Co-ordinate control of ammonium-scavenging enzymes in the fruit body cap of *Coprinus cinereus* avoids inhibition of sporulation by ammonium

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1. SUMMARY

Observation of stained nuclei coupled with determination of activity of NADP-linked glutamate dehydrogenase (NADP-GDH) and glutamine synthetase (GS) in the same tissue, showed that increase in activity of both enzymes was initiated as karyogamy became evident in normally-developing basidia of fruit bodies of the basidiomycete *Coprinus cinereus*. Derepression of the two enzymes was highly positively correlated (correlation coefficient 0.91), implying co-ordinated regulation. Application and injection of solutions of ammonium salts to *Coprinus* fruit body caps arrested further development. Other salts had no such effect, indicating a specific sensitivity to ammonium. Similar enzymic events occur during sporulation of *Saccharomyces cerevisiae*, which is also sensitive to inhibition by ammonium salts. It is concluded that meiosis generally includes ammonium-sensitive processes and that co-ordinated derepression of the ammonium scavenging NADP-GDH and GS is a device to ensure removal of ammonium ions from the meiotic cell.

2. INTRODUCTION

NADP-linked glutamate dehydrogenase (NADP-GDH; EC 1.4.1.4) is derepressed specifically, and only, in the cap of the mushroom fruit body of the basidiomycete fungus *Coprinus cinereus* (Schaeff: Fr.) S.F. Gray as it develops. No significant NADP-GDH activity is found in stipe tissues [1]. The enzyme activity is initially localised in basidia [2] and observation of stained nuclei coupled with determination of NADP-GDH activity in the same tissue has shown that increase in enzyme activity was initiated as karyogamy became evident [3]. Activity of the enzyme glutamine synthetase (GS; EC 3.5.1.2) also increases greatly in the developing cap while remaining at low levels in the stipe.

Similar enzymic events can be induced in a mycelium by transfer to a medium deficient in nitrogen [4]; increases in activity of NADP-GDH...
in such cultures resulted from de novo synthesis [5] and were positively correlated with GS activities (correlation coefficient 0.94), implying some form of co-ordinate control.

Co-ordinate regulation has not been tested in the fruit body because of problems in establishing a suitable time scale on which different analyses can be compared. However, the recent combination of cytological observation of meiosis with enzyme assay [3] provides a time base which is endogenously determined and entirely objective.

Here we report a study in which NADP-GDH and GS activities were measured together in tissue also assayed cytologically. The function of these enzymes in the developing cap of Coprinus is also considered.

3. MATERIALS AND METHODS

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

Fruit bodies were produced on cultures growing on horse dung using the techniques described by Moore and Ewaze [6]. 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 fluorescent lights giving an average intensity of 680 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 and Lu [7]. Enzyme assays have been described before [4,8]. Soluble protein was measured using the Lowry method.

4. RESULTS AND DISCUSSION

Activities of NADP-GDH and GS in developing caps are shown in Fig. 1. Figure 2 shows that, except for the later (post-meiotic) stages, the two activities are positively correlated. The correlation coefficient (0.91) compares well with that obtained in mycelium transferred to nitrogen-deficient medium (0.94). Thus there is a clear suggestion that the two enzymes are co-ordinately regulated both endogenously in the fruit body cap and in experimentally manipulated mycelia. The mycelial response to nitrogen deprivation can be easily understood as a possible example of nitrogen catabolite derepression; the two enzymes being produced to scavenge for and assimilate ammonium in order to relieve the imposed state of nitrogen starvation. However, it is extremely difficult to extend such an argument to the fruit body cap. This structure is always far removed from the substratum and the parent mycelium upon which the fruit body is produced. The nutritional environment of its constituent cells is there-
fore determined by their own internal activities and during meiosis large scale protein breakdown occurs [3]. Amino nitrogen must be obtainable readily from degraded proteins and the cells cannot be suffering nitrogen-starvation.

It is significant that similar enzymic events occur in sporulating yeast. Sporulation (i.e. meiosis and ascospore formation) in Saccharomyces cerevisiae is induced by transferring diploid vegetative yeast cells from a rich medium (usually with high levels of glucose and complex nitrogen sources) to a nitrogen-free medium [9–12]. Within 2 h of the transfer, NAD-GDH activity in the yeast cells in repressed to about 5% of its level in vegetative cells, but NADP-GDH activity remains unchanged [13]. The net result is a change in the ratio of the specific activities of the two enzymes from 2.04 : 1 (NAD-GDH : NADP-GDH) to 0.10 : 1. Glutamine synthetase activity initially declines by about 25% but within 2 h of transfer has increased to 140% of the activity of vegetative cells. Inclusion of 5 mM ammonium in the sporulation medium results in a decline in activity of both GDH enzymes and the glutamine synthetase. Meiosis and sporulation are inhibited by ammonia [14,15] and ammonium in the medium inhibits the considerable protein breakdown which normally occurs during meiosis in yeast [16].

Similar experiments with C. cinereus can be attempted by injecting materials into fruit body caps. Injection of 20 μl quantities of 0.5 M NH₄Cl into fruit bodies 20–30 mm tall consistently prevented further development of the whole fruit body. Like quantities of water or 0.5 M KCl had no effect, and the NH₄Cl injection had no effect on fruit bodies over 30 mm tall. When drops of solutions were placed onto fruit body caps, fruit bodies over 30 mm tall at the time of treatment were not affected but those less than 20 mm tall were always arrested in development by solutions of ammonium chloride, ammonium sulphate or ammonium acetate containing as little as 0.5 mM ammonium. Injection of 1–2 μl of 0.5 M NH₄Cl arrested the development of localised areas of the cap; resulting in white (spore-free) regions at the site of injection, in an otherwise normally-developing fruit body. Three or four such injections (totalling 5 μl) were sufficient to block further development in half of the cap, the other half developed normally. Measurement of enzyme activities in both the treated and untreated halves showed no significant differences in specific activities of NAD-GDH, NADP-GDH or GS.

Thus, in both Saccharomyces and Coprinus application of ammonium to sporulating cells inhibits sporulation. In both organisms the normal meiosis/sporulation programme includes both large scale intracellular protein degradation (implying release of large amounts of amino-nitrogen) and enhancement of NADP-GDH and GS activities, two enzymes which have high affinity for ammonium and are normally associated with ammonium assimilation.

We suggest that the role of the GS and NADP-GDH ammonium-scavenging is not to alleviate nitrogen deprivation, but to maintain an ammonium-free environment in a cell committed to sporulation processes which are inhibited by ammonium. In view of the considerable evolutionary gulf between yeast and the higher basidiomycetes, it seems likely that these processes are not just characteristic of fungi, but may well apply to eukaryotes as a whole.
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