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Induction of germination in *Blastocladiella emersonii* by cyclic AMP and inhibitors of cyclic AMP phosphodiesterase

Inducción de la germinación en *Blastocladiella emersonii* por AMP cíclico e inhibidores de la fosfodiesterasa de AMP cíclico

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Since K^+-induced germination of *Blastocladiella emersonii* is accompanied by a rapid decrease of a specific cyclic AMP phosphodiesterase (cAMP PDE) activity and a transient cyclic AMP accumulation, the effects of this compound as well as of some inhibitors of cAMP PDE on the induction of germination were tested.

Adenine and caffeine, competitive inhibitors of zoospore cAMP PDE, were able to elicit germination in substitution for K^+. Cyclic AMP is a poor inducer, but a synergistic effect was evident when non-effective concentrations of K^+ and cyclic AMP were added together to the medium. At the same concentration, cyclic GMP had no effect as compared with cyclic AMP.

Lanthanum, a specific antagonist of calcium in several biological systems, completely blocked the germination induced by potassium.

BLASTOCLADIELLA EMERSONII CYCLIC AMP CYTODIFFERENTIATION

The assexual life cycle of *Blastocladiella emersonii* is characterized by two transitional stages —germination and sporulation— during which conspicuous cellular transformations occur (1). Germination, the transition from zoospores to vegetative cells, occurs semi-synchronously in response to a simple shift in ionic environment, the total process requiring less than one hour (2). During this phase, the zoospore, flagellated and having no outer cell wall, rapidly converts into a sessile round cell (containing a chitin wall) and then into a vegetative cell, the germling, which is capable of prolific syncytial growth (cf. Fig. 1, top). Upon nutritional starvation, at any time during the growth phase, the vegetative cell initiates the sporulation process, which leads to the formation of new zoospores and their release into the surrounding medium.

In this report we will focus on zoospore germination. Some of the sequential cellular changes involved in this transition are summarized in Table I.

Studies with inhibitors of RNA and protein synthesis, experiments of leucine and uridine incorporation into whole cells and the kinetics of proliribosome formation, lead to the conclusion

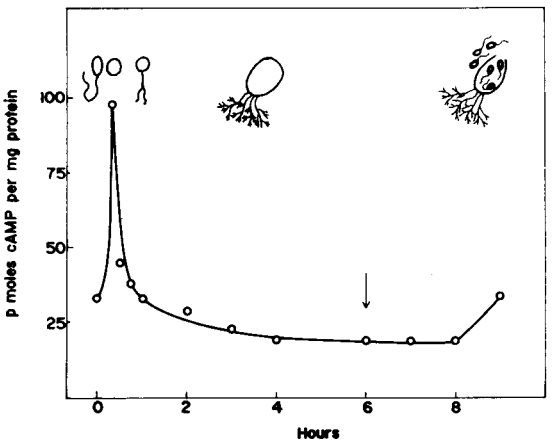

Fig. 1. Cyclic AMP levels during the life cycle of *B. emersonii*. The drawings at the top represent the different cell types during germination (zoospore, round cell and germling) as well as the vegetative and sporulating cells, identified by examination under phase microscopoe (16). Zoospores obtained according to Soll *et al.* (16) were inoculated (3.3×10^5 zoospores/ml) into 2 l-Fauerbach flasks containing DM_4 growth medium (9) and incubated in a giratory water bath shaker at 27°C and 200 rev/min. At the indicated intervals, cells were harvested by centrifugation and the cyclic AMP level determined as described (10). 20 min after inoculation of zoospores into DM_4 liquid medium, the % round cells in 4 experiments varied between 80% and 90%. The vertical arrow represents the time when growth medium was replaced by $CaCl_2$ to induce sporulation (9).

that most of the early structural and biochemical events of zoospore germination donot require either RNA or protein synthesis. In addition, it has been shown that the first protein synthesis takes place with stored messenger RNA and preformed ribosomes from the zoospore nuclear cap (3, 4, 5).

These results suggest that presumably post-translational control mechanisms are involved in this morphological transition. One area of intense current interest with respect to such control mechanisms is the metabolism and function of cyclic nucleotides-specially cyclic AMP. This nucleotide has been reported to be involved in the regulation of a number of metabolic process including cell proliferation and differentiation (6, 7, 8).

In *Blastocladiella,* zoospore germination is accompanied by a rapid decrease of a specific cyclic AMP phosphodiesterase (cAMP PDE) activity (9) and a transient cyclic AMP accumulation (10). Fig. 1 shows that there is a significant increase in the level of cyclic AMP during the early, translational-independet, stage of germination, reaching a maximum at 20 minutes (the round cell peak, in our conditions) and then declining gradually to a minimum level at 60 min of germination. The cyclic AMP

TABLA I

Some biochemical and morphological changes during zoospore germination

Changes	*Zoospore*	*Round cell*	*Germling*
Cell adhesion (16) (to dish bottom)	−	+	+
Resistance to lytic agents (16)	−	+	+
Cellular volume (17)		↓	
Germ tube (16)	−	−	+
Protein synthesis (1, 16)	−	+	
RNA synthesis (1, 16)	−	+	
Glycogen contents (18)		↓	
Lipid contets (19, 20)		↓	
O_2 uptake (18)		↑	
Rate of protein degradation (21)		↑	

Symbols:
+ or − indicate presence or absence respectively;
↑ & ↓ indicate decrease and increase respectively.

level remains low during the entire growth period and begins to rise again at the end of sporulation.

These data suggest that inducers of germination may be mediated by secondary messengers such as cyclic AMP. We have therefore tested the effects of this compound as well as of some inhibitors of cAMP PDE in the induction of germination.

The zoospore cAMP PDE which has an apparent K_m of 2-4 μm (11) is competitively inhibited by caffeine (K_i = 2.8 mM) as well as by adenine (K_i = 1.8 mM). Table II shows that these different drugs elicit germination, the cell types being morphologically identical, under light microscope examination, to those induced by potassium. The only kinetic difference which can be observed it that the peak of round cell formation occurs earlier in the presence of methyl-xanthines or adenine than in the presence of potassium.

Cyclic AMP or dibutyryl cyclic AMP are poor inducers. However, a synergistic effect was evident when non effective concentrations of potassium (12.5 mM) and cyclic AMP (0.75 mM), but not dibutyryl cAMP , were added together to the germination solution. The lack of effect of cyclic AMP alone may reflect a non uptake of this nucleotide in absence of potassium. An indirect corroboration of this assumption is the absence of germination in the presence of theophylline (0.6 mM) plus cyclic AMP (0.75 mM). Measurements of cyclic AMP uptake in the presence of potassium may answer this question. At the same levels, cyclic GMP does not induce germination either in presence or absence of potassium.

Several process which are dependent on cyclic AMP have been shown to require or involve calcium (12). We have therefore investigated the effect of lanthanum, a specific antagonist of calcium in a number of biological systems, on germination. Almost all of the effects of La^{3+} on calcium-dependent movements or reactions in intact cells have been explained by postulating that La^{3+} can replace calcium at well-defined sites on the outer cell membranes (13, 14). La^{3+}, at a concentration of 0.5 mM, completely blocked the germination induced by potassium (Table II). In these experiments, La^{3+} presumably

TABLE II

Effect of cyclic AMP and analogues on zoospore germination

Additions	*Concentration (mM)*	*% of germination*
KCl	50	100
KCl	12.5	3
KCl + $LaCl_3$	50 + 0.20	20
KCl + $LaCl_3$	50 + 0.50	7
Cyclic AMP	2.5	15
db cyclic AMP	0.75	1
Cyclic GMP	0.75	3
KCl + cyclic AMP	12.5 + 0.75	95
KCl + db cyclic AMP	12.5 + 0.75	16
KCl + cyclic GMP	12.5 + 0.75	23
Theophylline	2.5	100
Caffeine	2.5	95
Adenine	2.5	95
Theophylline	0.6	4
Theophylline + cyclic AMP	0.6 + 0.75	30
Theophylline + KCl	0.6 + 12.5	84

Germination was assayed essentially as described by Soll *et al.* (16). The initial zoospore concentration was 15 to 20 $\times$ 10^4 cells/ml of germination solution (10^{-3} M $CaCl_2$, 10^{-2} M $MgCl_2$, 5 $\times$ 10^{-2} M KCl in 10^{-3} M Tris-maleate, pH 6.7). The effect of the several drugs on induction of germination was tested substituting these compounds, at the concentration indicated, for K^+. % germination corresponds to % germs after a 60 min incubation.

prevented the depletion of intracellular calcium, which occur at germination (2) by blocking efflux of the cation from the cells. Indeed, La^{3+} is known to block both uptake and efflux of calcium in other intact cell systems (13, 15).

The actual mechanism by which germination is triggered by ionic environmental changes is not known. According to the evidence presented in this paper, cyclic AMP seems to stimulate the differentiative transition from zoospore to round cell. The mode of action of cyclic AMP in this system is not resolved. At the present, the value of the results consists in suggesting that cyclic AMP may be one of many possible endogenous mediators which could couple induction, that is, interaction of zoospore with their environment, with germination itself.

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