13

Differentiation and pattern formation in the fruit body cap of *Coprinus cinereus*

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Introduction

Over the past few years we have gained considerable insight into the metabolism involved in development of the fruit body cap of *Coprinus cinereus*. Most of this work has made use of homogenates of whole caps for biochemical analyses and there is now an urgent need to relate the findings to differentiation of individual cells and the creation of patterns that characterise the different tissues of the cap. The biochemical data identify specific enzyme regulatory mechanisms associated with developmental changes in the fruit body. However, proper understanding of the ways in which this regulation is integrated endogenously during morphogenesis requires detailed knowledge of the relationships between individual cells and of the differentiation processes leading to establishment of tissue domains. The existing literature is silent on the former and confusing and contradictory on the latter. In this chapter we review the biochemical data and indicate how we are attempting to extend the analysis to the cellular level. We concentrate, though, on an account of cap development derived from microscopical observations that leads to a unifying interpretation of tissue differentiation in the fruit body of this species and provides the basis for hypotheses about the integration of control systems during morphogenesis.

Metabolic control of morphogenesis

It appears that in *C. cinereus* the nitrogen metabolism of the developing cap has the most direct bearing on its morphology, though there is considerable interplay with carbohydrate and carboxylic acid metabolisms. The developmental sequence of the fruit body has been divided into a series of stages to facilitate analysis. This has been done in

a number of ways by different authors over the years (see Moore, Elhiti & Butler, 1979). The scheme used here is illustrated in Fig. 13.1.

Attempts to characterise the metabolism of the developing fruit body have concentrated on enzyme surveys and metabolite measurements. These investigations have revealed four enzymes which show large increases in activity in the developing cap while remaining at low levels (or declining in activity) in the stipe. These four enzymes are NADP-linked glutamate dehydrogenase (NADP–GDH), glutamine synthetase (GS), ornithine acetyltransferase (OAT) and ornithine carbamyltransferase (OCT). A fifth enzyme, urease, showed the reverse behaviour, being absent from the cap though present in the stipe and, indeed, being constitutive in mycelium (Table 13.1). Amplification of tricarboxylic acid (TCA) cycle activity is signalled by the observations that succinate dehydrogenase also shows much greater activity in the cap than in the stipe, and isocitrate dehydrogenase activity increases in both parts of the fruit body as development

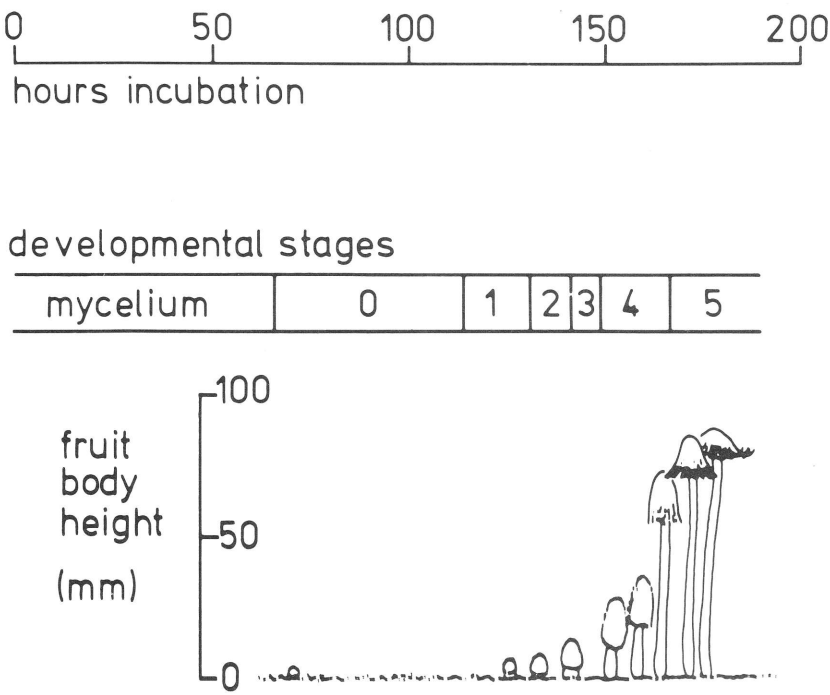

Fig. 13.1. Descriptions of developmental stages in *Coprinus cinereus* in relation to incubation period. Standardised culture conditions were described by Moore & Ewaze (1976) and the different stages are described by Moore, Elhiti & Butler (1979).

Table 13.1. *Some enzyme activities in developing fruit bodies of* Coprinus cinereus

Enzyme	Tissue	Fruit body developmental stage 3	4	5
NADP-linked glutamate dehydrogenase	cap	150	267	830
	stipe	12	15	103
Glutamine synthetase	cap	300	950	2067
	stipe	85	290	550
Ornithine acetyltransferase	cap	16	37	34
	stipe	8	7	5
Ornithine carbamyltransferase	cap	353	693	1240
	stipe	153	436	310
Urease	cap	55	70	38
	stipe	143	570	4300
NAD-linked glutamate dehydrogenase	cap	270	553	1183
	stipe	363	483	1287

Enzyme activities are shown as pmol substrate used min^{-1} (mg protein)$^{-1}$ for urease, and as nmol substrate used min^{-1} (mg protein)$^{-1}$ for all other enzymes.

proceeds (Stewart & Moore, 1974; Moore & Ewaze, 1976; Ewaze, Moore & Stewart, 1978) (Table 13.2).

Consideration of the metabolic pathways in which these enzymes are concerned leads to the inference that the increases in activity provide for amplification of the urea cycle, leading to accumulation of urea. Feeding of [U-^{14}C]-citrulline to live tissue slices confirms that urea synthesis and accumulation occurs *in vivo* in the cap but not in the stipe (the latter, of course, having a high urease activity). Urea is not the only compound which is accumulated. Arginine content increases by a factor of four (concentration by a factor of two) as the primordial cap develops to maturity, though both content and concentration decline in the stipe during this time. This situation can be interpreted as a means by which arginase activity is regulated. In *Coprinus* this enzyme has a K_m of 100 mM and it can be calculated that the flux through the arginase reaction is likely to increase by a factor of two to three in the cap while declining in the stipe as development proceeds from stage 3 (immature fruit body, meiosis ending but spore formation only just starting) to stage 5 (mature fruit body discharging spores). Indeed the arginine accumulation will lead to at least a six-fold greater flux through the arginase reaction in the cap than in the stipe even though there is little difference between the *in vitro* measurements

Table 13.2. *Some enzymes and metabolites in developing fruit bodies of* Coprinus cinereus

Enzyme or metabolite	Tissue	Fruit body developmental stage		
		3	4	5
NADP-linked glutamate dehydrogenase	cap	47	420	780
	stipe	29	11	20
Succinate dehydrogenase	cap	12	27	62
	stipe	28	16	10
Isocitrate dehydrogenase	cap	50	150	200
	stipe	90	200	220
Glycogen:				
μg per fruit body		1857	1361	97
% in cap		97	96	86
Ammonium	cap	32	11	9
	stipe	90	40	22

Enzyme activities are shown as nmol substrate used min^{-1} (mg protein)$^{-1}$; the amount of ammonium is shown as μmol (g fresh weight)$^{-1}$.

of arginase activity of the two tissues. This further strengthens the view that the urea cycle is specifically amplified in developing cap tissues.

The level of arginine increases whether quantified in terms of tissue fresh weight or dry weight; but while urea content on a dry weight basis increases by a factor of 2.5, the urea concentration (on a fresh weight basis) is essentially unchanged during cap development (Ewaze, Moore & Stewart, 1978). Among the compounds assayed urea was the only one to behave like this. The conclusion was drawn that during cap development urea accumulation drives water into the cells osmotically.

There is certainly a need for considerable water uptake during the later stages of cap development, for the hymenial cells particularly become greatly inflated (Moore *et al.*, 1979). This cell expansion is absolutely central to the whole morphogenesis of the developing cap. Mature gill hymenia are largely made up of paraphyses and these cells increase most dramatically in size. Expansion of these cells therefore increases the area of the gill plate but since this is bounded on its outer edge (i.e. the edge furthest from the stipe) by an inextensible, but flexible, layer of outer cap tissue, the increase in gill area is accommodated by a curling of the gill away from the stipe (Moore *et al.*, 1979). Thus the changes in cap morphology which characterise the maturation process can be accounted for by inflation of hymenial cells, and this depends on osmotic influx of

water driven by the substrate accumulations which are a consequence of the metabolic shift discussed above.

Experimentation with vegetative cultures has shown that the basic features of NADP–GDH are that induction occurs when acetyl-CoA accumulates in the mycelium in the virtual absence of ammonium (Moore, 1981). This correlates extremely well with observations made on fruit bodies (Table 13.2). Assays of normal fruit bodies show that glycogen is accumulated to high levels in the fruit body cap, but is metabolised as the cap matures (Moore *et al.*, 1979). During this process both isocitrate dehydrogenase and succinate dehydrogenase are elevated in activity in the cap, implying enhanced metabolism through the TCA cycle (Moore & Ewaze, 1976). There is a good evidence that the TCA cycle in *Coprinus cinereus* proceeds through the glutamate decarboxylation loop (Ewaze *et al.*, 1978). Thus 2-oxoglutarate amination is a necessary component of TCA cycle reactions and if the TCA cycle activity is to be amplified, this amination step must be amplified too. However, the cap always contains less free ammonium than does the stipe and the concentration of this metabolite declines drastically as the primordium develops into the mature fruit body (Ewaze *et al.*, 1978). Metabolism of up to 2 mg of glycogen per fruit body with little available ammonium could obviously lead to accumulation of acetyl-CoA to levels sufficient to induce NADP–GDH and associated enzymes because of the overall rate-limiting effect at the 2-oxoglutarate amination step normally carried out by NAD-linked glutamate dehydrogenase.

We believe that the NADP–GDH and glutamine synthetase together form an ammonium-scavenging system which is induced to safeguard the requirements of both the TCA and urea cycles under these metabolic conditions in the fruit body; NADP–GDH having a ten-fold higher affinity for ammonium than the NAD-linked enzyme (Al-Gharawi & Moore, 1977). Such an enzyme combination is a rather novel way of going about this task, but there is evidence that at least one other basidiomycete, *Sporotrichum*, employs a similar system (Buswell, Ander & Eriksson, 1982). In this discussion, however, we do not wish to take the metabolic description any further. Clearly, we have identified a system of enzymes which serve a distinct purpose during cap development. These enzymes exhibit a variety of levels of control; some are quite evidently derepressed by controls which must operate at the gene level, one is repressed in an apparently coordinated way, and there is substrate-level regulation too, including an allosteric control of NADP–GDH activity which is apparently related to accumulation of the substrate and which therefore again implies

that ammonium availability is rate-limiting for the TCA cycle (Al-Gharawi & Moore, 1977). These various phenomena are evidently integrated during development so as to provide for the maturation of the fruit body cap. Our major interest now is in the means by which the control of this metabolism is integrated in the differentiated cells of the fruit body cap.

Cytochemistry of developing gill tissues

Although the metabolism described seems to be specifically concerned with the expansion phase of fruit body maturation, the derepression of NADP–GDH (which is used as a model representative of this whole metabolic shift) occurs in quite young primordia. To understand the regulatory events themselves, we must look at the primordium and its mode of differentiation. The first efforts to examine the behaviour of individual cells were made some years ago using frozen sections of fruit body tissues (Elhiti, Butler & Moore, 1979). The sections were stained with tetrazolium and revealed a very distinct difference between the NAD-linked GDH (which is always present in cap and stipe tissues) and the NADP-linked enzyme, activity of which is found in caps but not stipes. The cytochemical observations paralleled those derived from assays of tissue homogenates. They also seem to show a new phenomenon, for the developing activity of NADP–GDH did not increase uniformly in all cells. Isolated islands of hymenial tissue showed positive enzyme staining. As successively older tissues were examined those islands enlarged and eventually coalesced, implying that the steadily increasing activity of NADP–GDH recorded in homogenates prepared from successively older primordia reflected a steady increase in the population of cells able to express this enzyme activity. These experiments used material frozen and sectioned at $-20\,^{\circ}C$. It is potentially possible to apply the tetrazolium reaction to living material. The technique involves stripping individual gills (or even individual hymenia) from primordia and incubating in a solution containing tetrazolium salt. After about 30 min, various patterns of staining are observed, and the most favourable give the impression of successful staining of individual cells to reveal NADP–GDH activity. Unfortunately, this staining reaction is non-specific (i.e. it is not dependent on the substrates of the glutamate dehydrogenase reaction included in the reaction mixture). However, it *is* interesting that for some reason something reacts differentially with tetrazolium in these immature hymenia. Nevertheless it is quite clear that frozen sections stained with tetrazolium salts reveal the localisation of NADP–GDH activity in basidia (Elhiti *et al.*, 1979); that in very young tissues only some of the basidia stain, and in older tissues

all of the basidia (indeed, eventually all of the cells) stain; and that these reactions *are* substrate-specific. So the question is, how is the regulatory signal communicated between these cells? Any attempt to answer this question will require detailed histological information.

Development of fruit body structure

To derive a developmental description at the biochemical and molecular levels we need an account of the histological structure and development of the fruit body. At the start of spore discharge, the mature cap encloses the top of the stipe and comprises a set of vertically oriented gills radiating inwards, towards the stipe, from the thin outer layer of cap tissue. As spore release proceeds, the cap margin curls outwards, the cap opens like a parasol as splits penetrate the tramal layer between adjacent hymenia, and the gills (or, at least, the gill remnants since autodigestion is occurring too) are eventually brought to an almost horizontal orientation. This terminating phase in development is well documented and the description is non-controversial. The enzyme control mechanisms are exercised in very young primordial tissues and the published literature lacks the clarity required for an unambiguous description of the way tissue domains are initially laid down. The difficulty seems to lie in the variety of developmental patterns observable in agarics (Watling: Chapter 11), in a controversy over interpretations which took place in the early decades of this century, and, in the fact that *C. cinereus* does not appear in the most authoritative account of *Coprinus* spp. (Reijnders, 1979) and has also been frequently misidentified over the years (Pinto-Lopes & Almeida, 1970; Moore *et al.*, 1979).

Accounts in the current literature

The existing literature claims that 'the lamellae originate as downward projecting salients of the palisade hymenophore fundament, in a series radiating outward towards the margin of the pileus, *the younger portions of the salients being towards the margin of the pileus and continuing to arise in a centrifugal direction*, following up the progressive development in the same direction of the palisade hymenophore, cavity, and pileus margin' (Atkinson, 1916, describing various species of *Coprinus*; the stress is ours). Thoughts on the direction of widening of the gill are variable. The prevalent belief is that the gills expand *towards* the stipe owing to growth of the trama hyphae at the inner edge (i.e. the edge closest to the stipe) and to intercalary growth of elements into the hymenium (Brefeld, 1877; De Bary, 1887; Atkinson, 1916; Reijnders, 1948, 1963). Levine (1914)

contradicts the popular belief that widening of the gill occurs from the outer to the inner edge of the gill (i.e. towards the stipe): 'The length of the ridges increases by the formation of new palisade cells from above...'; in this context the outer surface of the cap is 'above', so Levine seems to be indicating a widening from the inner, stipe-adjacent edge towards the outer edge.

Two different modes of development have been described for the origin of lamellae. Schmitz (1842) was one of the first to observe the presence of a general annular cavity around the stipe, the roof of which was lined with a continuous palisade layer, this latter being the young hymenophore. These observations were confirmed in Brefeld's (1877) study on *Coprinus lagopus*, Hoffman's (1860) work on *C. fimetarius* and Atkinson's (1906, 1914) descriptions of *Agaricus* spp. The second method of development was originally thought to be of limited applicability (Atkinson, 1914) until Levine (1914) reported it in *C. micaceus* and then in other species and concluded that this course of development prevailed in most Agaricaceae. According to Levine no annular prelamellar cavity is found. Instead, the palisade layer develops a series of groups or ridges which then elongate and split; halves of adjacent ridges unite to form the lamellae. Levine maintained that the protenchyme tissue between the ridged groups of palisade cells is continuous with the underlying stipe tissue from the earliest stage.

Atkinson (1916) refuted Levine's work in his treatise on *C. comatus*, *C. atramentarius* and *C. micaceus*. He remains adamant that, in Agaricaceae, there is first a general annular cavity with a continuous palisade layer which grows 'outward in a centrifugal direction over the undersurface of the pileus, following the centrifugal growth of the latter'. According to Atkinson, the unequal growth of areas of the palisade layer gives rise to folds which are the fundaments of the lamellae: as these widen in the cavity, they reach the underlying stipe or the fundamental plectenchyma surrounding the stipe. The stipe tissues and the gill trama therefore come to appear continuous. Atkinson argues that it is this secondary attachment of the gill trama to the stipe which led Levine to his 'erroneous' conclusions. Similarly, Chow (1934) reports palisade pockets with the interlying tissues continuous with the stipe, but, again, attributes this feature to the growth of the gills into the stipe.

Observations made since those already discussed have shown that both modes of development occur, but in different species (Reijnders, 1963, 1979). Owing to Levine's demonstration that cavities widened due to fixation artifacts and points-of-stress tearing, Reijnders fixed his material

in very mild Flemming solution. His results indicate that certain species do not have a continuous palisade layer or a general annular cavity and that pileus and stipe hyphae in those species are intimately intermingled in the regions between the palisade ridges. Reijnders (1979) examined a number of *Coprinus* species and found some to be rupthymenial (gill differentiation proceeding away from the stipe) while others were levhymenial (gills differentiate towards the stipe).

A considerable portion of the existing literature thus strongly suggests

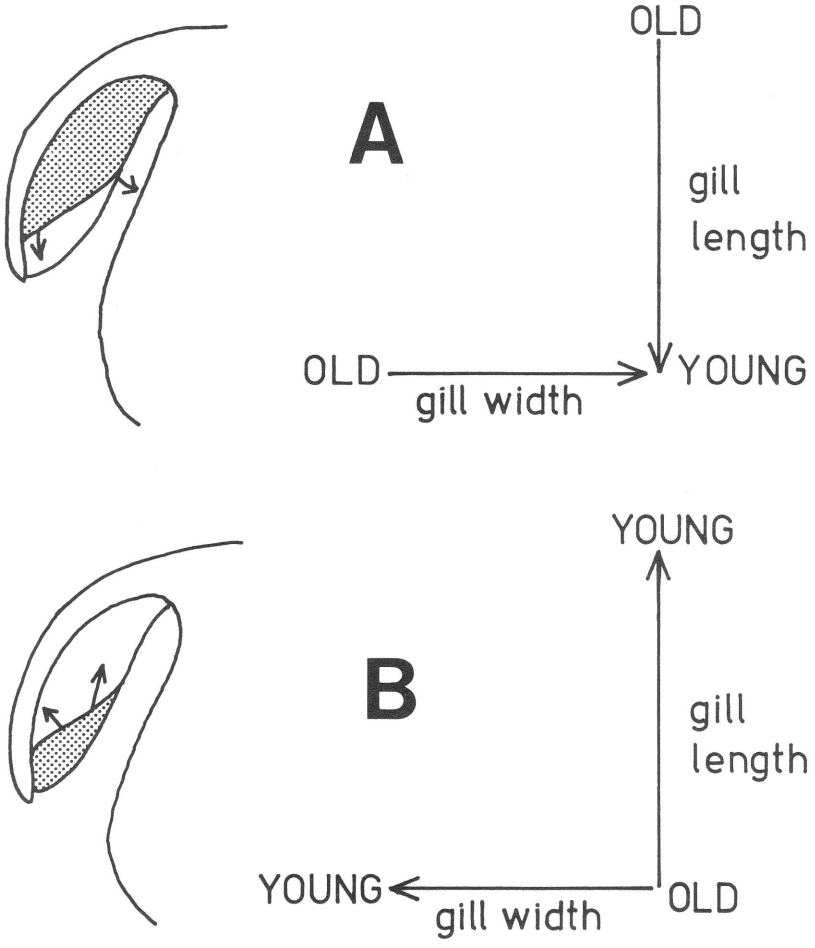

Fig. 13.2. Developmental polarity of the *Coprinus* fruit body cap. A depicts polarity of differentiation where development is levhymenial; B shows polarity in the rupthymenial mode of development.

that early development of the gills takes place in a direction towards the cap margin and towards the stipe. This implies that the youngest tissue will be that which is adjacent to the stipe and that which is closest to the cap margin (Fig. 13.2A). But what of the later stages of maturation? In *Coprinus cinereus*, nuclear fusion is immediately followed by chromosome pairing. This process is asynchronous, with the basal part of the gill (i.e. that at the cap margin) initiating the events. The difference, though, between the margin and the apex is less than an hour (Raju & Lu, 1970). Nevertheless, the implication is that it is cap margin tissue which first reaches the stage of development at which meiosis is initiated. Chow (1934) notes that the maturation of the basidia follows the same general order in *Coprinus* species and begins at the interior–inferior margin of each lamella. Thus, it is commonly agreed and a matter of simple observation that the production and pigmentation of spores is initiated at the edge of the gill closest to the stipe and the 'wave' of pigmentation travels from that edge towards the outer edge of the cap and from the cap margin towards the apex. These observations indicate that events associated with maturation progress in an upward direction, from the cap margin to the apex, and across the gill, from the inner edge (adjacent to the stipe) to the outer. Consequently, the oldest part of the gill (i.e. the part most advanced in development) appears to be at the cap margin *and* at the edge closest to the stipe (Fig. 13.2B).

There is quite obviously a clear contradiction in these accounts, and they could imply that morphogenetic polarity changes direction by 180 degrees at some stage during development. To determine whether this is so, one needs to know whether *Coprinus cinereus* shows a rupthymenial or levhymenial mode of origin of the hymenophore. Unfortunately, this species has often been misidentified and work has been published in the past under such names as *C. lagopus*, *C. fimetarius* and *C. macrorhizus* (Pinto-Lopes & Almeida, 1970). Furthermore, Reijnders (1979) showed that *C. macrorhizus* and *C. lagopus* differed in their mode of hymenophore origin, but he did not examine *C. cinereus*. We have therefore examined the situation in this species for ourselves.

Observations on the differentiation of Coprinus cinereus

Our observations were made on serial transverse sections cut from young primordia at three different stages of development – stages 1, 2 and 3 (see Fig. 13.1). The material was fixed in 5.75% glutaraldehyde, post-fixed in osmium tetroxide, then dehydrated through an alcohol series and embedded in a low-viscosity resin (Spurr, 1969). Serial sections 2 μm thick

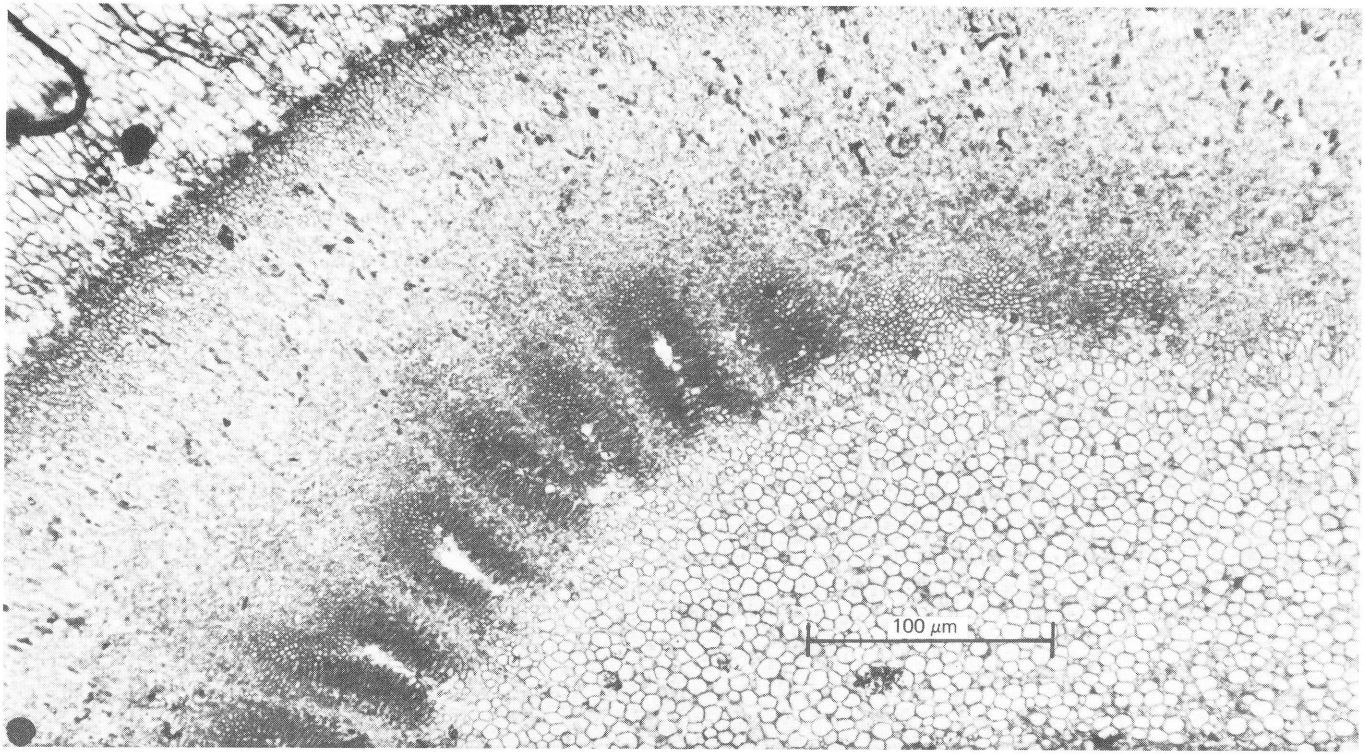

Fig. 13.3. A slightly oblique transverse section of the upper part of a Stage 1 fruit body cap. Various stages in the development of the gill cavities can be seen in this section. Note the absence of a general annular cavity and the continued connection between the stipe tissues and the tissue located between developing hymenia.

were cut on an ultramicrotome and mounted on glass microscope slides for observation with the light microscope. Sections were stained with a solution of 1% toluidine blue in 1% boric acid.

Examination of these serial sections shows that stipe differentiation can be recognised by the increased diameter and vertically parallel arrangement of the stipe cells. From the very earliest point at which such cells can be seen, the outer layers of the stipe are composed of hyphae which are thoroughly intermingled with the undifferentiated hyphae of the cap regions. The first evidence of cap differentiation is the formation of a wave-like contour around the stipe comprised of arched groups of cells (Figs 13.3 and 13.4). These are the palisade ridges, separated by protenchyme hyphae extending into the peripheral tissue of the stipe. Such 'Levine ridges' occur at the apical end of the gill and at the time they are first observed there is no evidence of a general annular cavity. The gill cavity

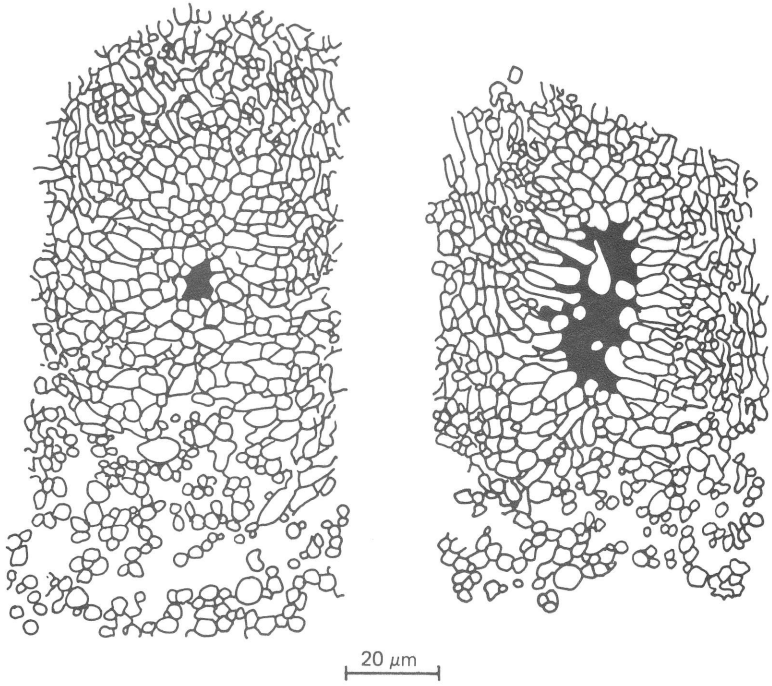

Fig. 13.4. Two successive stages in development of the gill cavity (black). The more loosely organised tissue in the bottom of the drawings corresponds in position to the 'annular cavity' region. Tracings made from photographs of transverse sections.

develops within the palisade ridge as its differentiation proceeds towards the outer edge of the cap. At about this time the region between the differentiating palisades and the stipe proper becomes more open and may represent the annular cavity region, but it is still traversed by a large number of intact hyphae. Moreover, the palisade layers (which clearly differentiate into cell plates that are to become the hymenia) have proto-tramal regions which are in full communication with the outer context of the stipe (Fig. 13.3). This means that the hymenium is discontinuous at the inner edge of each primary gill and the gill is physically connected to the stipe. In a stage 1 primordium this hymenial discontinuity at the inner edge of each gill is seen in all cross-sections down the length of the cap. Such connections between the primary gills and the stipe do not represent a secondary attachment; they arise as a result of the particular way in which the gill differentiates. In older stages, the hymenium discontinuity becomes less apparent with the formation of hymenial cells over the inner (stipe-adjacent) edge of the gill; and in older stages still, the appearance of an annular cavity is accompanied by tearing of the gills away from the stipe.

The discontinuity of the hymenium, forming an open inner edge to the gill, indicates that *C. cinereus* shares the rupthymenial mode of hymenophore development described for many other *Coprinus* species (Reijnders, 1979). With this mode of development the gill is envisaged as widening *towards* the periphery (outer edge) of the cap as a differentiating front moves into, and differentiates from, the protenchyme. Since the widest parts of the gills are those at the cap margin, it follows that the differentiating front is also moving *upwards*, towards the apex of the cap. Consequently, the morphogenetic polarities which initially establish the different tissue domains are the same as those that characterise the later, maturational, changes.

Detailed histological structure of the gill and the relationships of its component cells

While the protenchyme hyphae are continuously supplementing the palisade front along a defined acropetal polarity, the older palisades, near the inner edge, are differentiating into basidia. As a result of each ridge splitting in half behind the lateral advance of the palisade front, the gill cavity is formed, flanked by a hymenium of basidia (Fig. 13.4).

The young hymenium consists of poorly differentiated basidia and of conspicuous cystidia. The latter grow from the subhymenium and trama branches (Fig. 13.5). Cystidia insert into the opposite hymenium and their

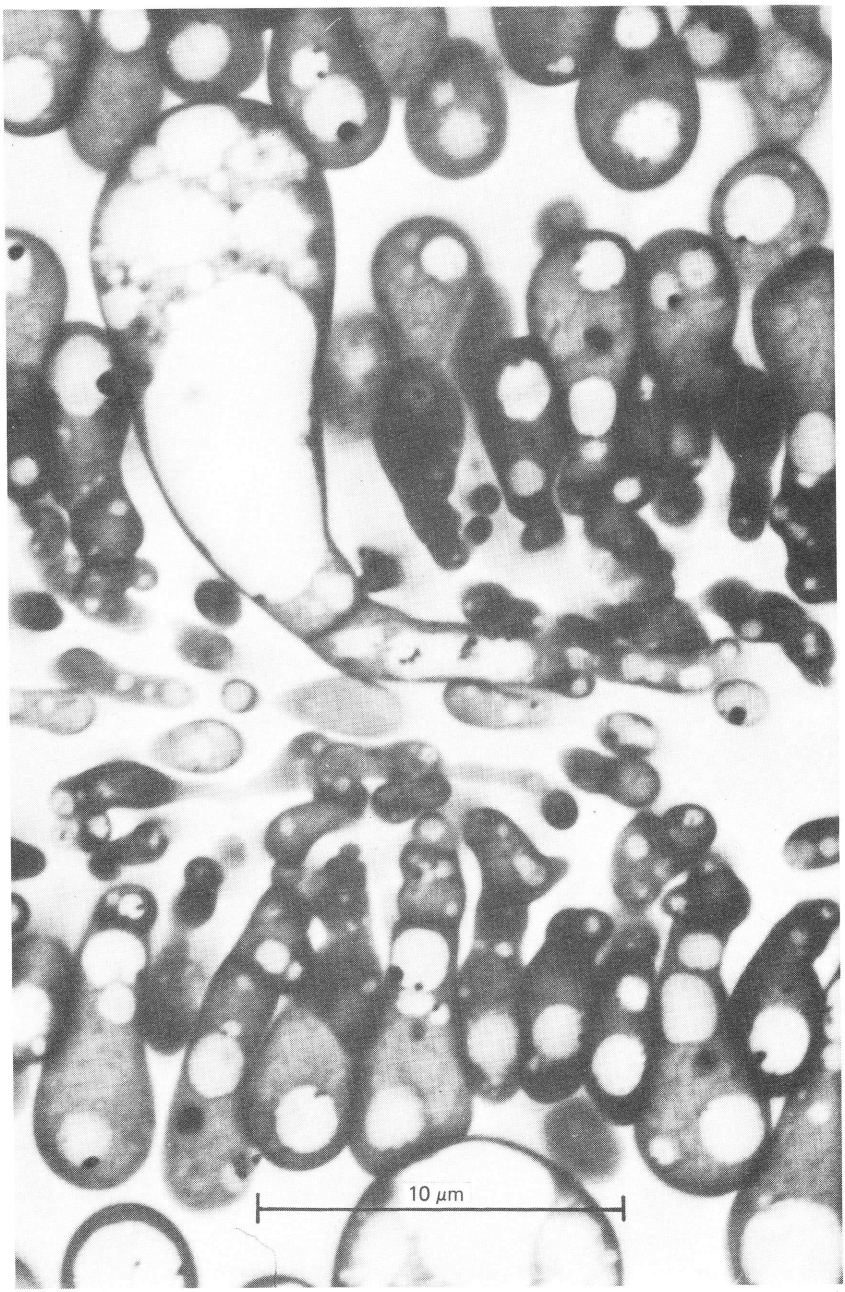

Fig. 13.5. Transverse section of the hymenium of a Stage 2 primordium. A cystidium emerges directly from the tramal hyphae. Paraphyses emerge as branches from sub-basidial cells and insert into the basidial layer.

turgidity prevents hymenium layers from touching and hindering sterigma and spore growth and spore discharge (Buller, 1924; Chow, 1934).

Most of the literature dealing with basidial ontogeny appears to ignore the initial stages of basidial formation and to picture basidia developing as outgrowths from subhymenial segments. In *C. cinereus*, basidia originate as slightly swollen chromophilic cells at the apex of protenchyme or pileus–trama hyphae. This apical differentiation follows the pattern of most basidiomycetes (Sundberg, 1978). Towards stage 2, cystidial cells and the apices of the basidia enlarge. The expansion of basidial apices is often associated with fruit bodies in which the hyphae of the trama, subhymenium and hymenium show similar expansion (Oberwinkler, 1982). The basidia are dimorphic with a slight preponderance of short basidia – a situation analogous to that in *C. sterquilinus* and *C. lagopus* (Buller, 1924).

During the early stages of hymenium development it is noticeable that adjacent basidia arise at the apex of sister branches from a parent hypha. This branching occurs at a distance away from the young hymenium and the intermediate cells give rise to subhymenial branches and to paraphyses. Paraphyses develop as outgrowths of sub-basidial cells during stage 2. Assuming that the chromophily of the tissue represents the distribution of carbohydrates, then at stage 1 the carbohydrate accumulation is observed in basidia and at stage 2 in the subhymenium and particularly the emerging paraphyseal branches (Fig. 13.5). This distribution appears to reflect the zone of the lamella which is in active 'extension' growth. In *C. micaceus* and *C. lagopus*, Chow (1934) observed a chromophilic pattern analogous to that found here in *C. cinereus* but concluded that basidia grew from the subhymenium and into a layer of paraphyses. We do not agree with this interpretation, believing that our observations show quite clearly that paraphyses emerge *from* sub-basidial cells and insert *into* a layer of young basidia. Our identification of the cells which insert into the existing layer as paraphyses is the basis of our identification of the cells that differentiate from the original palisade as 'poorly differentiated basidia'. Pukkila (see Chapter 22) puts the commencement of paraphyseal insertion at about the time that meiosis starts; with metaphase I of meiosis being about coincident with the start of the main phase of paraphyseal expansion. Statistical analysis of the geometrical relations between hymenial cells suggests that basidial numbers do not increase after the gill lamella is formed. About 60% of the paraphyseal population insert in this initial wave at about the time of meiosis, the rest inserting as gill maturation proceeds.

Elongation and enlargement of the gill is clearly dependent on this increase in the paraphyseal cell population, but more especially on an enormous increase in the constituent cell volumes. Paraphyses expand as much as 2.5 times their original volume, this expansion accounting for half the width of the gill, and most probably for the same proportion of its length. Basidial expansion accounts for the remaining increase in size. The ingress of water into the hymenium elements produces much vacuolation (Moore *et al.*, 1979). The exact identity of an osmoticum has not been established but, as discussed earlier, amplification of urea cycle activity, occurring specifically in the cap tissue, could contribute to such a force (Ewaze *et al.*, 1978).

The determination of developmental pattern

So far, although the direction of gill formation has been suggested, no attempts have been made to explain how this spatial and temporal sequence might be achieved. However, some discussion of a more general nature is in order. The tissue has an initial hyphal mass which is relatively homogeneous, but it then alters to form a well-defined spatial pattern. Some kind of specification has occurred. The specification of development occurs along two axes of polarity. These are from base to apex, and from the inner to the outer surface. The polarity is a unit vector which determines the direction in which the cells will become organised. The specification of information along these two axes of polarity results in the formation of a palisade layer and thus precipitates the establishment of basic tissue domains and the pattern of the cells within those domains.

The most popular ideas concerning the mechanism of polarity come from work on animal systems in which polarity is regarded as being determined by the concentration gradient of a substance, the so-called 'morphogen'. According to Wolpert (1969), this information is positional information and 'largely determines with respect to the cell's genome and development history the nature of its molecular differentiation'. Models of the regulation of cell differentiation have been analysed by Meinhardt & Gierer (1974) who illustrate how two-dimensional patterns very similar to those observed in the agaric hymenium may be generated in response to activators and inhibitors capable of diffusing through the tissues.

The concepts of positional information and polarity axes are capable of providing a basic understanding as to how the gill pattern develops, the specification of positional information being determined by the two axes of polarity. We presume that hyphal cells interpret this positional information and differentiate into palisades. These are the fundamental building

blocks of the hymenium and differentiate initially into basidia and cystidia. The differentiating agaric gill seems, on the face of it, to be an ideal candidate for interpretation along the lines of a diffusion gradient theory. The gill *does* have two developmental axes to which differentiation might be referred, and the hymenial cells *do* become positionally differentiated in a manner apparently analogous to epidermal cell layers (the usual classic examples) in higher plants and animals. There are problems, though. We are dealing with a structure whose construction is based on the hyphal organisation. As Read (1983) has put it, in the fungi '...cellular polarity resulting from polarised differentiation of the cytoplasm and/or the cell wall has only been found in hyphal like elements'. Dependence on hyphal organisation is well illustrated by the above description of the origin of paraphyses as branches formed beneath the hyphal tip cell which is differentiating into a basidium (other examples are mentioned by Reijnders & Moore: Chapter 27). Differentiation of the hymenium, therefore, which has the appearance and function of a plate-like layer of cells, owes a great deal to the *linearised* differentiation of the components which eventually come to comprise that cell layer. Much of the final pattern observable in the mature hymenial cell layer thus arises as a consequence of the sequential differentiation of compartments in the hyphae which terminate in the young hymenium. Nevertheless, some definition of positional significance in the hymenial layer must be involved since the basidium–paraphysis relation is an organised one, and cystidia certainly differentiate at regular intervals. In a broader sense, gill lamellae are organised at a constant distance from one another (Burnett, 1968) and the formation of other domains in the cap are also organised in ways that imply coordination of a spatially dispersed cell population may be achieved by the dissemination of regulatory signals.

This highlights another problem which exists in the application of currently favoured models relating to the definition of positional information. All of these models have been developed primarily from studies of differentiating animal tissues, though they can be applied to at least some higher plant systems. All of the models which have been developed depend on the existence of lateral cytoplasmic communications between cells in the differentiating tissue which extend over many cell diameters. Although the ultrastructure of many fungal structures has been carefully studied, there appears to be no evidence for any form of lateral communication between neighbouring hyphal compartments other than via lateral anastomoses. Nothing like gap junctions or plasmodesmata has ever been reported in structures which have arisen by hyphal aggregation. This does

not have disabling consequences for theoretical treatment of tissue pattern formation, but it does mean that a cytoplasmic route for coordinating regulatory signals might be excluded. Any regulatory molecule must be excreted across the membrane and wall of the 'sending' cell and absorbed across the wall and membrane of the 'responding' cell. This implies that membrane- and wall-associated processes, and perhaps transport through an extracellular matrix (Williams *et al.*: Chapter 18), may be the rate-limiting steps in the determination of fungal tissue organisation.

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