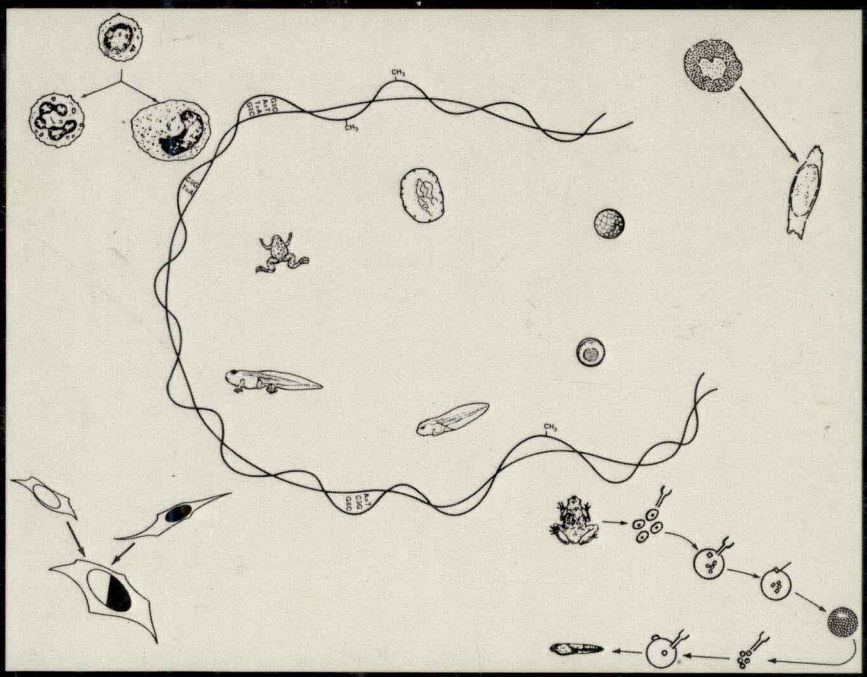
General Editor:
LEON W. BROWDER

Developmental Biology

A COMPREHENSIVE SYNTHESIS



Volume 6

Genomic Adaptability in Somatic Cell Specialization

Edited by MARIE A. DiBERARDINO
and LAURENCE D. ETKIN

Developmental Biology

A COMPREHENSIVE SYNTHESIS

Volume 6

Genomic Adaptability in Somatic Cell Specialization

Edited by
MARIE A. DiBERARDINO
The Medical College of Pennsylvania
Philadelphia, Pennsylvania

and

LAURENCE D. ETKIN
University of Texas M.D. Anderson Cancer Center
Houston, Texas

PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

(Revised for vol. 6)

Developmental biology.

Vol. 3 edited by Malcolm S. Steinberg; v. 4 edited by Ralph B. L. Gwatkin; v. 6 edited by Marie A. DiBerardino and Laurence D. Etkin.
Includes bibliographies and indexes.
Contents: v. 1. Oogenesis – v. 2. The cellular basis of morphogenesis – (etc.]–v. 6. Genomic adaptability in somatic cell specialization.
1. Developmental biology. I. Browder, Leon W.
QH491.D426 1985 574.3 85-3406
ISBN 0-306-43177-7

Cover illustration: Artist's rendition of the genetic control of development juxtaposed with modulations of the cell phenotype.

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

Printed in the United States of America

Contributors

S. C. Barton Department of Molecular Embryology, Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, England

Leon W. Browder University of Calgary, Calgary, Alberta, Canada T2N 1N4

Marie A. DiBerardino Department of Physiology and Biochemistry, The Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129

Christine Dreyer Max Planck Institute for Developmental Biology, Department for Cell Biology, D-7400 Tübingen, Federal Republic of Germany

Laurence D. Etkin Department of Molecular Genetics, University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030

Morgan Harris Department of Zoology, University of California–Berkeley, Berkeley, California 94720

John J. Heikkila University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

S. K. Howlett Department of Molecular Embryology, Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, England

David S. McDevitt Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6045

Robert Gilmore McKinnell Department of Genetics and Cell Biology, University of Minnesota, Saint Paul, Minnesota 55108-1095

Robert W. Nickells University of Calgary, Calgary, Alberta, Canada T2N 1N4

M. L. Norris Department of Molecular Embryology, Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, England

Michael Pollock University of Calgary, Calgary, Alberta, Canada T2N 1N4

David M. Prescott Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309-0347

W. Reik Department of Molecular Embryology, Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, England

M. A. H. Surani Department of Molecular Embryology, Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, England

Robert S. Winning University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Foreword

I am pleased to include this book as Volume 6 of *Developmental Biology: A Comprehensive Synthesis.* It has been edited by two of the foremost investigators in the study of genomic adaptability. I owe a special debt of gratitude to Dr. Marie A. DiBerardino, who developed the concept of the volume. Dr. DiBerardino is also a very active member of the editorial board for this series. Much of the success of this series is due to her valuable advice.

This series was established to create comprehensive treatises on specific topics in developmental biology. Such volumes serve a useful role in developmental biology, since it 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 intended to provide the conceptual basis for a comprehensive understanding of its topic as well as 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 field and be able to place that body of information in context to ascertain where additional research would be fruitful. At that point, the creative process generates new experiments. This series is intended to be a vital link in that process of learning and discovery.

Leon W. Browder

Preface

The intent of this volume is to focus on the genetic mechanisms of somatic cell specialization in animals and the ability to modulate their differentiated cellular phenotypes. We considered it timely to undertake this project because the fundamental question of genetic totipotency of somatic cells has never been answered; in fact, with the application of molecular techniques, more cases of irreversible genetic changes are being revealed. Thus, the extent of irreversible genetic changes concomitant with cell differentiation in animals remains unknown.

The contributors to this volume show that various genetic mechanisms have evolved that control somatic cell specialization (Chapters 1–4). While some cases involve alterations in the genome, most cell specializations appear not to involve genomic changes. This latter hypothesis is examined in various experimental systems (Chapters 5–9) that show that many silent genes can be activated in normal and cancer cells and that, in special cases, even nearly an entire genome can be activated. The collective studies indicate that genomic multipotentiality is widespread among the animal phyla. Future exploitation of the systems described, as well as others, should permit analysis of the regulatory factors involved in activating not only single genes but also those responsible for activating an entire genome. We thank the authors for contributing to our current knowledge of genomic adaptability in somatic cell specialization.

Marie A. DiBerardino
Laurence D. Etkin

Contents

Chapter 1

Introduction
Early Development and Cell Commitment

LAURENCE D. ETKIN

1. Introduction

The genome of most eukaryotic organisms is constant and perpetuates itself through numerous cell generations by replication. Yet, during embryogenesis, there is increasing cellular diversity, yielding a multitude of cell-specific phenotypes. Cells become different from one another, and their developmental potential is progressively restricted. The determined state and the overtly differentiated state are exceedingly stable and heritable during normal development. However, various perturbations, both natural and artificially induced, can result in a modulation of cellular phenotypes. It is the purpose of this volume to analyze the nature of the stability of the differentiated state and the ability to reverse the differentiated phenotype. It is not intended to be all-inclusive and, in fact, we have chosen certain biological systems that require further exploration but that have the potential for helping us to understand the cellular and molecular mechanisms underlying these processes.

2. The Genome during Development

Several lines of evidence suggest that the genome of most cells is not dramatically altered or rearranged during development in most eukaryotic organisms. These include biochemical analyses showing that the genome size remains quantitatively equivalent during development and differentiation, and functional studies, including nuclear transplantation and somatic cell hybrids. Nuclear transplantation studies show that the organism maintains a functionally near-complete genome in somatic cells throughout development, although there may be other restrictions imposed on the ability to activate all these genes in the proper sequence (for a review of this topic, see Chapter 8, *this*

LAURENCE D. ETKIN • Department of Molecular Genetics, University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030.

volume). Somatic cell hybrid studies have also demonstrated that it is possible to activate many genes that are not active in a differentiated cell type, providing further evidence that these genes are maintained and neither eliminated nor permanently altered.

There are, however, exceptions to the maintenance of an unaltered genome during development. Prescott (Chapter 2, *this volume*), describes many of these examples, including amplification of specific DNA sequences, such as chorion genes in insects and ribosomal RNA (rRNA) genes in many organisms. There are also numerous examples of rearrangements in the genome. An extreme example discussed in Chapter 2 is the developmental alteration of the macronuclear genome in ciliates. Other examples of rearrangements include the highly specific recombinational events in immunoglobulin (Ig) genes, which in themselves have varied mechanisms of achieving diversity (reviewed by Maizels, 1987; Okazaki *et al.*, 1987; Hesse *et al.*, 1987). Also, gene-conversion events take place during the switching of yeast mating types (Klar, 1987*a*), as well as trypanosome surface antigenic variation (Van der Ploeg, 1987). Thus, at the gross level in most eukaryotic organisms, the genome appears unchanged, but there are instances of rearrangements and gene-conversion events such as those cited above and in Chapter 2.

The process of gene conversion in the switching of yeast mating types is reversible, since a cell can switch back to the original mating type; thus, these types of changes can occur in the germ line without heritable consequences. Recombinational events and amplification that are part of the normal developmental program are not reversible and are probably limited to somatic cells. One exception is the amplification of rRNA genes in amphibian oocytes. However, the amplified copies of the genes are maintained as extrachromosomal nucleoli and are degraded during early embryogenesis. This would not affect the integrity of the germ-line genome.

In addition to the above-mentioned changes in the genome, methylation of CpG pairs occurs to varying degrees throughout most of the animal kingdom. Hypomethylation of sequences is associated with actively transcribing domains, whereas hypermethylation is associated with inactive domains. It is likely that the gross pattern of methylation is not influencing the expression of individual genes but that methylation of specific sites in and around a gene is important in its regulation (Busslinger *et al.*, 1983; Chandler and Jones, 1988).

3. Regulation of Early Development and Cell Specialization

A particularly attractive strategy related to the generation of cellular diversity is the prelocalization of either messenger RNA (mRNA) or proteins in the oocyte or egg. Classic experimental approaches to study this question involved deletion, ablation, or injury to specific regions or blastomeres of developing embryos and subsequent analysis of developmental defects (reviewed by Davidson, 1986). Recently, however, it has been possible to use modern technology in an attempt to assess the molecular nature of such localizations.

Amphibian oocytes and embryos have been used extensively for both embryological and molecular studies of developmental phenomena and more recently for molecular studies dealing with localization. Melton's group (Weeks *et al.*, 1985) have isolated complementary DNA (cDNA) clones for mRNAs localized in either the vegetal or animal pole regions of *Xenopus* oocytes. One of these mRNAs has been identified through sequence comparison encoding as a mitochondrial ATPase (Weeks and Melton, 1987*b*) and is localized in the animal pole region; another mRNA (vg 1) is localized in the vegetal pole region and codes for a transformation growth factor (TGF_β)-like factor (Weeks and Melton, 1987*a*). The activity of this growth factor-like molecule may synergistically amplify the mesoderm inducing activity of another maternal molecule, an fibroblast growth factor (FGF)-like molecule (Kimelman and Kirschner, 1987). *In situ* hybridization analysis of vg1 mRNA during development shows a localization of the mRNA in cells of the vegetal tier that are involved in mesoderm induction in the early frog embryo. Thus, although there is no formal evidence per se, it is possible that the localized TGF_β-like factor, along with other growth factors acting in synergism, may be involved in regional mesoderm induction.

Along with localized mRNA, there are localized distributions of proteins in eggs and oocytes of amphibians. Most proteins appear to be localized in an animal-to-vegetal hemisphere gradient (Moen and Namenworth, 1977; Dreyer *et al.*, 1982; King and Barklis, 1985). Dreyer *et al.* (1982) produced antibodies against proteins that originated in the germinal vesicle (GV; i.e., nucleus) of *Xenopus* oocytes and examined their fate and distribution during development and differentiation. Dreyer *et al.* detected unique patterns of tissue specific nuclear localization of some of these proteins much later in development (Chapter 3, *this volume*). It is quite possible that some of these proteins may be involved in the differentiation of these specific cell types.

One of the best examples of a localized cytoplasmic determinant is the bicoid gene product in *Drosophila*. Mutations of the bicoid gene result in perturbations in the position and establishment of anterior structures. The product of the bicoid gene is distributed in an exponential concentration gradient with the maximum at the anterior tip of the embryo. Genetic and experimental manipulations which affect the formation or the shape of the gradient result in corresponding changes in the position of anterior structures (Driever and Nusslein-Volhard, 1988).

Conceptually, localization of macromolecules is the most logical solution to the problem of how the embryo generates cell diversity necessary to establish embryonic cell lineages. An important question is the mechanism by which macromolecules become localized in oocytes and eggs. Recently, Kemphues *et al.* (1988) identified four genes (par 1, par 2, par 3, and par 4) in the nematode *C. elegans* that are required for normal cytoplasmic localization. Mutations in the genes result in phenotypes such as defects in localization of germ line-specific P granules, abnormal cleavage patterns, and abnormal timing of cleavage. In addition, there are indications that the cell cytoskeleton may be a fundamental component in the establishment of cytoplasmic localizations. The localization

phenomenon has begun to yield to molecular and genetic approaches but, for the most part, these analyses are still descriptive in nature.

4. Specification and Stability of Cell Lineages in Early Embryogenesis

An important component in a developing system is the perpetuation and transmission of an established cell phenotype or a determined state to daughter cells. Thus, once a cell is committed to a specific developmental pathway, a molecular mechanism must exist for heritable transmission of the phenotype to daughter cells and, in some cases, to permit one daughter cell to initiate a new cell lineage. An interesting model system for studying cell lineages that may be applicable to higher eukaryotic organisms is that of the mating type switch in yeast (Klar, 1987*a*). Single-celled yeast that divide by budding differentiate by producing developmentally nonequivalent daughter cells that have different mating types. There is a specific hierarchy or pattern to the switching in which only mother cells (older cells) produce switched progeny, whereas daughter cells (new buds) always remain the same. The switching or mating-type interconversion has a genetic basis involving a transposition substitution event from a silent a or α-locus (*HML* α or *HMRa*) to a site in the genome where it will be expressed (*MAT* locus). This gene conversion event involves recombination of DNA at the *MAT* locus by a double-stranded DNA break resulting from the action of a site-specific endonuclease encoded by the *HO* locus (Strathern *et al.*, 1982; Kostriken *et al.*, 1983). In essence, the daughter cells transmit information in a stable heritable manner, while the mother cell is capable of switching to a different cell lineage. This difference between mother and daughter cells is attributable to several factors, one of which is that the competence for *HO* expression is inherited by the mother cell, but not the daughter (bud). The property of competence is due to the differential operation of several genes that inhibit *HO* expression. In yeast that reproduces by fission instead of budding, it appears that the property of competence to switch is actually imprinted in the genome at the *MAT* locus during previous generations (Klar, 1987*b*). This suggests that the sister cells do not contain different cytoplasmic or nuclear factors, but that asymmetry is established through inheritance of nonequivalent parental chromosomes. Such an imprinting mechanism probably exists in mammalian cells (see Chapter 4, *this volume*).

Cell transfer experiments in early embryos show that, once established, the fate of a cell is determined and the cell will differentiate in a position-independent manner maintaining its original identity and fate. It is possible that the original determined state was initiated through the action of a localized determinant. In *Drosophila* there is an example in which the perpetuation of a determined state may be accomplished by an autoregulatory mechanism. This involves the autoregulation of a homeotic selector gene called deformed (Dfd) which functions in specifying the identity of the mandibular and maxillary segments (Kuziora and McGinnis, 1988).

The differentiation of the blood cell lineage during development provides

an example demonstrating the effects of various humoral agents, such as growth inducers, on growth and differentiation (reviewed by Sachs, 1987). Numerous proteins have been identified as playing a role in the differentiation of the myeloid cell lineage. These include various colony-stimulating factors (CSF), which are involved either directly or indirectly in the differentiation of macrophages or granulocytes, and interleukin-3 (IL-3), which is involved in the differentiation of macrophages, granulocytes, and other cell types. Several of these genes have been cloned, and it appears that their products represent a cascade or hierarchy of growth-inducing substances that act at specific stages of blood cell differentiation, biasing cells to follow specific cell lineages (Sachs, 1987).

There are also examples of the effects of cell–cell interactions on the establishment of cell lineages in the nematode *Caenorhabditis elegans*. During the second cleavage of *C. elegans* embryos, the AB blastomere divides into two sister blastomeres ABa and ABp that have different fates (Sulston *et al.*, 1983). ABa produces pharyngeal cells, such as muscle, glands, and neurons, while ABp produces γ-aminobutyric acid (GABA)-containing motor neurons in the ventral nerve cord (Sulston *et al.*, 1983). When the ABa cells are cultured alone, they do not produce pharyngeal muscle cells, suggesting that cell–cell interactions are necessary for proper differentiation. On the other hand, the ABp cell is able to produce pharyngeal cells after the positions of ABp and ABa are interchanged (Priess and Thompson, 1987). This demonstrates that cell–cell inductive interactions are important in the specification of cell lineages and that at least at this early developmental stage the fate of the ABp blastomere may be altered by exposing it to the influence of the neighboring cells normally needed to induce the ABa blastomere. It is quite apparent that the establishment of cell lineage is under genetic control involving specific molecular mechanisms, cell–cell interactions, and exposure to various growth inducers. In embryonic cells, it is still possible to modulate the developmental pathways by various experimental manipulations.

5. Changes or Modulations of Cell Phenotype in Determined and Differentiated Cells

The examples discussed so far all involve the establishment of determined or differentiated cell lineages during development. There are also numerous examples in which differentiated and determined cell phenotypes have been modulated, e.g., via transdifferentiation (see Chapter 7, *this volume*). These include such phenomena as lens regeneration involving the repopulation of crystallin producing lens cells from the dorsal iris epithelium, transdetermination of imaginal discs in *Drosophila*, and several other documented cases. These systems have been studied for many years and may only now be approachable at either the molecular or genetic level, or both. This is especially true of transdetermination of imaginal discs in light of the availability of molecular and genetic tools in *Drosophila*.

Cell lineages may be altered or changed during embryogenesis in the sea

urchin. In the sea urchin, the mesoderm forms the primary mesenchyme cells (PMC), which later form the larval skeleton, and the secondary mesenchyme cells (SMC), which form numerous other cell types. Primary mesenchyme cells are derived from the micromeres of the 16-cell embryo, whereas secondary mesenchyme cells are derived from a tier of vegetal cells (veg2) formed at the 64-cell stage (Horstadius, 1939). Ettensohn and McClay (1988) showed that a normal skeleton forms after surgical removal of the PMC and that the skeleton is formed by conversion of SMC into the skeletogenic phenotype. The converted cells not only form the skeleton but also exhibit cell-surface properties typical of PMC. Also, the number of SMC cells converted is directly proportional to the number of PMC that are surgically removed. This suggests that, under normal developmental conditions, the differentiating cell phenotype is extremely stable and heritable but that under the appropriate experimental conditions the SMC can convert to the function of the PMC.

In tissue culture cells, it is well documented that differentiated phenotypes may be lost for many cell generations but are reexpressed under the proper culture conditions (see Chapter 5, *this volume*). The molecular basis for this phenomenon is not known, but it probably involves heritable states of either DNA modification or chromatin conformation, or both.

The ability to modulate the differentiated phenotype is evident from studies using somatic cell hybrids and heterokaryons. When a hybrid is produced between a differentiated and undifferentiated partner (e.g., a hepatocyte cell producing liver-specific products and a fibroblast cell), the differentiated functions may be repressed. When this occurs, it has been termed extinction. In fact, extinction of the liver-specific product tyrosine aminotransferase has been correlated with the retention of a specific chromosome in the undifferentiated partner (Killary and Fournier, 1984).

Studies in heterokaryons, in which the nuclei remain independent in the same cytoplasm of fused cells, indicate that it is possible to activate muscle-specific functions of the nonmuscle partner in some heterokaryon fusions. This suggests that in addition to the negatively acting factors responsible for the extinction phenomenon, there are positively acting components to the system (Blau *et al.*, 1985). This also demonstrates the ability to modulate the phenotype of various cell types under the proper experimental conditions.

Modulation of the differentiated or determined cell phenotype is evident in the synthesis of heat-shock proteins under conditions of stress (see Chapter 6, *this volume*, for a detailed discussion of this topic). Browder *et al.* discuss how, under heat-shock stress, the heat-shock genes, which are normally repressed at the ambient temperature, are activated at either the transcriptional or translational level, or both. Under these conditions, a small number of heat-shock genes contribute to the primary expression pattern of the cell. Thus, the cellular phenotype is transiently modulated by an external factor.

Cancer cells usually are less differentiated than their normal counterparts and, under appropriate experimental conditions, may be induced to differentiate along different pathways. McKinnell (Chapter 9, *this volume*) discusses a number of these examples such as plant tumors, amphibian neoplasms, and

various germ layer tumors of mammals and the different inducers that elicit these differentiations.

Perhaps the most rigorous test of the ability to modulate a cellular phenotype is that of nuclear transplantation. The studies described in Chapter 8 (*this volume*) show that the genome of a terminally differentiated cell type such as an amphibian erythrocyte may be reactivated under the appropriate experimental conditions to support development through embryogenesis and early larval development.

6. Molecular Mechanisms of Gene Regulation and Heritability of the Determined or Differentiated State

Further elucidation of developmental principles will involve an understanding of the molecular basis by which genes are regulated both temporally and spatially in embryogenesis. The past 10 years have yielded a plethora of information regarding the molecular machinery involved in transcription. This includes identification of numerous *cis*-acting DNA elements that modulate the rate and specify the start and stop sites of transcription, and numerous *trans*-acting factors that interact with many of the *cis*-acting elements. The greatest challenge, however, is to begin to identify the factors and elements involved in regulating genes during development and during cellular differentiation and to understand the molecular basis by which the determined or differentiated state is inherited between cell generations (for review of transcription, see Maniatis *et al.*, 1987).

In *Drosophila*, the gene coding for alcohol dehydrogenase (ADH) has been cloned, and the role of both *cis*- and *trans*-acting elements in the temporal and spatial regulation have been partially characterized (Benyajati *et al.*, 1983; Posakony *et al.*, 1986; Heberlein and Tjian, 1988). The ADH gene is regulated by a two-promoter system. The proximal promoter is used during early embryogenesis with transcription increasing during the first and second larval instars and then decreasing; the distal promoter is expressed at low levels in mid-staged embryos and third-instar larvae. In adults, most of the transcription is directed by the distal promoter and is restricted to just a few tissues such as malphigian tubules and the fat body. The distal and proximal promoters are separated by a 700-base-pair (bp) region containing a large intron that is spliced from the mature transcript (Benyajati *et al.*, 1983). The *cis*-acting sequences have been identified by mutating the DNA and introducing the modified sequences into the germ line using p-element-mediated transformation (Posakony *et al.*, 1986). This analysis demonstrated that the control elements of the distal promoter are contained within a region 660 bp upstream of the distal start site, whereas proximal promoter function is regulated by sequences 400 bp upstream of the proximal start site. However, it appears that wild-type transcriptional levels by the proximal promoter are affected by sequences as far away as 2 kilobase (kb).

Using *in vitro* transcription extracts produced from different-staged *Drosophila* embryos, Heberlein and Tjian (1988) showed that the function of