Effects of U1 Nuclear RNA on Translation of Messenger RNA†

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ABSTRACT: U1 nuclear RNA has the 5′-terminal sequence $m_3^{2,2,7}$Gpp(p)AmUm*ACUU*ACCUGGCAGGGAGAU-ACC*AUG*- [Reddy, R., et al. (1974), *J. Biol. Chem. 249*, 6486; Ro-Choi, T. S., et al. (1974), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 33*, 1548]. The cap, $m_3^{2,2,7}$Gpp(p)Am, *ACUU*, and *AUG* are common to some mRNA species. Despite the presence of the cap and AUG initiator codon, U1 RNA is not translated in the wheat germ system but instead U1 RNA markedly inhibits translation of poly[A(+)] mRNA in the wheat germ system. On a molar basis the inhibitory effect of U1 RNA is 15 times greater (IC_{50} = 2.1 μM) than either pm^7G or $m^7GpppAm$ (IC_{50} pm^7G and $m^7GpppAm$ = 30 μM). Inhibition of translation by U1 RNA was noncompetitive with respect to mRNA and not reversed by increasing mRNA concentrations. Inhibition by U1 RNA was reversed by increasing amounts of wheat germ S-30 fraction. ^{125}I-labeled U1 RNA binds to 80S ribosomes under the conditions of initiation complex formation. The inhibitory effect of pm^7G on translation is correlated with its inhibition of the formation of the 80S initiation complex but U1 RNA did not inhibit the formation of the 80S complex at concentrations inhibitory to translation. Much higher concentrations of U1 RNA (7 μM) only inhibited formation of the 80S initiation complex by 40%. U1 RNA immediately stopped protein synthesis when added 20 min after the incubation started; a 10-min lag occurs with pm^7G. U1 RNA does not inhibit poly(U)-directed polyphenylalanine synthesis. These results show that, at low concentrations, U1 RNA binds to ribosomes and inhibits translation. This inhibition may result from interaction of oligonucleotides adjacent to the 5′ cap of U1 RNA with the messenger binding sites on ribosomes or with special factors necessary for protein synthesis.

The sequence of U1 RNA, a nuclear low-molecular-weight RNA, was defined by Reddy et al. (1974). The 5′ end of U1 RNA has $m_3^{2,2,7}$G in a pyrophosphate linkage to pAm in its "5′ cap" (Reddy et al., 1974; Ro-Choi et al., 1974).

In its 5′-terminal sequence ($m_3^{2,2,7}$Gpp(p)AmUm*ACUU*-ACCUGGCAGGGAGAUACC*AUG*-), both *ACUU* and initiation codon *AUG* are present. The tetranucleotide *ACUU* is also found in α- and β-globin mRNA (Lockard and Rajbhandary, 1976). Further, $m_3^{2,2,7}$G linked cap structure has also been found in Sindbis virus (Dubin et al., 1976). Similar "5′-cap" structures have been found in eukaryotic mRNAs as well as most of the viral RNAs (Furuichi, 1974; Rottman et al., 1974; Adams and Cory, 1975; Furuichi and Miura, 1975; Furuichi et al., 1975a,b; Rose, 1975; Wei and Moss, 1975; Zimmern, 1975; Busch, 1976; Rao et al., 1977).

Initially the role suggested for m^7G in the translational process was in formation of the initiation complex and in binding of mRNA to ribosomes. Muthukrishnan et al. (1975) showed that removal of m^7G from reovirus RNA by periodate oxidation and β elimination reduced the translational activity by 90%. Both et al. (1975) demonstrated that reovirus RNA lacking a methyl group on guanosine bound less efficiently to wheat germ ribosomes. Our studies showed that periodate oxidation of liver mRNA markedly decreased its translational efficiency (Rao et al., 1975; Busch et al., 1976). Hickey et al. (1976) observed that addition of 7-methylguanosine 5′-phosphate inhibited the translation of capped mRNA but not uncapped mRNA like STNV RNA in the wheat germ system. Weber et al. (1976) have further confirmed these observations using HeLa cell free systems. Roman et al. (1976) showed that pm^7G[1] inhibits the conversion of 40S-Met-$tRNA_{fMet}$ complex to the 80S-Met-$tRNA_{fMet}$-mRNA complex in the presence of 60S subunits.

The present study was designed to study the effects of U1 RNA on translation in the wheat germ system. U1 RNA inhibited translation of liver mRNA at much lower concentrations than either pm^7G or $m^7GpppAm$ (Busch et al., 1976). In the present study, the mechanism of inhibition was analyzed further.

Materials and Methods

Liver and Novikoff hepatoma mRNA was used for translational studies. pm^7G and m^7G(5′)pppAm were purchased from P-L Biochemicals. [^{3}H]Methyl-labeled reovirus RNA was a generous gift from Dr. A. Shatkin of the Roche Institute. [^{3}H]Leucine (61 Ci/mmol) and [^{3}H]phenylalanine (12 Ci/mmol) were obtained from Schwarz/Mann. Na^{125}I was from Amersham/Searle Co. (11–17 mCi/μg of I). Dimethylguanosine and polyuridylate were purchased from Sigma Chemical Co.

In Vitro Translation in Wheat Germ Cell-Free System. Analysis of translational activity was carried out essentially according to the method of Roberts and Paterson (1973). The incubation was carried out with 0.1 μg of mRNA and 0.2 mg (protein) of wheat germ S30 fraction in a volume of 41.5 μL along with the following constituents: ATP, 1 mM; GTP, 0.02 mM; creatine phosphate, 8 mM; creatine phosphokinase, 1.6 μg; KCl, 84 mM; magnesium acetate, 3 mM; 19 amino acids,

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[1] Abbreviations used: GMPPCP, guanosine 5′-(β,γ-methylene) triphosphate; GMPPNP, 5′-guanylylimido diphosphate; Tris, tris(hydroxymethyl)aminomethane; Hepes, *N*-2-hydroxylethylpiperazine-*N*′-2-ethanesulfonic acid; pm^7G, $pm^{2,2}G$, and $pm_3^{2,2,7}G$, 7-methylguanosine 5′-phosphate, N^2-dimethylguanosine 5′-phosphate, and N^2-dimethyl-N^7-methylguanosine 5′-phosphate, respectively.

TABLE I: Translational Activity of Liver Poly[A(+)] RNA and U1 RNA in the Wheat Germ Cell-Free System[a]

RNA (μg/mL)	[^{3}H]Leucine incorp (cpm/12.5 μL)				
	0	1.25	2.50	3.75	5.0
Liver poly[A(+)] RNA	2032	4300	8500	13800	16100
U1 RNA	2032	2206	2206	2196	2352

[a] The translational activities of mRNA and U1 RNA were determined in the wheat germ cell-free system as described in the text.

0.02 mM each, [^{3}H]leucine, 20 μCi, dithiothreitol, 2 mM; and Hepes buffer, pH 7.6, 28 mM. After incubation at 25 °C for 60 min, 12.5-μL samples were spotted on filter paper discs for radioactive analysis after washing two times with cold 10% Cl_3CCOOH, once with hot 5% Cl_3CCOOH, three times with cold 5% Cl_3CCOOH, and once each with 100% ethanol, ethanol-ether, and ether. For translation of poly(U), [^{3}H]phenylalanine was employed as a tracer.

80S Initiation Complex Formation. The incubation conditions for formation of the 80S initiation complex were the same as described above, except that [^{3}H]leucine was replaced by cold leucine and 2000 cpm of [^{3}H]methyl-labeled reovirus RNA (8000 cpm/μg of RNA) was used instead of liver mRNA. Anisomycin, an inhibitor of chain elongation, was present at a concentration of 0.125 mg/mL (Dasgupta et al., 1975). The incubation was carried out at 25 °C for 15 min; samples were layered on a 10–40% linear sucrose density gradient in 0.05 M Tris-HCl (pH 7.6), 0.05 M KCl, and 0.008 M magnesium acetate. Centrifugation was carried out at 26 000 rpm for 4 h in an SW-41 rotor; 0.3-mL fractions were collected and analyzed for radioactivity in a scintillation counter.

Synthesis of N^2-Dimethyl-N^7-methylguanosine 5′-Monophosphate. Phosphorylation of $m^{2,2}$guanosine was carried out according to the method of Rottman and Heinlein (1968). Three hundred micromoles of $m^{2,2}G$ was allowed to react with the pyridinium form of 2-cyanoethyl phosphate (150 μmol) in the presence of 0.5 g of dicyclohexylcarbodiimide, 2 mL of dry pyridine, and 1 mL of dry dimethylformamide. The reaction mixture was shaken for 65 h at room temperature in the presence of a few grains of Dowex-50 in the pyridinium form. Following the reaction, 3 mL of 50% aqueous pyridine was added and the mixture was extracted three times with cyclohexane, allowed to stand at room temperature for 24 h, and evaporated to dryness under a stream of air. The cyanoethyl group was hydrolyzed by treatment with 12 mL of 7 N NH_4OH at 70 °C for 6 h. The reaction mixture was evaporated to a small volume, cooled in ice, and filtered to remove cyclohexylurea.

The reaction products were applied to 1 × 5 cm column of Dowex-1 formate. The unreacted $m^{2,2}G$ was eluted by washing with water. After washing the column with 0.1 N formic acid, $pm^{2,2}G$ was eluted with 1 N formic acid and was concentrated by lyophilization. The purity of $pm^{2,2}G$ was checked both by electrophoresis at pH 3.5 and chromatography using the solvent system of Wyatt (Barrel, 1971).

The N^7 methylation of $pm^{2,2}G$ was carried out by the method of Saponora and Enger (1969). After the reaction with dimethyl sulfate and dimethylacetamide, the products were separated on 1 × 5 cm column of Dowex-1 formate. Because of the positive charge imposed by N^7-methyl group, $pm_3^{2,2,7}G$ could be eluted with 0.01 N formic acid, whereas unreacted $pm^{2,2}G$ was eluted by 1 N formic acid, and the 0.01 N formic acid eluate was concentrated by lyophilization; the purity of the product was determined by pH 3.5 electrophoresis and the two-dimensional system of Randerath and Randerath (1971).

Iodination of U1 RNA. Iodination of U1 RNA was carried out by a modification of the procedure of Commerford (1971) as described by Woo et al. (1975). Fifty micrograms of U1 RNA was incubated with 0.1 M sodium acetate (pH 5.0), 5 × 10^{-5} M potassium iodide, 7.5 × 10^{-4} M thallium chloride, and 0.75 mCi of carrier-free $Na^{125}I$ in a total volume of 50 μL. The reaction was carried out at 60 °C for 15 min after which 5 μL of 0.01 M tyrosine was added to remove the unreacted iodide. Ten microliters of 2.8 M sodium phosphate (pH 6.8) was added and the mixture was again incubated at 60 °C for 15 min. The reaction mixture was cooled to room temperature and passed through a 0.5 × 30 cm column of Sephadex G-50 equilibrated with deinoized water. Fractions (0.5 mL) were collected and those with radioactivity in the void volume were collected and the labeled U1 RNA was precipitated with 2 volumes of ethanol containing 2% potassium acetate at −20 °C. ^{125}I-labeled U1 RNA was purified by repeated dissolving and reprecipitation with ethanol. The integrity of ^{125}I-labeled U1 RNA was checked by electrophoresis on 8% polyacrylamide gels (Ro-Choi et al., 1973). ^{125}I-labeled U1 RNA detected by autoradiography comigrated with the stained pattern of unlabeled U1 RNA. The specific activity of U1 RNA thus obtained was 2.8 × 10^6 cpm/μg of RNA.

Results

Translational Activity of mRNA and U1 RNA. The translational activities of liver cytoplasmic poly[A(+)] mRNA and U1 RNA in the wheat germ cell-free system using [^{3}H]leucine as the tracer are shown in Table I. When the U1 RNA concentration was varied from 1.25 to 6.0 μg/mL assay mixture (25 to 100 nM), there was no significant stimulation of [^{3}H]leucine incorporation over the background. At similar concentrations, liver poly[A(+)] mRNA was very active in protein synthesis.

Inhibition of Translation by pm^7G and "Cap" Analogues. Figure 1A shows the effects of pG, pm^7G, $pm^7GpppAm$ on the liver poly[A(+)] RNA directed translational activity in the wheat germ cell-free system as a function of the inhibitor concentrations. The concentration at which both pm^7G and $m^7GpppAm$ produced 50% inhibition was 30 μM. At 100 μM, both pm^7G and $m^7GpppAm$ completely inhibited translation.[2] Previously, HeLa cell poly[A(+)] RNA translation in wheat germ and HeLa cell-free systems was shown to be markedly inhibited by pm^7G (Hickey et al., 1976; Weber et al., 1976; Canaani et al., 1976). Figure 1 also shows that the dialdehyde derivative of $m^7GpppAm$ is approximately half as inhibitory

[2] In recent experiments, the effect of pm^7G translation of mRNA has been variable from batch to batch of wheat germ S30 fraction prepared. The stimulation of translational activity by tRNA also depended upon the batch of wheat germ used.

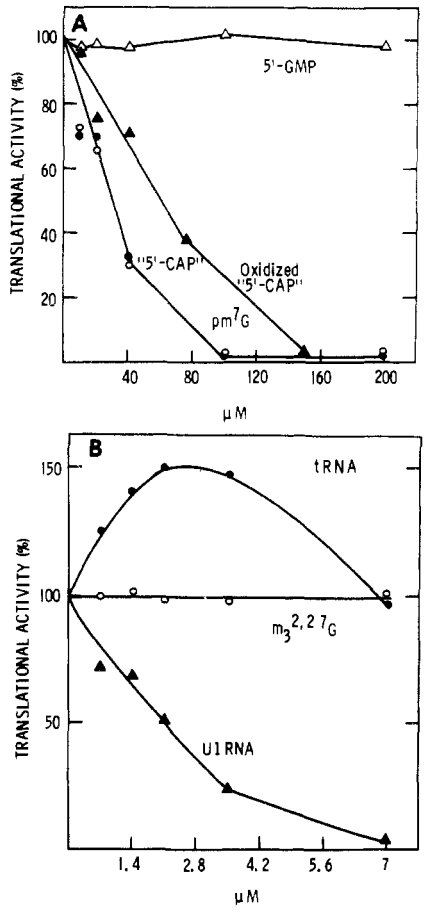

FIGURE 1: (A) Effect of pm^7G, $m^7GpppAm$, pG, and $m^7GpppAm$ oxidized with $NaIO_4$ on the translational activity of liver mRNA in the wheat germ system. Conditions for measuring the translational activity are described in the text. Incubation was for 60 min, and each value is an average of three independent determinations. (●—●) pm^7G, (○—○) $m^7GpppAm$, (△—△) pG, and (▲—▲) $m^7GpppAm$ oxidized with $NaIO_4$. (B) Effect of U1 RNA, $m_3^{2,2,7}G$, and tRNA on the translational activity of liver mRNA in the wheat germ cell-free system. Details of the experimental conditions described in the text. (▲—▲) U1 RNA; (○—○) $m_3^{2,2,7}G$; and (●—●) tRNA.

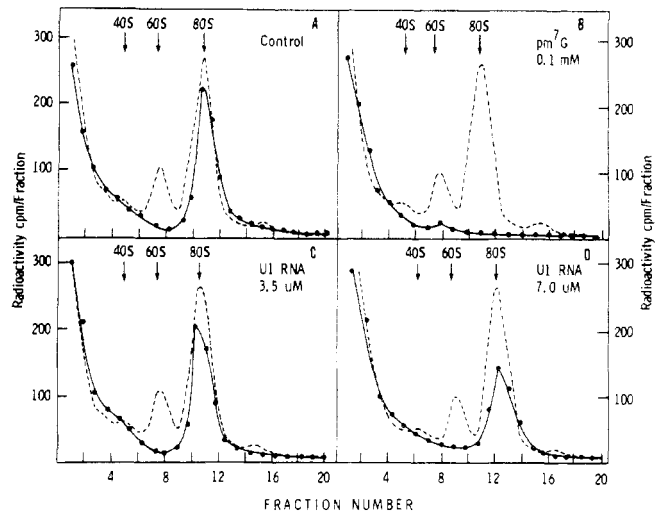

FIGURE 2: Effect of pm^7G and U1 RNA on 80S initiation complex formation in the wheat germ cell-free system. The incubation system was the same as for translation except that anisomycin was included at 0.125 mg/mL concentration and 0.25 μg of ^3H-labeled reovirus RNA (8000 cpm/μg) was used instead of liver mRNA. Incubation was for 15 min at 25 °C. The 80S complex was analyzed on 10–40% sucrose density gradients in 0.05 M Tris-HCl, pH 7.6, 0.05 M KCl, and 0.008 M magnesium acetate. The gradients were centrifuged at 26 000 rpm for 4 h, in a SW 41 rotor. Fractions (0.3 mL) were collected and counted using Bray's scintillator. (A) Control; (B) pm^7G, 100 μM; (C) U1 RNA, 3.5 μM; and (D) U1 RNA, 7.0 μM.

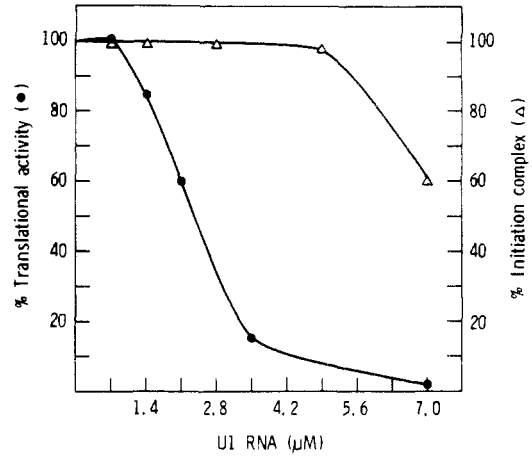

FIGURE 3: Comparison of the effects of U1 RNA on translation and 80S initiation complex formation. The data for translational effects are from Figure 1B. Each value is an average of three independent determinations.

as $m^7GpppAm$ (IC_{50} = 60 μM). This reduction of the inhibition correlates with the decreased translational activity of $NAIO_4$ treated mRNA (Rao et al., 1975; Busch et al., 1976).

In comparison, Figure 1B shows that U1 RNA inhibited the translational activity 15 times more on a molar basis than pm^7G or $m^7GpppAm$ (IC_{50} = 2.1 μM). As a control, purified tRNA was added. At similar concentrations, tRNA stimulated the translational activity by 40–50%.[2] The increased translational activity may result either from the limiting amounts of tRNA present in the wheat germ S30 fraction or suppression of RNase activity.

Effect of pm^7G and U1 RNA on 80S Initiation Complex Formation. Since pm^7G inhibits the formation of 80S initiation complex in the wheat germ S30 fraction, experiments were carried out to compare the effects of pm^7G and U1 RNA on this step of protein synthesis. For this purpose, the wheat germ S30 fraction was incubated with 0.25 μg of [^3H]methyl-labeled reovirus RNA (8000 cpm/μg) in the presence of 0.125 mg/mL anisomycin to inhibit chain elongation. Following incubation at 25 °C for 15 min, the initiation complex formed was analyzed on 10–40% sucrose density gradients. Figure 2 shows that approximately 40–50% of the input radioactivity was associated with the 80S region (Figure 2A). Addition of 0.1 mM pm^7G concentration completely inhibited formation of the 80S complex (Figure 2B). Figure 2C shows that addition of U1 RNA at a concentration of 3.5 μM did not inhibit formation of the 80S complex. At this concentration U1 RNA inhibited the translational activity by 75% (Figure 1B). Addition of 7.0 μM U1 RNA only inhibited formation of the 80S complex by 40%[3] (Figure 2D).

Figure 3 shows the comparative effects of U1 RNA on total translational activity and formation of the 80S complex. These results indicate the inhibitory effect of U1 RNA on translation

[3] Among possible explanations for this difference is that translation was carried out by liver mRNA and 80S complex formation with labeled reovirus RNA.

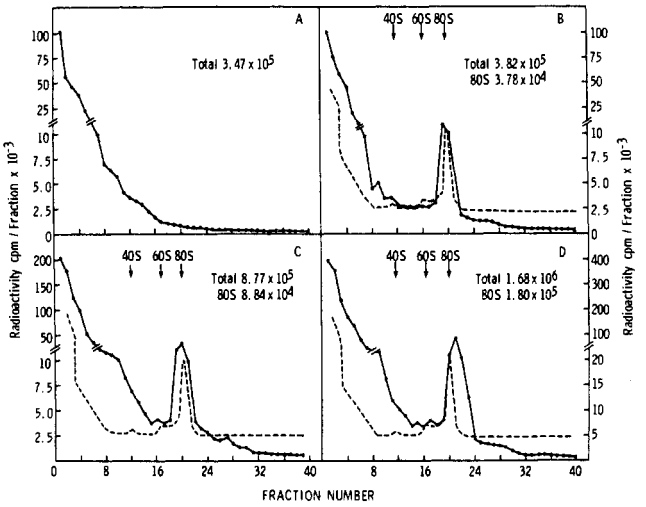

FIGURE 4: Binding of iodinated U1 RNA to wheat germ ribosomes. U1 RNA was iodinated as described in Materials and Methods. The specific activity of U1 RNA was 2.8×10^6 cpm/μg. Different concentrations of ^{125}I-labeled U1 RNA were incubated with wheat germ S30 fraction under the same conditions as described in the legend to Figure 2 and analyzed on 10–40% sucrose density gradients in 0.05 M Tris-HCl (pH 7.6), 0.05 M KCl, and 0.008 M magnesium acetate. The gradients were centrifuged at 26 000 rpm for 4 h in a SW41 rotor. Fractions (0.25 mL) were collected and isotope content was determined (solid line). OD_{260} was determined on a higher scale setting than in Figure 2. (A) ^{125}I-labeled U1 RNA (0.25 μg) without S30 fraction; (B) 0.25 μg of ^{125}I-labeled U1 RNA incubated with S30; (C) 0.625 μg of ^{125}I-labeled U1 RNA incubated with S30; and (D) 1.24 μg of ^{125}I-labeled U1 RNA incubated with S30.

is not on the initial phase of chain initiation. The inhibition of the formation of 80S complex at high concentrations of U1 RNA may be due to a weak affinity of the 5′ cap of U1 RNA for the "cap binding protein" (Shafritz et al., 1976; Filipowicz et al., 1976).

Binding of Iodinated U1 RNA to Wheat Germ Ribosomes. To study whether U1 RNA binds to wheat germ 80S initiation complex, U1 RNA was iodinated according to Commerford (1971) and was incubated in the wheat germ cell-free system under the conditions used for formation of the 80S initiation complex (Figure 2). After the incubation, samples were analyzed on 10–40% sucrose density gradients (Figure 4). A linear increase was found in the amount of iodinated U1 RNA bound to the 80S complex with increasing input of U1 RNA. Approximately 10% of the input radioactivity was found in the 80S region in each case. The efficiency of U1 RNA binding to wheat germ ribosomes was approximately one-fifth that of reovirus mRNA (Figures 2 and 4).

Evidence that Binding of U1 RNA to Ribosomes Involves Initiation Processes. To determine whether this binding of U1 RNA to ribosomes (Figure 4) involved normal initiation processes, the effects of pm^{7}G, aurintricarboxylic acid, and GMPPNP on U1 RNA binding were evaluated (Table II). In the control, approximately 10% of the U1 RNA was bound to the initiation complex. Both pm^{7}G and aurintricarboxylic acid inhibited 80S complex formation by 50 and 99%, respectively. GMPPNP inhibits the conversion of 40S initiation complex to the 80S initiation complex. Kozak and Shatkin (1976) showed that about 30% of the initiation complex with ^{3}H-labeled reovirus RNA remained in the 40S peak in the presence of GMPPCP. Similar results were obtained for binding of ^{125}I-labeled U1 RNA in the presence of GMPPNP (Table II).

Comparative Effects of pm^{7}G, pm^{2,2}G, and pm$_3^{2,2,7}$G on Translation and 80S Initiation Complex. To compare their

TABLE II: Effect of Inhibitors of Initiation on ^{125}I-labeled U1 RNA Binding to Wheat Germ Ribosomes.[a]

Inhibitor	^{125}I radioactivity (cpm) Total	80 S	40 S
None	36 000	3548	
pm^{7}G (100 μM)	34 736	1515	
ATA (100 μM)	37 083	105	
GMPPNP (200 μM)	50 185	2100	780
GMPPNP (500 μM)	49 450	1380	869

[a] Formation of 80S initiation complex was followed by measuring the ^{125}I radioactivity in the 80S peak as described in the legend to Figure 2. When GMPPNP was used as the inhibitor, centrifugation was done for 16 h instead of 4 h to get better separation of 40 S and 80 S from the top of the gradient.

TABLE III: Effect of pm^{7}G, pm^{2,2}G, pm^{2,2,7}G, and U1 RNA on 80S Initiation Complex in the Wheat Germ System.[a]

Inhibitor	% inhibition[b] 10 μM	50 μM	100 μM
pm^{7}G	29	62	100
pm^{2,2}G	0	0	12
pm^{2,2,7}G	0	0	12
U1 RNA	0[c]	0[d]	40[e]

[a] Formation of 80S initiation complex was followed by measuring the ^{3}H radioactivity in the 80S region as described in the legend to Figure 2. In the control incubation without any inhibitors, 50% of the total input radioactivity was associated in 80S region. Taking this as 100%, the radioactivity in the 80S region in the presence of varying concentrations of pm^{7}G, pm^{2,2}G, pm$_3^{2,2,7}$G, and U1 RNA are expressed as percent of the control. Each value is an average of three independent experiments. [b] For U1 RNA the concentrations were as follows. [c] 1.4 μM. [d] 2.8 μmM. [e] 7.0 μM.

effects on translation and formation of initiation complexes with those of pm^{7}G, pm^{2,2}G and pm$_3^{2,2,7}$G were synthesized from m^{2,2}G (Rottman and Heinlein, 1968; Saponora and Enger, 1969). pm^{2,2}G did not inhibit translational activity and pm$_3^{2,2,7}$G had an IC_{50} of approximately 200 μM. Both were only weakly inhibitory to formation of the 80S initiation complex (Table III). These experiments show a remarkable specificity of pm^{7}G and further that methylation at the N^2 position hinders its inhibitory effects.

Kinetics of mRNA Translation: Effects of pm^{7}G and U1 RNA. To compare the effects of pm^{7}G and U1 RNA on chain initiation, the wheat germ S30 fraction was allowed to initiate translation of liver mRNA. After 20 min of incubation, either pm^{7}G or U1 RNA was added to the system and the incubation was continued up to 60 min. Analyses of [^{3}H]leucine incorporation into Cl_3CCOOH precipitates were made at different time periods after addition of the inhibitor. By 20 min, initiation had occurred (Figure 5) (Dasgupta et al., 1975). If the inhibitor blocks initiation, a lag should occur before inhibition of [^{3}H]leucine incorporation into protein. Figure 5 shows that there was a lag of 10 min before 0.1 mM pm^{7}G inhibited translation. In studies on the effects of pm^{7}G in the HeLa cell-free system, Weber et al. (1976) also observed a 10-min lag in inhibition of translation. The inhibitory effect of pm^{7}G which occurs after chain initiation provides evidence for reinitiation in the wheat germ cell-free system. In contrast to pm^{7}G, U1 RNA immediately blocked further protein synthesis.

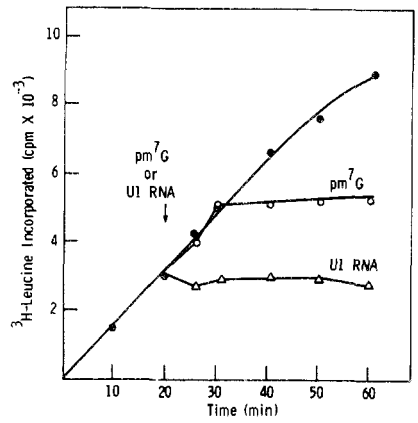

FIGURE 5: Kinetics of translation of liver mRNA and the effects of U1 RNA and pm^{7}G. The translational system is the same as described in Figure 1. The arrow indicates the time of addition of either pm^{7}G or U1 RNA. (●—●) Control; (○—○) pm^{7}G; and (△—△) U1 RNA.

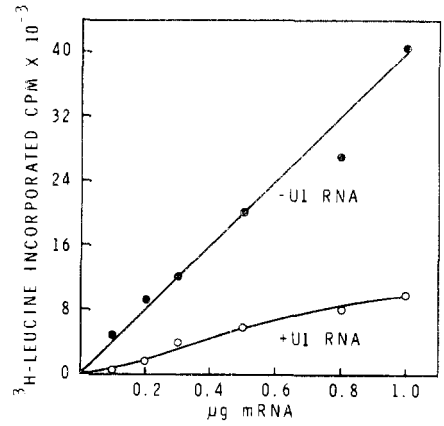

FIGURE 7: Competition of U1 RNA effect on translation with increasing concentrations of mRNA. In the competition assay U1 RNA was fixed at 2.1 μM and mRNA was varied from 0.1 to 0.5 μg. The incubations were done at 25 °C for 60 min.

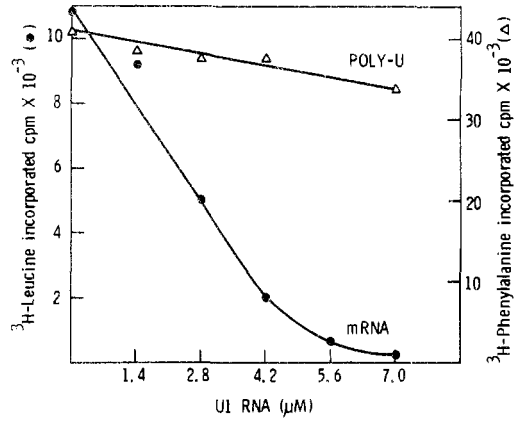

FIGURE 6: Comparative effects of U1 RNA effect on mRNA and poly(U) translation in the wheat germ system. Both poly(U) and mRNA were used at 0.1 μg/41.5 μL assay and the incubations were done at 25 °C for 60 min.

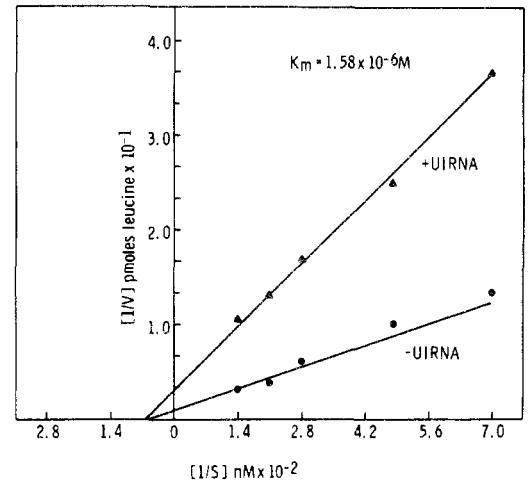

FIGURE 8: Lineweaver-Burk plot of the inhibition of U1 RNA of mRNA translation with respect to mRNA concentration. The experimental results obtained in Figure 9 have been replotted in this figure.

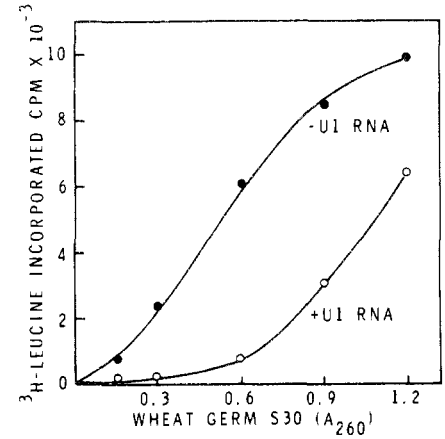

FIGURE 9: Competition of U1 RNA effect on mRNA translation with increasing concentrations of wheat germ S30 fraction. The study was done by using a fixed concentration of 2.1 μM U1 RNA and varying the S30 fraction added from 0.3 A_{260} unit to 1.2 A_{260} units.

Effects of U1 RNA on Poly(U)-Directed Polyphenylalanine Synthesis. To analyze the effects of U1 RNA on chain elongation, poly(U)-directed polyphenylalanine synthesis was studied. Translation of poly(U) occurs in the presence or absence of initiation factors (Crystal et al., 1974). In the absence of initiation factors, the Mg^{2+} optimum is shifted to 10 mM. For both natural mRNA and poly(U) translation the same elongation factors are required.

With the wheat germ S30 fraction which has all the necessary initiation and elongation factors, the Mg^{2+} optimum concentration was 3 mM. The translation of poly(U) was linear to concentrations of 0.2 μg. Figure 6 shows that, although U1 RNA markedly inhibited the translational activity of liver mRNA, it did not affect the translational activity of poly(U).

U1 RNA Affects the Ribosomes and Does Not Compete with mRNA. It was shown in Figure 4 that U1 RNA binds to the 80S initiation complex. The following study was designed to determine whether U1 RNA binds to ribosomal components competitively or noncompetitively with respect to mRNA.

The concentration of U1 RNA employed was the IC_{50} (2.1 μM). Figure 7 shows that, when mRNA was varied from 7 to 70 nM (0.1 to 1.0 μg/assay), the inhibitory effect of U1 RNA was not reversed. A Lineweaver-Burk plot (Figure 8) shows that the inhibition by U1 RNA was noncompetitive. U1 RNA did not affect the K_m of mRNA binding but affected the V_{max} of the reaction. The apparent K_m for binding of mRNA to the wheat germ ribosomes was 1.58×10^{-6} M.

Increasing the concentration of the wheat germ S30 fraction did reverse inhibition by U1 RNA (Figure 9). When 0.3 A_{260}

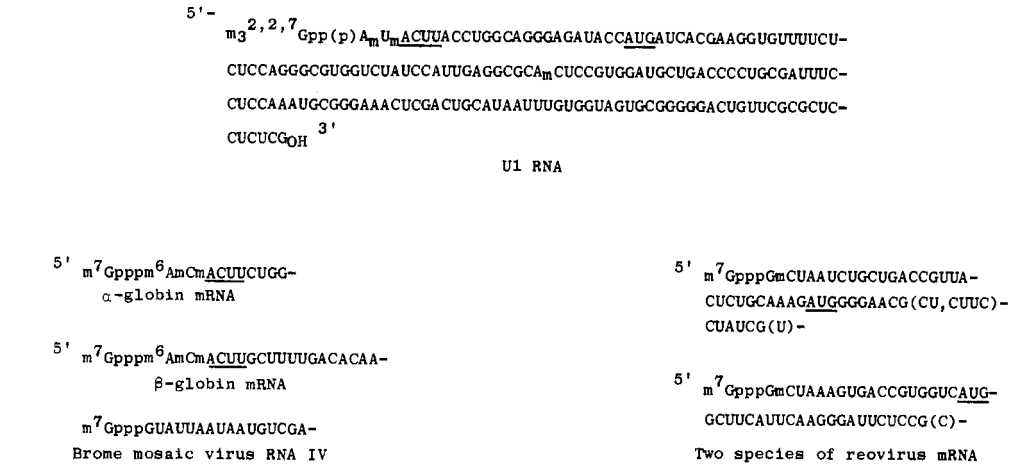

FIGURE 10: Nucleotide sequence of U1 RNA (Reddy et al., 1974), α- and β-globin mRNA (Lockard and Rajbhandary, 1976), reovirus mRNA (Kozak and Shatkin, personal communication), and Brome mosaic virus RNA IV (Dasgupta et al., 1975).

unit of the S30 fraction was added, the inhibition of translation by U1 RNA was 85%; at 1.2 A_{260} units of S30, the inhibition was only 36%.

Discussion

Structural Features of U1 RNA and Binding to Ribosomes. U1 RNA is an interesting species of LMWN RNA for studies in translational systems because in addition to its "5'-cap" linked in a pyrophosphate linkage to pAm on the 5'-terminal portion of the adjacent nucleotides, it also contains an AUG triplet 25 residues from the "5' cap" (Figure 10). Although U1 RNA shares these structural features of mRNA, it was not translated at concentrations ranging from 25 to 100 nM (Table I). Despite the presence of codons for leucine in the U1 RNA molecule, leucine incorporation was not detected. U1 RNA differs from mRNA in that it does not contain the termination codon UAG, UAA, or UGA near its 3' end.

U1 RNA also shares the tetranucleotide *ACUU* adjacent to the "5' cap" with those present in α- and β-globin mRNA (Lockard and Rajbhandary, 1976). Both α- and β-globin mRNA have $m^7Gpppm^6AmCmACUU$ (Figure 10), after which the nucleotide sequences diverge. The significance of this sequence homology at the 5' end between U1 RNA and globin mRNA is uncertain because *ACUU* is not present in reovirus mRNA or Brome mosaic virus RNA IV (Figure 10).

The finding that ^{125}I-labeled U1 RNA bound to 80S ribosome complexes shows that U1 RNA has structural features necessary for this binding. However, the binding affinity of U1 RNA for ribosomes was much less than that of mRNA (Figures 2 and 4), 10% of iodinated U1 RNA bound to the 80S complex and 40–50% of the labeled mRNA bound to the 80S complex. In addition, the ratio of the concentration of U1 RNA to that of mRNA at the IC_{50} was 250. The less efficient binding of U1 RNA to mRNA may be related to $pm_3^{2,2,7}G$ in the "cap" structure of U1 RNA.[3]

Inhibition of Translation. Table IV shows the comparative effects of pm^7G and U1 RNA on various parameters of translation. On a molar basis, U1 RNA was 15 times more inhibitory to translation than pm^7G. However, its "5'-cap" terminus, $pm_3^{2,2,7}G$, was only one-sixth as inhibitory as pm^7G (Table III). U1 RNA did not inhibit the formation of the 80S initiation complex at 2 μM, a concentration at which it produced a 50% inhibition of protein synthesis; pm^7G markedly inhibited formation of the 80S initiation complex (Figure 2).

TABLE IV: Comparative Effects of pm^7G and U1 RNA on Various Parameters of Translation.

	pm^7G	U1 RNA
IC50 for translation	30 μM	2.1 μM
80S complex formation	Inhibition	No inhibition
Poly(U) translation	No inhibition	No inhibition
Kinetics	10-min lag	Immediate

At a much higher concentration of U1 RNA (7 μM), there was partial inhibition of formation of the 80S initiation complex (Figure 3).

Neither pm^7G nor U1 RNA inhibits poly(U)-directed polyphenylalanine synthesis. As pointed out by Crystal et al. (1974), elongation factors EF1 and EF2 are required for translation of poly(U). Since U1 RNA does not inhibit mRNA binding to the 80S complex and does not inhibit peptide chain elongation at concentrations at which it blocks translation of mRNA, U1 RNA must either inhibit other factor(s) required for translation after the 80S complex is formed or exert some unusual effect on the translational system.[4]

U1 RNA differs from pm^7G at the rate of onset of inhibition protein synthesis. As noted by Weber et al. (1976) for HeLa cells and confirmed here for the wheat germ system, pm^7G inhibits after a 10-min lag period. A similar 10-min lag was observed in the reticulocyte cell-free system with aurintricarboxylic acid as an inhibitor of initiation (Lodish and Desalu, 1973). On the other hand, the effect of U1 RNA was virtually immediate. Assuming pm^7G specifically inhibits initiation of protein synthesis, the 10-min lag suggests this time is required for one complete round of translation.[5]

[4] At low concentrations (2–4 μg), U1 RNA had no effect on formation of the 80S initiation complex and differs in this respect from dsRNA (double-stranded RNA) which blocked IF-E2 in reticulocytes (Clemens et al., 1975). dsRNA did not inhibit mRNA translation in the wheat germ system (Grill et al., 1976).

[5] The average size of liver poly[A(+)] RNA is 16 S, which corresponds to a chain length of 1200 nucleotides (Rao et al., 1977). Assuming 300 nucleotides in untranslated regions contributed by poly(A) and adjacent nucleotides at the 3' end and "5' cap" and adjacent nucleotides at the 5' end, about 300 amino acids are incorporated into polypeptide chains resulting in a translational efficiency of one amino acid for each 2 s. This translational rate is much lower than the in vivo translational rate of 6–8 amino acids per second (Palmiter, 1975).

The findings that the inhibition of translation by U1 RNA is immediate, noncompetitive with respect to mRNA, and only reversed by the wheat germ S30 fraction suggests that some translational factors may be trapped by U1 RNA (Figure 5).

Function of U1 RNA. The physiological function of U1 RNA is unknown; several speculations have been made regarding its function (Ro-Choi and Busch, 1974; Ro-Choi et al., 1976). The present results show that U1 RNA has messenger-like structural features even though it is not translated. The abundance of U1 RNA in the nucleoplasm, its low turnover rate, and homogeneity support the suggestion that it may serve as part of a nuclear transport system such as the informosomes (Sekeris and Niessing, 1975).

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