Consequences of Expression of the "Relaxed" Genotype of the RC Gene

LIPID SYNTHESIS*

BURTON E. TROPP, LINDA C. MEADE,† AND PAUL J. THOMAS

From the Division of Science and Engineering, Richmond College of The City University of New York, Staten Island, New York 10801, and the Mayo Foundation, Rochester, Minnesota 55901

SUMMARY

Two pairs of strains that differ in the rel-1 locus were compared for their ability to synthesize protein, RNA, and lipid in the presence and absence of a required amino acid. In the absence of a required amino acid, protein synthesis ceased in both relaxed control (RCrel) and stringent control (RCstr) cells, and RNA synthesis ceased only in the RCstr cells. Lipid synthesis continued at an approximately equal rate in the presence or absence of a required amino acid in both RCrel and RCstr cells. Analysis of the lipid extracts showed a decrease in cardiolipin in RCrel cells deprived of a required amino acid. Otherwise, the distribution of the lipids was not affected by the absence of a required amino acid in either the RCrel or RCstr cells and was quite similar in both types of cells. The fatty acid distribution also appeared unaffected. These results are consistent with the view that the control of RNA synthesis exercised by the rel-1 locus cannot be simply at the level of nucleoside triphosphate availability.

When amino acid-requiring bacteria are cultured in media lacking necessary amino acids, there is a cessation of net RNA synthesis. The stringent dependence on amino acids for net RNA synthesis can be relaxed genetically by the presence of the rel-1 locus (1-3) or phenotypically by the presence of chloramphenicol in the growth medium (4, 5). It has recently been proposed that the difference in the control of net RNA synthesis exercised by the rel-1 locus cannot be simply at the level of nucleoside triphosphate availability. However, the observation that net lipid synthesis continues in RCrel cells deprived of amino acids but not RCstr cells (16) would certainly be consistent with control by nucleotides or by some other intermediate common to both synthetic pathways. We have therefore compared two pairs of strains that differ in the rel-1 locus for their ability to synthesize RNA, lipid, and protein in the presence and absence of a required amino acid and have characterized the lipids synthesized by one of the pairs cultured under these conditions.

MATERIALS AND METHODS

Chemicals—3H-6-Uracil (3.1 Ci per mole) was purchased from Schwarz BioResearch. 3H-1-Isoleucine (1.0 mCi/0.087 mg) was a product of New England Nuclear. 14C-2-Acetate was purchased from Mallinckrodt Nuclear (St. Louis, Missouri). The scintillation fluid used in all the experiments for monitoring 3H and 14H contained 2,5-diphenyloxazole (PPO), 16 g per gallon of toluene, and 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP), 0.8 g per gallon of toluene, both purchased from Sigma. All other chemicals were of reagent grade.

Bacteria and Culture Conditions

Escherichia coli PA1 (arg-, rel-) and E. coli PA2 (arg-, rel+), an isogenic pair except for the rel locus, belong to the TLB1 family of E. coli K-12. They were kindly provided by Dr. R. Lavallé. E. coli Tb6 (arg+, his-, rel-) and E. coli B77 (arg+, his-, rel+), an isogenic pair except for the arg and rel loci, are derivatives of E. coli B isolated by Dr. E. Ron. They were generously provided by Dr. B. Davis. The bacteria were cultured in standard Davis medium (17). The growth medium for PA1 and PA2 was supplemented with thiamine-HCl, 0.5 mg per liter, and where indicated with L-arginine, 100 mg per liter. The growth medium for Tb6 and B77 was supplemented with L-arginine, 100 mg per liter, and where indicated with L-histidine, 50 mg per liter. A fully supplemented overnight culture was diluted 25-fold into fresh supplemented minimal medium. Growth was followed on a Klett Summerson colorimeter. When the turbidity reached 80 to 90 Klett units, the cells were chilled in an ice bath and then harvested at 4°C in the Sorvall RC-2B centrifuge in the type SS-34 rotor at 15,000 rpm for 5 min. The cells were washed in 75% of the volume of cold

* This work was supported by a grant from The City University of New York.
† Recipient of a City University Predoctoral Research Assistantship.
minimal medium lacking the required amino acid. The cells were then resuspended in a volume of cold culture medium equivalent to 1.25 times the volume of the initial aliquot harvested.

**Assay for Protein Synthesis**

The culture medium was supplemented with L-isoleucine, 200 μg per ml, and "H-L-isoleucine, 0.1 μCi per ml (specific activity, 1.0 mCi/0.087 mg), as well as the necessary additions for growth. Incorporation of label into protein was determined by a slight modification of the procedure of Byfield and Scherbaum (18). At intervals, 0.10 ml was spotted onto Whatman No. 3MM filter paper discs. The discs were immediately placed in 5% trichloroacetic acid. The old trichloroacetic acid was poured out ½ hour after the last disc was collected, and the discs were washed for an additional 20 min with fresh 5% trichloroacetic acid. The process was repeated twice more, after which the filters were washed twice with acetone. Approximately 10 ml of 5% trichloroacetic acid were used for each of the discs in each wash and approximately half as much acetone was used. The dry discs were counted in toluene scintillator fluid in the Beckman model LS-150 scintillation counter.

**Assay for RNA Synthesis**

The culture medium was supplemented with uracil, 10 μg per ml, and "H-U-uracil, 0.1 μCi per ml (specific activity, 3.1 Ci per mmole) as well as the necessary additions for growth. The filter paper disc method described for measuring protein synthesis was used.

**Assay for Lipid Synthesis**

Two different procedures were used. The first method was essentially that of Sokawa et al. (16). The culture medium was supplemented with potassium acetate, 90 μg per ml and "C-2-acetate, 0.09 μCi per ml (specific activity, 17 mCi per mmole) as well as the necessary additions for growth. The samples were dried in scintillator vials either by overnight evaporation or by heating at temperatures below 50°C. The second method was a more extensive modification of the procedure of Sokawa et al. (16). Aliquots of 2 ml each were removed and mixed with chilled unlabeled carrier cells and centrifuged in the cold. After all the samples had been collected, the pellets were washed once with 2 ml of cold Davis medium. The washed pellets were then extracted overnight with 4 ml of a 3:1 chloroform-methanol solution instead of the 1:1 chloroform-methanol solution used in the first method. The remainder of the procedure was identical with the first method. The radioactivity was determined by dissolving in toluene scintillator fluid and counting on the Beckman LS-150 scintillation counter.

**Lipid Analysis**—After the bacteria were incubated for 80 min, the lipids were isolated for analysis by scaling up the second method to 40 ml and omitting the addition of unlabeled carrier cells. Total lipid extracts were examined by thin layer chromatography on Silica Gel H (S. Merck, Darmstadt, Germany), with chloroform-methanol-water (65:25:4). The distribution of labeled compounds on the plates was determined by the zonal scanning technique of Snyder (19). In this procedure, the silica is mechanically scraped from the plates into counting vials, in 2-mm increments, starting below the origin and proceeding through the solvent front. A 1-ml sample of 10% glacial acetic acid in absolute ethanol and 15 ml of toluene-base scintillation fluid is added to each vial for counting. A duplicate chromatogram, run on some of the samples, was sprayed with sulfuric acid and heated to visualize the separated compounds.

Fatty acid methyl esters were prepared from the total lipids.

---

**Fig. 1 (left).** RNA synthesis determined by "H-uracil incorporation by E. coli PA1 and PA2 in the presence and absence of arginine. See text for the details of the assay system. 

**Fig. 2 (center).** Protein synthesis determined by "H-isoleucine incorporation by E. coli PA1 and PA2 in the presence and absence of arginine. See text for the details of the assay system.

**Fig. 3 (right).** Lipid synthesis determined by "C-acetate incorporation by E. coli PA1 and PA2 in the presence and absence of arginine. See text for the details of the assay system.
TABLE I

Total fatty acids in strains PA1 and PA2 after incubation with and without arginine

| Strain | Zero time | After 80 min of incubation |
|--------|-----------|----------------------------|
|        |           | Minus arginine | Plus arginine |
|        | mg        | mg             | mg            |
| PA1    | 0.88      | 1.33           | 1.10          |
| PA2    | 0.97      | 1.20           | 1.23          |

by transesterification with boron trichloride-methanol, or by saponification, extraction, and treatment with diazomethane. Results with the two methods were comparable. The methyl esters were separated by gas chromatography at 180°, on a glass column (6 ft. x 1/2 in) packed with 10% EGSS-X on 100 to 120 mesh Gas Chrom P (Applied Science Laboratories, Inc., State College, Pennsylvania). Peak areas were measured with a CRS-104 digital integrator (Infotronics Corporation, Houston, Texas). When radioactivity was to be measured, the effluent gas of the chromatograph was bubbled into a flowing stream of toluene-base scintillation fluid and collected automatically in serial vials at 45-sec intervals, as described by Dutton (20). The vials were counted in a scintillation counter and the counts plotted to provide a profile of the distribution of 14C in the methyl esters.

RESULTS

In the absence of the required amino acid, arginine, E. coli PA1 continues to synthesize RNA but E. coli PA2 does not (Fig. 1). Both E. coli PA1 and PA2 stop making protein under these same conditions (Fig. 2). As expected, similar results were obtained in the absence of histidine when E. coli Tb6 and B77 were studied. Lipid synthesis was not affected by the removal of arginine in E. coli PA1 or PA2 (Fig. 3). Similar results were obtained in the absence of histidine when E. coli Tb6 and B77 were studied. Extraction of the lipids with either 3:1 or 1:1 chloroform-methanol yielded similar results regarding acetate incorporation.

Total fatty acids of E. coli PA1 and PA2, as measured by gas-liquid chromatography with an internal standard (Table I), show a definite increase for both mutants during arginine starvation. Lipid turnover was also measured directly by resuspending (14C-acetate)-labeled cells in cold media in the presence and absence of arginine. The loss of 14C was less than 10% in each case, after 80 min of incubation at 37°.

Thin layer chromatography and sulfuric acid charring of the lipid extracts of E. coli PA1 and PA2 indicated four components, a neutral component migrating with the solvent front and three polar or phospholipid components migrating with an Rf

TABLE II

Distribution of 14C from 14C-2-acetate in total lipids

| Strain | Total 14C recovered |
|--------|---------------------|
| PA1, plus arginine | % | % | % | % |
| PA2, plus arginine | % | % | % | % |
| PA1, minus arginine | % | % | % | % |
| PA2, minus arginine | % | % | % | % |

* Tentative identification.

TABLE III

Incorporation of 14C into individual fatty acids from 14C-2-acetate

Fatty acids synthesized from 14C-2-acetate, as in Table II, were analyzed in duplicate in two separate experiments. The results presented are averages of the duplicates in each experiment. See the text for a detailed description of the methods.
phospholipid ($R_F 0.4$) coincided with phosphatidylethanolamine and gave a positive reaction with ninhydrin. A minor component with $R_F 0.65$ coincided with cardiolipin obtained from mouse liver. The other minor component, $R_F 0.3$, was assumed to be phosphatidylglycerol. The distribution of radioactivity in these lipid classes is summarized in Table II. With the possible exception of cardiolipin, the distribution is virtually identical for *E. coli* PA1 and PA2, whether the essential amino acid was present or absent.

The distribution of $^{14}C$ in the fatty acids, shown in Table III, was considered identical within the error of the analysis, and was not significantly altered by the presence or absence of arginine in either *E. coli* PA1 or PA2.

**DISCUSSION**

The present studies were designed to investigate whether the rel locus controls lipid synthesis as well as RNA synthesis. It is clear that RNA synthesis abruptly ceases upon the removal of a required amino acid in RCstr cells but not in RCrel cells. Lipid synthesis continues in both types of cells. The results concerning lipid synthesis are in disagreement with those of Sokawa et al. (16). However, even in their studies, abrupt cessation of lipid synthesis was not observed. These workers dried their samples by heating under an infrared lamp which we at first thought might account for the differences observed in total lipid synthesis provided that some component of the lipid fraction made in RCstr cells was more volatile than that present in RCrel cells. The lipid analyses do not support this idea because the lipids made by RCstr and RCrel cells in the absence of a required amino acid appear to be nearly the same. Decreased cardiolipin incorporation for the relaxed cells during arginine starvation seems to be the only reproducible difference. We have no explanation for this deviation, and its significance is not apparent.

The observation that lipid synthesis continues in both RCstr and RCrel cells in the absence of the required amino acid would seem to make it unlikely that the control of RNA synthesis is at the level of nucleoside triphosphate synthesis. This is particularly true for ATP and CTP which are necessary for the continued lipid synthesis observed. Starvation of an RCstr cell for a required amino acid does not appear to affect the synthesis of cyclopropane fatty acids. This must mean that S-adenosylmethionine continues to be synthesized in these cells. RNA synthesis may be controlled by the combined effects of the concentration of several nucleotides including the mono- and diphosphates or be sensitive to the concentration of GTP (11). To date, no consistent picture concerning the role of the rel locus has emerged. The independence of lipid synthesis from the rel locus is consistent with recent observations concerning noncoordinate control of RNA synthesis (12–15) and the reports that nucleoside triphosphates are not the limiting factors for RNA synthesis (9, 10).

**Acknowledgments**—We acknowledge the excellent technical assistance of Mrs. Kay Murphy of the Mayo Foundation.

**REFERENCES**

1. STENT, G., AND BRENNER, S., Proc. Nat. Acad. Sci. U. S. A., 47, 2005 (1961).
2. ALFOLDI, J., STENT, G., AND CLAWES, R., J. Mol. Biol., 5, 348 (1961).
3. EDLIN, G., AND BRODA, F., Bacteriol. Rev., 32, 206 (1968).
4. AARONSON, A., AND SPIEGELMAN, S., Biochim. Biophys. Acta, 53, 70 (1961).
5. KIRKLAND, C., AND MAJÉ, O., J. Mol. Biol., 4, 193 (1962).
6. GALLANT, J., AND CASHEL, M., J. Mol. Biol., 25, 545 (1967).
7. CASHEL, M., AND GALLANT, J., J. Mol. Biol., 34, 317 (1968).
8. EDLIN, G., AND NEUHAUS, J., J. Mol. Biol., 24, 225 (1967).
9. BAGNARA, A., AND FINCH, L., Biochem. Biophys. Res. Commun., 33, 15 (1968).
10. EDLIN, G., AND STENT, G., Proc. Nat. Acad. Sci. U. S. A., 62, 475 (1969).
11. GALLANT, J., AND HARADA, B., J. Biol. Chem., 244, 3125 (1969).
12. NIEBRUCHEN, D., Proc. Nat. Acad. Sci. U. S. A., 62, 1345 (1969).
13. FORCHHAMMER, J., AND KJELGAARD, N. O., J. Mol. Biol., 37, 245 (1968).
14. EDLIN, G., STENT, G., BAKER, R., AND YANOFSKY, C., J. Mol. Biol., 57, 257 (1968).
15. LAVELLE, R., AND DEHAUDT, G., J. Mol. Biol., 37, 269 (1968).
16. SOKAWA, Y., NAKAO, E., AND KAZIRO, Y., Biochim. Biophys. Res. Commun., 33, 108 (1968).
17. DAVIS, B., AND MINGOLI, E., J. Bacteriol., 60, 17 (1950).
18. BYFIELD, J., AND SCHEIBAUER, O., Anal. Biochem., 17, 434 (1966).
19. SNYDER, F., in S. ROTHCHILD (Editor), Advances in tracer methodology, Vol. 2, Plenum Press, Inc., New York, 1969, p. 107.
20. DUTTON, H. J., \*J. Amer. Oil Chem. Soc.,\* 38, 631 (1961).
Consequences of Expression of the "Relaxed" Genotype of the RC Gene: LIPID SYNTHESIS
Burton E. Tropp, Linda C. Meade and Paul J. Thomas

*J. Biol. Chem.* 1970, 245:855-858.

Access the most updated version of this article at [http://www.jbc.org/content/245/4/855](http://www.jbc.org/content/245/4/855)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/245/4/855.full.html#ref-list-1](http://www.jbc.org/content/245/4/855.full.html#ref-list-1)