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Recent developments in morphogenetic studies of higher fungi

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Introduction

Study of plant and animal morphogenesis is well developed, but fungal developmental biology is grossly under-researched. Although fungi have been used as experimental organisms for some exciting research in biochemistry and molecular genetics (including cell differentiation), study of their multicellular structures contributes little to the main stream of research and thinking in developmental biology.

Higher fungi produce some extremely complex structures and their study is well worth pursuit. In the purely scientific sense, plants, animals and fungi are now seen as three quite distinct Kingdoms of eukaryotic organisms which must have separated in evolution at some protistan level long before the multicellular grade of organization was established. Comparative analysis of cell differentiation and pattern formation processes can reveal common strategies and conserved pathways, as well as alternative mechanisms. In a more utilitarian sense, many fungal pathogens employ multicellular structures in their infection, dispersal and survival strategies, whilst the commercial value of cultivated fungi obviously depends on their morphogenetic ability. Understanding the architecture and construction of fungal multicellular structures may lead to improved control and cropping methods.

Parallels between the morphogenesis of fungi and of higher organisms are worth seeking out so as to make use of the conceptual framework which has been established in the hope of formulating developmental models which can spur fresh experimentation with fungal material. However, the fundamental structure of the fungal cell differs in many important respects from animal and plant cells so significant differences might be anticipated in the way that the different cell types interact in the construction of organised tissues.

The origin of multicellularity

Fungal mycelia are capable of forming many different multicellular structures, each with its different function, ranging from sclerotia, strands, rhizomorphs and stromata, through to ascomata and basidiomata. Once the commitment is made to a particular fungal developmental pathway the major tissue domains are established very quickly. For example, in the basidiomycete *Coprinus cinereus,* a median section of a fruit body initial only 800 μm tall reveals clear differentiation into cap and stipe (Moore *et al.* 1979; Fig. 1) even though such an initial is only 1% of the size of the mature fruit body. It is this sort of example which brings to mind the analogy with animal embryology where the basic body plan is established very early in development of the embryo. The embryological analogy has not been far from the surface of mycological discussion for many years (see Burnett 1968, pp. 112-113). Ross (1979, pp. 257-258) even suggests that the vegetative mycelial growth phase of the fungus could be likened to a continuous embryonic blastula condition, the advent of differentiation into multicellular structures being analogous to gastrulation.

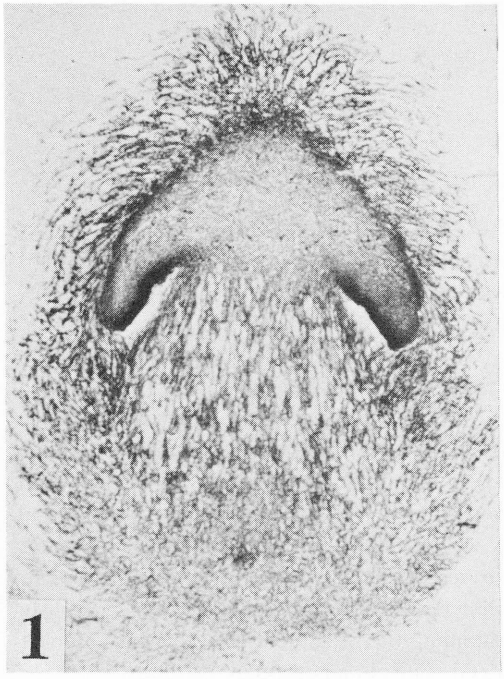

Fig. 1 Vertical section of a fruit body initial of *Coprinus cinereus* that was 0.8 mm tall when harvested. Note the clear mushroom-like organisation even though this specimen is only about 1% of the mature size.

Development of any organised structure in fungi requires reversal of the normal growth behaviour of the invasive, vegetative mycelium so that hyphae can grow towards one another, co-operating in formation of the differentiating organ. Although some light is being shed on the kinetics and characteristics of the altered growth and branching patterns involved (Prosser 1988) we are almost totally ignorant of the controlling elements which direct this change in the fundamental growth pattern of the hyphae. Autotropisms are known as phenomena, some sex-hormones are known in lower aquatic and terrestrial fungi, and detailed information has been obtained on molecules determining mating-type specific agglutination in yeasts; but that is about the limit of current knowledge (Moore 1984a). It seems likely that information about

the surface chemistry of hyphae will be prerequisite to any attempt to explain how they can co-operate in the formation of organs like the fruit body, sclerotium or rhizomorph. Indeed, fungi provide an extremely good system with which to study cell-to-cell tropisms and specific cell-to-cell adhesion since they change from one state to another as a part of their normal development (Reijnders & Moore 1985). Most models for cell surface interactions feature plant or animal cells cultured in unnatural conditions.

It is at, or immediately following, this step-over from the invasive vegetative mycelial to the co-operative protenchymal mycelial tuft (constituting the initial or primordium of whatever structure is arising) that developmental comparisons with other organisms can be made. Reference to basic reviews of animal egg development (e.g. Slack 1983) suggests that the component mechanisms in the overall process can be characterised as: development form a single cell; formation of inhomogeneous cell populations from homogeneous ones; regional specification of tissues (pattern formation) directed by organizers producing morphogens; specification and commitment of particular cells to particular fates; cell differentiation; and regulation of gene activity in ways specifically geared to morphogenesis. These phrases describe the major phenomena concerned in morphogenesis in both animals and plants. The following discussion will consider what evidence exists for such mechanisms in the development of fungal structures.

Fungal cells real or imagined?

The majority of the lower fungi have coenocytic hyphae; but then the lower fungi do not form multicellular structures. Fungal primary septa are formed in association with nuclear division (Talbot 1968) by a constriction process in which a peripheral belt of microfilaments interacts with microvesicles and other membranous cell organelles (Girbardt 1979). Girbardt (1979) emphasises the correspondence between fungal septation and animal cell cleavage. The completed septum has a pore which may be elaborated with the parenthesome apparatus in basidiomycetes or be associated with Woronin bodies in ascomycetes; in either case movement of cytoplasmic components between adjacent cells is under effective control. The cellular structure of the hypha cannot be doubted, at least to the extent of its being separated into compartments whose interactions are carefully regulated and which can exhibit contrasting patterns of differentiation. Furthermore, as stated by Griffin *et al.* (1974), in mycelial fungi branch formation, by increasing the number of growing points, is the equivalent of cell division; and the kinetic analyses of Trinci (1974, 1979, 1984) show clearly that fungal filamentous growth can be interpreted on the basis of a regular cell cycle. Thus, fungi can quite reasonably be considered to be cellular organisms producing differentiated tissues composed of cells which are the progeny of an initial cell or cell population which is induced to start multiplication and differentiation.

The crucial evolutionary steps permitting organised multicellularity were the development of mechanisms for dividing the cell and for controlling the placement of the plane of cell division in particular relation to the orientation of nuclear division.

Fungal cytokinesis

Common to normal morphogenesis in animals and plants alike is the concept of cellular polarity and the developmental consequences of precise positioning of he plane of cleavage (in

animals) or wall formation at the cell plate (in plants). The classic examples of embryology in both groups of organisms include instances of asymmetric divisions partitioning 'stem' cells in ways which result in the daughter cells expressing some sort of differentiation (not necessarily immediately expressed) relative to one another.

The characteristic fungal growth mode is, of course, the extreme cellular polarity which results from the apical growth of the fungal hypha. A comparison which immediately emphasises the fundamental difference between plants and fungi in their mechanism of generating 2- or 3-dimensional structures is the development of the protonema following bryophyte and pteridophyte spore germination. Here, a uniseriate filament of cells is formed as a result of the constraint of new cell walls to an orientation perpendicular to the long axis of the protonema. This growth pattern persists for a time which can be influenced by environmental and cultural conditions (Mohr 1964; Miller 1968), but eventually, and usually in the apical cell of the protonema, the division plane becomes reoriented so that the new cell walls are formed obliquely or parallel to the long axis of the protonemal filament and a flat plate of cells (the gametophyte prothallus) is formed. This transition, analysed in detail by Miller (1980), epitomises the importance in plant morphogenesis of the mitotic orientation but it is totally alien to the fungal approach to solution of the same problem.

Crosswalls in fungal hyphae are rarely formed other than at right angles to the long axis of the hypha. Except in cases of injury or in hyphal tips already differentiated to form sporing structures, hyphal tip cells are not subdivided by oblique crosswalls, nor by longitudinally oriented ones. Even in fission yeast cells producing irregular septation patterns under experimental manipulation, the plane of the septum was always perpendicular to the plane including the longest axis of the cell (Miyata *et al.* 1986). In general, then, the characteristic fungal response to the need to convert the 1-dimensional hypha into a 2-dimensional plate or 3-dimensional block is the formation of branches. Even casual observation shows that branching patterns differ greatly between the vegetative state and the multicellular structure. For example in the gill tissue of the *Coprinus cinereus* basidiome, successive branches may emerge from subhymenial hyphae to form hymenium elements at intervals of less than 5 μm, whereas the same dikaryon, growing as a vegetative mycelium on the surface of agar medium, forms branches at average intervals of 73 μm (Horner & Moore 1987).

In plants, morphogenesis depends on placement of the crosswall, in fungal hyphae the general absence of longitudinal walls means that the position of the branch tip must be specified and the new apex must have extended to some extent before the parental cytoplasm can be subdivided. Consequently, there are two fundamental processes involved in construction of fungal multicellular structures; the first is the origin of the branch (its appropriate placement and orientation on the parent hypha) and the second is the direction of growth of the new hyphal apex which is created by the branching event.

The former process seems to be the formal equivalent of determination of morphogenetic growth by orienting the plane of division and the new crosswall as is seen in plants, and the latter has much in common with the morphogenetic cell migrations that contribute to development of body form and structure in animals (as, for example, in the outgrowth of the growth cones of embryonic nerve fibres). Viewed in this light, therefore, the fungal Kingdom is seen as employing morphogenetic processes which have affinities with both of the other major kingdoms.

The initial orientation of the new growth axis is decided by the position of the new hyphal tip of the emerging branch and must, in turn, be specified by some prior intracellular event. Electrical current flow may be a component of this. Initial studies with *Achlya* (Kropf *et al.* 1983, 1984; Gow *et al.* 1984; Kropf 1986) revealed an ion current caused by influx of protons at the hyphal tip (as an amino acid symport in this organism) and their efflux from distal regions of the hypha. Generation of similar ion currents now seems to be a universal characteristic of fungal growth (Gow 1984) and related phenomena occur at the sites of pollen tube outgrowth from pollen grains and rhizoid emergence from *Pelvetia* zygotes (Nuccitelli 1978; Weisenseel & Kicherer 1981). The significant point is that, in *Achlya,* branch formation is preceded and predicted by a reversal of current and in young mycelia of several fungi applied electrical fields affect the site of branch formation and the direction of hyphal growth (McGillivray & Gow 1986); in *Neurospora crassa,* as well as orienting branch formation (causing branches to be formed on the anodal side) applied fields increased the rate of branch formation.

Septation in the main hypha is defined in some way by the position of the dividing nucleus (Girbardt 1979), but, for branch formation, there does not seem to be any dependence on orientation of the spindle during nuclear division. Existing observations suggest most strongly that the distribution of microvesicles has a close connection with branch initiation. Accumulations of cytoplasmic vesicles occur in growing hyphal apices (Grove 1978); during initial outgrowth of clamp connections of *Schizophyllum commune* these vesicles are displaced in the direction of curvature of the clamp cell soon after its emergence (Todd & Aylmore 1985); and localised accumulation of microvesicles has been suggested as a direct cause of branch initiation (Trinci 1978). The exact causal event is obscure, though it seems unlikely that electrophoretic transport via the transhyphal electrical fields is of great significance. Evidently, a considerable metabolic and ultrastructural architecture (including microtubule assemblies) is involved in the process of branch initiation.

Although the orientation of the branch tip decides the initial direction of the new growth, the hyphal tip is an invasive, migratory structure. Its direction of growth subsequent to initial formation determines the nature and relationships of the cells the hypha will form. This is clearly seen in hymenial layers which often appear to be constructed from branches which curve 'upwards' from the subhymenial tissues 'below' (Fig. 2). Images like this imply tropic response to something which may be located in the fluid (gaseous or liquid) above the protohymenium. In other cases surface or contact reactions seem to be operating, as is evident in structures constructed from closely appressed axially-arranged hyphae such as coremia, strands, rhizomorphs and ascomal necks (Rayner *et al.* 1985; Read 1983; Read & Beckett 1985). Indeed, since even the most open fungal tissues appear to be filled with an extracellular mucilaginous material (Williams *et al.* 1985), reactions akin to the cellmatrix interactions of animal tissues may guide and co-ordinate hyphal growth in tissues. Different tissues may synthesise and secrete specific polysaccharides and/or glycoproteins and provide an environment within which hyphae interact with great specificity.

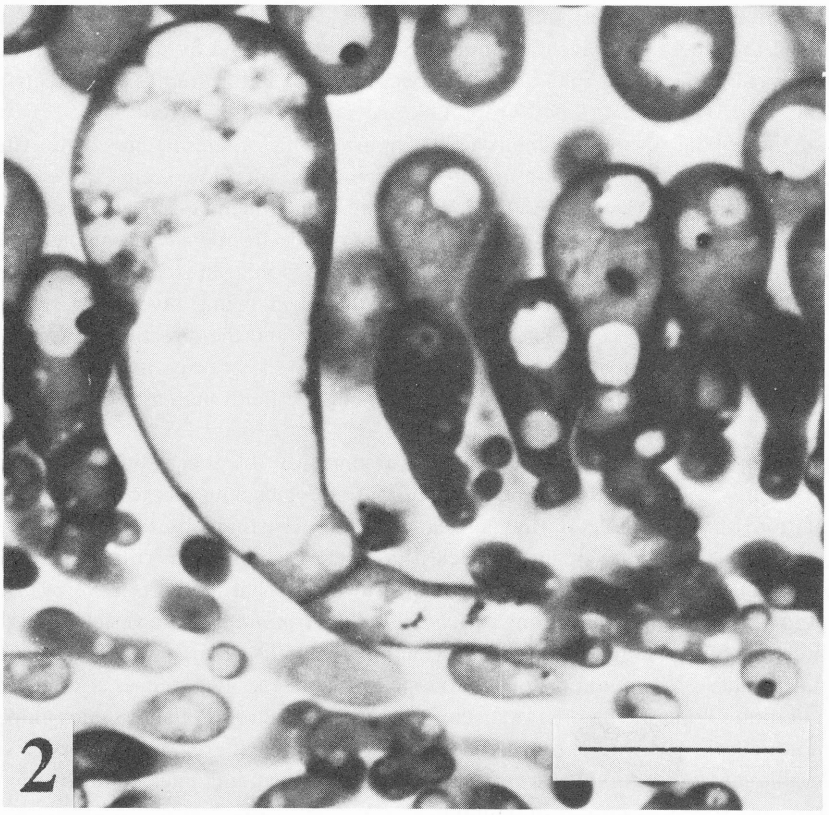

Fig. 2 Light micrograph of a section of the hymenium of *Coprinus cinereus*. The large cell is a young cystidium; note how it is curved towards the gill space above the hymenium. Scale bar = 5 μm.

Development from single cells?

Repeated suggestions have been made over the years that mushroom fruit bodies arise from single cells, but most of the illustrations are far from convincing (Fig. 3a & b). More recent research has shown that the initiation pathways of fruit bodies and sclerotia are genetically related (Moore 1981a) and both involve aggregation of cells from different sources as a result of hyphal congregation (Matthews & Niederpruem 1972; Waters *et al.* 1975; Van der Valk & Marchant 1978; Fig. 3c-f). This process of congregation readily accounts for the formation of chimeric fruit bodies. Kemp (1977) described how fruit bodies of *Coprinus* formed on horse dung collected in the field but incubated in the laboratory could consist of the two species, *C. miser* and C. pellucidus. The chimera extended throughout the fruit body as both species could be recovered by outgrowth from stipe segments placed on nutrient medium and the hymenium comprised a mixed population of basidia bearing the distinctive spores of the two species. Thus, fungal fruit bodies (unlike animal and plant embryos) do not normally consist of cells which are the progeny of a single progenitor but are assembled from contributions made by a number of co-operating hyphal systems which can be genetically distinct.

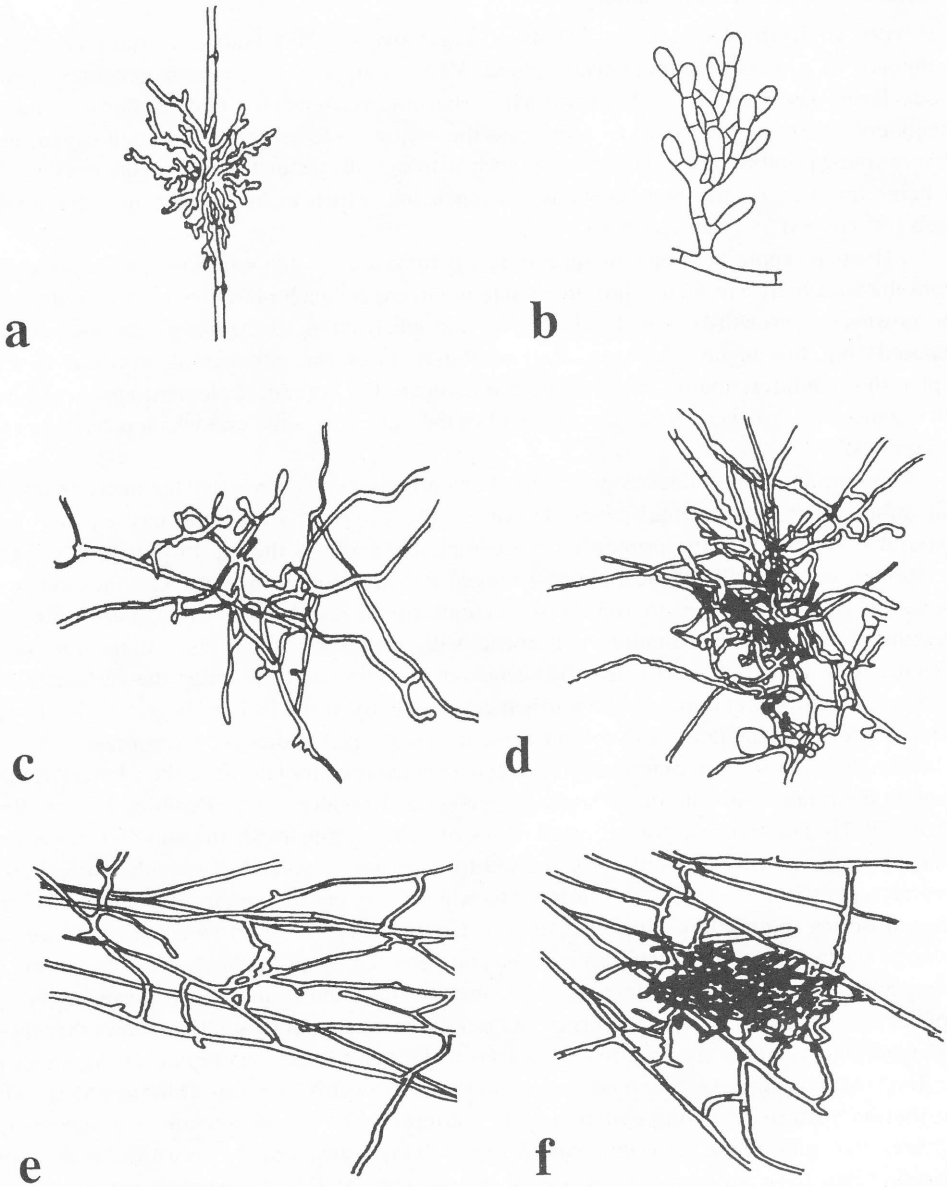

Fig. 3 Drawings depicting initiation of fruiting structures in *Coprinus*; a (Brefeld, 1877) and b (Chow 1934) claim to show how fruiting bodies arise from single cells; c & d were traced from photographs showing successive stages in development of a sclerotium (Waters *et al.* 1975), and e & f from photographs of fruit body initiation (Matthews & Niederpruem 1972).

Evidence for regional specification

The very young hyphal tufts or initials of vegetative or fruit body structures are simply composed of a mass of protenchymal tissue. Very soon, however, inhomogeneities appear; tissue layers involved in rapid cell formation become recognizable (called meristemoids by Reijnders 1979) and they quickly demarcate the major tissue layers of the adult organ, even in very young primordia (Fig. 1). To create such histologically distinct regions some organization is being imposed on the homogeneous 'protenchyme' which makes up the undifferentiated flesh (or 'context') of the fruit body.

The main spore-bearing structures of agaric fungi are the gills which are plates suspended from the fruit body cap tissue. Intuitively one might expect such plates to develop and extend by 'downward' growth of the distal edge of the gill (that is, the edge which is eventually exposed) but this in not the case. Rather, the roots of the gills extend into the steadily replenished undifferentiated tissue of the cap context. The formative element appears to be a gill organizer in the tissue at the extreme end of the gill cavity – the end which is buried in the cap context.

The direction of gill development has been a matter of controversy for many years and still awaits strict experimental proof. However, the *Coprinus* fruit body has a particularly favourable geometry as the primordial cap completely encloses the top of the stipe; the gills are formed as essentially vertical plates arranged radially around the stipe and their mode of formation can be followed in transverse sections of the whole fruit body. Such a section presents the cap as an annulus concentric with and surrounding the stipe, the inner circumference of the annulus being the surface of the stipe and the outer the surface of the cap tissue. These circumferences obviously increase greatly as the fruit body grows: the former shows a 9-fold and the latter a 15-fold increase as typical fruit bodies grow from one to 34 mm in height. This has some geometrical consequences since primary gills have their inner, tramal, tissue in continuity with the outer layers of the stipe (Reijnders 1979; Rosin & Moore 1985; Moore 1987). The intimate tramal connections of primary gills (with the stipe interiorly and with cap tissue exteriorly) would be expected to be prone to widening as both cap and stipe circumferences (topologically, the surfaces to which hymenia of primary gills are anchored) increase during maturation. However, mature *Coprinus* gills are narrow so this tendency to widen as the circumference increases must be compensated by gill replication i.e. formation of a new gill cavity and its bounding pair of hymenia *within* the trama of a pre-existing gill to produce a Y-shaped 'replicating structure. Observation of fruit body sections shows that these Y-shaped gill structures are oriented exclusively as though the new gill organizer originates at the level of the stipe surface and moves outwards towards the cap (Moore 1987). The distribution pattern of cystidia-inflated cells scattered over the hymenium, see below-also indicates that gills in the *Coprinus cinereus* fruit body grow radially outwards, their roots extending into the undifferentiated tissue of the cap context. The formative element appears to be in the tissue at the extreme end of the gill cavity where the change in structure occurs from the randomly intertwined hyphal context with large intercellular spaces to the highly compacted hymenial plates separated by the gill cavity.

Fig. 4 shows a series of successive serial sections which demonstrate the progress of development of a secondary gill in *Coprinus cinereus* (from Rosin & Moore 1985). The group of cells at the top of the gill cavity represents a transitional arrangement between the

protenchymal tissues of the undifferentiated cap (called the context) and the hymenial arrangements of the gills (the true hymenophores) which separate cap and stipe tissues. One can presume that there is an organizer in the vicinity of each gill arch which is responsible for the progression of the gill morphogenetic field outward, away from the stipe. Formation of the secondary gill (Fig. 4) is noteworthy for its implication that gill organizers arise set distances apart; only when the distance increases can a new organizer arise. This set of images gives presumptive evidence for two classic components of theoretical morphogenesis. On the one hand we can hypothesise radial diffusion of an activating signal which assures progression of the gill; on the other hand we may have tangential diffusion of an inhibitor which prevents formation of new organizers until the extent of the tissue domain exceeds the effective range of the inhibitor – at which stage a new organizer can arise in response to the radial activating signal. Note that interaction between the presumed gill organizer activator and the presumed gill organizer inhibitor is all that is necessary to control the spacing, number, thickness, and the perfectly radial orientation of gills.

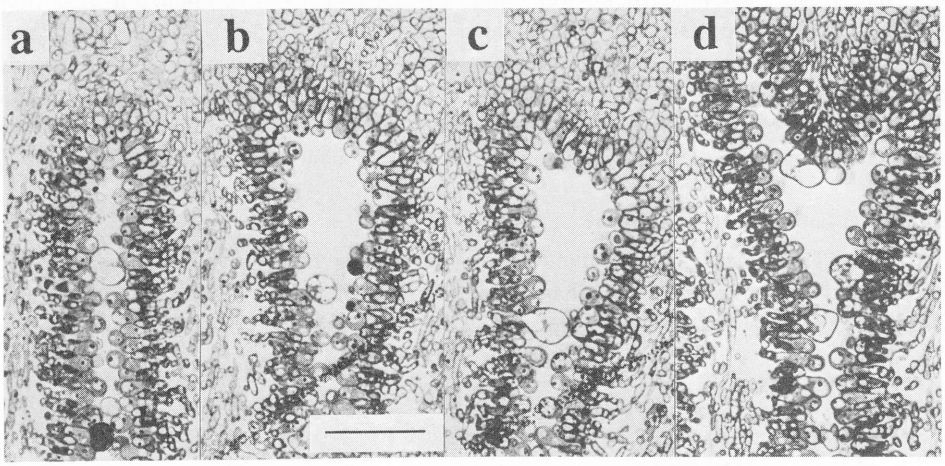

Fig. 4 Light micrographs of serial sections showing the origin of a secondary gill in *Coprinus cinereus*; a shows the original condition and b & c show the widening and lobing of the gill arch which eventually gives rise to the forked gill space shown in d. Scale bar = 20 μm.

Other evidence for local control of morphogenesis, this time of differentiation of a specific cell type, has been obtained from a comparison of the distributions of cystidia on adjacent hymenia in the *Coprinus* fruit body (Horner & Moore 1987). Cystidia are large, inflated cells which span the gill cavity in *Coprinus,* impinging upon the opposing hymenium of the neighbouring gill. There are, as a result, certain classes of cystidial relationship (as seen in microscope sections) which are open to numerical analysis. Thus, cystidia spanning the gill cavity may be 'distant', having other cells separating them, or 'adjacent', with no intervening cells; and, in any pair of neighbouring cystidia, both may emerge from the same hymenium (described as *cis*) or from opposite hymenia (*trans*). If the distribution of cystidia is entirely randomised then the frequency of adjacent pairs will depend on the population density (which can be measured as a function of the distance between neighbouring cystidia) and there will be an equal number of *cis* and *trans* in both the distant and adjacent categories. Quantitative data from serial sections of a primordium showed that *cis*-adjacents did not occur and in

general there were lower frequencies of close neighbours in the *cis* category, indicating that formation of a cystidium actively lowers the probability of another being formed in the immediate vicinity (Horner & Moore 1987). The extent of the inhibitory influence extended over a radius of about 30 μm and strictly limited to the hymenium of origin.

In this instance, as with the determination of gill development mentioned previously, the patterning process is open to analysis and simulation using the activator-inhibitor model developed by Gierer & Meinhardt (1972; Meinhardt 1982). This is an elegantly simple model which suggests that morphogenetic pattern results from interaction with just two compounds – an activator which autocatalyzes its own synthesis, and an inhibitor which inhibits synthesis of the activator. Both diffuse from the region where they are synthesised; the inhibitor diffusing more rapidly and consequently preventing activator production in the surrounding cells. A wide variety of patterns can be generated in computer simulations by varying diffusion coefficients, decay rates and other parameters (Meinhardt 1982). The model readily accounts for stomatal, cilial, hair and bristle distributions, and has been successfully applied to simulate leaf venation and phylotaxy (Meinhardt 1984); a belief that it can be applied to such fungal phenomena as have been outlined in this paper is implicit in the descriptions given of them. However, it is highly significant that a single model can be applied so successfully to animals, plants and fungi. Whether the model is right or wrong is immaterial; it concentrates attention on the fact that the distribution of stomata on a leaf, bristles on an insect and cystidia on a fungal hymenium have a great deal in common at a fundamental mechanistic level.

Cell differentiation and commitment

What the gill organizer leaves behind in the developing hymenophore of *Coprinus* is a protohymenium. The classic view is that the mature *Coprinus* hymenium is comprised of three cell types; basidia, cystidia and paraphyses (Fig. 5). Basidia and cystidia differentiate from the

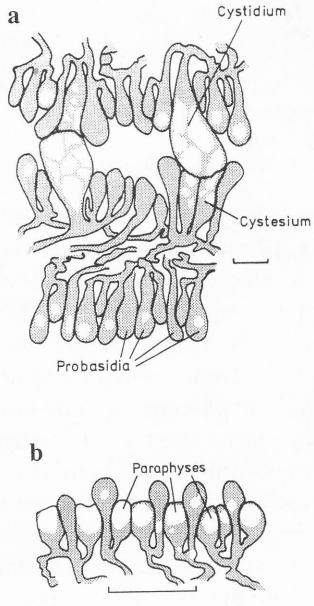

Fig. 5 Summary drawing of *Coprinus* hymenial structure as seen in sectional view; both diagrams are constructed from tracings of published micrographs (Rosin & Moore 1985; Horner & Moore 1987). Scale bars = 20 μm.

tips of branches which arise from the subhymenial tramal hyphae and together form the protohymenium as an 'epidermal layer' of the gill plate. A hymenial hyphal tip has a probability of about 40% of becoming a cystidium, but since a cystidium inhibits formation of further cystidia in the same hymenium (see above) only about 8% of these tramal hyphal branches become cystidia; the rest become probasidia (Horner & Moore 1987) which proceed to karyogamy and initiate the meiotic cycle ending with sporulation. Paraphyses arise as branches of sub-basidial cells and insert into the hymenium. About 75% of the paraphyseal population has inserted by the time meiosis is completed; the rest insert at later stages of gill development to expand the gills for active discharge of basidiospores from basidia to take place (Rosin & Moore 1985).

Here we have a defined temporal sequence of differentiation, probasidia appear first and then paraphyses arise as branches from sub-basidial cells. The tramal hyphae beneath the hymenium represent a fourth cell type; their apparent lack of differentiation being also an aspect of regional specification. It is important to appreciate that all the cell types so far mentioned arise as branches from the subhymenial hypha-like elements. Yet despite their relationship as sister branches of the same hyphal system, cells separated only by a dolipore septum will follow totally different pathways of differentiation (Moore 1984a). Although evidence for different dolipore superstructures has been found in the basidiomycete hymenium (Gull 1976, 1978; Moore 1985) it is not at all clear how alternate pathways of differentiation are regulated on the two sides of such a septum.

There is, however, a fifth cell type in the hymenium of *Coprinus cinereus* and its origin illustrates yet another phenomenon found in other organisms. At early stages in growth of the cystidium across the gill cavity the cell(s) with which the cystidium will come into contact in the opposing hymenium are indistinguishable from their fellow probasidia. However, when the cystidium comes firmly into contact with the opposing hymenium, the hymenial cells with which it collides develop a distinct granular and vacuolated cytoplasm, more akin to that of the cystidium itself than to the neighbouring probasidia. This suggests a contact stimulus setting in train an alternative pathway of differentiation leading to an adhesive cell type (called the cystesium; Horner & Moore 1987).

There is, then, a wealth of evidence for highly specific differentiation of individual cells in fungi. But there is very little direct evidence for that developmentally-important concept of commitment. This is the process whereby a cell becomes firmly committed to one of the developmental pathways open to it before expressing the phenotype of the differentiated cell type. The classic demonstration involves transplantation of the cell into a new environment; if the transplanted cell continues along the developmental pathway characteristic of its origin then it is said to have been committed prior to transplant. On the other hand, if the transplanted cell embarks upon the pathway appropriate to its new environment then it was clearly not committed at the time of transplant. Most fungal structures produce vegetative hyphae very readily when disturbed and 'transplanted' to a new 'environment' or medium. This is, of course, regenerative phenomenon, important in its own right and a very significant and experimentally attractive attribute of fungi. Yet it does create the impression that fungal cells express little commitment to their state of differentiation.

Unfortunately, very little transplantation experimentation has been reported with fungal multicellular structures and that which is in the literature is confused by conceptual uncertainties about the level of development equivalent to those embryological stages in which

commitment can be demonstrated. The clearest example of commitment of a developmental pathway has been provided by Bastouill-Descollonges & Manachère (1984) who demonstrated that basidia of isolated gills of *Coprinus congregatus* continued development to spore production if removed to agar medium 24 hours before the expected time of maturity, but immediately regressed to form vegetative mycelium if removed 12 hours earlier. Following this lead we have examined commitment in the different cell types of hymenial tissues fo *Coprinus cinereus* after explantation to agar medium (Chiu & Moore 1988a). Probasidia at the dikaryotic (prekaryogamy) stage at excision were arrested at that stage; explants made at later physiological ages did complete meiosis and/or sporulation, though at a slower rate than *in vivo*. Cystidia, paraphyses and tramal cells readily reverted to hyphal growth but this did not often happen to probasidia. Evidently, dikaryotic (prekaryogamy) probasidia are specified irreversibly as meiocytes and they become determined to complete the sporulation programme during meiotic prophase I. Once initiated, the maturation of basidia is an autonomous, endotrophic process which is able to proceed *in vitro*. Clearly, then, even if only to a limited extent, commitment to a pathway of differentiation some time before realization of the differentiated phenotype can occur in fungi.

Morphogenesis-specific regulation of gene activity

In few organisms are there, yet, specific examples of gene regulation events that control particular aspects of morphogenesis. Probably the most important point to be made here is that, despite their generally smaller genome size, the fungi share with other organisms the feature the activity of a relatively small proportion of the genome can be associated with a particular morphogenetic process. That is, the emphasis in gene regulation seems to be on differential integration of activity rather than on large scale replacement of one set of gene products by another. This has been demonstrated alike in *Schizophyllum, Coprinus and Saccharomyces*. The yeast example is of particular interest in view of the small size of this eukaryotic genome. *S. cerevisiae* has a haploid genome of about 1.4×10^4 kilobase pairs, which is less than four times the size of that of the genome of the bacterium *Escherichia coli*. The yeast genome is organised in a characteristic eukaryotic manner, with 17 linkage groups which are now known to be equivalent to chromosomes (Mortimer & Schild 1985); each of the chromosomes being a single, linear DNA molecule about the same size as the DNA molecule from a 'T' bacteriophage (Oliver 1987). Such a small genome might be anticipated to be largely devoted to defining the eukaryotic structures which distinguish yeast from the bacteria. Yet only between 21 and 75 of the estimated total of 12,000 genes in yeast are specific to sporulation, i.e. meiosis and ascospore formation (Esposito *et al.* 1972), and correspondingly few sporulation-specific polypeptides have been identified (Trew *et al.* 1979; Wright & Dawes 1979; Kraig & Haber 1980). Similar results have been reported for fruit body formation in the basidiomycetes *Coprinus cinereus* (Yashar & Pukkila 1985) and *Schizophyllum commune* (Zantinge *et al.* 1979).

Consideration of yeast in a discussion of multicellularity in higher fungi is not as eccentric as it may appear because recent work has revealed some startling similarities in the biochemistry of sporulation between yeast and higher fungi. Analysis of biochemical events accompanying fruit body formation in *Coprinus cinereus* has revealed that some enzymes are specifically derepressed in the fruit body cap; among these are the NADP-linked glutamate dehydrogenase (NADP-GDH) and glutamine synthetase (GS) (Moore 1984a). Cytochemical

examination shows that the NADP-GDH appears first in isolated islands of cells in the very young primordium (Elhiti *et al.* 1979) where the enzyme is localized to cytoplasmic microvesicles in the peripheral regions of specific cells (Elhiti *et al.* 1987). These cells are probasidia, and more enzyme analysis coupled with cytological observation clearly associates initial derepression of the enzyme with karyogamy and progress of meiosis (Moore *et al.* 1987) and shows NADP-GDH and GS regulation to be positively co-ordinated (Moore *et al.* 1987). This definitive correlation with meiosis has led to the realization that biochemical events surrounding this mode of division in *Coprinus* are remarkably similar to those which characterise sporulation (i.e. the meiotic cycle) in *Saccharomyces*. The most noteworthy findings with Coprinus are: (a) derepression of NADP-GDH in the mycelium involves *de novo* synthesis (Jabor & Moore 1984) and is caused by transfer into a medium lacking any nitrogen source but rich in carbon; (b) inclusion of as little as 2 mM NH_4^+ in the transfer medium prevents derepression of NADP-GDH, among many other nitrogenous compounds tested only those able to generate ammonia, and glutamine also inhibit derepression; (c) a mutant unable to synthesise acetyl-CoA failed to derepress NADP-GDH (Moore 1981b); (d) in the fruit body, derepression of NADP-GDH occurs in the cap, but not in the stipe (Stewart & Moore 1974) the accompanying NAD-GDH also increases in activity as the fruit body develops, but the ratio of the two enzymes alters from 10:1 (NAD-GDH: NADP-GDH) to 1.4:1, a considerable swing in favours of the enzyme with the 10-fold greater affinity for ammonium; (e) there is a high positive correlation between derepression of NADP-GDH and derepression of glutamine synthetase in both fruit body cap and mycelium (Ewaze *et al.* 1978; Moore *et al.* 1987); (f) large quantities (up to 2 mg dry weight per fruit body) of glycogen are accumulated and translocated specifically to the fruit body cap (Moore *et al.* 1979); (g) derepression of NADP-GDH occurs initially at karyogamy and is then re-amplified postmeiotically; glycogen utilization is initiated towards the end of meiosis and the polysaccharide is almost completely utilised within the few hours immediately following the meiotic division during which time basidiospore formation occurs (Moore *et al.* 1987).

Comparison of these results with published details about yeast brings to light a remarkable series of similarities which cannot all be coincidental. Study of sporulation in *Saccharomyces cerevisiae* is facilitated by a transfer process which induces synchronised sporulation in the treated cells. Sporulation can be induced by transferring diploid vegetative yeast cells from a rich medium to one which is nutritionally deficient, usually nitrogen-free (Fowell 1969, 1975; Tingle *et al.* 1973; Freese *et al.* 1982) [compare with (a) above]. This process is inhibited by ammonium and glutamine (Miller 1963; Piñon 1977) [compare with (b) above]. Within 2 hours of the transfer, NAD-GDH activity in the yeast cells is repressd to about 5% of its level in vegetative cells, but NADP-GDH activity remains unchanged (Delavier-Klutchko *et al.* 1980). The net result is a change in the ratio of the specific activities of the two enzymes from 2.04:1 (NAD-GDH:NADP-GDH) to 0.10:1 [compare with (d) above]. Glutamine synthetase activity initially declines by about 25% but within 2 hours of transfer has increased to 140% of the activity of vegetative cells [compare with (e) above]. Inclusion of 5 mM ammonium in the sporulation medium results in a decline in activity of both GDH enzymes and the glutamine synthetase [compare with (b) above]. Yeast mutants lacking the NADP-GHD were less sensitive to the meiotic-inhibitory effects of ammonia (Dickinson & Dawes 1983). Glycogen accumulates in sporulating yeast until the stage at which ascospore formation can be detected, but then glycogen levels decline [compare (f) & (g) above].

Glycogen accumulation in *S. cerevisiae* occurs in α / α and a/a diploids on transfer to sporulation medium (Hopper *et al.* 1974; Kane & Roth 1974); but the decline in glycogen level ocurred only in a/α cells, so appears to be sporulation-specific. Indeed, only DNA synthesis, glycogen breakdown, and extensive RNA and protein breakdown occur uniquely in a/α sporulating cells (Hopper *et al.*1974); and all of these processes seem to be inhibited by ammonium. Although ammonium does not inhibit the initiation of premeiotic DNA synthesis, DNA replication is arrested by ammonium after initiation; continued incubation in the presence of ammonium leads to massive DNA degradation (Piñon 1977). Degradation of accumulated glycogen is not observed in yeast cells incubated in medium supplemented with ammonium (Fonzi *et al.* 1979) and treatment with ammonium delays degradation of proteins at the onset of meiosis and inhibits protein and DNA synthesis (Croes *et al.* 1978). Note that the large scale protein breakdown which occurs in normally sporulating a/α yeast cells and in *Coprinus* basidia (Moore *et al.* 1987) means that it is unlikely that the enhanced GS and GDH activities which are also observed are involved in amination in order to assimilate nitrogen. Amino nitrogen must be obtainable readily from degraded proteins, so it is unlikely that the ammonium-scavenging GDH and glutamine synthetase activities appear to alleviate nitrogen deprivation by assimilating ammonium for metabolism, but rather that these enzymes are derepressed to maintain an ammonium-free environment in a cell committed to meiotic processes which are inhibited by ammonium.

Clearly, there are a great many biochemical similarities between these two contrasting fungi – one the most reduced and basic ascomycete, the other one of the most advanced and highly adapted basidiomycetes. The most recent research with *Coprinus* (Chiu & Moore 1988b) has emphasised this similarity by showing that basidiospore formation is inhibited by applications of ammonium or glutamine (and some of their structural analogues). Further, just as ammonium causes meiotic yeast cells to revert to vegetative (mitotic) growth, so, in *Coprinus,* application of ammonium causes vegetative hyphal apices to grow out of basidia in place of the sterigmata and spores which are expected (Fig. 6).

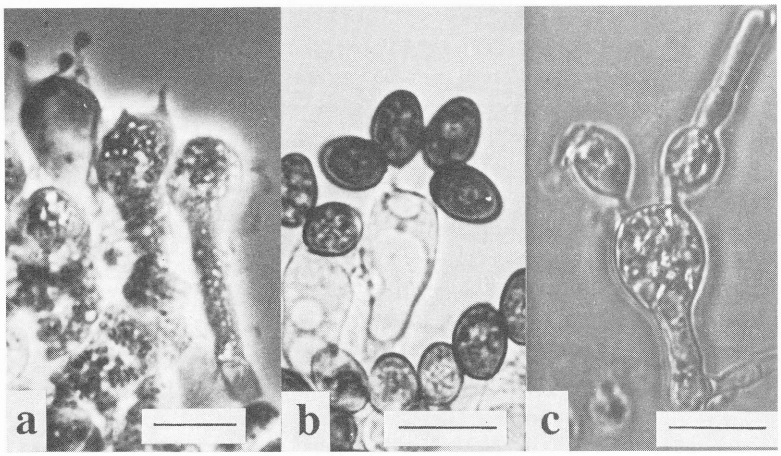

Fig. 6 *In vitro* experiments with hymenia of *Coprinus cinereus.* If tissue is excised from the parent fruiting body early in sporulation (a) and transferred to an agar medium, the process of sporulation is completed (b), but if ammonium is included in the medium sporulation is inhibited and the basidia revert to hyphal growth (c). Scale bars = 10 μm.

These observations suggest that some of the development-related gene control events might have been conserved through fungal evolution from the yeast to agarics. But whatever the exact significance and nature of these correspondences between yeast and *Coprinus* they emphasise that differentiation of fungal multicellular structures is genetically compartmentalised. The meiotic process, in part, may be conserved, but other aspects, including fundamentals like the structure of the cell wall, can be totally different. Indeed classical genetic studies show that fruiting structures are effectively 'genetically compartmentalised' in the sense that mature fruit bodies may be produced without spores (i.e. fruit body structure and meiosis are genetically distinct pathways) and fruit bodies with genetically abnormal stipes may have normal caps and vice versa (i.e. assembly of different parts of the same fruit body uses genetically distinct pathways). This, of course, re-emphasises the fact that morphogenesis, in fungi as in other organisms, involves integration of genetic capabilities by a relatively small number of genes, which are presumably control elements at some level.

Variation in the morphology of fruit bodies of higher fungi has been reported in many species, often appearing to be a strategy for adaptation to environmental stress. Detailed analysis of such developmental plasticity in *Volvariella bombycina* has given more evidence for normal morphogenesis being an assemblage of distinct developmental segments (Chiu *et al.* 1988). Although the fruiting structures observed varied from the normal agaric form to completely abnormal enclosed, puff-ball like structures, they were all actually or potentially functional as meiospore production/dispersal structures. On the basis of these observations it is suggested that normal fruit body development comprises a sequence of independent but co-ordinated morphogenetic subroutines, each of which can be activated or repressed as a complete entity. For example, the 'hymenium subroutine' in an agaric is normally invoked to form the 'epidermal' layer of the gill; the 'hymenophore subroutine' produces the classic agaric gill plates. That these (and others) represent independent developmental segments is indicated by the fact that abnormal fruit bodies consist of recognizable tissues. Thus a fruit body may, quite aberrantly, bear a functional supernumerary hymenium on the upper surface of the cap. The important point is that it *is* a functional and recognizable tissue, not a tumorous growth, which has been produced by correct execution of a morphogenetic subroutine which has been invoked in the wrong place.

Conclusions

I set out to look for evidence for a variety of phenomena and the outcome appears to us to be that, accepting the peculiarities imposed by the unique aspects of fungal structural organization, remarkable similarities in the orchestration of morphogenesis emerge between fungi, plants and animals. That there should be differences between developmental phenomena in fungi, plants and animals comes as no great surprise; that there should be such profound similarities as seem to exist is little short of amazing. There is no doubt that, in all three groups, multicellularity evolved long after the three evolutionary lines had diverged into their characteristic and totally separate modes of form, structure and behaviour. Thus, little, if any at all, of the organization which permits multicellular morphogenesis could have been possessed by any common ancestor. The parallels which do seem to exist in the basic regulation of morphogenesis in plants, animals and fungi imply convergent evolution; but further, and perhaps more interestingly, imply that there is only this limited number of ways of solving the

problems associated with organising populations of cells into particular patterns irrespective of the nature of those cells. The rules which govern morphogenesis appear to be natural laws owing more to the physical and chemical phenomena involved than to the biological entities that respond to them.

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