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5′-Terminal 7-methylguanosine and mRNA function: Influence of potassium concentration on translation *in vitro*

(vaccinia mRNA/unmethylated mRNA/wheat germ/reticulocyte lysate)

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ABSTRACT **Vaccinia mRNAs containing either 5′-terminal m^7G or unmethylated 5′-terminal structures were synthesized *in vitro* and their relative efficiencies of translation were compared in wheat germ and reticulocyte cell-free protein-synthesizing systems. The importance of the m^7G group for efficient translation increases as the K^+ concentration is raised. At K^+ concentrations optimal for translation of mRNA containing m^7G, unmethylated mRNA is translated at the same relative low efficiency in both cell extracts. The rate of binding of mRNA to ribosomes at K^+ concentrations close to those found in intact cells is strongly influenced by the presence of m^7G regardless of the source of the cell extract.**

Experiments designed to test the function of the 5′-terminal $m^7G(5')ppp$ cap structure of eukaryotic mRNAs have shown that either chemically removing the 5′-terminal m^7G or preventing the methylation involved in its formation reduces the ability of mRNAs to be translated *in vitro* (1). However, a low level of translation of these mRNAs remains in all cases, and the extent of this residual translation varies considerably when tested in different cell-free systems (1–6). It has been suggested that this variability might be due to characteristics of the protein-synthesizing machinery of different cell types (4–6), and to differences in the interaction between mRNA structures other than the 5′-terminal m^7G and components of the cell-free system (5). In view of these results, the role of 5′-terminal m^7G in mRNA translation has recently been questioned (7).

The experiments reported here show that the varying dependency on the 5′-terminal m^7G for translation of eukaryotic mRNAs *in vitro* can be the result of the conditions introduced during the assembly of the cell-free systems. We have examined the relative translational efficiency of the methylated and unmethylated forms of vaccinia virus mRNA over a range of K^+ concentrations in cell-free protein-synthesizing systems derived either from wheat germ or from rabbit reticulocytes. Our results show that the importance of the 5′-terminal m^7G for mRNA translation increases as the K^+ concentration in the cell-free system is increased, regardless of the source of the cell extract. By using KOAc rather than KCl, protein synthesis can be efficiently carried out *in vitro* at K^+ concentrations close to those found in intact cells (34). At these K^+ concentrations unmethylated mRNA is translated at 15–20% the efficiency of methylated mRNA in both reticulocyte and wheat germ cell-free systems. The difference in the relative translation of methylated and unmethylated mRNA results from a reduced rate of binding of unmethylated mRNA to ribosomes.

MATERIALS AND METHODS

Chemicals. pm^7G was purchased from P-L Biochemicals. Sparsomycin was a gift from Upjohn Co. Radioactive compounds were purchased from New England Nuclear.

Synthesis, Purification, and Characterization of Vaccinia Virus mRNA. Purified vaccinia virus (stain WR) was a gift of Enzo Paoletti, of the New York State Health Laboratories. The vaccinia virus was prepared from infected HeLa cells as described by Joklik (8). RNA was synthesized *in vitro* in reaction mixtures containing 2 A_{260} units of virus particles per ml (1 A_{260} unit is the amount of material having an A_{260} of 1 when dissolved in 1 ml and the light path is 1 cm), 50 mM Tris·HCl at pH 8.4, 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.05% Nonidet P-40, 2 mM of each nucleoside triphosphate, and 10 μM either AdoMet or AdoHcy. Following incubation for 1 hr at 37°, ethylenediaminetetraacetic acid (EDTA) was added to 20 mM and the virus particles were removed by centrifugation. The RNA remaining in the supernatant was extracted twice with phenol, precipitated three times with ethanol, dried, redissolved in distilled water, and used directly in translation assays.

Radioactive mRNA was synthesized *in vitro* in similar reactions with [^{3}H]UTP (0.2 mM; 0.25 Ci/mmol) in the presence of either AdoMet or AdoHcy. After phenol extraction and ethanol precipitation, the RNA was dissolved in buffer containing 0.5% sodium dodecyl sulfate, 0.1 M LiCl, 1 mM EDTA, and 10 mM Tris at pH 7.5, heated at 65° for 2 min, chilled, and fractionated on sucrose gradients. The peak fractions sedimenting between 7 S and 12 S were collected by ethanol precipitation and reprecipitated three times. Both the methylated and unmethylated mRNA had a specific activity of 240,000 cpm/μg.

Poly(A) content of vaccinia mRNA was determined on radioactive RNA samples synthesized *in vitro* using [^{3}H]ATP (0.2 mM; 0.025 Ci/mmol) as described above. The RNA was digested with T1 and pancreatic RNase and the fraction resistant to digestion was determined by precipitation with 10% trichloroacetic acid. The size of the poly(A) tract was determined by electrophoresis on 10% polyacrylamide gels (9).

Cell-Free Protein Synthesis. Wheat germ extract was prepared as described by Roberts and Paterson (10). The composition of reaction mixtures used for *in vitro* translation and the procedure used to determine incorporation of radioactive amino acids have been described (34). All reactions contained 36 mM KCl plus additional KOAc to arrive at the indicated K^+ concentration. Assays contained either 100–200 μCi/ml of [^{3}H]-lysine (40 Ci/mmol) or 1 mCi/ml of [^{35}S]methionine (390 Ci/mmol) as indicated in the figure legends. Where indicated, 0.32 mM AdoHcy or 0.01 mM AdoMet were added to the reactions to either inhibit or enhance methylation of unmethylated mRNA by the cell extract (11).

Reticulocyte lysates prepared as previously described (34)

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Abbreviations: pm^7G, 7-methylguanosine 5′-monophosphate; AdoMet, *S*-adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine; M_r, molecular weight; HSB, high-salt buffer (4); VSV, vesicular stomatitis virus.

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were made dependent on exogenous mRNA for protein synthesis according to Pelham and Jackson (12). The lysates were incubated 5 min at 20° with micrococcal nuclease (8000 units/ml; Boehringer) at 30 μg/ml and 1 mM $CaCl_2$. After inactivation of the nuclease with 2 mM ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA), the lysates were used in 50-μl assays assembled as previously described (34). KOAc was added to the final concentration indicated. Reactions contained [^{35}S]methionine at 400 μCi/ml as the radioactive amino acid. Incubations were carried out for 60 min at 30° and duplicate 5-μl samples were assayed for radioactivity as previously described (13).

Polyacrylamide Gel Electrophoresis. The products of *in vitro* translation of vaccinia mRNA in incubations containing [^{35}S]methionine were fractionated by electrophoresis on 15% polyacrylamide slab gels according to Laemmli (14). The gels were dried and autoradiographed. The molecular weights (M_r) of the bands in the autoradiographs were determined by running protein markers of known molecular weight in parallel tracks.

Binding of [^{3}H]mRNA to Ribosomes. Mixtures (50 μl) for binding reactions were assembled as described for protein synthesis, but contained 20 unlabeled amino acids, 0.1 mM sparsomycin, 0.32 mM AdoHcy, and 5000 cpm of radioactive mRNA. Lysates used in these experiments were not treated with nuclease. The reactions were incubated at 30°, terminated by the addition of 0.2 ml of ice-cold high-salt buffer (HSB) (4), and fractionated on 15–30% sucrose gradients in HSB buffer as previously described (15). The time course of binding reactions was determined by centrifuging samples 4 hr at 50,000 rpm through 4.8-ml 15–30% gradients in HSB in a Beckman SW-50 rotor. These conditions pellet 80S ribosomes quantitatively and leave 40S subunits and unbound mRNA in the supernatant. The pellets were resuspended in 0.5% sodium dodecyl sulfate and their radioactivities were measured in Scintiverse (Fisher). Percent of mRNA bound was calculated from the fraction of input radioactivity found in the pellet after subtraction of radioactivity pelleted in reactions held at 0°.

RESULTS

Chemical treatments that remove the 5'-terminal m^7G can cause nonspecific damage to mRNA (4). We have avoided this problem by synthesizing methylated and unmethylated mRNA from vaccinia virus, using the endogenous enzymes present in the viral cores (16). Vaccinia mRNA synthesized *in vitro* has previously been shown to be translated efficiently in several different cell-free systems (17–20), and to code for peptides made early during the course of infection of mammalian cells by vaccinia virus (18–20). Vaccinia RNA synthesized in the presence of AdoMet contains 5'-terminal $m^7G(5')ppp$ (16). The preparation used for translation does not serve as a substrate for the methyl transferase of solubilized vaccinia cores prepared according to Paoletti *et al.* (21), indicating that it is fully methylated. Vaccinia RNA synthesized in the presence of AdoHcy contains 5' termini of the type ppN and G(5')pppN (16). This RNA does serve as an efficient substrate for methyl transferase (data not shown). Both RNA preparations show identical sedimentation profiles on sucrose gradients and have identical poly(A) content, and the sizes of their poly(A) tracts are identical when analyzed on polyacrylamide gels as described in *Materials and Methods* (data not shown). Thus, unlike the *in vitro* RNA synthesis with vesicular stomatitis virus (VSV) (4, 6, 22), the addition of AdoHcy to vaccinia virus transcription reactions does not appear to alter the product RNA in any way other than by preventing methylation.

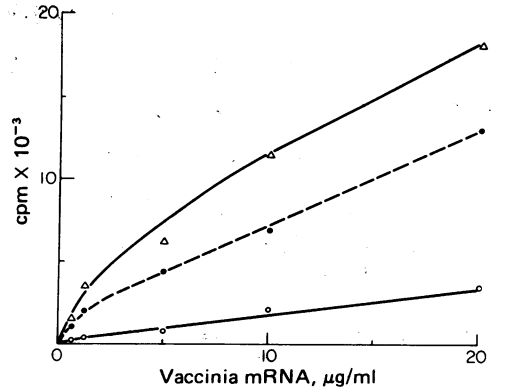

FIG. 1. Effect of methylation on the translation of vaccinia mRNA in the wheat germ cell-free system. Methylated mRNA was synthesized in the presence of AdoMet, and unmethylated mRNA, in the presence of AdoHcy (see *Materials and Methods*). The curves show incorporation of [^{3}H]lysine in 5-μl aliquots with: methylated mRNA (Δ—Δ); unmethylated mRNA with 0.32 mM AdoHcy included in the translation assay (O—O); unmethylated mRNA with 0.01 mM AdoMet included in the translation assay (●- - -●). Total K^+ concentration was 106 mM. Incorporation in control reactions with no added mRNA (2100 cpm) has been subtracted.

The functional integrity of unmethylated vaccinia mRNA is shown in Fig. 1. The methylated vaccinia mRNA is efficiently translated by wheat germ extracts and the efficiency of translation is not affected by the addition of either AdoHcy or AdoMet. The unmethylated mRNA is translated at a much lower efficiency when AdoHcy is included to prevent methylation by enzymes present in the wheat germ extract (11). However, translation of this mRNA is stimulated severalfold by AdoMet. Under conditions that allow methylation by the cell extract, approximately 70% of the translational efficiency of fully methylated mRNA is recovered.

It has been reported that analogues of the 5'-terminal cap structure, such as pm^7G, specifically inhibit translation of "capped" mRNAs (23, 24). Translation of natural mRNAs that lack the cap structure, such as picornavirus or satellite tobacco necrosis virus RNA, is resistant to inhibition by pm^7G (23, 24). The translation of unmethylated vaccinia virus mRNA is also completely resistant to inhibition by pm^7G, whereas translation of methylated mRNA is strongly inhibited (Fig. 2). The unmethylated vaccinia mRNA is therefore free of contamination with detectable amounts of methylated mRNA. We noticed,

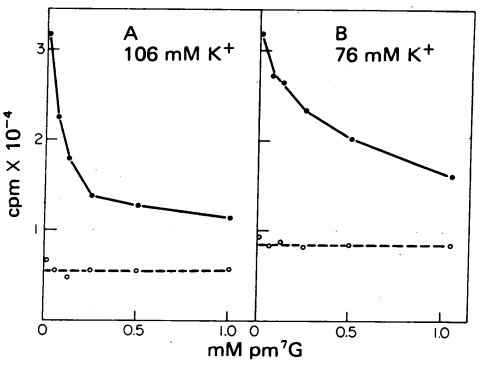

FIG. 2. Effect of pm^7G on the translation of methylated and unmethylated vaccinia mRNA in the wheat germ cell-free system. Reactions contained 106 mM K^+ (*A*) or 76 mM K^+ (*B*) and 0.32 mM AdoHcy. Each assay contained methylated (●—●) or unmethylated (O- -O) vaccinia mRNA at 20 μg/ml. The experiment in *B* had twice the concentration of [^{3}H]lysine. Endogenous incorporation at each concentration of inhibitor has been subtracted (5500–2000 cpm).

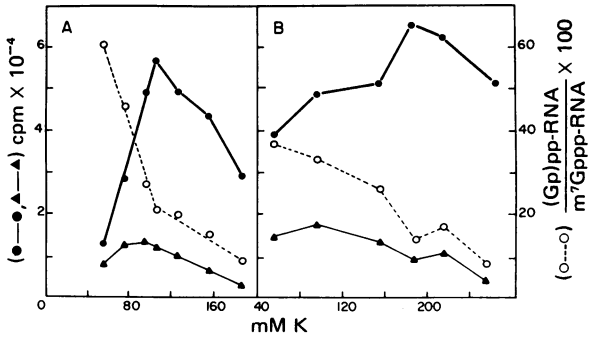

FIG. 3. Translation of methylated and unmethylated vaccinia mRNA at different K^+ concentrations. Incubations contained methylated (●—●) or unmethylated (▲—▲) mRNA at 20 μg/ml, 0.32 mM AdoHcy, and KOAc to give the final concentrations indicated. (*A*) Translation in the wheat germ cell-free system. (*B*) Translation in reticulocyte lysate. Incorporation obtained with unmethylated mRNA expressed as percent of the incorporation obtained with methylated mRNA is indicated (o- -o).

moreover, that the extent to which translation of methylated mRNA is inhibited by pm^7G is influenced by the K^+ concentration. A similar observation has been made by A. E. Smith (personal communication).

Forms of mRNA lacking the 5′-terminal m^7G are translated with significantly lower efficiency in wheat germ extracts than in reticulocyte lysates (1, 4–6). We considered the possibility that the effect of the m^7G on translation may be related to the K^+ concentration at which the assays were carried out. We have, therefore, investigated the relative efficiency of translation of methylated and unmethylated vaccinia mRNA and of the corresponding unmethylated mRNA at different K^+ concentrations in both protein-synthesizing systems (Fig. 3). Unmethylated mRNA is translated at a lower efficiency than methylated mRNA at all K^+ concentrations in both cell-free systems. In addition, the efficiency of translation of unmethylated mRNA relative to methylated mRNA decreases as the K^+ concentration is increased. At the lowest concentration tested unmethylated mRNA is translated at 60% and 40% the efficiency of methylated mRNA by the wheat germ and reticulocyte cell-free systems, respectively. At the K^+ optimum for vaccinia mRNA translation, unmethylated mRNA is translated at 15–20% the efficiency of methylated mRNA. Further increases in K^+ concentration reduce translation of both mRNAs, and the difference in translation between methylated and unmethylated mRNA becomes even more pronounced.

The same polypeptides are synthesized during translation of methylated or unmethylated vaccinia mRNA in both the wheat germ and reticulocyte lysates. These are shown in the autoradiographs of the translation products separated by polyacrylamide gel electrophoresis in Fig. 4. About 10 bands are discernible, indicating peptides with molecular weights ranging from 12,000 to 45,000. There is no apparent qualitative difference in the translation products of methylated and unmethylated mRNA synthesized at high or low K^+ concentration. This indicates that peptide synthesis is initiated at the same site on unmethylated as on methylated mRNA. The relative increase in translation of unmethylated mRNA at low K^+ concentration is therefore not a consequence of decreased fidelity of translation.

Binding of Methylated and Unmethylated mRNA to Ribosomes. The 5′-terminal m^7G in mRNA has previously been reported to function specifically in the initiation step of protein synthesis (1). Chemical elimination of the m^7G from reovirus or VSV mRNA reduces both the rate and the extent of mRNA binding to ribosomes (4–6). These mRNAs can be obtained in an unmethylated form by blocking methylation during *in vitro* transcription (25). The unmethylated mRNAs also bind poorly to ribosomes (25). These studies, and others in which pm^7G is used as an inhibitor (15, 23), have indicated that the 5′-terminal m^7G promotes a stable interaction of mRNA with the 40S ribosomal subunit (1).

The effect of K^+ concentration on the binding of radioactive vaccinia mRNA to ribosomes is shown in Figs. 5 and 6. Methylated mRNA binds to about the same extent after a 4 min incubation in reticulocyte lysates supplemented with either 40 mM or 120 mM KOAc (Fig. 5 *A* and *C*). Unmethylated mRNA, however, binds less well, particularly with 120 mM KOAc (Fig. 5 *B* and *D*). The reduced binding of unmethylated mRNA reflects a decrease in the rate of binding (Fig. 6). The unmethylated mRNA binds at a much slower rate at the higher salt concentration, whereas the methylated mRNA binds to ribosomes at similar rates with 40 or 120 mM KOAc.

Differential degradation of mRNA cannot account for the observed difference in ribosome binding. Degradation of radioactive mRNA was monitored in the reticulocyte cell-free system by oligo(dT)-cellulose chromatography (26). No endonucleolytic degradation could be detected during the short

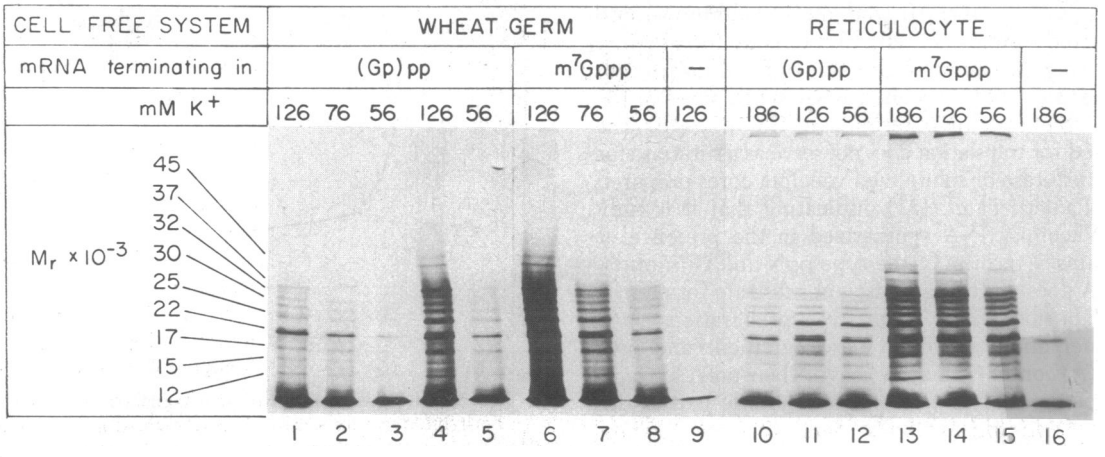

FIG. 4. Translation products of methylated and unmethylated vaccinia mRNA. The products of translation synthesized with [^{35}S]methionine in both the wheat germ and reticulocyte cell-free systems were analyzed by polyacrylamide gel electrophoresis followed by autoradiography. Unmethylated mRNA is designated as mRNA terminating in (Gp)pp; methylated mRNA is indicated as mRNA terminating in m^7Gppp. AdoHcy was omitted from the incubations whose products were run in slots 4 and 5.

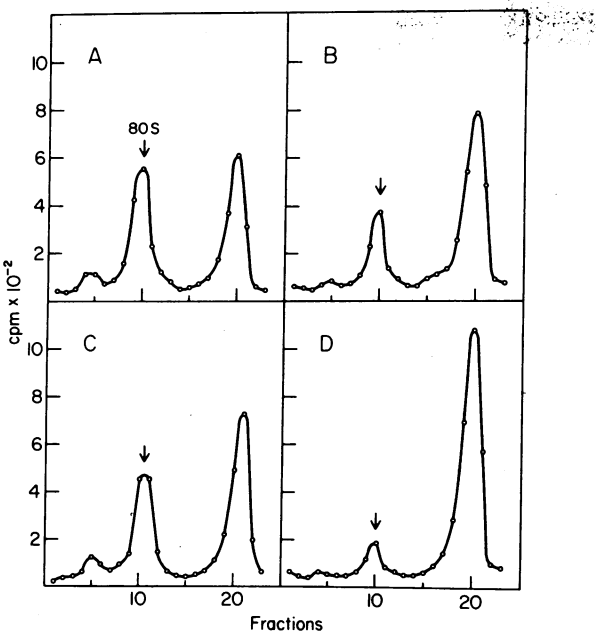

FIG. 5. Binding of labeled vaccinia mRNA to reticulocyte ribosomes at different K^+ concentrations. Binding reactions contained either methylated (*A* and *C*) or unmethylated (*B* and *D*) [^{3}H]mRNA and either 40 mM (*A* and *B*) or 120 mM (*C* and *D*) KOAc. The reactions were incubated 4 min at 30° and fractionated on sucrose gradients. Direction of sedimentation is from right to left and arrows indicate the position of 80S monosomes. The percentage of labeled mRNA bound to ribosomes is 47% (*A*), 26% (*B*), 43% (*C*), and 9% (*D*).

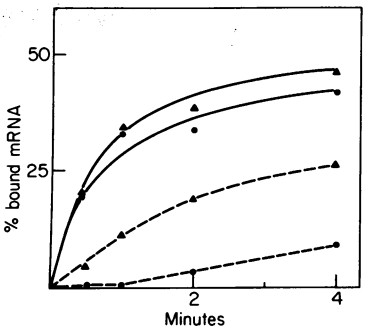

FIG. 6. Kinetics of binding of labeled methylated and unmethylated vaccinia mRNA to reticulocyte ribosomes at different K^+ concentrations. Incubations were performed as in Fig. 5 and terminated at the indicated times. The fraction of [^{3}H]mRNA bound to ribosomes was determined as described in *Materials and Methods*. Solid line: methylated mRNA binding at 40 mM (▲—▲) or 120 mM (●—●) KOAc. Broken line: unmethylated mRNA binding at 40 mM (▲- -▲) or 120 mM (●- -●) KOAc.

incubations used for binding experiments (our unpublished observations). Moreover, methylated and unmethylated mRNA are degraded with similar kinetics in reticulocyte lysates (27).

DISCUSSION

A functional role of the 5′-terminal m^7G was first indicated in experiments showing its requirement for efficient translation of reovirus and VSV mRNA by wheat germ extracts (11). Wheat germ ribosomes specifically bind mRNA molecules that have m^7G when incubated with reovirus mRNA containing a mixture of molecules with or without m^7G (25). Translation of cellular mRNAs by wheat germ extracts is also highly dependent on the presence of the 5′-terminal m^7G (28–30). In contrast, m^7G has been reported to be less important for translation of mRNA in reticulocyte lysates. Rose and Lodish (4) found that after removal of m^7G from VSV mRNA by β elimination, the efficiency of translation and the rate of ribosome binding was only reduced about 3-fold. These authors suggested that the decrease in biological activity after β elimination could result from chemical degradation of mRNA rather than from removal of m^7G and that a strong dependence on m^7G for initiation may be characteristic only of the wheat germ extract. In a more recent study, Lodish and Rose (6) compared the efficiency of translation of VSV mRNA having different 5′ termini in wheat germ and reticulocyte cell-free systems. They found that forms of VSV mRNA lacking 5′-terminal m^7G were translated with 6–7% the efficiency of control mRNA (containing m^7G) in wheat germ extracts but with 23–29% relative efficiency in the reticulocyte system.

In a similar study, Muthukrishnan *et al.* (5) report that β elimination reduces the binding of reovirus and VSV mRNA to wheat germ ribosomes from 80% to 5–7%. However, significant binding (17–34%) of these β-eliminated viral mRNAs occurs in reticulocyte lysates (5). These authors suggest that the reduced binding of wheat germ ribosomes may reflect an increased dependence upon m^7G for recognition of heterologous animal virus mRNA by plant ribosomes.

The results reported here show that the importance of m^7G for the translation of mRNA is strongly influenced by K^+ concentration in both the wheat germ and the reticulocyte cell-free system. It is important to consider this effect when evaluating data that indicate a greater or lesser dependence on m^7G in different cell-free systems. Translation of mRNA in wheat germ extracts is usually carried out at 90–100 mM K^+. At this K^+ concentration, the wheat germ extract shows a marked dependence on the presence of 5′-terminal m^7G in mRNA for initiation. Translation of mRNA by reticulocyte lysates is often carried out adding as little as 56 mM KCl (31). Differences in translational efficiency between methylated and unmethylated mRNA are reduced at the K^+ optimum determined with KCl in reticulocyte lysates. By using the acetate rather than the chloride salt of potassium, protein synthesis can be carried out in vitro at K^+ concentrations approaching levels present in cells (34). The difference in the dependence on 5′-terminal m^7G for translation of mRNA between wheat germ and reticulocyte extracts diminishes when protein synthesis is carried out at cellular K^+ concentrations in each cell-free system.

Changes in K^+ concentration presumably affect the activity of several components of the protein-synthesizing machinery. As the K^+ concentration is raised, protein synthesis reaches a maximum and then decreases (Fig. 3). However, the importance of m^7G for translation increases as the K^+ concentration is raised regardless of the overall effect on protein synthesis. This suggests that some step in the initiation process involving m^7G recognition is affected in a specific way. It is conceivable that an interaction between initiation factor(s) and the 5′-terminal m^7G is more important at high ionic strength because of increased mRNA secondary structure. Translation independent of m^7G at low ionic strength may reflect an increased conformational flexibility of mRNA that makes these interactions less important.

Our results emphasize that the interpretation of experiments done in cell-free systems requires consideration of how closely the experimental conditions approach the conditions in the cell. While certain mRNAs such as picornavirus mRNA do not contain m^7G (32, 33), and are translated efficiently, the 5′-

terminal m^7G may be essential for *in vivo* translation of those mRNAs that normally contain the cap structure.

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1. Shatkin, A. J. (1976) *Cell* **9**, 645–653.
2. Muthukrishnan, S., Filipowicz, W., Sierra, J. M., Both, G. W., Shatkin, A. J. & Ochoa, S. (1975) *J. Biol. Chem.* **250**, 9336–9341.
3. Shih, D. S., Dasgupta, R. & Kaesberg, P. (1976) *J. Virol.* **19**, 637–644.
4. Rose, J. K. & Lodish, H. F. (1976) *Nature* **262**, 32–37.
5. Muthukrishnan, S., Morgan, M., Banerjee, A. K. & Shatkin, A. J. (1976) *Biochemistry*, **15**, 5761–5768.
6. Lodish, H. F. & Rose, J. K. (1977) *J. Biol. Chem.* **252**, 1181–1188.
7. Griffin, B. (1976) *Nature* **263**, 188–190.
8. Joklik, W. K. (1962) *Biochem. Biophys. Acta* **61**, 290–301.
9. Loening, U. K. (1968) *J. Mol. Biol.* **38**, 355–365.
10. Roberts, B. E. & Paterson, B. M. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2330–2334.
11. Both, G. W., Banerjee, A. K. & Shatkin, A. J. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1189–1193.
12. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256.
13. Jacobs-Lorena, M. & Baglioni, C. (1973) *Eur. J. Biochem.* **35**, 559–565.
14. Laemmli, U. K. (1970) *Nature* **277**, 680–685.
15. Shafritz, D. A., Weinstein, J. A., Safer, B., Merrick, W. C., Weber, L. A., Hickey, E. D. & Baglioni, C. (1976) *Nature* **261**, 291–294.
16. Moss, B., Gershowitz, A., Wei, C. M. & Boone, R. (1976) *Virology* **72**, 341–351.
17. Beaud, G., Kirn, A. & Gros, F. (1972) *Biochem. Biophys. Res. Commun.* **49**, 1459–1466.
18. Fournier, F., Tovell, D. R., Esteban, B., Metz, D. H., Ball, L. A. & Kerr, I. M. (1973) *FEBS Lett.* **30**, 268–273.
19. Nevins, J. R. & Joklik, W. K. (1975) *Virology* **63**, 1–13.
20. Jaureguiberry, G., Ben-Hamida, F., Chapeville, F. & Beaud, G. (1975) *J. Virol.* **15**, 1467–1474.
21. Paoletti, E., Rosemond-Hornbeak, H. & Moss, B. (1974) *J. Biol. Chem.* **249**, 3273–3280.
22. Rose, J. K., Lodish, H. F. & Brock, M. L. (1977) *J. Virol.* **21**, 683–693.
23. Hickey, E. D., Weber, L. A. & Baglioni, C. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 19–23.
24. Weber, L. A., Feman, E. R., Hickey, E. D., Williams, M. C. & Baglioni, C. (1976) *J. Biol. Chem.* **251**, 5657–5662.
25. Both, G. W., Furuichi, Y., Muthukrishnan, S. & Shatkin, A. J. (1975) *Cell* **6**, 185–195.
26. Pemberton, R. E., Liberti, P. & Baglioni, C. (1975) *Anal. Biochem.* **66**, 18–28.
27. Furuichi, Y., LaFiandra, A. & Shatkin, A. J. (1977) *Nature* **266**, 235–239.
28. Muthukrishnan, S., Both, G. W., Furuichi, Y. & Shatkin, A. J. (1975) *Nature* **255**, 33–37.
29. Rao, M. S., Wu, B. C., Waxman, J. & Busch, H. (1975) *Biochem. Biophys. Res. Commun.* **66**, 1186–1193.
30. Kemper, B. (1976) *Nature* **262**, 321–323.
31. Villa-Komaroff, L. M., McDowell, M., Baltimore, D. & Lodish, H. F. (1974) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 30, pp. 709–721.
32. Hewlett, M., Rose, J. & Baltimore, D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 327–330.
33. Nomoto, A., Lee, Y. F. & Wimmer, E. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 375–380.
34. Weber, L. A., Hickey, E. D., Maroney, P. A. & Baglioni, C. (1977) *J. Biol. Chem.* **252**, 4007–4010.