Cytodifferentiation and Macromolecular Synthesis

Edited by

MICHAEL LOCKE

21st SYMPOSIUM OF THE SOCIETY FOR THE STUDY OF DEVELOPMENT AND GROWTH

Academic Press

Cytodifferentiation and Macromolecular Synthesis

Edited by

Michael Locke

Developmental Biology Center
Western Reserve University
Cleveland, Ohio

1963

ACADEMIC PRESS, New York and London

ACADEMIC PRESS INC.
111 Fifth Avenue, New York 3, New York

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
Berkeley Square House, London W.1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 63-14493

PRINTED IN THE UNITED STATES OF AMERICA

Cytodifferentiation and Macromolecular Synthesis

The Twenty-First Symposium
The Society for the Study of
Development and Growth

Asilomar, California, June 1962

EXECUTIVE COMMITTEE 1961–1962

CLIFFORD GROBSTEIN, Stanford University, *President*
ARMIN C. BRAUN, The Rockefeller Institute, *Secretary*
H. CLARK DALTON, Washington Square College, *Treasurer*
DOROTHEA RUDNICK, Albertus Magnus College, *Retiring Editor*
MICHAEL LOCKE, Western Reserve University, *Editor*
ARTHUR W. GALSTON, Yale University
MARCUS SINGER, Western Reserve University
JOHN G. TORREY, Harvard University

List of Contributors

Numbers in parentheses indicate the page on which the author's contribution begins.

JOSEPH G. GALL, Department of Zoology, University of Minnesota, Minneapolis, Minnesota. (119)

S. GRANICK, The Rockefeller Institute, New York, New York. (144)

PAUL B. GREEN, Division of Biology, University of Pennsylvania, Philadelphia, Pennsylvania. (203)

CLIFFORD GROBSTEIN, Department of Biological Sciences, Stanford University, Stanford, California. (1)

JEROME GROSS, Department of Medicine, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts. (175)

HEINZ HERRMANN, Institute of Cellular Biology, University of Connecticut, Storrs, Connecticut. (85)

FRANÇOIS JACOB, Services de Génétique microbienne et de Biochimie cellulaire, Institut Pasteur, Paris. (30)

CHARLES M. LAPIERE, Department of Medicine, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts. (175)

JAMES W. LASH, Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania. (235)

CLEMENT L. MARKERT, Department of Biology, The Johns Hopkins University, Baltimore, Maryland. (65)

JACQUES MONOD, Services de Génétique microbienne et de Biochimie cellulaire, Institut Pasteur, Paris. (30)

MARVIN L. TANZER, Department of Medicine, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts. (175)

CHARLES YANOFSKY, Stanford University, Stanford, California. (15)

Foreword

"... Our Microscope will easily inform us that the whole mass consists of an infinite company of small Boxes or Bladders of Air...

"... I told several ... of these small Cells placed endways in the eighteenth part of an inch, whence I concluded there must be ... in a Cubick Inch about twelve hundred Millions... a thing almost incredible, did not our microscope assure us of it by ocular demonstration; ... so prodigiously curious are the works of Nature, that even these conspicuous pores of bodies ... are yet so exceeding small, that the Atoms which Epicurus fancy'd would go neer to prove too bigg to enter them, much more to constitute a fluid body in them...

"... Now, though I have with great diligence endeavoured to find whether there be any such thing in those Microscopical pores... Yet have I not hitherto been able to say anything positive in it; though, methinks, it seems very probable, that Nature has in these passages, as well as in those of Animal bodies, very many appropriated Instruments and contrivances, whereby to bring her designs and end to pass, which 'tis not improbable, but that some diligent Observer, if help'd with better Microscopes, may in time detect ..."

Observ. XVIII. Of the Schematisme or Texture of Cork, and of the Cells and Pores of some other such frothy Bodies.
Robert Hook, 1665

At Asilomar in June, 1962, a group of latter day diligent observers reported progress to the Society in their studies on the means whereby nature brings her designs and ends to pass, helped by the National Science Foundation and the local committee, to whom all thanks.

M.L.

Contents

Cytodifferentiation and Macromolecular Synthesis

CLIFFORD GROBSTEIN

Department of Biological Sciences, Stanford University, Stanford, California

The title of this essay, and this volume, was the theme of the 21st Growth Symposium. The number of the symposium may be significant, for there is a sense in which the Growth Symposium, its sponsoring Society, and the science of development which is the Society's focus, all have been coming of age. After a long period of successful operation provided by the impetus of its founders, the Society has been re-evaluating and redefining its role. The appearance of this volume, under a new publisher and a new editor, comes only after careful soul-searching by the Society's Publication Committee. Despite altered circumstance since the Growth Symposium was founded, it was decided that its publication remains a valuable contribution to the literature of developmental biology, and that it is the responsibility of the Society to make it an even greater one. To accomplish this, changes—involving both procedures and content—were suggested. Among these was looser "coupling" of the orally presented symposium and the published one, and inclusion of a synthetic article on the theme of the symposium.

Traditionally, the President of the Society for the Study of Development and Growth, in consultation with the Executive Committee, has been the chief organizer of the symposium. Having suggested the theme it is reasonable that the President should comment on it, or should invite some one else to do so whose views may prove illuminating. The transitional status of the 21st Symposium, planned during the deliberations of the Publications Committee, provided no opportunity to select an alternative commentator. Hence, responsibility fell to the President and this essay was written after the symposium had occurred and with full knowledge of its content.

It is no disparagement of speakers to note that symposium organizers frequently find their chosen theme honored, if at all, more in the introduction than in the body or conclusions of the papers. This seems inevitable, since the speakers do not necessarily hold the same conception of the theme as the organizer, and sometimes are dragged in, bitterly protesting, under its umbrella. Occasionally a speaker sees the organizer as tyrannical, and the

organizer sees the speaker as recalcitrant and resentful in the face of integrating guidance. For these and other reasons, the preconceived theme may be less sharply delineated than hoped. The present symposium is no exception; its papers as a set clearly fit the theme, but the treatment is neither as focused nor as complete as might be provided by a single author pursuing a single conception. In this lies opportunity and justification for an effort at synthesis. To restate the theme as conceived, and to round out the treatment by the authors from the point of view of the organizer—these are the objectives which, if they accomplish little else, at least provide opportunity for a last word for the organizer.

The theme may be rephrased as a hypothesis, which indeed it is. A number of authors in recent years (e.g., Spiegelman, 1948; Markert, 1956, 1960) have suggested that the differentiation of cells is fundamentally a switching of biosynthetic activity, leading to the appearance of new macromolecular species whose accumulation or export is manifested as specialized structure or activity. Whether or not differentiation is solely or primarily such switching of biosynthesis may be questioned (Ebert, 1955), but certainly it is involved in many instances. In the context of spectacular advances in knowledge of biosynthesis, particularly of proteins, the hypothesis has been given new content. Biosynthesis of proteins is now firmly linked to the gene at one end, and to cytoplasmic assembly centers somewhere near the other end. Between the fixed genotypic nucleotide sequence of deoxyribonucleic acid (DNA), and the alterable phenotypic expression in a characteristic protein, lie messenger ribonucleic acid (RNA), amino acid activation, the yet to be clarified order within the ribosome, and processes of polymerization, folding, and complexing of macromolecules whose importance increasingly is emphasized. If a new molecular species is to appear, whether *de novo* or through increase in amount, some alteration must be effected along this chain from gene to final product. Induction of differentiation thus would be effected through control exerted along the chain of biosynthesis. With the relative paucity of information about control of intermediate steps in the chain, particularly in complex organisms which most characteristically differentiate, it has been natural to concentrate attention on the point of most precise information, on the relationship between gene and biosynthesis. Yanofsky in his chapter in this volume reviews some of the exciting new information in this area, indicating that amino acid sequence of particular proteins is coded in, and ultimately determined by, nucleotide sequence in the DNA of the gene. Given the genetic conception of replicating nucleotide sequences, and the assumption of a system for transcribing these into sequences of other building blocks such as amino acids, cytodifferentiation can be, and has

been, visualized as the switching on and off of synthesis at particular genetic sites (see chapter by Markert). Precedent for this exists in microbial systems, and Jacob and Monod in their paper present a number of models based on enzyme induction, which could operate as controls. What is important to the general hypothesis is not so much the details of the models, which can be adjusted to yield anything desired, but the provision of regulation, i.e., alteration of genomic product without alteration of nucleotide sequence. The genome is assumed to undergo "functional" change, without sacrifice or modification of properties detected by allowing it to replicate. Differentiative macromolecular synthesis thus stems from control of the functional or transcriptive behavior of the genome, without alteration of its genetic behavior as classically conceived in breeding experiments.

This is a provocative and powerful hypothesis and, of course, deals perfectly with what has been called the dilemma of differentiation, how nuclei, classically assumed to be genetically equivalent, can be controlling cells so different as neuron, macrophage, and melanocyte. This so-called dilemma, man-made rather than cell-made, has never been as sharp or fearsome as sometimes has been portrayed. It has several possible resolutions not yet excluded by the evidence, viz., the nuclei of differentiated cells may not remain genetically equivalent, some extranuclear genetic change may occur in differentiation, or differentiation may not involve a genetic change at all. The first possibility has been raised seriously by nuclear transplantation studies (Briggs and King, 1959), the second by data suggesting genetic stability in cytoplasmic particles (see chapter by Granick), the third by challenge to the assumption that differentiated states are replicatively transmitted through successive cell divisions (Trinkaus, 1956; Grobstein, 1959). The Jacob and Monod model, applying primarily the newer understandings of high-resolution microbial genetics, dissolves the dilemma in yet another way. It distinguishes two kinds of genetic mechanism, that involved in replication of the code and that governing its transcription. Nuclei may have a complete set of replicative sites, but be transcribing only from particular ones at particular times. Moreover, and here the model advances beyond earlier speculations and reaches out to link genetic and physiological approaches to cell function, transcription of structure-determining sites of synthesis may be controlled by other replicative sites (regulators) through the intervention of epigenetic inducers and repressors. By defining *genetic* to include both replicative and transcriptive operations, nuclei simultaneously may be genetically equivalent (when tested by breeding behavior for replicative properties) and genetically nonequivalent (when tested by synthetic behavior for metabolic properties).

This interesting resolution has an additional feature of possible significance. It has long been recognized that most differentiations in higher organisms involve more than a single new biosynthetic pathway; that a given cell type must be defined in terms of a constellation of syntheses and properties. The concept of the operon, of transcriptively coupled syntheses turned off and on by a single operator gene, conceivably in response to a single external intervention, may prove helpful in this connection. Compounding simplifications, one might think of the number of differentiated cell types in a higher organism as some function of the number of operons in its genotype.

Thus, the hypothesis of macromolecular synthesis as the key to cytodifferentiation has acquired a codicil, close impingement of genetic control. This increases its interest and attractiveness in relation to a general theory of cell heredity and function. It does not make easier, however, its evaluation as a specific theory of cytodifferentiation, for very few data currently are available to test critically the specifically genetic aspect. The closest applicable information comes from the remarkable cytological observations, summarized by Gall in his article, which are correlating details of chromosome structure with cell type. The evidence for localized synthetic activity along the length of the chromosome, in patterns characteristic for a differentiated cell type, clearly conforms with the hypothesis. Without greater resolution, however, whether chemical, genetic, or morphological, it cannot yet be said that what are observed are actual functioning genetic sites. Nor is anything known of the details of control of these sites. It is not clear whether the localized changes are first steps leading to differentiation, or early consequences of differentiation initiated elsewhere. Certainly nothing can be said as to the possible involvement of regulator genes, or of operators. Nonetheless, there now has emerged out of an impressive array of genetic, biochemical, cytological, and embryological data, a working hypothesis of cytodifferentiation at the molecular level. Particularly in the genetic version of Jacob and Monod, it is based largely on microbial data and its applicability to multicellular systems—whose behavior is more traditionally and spectacularly differentiative—is only beginning to be tested. It seems worthwhile to evaluate and comment on the applicability of the working hypothesis to embryonic differentiation, both to emphasize implications for future work and to reduce terminological and other differences between the several parties of investigators who are now converging from different directions on a common problem area.

The question of definition of differentiation deserves brief consideration. What is under discussion, of course, is not differentiation of the whole organism but cytodifferentiation, i.e., the cell changes which accompany and reflect the increasing heterogeneity which is one of the hallmarks of develop-

ment in complex organisms. The changes are in the direction of cell specialization—concentration on certain activities and syntheses at the expense of others. Jacob and Monod propose to define differentiation in a manner which is entirely appropriate for microbes, viz., "Two cells are differentiated with respect to each other if, while they harbor the same genome, the pattern of proteins which they synthesize is different." Several points should be kept in mind, however, in judging applicability to higher organisms.

First, in multicellular systems the only differentiations demonstrably admissible under a standard of genomic equivalence are those of gametogenesis. It is often overlooked that in gametogenesis cytodifferentiations of extreme degree occur without alterations of the genome in the replicative sense. Taken by itself gametogenesis strongly implies that specialized syntheses *can* proceed without alteration of nucleotide sequence. Apart from this case, however, a flat assumption of genomic equivalence will seem to some to beg the question for multicellular organisms. Hope that it may be possible to test the genome of differentiated cells is higher than seemed justified some years ago, but it has not yet been done unequivocally. Nonetheless, the new insights into the nature of genetic coding, and the discrimination of a separately regulated process of transcription, are widely felt to make message changes less attractive than reading changes as a mechanism of differentiation. Pending evidence to the contrary, and since it constitutes a basis for a working hypothesis of considerable heuristic value, it seems reasonable to accept genomic equivalence provisionally.

Second, in multicellular systems (as probably in microbial) acceptable differentiative changes are not limited to biosynthesis of proteins. Behavioral changes such as motility and phagocytosis, as well as synthesis of complex polysaccharides, lipids, or steroids, are distinguishing criteria of certain differentiations. It is not unreasonable to assume, however, that underlying these is the synthesis of specific proteins if only in catalytic amounts. Again as a working hypothesis, the appearance of a new pattern of synthesis of proteins seems a reasonable criterion of differentiation.

Third, a number of cyclical changes in cellular synthesis, illustrated by hormonally controlled changes in secondary sex structures, have not usually been regarded as differentiative. The term modulation has been applied to such changes when it is clear that they are without stability in the absence of the initiating circumstance. However, the borderline between these changes and "true" differentiations has never been sharp, and there seems to be no good reason why they should not be grouped under the hypothesis as differentiations of minimal intrinsic stabilization.

In view of these considerations the proposed definition of Jacob and Monod

perhaps may be rephrased as follows. There is a class of phenomena involving change of some stability in the cellular pattern of protein synthesis, without known change in the replicative properties of the genome. The class includes at least several subclasses which may have mechanisms in common: bacterial induction-repression, mating types in *Paramecium* (Sonneborn, 1960), and embryonic cytodifferentiation. In applying the term differentiation to the class, one emphasizes the likelihood of important similarities in the subclasses without intending to minimize the equally important differences which may exist as well.

More interesting than definition, however, is the question whether properties implied by the model are common to the subclasses. For example, is it the case that embronic differentiation appears to involve genetic transcription? A whole series of examples, both classical and recent, answer affirmatively. The results of heteroplastic and xenoplastic developmental interactions, together with the occurrence of mutations affecting the structure of such specialized proteins as hemoglobin, show incontrovertibly that genetic sites are operative during differentiation, and hence must be coupled in some fashion with its controls.

More difficult to analyze is the specific implication that differentiative controls impinge on genetic sites through combination with repressors of structural genes. The matter has two aspects, whether repressors of structural genes actually exist in cells of higher organisms, and, if so, whether factors known to control embryonic cytodifferentiation interact with such elements of the genetic system. Concerning the first aspect there is as yet only scanty indirect evidence, which has been referred to elsewhere (Jacob and Monod, 1961) and to which nothing can be added here. Concerning the second aspect there is more evidence, enough to put the matter in perspective, though not enough to draw firm conclusions. The evidence comes from the study of developmental induction, in the course of which new differentiative pathways are established. Since the discovery that differentiative processes in one tissue are controlled through intimate association with another, embryologists have wrestled with the nature of the mechanisms involved. Though these mechanisms cannot yet be specified, the current status of the problem has two implications for the Jacob-Monod model: it suggests that dependent differentiation as seen in developmentally coupled tissues is favorable test material, and it warns that there may be several levels of control between an inducer as observed in embryos and the primary effector, *F*, postulated for microbial genetic systems.

With respect to favorable test material, it is clear that one would like a population of cells, as large and homogeneous in behavior as possible, which

can be controlled developmentally so as to initiate synthesis of well-characterized specialized proteins under defined nutritional conditions. It would be useful if genetic variants affecting the character of the proteins were available or could be readily produced. No such ideal system has yet been described, but a number of laboratories have these requirements in mind and advancing tissue culture technology is providing promising candidate-systems. Particularly attractive are the differentiation of muscle, cartilage, melanocytes, erythrocytes, fibroblasts, lens, and various epithelial cells. In our laboratory we are especially impressed by the potentialities of epithelio-mesenchymal rudiments, where presence or absence of mesenchyme provides control over epithelial differentiation and, in such rudiments as the pancreas, the epithelium produces a number of characterizable proteins.

Investigation of such systems, along with much other data on embryonic induction *in vivo* and *in vitro*, has not encouraged the view that differentiative control of this kind involves direct impingement of products of one tissue on the genetic system of another. In discussing this we may treat the regulator-operator-structural gene complex of Jacob and Monod as a component (black box), and ask whether its input seems likely to be a molecular species emitted by, and coming directly from, a second tissue acting as an embryonic inductive source. Or asked in another way, does it seem likely that embryonic induction involves a single step, the direct initiation of synthesis of differentiated product?

In my own view this does not seem likely, though the possibility is not excluded for some instances. I am impressed by the following considerations:

First, in many inductions the response involves the appearance of several cell types, and these in a complex temporal and spatial pattern. Primary induction by chorda mesoderm is a good example, but many secondary inductions are simpler only in degree, e.g., the response of metanephrogenic mesenchyme to ureteric bud involves appearance of glomeruli and secretory tubules containing a number of cell types. It is hard to see how the inducer could directly turn on biosyntheses of so many different sorts in so many cell types. Either a battery of molecular species, or intermediate relays, seem likely.

Second, some inductive responses can be initiated by a number of chemically unrelated molecular species. Hence, specific chemical information does not appear to be required to be fed in by the inducer. Allosteric induction, as emphasized by Jacob and Monod, provides a degree of freedom in the regulation of microbial biosynthesis. In embryonic induction there appears to be even less coupling of effector and response, implying the existence of yet additional intermediate steps.