# Methods in Enzymology

## Volume XXIX

# Nucleic Acids and Protein Synthesis

## Part E

#### EDITED BY

### Lawrence Grossman

GRADUATE DEPARTMENT OF BIOCHEMISTRY
BRANDEIS UNIVERSITY
WALTHAM, MASSACHUSETTS

# Kivie Moldave

DEPARTMENT OF BIOLOGICAL CHEMISTRY
CALIFORNIA COLLEGE OF MEDICINE
UNIVERSITY OF CALIFORNIA
IRVINE, CALIFORNIA

1974



ACADEMIC PRESS New York and London

A Subsidiary of Harcourt Brace Jovanovich, Publishers

COPYRIGHT © 1974, BY ACADEMIC PRESS, INC. ALL RIGHTS RESERVED.

NO PART OF THIS PUBLICATION MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM OR BY ANY MEANS, ELECTRONIC OR MECHANICAL, INCLUDING PHOTOCOPY, RECORDING, OR ANY INFORMATION STORAGE AND RETRIEVAL SYSTEM, WITHOUT PERMISSION IN WRITING FROM THE PUBLISHER.

ACADEMIC PRESS, INC. 111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by ACADEMIC PRESS, INC. (LONDON) LTD. 24/28 Oval Road, London NW1

Library of Congress Cataloging in Publication Data Main entry under title:

Nucleic acids, part A-F.

(Methods in enzymology, v. 12, 20, 21, 29, 30)

Parts C, E-F have title: Nucleic acids and protein synthesis, edited by K. Moldave and L. Grossman.

Includes bibliographical references.

Protein biosynthesis. Nucleic acids. I. Grossman, Lawrence, Date ed. Moldave, Kivie, Date ed. IDNLM: l. Nucleic acids-Biosynthesis. 2. Proteins-Biosynthesis: W1 ME9615K v. 30 1974/QU55 N964 1974] QP601.C733 vol. 12, etc. 574.1'925'08s [574.8'732] ISBN 0-12-181892-6 (pt. E) (v. 29) 74-26909

PRINTED IN THE UNITED STATES OF AMERICA

#### Contributors to Volume XXIX

Article numbers are in parentheses following the names of contributors.

Affiliations listed are current.

- Shohei Aoyagi (45), Institute of Natural Color Technology, Faculty of Engineering, Chiba University, Chiba, Japan
- DAVID BALTIMORE (13), Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts ROBERT BAMBARA (21), Section of Bio-

chemistry and Molecular Biology, Cornell University, Ithaca, New York

- P. Bartl. (33), Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey
- A. A. BAYEV (51), Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow, USSR
- GIORGIO BERNARDI (27, 28), Laboratoire de Génétique Moléculaire, Institut de Biologie Moléculaire, Paris, France
- UMBERTO BERTAZZONI (28), Laboratoire de Génétique Moléculaire, Institut de Biologie Moléculair, Paris, France
- OPINDER S. BHANOT (45), Department of Biochemistry, School of Medicine, New York University Medical Center, New York, New York
- J. BIERBAUM (54), Department of Biochemistry, Roche Institute of Molecular Biology, Nutley, New Jersey
- HANS BLOEMENDAL (47), Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands
- F. J. BOLLUM (8), Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky
- KALLOL K. Bose (43), Department of Chemistry, University of Nebraska, Lincoln, Nebraska
- Don J. Brenner (29a), Division of Biochemistry, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.
- ROY J. BRITTEN (29), California Institute of Technology, Kerckhoff Marine Laboratory, Corona del Mar, California;

- and Department of Terrestrial Magnetism, Carnegie Institute of Washington, Washington, D.C.
- RONALD D. BROWN (15b), Laboratory of Cell Biology, CNR, Rome, Italy
- ROBERT W. CHAMBERS (45), Department of Biochemistry, School of Medicine, New York University Medical Center, New York, New York
- Lucy M. S. Chang (8, 9), Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut
- NANDO K. CHATTERJEE (43), Department of Chemistry, University of Nebraska, Lincoln, Nebraska
- W. W. CLELAND (49), Department of Biochemistry, University of Wisconsin, Madison, Wisconsin
- NICHOLAS R. COZZARELLI (4), Department of Biochemistry, The University of Chicago, Chicago, Illinois
- STEPHEN O. CUNNION (57), Department of Biochemistry and Biophysics, University of California School of Medicine, San Francisco, California
- P. Delaney (54), Department of Biochemistry, Roche Institute of Molecular Biology, Nutley, New Jersey
- MURRAY P. DEUTSCHER (46, 58), Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut
- RAVI DHAR (22), Department of Internal Medicine, Yale University, New Haven, Connecticut
- B. P. Doctor (29a, Appendix), Division of Biochemistry, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.
- J. W. Dorson (8), Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky

300

- MARY EDMONDS (30), Department of Biochemistry, Faculty of Arts and Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania
- B. Z. Egan (34, 37), Chemical Technology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee
- STANISLAV D. EHBLICH (27, 28), Laboratoire de Génétique Moléculaire, Institut de Biologie Moléculaire, Paris, France
- ELIZABETH ANN EIGNER (48), 142 Marlboro Street, Boston, Massachusetts
- P. T. ENGLUND (23), Department of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland
- Bradford S. Fansler (7), The Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania
- DIETRICH FISCHER (26), Genetik Molekularbiologie, und Biophysik, Institut für Biologie III, Universität Freiburg, Freiburg, Germany
- Kirk Fry (25), Department of Biology, University of California, Los Angeles, California
- Kenneth B. Gass (4), Department of Biochemistry, The University of Chicago, Chicago, Illinois
- MALCOLM L. GEFTER (3), Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts
- E. P. Geiduschek (18), Department of Biology, University of California, San Diego, La Jolla, California
- DALE E. GRAHAM (29), California Institute of Technology, Kerckhoff Marine Laboratory, Corona del Mar, California
- NABA K. GUPTA (43), Department of Chemistry, University of Nebraska, Lincoln, Nebraska
- LESTER D. G. HAMILTON (5), Graduate
  Department of Biochemistry, Brandeis
  University, Waltham, Massachusetts
- C. W. HANCHER (41), Chemical Technology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee
- Boyd Hardesty (60), Clayton Foundation Biochemical Institute, Depart-

- ment of Chemistry, The University of Texas, Austin, Texas
- D. E. Heatherly (37), Chemical Technology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee
- D. W. Holladay (42), Chemical Technology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee
- J. Hurwitz (15), Department of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York
- Ross B. Inman (32), Biophysics Laboratory and Biochemistry Department, University of Wisconsin, Madison, Wisconsin
- K. R. ISHAM (36), Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee
- G. G. Johnson (18), Department of Biology, University of California, San Diego, La Jolla, California
- D. L. KACIAN (15a), Institute of Cancer Research, College of Physicians and Surgeons, Columbia University, New York, New York
- CHIL-YONG KANG (12), McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin
- T. J. Kelly (24), Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland
- A. D. Kelmers (34, 37, 41, 42), Chemical Technology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee
- SYLVIA J. KERR (59), Department of Surgery, University of Colorado Medical Center, Denver, Colorado
- Hans Kössel (26), Genetik, Molekularbiologie, und Biophysik, Institut für Biologie III, Universität Freiburg, Freiburg, Germany
- DAVID KORN (10), Pathology Department, Stanford University Medical Center, Stanford, California
- THOMAS KORNBERG (3), Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

- ŽELJKO KUĆAN (45), Institute "Rudjer Boskovic," Bijenicka, Zagreb, Yugoslavia
- YEHUDA LAPIDOT (55, 56), Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel
- PAUL LEBOWITZ (22), Department of Internal Medicine, Yale University, New Haven, Connecticut
- C. S. Lee (31), Department of Zoology, The University of Texas, Austin, Texas
- FLORENCE C. LEE (45), Department of Biochemistry, St. Louis University, St. Louis, Missouri
- I. R. LEHMAN (6), Department of Biochemistry, Stanford University School of Medicine, Stanford, California
- J. Leis (15), Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York
- LAWRENCE A. LOEB (7), The Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania
- ROBERT B. LOFTFIELD (48), Department of Biochemistry, University of New Mexico School of Medicine, Albuquerque, New Mexico
- CHARLES A. MAROTTA (22), Departments of Internal Medicine and Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut
- ALAN H. MEHLER (50), Department of Biochemistry, Medical College of Wisconsin, Milwaukee, Wisconsin
- CHRISTIAN F. MIDELFORT (50), Department of Biochemistry, Medical College of Wisconsin, Milwaukee, Wisconsin
- CHRISTINE MILCAREK (16), Department of Microbiology, The Johns Hopkins University, Baltimore, Maryland
- W. L. MILLER (29a), Division of Biochemistry. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.
- A. D. MIRZABEKOV (51), Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow, USSR
- SATOSHI MIZUTANI (12), McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

- JERRY MORRISEY (60), Clayton Foundation Biochemical Institute, Department of Chemistry, The University of Texas, Austin, Texas
- ROBB E. Moses (2, 19), Department of Biochemistry, Baylor College of Medicine, Texas Medical Center, Houston, Texas
- HIROSHI NAKAZATO (30), Department of Biochemistry, Faculty of Arts and Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania
- Berney R. Neufeld (29), California Institute of Technology, Kerckhoff Marine Laboratory, Corona del Mar, California
- James Ofengand (52, 54), Department of Biochemistry, Roche Institute of Molecular Biology, Nutley, New Jersey
- Angela Otto (26), Genetik, Molekularbiologie, und Biophysik, Institut für Biologie III, Universität Freiburg, Freiburg, Germany
- R. Padmanabhan (21), Section of Biochemistry and Molecular Biology, Cornell University, Ithaca, New York
- WADE P. PARKS (14), Viral Carcinogenesis Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland
- R. L. Pearson (41, 42), Chemical Technology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee
- P. PHILIPPSEN (35), Institut für Physikalische Biochemie der Universität München, Munich, Germany
- S. S. PRICE (23), Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland
- SARA RAPPOPORT (55, 56), Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel
- P. H. Roy (24), Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland
- RANAJIT ROYCHOUDHURY (26), Genetik, Molekularbiologie, und Biophysik, In-

- stitut für Biologie III, Universität Freiburg, Freiburg, Germany
- WINSTON SALSER (25), Department of Biology, University of California, Los Angeles, California
- DANIEL V. SANTI (49, 57), Department of Biochemistry and Biophysics, University of California School of Medicine, San Francisco, California
- NEAL SCHERBERG (38), The Franklin McLean Memorial Research Institute, The University of Chicago, Chicago, Illinois
- EDWARD M. SCOLNICK (14), Viral Carcinogenesis Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland
- W. DAVID SEDWICK (10), Department of Pathology, Stanford University Medical Center, Stanford, California
- PETER SETLOW (1), Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut
- LEE SHUGARY (39), Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee
- A. SKALKA (33), Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey
- H. O. SMITH (24), Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland
- MARY ANN SODD (Appendix), Division of Biochemistry, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.
- S. SPIEGELMAN (15a), Institute for Cancer Research, College of Physicians and Surgeons, Columbia University, New York, New York
- WENDELL M. STANLEY, JR. (44), Department of Molecular Biology, School of Biological Sciences, University of California, Irvine, California
- M. P. STULBERG (36, 39), Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

- R. H. SYMONS (11), Department of Biochemistry, University of Adelaide, Adelaide, South Australia
- Howard M. Temin (12), McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin
- JEAN-PAUL THIERY (27), Laboratoire de Génétique Moléculaire, Institut de Biologie Moléculaire, Paris, France
- C. A. THOMAS, JR. (31), Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts
- Giauco P. Tocchini-Valentini (15b), Laboratory of Cell Biology, CNR, Rome, Italy
- C. M. TSIAPALIS (8), Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky
- CLAUDIA VENNEGOOR (47), Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands
- INDER M. VERMA (13), Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts
- BARBARA S. VOLD (40), Department of Microbiology, Scripps Clinic and Research Foundation, La Jolla, California
- JAMES C. WANG (17), Department of Chemistry, University of California, Berkeley, California
- Teresa Shu-Fong Wang (10), Pathology Department, Stanford University Medical Center, Stanford, California
- ROBERT W. WEBSTER, JR. (49), Department of Biochemistry and Biophysics, University of California School of Medicine, San Francisco, California
- P. H. WEIGEL (23), Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland
- Bernard Weiss (16), Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland
- J. F. Weiss (42), Chemical Technology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee
- Samuel B. Weiss (38), The Franklin McLean Memorial Research Institute.

- The University of Chicago, Chicago, Illinois
- SHERMAN M. WEISSMAN (22), Departments of Internal Medicine, Human Genetics, and Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut
- PHILIP WHITCOME (25), Department of Biology, University of California, Los Angeles, California
- REED B. WICKNER (20), Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York

- W. WINTERMEYER (53), Institute for Cell Research, Karolinska Institute, Stockholm, Sweden
- RAY Wu (21), Section of Biochemistry and Molecular Biology, Cornell University, Ithaca, New York
- H. G. ZACHAU (35, 53), Institut für Physikalische Biochemie der Universität München, Munich, Germany
- B. SAYEEDA ZAIN (22), Department of Internal Medicine, Yale University, New Haven, Connecticut

#### **Preface**

The introduction of two additional volumes dealing with nucleic acids and protein synthesis (Volume XXIX, Part E and Volume XXX, Part F) attests to the remarkable progress that continues to be made in these fields of research.

In this volume detailed descriptions for the isolation, purification, and properties of DNA polymerases from a variety of prokaryotic and eukaryotic organisms are given. In addition, a description of those proteins that may participate in the replication process in an ancillary manner is presented. Techniques with which the activities of DNA polymerases may be assessed under conditions in vivo by virtue of the employment of cells with modified permeability properties are also included. The isolation and characterization of those DNA polymerases from animal cells and viruses capable of transcribing RNA are described in detail.

The formidable task of DNA sequencing is currently being approached through the use of a variety of techniques. Repeating DNA sequences analyzed by reassociation kinetics, visualization of reassociated ring formation, and denaturation mapping by electron microscopy are detailed in this volume. Primary sequencing methods of DNA are currently approached through the combined use of restriction enzymes, DNA and RNA polymerase-catalyzed transcripts, and ribonucleotide substitution into DNA. It is anticipated that the availability of these novel methods will facilitate the solution of this arduous task.

One section of this volume deals with the preparation, resolution, and characterization of tRNA's, of some derivatives of tRNA's, and of some enzymes that use tRNA as a substrate.

Volume XXX, Part F deals with the preparation and characterization of initiation, elongation, and termination factors, of ribosomes and ribosomal subunits, of messenger RNA's, and of *in vitro* systems capable of translating information in mRNA into proteins.

We thank the many colleagues who have so generously acknowledged the influence of these volumes and the reliability of the methods. The credit belongs to the numerous authors who have contributed so ably. The methods presented in these two new volumes reflect the most recent advances in the methodology with which problems in molecular biology are currently under investigation; we hope that they will be found equally useful. We also wish to acknowledge the valuable assistance and cooperation of the very capable staff of Academic Press.

LAWRENCE GROSSMAN KIVIE MOLDAVE

## METHODS IN ENZYMOLOGY

#### EDITED BY

# Sidney P. Colowick and Nathan O. Kaplan

VANDERBILT UNIVERSITY SCHOOL OF MEDICINE NASHVILLE, TENNESSEE DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA
AT SAN DIEGO
LA JOLLA, CALIFORNIA

- I. Preparation and Assay of Enzymes
- II. Preparation and Assay of Enzymes
- III. Preparation and Assay of Substrates
- IV. Special Techniques for the Enzymologist
- V. Preparation and Assay of Enzymes
- VI. Preparation and Assay of Enzymes (Continued)
  Preparation and Assay of Substrates
  Special Techniques
- VII. Cumulative Subject Index

## METHODS IN ENZYMOLOGY

#### EDITORS-IN-CHIEF

Sidney P. Colowick Nathan O. Kaplan

Volume VIII. Complex Carbohydrates

Edited by Elizabeth F. Neufeld and Victor Ginsburg

Volume IX. Carbohydrate Metabolism Edited by Willis A. Wood

Volume X. Oxidation and Phosphorylation Edited by Ronald W. Estabrook and Maynard E. Pullman

VOLUME XI. Enzyme Structure Edited by C. H. W. Hirs

Volume XII. Nucleic Acids (Parts A and B)

Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XIII. Citric Acid Cycle Edited by J. M. LOWENSTEIN

VOLUME XIV. Lipids
Edited by J. M. LOWENSTEIN

VOLUME XV. Steroids and Terpenoids Edited by RAYMOND B. CLAYTON

VOLUME XVI. Fast Reactions Edited by Kenneth Kustin

Volume XVII. Metabolism of Amino Acids and Amines (Parts A and B) Edited by Herbert Tabor and Celia White Tabor

VOLUME XVIII. Vitamins and Coenzymes (Parts A, B, and C) Edited by Donald B. McCormick and Lemuel D. Wright

Volume XIX. Proteolytic Enzymes

Edited by Gertrude E. Perlmann and Laszlo Lorand

VOLUME XX. Nucleic Acids and Protein Synthesis (Part C)

Edited by KIVIE MOLDAVE AND LAWRENCE GROSSMAN

Volume XXI. Nucleic Acids (Part D)

Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

VOLUME XXII. Enzyme Purification and Related Techniques Edited by WILLIAM B. JAKOBY

VOLUME XXIII. Photosynthesis (Part A)

Edited by Anthony San Pietro

VOLUME XXIV. Photosynthesis and Nitrogen Fixation (Part B) Edited by Anthony San Pietro

Volume XXV. Enzyme Structure (Part B)

Edited by C. H. W. Hirs and Serge N. Timasheff

VOLUME XXVI. Enzyme Structure (Part C)

Edited by C, H. W. Hirs and Serge N. Timasheff

VOLUME XXVII. Enzyme Structure (Part D)

Edited by C. H. W. HIRS AND SERGE N. TIMASHEFF

Volume XXVIII. Complex Carbohydrates (Part B) Edited by Victor Ginsburg

VOLUME XXIX. Nucleic Acids and Protein Synthesis (Part E)

Edited by LAWRENCE GROSSMAN AND KIVIE MOLDAVE

Volume XXX. Nucleic Acids and Protein Synthesis (Part F)

Edited by Kivie Moldave and Lawrence Grossman

VOLUME XXXI. Biomembranes (Part A)

Edited by S. Fleischer, L. Packer, and R. W. Estabrook

VOLUME XXXII. Biomembranes (Part B)

Edited by S. Fleischer, L. Packer, and R. W. Estabrook

## Table of Contents

| · · · · · · · · · · · · · · · · · · ·   | •   |     |
|---|---|-----|
| CONTRIBUTORS TO VOLUME XXIX   |   |     |
| Preface   |   |     |
| Volumes in Series   |   |     |
|   |   |     |
| Section I. Nucleic Acid Synth   | nesizing Systems  |     |
| A. DNA Directed DNA I   | - ·   |     |
| 1. DNA Polymerase I from Escherichia coli   | Peter Setlow  | 3   |
| 2 The Isolation and Properties of DNA Polymerase II from Escherichia coli   | Robb E. Moses   | 13  |
| 3. Deoxyribonucleic Acid Polymerase III (Escherichia coli K12)  | THOMAS KORNBERG AND<br>MALCOLM L. GEFTER                          | 22  |
| 4. Bacillus subtilis DNA Polymerases  | Kenneth B. Gass and<br>Nicholas R. Cozzarelli                     |     |
| 5. The Purification of the DNA Polymerase from <i>Micrococcus luteus</i>  | LESTER D. G. HAMILTON   | 38  |
| 6. T4 DNA Polymerase  | I. R. LEHMAN  | 46  |
| 7. Sea Urchin Nuclear DNA Polymerase  | Bradford S. Fansler and<br>Lawrence A. Loeb                       | 53  |
| 8. Nucleotide Polymerizing Enzymes from Calf<br>Thymus Gland  | F. J. BOLLUM, LUCY M. S. CHANG, C. M. TSIAPALIS, AND J. W. DORSON | 70  |
| <ol> <li>Purification and Properties of Low Molecular<br/>Weight DNA Polymerase from Mammalian.<br/>Cells</li> </ol>                  | LUCY M. S. CHANG  | 81  |
| 10. The DNA Polymerases of KB Cells   | W. David Sedwick, Teresa  | 01  |
|   | SHU-FONG WANG, AND<br>DAVID KORN                                  | 89  |
| <ol> <li>Synthesis of [α<sup>2</sup>*P]Ribo- and Deoxyribonucle-<br/>oside 5'-Triphosphates</li> </ol>                                | R. H. Symons  | 102 |
| B. RNA Directed DNA P   | olymerases  |     |
| 12. Endogenous RNA-Directed DNA Polymerase<br>Activity in Virions of RNA Tumor Viruses<br>and in a Fraction from Normal Chicken Cells | SATOSHI MIZUTANI,<br>CHIL-YONG KANG, AND<br>HOWARD M. TEMIN       | 119 |

DNA

| 13. Purification of the RNA-Directed DNA Polymerase from Avian Myeloblastosis Virus and Its Assay with Polynucleotide Templates                | INDER M. VERMA AND<br>DAVID BALTIMORE                   | 125 |
|--|---|-----|
| 14. Identification of Viral Reverse-Transcriptase  | EDWARD M. SCOLNICK AND WADE P. PARKS                    | 130 |
| 15. RNA-Dependent DNA Polymerase from Avian Myeloblastosis Viruses   | J. Leis and J. Hurwitz                                  | 143 |
| 15a. Purification and Detection of Reverse Transcriptase in Viruses and Cells  | D. L. KACIAN AND<br>S. SPIEGELMAN                       | 150 |
| 15b. The Preparation of RNA-Directed DNA Polymerase from Ovaries of Xenopus laevis   | RONALD D. BROWN AND<br>GLAUCO P. TOCCHINI-<br>VALENTINI | 173 |
| C. Mass Screening for  | Mutants   |     |
| 16. Mass Screening for Mutants with Altered DNases by Microassay Techniques  | BERNARD WEISS AND<br>CHRISTINE MILCAREK                 | 180 |
| D. Proteins Affecting the Conformations  | of Nucleic Acid Structur                                | е   |
| 17. Protein $\omega$ from Escherichia coli   | JAMES C. WANG   | 197 |
| 18. Purification of TF1—A Template-Specific DNA-Binding Protein and Transcription Inhibitor from Bacteriophage SPO1-Infected Bacillus subtilis | G. G. Johnson and<br>E. P. Geiduschek                   | 204 |
| E. Cells with Altered Permeab  | ility Properties  |     |
| 19. DNA Synthesis in Toluene-Treated Cells of Escherichia coli   | ROBB E. MOSES   | 219 |
| 20. DNA Replication in Plasmolysed Escherichia coli  | REED B. WICKNER   | 224 |
| Section II. Structural Analysis  | of Nucleic Acids  |     |
| A. Primary Sequencing  | Methods   |     |
| 21 Nucleotide Secuence Analysis of Bastarianhage   | DAY WIL D. DADMANADHAN                                  |     |

AND ROBERT BAMBARA

231

| 22.         | Preparation of RNA Transcripts of Discrete<br>Segments of DNA  | CHARLES A. MAROTTA, PAUL<br>LEBOWITZ, RAVI DHAR, B.<br>SAYEEDA ZAIN, AND<br>SHERMAN M. WEISSMAN | 254 |
|-------------|--|---|-----|
| 23.         | The Use of the T4 DNA Polymerase in Identification of 3' Terminal Nucleotide Sequences of Duplex DNA       | P. T. ENGLUND, S. S. PRICE,<br>AND P. H. WEIGEL   | 273 |
| 24.         | Enzymatic Methods for Sequence Analysis Applied to DNA Restriction and Methylation Sites                   | H. O. SMITH, T. J. KELLY,<br>AND P. H. ROY  | 282 |
| <b>25</b> . | The Use of Ribosubstitution Techniques for Determining DNA Sequences                                       | PHILIP WHITCOME, KIRK<br>FRY, AND WINSTON SALSER  | 295 |
| 26.         | 3' End-Group Labeling and Partial Sequence<br>Determination of Oligodeoxynucleotides                       | Hans Kössel, Ranajit<br>Roychoudhury, Dietrich<br>Fischer, and Angela Otto                      | 322 |
| 27.         | A New Approach to the Study of Nucleotide<br>Sequences in DNA: the Analysis of Termini<br>Formed by DNases | Giorgio Bernardi, Stanislav<br>D. Ehrlich, and Jean-Paul<br>Thiery                              | 341 |
| 28.         | Analysis of Labeled 3' Terminal Nucleotides of<br>DNA Fragments  | Umberto Bertazzoni,<br>Stanislav D. Ehrlich,<br>and Giorgio Bernardi                            | 355 |
|             | B. Repeating Primary Seque   | ence Analysis   |     |
| 29.         | Analysis of Repeating DNA Sequences by<br>Reassociation  | ROY J. BRITTEN, DALE E.<br>GRAHAM, AND BERNEY R.<br>NEUFELD                                     | 363 |
| <b>29</b> a | . Isolation of Transfer Ribonucleic Acid Cistrons and 5S Ribonucleic Acid Cistrons from Escherichia coli   | B. P. Doctor, Don J.<br>Brenner, and W. L. Miller   | 419 |
| 30.         | Purification of Messenger RNA and Hetero-<br>geneous Nuclear RNA Containing Poly(A)<br>Sequences           | Hiroshi Nakazato and<br>Mary Edmonds  | 431 |
| 31.         | Formation and Visualization of Rings from Eukaryotic DNA Fragments   | C. S. LEE AND C. A.<br>THOMAS, JR.  | 443 |
| <b>32</b> . | Denaturation Mapping of DNA  | Ross B. Inman   | 451 |
| <b>33</b> . | Identification of Repeating Units in DNA Concatemers   | A. SKALKA AND P. BARTL  | 459 |

|             | Section III. tRNA and Enzyme   | s Acting on tRNA  |     |
|-------------|--|---|-----|
| 34.         | Improved Separation of Oligonucleotides by Reversed-Phase Chromatography   | B. Z. EGAN AND<br>A. D. KELMERS                                     | 469 |
| .35.        | Preparation of Transfer RNA Fragments by<br>Limited Degradation with Spleen Exonu-<br>clease   | P. PHILIPPSEN AND<br>H. G. ZACHAU                                   | 473 |
| 36.         | The Production of Limit-Oligonucleotide Di-<br>gests for Phenylalanine tRNA (Escherichia<br>coli) with Snake Venom Phosphodiesterase               | M. P. STULBERG AND<br>K. R. ISHAM                                   | 477 |
| 37.         | Miniature Reversed-Phase Chromatography<br>Systems for the Rapid Resolution of Trans-<br>fer RNA's and Ribosomal RNA's                             | A. D. KELMERS, D. E.<br>HEATHERLY, AND B. Z. EGAN                   | 483 |
| 38.         | The Qualitative Analysis and Preparation of Phage Transfer RNA   | NEAL SCHERBERG AND<br>SAMUEL B. WEISS                               | 487 |
| 39.         | Isolation, Purification, and Methylation of Undermethylated tRNA <sup>Phe</sup> from an RC <sup>rel</sup> Mutant of Escherichia coli               | LEE SHUGART AND<br>M. P. STULBERG                                   | 492 |
| 40.         | Preparation of tRNA's and Aminoacyl-tRNA<br>Synthetases from <i>Bacillus subtilis</i> Cells at<br>Various Stages of Growth and Spores              | Barbara S. Vold   | 502 |
| 41.         | Preparation of Crude tRNA and Aminoacyl-<br>tRNA Synthetase from Calf Liver  | C. W. HANCHER, R. L.<br>PEARSON, AND A. D.<br>KELMERS               | 510 |
| 42.         | Purification of Calf Liver tRNA's  | R. L. Pearson, J. F. Weiss,<br>D. W. Holladay, and<br>A. D. Kelmers | 514 |
| 43.         | Fractionation of Rabbit Liver Methionyl-tRNA Species   | KALLOL K. BOSE, NANDO K. CHATTERJEE, AND NABA K. GUPTA              | 522 |
| 44.         | Specific Aminoacylation of the Methionine-<br>Specific tRNA's of Eukaryotes  | Wendell M. Stanley, Jr.   | 530 |
| 45.         | Purification of Tyrosine: tRNA Ligase, Valine: tRNA Ligase, Alanine: tRNA Ligase, and Isoleucine: tRNA Ligase from Saccharomyces cerevisiae a8288C | 'Kućan, Shohei Aoyagi,  | 547 |
| 46.         | Aminoacyl-tRNA Synthetase Complex from Rat Liver   | MURRAY P. DEUTSCHER   | 577 |
| <b>4</b> 7. | Particle Character of Aminoacyl-tRNA Syn-<br>thetases Isolated from a Postmicrosomal<br>Fraction   |   | 584 |

| 48. Kinetic Techniques for the Investigation of Amino Acid:tRNA Ligases (AminoacyltRNA Synthetases, Amino Acid Activating Enzymes)   | ELIZABETH ANN EIGNER AND<br>ROBERT B. LOFTFIELD                  | 601 |
|--|--|-----|
| 49. Kinetics of Aminoacyl-tRNA Synthetases Catalyzed ATP-PP Exchange   | DANIEL V. SANTI, ROBERT<br>W. Webster, Jr., and<br>W. W. Cleland | 620 |
| 50. Applications of Kinetic Methods to Amino-<br>acyl-tRNA Synthetases   | CHRISTIAN F. MIDELFORT<br>AND ALAN H. MEHLER                     | 627 |
| <ol> <li>Investigation of Recognition Sites in Valine<br/>tRNA I (Baker's Yeast) by Dissected Mole-<br/>cule Method</li> </ol>       | A. D. MIRZABEKOV AND<br>A. A. BAYEV                              | 643 |
| 52. Assay for AA-tRNA Recognition by the EFTu-GTP Complex of Escherichia coli  | James Ofengand   | 661 |
| 53. Replacement of Odd Bases in tRNA by Fluorescent Dyes   | W. WINTERMEYER AND<br>H. G. ZACHAU                               | 667 |
| 54. Photo-Induced Cross-Linking of 'Srd and Cyd<br>Residues in <i>Escherichia coli</i> tRNA and Its<br>Use as a Conformational Probe | J. Ofengand, P. Delanby,<br>and J. Bierbaum                      | 673 |
| 55. The Chemical Preparation of Acetylamino-<br>acyl-tRNA  | SARA RAPPOPORT AND<br>YEHUDA LAPIDOT                             | 685 |
| 56. The Synthesis of Oligopeptidyl-tRNA  | YEHUDA LAPIDOT AND<br>SARA RAPPOPORT                             | 688 |
| 57. Peptidyl-tRNA Analogs as Affinity Labeling Agents  | Daniel V. Santi and<br>Stephen O. Cunnion                        | 695 |
| 58. Rabbit Liver tRNA Nucleotidyltransferase   | MURRAY P. DEUTSCHER  | 706 |
| 59. tRNA Methyltransferases  | SYLVIA J. KERR   | 716 |
| 60. Assay and Purification of Met-tRNA Hydro-<br>lase from Rabbit Reticulocytes  | JERRY MORRISEY AND<br>BOYD HARDESTY                              | 726 |
| Appendix: Nucleotide Sequences of Transfer<br>Ribonucleic Acids  | MARY ANN SODD AND<br>B. P. DOCTOR                                | 741 |
| Author Index   |  | 757 |
| Subject Index  |  | 775 |