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Fungal Differentiation

A Contemporary Synthesis

edited by
John E. Smith

FUNGAL DIFFERENTIATION

A Contemporary Synthesis

Edited by John E. Smith
Strathclyde University
Glasgow, Scotland

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Series Introduction

Mycology is the study of fungi, that vast assemblage of microorganisms which includes such things as molds, yeasts, and mushrooms. All of us in one way or another are influenced by fungi. Think of it for a moment – the good life without penicillin or a fine wine. Consider further the importance of fungi as pathogens to plants and to humans. Yes, fungi are ubiquitous and important.

Mycologists study fungi either in Nature or in the laboratory, and at different experimental levels ranging from descriptive to molecular and from basic to applied. Since there are so many fungi and so many ways to study them, mycologists often find it difficult to communicate their results even to other mycologists, much less to other scientists or to Society in general.

This series establishes a niche for publication of works dealing with all aspects of mycology. It is not intended to set the fungi apart, but rather to emphasize the study of fungi and of fungal processes as they relate to mankind and to science in general. Such a series of books is long overdue. It is broadly conceived as to scope, and should include textbooks and manuals as well as original and scholarly research works and monographs.

The scope of the Series will be defined by, and hopefully will help define, progress in mycology.

Paul A. Lemke

Preface

Fungi exhibit a bewildering array of forms, ranging from simple unicellular types such as *Blastocladiella* and yeast to the complex and macroscopic Basidiomycetes. Between these extremes are the filamentous mold fungi that exert such major influences on mankind by way of biodeterioration, toxicology, and industrial mycology.

Morphogenesis can be considered as the development of an organism to its specific form. How this form is achieved at a biochemical and cellular level in a wide range of fungal types is the subject of this volume.

To cover the field of fungal morphogenesis would require the writing of several voluminous treatises. However, by the skill and application of the contributors, a volume has been produced which is a concise and advanced analysis of this absorbing subject.

The contributors were asked to consider the state of the art of their specific subject and also to indulge in creative speculation for the future. In this way it is hoped that this volume will act not only as a source of reference for what has already been accomplished, but also as a guideline for future directions of research.

John E. Smith

Contributors

Knud Allermann, M.D. Senior Lecturer, Institute of Plant Physiology, University of Copenhagen, Copenhagen, Denmark

David R. Berry, Ph.D., M. Sc., M.A. Senior Lecturer, Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow, Scotland

Jean-Paul Bret, M.D. Laboratoire de Physiologie Végétale, Université Lyon 1, Villeurbanne, France

Edward C. Cantino, Ph.D., A.B. Professor, Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan

Mariette Carels, Research Assistant, Linor-Food Products Development Center, Nestlé Products Technical Assistance Company, Ltd., Orbe, Switzerland

Roderic Cooke, Ph.D., D.Sc. Reader in Mycology, Department of Botany, University of Sheffield, Sheffield, England

Kurt R. Dahlberg, Ph.D., M.S., B.S.* Postdoctoral Research Associate, Department of Plant Pathology, University of Nebraska - Lincoln, Lincoln, Nebraska

Roger Durand, M.D. Laboratoire de Physiologie Végétale, Université Lyon 1, Villeurbanne, France

Present affiliation:

*Project Leader, Microbiology Department, Ralston Purina Company Central Research, St. Louis, Missouri

Ruth Emyanitoff, Ph.D.* Research Associate, Department of Developmental Biology, Boston Biomedical Research Institute, Boston, Massachusetts

Karl Esser, Dr. Phil., Dr. h.c. Professor, Lehrstuhl für Allgemeine Botanik, Ruhr-Universität, Bochum, Federal Republic of Germany

Michel Fèvre, D.Sc. Laboratoire de Physiologie Végétale, Université Lyon 1, Villeurbanne, France

Graham W. Gooday, Ph.D. Reader, Department of Microbiology, Marischal College, University of Aberdeen, Aberdeen, Scotland

David Lloyd, D.Sc, Ph.D., B.Sc. Professor and Head of Department, Department of Microbiology, University College, Cardiff, Wales

James S. Lovett, Ph.D., A.B. Professor of Biology, Department of Biological Sciences, Purdue University, West Lafayette, Indiana

Gèrard Manachère, D.Sc. Professor, Laboratoire de Physiologie Végétale, Université Lyon 1, Villeurbanne, France

Friedhelm Meinhardt, Dr. rer. nat., Dipl. Biol. Hochschulassistent, Lehrstuhl für Allgemeine Botanik, Ruhr-Universität, Bochum, Federal Republic of Germany

Gary L. Mills, Ph.D. Visiting Assistant Professor, Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan

Peter C. Newell, M.A., D. Phil. University Lecturer, Department of Biochemistry, University of Oxford, Oxford, England

Jørgen Olsen, M.D., Cand. scient. Senior Lecturer, Institute of Plant Physiology, University of Copenhagen, Copenhagen, Denmark

John F. Peberdy, Ph.D., B.Sc. Reader in Plant Microbiology, Department of Botany, University of Nottingham, Nottingham, England

Gerard Pierron† Research Associate, Zoologisches Institut, Universität Würzburg, Würzburg, Federal Republic of Germany

Present affiliations:

*Senior Staff Scientist, Technology Assessment, Genex Corporation, Rockville, Maryland
†Biology Department, Texas A & M University, College Station, Texas

J. I. Prosser, Ph.D., B.Sc. Department of Microbiology, Marischal College, University of Aberdeen, Aberdeen, Scotland

Jean-Claude Robert, D.Sc. Laboratoire de Physiologie Végétale, Université Lyon 1, Villeurbanne, France

Peter J. Rogers, Ph.D., M.Sc., B.Sc. Senior Lecturer, School of Science, Griffith University, Brisbane, Queensland, Australia

Geraldine M. Russo, M.S., B.S. Research Assistant, Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, Nebraska

Helmut W. Sauer, Dr. rer. nat.* Professor, Zoologisches Institut, Universität Würzburg, Würzburg, Federal Republic of Germany

David Shepherd, Ph.D., M.Sc., B.Sc. Section Manager, Linor-Food Products Development Center, Nestlé Products Technical Assistance Company, Ltd., Orbe, Switzerland

John E. Smith, Ph.D., D.Sc., M.Sc., B.Sc., F.R.S.E., F.I. Biol. Professor and Chairman, Department of Bioscience and Biotechnology, Strathclyde University, Glasgow, Scotland

Peter R. Stewart, Ph.D., B. Agr. Sc. Reader in Biochemistry and Microbiology, Department of Biochemistry, Faculty of Science, Australian National University, Canberra, Australia

Gilbert Turian, D.Sc. Professor, Laboratory of General Microbiology, Department of Plant Biology, University of Geneva, Geneva, Switzerland

Herman van den Ende, M.D. Professor, Department of Plant Physiology, University of Amsterdam, Amsterdam, The Netherlands

James L. Van Etten, Ph.D., M.S., B.A. Professor, Department of Plant Pathology, University of Nebraska - Lincoln, Lincoln, Nebraska

Barbara E. Wright, Ph.D.† Director, Department of Developmental Biology, Boston Biomedical Research Institute, Boston, Massachusetts

Present affilations:

*Biology Department, Texas A & M University, College Station, Texas
†Research Professor, Department of Microbiology, University of Montana, Missoula, Montana

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1
Concepts of Fungal Differentiation

GILBERT TURIAN *University of Geneva, Geneva, Switzerland*

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I. GENERAL DEFINITIONS

Morphogenesis can be defined as the development of an organism to its specific form. This implies differentiation of its cells into types differing in their shape, size, structure, and chemical composition. In unicellular forms, internal differentiation also results in morphogenetic processes leading to a change in form. Thus endospores in bacteria or asci in yeasts are obviously different in form from the normal vegetative cells (Smith and Berry, 1974). Such cases of individual cells exhibiting differences in structure and function at different stages of their life cycle are examples of time-dependent or *temporal* differentiation, in contrast to the more usual *spatial* differentiation typical of conidiogenous fungi, not to mention embryos (Bonner, 1974).

The connection between cell division and cell differentiation is not only a considerable theoretical problem, but also quite a technical nuisance, for it is often difficult to distinguish between events which are really a consequence of differentiation and those that are a consequence of the cell cycle when both sets of events are occurring simultaneously. These difficulties can be overcome with the cellular slime mold, because in its life cycle growth and differentiation are mutually exclusive phenomena (Ashworth, 1973). Another attractive feature of this eukaryotic microbe is that it can separate cellular differentiation (spores plus stalk cells) from morphogenesis (culmination into a

sorocarp), as has recently been shown in an aggregateless mutant (Ishida, 1980).

It is generally believed that the process of differentiation is associated with the activation of different genes in different cells at different stages of development. Controls of gene functioning can intervene at *both* transcriptional *and* translational levels; in the latter case the process would proceed only until a specific "inducer" (or stimulator; see Gross, 1968) arrived which would induce at the translational level. Indeed, where hormones appear to control differentiation by influencing transcription, translation-level controls have been shown to play an important role in the orderly expression of hormone-induced changes, as stated by Gross (1968), who provided a few examples drawn from animal cells, as well as from the evolution of the patterns of synthesis and decay during differentiation of cellular slime mold (timing of relevant transcriptional and translational events).

Experimental facts generally suggest that cells become committed to a particular differentiated state before the existence of that state is detectable. The high degree of fixity in visible, final differentiated states would thus contrast with the lability, gradually reduced with time, of the initial determination process. However, there is no evidence, experimental or logical, in favor of the idea that commitment to a cause of change involves molecular mechanisms distinct from the final realization of that change. Hence, according to Gross (1968), the biochemical analysis of determination must be a part of the analysis of differentiation, which may differ from determination only in that some change in affected cells becomes more easily detectable and appears to be reversible only with difficulty.

The question arises as to whether even a complete biochemical description of the pathway to terminal chemodifferentiation could contribute to an understanding of the shape changes and movements of cell groups implicated in morphogenesis. Analysis of the supra- and inframolecular forces acting on cells with that of biosynthetic controls could contribute to such understanding, but "the visible events of morphogenesis might be a spontaneous consequence of biochemical differentiation, creative of differential cell surface properties, adhesiveness, motility, response to the environmental composition" (Gross, 1968).

A most restrictive and operational definition of differentiation depends upon foreknowledge of the adult condition of cell types. The study of their cytodifferentiation requires identification of a particular biochemical pathway, a catalytic or structural macromolecule, or a morphological feature uniquely characteristic of the adult cell type (Gross, 1968). A relevant case in fungi is that of the male cells in the sexual species of *Allomyces*, which elicit the bright yellow color of γ-carotene indicative of a selective deviation of terpenoid biosyntheses toward unsaturated carotenoids at their level (Turian, 1969).

Assuming that whole differentiation embracing the succession of determining, differentiating, and terminally differentiating systems is the result of differential gene action permitted Gross (1968) to establish the following categories of the process of gene action at differentiation: replication, transcription, intranuclear processing, and translation, beyond which is "epigenetic control."

More recently, a differentiated cell has been considered as poised in a state of dynamic equilibrium between the nucleus and cytoplasm, in which signals from the cytoplasm are necessary for the maintenance of the nuclear gene expression which characterizes that state (Maclean, 1977). The limited number of possible types of differentiated cells in a complex organism implies that a very limited number of "tissue-master" genes may exist, each one responsible for the release of a particular genetic program. This conception of differentiation (Maclean, 1977) provides an easy understanding of the distinction between determination and differentiation. The first is the assumption of the stable state between the nucleus and cytoplasm, which involves choice of one master gene, while the second is the actual expression of the program under the direction of that gene. Clearly, additional factors may often be necessary in order to release the activity of an already selected master gene. The factors controlling and determining the activity or inactivity of tissue-master genes are presumably often cytoplasmic, may well be partitioned during embryonic cell division, and are really the decisive factors in cell differentiation. Their identity remains a mystery (Maclean, 1977).

Essential as selective gene activation and sequential genome readout are for differentiation, they necessarily interplay with other equally important extranuclear components and processes. Wright (1973) has explored the consequences of metabolite availability on the process of differentiation and has proposed that in primitive systems such as slime molds, changes in the rate of availability, or flux, of endogenous metabolites could control the activity of essential metabolic pathways essential to differentiation. This would involve, for example, the rate of substrate-limited reactions critical to differentiation; the concentration of enzymes, by changing their rate of synthesis (induction) or degradation (stabilization); enzyme activity, by allosteric modulation and end-product inhibition; and the duration and stability of differentiated structures. From such critical variables, Wright (1973) has been led to construct kinetic models which simulate long-term change in metabolism (e.g., carbohydrates in *Dictyostelium*) such as occur during differentiation.

Differentiation can be studied at several levels of structural and functional organization. Even in prokaryotic organization it is possible to recognize some anticipation of the process of eukaryotic differentiation. Thus the switching on and off of bacterial operons constitutes an altered commitment on the part of the cell and its metabolic machin-

ery in response to the changing environment. By contrast, both division and sporulation demand an even closer commitment to a particular pathway.

Two cells are differentiated from each other if they have the same genome but the pattern of other molecules which they possess and synthesize is different. It follows from this statement that very simple cells may have a greatly reduced potential for differentiation. Indeed, a population of cells might exist in which the genome was so simple that no cell could possibly survive without constantly expressing the full genetic complement. Such cells probably do not exist, but certainly within populations of bacteria the possibilities of differentiation are severely limited. Some bacteria may be induced by a substrate to transcribe a particular operon, while their sister cells, not exposed to the substrate, fail to express these genes. But such differences in metabolism are temporary and do not involve gross changes of cellular morphology. We must therefore try to distinguish between changes of an adaptive type, which are simply part of the normal cell cycle, and changes which lead to more permanent differences between cells in the same population.

As selective gene activation might not be the only way to control differentiation, a scheme of progressive repression of previously active genes has appeared as most consistent, with observations at least from experimental embryology, as well as from more recent biochemical experimentation (Caplan and Ordahl, 1978). How far the model proposed, in which each initial pluripotent embryo cell progressively becomes irreversibly restricted to the expression of a single phenotype, can be transposed to sporulating fungi remains conjectural. As yet, the "activation-repression" rather than the "irreversible repression" mechanism seems to fit most generally to the several progressive sporulation types of differentiation. According to the first mechanism, genes that have not previously been transcribed are activated (or derepressed), and their transcripts and subsequent translational products appear for the first time in the newly differentiated spore; part, at least, of the previously transcribed "vegetative genes" are then repressed. The newly available plasmid technology or "genetic engineering" to obtain phenotype specific probes should help to further assess the value of that conceptual framework concerned with the role of genes in differentiation (Paul, 1971). Such an approach involving the cloning of selected pieces of DNA (specific gene isolation) has been aimed at a better understanding of the temporal control of genes turned on during the yeast cell cycle (Halvorson, 1977) and is currently being further tested in sporulating filamentous fungi (Dutta et al., 1981).

Reversibility of differentiation is evident in microorganisms. Thus spores do change back into normal, dividing, vegetative cells by the process of germination. Should spore formation, then, be considered

a process of adaptation rather than of differentiation? Either case could be argued according to Pasternak (1970), who takes the following point to consider sporulation (or sporogenesis) as a differentiation process:

> One of the stimuli for the formation of bacterial spores is the exhaustion of nutrients. If nutrients are restored to the culture immediately after depletion, spore formation does not take place; the culture is in the reversible, adaptive state. But if nutrients are added some time after spore formation has already begun, sporulation continues despite the presence of nutrients. In other words, cells are now *committed* to sporulation. Likewise spores become committed to germination on the return to more favourable conditions. It is the presence of a commitment stage, also referred to as *determination* during embryological development, which allows one to distinguish between differentiation and adaptation.

Differential gene expression, although no doubt important in cell differentiation, does not, of itself, explain how the initial commitment is made. This initial commitment need only involve a choice between perhaps a hundred or so separate programs of gene expression. The striking similarity of different cells of the same differentiated type probably reflects the identical nature of the program selected within them (Maclean, 1977).

From the above considerations, we can consider differentiation as the stable development of altered structure and function. Its stimulus is sometimes environmental, as in the formation of spores. Agents that cause it are known as initiators or inducers and their action as *initiation* or *induction* (Pasternak, 1970). Biochemical differentiation is a manifestation of an altered synthesis of specific proteins; these function either as structural units, such as actomyosin of muscle or other motile cells, or as enzymes catalyzing the synthesis and transport of a variety of molecules.

Differentiation is generally irreversible in animals (liver cells cannot change back to an ovum, etc.). It is, however, not always based on the loss of various potentialities by the cell; these particular potentialities can merely be masked by others. This is well exemplified in higher plants, in which certain cells in the phloem of the carrot are capable of *dedifferentiation*, with eventual production of a complete new plant; moreover, many differentiated plant tissues lose much of their diagnostic characters when grown in tissue culture (Gautheret, 1966). In germinating spores of fungi, dedifferentiation first occurs during the isometric growth (Turian, 1969), followed by redifferentiation into a germ tube that becomes a new vegetative hypha.

Regarding this question of the *stability* of differentiation, we could follow the suggestion of Abercrombie (1967), that with his kind of

approach in terms of the epigenotype, "one is relieved of the necessity of postulating any sort of stability of differentiation: the stability is in the epigenotype and the differentiation is the phenotype; and this can fluctuate and change." The complicated term *epigenotype* could, however, be avoided if we use Gurdon's (in Abercrombie, 1967) following simple assumption:

> ...in any differentiated cell the products of its genes continually influence these same genes to be active; consequently, any cell will necessarily stay the same until something changes it. This would account in early development for how the different cytoplasmic substances cause nuclei in different parts to behave differently, and we have many examples of induction where we have only to suppose that the influences coming from outside interfere in some way with this continual process.

II. FUNGAL DIFFERENTIATION

Fungi as simple eukaryotic organisms have been considered as favorable for studies of differentiation (Turian, 1969; Smith and Berry, 1974), as they provide relatively simple morphological systems which are genetically controlled and amenable to biochemical investigation.

A. Dimorphism

Dimorphism corresponds to the easily reversible alternative between a mycelial (M) and a yeast (Y) phase, as shown by many fungal types exposed to certain physical (temperature, etc.), chemical (O_2-CO_2 tensions, sugar concentration, etc.), or environmental factors (Stewart and Rogers, 1978). It does not, however, fully meet the basic criteria of differentiation. At the limit, it could be viewed as an adaptation in Pasternak's sense or environmental modulation (Smith and Berry, 1974). It could rather be considered as a relatively simple morphogenetic event, as it involves a contrasted change in the shape of growth, from the normally anisometrical, polarized, cylindrical type of the standard fungal hyphae, to an isometrical, spherical or subspherical yeast type with its unpredictable peripheral budding of new spherical or ellipsoidal cells.

The changed vegetative shape has initially been explained by Nickerson's group (Baldwin and Rusch, 1965) as being due to chemical changes in the macromolecular components of the cell wall (disulfide cross-linking of a manno-protein complex and/or changes in the polysaccharide components). However, primary disturbance in the polarity of growth has more recently been envisaged: The wall-making vesicles could no longer be polarly directed toward the apex of a normally elongating hypha (Bartnicki-Garcia, 1981). A newly differentiated

structure found during the isodiametrical growth phase of spore germination is the vegetative wall, which is deposited anew under the existing spore wall around the germ sphere. In this reconversion Y → M, the initial appearance of a hyphal tip from the yeast form can be considered as homologous to the appearance of the germ tube from a spore. It then becomes crucial to investigate further the transitory processes initiating polarized growth of the germ tube emerging from swollen (initial spherical growth) spores.

B. Polarity and Gradients

There is no differentiation without *polarity* (Bloch, 1943; Bünning, 1952; Sinnott, 1960). Such a statement, that differentiation is impossible without protoplasmic polarity, is based not only on theoretical considerations, but also on experimental facts. Polarity is indeed the first step in the differentiation of an egg cell or a spore. The eggs of *Fucus* and other marine algae that are discharged into the surrounding medium, as well as the spores of mosses and ferns, are unpolarized at first. Their polarization is the result of the unilateral influence of external factors, such as light, gravity, or substratum (Bonner, 1974).

A specific protoplasmic structure providing the necessary physical asymmetry has been assumed to be the ultimate cause of polarity (Bünning, 1952). Such structural asymmetry may then lead to chemical gradients and electrical potential differences. This last has also been ascribed to the selective entrance of positive ions (Ca^{2+}) across the plasma membrane of the prospective pole (Jaffe and Nuccitelli, 1977). Stability of polarity during dormancy periods can be ensured by the asymmetry of protoplasm, despite momentary cessation of all the metabolic energy-yielding processes necessary for the maintenance of the gradients. As a consequence, it can be shown that visible polarity can only be temporarily reversed by its overcompensation by changing external (centrifugal, electrical, hormonal, etc.) factors. The concentration gradients produced by polarity offer the requisite ground for intercellular differentiation following unequal division, provided that the new cell wall is at right angles to the axis of polarity. A well-known example is the division in pollen grains, with their mitotic spindle polarity oriented along a gradient of protoplasmic density (Bünning, 1952) probably corresponding to the gradient distribution of ribonucleoprotein (Browder, 1980).

The origin of *spatial* patterns therefore appears to be related to the existence of physiological gradients (Child, 1941) providing a basis for difference in gene action. The recognition of such an important role of gradients finally led to the concept of positional information developed by Wolpert (1971), which implies the existence of a coordinate system within which all positions are specified. This results in a spatial differentiation that precedes, and is independent of, mole-