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Bacterial adaptation: macromolecular biosynthesis during diauxic growth of *Escherichia coli*

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1. SUMMARY

Synthesis of DNA, RNA and protein was studied during a diauxic adaptation (glucose- to acetate-utilization) of *Escherichia coli* B. The multiphasic transition described illuminates certain features of the mechanisms regulating metabolism of the 3 macromolecules involved in the genetic flow of information.

2. INTRODUCTION

Many bacterial species are more versatile than the gram-negative *Enterobacteriaceae* and are better able to adapt to external environments. The gram-positive soil bacterium *Bacillus subtilis*, for example, can form spores that are highly resistant to various chemical and physical agents upon encounter of adverse conditions [1]: it excretes during sporulation specific antibiotic materials; it avoids flagellation during exponential growth in rich media [2]; it suppresses cell division under such conditions and hence grows helically to form macrobes [3]; it maintains a constant protonmotive force by decreasing its membrane electrical potential at low pH [4]. None of these abilities is displayed by *Escherichia coli*, but this organism exhibits a biphasic type of growth (diauxie) due to successive adaptations when limiting concentrations of two carbohydrates supplement a culture medium [1]. The more rapidly adapting system for utilization is formed first and exerts suppression on the formation of the other. Hence one observes a rapid growth of the culture until utilization of the first substrate is complete and then, after a plateau in the growth curve, a rise albeit slower growth rate, due to utilization of the second substrate. Complete exhaustion of the first carbon source precedes depression of the second system and the length of the intermittent lag period reflects difficulties in producing the enzymes involved in utilizing the second carbon source without their presence to catabolize it [5].

The mechanisms responsible for the diauxic adaptation are of great interest (e.g., [6,7]). Here we describe changes in composition and synthesis rates of the macromolecules involved in the genetic flow of information during such a transition in *E. coli* B.

3. MATERIALS AND METHODS

E. coli B, strain KM98 (derived from AS19 [8]), was exploited in this study. It is polyauxotrophic (Thy^-, Ura^-, Leu^-); i.e., it requires a necessary building block specific for each of the 3 macromolecules, thus allowing direct determinations of their relative amounts or synthesis rates simply by following incorporation of radiolabelled precursors (circumventing the complication of relaxed

repression of the pathways through which they are produced).

Cells were cultivated in AB minimal salts solution [9] supplemented with 0.4% of the appropriate carbon source. The cultures were vigorously aerated at 37°C in a New Brunswick gyratory shaker, and growth monitored by absorbance increment at 450 nm (A_{450}) (Gilford microsample spectrophotometer). For diauxic studies, the lag was reached at $A_{450} \cong 0.5$, by using 0.015% glucose as the first carbon source.

Relative macromolecular contents were determined by following incorporation into the cold trichloroacetic acid insoluble material of the appropriate labeled precursor, using the following conditions: 0.05 μCi/(5 μg/ml) [^{14}C]thymine, 0.02 μCi/(5 μg/ml) [^{14}C]uracil and 0.1–1.5 μCi/(20 μg/ml) [^{3}H]leucine.

4. RESULTS AND DISCUSSION

The most efficient carbon source for fast growth and catabolite repression is glucose. The second was chosen among 4 candidates (Fig.1A): proline did not allow further multiplication for at least 48 h after glucose exhaustion; the lag period in glycerol as second carbon source was too short (about 25 min) for detailed studies; succinate resulted in at least 5 h of diauxic lag, but a more convenient period (about 120 min) was obtained with acetate (Fig.1A).

The differential accumulation per mass (expressed as A_{450}) of DNA, RNA and of protein during the transition chosen (glucose- to acetate-utilization) is shown in Fig.1B. The transition was multiphasic with respect to each of these macromolecules. DNA synthesis continued at a decelerating rate for about 80 min and started again soon after the rise in absorbance. The increment of DNA in the initial period (some 50%) was similar to that during glucose starvation alone (unpublished observations). Assuming that all rounds of chromosome replication in progress before glucose depletion went to completion but no further rounds were initiated, as is the case following inhibition of protein synthesis [10], this value is consistent with a replication time of 60 min, in good agreement

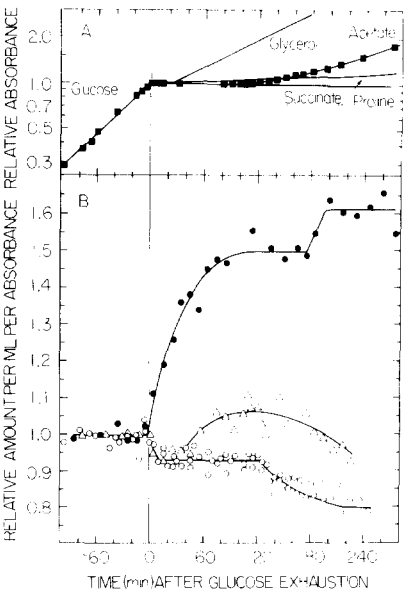

Fig. 1. (A) Growth (■); (B) relative macromolecular composition (other symbols) of *Escherichia coli* B cells, during a diauxic transition in a minimal medium supplemented by a mixture of acetate and a limiting concentration of glucose. Other lines in (A) represent different secondary carbon sources, as indicated; other symbols represent DNA (●), RNA (○) and protein (△). Growth is described in terms of absorbance at 450 nm. Data points were normalized to the respective values at the start of the transition.

with the values obtained for several other Thy$^-$ strains [11,12]. The further rise in DNA/mass ratio at onset of growth in acetate could be due to partial synchrony, in rounds of chromosome replication, that sometimes occurs upon dilution of early stationary cultures [13], but detailed studies have not been attempted. The transition has been completed by 4 h, but average DNA concentration (G/M)) [11] did not decline afterwards. Such a big difference in G/M between the two growth conditions is consistent with the conclusion [14–16] that cell mass/chromosome origin at initiation is smaller at slower growth rates. The only other explanation for this observation would assume an extremely fast chromosome replication in acetate. However, replication rate has been reported to slow down under slow growth [17], which rules out this theoretical explanation.

The fact that propagation of DNA synthesis continued at a normal rate before acetate is used for RNA or protein accumulation (Fig.1B) indi-

cated that the CO- or KCN- sensitive reaction involved in chromosome replication [18] was not inhibited by glucose starvation. It is not known whether the DNA elongation process does not demand a substantial amount of energy supplied from breakdown of storage macromolecules [19]. Another possible source here was breakdown products of RNA and protein, which did not seem to be utilized immediately. Ribonucleoside-diphosphates, products of RNA degradation, were likely also to serve as precursors for the ensuing DNA replication later (at about 120 min), after being converted to their deoxy counterparts by the responsible enzyme [20].

Accumulation of both RNA and protein ceased immediately after glucose exhaustion (Fig.1B) Moreover, during the first 10 min they were degraded to a certain extent. The degree of RNA degradation (5–9%) was somewhat higher than the relative amount that is mRNA (3–4.5%) [5], suggesting that some of the rRNA and tRNA lost stability under the shift-down, in accord with earlier findings [5]. The further decrease in cellular RNA content upon resumption of growth was probably a consequence of partial repression of ribosomal biogenesis during slow growth. The value

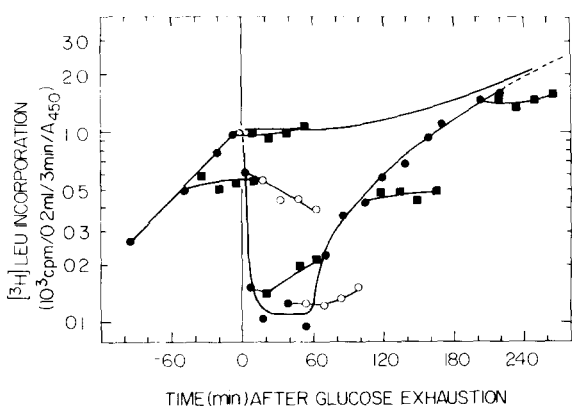

Fig. 2. Relative rate of protein synthesis (●) and degree of degradation of newly-made protein (other symbols) during a diauxic adaptation, similar to that described in Fig.1. Determinations were made by incorporation of [^{3}H]leucine during a 3 min pulse, and retention of the label as TCA-insoluble material during the subsequent chasing period, respectively. The upper line represents relative growth, transcribed from Fig.1A. Data points were normalized to the respective values at the start of the transition.

of relative RNA/mass reached at 4 h (0.8, Fig.1B) is still higher than that (about 0.7) obtained under steady-state growth in acetate (H. Jacobson, cited in [5]), demonstrating lack of completion of the transition under study.

Knowledge concerning protein turnover is less established [21]. The degradation observed during the initial 15 min (about 5%) was much higher than that under fast growth in rich media (1–2%/h), but closer to that under carbon source starvation (about 5%/h) [22]. Relative protein/mass had been expected to rise eventually to about 1.05 [5], as it actually did at around 2 h (Fig.1B), but the reason for its decrease later is unclear. The decrease in protein concentration can be explained by an increased degradation rate or by a decreased synthesis rate.

In order to discriminate between the two possibilities, portions of an unlabeled culture were pulsed with [^{3}H]leucine [3 μCi/(20 μg/ml)] for 3 min, and cold TCA-insoluble material determined (Fig. 2). A 100 fold concentration (2 mg/ml) was used for the chase, to determine degradation of newly-made protein. The rate of protein synthesis, increasing exponentially during the preshift (at a rate constant equivalent to the growth rate in glucose), fell sharply to 10% of its preshift level within 10 min after glucose exhaustion. Protein synthesis resumed at about 60 min with an accelerating rate, concommittant with the onset of rise in protein concentration (Fig.1B). However, degradation of newly-made protein was negligible (Fig.2). It remains to be seen whether pre-existing protein is later preferentially turned-over.

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REFERENCES

[1] Oginsky, E.L. and Umbreit, W.W. (1954) An Introduction to Bacterial Physiology, pp. 341–371, W.H. Freeman and Company, San Francisco.

[2] Zaritsky, A. and Macnab, R.M. (1981) J. Bacteriol. 147, 1054–1062.

[3] Mendelson, N.H. (1978) Proc. Natl. Acad. Sci. USA 75, 2478–2482.
[4] Khan, S. and Macnab, R.M. (1980) J. Mol. Biol. 138, 599–614.
[5] Maaløe, O. (1979) In: Biological Regulation and Development (Goldberger, R.F. Ed.) Vol. 1, pp. 487–542, Plenum press, New York.
[6] Harshman, R.B. and Yamazaki, H. (1971) Biochemistry 10, 3980–3982.
[7] Braedt, G. and Gallant, J. (1977) J. Bacteriol. 129, 564–566.
[8] Pato, M.L and v. Meyenburg, K. (1970) Cold Spring Harbor Symp. Quant. Biol. 35, 497–504.
[9] Clark, D.J. and Maaløe, O. (1967) J. Mol. Biol. 23, 89–112.
[10] Lark, K.G., Repko, T. and Hoffman, E.J. (1963) Biochim. Biophys. Acta 76, 9–24.
[11] Zaritsky, A. and Pritchard, R.H. (1971) J. Mol. Biol. 60, 65–74.
[12] Ephrati-Elizur, E. and Borenstein, S. (1971) J. Bacteriol. 106, 58–64.
[13] Cutler, R.G. and Evans, J.E. (1966) J. Bacteriol. 91, 469–476.
[14] Churchward, G., Estiva, E. and Bremer, H. (1981) J. Bacteriol. 145, 1232–1238.
[15] Pritchard, R.H. (1978) In: DNA Synthesis: Present and Future (Molineux, I. and Kohiyama, M. Eds.) pp. 1–26, Plenum press, New York.
[16] Zaritsky, A. and Zabrovitz, S. (1981) Mol. Gen. Genet. 181, 564–566.
[17] Helmstetter, C.E. and Pierucci, O. (1976) J. Mol. Biol. 102, 477–486.
[18] Cairns, J. and Denhardt, D.T. (1968) J. Mol. Biol. 36, 335–342.
[19] Govons, S., Vinopal, R., Ingraham, J.L. and Preiss, J. (1969) J. Bacteriol. 97, 970–972.
[20] Filpula, D. and Fuchs, J.A. (1978) J. Bacteriol. 135, 429–435.
[21] Goldberg, A.L. and St. John, A.C. (1976) Annu. Rev. Biochem. 45, 747–803.
[22] Yen, C., Green, L. and Miller, C.G. (1980) J. Mol. Biol. 143, 21–33.