Eucaryotic Microbes as Model Developmental Systems

Volume 2

edited by

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PREFACE

When we compiled this volume we were motivated by several stimuli. We felt that previous volumes on microbial development were often preoccupied with procaryotic cells and were of limited value to those interested in aspects of development, its control, and its maladies in eucaryotic cells. Not taking away from the inherent beauty of the organisms themselves, we also felt, in these times of relevance, that it was essential to reveal the merits of studying lower nucleated cells. The cellular systems discussed in this volume offer numerous advantages not afforded by higher plant and animal cellular systems, especially for approaching complex developmental questions. This volume focuses on many of the major problems of developmental biology and shows how the study of eucaryotic microbes is helping to elucidate these problems.

The book is divided into three major parts: Growth and Cellular Differentiation, Cell Communication and Morphogenesis, and Dormancy and Germination. Each part begins with an Editors' Introduction, which puts the contained articles into a general perspective but which is not intended as a comprehensive review. The volume will appeal mainly to senior undergraduates, graduate students, and scientists already working on the development of eucaryotic microbes. However, since each article begins with an introduction to the respective experimental organism, its morphology, and its life cycle, the book should have wider appeal to a more general readership.

The authors are indebted to Elinor Foden for her assistance in all phases of compilation of this volume and for her unending patience.

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PART I

Growth and Cellular Differentiation

EDITORS' INTRODUCTION

The majority of the current scientific investigations into the mechanisms that determine how a cell is transformed from one state into another have focused on the regulation of genomic expression and on the accumulation and function of specific gene products. Eucaryotic microbes lend themselves admirably to such studies because it is often possible to obtain large amounts of genetically identical cells which, by simple manipulation of environmental parameters, can be induced to grow and divide or to follow alternative pathways of development. For example: plasmodia of *Physarum* can either develop into sporangia or into thick-walled cysts; amoebas of *Naegleria* can differentiate into flagellates or into cysts; and cellular slime mold amoebas may embark on either a multicellular developmental pathway to form fruiting bodies or macrocysts or undergo unicellular differentiation to form microcysts. Thus, like the fertilized egg of animals or plants which develop into the multifarious cells of the mature organism, the cells of lower eucaryotes may undergo diverse kinds of differentiation. The advantage these microbes provide, however, is that each differentiation may be studied alone or, at most, in the presence of but a few other simultaneous events rather than in the complex, heterogeneous cellular environment that characterizes the multi-

cellular embryo. The contributors to this part reveal how eucaryotic microbes are being used to solve some of the most perplexing problems of cellular differentiation.

GENETIC ANALYSIS OF DEVELOPMENTAL EVENTS

The discovery of the sexual cycle (macrocyst formation) of cellular slime molds [1] allows for the possibility of precise genetic mapping in this important group of eucaryotic microbes. David W. Francis and Robert M. Eisenberg review the history behind this discovery and show how developmental genetics, using both the parasexual and sexual cycle, is beginning to yield important information on the program of development in cellular slime molds.

In the yeasts, genetic analysis has been realized for many years. Since the mating-type locus has been shown to play a critical role in controlling sporulation, it has been the subject of much investigation. James E. Haber's group is pursuing the problem by employing conditional mutants which affect the mating-type locus. Their work with temperature-sensitive mutants suggests that an amber mutation in one (α) allele converts one mating type (α) to the other mating phenotype (a).

THE GENOME AND TRANSCRIPTION

If we are to understand the way the information that is stored in the genome is retrieved and utilized during the differentiation process, we must understand the organization of the eucaryotic chromosome. In the eucaryotic chromosome, the histone proteins are implicated as general repressors of gene function while certain nonhistone (acidic) proteins have been suggested as specific gene regulators [2,3]. Wallace M. LeStourgeon's early work with *Physarum* provided the first correlation between changes in the complement of nonhistone chromosomal proteins and altered patterns

of genetic activity during cellular differentiation [4]. In this part, LeStourgeon reviews the current model for the organization of the basic chromatin fiber of the chromosome [5] and shows why *Physarum* is an excellent system for characterizing the residual nonhistone proteins of chromatin. His work demonstrates a remarkable correlation between the developmental changes in the nonhistone proteins of *Physarum* and mammalian cells.

In addition to regulatory proteins, the complex group of nonhistone proteins also includes enzymes such as DNA and RNA polymerases. There are three major species of RNA polymerase in eucaryotic cells, and these may contain a couple of subspecies [6]. How so few enzymes can selectively produce the specific RNA transcripts that characterize a specific kind of cellular differentiation is a perplexing problem. In procaryotes it is suggested that highly phosphorylated nucleotides may regulate RNA polymerase function [7]. Herb B. LéJohn and his co-workers demonstrate the appearance of polyphosphate compounds during the development of *Achlya*. The three polyphosphates of *Achlya* show complex activating and inhibiting effects on the various RNA polymerase species isolated from this organism, suggesting a role for these compounds in eucaryotic development.

Since the first products of gene action are various classes of RNA, the developmental appearance of these molecules has received much attention. Shuhei Yuyama has examined the patterns of RNA synthesis during heat-synchronized cell division in *Tetrahymena*. By starving synchronized cells, he was able to dramatically reduce RNA synthesis to one-fiftieth of that of control cells without significantly altering the time of the first synchronous cell division. Characterizing the species of RNA synthesized, he revealed that the synthesis of rRNA is not essential for cell division while the synthesis of certain species of mRNA is essential.

During sporulation in *Saccharomyces*, which occurs under conditions of pseudostarvation, rRNA synthesis appears to be significant and important. Using temperature-sensitive mutants for rRNA

synthesis, James E. Haber's group provides data that suggest that different controls regulate rRNA synthesis during vegetative growth and sporulation. They also provide evidence indicating that the length of the poly(A) moiety of mRNA is shorter in sporulating cells as compared to vegetatively growing cells.

ENZYME ACCUMULATION AND FUNCTION

A cell is transformed from one state to another as a consequence of the accumulation of new, specific gene products. Generally, the intracellular accumulation of specific enzymes is accepted as the driving force of cellular differentiation. In this part, William F. Loomis and his co-workers show how the selection of enzyme-deficient mutants of *Dictyostelium* is providing information on the physiological roles of certain stage-specific enzymes. Through the accumulation of large amounts of information on developmental mutants, they are also able to show that certain biochemical events are independent of previous events while others are dependent on previous biochemical differentiations. This concept of sequence of events or timing sequences is also pursued by David W. Francis and Robert M. Eisenberg in this part.

The work of Byron F. Johnson, G. B. Calleja, and Bong Y. Yoo is concerned with the role enzymes play in the morphogenesis of fission yeasts. They propose a model of coordinated enzyme activities involving both autolytic and synthetic enzymes, which can explain the cell extension in yeast. With certain modifications, this model has also been used to explain cell division, conjugation, and spore liberation.

Barbara E. Wright and David A. Thomas acknowledge the importance of enzymes but indicate that the mechanism of enzyme accumulation is less important than the functional role of the enzyme in the differentiation process. The accumulation of activity of an enzyme is only important if that enzyme plays a key role in the developmental process and is rate-limiting. (Of five enzymes that

have been shown to accumulate during the development of *Dictyostelium*, only the accumulation of glycogen phosphorylase seems to be the product of differential gene activation.) Their data reveal the value of employing kinetic models which integrate many different kinds of information relevant to enzyme action and substrate availability.

THE PRIMARY CONTROL OF CELLULAR DIFFERENTIATION

One of the primary questions of developmental biology is exemplified by the following simple question: what is the initial stimulus that tells a cell how it should differentiate? Current models of cellular differentiation suggest pivotal roles for ions and cyclic AMP [8,9]. In this part, Allan Dingle shows how *Naegleria* is a useful organism for pursuing such problems. Of special interest is the synchrony of the developmental sequence and the precision with which the independent events of the amoeba-flagellate transformation can be timed.

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MACROMOLECULAR SYNTHESIS AND CELL DIVISION IN *TETRAHYMENA*

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I. INTRODUCTION

In studying any cellular morphogenetic events, four categories of problems may be discerned: (1) the problem of the triggering (stimulation or activation) mechanism; (2) the problem of control of macromolecular synthesis which is involved in that particular developmental event; (3) the problem of structural changes (assembly process) that are brought about by the products of macromolecular synthesis; and (4) the problem of relationships between cell growth (cell cycle) and developmental events. The present paper will deal mainly with the problem of the fourth category, i.e., the relationships between growth and division in heat-synchronized *Tetrahymena*, with emphasis on RNA synthesis. Other aspects of heat-synchronized *Tetrahymena* have been comprehensively reviewed by Zeuthen [1-3].

Tetrahymena pyriformis GL is a hymenostome ciliate protozoan (70 × 40 μm) that is known only to grow and divide, although some other species of *Tetrahymena* are known to be polymorphic or to

differentiate into cysts [4]. The GL strain lacks micronuclei and does not undergo sexual differentiation [5]. The ciliates divide transversely during cytokinesis, in spite of the fact that all ciliates have a distinct polarity with quite different structures associated with the posterior and anterior halves. Complex morphogenetic events take place during the cell cycle in order for a cell to generate two essentially identical daughter cells with their full complement of organelles. One marked feature of these morphogenetic events is the development of a new oral apparatus [6]. A diagrammatic representation of morphogenetic changes in heat-synchronized *Tetrahymena* is shown in Fig. 1.

T. pyriformis possesses many advantages for studying the relationships between macromolecular synthesis and morphogenetic events, i.e., either cortical morphogenesis or cell division, or both. The cells can be grown axenically in simple [5] or defined [7] medium, have a short generation time (2.5 h), take up nutrients, labeled precursors, and metabolic inhibitors readily, and can be

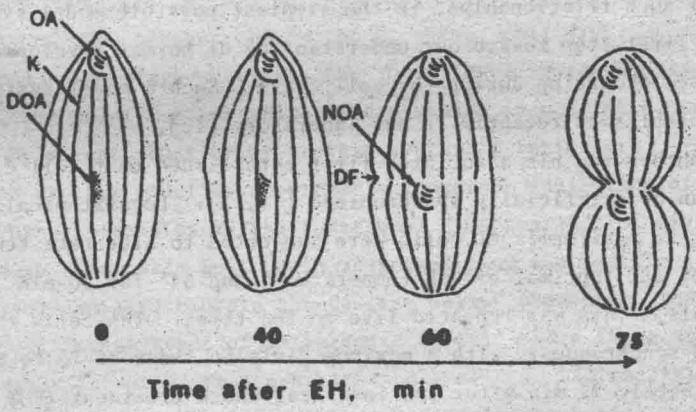

FIG. 1. Diagrammatic representation of heat-synchronized *Tetrahymena pyriformis* GL cells after the end of the last heat shock treatment. EH = end of the last heat shock; OA = oral apparatus; DOA = developing oral apparatus; K = kinetics; DF = division furrow; note that no detectable change in cell size occurs in the starvation medium.

synchronized by a G_2-sensitive event [8]. They exhibit a complex but clearly defined cortical pattern and are ideally suited for studying pattern formation of the cell surface [6]. The surface patterns can also be used as a marker to identify different regions of the cell. In addition, we already possess diversified and detailed information on the taxonomy, morphology, nutrient requirements, metabolic pathways, and growth characteristics (for reviews see Refs. 9 and 10), which enable us to continue investigating important contemporary issues without being hampered by numerous technical problems.

For studying biochemical aspects of developmental events, synchronous cultures must be available. Synchrony in *Tetrahymena* can be obtained by certain selection methods or by various induction methods (see Ref. 11). For studying a normal growth process, the use of a selection synchrony system is imperative, while various induction synchrony systems have advantages for answering other kinds of questions. The classic heat shock-induced synchrony [11] is well suited for studying the relationships between macromolecular synthesis and cell division. In cell cycle studies, elucidation of such relationships, in the simplest possible model system, is the first step toward our understanding of normal developmental processes occurring during the cell cycle. Such a model system has been established recently in our laboratory [12].

Zeuthen and his associates first established that cell division can be artificially synchronized [11,13]. Logarithmically growing *T. pyriformis* GL cells were subjected to alternate temperatures of 28° (optimal growth temperature) and 34° for 30-min intervals. This was repeated five to ten times. The cells then divided synchronously with a maximum division index of 70 to 80% approximately 72 min after the last heat shock treatment (EH) was completed. The 72-min interval between EH and division is shorter than the normal G_2 period. The induction of division synchrony can be explained by the fact that single cells isolated at different stages of the cell cycle display an increasing duration of division