

Developmental Biology

A COMPREHENSIVE SYNTHESIS

Volume 5

The Molecular Biology of Cell Determination and Cell Differentiation

Edited by

LEON W. BROWDER

*University of Calgary
Calgary, Alberta, Canada*

PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

(Revised for vol. 5)

Developmental biology.

Vol. 4 edited by Ralph B. L. Gwatkin.

Includes bibliographies and indexes.

Contents: v. 1. Oogenesis—v. 2. The cellular basis of morphogenesis—[etc.]—v. 5. The molecular biology of cell determination and cell differentiation.

1. Developmental biology—Collected works. I. Browder, Leon W.

QH491.D426 1985

574.3

85-3406

ISBN 0-306-42735-4

Cover illustration: Drosophila embryo that is homozygous for a strong mutation at the Krüppel locus. The embryo has been stained with an antibody to the fushi tarazu (ftz) segmentation protein. The Krüppel mutation causes an altered pattern of ftz stripes. (Micrograph courtesy of Dr. Sean Carroll, University of Wisconsin, Madison.)

© 1988 Plenum Press, New York
A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

Printed in the United States of America



Grandma Kröte is enjoying the balmy summer night at the shores of her beloved pond. Unconcerned about science and scientists, she stubbornly keeps her secrets to herself. (Print by Dr. Johannes F. Holtfreter.)

Contributors

T. Bisseling Department of Molecular Biology, Agricultural University, De Dreijen 11/6703 BC, Wageningen, The Netherlands

Lois A. Chandler Urological Cancer Research Laboratory, Comprehensive Cancer Center and Department of Biochemistry, University of Southern California, Los Angeles, California 90033

Caren Chang Division of Biology, California Institute of Technology, Pasadena, California 91125

Arthur Chovnick Molecular and Cell Biology Department, The University of Connecticut, Storrs, Connecticut 06268

William R. Crain, Jr. Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

E. Bryan Crenshaw III Howard Hughes Medical Institute, Eukaryotic Regulatory Biology Program, School of Medicine, University of California, San Diego, La Jolla, California 92093

Martha L. Crouch Department of Biology, Indiana University, Bloomington, Indiana 47405

F. Lee Dutton, Jr. Molecular and Cell Biology Department, The University of Connecticut, Storrs, Connecticut 06268

Ronald Emeson Howard Hughes Medical Institute, Eukaryotic Regulatory Biology Program, School of Medicine, University of California, San Diego, La Jolla, California 92093

Laurence D. Etkin Department of Molecular Genetics, The University of Texas Cancer Systems Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Jeffrey Guise Howard Hughes Medical Institute, Eukaryotic Regulatory Biology Program, School of Medicine, University of California, San Diego, La Jolla, California 92093

- Johannes F. Holtfreter** Department of Biology, University of Rochester, Rochester, New York 14627
- William R. Jeffery** Center for Developmental Biology, Department of Zoology, University of Texas, Austin, Texas 78712
- Peter A. Jones** Urological Cancer Research Laboratory, Comprehensive Cancer Center and Department of Biochemistry, University of Southern California, Los Angeles, California 90033
- Stuart Leff** Howard Hughes Medical Institute, Eukaryotic Regulatory Biology Program, School of Medicine, University of California, San Diego, La Jolla, California 92093
- Sergio Lira** Howard Hughes Medical Institute, Eukaryotic Regulatory Biology Program, School of Medicine, University of California, San Diego, La Jolla, California 92093
- Elliot M. Meyerowitz** Division of Biology, California Institute of Technology, Pasadena, California 91125
- N. A. Morrison** Department of Biology, Centre for Plant Molecular Biology, Montreal, Quebec H3A 1B1, Canada
- Charles Nelson** Howard Hughes Medical Institute, Eukaryotic Regulatory Biology Program, School of Medicine, University of California, San Diego, La Jolla, California 92093
- Christian Nelson** Howard Hughes Medical Institute, Eukaryotic Regulatory Biology Program, School of Medicine, University of California, San Diego, La Jolla, California 92093
- Michael G. Rosenfeld** Howard Hughes Medical Institute, Eukaryotic Regulatory Biology Program, School of Medicine, University of California, San Diego, La Jolla, California 92093
- Andrew Russo** Howard Hughes Medical Institute, Eukaryotic Regulatory Biology Program, School of Medicine, University of California, San Diego, La Jolla, California 92093
- Matthew P. Scott** Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309-0347
- J. C. Smith** Laboratory of Embryogenesis, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, England
- Jamshed R. Tata** Laboratory of Developmental Biochemistry, National Institute for Medical Research, Mill Hill, London NW7 1AA, England
- D. P. S. Verma** Department of Biology, Centre for Plant Molecular Biology, Montreal, Quebec H3A 1B1, Canada

William B. Wood Department of Molecular, Cellular, and Developmental
Biology, University of Colorado, Boulder, Colorado 80309-0347

W. Michael Wormington Department of Biochemistry, Rosenstiel Basic
Medical Sciences Research Center, Brandeis University, Waltham, Mas-
sachusetts 02254

Preface

This series was established to create comprehensive treatises on specific topics in developmental biology. Such volumes serve a useful role in developmental biology, which is a very diverse field that receives contributions from a wide variety of disciplines. This series is a meeting ground for the various practitioners of this science, facilitating an integration of heterogeneous information on specific topics.

Each volume is comprised of chapters selected to provide the conceptual basis for a comprehensive understanding of its topic as well as an analysis of the key experiments upon which that understanding is based. The specialist in any aspect of developmental biology should understand the experimental background of the specialty and be able to place that body of information in context, in order to ascertain where additional research would be fruitful. The creative process then generates new experiments. This series is intended to be a vital link in that ongoing process of learning and discovery.

The application of the techniques and principles of molecular biology to problems of development has resulted in the most intense period of investigation and discovery in the history of developmental biology. Today, the major thrust of molecular biology is to understand the controls over differential gene expression. The identification of *cis*-acting regulatory sequences and demonstration of their potentiation by *trans*-acting factors have provided us with the framework for understanding how selective transcription of individual genes is mediated. Much remains to be learned, however, about the molecular mechanisms involved in activation and maintenance of transcriptional activity.

Developmental biologists have a broader mission, which is to explain how the complex program of differential gene expression is established and utilized during embryonic development. We recognize two general mechanisms for establishment of cellular domains having unique patterns of gene expression: segregation of cytoplasmic determinants into discrete mitotic derivatives and exposure of cells to extrinsic signals (i.e., embryonic induction). The roles of cytoplasmic determinants are discussed in Chapters 1 and 2. Embryonic induction has been studied most intensively in amphibians, beginning with Spemann's pioneering work. Johannes Holtfreter, Spemann's student, presents

a personal perspective on Spemann's organizer in Chapter 4. Contemporary research on embryonic induction is discussed in Chapter 3.

Study of the expression of specific genes during development promotes the understanding of differential gene expression in a broader context, and the developmental systems highlighted in this book have been chosen with that aim in mind. Actin genes, discussed in Chapter 6, are expressed in lineage-specific patterns during sea urchin development. The coincidence of expression of specific actin genes and the establishment of lineages provide the opportunity to study cell determination at the molecular level with a precision never before possible. *Xenopus* ribosomal protein synthesis, discussed in Chapter 8, is subject to both transcriptional and posttranscriptional regulation during early development. Since selective transcription and delayed utilization of messenger RNA are both important strategies in animal development, this system provides an opportunity to learn how the embryo exploits these two regulatory modes to control protein synthesis.

Regulation of protein synthesis by steroids provides a means for ensuring that physiologically significant proteins will be rapidly produced by specific cells when needed by the organism. This strategy is particularly important for regulation of the reproductive cycle. This mode of regulation also provides the investigator with an important experimental advantage: the ability to modulate gene expression either *in vivo* or *in vitro* by the administration of exogenous hormone. The vitellogenin genes of *Xenopus*, discussed in Chapter 9, provide one of the best-studied examples of genes under steroid regulation.

There are two general strategies for studying gene control of development. One is to begin with the phenomenon and use molecular techniques to isolate the genes that are involved in its control. The cloned genes can then be experimentally modified and the effects of these modifications assessed. The other approach is to begin with a mutant gene that influences a developmental event. By comparison of the wild-type and mutant genes, the genetic basis for the defect and the regulation of the normal process can be determined. The latter approach has been particularly well exploited by investigators of *Drosophila* development. Genes affecting pattern formation of this insect have yielded a massive amount of information, which is masterfully discussed by Scott in Chapter 5. One of the best-studied gene loci in eukaryotes is the *rosy* locus of *Drosophila*, which encodes xanthine dehydrogenase (XDH). Mutant genes that have tissue-specific effects on XDH synthesis have been cloned, and the nucleotide sequences that exert this effect have been pinpointed. This process is discussed in Chapter 10. Further exploitation of this system should yield valuable information regarding the molecular mechanisms involved in tissue-specific gene expression.

In *Xenopus* development, the zygote is initially transcriptionally inert. Transcription is first detected at the mid-blastula stage. As discussed in Chapter 7, the rate of cell division influences the onset of transcription during *Xenopus* development. The relationship between mitosis and cell differentiation has fascinated developmental biologists for many years and is now under

intense investigation. We shall soon learn a great deal about this relationship as it pertains to both normal development and malignant transformation.

One of the most surprising recent findings of molecular biology is that individual gene sequences may contain information that can be utilized to produce different proteins in different cells. The mammalian neuroendocrine system provides a remarkable example of differential processing of messengers to produce tissue-specific products, as is discussed in Chapter 11.

An important characteristic of cell determination is the stability of the determined state. Once determined to differentiate, cells normally retain their specificity, which they can transmit to their progeny at cell division. Any hypothesis of differential gene regulation must account for this fact. One means of achieving stability might involve heritable modifications to the nucleotides themselves. A correlation between hypomethylation of cytosine residues and transcriptional activity exists in higher animals and appears to play a role in determination and stability of differential transcription, as is discussed in Chapter 12.

Plant molecular biology has not yet yielded as much information as animal molecular biology, but it is emerging as an extremely important and active area of investigation, particularly due to the potential economic and social impact of the manipulation of plant gene expression. Mankind relies on plant proteins for food, clothing, and shelter. The ability to manipulate production of plant proteins has the potential to revolutionize the economies of both the developed and underdeveloped nations of the world. Part III of this book presents three of the finest systems for the study of differential gene expression in plants: Chapter 13 describes *Arabidopsis*, which has emerged as an outstanding organism for the conduct of genetic and molecular biological research; Chapter 14 discusses gene expression during seed development in flowering plants; and Chapter 15 deals with the synergistic relationship between plants and bacteria, which is necessary for nitrogen fixation. Not only is nitrogen fixation vital for nutrient enrichment, but it also provides an excellent experimental system for studying induced gene expression.

The publication of a book during a period of rapid progress, in which scientific journals report spectacular discoveries with incredible frequency, is risky. However, the authors have not attempted to present definitive answers to the problems of cell determination and differentiation. Rather, they bring us to the present level of understanding and lead us to the future, which is indeed going to be very exciting. I think they have succeeded admirably. I am proud of their achievements and I am confident that this book will serve both to inform its readers and to stimulate investigators to supplement our rapidly growing body of knowledge.

Leon Browder

Calgary, Alberta

Contents

I. Specification of Regional Patterns of Cell Fate in Animal Development

Chapter 1 • The Role of Cytoplasmic Determinants in Embryonic Development

William R. Jeffery

1. Introduction	3
2. Evidence for the Existence of Cytoplasmic Determinants	8
3. Identity of Cytoplasmic Determinants	18
4. Localization and Segregation of Cytoplasmic Determinants	25
5. Mechanism of Cytoplasmic Determinant Localization	37
6. Mechanism of Cytoplasmic Determinant Action	45
7. Cytoplasmic Determinants Revisited	48
References	50

Chapter 2 • Determination of Pattern and Fate in Early Embryos of *Caenorhabditis elegans*

William B. Wood

1. Introduction	57
2. Overview of <i>Caenorhabditis elegans</i> Embryology	58
3. Description of Cellular Events	59
4. Determination of Cell Fates and Patterns in the Embryo	67
5. Mutations That Affect Embryogenesis	72
6. Possible Mechanisms of Determination	75
References	76

Chapter 3 • Cellular Interactions in Establishment of Regional
Patterns of Cell Fate during Development

J. C. Smith

1. Introduction	79
2. Normal Development of <i>Xenopus laevis</i>	80
3. Fate Maps, Specification, and Determination	87
4. Regional Molecular Markers	91
5. Origins of Polarity in the Fertilized Egg	91
6. Mesoderm Induction	95
7. Dorsalization	102
8. Neural Induction	105
9. Summary of Inductive Interactions in Early Amphibian Development	112
10. Molecular Mechanisms of Induction	112
11. Conclusions	119
References	120

Chapter 4 • A New Look at Spemann's Organizer

Johannes F. Holtfreter

1. Introduction	127
2. The Experiment of Spemann and Hilde Mangold Reconsidered ...	129
3. Misinterpretations That Led to Misconceptions	132
4. Further Reexamination of Spemann's Concept of the Organizer ...	134
5. Questions of Priority	138
6. Other Investigations of the 1920s	139
7. Exogastrulation and Its Consequences	141
8. Head and Trunk-Tail Inductors	144
9. In Quest of the Nature of the Inducing Agents	145
10. Conclusions	149
References	149

Chapter 5 • The Molecular Biology of Pattern Formation in the Early
Embryonic Development of *Drosophila*

Matthew P. Scott

1. Central Problems of Early Development	151
2. Early <i>Drosophila</i> Development	158
3. Determination of Blastoderm Cell Fates	159
4. Molecular Components of the Cytoskeleton of the Early Embryo ..	161
5. Segmentation Genes	162
6. Homeotic Genes	168
7. Complex Patterns of Expression Require Complex Genes	171
8. Protein Products of the Homeotic and Segmentation Genes	177

9. Conclusions	179
References	180

II. Developmental Regulation of Gene Expression in Animals

Chapter 6 • Regulation of Actin Gene Expression during Sea Urchin Development

William R. Crain, Jr.

1. Introduction	189
2. Actin Synthesis during Embryogenesis	190
3. Actin mRNA Levels during Embryonic Development	191
4. The Sea Urchin Actin Genes Comprise a Small Gene Family	195
5. Distinct and Characteristic Patterns of Specific Actin Gene Expression in Embryos and Adult Tissues	198
6. Embryonic Expression of at Least Three Actin Genes Results from Transcriptional Activation	200
7. Cell Lineage-Specific Expression of Actin Genes	202
8. Summary	206
References	206

Chapter 7 • Regulation of the Mid-Blastula Transition in Amphibians

Laurence D. Etkin

1. Introduction	209
2. Development from Oogenesis through the Mid-Blastula Stage	210
3. General Events Associated with the Mid-Blastula Transition	212
4. Mechanisms for Regulation of the Mid-Blastula Transition	221
5. Summary	222
References	223

Chapter 8 • Expression of Ribosomal Protein Genes during *Xenopus* Development

W. Michael Wormington

1. Introduction	227
2. Isolation and Structure of <i>Xenopus</i> Ribosomal Protein Genes	228
3. Ribosomal Protein Gene Expression during Oogenesis	229
4. Ribosomal Protein Gene Expression during Embryogenesis	232
5. Expression of Exogenous Ribosomal Protein Genes and mRNAs in Oocytes	236

6. Summary 237
 ·References 238

Chapter 9 • Regulation of Expression of *Xenopus* Vitellogenin Genes

Jamshed R. Tata

1. Vitellogenesis 242
 2. The Vitellogenin Multigene Family 244
 3. Hormonal Regulation of Expression of *Xenopus* Vitellogenin Genes 247
 4. The Role of Estrogen Receptor in Vitellogenin Gene Transcription 255
 5. Switching on Silent Vitellogenin Genes in Vitro 257
 6. General Conclusions 260
 References 262

Chapter 10 • Developmental Regulation of the *rosy* Locus in *Drosophila melanogaster*

F. Lee Dutton, Jr., and Arthur Chovnick

1. Introduction 267
 2. Phenotypes of *rosy* Mutants 268
 3. The *rosy* Gene Product 269
 4. *rosy* Gene Organization 273
 5. Transcription of *rosy* 277
 6. *rosy* Expression in Standard Strains 283
 7. Regulation of *rosy* Expression 291
 8. Denouement 309
 Appendix I 311
 Appendix II 312
 References 314

Chapter 11 • Transcriptional and Post-Transcriptional Strategies in Neuroendocrine Gene Expression

Michael Rosenfeld, E. Bryan Crenshaw III, Ronald Emeson, Stuart Leff, Jeffrey Guise, Sergio Lira, Christian Nelson, Charles Nelson, and Andrew Russo

1. Regulation of Gene Expression at the Level of Alternative RNA Processing as a Mechanism for Generating Diversity 317
 2. Calcitonin/CGRP Gene Expression: Developmentally Regulated Tissue-Specific Control of Alternative RNA-Processing Events 319
 3. Developmental Regulation of Neuroendocrine Gene Expression Based on Heritable Patterns of Cell-Specific Gene Transcription .. 329
 References 331

Chapter 12 • Hypomethylation of DNA in the Regulation of Gene Expression

Lois A. Chandler and Peter A. Jones

1. Introduction	335
2. Nonrandom Distribution of 5-Methylcytosine in Eukaryotic Genomes	335
3. Clonal Inheritance of Methylation Patterns	336
4. Evidence for Gene Regulation by DNA Methylation	338
5. 5-Azacytidine and Gene Expression	340
6. DNA Methylation and Cellular Differentiation	342
7. DNA Methylation and Cancer	344
8. Summary	345
References	346

III. The Molecular Biology of Plant Growth and Development

Chapter 13 • Molecular Biology of Plant Growth and Development: *Arabidopsis thaliana* as an Experimental System

Elliot M. Meyerowitz and Caren Chang

1. Introduction	353
2. General Description	354
3. Classical Genetics	357
4. The <i>Arabidopsis</i> Genome	360
5. Molecular Genetics	361
6. The Future	363
References	364

Chapter 14 • Regulation of Gene Expression during Seed Development in Flowering Plants

Martha L. Crouch

1. Introduction	367
2. The Significance of the Seed	367
3. Fertilization: The Trigger of Seed Development	369
4. Development of Maternal Tissues	370
5. Endosperm	372
6. Embryo Development	384
7. Concluding Remarks	398
References	398

**Chapter 15 • Development and Differentiation of the Root Nodule:
Involvement of Plant and Bacterial Genes**

N. A. Morrison, T. Bisseling, and D. P. S. Verma

1. Introduction	405
2. Specificity of Infection	406
3. Induction of Host Cell Division	408
4. Intercellular and Intracellular Compartmentalization	412
5. Induction of Host Genes during Nodulation	416
6. Perspectives	421
References	422
Index	427

I

Specification of Regional Patterns of Cell Fate in Animal Development

