SHORT COMMUNICATION

Mis-transcription During Uridine Starvation in
Escherichia coli K12

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Although no β-galactosidase activity could be induced in Escherichia coli K12 during uridine starvation, material which cross-reacted with antiserum against β-galactosidase could be detected. The synthesis of enzymically inactive proteins during uridine starvation appeared to be due to errors in transcription.

INTRODUCTION

During pyrimidine and purine deprivation of the corresponding Escherichia coli auxotrophs, RNA synthesis continues at a reduced rate, though net RNA accumulation stops (Lazzarini et al., 1969; Erlich et al., 1975). All species of RNA (messenger, ribosomal and transfer) are synthesized and the required nucleotide is made available through RNA degradation. During uracil starvation of E. coli 15 TAU, Lazzarini et al. (1969) showed that protein synthesis, as measured by the incorporation of radioactive amino acids, also continued at a rate comparable to that of RNA synthesis; the rates of protein and RNA synthesis were 25% and 16% of the non-starved rate, respectively, after 1 h starvation.

We were interested to determine whether starved bacteria could synthesize specific enzymes since this would give information about the qualitative nature of transcription during purine or pyrimidine starvation. Nakada (1963) and Lazzarini et al. (1969) found that uracil-starved E. coli could synthesize β-galactosidase if grown in the absence of glucose to avoid catabolite repression. However, it is important to note that when growth is restricted by carbon source depletion, the requirement for uracil may be reduced so much that the cells may no longer be effectively starved of uracil. Indeed, we found that uridine auxotrophs in glucose-depleted medium synthesized β-galactosidase at the same rate whether or not uridine was added to the medium. We therefore decided to overcome catabolite repression by growing the cells with glycerol instead of glucose as the carbon source, and inducing β-galactosidase in the presence of adenosine 3':5'-monophosphoric acid (cyclic AMP). The results suggest that transcription during uridine starvation in E. coli K12 may be error prone.

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Fig. 1. Protein synthesis in uridine-starved and non-starved cells.

(a) A few minutes before the cells were to starve (0 min), one 20 ml portion of the culture was added to 2 μCi [14C]leucine and another 20 ml portion to 2 μCi [14C]leucine plus unlabelled uridine (9 μg ml⁻¹). Samples (1-0 ml) were taken periodically into trichloroacetic acid and the radioactivity incorporated into proteins was determined. The arrow indicates the time of onset of starvation as determined from $A_{420}$. Radioactivity incorporated into proteins of non-starved cells (○—○), starved cells (●) and non-starved cells corrected for increase in cell density (○——○).

(b) A few minutes before the cells were to starve (0 min), 5 ml portions of the culture were induced for β-galactosidase. Other 5 ml portions to which uridine was restored (3 μg ml⁻¹) were similarly induced. Samples (0.5 ml) were removed periodically for enzyme assay. The arrow indicates the time of starvation. Enzyme activity in non-starved cells (○) and starved cells (●).

(c) To assay for β-galactosidase cross-reacting material (CRM), a 200 ml portion of a culture starved of uridine for 10 min was induced for β-galactosidase. Protein synthesis was stopped by adding chloramphenicol (100 μg ml⁻¹) to this culture after the desired period of induction and also to a parallel uninduced culture. Cells were harvested by centrifuging, washed and resuspended in 1-0 ml 0.1 M-sodium phosphate buffer, pH 7.0, and lysed. The broken-cell suspension was clarified by centrifuging at 20000 g for 20 min. The supernatant solution was used for assay of CRM. The lysates tested were from uninduced cells (○), cells induced for 10 min (●) and cells induced for 30 min (△).

(d) After 10 min starvation, one 20 ml portion of the culture was induced for β-galactosidase while another 20 ml portion remained uninduced. A 200 ml portion of the culture to which uridine had
METHODS

Organism and growth conditions. A leucine-proline-uridine auxotroph of E. coli K12 (W3110) was grown essentially as described by Goldstein et al. (1959). For determination of the nucleotide composition of RNA, the medium was adapted from Volkin & Astrachan (1956). For non-starved cells the phosphate concentration used was one-tenth of that specified, while for starved cells, it was one-fifth. Glucose was used as carbon source for all experiments, except those involving assay of β-galactosidase (EC 3.2.1.23), δ-serine dehydratase (EC 4.2.1.14) and tryptophan decarboxylase (EC 4.1.1.28), where glycerol was used instead. For assay of δ-serine dehydratase the growth medium was supplemented with 0.2 % Casamino acids. All experiments were performed at mid-exponential growth phase.

Induction and assay of enzymes. Cells were induced for β-galactosidase by adding isopropyl β-D-thiogalactopyranoside and cyclic AMP, both at a final concentration of 0.5 mM. For kinetic studies, 0.5 ml samples were transferred periodically to chilled tubes containing 0.01 ml toluene, the contents were mixed vigorously for 30 s and left in ice. β-Galactosidase was assayed as described by Pardee et al. (1959); one unit of enzyme is the quantity which produces 1 nmol o-nitrophenol min⁻¹. The assay for δ-serine dehydratase was based on that of Pardee & Prestidge (1955); cells were induced by the addition of δ-serine (500 μg ml⁻¹) and cyclic AMP (0.5 mM). The assay for tryptophan decarboxylase was based on that of Pollard & Davis (1970); cells were induced by adding L-tryptophan (500 μg ml⁻¹). The assay for acetate kinase (EC 2.7.2.1) was based on the method of Rose et al. (1954).

Assay for β-galactosidase cross-reacting material (CRM). The assay for CRM was based on the method of Berg & Zabin (1964). Protein was determined by the Lowry method, using bovine serum albumin as standard.

Determination of nucleotide composition of RNA. Incorporation of [³²P]orthophosphate was terminated by pouring the cells into ice-cold 0.1 M-sodium phosphate buffer, pH 7.0, containing 0.02 M-Na₂SO₄. The cells were immediately harvested by centrifuging at 4 °C, washed three times with ice-cold 0.1 M-sodium phosphate buffer and lysed ultrasonically. The lysate was treated with DNAase (ribonuclease free; 50 μg ml⁻¹) at 0 °C for 10 min, 0.1 ml 15 % (w/v) sodium dodecyl sulphate was added. RNA was extracted by a modification of the usual phenol procedure (Goldstein et al., 1964). The RNA was further purified by passage through a column of Sephadex G-25. The method of Bautz & Hall (1962) was followed for nucleotide analysis. Non-radioactive E. coli stable RNA (1.5 mg) was mixed with radioactive RNA and hydrolysed with KOH. The mixture of 2',3'-ribonucleotides was applied to a column of Dowex-1-formate and the individual nucleotides were eluted sequentially. Completion of elution of each nucleotide was determined from the absorbance at 260 nm. Radioactivity in five peak fractions of each nucleotide was determined from Cerenkov radiation (Gould et al., 1972). Frequently, a large contamination of [³²P]orthophosphate co-eluted with UMP from Dowex-1-formate column. This was especially so with RNA from starved cells. The five peak fractions of UMP were pooled, brought to pH 6.5 with solid Na₂CO₃, and [³²P]orthophosphate was separated from UMP by the method of Cohn (1950), using a Dowex-1-chloride column. The fractions from this column were assayed for absorbance and radioactivity as before.

RESULTS AND DISCUSSION

Whereas incorporation of [¹⁴C]leucine into trichloroacetic acid precipitable protein proceeded, after the onset of uridine starvation, at a linear rate which was 30 to 40 % of the non-starved control, β-galactosidase activity levelled off completely within 10 min (Fig. 1a, b). This cessation of active enzyme synthesis during starvation was not peculiar to β-galactosidase, since two other inducible enzymes tested (δ-serine dehydratase and tryptophan decarboxylase) and one constitutive enzyme (acetate kinase) showed similar kinetic patterns (data not shown). Two events could lead to this result. (i) A different class of proteins is synthesized during starvation and the enzymes tested do not belong to it. (ii) The

been restored was also induced for β-galactosidase. After 30 min induction, protein synthesis was terminated in all cultures and cell extracts were prepared as described above. The indicated amounts of extract from non-starved, induced cells were mixed with extract from starved, uninduced cells containing 18 μg protein (O) or with extract from starved, induced cells containing 18 μg protein (●) and incubated with a fixed amount of antiserum to assay for CRM. (The point● representing enzyme activity in the supernatant as a result of 25 μg protein input was too high to be accommodated within the scale).
Table 1. Nucleotide composition of pulse-labelled RNA* and stable RNA†

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* Non-starved cells (5 ml) were allowed to incorporate 4 mCi [32P]orthophosphoric acid for 15 s at 37 °C and starved cells (5 ml) were allowed to incorporate 6.5 mCi for 2 min at 37 °C. Other experimental details are given in Methods.

† Non-starved cells and cells starved of uridine for 10 min were allowed to incorporate 1 mCi [32P]-orthophosphoric acid for 40 min at 37 °C. Rifampicin (300 µg ml⁻¹) was added and incubation was continued for 20 min to get rid of unstable RNA. The stable RNA was then extracted and nucleotide composition determined as described in Methods.

†† Cells resuspended in medium lacking phosphate.

§ Phosphate not removed from the medium.

proteins tested are synthesized, but, due to errors, they are enzymically inactive. If the second possibility is true, the inactive proteins may still retain antigenic determinants. Starved cells were therefore tested for the presence of β-galactosidase cross-reacting material (CRM). Increasing amounts of authentic β-galactosidase were added to a fixed amount of antiserum and extract from starved cells, the antigen–antibody precipitate was removed by centrifugation and the enzyme activity appearing in the supernatant solution was measured (Fig. 1 c). If extract from starved cells induced for β-galactosidase contained CRM, it should compete with the added enzyme for the same sites on the antibody. Consequently, enzyme activity should appear in the supernatant at lower enzyme inputs where extract from starved, induced cells was present, compared with a control where the extract was from starved, uninduced cells. Figure 1(c) shows that a significant amount of β-galactosidase CRM was present in starved, induced cells. To determine the amount of CRM in starved cells relative to that in non-starved cells induced for the same time period, the experiment described in Fig. 1(d) was performed. When extract from starved, uninduced cells containing 18 µg protein was present in the reaction mixture, extract from non-starved, induced cells containing 14.5 µg protein was required to saturate the input antiserum. However, when extract from starved, induced cells containing 18 µg protein was present,
saturation could be achieved with extract from non-starved, induced cells containing only 9.5 µg protein. Therefore, the ratio of CRM in starved, induced cells to that in non-starved, induced cells is of the order of 5:18.

Nakada & Magasanik (1964) found that E. coli induced for β-galactosidase in the presence of 5-fluorouracil synthesized enzymically inactive protein which could cross-react with β-galactosidase antiserum. They inferred that this was due to the incorporation of 5-fluorouracil into β-galactosidase mRNA.

If, during uridine starvation, enzymically inactive proteins are synthesized as a result of transcriptional errors, these might be reflected in the nucleotide composition of RNA from starved cells. Our measurements of the nucleotide composition of pulse-labelled and stable RNA from both starved cells and non-starved ones are presented in Table 1. When the t-test was applied to the results, it became evident that the UMP content of pulse-labelled RNA from starved cells was significantly decreased (confidence level > 99%) while the amounts of CMP, AMP and GMP was not altered significantly. In the case of stable RNA from starved cells, the UMP content was significantly decreased (confidence level > 99.8%) while AMP was significantly increased (confidence level > 98.9%). The amount of the other two nucleotides was not significantly different.

It therefore appears that during uridine starvation, the RNA transcripts are not made accurately and this leads to the synthesis of enzymically inactive proteins.

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REFERENCES


