Schimke, R. T. (1973), *J. Biol. Chem. 248,* 7530-7539.
Tsai, M.-J., Schwartz, R. J., Tsai, S. Y., and O'Malley, B. W. (1975a), *J. Biol. Chem. 250,* 5165-5174.
Tsai, S. Y., Tsai, M.-J., Schwartz, R. J., Kalimi, M., Clark, J. H., and O'Malley, B. W. (1975b), *Proc. Natl. Acad. Sci. U.S.A. 72,* 4228-4232.
Wetmur, J. G., and Davidson, N. (1968), *J. Mol. Biol. 31,* 349-370.
Woo, S. L. C., Monahan, J. J., and O'Malley, B. W. (1977), *J. Biol. Chem. 252,* 5789-5797.
Woo, S. L. C., and O'Malley, B. W. (1975), *Life Sci. 7,* 1039-1048.
Woo, S. L. C., Rosen, J. M., Liarakos, C. D., Robberson, D. L., Choi, Y. C., Busch, H., Means, A. R., and O'Malley, B. W. (1975), *J. Biol. Chem. 250,* 7027-7039.
Woo, S. L. C., Smith, R. G., Means, A. R., and O'Malley, B. W. (1976), *J. Biol. Chem. 251,* 3868-3874.
Young, B. D., Harrison, P. R., Gilmour, R. S., Birnie, G. D., Hill, A., Humphries, S., and Paul, J. (1974), *J. Mol. Biol. 84,* 555-568.

Dependence on Potassium Concentration of the Inhibition of the Translation of Messenger Ribonucleic Acid by 7-Methylguanosine 5′-Phosphate†

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ABSTRACT: The effects of potassium on the inhibition by 7-methylguanosine 5′-phosphate of the translation in the wheat germ cell-free system of globin mRNA and satellite tobacco necrosis virus (STVN) RNA are examined. The concentration of potassium ion and the anion of the potassium salt both influence the effects of 7-methylguanosine 5′-phosphate on the translation of globin mRNA. If potassium concentrations are less than the optimum for protein synthesis, the inhibition of the translation of globin mRNA by 7-methylguanosine 5′-phosphate is greatly diminished. The inhibition increases as the potassium concentration is increased even after the optimal concentration for protein synthesis is exceeded. At equivalent potassium concentrations, the inhibitory effect of 7-methylguanosine 5′-phosphate on translation of globin mRNA is substantially decreased if potassium acetate is substituted for KCl. However, the optimal concentration for protein synthesis is considerably greater for potassium acetate than for KCl and, if examined at the respective optimal concentrations of these salts for protein synthesis, the inhibiting effects of 7-methylguanosine 5′-phosphate are equivalent. The relative importance of the 7-methylguanosine cap for translation of globin mRNA is apparently not decreased at low potassium concentrations since the inhibition of translational activity of globin mRNA resulting from chemical removal of the cap is similar at all potassium concentrations. As expected, inhibition by 7-methylguanosine 5′-phosphate of the translation of STNV RNA, which does not contain a 7-methylguanosine cap, is considerably less than that observed with globin mRNA. However, the inhibition that is observed with STNV RNA exhibits qualitatively the same dependence on the concentration of potassium as that observed with globin mRNA and no inhibition by guanosine 5′-monophosphate is observed. These results illustrate the necessity of optimizing reaction conditions for individual mRNAs if the inhibition of translation of the mRNA by 7-methylguanosine 5′-phosphate is used as a criterion for the presence of a 7-methylguanosine cap.

The 5′ termini of most eukaryotic mRNAs have a unique structure of 7-methylguanosine (m^7G)[1] linked through its 5′-hydroxyl via a triphosphate to the penultimate nucleoside (Shatkin, 1976). The m^7G group is required for maximal translational activity in the wheat germ cell-free system of mRNAs for vesicular stomatitis virus, reovirus, hemoglobin, and parathyroid hormone (Both et al., 1975; Muthukrishnan et al., 1975; Kemper, 1976). Hickey et al. (1976a) observed that, in the wheat germ cell-free system, 7-methylguanosine 5′-phosphate (pm^7G) inhibited the translation of mRNAs containing m^7G but did not inhibit the translation of satellite tobacco necorsis virus (STNV) RNA which does not contain m^7G. Canaani et al. (1976) similarly observed that translation in the wheat germ system of the capped mRNAs, globin mRNA, and SV40 mRNA was inhibited to a much greater extent than that of uncapped mRNAs. However, translation of total poly(A)-containing mRNA from either HeLa cells or sea urchin eggs was inhibited only 75% to 80% by pm^7G compared with greater than 90% for globin mRNA (Weber et al., 1976; Hickey et al., 1976b), suggesting that the translation of some or all of these mRNAs is less sensitive to pm^7G than globin mRNA.

Since different mRNAs have different optimal ionic conditions for translation in the cell-free systems (Mathews, 1972; Tse and Taylor, 1977), it is possible that the sensitivities of presumed capped mRNAs to pm^7G are also a function of ionic conditions. In this report we show that the inhibition of the translation of globin mRNA by pm^7G is enhanced by increasing concentrations of potassium in the wheat germ cell-free system.

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[1] Abbreviations used are: Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; m^7G, 7-methylguanosine; pm^7G, 7-methylguanosine 5′-phosphate; STNV, satellite tobacco necrosis virus; ATA, aurintricarboxylic acid; poly(A), poly(adenylic acid); poly(U), poly(uridylic acid); EDTA, ethylenediaminetetraacetic acid.

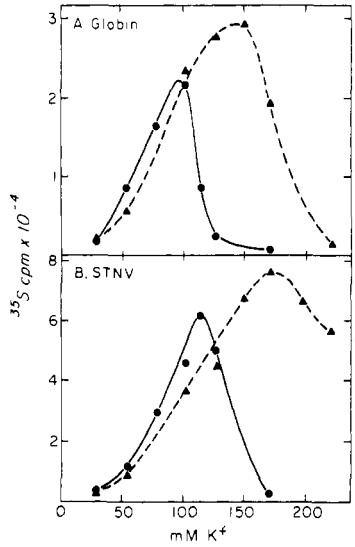

FIGURE 1: Dependence on the concentration of potassium of translation of globin mRNA and STNV RNA. Either KCl or potassium acetate was added to adjust the total potassium concentration to that indicated on the ordinate. A potassium ion concentration of 30 mM was contributed by 6 mM K^+-Hepes and 24 mM KCl from the buffer of the wheat germ extract. (● — ●) Addition of KCl; (▲ - - - ▲) addition of potassium acetate.

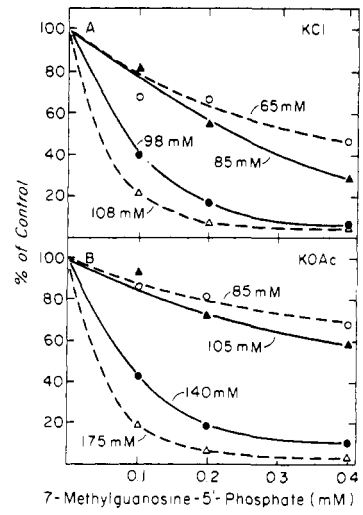

FIGURE 2: Inhibition by pm^{7}G of the translation of globin mRNA at varying concentrations of KCl and potassium acetate. The concentration of total potassium for each curve is indicated in the figure. The trichloroacetic acid insoluble radioactivities determined in a 1-μL aliquot of the reactions without pm^{7}G added were: for KCl, 12 045, 15 077, 19 767, and 13 715 cpm for 65, 85, 98, and 108 mM, respectively; and for potassium acetate, 12 568, 15 930, 23 264, and 21 191 cpm for 85, 105, 140, and 175 mM, respectively.

Methods

Isolation of RNA and Protein Synthesis. Globin mRNA was isolated by sedimentation on sucrose gradients as described by Labrie (1969). STNV RNA, provided by Dr. John M. Clark, Jr., was isolated as described previously by Clark and Klein (1974). Conditions for the translation of mRNA in the wheat germ cell-free system have been described previously (Kemper et al., 1974). To each reaction of 25 μL, 0.2 μg of globin mRNA or 1.0 μg of STNV RNA was added unless otherwise indicated. Incorporation of [^{35}S]methionine into protein is nearly directly proportional to added RNA up to these amounts (Kemper, 1976). In experiments in which the potassium concentration was varied by the addition of potassium acetate or KCl, an initial potassium concentration of 30 mM was contributed by 6 mM potassium-Hepes in the buffer and 24 mM KCl added with the wheat germ extract. All data are presented as the total potassium ion concentration in the reaction. [^{35}S]Methionine (5 μCi; 200–400 Ci mmol^{-1}, New England Nuclear) was added to each reaction. 7-Methylguanosine 5'-phosphate (P-L, Biochemical, Inc.) and *S*-adenosylhomocysteine (Sigma Chemical Co.) were added as indicated. Total trichloroacetic acid insoluble radioactivity was determined in 1-μL aliquots of the reaction.

Acrylamide Gel Electrophoresis of Proteins. Ten microliters of each reaction was analyzed on 15% acrylamide slab gels containing sodium dodecyl sulfate according to Laemmli (1970), as described previously (Kemper et al., 1976). Autoradiograms were prepared by exposing the dried gels to Kodak RP Royal x-ray film.

β Elimination of Globin mRNA. The m^{7}G cap was removed from 20 μg of globin mRNA by treatment with 1 mM sodium periodate in 0.1 M sodium acetate, pH 5.3, 1 mM EDTA for 2 h followed by treatment with 0.33 M aniline hydrochloride, pH 5.0, for 2 h as described previously (Kemper, 1976). Unmodified mRNA was incubated as above without sodium periodate and aniline.

Results

The optimal concentrations of potassium for the translation of both globin mRNA and STNV RNA were strongly dependent on the anion of the potassium salt (Figure 1). In both cases, the optimal concentration of potassium acetate was about 50% greater than the optimal concentration for KCl. At concentrations of potassium less than the optimal KCl concentration, translation was more efficient in KCl than potassium acetate, but, if compared at the respective optima of the two salts, incorporation was about 25% higher in potassium acetate. The simplest explanation of these unusual observations is that the chloride salt inhibits protein synthesis at the higher concentrations. The data in Figure 1 also illustrate that the optimal concentration of potassium was different for different mRNAs. The optimal potassium concentrations for globin mRNA and STNV RNA, respectively, were 95 mM and 115 mM for KCl and 140 mM and 175 mM for potassium acetate. For both mRNAs, analysis by electrophoresis in acrylamide gels containing sodium dodecyl sulfate revealed that the major translation product at all potassium concentrations was the expected complete polypeptide indicating that normal initiation of protein synthesis occurred even at low concentrations of potassium (not shown).

The effects of potassium on total protein synthesis suggest that both the concentration of potassium and the nature of the anion affect the interaction of the ribosome with mRNA. Since the m^{7}G cap of mRNA probably is involved in the binding of mRNA to ribosomes, we examined the effects of potassium on the inhibition of the translation of globin mRNA by pm^{7}G. At concentrations of potassium below the optimum for protein synthesis, the inhibiting effect of pm^{7}G was greatly reduced (Figure 2). As the concentration of potassium increased, the inhibition by pm^{7}G increased even when the optimal potassium concentration for protein synthesis was exceeded. Although there was considerable difference in the potassium optima for the chloride and acetate salts, the inhibition at the respective optima for the two salts was approximately the same. On the other hand, at the same potassium concentrations translation was inhibited much more for the chloride salt than the acetate salt. For example, 0.2 mM pm^{7}G inhibited translation 90% at 108 mM KCl, but only 30% at the nearly equivalent 105 mM potassium acetate.

Since pm^{7}G probably acts as an inhibitor of initiation, it is

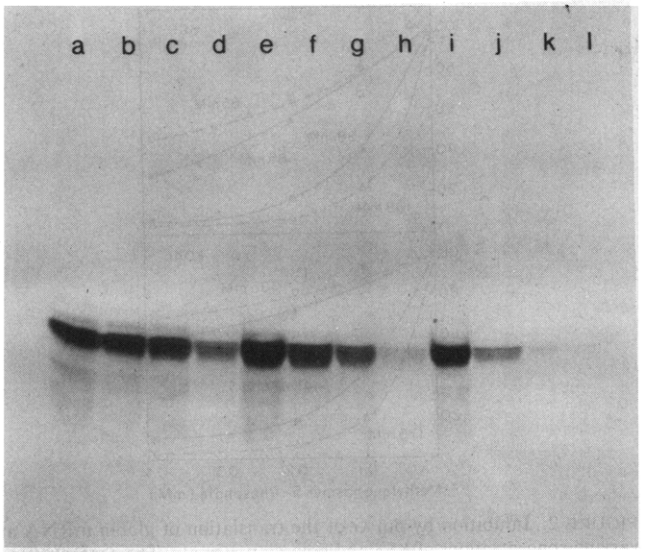

FIGURE 3: Sodium dodecyl sulfate gel electrophoresis of the ^{35}S-labeled polypeptide products of the translation of globin mRNA in the presence of pm^7G at varying potassium concentrations. Samples were analyzed as described in the legend to Figure 2 and the dried gels were exposed to x-ray film for 20 h. The major radioactive band comigrates with globin. (a–d) KCl, 65 mM; (e–h) 98 mM KCl; (i–l), 108 mM KCl. (a, e, i) No pm^7G; (b, f, j) 0.1 mM pm^7G; (c, g, k) 0.2 mM pm^7G; (d, h, l) 0.4 mM pm^7G.

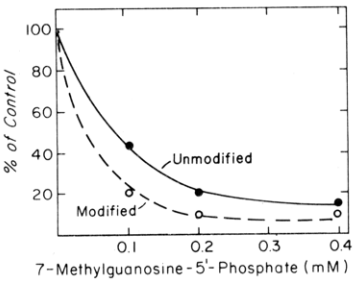

FIGURE 4: Inhibition by pm^7G of the translation of globin mRNA treated with periodate and aniline. Modified globin mRNA is globin mRNA in which the m^7G is removed by treatment with periodate and aniline. One microgram of the mRNA was translated in 25-μL reactions containing 98 mM KCl and 400 μM *S*-adenosylhomocysteine. The trichloroacetic acid insoluble radioactivity in 1-μL aliquots of the reactions without pm^7G were 24 208 cpm and 9170 cpm for the unmodified and modified globin mRNA, respectively. (● — ●) Unmodified globin mRNA; (○ - - - ○) modified globin mRNA.

TABLE I: Effect of Potassium Chloride Concentration on the Translation of Globin mRNA in Which the m^7G Cap Was Removed Chemically.[a]

Amount of mRNA added (μg)	KCl concn (mM)	Protein synthesis: Globin mRNA (cpm × 10^{-3})	Protein synthesis: Modified globin mRNA (cpm × 10^{-3})	Modified globin mRNA/ globin mRNA
0.2	75	10.1	1.2	0.12
0.2	98	14.9	2.4	0.16
0.2	108	13.2	1.8	0.14
0.4	75	7.6	2.2	0.29
0.4	98	14.6	4.0	0.27
0.4	108	16.8	2.9	0.17
1.0	75	5.2	4.0	0.77
1.0	98	13.3	6.3	0.47
1.0	108	17.0	6.9	0.41

[a] Modified globin mRNA is globin mRNA that was treated with periodate and aniline to remove the m^7G cap by a β-elimination reaction. The mRNA was translated in 25-μL reactions and the total trichloroacetic acid insoluble radioactivity in 1-μL aliquots was determined. The amount of mRNA is the total amount of mRNA added to the 25-μL reaction. The incorporation of radioactivity has been corrected by substrating the endogenous incorporation in the absence of added mRNA of 1155 cpm, 790 cpm, and 609 cpm for 75 mM, 98 mM, and 108 mM KCl, respectively.

possible that the resistant incorporation at lower potassium concentrations is the result of abnormal initiation. If examined by electrophoresis in sodium dodecyl sulfate–acrylamide gels, the major radioactive products of the reactions in the presence of pm^7G at low potassium concentrations were full length globin molecules (Figure 3). This suggested that initiation was occurring at or near the appropriate initiator codon and that pm^7G was not selectively inhibiting the synthesis of full length chains and allowing the increased production of the smaller radioactive products at low potassium concentrations.

As a consequence of the decreased inhibition of the translation of globin mRNA by pm^7G at lower potassium concentrations, the optimal potassium concentration for protein synthesis was changed. As the concentration of pm^7G in the reaction mixture increased, the optimal concentration for protein synthesis for both KCl and potassium acetate decreased. This result suggested the possibility that the m^7G cap could be less important for the translation of globin mRNA at the lower potassium concentrations. If this is true then the removal of the m^7G cap should have a smaller effect on the translational activity of globin mRNA at the lower concentrations of potassium. In Table I the translational activity of unmodified globin mRNA is compared with that of globin mRNA modified by treatment with periodate and aniline to remove the m^7G cap. The inhibition of translational activity by this treatment was essentially the same at the three concentrations of KCl examined. The only exception was an apparent decreased inhibition observed at 75 mM KCl if 1 μg of RNA was added to the reaction. This result, however, seemed to be due to decreased translational activity of unmodified mRNA under these conditions rather than increased translational activity of the modified mRNA. For all the concentrations of added mRNA, the translational activity of the modified globin mRNA was lower at 75 mM KCl than at 98 mM KCl. These results therefore do not support the hypothesis that the m^7G cap is less important for translation of globin mRNA at lower potassium concentrations.

The nature of the translational activity of the modified globin mRNA was examined further by determining its sensitivity to pm^7G. To prevent the possible enzymatic addition of a m^7G cap to the modified globin mRNA by wheat germ enzymes, 400 μM *S*-adenosylhomocysteine was added to the reaction. Both et al. (1975) demonstrated that, for reovirus RNA, methylation of the m^7G cap that is dependent on *S*-adenosylmethionine was inhibited by 160 μM *S*-adenosylhomocysteine in wheat germ extracts. As shown in Figure 4, the translation of the modified globin mRNA was actually inhibited slightly more than that of unmodified globin mRNA by pm^7G. These results thus indicate that the residual 10% to 15% translational activity of the modified globin mRNA is due to a fraction of the mRNA which retained its m^7G cap during the chemical treatment. These data further suggest that the m^7G cap is required for translation of globin mRNA in the wheat germ cell-free system.

STNV RNA does not contain a m^7G cap, but is actively translated in the wheat germ cell-free system (Kemper, 1976; Roman et al., 1976; Leung et al., 1976). If pm^7G inhibits initiation of protein synthesis by competitively inhibiting binding

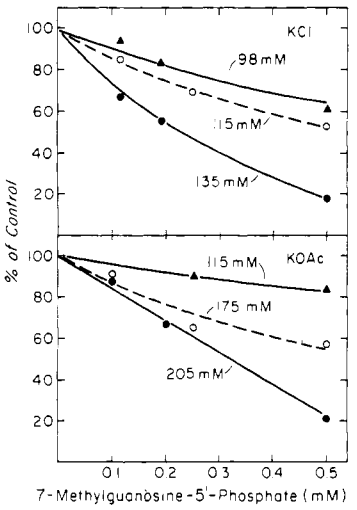

FIGURE 5: Inhibition by pm^{7}G of the translation of STNV RNA at varying concentrations of KCl and potassium acetate. The concentration of total potassium for each curve is indicated in the figure. The trichloroacetic acid insoluble radioactivities in a 1-μL aliquot of the reaction without pm^{7}G added were: for KCl, 41 438, 53 942, and 31 632 cpm for 98, 115, and 135 mM, respectively; and for potassium acetate, 35 851, 67 965, and 51 830 cpm for 115, 175, and 205 mM, respectively.

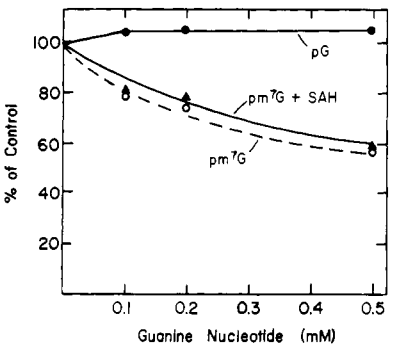

FIGURE 6: Inhibition by pm^{7}G of the translation of STNV RNA in the presence of *S*-adenosylhomocysteine and the effect of guanosine 5'-monophosphate on translation. The reaction mixture contained 115 mM KCl. *S*-Adenosylhomocysteine (SAH) was added to a concentration of 400 μM. The trichloroacetic acid insoluble radioactivities in a 1-μL aliquot of the reactions without pm^{7}G or guanosine 5'-monophosphate (pG) added were 66 679 and 71 913 cpm with and without SAH, respectively. (● — ●) Guanosine 5'-monophosphate added; (○ - - - ○) pm^{7}G added; (▲ — ▲) pm^{7}G + *S*-adenosylhomocysteine added.

of the m^{7}G cap to a specific protein, then translation of STNV RNA should not be inhibited and can be used as a control for nonspecific inhibition (Hickey et al., 1976). The translation of STNV RNA was inhibited by pm^{7}G but to a considerably lesser extent than globin mRNA (Figure 5). At the optimal potassium concentrations for protein synthesis for each mRNA, STNV RNA translation was inhibited only about 25% compared with about 80% for globin by 0.2 mM pm^{7}G. As with globin, however, there was increasing inhibition of STNV RNA translation with increasing concentrations of potassium.

The inhibition of the translation of STNV RNA by pm^{7}G could result because of the enzymatic addition of a m^{7}G cap to the STNV RNA. Roman et al. (1976) and Leung et al. (1976) have reported that *S*-adenosylhomocysteine does not inhibit the translation of STNV RNA in the wheat germ system and Leung et al. (1976) have also shown that radioactivity from [^{3}H]GTP is not detectable in initiation complexes containing STNV RNA as would be expected if m^{7}G is added to the RNA in the wheat germ system. However, to rule out this possibility under our conditions we examined the effect of pm^{7}G on the translation of STNV RNA in the presence of *S*-adenosylhomocysteine. As illustrated in Figure 6, *S*-adenosylhomocysteine had essentially no effect on the inhibition of the translation of STNV RNA by pm^{7}G at 115 mM KCl. Similar results were obtained if 135 mM KCl was present in the incubation buffer. Also if guanosine 5'-monophosphate was added instead of pm^{7}G, no inhibition of STNV RNA translation resulted (Figure 6).

Discussion

The inhibition of the translation of mRNAs by pm^{7}G has been proposed as a means for determining whether a particular mRNA contains a m^{7}G cap (Hickey et al., 1976a). The data in the present report illustrate several potential technical problems in this method. The most important is the great reduction in the pm^{7}G-dependent inhibition of the translation of globin mRNA at concentrations of potassium below the optimum for protein synthesis. Translation of mRNAs at suboptimal concentrations of potassium, therefore, could result in the false conclusion that the mRNA was not capped. This could also result in incomplete pm^{7}G-dependent inhibition of the translation of a heterogeneous mixture of mRNAs since they are not likely to all have the same optimal concentrations of potassium for protein synthesis. A second consideration is that STNV RNA and globin mRNA have different optimal potassium concentrations for protein synthesis. If STNV RNA is used as a control and is translated at the optimal potassium concentration for an mRNA such as globin mRNA, then STNV is translated at suboptimal potassium concentrations. In this case, the apparent "nonspecific" inhibition by pm^{7}G of the translation of STNV RNA will probably be underestimated. Finally, the fact that KCl and potassium acetate have different optima for both protein synthesis and inhibition of protein synthesis by pm^{7}G demonstrates that the optimal concentration must be determined if another potassium salt is substituted for a standard one. However, if both STNV RNA and globin mRNA are translated under their respective optimal conditions for protein synthesis, globin mRNA is considerably more sensitive to inhibition by pm^{7}G than STNV RNA in the wheat germ cell-free system. This method, thus, under optimal conditions is a valid criterion for determining whether or not an mRNA contains a m^{7}G cap.

The mechanism by which the effect of pm^{7}G is decreased at lower potassium concentration is not clear in part because the mechanism by which pm^{7}G inhibits translation of mRNA is not completely understood. The primary effect of pm^{7}G appears to be an inhibition of the initiation of protein synthesis (Roman et al., 1976; Hickey et al., 1976a; Shafritz et al., 1976) by competitively binding to a ribosomal initiation factor (Shafritz et al., 1976) or to a cytoplasmic protein involved in binding of the mRNA to the ribosome (Filipowicz et al., 1976). Initiation probably involves the interaction of multiple regions of mRNA with the ribosome including the AUG initiator codon, the m^{7}G cap, and a ribosomal binding region (Shih et al., 1976; Leung et al., 1976; Shine and Delgano, 1974; Steitz and Jakes, 1975). The effect of potassium on the action of pm^{7}G might then be explained either by a decreased importance of the m^{7}G cap at lower potassium concentrations or by an increased affinity for a specific protein by the intact cap structure relative to that of the small molecule pm^{7}G at lower potassium concentrations. Suggestive evidence against the first possibility includes the facts that compared with globin mRNA

the uncapped STNV RNA has a higher rather than lower optimum potassium concentration for protein synthesis and the globin mRNA from which the m^7G has been removed is not relatively more active at low potassium concentrations. With regard to the second possibility the intact cap structure apparently binds more tightly to the specific protein than pm^7G since a 1000-fold molar excess of pm^7G is required for inhibition. Thus, it is reasonable to suggest that relative changes in binding affinities may result from changes in the potassium concentration.

We found that the translation of an mRNA without an m^7G cap, STNV RNA, is inhibited by pm^7G, although to a considerably lesser extent than globin nRNA. This effect is similar to that observed by Hickey et al. (1976a), but they observed an initial stimulation of the translational activity of STNV RNA at low concentrations of pm^7G. For unknown reasons, we did not observe this increase even at concentrations of pm^7G ranging from 0.025 mM to 0.100 mM. The translation of another uncapped mRNA, encephalomyocarditis RNA, is also inhibited by pm^7G, but again to a lesser extent than the translation of mRNAs containing an m^7G cap (Canaani et al., 1976). The inhibition of STNV RNA is not the result of enzymatic addition of a m^7G cap by the wheat germ extract because the translation of STNV RNA is unaffected by *S*-adenosylhomocysteine (Romans et al., 1976; Leung et al., 1976) and *S*-adenosylhomocysteine does not alter the effect of pm^7G on the translation of STNV RNA. Furthermore, the translation of STNV RNA is not affected by guanosine 5′-monophosphate and the inhibition of translation by pm^7G exhibits a dependence on the potassium concentration qualitatively similar to that of globin mRNA. These observations raise the possibility that pm^7G may alter the structure of the ribosome so that initiation of mRNAs without m^7G caps is also inhibited. This possibility is supported further by the observation that the translation of poly(U) in the wheat germ system was not inhibited by pm^7G (Hickey et al., 1976a) suggesting that inhibition of elongation is not occurring.

These results illustrate the complexities of the effects of potassium on protein synthesis. Different concentrations of potassium are required for maximal total protein synthesis, for maximal synthesis of completed polypeptide chains (Benveniste et al., 1976; Harwood et al., 1975; Davies and Kaesberg, 1974; Thang et al., 1976; Tse and Taylor, 1977) and for maximal pm^7G-dependent inhibition of protein synthesis. In addition, the nature of the anion influences the optimal concentrations. The molecular mechanisms involved in these effects are obscure but they presumably involve changes in ribosomal structure caused by different potassium concentrations. The potassium-dependent properties of protein synthesis may be at least partially related to each other. For example, the inhibition of protein synthesis by pm^7G is related to the relative efficiency of total protein synthesis since equivalent inhibition with pm^7G is observed at the optimal concentrations of KCl and potassium acetate for protein synthesis even though the concentration of potassium ion for each salt is quite different. Regardless of the mechanisms involved, these results demonstrate the necessity of reoptimizing conditions for cell-free protein synthesis whenever a different component is used in the system, such as a different mRNA or even something seemingly as trivial as a different salt of potassium.

Acknowledgments

We thank Dr. John M. Clark, Jr., for helpful and critical discussions and A. Dorner for technical assistance.

References

Benveniste, K., Wilczek, J., Ruggieri, A., and Stern, R. (1976), *Biochemistry 15,* 830.

Both, G. W., Banerjee, A. K., and Shatkin, A. J. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 1189.

Canaani, D., Revel, M., and Groner, Y. (1976), *FEBS Lett. 64,* 326.

Clark, J. M., Jr., and Klein, W. H. (1974), *Methods Enzymol. 30,* 754.

Davies, J. W., and Kaesberg, P. (1974), *J. Gen. Virol. 25,* 11.

Filipowicz, W., Furuichi, Y., Sierra, J. M., Muthukrishnan, S., Shatkin, A. J., and Ochoa, S. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73,* 1159.

Harwood, R., Grant, M. E., and Jackson, D. S. (1975), *FEBS Lett. 57,* 47.

Hickey, E. D., Weber, L. A., and Baglioni, C. (1976a), *Proc. Natl. Acad. Sci. U.S.A. 73,* 19.

Hickey, E. D., Weber, L. A., and Baglioni, C. (1976b), *Nature (London) 261,* 71.

Kemper, B. (1976), *Nature (London) 262,* 321.

Kemper, B., Habener, J. F., Ernst, M. D., Potts, J. T., Jr., and Rich, A. (1976), *Biochemistry 15,* 20.

Kemper, B., Habener, J. F., Mulligan, R. C., Potts, J. T., Jr., and Rich, A. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 3731.

Labrie, F. (1969), *Nature (London) 221,* 1217.

Laemmli, U. K. (1970), *Nature (London) 227,* 680.

Leung, D. W., Gilbert, C. W., Smith, R. E., Sasavage, N. L., and Clark, J. M., Jr. (1976), *Biochemistry 15,* 4943.

Mathews, M. B. (1972), *Biochim. Biophys. Acta 272,* 108.

Muthukrishnan, S., Both, G. W., Furuichi, Y., and Shatkin, A. J. (1975), *Nature (London) 255,* 33.

Roman, R., Brooker, J. D., Seal, S. N., and Marcus, A. (1976), *Nature (London) 260,* 359.

Shafritz, D. A., Weinstein, J. A., Safer, B., Merrick, W. C., Weber, L. A., Hickey, E. D., and Baglioni, C. (1976), *Nature (London) 261,* 291.

Shatkin, A. J. (1976), *Cell 9,* 645.

Shih, D. S., Dasgupta, R., and Kaesberg, P. (1976), *J. Virol. 19,* 637.

Shine, J., and Delgano, L. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 1342.

Steitz, J. A., and Jakes, K. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 4734.

Thang, M. N., Dondon, L., and Mohier, E. (1976), *FEBS Lett. 61,* 85.

Tse, T. P. H., and Taylor, J. M. (1977), *J. Biol. Chem. 252,* 1272.

Weber, L. A., Fenan, E. R., Hickey, E. D., Williams, M. C., and Baglioni, C. (1976), *J. Biol. Chem. 251,* 5657.