# The stage in sporulation between the end of meiosis and emergence of sterigmata is most sensitive to ammonium inhibition in *Coprinus cinereus*

David Moore*, Farida Hammad and Junxia Ji

Microbiology Research Group, School of Biological Sciences, 1.800 Stopford Building, The University, Manchester M13 9PT, Great Britain (*Reprint address)

*Key words*: differentiation, regulation, spore formation, metabolism, nuclear division

## Abstract

From experiments designed specifically to test the degree of similarity between the behaviour of *Coprinus* and published information about yeast and *Dictyostelium*, it emerges that similarities in the metabolism surrounding sporulation events in these organisms are coincidental. The very early stages in the outgrowth of sterigmata from the basidium of *Coprinus* are most sensitive to inhibition by ammonium. It seems likely that ammonium ions interfere with the cytoskeletal architecture which defines the number, position and nature of the outgrowths which become sterigmata.

## Introduction

Basidia of *Coprinus cinereus* (Schaeff., Fr) S. F. Gray *sensu* Konr. continue through meiosis and sporulation when explanted to defined media *in vitro* (Chiu and Moore, 1988a). However, inclusion of ammonium salts in the medium inhibits differentiation of basidia, causing vegetative hyphal tips to grow out from regions of the basidial apparatus which are in active growth during sporulation (Chiu and Moore, 1988b).

A number of processes apparently similar to those occurring in *C. cinereus* have been reported in other organisms. Sporulation of *Saccharomyces cerevisiae* is inhibited by ammonium (Miller, 1963; Piñon, 1977). Indeed, ammonium treatment delays protein degradation at the onset of meiosis, inhibits protein and DNA synthesis (Croes *et al.*, 1978), and degradation of glycogen (Fonzi *et al.*, 1979). Glycogen usage in *S. cerevisiae* appears to be sporulation-specific. Glycogen accumulation occurs in $\alpha/\alpha$ and a/a diploids on transfer to sporulation medium but the decline in glycogen level occurred only in a/$\alpha$ cells (Hopper *et al.*, 1974; Kane and Roth, 1974). The pileus of the *C. cinereus* fruit body also accumulates glycogen which was reported to be degraded post-meiotically by Moore *et al.*, (1987). Ammonium inhibits reinitiation of meiosis in starfish oocytes (Doree *et al.*, 1982), and gametogenesis in *Chlamydomonas reinhardtii* (Kates and Jones, 1964; Martin and Goodenough, 1975). Ammonium also determines choice of developmental pathways in *Dictyostelium discoideum* (Schindler and Sussman, 1977).

The frequent observation of ammonium-inhibitions may suggest action against evolutionarily conserved components of the cell (Chiu and Moore, 1988b). In the observations reported here we have carried out a series of experiments designed specifically to test the degree of similarity between the

behaviour of *Coprinus* and published information about yeast and *Dictyostelium*. We find that as more detail is assembled the degree of similarity declines.

## Materials and methods

The 'Meathop' dikaryotic strain of *Coprinus cinereus* was grown and fruit bodies produced as described previously (Moore and Ewaze, 1976). Gill lamellae were excised from a fruit body after the cells of the veil had been removed from the cap surface. A segment consisting of two or three gills was explanted to agar media in conventional 9 cm Petri dishes, or to Nunclon Delta SI 24-well 'multidishes' in which the wells contained 0.35 ml of either liquid or agar-solidified medium (Chiu and Moore, 1988a).

The developmental stage of the tissue at explantation was determined by microscopic examination of silver-stained (Pukkila and Lu, 1985) squash preparations. The exact time-scale of sporulation in *C. cinereus* has already been characterized in this way (Hammad *et al.*, 1993). To determine the stage of maximum sensitivity, batches of gills were excised at various stages during meiosis and sporulation for explantation to buffer agar containing 80 mM $NH_4Cl$. After different lengths of time of exposure to the ammonium-medium, the segments were removed to buffer-agar and their further development recorded.

Glycogen and chitin contents were determined in adjacent samples from cap tissues of varying developmental stages, from the dikaryotic stage, through to karyogamy, meiosis and sporulation. Glycogen was assayed using the iodine reagent (Jirjis and Moore, 1976). This technique involves ethanol-precipitation of extracted polysaccharides prior to assay and consequently measures the content of high molecular weight polysaccharide. Chitin was indirectly assayed colorimetrically (Ride and Drysdale, 1972). The method is based on the alkaline deacetylation of chitin to chitosan. The glucosamine residues of the chitosan are then deaminated using nitrous acid and this leads to the production of an aldehyde which is determined colorimetrically.

## Results and discussion

Chiu and Moore (1988b) described a small scale bioassay for the effects of chemicals on sporulation in explanted gills of *Coprinus*. We modified this technique to establish the minimum length of time for exposure to 80 mM $NH_4Cl$ to be inhibitory to sporulation, and the most sensitive time period during the course of meiosis. The time of exposure required varied directly with the stage in meiosis in the tissue at the time of excision. Tissue excised as karyogamy was occurring required 7 to 8 h exposure to $NH_4Cl$ for sporulation to be halted, but tissue excised during meiotic division I required only 2 to 3 h exposure. Observations made with sixteen different fruit bodies

established that the stage just after completion of the second meiotic division but before the appearance of sterigmata (spanning 60 to 90 min) was most sensitive to inhibition by ammonium.

This result contrasts with the situation in yeast in which Piñon (1977) claimed the most sensitive period to be at the end of premeiotic DNA synthesis and meiotic prophase. There may be some parallel, however, for Dickinson and Dawes (1983) identified two ammonium-sensitive stages, one early in meiosis, and the other later, concerned with organization and delimitation of spores. The ammonium-sensitive phase in *Coprinus* may be analogous to the second of these.

Ammonium depletion triggers a *Dictyostelium* slug to transform into a fruit body (Schindler and Sussman, 1977) and ammonium acts as an inhibitor of stalk-cell differentiation (Gross *et al.*, 1983). The inhibitory effect of ammonium is antagonized by the morphogen DIF-1, and 1 to 2 $\mu$M diethylstilboestrol and zearalenone, which inhibit plasma membrane ATPase proton pumps and mimic the action of DIF-1 (Gross *et al.*, 1983). We have used the excised gill bioassay to test the ability of zearalenone and diethylstilboestrol

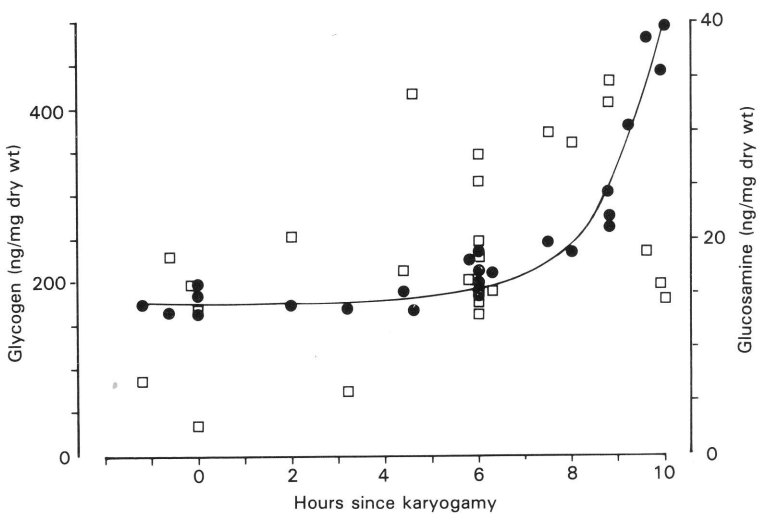


**Figure 1** Glucosamine (●) representing chitin, and glycogen (□) contents of basidiome gill tissue throughout development. Each data point represents the mean of assays performed on five to seven samples of gill tissue removed from a single fruit body. Parallel assays were performed for both polymers on each fruit body. The stage of development was determined by making regular cytological observations of the progress of meiosis. Zero h was arbitrarily chosen to be when nuclear fusion (karyogamy) starts. Other timings emerged from the observations: meiosis I occurred at 5 h, meiosis II at 6 h, sterigmata appeared at 7.5 h, basidiospores started to appear at 9 h and were well developed (but still unpigmented) at 10 h.

to influence the effect of ammonium ions on sporulation in *Coprinus* by transplanting gill segments to medium containing ammonium plus diethylstilboestrol (1 to 800 $\mu$M) or zearalenone (100 to 600 $\mu$M). No antagonism between the additives was observed. We conclude that although in both *Coprinus* and *Dictyostelium* ammonium determines a choice between differentiation states, the mechanism of action is different in the two organisms.

If glycogen accumulation and degradation were an integral part of meiosis/sporulation, close synchrony between the two processes might be expected. Ji and Moore (1993) have recently compared how the content of glycogen varies with time in normally-developing fruit bodies and concluded that glycogen metabolism and 'meiosis' (meaning the whole programme of meiosis + sporulation) may not be coupled together in *Coprinus* as closely as they are in yeast.

We have tested this proposition by comparing chitin and glycogen contents of parallel samples from the same tissues (Figure 1). As is to be expected from the wall synthesis associated with spore formation, increase in chitin content was closely correlated with progress through sporulation, but the glycogen content of the same tissues showed no such correlation. Glycogen degradation is not coupled to sporulation in *Coprinus* and similarities in the metabolism surrounding sporulation events in *Coprinus*, yeast and *Dictyostelium* are coincidental.

The very early stages in the outgrowth of sterigmata from the basidium of *Coprinus* are sensitive to inhibition by ammonium. Since the sterigmata are located on the basidium with great precision, it seems likely that ammonium ions interfere with the cytoskeletal architecture which defines the number, position and nature of the outgrowths which become sterigmata.

## Acknowledgements

This research was made possible by the award of a SERC Research Studentship to F.H. and a Manchester University Research Studentship in Science to J.J., supplemented by grants from the Henry Lester Trust and the Great Britain-China Educational Trust.

## References

CHIU S. W. and Moore D. 1988a. Evidence for developmental commitment in the differentiating fruit body of *Coprinus cinereus*. *Trans. Br. Mycol. Soc.* **90** 247–53.

CHIU S. W. and Moore D. 1988b. Ammonium ions and glutamine inhibit sporulation of *Coprinus cinereus* basidia assayed *in vitro*. *Cell Biol. Intern. Repts* **12** 519–26.

CROES A. F., Steijns J. M., De Vries G. J. M. L. and van der Putte T. M. J. A. 1978. Inhibition of meiosis in *Saccharomyces cerevisiae* by ammonium ions: interference of ammonia with protein metabolism. *Planta* **141** 205–9.

DICKINSON J. R. and Dawes I. W. 1983. Ammonium ion repression of sporulation in *Saccharomyces cerevisiae*. *J. gen. Microbiol.* **129** 1883–8.

DOREE M., Sano K. and Kanatani H. 1982. Ammonia and other weak bases applied at any time of the hormone-dependent period inhibit 1-methyladenine-induced meiosis reinitiation of starfish oocytes. *Dev. Biol.* **90** 13–7.

FONZI W. A., Shanley M. and Opheim O. J. 1979. Relationship of glycolytic intermediates, glycolytic enzymes and ammonia to glycogen metabolism during sporulation in the yeast *S. cerevisiae. J. Bact.* **137** 285–94.

GROSS J. D., Bradbury J., Kay R. R. and Peacey M. J. 1983. Intracellular pH and the control of cell differentiation in *Dictyostelium discoideum. Nature* **303** 244–5.

HAMMAD F., Ji J., Watling R. and Moore D. 1993. Cell population dynamics in *Coprinus cinereus*: co-ordination of cell inflation throughout the maturing basidiome. *Mycol. Res.* **97** 269–74.

HOPPER A. K., Magee P. T., Welch S. K., Friedman M. and Hall B. D. 1974. Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. *J. Bact.* **119** 619–28.

JI J. and Moore D. 1993. Glycogen metabolism in relation to fruit body maturation in *Coprinus cinereus. Mycol. Res.* **97** 283–9.

JIRJIS R. I. and Moore D. 1976. Involvement of glycogen in morphogenesis of *Coprinus cinereus. J. gen. Microbiol.* **95** 348–52.

KANE S. M. and Roth R. 1974. Carbohydrate metabolism during ascospore development in yeast. *J. Bact.* **118** 8–14.

KATES J. R. and Jones R. F. 1964. The control of gametic differentiation in liquid cultures of *Chlamydomonas. J. Cell. Comp. Physiol.* **63** 157–64.

MARTIN N. C. and Goodenough U. W. 1975. Gametic differentiation in *Chlamydomonas reinhardtii*. I. Production of gametes and their fine structure. *J. cell Biol.* **67** 587–605.

MILLER J. J. 1963. The metabolism of yeast sporulation. V. Stimulation and inhibition of sporulation and growth by nitrogen compounds. *Can. J. Microbiol.* **9** 259–77.

MOORE D. and Ewaze J. O. 1976. Activities of some enzymes involved in metabolism of carbohydrate during sporophore development in *Coprinus cinereus. J. gen. Microbiol.* **97** 313–22.

MOORE D., Liu M. and Kuhad R. C. 1987. Karyogamy-dependent enzyme derepression in the basidiomycete *Coprinus. Cell Biol. Intern. Repts* **11** 335–41.

PIÑON R. 1977. Effects of ammonium ions on sporulation of *Saccharomyces cerevisiae. Expl Cell Res.* **105** 367–78.

PUKKILA P. J. and Lu B. C. 1985. Silver staining of meiotic chromosomes in the fungus, *Coprinus cinereus. Chromosoma* **91** 108–12.

RIDE J. P. and Drysdale R. B. 1972. A rapid method for the chemical estimation of filamentous fungi in plant tissue. *Physiol. Plant Pathol.* **2** 7–15.

SCHINDLER J. and Sussman M. 1977. Ammonia determines the choice of morphogenetic pathways in *Dictyostelium discoideum. J. molec. Biol.* **116** 161–9.

Accepted 11 October 1993