated by the arrows at the top and bottom of the diagram. For the sake of clarity, the molecular structure of the sugar-phosphate backbone is shown only over small regions. The two ribbons serve to represent the continuity of the two chains, the shaded regions being closest to the viewer.

The hydrogen-bonded base pairs, represented by horizontal heavy lines, are planar molecules occupying the central core of the helix (the region indicated in cross section within the dotted rectangles at the right of the diagram). Only the bases lie in the plane of the cross sections, and thus only the base pairs are drawn, with attachment to the sugar merely being indicated. The position of each ribbon at either of the two cross-sectional levels is indicated.

The broken line forming a circle serves to indicate the outer edge of the double helix that would be observed when the molecule is viewed end on. An adenine (A)-thymine (T) pair is shown as the pair representative of level A, whereas a guanine (G)-cytosine (C) pair is shown to represent level B.

The surface planes of the bases are perpendicular to the vertical axis and are separated from each neighboring base pair by a vertical distance of 0.34 nm. There are 10 base pairs per complete turn of the helix so that each turn of the helix has a vertical length of 3.4 nm, and each base pair is rotated 36° relative to its nearest neighbors. As a result of this rotation, the successive side views of the base pairs appear as lines of varying lengths depending upon the viewing angle. The hydrogen bonding between the bases and the hydrophobic interactions resulting from the parallel "stacking" of the bases serve to stabilize the helical structure. Reprinted with permission from Kelln, R.A., Gear, J.R. 1980. A diagrammatic illustration of the structure of duplex DNA. BioScience 30:110-111. © American Institute of Biological Sciences.

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was that it was possible to extract intact bacterial genophores (also called **nucleoids** or **folded chromosomes**) from *E. coli* and examine them with an electron microscope. Figure 1-6 shows a photograph of one such structure. Similar structures have been isolated from many other bacteria. Worcel and Burgi have demonstrated that this complex structure consists of a DNA molecule which is held in some 50 superhelical coils[1] by RNA and protein

[1]A supercoil is prepared by taking a double helical DNA molecule and twisting the entire coil, rather like taking a rope and coiling it on the ground, and then gluing the ends together so that the extra coils are an integral part of the structure. In the case of DNA, the process is catalyzed by the enzyme DNA gyrase. An example of the resultant DNA molecule can be seen in Figure 13-1.

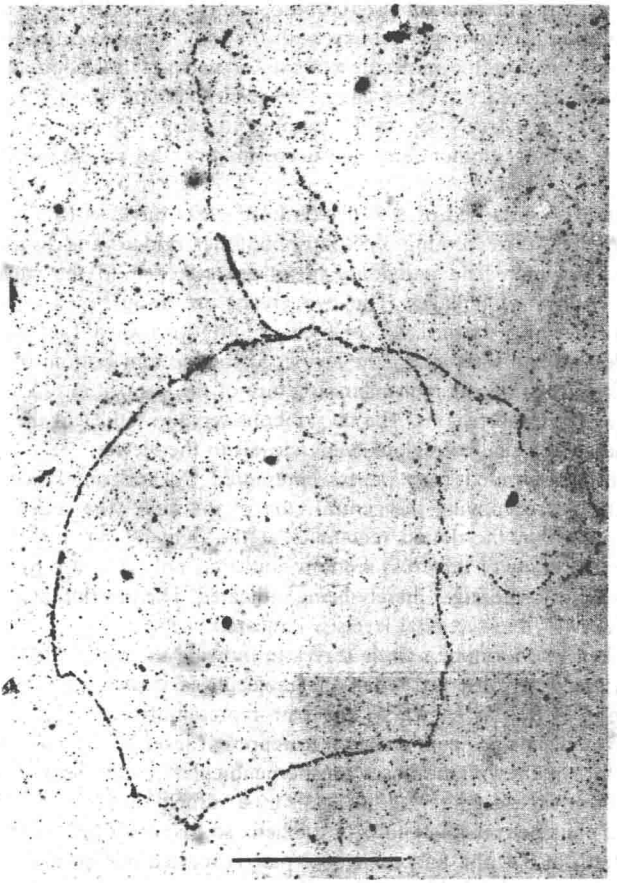

Figure 1-5. Autoradiograph of the genophore of *E. coli* Hfr3000. The DNA was labeled with tritiated thymidine for two generations and then extracted from the cell by the use of the enzyme lysozyme, which attacks the cell wall. A photographic emulsion was overlayed on the DNA and exposed to the radioactive atoms for 2 months. As the tritium decayed, grains of silver were deposited in the emulsion, and these grains indicate the position of the DNA molecule. The scale at the bottom represents a length of 100 μm; the length of the DNA, discounting the replicated portion, is estimated as about 1.1 mm. It should be remembered that the cell from which this DNA molecule was extracted was probably only a few micrometers in length. From Cairns, J. 1963. The chromosome of *E. coli*. Cold Spring Harbor Symposia on Quantitative Biology 28:43-45.

Figure 1-6. Membrane-attached *E. coli* genophore. Cells were gently lysed with lysozyme and detergent. The DNA was separated from cellular debris by sedimentation through increasing concentrations of sucrose and then mounted for electron microscopy by the use of a monolayer of cytochrome C molecules on the surface of a formamide solution. The DNA was stained with uranyl acetate and coated with platinum to increase contrast.