ON THE RELATIONSHIP BETWEEN CELL DIVISION AND CYCLIC ADENOSINE-3',5'-MONOPHOSPHATE IN *ESCHERICHIA COLI*

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1. Introduction

A number of reports have indicated that cyclic adenosine-3',5'-monophosphate (cyclic AMP) has a regulatory function in the growth of mammalian cells [1–2]. A similar phenomenon seems to occur in procaryotic organisms. Addition of cyclic AMP to the culture medium decreases the growth only with carbon sources that cause catabolite repression.

In mouse 3T3 cells and in fibroplasts, it has been observed that when growth stops at confluency the endogenous level of cyclic AMP increases [2]. A similar effect is observed when growth of mammalian cells is inhibited by prostaglandin E_1 [2]. The same effect might occur in procaryotic organisms when cell division is inhibited, even though the cyclic AMP level is regulated in a different way and contact inhibition of growth does not exist. Therefore, we have studied the effect of specific inhibition of cell division on intracellular levels of cyclic AMP in *E. coli* grown under conditions of catabolite repression.

2. Materials and methods

P4 X 8 ts 84, a filament-forming temperature sensitive derivative of P4 X 8, was a generous gift of Dr. Y. Hirota. The cultures were incubated in medium 63 [4] supplemented with 0.5% casamino acids. Glycerol 0.4% was used as a carbon source. Protein synthesis was followed by incorporation of [^{3}H]leucine into trichloroacetic acid precipitates as previously described [5]. Cell numbers were counted in a Coulter counter model F (Coultronic France). Samples were diluted a thousand times in a buffer 0.1 M sodium phosphate, pH 7, containing 1g/l sodium citrate and 0.4% formaldehyde, filtered through a millipore filter (0.1 μm). For cyclic AMP assay, samples (4.5 ml) of the culture were filtered rapidly through membrane filters (Millipore HA 0.45 μm). The filters, without washing, were suspended immediately in 1 ml of 1 N hot perchloric acid and kept for 10 min at 100°C. For assaying cyclic AMP, the heated extract was homogenized with a micro-potter and centrifuged. The supernatant was collected, neutralized with potassium carbonate and then appropriate dilutions were used for the assay. A highly sensitive radioimmunoassay of cyclic AMP was employed. In this new assay, reported by H. Cailla et al. [6], cyclic AMP is first converted to 2-*O'*-succinyl cyclic AMP. Like an earlier assay [7] this one is based on the competition between iodinated and cold antigen for the binding site of an antibody directed against succinyl cyclic AMP. The data obtained from the auto gamma scintillation counter on a perforated tape, were analyzed by a computer. Duplicate assays differed rarely by more than 5%. Calculation of intracellular concentrations of cyclic AMP in units of molarity is based on an accessible volume of 7.5×10^{-13} ml/bacterium.

3. Results and discussion

Cell division can be inhibited in *E. coli* by chemicals such as penicillin [8] or diazouracil [9]. However, these drugs might have pleiotropic effects and the use of a thermosensitive filament forming mutant P4 X

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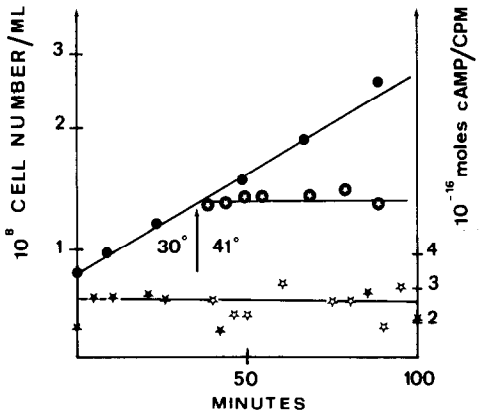

Fig. 1. Cell division and cyclic AMP level – An exponential culture of P4 X 8 ts 84 was divided into 2 subcultures at the arrow. One was kept at 30°C as a control (•) the other one shifted to 41°C (⊛) The ratio of intracellular cyclic AMP concentration to the radioactivity incorporated into proteins was determined for the cells kept at 30°C (⋆) and 41°C (✩).

8 ts 84 was preferred. This strain is deficient in septum formation at 41°C, but continues other cellular functions and maintains its ability to survive even after two hours at the non-permissive temperature [10,11].

We first checked the parental strain P4 X 8 to make sure that the temperature shift from 30°C to 41°C did not cause any change in cyclic AMP level under the conditions used (unpublished result).

When a well-balanced culture of the mutant is shifted from 30°C tot 41°C, cell division stops immediately (fig. 1). The biosynthesis of RNA, DNA, and protein are not affected at the restrictive temperature [10,11]. The ratio of intracellular cyclic AMP concentration per ml of culture to the protein content was followed before and after the temperature shift. The rate of protein synthesis followed by incorporation of radioactive leucine is increased by a factor 1.41 at 41°C compared to the rate at 30°C (fig. 2). The ratio of cyclic AMP concentration to the radioactivity incorporated into protein remains constant after cessation of cell division at 41°C (fig. 1). This result has two implications: 1) cessation of cell division has no influence on the intracellular level of cyclic AMP; 2) the adjustment of the rate of synthesis of this nucleotide and protein synthesis is very fast. With the parental strain (not shown) as well as with the mutant P4 X

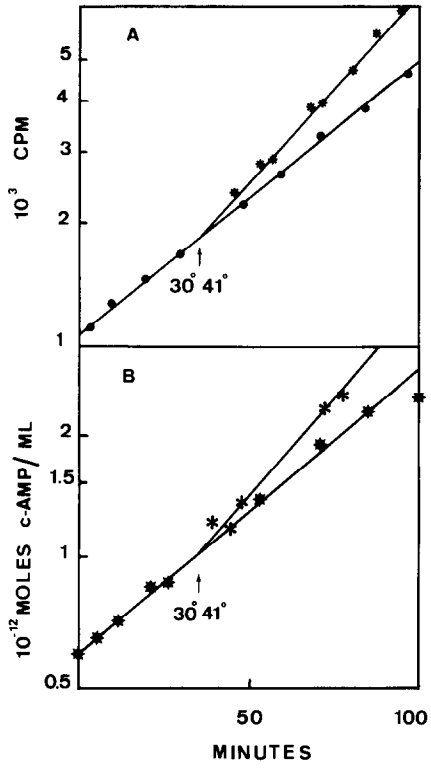

Fig. 2. (A) Protein synthesis at 30°C (•) and 41°C (⁎) P4 X 8 ts 84 was grown in medium 63 supplemented with 0.5% casaminoacids and 0.4% glycerol and the incorporation of [^{3}H]leucine was measured as a function of time. (B) Intracellular cyclic AMP concentration per ml of culture was determined at 30°C (⋆) and 41°C (⁎) was described in Material and methods.

8 ts 84, within 5 min after the shift the ratio is already adjusted, whereas the rate of protein synthesis is increased by a factor 1.41 (fig. 2). This suggests that a similar increase of cyclic AMP concentration has occurred and that the energies of activation of adenyl·cyclase and of cyclic AMP exit from the cell are of the same order. These are the two main parameters involved in the control of cyclic AMP levels in *E. coli* [12]. Such a result is in fact found when figs. 2A and 2B are compared.

Intracellular molarity of cyclic AMP can be evaluated at any time during growth since we know both the intracellular cyclic AMP concentration and the cell number per ml of culture. At the time of the temperature shift from 30 to 41°C, the molarity was 1.02×10^{-5}M. This concentration was achieved by

adding 0.5% casamino-acids to the culture medium. It is identical with the cyclic AMP level obtained on minimal medium supplemented with 10 mM glucose [12]. Under these conditions, addition of cyclic AMP to the culture medium decreases the growth rate of *E. coli* [3]. Similarly, the addition of dibutyryl cyclic AMP to mammalian cells has the same effect. Therefore, in this respect, the control mechanism mediated by the cyclic nucleotide is similar in procaryotic and mammalian cells. Concerning the relationships between cell division and cyclic AMP level the situation is quite different. For mammalian cells, inhibition of cell growth, either by contact effect or by prostaglandin E_1, is accompanied by an increase of endogenous level of cyclic AMP. In this report we show that when cell division is prevented in *E. coli* the cyclic AMP level is not altered at all.

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