MICROBIAL DIFFERENTIATION

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SOCIETY FOR GENERAL MICROBIOLOGY
HELD AT
IMPERIAL COLLEGE LONDON
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EDITORS' PREFACE

We sometimes forget that although cell differentiation is a characteristic of higher organisms it is by no means their exclusive prerogative. In this Symposium we have set out to show, in fact, that microbial organisms can have complex and sophisticated patterns of differentiation and indeed, in some cases, construct multicellular or macroscopic structures which are fully comparable with the 'bodies' of higher organisms. For this reason much interest has recently arisen in the possible use of microbes as 'model systems' for the study of cell differentiation. However, as pointed out by J. T. Bonner in his Introduction, what is important is 'not what one organism tells you about another, but what it tells you about itself'. There can be few, in fact, who do not feel that 'their' organism is much more than a model – whatever their initial motives for choosing to work with that organism might have been. Thus, although many of our contributors (e.g. A. Keynan) have stressed the wider applicability of the work they have surveyed, the particular appeal of the system they study is still very apparent.

Nor must it be forgotten that cell differentiation is far from being restricted to the eukaryotes. Indeed the transformation of a vegetative bacterial cell into a spore discussed by J. Szulmajster and the converse process discussed by A. Keynan involve as extensive and dramatic a change as any seen in the eukaryote world. These two processes are connected with changes in the cellular environment which affect the capacity of the cell to grow and divide. In these, and many other similar examples, there must be a close connection between the controls which regulate the division cycle of the cell discussed by W. D. Donachie and the controls which initiate the differentiation event(s).

Prokaryotes are often thought of as unicellular organisms, and indeed so they often are, but M. Dworkin reminds us that this is by no means always the case and the sociable behaviour of the *Myxobacteriaceae* which he documents shows how complex apparently simple systems can be. However, the most complex example of differentiation amongst the prokaryotes must be either the blue-green algae discussed by N. G. Carr, where the filamentous species possess a variety of differentiated structures and a complex life cycle, or the *Streptomyces* described by K. F. Chater & D. A. Hopwood. In both these cases there is clearly going to be a rapid deployment of the formidable techniques of genetic analysis and as these organisms come to be studied more extensively they will no doubt cause many surprises.

The simplest eukaryotes show differentiation phenomena which are very similar to those shown by the prokaryotes, and H. O. Halvorson in his article on sporulation in yeasts points out how close and yet how different this organism is from a prokaryote.

J. M. Mitchison challenges the old-established proposition that growth and differentiation are antithetic properties of a cellular system and clearly shows that the vegetative cell cycle in yeast encompasses two of the most important characteristics of a differentiating system – morphogenesis and the periodic synthesis which are the manifestations of ordered gene expression.

The filamentous fungi present yet another aspect of differentiation in that in most cases growth is limited to the apical tip area of the hyphae. The recent advances in cytology, physiology and biochemistry of fungal hyphae are discussed by S. Bartnicki-Garcia and it is clear that these studies will permit a more precise formulation of the mechanisms responsible for apical growth and therefore hyphal morphology.

The involvement of hormones in eukaryotic differentiation has been widely considered. G. W. Gooday describes the historical and present-day status of hormones in fungal differentiation. The identification and chemical characterisation of the trisporic acids undoubtedly emphasises the advantages of a multidisciplinary approach to problems of differentiation.

Reading these articles we have been struck by the tremendous amount of work which is going on in this field at the moment and the surprising absence of any widespread industrial application of the knowledge which is being acquired. This one-sided situation cannot last long and undoubtedly the ability to control the form and function of micro-organisms and particularly the mould fungi, must surely lead to a wider involvement of such studies with industrial processes.

We thank our contributors for producing manuscripts which were such a pleasure for us to read and put together and the staff of the Cambridge University Press who made the putting together as painless as possible.

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CONTENTS

DEVELOPMENT IN LOWER ORGANISMS

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For various reasons the development of a human being makes a rather poor 'model system' (to use the current phrase) to study the development of a microbe. The principal difficulty is that *Homo sapiens* hardly lends itself to easy experimentation: in general, the lowlier the organism the more we know about its molecular biology. The main point, of course, is not what one system tells you about another, but what it tells you about itself. And once we know something of a variety of different organisms up and down the evolutionary scale, it is possible to make some interesting statements about the 'comparative anatomy' of development. I mean anatomy at the molecular level and of course most particularly at the level of molecular control.

The fact that we know much more about such mechanisms in microbial systems has tended to give a considerable asymmetry to our 'comparative anatomy'. There has also been some confusion as to what relation certain phenomena in one group mean in terms of another. Let me begin this discussion by indicating a few basic concepts of development and what they mean at the different levels of organization. It will not be a discourse on the definition of words: not an old-fashioned grapple with semantics, but an attempt to describe the phenomena that occur at the different levels in such a way that genuine comparisons can be made. With this background we can then go on to look at the variety of different kinds of lower organisms, and not only compare them, but show how each has certain virtues for the study of particular aspects of development.

This symposium is called 'microbial differentiation'. We all agree that the formation of a fruiting body in a mould or the formation of a heterocyst in a blue-green alga constitutes differentiation. But if we confine ourselves to single cells the problems begin: is spore formation in a bacterium, where the whole cell turns from one state to another, the same thing as heterocyst formation in a multicellular filament? The answer is simply that one has two kinds of differentiation: temporal and spatial. In the former there is a change in time only (e.g. bacterial spore differentiation), while in the latter some cells undergo temporal differentiation and the others not, so that one can have one or more types

of cells existing together in one multicellular organism (e.g. heterocyst formation in blue-green algae).

The far more difficult case (as J. M. Mitchison has pointed out to me) is the cell cycle. There are changes going on from one moment to the next, so in a sense this is a simple case of temporal differentiation. But since the cell always returns to its initial state it does not seem to be quite the same thing as, for instance, spore formation. I think it quite wrong to argue that the cell cycle is fundamentally different, but that it nevertheless provides a useful 'model system'. It is quite likely that certain control events take place in the cell cycle which also play a role in other differentiations, but the mechanisms of the cell cycle are especially interesting in their own right. However, there still remains the question of how cell cycle events relate to the more generally accepted examples of differentiation.

Before answering this question, there is one further difficulty in using the concept of differentiation in single cells. It is usually imagined that a single cell is differentiated into parts: for instance, in a eukaryotic cell not only does it have a nucleus with all its structure, but also ribosomes, basal bodies, mitochondria, Golgi apparatus, plastids, and so forth. The question can be put in this way: are cells ever undifferentiated? It is true that some patterns of organelles may come and go, but the majority of the cell parts, for example the chromosomes or the mitochondria, stem directly from identical cell parts by multiplication or replication.

The way around these difficulties is to remember that development is synonymous with reproduction in its most general sense. All organisms have life cycles and each cycle is a direct reproduction, a direct copy of the previous cycle. If the organism is a single cell then the life cycle and the cell cycle may be one and the same. If the organism is multicellular then the life cycle is made up of a series of cell cycles. In this sense differentiation becomes a condition (a molecular composition in time and space) which is simply a copy of that same condition in the previous life cycle. It can be a metaphase plate, a bacterial spore, a heterocyst-studded filament in a blue-green alga, or the central nervous system of a man. What we seek, then, is to understand how protoplasm is controlled so that these structures can recur with such cyclic precision.

From the point of view of experimental analysis we must look for three different kinds of control mechanisms: (1) the control of the production of substances, (2) the control of the time of appearance of certain substances, and (3) the control of the spacing of the substances, that is, their localization. Again our existing knowledge is quite lopsided. We know much more about the control of substance production

than we do about the control of timing or substance localization. In fact, the whole exposing of the methods of transcriptional control, from repressors to sigma factors, has been one of the most successful and important advances of microbiology. The continued and increasing interest in translational controls and their importance in development is a matter of special concern at the moment, not only to the microbiologist, but also to those interested in higher organism development. Finally, and here is the greatest unexplored area of all, there are clearly a large number of secondary controls, such as feed-back inhibitions and hormone-mediated reactions. Often these link back to the genome at some point in their complex series of enzyme-controlled steps so that the secondary reactions are never totally divorced from the primary activity of the genome.

Unfortunately, timing mechanisms are surrounded by much greater mystery. In prokaryotes there is reason to believe that the life span of the *messenger* RNA may play an important role, but this is less likely to be the case in eukaryotes where, in some instances, it is believed that the messengers are stable and persist for long periods of time. Enzymes are catalysts and will therefore control the rate of reactions; so clearly this is a factor, especially when one has a series of enzyme-controlled reactions in a cascade. This is important in those cases where one reaction depends upon the product of a previous reaction; where there is a set sequence of events. No doubt many examples of such timing mechanisms will be given in the pages that follow, but one last point should be stressed. Much work has been done in recent years on circadian rhythms or biological clocks, which are known to exist in many single-celled organisms. There is, however, no evidence so far that these rhythms directly control developmental processes. Rather they seem to be a mechanism whereby the organism can time its stages of development with environmental changes such as night and day, or spring and fall (by responding to increasing or decreasing day lengths). But I feel sure we still have much to learn about the role of biological clocks.

The problems of the localization of substances in space and its control is one of the most pressing ones in modern developmental biology. Here we are not only concerned with the forces that distribute substances within cells, but also the distribution of substances in multicellular systems. Besides the localized production of a substance, one can also move substances by diffusion and even by cell locomotion (morphogenetic movement). There is, in fact, a considerable variety of ways in which localization can occur, and in many instances where we have some understanding of the control mechanisms, certain key substances

(e.g. hormones, or what are more generally referred to as morphogens) play an essential role in the pattern formation. It is not possible, in this brief introduction, to illustrate all the different kinds of localization, and what evidence we have for mechanism. Let it be said that there is little reason to doubt that ultimately the control of localization may be ascribed to the DNA of the genome, although the chain of events between the initial protein synthesis and the ultimate spacing may be exceedingly tortuous and indirect.

A few examples of differentiation in microbes may be helpful in illustrating the framework I have set forth in the above discussion. To consider microbes or lower organisms as an entity is, of course, quite illogical. With certain exceptions (especially the lack of development of a nervous system) these lower forms have all the main features of higher animals and higher plants. Their great virtue is that because of their relative simplicity they make available for experimental analysis some aspect that may be quite hidden and unavailable in a complex higher organism. One of the most striking examples of this comes from *Acetabularia*, a green alga with an anatomy so singular that it has permitted Hämmerling and those who have followed him to discover all sorts of important developmental information concerning the role of the nucleus and the cytoplasm.

The control of the production of substances has been studied (as we have already said) with exceptional success in single-celled organisms. This is not only true of *E. coli* but also other prokaryotes, and eukaryotes, such as yeast and other fungi, slime moulds of all sorts, protozoa, and algae. The initial step is to describe the appearance of new substances in the life cycle, enzyme synthesis being a particularly desirable kind of measure. In this way one can make a catalogue of the substances produced and even a time sequence for their production. This raises two problems: what controls the production of each substance, and what does the substance do? The former problem is usually attacked by the use of protein synthesis inhibitors to find the moment of translation and RNA synthesis inhibitors to find the moment of transcription. Furthermore one wants to discover what factors, internal or external, will affect the period of translation and transcription. The substance may be an enzyme and one wants to know its substrate and its product, and what controls the activity of the enzyme; are there feed-back controls? The product of the reaction may now be important in a subsequent reaction, so in this way one must piece together a series of controlled events.

The timing of these sequences soon becomes a matter of central concern. What starts one event or stops another? All aspects of the cell

cycle revolve around this problem: what sequences are rigid and is it possible to dissociate one sequence from another? What controls the sequence: is it chromosomal events, or substrate-product feed-back events, or both? The very same questions may be asked for spore formation in bacteria, or fruiting body differentiation in fungi, myxomycetes and cellular slime moulds.

Most single cells do not provide easy material for the all-important developmental problem of localization, although there are some notable exceptions such as the formation of cortical patterns in Protozoa, or the beginning of polarity of the eggs of the brown alga, *Fucus*. Some of the best examples come from multicellular lower organisms and I would like to mention four cases which have certain basic similarities.

One example is found among the soil fungi. The first indication of spatial arrangements may be seen as a mycelium spreads through the soil (or an agar plate) for it is obvious that the advancing tips of the hyphae repel one another so that there is an optimal spacing of the feeding tips. This is apparently effected by a chemical which is given off by the growing hyphae that inhibits the growth of neighbouring hyphae and thereby causes their spatial distribution. The beauty of this system is that one has action at a distance in two dimensions and therefore it is admirably suited for experimentation. But besides this hormone-controlled spacing mechanism, there are a number of other such hormone effects. During fruiting in certain ascomycetes or basidiomycetes where there is a compound fruiting body (e.g. a mushroom) there can be specific sites where the hyphae are attracted to each other rather than repelled. The same phenomenon is seen in the hyphal anastomoses that produces heterokaryons. Finally when the mushroom itself forms there must be an internal system of gradients or patterns of substances which is responsible for the shaping of the fruiting body. In this case the morphogens need not act at a distance, but directly from cell to cell. It not only means that large molecules might be involved, but also more complex variations of simple diffusion mechanisms are possible. The gradients can be established by a mutual stimulation of morphogen production or suppression in cell–cell contacts of actively metabolizing cells. There is even evidence, in mushrooms, of a hormone produced in the primordial gill region which stimulates and controls the elongation of the stalk. So the pattern of the vegetative hyphae and the pattern of the fruiting body are controlled by morphogens which are themselves localized and in turn cause further localization of substances, in this instance by stimulating or inhibiting growth movements. The question of how the morphogen is asymmetrically distributed in the first

place can also be answered: its pattern was either passed directly from the previous generation, or due to some instability phenomenon; as Turing showed, an even distribution of a morphogen can break up into a non-uniform pattern. Finally, the fruiting bodies themselves may be spaced in relation to one another. This is again action at a distance by some morphogen: one fruiting body may inhibit the formation of others in its immediate vicinity. This then is a spacing between multicellular organisms: it is a localization, a pattern on the population or social level. To say that micro-organisms have many of the developmental capabilities of higher animals and plants is no exaggeration.

A very similar example of localization may be found in the cellular slime moulds. The feeding amoebae are separate and repel one another, but once the food is gone they form central collection points and the cells stream towards these points. Not only is the non-random distribution of these centres caused by an inhibitory morphogen, but the orientation of the cells towards the centres is caused by the diffusion gradient of a chemotactic agent (acrasin). Once the cell mass is formed there is a redistribution or sorting out of the cells with the result that there is a strictly proportional relation between the number of anterior cells which will form stalk cells and the number of posterior cells which will form spores. It is suspected that this difference is achieved by the unequal distribution of key substances, but the facts are still wanting. Finally the fruiting bodies, as they rise into the air, give off a volatile substance that affects the orientation. If two fruiting bodies rise close to one another they lean away from each other as they rise; if a single fruiting body is found in a confined place it will, by means of this volatile morphogen, orient itself so as to be precisely in the middle of the cavity or cleft. Again this shows a communication between multicellular individuals at a distance and it accounts for the fact that the fruiting bodies always stand at right angles from the substratum regardless of the orientation with respect to gravity, for they are too small to be affected by gravity.

Some of the earlier workers at the turn of the century pointed out the remarkable similarities between the formation of fruiting bodies in the multinucleate or plasmodial myxomycetes, the uninucleate, amoeboid cellular slime moulds which we have just described, and the prokaryotic myxobacteria. In the latter one has aggregation of bacterial rods by chemotaxis and in some of the more elaborate forms there is a differentiation of multicellular cysts. To these three one can add the soil fungi with their spreading mycelial network. One assumes that in each of these cases there must have been strong selection pressure for these

small fruiting bodies; they must have a form which ensures effective spore dispersal.

The fact that the cell building block can consist of a bacterial cell, a uninucleate amoeba, a syncytial plasmodium, or a rigid-walled hypha, and yet achieve basically similar kinds of evenly dispersed, small fruiting bodies, is indeed a remarkable fact. By having such an array of similar structures built in totally different ways, one has an ideal opportunity to study their comparative molecular anatomy. This can be done not only on their systems for the localization of substances during development, but on their systems of producing and timing the production of substances during development. If one now adds all the other diverse bacteria, fungi, algae and protozoa, the raw material available, among lower forms, for significant studies on development seems almost unlimited.

Note: Detailed examination of the points covered in this brief introduction, including references, will be found in a forthcoming book now in preparation.

THE BACTERIAL CELL CYCLE

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INTRODUCTION

The growth and development of all higher animals and plants involves the growth and division of their component cells. This growth and division may be accompanied by progressive cell differentiation as the initial cell line develops into the various tissues that comprise the whole organism. Nevertheless, since the growth and division of single cells is a common denominator in all such developmental sequences, it is appropriate to include studies of the cell cycle itself in any general consideration of differentiation.

In addition to the fact that the cell cycle is an obligatory component of most embryological development, it is also true that the growth of a single cell, from its creation at the division of its parent through the steps required to enable it to divide in its turn, is a process of differentiation. The newly formed cell differs from a cell about to divide not only in size but, as we shall see, in its composition, and this composition changes qualitatively in a fixed sequence throughout the cell cycle.

In general the process of cellular differentiation can be studied more easily in bacteria than in higher cells. *Escherichia coli* is probably the best understood organism in terms of its molecular biology and this makes the investigation of the cell cycle easier than in organisms where the nature of fundamental molecular processes, such as the regulation of gene activity, is still ill understood. We believe therefore that the investigation of the cell cycle in *E. coli* and other bacteria should be able to proceed much more rapidly than in other organisms. Although this process may prove to differ in some ways from that of higher cells (as it obviously must when the relative simplicity of the bacterial cell is considered) it will probably, like earlier work on the molecular biology of bacteria, prove illuminating and helpful to the investigation of eukaryotic systems.