# METHODS IN ENZYMOLOGY

Volume XXI

NUCLEIC ACIDS

Part D

*Methods in Enzymology*

*Volume XXI*

# *Nucleic Acids*

*Part D*

EDITED BY

*Lawrence Grossman*
GRADUATE DEPARTMENT OF BIOCHEMISTRY
BRANDEIS UNIVERSITY
WALTHAM, MASSACHUSETTS

*and*

*Kivie Moldave*
UNIVERSITY OF CALIFORNIA
IRVINE, CALIFORNIA

1971

ACADEMIC PRESS New York and London

COPYRIGHT © 1971, BY ACADEMIC PRESS, INC.
ALL RIGHTS RESERVED
NO PART OF THIS BOOK MAY BE REPRODUCED IN ANY FORM, BY PHOTOSTAT, MICROFILM, RETRIEVAL SYSTEM, OR ANY OTHER MEANS, WITHOUT WRITTEN PERMISSION FROM THE PUBLISHERS.

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

*United Kingdom Edition published by*
ACADEMIC PRESS, INC. (LONDON) LTD.
Berkeley Square House, London W1X 6BA

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 54-9110

PRINTED IN THE UNITED STATES OF AMERICA

# Contributors to Volume XXI

Article numbers are shown in parentheses following the names of contributors. Affiliations listed are current.


BRUCE ALBERTS (11), *Biological Sciences Division, Princeton University, Princeton, New Jersey*

NAOYO ANRAKU (21), *Faculty of Science, Osaka University, Osaka, Japan*

K. BARRETT (43), *Virus Laboratory, University of California, Berkeley, California*

M. BEER (32), *Department of Biophysics, The Johns Hopkins University, Baltimore, Maryland*

CLAIRE M. BERG (35), *Genetics and Cell Biology, University of Connecticut, Storrs, Connecticut*

D. BERG (43), *Department of Neurobiology, Harvard Medical School, Boston, Massachusetts*

GIORGIO BERNARDI (3), *Laboratoire de Genetique Moleculaire, Institut de Biologie Moleculaire, Faculte des Sciences, Paris, France*

K. BEYREUTHER (39), *Institut für Genetik, der Universität zu Köln, Köln, Weyertal, Germany*

SUZANNE BOURGEOIS (41), *The Salk Institute for Biological Studies, San Diego, California*

K. BØVRE (29), *Bakteriologiske Institutt, University of Oslo Rikshospitalet, Oslo, Norway*

RICHARD R. BURGESS (42), *Institut de Biologie Moléculaire, Université de Genéve, Genéve, Switzerland*

LUCIEN CARO (35), *Institut de Biologie Moléculaire, Université de Genéve, Genéve, Switzerland*

W. L. CARRIER (13), *Biology Division Oak Ridge National Laboratory, Oak Ridge, Tennessee*

B. L. A. CARTER (37), *Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisconsin*

M. CHAMBERLIN (43), *Virus Laboratory, University of California, Berkeley, California*

PRISCILLA K. COOPER (12), *Department of Genetics, Stanford Medical Center, Stanford, California*

NORMAN DAVIDSON (31), *Chemistry Department, California Institute of Technology, Pasadena, California*

RONALD W. DAVIS (31), *Department of Biochemistry, Stanford University School of Medicine, Stanford, California*

R. DE WACHTER (7), *Laboratory of Physiological Chemistry, University of Ghent, Ghent, Belgium*

DAVID DUBNAU (33), *Public Health Research Institute of the City of New York, Inc., New York, New York*

J. EISINGER (2), *Bell Telephone Laboratories, Inc., Murray Hill, New Jersey*

W. FIERS (7), *Laboratory of Molecular Biology, University of Ghent, Ghent, Belgium*

DAVID FREIFELDER (6, 34), *Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts*

JOSEPH G. GALL (38), *Department of Biology, Yale University, New Haven, Connecticut*

MARTIN GELLERT (26), *National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland*

W. GILBERT (39), *Department of Physics and The Biological Laboratories, Harvard University, Cambridge, Massachusetts*

P. T. GILHAM (10), *Department of Biological Sciences, Purdue University, Lafayette, Indiana*

LAWRENCE GROSSMAN (15, 16), *Graduate*

*Department of Biochemistry, Brandeis University, Waltham, Massachusetts*

H. O. HALVORSON (37), *Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisconsin*

PHILIP C. HANAWALT (12), *Department of Biological Sciences, Stanford University, Stanford, California*

GLENN HERRICK (11), *Biochemistry Department, Princeton University, Princeton, New Jersey*

NANCY HOPKINS (40), *Harvard University, Biology Laboratories, Cambridge, Massachusetts*

PAUL HOWARD-FLANDERS (14), *Yale University, New Haven, Connecticut*

Z. HRADECNA (30), *Institute of Biophysics, Brno, Czechoslovakia*

THOMAS M. JOVIN (8), *Max-Planck Institut für Physikalische Chemie, Göttingen, Germany*

JOAN C. KAPLAN (16), *Department of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts*

J. S. KRAKOW (9, 44), *Space Science Laboratory, University of California, Berkeley, California*

H. KUBINSKI (30), *Department of Surgery, University of Wisconsin Medical School, Madison, Wisconsin*

SIDNEY R. KUSHNER (15), *Department of Molecular Biology, University of California, Berkeley, California*

A. A. LAMOLA (2), *Bell Telephone Laboratories, Inc., Murray Hill, New Jersey*

JEAN BERNARD LE PECQ (17), *Laboratoire de Physico-Chimie Macromoleculaire, Institut Gustave Roussy, Villejuif, France*

TOMAS LINDAHL (5, 27), *Department of Chemistry II, Karolinska Institutet, Stockholm, Sweden*

MATTHEW MESELSON (18), *Biological Laboratories, Harvard University, Cambridge, Massachusetts*

B. MÜLLER-HILL (39), *Institut für Genetik, der Universität zu Köln, Köln, Weyertal, Germany*

L. D. MURTHY (9), *Space Science Laboratory, University of California, Berkeley, California*

M. OISHI (4, 23), *Department of Genetics, The Public Health Research Institute of the City of New York, Inc., New York, New York*

REIJI OKAZAKI (22), *Institute of Molecular Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya, Japan*

BALDOMERO M. OLIVERA (24), *Department of Biochemistry, College of Medicine, University of the Philippines, Manila, Philippines*

EMIL PALEČEK (1), *Institute of Biophysics, Czechoslovak Academy of Sciences, Brno, Czechoslovakia*

C. PAOLETTI (17), *Laboratoire de Biochimie et Enzymologie, Institut Gustave Roussy, Villejuif, France*

MARY LOU PARDUE (38), *Institute of Animal Genetics, Kings Buildings, Edinburgh, Scotland*

VINCENZO PIRROTTA (40), *Harvard University, Biology Laboratories, Cambridge, Massachusetts*

MARK PTASHNE (40), *Department of Biology, Harvard University, Cambridge, Massachusetts*

CHARLES M. RADDING (19, 36), *Departments of Internal Medicine and Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut*

W. DEAN RUPP (14), *Department of Radiology, Yale University School of Medicine, New Haven, Connecticut*

R. B. SETLOW (13), *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee*

MARTHA SIMON (31), *Department of Biochemistry, State University of New York at Stony Brook, Stony Brook, New York*

A. SKALKA (28), *Roche Institute of Molecular Biology, Nutley, New Jersey*

W. C. SUMMERS (30), *Department of Radiology, Yale University School of Medicine, New Haven, Connecticut*

此为试读,需要完整PDF请访问: www.ertongbook.com

W. SZYBALSKI (29, 30), *McArdle Laboratory, University of Wisconsin, Madison, Wisconsin*

P. TAURO (37), *Department of Microbiology, Haryana Agricultural University, Hissar, Haryana State, India*

JUN-ICHI TOMIZAWA (21), *Faculty of Science, Osaka University, Toyonaka, Osaka, Japan*

ANDREW A. TRAVERS (42), *MRC Laboratory of Molecular Biology, University Postgraduate Medical School, Cambridge, England*

BERNARD WEISS (25), *Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland*

ARTHUR WEISSBACH (20), *Roche Institute of Molecular Biology, Nutley, New Jersey*

ROBERT YUAN (18), *Harvard University, Biological Laboratories, Cambridge, Massachusetts*

# Preface

Since the publication of the previous two volumes of "Methods in Enzymology" dealing with nucleic acids, this field of research has seen continued and rapid development. In order to maintain a comprehensive coverage of pertinent methodology for workers in the biological sciences, this compilation is extended in the form of two additional volumes: "Nucleic Acids and Protein Synthesis," Volume 20, Part C, and "Nucleic Acids," Volume 21, Part D.

Part D deals with the resolution of nucleic acids and their involvement in repair, restriction, recombination, and replication. Portions of this volume deal with the transcription control of DNA by repressors and initiators as well as the methods currently employed for specific localization genes at the molecular and biological levels of enzyme investigation.

We wish to express our appreciation to the numerous authors who contributed so ably and generously and to the very capable staff of Academic Press for their valuable assistance and cooperation.

We dedicate this volume to Drs. Maurice R. Atkinson and Daniel F. Bradley.

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

# 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 (Part A)
*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

*In Preparation:*

VOLUME XVII. Metabolism of Amino Acids and Amines (Part B)
*Edited by* HERBERT TABOR AND CELIA WHITE TABOR

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

VOLUME XXIII. Photosynthesis (Part A)
*Edited by* ANTHONY SAN PIETRO

# Table of Contents





此为试读,需要完整PDF请访问: www.ertongbook.com

## Section VI. Gene Localization Techniques



## Section VII. Factors Affecting Gene Expression

Section I

# Techniques for Structural Analysis

# [1] Nucleic Acid Structure Analysis by Polarographic Techniques

*By* EMIL PALEČEK

## Principles of Polarography

Polarography is an electrochemical method that deals with the relations between the potential of a mercury indicator electrode in an electrolytic cell and the current that flows through it.[1,2] The principle of classical direct-current polarography is shown in Fig. 1. The mercury indicator electrode most frequently used in polarography is the dropping electrode ($P$); this consists of a capillary ($C$), one end of which is connected to the reservoir of mercury ($M$) and the other immersed in the solution being investigated. Because of the hydrostatic pressure of the mercury column, mercury flows through the capillary, forming droplets at the capillary tip. Thus the surface of the polarizable indicator electrode is continually renewed; the measured currents are accurately reproducible and are independent of the previous history of the experiment. As the nonpolarizable reference, electrode ($R$) serves either as a pool of mercury on the bottom of the vessel ($V$) or as a separate electrode (e.g., a calomel one) with a surface many times larger than that of the indicator electrode.

A gradually increasing (or decreasing) voltage is applied to the electrodes from an external source ($G$), and at the same time the current is measured. As long as the potential does not reach a value at which electron transfer between the electrode and a substance in the solution starts, no substantial current appears (the so-called charging or capacity currents observed under these conditions are due to the fact that each drop of mercury falling from the capillary must be charged to a potential corresponding to the applied voltage; these currents do not, as a rule, exceed $10^{-7}$ A). When the potential reaches a value at which some substance in the solution is reduced or oxidized (such a substance is called a depolarizer), a sharp current increase occurs (Fig. 1b). The increase continues until a potential is reached when all the depolarizer in the neighborhood of the electrode is exhausted and the current is limited by the transport rate of the depolarizer. A polarographic step[3] appears, the height ($H$) of which depends on the

[1] L. Meites, "Polarographic Techniques." Wiley (Interscience), New York, 1965.

[2] J. Heyrovský and J. Kůta, "Principles of Polarography." Publ. House Czechoslovak Acad. Sci., Prague, 1965.

[3] The term "step" refers to the curve obtained by means of direct-current polarography and *normal* pulse polarography; *derivative* pulse-polarographic curves are called "waves."

此为试读,需要完整PDF请访问: www.ertongbook.com