STIMULATION OF CELL-FREE PROTEIN SYNTHESIS BY HOMOLOGOUS AND HETEROLOGOUS MESSENGER RIBONUCLEIC ACID

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The ability of messenger RNA to stimulate the incorporation of amino acids into protein in a cell-free system has been widely used to demonstrate the presence of RNA with messenger activity (1,2). In most cases, the protein synthesized by cell-free systems has not been characterized and synthesis of a well-defined heterologous protein has been obtained only with RNA from f2 and related bacteriophages (3). In the case of RNA from animal cells, it has been found that reticulocyte RNA stimulates hemoglobin synthesis in a homologous test system (4,5) but RNA from other tissues such as liver, kidney or intestine is also active (6). In some experiments reticulocyte RNA apparently failed to bring about the synthesis of hemoglobin by cell-free extracts from Escherichia coli (7). However, a more recent report indicates that such a heterologous system may indeed be capable of synthesizing hemoglobin (8).

The experiments reported in this communication demonstrate that the nature of the protein synthesized and released from reticulocyte ribosomes in a cell-free system stimulated by either homologous or heterologous RNA was dependent on the species of m-RNA employed.

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METHODS AND RESULTS

Since preparations from rabbit reticulocytes respond well to synthetic polynucleotides such as polyuridylic acid or to messenger RNA (9,4,10), this system was employed with reticulocyte RNA as the homologous and turnip yellow mosaic virus (TYMV) RNA as the heterologous messenger.

The isolation of subcellular fractions from reticulocytes of anemic rabbits and details of the cell-free system have been previously described (10), but the incubation was carried out on a larger scale, each tube containing 1.25 mg. of L-ribosomes and 8 mg. of "pH 5 enzymes" in a final volume of 2.5 ml. L-^{14}C-valine (specific activity 161 μC/μmole) and DL-^{3}H-isoleucine (specific activity 382 μC/μmole) were used as labelled precursors together with a mixture of 18 unlabelled protein amino acids. Either 0.125 mg. of rabbit reticulocyte RNA (prepared as described by Nair & Arnstein (10)) or 0.125 mg. TYMV RNA was added as messenger. After incubation at 37° for 1 hour, the ribosomes were removed by centrifugation at 105,000 g for 1 hr. at 4°C. Aliquots of the supernatants (0.05 ml.) were treated with alkali and the protein precipitated with trichloroacetic acid. The protein was filtered onto oxoid membranes and the ^{14}C activity determined in a low background ultra-thin window counter (Nuclear Chicago Corp.). The radioactivity of the aliquot from the control incubation mixtures was 53.6 cpm, the sample which had been incubated with reticulocyte RNA was 155.6 cpm and that incubated with TYMV RNA was 118.6 cpm. The ratios of ^{14}C-valine incorporated in the presence of RNA to that in the control were 2.89 with reticulocyte RNA and 2.20 with TYMV RNA.

The ribosome-free supernatants were dialyzed against three changes of 0.05M potassium phosphate, pH 6.5 (250 ml.), carrier valine and isoleucine being added to the second change of buffer. Finally, the volume was reduced by placing the dialysis tubes in polyvinylpyrrolidone (MW 38,000). Aliquots (0.2 ml.) were fractionated by electrophoresis for 16 hrs. in 0.05M potassium phosphate, pH 6.5 at room temperature, using as the supporting medium fine bead Sephadex G-50 on a flat bed (20 x 7 x 0.12 cm.). The

samples were applied to filter paper strips equal in width to the thickness of the gel. The loaded paper strips were placed in the gel 2-3 cm. from the anodal end, and the electrophoresis was run with a voltage across the gel of 35 - 40V and a current of 9 - 9.5 mA. Hemoglobin migrated 4-5 cm. towards the cathode as a 1 - 1.5 cm. wide band. The gel was divided into 0.5 cm. wide segments, transferred to vials, mixed with 0.2 ml. water and 10 ml. dioxane scintillation fluid (180 g. naphthalene, 4 g. PPO[1], 0.1 g. POPOP[1], 1 l. dioxane A.R.) and counted in a Tricarb scintillation spectrometer (Packard Instrument Company, Inc.). Suitable standards were included and the appropriate correction made to exclude ^{14}C from the ^{3}H counts. The total radioactivity (cpm) recovered after electrophoresis was as follows: control (no RNA added): ^{14}C, 956; ^{3}H, 637; reticulocyte RNA added: ^{14}C, 1944; ^{3}H, 984; TYMV RNA added: ^{14}C, 1293; ^{3}H, 1224. The $^{14}C/^{3}H$ ratios were thus 1.50, 1.97 and 1.06, respectively. Since the concentration of radioactive protein after dialysis varied from that originally present due to volume changes, these results have been normalized using the ^{14}C counts in the protein from the control sample (956 cpm) as the base line and calculating the corrected ^{14}C counts from the ratios of ^{14}C activity in the control and RNA-stimulated samples that were determined directly on aliquots of the supernatants prior to dialysis. The corrected ^{3}H counts were then calculated by multiplying the ratio of observed ^{3}H to observed ^{14}C counts by the corrected ^{14}C counts.

In Table I is shown the corrected radioactivity in the total protein after electrophoresis and in the protein present in the zone to which hemoglobin migrated. The stimulation of the incorporation of ^{14}C-valine and ^{3}H-isoleucine by messenger RNA has been calculated by subtracting the appropriate control incorporation.

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1. PPO, 2,5-diphenyl oxazole; POPOP, 1,4-bis-2-(5 phenyloxazolyl)-benzene

TABLE I

The Effect of Reticulocyte RNA and TYMV RNA on the Incorporation of ^{14}C-Valine and ^{3}H-Isoleucine into Soluble Proteins by a Cell-Free System from Rabbit Reticulocytes

RNA Addition	Incorporation cpm*		RNA Stimulated Incorporation, cpm*		
	^{14}C	^{3}H	^{14}C	^{3}H	Ratio $^{14}C/^{3}H$
	Into Total Protein				
Control (no RNA added)	956	637	-	-	-
Reticulocyte RNA	2760	1399	1804	762	2.37
TYMV RNA	2105	1995	1149	1359	0.85
	Into Hemoglobin Zone				
Control (No RNA added)	403	164	-	-	-
Reticulocyte RNA	1735	640	1332	476	2.8
TYMV RNA	1040	740	637	576	1.1

* The observed radioactivities have been corrected as described in the text in order to compare different samples.

DISCUSSION

It is evident from the data in Table I that reticulocyte RNA stimulates the incorporation of valine more than that of isoleucine whereas TYMV RNA stimulates the incorporation of proportionately more isoleucine. New protein synthesized as a result of added TYMV RNA thus appears to differ from that synthesized by reticulocyte RNA. Most of the latter is probably hemoglobin as shown previously by the relative incorporation of different amino acids as well as by chromatography and end group analysis of the product (4). Although the absolute incorporation of valine relative to isoleucine has not been determined in the present experiments (allowance would have to be made not only for differences in counting efficiencies of ^{14}C and ^{3}H, but also for dilution of the high specific activity precursor amino acids by the respective amino acid pools (10)), a comparison of the

$^{14}C/^{3}H$ ratios indicates that the protein synthesized by TYMV RNA contains 2.5 to 3 times as much isoleucine relative to valine than does hemoglobin, even in the case of the protein fraction that migrates with hemoglobin on electrophoresis. Since the coat protein of TYMV is known to be relatively rich in isoleucine (11), and other proteins that may be coded for by this viral RNA are also likely to contain more isoleucine than the unusually small amount present in hemoglobin, these results and others obtained in this laboratory are consistent with the stimulation of synthesis of hemoglobin by reticulocyte RNA and of viral proteins by TYMV RNA. Further characterization of the products is, however, required to establish their identity conclusively.

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