STIMULATION OF AMINO ACID INCORPORATION BY RAUSCHER MURINE LEUKEMIA VIRUS RNA IN A CELL-FREE SYSTEM

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SUMMARY: RNA from purified Rauscher murine leukemia virus (RLV) stimulated L-^{14}C-leucine incorporation into protein some three-fold using a cell-free system derived from animal cells. The stimulation was completely dependent on energy, ribosomal subunits and factors present in a high salt wash of polyribosomes isolated from infected, transformed JLS-V5 cells; and the stimulation was prevented by puromycin. Heated 60-70S RNA and the lower s-rate RLV-RNA fractions were more active per μg of RNA than the intact 60-70S RNA.

INTRODUCTION

Advances in protein biosynthesis in mammalian systems have made it possible to study the translation of genetic messages in cell-free systems (1-3). Several reports have appeared on the stimulation of amino acid incorporation by animal virus RNA (2-5), and recently it has been reported that RNA synthesized in the test tube by the Reovirus-containing RNA polymerase can stimulate amino acid incorporation in a cell-free system (6). The work of Říman, et al. demonstrated template activity with avian myeloblastosis virus RNA in an E. coli amino acid incorporation system (7). Studies on factors isolated from polyribosomes with high salt (0.5M - 1.0M KCl) showed that these factors are required for the initiation of protein biosynthesis (1,2,8,9).

METHODS

The JLS-V5 cell line was used as a source of Rauscher leukemia virus (RLV). This JLS-V5 line is an RLV-infected and transformed cell line (10) which continually produces RLV particles. The virus was purified in the presence of the RNase inhibitor, sodium dextran sulfate 500, from culture fluids by 6% polyethylene glycol precipitation and isopycnic sedimentation in a sucrose gradient buffered in 0.01M Tris, pH 7.5, 0.1M NaCl, 0.001M EDTA (TNE). The viral RNA was

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extracted from the purified virus with sodium dodecyl sulfate (SDS)-phenol at 20°C. Polyribosomes, ribosomal subunits and the high salt wash fraction were prepared from JLS-V5 cells using procedures previously described (2,9,11,12). The preparation of the high salt wash fraction by a 0.85M KCl wash of polyribosomes is a modification of the Miller and Schweet procedure (1). The cell-free system contained the basic components as described by Allen and Schweet (13) except yeast t-RNA (100 μg/ml), JLS-V5 ribosomal subunits (1.0 mg/ml), and 250 μg (protein) per ml of the high salt wash fraction were used. Ribosomal subunits were pre-incubated for 60 min at 37°C in the complete cell-free system minus isotope, high salt wash fraction, and RLV-RNA. The L-^{14}C-leucine at specific activity 20 μc/μmoles, high salt wash fraction, and 10-13 μg of RLV-RNA were added and the incubation was continued for an additional 60 min. Samples were washed with 5% CCl_3COOH following NaOH treatment (13) prior to counting.

RESULTS

Polyribosomes from JLS-V5 cells were quite active for L-^{14}C-leucine incorporation with the values ranging from 200-300 pmoles per mg of ribosomes. When RLV-RNA (13 μg) extracted from purified virus was added to these polyribosomes in the complete cell-free system, there was approximately a 25% inhibition of ^{14}C-leucine incorporation. Similar inhibition of cell-free protein synthesis by addition of messenger RNA or ribohomopolymers has been reported (14-16). However, when pre-incubated ribosomal subunits were used, the RNA (10 μg) stimulated L-^{14}C-leucine incorporation in the presence of a high salt wash fraction obtained from polyribosomes (Table 1). The stimulation increased linearly with the concentration of RLV-RNA in the range tested (1-15 μg/assay), and was completely dependent on the high salt wash fraction, ribosomal subunits, and energy. No stimulation was observed in the presence of 0.001M puromycin. Other viral RNAs did not stimulate as well. Mengovirus RNA gave less stimulation than RLV-RNA, and MS-2 RNA had little effect.

Addition of an optimal amount of the high salt wash fraction to the pre-incubated system in the absence of added RLV-RNA always stimulated amino acid incorporation. The response was somewhat variable but the average response

TABLE 1

REQUIREMENTS FOR RLV-RNA STIMULATED PROTEIN BIOSYNTHESIS

	Additions	[a]pmoles L-^{14}C-Leucine Incorporation/mg Ribosomes
1.	Complete Minus high salt wash fraction	8.2
2.	Complete	13.6
3.	Complete Plus RLV-RNA (13 μg)	21.5
4.	No. 1 Plus RLV-RNA (13 μg)	8.6
5.	No. 3 Minus Ribosomes	0
6.	No. 3 Minus Energy	0.9
7.	No. 3 Plus Puromycin, 0.001M	2.8
8.	Complete Plus 13 μg of Mengovirus RNA	14.1
9.	Complete Plus 13 μg of MS-2 RNA	9.5

[a]1 pmole = 26 cpm at SA 20 mμc/mμmole.

The ribosomal subunits were pre-incubated in the complete system without isotope, high salt wash fraction, and RLV-RNA for 60 min at 37°C. L-^{14}C-leucine was added to SA 20 mμc/mμmole and the other additions were made as indicated. The tubes were re-incubated for an additional 60 min at 37°C. Minus energy indicates that ATP, GTP, PEP, and pyruvate kinase were omitted from both the pre-incubation and the incubation. Samples were treated with 0.1N NaOH for 5 min at 25°C, and precipitated with 5% CCl_3COOH as described in Methods.

was 150-200% of the endogenous level.

To determine which fraction of RLV-RNA was responsible for L-^{14}C-leucine incorporation, RLV-RNA was fractionated on a sucrose gradient (Fig. 1) and 3 regions of the gradient were tested for activity. Fractions 15-25, 7-14, and 1-6 were pooled separately and re-isolated. The pooled fractions were designated 60-70S RNA, 20-40S RNA, and 4-20S RNA, respectively. The results show that all three fractions stimulated amino acid incorporation (Table 2). However, the 20-40S fraction was twice as effective as the 60-70S RNA per μg of RNA. The 4-20S RNA had intermediate activity. Note the four-fold stimulation by the 20-40S RNA fraction above the complete system (Table 2, line 2 and 5).

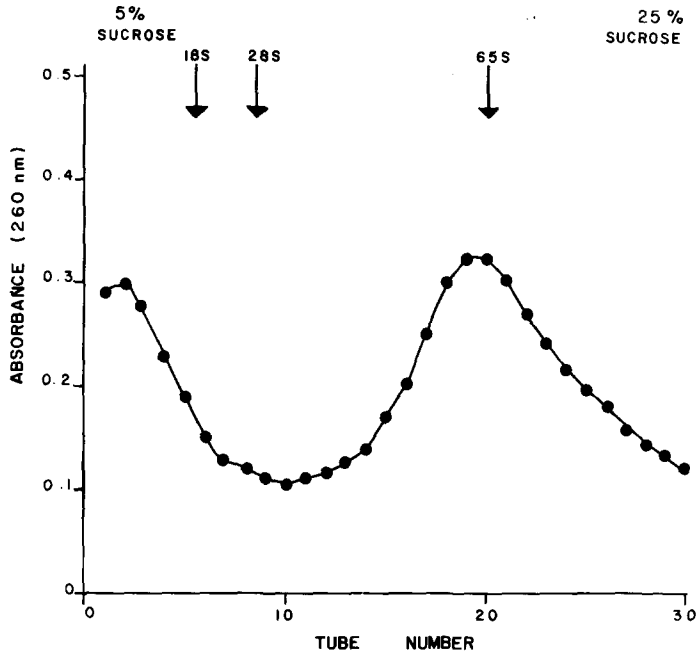

Figure 1 - The fractionation of RLV-RNA by velocity sedimentation in a sucrose gradient. Rauscher leukemia virus RNA was extracted from purified virus obtained from 10 liters of JLS-V5 culture fluids. The RNA was extracted with SDS-phenol yeilding about 375 μg of RNA. The entire sample was applied to 5-25% linear sucrose gradient buffered in TNE, and the sample was centrifuged for 16 hr at 15,000 rev/min in the SW-27 rotor at 4°C. The direction of sedimentation is from left to right. Fractions 1-6, 7-14, and 15-25 were separately pooled and precipitated with 2 volumes alcohol after addition of 1/10 vol. of 2M K acetate, pH 5.3. The RNA was dissolved in 0.01M Tris-HCl, pH 7.5 and then tested for L-^{14}C leucine incorporation (Table 2). A 1% solution of RNA was assumed to have an absorbance of 230 optical density units at 260 nm.

A portion of the 60-70S fraction of RLV-RNA obtained in Fig. 1 was heated at 80°C for 2.5 min as described previously by Blair and Duesberg (17). This fraction had 50% more activity per μg of RNA than 60-70S unheated RNA (Table 2).

Studies with ^{3}H-labeled RLV-RNA showed that such heat treatment dissociated the 60-70S RLV-RNA producing RNA with s-rate ranging from 4-45S. ^{14}C-mengovirus RNA was included in this experiment as a control for RNase degradation and very little degradation was seen.

DISCUSSION

Our results show that the RLV-RNA can stimulate amino acid incorporation into acid-precipitable polypeptides using a system derived from the RLV-infected

TABLE 2

STIMULATION OF AMINO ACID INCORPORATION BY FRACTIONS OF RLV-RNA

Addition	pmoles of ^{14}C-Leucine Incorporation/mg rib/10 μg RNA	
	Incorporation	Stimulation by RNA
Complete Minus High Salt Wash	7.5	-
Complete	15	-
Complete plus total RLV-RNA	23	8
Complete Plus 60-70S RNA	38	23
Complete Plus 20-40S RNA	66.5	51.5
Complete Plus 4-20S RNA	49	34
Complete Plus Heated 60-70S RNA	52	37

Ribosomal subunits were pre-incubated in the complete cell-free system as described in Table 1. RNA fractions were prepared as described in Fig. 1.

JLS-V5 cells. The 60-70S RLV-RNA stimulated L-^{14}C-leucine incorporation 2-3 fold above the ribosome-high salt wash fraction tube (Table 2, line 2). However, all other RNA fractions had better stimulating activity per μg of RNA, and the 20-40S RNA fraction contained the best activity. Even heated 60-70S RLV-RNA which contains 4-45S RNA had better stimulation per μg of RNA than the 60-70S fraction. Říman, et al. (7) also showed that avian myeloblastosis virus RNA and a 30-40S RNA fraction stimulated amino acid incorporation in an E. coli cell-free system.

Blair and Duesberg (17) have shown that the 60-70S RNA of RNA tumor viruses is a highly structured molecule and is quite different from the RNA of other types of RNA viruses, such as poliovirus or Newcastle disease virus. The 60-70S RLV-RNA is converted to a 30-35S RNA by heating at 80°C or with 90% dimethyl sulfoxide at 37°C. It has been suggested that there are 3-4 molecules of 35S RNA per molecule of 60-70S RNA. Our results indicate that the highly structured 60-70S RNA is less active as a template for protein synthesis than either heat-denatured 60-70S RNA or lower s-rate fractions of RLV-RNA. However, considering

the number of RNA molecules per μg of RNA, the 60-70S RNA is the most active component on a molecular basis. The crucial point in considering template activity is the number of ribosome binding sites accessible in the intact highly structured 60-70S RNA as compared to the lower s-rate RNA or heat-denatured RNA. It is possible that denaturation of the 60-70S RLV-RNA exposes more ribosomal binding sites.

For some RNA viruses it is clear that the viral genome is the genetic message (18-21). In cells infected with paramyxoviruses (22) and vesicular stomatitis virus (23), RNA complementary to the virion RNA may be translated on polyribosomes. Green, et al. (24) show that smaller fragments of viral RNA (+ strands) are present in the cytoplasm of RLV-infected cells. The data reported here and that of Green, et al. (24) considered together suggest that the positive strand of RLV-RNA, or a fragment thereof, might act as true messenger RNA in RNA tumor virus infected cells.

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