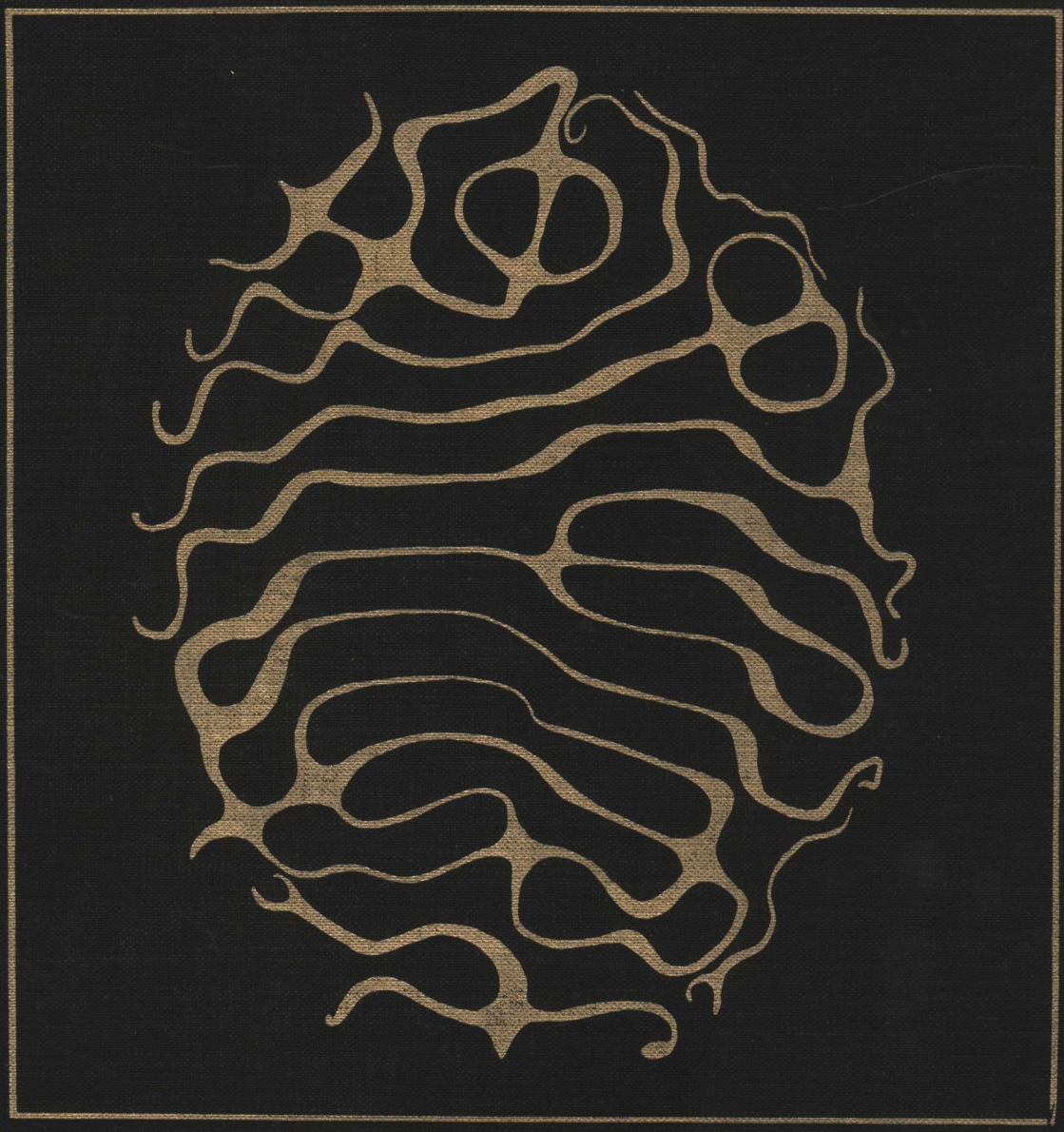
CELL DIFFERENTIATION



Ole A. Schjeide / Jean de Vellis

CELL DIFFERENTIATION

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Preface

Differentiation of a cell may be defined as the process whereby such a unit changes from a pre-existing condition into one of increased complexity and specialization. If all cells of an individual differentiated equally, there would seem to be little advantage in multicellularity. The fact that they do not, together with the processes of natural selection, has resulted in the emergence of highly evolved organisms. Control—or even substantial understanding—of the mechanisms involved in cell differentiation would be an achievement of overwhelming implications for the future of mankind.

Whether such realization is constantly kept in mind or not, a large segment of the scientific community is inherently interested in the subject of cell differentiation. For biology it is the heart of the matter—the apex of a large cone of generally oriented research that has gone on for centuries, but which has increased fantastically in depth and range in the last decade. Documented information and ideas about cell differentiation have accordingly become increasingly available. Since both instructors and researchers seem anxious to fit these facts and concepts into the context of their own endeavors, the time has come for organized texts on the subject.

Knowledge of the general subject of cell biology (including molecular biology) is prerequisite for concentration on cell differentiation. There is, nevertheless, still so much to be learned about intracellular structure and functions of the cell's organelles that the mechanisms directing their appearance and ordering have seemed to many cell biologists to be topics for future attention. In any event, texts on Cell Biology must, because of space considerations alone, present diluted information on topics more directly related to cell differentiation.

The editors decided to fuse the authority and experience of available experts with their own concept of the subjects which should comprise a text on Cell Differentiation. Acting as organizers, even more than as contributors, they have sought to bring pertinent people into the act. As may be seen from the Contents page, they were fortunate in the quality of their recruitment. Because of the unique insights of these scientists and because the text is intended primarily for advanced students, instructors, and working scientists,

each author was asked to introduce as much information on the subject as possible while maintaining the perspective (or bias) that seemed most reasonable and useful to him. Other authors, given similar instructions, might orient their material somewhat differently. This is because the discussions included herein are taking place on the fringe of knowledge. Who is to say, at this time, that the concensus of today will not be the retraction of tomorrow!

It is in this same spirit that the authors have been urged not only to include in their discussions pertinent accounts of their own work but also to make leading interpretations and to propose far-reaching concepts for the scrutiny of an audience that will profit from them even if they turn out to be wrong in the enlightened future. Editing has been carried out chiefly in the framework of general organization and form rather than content. Some issues have been sufficiently inflammatory to result in considerable discussion and correspondence between authors and editors. Likewise, the reader will probably be able to pick out some items in which his experience and views differ from those of the authors. It is hoped that this will provide the stimulation for constructive thought and definitive research that will eventually lead to elucidation of these issues.

Ole A. Schjeide
Jean de Vellis

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I GENERAL ASPECTS

1 Introduction

OLE A. SCHJEIDE AND JEAN DE VELLIS

Before the reader becomes involved in the specific issues of this text it may be useful to discuss briefly some of the general concepts within this area of biology that will be taken up in considerably more detail in the chapters that follow. Also, in this preliminary discussion, an attempt will be made to define some of the terms used in describing differentiation.

First it must be emphasized that *all* of the cells to be discussed henceforth are differentiated. By definition, *there are no undifferentiated cells*. Some cells may be more differentiated than others, but all exhibit certain morphological features and perform certain functions that permit their identification as cells, if not their complete characterization. On the other hand, relatively *uncommitted cells* do exist—in the sense that these are capable of further differentiation in one of several directions. An example is the hematopoietic stem cell which, apparently, can give rise to erythrocyte, lymphocyte, or granulocyte (see Chapter 15).

There are, of course, many instances in which cells that look very much alike are different in their capacity (*competence*) for further differentiation. This being so, their present states of differentiation cannot be identical because their further differentiation must be based on a pre-existing situation. In embryology, a cell that has been led along such a specific path of differentiation, which will generally lead to a given *expression* despite translocation, is said to be *determined* (see Chapter 4).

I. WHAT IS CELL DIFFERENTIATION?

A discussion of cell differentiation brings up the question as to the ways in which a cell can be differentiated. The possibility of any two cells being alike is very remote, but some differences are relatively insignificant, whereas others form the major bases for variation in phenotype and function.

A. CHROMOSOMAL DIFFERENTIATION

A key factor in the differentiation of a cell may be the composition and *functional status* of its genetic material, most of which is located in the nucleus in the form of chromosomes. Different species possess different numbers of chromosomes, and

individuals of a species contain different genes, although the number of chromosomes in cells of one individual is normally the same as in other representatives of that species. Some exceptions to the general rule of equal numbers of chromosomes in a species, or within different cells of a single multicellular organism, do exist, and indeed the presence of more or fewer chromosomes in a cell may have profound influence therein. It is commonly accepted among plant geneticists that polyploidy results in improved varieties of plants. It should also be noted that after the fusion of myoblasts to form a larger functional entity, at least most of the nuclei are preserved (see Chapter 17). The presence of a single extra chromosome, as in the case of Mongolism, appears to have a modifying effect on the course of differentiation of some cells. Another intriguing observation is the existence of polyploidy in Purkinje's cells of the brain at the time of their differentiations (see Chapter 19). This suggests that the doubling of nuclear DNA (not necessarily a doubling of the number of chromosomes) may have a special functional significance.

Within a given organism it is generally accepted that *most* of the somatic cells harbor identical genomes (Chapter 4). Chromosomal differentiation in the case of various organs may therefore reside either in the nature and extent of the repression of expression of the genetic material or in the manner in which the nonchromosomal portion of the cell reacts to the gene products. Although these possibilities have not been completely resolved, considerable evidence has accumulated, largely as a consequence of the stimulating studies of Jacob and Monod,[1] for the existence of proteins or nucleoproteins[2] which, by virtue of their association with specific portions of the DNA of the chromosomes, prevent the transcription of RNAs that can direct synthesis of specific proteins (see Chapters 3 and 4). Removal of the *repressor* is one step in the direction of synthesis of a protein. Of course, the synthesis of specific proteins, both enzymes and structural moities, determines most specifically the nature of the functional differentiation of the cell (see Chapter 12).

Busch[3] and others have made the interesting proposal that in every cell of evolved organisms various groups of operons (*polyoperons*) exist. According to their view, during any given period, one or more polyoperons is actively transcribing messenger RNAs (mRNAs) and thus providing information for:

I. Basic Cell Metabolism
II. A Specialized Cell Function
III. Cell Growth
IV. Cell Division
V. Cancer

Weber *et al.*[4] have suggested that the enzymes regulating gluconeogenesis in the liver are encoded in one polyoperon, cortisol acting as a signal for induction and insulin as a signal for repression of this genome unit.

It has been proposed further that more than a single polyoperon for specialized cell function exists in a cell—i.e. the DNA of any cell of the same individual could, under the proper circumstances, support the development of a muscle or liver as well as that of a nerve cell. This was, of course, much in the minds of classical embryologists at the turn of the century. More recent support for this idea is the finding by Gurdon[5] that nuclei taken from various specialized tissues of the frog and substituted for the original nucleus in a frog egg exhibit the potential for development of a whole embryo (see Chapters 4 and 7). The concept of only certain polyoperons functioning at a given time, the others being repressed, is a very

useful model for thinking about mechanisms of cell differentiation. However, it should be emphasized that not all biologists agree that sufficient evidence has been forthcoming to support the concept of operation of evolved cells along the exact lines proposed by Jacob and Monod for bacterial cells.

Chromosomal differentiations, resulting in modifications in rates of transcription of various genes, are expressed in ways other than differences in numbers per nucleus and damping by repressor molecules. As will be discussed in Chapter 4, chromatin diminution occurs in somatic cells of *Ascaris*. In other cases, regions of chromosomes or, as in the case of mammalian X chromosomes, an entire chromosome, may remain heterochromatic (tightly coiled) throughout most of the lifetime of a cell. Other positional and geometric factors may be important in gene expression—e.g. enlargement of a nucleolus may inhibit transcription of genes close to the nucleolar organizer. Scarano* has submitted evidence suggesting that increased methylation of DNA takes place during development. Such alterations might result in failure to transcribe certain RNAs. Byfield† has suggested that certain mRNA transcriptions (e.g. some resulting in enzymes necessary for cell division) may take place only on newly replicated DNA.

Several studies suggest that in higher forms rates of synthesis of proteins are not directly proportional to rates of mRNA synthesis (as is true generally of bacteria). Control of differentiation of evolved cells may operate at the levels of translation as well as transcription as illustrated by the case of hemoglobin in the chick (see Chapter 12). Another example is the activation of masked mRNAs (*m*mRNAs) following fertilization of the egg (Chapter 4). Studies of syntheses by individual enzymes further indicate the possibility of translational control (Chapter 11), and a lack or deficiency of certain transfer RNAs may determine which mRNA is actually translated (Chapters 10 and 11). In developing cells, a large amount of mRNA synthesized in the nucleus never reaches the cytoplasm. It is possible that some degree of translational control is thus effected by the nuclear membrane (Chapters 5 and 7). Weiss‡ has indicated that ribosomes, even of the same cell type, may vary in their compositions and abilities to support protein synthesis.

B. NONCHROMOSOMAL GENETIC DIFFERENTIATION

As several chapters in this book will show (see Chapters 3, 4, and 5), the notion that the genetic material residing in the nucleus of a cell is the keyboard on which the theme of cell differentiation is played, is based on considerable experimental evidence and logical deduction. Indeed, no other equally convincing scheme of control of differentiation has been introduced. However, such an attractive hypothesis of the role of chromosomal genes in protein synthesis, and hence cell differentiation, draws attention away from legitimate roles that other sources of information in the cell may also play. Some such information appears to be present in the form of cytoplasmic DNA (see Chapters 4, 9, and 14). The DNA present in mitochondria and chloroplasts has been especially well characterized. It is usually different in density from the bulk of chromosomal DNA in the species studied; it is often circular, as is the case for bacterial chromosomes (see Chapter 4). It appears that, to a certain extent, synthesis in these organelles is independent of information originating in the chromosomes of the nucleus. The DNA in

*E. Scarano (Naples). Address presented at VIII International Embryological Conference, Interlaken, Switzerland, September (1967).

†J. E. Byfield. Transcription of division related messenger RNA. Biophysics-Nuclear Medicine Seminar, Lab. Nuc. Med. Rad. Biol, UCLA, March 25 (1969).

‡S. B. Weiss, Argonne Cancer Research Hospital. In a seminar entitled "RNA Synthesis in Viral Infected Bacteria" at UCLA, July 10, 1969.