CYCLIC GMP AND CYCLIC GMP PHOSPHODIESTERASE IN THE CELL CYCLE OF *TETRAHYMENA PYRIFORMIS*

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1. Introduction

In a recent report on the cyclic AMP metabolism in the cell cycle of *Tetrahymena pyriformis* we found a large peak in intracellular cyclic AMP coincident with cell division [1]. Both cyclic AMP and cyclic GMP have been implicated as important controlling elements in the eukaryotic cell cycle [2]. For *Tetrahymena pyriformis* if has been reported that exogenous cyclic GMP can induce thymidylate synthetase activity [3]. Furthermore cyclic GMP-dependent Protein Kinase activity has been observed in this organism [4]. It was thus of interest to determine whether *Tetrahymena pyriformis* contains cyclic GMP.

We report for the first time the existence of cyclic GMP and cyclic GMP phosphodiesterase activity in *Tetrahymena pyriformis* and their variation throughout the natural cell cycle. Cyclic GMP reached a maximum during cell division, then decreased to a minimum which occurred immediately before the next cell division. Cyclic GMP phosphodiesterase activity was consistent with this result.

2. Materials and methods

2.1. *Selection of a synchonous population of* Tetrahymena pyriformis *strain W*

The growth, method of selection of dividing cells for experimentation and cell counting techniques have all been described previously [1].

2.2. *Assay of cyclic GMP phosphodiesterase activity*

Cells were harvested at 2000 × *g* for 5 min. at growth temperature, washed with buffer A (40 mM Tris–HCl, pH 7.5, containing 2 mM $MgSO_4$), then resuspended to 1 ml in this buffer, cooled to 0°C and sonicated in a MSE 150 W sonicator at maximum power and amplitude for 15 s. This cell homogenate was then diluted as appropriate with buffer A and used immediately to initiate the assay which comprised 1 mM cyclic GMP in buffer A in a total volume of 1 ml. Incubation was at 30°C for 30 min. After the first 20 min. of the incubation, 0.1 ml of a solution of *Ophiophagus hannah* venom in buffer A was added containing 0.1 mg of venom. The snake venom hydrolyzes the product to release inorganic phosphate. The reaction was terminated by the addition of 0.1 ml of cold 55% trichloroacetic acid. After addition of trichloroacetic acid, the precipitate was removed by centrifugation, and aliquots of the supernatant analyzed for inorganic phosphate. The assay is based on that described by Butcher and Sutherland for the assay of cyclic AMP phosphodiesterase [5].

2.3. *Extraction and assay of cyclic GMP*

The extraction procedure was essentially the same as that described previously for cyclic AMP [1]. Cyclic GMP was separated from cyclic AMP and other nucleotides by chromatography on columns of neutral alumina using 10 mM Tris–HCl, pH 7.4, as eluting buffer. Cyclic GMP eluted much later from the column than cyclic AMP. Cyclic GMP was then concentrated-up prior to assay by freeze-drying and subsequent resuspension in a smaller volume. Cyclic GMP was assayed using a commercially available cyclic GMP-assay kit (Boehringer) according to the manufacturer's recommended routine. Since this

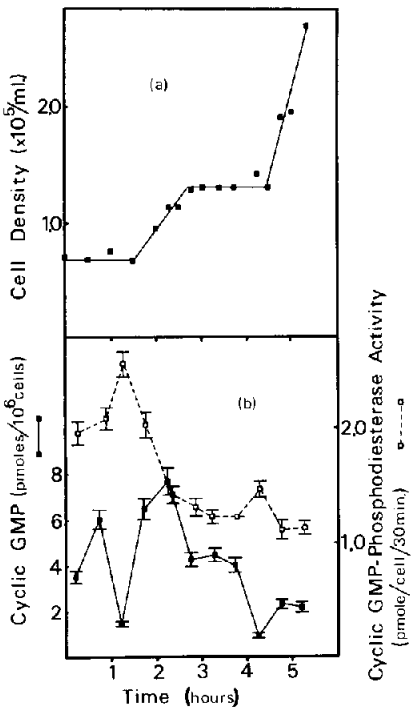

Fig.1. A typical cell cycle study. (a) Cell density. (b) Cyclic GMP measured per 10^6 cells and cyclic GMP phosphodiesterase activity measured at 1 mM substrate concentration. These results represent the mean of at least two experiments, ± S.E.M.

assay uses a cyclic GMP-binding protein, all cyclic GMP standard curves were constructed containing similarly concentrated-up elution buffer in equivalent amounts to those present in 'unknowns'. This procedure removes 'non-specific' effects that otherwise result. Material that was assayed as cyclic GMP was destroyed in the same way as bona fide cyclic GMP by beef heart cyclic nucleotide phosphodiesterase (Boehringer). Multiple amounts of samples resulted in the values expected from the calibration curve, as did addition of defined amounts of bona fide cyclic GMP to 'unknowns'. Recovery of cyclic GMP was 65.0 ± 1.3%.

3. Results and discussion

We measured a nearly ten-fold variation in cyclic GMP throughout the cell cycle. The range of cyclic GMP concentrations is similar to that we reported for cyclic AMP [1].

Cyclic GMP was highest during cell division and declined progressively to its lowest level at a point immediately before the next cell division. The peaks in cyclic GMP-phosphodiesterase activity coincided with the cyclic GMP minima.

From this and our previous report [1] it appears that the peaks in cyclic AMP and cyclic GMP occur at very nearly the same point in the cell cycle, although the pattern of modulation of the two nucleotides is different. Regulation of important cell cycle events could be accomplished by the independent activation of cyclic GMP-dependent and cyclic AMP-dependent protein kinases.

Perturbed cell cycles are now being studied in order that we may elucidate the role of these cyclic nucleotides.

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