CYCLIC AMP METABOLISM IN THE CELL CYCLE OF TETRAHYMENA PYRIFORMIS

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1. Introduction

It is now widely recognised that cyclic nucleotides are of fundamental importance in regulating the many biochemical processes that comprise the eukaryotic cell cycle. Many of the studies to date have used cells derived from multicellular organisms. A major feature of such cells is their ability to respond to extracellular stimuli by processes which can involve cyclic nucleotides. *Tetrahymena pyriformis,* a free-living unicellular eukaryote, should be largely devoid of such behaviour, and thus represents a more suitable cell system for the study of the regulation of intracellular events by cyclic nucleotides. Moreover, mass cultures of this organism are amenable to selection synchrony methods [1–3], thus avoiding possible artefactual results that could be caused by induction synchrony techniques.

Cyclic AMP, adenyl cyclase, cyclic AMP phosphodiesterase and cyclic AMP-dependent protein kinase activity have all been demonstrated in *Tetrahymena pyriformis.* These studies [4–14] have implicated cyclic AMP as a possible regulator of the cell cycle of *Tetrahymena pyriformis.* However, no direct measurement of cyclic AMP in the cell cycle of this organism has been made. We report for the first time the measurement of cyclic AMP, adenyl cyclase and cyclic AMP phosphodiesterase activities in the natural cell cycle of *Tetrahymena pyriformis* strain W. A large peak of intracellular cyclic AMP was associated with cell division. The observed activities of adenyl cyclase and cyclic AMP phosphodiesterase can account for the fluctuations of this cyclic nucleotide.

2. Materials and methods

2.1. *Selection of a synchronous population of* Tetrahymena pyriformis

Tetrahymena pyriformis strain W, was grown in conical flasks filled up to 20% total volume with a medium containing 2% proteose peptone (Difco), 0.1% yeast extract, 0.5% w/v glucose and 5 μg/ml $FeCl_3 \cdot 6\ H_2O$. Incubation was at $28 \pm \frac{1}{2}$°C in an orbital shaker at a rate of 180 rev/min.

The cell density at times throughout the cell cycle was determined by fixing aliquots of cells in equal volumes of 20% formaldehyde neutralised with 0.01 M phosphate buffer and counting in a Neubauer haemocytometer. More than 200 cells were counted at each time of sampling. Late division cells were scored as 'furrowing cells' because the prominent furrow girdling the cell made identification of this stage unequivocal.

Mitotic cells were selected on the basis of their inability to inject particulate material by using the procedure of Hildebrandt and Duspiva [1] with slight modifications. This generates a synchronous population of recently divided cells for cell cycle analysis.

2.2. *Assay of adenyl cyclase activity*

Cells were harvested at 2000 *g* for 5 min at growth temperature, washed with 25 mM Tris·HCl buffer, pH 7.5 (containing 0.25 M sucrose, 2 mM EDTA and 0.2 mM dithiothreitol), 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

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for 10 sec. This cell homogenate was then used immediately in the assay of adenyl cyclase which comprised: 40 mM Tris·HCl, pH 7.5, 5 mM cyclic AMP, 5 mM $MgCl_2$, 10 mM theophylline, 1.2 mM [2-^{3}H]ATP (17.5 μCi per μmole), 25 mM phosphocreatine, 50 units/ml creatine phosphokinase and 0.05 ml of sonicate in a final volume of 0.1 ml. The reaction was terminated after 10 min at 30°C by addition of 1 ml of 3 mM EDTA pH 7.5 and heating rapidly to 100°C for 4 min. Protein was removed by centrifugation and the supernatant submitted to sequential chromatography on Dowex 50 W and neutral alumina [15]. The product of this purification has been identified as cyclic AMP by hydrolysis with beef heart cyclic AMP phosphodiesterase (Boehringer Corp. (London) Ltd., London). Liquid scintillation counting was used to estimate the conversion of [2-^{3}H]ATP to cyclic AMP.

Incubation blanks (terminated prior to the addition of enzyme) contained less than 0.005% of total radioactivity. The recovery of cyclic AMP, which was 74.2 ± 2.2% in ten separate experiments, was not affected by the presence of *Tetrahymena* homogenate containing up to 0.6 mg protein per assay.

2.3. *Assay of cyclic AMP phosphodiesterase*

A cell homogenate was prepared as described in 2.2 and diluted appropriately with 40 mM Tris·HCl buffer pH 7.5 containing 2 mM $MgSO_4$. Cyclic AMP phosphodiesterase was then assayed essentially according to the methods of Butcher and Sutherland [16].

2.4. *Extraction and assay of cyclic AMP*

Cells were harvested as described in 2.2 and then sonicated at maximum power in 10% trichloroacetic acid at 0°C. After standing at this temperature for 1 h denatured cellular material was removed by centrifugation. The supernatant was then washed five times with two volumes of water-saturated diethyl either to remove the trichloroacetic acid. After freeze-drying, the material was resuspended in 10 mM Tris·HCl pH 7.4, and cyclic AMP separated by chromatography on columns of neutral alumina (according to the method of Ramachandran [17]), using the same buffer for elution. Cyclic AMP was then concentrated-up prior to assay by further freeze-drying and subsequent resuspension in a smaller volume. Cyclic AMP was then assayed by the method of Brown et al. [18], using all the controls necessary in this assay method [19]. Material that was assayed as cyclic AMP was destroyed in the same way as bona fide cyclic AMP by beef heart cyclic AMP phosphodiesterase (Boehringer). Addition of defined amounts of bona fide cyclic AMP to 'unknowns' resulted in the values expected from the calibration curve. Recovery of cyclic AMP was 71.6 ± 1.5%.

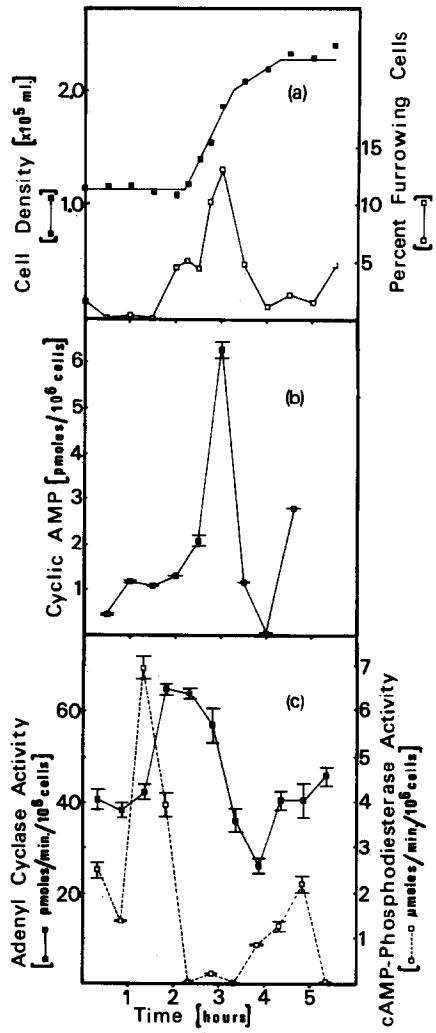

Fig.1. The time course of a typical synchronous culture of *T. pyriformis* W. (a) Cell numbers and percentage dividing cells. (b) Cyclic AMP measured per 10^6 cells. (c) Adenyl cyclase and cyclic AMP phosphodiesterase activities measured at approx. physiological substrate concentrations; (1.2 mM ATP, and 5 × 10^{-7} M cyclic AMP respectively). These results represent the mean of at least 2 experiments, ± S.E.M.

3. Results and discussion

The major observation was the large peak in intracellular cyclic AMP coincident with cell division. Shortly after division the cells contained little cyclic AMP. The fluctuations in adenyl cyclase and cyclic AMP phosphodiesterase activities can readily account for the observed levels of cyclic AMP. High cyclic AMP levels were associated with high adenyl cyclase activity and low phosphodiesterase activity. Conversely, low cyclic AMP occurred where adenyl cyclase was at a minimum and phosphodiesterase activity had increased.

Many workers using cells derived from higher eukaryotes report elevated cyclic AMP during the G_1 phase of the cell cycle. [20]. This has been correlated with high levels of the cyclic nucleotide in quiescent (or stationary phase) cells, the G_0 phase. A subsequent fall in cyclic AMP would then commit the cells to the next division. Furthermore, in *Tetrahymena pyriformis* stationary phase cells have been shown to contain higher levels of cyclic AMP than logarithmic phase cells. [4,5,7]. However, our observations show a transitory high level of cyclic AMP before the cells progress into G_1. This point in the cell cycle would seem to be a good candidate for the location of a decision-making process, whereby the cell may initiate a further cell cycle or become quiescent. The decline in cyclic AMP reported here may be required for cells to progress into G_1. Hence, cells which maintain a high cyclic AMP level will enter stationary phase.

We are currently investigating the modulation of cyclic GMP in the cell cycle of *Tetrahymena pyriformis*. These studies may clarify further the control points within the cell cycle.

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