Advances in Cell and Molecular Biology

Edited
by
E. J. DuPraw

Volume 1

ACADEMIC PRESS

ADVANCES IN CELL AND MOLECULAR BIOLOGY

Edited by E. J. DuPraw

STANFORD UNIVERSITY
SCHOOL OF MEDICINE
STANFORD, CALIFORNIA

VOLUME 1

ACADEMIC PRESS New York and London 1971

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: 73-163767

PRINTED IN THE UNITED STATES OF AMERICA

This volume is dedicated to Sophia DuPraw
who typed the manuscript and gave birth to the editor,
not necessarily in that order

LIST OF CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

R. D. ALLEN (153), Department of Biological Sciences, State University of New York at Albany, New York

G. F. BAHR (267), Armed Forces Institute of Pathology, Washington, D. C.

A. BAJER (213), Department of Biology, University of Oregon, Eugene, Oregon

JAMES BONNER (1), Division of Biology, California Institute of Technology, Pasadena, California

SARAH C. R. ELGIN (1), Division of Biology, California Institute of Technology, Pasadena, California

STANLEY C. FROEHNER (1), Division of Biology, California Institute of Technology, Pasadena, California

B. A. KIHLMAN (59), Department of Genetics and Plant Breeding, Agricultural College, Uppsala, Sweden

FRITZ LAMPERT (185), Department of Pediatrics, University of Munich, Munich, Germany

S. M. MCGEE-RUSSELL (153), Department of Biological Sciences, State University of New York at Albany, Albany, New York

J. MOLÈ-BAJER (213), Department of Biology, University of Oregon, Eugene, Oregon

JOHN E. SMART (1), Division of Biology, California Institute of Technology, Pasadena, California

J. ROBERTO SOTELO (109), Departamento de Ultraestructura Celular, Instituto de Investigación de Ciencias Biológicas, Montevideo, Uruguay

RODOLFO WETTSTEIN (109), Departamento de Ultraestructura Celular, Instituto de Investigación de Ciencias Biológicas, Montevideo, Uruguay

PREFACE

Advances in Cell and Molecular Biology has been designed as a research-oriented serial, which will provide scholarly yet readable communication among those interested in the cell–molecular sciences. In particular, we hope to provide a meeting ground between biochemically and ultrastructurally oriented researchers. Each article is divided about equally between original information from the author's own laboratory and a readable review of its importance relative to research from other laboratories.

Since this first volume keynotes the series, we are particularly fortunate to have brought together a group of authors, every one of whom represents a laboratory ranked among the best in the world in its particular specialty. The first article, from Professor James Bonner and his colleagues at California Institute of Technology, leads off with an important reinterpretation of histones and other chromosomal proteins in light of the complete amino acid sequences recently published for histones II and IV. Bonner's group has long been a pacesetter for other laboratories in this field, by designing and executing experiments unparalleled in their combination of daring, incisiveness, and impeccable biochemical expertise. The subject itself is widely recognized as a key frontier area, which in due course may reveal the secrets of gene regulation.

Certainly no less exciting is the contribution from Professor Bengt Kihlman, who writes about "Molecular Mechanisms of Chromosome Breakage and Rejoining." Dr. Kihlman is director of the Department of Genetics at the University of Uppsala, Sweden, and for some years his book, *The Actions of Chemicals on Dividing Cells,* has been a core reference in this field. His article for this volume goes far toward introducing coherence and logic to a sprawling subject, which in the past has embraced a whole menagerie of radiation, biochemical, and autoradiographic studies done at the light microscope level with a wide variety of plant, animal, and human species. It is amazing that this is the first time an expert in any field dealing with chromosome anomalies has attempted to relate that science to the ultrastructure of chromosomes, as known from electron microscope studies.

Fully as significant as the preceding is a new article by Professor

Andrew Bajer and his close colleague, Dr. Molè-Bajer, who continue the unique collaboration that has given the world both a vast amount of information about the mitotic spindle, and two beautiful little girls. Those familiar with the history of this field know that the Bajers have continually set higher standards of excellence by their skillful use of time-lapse cinemicrography combined with Nomarski phase contrast instruments, and more recently by the use of the electron microscope to examine individual cells filmed during normal mitosis up to the moment of fixation. In this way they have approached more closely than any other investigators toward the study of *living* cells by electron microscopy.

Turning attention from mitosis to meiosis, we are fortunate in having an important new article about synaptonemal complexes from the laboratory of Dr. J. Roberto Sotelo and his colleague, Rodolfo Wettstein. As director of the Department of Cell Ultrastructure at the Institute for Investigation in the Biological Sciences, Montevideo, Uruguay, Dr. Sotelo has directed research that is famous throughout the world for its painstaking excellence. It is thanks to this laboratory alone that we know: every pair of homologous chromosomes has its own synaptonemal complex (SC); every SC is attached at either end to the nuclear envelope; the trajectories of the SC's vary from nucleus to nucleus; supernumerary SC's occur independently of paired homologs; and the fine structure of the SC's changes characteristically from species to species. In this volume, Sotelo and Wettstein take a new look at the structure and function of synaptonemal complexes at a time when many of their earlier inferences have been dramatically confirmed by the first electron micrographs showing intact SC's prepared as whole mounts.

Still another beautiful contribution comes from the laboratory of Professor R. D. Allen, Chairman of the Department of Biological Sciences at the State University of New York in Albany. Long a student of amoebae, and father of the presently accepted fountain zone model of amoeboid movement, Dr. Allen and his collaborator, Dr. McGee-Russell, report new methods for reversibly fixing the structure of unusual pseudopods called filopodia. This variant of amoeboid movement has been difficult to fit into a general concept of protoplasmic flow; however, the innovations which they now report, dramatically clarify the ultrastructure of filopodia and help to settle a long debate about the importance of microtubules in filopod formation.

The application of fundamental research to clinical medicine is very much a part of the *Advances in Cell and Molecular Biology*. In this volume, we are pleased to have an article by Dr. Fritz Lampert, Chairman of Hematology at the University of Munich's Children's Hospital.

Dr. Lampert is a talented physician, dedicated to eradicating the primary life-threatening disease of children, acute leukemia. His approach has been the use of quantitative electron microscopy to detect alterations in the dry mass and structure of chromosomes from malignant cells. Here he reports impressive progress with this exciting method.

The scientist who originated quantitative electron microscopy is Dr. Gunther Bahr, Chief of Biophysics at the Armed Forces Institute of Pathology in Washington, D.C. Dr. Bahr has also provided an important article in this volume, in which for the first time he presents evidence correlating the DNA content of mitochondria with their dry mass. This is particularly significant because mitochondrial DNA occurs in the form of circular molecules having a very uniform size. The suggestion is that a mitochondrion containing only one such molecule must have a minimum dry mass, corresponding to a "unit mitochondrion."

To all of the authors who contributed to this first volume of the *Advances in Cell and Molecular Biology,* thanks are due for highly successful labor and delivery. Credit for easing their pains goes to the many people within Academic Press who attended the birth of this new serial. As for the *Advances'* paternity, responsibility is sure to fall on the shoulders and other parts of the Editor, who can hardly deny cross-antigenicity with his prior offspring, called just plain *Cell and Molecular Biology.* All of us hope that our *Advances* will find good friends everywhere, and that in its journeys to distant parts of the earth, it will bring insight and new knowledge to everyone who values them.

April, 1971

E. J. DuPraw
Stanford, California

CONTENTS

Reversible Stabilization of Labile Microtubules in the Reticulopodial Network of *Allogromia*

S. M. McGee-Russell and R. D. Allen

Chromosome Alterations in Human Carcinogenesis

Fritz Lampert

Architecture and Function of the Mitotic Spindle

A. Bajer and J. Molè-Bajer

A Unit Mitochondrion: DNA Content and Response to X-Irradiation

G. F. Bahr

THE BIOLOGY AND CHEMISTRY OF CHROMOSOMAL PROTEINS*

Sarah C. R. Elgin,
Stanley C. Froehner,
John E. Smart, and
James Bonner

DIVISION OF BIOLOGY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

* Supported in part by NSF Predoctoral Fellowship and U.S. Public Health Service Grants GM 86 and 13762. We wish to acknowledge the helpful counsel of Drs. John Mayfield and David McConnell.

Recent attempts to dissect the chromosomes of eukaryotes and to study their constituent molecules have led to several achievements which have made "histones respectable again" (*Nature* **223**:892, 1969). However, serious studies on nonhistone chromosomal proteins are at an early stage. We propose here to discuss both types, but to emphasize those recent studies which have clearly increased our understanding of these proteins and their functions. We will discuss only a minority of the one thousand plus papers that have appeared on these subjects in the last ten years. For other reviews of the recent literature the reader is referred to Stellwagen and Cole (1969b) and to Hearst and Botchan (1970).

I. Characterization of Histones

A. Characteristics and Heterogeneity

There are three classes of histones: lysine-rich, slightly lysine-rich, and arginine-rich. Each class consists of two or more protein species, for which several different systems of nomenclature have been devised. The relationships between these systems and the general characteristics of each protein are presented in Table I. Their amino acid compositions are presented in Table II. Unless otherwise noted, discussions in this article will center around the histones of calf thymus.

Diversity of lysine-rich histones has been studied extensively in several tissues and organisms. Kinkade (1969) demonstrated qualitative differences in the lysine-rich histones of calf, cat, rat, and chicken, but he found only quantitative differences among various tissues, including liver, kidney, spleen, thymus, and erythrocytes. The number of components varied: three in calf spleen, four in cat spleen, and five in rat spleen. Of particular interest was the finding that one of the histone I components in rat contains methionine, an amino acid which is rare in histones; this is a clear example of species specificity at the level of primary structure. Bustin and Cole (1969a) separated histone I from rabbit thymus into nine fractions, eight of which seem to be homo-

TABLE I

PRINCIPAL COMPONENTS OF CALF THYMUS HISTONE AND THE TWO COMMONLY USED SYSTEMS FOR THEIR NOMENCLATURE

Class	Subclass; nomenclature of		Lys/Arg ratio	Molecular weight	Moles/100 moles total histone[c]	N-terminal[d]	C-terminal[d]
	Rasmussen *et al.*[a]	Johns and Butler[b]					
Lysine-rich	Ia	f1	22[e]	21,000[e]	6.7	Blocked	Lysine
	Ib	f1	22[e]	21,000[e]	6.6	Blocked	Lysine
Slightly lysine-rich	IIb1	f2a2	~2.5[f]	13,000–15,000[g]	20.0	Blocked	Lysine
	IIb2	f2b	2.5[h]	13,774[h]	24.6	Proline	Lysine
Arginine-rich	III	f3	0.8[f]	13,000–15,000[g]	18.3	Alanine	Alanine
	IV	f2a1	0.7[i]	11,282[i]	23.8	Acetylserine	Glycine

[a] Rasmussen *et al.* (1962).
[b] Johns and Butler (1962).
[c] Data of Panyim and Chalkley (1969a).
[d] Fambrough and Bonner (1969); Phillips and Simson (1969).
[e] Bustin *et al.* (1969).
[f] Fambrough and Bonner (1966).
[g] Values estimated by comparison with electrophoretic and chromatographic characteristics of histone IIb2.
[h] Iwai *et al.* (1969).
[i] Smith *et al.* (1970).

geneous. Although histone I is more susceptible than other histones to degradation, the authors showed conclusively that these fractions were not degradation products because they all possessed the lysyl-lysine C-terminal and blocked N-terminal residues which characterize histone I. Nevertheless, the differences among the proteins were small; they

TABLE II
AMINO ACID COMPOSITIONS[a] OF HISTONES

Amino acid	Histone I[b]	Histone IIb2[c]	Histone III[d]	Histone IV[e]
Lys	61	20	12	11
Arg	3	8	18	14
His	0	3	2	2
Asp	5	6	6	3
Glu	8	10	15	4
Ser	12	13(14)	5	2
Thr	8	8	10	7
Asn	—	—	—	2
Gln	—	—	—	2
Ala	56	13	18	7
Val	9	9	5	9
Ilu	2	6	6	6
Leu	9	6	12	8
Met	0	1(2)	0	1
Phe	1	2	4	2
Tyr	1	4(5)	2	4
Try	0	0	0	0
Pro	22	6	5(6)	1
Gly	15	7	8	17
Cys	0	0	2	0
Total	212	122(125)	130(131)	102

[a] Compositions are in moles of amino acid per mole of protein.
[b] Bustin *et al.* (1969) for rabbit thymus.
[c] Iwai *et al.* (1969) for calf thymus.
[d] Calculated from data of Fambrough and Bonner (1968b) for calf thymus.
[e] DeLange *et al.* (1969a) for calf thymus.

all had essentially the same size, charge, and amino acid composition. Consequently the heterogeneity may have resulted from differential methylation, acetylation, or phosphorylation (see Section IV). Phosphorylation at a single point in a histone can produce a very altered chromatographic profile on CG-50 Amberlite (Marushige *et al.*, 1969). Therefore, determination of amino acid sequences is generally necessary to show conclusive species specificity in histone primary structure.

The characteristics of the histones II (slightly lysine-rich) are less well determined than those of the other histones, due to difficulties in obtaining pure proteins. However, it is known that there are two subclasses, histone IIb1, and IIb2, and their properties are listed in Table I. The amino acid sequence of histone IIb2 has been determined (Iwai *et al.*, 1969) and will be discussed below. It is important to note that histone f2a1 in the nomenclature of Johns is not a slightly lysine-rich histone, but is of the arginine-rich class, and is referred to in this review as histone IV.

The heterogeneity of histone III, an arginine-rich histone, has been the subject of much controversy. Early experiments (Hnilica and Bess, 1965; Mauritzen *et al.*, 1967; Johns, 1968a) suggested that histone III might consist of as many as 12 components, separable by polyacrylamide gel electrophoresis and other analytical methods based on molecular weight. However, views have changed since the discovery that histone III contains cysteine, an amino acid previously thought not to be present in histones (Blazek and Bukaresti, 1964; Phillips, 1965; Ord and Stocken, 1966c; Jellum, 1966; Sadgopal, 1968; Fambrough and Bonner, 1968b). Calf thymus histone III contains two moles of cysteine per mole of protein and possesses the ability to form polymers of various molecular weights by oxidation of the sulfhydryl groups to form intermolecular disulfides (see Fig. 1). It has been shown by Fambrough and Bonner (1968b) and by Phillips (1967) that the supposed heterogeneity of histone III can be eliminated if pure preparations are reduced with mercaptoethanol prior to gel electrophoresis. Although histone III contains two cysteine residues in calf and HeLa cells (Sadgopal, 1968), in plants it generally contains only one and can form only dimers (Smith *et al.*, 1970; see Fig. 1). Polymerization of histone III seems to occur only in relatively purified preparations; under other conditions, incubation of whole calf thymus histone in a denaturant such as urea results in the disappearance of sulfhydryl groups without formation of polymers, suggesting that intramolecular disulfide bonds have formed (Froehner, 1969). The biological significance of the oxidation state of histone III sulfhydryl groups has been considered by Hilton and Stocken (1966) and by Ord and Stocken (1966a, 1968a,b, 1969). Sadgopal and Bonner (1970b) found that in interphase chromatin these sulfhydryls occur mainly in the reduced form, while in metaphase chromosomes they are oxidized, and histone III is either polymerized or complexed with acid-soluble nonhistone proteins. A very sensitive colorimetric assay for estimating histone thiol directly in acid, recently developed from Saville's method (nitrous acid reaction), should be of use in further studies (Todd and Gronow, 1969).

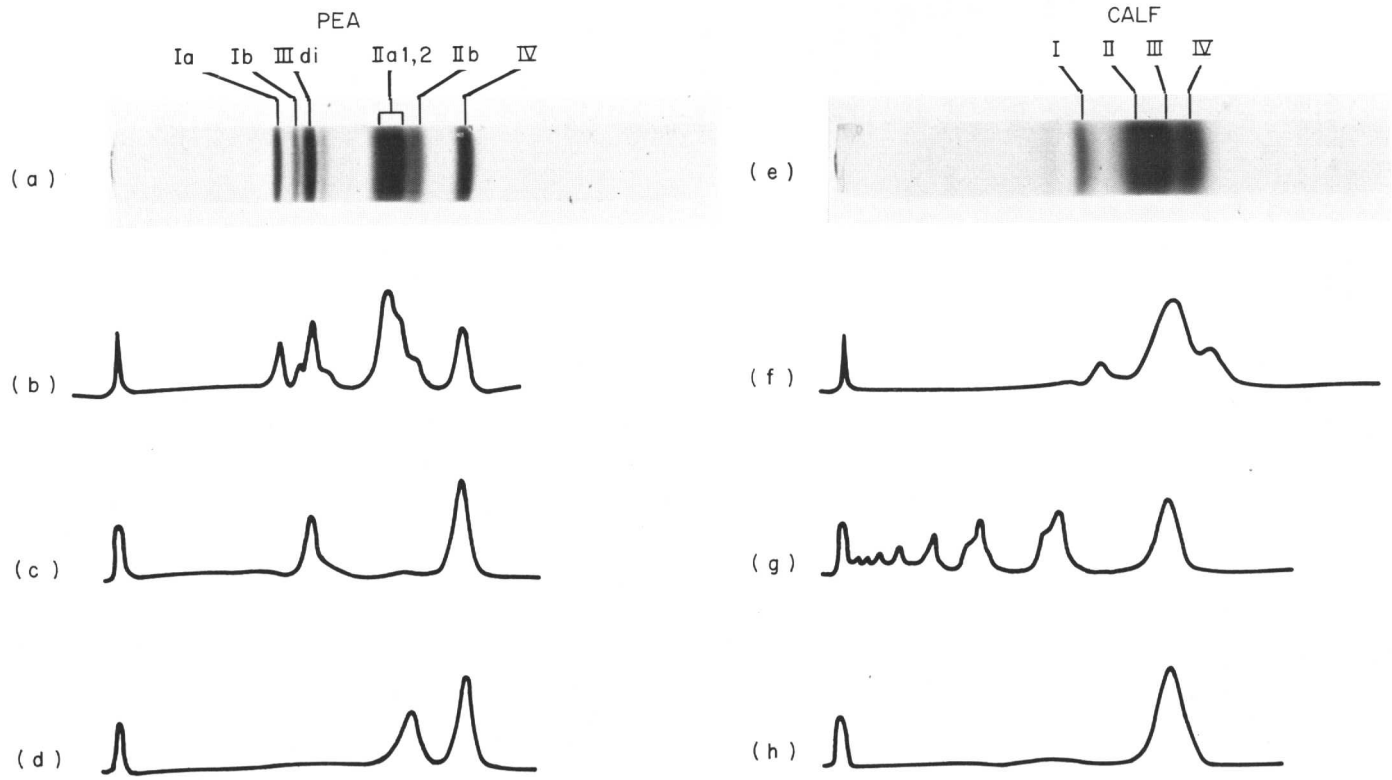

FIG. 1. (a) Polyacrylamide-urea gel of pea whole histone. (b) Densitometer tracing of pea whole histone gel. (c) Pea histone IV + III (dimer). (d) Pea histone IV + III (monomer). (e) Polyacrylamide-urea gel of calf thymus whole histone. (f) Densitometer tracing of calf thymus whole histone gel. (g) Calf thymus histone III (polymers). (h) Calf thymus histone III (monomer).