5

Positional control of development in fungi

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Filamentous fungi must be unique in offering, as part of the normal pattern of growth of one organism, the opportunity to study the behaviour of independent cells undergoing vegetative growth as well as the behaviour of cells which are contributing to the structure and development of complex organs and tissues. The free-living microbial cell shows great flexibility and adaptability in responding to its environment but the contact with the environment is so close that the concept of the behaviour of the cell has no meaning unless the whole system of cell plus environment is considered. This is not the case for the cells of a tissue where the organization and metabolism of the cells which constitute the tissue can in large measure determine the environment of individual cells. However, the contribution to a tissue must be viewed as carrying with it some loss of freedom of response – a diminution of the flexibility and adaptability of which the individual cell may be capable – in favour of the concerted action required of the tissue as a whole.

In most organisms the behaviour of independent cells can only be studied under the most artificial conditions by investigation of cell cultures. For the fungi the vegetative growth of 'undifferentiated' cells in the form of a mycelium is a normal part of the life cycle. Yet that same mycelium may give rise to fruiting bodies which are as complex in structure and every bit as massive (some Zambian mushrooms can approach one metre in cap diameter (Piearce, 1981)) as are the organs and tissues of higher organisms. This, of course, is a very considerable experimental advantage. The only other group of organisms which offers the same sorts of experimental attractions are the slime moulds, and even these, as the class Myxomycetes, have been claimed as kindred of the true fungi.

Such uniqueness, though, does bring with it some difficulties. One such has already been encountered by the use of the word 'cell' in the first paragraph. In this essay 'cell' will be used to refer to discrete compartments; whether those compartments are in free cytoplasmic contact with

one another, and whether they are uninucleate or multinucleate, will vary with the situation.

The initial emphasis of this essay will be on the growth and structure of the mycelium. The sorts of controls which operate between the constituent cells to determine the pattern of growth of mycelial hyphae will be discussed. The form and structure of the complex organs and tissues will then be illustrated and an attempt made to indicate how the behaviour of mycelial hyphae may be modified to direct morphogenesis of these structures.

Organization of mycelial growth

Control of hyphal growth

Filamentous fungi are well adapted to the colonization of solid substrata. By hyphal extension and regular branching the mycelium can increase in size without disturbing the cell volume/surface area ratio so that metabolite and end-product exchange with the environment can involve translocation over very short distances. Growth of the mycelium, of course, is not haphazard and Bull & Trinci (1977) have identified three mechanisms involved in regulating the growth pattern of undifferentiated mycelia. These are: the regulation of hyphal polarity, the regulation of branch initiation, and the regulation of the spatial distribution of hyphae.

Fungal hyphae are as variable between species as is any other aspect of fungal biology, but generally speaking the hyphal filament, when separated into compartments by cross-walls, has an apical compartment which is perhaps up to ten times the length of the intercalary compartments. The septa which divide hyphae into cells may be complete (imperforate), penetrated by plasmodesmata, or perforated by a large central pore. The latter may be open (and offer little hindrance to the passage of cytoplasmic organelles and nuclei), or protected by a complex cap structure derived from the endoplasmic reticulum (the dolipore septum of basidiomycetes). Septal form and function have recently been reviewed by Gull (1978), and it is worth noting, in relation to problems associated with the communication of signals, the conclusion that septal form may be modified by the hyphal cells on either side of the septum, and may vary according to age, position in the mycelium, or position in the tissues of a differentiated structure.

Growth of the hypha involves the integration of cellular growth processes so as to produce an ordered sequence of events contributing to a duplication cycle which is exactly analogous to the cell cycle of uninucleate cells (Trinci, 1979). Although it is the case that the hypha is a filament

composed of numerous cells connected end to end, it is essential to appreciate that hyphal growth is highly polarized, true extension growth being absolutely limited to the hyphal tip, so the whole morphology of the hypha depends on events taking place at its apex (Grove, 1978). It follows from this that the pattern of hyphae in a mycelium, which is largely a consequence of the distribution of hyphal branches, depends on the pattern of formation of the hyphal tips which initiate the branches. It seems now to be generally accepted that the materials necessary for hyphal extension growth are produced at a constant rate (related to the specific growth rate) throughout the mycelium. Under the influence of a mechanism which achieves polarized transport (Trinci, 1978*a*), these materials are transported towards the tip of the growing hypha. Among the materials taking part in this polarized transport are the cytoplasmic vesicles, which are thought to contain wall precursors and the enzymes needed for their insertion into the existing wall, that seem always to be involved in primary wall growth in the hypha (Bartnicki-Garcia, 1973). Trinci (1974, 1978*b*, 1979) has argued that lateral branches are formed at locations where these vesicles (and other components) affect the rigidified wall of the hypha so as to produce a new 'hyphal tip'. What specifies the site of the branch initiation is not entirely clear, though it has been shown that changes in the ion flow pattern accompany branch initiation in *Achlya* (Kropf, Lupa, Caldwell & Harold, 1983). Anything which interferes with the polarized flow of vesicles and other materials towards the established tip would promote a localized accumulation which may initiate a branch. In septate moulds there is often a correlation between septation and branch initiation such that branches arise just behind septa. This implies that the apical flow of vesicles is interrupted by the septum and accumulated vesicles interact with the lateral wall of the hypha. Trinci (1974) showed that a new branch is initiated when the mean volume of cytoplasm per hyphal tip (the hyphal growth unit) exceeds a particular critical value. For a range of fungi the hyphal growth unit increased following spore germination but then exhibited a series of damped oscillations tending towards a constant value (Trinci, 1974). Such constancy demonstrates that over the mycelium as a whole, and not just in single hyphae, the number of branches is regulated in accord with increasing cytoplasmic volume.

Control of hyphal branching

Initiation of a hyphal branch amounts to the initiation of a new gradient of polarity by disturbance of the original gradient. Basically similar events

must occur during spore germination when the polarity of the emerging hypha is first established. Most fungal spores swell during germination. In part this is due to imbibition of water but it is also nutrient dependent and involves active 'spherical growth' (Bartnicki-Garcia, Nelson & Cota-Robles, 1968) which can increase the spore diameter by up to three times. During spherical growth the vegetative wall is synthesized beneath the spore wall by the uniform, isotropic, deposition of wall materials (Bartnicki-Garcia, 1973). This non-polarized pattern of wall growth is in some way altered so that in one, or perhaps a few, regions vesicles accumulate and germ tube outgrowths occur which eventually become vegetative hyphae. The nature of the polarizing influence which converts isotropic spherical growth to non-isotropic germ-tube growth is unknown, but germ tubes and hyphae are alike in that their extension growth must be organized by reference to the growth of other germ tubes or hyphae around them.

The mechanism by which this organization is effected has been described as an autotropism – a growth response to a unidirectional stimulus emitted by the same organism or by a separate individual of the same species. Spores express autotropism in the directions of growth of germ tubes produced by neighbouring spores. Three types of interaction may occur: adjacent spores may be neutral with respect to one another, or may show positive, or negative, autotropic behaviour. Emerging germ tubes of paired spores of *Botrytis cinerea* germinating in liquid medium tend to grow towards one another – positive autotropism (Jaffe, 1966), although spores of the same species show neutral autotropism when grown on the surface of solid medium (Robinson, Park & Graham, 1968). Species of *Mucor*, *Rhizopus*, and *Trichoderma* exhibited various degrees of negative autotropism (Robinson *et al.*, 1968). Only a limited number of fungi have been examined and though there are some differences between them, the majority show negative autotropism. Broadly speaking, this is also true for hyphae. Although the early growth in a large spore population may be erratic overall, by the time a young mycelium is established the peripheral hyphae grow in a direction which is diametrically away from the main mass of mycelium. These phenomena have usually been explained by the assumption that they result from a chemotropic response to some (unknown) factor in the environment. Autotropism between spores has generally been studied from the point of view of investigating the relationships between adjacent spores. Hence the distances involved have usually been small – in the region of a few micrometres – and as autotropic effects become less marked with increasing distance, labile chemical species have

been postulated to account for the observed effects. Thus, Jaffe (1966) accounted for the positive autotropism exhibited by *Botrytis cinerea* spore pairs (the spores of a pair either touching or being up to 10 μm apart) by postulating '... a diffusible, unstable, locally effective, macromolecular growth stimulator ...' which '... while initially emitted uniformly by each spore, comes to be emitted most rapidly by the very presumptive growing points that it favors' (Jaffe, 1966). It was noted that the spores became negatively autotrophic when the medium was equilibrated with 0.3 to 3% CO_2, and Jaffe (1966) concluded that under these conditions interaction between the spores was dominated by a locally effective growth inhibitor, although the growth stimulator was still thought to be having some effect. A diffusible inhibitor of germ-tube growth was also thought likely to be responsible for the negative autotropic effects described by Robinson *et al.* (1968) between paired spores of *Rhizopus*, *Mucor* and *Trichoderma*. Here again there was a decline in the degree of response as spore pairs separated by greater distances (up to 40 μm in this study) were observed.

Stadler (1952) described experiments in which a dense suspension of spores of *Rhizopus stolonifer* (in agar medium) induced negative autotropism in a less-dense suspension some 2.5 mm away. Arthrospores of *Geotrichum candidum* behaved similarly (Robinson, 1973*a*). Over these sorts of distances it is unlikely that compounds as labile as that suggested by Jaffe (1966) can have much effect. Although Stadler (1952) was unable to demonstrate any chemotropism caused by cell-free filtrates of dense spore suspensions and therefore suggested that the active metabolite is labile, germinating spores do produce stable metabolites which can inhibit germination of spores of the same species. Spores also produce metabolites which can stimulate germination (Robinson *et al.*, 1968; Robinson, 1973*b*), and Stadler (1952) detected production of a stable promotor of hyphal extension growth by germinating spore suspensions of *Rhizopus stolonifer*.

There is, therefore, considerable suggestive evidence to the effect that substances may be produced which are able to organize the growth pattern of neighbouring spores or hyphae. The chemical nature of the compounds involved is unknown. Exhaustion of metabolites and production of staling substances by metabolism of the nutrients of the medium have both been proposed as ways in which a gradient of chemotropic activity could be established. Robinson (1973*c*) has suggested that a gradient of oxygen, in an environment where oxygen is the metabolite most likely to be growth limiting, could account for a variety (but not all) of the autotropic reactions which have been reported. It is envisaged that a gradient

in respiratory activity across a spore or hyphal tip could polarize the accumulation of vesicles and thereby determine the direction of germ-tube emergence or growth of the hyphal apex. Further, the position of branch initiation can be correlated with the oxygen concentration gradient in a particular experimental system (Robinson, 1973*b*), so it is feasible that the arrangement of leading and branch hyphae is also influenced by such a gradient. Since oxygen is only sparingly soluble and has a rate of diffusion in water some four orders of magnitude less than that in air, steep oxygen gradients are frequently encountered in nature. Similarly, uptake and accumulation processes in fungi are sufficiently rapid and effective for the cell to act as a 'sink' for many metabolites. It is consequently relatively easy to account for any autotropic response by assuming that growth is directed in one or other direction along these nutrient gradients. Some of the observed tropisms must be based on this sort of response, but there are other observations which imply a degree of specificity which argues against total dependence on nutrient gradients.

If the patterns of hyphal growth and branching were entirely dependent on their immediate environment then mycelial form would be determined rather mechanically. Yet, for one thing, there is plenty of evidence of a genetic control being exercised. An interesting example is the considerable difference between mycelial morphology of monokaryons and dikaryons of *Coprinus cinereus*. The monokaryon is generally comprised of narrow hyphae (4 μm in diameter) which have branches emerging virtually at right angles to the main axis. Dikaryons, as well as having thicker hyphae (7 μm in diameter) which grow much faster than those of the monokaryon, show a very acute angle of branching and, additionally, have clamp connections. The latter are specialized branches that grow in the opposite direction to their parental hyphae before fusing with the latter (Casselton, 1978). These differences arise as a result of the genotypic change occasioned by the co-existence of two compatible nuclei in the same cytoplasm. Clutterbuck (1978) has reviewed the variety of mutants affecting hyphal morphology, while Lysek (1978) considers another phenomenon – mutants causing morphological rhythms. A variety of strains show spontaneous rhythmic alterations in branching patterns which give rise to successive zones in the extension growth of the colony, forming concentric bands. Radially-placed barriers interrupt the regularity of the bands, implying that the periodicity of branching in adjacent hyphae depends on a synchronization of metabolic activities which requires lateral communication.

It seems reasonable to expect that nutrient depletion in the vicinity of a

hypha will create gradients which will ensure that other hyphae and hyphal branches grow away from it (negative autotropism). The formation of complex structures, like fruit bodies, involves positive autotropism and very specific systems may therefore have arisen to provide the attractants and responses necessary to secure the growth of hyphae towards one another and across each other's gradients of nutrient depletion. Nothing is known of the phenomena involved in the aggregation of hyphae during the initial stages of organ development. But there are some other behaviour patterns which indicate the sorts of factors which could be involved.

When oidiospores of certain basidiomycetes are spread on solid medium just in front of an advancing mycelium the mycelial hyphae often show a positive tropic response towards the oidia with which the hyphae eventually fuse. This 'homing reaction' is often not very specific but extensive and rapid homing can be followed by vacuolization and death of the homing hyphae. Such a lethal reaction occurs between species which seem on other criteria to be very closely related, and studies of the homing and lethal reactions have been used to distinguish relationships between species of basidiomýcetes (Kemp, 1975, 1977). Kemp (1970) and Bistis (1970) showed that the oidia produced a growth substance which elicited a positive chemotropic response in the hyphae. It is highly unlikely that such a mechanism would employ a simple metabolite as chemotropic agent since such a strategy would lack the taxonomic specificity which is observed. So the implication is that the process depends on production of a very specific chemical attractant.

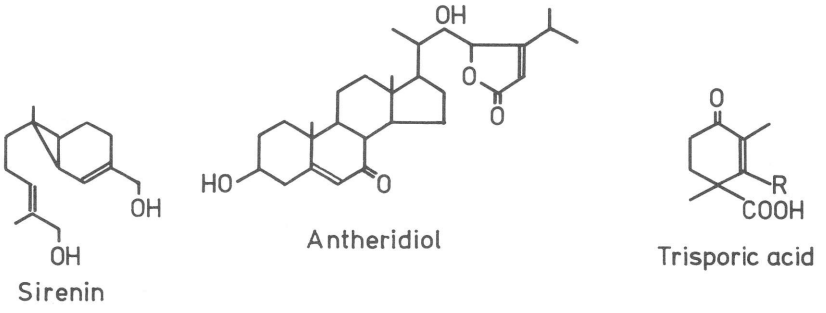
Characterized chemo-attractants

Aquatic fungi

A number of lower fungi produce chemo-attractants involved in growth processes leading up to sexual reproduction which can quite properly be described as sex hormones (Bu'lock, 1976). *Allomyces* is an aquatic fungus which produces uniflagellate motile gametes. These are differentiated as male and female although they arise on the same haploid thallus and consequently have the same genotype. The female gametes and gametangia produce a substance called sirenin to which the male gametes show strong chemotaxis. Sirenin is a bicyclic sesquiterpene diol (Fig. 5.1) which is active at concentrations less than 10^{-10} g ml^{-1}. Female gametes are only sluggishly motile but male gametes swim in random smooth arcs interrupted by stops after which the cell swims in a different direction.

Sirenin organizes the direction of swimming by shortening the run between interruptions if the cell moves away from the source of hormone and diminishing the number of stops if the cell is moving towards the source. Neither the metabolic origin nor the mode of action of sirenin are known. Inactivation of the hormone by the male gamete is essential to the overall activity but it is not known whether this results from enzymic breakdown or irreversible binding to some component of the cell. This chemotaxis thus leads to cell contact which is a prelude to plasmogamy. Bu'lock (1976) suggests that the specific cell adhesion which follows establishment of contact involves special surface polysaccharides. More is known about cell adhesion in yeasts and this will be discussed later. The female sex hormone of another water mould, *Achlya*, has also been characterized in some detail. This material, called antheridiol, is a steroid (Fig. 5.1) the activity of which can be detected by bioassay in 10^{-11} M solution (Raper, 1952; Barksdale, 1969). The mating sequence reported by Raper (1966) consisted of the development of antheridial hyphae on the male, the production of oogonial initials on the female, growth of antheridial hyphae towards oogonial initials, formation of cross-walls separating off oogonia and antheridia, and finally, after the two made contact, the antheridium grew through a lysed portion of the oogonial wall, after which its own wall was dissolved. Antheridiol is produced continuously by the female and under the influence of the hormone, branches on the putative male thallus which might otherwise grow out as vegetative branches are caused to elongate rapidly and differentiate into antheridia. The male is also induced to excrete a second hormone, hormone B or oogoniol, and it is in response to hormone B that the female initiates oogonial differentiation and amplifies antheridiol levels to those which attract antheridial hyphal growth. There are thus at least two

Fig. 5.1. Structural formulae of some sex hormones of lower fungi.



contributors to this hormonal 'conversation'; the female produces antheridiol but takes up very little itself, and does not synthesize hormone B though it does have a receptor for this hormone. On the other hand, the male makes no antheridiol but is sensitive to it, and one of the responses is to produce hormone B to which the male is insensitive. Antheridiol and hormone B are thought to be alternative products of a branched biosynthetic pathway. In the male, antheridiol amplifies that branch of the pathway which leads to hormone B synthesis and also increases respiration, induces breakdown of glucan reserves in the cytoplasm and triggers the *de novo* synthesis of cellulase. These metabolic changes contribute to processes involved in antheridium initiation – including aggregation of vesicles at the sites where initials develop (Mullins & Ellis, 1974). It is likely that broadly similar responses are elicited in the female by hormone B. It is not known how these sterols influence gene regulation but the available data seem to indicate a system very much akin to sterol regulation in animals (Horgen, 1977).

Mucorales

The only other fungi in which the activity of known hormones has been well characterized are some members of the Mucorales. These are filamentous, terrestrial, lower fungi with mycelia typically composed of unbranched coenocytic hyphae. A little way behind the advancing hyphal tips of vegetative mycelia asexual sporangiophores are produced. However, in the vicinity of a mycelium of opposite mating type sporangiophore formation is suppressed and sexual differentiation takes place, involving formation of sexual hyphae (zygophores) which grow towards each other, fusing in pairs to eventually form gametangia. Zygophore formation is determined by trisporic acid (Fig. 5.1); if this chemical is added to pure, unmated, cultures sporangiophore formation ceases and zygophores form instead (Gooday, 1973, 1974*a*; Bu'lock, 1976). Although there are a number of trisporic acid-related compounds, some of them corresponding to intermediates in the pathway, both mating types produce and respond to the same hormone. The trisporic acids are synthesized from β-carotene: the molecule is cleaved to retinal, a C_2 fragment is lost, and then there is a series of oxidations. The complete reaction sequence occurs only when both plus and minus mating types are grown in mixed culture or in an experimental set-up in which they are separated by a membrane permeable to small molecules. Both mating types have the genetic capacity to produce the enzymes of the complete pathway, but the

alleles which determine the mating type repress complementary steps in the later stages of trisporic acid synthesis. Thus in plus strains synthesis of enzymes needed to form the 4-keto group is repressed by the MT^+ allele while enzymes involved in forming the 1-carboxylic acid group are repressed by MT^- (Bu'lock, Jones, Quarrie & Winskill, 1973; Bu'lock, 1975). Each mating type thus produces a precursor which only the opposite mating type can convert to trisporic acid. The precursors diffuse between the strains and have the status of prohormones which stimulate trisporic acid synthesis. Early steps in the pathway are repressed to a rate-limiting level by a mechanism which allows activation by trisporic acid. When plus and minus strains come together, therefore, the complementary synthesis of trisporic acid consequent on the co-diffusion of the prohormone precursors leads to derepression of the early part of the pathway and an amplification of overall trisporic acid synthesis. The increasing gradient of prohormone diffusing from each zygophore induces a chemotropic response. The zygophores can grow towards one another from distances of up to two millimetres. When the zygophores make contact they adhere firmly in a way that implies that mating type- and species-specific substances are formed on the zygophore surface. These features are clearly an aspect of the mating type phenotype and are necessary for completion of the mating programme – without adhesion the zygophores continue unproductive extension growth – but the nature of the substances involved is unknown.

Yeasts

Some idea of the sorts of molecules which might be involved in cell to cell contact comes from work with a number of yeast species. Many release diffusible sex hormones ('pheromones') as a prelude to the cell fusion that leads to conjugation. These substances are outside the scope of this discussion apart from noting that they prepare the cells for conjugation and contribute to the recognition of different mating types. However, the major step in the recognition of compatible cell types involves macromolecules on the cell surfaces which cause cells to agglutinate. Some of these are constitutive (i.e., cells agglutinate immediately the different clones are mixed) while others are inducible, the cells only acquiring the ability to agglutinate after growth in mixed culture. In both *Hansenula wingei* and *Saccharomyces cerevisiae* there is evidence that the molecules directly involved in agglutination – the agglutinins – are probably glycoproteins. In *H. wingei* one of the agglutinin components consists of 28 amino acids and about 60

mannose residues. The agglutinins seem to be located on surface filaments external to the cell wall. The function of the agglutinins is to bring cells of opposite mating type together. They do this by virtue of their ability to bind in a complementary manner, the agglutinin of one mating type binding specifically to that produced by the compatible mating type. Following this adhesion of yeast cells by complementary binding, protuberances grow out from the cell walls and when these meet cytoplasmic communication is established by dissolution of the walls. These phenomena (reviewed by Crandall, Egel & Mackay (1977)) are obviously specifically part of the mating process in yeast; yet they clearly demonstrate that fungal cells are capable of producing surface glycoproteins which, by a sort of antigen–antibody reaction, can achieve a very specific adhesion. It is exactly this sort of specific cell binding which one might expect to be part of the cell-to-cell communication which contributes to the construction of differentiated multicellular structures.

The possible role of cyclic-AMP

The aspects of sexual reproduction in a few lower fungi which have just been described represent the only cases (along with examples from related species not referred to here) in which fungal hormonal chemical attractants have been characterized. In a real sense, therefore, they exemplify the only agents of positional signalling in fungi which are known, even if imperfectly. Their service to a discussion centred on morphogenesis and its organization is that such processes, involving as they do the mutual attraction and adhesion of differentiating hyphal branches, illustrate ways in which metabolic signals can be generated and used. I have already described the vegetative hyphal growth form as one which shows a characteristically spreading growth habit; yet all complex fungal structures require that hyphae grow together and this must involve both chemotropism and adhesion. How such concerted growth is initiated and organized in filamentous fungi is completely unknown and the closest approach we have to any indication is provided by the slime mould *Dictyostelium discoideum* (Newell, 1978).

Slime moulds

When the free-living amoebae of *D. discoideum* begin to starve the cell aggregation phase is initiated; this eventually leads to the formation of the multicellular slug (pseudoplasmodium). Aggregation depends on the

emission of a chemical – given the generic name of acrasin – which acts as an attractant. In the most widely known case, that of *D. discoideum* itself, the chemical attractant is the nucleotide cyclic-AMP (cAMP). Newell (1978) divides the aggregation process into seven stages. In the first stage the signal is generated. The evidence indicates that signalling is pulsatile rather than forming a continuous gradient. The primary source of the oscillations is enzymic; it may be the adenylate cyclase or the cytochrome chain. The second stage is reception of the signal which seems, at least temporarily, to be the responsibility of receptor molecules on the surface of the amoebae. The characteristics of these receptors are similar to those of some hormone receptors in animals. Thirdly, the receiving cell destroys the incoming signal – via a membrane-bound and a soluble extracellular phosphodiesterase – and, fourthly, relays the signal. There is a delay of about 12 s between receipt of the incoming signal and generation of a new pulse of cAMP, and in part this delay determines the rate of propagation of the signal through the population. The fifth step is the chemotactic response itself. Receipt of a pulse of cAMP causes the amoebae to move towards the signal source for about 100 s, during which time the cells move about two cell diameters. As well as causing the chemotactic response, the pulse of cAMP initiates cellular changes associated with differentiation including the synthesis of cell-surface glycoproteins which are involved in cell adhesion during the final stage – the association of the cells into a multicellular aggregate. Once the aggregate is formed proper progress of the developmental program requires the maintenance of cell contacts; and the consequences of mechanical separation of the cells imply that intercellular signalling is required continuously for completion of development. There is some evidence that cAMP continues to play a role as a morphogen in the aggregated slug, but ammonia has also been claimed to regulate differentiation (Schindler & Sussman, 1977; Sussman, Schindler & Kim, 1977).

Basidiomycetes

Not surprisingly, a great deal of interest has been focused on the likely role of cAMP in controlling differentiation in other organisms. Before discussing one of these cases it is worth emphasizing that this 'magic bullet' does not have the same role in all slime moulds; in *Polysphondylium violaceum* the acrasin (aggregation signal) is a small peptide (Wurster, Pan, Tyan & Bonner, 1976; Bonner, 1977). Nevertheless it is inevitable, considering the widespread degree of interest in cAMP, that attempts have been made to

find a developmental role for the compound in filamentous fungi. The most assiduous search has been conducted by Uno and Ishikawa in their work with the basidiomycete *Coprinus cinereus* (using the name *C. macrorhizus*). In a long series of papers these workers have investigated the metabolism of cAMP during fruit-body development in *Coprinus*. Superficially, the developmental problem in a basidiomycete like *Coprinus* is rather similar to that faced by *Dictyostelium*. The spreading growth of the vegetative mycelium can be likened to the migrating slime mould amoebae. The analogue of amoebal aggregation would then be the aggregation of hyphae into the initials of structures such as fruit bodies. Additions of cAMP to preparations of '*Coprinus lagopus*' (= *C. cinereus*) have been reported to accelerate the production of fruit-body primordia (Matthews & Niederpruem, 1972). A fruit-body-inducing substance (FIS) which was able to induce fruiting in certain mutant strains of *C. cinereus* has been extracted from various tissues and identified as cAMP (Uno & Ishikawa, 1973). Although most of the work done by Uno and Ishikawa has been concentrated on the phenomenon of monokaryotic fruiting (which is an abnormality of certain mutant monokaryons) they have included the dikaryon (the normal mycelial origin of fruiting bodies) in sufficient of their work for the picture to be reasonably clear. They have shown that the mycelium accumulates cAMP at the onset of fruiting, the accumulation being first evident at about the time that fruit-body formation is first initiated. The accumulation proceeds to a late primordial stage, but then the amount of cAMP declines as the primordium matures (Uno, Yamaguchi & Ishikawa, 1974). However, supplementation of the medium with cAMP did not induce fruiting in these Japanese strains of *C. cinereus* (Uno & Ishikawa, 1973). Some involvement of cAMP in the phenomenon of catabolite repression has also been demonstrated (Uno & Ishikawa, 1974) and is significant since fruiting is usually initiated as the carbohydrate supply of the medium is becoming exhausted. The nucleotide has also been shown to activate glycogen phosphorylase and inhibit glycogen synthetase (Uno & Ishikawa, 1976, 1978). It is quite clear from the extensive work done by Uno and Ishikawa that cAMP is closely involved in fruit body development in *C. cinereus*; however, despite the amount of work done, the role of the chemical remains obscure. Throughout their analyses Uno and Ishikawa made their extractions from mycelia together with any fruit bodies they may have produced. No attempt was made to separate the two structures. Preliminary attempts to partition the cAMP between the different parts of the organism indicate that in the primordium all of the cAMP is located in the fruit-body cap whereas in more

mature fruit bodies it is found in the upper part of the stipe (Darbyshire, 1974). Moreover, when separate analyses are made of the mycelium and its fruiting structures, the cAMP is found to be accumulated in the fruiting bodies and particularly heavy accumulations are observed in the very earliest stages (Milne, 1977). In the fruit body initials cAMP is accumulated to levels up to four times the highest recorded by Uno & Ishikawa (1973). These peaks of cAMP concentration seem to be located in the parts where metabolic changes, especially the mobilization and utilization of reserve materials, occur most rapidly (Moore, Elhiti & Butler, 1979). A particularly interesting point is that, in the first case, the fruit body initial, and subsequently, the cap of the primordium are the recipients of very large quantities of reserve materials channelled to these locations from elsewhere. In the average fruit body about one milligramme of glycogen, first accumulated in the stipe base, is relocated into the cap of the primordium (Moore *et al.*, 1979), while polysaccharide, presumably glycogen too, is mobilized from the mycelium and relocated in the developing fruit body initials (Madelin, 1960). Thus it may be that cAMP serves to organize the directionality of these translocation streams. The evidence does seem to suggest that reserve materials, notably glycogen but probably other compounds as well, are translocated through the hyphae of the mycelium and of the primordium along cAMP gradients. This process, rather than the autotropic growth of the hyphae, may be the broad equivalent of the chemotropic effect which cAMP has on *Dictyostelium* amoebae. *Coprinus* hyphae are unable to migrate like the amoebae but their contents can migrate.

Basidiomycete 'social organization'

This inward translocation of materials towards centres of development highlights a feature of the mycelium which may influence the form and nature of positional control signals. Buller (1931) was the first to describe what he called 'social organization' in the basidiomycete *Coprinus sterquilinus*. He indicated that the numerous hyphal fusions which interconnect the hyphae as they grow through the dung substrate provide the direct routes through which nutrients flow towards the developing fruit. He pointed out that where the substrate has been inoculated with a number of spores the hyphal fusions between the individual mycelia to which they gave rise still allow such nutrient flow and ensure that a fruiting body is still produced on that substrate, although the mycelia may individually have invaded an amount of substrate which is insufficient to provide for

formation of a fruit body. In other words, it appears that in these circumstances the mycelia co-operate rather than compete. Such a compound (or 'unit') mycelium, which is genetically heterogeneous but acts as a physiological unit, has been seen as a consequence of the readiness with which hyphal fusions occur (Burnett, 1976). In many ways such a concept views the mycelium like a whole organism in which the reproductive capacity is concentrated in a limited section of the body while being nutritionally supported by the activities of the whole organism. Of course the nuclear heterogeneity sets the situation apart from that evident in higher organisms where the 'division of labour' between cells and tissues is most highly developed. Nevertheless, there is a clear implication that in some way in these compound mycelia the developmental potential of one part is expressed at the expense of the others and this must reflect a degree of organization which requires the dispatch and receipt of some sort of positional control signal.

It must be stated that promiscuous hyphal fusion and the unit mycelium are not universally observed in fungi. Rayner & Todd (1979) review situations in which the opposed relation between mycelia (specifically, between different dikaryons) of the same species is observed – a relationship in which neighbouring mycelia display mutual antagonism. Indeed, these authors provide an authoritative discussion of the reality of the individuality of fungal mycelia. In their view the unit mycelium concept could well only apply in limited circumstances where the mycelia are closely related (a criterion likely to be met in the circumstances envisaged by Buller (1931)). In wood-decaying fungi certainly, and probably in others, Rayner & Todd (1979) believe that the population is made up of dikaryotic individuals analogous to the diploid individuals which make up the populations of higher organisms. If this is the case then it may have implications for the nature and need for signals to pass between neighbouring individuals in the population.

Differentiation of multicellular structures

I have so far discussed events which essentially can be described as involving hyphal interactions within the mycelium. But in the various complex structures produced by the higher fungi in particular, the interactions are much more of the sort that might be anticipated between differentiating cells in organized tissues. The fungi produce many organized structures and only a few of these will be illustrated to give some idea of the level of complexity that these supposedly lower organisms can attain. The selected

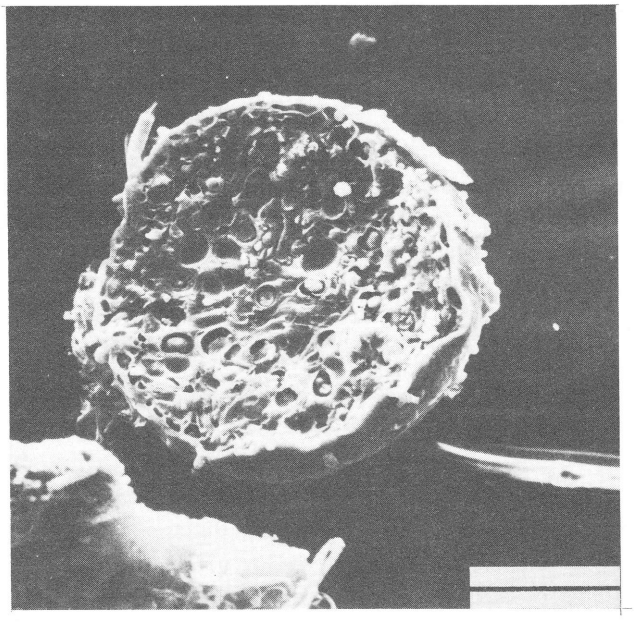
illustrations are not exhaustive; they happen to be the examples with which I am most familiar, but I believe them to be representative.

Basidiomycete structures

Vegetative organs

The most highly organized structures are the mushroom fruit bodies of the Basidiomycetes, but the vegetative mycelium is capable of elaborating a range of other organs. Among these are sclerotia, strands and rhizomorphs. These represent essentially alternative strategies. The sclerotium is a resting structure with cells specialized to withstand adverse environmental conditions; it tends to be spherical, or at least globose, and fairly small (a few millimetres or less in diameter). Sclerotia generally show some degree of radial symmetry with an outer layer of closely packed rind

Fig. 5.2. Scanning electron micrograph of a broken sclerotium of *Coprinus cinereus*. Harvested sclerotia were frozen in liquid nitrogen and fractured by impact while still frozen. After critical-point drying they were scattered onto an adhesive-covered stud and coated with gold. The image shows the compact internal medulla surrounded by the smooth, thick-walled rind. Scale bar represents 20 μm.

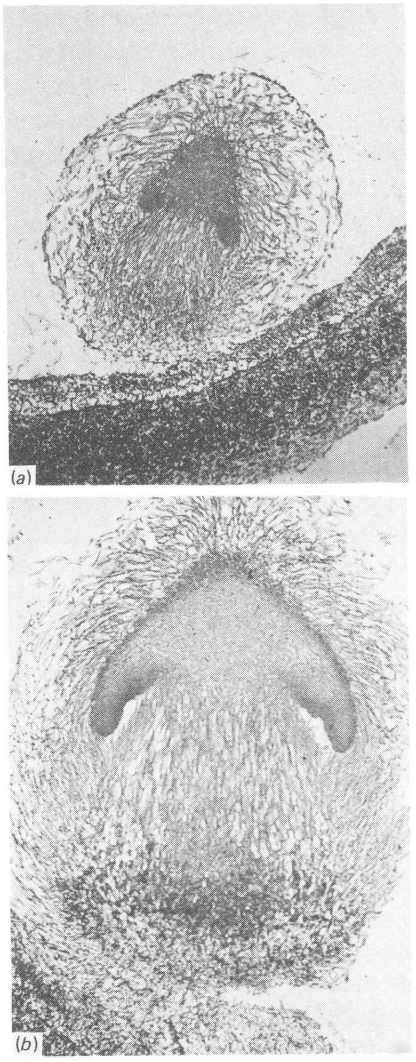


cells which have thickened and pigmented walls surrounding a medullary region of interwoven hyphae (Fig. 5.2). The sclerotium is non-motile; it remains where it is formed to withstand adversity and then germinates when conditions improve. Strands and rhizomorphs, on the other hand, are root-like structures composed of parallel-running hyphae which are interwoven and held together by fusions. Strands increase in diameter by the development of further hyphae from the origin. Rhizomorphs are more highly organized constructions consisting of thousands of hyphae; they are internally differentiated and grow apically from something analogous to an apical meristem. The difference between strands and rhizomorphs may simply be one of degree of co-ordination between the component parts; both are organs of mycelial migration and nutrient translocation. They grow out from colonies on a 'food base' and will grow across non-nutrient surfaces for considerable distances. Low nutrient levels tend to promote strand and rhizomorph initiation, but in *Armillaria mellea* the initiation of a rhizomorph tends to inhibit the formation of further such structures in the vicinity (Garrett, 1953). There is presumably some way in which the growth of one structure controls the initiation of similar structures in the neighbourhood. Garrett (1953) suggested that this is a nutritional phenomenon, the inception of independent rhizomorphs being inhibited by the translocation of nutrients into the existing rhizomorph; but even if this is so there must be some sort of signal which directs the distribution of nutrients. The organization of the rhizomorph is in some way positively maintained. This, too, may be based on a dependence on nutrient gradients since Watkinson (1971*a*, *b*) has shown that translocated nutrients leak from strands growing over a nutrient-depleted medium and Garrett (1960) notes that the hyphae composing the strand separate from one another when they grow over a nutrient-rich medium. Both these observations support the idea (Garrett, 1960) that the hyphae of the strand are positively chemotropic towards nutrients, but since the strand grows away from the food base this cannot be true for extension growth of the strand as a whole.

Burnett (1976) identified three factors which are involved in these vegetative aspects of differentiation. These are: synchronization of the behaviour of adjacent hyphae; regulation of the balance between extension growth and branch formation; and the internal control of localized growth through competition for nutrients. Very little is known about the ways these parameters are regulated, but such mechanisms must operate with even greater sophistication in determining the structure of fruit bodies. These, too, are initiated by the localized aggregation of hyphae.

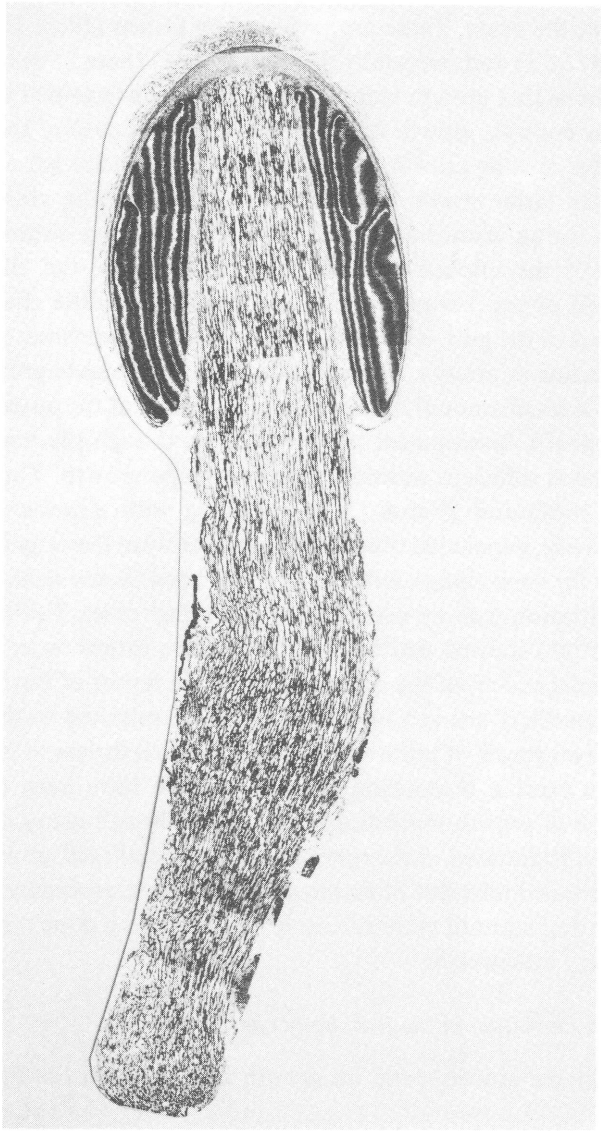
In C. cinereus there is evidence that the same initial hyphal aggregate can serve as the starting point for either a sclerotium or a fruit body (Moore, 1981*a*), but whereas the sclerotium develops a radial symmetry, the fruit-body developmental sequence imposes a polarizing influence on the aggregate, and from a very early stage the shape of the developing mushroom is clearly evident. This is illustrated for *C. cinereus* in Fig. 5.3. Such images

Fig. 5.3. Vertical sections of initials of *C. cinereus* fruit bodies. The structure shown in (*a*) was 0.8 mm tall, that in (*b*), 1.2 mm tall. From Moore *et al.* (1979).



are fairly representative of most gilled mushrooms (Taber, 1966), though the 'bud' or 'button' varies in physical size and rate of development. Further growth of the primordium leads to the delimitation of its various tissues and establishment of the unmistakable mushroom form (Fig. 5.4).

Fig. 5.4. Section of a primordium of *C. cinereus* which was 15 mm tall. The figure is a photograph of a montage of light micrographs. From Moore *et al.* (1979).



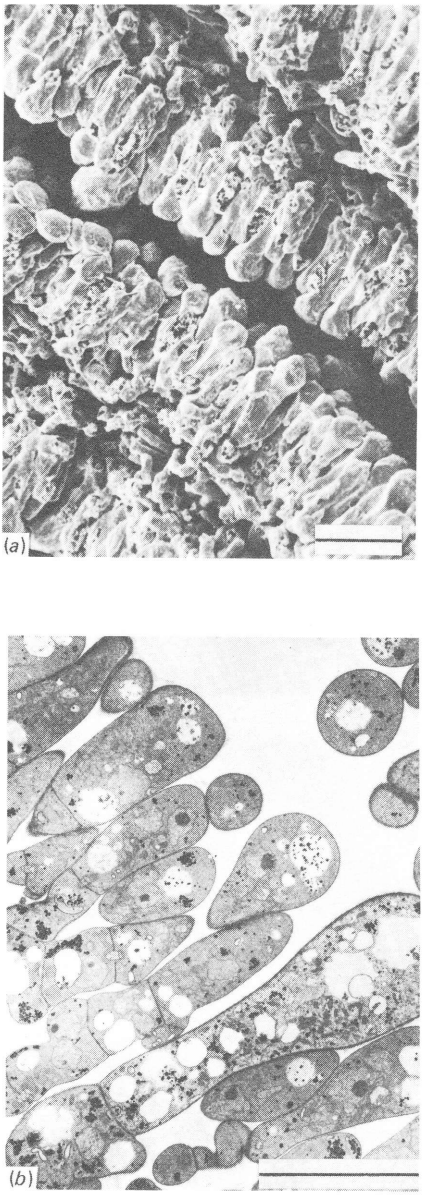
Mushroom development

Development of such a highly organized structure can proceed only under the most rigid control, but we are almost completely ignorant of the means by which such control may be exercised. For nearly 150 years, though, efforts have been made to understand the regulation of mushroom growth by using experiments involving surgical removal of portions of the developing fruit body. A number of such studies have been carried out over the years. These are reviewed by Gruen (1963, 1967), Gruen & Wu (1972*a*, *b*) and, especially, Burnett (1976). There have been consistent indications that growth factors produced by the fruit-body cap influence, perhaps control, growth of the stipe. Total removal of the cap leads to cessation of stipe growth. When segments of cap are left attached to the stipe, the latter shows a growth curvature with the greatest extension growth being immediately beneath the remaining sector of cap. The source of the implied growth factor seems to be the gills themselves. Removal of cap tissues other than the gills had little effect on growth. Removal of the gills, leaving the rest of the cap tissue intact, was followed by a decline in growth. The gills also seem to promote growth of the cap since if a small amount of gill tissue is left intact at the outer edge it is able to safeguard development of the cap even though the amount of tissue removed is sufficient seriously to reduce stipe growth. Thus both Gruen (1963, 1967) and Borriss (1934), working with *Agaricus* and *Coprinus* respectively, concluded that the gill lamellae were the origin of controlling factors for stipe elongation and cap expansion. Some qualification of this generalization may be necessary in individual cases. For example, Gooday (1974*b*) showed that later stages of stipe extension in *Coprinus* were quite independent of the cap, confirming the report of Borriss (1934) that the influence of the cap in this organism is restricted to the earliest (but formative) stages of primordium growth. Nevertheless, it is clear that the gills do exert a controlling influence. There have been claims for the isolation of growth-regulating substances although many of these remain to be substantiated. Attempts to identify the alleged growth promoters have revealed mixtures of amino acids including, especially, derivatives of glutamate, but until more extensive work has been done these suggestions can carry little weight.

Pattern formation in the fruit body cap

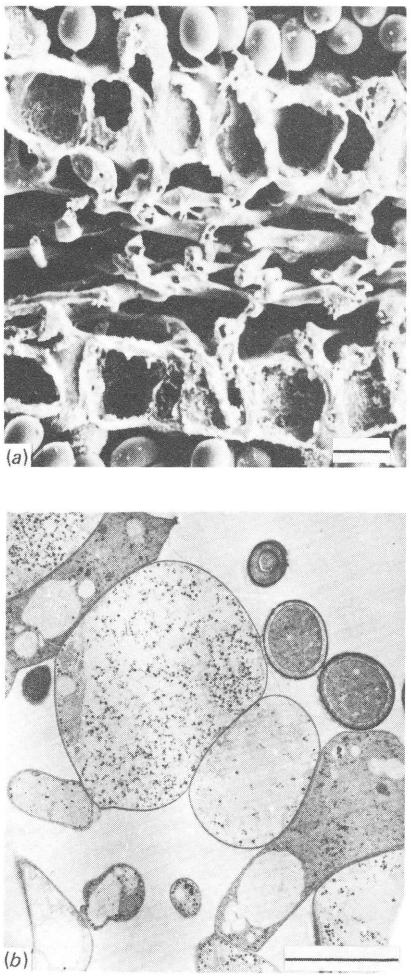
Most of the studies done on growth regulation in the mushroom fruit

Fig. 5.5. Structure of the gill surfaces (hymenia) of primordia similar to that shown in Fig. 5.4, as revealed by scanning (*a*) and transmission (*b*) electron microscopy. Scale bars represent 10 μm. From Moore *et al.* (1979).



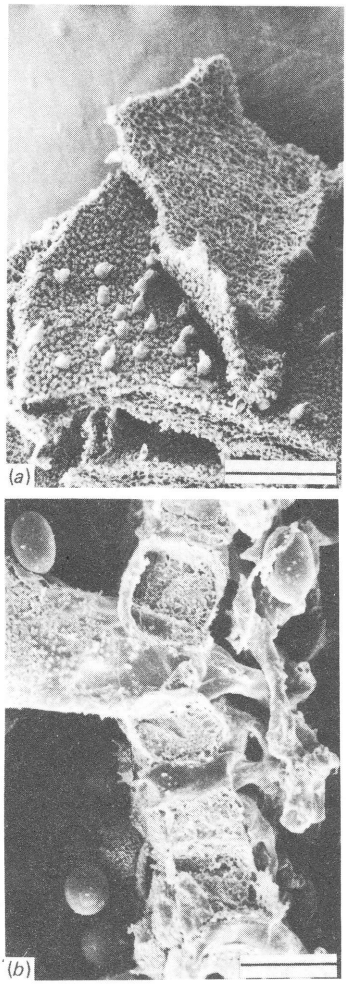
body have concentrated on growth of the stipe, yet they have revealed that some factors do influence cap expansion. It is the cap which displays the greatest morphological, and consequently morphogenetic, complexity. Burnett (1976) has noted that the work which has been done points to the regulation of such features as gill initiation, gill spacing, cell morphology and cell packing. The cell differentiation which occurs can be illustrated

Fig. 5.6. Structure of the hymenium of a mature *C. cinereus* fruit body as revealed by scanning (*a*) and transmission (*b*) electron microscopy. Scale bars represent 10 μm. Note the close-packed pavement of box-like paraphyses and the regularly interspersed club-shaped basidia. From Moore *et al.* (1979).



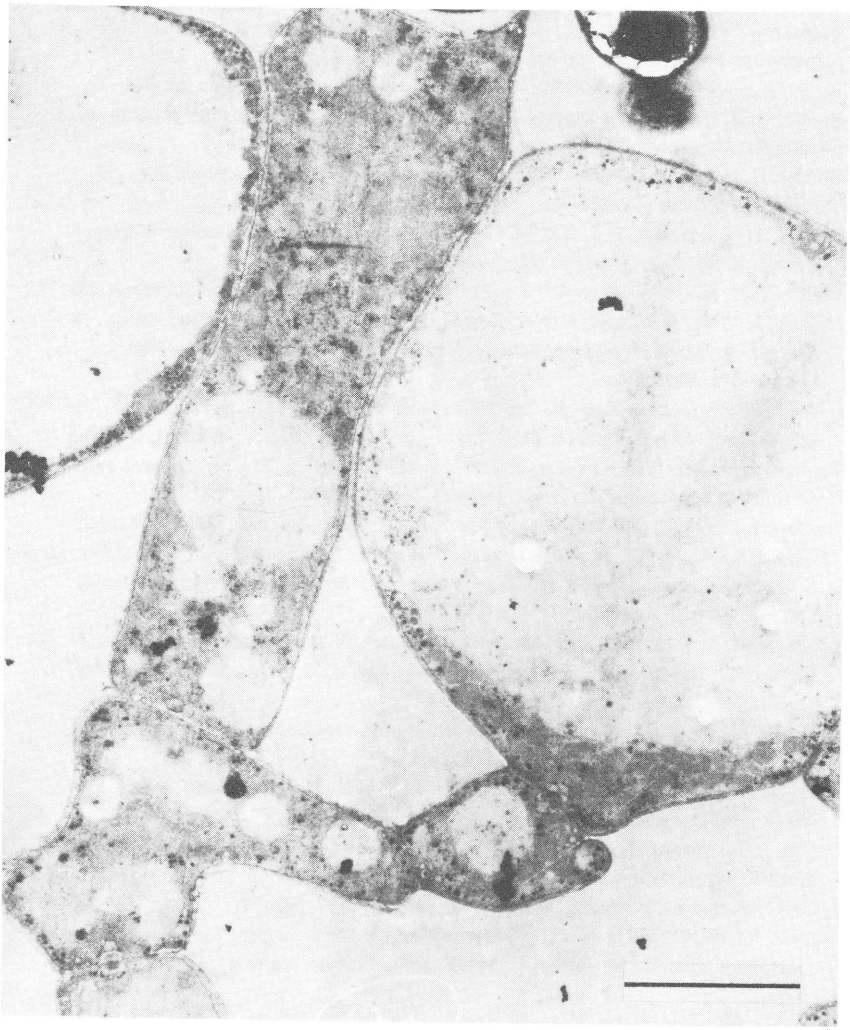
by reference to *Coprinus*. Early in development the gill surface (hymenium) is made up of a loosely-packed array of essentially undifferentiated hyphal tips (Fig. 5.5). By the time the cap has matured three morphologically distinct cell types have been produced, for at maturity the hymenium consists of a pavement of highly inflated paraphyses within which are embedded a great many basidia and a scattering of cystidia (Figs. 5.6,

Fig. 5.7. (*a*), Scanning electron micrograph of a torn gill surface of *C. cinereus* showing the large bulbous cystidia scattered in the regular carpet of spore-bearing basidia. Scale bar represents 200 μm. (*b*), Edge of the cut gill surface of a mature hymenium showing the point of emergence of a cystidium. Scale bar represents 10 μm. From Moore *et al.* (1979).



5.7). The development of these structures could provide a classic example of a morphogenetic field, the more so since the gill plate grows in two directions (inwards, towards the stipe, and along the length of the cap as the cap increases in diameter) which are essentially at right angles and which could contribute to a co-ordinating system to which cell differentiation could be referenced. Models have been proposed which account for the development of such morphologies by assuming that the progressive distribution of a substance – the morphogen – is the factor which regulates cell differentiation. The models have been most effectively analysed by Meinhardt & Gierer (1974) who show how two-dimensional patterns closely similar to those observed in the basidiomycete hymenium may be generated, based on activators and inhibitors capable of diffusing through the tissues. However, the developing mushroom is faced with a problem which may be of a different order of difficulty to that met by higher plants and animals. Models of morphogenetic processes based on distribution of morphogens are very dependent on adequate communications within the tissue, communication which must extend over many cell diameters. Higher animals and higher plants are well provided – via cell processes, gap junctions, plasmodesmata and the like – with avenues for such communication, but the developing basidiomycete hymenium is an array of separate cells (Fig. 5.5). There is no evidence for any lateral cytoplasmic contact between neighbouring cells. Indeed, the electron micrographs show considerable space between hymenial cells of primordial tissues at about the time that the morphogenetic pre-patterning might be expected to be taking place. Of course, the cells of the hymenium are branches from the subhymenial hyphae, but any suggestion that communication of morphogens takes place through the subhymenium must account for the fact that adjacent branches from the same subhymenial hypha can have different morphogenetic fates (Fig. 5.8). Models involving gaseous or volatile morphogens could clearly be constructed, and might have some basis in known metabolic events (Ewaze, Moore & Stewart, 1978; Moore 1981*b*), but it is a sad fact that we are largely ignorant of the detailed aspects of tissue construction – the relationships between adjacent cells are known in only the vaguest way – and the knowledge we do have about the possible effects of growth factors is woefully inadequate. Nevertheless, I believe the pursuit of this knowledge is worthwhile. Understanding of the ways in which these lowly organisms have solved the organizational problems associated with their morphogenesis could tell us a great deal about the more highly-regarded candidates for developmental studies.

Fig. 5.8. Transmission electron micrograph of the mature gill hymenium of *C. cinereus* showing a basidium and paraphysis arising as branches from the same subhymenial hypha. Scale bar represents 5 μm.



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Reprinted from Positional Controls in Plant Development
Edited by P.W. Barlow and D.J. Carr
Canbridge University Press