ON THE NATURE OF THE CONTROL BY RC GENE IN E. COLI: AMINO ACID-DEPENDENT CONTROL OF LIPID SYNTHESIS

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Most amino acid auxotrophs of E. coli fail to synthesize RNA when deprived of any required amino acid from the culture medium(Pardee and Prestidge, 1956). On the other hand, it was known that a certain methionine-requiring mutant of E. coli(K12 W-6 or 58-161) accumulates RNA in the absence of methionine(Borek, Ryan and Rockenbach, 1955). From the genetic studies by Stent and Brenner(1961), it was demonstrated that the locus "RC" on E. coli chromosome is responsible for this amino acid-dependent control of RNA synthesis. The normal allele of this gene was designated "RC^{str}" for "stringent" amino acid control of RNA synthesis, and the mutant allele, "RC^{rel}" for its "relaxed" control.

Subsequent studies on "RC" control system has therefore been focused mainly on the regulation of RNA(Maaløe and Kjeldgaard, 1966). However, no evidence has so far been obtained indicating that biosynthesis of RNA is the only pathway which is selectively regulated by the product of RC gene. Accordingly, it seemed of interest to examine the possibility as to whether any other metabolic pathway in amino acid auxotrophs of E. coli is also under the rule of RC control.

In this paper, we wish to report some of the results of our experiments demonstrating that the incorporation of 1-^{14}C-acetate into lipid fraction is greatly reduced in the absence of required amino acid in RC^{str} strains but not

in RC^{rel} strains. Furthermore, as in the case of RNA synthesis(Aronson and Spiegelman, 1961), the addition of chloramphenicol(CM) eliminates the inhibition of lipid synthesis in amino acid starved cells and the rate of incorporation of the radioactive acetate is restored to the same level as in the presence of required amino acid.

MATERIALS AND METHODS

E. coli K12 strains W677(Leu^-, Thr^-, B_1^-, RC^{str}) and 58-161(Met^-, RC^{rel}) were supplied from Dr. J. Tomizawa of the National Institute of Health, Tokyo. CP78(Leu^-, Thr^-, Arg^-, His^-, B_1^-, RC^{str}) and CP79(Leu^-, Thr^-, Arg^-, His^-, B_1^-, RC^{rel}), originally isolated by Dr. N. Fiil of the University Institute of Microbiology, Copenhagen, were kindly given by Dr. P. Lengyel of the Department of Molecular Biophysics, Yale University, New Haven. These two strains are derivatives of W677, and isogenic except RC locus(Fiil and Friesen, 1968).

The cells were grown at 37° with shaking in M9 medium supplemented with amino acid requirements(each 20 ug/ml) and thiamine(2 ug/ml) to a density of 1.5-2.5 x 10^8 cells/ml. The bacteria were harvested by centrifugation, washed once with M9 medium without amino acid and suspended in the same medium to half of the original volume.

The synthesis of lipid was followed by incorporation of 1-^{14}C-acetate into lipid fraction as follows: To 2.6 ml of the above cell suspension were added 0.21 umole of 1-^{14}C-acetate(2 uC/umole), and amino acids and CM at the final concentrations of 40 ug/ml when specified. The final volume was adjusted to 2.8 ml. After incubation at 37°, 0.5-ml aliquots were taken at various times, pipetted into 3 ml of ice-cold distilled water containing carrier cells, and centrifuged. The pellets were suspended in 4 ml of chloroform-methanol (1:1, v/v), allowed to stand at room temperature overnight. The extracts were then washed twice with 1.0 ml and 0.5 ml of distilled water, placed in alumina planchets and dried under an infrared lamp. The radioactivity was counted with an Aloka windowless gas-flow counter. 1-^{14}C-acetate was purchased from C. E. A., Sacley.

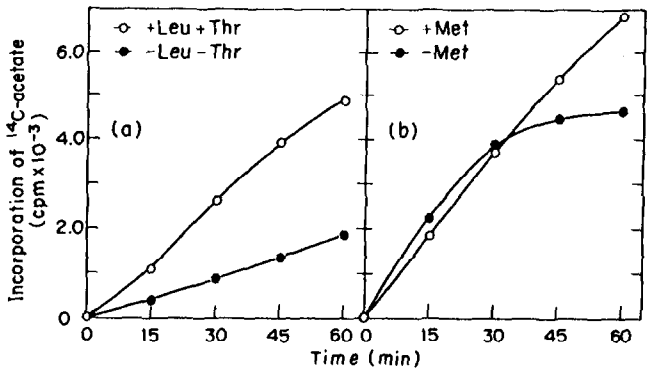

Fig. 1. Incorporation of 1-^{14}C-acetate into lipid in stringent and relaxed auxotrophs of E. coli in the presence and absence of the required amino acids. (a) W677(stringent strain), requiring leucine and threonine; (b) 58-161(relaxed strain), requiring methionine. Procedures were as shown in MATERIALS AND METHODS.

RESULTS

The effect of the removal of amino acid on the rate of incorporation of 1-^{14}C-acetate into lipid fraction was compared with two E. coli auxotrophs W677(Leu$^-$, Thr$^-$, B_1^-, RC^{str}) and 58-161(Met$^-$, RC^{rel}). As shown in Fig. 1, lipid synthesis in the stringent strain W677 was reduced when leucine and threonine were removed from the medium, whereas the relaxed strain 58-161 continued the synthesis of lipid in the absence of methionine.

In order to exclude the possibility that the above result was due to difference of the amino acid requirements rather than difference of the RC alleles, the similar experiment was repeated using the strain CP78(RC^{str}) and CP79(RC^{rel}). These two strains are isogenic except RC locus and require leucine, threonine, arginine and histidine. As illustrated in Fig. 2, the elimination of any of the above amino acids from the medium resulted in decrease of the rate of synthesis of lipid in the stringent strain CP78, whereas in the relaxed mutant CP79, the rate was practically unaffected by removal of either any or all of the required amino acids.

These results indicate that, in the stringent strains of E. coli, the

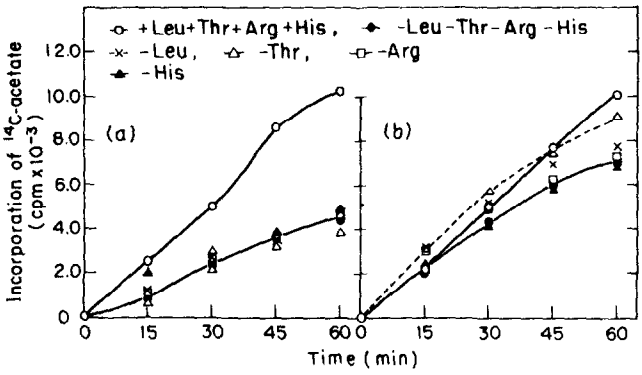

Fig. 2. Effect of amino acid deprivation on 1-^{14}C-acetate incorporation into lipid in isogenic stringent and relaxed strains. These strains require leucine, threonine, arginine and histidine. (a) CP78(stringent); (b) CP79 (relaxed). For details see MATERIALS AND METHODS.

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biosynthesis of lipid as well as RNA is under amino acid-dependent control. This conclusion is supported by the following experiment in which the effect of CM on the amino acid control of lipid synthesis was studied.

It has been known that CM induces RNA synthesis in amino acid auxotrophs of E. coli in the absence of essential amino acids(Aronson and Spiegelman, 1961). Therefore, it was expected that CM could eliminate the inhibition of lipid synthesis. The result in Fig. 3 shows that this was indeed the case.

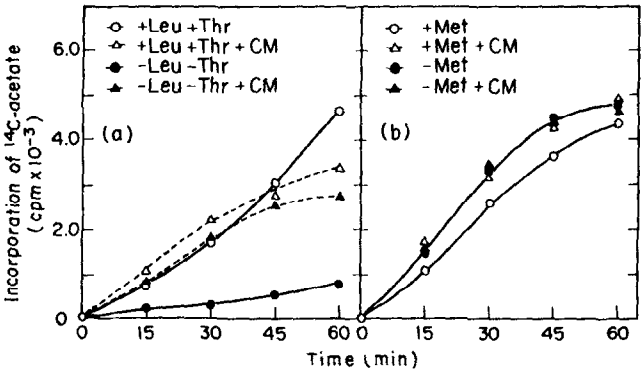

Fig. 3. Effect of chloramphenicol(CM) on 1-^{14}C-acetate incorporation into lipid in stringent and relaxed auxotrophs in the presence and absence of the required amino acids. (a) W677(stringent); (b) 58-161(relaxed). Experimental details were as indicated in MATERIALS AND METHODS.

In the absence of the essential amino acids but in the presence of CM, the synthesis of lipid in the stringent strain W677 proceeded almost at the same rate as when the medium was saturated with the required amino acids. In the relaxed mutant 58-161, the rate of the uptake of 1-^{14}C-acetate into lipid was similar either in the presence or absence of methionine irrespective to the addition of CM to the medium.

DISCUSSION

It has been believed that in amino acid auxotrophs of E. coli the function of the product of RC gene is the regulation of the synthesis of RNA. However, the present findings revealed that the lipid synthesis is also controlled by RC gene. It is also possible that metabolic pathways other than the syntheses of RNA and lipid are likewise subject to amino acid-dependent control. Our preliminary results indicate that the syntheses of total carbohydrates and acid-soluble nucleotides are controlled by RC gene, and that the addition of CM restores their syntheses in the absence of required amino acids(manuscript in preparation). Edlin and Neuhart(1967) and Cashel and Gallant(1968) have recently reported that RC gene regulates the level of precursors for RNA synthesis, i.e., the level of nucleoside triphosphates.

From these results it is obvious that many biosynthetic reactions in auxotrophs of E. coli are under amino acid control.

The elucidation of the detailed mechanism of this intriguing regulation, including the identity of the product of RC gene, must await further investigation.

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