Presidential Address

Mushrooms upright, sideways and inside-out

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Morphogenesis is not simply a matter of playing out a predefined genetic programme. Expression of developmentally important genes is epigenetic and place- and time-dependent relying on previously-formed tissue structures. Most differentiated hyphal cells require reinforcement of their differentiation 'instructions'. This reinforcement is part of the context (chemical, electrical and structural/mechanical environment) within which they normally develop. Key words at each stage of development in fungi are: competence, induction and change. Fungal morphogenesis is compartmentalized into a collection of 'sub-routines' which are distinct genetically and physiologically. Flexibility in expression of developmental sub-routines illustrates that tolerance of imprecision is an important attribute of fungal morphogenesis.

The proposition I want to put to you in this address is that morphogenesis is not simply a matter of playing out a predefined genetic programme. Rather, expression of developmentally-important genes is (*a*) epigenetic, (*b*) place-dependent, and (*c*) time-dependent. Ultimately, I would claim that the key to understanding fungal developmental biology is to accept the extent to which the expression of morphogenesis is dependent upon the environment. This I will discuss in detail, but before discussing the keys to form and structure in fungi I would first like to indulge in a tangential discussion of the keys to development in mycologists by stressing how a mycologist's interpretations are likely to be governed by his or her origins and environment.

William Wordsworth's aphorism is pertinent here: 'The Child is father of the Man' (*Intimations of Immortality*, 1807), though I do not intend a remorseless account of a childhood spent in Liverpool's Toxteth district. Suffice to say that even in that deprived area there was a family friend whose interest in fine engineering was expressed in a collection of gleaming brass microscopes. The wonders they revealed to me in drops of pond water and in diatoms were enough to drive me, even as a young boy in the mid-1950s, towards biology. The sculpturing on diatom valves was regularly used in club competitions designed to test the resolution of dry-, water- and oil-immersion objectives. Can you think of a more arcane activity for what, for the most part, were off-duty marine engineers? Or, for that matter, a better way for a microscopical neophyte to be introduced to the relation between magnification and resolution and ways of getting the best performance out of light microscopes?

I suppose, though, that it is the Ph.D. project which is the more realistic analogue of a scientific 'childhood'. My Ph.D. research dealt with the biochemical genetics of *Coprinus*

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cinereus (then called *C. lagopus*) and I can readily appreciate how that training still influences the way in which I interpret information. This research was done in the mid-1960s, in the days of biochemical markers and auxotrophic mutants, and was a study of Mendelian 'formal' genetics, aimed at extending the chromosomal linkage map. Consequently, the work was highly numerate and involved extensive intercrossing, progeny counting, linkage calculations and data interpretation (Moore, 1967*a*).

I continued this traditional approach into my immediate postdoctoral work in which I prepared an allele map of a single (but complex) gene which we subsequently found to be the structural gene for the main sugar transporter (Moore, 1972; Taj-Aldeen & Moore, 1982). Results of crosses between alleles were quite variable so this experience added to my scientific prejudices the need for application of good statistical techniques to results from many replicate experiments. Only this allowed extraction of recombination values which contributed to an internally consistent gene map which finally featured 52 alleles, was based on over 400 crosses between those allelic mutants (Moore, 1972), and prompted speculation on the mechanism of recombination (Moore, 1974).

These experiences schooled me in the formal genetical approach of interpreting the behaviour of cellular organelles (chromosomal segments in this case) from entirely indirect experiments. The geneticist's cycle of cross, count, calculate, conjecture, then check. But this was not all. In my Ph.D. research I dealt with nutritional mutants which needed biochemical analysis to identify their metabolic basis. In terms of simple nutritional tests, one methionine-requiring auxotroph is much like any other methionine-requiring mutant until the defective steps in metabolism can be identified. The same is true for mutants auxotrophic for adenine, although these provided a particular challenge by demanding purely chemical approaches to identification of precursors and intermediates as well as biochemical and enzymological analyses (Moore, 1967*b*).

By the end of my Ph.D. project I was adept at genetical and general data analysis and biochemical techniques. Add the fascination with microscopy that had brought me into biology at school and the stage was set for my first exposure to developmental biology. In the early 1970s my Manchester colleague, George Stewart, discovered that NADP-linked glutamate dehydrogenase (NADP–GDH) was derepressed specifically during development of caps of fruit bodies of *C. cinereus* but was absent from the stems of those same fruit bodies (Stewart & Moore, 1974). During more-or-less the same time period, my first postgraduate student, Henry Waters, co-supervised by Ron Butler, undertook a study of sclerotium structure and development in *C. cinereus* (Waters, Butler & Moore, 1975; Waters, Moore & Butler, 1975).

The study of sclerotia acquired a genetical twist before long (Moore, 1981) and led directly to extensive study of fruit body development, much of which will be described below. Discovery of the tissue-specific derepression of NADP–GDH in 1972 was the start of a long-running saga of biochemical investigation of carbon and nitrogen metabolism which does not have much place here. Along the way, we revealed a major role for urea as an osmotic metabolite in the *C. cinereus* fruit body cap (and only the cap). This and other aspects of the study was reviewed in 1984 (Moore, 1984) but it was not until 1988 that we found a cap-specific function for GDH and a likely function for the NADP-linked enzyme important enough to warrant its derepression during cap development (Chiu & Moore, 1988*a*, *b*). I will return to this later, now I want to deal with more recent work.

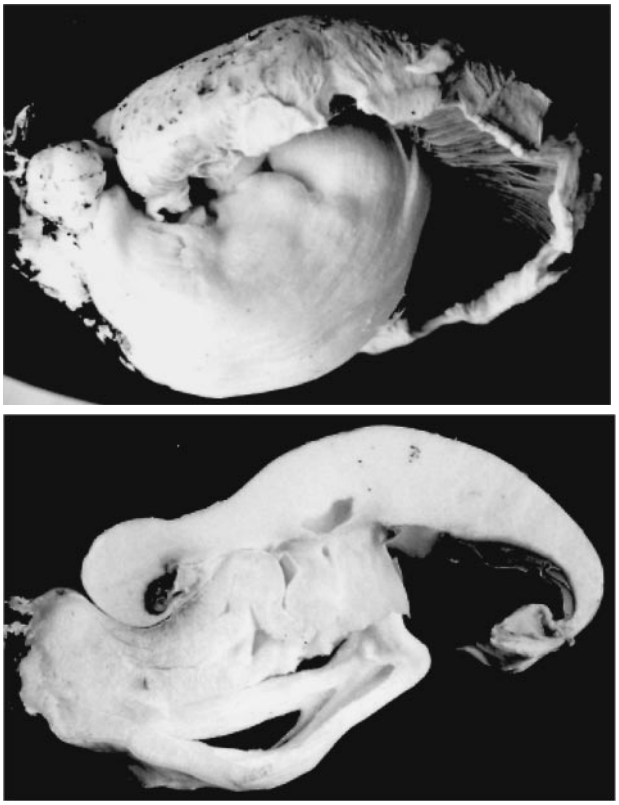

Fig. 1. Gravitropism of *A. bisporus*. An inverted fruit body is able to regain the vertical (top). Cutting the fruit body down the centre (bottom image) shows that this has been achieved at the expense of other aspects of fruit body morphogenesis, like the poorly-developed gills on the inside of the curved stem and the structural disruption of the stem itself. Images kindly provided by Dr M. H. Umar, Mushroom Experiment Station, Horst, The Netherlands.

THE KEYS TO FORM AND STRUCTURE IN FUNGI

Novel experimental approaches to study of development which I have recently introduced include (*a*) study of gravitropism as a morphogenetic model; (*b*) quantitative analysis of cell sizes and distributions during development; and (*c*) *in vitro* tissue transplantations.

Gravitropism as a morphogenetic model

Fig. 1 shows some recent photographs of an *Agaricus bisporus* fruit body which has restored itself to the vertical after being inverted. These photographs alone show that the gravitropic reaction is an imperative. The section in Fig. 1 (lower image) shows that development of the cap on the inside of the curved stem has been aborted to enable the stem to bend sufficiently, and the bending has pulled apart the structure of the stem. Such radical re-engineering of fruit body structure is permitted

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Table 1. Cell morphometrics in sections of gravitropically-responding stems of *C. cinereus* at the point of maximum curvature

	Lower flank of bend	Upper flank of bend
Mean width of hyphae (μm)	20·9	19·9
Narrow hyphae (%)	30·5–39·1	28·8–41·5
Packing density	0·44	0·47
Cell length (μm)	542	116

Data from Greening *et al.*, 1997.

because vertical orientation of the fruit body is essential for effective spore release and it may be achieved even at the expense of other aspects of fruit body morphogenesis (Moore, 1991). These images of *A. bisporus* gravitropism dramatise the process but this is a difficult organism to work with routinely and our work has concentrated on *C. cinereus* gravitropism. This, together with research by our colleagues on *Flammulina velutipes* gravitropism, was the subject of a Centenary Review (Moore *et al.*, 1996) which I do not intend to repeat here. I do, however, want to emphasise two points.

First, I am using gravitropism as a convenient morphogenetic process. Obviously, it is interesting in its own right, but my main interest is that simply placing a stem of *C. cinereus* on its side initiates a sequence of events which results in that stem bending through a right angle to bring its apex back to the vertical. The process is non-invasive because the gravity field is uniform; it is concluded within a matter of 3–4 h; can be controlled absolutely by the experimenter; and is eminently suitable for replication, enumeration and measurement. Yet it is a morphogenetic process.

Second, our latest observations have shown that gravitropic bending results solely from an increase in the length of cells in the lower half of the horizontal stem (Greening, Sánchez & Moore, 1997). None of the other parameters measured differed significantly between the upper and lower flanks of the bending stem (Table 1). Change in packing density of cells may be a factor in accommodating differential growth in plant organs, and it might be expected that the more open distribution of fungal cell populations would react to changes in tension. So it is particularly interesting that even when the upper flank is under compression during bending the packing density remains unchanged (Table 1). Perhaps this reflects the importance of maintaining organization in the stem during the morphogenetic change. Nevertheless, the most remarkable feature is that the morphogenetic change results from highly localized activity. The upward bending of the stem clearly results from localization of the cellular response to the lower flank of the stem, i.e. not just to one side of the stem but to the correct side to assure upward curvature. Then, at the cell level, there is highly localized wall synthesis which is directed to increase in length without increase in cell diameter. Tropic bending itself seems to imply interhyphal signalling mechanism(s) which can operate over the sorts of distances which encompass the stem diameter (several mm). The results shown in Table 1 imply signalling at the cellular level which can orchestrate longitudinal wall extension without circumferential extension. We are entirely ignorant of the sorts of inter- and intra-cellular machinery which can determine such localizations. The cell morphometric approach used here, however, introduces another procedure which has recently been applied successfully to describing fruit body development.

Quantitative analysis of cell sizes and distributions during development

Most mycologists must be well aware of the description of tissue construction in mushrooms and toadstools which is called hyphal analysis. This is a procedure whereby the range and type of differentiated cell types are catalogued and used as taxonomic criteria. Hyphal analysis was introduced by Corner (1932*a*, *b*), who coined the terms monomitic, dimitic and trimitic to describe tissues consisting of one, two or three kinds of hyphae. Later, the words sarcodimitic and sarcotrimitic were used to describe fruit bodies having two or three types of hyphae of which one is inflated and has thickened walls (Corner, 1966; Redhead, 1987). Hyphal analysis in this sense is an almost entirely descriptive study, aimed at establishing structural features as taxonomic criteria. Many of the cell types which hyphal analysis usefully identifies are named for their morphological features alone (the essence of taxonomic description), yet the names carry functional overtones (words like 'generative' and 'skeletal' are used) though this is entirely a matter of presuming a function without proof or even evidence beyond the morphology. Walls which are seen to be unusually thick by light microscope observation are almost always assumed to be mechanically strengthening (= skeletal or ligative hyphal characters). Yet fungal wall structure is modified both chemically and physically to serve as a transient nutrient store, so wall appearance is not a reliable guide to wall function. Nevertheless, the taxonomic importance of hyphal analysis is immense (Pegler, 1996). It can even be applied *in vitro* to identification of fungi (especially wood rotting species) in culture (Nobles, 1958, 1965, 1971; van der Westhuizen, 1958, 1963, 1971; Stalpers, 1978; Rajchenberg, 1983; Lombard, 1990; Nakasone, 1990). My interest, however, is in the contribution it makes to our appreciation of two features. First, the analysis demonstrates the range of functional differentiation of which the hypha is capable. Second, the varied appearance of the hyphal types and the intergradations between them illustrates the adaptability, and versatility, the fungi express in constructing their fruit bodies.

The functional and morphogenetic purposes of the hyphal differentiation which hyphal analysis describes have only rarely been considered. Listing the presence or absence of a cell type is considered adequate. Consideration of its function, how it arises, how its position may be regulated, and its prevalence are seldom considered. The first quantitative hyphal analysis was done by Hammad *et al.* (1993*a*) and Hammad, Watling & Moore (1993*b*) who showed that enumerating cell types at different stages of development (in the fruit bodies of *C. cinereus*) is a powerful way of revealing how the macroscopic aspects of fruit body structure emerge during morphogenesis as a result of changes in hyphal type and distribution.

Details of the procedure, which uses computer-aided image analysis of microscope images, appear in Hammad *et al.*

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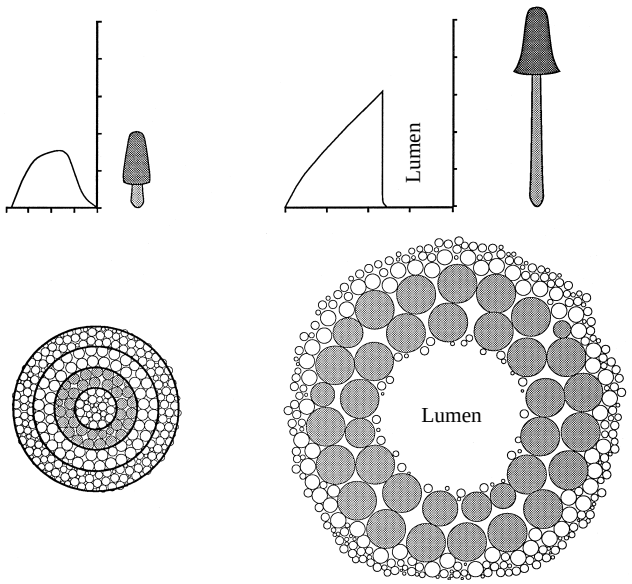

Fig. 2. Interpretation of the geometrical consequences of the cell size changes during development of the stem of *C. cinereus*. The graphs in the upper part of the figure show the lines of best fit for data relating hyphal cross sectional area to position across radii of 27 and 70 mm stems, together with scale drawings of the fruit bodies. The diagrams in the lower part of the figure are transverse sections of the stems, drawn to scale. The diagrammatic transverse section of the stem of a 27 mm tall primordium (on left) is composed of solid tissue which is divided into four zones corresponding to the zones in the radius in the graph above. The central (zone 4) and outermost (zone 1) zones are comprised of rather smaller cells than the two cortical zones. During further growth the most dramatic cell inflation occurs in the cells of zone 3 which are here shown shaded. Growth from 27 mm to 70 mm in height is accompanied by a 3·6-fold increase in cell area in zone 3 and a 1·6-fold increase in zone 2. The cells in the other zones have to be rearranged to accommodate the inflation of zone 3 and a major consequence is that a lumen appears in the centre of the stem. Changes in area are shown to scale in these diagrams, though only a total of 327 cell profiles are illustrated. As this is only a tiny proportion of the cells involved *in vivo*, the diagrams inevitably distort the apparent relationship between cell size and stem size. Narrow hyphae have been ignored in these diagrams, though they are distributed throughout the tissue and their conversion to inflated hyphae contributes to stem expansion. From Moore, 1996.

(1993*a*, *b*). They discovered that the stem of *C. cinereus* comprises two cell populations and that cell inflation is accentuated in cells occupying a specific zone of the stem. Differential expansion of cells in this zone readily explains how the stem changes from a solid cylinder to a hollow tube during its development.

Narrow and inflated hyphae in the fruit body stem of *C. cinereus*. Although *Coprinus* is one of the most frequently studied genera there is surprisingly little information concerning the structure of the stem of its fruit body other than that the stem is composed of greatly inflated and elongated cells. Most observers would consider the main body of the stem to be basically uninteresting, being made up exclusively of inflated cells. Hammad *et al.* (1993*b*) measured cross-sectional areas of hyphal profiles in 5 μm thick transverse sections cut from stems of fruit bodies of a range of developmental ages. For each section of each piece of stem, the area of every cell within two randomly chosen radial transects 12 μm wide was measured. On the basis of frequency distributions two distinct populations of hyphae were identified and categorised as narrow hyphae, with cross-sectional area $< 20\ \mu m^2$, and inflated hyphae, with cross-sectional area $\geqslant 20\ \mu m^2$. Narrow hyphae constituted 23–54% of the cells in transverse sections of the stem but only contributed 1–4% of the overall cross-sectional area.

All the available evidence indicates that narrow hyphae in stems of *C. cinereus* have diverse functions. They tend to be particularly concentrated at the exterior of the stem where they may serve as an insulating layer, and as a lining to the lumen where they may represent the remnants of the initially central core of dikaryotic hyphae.

What makes some hyphae become inflated and multinucleate while others remain morphologically similar to the vegetative mycelial hyphae is not known although the even (i.e. non-random) distribution of the former (see Hammad *et al.*, 1993*b*) implies some form of organisational control. This differentiation occurs at an extremely early stage as both narrow and inflated hyphae can be seen in primordia 3 mm tall. During stem elongation, however, the numerical proportion of narrow hyphae decreased. About 25% of the narrow hyphae were recruited to the inflated category as the fruit body developed.

The cell measurements of Hammad *et al.* (1993*a*) in *C. cinereus* revealed little increase in cell length between a 3 mm fruit body and an 8 mm tall fruit body (both of which were at pre-meiotic developmental stages). Presumably any stem elongation occurring at these stages is due primarily to cell proliferation rather than cell elongation. By contrast, there was a large increase in cell length between the stems of the 8 mm fruit body (pre-meiotic) and that of a 25 mm fruit body undergoing meiosis. Initially the cells in the basal and middle regions of the stem lengthened. Cells in the extreme basal and apical regions were always shorter than those in other regions of elongated stems. For example, cells near the cap/stem junction at the extreme apex of an 83 mm fruit body (fully elongated) had a typical length of 150 μm compared with an average for the whole of the apical section examined (about 10 mm long) of 313 μm. The most elongated cells were found in the upper mid-region of the stem. Ratios of length to width were about two in pre-meiotic stems (3 and 8 mm fruit bodies), but increased after meiosis, particularly in the upper middle regions, to 10, 20 and approximately 35 in 48 mm, 55 mm and 83 mm tall fruit bodies respectively.

Overall, therefore, stem extension of *C. cinereus* involves increase in length and cross-sectional area of inflated hyphae and recruitment of narrow hyphae into the inflated population.

Developmental changes in the distribution of hyphal sizes within the stem. Comparing cell size with absolute position within transects of fruit bodies of different size revealed a progressive change in the distribution of inflated hyphae. In 6 mm and 27 mm tall fruit bodies the inflated hyphae increased in cross-sectional area up to halfway across the cortex but then their size declined towards the centre. In

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the 45 mm tall fruit body the cross-sectional area of inflated hyphae increased gradually from the exterior to the centre, and this pattern was even more pronounced in the 70 mm tall fruit body, where the peak cell area was adjacent to the lumen rather than in the mid-cortex (Fig. 2).

These data obtained by Hammad *et al.* (1993*b*) show that expansion of the stem is mainly due to increase in cross-sectional area of inflated hyphae in the region internal to the mid-cortex. Inflated hyphae around the periphery of the stem do not enlarge much. The mechanical consequences of this pattern of cell inflation are obvious. Increase in cross-sectional area of inflated hyphae in such a deep-seated zone will: (*a*) result in the central axis being torn apart, leaving its constituent cells as a remnant around the inner wall of the lumen so created; and (*b*) reorganize and stretch the tissues in the outer zones of the stem (Fig. 2). The outcome of these processes is a hollow cylinder with an outer 'skin' stretched over inner compressed tissue. In engineering terms, an ideal structure for a columnar support.

Consequently, formation of the mature stem in its optimum mechanical construction is entirely a result of a specific pattern of cell inflation within the stem as it develops. This particular pattern of inflation must be organized by signalling molecules which determine differential cell inflation across the stem radius.

Such observations concentrate attention on the integration of control of cell expansion throughout the fruit body. Expansion of the different cell types in the cap as well as inflation of cells of the stem began immediately post-meiotically (Hammad *et al.*, 1993*a*). Such co-ordination may be achieved by some sort of signalling system that 'reports' the end of meiosis to spatially distant parts of the fruit body. The route such a signal might take is not clear, but primary gills are attached to the stem, with their tramal regions in full hyphal contact with stem tissues, so the connection between tissues undergoing meiosis and the upper (most reactive) regions of the stem may be fairly direct.

The overall implications of these studies are that positional information in otherwise homogeneous cell populations is imparted by some sort of signalling molecule(s) and that such signalling may occur over short ($<$ µm) and long ($>$ mm) distances.

In vitro *tissue transplantations*

Commitment to pathways of differentiation in the *Coprinus* hymenium has been studied by Chiu & Moore (1988*a*) who removed gill lamellae from caps at various stages of development and placed them onto the surface of nutrient agar explantation medium or 1% water agar. Their further development was then observed during incubation at 27 °C.

Cytological examination of 16 specimens of explanted lamellae taken at the dikaryotic stage (prior to meiosis) showed that only very few probasidia in some samples proceeded to prophase I, even after 2 d incubation on explantation medium. The majority of probasidia in such samples remained at the stage which they had reached at the time of explantation despite the fact that surrounding cells (at this stage of development, largely tramal hyphae) formed hyphal outgrowths. Probasidia of samples taken at or after prophase I all completed meiosis and sporulation after explantation (25 specimens were tested). In contrast, paraphyses, cystidia and tramal hyphae in the same samples reverted to hyphal growth by formation of one to many hyphal apices.

Thus, although young probasidia are unable to continue development on explantation; they are somehow inhibited from reversion to the vegetative state; i.e. they are specified as meiocytes but not yet determined for sporulation. Paraphyses, although highly differentiated by being much swollen, retain the ability to revert immediately to (dikaryotic) vegetative growth on explantation. All the evidence suggests that prophase I is the critical stage at which basidia become determined for the division programme. In the experiments of Chiu & Moore (1988*a*), similar results were obtained whether water-agar or nutrient-agar was used as the explantation medium. Thus, meiosis in basidia, once initiated, is endogenously regulated and proceeds autonomously.

Although basidia appeared to be specified for meiosis and sporulation, these processes were slowed in the explants. Some gills isolated at prophase I had formed only sterigmata after one day, producing spores after 2 d although karyogamy through to spore maturation normally occupies only 10 h. Thus, explantation and isolation from the environment of the fruit body cap slows the rate of maturation quite considerably. Similarly, an important implication of the ready ability of paraphyses (and tramal hyphae) to revert to vigorous hyphal growth on explantation is that this growth mode must be actively and continually inhibited *in vivo* to ensure the orderly formation and development of the fruit body. Thus, it is extremely important that a differentiating 'environment' is maintained within the intact tissue, possibly via morphogens, to ensure fulfilment of development. Primordia are often enveloped in an extracellular matrix of mucilage which could serve as the medium through which morphogens could maintain the differentiated state and avoid the dedifferentiation which so readily occurs upon transplantation.

Breaking commitments. The explantation technique of Chiu & Moore (1988*a*) has been developed into a rapid small-scale bioassay which can be used to study the effects of exogenous compounds on the progress of differentiation of basidia after removal from their parent fruit body. Chiu & Moore (1988*b*) showed that ammonium ions and glutamine halt meiocyte differentiation. In such experiments sporulation was terminated and vegetative hyphae emerge from those parts of the basidium which were in active growth at the time of exposure to the inhibitor. Ammonium salts injected into the caps of young fruit bodies with a microsyringe also terminated further development. Very young primordia (prekaryogamy) were not able to withstand the damage caused by injection and in most cases aborted. From the meiotic division stage onwards, very small volumes could be injected without causing non-specific damage. Injections of 2·5 µl of 1 M ammonium salt solutions (buffered to pH 7) were effective in locally suppressing sporulation if injected in post-meiotic and early sporulation stages, causing the occurrence of white zones around the point of injection as the rest of the cap matured

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and produced its crop of blackened spores. Similar injections of water or buffer had no visible effect on fruit body maturation.

Some ammonium-treated basidia were apparently merely arrested, but some reverted to hyphal growth. Remember that this behaviour is quite unusual for basidia which characteristically continue sporulation if explanted to buffer agar after karyogamy, and remain arrested but without reverting if explanted before karyogamy (Chiu & Moore, 1988*a*). Thus a further direct effect of ammonium treatment is the rapid and regular promotion of reversion to hyphal tip growth among the basidial cells. This constitutes a breakdown of the commitment normally shown by these cells to their developmental pathway. The pattern of reversion was also interesting as the new hyphal apices were not distributed randomly, rather they formed at sites expected to be involved in active wall synthesis during the normal progress of development. When the tissue exposed to ammonium treatment was in post-meiotic and early sporulation stages the reversion hyphae grew out at the sites of sterigma formation; if the basidia had formed sterigmata, hyphae, instead of basidiospores, grew from their apices; if spores were in process of formation, exposure to ammonium caused termination of spore formation and outgrowth of hyphal tips. Hyphae also emerged from basal regions of the basidium.

Ammonium inhibited the meiocyte development pathway *in vitro* when applied at any time during meiosis (stages prophase I through to the second meiotic division were tested). When applied at similar stages *in vivo*, ammonium retarded the rate of progress through meiosis but did not suppress sporulation. When applied at later sporulation stages (sterigma formation, spore formation, spore pigmentation), ammonium arrested sporulation completely both *in vivo* and *in vitro*. Thus, exposure to ammonium causes termination of the normal developmental sequence of the basidium; the meiotic process shows some sensitivity to ammonium-arrest but by far the most obvious ammonium-sensitive stages are the post-meiotic sporulation processes of sterigma and spore formation.

Since ammonium ions cause basidia, the only committed cells of the hymenium, to abort sporulation and revert to hyphal growth, normal sporulation may require some form of protection from the inhibitory effects of metabolic sources of these metabolites. Significantly, the ammonium assimilating enzyme NADP–GDH is derepressed specifically in basidia (Stewart & Moore, 1974), being localized in microvesicles associated with the cell periphery (Elhiti, Moore & Butler, 1987) where it serves as a detoxifying ammonium scavenger.

CONTROL OF FUNGAL MORPHOGENESIS

The examples I have discussed so far indicate that expression of the genes which contribute to morphogenesis relies on previously-formed cell and tissue structures. Further, developmental gene expression depends on the environment as this provides the 'context' or 'network' within which new gene products must work. This 'context' includes chemical, electrical and structural or mechanical tensions as well as cell and organelle structures.

Homologues and analogues of all of the developmental mechanisms known in animals and plants can be found in fungi, including (*a*) mechanical effects, (*b*) temporal sequencing, (*c*) pattern formation and morphogenetic fields, (*d*) programmed cell death, (*e*) reaction with an extracellular matrix.

Mechanical effects

The role of mechanical stresses in producing the stem structure is one example already discussed. Another example is the regular radial arrangement of gills. In both *C. cinereus* and *Volvariella volvacea* embryonic gills are contorted (Chiu & Moore, 1990*a*, *b*). In both species the first-formed gills were radially arranged, but as the cap expands more gills are formed. In *V. bombycina*, new gills were formed in two ways (Chiu & Moore, 1990*a*). First, by bifurcation of an existing gill near its free edge. Initiation of the folding which produced bifurcations on existing gills was localized and irregular, resulting in sinuous, contorted gills. The formation of two daughter gills depended on completion of the bifurcation along the entire edge of the parental gill. New generations of gills appeared as ridges in the region between existing gill roots, creating new folds on the cap context representing the free edges of new secondary or tertiary gills, the gill spaces on either side extending into the cap context as the gill grew by its root differentiating from the context.

The sinuous 'embryonic' gills of a *V. bombycina* fruit body are inflated into their final shape by the inflation of the cells of the trama. The hymenium of *V. bombycina* is a skin-like layer of tightly appressed cells, and the trama of the gill becomes filled with greatly inflated cells as maturation proceeds. These features suggest that expansion of tramal cells in gills enclosed by the hymenial 'epidermis' generates compression forces which effectively inflate, and so stretch, the embryonic gills to form the regularly radial pattern of the mature cap. When complete, this stressed-skin construction exhibits great similarity to the stretched skin constructions which is used for light-weight, high-strength engineering structures.

In *C. cinereus*, more gills are added as the fruit body enlarges by bifurcation of existing gills either on one side or at the stem-gill junction, and by division of gill organizers at the roots of existing gills. Consequently, *Coprinus* gills are also formed as convoluted plates. Subsequently, tensions generated by growth of other parts of the fruit body place geometrical stress on the 'embryonic' gills, like a folded cloth being straightened by stretching. Such a mechanism requires that the folded elements (in this case the gills) are anchored. The connection of primary gills to the stem provides the initial anchorage; subsequently cystidium–cystesium pairs interconnect gill plates around the stem. Tensions generated by expansion of the cap will then be communicated and balanced throughout the structure. Cystidium–cystesium pairs act as tension elements whose function is to hold adjacent hymenia together as cap expansion pulls the gills into shape. The strength of the adhesion between cystidia and the opposing hymenium (see discussion on p. 486 in Horner & Moore, 1987) is essential to this mechanical function; when they do not exist, as in the *revoluta* mutant, the gills are not straightened (Chiu & Moore, 1990*b*). This is why cystidia

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must be thought of as tension elements and not buttresses. An engineering comparison here, would be the bracing (tensioning) wires which are strung between the wings of a biplane and which serve to keep the wings together in flight.

As in *Volvariella*, the stress which drives the straightening of the convoluted gills of *Coprinus* is a function of the expansion of the maturing primordium. A crucial aspect of understanding how the final structure of the fruit body is attained is appreciation of the geometrical consequences of the differential growth of the primordium. For example, as a typical fruit body of *C. cinereus* grows from 1 to 34 mm in height, the circumference of the stem increases nine-fold and the circumference of the outer surface of the cap increases 15-fold; this latter corresponds to more than a 3000-fold increase in volume. The differential growth which generates primordium enlargement exerts enormous mechanical effects on relationships between tissue layers which are often concentrically arranged. Mechanical forces themselves generate many of the patterns which characterise the form and structure of the mature mushroom fruit body.

Temporal sequencing: hymenium assembly in Coprinus

Construction of the *Coprinus* hymenium seems to depend on a series of events occurring in a particular temporal sequence. First, is the establishment of a cell layer comprised of basidia with a scattering of cystidia, formed by a synchronized cessation of apical growth by the probasidia (the majority cell type). The distribution of cystidia is determined by each exercising control over a morphogenetic field extending over a radius of about 35 μm which inhibits emergence of a neighbouring cystidium. Cessation of apical growth by the apical (probasidial) hyphal compartment seems to remove a constraint on branching and lateral proliferation of the subbasidial cells. The branches which then arise from the subbasidial compartments insert themselves into the hymenium as paraphyses.

One can accordingly write a sequential list of major controls which have to be exercised to construct the hymenium: specification of determination of the apical differentiation of tramal hyphae into basidia and determination of cystidial distribution and, as a consequence of basidial differentiation (including arrestment of extension growth of the basidial hyphal apex), emergence of paraphyses as subbasidial branches which insert between the basidia before arresting apical wall growth and initiating the spherical wall growth which then inflates the paraphyses so that they become the main structural component as a pavement from which basidia and cystidia protrude (Rosin & Moore, 1985; Rosin, Horner & Moore, 1985; Horner & Moore, 1987).

Programmed cell death

Morphogenesis can require the removal of tissue as well as tissue growth and the cell death responsible for this must be controlled in time and position. This is programmed cell death; it is an important aspect of development in plants and animals. Recent observations have shown that in fungi, too, hyphal compartments are sacrificed to trim hyphae to shape and tissues are sculptured by extensive sacrifice of hyphae.

If the death of cells can be regulated and, more importantly in a hyphal system, if the death of hyphal compartments can be strictly localized, then cell death could be used as a morphogenetic process. Such a mechanism occurs in animal development and there is probably a need for a similar mechanism in fungal morphogenesis. In animals there are two patterns among dying cells (Sen, 1992). Traumatic or necrotic death occurs when the cell is suddenly confronted with extreme non-physiological conditions and loses control of its ionic balance. As a result calcium enters the mitochondria (causing swelling and dilation) and the cytoplasm becomes hypertonic. Uncontrolled water influx causes the cell and its organelles to swell and lyse. On the other hand, apoptotic or programmed cell death occurs in physiological conditions, often in response to effectors which are not lethal to other cells in the vicinity. Indeed, some mammalian cells seem to be programmed for apoptotic suicide unless suppressed by signals from other cells. Apoptotic death is relatively slower than necrosis and involves a programme of well regulated processes (including synthetic ones) which lead to internal cell degeneration and eventual removal of the dying cell by phagocytosis. Apoptosis is of enormous importance in organ development during embryogenesis where cell elimination is a key feature in morphogenesis (though only a very small minority of cells, less than 1%, may be undergoing the process at any one time). Apoptotic cell death is also important in higher animals as a mechanism whereby autoimmunity can be avoided or minimized. Necrotic cell death releases all of the cell machinery to become potential antigens. In apoptotic death the components of the dying cell are digested within its membrane prior to phagocytosis so in most cases no antigens escape. Obviously, this last point is not a consideration in fungi. But it is particularly interesting and significant that Umar & Van Griensven (1997*a*) identified two modes of cell death in *A. bisporus* fruit bodies; one impacting as a trauma from the outside which can be likened to necrotic death, and the other which is more like a true, internally driven, senescence. This latter can be considered a fungal type of programmed cell death.

Umar & Van Griensven (1997*a*) found that the life span of fruit bodies of *A. bisporus* was 36 days when grown in a cultivation environment which protected the culture from pests and diseases. Senescence first became evident around day 18, with cytological indications of localized nuclear and cytoplasmic lysis. These changes were followed by increased permeability of the cytoplasmic membranes and by structural changes to the cell wall. Remains of the lysed cells aggregated around and between the remaining hyphal cells. Most of the stem hyphae became empty cylinders. Other cells within the fruit body collapsed irregularly. Electron microscopy showed that most of the cells throughout the fruit body were severely degenerated and malformed after 36 days, yet a number of basidia and subhymenial cells cytologically remained alive even on day 36. When mushrooms were cultivated using conventional mushroom farming procedures, about 50% of the fruit bodies were found to have been infected by *Trichoderma harzianum* and/or *Pseudomonas tolaasii* by day 18.

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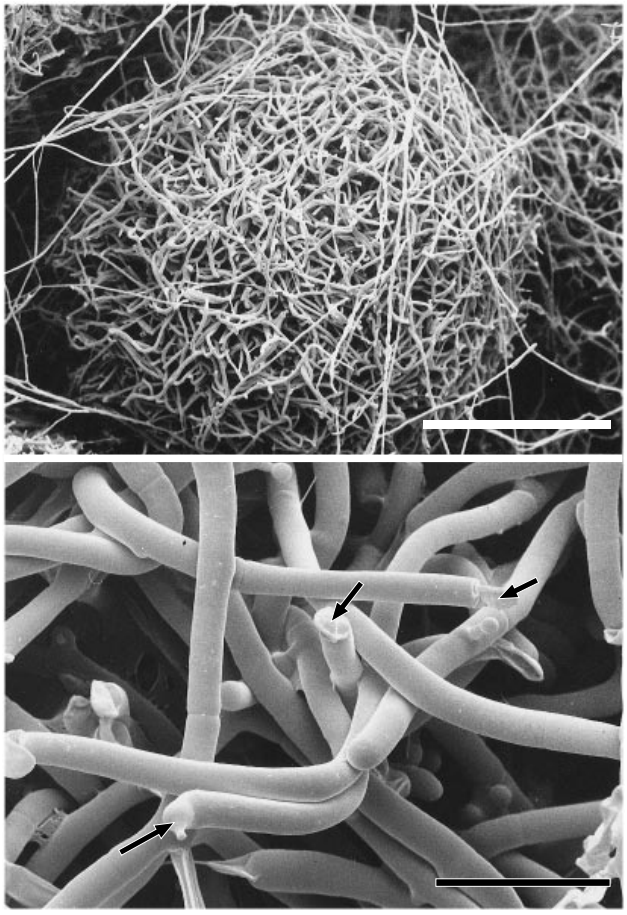

Fig. 3. Cryo-SEM images of the outer layers of fruit body initials of *P. pulmonarius*. Many of the hyphae were alive when flash-frozen and remained fully inflated. Cells undergoing programmed cell death, however, tend to collapse. In the lower image a number of the hyphae (some indicated by arrows) show abrupt terminations where the hyphal filament has been severed by programmed death of one or more hyphal compartments. Photographs kindly provided by Ms Carmen Sánchez. Scale bars, top = 200 μm, bottom = 20 μm.

All such fruit bodies died on day 24 due to generalised severe bacterial and fungal infections leading to tissue necrosis and decay of the caps and stems.

In harvested *A. bisporus* fruit bodies (stored under various conditions) diffuse cell wall damage was observed first, this only later being accompanied by cytoplasmic degeneration. Consequently, Umar & Van Griensven (1997*a*) emphasise that the morphological changes which occur in naturally-senescent and post-harvest fruit bodies of *A. bisporus* are different. Post-harvest physiology and morphology of mushrooms is of paramount importance for mushroom marketing and has been extensively studied, but post-harvest behaviour is usually described as senescence or as an ageing process. It is quite clear from the detailed analysis of Umar & Van Griensven (1997*a*) that this is not the case. The harvested mushroom has suffered a traumatic injury and its post-harvest behaviour stems from that. A major factor must be that it has no way of replacing water lost by evaporation. Consequently, exposed surfaces become desiccated and are damaged first. Thus, in what might be called 'post-harvest stress disorder', further damage is inflicted on the cell inwards, from the outside. In complete contrast, during the senescence which accompanies normal ageing, the damage is inflicted first on the genetic architecture (this to include nuclear and organelle genomes) and subsequently on cytoplasmic integrity, so that cell wall damage occurs as an aspect of the eventual necrosis suffered by the lysing cell. That is, in senescence the damage starts inside the cell and proceeds outwards, from the inside.

The most obvious example of programmed cell death is the autolysis which occurs in the later stages of development of fruit bodies of many species of *Coprinus*. Buller (1924, 1931) described these in detail and interpreted the autolysis to be part of the developmental programme. The well-known grosser aspects of gill autolysis serve to remove spent gill tissue from the bottom of the cap upwards to avoid interference with spore discharge from regions above. That is, when the hymenium has discharged its basidiospores, autolysis is initiated to destroy that part of the gill so that it will not be a physical barrier to discharge of spores by the remaining part of the gill. The enzymology of this gross autolysis has been examined in detail in *C. cinereus* and found to be due to the release of chitinase, acid and alkaline proteinase, RNA-ase, phosphatase and β-glucosidase enzymes which had previously been localized in intracellular vacuoles (Iten, 1970; Iten & Matile, 1970). Vacuoles containing acid and alkaline proteinase, RNA-ase, phosphatase and β-glucosidase were found in vegetative mycelium as well as fruit body gill tissues, so they appear to be part of the normal turnover-metabolism of the cells. Vacuoles with chitinolytic activity were, however, newly formed shortly before spore release was initiated. Iten & Matile (1970) suggested the chitinolytic enzymes were passively released by cells whose metabolic activity had ceased; their growth thereby becoming unbalanced.

Taken together these observations show that autolytic modification of the fruit body in later stages of its development in *Coprinus* involves specific production of new enzymes in particular cells at a particular time. The autolytic destruction of those cells is clearly part of the morphogenesis of the fruit body; it is a programmed cell death. The process cannot be dismissed as simply the final step of some other developmental programme. Buller (1924) also described a much more localized autolysis of cap flesh immediately above the gill trama in small species of *Coprinus* which enabled the gills to split and their two hymenia to be stretched apart as the cap opened like an umbrella. In this case the same autolytic programme is being exercised at an earlier stage in development and in cells which are specifically placed to achieve a particular fruit body morphology.

Umar & Van Griensven (1997*b*, 1998) have indicated that there may be a more general involvement of a fungal type of programmed cell death in fruit bodies of higher fungi. It is shown that in very early primordia of *A. bisporus* the first gill spaces are formed as a result of cell death. The authors point out that the exact timing (prior to basidial differentiation) and exact positioning (in an annulus close to the junction of cap and stem) implies that cell death is genetically programmed as part of the morphogenetic process. It would seem that fungal programmed cell death plays a role at many stages in development of many species (Umar & Van Griensven, 1998). A need for such a mechanism is evident from the morphology of fruit body initials (Fig. 3). These appear to be tangled

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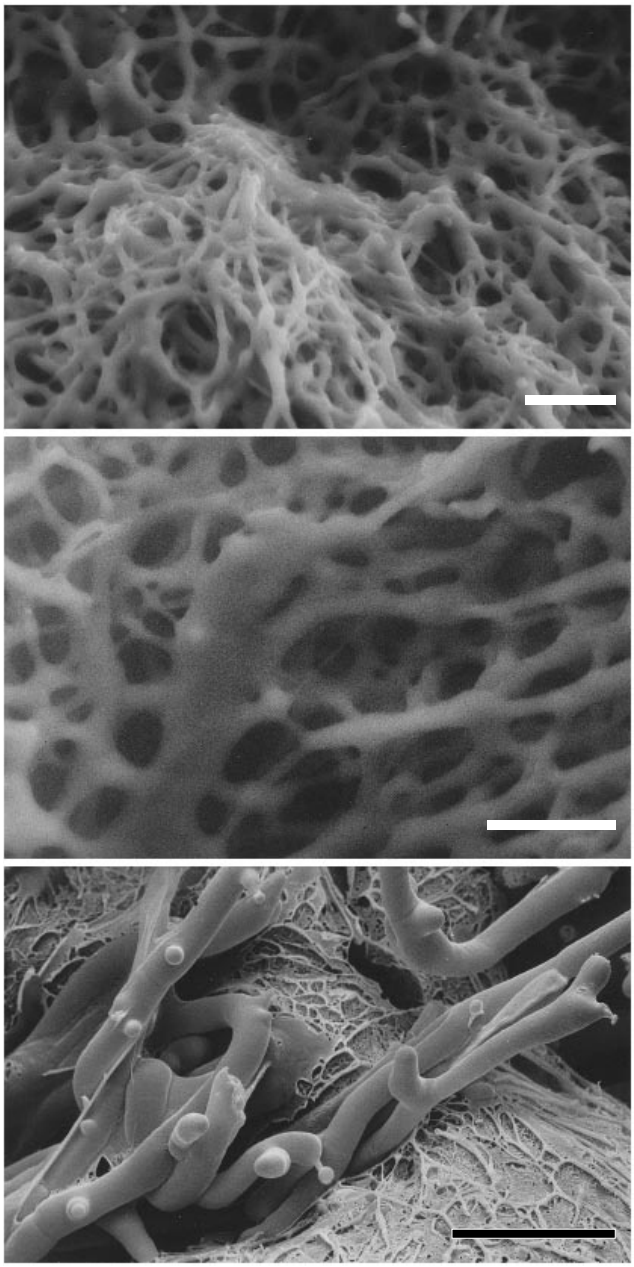

Fig. 4. Fruit body initials of *P. pulmonarius*. An entire initial is shown at the top, similar to that shown in the upper image of Fig. 3, but here the specimen has been imaged using an Ectroscan environmental SEM which operates at a sufficient pressure to enable imaging of live tissue. This photograph shows that living hyphae are surrounded by a sheath of mucilage, shown in higher magnification in the middle micrograph. Frozen and partially-desiccated mucilage can be imaged in conventional cryo-SEM preparations (bottom micrograph). Photographs kindly provided by Ms Carmen Sánchez. Scale bars = 20 μm.

masses of long hyphae, yet very early in fruit body development compact structures emerge with clearly demarcated surfaces. In the initials, hyphae extend in every direction so demarcated surfaces can only arise if pre-existing hyphae which cross the boundary before it is established can be severed. The cytological evidence seems to indicate that individual hyphal compartments can be sacrificed (Fig. 3) in order to trim hyphae to create a particular shape. Programmed cell death is used, therefore, to sculpture the shape of the fruit body from the raw medium provided by the hyphal mass of the fruit body initial and primordium.

Extracellular matrix

In several examples detailed by Umar & Van Griensven (1998) the programme leading to cell death involves the sacrificed cells over-producing extracellular matrix and then lysing to release it. Evidently, the process is not simply one of killing cells at particular times and in particular places. The cell contents which are released when the cells die seem to be specialized to particular functions too. In the autolysing *Coprinus* gills the cell contents released on death contain heightened activities of lytic enzymes. The cell deaths which carve out the shapes of fruit body primordia release mucilaginous materials. It seems, therefore, that this cell death programme includes a subroutine which causes the cells to synthesise large quantities of extracellular matrix for release when the cell lyses.

The fungal extracellular matrix is extensive and complex (Fig. 4). It provides the environment within which reinforcement of differentiation can occur. Hyphal cells require reinforcement of their differentiation 'instructions'. This reinforcement is part of the context within which they normally develop and may involve production of location- and/or time-specific extracellular matrix molecules and/or smaller molecules serving as hormones or growth factors. It is important to note that uptake is not necessary because the molecules may react with integrins in the plasmalemma and the existing extracellular matrix. Such direct effects on the cytoskeleton can cause immediate metabolic changes in one or more cellular compartments and may directly influence gene transcription. This phenomenon is well established in many animal systems and is known as 'outside-in' signalling.

GENETIC ASPECTS OF FUNGAL DEVELOPMENTAL BIOLOGY

It must be evident from the discussion so far that patterning genes may be difficult to find because the pattern under investigation may simply be an inevitable consequence of events which have gone before. A specific gill pattern in an agaric, say, may result because the application of general rules within the context of its development 'invariably' results in that pattern. Clearly, genetic components are essential but recognising their precise phenotype may not be straightforward.

Another common feature is that fungal morphogenesis is compartmentalized into a collection of distinct developmental processes (called 'subroutines'; Chiu, Moore & Chang, 1989). These are recognizable at all levels: including particular organs (e.g. cap, stem, veil, etc.); tissues (e.g. hymenophore, context, pileipellis, etc.); cells (e.g. basidium, paraphysis, cystidium, etc.); and cellular components (e.g. wall growth may be uniform, in girth, in length or in thickness).

Developmental subroutines are distinct genetically and physiologically and may run in parallel or in sequence. When played out in their correct arrangement, 'normal' morphology results, but if some subroutines are disabled (genetically or through physiological stress), the rest may still proceed. Such

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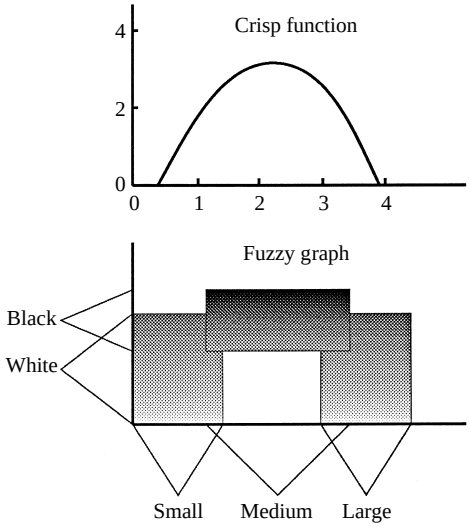

Fig. 5. Comparison of a crisp function (top) with a fuzzy graph (bottom). The peak values of the top graph are specified by a precise relation between x and y, possibly with some definition of the extent of stochastic variation. On the other hand, the peak of the fuzzy graph is indicated adequately by the phrase 'about medium size and very dark in colour'. Adapted from Zadeh, 1996.

partial execution produces an abnormal morphology, but in many instances spores are still produced. This flexibility in expression of developmental subroutines allows the fruit body to react to adverse conditions and still produce a crop of spores. It also illustrates that tolerance of imprecision is an important attribute of fungal morphogenesis.

Fungal development uses fuzzy logic rather than crisp logic. Discussion of differentiation in fungi often involves use of words like 'switch' in phrases which imply wholesale diversion at some stage between alternative developmental pathways. There are now many examples, some of which have been described above, which suggest that fungal cells behave as though they assume a differentiation state even when all conditions for that state have not been met. Rather than rigidly following a prescribed sequence of steps, these fungal differentiation pathways appear to be based on application of rules which allow considerable latitude in expression. What might be thought of as 'decisions' between developmental pathways are made with a degree of uncertainty, as though they are based on probabilities rather than absolutes. For example, facial cystidia of *C. cinereus* are generally binucleate, reflecting their origin and the fact that they are sterile cells, yet occasional examples can be found of cystidia in which karyogamy has occurred (Chiu & Moore, 1993) or of cystidia bearing sterigmata.

Development of a cystidium represents expression of a perfectly respectable pathway of differentiation and commitment of a hyphal tip to the cystidial as opposed to the basidial pathway of differentiation. The commitment must occur very early in development of the hymenium because young cystidia are recognizable in the very earliest stages (Rosin & Moore, 1985). The controls which determine formation of a cystidium, instead of a basidium, by a particular hyphal apex need to be established. It is certainly the case that the basidial developmental pathway (in *A. bisporus*) can be interrupted to allow this cell type to serve a structural rather than spore-producing function (Allen, Moore & Elliott, 1992), though this is clearly arrested meiosis, not sterility. Conceptually, this is similar to the cystidia of *Coprinus* which show evidence of entry into meiosis (Chiu & Moore, 1993) and which suggest that entry to the cystidial pathway of differentiation does not totally preclude expression of at least part of the meiocyte differentiation pathway. Similarly, the fact that a large fraction of the *in situ* basidial population of *A. bisporus* remains in arrested meiosis indicates that entry to the meiotic division pathway does not guarantee sporulation; a fact also demonstrated with excised gills of *C. cinereus in vitro* (Chiu & Moore, 1990*a*).

Chiu & Moore (1993) discuss the possibility that fungal differentiation pathways exhibit what would be described as 'fuzzy logic' in cybernetic programming terms. The phrase has a particular meaning, which has been equated with 'computing with words' by Zadeh (1996). It is a methodology which is useful for dealing with situations which must tolerate imprecision; effectively, where the programming term 'either/or' must become 'maybe'. It is probably easiest to illustrate by contrasting a graph based on conventional real numbers, which describes what is called a crisp function, with a fuzzy graph (Fig. 5). Clearly, the imprecision of the input to the fuzzy graph greatly expands the fuzzy constraints beyond those defined by the crisp function. It is this notion of tolerance of imprecision which can be applied to fungal morphogenesis.

Instead of viewing fungal cell differentiation as involving individual major 'decisions' which switch progress between alternative developmental pathways which lead inevitably to specific combinations of features, this idea suggests that the end point in fungal differentiation depends on the balance of a number of minor 'decisions'. Observation shows that developmental decisions between pathways of differentiation are able to cope with a degree of uncertainty, allowing hyphae to assume a differentiation state even when all conditions of that state have not been met. So, rather than rigidly following a prescribed sequence of steps, fungal differentiation pathways must be based on application of rules which allow considerable latitude in expression – fuzzy constraints.

Plausible genetic mechanisms

Epigenetics is not often discussed in relation to fungal development, and when it is the more restricted interpretation of epigenetic modification of the genome is the usual topic, with the implication that gene expression takes pride of place in morphogenesis. This comment is not intended to underestimate any role played by such mechanisms. There are a number of processes that silence genes in filamentous fungi (Irelan & Selker, 1996). One of these is transcriptional silencing of genes by methylation of cytosines in the DNA which seems to be common in fungi and plants (Meyer, 1996). Pre-meiotic methylation occurs during the sexual cycle in *Ascobolus immersus* and *C. cinereus* to silence repeated sequences reversibly. The mechanism involves homologous pairing to sense the presence of duplications. Differences in methylation

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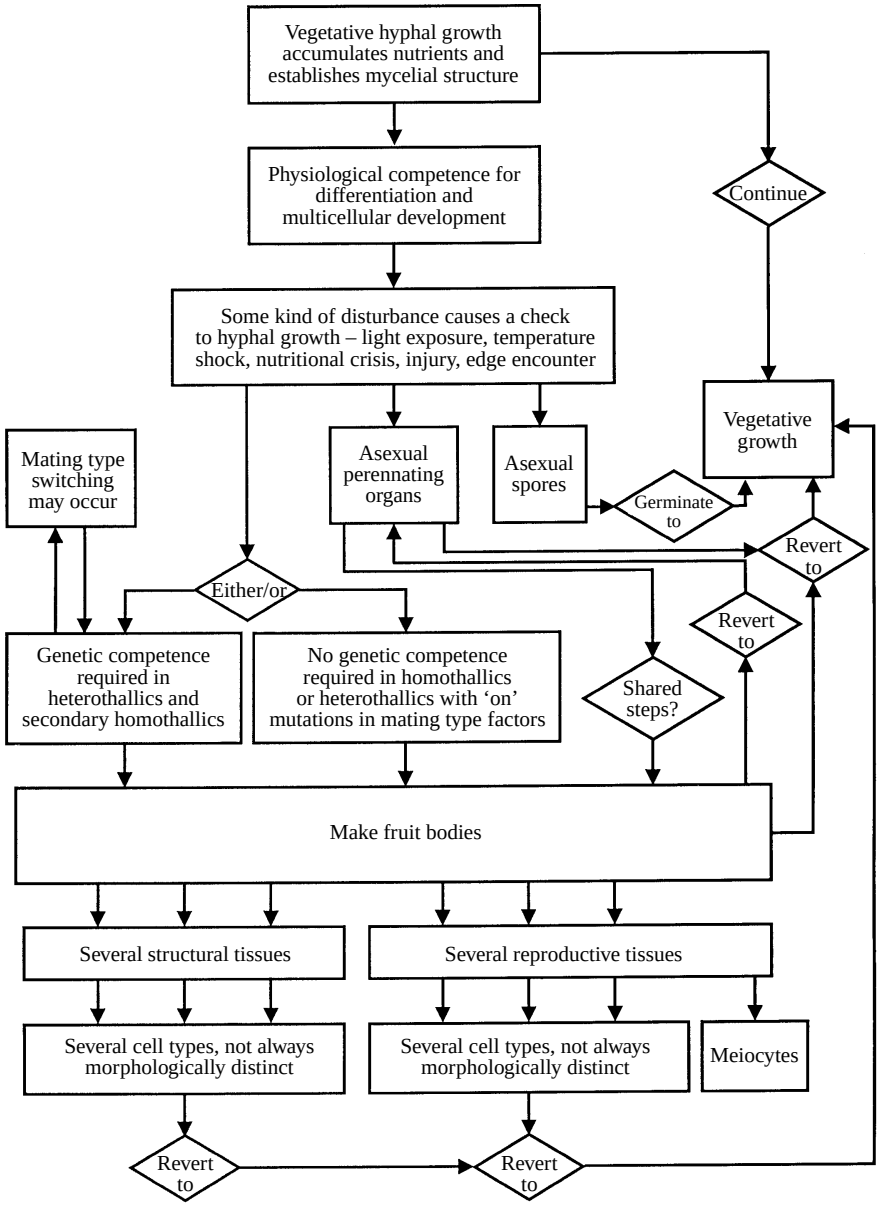

Fig. 6. Flow chart showing a simplified view of the processes involved in development of fruit bodies and other multicellular structures in fungi. From Moore, 1998.

patterns have been detected between yeast-phase and filamentous growth in some dimorphic fungi (Reyna Lopez, Simpson & Ruiz-Herrera, 1997). It is this reversible differential DNA methylation which might be related to fungal morphogenesis. This is an attractive proposition in general theoretical terms (Bestor, Chandler & Feinberg, 1994). There is, however, also evidence that methylation plays a role in the control of chromosome behaviour during meiosis in *Neurospora crassa* (Foss *et al*., 1993). This specific possibility argues against DNA methylation being involved generally in morphogenesis. A similar conclusion is indicated by the facts that methylation occurs to a lesser extent in the mushroom *C. cinereus* than it does in *Ascobolus* or *Neurospora* (Freedman & Pukkila, 1993), and that there was no evidence for development-related differences in methylation in *A. bisporus* (Wilke & Wach, 1993).

Neither methylation nor any other single mechanism provides an adequate foundation for a developmental network at the gene expression level. My preference is for a more holistic approach to identifying factors with the potential to influence morphogenesis.

Key words at each stage of development in fungi seem to be competence, induction and change. Competence is repeatedly encountered. Hyphae must be able to initiate the next step, but the next step is not inevitable. Competence may be genetic (e.g. mating types) but is primarily a physiological state. Induction is the process by which the competent tissue is exposed to conditions which overcome some block to progress and allow the next stage to proceed. Change occurs when the competent tissue is induced. The next stage always involves change in hyphal behaviour and physiology. This is usually quite drastic and represents an additional property to those already expressed. That is, each developmental step takes the tissue to a higher order of differentiation. The state of competence represents the establishment of a network in the sense of Trewavas (1986, and see below), but expanded to

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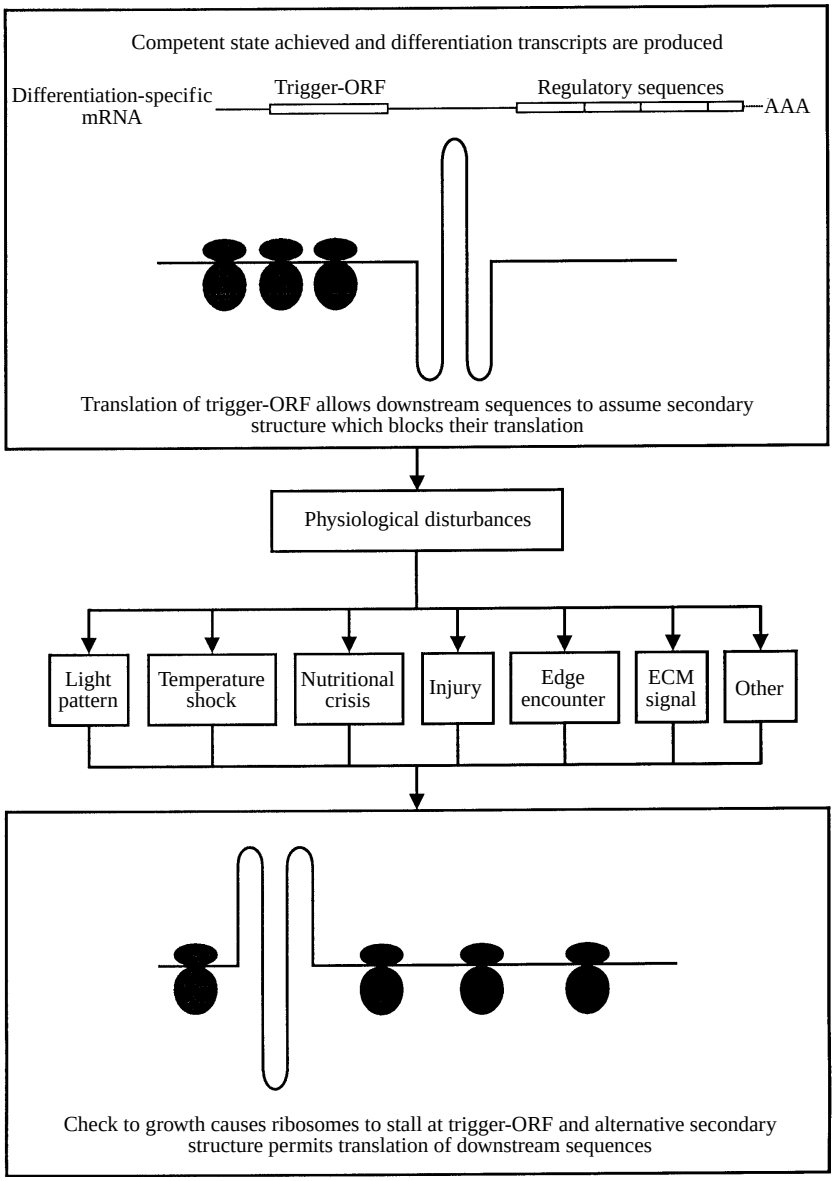

Fig. 7. Translational triggering adopted as a general model for entry of competent tissues into fungal pathways of differentiation. From Moore, 1998.

include the full range of physical and physiological components.

Differentiated hyphal cells require reinforcement of their differentiation 'instructions'. This reinforcement is part of the context within which they normally develop (that is, it is part of their network), but when removed from their normal environment most differentiated hyphae revert to the mode of differentiation which characterizes vegetative hyphae. Hyphal differentiation is consequently an unbalanced process in comparison with vegetative hyphal growth. In most hyphal differentiation pathways the balance must be tipped in the direction of 'differentiation' by the local microenvironment which is, presumably, mainly defined by the local population of hyphae.

Another common feature is that morphogenesis is compartmentalized into a collection of distinct developmental processes, the 'subroutines' discussed above, which give rise to the 'normal' morphology when played out in their correct arrangement. The flexibility in expression of developmental subroutines, which allows the fruit body to react to adverse conditions and still produce a crop of spores, provides the tolerance of imprecision referred to above. The ultimate flexibility, of course, is that the differentiation process can be abandoned in favour of vegetative hyphal growth and reversion to the invasive mycelium ensues. A lesser level of flexibility may be that a particular function is carried out by an incompletely adapted cell type. The flow chart in Fig. 6 summarizes all of these notions.

When it comes to searching for mechanisms which might control fungal morphogenesis there is no shortage of candidates. Homologues and analogues of all of the mechanisms known in animals and plants can be found in fungi. For control at the genetic level the mating type factors provide prime examples of transcriptional control elements able to regulate specific morphogenetic subroutines. The regulation involves transcriptional activation and repression

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and further 'complication' can be introduced, if necessary, by using intrachromosomal recombination to interchange regulatory cassettes.

Given the prevalence of data which indicate that hyphal systems (*a*) need to develop a state of competence before they are able to undertake a developmental pathway, and (*b*) can be precipitated into embarking upon a particular morphogenesis by a variety of environmental signals, it is difficult to believe that translational triggering and feedback fixation (Timberlake, 1993) are not widely used as regulators throughout the higher fungi.

Translational triggering

Translational triggering is a mechanism which can relate a morphogenetic pathway to the development of competence on the one hand, and to initiation in response to environmental cue(s) on the other hand. There are indications from a wide range of physiological studies that nitrogen metabolism may be crucial in regulating morphogenesis. There would certainly be scope for associating particular differentiation pathways with particular aspects of metabolism, so that supply of specific aminoacyl-tRNA molecules might regulate entry into differentiation pathways by affecting translation of a controlling reading frame (trigger-ORF in Fig. 7).

If the trigger-ORF contained adjacent codons for aminoacyl-tRNAs subject to variation in supply, stalling/non-stalling of translation of trigger-ORF might determine whether the messenger transcript forms secondary structures which permit/do not permit translation of down-stream reading frames. Note that either one or both components of the aminoacyl-tRNA may be the limiting factor and the limitation may be imposed by a compartmentalization. That is, amino acid or a specific tRNA (or, presumably, an aminoacyl-tRNA synthetase) may be compartmentalized, regulated in local concentration, or both.

The interpretation offers a way by which a competent tissue can be released to undertake differentiation by a range of physiological events. Competence is interpreted to mean that messenger transcripts for the necessary regulators (and perhaps some key structural genes) are produced but not fully translated because an upstream sequence (trigger-ORF) prevents translation. There may be a number of different such transcripts with regulators corresponding to the different pathways upon which the competent cell can embark, their trigger-ORFs responding to separate physiological events. On the other hand, there may be a number of similar transcripts in different cellular compartments so that the translational trigger can be released by the particular activities of those compartments with the result that one differentiation process may be triggered by different physiological events. It could also be that such a transcript was limited to one compartment, even one type of vesicle, perhaps, from which the trigger molecule can be excluded until some highly specific and/or localized physiological change occurs. Unfortunately, there is no evidence for any of these speculations. The only possibly relevant data is the finding that depletion of carbon source available to *Saccharomyces cerevisiae* blocks translation but not transcription (Martinez-Pastor & Estruch, 1996). This may not even be relevant, but it does at least indicate that a major physiological crisis can have more immediate impact on translation and it emphasises that carbon metabolism is important, too, even if nitrogen metabolism is more often associated with morphogenetic change. Despite the lack of direct evidence at this time, I suggest that a variety of physiological signals and stresses cause translation-level controls to direct competent fungal tissues to undertake specific differentiation processes. Comparison with the operation of mating type factors makes it reasonable to suggest that the translational trigger could immediately lead to translation of components of highly specific transcription activators and inhibitors which then regulate gene sequences required for the differentiation which has been initiated. These, or their eventual products, may be involved in feedback fixation of the differentiation pathway.

Feedback fixation

Feedback fixation is the outcome of feedback activation and autoregulation which together reinforce expression of the whole regulatory pathway to make it independent of the external environmental cues which initiated it. Feedback fixation results in developmental determination in the classic embryological sense. The epigenetic aspects of the network governing fungal morphogenesis starts with feedback fixation, but also includes signals from outside the cell (Fig. 8). The fungal extracellular matrix is extensive and complex. Its reaction to, and interaction with the environment can be communicated to the intracellular environment to modify cytoplasmic activity. Since neighbouring cells are components of the external environment, it must be the case that the activity of one hyphal cell is modulated by changes made to the extracellular matrix by a neighbouring hyphal cell.

On this interpretation, therefore, continued progress in differentiation for most fungal cells requires continued reinforcement from their local microenvironment. This may involve production of location- and/or time-specific extracellular matrix molecules, or any of a range of smaller molecules which might be classed as hormones or growth factors. Smaller molecules might exert their effects by being taken up into the cell. But uptake is not necessary. Any of these molecules may also affect relations between integrins and the existing extracellular matrix. As a result there could be direct effects on the cytoskeleton which are able to cause immediate metabolic changes in one or more cellular compartments, or directly influence gene transcription.

Connections to the extracellular matrix may also be involved in that other great enigma: the control of hyphal branching. By varying extracellular matrix/membrane or wall/membrane connections external signals may be able to specify branch initiation sites. Similarly, internal cytoskeletal architecture could also arrange specific membrane/wall connections to become branch initiation sites. Branch initiation sites specified in these ways may then become gathering sites for the molecules which create a new hyphal tip. First among these could be molecular chaperones (heat shock proteins; Hartl, Hlodan & Langer, 1994) which rearrange existing wall and membrane proteins. The chaperones could arrange for a

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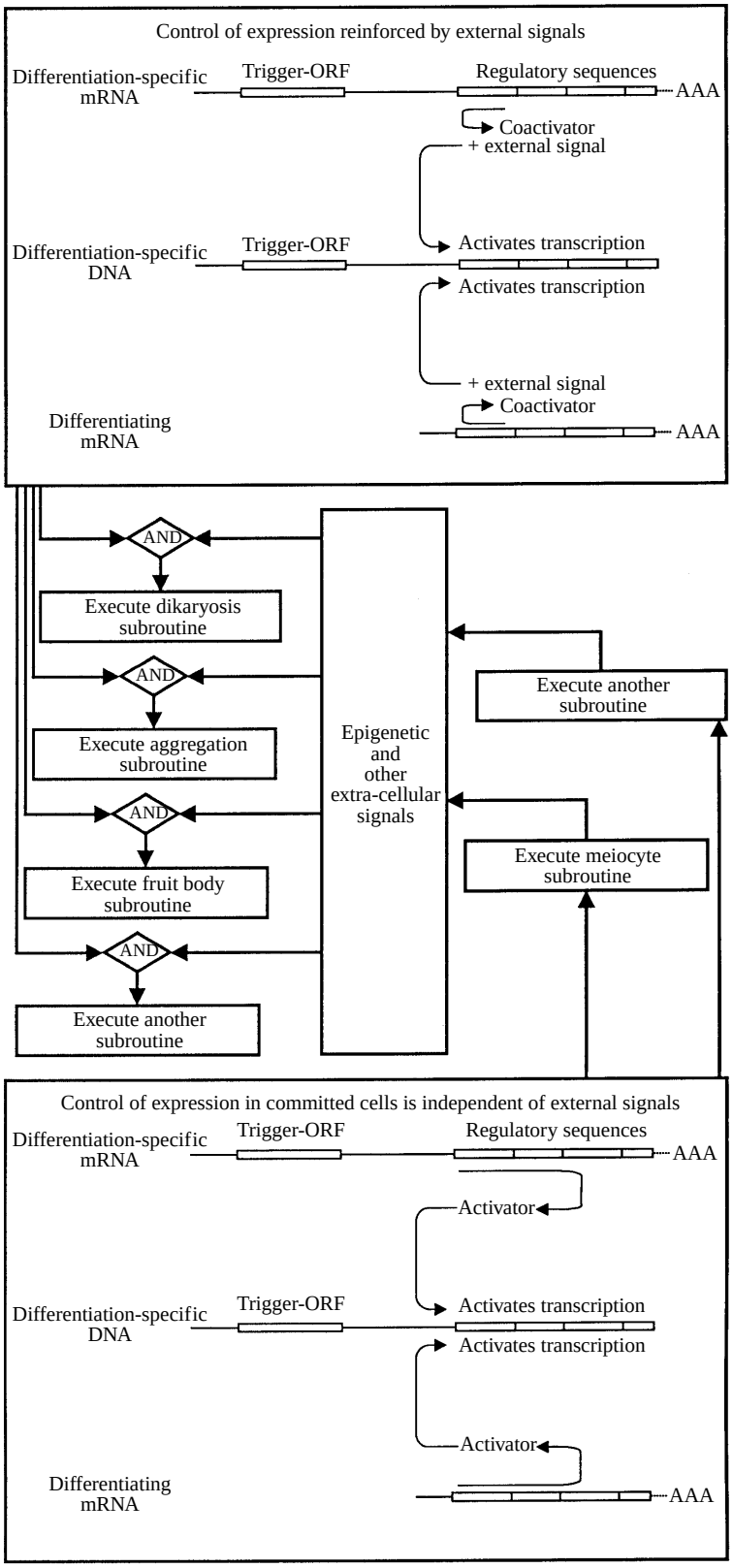

Fig. 8. Feedback fixation adopted as a general model for maintaining progress through fungal pathways of differentiation. In this flow chart the box at the top shows the type of feedback fixation process envisaged to apply to most developmental subroutines in which epigenetic reinforcement from the local microenvironment is needed to interact with coactivators in order to maintain the feedback activation loop. In the bottom panel, the alternative of direct feedback fixation independently of other signals is shown as being applicable to cell types which show developmental commitment; only meiocytes are known to be committed, but there may be other committed cell types. From Moore, 1998.

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mature wall region to become juvenile again by altering the conformation of existing polypeptides rather than by severing any covalent bonds. Local activation of resident chitin synthase (Gooday & Schofield, 1995) and initiation of a localized resumption of wall synthesis would then establish the new hyphal tip. The branch would consequently emerge in a position precisely defined by the stimulation of generalized cytoskeleton/membrane/wall connections by a positional stimulus.

The focus of these hypothetical regulatory activities is, obviously, the hyphal wall, its surface and the immediate extracellular environment. These are features about which we are very ignorant and urgent and extensive research on these topics is necessary.

The epigenetic context

The main lesson to learn from other organisms bears on the question of the ultimate level of morphogenetic control. It is clear that in animals and plants morphogenesis is not simply a matter of playing out a predefined genetic programme and it is likely that this will be true for fungi also. Goodwin (1984) states that the '… developmental process involves principles of organization … which are not caused by genes in the way that computer output is caused by a program.' Instead, he argued that macromolecular and cellular structures, such as the cytoskeleton and endomembrane system, provide a more promising foundation for describing the forces which define morphogenetic fields during animal embryogenesis. Those fields of force, thought to include electrical, chemical and mechanical effects, are modified by, and themselves modify, the expression of specific gene products.

Trewavas (1986) developed a similar argument about the involvement of growth substances in plant development. Rather than the abstraction 'fields of force', Trewavas (1986) likened plant development to a network of metabolic processes. Interactions between metabolic pathways create the network and the form of the tissue or organ changes in response to introduction of new components and/or new links within the network. Trewavas (1986) was keen to argue against the common notions that growth substances in plants serve as limiting factors which act in direct 'cause and effect' pattern. He argued that these ideas are best replaced by the concept of sensitivity to control. This sees the network as reaching a state which is able to react to the growth substance (or the inverse interpretation: the growth substance is such that it alone can disturb an otherwise stable network). Edelman (1992) emphasized three observations which are key to his own morphoregulator hypothesis. These three points are: (i) expression of developmentally important genes is epigenetic and place-dependent, relying on previously-formed tissue structures; (ii) the driving forces of morphogenesis are cellular in origin, namely cell division, movement (Edelman is concerned with animal embryogenesis), adhesion and death; (iii) the links between gene expression and the mechanical driving forces are molecular, proteins, growth factors and adhesion molecules. Edelman's (1992) morphoregulator hypothesis is essentially a network in which he places particular importance on adhesion molecules (cell-to-substratum and cell-to-cell adhesion) and cell junctional molecules. These molecules are the products of differentially-expressed (that is, developmentally regulated) genes, but the gene products interact epigenetically to modulate the cell surface. The cell surface phenotype which results may then itself induce further differentiation and/or development-specific changes. In other discussions the part played by mechanical tensions in animal morphogenesis has also been stressed. Bard (1990) provides a general discussion, but Van Essen (1997) concludes his review of morphogenesis of the mammalian central nervous system with this paragraph: '… Morphogenesis entails an intricate choreographing of physical forces that cause differential tissue growth and displacement. Does this require an elaborate set of developmental instructions, transcending those needed to regulate the processes of neural proliferation, migration, axonal pathfinding, and synapse formation? If morphogenesis is driven largely by tension, the answer is no. Instead, the specificity of shape changes would largely be a by-product of factors that dictate the connectivity and topology of the underlying neural circuitry. This constitutes an efficient strategy for sharing the instructions that guide neural development.' Even though he places his emphasis on mechanical tension, Van Essen's argument is clearly fundamentally epigenetic and of exactly the same sort as those featured earlier in this section.

It is interesting that the embryologist (Edelman, 1992) emphasizes externalized molecules involved in adhesion, whilst the botanist (Trewavas, 1986) emphasises interactions between internal metabolic pathways (albeit influenced by growth substances from outside the cell). Goodwin (1984) is more inclusive, mentioning mechanical and electrical as well as chemical fields as components of the 'organized context' within which developmental genes operate. Despite these cultural differences, all of these interpretation stress the role of complex interactions in specifying morphogenetic stages. In such contexts, properties are shared throughout the network because of the interconnections. Thus, change in one part results in a response by the whole. The more complex the interactions, the more stable the state. The concept also envisages, however, that introduction (or removal) of a component will have sufficient influence to alter the state of the network. This helps to explain how changes in morphogenesis can depend on discrete events such as differential expression of just a few genes, or one specific environmental variable. Genes which can be characterized as developmental genes are those whose products have sufficient epigenetic impact to shift the network to a new state. Similarly, a network sensitized by its intrinsic structure to a particular environmental variable will shift in response to that variable but will be insensitive to other variables.

Again, the common observation of numerous (sometimes small) changes characterizing an altered state of differentiation can also be accommodated. The assumption in this case is that these phenotypic features comprise a collection of characters which result from the interactions within the network. When the network shifts in response to a signal to which it is sensitive, all those interactions change. As a consequence, all of the characters in the collection which defines the present state of differentiation are likely to alter as the network shifts

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to a new state of differentiation. The differentiation signal may be correlated with many of the morphogenetic changes it induces, but may not cause them directly. Their cause lies within the network of interactions. If the signal and the morphogenetic change are studied in isolation, away from the network, it may not be possible to demonstrate any connection between them at all.

The key to fungal morphogenesis lies in understanding how that which is outside a hypha can influence that which goes on inside the hypha in a time- and place-dependent manner. Exactly the same can be said for animals and plants. The fungal situation is no less complex, and certainly no less interesting, than these other eukaryotes. Mycologists must learn to think much more in developmental terms; the fungi had it all sorted out several hundred million years ago!

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