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REVIEW

Developmental Biology of the *Coprinus cinereus* Carpophore: Metabolic Regulation in Relation to Cap Morphogenesis

DAVID MOORE

Department of Botany, The University, Manchester M13 9PL, United Kingdom

MOORE, D. 1984. Developmental biology of the *Coprinus cinereus* carpophore: Metabolic regulation in relation to cap morphogenesis. *Experimental Mycology* 8, 283–297. Maturation of the cap of *Coprinus cinereus* is accompanied by a specific pattern of changes in enzyme activities and metabolite levels. The most significant changes result in amplification of activity in the tricarboxylic acid cycle and the urea cycle, as judged from the observation that succinate dehydrogenase, NADP-linked glutamate dehydrogenase, glutamine synthetase, ornithine acetyltransferase, and ornithine carbamyltransferase are elevated to levels in excess (in some cases greatly in excess) of those found in the stipe, while activity of the enzyme urease is absent from the cap though present in both stipe and mycelium. Regulation of NADP–glutamate dehydrogenase depends on a circuit involving accumulation of acetyl-CoA in tissues where ammonia is limiting. This enzyme, together with glutamine synthetase, probably contributes to an ammonium scavenging system. The net result of the shift in metabolism is accumulation of urea, and probably other nitrogenous metabolites, as osmotic solutes which drive water into the cells of the gill hymenium. This leads to inflation of these cells and their expansion can account for the changes in form through which the cap progresses as maturation proceeds. The system exemplifies different sorts of regulation, from substrate level to the gene level, and is an ideal model for study of the causative events that give rise to metabolic shifts which direct differentiation processes.

INDEX DESCRIPTORS: *Coprinus;* developmental biology; metabolic regulation; fruit body morphogenesis; enzymology; enzyme control; differentiation; nitrogen metabolism.

This paper describes a metabolic system which is involved in the final stages of maturation of the fruit body cap of the basidiomycete *Coprinus cinereus*. Although the enzyme systems involved appear to contribute to metabolic processes which provide for the changes in shape of the cap during basidiospore release, some of the regulatory events occur in very young tissues. The results which have been obtained over the past several years are potentially significant in a number of respects. First, the phenomena which have been observed represent a wide selection of regulatory events, so the system could make a real contribution to our understanding of the control of morphogenesis in eucaryotes generally. Second, since we are essentially dealing with fruit body maturation, the knowledge gained with *Coprinus* may well assist in understanding maturation processes in commercially important mushrooms, and this could help in attempts to improve cropping techniques or attempts to control shelf life. This paper reports some previously unpublished data and reviews the work already published.

This account will deal exclusively with *C. cinereus* but it is an unfortunate fact of life for those who work with this fungus that it has been misidentified on a number of occasions, so the literature is peppered with other, erroneous, specific names. That the true name is *Coprinus cinereus* (Schaeff. ex Fr.) S. F. Gray was firmly established by Pinto-Lopes and Almeida (1970). However, the first English collections, for research on incompatibility control, were made by D. Lewis in 1952. The material was, and still is, very similar to the

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description of *C. lagopus* given by Buller (1924) and so this name was used to describe the species in England for many years. At about the same time that Lewis was collecting in England, and for similar purposes, Kimura collected the species in Japan but called it *C. macrorhizus* f. *microsporus*. Pinto-Lopes and Almeida (1970) corrected the identification but it takes a long time for correct names to be adopted. Some of the problems of identification are discussed by Moore *et al.* (1979), who also give a general description of the morphological aspects of fruit body development in the organism.

DEVELOPMENT-SPECIFIC REGULATION OF GLUTAMATE DEHYDROGENASE

Interest in the enzymology of developmental processes in *Coprinus* began in the early 1970s with the observation that activity of the NADP-linked glutamate dehydrogenase (NADP-GDH; EC 1.4.1.4) was found only in the cap and, indeed, that the activity increased as the cap developed. Although both cap and stipe exhibited activity of the NAD-linked enzyme (NAD-GDH;[1] EC 1.4.1.2), no significant NADP-GDH activity was ever found in stipe tissues (Stewart and Moore, 1974).

Of course *Coprinus* is not alone in having two GDH enzymes linked specifically to NAD or NADP. Many filamentous fungi and yeasts have been shown to be similarly equipped. It is often considered that NAD-GDH has a catabolic role (glutamate = 2-oxoglutarate + ammonium) while the NADP-linked enzyme is anabolic (2-oxoglutarate + ammonium = glutamate). This suggestion was first made for *Neurospora crassa* (Sanwal and Lata, 1961) and *Saccharomyces cerevisiae* (Holzer *et al.*, 1965), and Sanwal and Lata (1962) further developed the idea by suggesting that these two enzymes are reciprocally (or concurrently) regulated. These concepts have almost reached the stage of being accepted as truisms, so it is necessary to point out that the situation is far more complex than such confident generalizations would imply. In particular, Stine (1968) concluded that reciprocal (concurrent) regulation of these two enzymes did not occur in germinating conidia of *N. crassa;* while Dennen and Niederpruem (1967) showed that NADP-GDH activity of *Schizophyllum commune* monokaryotic mycelium was depressed by transfer from glutamate medium to ammonium medium but increased by the reciprocal transfer, the NAD-linked enzyme being largely unaffected by these treatments (which is not expected if NADP-GDH is devotedly anabolic and NAD-GDH catabolic). The position has been reviewed by Casselton (1969) although the identification of a glutamate synthase/glutamine synthetase ammonium assimilation system in many of these fungi further complicates the issue and a reappraisal of these data is long overdue.

For *Coprinus* we can say that, despite careful search, no evidence has been obtained for a glutamate synthase activity. Furthermore, the NAD-GDH of the mycelium shows high activity whether the growth conditions demand amination or deamination (Table 1). Data shown below indicate that the enzymes are not reciprocally regulated, so the view which has been taken of the *Coprinus* system is that the NAD-GDH serves the mycelium for amination or deamination as nutritional conditions require, the NADP-linked enzyme being reserved for metabolic purposes related specifically to morphogenesis.

THE METABOLIC CONTEXT OF GDH REGULATION

The seemingly ubiquitous occurrence of NAD-GDH is probably related to the re-

[1] Abbreviations used: NAD-GDH, NAD-linked glutamate dehydrogenase; TCA, tricarboxylic acid; GS, glutamine synthetase; OAT, ornithine acetyltransferase; OCT, ornithine carbamyltransferase; MSO, methionine sulfoximine.

TABLE 1
Comparison of Specific Activities of Glutamate Dehydrogenases and Mycelial Growth of Monokaryon BC9/6,6 of *Coprinus cinereus* Cultured on Contrasting Media

Medium composition	Enzyme activity[a]		Mycelial dry wt (mg/100 ml)
	NAD-GDH	NADP-GDH	
25 m*M* Acetate + 25 m*M* urea	177	5	24.4
25 m*M* Acetate + 25 m*M* glutamate	207	7	25.9
Dung extract	219	0	43.2

[a] Enzyme activity is shown as nmol substrate used/min/mg protein. Mycelia were grown in still culture and assayed after 4 days incubation at 37°C. Data from Stewart and Moore (1974).

quirement for 2-oxoglutarate amination for completion of the tricarboxylic acid (TCA) cycle in *Coprinus* (Moore and Ewaze, 1976). Activity of 2-oxoglutarate dehydrogenase (EC 1.2.4.2) could not be demonstrated despite trials with a variety of extraction methods, so what might be considered to be the conventional TCA cycle cannot operate. Activity of isocitrate lyase (EC 4.1.3.1) in the fruit body did not exceed the repressed levels characteristic of glucose-grown mycelium (Casselton *et al.*, 1969). Indeed, crude extracts of fruit body caps (but not stipes) contained a low-molecular-weight inhibitor of this enzyme. The identity of this inhibitor is not known, but its presence coupled with the very low enzyme activity detected when the inhibitor was removed by gel filtration make it most unlikely that isocitrate lyase, and hence the glyoxylate shunt, has any major function in fruit body metabolism.

However, high activities, especially in the cap, of glutamate decarboxylase (EC 4.1.1.15) and 4-aminobutyrate aminotransferase (EC 2.6.1.19) have been found in *Coprinus* fruit bodies (Moore and Ewaze, 1976). These enzymes contribute to the "glutamate decarboxylation loop" which operates in the central nervous system of higher animals. In this variant of the TCA cycle 2-oxoglutarate is aminated to glutamate rather than being oxidatively decarboxylated to succinate. The glutamate is decarboxylated to 4-aminobutyrate, transamination between the latter and 2-oxoglutarate yielding succinate semialdehyde which, an oxidation, feeds back into the TCA cycle as succinate. Since the enzymes for this loop exist in *Coprinus* it is believed to be the normal route of TCA metabolism in this organism. Evidence has also been presented that the glutamate decarboxylation loop operates in *Agaricus bisporus:* the radioactivity of [U-^{14}C] glutamate appeared in 4-aminobutyrate, succinate, and malate within 2 h of exposure of fruit body material to the labeled substrate (Piquemal *et al.*, 1972) and the enzymes of the glutamate decarboxylation loop occur in *Agaricus* spores (Rast *et al.*, 1976).

If the NAD-GDH is effectively a normal component of the TCA cycle in *Coprinus*, then what of NADP-GDH? The two enzymes are kinetically quite different (Al-Gharawi and Moore, 1977). The NADP-linked GDH exhibited positively cooperative interactions with the substrates 2-oxoglutarate and NADPH, negatively cooperative kinetics with NADP+, and was extremely sensitive to inhibition of deamination activity by ammonium. On the other hand, NAD-GDH showed positive cooperativity with NADH but Michaelis–Menten kinetics with all other substrates and was only mildly inhibited by reaction products. Figure 1 illustrates the substrate cooperativity observed in the NADP-GDH amination reaction; this phenomenon is thought to be an example of substrate coop-

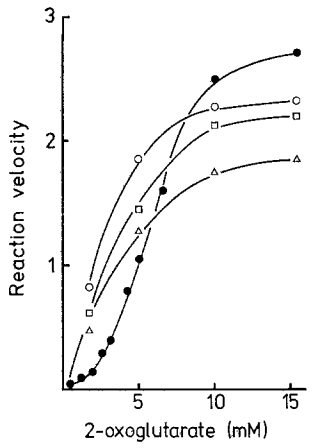

FIG. 1. Kinetics of the amination reaction promoted by the NADP-linked glutamate dehydrogenase of *Coprinus cinereus*. The figure shows plots of reaction velocity (in arbitrary units) against substrate (2-oxoglutarate) concentration for the normal assay mixture (●) and for mixtures which contain 15 m*M* quantities of the activators glutarate (○), L-2-hydroxyglutarate (△), and D-glutamate (□). Comparison of the structures of analogs tested for their effects on amination activity leads to the conclusions that the most effective allosteric activators are 5-carbon chains (2-aminobutyrate and 4-aminobutyrate were ineffective), which have two carboxylic acid groups (*n*-valerate was much less effective than glutarate) and little or no substitution, particularly in the L-orientation, at the 2-carbon position. In the normal metabolic environment of NADP-GDH, 2-oxoglutarate would best satisfy these requirements. Redrawn from Al-Gharawi and Moore (1977).

erativity being used as a switch mechanism which allows substrate (2-oxoglutarate) to accumulate to a threshold level of about 4 m*M* before maximum enzyme activity is released. An unresolved problem is the origin of NADPH+ required by the NADP-GDH reaction. On the basis of enzyme activity measurements *in vitro* Moore and Ewaze (1976) concluded that the pentose phosphate pathway plays only a minor role in carbohydrate metabolism in the developing fruit body cap because glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity declined drastically as the cap matured. Since the TCA cycle activity is considered to be greatly amplified as the cap develops (see below), NADH+ could be used to regenerate NADPH+. Enzymes responsible for this cycling may well be found among the numerous NAD-dehydrogenase and NADP-dehydrogenase isozyme activities (some of which seem to show development-related changes in level) detected by specific enzyme staining methods following electrophoresis in polyacrylamide (Moore and Jirjis, 1981). This point has not been examined further.

Attempts to define the metabolic context within which NADP-GDH is derepressed in the developing fruit body have concentrated on enzyme surveys and metabolite measurements (Ewaze *et al.*, 1978). These investigations revealed three enzymes which, like NADP-GDH, showed great increases in activity in the developing cap while remaining at low levels (or declining in activity) in the stipe: these three enzymes are glutamine synthetase (GS; EC 3.5.1.2), ornithine acetyltransferase (OAT; EC 3.5.1.16), and ornithine carbamyltransferase (OCT; EC 3.5.1.20). A fourth enzyme, urease (EC 3.5.1.5), showed the reverse behavior, being absent from the cap though present in the stipe (Table 2) and, indeed, being constitutive in mycelium (Table 3). Amplification of TCA cycle activity is signaled by the facts that succinate dehydrogenase (EC 1.3.99.1) also shows much greater activity in the cap than in the stipe, and isocitrate dehydrogenase (EC 1.1.1.41) activity increases in the fruit body as development proceeds (see Table 11).

A clue to the significance of these changes in enzyme activity was obtained when the concentrations of some amino and amido compounds were measured in developing fruit body tissues (Table 4). Such analyses showed that, most significantly, arginine and urea accumulated in the cap as development proceeded. The level of arginine increased whether quantified in terms of tissue fresh weight (Table 4a) or dry weight (Table 4b), but while urea content on a dry weight basis increased by a factor of 2.5, the urea concentration (on

TABLE 2
Some Enzyme Activities in Developing Fruit Bodies of *Coprinus cinereus*

Enzyme	Tissue	Enzyme activity[a] 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
NAD-linked glutamate dehydrogenase	Cap	270	553	1183
	Stipe	363	483	1287
Urease (note different units)	Cap	55	70	38
	Stipe	143	570	4300

[a] Except for urease, enzyme activities are shown as nmol substrate used/min/mg protein; urease activity is shown as pmol substrate used/min/mg protein. The stage 3 primordium is beginning to make spores, the stage 4 fruit body has a full complement of (mostly unpigmented) spores, and the stage 5 fruit body is releasing mature spores. Data from Ewaze *et al.* (1978).

a fresh weight basis) was essentially unchanging during cap development. Among the compounds assayed (and it is not claimed that the analyses were exhaustive) urea was the only one to behave like this. Obviously the concentrations of many metabolites change drastically during development (and note the breakdown of large quantities of glycogen shown in Table 11), but the close correlation between water uptake and urea content suggests that urea serves a special function. The conclusion was drawn, therefore, that during cap development synthesis of urea is amplified, urea accumulates, and water is driven osmotically into the cells in which urea accumulation is taking place. 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. The gill hymenia are largely made up of paraphyses—the cells which 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 the inextensible, but flexible,

TABLE 3
Specific Activity of the Enzyme Urease in Monokaryon (Strain BC9/6,6) and Dikaryon (BC9/6,6 × H1) Cultures of *Coprinus cinereus*

Nitrogen source in the medium	Enzyme activity[a] Monokaryon	Dikaryon
50 m*M* Urea	3.1	1.5
50 m*M* Ammonium tartrate	2.1	3.2
25 m*M* Urea + 25 m*M* ammonium tartrate	4.8	3.6

[a] Mycelium was incubated in a medium containing 222 m*M* glucose as carbon source without agitation for 4 days at 37°C. Urease activity is expressed as nmol $^{14}CO_2$ liberated from [^{14}C]urea/min/mg protein. Data from Ewaze *et al.* (1978).

TABLE 4
Concentrations of Some Metabolites in Fruit Body Tissues of *Coprinus cinereus*

Metabolite	Fruit body stage 3		Fruit body stage 5	
	Cap	Stipe	Cap	Stipe
(a) Quantified in terms of μmol per gram fresh wt				
Alanine	0.8	0.5	3.9	0.8
Arginine	2.0	1.8	4.3	0.8
Glutamate	1.9	1.4	3.8	0.9
Ornithine	0.5	0.5	1.1	0.2
Urea	2.1	1.2	2.8	1.1
(b) Quantified in terms of μmol per average fruit body (dry wt basis)				
Alanine	0.3	0.2	3.2	0.4
Arginine	0.9	0.4	3.5	0.3
Glutamate	0.8	0.3	3.1	0.4
Ornithine	0.2	0.1	0.9	0.1
Urea	0.9	0.2	2.3	0.5

TABLE 5
Metabolism of [U-^{14}C]Citrulline by Intact Fruit Body Tissues of *Coprinus cinereus*

Incubation time (min)	Tissue	Radioactivity (cpm/mg dry wt) recovered in		
		Citrulline	Arginine	Urea
30	Cap	17	425	44
60	Cap	707	1222	351
120	Cap	172	731	526
30	Stipe	33	219	19
60	Stipe	565	964	101
120	Stipe	395	860	95

layer of outer cap tissue the increase in gill area is accommodated by a curling of the gill away from the stipe. Thus the changes in cap morphology which characterize 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 here.

The suggestion is, then, that the changes in enzyme activity already mentioned occur specifically in the cap in order to provide for amplification of the urea cycle leading to accumulation of urea as an osmotic metabolite. Feeding of live tissue slices with [U-^{14}C]citrulline (Table 5) confirms that urea synthesis occurs *in vivo* and further illustrates its accumulation in the cap but not in the stipe (the latter, of course, having a high urease activity). Urea is not the only compound to accumulate, however. Arginine content increases by a factor of 4 (concentration by a factor of 2) 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 the activity of arginase (EC 3.5.3.1) is regulated. In *Coprinus* this enzyme has a K_m of 100 mM and a V_{max} of 1.6 μmol substrate used min^{-1} (mg protein)$^{-1}$. If the accumulated arginine occupies the same metabolic compartment as the enzyme, it can be calculated that the flux through the arginase reaction is likely to increase at least by a factor of 2 to 3 in the cap while declining in the stipe as development proceeds from stage 3 (immature fruit body, postmeiotic but spore formation only just starting) to stage 5 (mature fruit body discharging spores). Indeed the arginine accumulation will lead to at least a sixfold greater flux through the arginase reaction in the cap than in the stipe even though there is little difference between the *in vitro* measurements of arginase activity in the two tissues. This further strengthens the view that the urea cycle is very specifically amplified in developing cap tissues. The belief that these mechanisms operate *in vivo* is supported by the observation that after 1 h incubation with [U-^{14}C]glutamate, 22% of the radioactivity recovered from cap tissues appeared as 4-aminobutyrate, 54% as malate + succinate + citrate, and 6% as arginine. The corresponding figures for the stipe were, respectively, 38, 35, and 2% (Ewaze *et al.*, 1978).

EXPERIMENTAL ANALYSIS OF ENZYME REGULATION IN MYCELIA

All of the results discussed so far derive

from analyses of normally developing fruit bodies. They are observations more than experiments. Fruit body maturation seems to be an endotrophic process (Gooday, 1974), at least in its later stages, and so far it has proved virtually impossible to manipulate the developmental process in the fruit body in any effective manner. We are fortunate, however, in having a monokaryotic strain in which the same enzymatic events can be induced. Valuable information has been obtained by growing the mycelium in media containing different carbon and nitrogen sources (Stewart and Moore, 1974; Al-Gharawi and Moore, 1977) but this involves extensive incubation and more meaningful data have been obtained from a shorter term experiment which is a medium transfer experiment derived from a technique used by Fawole and Casselton (1972). The mycelium of the monokaryotic strain number BC9/6,6 (ATCC 42725) is grown in a rich medium for 4 to 5 days to obtain a good mycelial yield and is then harvested, washed, and resuspended for further incubation in a salts medium containing 100 m*M* pyruvate and no nitrogen source. The result of this treatment is that BC9/6,6 produces high activities of NADP-GDH, GS, OAT, and OCT and shows diminished activities of NAD-GDH and urease (Table 6). The significant observation here is that the increases in activity of NADP-GDH and GS are highly correlated (correlation coefficient 0.94) implying some form of coordinate control. The response to the medium transfer is sensitive to inhibition by cycloheximide and recent experiments using differential isotopic labeling before and after transfer have shown that the NADP-GDH protein is synthesized *de novo* (Jabor and Moore, unpublished). The time course of this derepression shows no lag period (Fig. 2) but does reveal that during the first 5 h after transfer, NAD-GDH also increased in activity (by about 40%); only when incubation was continued overnight did the recorded activity of NAD-GDH prove to be less than that measured for NADP-GDH. Categorically, therefore, the NAD- and NADP-linked glutamate dehydrogenases in *Coprinus* are not reciprocally regulated. This medium transfer technique has provided the opportunity to establish which of the metabolites are involved in the regulation of NADP-GDH. The approach provides us with a method whereby the metabolism comparable to a particular stage in the development of the fruit body can be studied in isolation from the bulk of fruit body metabolism.

By replacing pyruvate with other compounds a catalog of effective and ineffective materials has been established and by adding compounds to the pyruvate medium the effect of potential repressors or inhibitors has been determined (Table 7). The most significant feature of this table is that so few compounds are effective in either direction. Among potential inducers only

TABLE 6
Specific Activities of Selected Enzymes in Mycelia of Strain BC9/6;6 of *Coprinus cinereus* before and after Transfer to Medium Containing 100 m*M* Pyruvate with no Nitrogen Source[a]

Treatment	Enzyme activity			
	Urease	GS	NADP-GDH	NAD-GDH
Initial control	8.0	16	36	550
3 h after transfer	7.7	160	140	524
18 h after transfer	2.1	330	260	350
24 h after transfer	1.7	440	290	260

[a] Data from Ewaze *et al.* (1978) and Moore (unpublished).

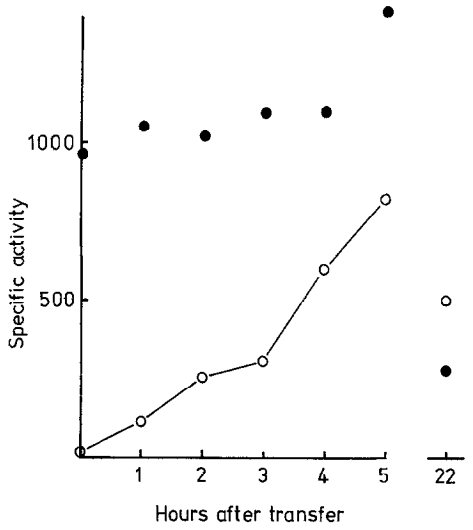

FIG. 2. Time course of induction of NADP-linked glutamate dehydrogenase (○—○) following transfer of mycelium of strain BC9/6,6 to a nitrogen-free medium supplemented with 100 m*M* pyruvate. The NAD-linked GDH was also assayed (●). Redrawn from Moore (1981a).

glucose, fructose, dihydroxyacetone, acetate, and propan-1-ol share with pyruvate the ability to cause significant increases in NADP-GDH activity. Note particularly that alanine (= 2-aminopyruvate) is ineffective, as are the TCA cycle intermediates. Among compounds thought likely to prevent the induction caused by pyruvate only ammonium, urea, arginine, and citrulline (and possibly ornithine) were effective. Urea and urea-cycle intermediates are most probably rapidly metabolized to ammonium, so the conclusion is that it is ammonium which is the active repressor or inhibitor.

Further tests using different concentrations of ammonium supplementation show that this molecule is effective at very low concentrations (Fig. 3). A noteworthy feature of the data in Table 7 is that neither methylamine nor hydroxylamine has any inhibiting effect on the pyruvate-promoted induction of NADP-GDH. Since both these compounds are useful structural analogs of the ammonia–ammonium molecule, the im-

TABLE 7
Activity of NADP-Linked Glutamate Dehydrogenase following Transfer to Medium Containing Supplements Other than Pyruvate

Identity of supplement	NADP-GDH specific activity[a]
(a) Single supplements at 100 m*M* concentration	
Glucose	769
Fructose	482
Dihydroxyacetone	110
Acetate	287
Propan-1-ol	286
Propan-2-ol	85
Oxaloacetate	31
Citrate	5
2-Oxoglutarate	36
Succinate	22
Malate	19
Lactate	87
Alanine	15
Aspartate	16
(b) Mixed supplements: Medium contains 100 m*M* pyruvate plus 50 m*M* concentrations of the following:	
Oxaloacetate	479
Citrate	717
2-Oxoglutarate	1373
Succinate	1270
Malate	1124
Alanine	279
Aspartate	1205
Glutamate	298
Ornithine	138
Potassium nitrate	737
Methylamine	444
Hydroxylamine	262
Ammonium chloride	54
Arginine	52
Citrulline	97
Urea	44

[a] Enzyme activity is shown as nmol substrate used/min/mg protein. The experiments shown in part (a) test for the ability of the supplement named to promote induction of NADP-GDH and those in part (b) for the ability of the named compound to prevent the induction usually caused by pyruvate. Strain number BC9/6,6 (= ATCC 42725) was used in these tests. Compare with Tables 8 and 9 for the normal response of this wild-type strain to the pyruvate transfer. Data from Moore (1981a).

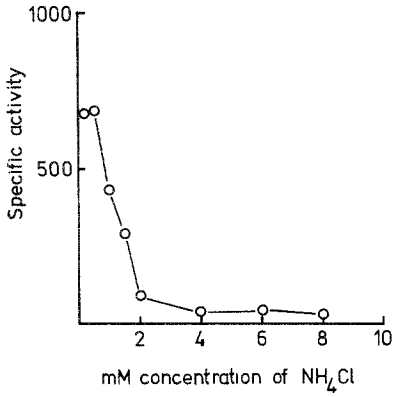

FIG. 3. Effect of the inclusion of ammonium chloride in the pyruvate transfer medium on activity of NADP-linked glutamate dehydrogenase in mycelia of strain BC9/6,6. Mycelia were harvested for assay 20 to 22 h after transfer. Redrawn from Moore (1981a).

plication could be that it is not ammonium which is itself effective in preventing induction but some metabolic derivative of it. Comparison with the highly developed models of ammonium repression in *Aspergillus* and *Neurospora* (Arst and Cove, 1973; Hynes, 1975; Grove and Marzluf, 1981) would suggest glutamine as the effective intracellular molecule, and this would be an attractive proposition in view of the close correlation between induction of NADP-GDH and GS in *Coprinus* (Table 6). It is known that addition of 50 m*M* glutamine to the pyruvate medium does prevent NADP-GDH induction but the instability of glutamine and consequent contamination of its solutions with appreciable quantities of ammonia, and the extreme sensitivity of the induction process to inhibition by ammonia, combine to make it impossible to distinguish between the relative effectiveness of glutamine and ammonium in experiments requiring the sorts of incubation periods needed to demonstrate induction. Work with structural analogs of glutamine may clarify this point but it has not so far been pursued. It has been shown that the irreversible inhibitor of GS, methionine sulfoximine (MSO), has no effect on NADP-GDH regulation. When MSO was included in the pyruvate transfer medium, NADP-GDH was induced. More significantly, when MSO was included in a pyruvate + ammonium transfer medium, induction of NADP-GDH did not occur. These results argue against GS activity being involved in expression of ammonium repression, but more quantitative kinetic analyses are required to verify this conclusion and determine whether MSO itself (as an analog of glutamine) influences the regulatory process.

The remaining question, of course, is what do the alleged inducers have in common? They represent two diverse metabolic pathways: glucose, fructose, dihydroxyacetone, and pyruvate can be considered to be glycolytic intermediates; acetate and probably propan-1-ol represent a different carbon metabolism requiring the glyoxylate cycle for its operation. The only metabolite which is common to both glycolysis and early acetate metabolism is acetyl-CoA, for in both glycolysis and acetate metabolism acetyl-CoA must be formed to gain entry into the TCA or glyoxylate pathway, respectively. One might suppose, therefore, that transfer to a medium containing very high concentrations of pyruvate or acetate but no nitrogen source leads to accumulation of acetyl-CoA because the lack of a nitrogen source severs the glutamate decarboxylation loop between 2-oxoglutarate and succinate.

The ideal test of this proposition is to see how mutants unable to synthesize acetyl-CoA behave in the transfer medium. Unfortunately there are no mutants of *Coprinus* which are defective in the pyruvate dehydrogenase complex so the pyruvate response cannot be tested. However, acetate metabolism has been carefully studied and an acetyl-CoA synthetase (EC 6.2.1.1)-deficient mutant identified (Casselton and Casselton, 1974). Tests with this mutant (Table 8) showed that there was no induction on transfer to either acetate or propan-1-ol media, but the usual response was ob-

TABLE 8
Activities of NADP-Linked Glutamate Dehydrogenase before and after Transfer in Mycelia of a Wild-Type Strain and of a Mutant Which Is Deficient in Acetyl-CoA Synthetase Activity

Treatment	NADP-GDH specific activity[a]	
	Wild-type mycelium	Mutant mycelium
Untransferred control	29	46
Transferred to		
100 m*M* Pyruvate	707	557
100 m*M* Glucose	647	640
100 m*M* Acetate	290	14
100 m*M* Propan-1-ol	491	23

[a] Enzyme activity is shown as nmol substrate used/min/mg protein. Data from Moore (1981a).

TABLE 9
Activities of NADP-Linked Glutamate Dehydrogenase in Mycelia of Different Wild-Type Monokaryons of *Coprinus cinereus* following Transfer to Pyruvate Induction Medium

Culture	Geographical origin	NADP-GDH specific activity[a]	
		Before transfer	After transfer
BC9/6,6	UK (laboratory)	58	222
H9	UK	32	287
H1	UK	50	33
H6	UK	32	27
TC-4	UK (laboratory)	25	14
Brum-b	UK	92	14
ATCC 18450	UK	11	21
ATCC 42730	Czechoslovakia	36	4
ATCC 42729	Singapore	19	98
Singapore-12	Singapore	34	40
Java-a	Indonesia	18	14
Java-b	Indonesia	14	26
ATCC 42728	Poland	88	15
ATCC 42727	USA	62	58
Penn-a	USA	62	16
ATCC 24926	Canada	35	27
ATCC 20120	Japan	8	2

[a] Enzyme activity is shown as nmol substrate used/min/mg protein. Data from Moore (1981a).

served on transfer either to glucose or pyruvate (i.e., the mutant did respond when the mutational lesion was bypassed through the pyruvate dehydrogenase pathway).

The conclusion, then, is that synthesis of acetyl-CoA is necessary for the induction of NADP-GDH. Bearing in mind the inhibitory effects of the presence of ammonium, the regulatory situation for NADP-GDH has been stated as being such that a high concentration of acetyl-CoA is able to induce the enzyme providing the ammonium concentration is low (Moore, 1981a).

The mycelium used in these analyses, strain number BC9/6,6, has been used for a wide variety of studies over the years. It is a laboratory-produced prototroph derived from a wild isolate (called H9 = ATCC 18064) collected by Peter Day (Day and Anderson, 1961). It has been used in the belief that it is representative of the wild population of this species as a whole, but comparative tests using the medium-transfer technique reveal that it is not (Table 9). Of the 17 strains listed in this table only BC9/6,6 and its parent H9 respond to the pyruvate transfer by producing enhanced NADP-GDH activity. Among the cultures which failed to respond were H1 and H6, which were derived from the same fruit body as H9. This gives rise to the suspicion that the "pyruvate response" may segregate and when progeny from a cross between BC9/6,6 and one of the nonresponders (Singapore-7 = ATCC 42729) were tested they showed the segregation pattern expected of a single major gene (Table 10). Only four progeny are shown in this table, but they all belong to the same tetrad (i.e., represent the four products of a single meiosis) and similar data have been repeatedly obtained with other tetrads from this cross. The nature of the feature which is segregating is not yet known but comparisons are being made of the physiology and biochemistry of BC9/6,6 other wild types, and progeny of the cross in the hope that it can be identified.

CORRELATION OF MYCELIAL EXPERIMENTS WITH ENDOGENOUS EVENTS IN THE FRUIT BODY

This genetic distinction between closely

TABLE 10
Activities of NADP-Linked Glutamate Dehydrogenase in Mycelia of a Tetrad of Progeny from the Cross BC9/6,6 × ATCC 42729 before and after Transfer to Pyruvate Medium

Progeny code number	NADP-GDH specific activity[a]	
	Before transfer	After transfer
T1a	21	45
T1b	54	479
T1c	10	73
T1d	39	488

[a] Enzyme activity is shown as nmol substrate used/min/mg protein. Data from Moore (1981a).

TABLE 11
Some Enzyme and Metabolite Levels in Developing Fruit Bodies of *Coprinus cinereus*

Measured characteristic[a]	Developmental stage		
	3	4	5
NADP-GDH activity			
Cap	47	420	780
Stipe	29	11	20
NAD-linked isocitrate dehydrogenase activity			
Cap	50	150	200
Stipe	90	200	220
Succinate dehydrogenase activity			
Cap	12	27	62
Stipe	28	16	10
Glycogen content			
μg per fruit body	1857	1361	97
% in the cap	97	96	86
Ammonium content (μmol/g fresh wt)			
Cap	32	11	9
Stipe	90	40	22

[a] Enzyme activities are shown as nmol substrate used/min/mg protein. Note that mycelia and fruit body primordia contain approx 125 μmol of ammonium/g fresh wt. The stage 3 primordium is beginning to make spores, the stage 4 fruit body has a full complement of (mostly unpigmented) spores, and the stage 5 fruit body is releasing mature spores.

related cultures provides yet another source of material for experimental investigation of enzyme regulation. However, if strain BC9/6,6 really is so unique, the question which arises is whether the results obtained using this strain have any relevance to the endogenous processes taking place in the normally developing fruit body. The answer to this question seems quite definitely to be yes. Recall that the basic features of NADP-GDH control in BC9/6,6 are that induction occurs when acetyl-CoA accumulates in the virtual absence of ammonium. Assays of normal fruit bodies show that glycogen is accumulated to high levels in the fruit body cap, but is metabolized as the cap matures (Moore *et al.*, 1979); during this process both isocitrate dehydrogenase and succinate dehydrogenase are elevated in activity in the cap (relative to the stipe), implying enhanced metabolism through the TCA cycle (Moore and Ewaze, 1976). Furthermore, the cap always contains less free ammonium than does the stipe and the concentration of this metabolite drastically declines as the primordium develops into the mature fruit body (Ewaze *et al.*, 1978). These results are summarized in Table 11. Metabolism of what amounts to almost 2 mg of glycogen per fruit body with so little available ammonium could obviously lead to accumulation of acetyl-CoA to levels sufficient to induce NADP-GDH and associated enzymes.

A fundamental question is why NADP-GDH needs to be induced at all in the developing cap. NAD-GDH appears always to be present in the tissues which acquire the NADP-linked enzyme, so NADP-GDH is introduced as an additional component of nitrogen metabolism. It seems likely that NADP-GDH contributes to an ammonium scavenging system. Some of the reactions discussed above, particularly the glutamate decarboxylation loop, can clearly cycle ammonium in the sense that successive reactions in the same pathway involve both amination and deamination. However, the evidence points to a considerable am-

plification of TCA cycle activity and, moreover, nitrogen is being removed from availability by being accumulated as urea and other nitrogenous compounds. Thus there will be a need for enhanced ammonium assimilation, and as the K_m for ammonium of NADP-GDH (2 m*M*) is some 10 times lower than that of NAD-GDH (18.8 m*M*), the former enzyme is a better candidate for the task of ammonium scavenging than the latter.

Since induction of NADP-GDH is very closely correlated with induction of GS (Ewaze *et al.*, 1978) it is possible that in *Coprinus* NADP-GDH and GS together form an ammonium scavenging system. Now, the conventional interpretation of ammonium assimilation in both microorganisms and higher plants is that there are two systems: at relatively high ammonium levels NADP-GDH is thought to be responsible for assimilation, but at low levels of available ammonium this enzyme tends to be replaced by a new process involving the combined operation of glutamate synthase and GS (Brown *et al.*, 1974). The results obtained with *Coprinus* imply an intermediate arrangement in which NADP-GDH and GS operate together. There is no direct evidence for this yet, although the suggestion carries with it implications about substrate specificities and affinities which could be tested by analysis of the purified enzymes. Other work suggests that *Coprinus* may not be the only basidiomycete to have this novel ammonium scavenging process; cultures of *Sporotrichum pulverulentum* grown under nitrogen-limiting conditions also showed simultaneously increased levels of both NADP-linked glutamate dehydrogenase and glutamine synthetase (Buswell *et al.*, 1982).

GENETIC ASPECTS OF FRUIT BODY MORPHOGENESIS

It would be advantageous to relate these metabolic studies to investigations of the genetic control of fruit body morphogenesis, but although developmental variants are known in *C. cinereus* their analysis has not yet reached the stage where valid connections can be made between the different approaches. Evidence has been presented which shows that segregating genetic factors identified in monokaryons for their ability to prevent formation of vegetative resting structures (sclerotia) also prevent formation of the fruit body in homozygous dikaryons (Moore, 1981b). Sclerotia are small globose structures with a distinctive radially symmetrical internal differentiation (Waters *et al.*, 1975a; Hereward and Moore, 1979). The early stages of the developmental pathway leading to formation of the fruit body primordium (Matthews and Niederpruem, 1972) are strikingly similar to events described for the initiation of sclerotia (Waters *et al.*, 1975b) and the genetic evidence can best be interpreted as implying that the two structures share a common pathway of initiation which subsequently branches into the two separate developmental directions (Moore, 1981b). Commitment to either branch of the pathway when it bifurcates depends on temperature, illumination and nutrition, and maturation of sclerotium initials is particularly sensitive to the C:N ratio (Moore and Jirjis, 1976).

The broadest study of developmental mutants in this organism has been done with the Japanese strains of *C. cinereus* (under the name *C. macrorhizus*). Takemaru and Kamada (1972) isolated over 1500 developmental variants following mutagen treatment of a dikaryon. Although some further analysis of a few of these variants has been accomplished (see below), they have not been systematically studied. An observation relevant to the present discussion, though, is that among the variants obtained there were classes in which stipe elongation was defective but cap expansion normal, those in which cap expansion was defective but stipe elongation was normal,

and those in which fruit body structure was normal but no spores were formed. These observations indicate that development of the cap is genetically distinct from development of the stipe and of the spores, and this in turn may suggest that, at least in part, development of these different tissues employs different metabolic sequences. This conclusion is certainly in accord with the biochemical data from various sources reviewed by Moore *et al.* (1979). While, as we have seen above, there is every reason to believe that accumulation of amino compounds and urea has osmoregulatory significance in the cap of *C. cinereus,* low and steadily declining levels of amino nitrogen reported for developing stipes (Ewaze *et al.,* 1978) argue for a different mechanism in this tissue. In fact, simple carbohydrates appear to be the best candidates as the "adjustable" stipe osmoticum. Rao and Niederpruem (1969) and Gooday (1982) showed that trehalose could amount to almost 18% of the final stipe dry weight. Combining these observations with data for reducing sugars (Ewaze *et al.,* 1978), it appears that trehalose and alcohol-soluble reducing sugars amount to about 30% of the dry weight of the mature stipe. Polyols never exceed 6% of the stipe dry weight and decline in quantity as the fruit body develops (Darbyshire, 1974) so there is a sharp contrast here with *Agaricus* in which mannitol can represent as much as 50% of the fruit body dry weight (Hammond and Nichols, 1976).

Kamada and Takemaru (1977) have investigated both the mechanical and biochemical properties of stipe cell walls in the wild type of *C. cinereus* and in an elongationless mutant. Protein and polynucleotide levels have been studied in the same mutant and in a sporeless mutant (Kamada *et al.,* 1976). Other sporeless mutants have been used in surveys of NADP-GDH, chitinase, and glucanase activities during fruit body development (Miyake *et al.,* 1980). Unfortunately, none of these careful studies has identified a metabolic lesion which can be ascribed to the mutations.

The same disappointing statement can be repeated in reverse with respect to the detailed metabolic studies described earlier. Although the work clearly associates NADP-GDH and related enzymes of urea metabolism with maturation processes in the fruit body cap, no mutants with defects in this area of metabolism have been identified so far. However, techniques under development will allow application of recombinant DNA methods to the study. Wu *et al.* (1983) have already developed a genomic library of the organism and used it to study DNA polymorphisms, and analysis of genomic sequences which are differentially expressed during fruit body development is also underway (Pukkila *et al.,* 1984). The system promises to be an ideal candidate for study of the molecular relationships between metabolism and morphogenesis and could contribute greatly to our understanding of the relationships between adjacent cells in the tissues of the cap and the way those relationships are modified during cap development.

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