TRANSIENT CYCLIC AMP ACCUMULATION IN GERMINATING ZOOSPORES OF *BLASTOCLADIELLA EMERSONII*

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1. Introduction

Blastocladiella emersonii, a unicellular water mold, represents a useful organism with considerable potential for developmental studies [1]. In its cell cycle, the growth period is both preceded (germination) and followed (sporulation) by periods of non growth, which are characterized by dramatic changes in cell structure and function.

During germination of *B. emersonii* zoospores, a sequence of biochemical and morphological changes occurs. Most of this sequence does not appear to require either concommitant transcription or concommitant translation [2–7]. Thus, intracellular reorganization and reactivation of metabolism, during this rapid cellular transition in the cell cycle, presumably involve control mechanisms not directly related to gene expression. These conditions led us to speculate that cyclic adenosine 3′,5′- monophosphate (cAMP) could play a role in regulating the germination process in *B. emersonii*. We have demonstrated that zoospores contain independent specific enzymes involved in the hydrolysis of cyclic adenosine 3′,5′-monophosphate and cyclic guanosine 3′,5′-monophosphate [8]. cAMP phosphodiesterase activity is at its highest level in zoospores and drops precipituously during the first 20 min of germination [7]. The present paper shows that this drop in activity of cAMP phosphodiesterase is accompanied by an increase of the intracellular cAMP levels during the germination process, reaching a maximum at 20 min, when most zoospores have been transformed in round cells.

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2. Materials and methods

2.1. *Cell growth conditions*

Zoospores were obtained according to Soll et al. [9]. For the germination experiments, they were inoculated (3.3×10^5 spores/ml) into 2 litre Fauerbach flasks containing DM_4 growth medium [7] and incubated in a gyratory water bath shaker at 27°C and 200 rev/min.

2.2. *cAMP extraction and analytical methods*

Zoospores or cells of cultures at different stages of the germination phase were harvested by centrifugation (approx. 2×10^7 cells). The medium was removed by aspiration and the cell pellets were immediately frozen in an acetone–dry ice bath, and thawed in the presence of 0.5 ml of cold 5% TCA. The suspension was sonicated for no more than 100 sec in a Sonifier cell disruptor. The homogenates were centrifuged for 5 min at 1 000 *g* and the supernatants saved. The pellets were again extracted in 5% TCA, centrifuged and the supernatants added to the prior ones. The treatment of TCA-extracts and the assay of cAMP were made according to Gilman [10]. Binding protein was prepared according to Miyamoto et al. [11] and Gilman [10] and protein kinase inhibitor by the method of Appleman et al. [12], both from fresh bovine heart muscle.

As a control [^{3}H] cAMP (New England Nuclear) was added to selected samples before addition of TCA to the frozen pellets. Recovery of [^{3}H] cAMP was 85–90%.

Protein determination [13] was made in the pellets (from the TCA extracts) after solubilization in 0.5 M NaOH.

3. Results and discussion

Material assessed as cAMP by a competitive reaction [10] and obtained from extracts of different cell types was examined for its sensitivity to cyclic nucleotide phosphodiesterase from different sources, in order to assure that the substance measured was authentic cAMP. The results in table 1 show that the putative cAMP of the extracts was about 90% hydrolysed by cyclic nucleotide phosphodiesterase.

Zoospores contain an average concentration of 33.2 (± 1.95) pmol cAMP/mg protein. This value corresponds to mean (± SEM) value of 30 assays. If we assume the zoospore as roughly being a sphere with 4 μm radius and containing 20 pg of protein [2], this level would correspond to an intracellular concentration of about 2.5 μM, which is comparable to the levels reported by Silverman and Epstein [14].

Zoospores inoculated in liquid synthetic medium undergo a sequence of cellular changes, known as zoospore germination, resulting in the formation of germling cells. During this phase of the life cycle three distinct cell types can be easily recognized under phase contrast microscope (cf. fig.1 top, and ref. [9]): zoospores (zero time), round cells (T_{50} = 12.6 min) and germling cells (T_{50} = 31 min). In the presence of cycloheximide zoospores complete all transformations occurring during germination except the disappearance of the internal flagellar axoneme and rhizoid emergence [3].

During germination, we observed a significant increase in the intracellular levels of cAMP (fig.1). The increase in cAMP contents occurs during the early, translation-independent, stage of germination, reaching a maximum (approx. 100 pmol/mg protein) when the bulk of the population has completed the transformation from zoospores to round cells. From then on a gradual decline in cAMP levels was observed, this being closely paralleled by the curve of round cell disappearance.

Table 1
Hydrolysis of cAMP by beef heart and *B. emersonii* cyclic nucleotide phosphodiesterases

Cells	cAMP (pmoles)		
	Control	Beef heart PDE (40 μg)	*B. emersonii* PDE[a] (38 μg)
Zoospore	3.8	0.38	0.36
Round Cell	4.6	0.33	0.36
Germling	2.4	0.33	–

Aliquots (50 μl) of treated extracts were added to an incubation mixture containing 40 mM Tris-HCl buffer, pH 7.2, 5 mM $MgCl_2$, 10 mM KCl, 1 mM β-mercaptoethanol, 1 mg/ml bovine serum albumin, with or without cAMP phosphodiesterase (PDE). The reaction mixture was incubated at 30°C for 3 h, boiled for 2 min, centrifuged and the supernatant assayed for cAMP contents as described in Materials and methods.

[a] *B. emersonii* PDE was purified in our laboratory and is specific for cAMP [8].

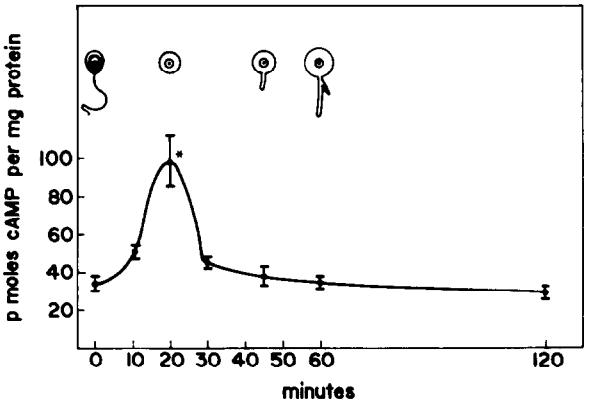

Fig.1. cAMP levels during germination of *B. emersonii*. The drawings at the top represent the sequential appearance of the three cell types during germination, as described in the text and identified by examination under phase contrast microscope [9]. 20 min after inoculation of zoospores into DM_4 liquid medium the % of round cells in 4 experiments varied between 80% and 90%. At the indicated intervals, cells were harvested and the cAMP level determined. The values represent the mean of 4 experiments. Bars indicate s.e.m. * $P < 0.05$.

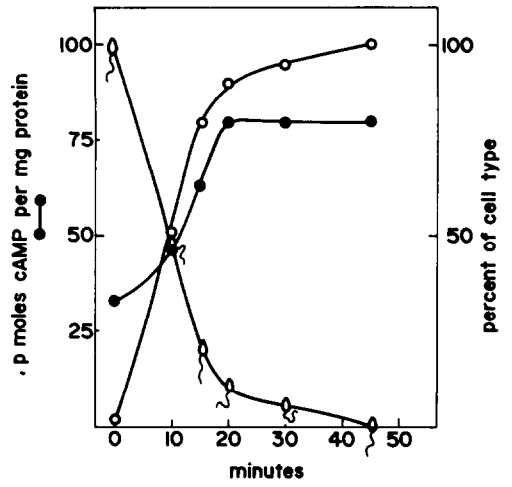

Fig.2. cAMP levels during germination in the presence of cycloheximide. Same procedure as in fig.1. Cycloheximide (2 μg/ml) was added at zero time. (○——○) Zoospores; (○——○) round cells; (●——●) cAMP.

The changes in the endogenous levels of cAMP during germination correlate well with the variations of the specific activity of cAMP phosphodiesterase in this phase, which is high in zoospores and diminishes by 15-fold as zoospores convert to round cells [7].

In another experiment, germination was induced in the presence of cycloheximide (fig.2) and in contrast to what has been found in fig.1, cAMP contents, after reaching the maximum, remain at high levels. This result suggests that the process of conversion of round cells to germlings is somewhat linked to the decrease in cAMP levels observed after 20 min, when germination is unblocked.

Our results are at variance with those reported by Silverman and Epstein [14]. These authors did not find an increase in cAMP levels during encystment, as opposed to our results. A possible explanation for this discrepancy is that the above mentioned authors have not measured the levels of the nucleotide in shorter intervals of time. However, one cannot find a reasonable explanation for the fact that in their experiments the cAMP, after 45 min, decrease to levels much lower than those found in spores, contrary to our findings.

Although the physiological role of cAMP in fungi has not been studied in detail, in *B. emersonii* the rise in cAMP contents during encystment might be related to the concomitant activation of metabolism observed in this phase [2–6, 15–17], as expected from the large body of evidence suggesting the participation of this nucleotide in the regulation of both glycogen [18] and protein [19] metabolism in other systems. This rise is also in accordance with the proposal of Filosa et al. [20], whereby high levels of cAMP are required for a differentiative transition.

The results presented in this work show that *B. emersonii* is a suitable organism for studies on the interrelationship between cAMP and morphogenesis.

Acknowledgements

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