PROGRESS IN NUCLEIC ACID RESEARCH AND MOLECULAR BIOLOGY

Edited by

J. N. Davidson

Waldo E. Cohn

VOLUME 3

PROGRESS IN
Nucleic Acid Research and Molecular Biology

edited by

J. N. DAVIDSON
Department of Biochemistry
The University
Glasgow, Scotland

•

WALDO E. COHN
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Volume 3

1964

ACADEMIC PRESS
New York and London

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 W.1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 63-15847

PRINTED IN THE UNITED STATES OF AMERICA

PROGRESS IN

Nucleic Acid Research and Molecular Biology

Volume 3

Contributors to Volume 3

FUJIO EGAMI

I. H. GOLDBERG

K. S. KIRBY

P. MANDEL

FREDERICK C. NEIDHARDT

B. NISMAN

J. PELMONT

DAVID M. PRESCOTT

E. REICH

KENJI TAKAHASHI

TSUNEKO UCHIDA

JOSEPH J. WEISS

List of Contributors

Number in parentheses refers to the page on which the author's contribution begins.

FUJIO EGAMI (59), *Department of Biophysics and Biochemistry, Faculty of Science, The University of Tokyo, Hongo, Tokyo, Japan*

I. H. GOLDBERG (183), *Departments of Medicine and Biochemistry, University of Chicago, Chicago, Illinois*

K. S. KIRBY (1), *Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, England*

P. MANDEL (299), *Institut de Chimie Biologique, Faculté de Médecine, Strasbourg, France*

FREDERICK C. NEIDHARDT (115), *Department of Biological Sciences, Purdue University, Lafayette, Indiana*

B. NISMAN (235), *Laboratoire d'Enzymologie Microbienne, C. N. R. S., Gif-sur-Yvette (Seine-et-Oise), France*

J. PELMONT (235), *Laboratoire d'Enzymologie Microbienne, C. N. R. S., Gif-sur-Yvette (Seine-et-Oise), France*

DAVID M. PRESCOTT (33), *Department of Anatomy, University of Colorado Medical Center, Denver, Colorado*

E. REICH[1] (183), *The Rockefeller Institute, New York, New York*

KENJI TAKAHASHI (59), *Department of Biophysics and Biochemistry, Faculty of Science, The University of Tokyo, Hongo, Tokyo, Japan*

TSUNEKO UCHIDA (59), *Department of Biophysics and Biochemistry, Faculty of Science, The University of Tokyo, Hongo, Tokyo, Japan*

JOSEPH J. WEISS (103), *School of Chemistry, University of Newcastle upon Tyne, England*

[1] Present address: Department of Medicine, Harvard Medical School, and Beth Israel Hospital, Boston, Massachusetts

Preface

This series of publications, of which Volume 3 is now presented, is an attempt to meet the need for a continuing, periodical assessment or reassessment to those areas in the field of nucleic acids and molecular biology that have arisen or advanced significantly since the publication of the last of the three volumes of "The Nucleic Acids," edited by E. Chargaff and J. N. Davidson.

It is not our intention to sponsor an annual or fixed-date publication in which literature appearing in a given period of time is summarized, as in bibliographic reviews or literature surveys. We seek rather to present "essays in circumscribed areas" in which recent developments in particular aspects of the nucleic acid field are discussed by workers provided with an opportunity for more personal expression than is normally met in review articles. To this end we have encouraged discussion, argument and speculation, and the expression of points of view that are individualistic and perhaps controversial. It is to be expected that different authors will interpret this charge in different ways, some essaying a broad and philosophical vein, some developing or describing new theories or techniques, some taking the opportunity to assemble a number of fragmentary observations into a coherent pattern, and some reviewing a field in a more conventional manner. We have not attempted to define or restrict any author's aproach to his chosen subject and have confined our editing to ensuring maximum clarity to the reader, whom we envisage to be a person himself active in or concerned with the general field of nucleic acids and molecular biology. Needless to say, we do not necessarily share all the opinions or concepts of all the authors and accept no responsibility for them. We seek to provide a forum for discussion and debate and we will welcome suggestions from readers as to how this end may best be served.

So many abbreviations used by writers on nucleic acids are now standard that they are not listed at the beginning of each chapter. A note on the system of abbreviations employed throughout the book is included immediately after this Preface.

September, 1964

J.N.D.
W.E.C.

Abbreviations and Symbols

The abbreviations used without definition are those listed in the Instructions to Authors in the *Journal of Biological Chemistry* and in the Tentative Rules of the Commission for the Nomenclature of Biological Chemistry of the International Union of Pure and Applied Chemistry (reprinted in *J. Biol. Chem.* **237**, 1381–1387, 1962). The most commonly used are:

RNA	Ribonucleic acid or ribonucleate
DNA	Deoxyribonucleic acid or deoxyribonucleate
RNase (*not* RNAase)	Ribonuclease
DNase (*not* DNAase)	Deoxyribonuclease
AMP, GMP, IMP, UMP, CMP, rTMP	5′-phosphates of ribosyl adenine, guanine, hypoxanthine, uracil, cytosine, thymine
dAMP, etc.	5′-phosphate of 2′-deoxyribosyl adenine, etc.
2′-AMP, 3′-AMP, (5′-AMP), etc.	2′-, 3′-, (and 5′-, where needed for contrast) phosphate of adenosine, etc.
ADP, etc.	5′-(pyro)diphosphate of adenosine, etc.
ATP, etc.	5′-(pyro)triphosphate of adenosine, etc.
P_i, PP_i	Orthophosphate and pyrophosphate

The following abbreviations are also used:

sRNA; mRNA	Soluble or "transfer" RNA; "messenger" RNA
rRNA; nRNA	Ribosomal RNA; nuclear RNA
poly X, or (X)	3′→5′ polymer of nucleotide X
poly dX, or (dX)	3′→5′ polymer of deoxynucleotide X
(X–Y), or r(X–Y), or (rX–rY)	3′→5′ copolymer of X–Y–X–Y– in alternating *known* sequence
d(X–Y), or (dX–dY)	3′→5′ copolymer of dX–dY–dX–dY– in alternating *known* sequence
(X,Y)	3′→5′ copolymer of X and Y in *random* sequence
(A):(B)	Two chains, generally or completely associated
(A),(B)	Two chains, association unspecified or unknown
(A) + (B)	Two chains, generally or completely unassociated

In naming enzymes, the recommendations of the Commission on Enzymes of the International Union of Biochemistry (1961) are followed as far as possible.

Contents of Volume 1

Contents of Volume 2

Articles Planned for Future Volumes

The Topography of sRNA
J. R. Fresco

Mammalian Polymerases
H. M. Keir

Effects of Some Chemical Mutagens and Carcinogens on Nucleic Acids
P. D. Lawley

The Fractionation and Biological Utilization of DNA Preparations
L. Ledoux

Fluorouracil Derivatives
C. Heidelberger

The Evolution of Base Sequences in Polynucleotides
B. J. McCarthy

Biosynthesis of Ribosomes in Bacterial Cells
S. Osawa

5-Hydroxymethyl Pyrimidines and Their Derivatives
T. L. V. Ulbricht

Biochemical Mechanisms for Recombination
E. Volkin

Amino Acid Esters of RNA, Nucleosides, and Related Compounds
H. G. Zachau and H. Feldmann

In addition, articles by the following authors are forthcoming:

E. Borek
E. Chargaff
S. S. Cohen
E. Katchalski
A. M. Michelson
S. Ochoa
J. R. Tata
P. C. Zamecnik

Contents

Isolation and Fractionation of Nucleic Acids

K. S. Kirby

Cellular Sites of RNA Synthesis

David M. Prescott

Ribonucleases in Taka-Diastase: Properties, Chemical Nature, and Applications

FUJIO EGAMI, KENJI TAKAHASHI, AND TSUNEKO UCHIDA

Chemical Effects of Ionizing Radiations on Nucleic Acids and Related Compounds

JOSEPH J. WEISS

The Regulation of RNA Synthesis in Bacteria

FREDERICK C. NEIDHARDT

Actinomycin and Nucleic Acid Function

E. REICH AND I. H. GOLDBERG

De Novo Protein Synthesis *in Vitro*

B. NISMAN AND J. PELMONT

Free Nucleotides in Animal Tissues

P. MANDEL

Isolation and Fractionation of Nucleic Acids

K. S. KIRBY
Chester Beatty Research Institute, of the Institute of Cancer Research, Royal Cancer Hospital
London, England

I. Introduction

Nucleic acids occur naturally in association with proteins and with lipoprotein organelles and it is the separation from these and any polysaccharide in the cell that constitutes the problem of isolation. Practically the problem is limited by the sensitivity of nucleic acids to rupture by changes in pH, by physical damage, and by enzymes present in

the cell. It is essential therefore to choose reagents that inhibit or, preferably, put out of commission the cellular nucleases and at the same time leave the native structure of the nucleic acids intact. The problem is further complicated by the presence of two kinds of nucleic acid in the cell: DNA and RNA, with the RNA further subdivided into "soluble" or "supernatant" RNA (sRNA), cytoplasmic or ribosomal RNA (rRNA), nuclear RNA (nRNA), and "messenger" RNA (mRNA) species, apart from any nucleic acid that may be present in viruses. In order to understand completely the relationships between the various groups it is necessary to separate them into their molecular species and ultimately to determine the sequence of bases along the polynucleotide chains.

The nature of the intermolecular bonding and associations and the amount of nucleases vary with the organ or organism, so that no general method for the isolation and purification of the nucleic acids is to be expected. However the application of a particular method to a given species is itself a clue to the molecular architecture present in the cells investigated.

II. Isolation of Deoxyribonucleic Acids

A. Isolation from Vertebrate Tissue

DNA constitutes the genetic material of all cells, but the ease with which it is liberated varies with the nature of the tissue. For example, salmon sperm heads are completely dissociated into DNA and protamine by the action of concentrated solutions of sodium chloride while some mammalian sperms are unaffected by this treatment.

The use of detergent for removing proteins and inhibiting enzymes was introduced by Marko and Butler (*1*), and a method using sodium dodecyl sulfate was developed by Kay *et al.* (*2*), while sodium xylene sulfonate was used by Simmons *et al.* (*3*). These methods consist in separating nuclei, rupturing DNA-protein bonds with strong salt, and removing protein with the detergent. Final deproteinization is achieved by several treatments with chloroform and amyl alcohol. This procedure has been described in detail by Zamenhof (*4*). Colter *et al.* (*5*) compared the two methods using Ehrlich ascites tumor cells and decided that sodium xylene sulfonate gave the better product as judged by the viscosity of the DNA. Zahn *et al.* (*6*) have listed carefully the various points at which degradation might occur during the isolation and introduced warming the extract at $(T_m - 10)$°C [usually 62°C, cf. Marmur and Doty (*7*)] for 15 minutes to inactivate nucleases and removing added ribonuclease by treatment with bentonite [cf. Brownhill *et al.* (*8*)].

The DNA prepared from Ehrlich ascites tumor cells by this method was judged free from protein.

Phenol is an alternative to the use of detergent for removing proteins (*9–11*). This reagent inactivates nucleases and the products are low in protein contamination without a large number of chloroform deproteinization steps. However, the yield of DNA and the amount of residual protein depend upon the salt used in association with the phenol. At 0.3 *M* concentration, hydrophilic salts release very little DNA from rat liver while a good yield of DNA is obtained when lipophilic salts are used. Some residual protein remains with the DNA in the latter case but this can be reduced to a minimum by using a salt combining lipophilic with chelating properties; empirically, the best result is obtained with 4-aminosalicylate. However, combinations of salts are sometimes equally good and naphthalene-2-sulfonate with ethylenediaminetetraacetate gives a good yield of DNA from mouse liver with a smaller amount of residual protein than when 4-aminosalicylate is used. Addition of sodium dodecyl sulfate to the mixture has no effect on the amount of residual protein associated with the DNA.

Recently we added 8-hydroxyquinoline to the phenol (*12, 13*) to prevent oxidation and discoloration of phenol, and found that, with phenol and 8-hydroxyquinoline, some salts (e.g., *p*-hydroxybenzoate and cholate), previously reported to be ineffective at 0.15 *M*, do release DNA from rat liver. The difference is probably related to the removal of metal linkages from the nucleoprotein structure. It is possible that the chromosome is covered by a lipoprotein held together, to some extent, by metal linkages and this covering may be disrupted either by lipophilic anions or by chelating agents.

Dithiocarbamate is about as effective as 4-aminosalicylate for the removal of protein from rat liver DNA and this compound (0.3 *M*) in association with phenolphthalein diphosphate (0.015 *M*) (*12*) has been tested with mammalian tissues. This mixture releases DNA very effectively from both rat and mouse livers and is more effective than 4-aminosalicylate in releasing DNA from rat and mouse spleen. The yield of DNA is higher and the protein contamination is less. Previously, rat spleen DNA with about 3 to 4% residual protein and a sedimentation content of about 24 to 26S had been isolated, whereas the DNA isolated with dithiocarbamate and phenolphthalein diphosphate had a sedimentation constant of 18 to 20S.

Phenolphthalein diphosphate (*14*) has proved particularly useful in combination with phenol in that it is possible to separate DNA from RNA without the use of enzymes. When tissues are broken in phenolphthalein diphosphate (pH 6.0, 0.15 *M*) and extracted with phenol, the