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Karyogamy-dependent enzyme derepression in the basidiomycete Coprinus

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ABSTRACT

Observation of stained nuclei coupled with determination of activity of NADP-linked glutamate dehydrogenase (NADP-GDH) in the same tissue, showed that increase in enzyme activity was initiated as karyogamy became evident in normally-developing fruit bodies of the basidiomycete Coprinus cinereus. Enzyme activity stabilised for about 4 hours during meiosis, but resumed after meiosis II, and continued to increase until spore maturation. When the time of exposure to light was varied, karyogamy occurred only in tissues which received at least 24 h light exposure and, most significantly, derepression of NADP-GDH was apparent at the time of or very soon after karyogamy. It is concluded that expression of NADP-GDH in the fruit body cap of Coprinus cinereus is either a component part of the cellular programme involved in karyogamy, or is directly triggered by that programme. Parallel assays showed that protein content of fruit body cap tissue declined during development; the decline started before meiosis and also arrested during the division. A major flux in cyclic-AMP content occurred at a much earlier stage, a large accumulation in fruit body initials being rapidly reduced as these developed into primordia. Levels of cAMP similar to those recorded in vegetative cells were approached prior to meiosis, suggesting that this nucleotide has little involvement in development of the fruit body after formation of initials. Onset of large-scale utilization of accumulated glycogen proved to be a post-meiotic event.

INTRODUCTION

Morphogenesis of the basidiomycete mushroom fruit body is an excellent model for a variety of eukaryotic differentiation processes. The fruit body of Coprinus cinereus is of particular value since development is rapid and meiosis is quite well synchronised across a large population of cells (basidia) which represent a high proportion of the tissue mass of the fruit body cap. Since fruit body tissue is dikaryotic - its cells containing two haploid nuclei derived from the original parents - the meiotic process in the fruit body includes a nuclear fusion or 'fertilisation' event. Thus, all of the meiotic processes are open to study.

It has been shown that a number of enzymes are specifically derepressed as tissues of the Coprinus mushroom cap begin to form (Moore, 1984). Among these is NADP-linked glutamate dehydrogenase (NADP-GDH; EC 1.4.1.4.) the regulatory circuit of which involves derepression by accumulated acetyl-CoA (Moore, 1981) which could be derived from the large quantities of glycogen (up to 2 mg per average fruit body) which are consumed during spore formation (Moore et al., 1979). This enzyme activity has been localised in basidia (Elhiti et al., 1979), the only cells which undergo karyogamy and meiosis.

In this paper we report the results of a study in which glutamate dehydrogenase activity, together with soluble protein, glycogen and cyclic-AMP levels have been determined in tissue samples which have also been analysed cytologically in order to relate the enzyme control processes to progress of the meiotic division.

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MATERIALS AND METHODS

A dikaryon of Coprinus cinereus (Schaeff.: Fr.) S.F. Gray sensu Konr. was used throughout; it is called 'Meathop' and was originally isolated, by R.A. Johnson, from a compost heap at Lower Meathop Hill in Cumbria, UK.

Fruit bodies were produced on cultures growing on horse dung using the techniques described by Moore & Ewaze (1976). For routine purposes, dung cultures were inoculated with pieces of the dikaryon taken from petri-dish cultures, incubated in the dark for 3 days at 37°C and then transferred to an illuminated incubator at 26°C, in which illumination was provided by 'warm white' fluorescent lights giving an average illuminance of 800 lux with a day length cycle of 16 h light/8 h dark.

Meiotic division stages were examined using the silver staining technique described by Pukkila & Lu (1985). Spectrophotometric assays for glutamate dehydrogenases were based on those described by Al-Gharawi & Moore (1974). Soluble protein was measured using the Lowry method. Extraction and assay of glycogen were done using the methods described by Jirjis & Moore (1976); authentic rabbit liver glycogen being used to prepare a standard curve. Assays of cAMP were done in triplicate, using an extraction method adapted from Brown et al. (1971). Tissue was pulverised under liquid nitrogen, then deproteinised with ice-cold 0·5 M perchloric acid in 25% (v/v) ethanol. After standing for one hour in an ice-bath the slurry was filtered. The filtrate was brought to pH 7·5 with 1 M KOH; the precipitate removed by centrifugation and 2 ml portions of the supernatant were lyophilised in multi-dose vials and stored under vacuum at -20°C. Shortly before assay, 2 ml of 0·05 M Tris/EDTA buffer, pH 7·5, was added to the sample vial. The cAMP content of 50 ul quantities of the resulting solution was measured with an Amersham cAMP assay kit; scintillation counting being done with a Packard 300CD spectrometer.

RESULTS AND DISCUSSION

Observation of stained nuclei and determination of NADP-GDH activity in the same tissue shows that increase in enzyme activity was initiated as karyogamy became evident (Figs. 1 and 2). Enzyme activity stabilised for about 4 hours during the progress of the meiotic division (Fig. 2), presumably reflecting cessation of transcription in favour of replication. Increase in NADP-GDH activity resumed after meiosis was completed, marked by the emergence of sterigmata from the basidia, and this increase continued until maturation of the spores. Protein content of fruit body cap tissue declined during development; this decline was also arrested temporarily during the progress of the meiotic division (Fig. 2, lower panel).

The major flux in cyclic-AMP content occurred at a much earlier stage (Table 1), the large accumulation in fruit body initials rapidly declining

Table 1. Level of cyclic-AMP in fruit body cap tissue of Coprinus cinereus at different stages of development

Material and nuclear stage	cAMP content (pmole/mg dry wt)
initials, prekaryogamy	50·9±22·5
primordium, prekaryogamy	13·5±6·9
primordium, karyogamy	7·3±4·0
fruit body, meiotic division stages	0·4±0·4
fruit body, post meiotic, immature spores	0·4±0·3
fruit body, mature spores being released	0·2±0·2

as these developed into primordia. A level approximating to the amounts recorded in vegetative dikaryotic mycelium (2 to 8 pmol cAMP per mg dry wt; Uno & Ishikawa, 1974) was approached prior to meiosis suggesting that

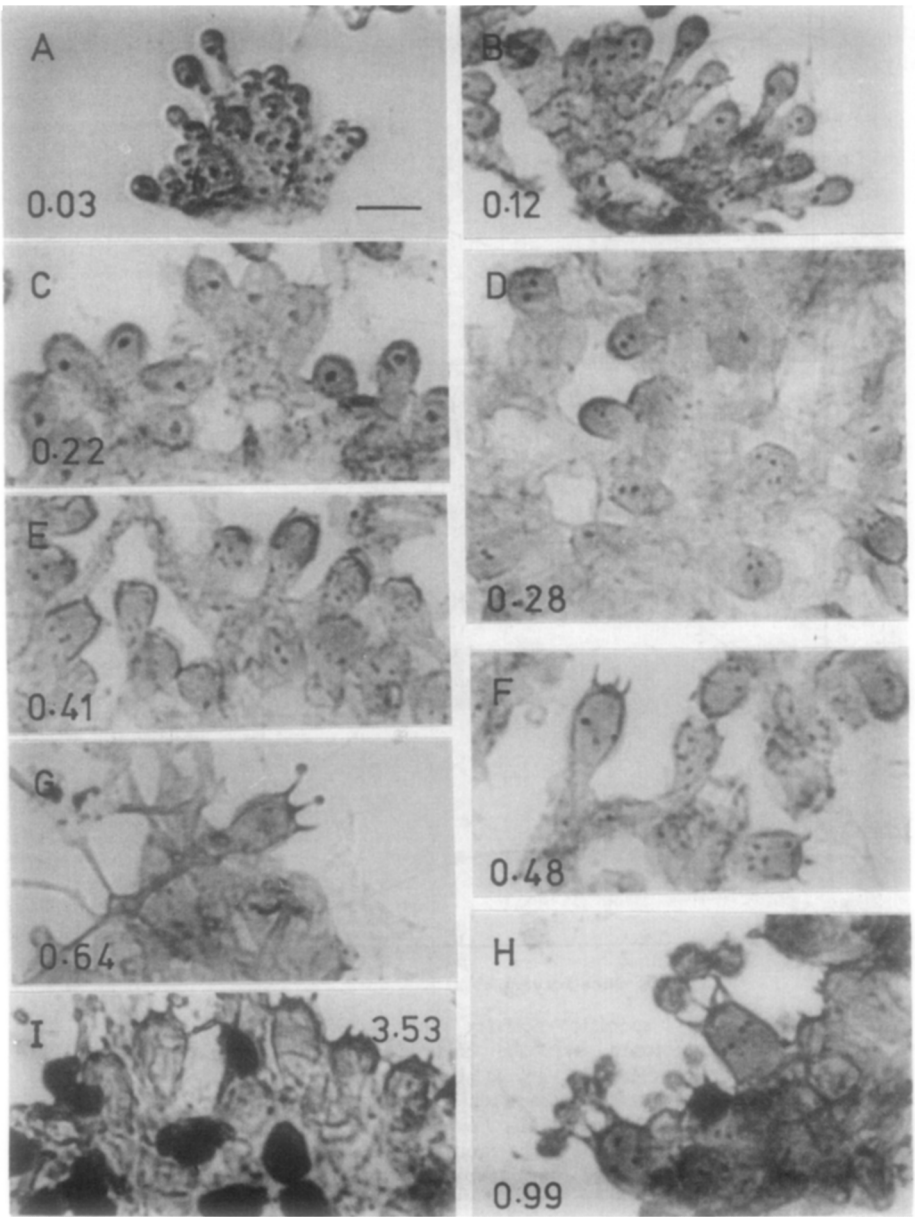

Fig. 1. Squashes of fruit body cap samples also assayed for NADP-GDH activity. The technique used stains, particularly, the persistent nucleoli. A is prekaryogamy, the young basidia being dikaryotic; B shows some karyogamy, which is complete in C; D, basidia with two (end of MI) and four (end of MII) meiotic products; E, sterigmata begin to emerge; F, sterigmata extend, and spores begin to form in G; H, spores well developed but nuclei still within basidium; I, mature, dispersed spores. The numbers on each picture show the NADP-GDH specific activity (umol/min/mg protein) of that tissue. All to the same scale; scale bar = 10 μm.

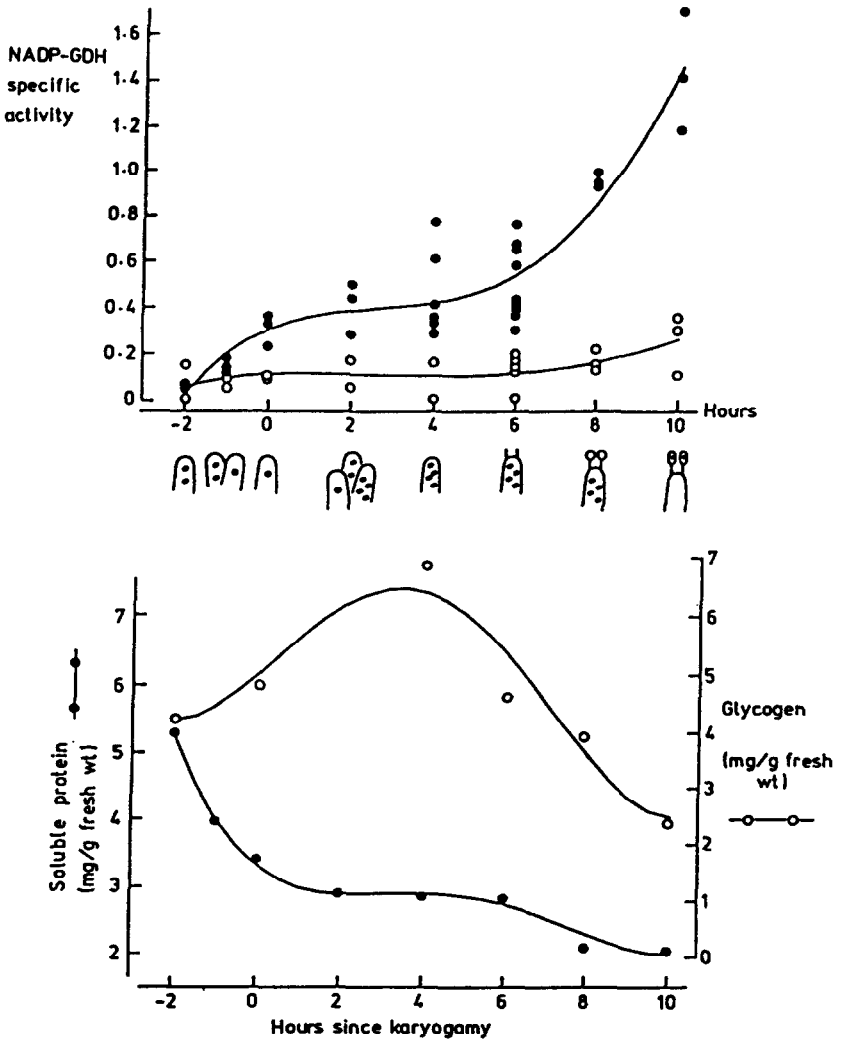

Fig. 2. **Upper panel:** specific activity of NADP-linked glutamate dehydrogenase in caps (closed symbols) and stipes (open symbols) of Coprinus cinereus fruit bodies of various stages of development. Enzyme activity was determined spectrophotometrically in tissue samples of which portions were also examined cytologically to determine the nuclear status of their basidia. These are shown as cartoons beneath the plot depicting: the dikaryotic prekaryogamy stage, karyogamy, meiotic stages with two and four daughter nuclei, post-meiotic stages with and without sterigmata, possession of young spores prior to nuclear migration, and possession of nucleated spores. The exact timing of these events was determined in separate fruit body samples from which small gill segments were taken at successive intervals for cytological examination from individual fruit bodies as they developed. Karyogamy is arbitrarily chosen as time zero. Lines of best fit were computed using the least squares method.

Lower panel: content of soluble protein (closed symbols) and glycogen (open symbols) in fruit bodies of Coprinus cinereus at various stages of development. The time scale refers to the progress of nuclear fusion and meiosis in the basidia of the samples dealt with and is the same as that used for the upper panel.

cyclic-AMP has little involvement in development of the fruit body after formation of the initials.

Onset of large-scale utilization of accumulated glycogen proved to be a post-meiotic event (Fig. 2, lower panel). In these experiments an average of 1·7 mg of glycogen per fruit body was used in the 8 hours between completion of meiosis and release of mature spores. It is significant that onset of this large-scale glycogen utilisation is correlated with the second major increase in NADP-GDH activity as it supports the view that transient accumulation of acetyl-CoA may be part of the NADP-GDH control circuit in fruit bodies as it is in mycelium (Moore, 1981).

The nature of the control process which causes the pre-meiotic derepression of NADP-GDH is still obscure. A light signal may be involved. In common with other strains, when grown in the dark the Meathop dikaryon of Coprinus cinereus will produce elongated stipe-like structures formed by elongation of the stipe base beneath very rudimentary fruit body primordia (Buller, 1931); they are illustrated by Lu (Fig. 1 in Lu, 1974) who called them primordial buds. Primordial buds do not mature unless exposed to further illumination, yet the basic tissue patterning (stipe, cap - including gill hymenia) is established within them. To determine whether regulation of NADP-GDH was related to the onset of meiosis or these other aspects of cap morphogenesis, we varied exposure to light. In the normal case, the illumination cycle was 16 h light [L] and 8 h dark [D] at 26°C. Cultures were grown initially in the dark at 37°C to produce crops of primordial buds and were then randomly assigned to groups for different light treatments. All cultures were subjected to the temperature shift-down from 37°C to 26°C and all were harvested for NADP-GDH assay after 48 h in the first experiment, and after 68 h in the second. Light exposure was varied by wrapping the cultures in aluminium foil. Thus, zero-light exposure samples were wrapped throughout the experiment; 32 h exposure were unwrapped for two cycles; 24 h exposure received one complete cycle but were wrapped halfway through the light period of the other [and there were two versions of this test, one in which the first cycle was truncated and one in which the light period of the second cycle was halved]; etc. Results of enzyme assays and cytological observations are summarised in Fig. 3.

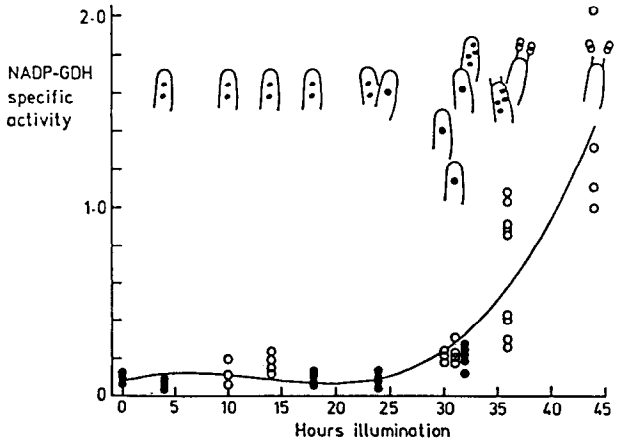

Fig. 3. Relationship between length of light exposure, the progress of meiosis (shown as cartoons) and NADP-GDH specific activity in the Coprinus fruit body cap. Karyogamy became evident in samples exposed to 24 h light, and meiosis was completed in those exposed for 32 h. There were two treatment patterns in which illumination lasted for 36 h and they gave significantly different results (see text), but all data were combined in computing the line shown here.

Some cultures from all of these tests were allowed to continue development, without harvest for enzyme assay, to verify that development was normal. With the exception of those which received no light exposure, all cultures did produce mature fruit bodies. Interestingly, although the total length of time at 26°C required for fruit body maturation varied between 68 and 135 h (mean = 93·7 ±23·5 h), the length of the required light exposure within that time was much more constant, varying between 41 and 53 h (45·3 ±4·3 h), showing that the rate of maturation depends on the total light exposure and is not synchronised by the temperature shift-down.

Onset of meiosis in the primordial buds was also quite rigidly related to the total illumination received irrespective of the length of time over which the light period(s) were given. Karyogamy occurred only in tissues which received at least 24 h light exposure and, most significantly, derepression of NADP-GDH was apparent only in those samples showing evidence of karyogamy and at the time of, or very soon after, nuclear fusion (Fig. 3). Most significant is that derepression of NADP-GDH was delayed for the first 24 to 30 h of the experiments summarised in Fig. 3. This cannot be due merely to limitations imposed by the intrinsic rate of expression of the gene(s) involved, since derepression of NADP-GDH in mycelium was evident within one to two h of exposure to the appropriate medium (Moore, 1981). Repression was, therefore, positively maintained until about the time that karyogamy occurred.

Some influence of elapsed time was evident in experiments featuring longer light exposure times. There were two treatment patterns giving 36 h light exposure: (a) 16 h [D] - 16 h [L] - 8 h [D] - 16 h [L] - 8 h [D] - 4 h [L], the six samples of which gave a mean NADP-GDH specific activity of 0·515 ±0·28 μmol min^{-1} (mg protein)$^{-1}$ and were, at the time of harvest, cytologically post-meiotic, though the basidia had only just developed sterigmata; and (b) 16 h [L] - 24 h [D] - 16 h [L] - 8 h [D] - 4 h [L] in which four samples had an activity of 1·01 ±0·07 μmol min^{-1} (mg protein)$^{-1}$ and hymenial development had proceeded to spore nucleation and maturation. The two enzyme activities quoted here are significantly different. This sort of distinction shows the expected dependence of the progress of meiosis on elapsed time: the earlier first exposure to light in the latter samples permitting enhanced development by the time they were harvested for examination. More importantly, the higher NADP-GDH activity in the latter set demonstrates that the time of first light exposure synchronises not only the meiotic division, but derepression of NADP-GDH also, so the two phenomena must be very positively correlated. Again, it is evident that the temperature shift-down (37°C to 26°C), which seems to be essential to fruiting in agarics, is not involved in the timing of such processes. Presumably, because some steps involved in fruiting are temperature sensitive, the temperature shift down enables their occurrence, but without inducing it.

As NADP-GDH derepression occurred only at or immediately after nuclear fusion in all these experiments and observations, we conclude that expression of NADP-GDH in the fruit body cap of Coprinus cinereus is either a component part of the cellular programme involved in karyogamy, or is directly triggered by that programme. Further study of this system will be an important contribution to understanding of the immediate metabolic impact of nuclear fusion events like fertilisation.

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