RECEPTORS AND HORMONE ACTION

I

Edited by Bert W. O'Malley and Lutz Birnbaumer

ACADEMIC PRESS, INC. A Subsidiary of Harcourt Brace Jovanovich, Publishers

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VOLUME I

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Bert W. O'Malley

Lutz Birnbaumer

Department of Cell Biology
Baylor College of Medicine
Houston, Texas

Academic Press New York San Francisco London 1977

A Subsidiary of Harcourt Brace Jovanovich, Publishers

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:

Receptors and hormone action.

1. Hormones. 2. Hormone receptors. I. O'Malley, Bert W. II. Birnbaumer, Lutz.
QP571.R4 591.1'42 77-74060
ISBN 0-12-526301-5 (v. 1)

PRINTED IN THE UNITED STATES OF AMERICA

List of Contributors

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

ROBERT C. ANGERER (1), Division of Biology, California Institute of Technology, Pasadena, California

RONALD E. BARNETT* (427), Department of Chemistry, University of Minnesota, Minneapolis, Minnesota

LUTZ BIRNBAUMER (485), Department of Cell Biology, Baylor College of Medicine, Houston, Texas

HARRIS BUSCH (31), Department of Pharmacology, Baylor College of Medicine, Houston, Texas

JEN-FU CHIU (151), Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee

JAMES H. CLARK (383), Department of Cell Biology, Baylor College of Medicine, Houston, Texas

HIDEO FUJITANI (151), Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee

DAVID L. GARBERS (549), Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee

R. STEWART GILMOUR (331), Beatson Institute for Cancer Research, Wolfson Laboratory for Molecular Pathology, Bearsden, Glasgow

M. W. GOLOMB (195), Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri

KENNETH HARDY (151), Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee

STEPHEN E. HARRIS† (297), Department of Cell Biology, Baylor College of Medicine, Houston, Texas

* Present address: Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

† Present address: National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

LUBOMIR S. HNILICA (151), Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee
LOWELL E. HOKIN (447), Department of Pharmacology, University of Wisconsin Medical Center, Madison, Wisconsin
BARBARA R. HOUGH-EVANS (1), Division of Biology, California Institute of Technology, Pasadena, California
RICHARD L. JACKSON (411), Department of Medicine, Baylor College of Medicine, Houston, Texas
J. A. JAEHNING (195), Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri
ROGER A. JOHNSON (549), Department of Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee
HSUEH JEI LI (103), Division of Cell and Molecular Biology, State University of New York at Buffalo, Buffalo, New York
JOHN J. MONAHAN* (298), Department of Cell Biology, Baylor College of Medicine, Houston, Texas
EVA J. NEER† (463), The Biological Laboratories, Harvard University, Cambridge, Massachusetts
S. Y. NG (195), Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri
BERT W. O'MALLEY (267, 297, 359), Department of Cell Biology, Baylor College of Medicine, Houston, Texas
C. S. PARKER (195), Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri
ERNEST J. PECK, JR. (383), Department of Cell Biology, Baylor College of Medicine, Houston, Texas
R. G. ROEDER (195), Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri
JEFFREY M. ROSEN (237), Department of Cell Biology, Baylor College of Medicine, Houston, Texas
L. B. SCHWARTZ‡ (195), Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri

* Present address: Roche Institute of Molecular Biology, Nutley, New Jersey.
† Present address: Harvard Medical School and Peter Bent Brigham Hospital, Boston, Massachusetts.
‡ Present address: Department of Medicine, Barnes Hospital, St. Louis, Missouri

V. E. F. SKLAR (195), Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri

H. C. TOWLE* (359), Department of Cell Biology, Baylor College of Medicine, Houston, Texas

M. J. TSAI (359), Department of Cell Biology, Baylor College of Medicine, Houston, Texas

R. WEINMANN (195), Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri

SAVIO L. C. WOO (267), Department of Cell Biology, Baylor College of Medicine, Houston, Texas

* Present address: Department of Medicine, University of Minnesota, Minneapolis, Minnesota.

Preface

The field of hormone action is undoubtedly one of the fastest growing areas of biological science. A rough assessment of the rate of growth of this field as determined from an evaluation of journal articles and programs of national meetings leads us to the surprising conclusion that an approximate tenfold expansion of this field has occurred over the last decade. Research in hormone action not only has grown into a dominant effort in endocrinology and reproductive biology, but also has captured a large share of the more general disciplines of biochemistry, cell biology, and molecular biology. This development has occurred because of the dynamic aspects of the field and the increasing interest inherent to the new discipline of regulatory biology.

The creation of a series of volumes summarizing the advances in the field of hormone action has been a major undertaking. Nevertheless, the investment of time required for this project on the part of the contributors and editors appears to be justified since the compilation of a series of volumes on receptors and hormone action should prove useful to those interested in studying the regulatory biology of the eukaryotic cell. The articles contained in these books are oriented toward a description of basic methodologies and model systems used in the exploration of the molecular bases of hormone action and are aimed at a broad spectrum of readers including those who have not yet worked in the field as well as those who have considerable expertise in one or another aspect of hormone action. In the initial three volumes we therefore compiled articles that present not only a rather extensive description of hormone receptors and their properties, but also basic aspects of structure and function of chromatin and membranes, the sites at which hormones and their receptors exert their action. The receptors discussed include soluble cytoplasmic and nuclear receptors for steroid hormones and vitamins, membrane-bound receptors for protein hormones and biogenic amines, and nuclear receptors for thyroid hormones. It seemed appropriate to cover receptor types, in view of the large body of literature accumulated recently dealing with the various functions of these

fascinating but elusive molecules. Thus, while steroid hormone receptors have been isolated and purified, this has not yet been possible for other types of hormone receptors, a fact that clearly highlights a hiatus in our knowledge and demarcates an area for intense future work. We hope that the background and recent advancements presented here will stimulate further experimentation. Future volumes will deal more with the detailed molecular and biochemical processes regulated by these hormones.

Certain omissions have inevitably occurred in the compilation of these initial volumes. Some are due to the fact that certain authors were overcommitted or unable to meet the present deadlines. Other omissions were due to editorial oversight. Nevertheless, we hope that the completion of future volumes will permit this series to stand as a reference of the complete works of the major laboratories working in the field of receptors and hormone action.

Bert W. O'Malley
Lutz Birmbaumer

Contents

List of Contributors xi

Preface xv

1 Sequence Organization of Eukaryotic DNA
ROBERT C. ANGERER and BARBARA R. HOUGH-EVANS

I. Introduction 1
II. Techniques for Determining Interspersion Patterns 6
III. The Short and Long Interspersion Patterns 19
IV. Generality of the Interspersion Patterns 22
V. Conclusions 26
References 28

2 The Eukaryotic Nucleus
HARRIS BUSCH

I. Introduction 32
II. The Nuclear Structures 38
III. Chromatin 46
IV. The Nucleolus 76
References 97

3 Chromatin Structure
HSUEH JEI LI

I. Introduction 104
II. Histone–DNA Interactions 105
III. Histone–Histone Interactions 113
IV. Structure of Histone-Bound Regions in Chromatin 119
V. Structure of Chromatin Subunits 128
VI. Chromatin Structure and Its Relation to Biological Functions 136
References 143

4 Functional Organization of Chromatin

KENNETH HARDY, HIDEO FUJITANI, JEN-FU CHIU, and LUBOMIR S. HNILICA

I.	Introduction	151
II.	Histones as Gene Repressors and Structural Elements	152
III.	Nonhistone Proteins in DNA Transcription	154
IV.	Immunospecificity of Chromosomal Components	157
V.	Chromsomal Proteins with Affinity for DNA	163
VI.	Biological Properties of the Chromosomal Nonhistone Protein Fraction NP	173
VII.	Nonhistone Proteins in Chromatin Fractionation	180
VIII.	Discussion and Conclusions	188
	References	190

5 Animal Nuclear RNA Polymerases

R. G. ROEDER, M. W. GOLOMB, J. A. JAEHNING, S. Y. NG, C. S. PARKER, L. B. SCHWARTZ, V. E. F. SKLAR, and R. WEINMANN

I.	Introduction	196
II.	Isolation and Diversity of Nuclear RNA Polymerases	197
III.	General Properties of Nuclear RNA Polymerases	199
IV.	General Functions of Nuclear RNA Polymerases	202
V.	Molecular Structures of Nuclear RNA Polymerases	207
VI.	Levels of Nuclear RNA Polymerases during Alterations in Gene Activity	214
VII.	Components Which Regulate the Activity or Selectivity of Nuclear RNA Polymerases	220
VIII.	Selective Gene Transcription by Nuclear RNA Polymerases in Reconstructed Systems	221
IX.	Conclusions	232
	References	234

6 Synthesis and Processing of Eukaryotic Messenger RNA

JEFFREY M. ROSEN

I.	Introduction	237
II.	Size and Sequence Organization of Primary Transcripts	240
III.	Posttranscriptional Modifications	248
IV.	Synthesis and Processing of Specific Gene Sequences	257
V.	Conclusions: Future Approaches	261
	References	263

7 Purification and Characterization of Eukaryotic RNA and Unique Sequence Genes

SAVIO L. C. WOO and BERT W. O'MALLEY

I.	Introduction	268
II.	Translation of Messenger RNA *in Vitro*	269
III.	Preparation of RNA from Tissue	274
IV.	Purification of Messenger RNA	276
V.	Characterization of Messenger RNA	282
VI.	Synthesis and Amplification of Structural Genes	287
VII.	Isolation of Intact Genes from Natural Eukaryotic DNA	289
VIII.	*Purification of Eukaryotic Messenger RNA's Present in Low Concentrations*	291
	References	292

8 Analysis of Cellular Messenger RNA Using Complementary DNA Probes

JOHN J. MONAHAN, STEPHEN E. HARRIS, and BERT W. O'MALLEY

I.	Introduction	298
II.	Synthesis of cDNA's	299
III.	Hybridization Experiments with cDNA's	305
IV.	Interpretation of Hybridization Data	309
V.	Uses of cDNA Probes to Isolate Hormone- or Tissue-Specific RNA Sequences	316
VI.	Incorporation of cDNA's into Bacterial Plasmids	317
VII.	Conclusions	318
VIII.	Appendix: A Computer Program for Analysis of Nucleic Acid Hybridization	318
	References	328

9 Gene Expression in the Eukaryotic Cell

R. STEWART GILMOUR

I.	Introduction	331
II.	Evidence for Differential Gene Transcription	332
III.	Possible Mechanisms for Selective Gene Expression	337
IV.	The Role of Nonhistone Proteins in Gene Regulation	342
V.	Problems and Perspectives	349
	References	352

10 Regulation of Gene Expression in the Eukaryotic Cell

B. W. O'MALLEY, M. J. TSAI, and H. C. TOWLE

I.	Introduction	359
II.	Subreactions of Transcription and Kinetics	360

III. Measurement of Chromatin Initiation Sites during Estrogen Mediated Oviduct Differentiation 363
IV. *In Vitro* Transcription of the Ovalbumin Gene 367
V. Fidelity of *In Vitro* Transcription of the Ovalbumin Gene 370
VI. Role of Chromatin Proteins in the Regulation of the Ovalbumin Gene 374
References 379

11 Steroid Hormone Receptors: Basic Principles and Measurement
JAMES H. CLARK and ERNEST J. PECK, JR.

I. Introduction 383
II. Receptor Criteria and Measurement 388
III. Determination of Receptor Parameters: Theory and Practice 392
IV. Receptor States and Measurement by [^{3}H]Steroid Exchange 403
References 409

12 Current Views on the Organization of Lipids and Proteins in Plasma Membranes
RICHARD L. JACKSON

I. Introduction 411
II. Organization of Membrane Lipids 412
III. Organization of Membrane Proteins 416
IV. Membrane Lipid–Protein Association 420
V. Regulation of Membrane Function 421
References 424

13 Fluidity in Membranes
RONALD E. BARNETT

I. Introduction 427
II. Probes of Membrane Structure 430
III. Applications to Natural Membranes 438
References 444

14 Reconstitution of the Coupled Transports of Na^+ and K^+ from Purified Na^+K^+-ATPase
LOWELL E. HOKIN

I. Purification of the Na^+K^+-ATPase 447
II. Properties of Na^+K^+-ATPase 451
III. Reconstitution of Coupled Na^+ and K^+ Transport in Vesicles Containing the Purified NA^+K^+-ATPase from the Rectal Gland of *Squalus acanthias* 452

IV. Role of Phospholipids in the Coupled Transports of Na^+ and K^+ in Vesicles 457
V. Exchange Diffusion of Na^+ 458
VI. Exchange Diffusion of K^+ 459
VII. Other Studies on the Reconstitution of K^+ Transport 459
References 460

15 Solubilization and Characterization of Adenylyl Cyclase: Approaches and Problems
EVA J. NEER

I. Solubilization of Adenylyl Cyclase 463
II. Characterization of Soluble Adenylyl Cyclase 469
III. Conclusions 480
References 482

16 The Actions of Hormones and Nucleotides on Membrane-Bound Adenylyl Cyclases: An Overview
LUTZ BIRNBAUMER

I. Introduction 485
II. General Properties of Adenylyl Cyclases 486
III. Stimulation by Hormones 494
IV. Coupling 502
V. Effects of GMP-P(NH)P 531
VI. Alternate Models for Action of Guanyl Nucleotides 536
VII. Modes of Action of Hormones 538
VIII. Concluding Remarks 542
References 543

17 An Approach to the Study of the Kinetics of Adenylyl Cyclase
ROGER A. JOHNSON and DAVID L. GARBERS

I. Introduction 549
II. Experimental Procedures 551
III. Results 553
IV. Discussion and Conclusions 569
References 571

Index 573

1

Sequence Organization of Eukaryotic DNA

ROBERT C. ANGERER AND
BARBARA R. HOUGH-EVANS

I. Introduction 1
II. Techniques for Determining Interspersion Patterns 6
A. Hydroxyapatite Binding of Reassociated DNA of Increasing Fragment Lengths; Interspersion Curves 6
B. Direct Kinetic Assay for Repetitive and Single Copy Sequences on the Same Fragment 10
C. Demonstration of Interspersion by Optical Hyperchromicity Measurements 13
D. Measurement of Repeat Lengths Using Single-Strand-Specific Nucleases 14
E. Electron Microscope Measurement of Interspersion Pattern Parameters 15
F. Molecular Cloning 17
III. The Short and Long Interspersion Patterns 19
IV. Generality of the Interspersion Patterns 22
V. Conclusions 26
References 28

I. INTRODUCTION

The differential expression of eukaryotic genes is a widely recognized phenomenon. However, the molecular reactions which control differential expression are still unknown. It is likely that specific nucleotide sequence arrangements are involved in the process, just as prokaryotic DNA sequences are involved in the regulation of adjacent structural genes (Gil-

bert and Maxam, 1973). In the laboratories of Eric Davidson and Roy Britten, we are interested in gene control and, therefore, in the arrangement of nucleotide sequences in the DNA of higher organisms. According to a model proposed by Britten and Davidson (1969) and Davidson and Britten (1973), groups of structural genes are transcribed coordinately in response to activators which recognize the particular repetitive DNA sequences adjacent to each protein coding sequence. Other aspects of genome function might also require signals which involve repetitive nucleotide sequences. In order to understand the structural relationships which may mediate transcriptional control, the sequence content and organization of eukaryotic DNA has been studied in detail.

The discovery that eukaryotic DNA's include many nucleotide sequences which are repeated a number of times in the genome was made in the 1960's (Bolton *et al.*, 1966; Waring and Britten, 1966; Britten and Kohne, 1967, 1968; Britten, 1969). The existence of these repeated sequences was demonstrated in DNA reassociation experiments. A fraction of each eukaryotic genome examined was found after melting to reanneal at a rate greater than that predicted from the size of the genome. In these studies DNA was sheared to short fragments, denatured, and allowed to reassociate under conditions which establish a criterion for base pairing fidelity. A standard incubation condition or criterion is 60°C and 0.18 *M* sodium ion. The extent of incubation is quantitated in units of C_0t, the product of initial DNA concentration (nucleotide molarity) and time in seconds. The extent of reassociation, i.e. the fraction of fragments bearing duplex regions, can be measured by passing the samples over hydroxyapatite columns at 60°C. Double-stranded DNA binds, while purely single-stranded fragments pass through the column. The results of such analyses can be plotted as the percent of DNA fragments bound as a function of C_0t. The upper curve in Fig. 1 is a C_0t curve for calf DNA (Britten and Smith, 1970).

The reassociation of any DNA in which each sequence is present in a single copy per haploid genome (as, for example, in *E. coli*) can be described by a second-order rate equation (Wetmur and Davidson, 1968; Britten and Kohne, 1968). For eukaryotic DNA's which contain repeated sequences the overall reassociation curve is described by the sum of several second-order components (Britten *et al.*, 1974). Each component includes sets of sequences which are present in approximately the same number of copies. Within each set the nucleotide sequences are similar enough to form stable duplexes at the criterion of incubation. Such a set of sequences has been termed a "family" of repetitive sequences. The repetition frequency of the families in a component can be determined from the second-order rate constant for that component. The nucleotide sequence complexity, or

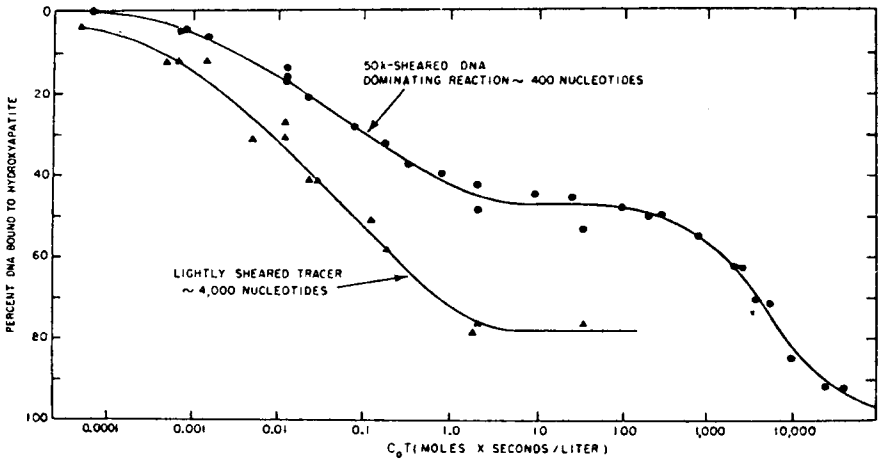

Fig. 1. The demonstration of fine-scale intermixing of repeated and nonrepeated sequences in the calf genome. The upper curve shows the reassociation of calf DNA fragments sheared to about 400 nucleotides. Samples were incubated at 60°C in 0.12 *M* phosphate buffer and assayed by hydroxyapatite under the same conditions. The lower curve shows the reassociation of a small quantity of labeled 4000 nucleotide long fragments with an excess of 400 nucleotide long fragments. For the upper curve, data have been included from a number of other measurements in order to give a more complete picture of calf DNA reassociation kinetics. From Britten and Smith (1970).

number of nucleotides of diverse sequence present in each component, is calculated by multiplying the genome size (in nucleotide pairs) by the fraction of the DNA which belongs to the component, and dividing by the repetition frequency. The complexity of a component is the sum of the complexities of all the families which make up that component. The physical length in the genome of members of different families cannot be determined from this analysis.

Repetitive components of a variety of frequencies have been demonstrated by analysis of the reassociation kinetics of eukaryotic DNA's. In many DNA's a small percentage of fragments binds to hydroxyapatite after incubation to values of C_0t less than those required for interstrand reassociation. Such fragments have been shown to contain foldback or inverted repeat sequences (Wilson and Thomas, 1974). Intrastrand reassociation of these sequences forms duplex regions which bind to hydroxyapatite. The sequences appear to occur within or near both single copy and repetitive sequences (Davidson *et al.*, 1973; Schmid *et al.*, 1975; Deininger and Schmid, 1976) (see Section II). The function of these sequences is unknown at present.

DNA sequences which reassociate very rapidly but not instantaneously are generally satellite DNA components. Satellites typically consist of short

nucleotide sequences that are repeated as many as a million times in tandem (Southern, 1970; Peacock *et al.*, 1973). Because of their clustered arrangement and simple nucleotide sequence composition, they frequently, but not always, have a buoyant density in cesium chloride gradients which is different from that of the rest of the DNA. As far as can be determined, satellite sequences are not transcribed into RNA (Brutlag and Peacock, 1975). They exist in large blocks on chromosomes, in particular at the centromeres (Pardue and Gall, 1970), and comprise part of the constitutive heterochromatin (Brutlag and Peacock, 1975). The function of these simple, very highly repeated sequences may be related to chromosomal events, i.e. mitosis or meiosis, rather than gene expression or its regulation (Goldring *et al.*, 1975). In view of the low nucleotide sequence complexity, and therefore low information content of the satellite sequences, it is doubtful that they could be involved in specific gene control interactions.

Sequences repeated from ten to a few thousand times (the moderately repetitive or middle repetitive families) have been found in most eukaryotic genomes. A fraction of this class of sequences is composed of identified repetitive genes, including those for ribosomal RNA's (Birnstiel *et al.*, 1969) and for histones (Kedes *et al.*, 1975). The repeats of these genes are arranged tandemly in large blocks, and at least in the case of the ribosomal genes they are separated by repetitive spacer sequences. The function of the majority of repetitive sequences is not clear. However, specific sets of repetitive sequences are transcribed in different tissues and at different stages of development (e.g., McCarthy and Hoyer, 1964; Davidson *et al.*, 1968). Repetitive sequences are expressed in heterogeneous nuclear RNA (HnRNA) (Holmes and Bonner, 1974; Smith *et al.*, 1974) and comprise a minor fraction of cytoplasmic messenger RNA. The arrangement in the DNA of these middle repetitive sequences has been determined in the interspersion studies to be described below.

Nonrepeated nucleotide sequences reassociate at a rate inversely proportional to the size of the genome. The complexity of these single copy sequences is always much higher than that of repetitive components, and represents a vast quantity of potential genetic information. For example, the single copy DNA of calf (Fig. 1) has a complexity of 1.9×10^9 nucleotide pairs, enough to code for about a million average-sized proteins. Single copy sequences are the templates from which most messenger RNA's are transcribed. This has been shown in experiments with total polysomal messenger RNA (Goldberg *et al.*, 1973; Klein *et al.*, 1974) and in investigations of the messenger RNA's which code for specific proteins (Suzuki *et al.*, 1972; Bishop and Rosbash, 1973; Harris *et al.*, 1973). We do not know how much of the single copy DNA of an organism actually codes for proteins. Measurements of the sequence complexity of sea urchin messenger