EVIDENCE THAT PROTEIN SYNTHESIZED IN A HETEROLOGOUS CELL-FREE SYSTEM IS NOT FUNCTIONAL

B. R. Chatterjee and R. P. Williams

Department of Microbiology
Baylor University College of Medicine
Houston, Texas

Received August 7, 1962

Recent reports have described synthesis of proteins by Escherichia coli ribosomes in a cell-free system when ribosomal or "messenger" ribonucleic acid (RNA) is supplied (Nirenberg and Matthei, 1961; Ofengand and Haselkorn, 1962). It has been claimed that the genetic code for protein synthesis carried by the "messenger" RNA is universal and, when supplied with this information, a ribosomal system can synthesize any protein. The criterion for protein synthesis in these experiments was incorporation of labeled amino acids into the acid-precipitated proteins. We have planned the following experiments to determine if a functional protein could be synthesized in such a system, and if this could be accomplished in a heterologous system. Ribosomal RNA from E. coli cells induced to form β-galactosidase was used in E. coli (homologous) and Pseudomonas pyocyaneus (heterologous) cell-free systems. The results are reported in this paper.

MATERIALS AND METHODS

E. coli strains ML 35 and 15 T-U- (thymine and uracil dependent) were obtained through the courtesy of Dr. T. D. Brock, Department of Bacteriology, Indiana University. Strain ML 35 is at least three times more efficient than 15 T-U- in the induced formation of β-galactosidase. A locally isolated strai of P. pyocyaneus was used, and no induction of β-galactosidase could be demonstrated in the organism. All the strains were grown in nutrient broth. The β-galactosidase activity was assayed with the chromogenic substrate, O-nitrophenyl-β-galacto-pyranoside (ONPG, Calbiochem).

Preparations of buffer, ribosomes, soluble fraction and extraction of RNA from these fractions were done as described by Nomura et al. (1960). After centrifugation at 105,000 X g the supernatant or soluble fraction, designated as S-40, was incubated with 2 ug/ml of DNase (Nutritional Biochemical Corporation) and stored at -15°C. The ribosomal suspensions and extracted RNA solutions were also stored at -15°C.

Induced formation of β-galactosidase was carried out by suspending washed cells of E. coli ML 35 in a chemically defined medium with melibiose (Calbiochem) replacing the usual carbon source, maltose. After the necessary period of induction, ribosomal ("messenger") RNA was extracted from the cells as described (Nomura et al., 1960).

Glycine-1-C^{14} was used for radioactivity incorporation experiments, and in these cases was omitted from the complete amino acid mixture in the system to be described. Counting was done on the washed protein fraction precipitated by 10% TCA. Protein was estimated by the Lowry method (Lowry et al., 1951) and RNA, by the orcinol method (Hurlbert et al., 1954).

RESULTS AND DISCUSSION

The data in Table 1 demonstrate that β-galactosidase synthesis occurs and can be measured under these relatively simple conditions. Addition to the cell-free systems of the substrate, ONPG, for β-galactosidase induction resulted in prompt synthesis of the enzyme. Comparing the tempo of hydrolysis of ONPG in tubes No. 1 and 2, it is seen that while a mixture of ribosomes and supernatant fraction (S-40) from a competent strain (ML 35) can be promptly induced to synthesize the enzyme, β-galactosidase, addition of ML 35 ribosomal RNA markedly accelerated the reaction. Ribosomal RNA from E. coli 15 T-U- was less effective as a promoter of β-galactosidase synthesis in the E. coli ML 35 cell-free system (tube No. 3). As shown by tube No. 6, ML 35 ribosomal RNA is more effective in accelerating β-galactosidase synthesis in the E. coli 15 T-U- system than 15 T-U- ribosomal RNA itself (tube No. 5). Comparison of the cell-

TABLE 1

Induction of β-Galactosidase Activity in Various Cell-free Systems

Tube No.	Additions	ug ONPG hydrolyzed in minutes 2	5	10	20
1	*E. coli* ML 35 ribosomes " " S-40	210	650	1000	–
2	*E. coli* ML 35 ribosomes " " S-40 " " rib-RNA	370	890	1000	–
3	*E. coli* ML 35 ribosomes " " S-40 " 15 T-U- rib-RNA	200	600	1000	–
4	*E. coli* 15 T-U- ribosomes " " S-40	140	195	320	810
5	*E. coli* 15 T-U- ribosomes " " S-40 " " rib-RNA	170	280	410	890
6	*E. coli* 15 T-U- ribosomes " " S-40 " ML 35 rib-RNA	250	390	460	840
7	*P. pyocyaneus* ribosomes " S-40 *E. coli* ML 35 rib-RNA	0	0	0	0

The reaction mixtures contained 0.5 ml each of ribosomes (*E. coli* ML 35, 5 mg protein/ml; *E. coli* 15 T-U-, 4.5 mg protein/ml; *P. pyocyaneus*, 4.2 mg protein/ml), and S-40 fraction (ML 35, 2.1 mg protein/ml; 15 T-U-, 2.2 mg protein/ml; *P. pyocyaneus*, 1.8 mg protein/ml); 0.25 ml each of ribosomal RNA (6 mg/ml) where indicated (rib-RNA); ML 35 S-RNA (4 mg/ml); ONPG (4 mg/ml); 5 uM/ml phosphoenol pyruvate; 1 uM/ml ATP; 0.1 ug each of 20 L- or DL-amino acids; 30 ug of soluble pyruvate kinase; and 0.05 ug each of GTP, CTP and UTP. Total volume was 2.3 ml. The substrate, ONPG, was added at zero time.

free systems in tubes No. 1 and 4 also demonstrates that *E. coli* 15 T-U- is more sluggishly inducible than strain ML 35.

These experiments demonstrate the simplicity of our cell-free system, and the ability of the system to detect differences in β-galactosidase activity. Using this system different degrees of efficiency for synthesis of the enzyme by ribosomal RNA obtained from different strains of the same bacteria can be detected. It is reasonable to suppose that if the code for protein synthesis

borne by the "messenger" or ribosomal RNA is indeed universal, this RNA extracted from ribosomes of E. coli ML 35 induced to form β-galactosidase should be able to synthesize at least small amounts of the enzyme in a cell-free system of a different bacterium. However, contrary to expectations, addition to a P. pyocyaneus cell-free system of E. coli ML 35 ribosomal RNA from β-galactosidase-induced cells completely failed to register any β-galactosidase activity as measured by hydrolysis of ONPG.

The latest ingenious experiments of Tsugita et al. (1962) to demonstrate functional protein synthesis in an E. coli system with tobacco mosaic virus (TMV) RNA as the code suggest that only about 10% of the protein synthesized was virus specific TMV protein. They concluded that a part of this virus specific protein might actually be functional, but that it was not identical to the native TMV protein. The important criteria for the identical nature of the synthesized protein were similarity of a few peptides of the newly synthesized protein with that of native TMV protein, and incorporation into the virus coat of some newly synthesized, labeled protein in virus reconstitution experiments. we feel that the system we have employed to test functional protein synthesis has some advantages over the one described above since the newly synthesized protein is an active enzyme which can be easily assayed, and thus its presence determined. From our experiments we conclude that functiona β-galactosidase cannot be synthesized in the P. pyocyaneus cell-free system when ribosomal RNA from induced E. coli cells is used as the "messenger".

We examined the ability of ribosomal RNA from E. coli ML 35 to direct incorporation of labeled amino acids into proteins. The data for these experiments are shown in Table 2. While the induced E. coli ribosomal RNA did not promote synthesis of β-galactosidase in the cell-free P. pyocyaneus system, the "messenger" RNA does promote incorporation of glycine-1-C^{14} into protein almost to the same extent as in the E. coli cell-free system. When ribosomal RNA was not added to the systems, much less radioactivity was incorporated into protein.

TABLE 2

Efficiency of Glycine-1-C^{14}

Incorporation into the Protein of Various Cell-free Systems

Experiment No.	Addition	Percent of added glycine-1-C^{14} taken up in 30 min in the acid-precipitated fraction
1	*E. coli* ML 35 ribosomal system with *E. coli* ML 35 ribosomal RNA	Sample 1 18
		" 2 24.5
2	*E. coli* ML 35 ribosomal system without ribosomal RNA	" 1 7
		" 2 9
3	*P. pyocyaneus* ribosomal system with *E. coli* ML 35 ribosomal RNA	" 1 22
		" 2 17.5
4	*P. pyocyaneus* ribosomal system without ribosomal RNA	" 1 4
		" 2 6.5

The reaction mixtures contained the same ingredients as listed in Table 1 except without the addition of ONPG. Glycine in the amino-acid mixture was replaced by glycine-1-C^{14}. Acid-precipitated fraction was prepared by adding 10% TCA to the reaction mixtures.

These experiments demonstrate that protein synthesis, as indicated by incorporation of a radioactive amino acid, can occur in a cell-free system when ribosomal or "messenger" RNA is added from a heterologous source. However, the protein synthesized is not necessarily functional, as indicated by the failure of the system to show β-galactosidase activity.

ACKNOWLEDGMENT

This investigation was supported by research grant E-1535 (CRI) from the National Institutes of Health, U. S. Public Health Service.

REFERENCES

Hurlbert, R.B., Schmitz, H., Brumm, A.F., and Potter, V.R., J. Biol. Chem., 209, 23 (1954).

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem., 193, 265 (1951).

Nirenberg, M.W., and Matthei, J.H., Proc. Nat. Acad. Sci. (U.S.), 47, 1588 (1961)

Nomura, M., Hall, B.D., and Spiegelman, S., J. Mol. Biol., 2, 306 (1960).

Tsugita, A., Fraenkel-Conrat, H., Nirenberg, M.W., and Matthei, J.H., Proc. Nat. Acad. Sci. (U.S.), 48, 846 (1962).