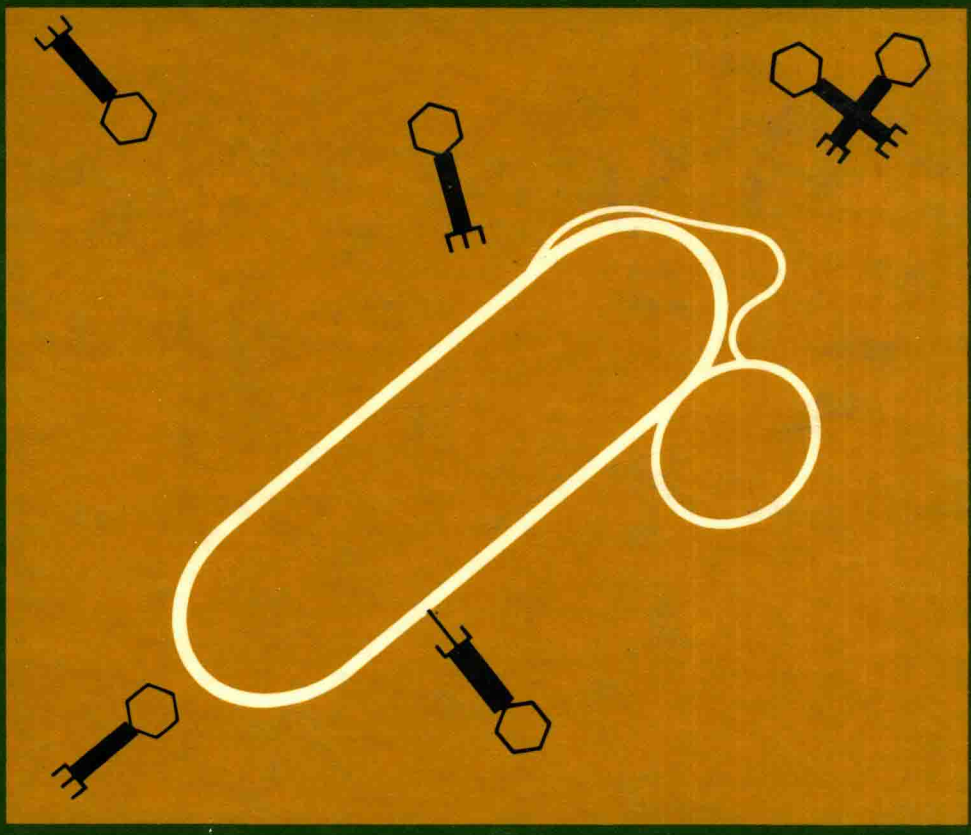

EDWARD A. BIRGE

Bacterial and Bacteriophage Genetics

An Introduction

SECOND EDITION

Springer-Verlag

Edward A. Birge

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An Introduction

Second Edition

With 150 Figures

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For Lori, Anna, and Colin

Preface to the Second Edition

It is with real pleasure that I offer some introductory remarks to this second edition of *Bacterial and Bacteriophage Genetics*. The reception of the first edition was very good, and most of the criticisms offered have only served to strengthen this new edition. The majority of the suggestions for revision of material included requests for more molecular detail. I have tried to provide this throughout the text but especially in the heavily revised Chapter 1 and completely rewritten Chapters 2 and 10. The former Chapter 2 has been repositioned as an appendix so as to offer an uninterrupted flow of material in the main body of the text.

The tremendous increase in our knowledge of the genetics of eukaryotic microorganisms has permitted another sort of change in coverage. Instead of general discussions about eukaryotic organisms, wherever possible I have tried to offer specific details about *Saccharomyces cerevisiae*, whose wealth of genetic detail will soon rival that of *Escherichia coli*. Although this material is not intended as a substitute for a course in yeast genetics, it is my hope that it will enable the beginning student to comprehend some of the similarities and differences between these two popular microorganisms.

As before this book is intended for the student who is taking a first course in bacterial and bacteriophage genetics and who brings to it some background in genetics. The best background would be an introductory course in general genetics, but extensive coverage of genetics in an introductory biology course might well prove sufficient. As an example, it is assumed that the student is familiar with the standard Watson and Crick model for DNA structure. A broad knowledge of microorganisms is helpful but not required to understand the material presented. In general the material from a good introductory biology course should be adequate.

Several books can be noted as being particularly useful sources of detailed supplementary information. David Freifelder's *Physical Biochemistry*, Second Edition, is particularly valuable as a resource for methods used in analyzing macromolecules. Additional information about *Saccharomyces* can be found in *Yeast Genetics: Fundamental and Applied Aspects* edited by J.F.T. Spencer, D.M. Spencer, and A.R.W. Smith. Cold Spring Harbor Laboratory constantly publishes many monographs dealing with various aspects of bacterial and viral genetics. Their current book list should be consulted for details. Probably the most important one for this book is *Genetic Maps*, Volume 4, edited by S.J. O'Brien. The American Society for Microbiology also is a good source for monographic literature. Their recent publications include *Escherichia coli and Salmonella typhimurium: Cell and Molecular Biology*, edited by F.W. Neidhardt. Most references to classic papers have been omitted, and the reader is referred to collections of papers that have been reprinted such as the volume edited by Abou-Sabe.

Acknowledgments

I am indebted to many people for helpful suggestions and comments about the first edition. I would particularly like to acknowledge the following individuals who assisted in correcting errors in the first edition: Dr. Paul A. Lemke, Auburn University; Dr. Margarita Salas, Universidad Autonoma de Madrid; and Dr. T.A. Trautner, Max Planck Institut, Berlin. Dr. W. Scott Champney's suggestions on reorganizing the material were most helpful. Dr. Martha Howe graciously volunteered some assistance with the material on phage Mu. However, any errors that remain must be attributed to me.

It has once again been a pleasure to work with the people at Springer-Verlag who have been very helpful during all phases of this revision.

Linkage Maps

Inside the front cover The illustrations on the front inside cover are linear scale drawings representing the circular linkage map of *E. coli* K-12. The time scale of 100 minutes, beginning arbitrarily with zero at the *thr* locus, is based on the results of interrupted-conjugation experiments. Major genetic symbols used in this figure are defined in Table 2-2. Parentheses around a gene symbol indicate that the position of that marker is not well known and may have been determined only within 5 to 10 minutes. An asterisk indicates that a marker has been mapped more precisely but that its position with respect to nearby markers is not known. The small vertical arrows indicate the directions of transcription of certain well-studied loci. Parentheses around an operon indicate that, although the direction of transcription of the genes in the operon is known, the orientation of the operon on the chromosome is not known. A similar map for *Salmonella typhimurium* may be found inside the back cover. From Bachmann, B.J. (1983) Linkage map of *E. coli* K-12, edition VII. Microbiological Reviews 47:180–230.

Inside the back cover The illustrations on the back inside cover are linear scale drawings representing the circular linkage map of *Salmonella typhimurium*. The scale of 100 units has been chosen to emphasize the similarities to the *E. coli* map (inside the front cover). A length of one unit represents the amount of DNA carried by P22, KB1, or ES18 transducing phage particles, while a length of two units represents the amount of DNA carried by a P1 transducing phage particle (see Chapter 7). The segmented lines to the right of the gene symbols indicate genes that are jointly transduced and the linear distances determined from these data. Major genetic

symbols used in this figure are defined in Table 2-2. Parentheses around a gene symbol indicate that the location of the gene is known only approximately, usually from conjugation studies. An asterisk indicates that a marker has been mapped more precisely, usually by phage-mediated transduction, but that its position relative to adjacent markers is not known. Arrows to the extreme right of operons indicate the direction of mRNA transcription at these loci. From Sanderson, K.E., Roth, J.R. (1983). Linkage map of *Salmonella typhimurium*, edition VI. Microbiological Reviews 47:410–453.

Contents

Chapter 1
Prokaryote Molecular Biology

When beginning a 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 and molecular processes, and the ways in which these processes differ from those used by eukaryotic organisms. This chapter provides a brief overview of major cell activities with comparisons specifically between the eubacteria, primarily represented by *Escherichia coli*, and the "lower" eukaryotes, primarily represented by *Saccharomyces cerevisiae*. The focus in this chapter is on the major molecular biologic processes of DNA replication, transcription, and translation. Specific genetic considerations are the subject of the next chapter. Some of the material in these chapters may be familiar from introductory biology classes, but all of it forms a necessary foundation for the topics to be presented later. The many varied replication and transcription mechanisms found among the bacteria, their plasmids, and their viruses refer back to this material.

Prokaryotic Cells and Eukaryotic Cells

Structure

The key feature that distinguishes prokaryotic organisms from eukaryotic organisms is their lack of an organized nucleus. They are also typically smaller than eukaryotic cells. For example, an average, rapidly growing *E. coli* cell is a cylinder about 1×0.5 μm, whereas a typical *S. cerevisiae* cell is round to ovoid and about 3 to 5 μm in diameter. The approximately 64-fold difference in cell volume is reflected in their internal cytoplasmic

complexity, with *S. cerevisiae* containing the usual intracellular organelles such as mitochondria and endoplasmic reticulum, and prokaryotic cells having no real compartmentalization of function. In all the comparisons that follow, only the nuclear activity in the eukaryotic cells is considered, as modern evolutionary theory assumes that mitochondria and chloroplasts are descended from prokaryotic ancestors.

Another difference between the cell types is found in the way in which the cells carry out the processes of cell division and segregation of DNA. In both cases the cell volume increases during metabolism until a particular size is attained. At that point a complex series of events begins that culminates in the production of two daughter cells, each containing an exact copy of the DNA found in the parent cell.

Eukaryotic cells in general divide by a simple fission process coupled to mitosis that yields two equal-sized cells. However, in the case of many of the yeasts and *Saccharomyces* in particular, the process of cell division is called **budding,** because the new cell is produced as a small protrusion from the surface of the parent cell that rapidly enlarges and eventually pinches off (Fig. 1-1). During formation of the bud, the process of mitosis occurs. Spindle fibers form and attach to the centromeres on the already duplicated yeast chromosomes. The pairs of chromosomes line up and then are separated, moving along the spindle toward the poles of the elongating nucleus. The nuclear membrane persists at all times, unlike the situation in animals and plants. The nucleus continues to elongate, eventually entering the already large bud. When the nucleus splits in two, cytokinesis can occur to form the new cells.

The eubacteria reproduce by binary fission (Fig. 1-2), in which cell mass and volume enlarge linearly until the cell undergoes cytokinesis to yield two equal-sized daughters. The cell density remains roughly constant throughout the cycle. This mechanism is grossly similar to that in eukaryotic cells, but the process of mitosis is unknown in prokaryotic organisms. Moreover, there is no prokaryotic structure that is physically analogous to a centromere, and no microtubules have been seen. Therefore it is obvious that the cells must employ some other means to ensure proper segregation of their DNA molecules. The generally accepted theory, formulated by Jacob and co-workers, is that the replicating DNA molecules are attached to the plasma membrane, presumably at a site near the origin of replication. 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 line of the cell membrane and that are therefore initially close together gradually separate as the membrane grows (Fig. 1-3). 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

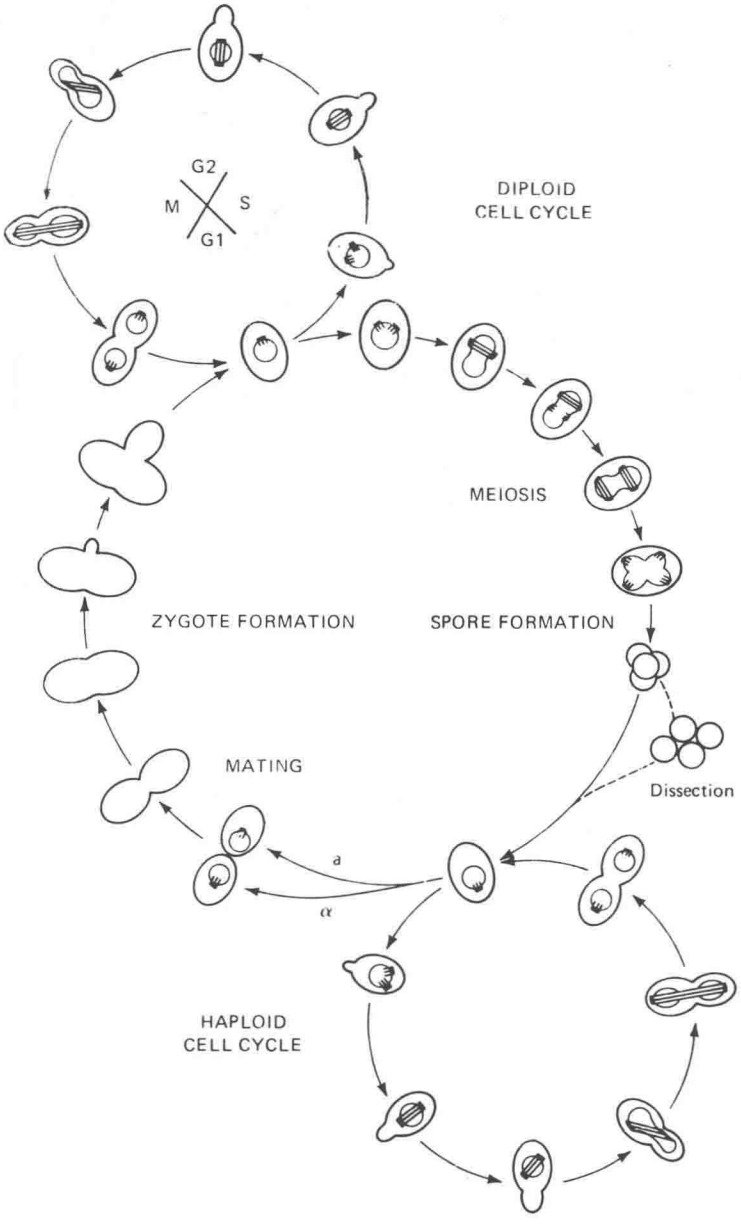

Figure 1-1. Possible life cycles of heterothallic strains of *S. cerevisiae*. In the diploid cycle there are two growth phases separated by DNA synthesis (S) and mitosis (M). The nuclear configuration is indicated for all dividing cells. From Dawes, I.W. (1983). Genetic control and gene expression during meiosis and sporulation in *Saccharomyces cerevisiae*, pp. 29–64. In: Spencer, J.F.T., Spencer, D.M., Smith, A.R.W. (eds.) Yeast Genetics. New York: Springer-Verlag.