CHAPTER 3

CONTROL OF PATTERN AND FORM IN MUSHROOM MORPHOGENESIS

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1. INTRODUCTION

Major tissue domains in the mushroom fruit body (pileus, stipe, basal bulb, etc.) are established very early in the development of the structure, and the controlling events which determine tissue differentiation and distribution are completely obscure.

Some observations provide evidence for control of patterning by diffusion of chemical morphogens in morphogenetic fields around differentiating cells. Even the generation of the agaric gill can be understood in terms of organising centres which interact with one another by production of (and response to) freely diffusing activator and inhibitor molecules.

A major problem is our ignorance of the anatomy of the fruit body. Recent analyses of the stipe in *Coprinus* will be described to illustrate the new sorts of information which can be obtained through application of simple numerical methods to otherwise conventional microscopic analysis. Knowledge of the seat of the growth which drives morphogenesis and appreciation of the balance between physical and biological phenomena are also necessary. Research on these aspects shows that (a) agaric gills grow at their base, not their margins; and (b) agaric gills are initially convoluted, being stressed into their regular radial arrangement later.

We have chosen stipe gravitropism as a model representing a controllable morphogenetic change. Some of the new findings and the new ideas which are beginning to emerge from this work will be discussed.

2. PATTERNS IN THEORY

Morphogenesis is the development of the shape, form and structure of an organism. The most extensive research has been done with animals and from this a vocabulary has been developed which describes morphogenetic events without prejudging the mechanisms which may be involved. It is important to appreciate that the basic 'body plan' of the developing structure (whether fungal, plant

or animal) is not specified all at once. Rather, the shape and form arises as a result of a sequence of developmental 'decisions'. Each decision is irreversible within its morphogenetic sequence although often reversible by some gross disturbance; e.g. differentiated cells being put into tissue culture, nuclear & cell transplants, regeneration after injury, etc.

The states which precede terminal differentiation each embody reduction of potential compared with the previous state. Each decision is made by cells already ***specified*** by earlier decisions to belong to a particular developmental pathway. Consequently, each decision is made from among progressively smaller numbers of alternatives until the particular structure to which the cell will contribute is finally ***determined***. Classic embryological transplantation experiments revealed these states. Where the explant differentiated in accord with its old position then it was said to have been determined prior to transplantation. If it developed in accord with its new position, then it had not been determined, but may have been specified.

At the cellular level, the competent tissue becomes differentially determined in response to chemical signals from other regions of the developing structure. These chemicals (none truly identified yet) may be termed organisers, inducers or morphogens, and seem to inhibit or stimulate entry to particular states of determination. They may contribute to a ***morphogenetic field*** around a structure (cell or organ) which permits continued development of that structure but inhibits formation of another structure of the same type within the field.

All of these phenomena contribute to the ***pattern formation*** which characterises the 'body plan' or, more formally, the distribution of differentiated tissues in the structure (organ or individual). Pattern formation depends on ***positional information***, which allows the cell to differentiate in a way characteristic of its position in the structure. Positional information is usually understood to be imparted by the concentration of one or more morphogens emitted from one or more spatially distinct organisers. Effectively the cell 'triangulates' on the incoming signals and adjusts its morphogenetic response in accord with its position relative to the controlling organisers. Populations of cells which respond like this are said to show ***regional specification***. The operation can be divided into an ***instructive process*** which imparts positional information, and an ***interpretive process*** in which the competent tissue responds.

The basic rules of pattern formation seem to be that regional specification (directed by organisers producing morphogens) occurs first, regulating gene activity in ways specifically geared to morphogenesis so that particular cells are first specified (a state which is still flexible) and then determined (a state which is inflexible) to their differentiated fates. Cell differentiation is a consequence of these events - cells which are either specified or determined are not necessarily morphologically different from their neighbours or predecessors.

These statements highlight the major events contributing to animal and plant morphogenesis. Our challenge is to establish whether evidence exists for such mechanisms in the development of fungal structures. The great bulk of the published research on fungal morphogenesis has been done with taxonomic intentions. It has great value for its descriptive and comparative content, but precise developmental accounts are extremely rare and ***experimental*** approaches rarer still. Nevertheless, as I hope to show in what follows, there is a *prima facie* case for similar events (and perhaps similar mechanisms) being involved in establishing the patterns which result in formation of defined tissues in mushroom fruit bodies.

3. PATTERNS IN TIME

Descriptions and definitions of development, differentiation and morphogenesis always

emphasise ***change***. Morphogenesis has a time dimension and it is crucial to appreciate that developmental processes are dynamic ones which involve interactions in time as well as space. In the beginning, the normal invasive growth of vegetative mycelium is modified. Hyphae grow towards one another and a population of cells is assembled from contributions of a number of co-operating hyphal systems to form the community of hyphae and their branches which creates the mass of undifferentiated prosenchymal tissue described as the ***fruit body initial***.

Microscope sections of even extremely small fruit body initials can be resolved into regions of recognisable pileus and stipe and, since the creation of such histologically distinct regions requires that some organisation is imposed upon the homogeneous prosenchyma, these images provide *prima facie* evidence for regional specification. Reijnders (1948, 1963, 1979) has stressed the importance of: (a) development and nature of the veil and **pileipellis** (the 'epidermis' of the pileus) in relation to covering the developing hymenophore (the **hymenophore** carries the **hymenium**, a cell layer responsible for eventually producing the basidiospores); (b) the sequence of development of the stipe, pileus and hymenophore, which are the major functional zones of the basidiome; (c) the mode of development of the hymenophore. The terminology has been discussed by Watling (1985). The most highly differentiated cells are found at the boundaries of tissue regions (Williams, 1986). In the youngest specimens these boundaries are occupied by layers of parallel hyphae (called meristemoids by Reijnders, 1977) which seem to be involved in rapid cell formation in the sense that the distance between successive hyphal cross walls is minimised. But these are **NOT** meristems. Meristems do not occur in fungi.

Major tissue domains are demarcated very early in fruit body development. For example, in *Coprinus cinereus*, fruit body initials only 800 μm tall are clearly differentiated into pileus and stipe (Moore *et al.*, 1979) though this size represents only 1% of the size of a mature fruit body. As the different tissues do not grow uniformly, the ***differential growth*** of the primordium as it matures causes inevitable geometrical changes. As a typical fruit body of *C. cinereus* grows from 1 to 34 mm in height (i.e. a vertical linear change of 34x), the circumference of the stipe increases 9x, the outer circumference of the pileus increases 15x, but the volume increases more than 3000x.

The mechanical consequence of primordium enlargement for relationships between tissue layers which are often concentrically arranged is extremely important. For example, the internal structure of the *Coprinus* primordium is uniformly solid at the time that gills begin to arise, so gills and gill space arise together, but the enormous increase in size of the fruit body primordium helps explain where the space comes from to form a gill cavity. When the opposing hymenia of neighbouring gills differentiate they form a fracture plane which can be opened out into a cavity when the expansion of the underlying tissue puts tension across the 'fracture' and pulls the hymenia apart. This is a process I call 'cavitation' (Moore, 1994) and the argument applies to cavitation in all differentially expanding cellular structures. Variations on the theme can be imagined in other organisms. If the 'fracture planes' form an annulus around the top of the stipe (one tissue layer might be the stipe apical meristemoid, the other the hymenophore meristemoid), then an annular cavity could arise before gill formation.

The meristemoid of the developing hymenophore of *Coprinus* is a protohymenium in which probasidia which proceed to karyogamy and initiate the meiotic cycle ending with sporulation. There is a defined temporal sequence: probasidia appear first and then paraphyses arise as branches from sub-basidial cells and insert into the hymenium (Rosin & Moore, 1985b).

4. PATTERNS IN SPACE

About 8% of the hyphal tips in the protohymenium of *C. cinereus* become cystidia, but when a cystidium does arise, it inhibits formation of further cystidia within a radius of about 30 μm (Horner & Moore, 1987); this is the cystidial morphogenetic field. Its influence is limited strictly to the hymenium from which the cystidium arises and it determines the distribution of cystidia over the hymenial surface.

Perhaps the most obvious pattern, though, is the distribution of the gills of agaric fungi. Essentially the gills are plates suspended from the fruit body pileus tissue. In *Coprinus*, the pileus of the fruit body primordium encloses the top of the stipe and gills are formed as essentially vertical plates arranged radially around the stipe. There are two types of gill: primary gills which, from formation, have their inner, tramal tissue in continuity with the outer layers of the stipe, and secondary (and lesser ranked) gills in which the hymenium is continuous over the gill edge (Reijnders, 1979; Rosin & Moore, 1985a; Rosin *et al.*, 1985; Moore, 1987).

Primary gills are connected with pileus tissue at their outer edge and with the stipe at their inner edge; the tendency to widen as the stipe circumference increases is compensated by gill replication, and specifically by formation of a new gill cavity and its bounding pair of hymenia ***within*** the trama of a pre-existing gill. This clearly sets the direction of development as outwards ***from the stipe***; i.e. gills in the *C. cinereus* fruit body grow radially outwards, their roots extending into the undifferentiated tissue of the pileus context.

The formative element is an **organiser** in the tissue at the extreme end of the gill cavity. The gill organiser is responsible for the progression of the gill cavity radially outwards, away from the stipe. It directs the prosenchyma/protohymenium transition - an increase in branch frequency to produce branches of determinate growth which are mutually 'attracted' so that they form the opposing palisades of a fracture plane (see above). Pileus expansion separates the two protohymenia, thus extending the gill cavity. Since they are progressing radially outwards, neighbouring organisers become further and further separated from one another as development proceeds and as the distance between neighbouring organisers increases a new one can arise between them (Rosin & Moore, 1985a); when a new gill organiser emerges, the margin of a new (but 'secondary') gill is formed. It is extended not by growth of its margin, but by continued radial outward progression of the two gill organisers on either side of its root.

In the origin of the gills we seem to have operating two classic components of theoretical morphogenesis - activation and inhibition by diffusing morphogens. First, we can suggest that diffusion of an activating signal along the fruit body radius assures progression of the gill organiser along its radial path. Second, each organiser can be assumed to produce an inhibitor which prevents formation of a new organizer within its diffusion range (i.e. the gill organiser uses this inhibitor to control its morphogenetic field). As radial progression into the extending pileus context causes neighbouring organisers to diverge, a region appears between them which is beyond the range of their inhibitors -- at this point a new organizer can arise in response to the radial activating signal. Interaction between the diffusion characteristics of the activator and the inhibitor is all that is necessary to control gill spacing, gill number, gill thickness, and the radial orientation of the gill field.

Gill development of *Volvariella bombycina* seems to be exactly homologous with the process in *Coprinus*; i.e. growth of any one gill occurs by outward progression of gill organisers on either side of the root of the gill into outwardly expanding pileus context. We have demonstrated that the gill margins remain essentially intact by painting black ink marks on the tissues in a primordium (Chiu & Moore, 1990a). During further fruit body development, ink marks placed on the pileus margin and those placed on the edges of the gills ***remained at the margin or the gill edges, respectively***. The

growth increment here is quite considerable, the radius of the pileus increasing from 0.5 to 2.5 cm and the depth of the gills from 1.5 to 5 mm. If growth of the pileus and gill margins resulted from apical growth of the hyphal tips which occupied the margin, then ink particles placed on those hyphal tips would be left behind as the hyphal apices extended which would consequently have resulted in the ink marks being buried beneath 4 to 20 mm of newly formed tissue by the end of the experiment. It follows, therefore, that the hyphal tips which first form the pileus margin, and those which form the gill margin, always remain ***at*** the margin. They do not continue to grow apically to extend the margin radially, nor are they overtaken by other hyphae; instead they are 'pushed' radially outwards by the press of fresh growth behind, and they are joined by fresh branches appearing alongside as the circumference of the margin is increased.

In both *C. cinereus* and *V. bombycina* gills are formed as convoluted plates (Chiu & Moore, 1990b). A sinuous, labyrinthiform hymenophore appears to be a normal 'embryonic' stage in basidiome development in agarics, yet a regular radial arrangement of the gills is characteristic of the mature basidiome. How this is achieved is a function of the expansion of the maturing primordium generating stresses between tissue layers which stretch (in *C. cinereus*) or inflate (in *V. bombycina*) the convoluted gills into strict radii.

Until recently there was surprisingly little information concerning the structure of the stipe of the *Coprinus* fruit body. Hammad, Watling & Moore (1993) have now demonstrated that the stipe contains both narrow and inflated hyphae. Narrow hyphae (cross-sectional area <20 μm^2) always comprise a significant numerical proportion (23% to 54%) of the cells seen in microscope sections of stipe tissue, although they only contribute 1% to 4% to the overall cross-sectional area of the stipe. During normal stipe growth the greatest cell expansion is seen in the inflated cells situated between the mid-cortex and the lumen rather than at the periphery of the stipe. Such a distribution of expansion would actually generate the lumen in the first instance and would obviously contribute to the stretching mechanisms (remember that the pileus surrounds the stipe in *Coprinus*) referred to in the previous paragraph.

Nearest neighbour analysis of cell distributions in *Coprinus* stipes shows that inflated hyphae are evenly rather than randomly distributed so, presumably, there is some sort of control over the pattern of inflation. This is yet another example of indirect evidence for local control of morphogenesis to be added to those provided by the distribution pattern of cystidia, the formation of groups of paraphyses around basidia, and the patterning process in gill development. Very similar aggregations of hyphae, termed hyphal knots (Reijnders, 1977) have been observed in a wide range of species (Reijnders, 1993). The common features of Reijnders' hyphal knots seem to be a central hypha (which remains hyphal) and an immediately-surrounding family of hyphae which differentiate in concert. Perhaps, in all multihyphal fungal structures, the ultimate morphogenetic regulatory unit is the Reijnders hyphal knot - a little community comprising an induction hypha (or hyphal tip, or hyphal compartment) and the immediately surrounding hyphae (or tips, or compartments) which can be brought under its influence. Larger scale morphogenesis could be co-ordinated by 'knot-to-knot' interactions.

Unfortunately, there are no clues to the nature of the **morphogens** which might serve as the growth factors involved in these phenomena. Also, ***lateral*** contacts between fungal hyphae are extremely rare, being represented only by lateral hyphal fusions. The constituent cells of plant and animal tissues are interconnected laterally by frequent plasmodesmata, gap junctions and cell processes. The absence of similar structures connecting adjacent hyphae suggests that any morphogens which do exist are likely to be communicated exclusively through the extracellular environment (Reijnders & Moore, 1985). Although it is abundantly clear that co-ordination of developmental processes is successfully achieved in fungal multicellular structures, the evidence for chemicals able

to perform the signal communication involved is sparse and disappointingly unconvincing (discussed in Moore, 1991).

5. PATTERNS IN EXPERIMENT

Most of the phenomena described so far are poor candidates for experiment because their control processes are entirely endogenous. We have recently begun to experiment with gravitropism in *Coprinus* as a morphogenetic model system. Mushrooms have a very sensitive gravity detection system and if reoriented they regain the vertical by bending the stem. The gravitropic response is a simple developmental pattern forming process; its control demands that the organism has a gravity perception system and a means of coupling this to stipe growth. The perception system must establish a new morphogenetic pattern to which the organ is caused to adjust by differential growth. Study of gravitropism is therefore a natural, non-invasive means of generating a particular morphogenetic change on demand in a specific location.

We have used video recording and computer-based video-image analysis to complete the first kinetic analysis of mushroom stem gravitropism (Kher *et al.*, 1992). Completion of meiosis in the cap coincides with the stem becoming competent to react gravitropically. Within 30 min of disorientation the negatively gravitropic bend initially appears within the apical 15% of its length. The bend then becomes more acute and progresses basally, traversing 40% of the initial length of the stem. Gravitropic bending is most likely the result of asymmetric distribution of growth, as represented by cell expansion, being stimulated by a diffusing, extracellular growth factor produced by the apical region of the stem. Continued exposure to the unilateral gravity vector is necessary for continued bending (Hatton & Moore, 1992). Bending raises the apex and as this approaches an angle of about 35° to the horizontal curvature compensation begins to adjust the degree of bending so that the apex can be brought exactly vertical. Curvature compensation requires that the apical region is free to move towards the vertical. The mechanism involved is uncertain, but it may involve a second diffusing signal.

Further work will concentrate on the nature of the signalling processes, biochemical, structural and ultrastructural aspects of curvature and curvature compensation, and sensitivity to gravitational acceleration.

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With best regards
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With best wishes,
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