GENE ACTIVITY IN EARLY DEVELOPMENT

SECOND EDITION

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Preface

Knowledge of the molecular biology of early development derives from a tangled skein of measurements carried out on a number of diverse organisms. My ultimate objective in writing this second edition of "Gene Activity in Early Development" has been to review critically the many observations which are now available in order that a coherent view of at least some areas of this field might emerge. This is a nearly impossible endeavor, and at best can be only partially successful. In order to achieve a comprehensive picture, it has been necessary in many places to rely on my interpretations where direct knowledge is lacking and to choose between incompatible data. I have not shied away from this, for though I believe the book provides a documented review of certain areas of the literature, it is basically a work which is organized according to my own views of this subject. Many have of course changed since the first edition was written in 1967.

A major aim in this edition has been to develop the outlines of a quantitative treatment of some of the key classes of macromolecules in early embryos and oocytes. Thus I have devoted considerable space to estimates of RNA and protein synthesis rates, complexities, and amounts. Such information must underlie a molecular level resolution of the basic process with which development begins.

My hope is that this book will be useful to the friends, colleagues, and advanced students with whom I have spent so much time arguing the various subjects considered, and to others like them.

It is important and pleasurable for me to acknowledge the essential contributions of several of my colleagues and associates. The manuscript

in its various drafts was reviewed critically and perceptively by Dr. Barbara R. Hough-Evans, Dr. William H. Klein, and Dr. Glenn A. Galau of our research group at Caltech, and I am particularly grateful for their detailed assistance. My partner, Dr. Roy J. Britten, encouraged me to carry out this project and suggested many important improvements. Professor Fotis Kafatos of Harvard University and Professor L. Dennis Smith of Purdue University each reviewed a major portion of the book, and Professor Gary Freeman of the University of Texas reviewed Chapter 7. I owe to these excellent scientists a large number of essential corrections, additions, and suggestions. I wish to extend my gratitude and thanks to these people and to the members of my research group who frequently assisted me in this project, and from whom my time and attention were often diverted. I would also like to thank Ms. Brooke Moyer who assisted with the cover design. This book is dedicated to Jane Rigg who transformed my imperfect drafts into a book and who so often remembered what I forgot.

ERIC H. DAVIDSON

Contents

2 The Onset of Genome Control in Embryogenesis

3 First Indices of Differential Embryo Cell Function

4 Quantitative Aspects of Protein Synthesis in Early Embryos: The Role of Maternal Components

1

Introduction: The Variable Gene Activity Theory of Cell Differentiation

The basic arguments leading to the proposal of transcription level regulation in animal cells are reviewed, and their history is briefly outlined. Nineteenth century cell biologists considered the possibility that differentiation can be accounted for by qualitative division of the genome during development. This view was rejected on the basis of classical experiments suggesting that the potentialities of embryonic cells are equivalent. A large amount of later evidence demonstrates genomic equivalence in differentiated cells within the same organism. The main forms of evidence include cases in which given cells or cell lineages are shown to carry out diverse functions successively ("transdifferentiation"), the observation that differentiated cells usually contain equal quantities of DNA and the same complements of DNA sequence, and proof that differentiated cell nuclei may contain all the genetic information necessary to program the development of a whole organism. Nor in general do differentiated cells which intensively express given genes contain extra copies of these genes. Current experiments show that only minor fractions of the genome are represented in the RNA of differentiated cells and that when various differentiated cells are compared, the transcribed regions constitute distinct, though overlapping, sets of DNA sequences. In addition, transcriptionally inactive DNA exists in all differentiated cells. Direct evidence for variable gene activity, i.e., transcriptional control, comes from mea-

surements of specific messenger RNA accumulation. These show in general that when given messenger RNA's are present in the cytoplasmic polysomes, the structural genes from which they are derived are transcribed, while at other times or in other cell types, these genes are transcribed less often. The initial level of control is at the transcriptional, rather than post-transcriptional level. Thus, at least in some examples so far studied, structural gene sequences can be transcribed in chromatin only from cells in which the gene is being expressed, and sequences not represented in polysomal RNA are also undetectable in nuclear RNA. However, many levels of control are possible, and probably all are utilized to some extent. The molecular basis of transcription level regulation in animal cells is not understood, but its mechanism seems likely to depend on the way(s) in which DNA sequences are organized in the genome. Recent discoveries, showing that there exists an ordered pattern of interspersion of repetitive and nonrepetitive sequence in animal DNA, are briefly reviewed. At least some of the interspersed repetitive sequences probably play a role in structural gene function. The evidence for this is that structural genes are located in the immediate vicinity of interspersed repetitive sequences and that special subsets of repetitive sequences are contiguous to those structural genes expressed in a given state of differentiation. The view taken in this book is that transcription level regulation is the fundamental process underlying differentiation and development.

Two premises are required in arriving at the proposition that differentiation is a function of variable gene activity. The first of these is the well-understood relationship between the nucleotide sequence of the DNA in the genome and the amino acid sequence of the various proteins found in the cell. Since the structural and functional characteristics of the cell depend on its proteins, the cell requires the expression of genetic information specifying its proteins in order for these characteristics to materialize. Therefore, the differentiated state ultimately depends on the transcription of genomic information.

Early Evidence for the Informational Equivalence of Differentiated Cell Genomes

A second premise of the argument for the variable gene activity theory is that every living cell nucleus in a metazoan organism contains the same

complete genome as was present in the zygote nucleus. The opposite view was proposed by Roux in 1883. Roux's idea was that differentiation of cell function results from the partition of qualitatively diverse genetic determinants into different cell nuclei. Thus, each cell would contain in its nucleus only those genes needed for the programming of its particular set of functional activities, so that developmental specialization would stem from the establishment of a mosaic of diverse partial genomes. Experiments designed specifically to test this point were carried out by Driesch (1892) and later by various other experimental embryologists (Morgan, 1927). In Driesch's experiments the normal pattern of distribution of cleavage stage nuclei into the diverse sectors of egg cytoplasm was transiently altered by forcing cleavage to occur under the pressure of a flat glass plate. When the plate was removed it was found that given nuclei had been partitioned into cells other than those normally inheriting them, but that normal development could still occur. Since nuclei normally assigned to endoderm cells could also direct the development of mesoderm, and vice versa, it was argued that these nuclei must contain the genes for mesoderm as well as those for endoderm properties. It follows that any cleavage-stage nucleus contains all the zygote genes.

The contemporaries of Driesch and his followers believed that the pressure plate experiments showed the theory of qualitative nuclear division to be incorrect (see, e.g., Wilson, 1925). However, it can be argued that these experiments demonstrate the genomic equality of nuclei only at a period of development which long precedes either the onset of cell differentiation or the onset of direct control over morphogenesis by the embryo nuclei. On the other hand, a variety of other observations suggest that even highly differentiated cells contain a complete genome equal to that contained in the zygote nucleus. It was recognized very early that the cells of an organism are normally equal in the number of distinct chromosomes which they possess. A significant early clue came from the study of dipteran polytene chromosomes, where chromosomal abnormalities associated with mutations affecting the structural characteristics of one tissue can be observed in the chromosomes of another tissue. An example was furnished by the *Bar* gene in *Drosophila*, which effects the morphogenesis of the eye. Bridges (1936) showed that a duplication in band 16A of the X chromosome is visible in the polytene chromosomes of salivary gland cells in flies bearing this mutation. Yet the salivary gland cells are evidently not responsible for the details of eye morphogenesis. Another early example was the *Notch* mutation in *Drosophila*, which in heterozygotes causes peripheral incisions and other morphological abnormalities in the wings. This phenotype was associated with a heterozygous deficiency in salivary chromosome band 3C7 (Demerec *et al.*, 1942). The nuclei of one differentiated cell type (the salivary gland) thus

seem to bear genetic information required for the differentiated function of other kinds of cells, such as wing and eye forming cells.

Transdifferentiation

An interesting test of the idea that differentiated cells carry information normally expressed only in other cell types can be found in altered cell fate experiments, in which obviously differentiated cells are shown to change their specialized roles and to assume a new state of differentiation. This phenomenon is termed "transdifferentiation." For example, it was shown by Stone (1950) that in the regenerating newt eye neural retinal cells derive directly from cells which were formerly pigment cells. Changes in state of cellular differentiation also occur in the regeneration of the eye lens (reviewed by Yamada, 1967) and in other cases of regeneration, such as limb regeneration (for instance, see Namenwirth, 1974; reviewed by Hay, 1968). It has long been known that extensive changes in cell state also take place during regeneration in simple metazoa such as *Hydra* (e.g., Burnett *et al.*, 1973; Lowell and Burnett, 1973).

A great number of examples of transdifferentiation probably occur in the normal embryological development of higher animals, where cells performing a given specialized function at one stage later perform other functions. In developmental cases, however, it is often difficult to prove that the same cells or their lineal descendants are responsible for the new state of differentiation rather than clones descended from previously undifferentiated cell types. Several developmental examples have now been well described. A clear case is the transdifferentiation of larval silk gland cells in the moth. Selman and Kafatos (1974) have shown that in this animal the cuticular cells of the silk gland later redifferentiate into cells specialized for the secretion of comparatively huge volumes of $KHCO_3$ solution, which is used as a solvent for the hatching enzyme cocoonase. Another example from the same silk moth concerns cells of the labial gland. During the pupal stage these cells produce a thick cuticle, but as metamorphosis proceeds they synthesize and secrete cocoonase zymogen (Selman and Kafatos, 1975). A classic case of transdifferentiation claimed to occur many years ago by Maximow (1927) was the transformation of blood lymphocytes into phagocytic macrophages and then into collagen-secreting fibroblasts. Petrakis *et al.* (1961) studied this transformation, and showed that a culture of circulating mononuclear leukocytes sealed into a diffusion chamber is indeed able to give rise to a sheet of collageneous connective tissue fibroblasts after passing through an intermediate macrophage stage. The identity of the collagenous fibroblasts with their mac-

rophage precursors was certified by their retention of India ink particles originally incorporated by the macrophages.

The occurrence of transdifferentiation in normal development, in regeneration, and in various other special experimental circumstances shows that differentiated cells contain genomic information other than that needed for their current specialized activities. However, it can be argued that each such case involves only a small fraction of the total genomic information possessed by the organism, since it concerns only a few functional traits. Such traits could be regarded as "closely related," *de facto*, since they belong to the repertoire of functions which are demonstrable in a single cell type. From a biochemical point of view this argument seems arbitrary, since the differences between a cell specialized for pigment synthesis and a neuron, between a leukocyte and a collagen-secreting fibroblast, or between a cuticle- and a salt-secreting cell would seem no less than those between a liver and a kidney cell. Nonetheless, it requires a considerable act of generalization to conclude that because transdifferentiation can occur, a differentiated cell nucleus actually contains the *whole* genome, and the case for this now rests to a large extent on other evidence.

DNA Constancy and Nuclear Transplantation

A critical element of evidence is the presence of twice the haploid amount of DNA in the nucleus of *every differentiated cell* (a few particular exceptions aside), except for the gametes, which contain half the somatic cell quantity. The constancy of DNA content among diploid cells was discovered by Boivin *et al*. (1948) and Mirsky and Ris (1949), and provided one of the major reasons for regarding DNA as the genetic material. Equality of DNA content among differentiated cell nuclei means that differentiation cannot in general be explained through the selective *loss* of massive fractions of unused genes from the nucleus, but this does not preclude the possibility that differentiation involves the inactivation of DNA coding for properties not manifest in a given cell type by means of chemical alterations in the genetic material. Furthermore, animal genomes are so large that the DNA of a large number of structural genes could be deleted without detectably affecting the total DNA content. It is now clear, however, that developmental alterations in the genomic DNA either do not occur or are reversible. This important conclusion rests to a large extent on nuclear transplantation experiments in which nuclei from differentiated cells are injected into mature eggs and are shown to possess the capacity to direct the complete course of development.