VIRAL RNA-DEPENDENT INCORPORATION OF AMINO ACIDS INTO PROTEIN BY CELL-FREE EXTRACTS OF E. COLI

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Recent work in several laboratories (1-3) is consistent with the proposal of Jacob and Monod (4) that the information for specifying protein structure is carried from DNA to the site of protein synthesis, the ribosome, by a class of RNA molecules termed "messenger" RNA. Their proposal further requires that the ribosome be an unspecialized structure capable of making any protein if provided with a suitable messenger RNA. Messenger RNA is currently thought to obtain its information from DNA by a copying process in which DNA acts as a complementary template (5-7). It occurred to us that RNA from an RNA-containing plant virus might be able to act directly as its own messenger, and furthermore would provide an interesting test of the supposed ability of the ribosome to make any kind of protein. Accordingly, we have examined the effect of added turnip yellow mosaic virus (TYMV) RNA upon amino acid incorporation into protein by cell-free extracts of E. coli. In this communication we describe some of the characteristics of the RNA-dependent incorporation observed and in a subsequent paper some of the requirements for an active RNA will be reported. During the course of this work, the report of Nirenberg and Matthaei (8) appeared, which describes a similar stimulation of protein synthesis by RNA from E. coli ribosomes and from tobacco mosaic virus.

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MATERIALS AND METHODS

Ribosomes: Exponential phase cells of E. coli B were rapidly chilled, washed, and suspended in buffer consisting of 0.25 M sucrose, 0.02 M KCl, 0.01 M Tris pH 7.6, and 0.01 M MgOAc. Extracts were prepared by grinding with alumina according to Tissieres et al. (9) without DNase or by passage through a French pressure cell (10). After addition of sodium deoxycholate to 0.1% and Tris buffer pH 8.0 to 0.05 M, the ribosomes were isolated by two cycles of differential centrifugation and stored frozen in sucrose buffer.

Supernatant: Fully grown cells of E. coli B were harvested, washed, and disrupted by the methods described above. Ribosomes were removed by centrifugation of the supernatant two times at 105,000 x g for 180 minutes and the resulting supernatant fraction was dialysed overnight. After a five-fold concentration by lyophilization and re-dialysis, the supernatant was stored frozen in 0.01 M Tris, pH 7.6 and 0.0005 M glutathione.

Viral RNA: TYMV (11) was prepared by differential centrifugation of sap from infected Chinese cabbage leaves. RNA was separated from the protein of purified virus by brief shaking with water-saturated phenol and was isolated by precipitation with isopropanol followed by dialysis. This procedure has been shown to yield infectious RNA from TYMV (12).

C^{14}-amino acid mixture: An acid hydrolysate of C^{14}-Chlorella protein was used, supplemented with C^{12}-histidine, -methionine, -cysteine, -tryptophan, -glutamine, and -asparagine, which were absent from the algal hydrolysate. The final specific activity was approximately 9×10^6 cpm/mg amino acid mixture.

Incorporation assay: Reactions were stopped by addition of perchloric acid to 3.5% and then boiled for 15 minutes. The resulting precipitate was collected, washed twice with 3.5% perchloric acid at room temperature, dissolved in 1.7 N NH_3, and the radioactivity determined.

RNA concentration: RNA was measured by its absorbancy at 260 mμ and expressed as μmoles of nucleotide, weighted for the distribution in TYMV-RNA.

RESULTS

Characteristics of the incorporating system: An approximate 20-fold increase in ribosome-dependent amino acid incorporation

could be obtained by the addition of TYMV-RNA to the E. coli system (Table I). The amount of incorporation was dependent upon and proportional to the number of ribosomes present, required the presence of supernatant, energy source, and a complete mixture of amino acids, and was inhibited by chloramphenicol, puromycin, and ribonuclease. Amino acid acceptor RNA from E. coli (13) could not replace virus RNA nor did it stimulate the system when added in addition to viral RNA. Similarly, UTP + CTP had no effect when added in the presence or absence of viral RNA. A sharp optimum of Mg^{++} concentration was observed at 0.01 M, which varied slightly with different preparations of ribosomes. This result is similar to that reported for the non-RNA requiring E. coli system described by Tissieres et al (9).

Table I. Properties of Amino Acid Incorporation System

Exp.	Additions	cpm incorporated
I	Complete	1353
	Complete (2 x ribosomes)	3050
	-RNA	140
	-Ribosomes, -RNA	90
	-Ribosomes	80
	-Supernatant	0
	-GTP, ATP, PEP, PK	8
	-GTP	743
	+Chloramphenicol ($1.3_4 \times 10^{-4}$ M)	146
	+Puromycin (1.0×10^{-4} M)	71
	+RNase (0.4 µg/ml)	75
II	Complete	865
	-C^{12}-amino acids	93

The complete system contained in 0.5 ml, 25 µmoles Tris buffer pH 7.6, 35 µmoles KCl, 5 µmoles MgOAc, 0.5 µmole ATP, 0.1 µmole GTP, 2.1 µmoles phosphoenolpyruvate (PEP), 0.02 mg phosphoenolpyruvate kinase (PK), 5 µmoles mercaptoethanol, a saturating amount (3 mg) of supernatant fraction, 0.5 mg ribosomes, and a saturating amount (70 mµmoles) of TYMV-RNA. The reaction was started by addition of 0.5 mg of the C^{14}-amino acid mixture (Exp. I), and allowed to proceed for 30 minutes at 30°C. This time gave complete reaction. Deproteinization at zero time gave a blank of 104 cpm, which was subtracted. In Exp. II, 0.1 µmole of C^{14}-L-phenylalanine was used (5 x 10^6 cpm/µmole) plus 0.05 µmole of each of the other C^{12}-amino acids.

The effect of varying the amount of RNA in the system is shown in Figure 1. In the absence of RNA there was little incorporation. When RNA was added, the amount of incorporation observed was proportional to the amount of RNA added, until

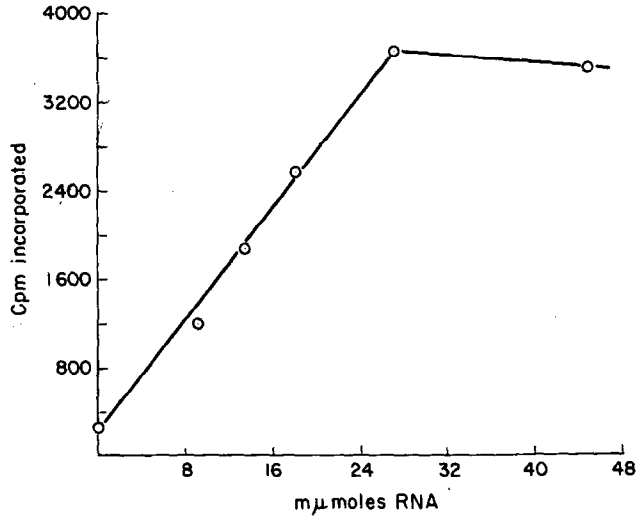

Fig. 1. Titration of Added RNA. Conditions as in Table I.

saturation with respect to RNA was reached. This observation enabled us to assay various RNA preparations and to show that the stimulation by viral RNA was not general for any polynucleotide. Thus, ribosomal RNA from liver was found to be completely inactive although of a size comparable to the active viral RNA samples. This data will be published shortly in a subsequent paper.

In order to show that the activity in these preparations was due to RNA itself and not to some accompanying factor, the following experiment was performed: RNA was treated with pancreatic ribonuclease and the enzyme was subsequently removed by adsorption onto bentonite (14). In Table II, comparison of lines 1 and 4 shows that this treatment was effective in removing ribonuclease. Although incubation of the RNA alone (line 2) had little effect, addition of 0.01 μg/ml of RNase completely abolished activity (line 3), confirming that the active principle was indeed RNA.

Nature of the reaction: Of immediate interest for an understanding of the way in which viral RNA acts to stimulate the incorporation of amino acids is the finding that obligate synthesis of RNA does not appear necessary since no stimulation of incorporation was observed when all four ribonucleoside triphosphates were added to the system described in Table I. More recently, we have extended these observations to the purified

Table II. Effect of RNase Treatment on RNA Activity

	Conditions	Specific activity*
1.	Untreated RNA	50.9
2.	RNA incubated 37°, 30 min	41.6
3.	RNA incubated 37°, 30 min with 0.01 µg/ml RNase	<0.8
4.	(1) plus (3)	48.0

*cpm incorporated per mµmole of RNA added

After incubation of the RNA as described above 3 mg of bentonite were added to all tubes to adsorb RNase. After 5 min adsorption, the bentonite was removed by centrifugation and the supernatant solutions tested for activity in the assay system described in Table I. 20 to 50 mµmoles of RNA from each incubation were used.

system described by Nathans and Lipmann (15) consisting of transfer factor, deoxycholate-treated ribosomes, and amino acyl RNA where the chances of nucleotide contamination are much less. TYMV-RNA still gave a five-fold stimulation, and there was no effect when ATP, CTP, and UTP were added, suggesting that viral RNA does not act as a complementary template for the synthesis of messenger RNA but is instead its own messenger.

From the data presented in Figure 1, we may calculate that one amino acid was incorporated for every nine nucleotides added as TYMV-RNA. Since preliminary results indicate that only a fraction of the RNA is active, the true "coding ratio" is probably lower still, although we cannot yet say if a number lower than the theoretical value of three can be obtained.

Studies on this system are continuing in attempts to further define the mechanism of viral RNA stimulation and the nature of the product protein formed.

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