EFFECT OF *dna*-B MUTATION ON INTRACELLULAR CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE IN *ESCHERICHIA COLI*

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1. Introduction

Replication of the *Escherichia coli* chromosome requires the activity of several genes products. Temperature sensitive mutants have been isolated that do not replicate DNA at non-permissive temperatures. The genes defined by these mutants have been designated *dna*-A to *dna*-P [1]. The enzymatic activity of the *dna*-E gene product has been identified as DNA polymerase III [2,3] and that of *dna*-B as ribonucleoside triphosphatase [4].

On the other hand, *dna*-B mutants are also known to have pleiotropic effects; other cellular processes beside DNA synthesis are affected in them. Among these are: the inability of some of them to permit growth of wild-type bacteriophage lambda [5], DNA breakdown and loss of cell viability [6], an alteration in the stability of the replicating complex [7], an alteration in the membrane protein composition [8]. It has also been reported that bacteriophage P_1 determines a product that can substitute in DNA replication for the protein specified by cistron *dna*-B of *E. coli* [9].

The physiology of the host cell can influence the plating of temperature bacteriophage. In particular, intracellular cyclic AMP level has been shown to regulate the viral decision between lysogeny and lysis [10]. Therefore, we have followed the synthesis of cyclic AMP at permissive and restrictive temperatures in a *dna*-B mutant.

The results reported here suggest an indirect effect of the thermosensitivity of the *dna*-B gene product on the endogenous level of this cyclic nucleotide.

The specificity of this effect is ascertained by using another temperature sensitive mutant *dna*-E that has a heat-sensitive polymerase III [2,3] and does not show any alteration in intracellular cyclic AMP concentration at permissive and restrictive temperatures.

2. Materials and methods

2.1. *Chemicals*

[^{14}C]Thymidine (40 mCi/mM) and [^{3}H]leucine (0.3 Ci/mM) were purchased from the Departement des Radioéléments CEA France. All other products were reagent grade.

2.2. *Bacterial strains*

The following strains were used throughout this work: CR 34; F^-, Thr^-; Leu^-, Thy^-, B_1^-, lac y^-, λ^s; CR 34-BT 313 same as CR 34 but *str*-r and *dna*-B BT 313, originally from Dr Bonhoeffer.

2.3. *Media*

The bacterial strains were grown at 30°C or 41°C in minimum salt medium 63 [11] supplemented with glycerol 0.4%, thymine (50 μg/ml), and the required amino acids (30 μg/ml each).

2.4. *Macromolecular biosynthesis*

Incorporation of [^{3}H]leucine (1 μCi/ml) and [^{14}C]-thymidine (0.1 μCi/ml) into trichloracetic acid-insoluble material was used as an index of protein and deoxyribonucleic acid (DNA) synthesis, respectively. The concentration of precursors was reduced to 20 μg/ml, and the protocol used was as described [12].

2.5. *Measurement of cyclic AMP levels and cell numbers*

Cyclic AMP levels and cell numbers were measured as described [13].

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3. Results

3.1. *Effect of the temperature of growth of CR 34-BT 313 on cell mass increase, cell number, cyclic AMP level and protein and DNA synthesis*

The growth parameters of the *dna*-B strain were monitored at 30°C and 41°C. At 30°C (permissive temperature) DNA synthesis is already affected since the mutant synthetizes only 36% as much DNA as compared with the parental strain CR 34 (table 1). This results in a partial defect of cell division and cell mass increases twice as fast as cell number (table 1 and fig.1a, 1b). The amount of DNA synthesis at 30°C can be increased by addition to the medium of 12% sucrose. Such an osmotic effect has been previously described [14]. At 41°C (restrictive temperature) the DNA synthesis stops immediately after the temperature shift (fig.2b). This results in a blocking of cell division after a few minutes (fig.1b). At 30°C and 41°C, the protein synthesis is normal as shown by [^{3}H]leucine incorporation (fig.2c). The intracellular level of cyclic AMP was determined at both temperatures. Shifting the cells from 30°C to 41°C causes an almost immediate stop in the increase of cyclic AMP concentration (fig.2a).

We have previously shown that under normal conditions, during logarithmic growth, the synthesis of cyclic AMP and proteins are balanced [15]. This

Table 1
The mutation *dna*-B in strain CR 34-BT 313 is partially expressed at 30°C

Growth parameters	Growth at 30°C	Growth at 41°C
Cell mass doubling time	135 min	108 min
Cell number doubling time	260 min	No division
DNA synthesis[a]		
CR 34	100%	136%
CR 34-BT 313	36%	0%

[a] The percentage of DNA synthesis was calculated from the ratio of [^{14}C]thymidine incorporated into DNA to the optical density of the culture at 600 nm

The figure obtained with the parental strain CR 34 at 30°C was taken as 100%

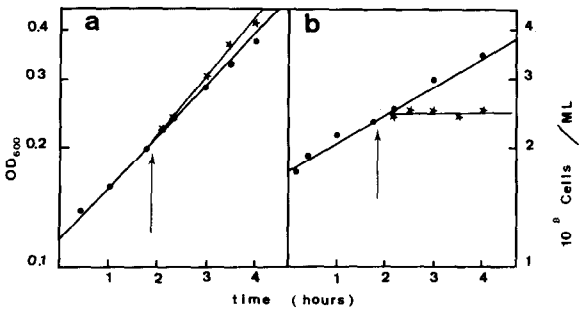

Fig.1. Effect of the temperature of the growth of the strain CR 34-BT 313 on cell mass increase and cell number. An exponential culture growing at 30°C was divided into 2 subcultures at the arrow. One was kept at 30°C as a control (•), the other one was shifted to 41°C (⋆). The cell mass ($A_{600\,nm}$) (a) and the cell number (b) were determined at both temperatures.

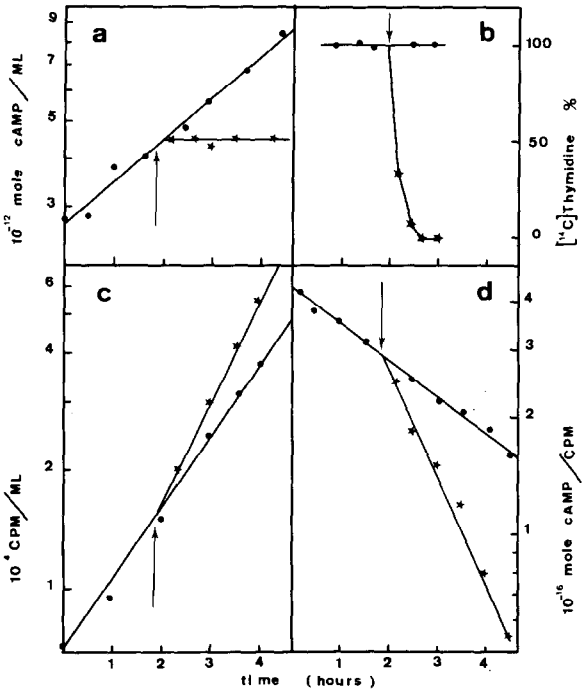

Fig.2. Effect of the mutation *dna*-B on protein and DNA synthesis and on cyclic AMP level. An exponential culture of CR 34-BT 313 growing at 30°C was divided into two subcultures at the arrow. One was kept at 30°C as a control (•); the other one was shifted to 41°C (⋆). Intracellular cyclic AMP concentration per ml of culture (a); percentage of [^{14}C]thymidine incorporation compared to the parental strain (CR 34) (b); the radioactivity incorporated into protein per ml of culture (c) and the ratio of intracellular cyclic AMP concentration to the radioactivity incorporated into proteins (d) were determined at both temperatures.

results in a parallel to the abcissa in a plot like that of fig.2d. Here we find with mutant CR 34-BT 313 that, at 30°C, cyclic AMP increases more slowly than protein. This is reflected by a linear decrease in the ratio of intracellular cyclic AMP concentration to the radioactivity incorporated into proteins (fig.2d). This decrease is much faster at 41°C since there is no more accumulation of cyclic AMP.

3.2. *Inhibition of DNA synthesis is not the primary cause of the alteration of the endogenous cyclic AMP level*

Cells of *E. coli* are able to regulate the synthesis of deoxy-ribonucleoside triphosphates in such a way that the triphosphate pools remain nearly constant, even when DNA synthesis is inhibited by specific drugs [16]. Thus, inhibition of DNA synthesis by itself would not seem to be the primary cause of the alteration of the cyclic AMP level. To check this point under the most similar conditions, we used another mutant of *E. coli* that is temperature-sensitive for DNA synthesis but its mutation is in the *dna*-E locus. The strain 4862 *dna*-E grows normally at 30°C. When cells are shifted to 41°C the DNA synthesis stops immediately (fig.3). As a consequence, cell division also stops after a short time (fig.3b). However, synthesis of cyclic AMP and protein are normal at 41°C (as well as at 30°C, fig.3a, 3c). Thus the endogenous cyclic AMP level is constant at both temperatures unlike in the *dna*-B strain. When the results are expressed as the concentration of cyclic AMP per cell, this concentration remains constant at 30°C during the part of the logarithmic growth followed; it increases of course at 41°C since the cells are not dividing any more (fig.4a), but the molarity within filaments and the ratio of intracellular cyclic AMP concentration to the radioactivity incorporated into proteins remain constant (fig.4b). This ratio appears to be lower than that observed with *dna*-B cells (fig.2d), but this is only the result of a much higher specific radioactivity of proteins in *dna*-E cells.

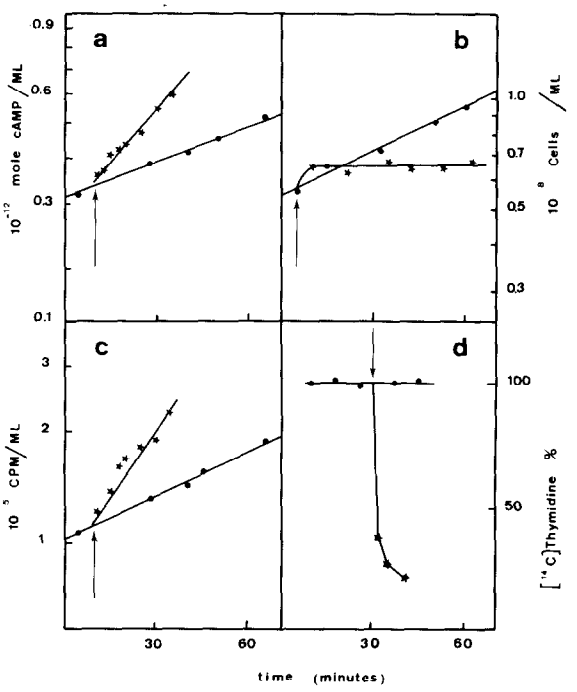

Fig.3. Effect of the temperature of growth of the *dna*-E strain on intracellular concentration of cyclic AMP, on cell number and on protein and DNA synthesis. An exponential culture growing at 30°C was divided into two subcultures. One was kept at 30°C as a control (•); the other one was shifted to 41°C (⋆). The intracellular concentration of cyclic AMP per ml culture (a), the cell number (b), the radioactivity incorporated into proteins per ml culture (c), and the percentage of [^{14}C]thymidine incorporation compared to the parental strain (d).

4. Discussion

Our results suggest an indirect relationship between the *dna*-B mutation and the synthesis of cyclic AMP. In the mutant CR 34-BT 313 that we used, the *dna*-B phenotype is partially expressed at 30°C since DNA synthesis is less than half of that of the parental strain. However, the coupling between DNA replication and cell septation [17,18] is conserved, and mutant cells are dividing half as often as parental cells (fig.1a, 1b).

It is striking to observe that this results at 30°C in an unbalance between intracellular cyclic AMP and protein content (fig.2d). At 41°C, the *dna*-B phenotype is totally expressed and there is a complete cessation of cyclic AMP accumulation in *dna*-B cells. Thus, it appears the impairment to cyclic AMP accumulation in this mutant is proportional to the extent of the expression of the *dna*-B mutation.

The results obtained with the *dna*-E mutant show

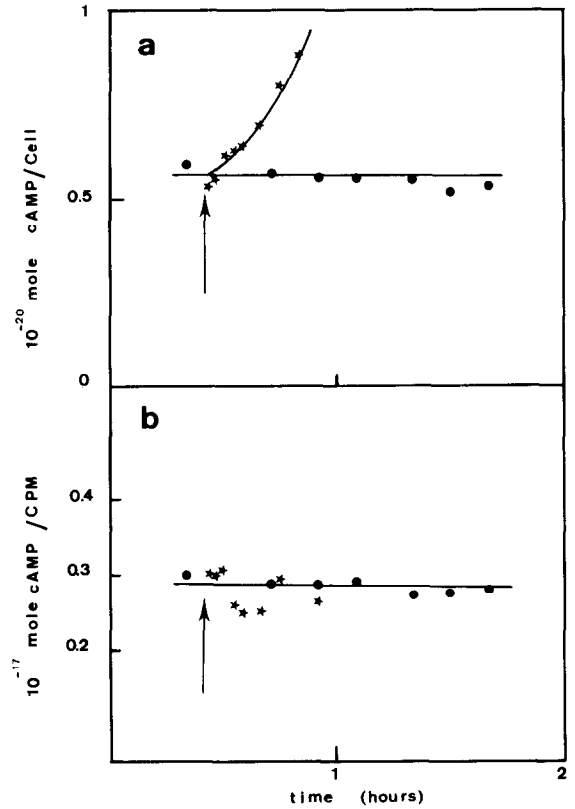

Fig.4. Mutation in *dna*-E locus have no effect on cyclic AMP level. The results contained in fig.3 have been plotted for comparison with *dna*-B mutant at 30°C (•) and 41°C (⋆) as the ratio of intracellular cyclic AMP concentration to the cell number (a) and to the radioactivity incorporated into proteins (b).

unambiguously that cessation of DNA synthesis by itself is not the primary cause of the observed phenomenon. As working hypothesis, we can propose two different explanations. A first possible explanation for the effect of *dna*-B mutation on cyclic AMP synthesis is that, as a consequence of the thermosensitivity of the ribonucleoside triphosphatase (*dna*-B gene product), the sizes of ribonucleoside triphosphate pools and their deoxy-counterparts increase as shown with the *dna*-B mutant FA 22 [19]. These triphosphates might then compete successfully with ATP for adenyl-cyclase and prevent further synthesis of cyclic AMP, since it has been shown that cyclic AMP synthesis is limited by the ATP pool size [20]. Such a situation would not occur in the *dna*-E mutant cells since the *dna*-E gene product is polymerase III [2,3]. A second possible explanation for the effect of *dna*-B mutation on the endogenous cyclic AMP level is that the permeability of the cell envelope to cyclic AMP is altered as a consequence of the mutation. The report that protein composition and turnover rates of the membrane proteins in *dna*-B mutants are altered at restrictive temperature [8], lends support to this second hypothesis.

In order to understand the physiological role of the *dna*-B gene product which has been shown to have a ribonucleoside triphosphatase activity [4], it is useful to know all the in vivo effects on the mutation and to sort out primary from secondary physiological effects. There is evidence that some of these effects are related to an alteration of the cell membrane [8,21]. We suggest that some effects might also be related to an alteration in the endogenous level of cyclic AMP. As mentioned above, this alteration might itself be a consequence of an envelope defect. We are currently investigating these hypotheses.

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