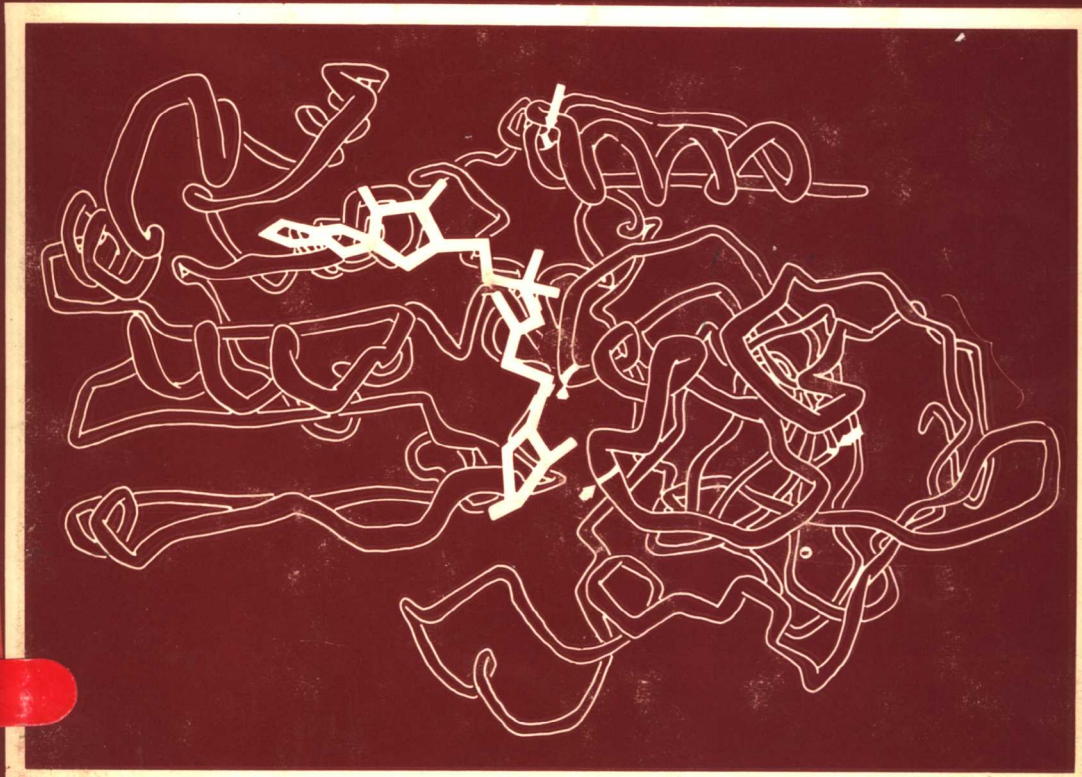
MONOGRAPHS IN EVOLUTIONARY BIOLOGY

MICROORGANISMS AS MODEL SYSTEMS FOR STUDYING EVOLUTION

Edited by

Robert P. Mortlock



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Preface

The microorganisms present on the earth today possess a vast range of metabolic activities and are often able to demonstrate their surprising versatility by gaining both new enzyme activities and new metabolic pathways through mutations. It is generally assumed that the earliest microorganisms were very limited in their metabolic abilities, but as time passed they gradually expanded their range of enzymatic activities and increased both their biosynthetic and catabolic capacity. It is also believed that these primitive microorganisms increased the amount of genetic material they possessed by duplicating their existing genes and possibly by acquiring genetic material from other organisms.

A small group of scientists has been exploring the means by which existing microorganisms are capable of mutating to expand their biochemical abilities. In recent years, more attention has been focused on this type of research, sometimes called "evolution in a test tube." The recent advances in biotechnology and modern techniques of genetic transfer have generated new interest in the methods by which a microorganism's metabolic activities can be improved or deliberately changed in some specific manner.

The work reported in this book describes a type of "genetic engineering" whereby microorganisms are challenged to develop new metabolic activities, not by the acquisition of genetic material from external sources, but by altering their own existing genetic information. This approach to studying evolution has been surprisingly fruitful and has clearly illustrated, for example, the importance of regulatory mutations in the establishment of new metabolic pathways. This area of research holds much more promise than previously realized, and it is hoped and anticipated that as we learn more about the process by which mutations create new metabolic abilities we will be better able to control and direct that process.

Many of the early studies involved the use of novel sugars of five-carbon structure as potential growth substrates and were inspired by the observation in several laboratories that the genetic mechanism establishing the growth of *Klebsiella* strains on the pentitol xylitol, was a single mutation in the regulation of the inducible pathway for another pentitol, ribitol. The first three chapters in this book describe investigations into how bacteria, especially those in the genera *Klebsiella* and *Escherichia*, are able to gain the ability to establish and then improve a pathway for the catabolism of xylitol.

Chapter 1 contains a general description of the routes of pentitol degradation by coliform bacteria and shows how such bacteria can acquire, through mutation, the ability to utilize some of the less common pentitols as substrates for both carbon and energy. Once the genetic and biochemical changes leading to the establishment of growth on xylitol were clearly understood, further experiments were designed to improve the new xylitol pathway, and these experiments are described by Hartley in Chapter 2. Two different approaches for increasing the activity of a newly established enzymatic reaction are discussed. For an enzyme "borrowed" from an existing pathway for use in catalyzing a similar reaction for a novel substrate in a new pathway, the activity of the enzyme for the new substrate may be poor and rate limiting, as it is for the new xylitol pathway. In such a case it can be demonstrated that two types of genetic change, either altering the enzyme to make it more efficient for the new substrate or making larger amounts of the unaltered enzyme by mechanisms such as gene amplification, will both improve the growth rate on the novel substrate. Chapter 3, also by Hartley, continues the discussion of the mutations permitting growth on xylitol with an elegant study of the sequence of the regulatory and structural genes involved in the establishment of the new xylitol pathway in the xylitol-positive mutants.

Chapter 4 examines some of the five-carbon aldopentose sugars and the mutations which establish new catabolic pathways for the degradation of such carbohydrates as D-arabinose, D-lyxose, L-xylose, and L-lyxose. For most of these new pathways the critical enzyme activities needed for the metabolism of the novel substrate are shown to be borrowed, via regulatory mutations, from pathways established by evolution for the degradation of more common substrates. For one of the uncommon aldopentoses, however, D-lyxose, the new enzyme activity mobilized to initiate the D-lyxose catabolic pathway may indeed come from what was previously an unexpressed and unknown "silent gene."

Chapter 5, by Lin and Wu, also shows how enzymes can be "borrowed" from an existing pathway to establish a new catabolic pathway. It describes how the establishment of a new pathway for the degradation of propanediol results from a regulatory mutation that converts an enzyme normally employed as an anaerobic reductase into a dehydrogenase that can function under aerobic conditions. Chapter 5 further illustrates how mutations leading to the establishment of a new metabolic pathway can result in the elimination of another pathway.

Chapter 6 describes a different approach to studying the means by which microorganisms can establish new metabolic activity. In these experiments, an existing ability for the utilization of a substrate (lactose) is removed by genetic deletion and the mutant cells are challenged to find and modify other genes to replace the function of those deleted. In his studies reported in this chapter, Hall shows the step-by-step development of an entire new operon to replace the *lac* operon, an operon that responds to new regulatory elements. The location of the new operon suggests the recruitment of "silent genes" on the cell chromosome to replace those of the normal *lac* operon.

In Chapter 7, Clarke describes experiments designed to expand the range of amide substrates for the aliphatic amidase enzyme of *Pseudomonas aeruginosa*, experiments that led to the establishment of new catabolic pathways. She examines the role of both regulatory and structural gene mutations in establishing pathways for new amide substrates. In Chapter 8, Wills documents how selective pressure can be placed upon an enzyme, in this case an alcohol dehydrogenase of yeast, to select for deliberate alterations in the enzyme's kinetic properties. Chapter 9, by Kemper,* describes the most interesting situation, where the loss of a subunit of one enzyme actually results in the replacement of a missing subunit for a different enzyme. In such a manner, mutants auxotrophic for the leucine biosynthetic pathway regain the ability to synthesize leucine as the result of a deletion in a different location of the chromosome that makes available a product of another gene, the *new leu* gene, to take the place of the missing subunit.

Chapter 10 is slightly different from the others, for there Riley describes her fascinating studies on the current structure of the *E. coli*

*As this volume was in the final stages of preparation I learned, to my great sorrow, of the death of Dr. Jost Kemper. He will be deeply missed by all of his colleagues.

chromosome, studies that not only suggest how silent genes may originate but also illustrate the role past duplications have played in the evolutionary development of the modern cell's DNA.

It is not an exaggeration to state that the work presented in this volume, most of it the result of the investigations of the authors themselves, represents the pioneering studies on the means by which microorganisms can mutate to increase their metabolic capabilities.

Robert P. Mortlock

Ithaca, New York

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CHAPTER 1

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ROBERT P. MORTLOCK

1. Introduction

Microbiologists generally agree that the earliest microorganisms to evolve were simple in their metabolic capabilities and had to be supplied with many preformed, complex molecules to satisfy their nutritional requirements. As time passed, certain organisms developed new metabolic pathways to synthesize such required compounds from smaller, less complex molecules and thus became more versatile in their nutritional requirements, utilizing a wider range of food sources. Changes in the metabolism of higher organisms must have produced new types of organic compounds that entered the environment upon the death of the organisms. Microorganisms, in turn, evolved metabolic pathways for the degradation of these new molecules and used them to obtain carbon and energy to satisfy their growth requirements. The extra DNA provided by the duplication of previously existing genes may have been modified through mutation to eventually become the structural and regulatory genes for new degradative pathways.

Although studies comparing the amino acid sequences in proteins or nucleotide sequences in nucleic acids can provide some information about the evolutionary relationship between enzyme pathways, they do not provide details on the exact mutational events that led to the differentia-

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