Benchmark Papers in Microbiology

V. 10

MOLECULAR BIOLOGY AND PROTEIN SYNTHESIS

Edited by
ROBERT A. NIEDERMAN

Dowden, Hutchinson & Ross, Inc.

Distributed by
HALSTED PRESS

Benchmark Papers
in Microbiology / 10

A BENCHMARK® Books Series

MOLECULAR BIOLOGY AND PROTEIN SYNTHESIS

Edited by

ROBERT A. NIEDERMAN
Rutgers—The State University

Dowden, Hutchinson & Ross, Inc.
STROUDSBURG, PENNSYLVANIA

Distributed by
HALSTED PRESS
A Division of
John Wiley & Sons, Inc.

Benchmark Papers in Microbiology, Volume 10
Library of Congress Catalog Card Number: 76-1388
ISBN: 0-470-15055-6

78 77 76 1 2 3 4 5
Manufactured in the United States of America.

LIBRARY OF CONGRESS CATALOGING IN PUBLICATION DATA

Exclusive Distributor: **Halsted Press**
A Division of John Wiley & Sons, Inc.

ACKNOWLEDGMENTS AND PERMISSIONS

ACKNOWLEDGMENTS

NATIONAL ACADEMY OF SCIENCES—*Proceedings of the National Academy of Sciences of the United States of America*
- Analysis of DNA Polymerases II and III in Mutants of *Escherichia coli* Thermosensitive for DNA Synthesis
- Mechanism of Activation of Catabolite-Sensitive Genes: A Positive Control System
- N-Formylmethionyl-sRNA as the Initiator of Protein Synthesis
- Replication and Repair of DNA in Cells of *Escherichia coli* Treated with Toluene
- The Replication of DNA in *Escherichia coli*
- RNA-Linked Nascent DNA Fragments in *Escherichia coli*
- Structure and Function of *E. coli* Ribosomes: V. Reconstitution of Functionally Active 30S Ribosomal Particles from RNA and Proteins
- Synthesis of a Large Precursor to Ribosomal RNA in a Mutant of *Escherichia coli*

PERMISSIONS

The following papers have been reprinted or translated with the permission of the authors and copyright holders.

AMERICAN CHEMICAL SOCIETY—*Biochemistry*
Effect of Aminoacyl Transfer Ribonucleic Acid on Competition Between Guanosine 5′-Triphosphate and Guanosine 5′-Diphosphate for Binding to a Polypepetide Chain Elongation Factor from *Escherichia coli*
Isolation and Physical Properties of the Ribosomal Ribonucleic Acid of *Escherichia coli*
Stoichemistry of the 30*S* Ribosomal Proteins of *Escherichia coli*

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS—*The Journal of Biological Chemistry*
The Enzymic Synthesis of Amino Acyl Derivatives of Ribonucleic Acid: I. The Mechanism of Leucyl-, Valyl-, Isoleucyl-, and Methionyl Ribonucleic Acid Formation

COLD SPRING HARBOR LABORATORY—*Cold Spring Harbor Symposia on Quantitative Biology*
On the Catalytic Center of Peptidyl Transfer: A Part of the 50*S* Ribosome Structure
Peptide Chain Termination, Codon, Protein Factor, and Ribosomal Requirements
Polynucleotide Synthesis and the Genetic Code
The RNA Code and Protein Synthesis
In Vivo Mechanism of DNA Chain Growth

ELSEVIER SCIENTIFIC PUBLISHING CO.—*Biochimica et Biophysica Acta*
On the Presence of Low-Molecular-Weight Ribonucleic Acid in the Ribosomes of *Escherichia coli*

MACMILLAN (JOURNALS) LTD.—*Nature*
Conformation of the Anticodon Loop in tRNA
Factor Stimulating Transcription by RNA Polymerase
Genetical Implications of the Structure of Deoxyribonucleic Acid
Isolation of an *E. coli* Strain with a Mutation Affecting DNA Polymerase
Nucleotide Sequence of 5*S*-Ribosomal RNA from *Escherichia coli*
Polypeptide Chain Initiation: Nucleotide Sequences of the Three Ribosomal Binding Sites in Bacteriophage R17 RNA
Termination Factor for RNA Synthesis
Two Compounds Implicated in the Function of the *RC* Gene of *Escherichia coli*
Unstable Ribonucleic Acid Revealed by Pulse Labelling of *Escherichia coli*

SERIES EDITOR'S PREFACE

In the past decade there has clearly been no more exciting and advancing field than molecular biology; yet, to the novice, it may appear excessively complex. Dr. Niederman has prepared this volume with the needs of the novice in mind. So much information and such a wealth of literature exist that it has been important to select those papers which provide not merely superficial understanding but real knowledge of how and why and on what evidence we know how DNA is replicated, how proteins are synthesized, and how the complex, yet exquisitely tuned, machinery is controlled. Previous collections were designed for workers in the field, to whom the methods and the language were familiar. This volume has as its purpose to provide a knowing and useful guide to the fundamental papers upon which modern molecular biology is based. In this task, Dr. Niederman has brought not only his experience as an operator of a research laboratory actively involved in this field but also his experience as a teacher of undergraduates (and graduates) in a course that is a model of its kind. As such, we have here a volume useful to the novice—as well as to the advanced investigator whenever the latter wishes to refer to the major contributions to the field.

The volume is composed of five parts. In the first, eight papers cover the field of DNA structure and replication and introduce the novice to polymerase III and Okazaki pieces. Part II provides six papers on messenger RNA formation which introduce the novice to core enzyme, sigma factor, and other transcription factors (M, PSI, and rho), as well as information regarding the action of the promising antibiotic rifampicin and the beginnings of an explanation for catabolite repression. There is also a discussion of ppGpp and pppGpp, originally known as "magic spots."

The third part contains eight papers on the nature and the assembly of the ribosome, the organelle that translates messenger RNA into protein. The proteins, especially, seem to be exceedingly numerous and complex, and the RNA that they contain may well come from cleavage of a much larger molecule. Part IV, with eight papers, covers the actual mechanism of peptide-bond formation, and for persons brought up in an earlier day, the number of external, or at least dissociable, factors

seems tremendous. Actually it appears that peptide-bond formation is considerably more complicated than originally supposed. In the fifth part, seven outstanding papers deal with the genetic code and transfer RNA, the latter known in such detail that one can begin to visualize three-dimensional structures.

Although a glance at the contents will reveal this arrangement, it seems sensible to outline it here so as to point out the unusual and illuminating findings that these sections report.

WAYNE W. UMBREIT

CONTENTS BY AUTHOR

CONTENTS

PART V: TRANSLATION: THE GENETIC CODE AND TRANSFER RNA

INTRODUCTION

An assessment of the explosive growth in molecular biology that has occurred during the last two decades reveals many significant contributions from a variety of disciplines. Indeed, biochemistry, biophysics, crystallography, genetics, and microbiology have each provided major contributions. However, the demonstration of deoxyribonucleic acid (DNA) rather than protein as the carrier of the cell's genetic information by Avery et al. (1944) marks the beginning of this scientific revolution. These investigators identified highly polymerized DNA as the constituent that caused the transformation of one strain of bacterium to another. This startling finding stimulated a number of attempts at elucidating the structure of DNA in the hope that deciphering its molecular architecture would provide a basis for understanding how this molecule functions as the primary hereditary material within the cell. This hope was finally realized with the advent of the double-helical model of Watson and Crick (1953) for the structure of DNA. The double helix represented a series of brilliant insights by the latter investigators that were largely based upon X-ray diffraction analysis (Wilkins et al., 1953; Franklin and Gosling, 1953) and the purine and pyrimidine contents of a variety of DNA molecules (described in Part I). The complementarity of the two DNA chains suggested that, upon strand separation, each serves as a template for the synthesis of a new complementary strand. This provided a plausible model for replication of the chromosome, thus maintaining continuity in the transfer of genetic information from one generation to another. The area of molecular biology encompassed within this volume, that is the biochemical aspects of DNA replication and the processing of this genetic information into protein gene products, has largely stemmed from the funda-

mental discoveries made in these early investigations. The mechanism of DNA replication is the subject of Part I and has recently been extensively reviewed (Klein and Bonhoeffer, 1972; Kornberg, 1974; Schekman et al., 1974).

Another crucial aspect of the Watson–Crick double helix was the lack of restriction on the sequence of bases within the interior of the DNA helix. This suggested a coding mechanism for the expression of hereditary information. The manner in which this code, embodied within the base sequence of the DNA, is transcribed and translated into a specific linear array of amino acids in the protein gene products thus became the central question of molecular biology. These studies, in little more than a decade, led to the complete elucidation of the genetic code. In addition, many components of the transcription and translation apparatus have now been elucidated. However, very little is presently known about the manner in which the cell controls the selective transcription of specific regions of DNA templates into the various ribonucleic acid (RNA) species. (For a recent review on the selectivity of transcription see Chamberlin, 1974.) Part II deals with the transcription process and several of its regulatory aspects.

The overall process in which the specific information contained within the sequence of bases in the RNA messenger species is translated on the ribosome into a linear protein sequence can now be looked upon as a series of partial reactions involving initiation, elongation, and termination of peptide chains. The mechanisms of the individual steps and the details of the role that the ribosome plays in the translation sequence are presently the object of extensive investigations (Nomura et al., 1974). Several aspects of translation, for instance, the structure and function of the ribosome, the mechanism of peptide bond formation, the genetic code, and the role of transfer RNA, form the subject matter of Parts III, IV, and V.

This Benchmark volume has been designed primarily as an introduction for the beginning student of molecular biology. Accordingly, the comments at the beginning of each chapter and their accompanying bibliographies are mainly intended as a guide for the advanced undergraduate and beginning graduate student. These introductory comments are by no means exhaustive reviews of the literature (references to appropriate reviews are provided in each section). Instead, it is hoped that they will aid in bringing the collected papers within each section into a more coherent framework. In addition, they should serve both as an overview and a critical guide for the student. Where appropriate, they also function in updating the selected papers.

In that the papers in this collection represent diverse areas of molecular biology, it is also hoped that they will prove useful to investigators in the field who might appreciate having them compiled in a

single volume. In addition to many of the classical papers of molecular biology, several recent papers of unusual impact have been included. Where essentially the same findings have been reported simultaneously by more than one research group, the task of choosing a single benchmark paper has been difficult. In these cases, largely because of space limitations, the papers not reprinted have been cited in the introductory comments preceding each section. Since this volume forms part of a series of classical papers in microbiology, it has been confined to studies that broadly fit into this overall discipline, and, in general, to those with prokaryotic microorganisms. In many cases, these studies have provided a basis for understanding the various aspects of molecular biology in higher forms.

It is a pleasure to thank Sewell P. Champe, Sam Kaplan, K. Sieglinde Neuhauser, John P. Reeves, and W. W. Umbreit (series editor) for their critical reading of the manuscript and for their many valuable suggestions. I also wish to express my appreciation to M. L. P. Collins, John A. Distasio, and Lawrence C. Parks, who also served in this capacity. I am especially grateful to K. S. Neuhauser for her excellent English translation of Paper 16, which appeared originally in French.

REFERENCES

Avery, O. T., C. M. MacLeod, and M. McCarty (1944). Studies on the chemical nature of the substance inducing transformation of pneumococcal types: I. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. *J. Exptl. Med., 79, 137–158.*

Chamberlin, M. J. (1974). The selectivity of transcription. *Ann. Rev. Biochem., 43,* 721–775.

Franklin, R. E., and R. G. Gosling (1953). Molecular configuration in sodium thymonucleate. *Nature (London), 171,* 740–741.

Klein, A., and F. Bonhoeffer (1972). DNA replication. *Ann. Rev. Biochem., 41,* 301–332.

Kornberg, A. (1974). *DNA Synthesis.* W. H. Freeman, San Francisco.

Nomura, M., A. Tissières, and P. Lengyel (1974). *Ribosomes.* Cold Spring Harbor Monograph Series, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Schekman, R., A. Weiner, and A. Kornberg (1974). Multienzyme systems of DNA replication. *Science, 186,* 987–993.

Watson, J. D., and F. H. C. Crick (1953). The structure of DNA. *Cold Spring Harbor Symp. Quant. Biol., 18,* 123–131.

Wilkins, M. F. H., A. R. Stokes, and H. R. Wilson (1953). Molecular structure of deoxypentose nucleic acids. *Nature (London), 171,* 738–739.