Volume 2 number 8 August 1975 Nucleic Acids Research

$m^7G^{5'}ppp^{5'}$GmpCpUp at the 5' terminus of reovirus messenger RNA

M. Faust*, K. E. M. Hastings and S. Millward+

Department of Biochemistry, McGill University, Montreal, Canada

Received 18 June 1975

ABSTRACT

In the presence of S-adenosyl methionine the 5' terminal guanosine residue of in vitro synthesized reovirus mRNA becomes methylated at the 2'-OH position. In addition, 7-methyl guanylic acid is condensed covalently at the 5' terminus resulting in the formation of a 5' to 5' triphosphate bridge. Analysis of the 5' terminal sequence of methylated reovirus mRNA revealed that it has the structure $m^7G^{5'}ppp^{5'}$GmpCpUp

INTRODUCTION

Reovirions possess a methyl transferase which catalyzes the transfer of methyl groups from S-adenosyl methionine (SAM) to the 5' terminus of in vitro synthesized mRNA[1,2]. This type of reaction was first reported for cytoplasmic polyhedrosis virus[3] and has since been reported for a number of other viral systems including vaccinia virus[4] and vesicular stomatitis virus[5]. Methylated sequences have also been observed in mRNA isolated from eukaryotic cells[6,7].

In earlier experiments we found that the 5' terminal phosphates of methylated mRNA were resistant to hydrolysis with alkaline phosphatase[2]. This observation has since been confirmed and in this report we have established that the 5' terminus of methylated reovirus mRNA has the unusual structure $m^7G^{5'}ppp^{5'}$GmpCpUp. A similar conclusion has been reached recently by Furuichi et al. who derived the structure via independent methods[8].

MATERIALS AND METHODS

Cells and virus. Reovirus type 3 (Dearing strain) was grown in mouse L-cells and purified as described previously[9].

Synthesis of methylated mRNA and analysis of alkali-derived methylated oligonucleotides. The synthesis and purification of methylated mRNA and the analysis of methylated oligonucleotides on DEAE-cellulose columns have been described previously[2].

Oxidation with sodium periodate and β elimination. The procedure used was essentially that described by Fraenkel-Conrat[10]. Oxidation of oligonucleotides was carried out in 200 μl of 40 mM sodium acetate buffer, pH 5.2 in the presence of 50 nmoles of sodium periodate. The reaction was allowed to proceed in the dark at 24°C for 60 minutes. Excess periodate was consumed with propylene glycol and after ten minutes, 0.33 M aniline (adjusted to pH 5.0 with HCl), was added to a final concentration of 0.25 M. The mixture was placed in the dark at 24°C and allowed to react for six hours. After diluting the reaction mixture fifty-fold with buffer containing 5 mM Tris-HCl pH 8.0 and 7 M urea, the products were analyzed on DEAE-cellulose columns.

Paper chromatography of 2'-O-methylated nucleosides. The method used was that of Al-arif and Sporn[11]. Chromatography (ascending) was carried out on Whatman 3 MM paper. The solvent system used was n-butanol (2000 ml)/0.8 M boric acid (270 ml)/concentrated (28-30%) ammonia (8 ml). Authentic 2'-O-methylated nucleosides were obtained from wheat germ ribosomal RNA[12], and identified by eluting the spots from the chromatogram in 0.1 N HCl and taking their absorption spectra at acidic, neutral and alkaline pH. In our hands the order of migration of the 2'-O-methylated nucleosides was Am > Cm > Gm > Um which differs from that described previously[11].

Acid hydrolysis of methylated mRNA and column chromatography of 7-methyl guanine. Material to be analyzed was mixed with 1-2 μmoles each of guanosine, adenosine and 7-methyl guanosine and the mixture was hydrolyzed in 1 N HCl at 100°C for 1 hr. The hydrolysate was then loaded on a 1 x 15 cm column of Bio-Rad AG 50W x 4 (H^+) and the column developed with 2 N HCl at a flow rate of 1 ml/min[13]. The effluent was monitored continuously at 260 nm. Fractions

of 8 ml each were collected and aliquots were counted in Hydromix (Yorktown Chemicals).

Chemicals. S-adenosyl-L-[methyl-^{3}H]methionine (specific activity, 8.1 Ci/mmole) and [α-^{32}P]ribonucleoside triphosphates were obtained from New England Nuclear Corp. [γ-^{32}P]GTP (specific activity, 0.7 Ci/mmole) was from Amersham/Searle while [β,γ-^{32}P]GTP (specific activity, 2.5 Ci/mmole) was from International Chemical and Nuclear Co. Bacterial alkaline phosphatase and snake venom phosphodiesterase were products of Worthington Biochemical Corp. and pyrophosphatase was obtained from Sigma Chemical Company Ltd.

RESULTS

Unusual properties of the 5' terminus of methylated mRNA. The presence of an unusual structure at the 5' terminus of methylated mRNA was suggested to us by the following series of experiments. After alkaline hydrolysis of methylated mRNA and subsequent DEAE-cellulose chromatography the methyl groups were recovered quantitatively as highly charged oligonucleotide derivatives (Fig. 1a), indicating that they were derived from the 5' terminus of the mRNA molecules[2]. Considering that the 5' terminus of unmethylated mRNA was known to have the configuration ppGpNp[14], it seemed likely that the methylated derivative was simply ppGmpNp. This would be consistent with the estimate of 1-2 methyl groups per RNA chain[1,2] and would account for the alkali stability of the methylated species. However, treatment of the alkali-derived oligonucleotides (Fig. 1a) with alkaline phosphatase caused them to elute between the dinucleotide (-3 charge) and trinucleotide (-4 charge) markers on DEAE-cellulose columns (Fig. 1b). This result suggested a reduction of -2 in the net negative charge of the oligonucleotide consistent with the loss of the phosphomonoester generated during alkaline hydrolysis. Moreover, the 5' terminal phosphate groups must be blocked to the action of alkaline phosphatase, possibly in the form X(p)$_n$GmpNp where X is the blocking group.

In a separate experiment the 5' terminal oligonucleotide was oxidized

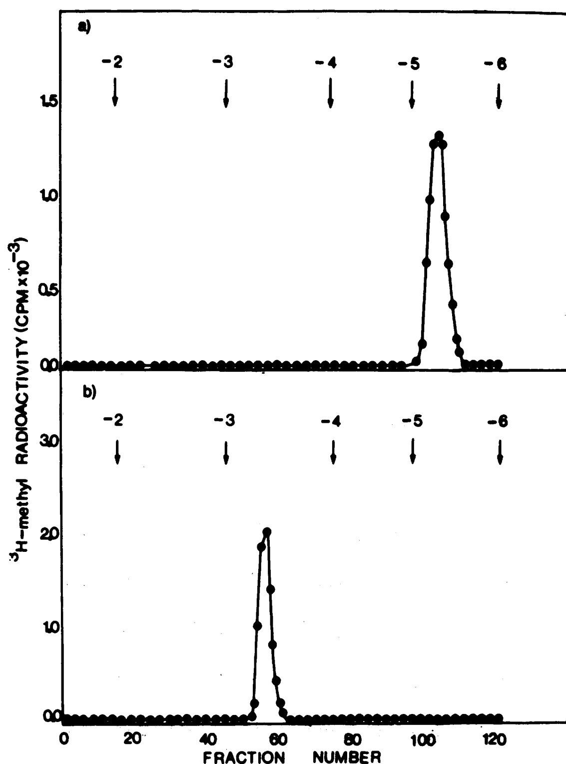

Figure 1. a) DEAE-cellulose chromatography of an alkaline hydrolysate of ^{3}H-methyl mRNA. b) Re-chromatography of the material in a) after treatment with alkaline phosphatase, 10 µg/ml, for 3 hr at 40°C. The arrows represent the elution positions of nucleotides generated by pancreatic RNase digestion of wheat germ ribosomal RNA.

with periodate and subjected to β elimination prior to alkaline phosphatase digestion. The methyl-labeled products of this sequence of reactions eluted from a DEAE-cellulose column before the mononucleotide region, indicating a net charge of less than -2 (data not shown). Thus, β elimination renders the 5' terminal phosphates susceptible to alkaline phosphatase suggesting that the blocking group has vicinal hydroxyl groups linked β to the 5' phosphate.

When the alkali-derived oligonucleotide was subjected to oxidation with $NaIO_4$ and subsequent β elimination, and the products analyzed directly on DEAE-cellulose (Figure 2), one-half of the methyl label no longer bound to the column, while the remainder eluted between the tetranucleotide (-5 charge)

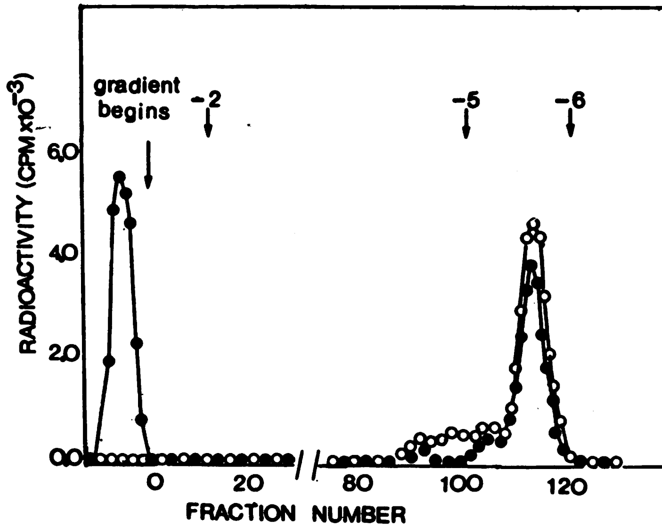

Figure 2. DEAE-cellulose chromatography following β elimination of the alkali-derived oligonucleotide. The oligonucleotide was obtained from mRNA doubly labeled with ^{3}H-methyl SAM and a mixture of the four [α-^{32}P]ribonucleoside triphosphates (final specific activity = 0.71 Ci/mmole). ^{32}P, open circles; ^{3}H, closed circles.

and pentanucleotide (-6 charge) markers. Since β elimination resulted (i) in the loss of one-half of the ^{3}H-methyl label and (ii) in the release of the blocking group, we tentatively conclude that the blocking group is methylated. In light of the reactants present during in vitro synthesis of the mRNA the results further suggest that the blocking group is a base-methylated nucleotide necessarily linked 5'-5' to the methylated mRNA.

Chromatographic analysis of methylated bases and nucleosides. Since certain base methylated nucleosides are altered under alkaline conditions[15], ^{3}H-methyl mRNA was reduced to nucleosides enzymatically using a mixture of snake venom phosphodiesterase, alkaline phosphatase and pyrophosphatase. The products were analyzed by paper chromatography in a borate solvent system which showed that at least two methylated species were present in approximately equal amounts (Fig. 3). One co-chromatographed with 2'-O-methyl guanosine while the second species remained near the origin suggestive of a base methylated derivative. The latter species was recovered, hydrolyzed in acid

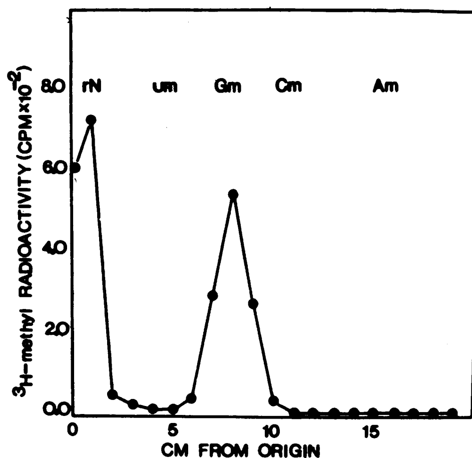

Figure 3. Identification of 2'-O-methyl guanosine among the nucleosides generated enzymatically from ^{3}H-methyl mRNA. Analysis was carried out by paper chromatography in a borate solvent system.

and the hydrolysate analyzed by column chromatography as described in Materials and Methods. Approximately 90% of the ^{3}H-methyl label co-eluted with 7-methyl guanine (Fig. 4b) and this identification was confirmed by chromatography in two additional solvent systems (Figs. 5a, b). In a separate experiment ^{3}H-methyl RNA was subjected to acid hydrolysis and the products analyzed again by column chromatography (Fig. 4a). Two distinct methylated species were detected with one-half the radioactivity corresponding to authentic 7-methyl guanine. From the results of Figures 3a and 4a we conclude that the ^{3}H-methyl mRNA contains 7-methyl guanine and 2'-O-methyl guanosine in approximately equimolar amounts. Furthermore, since methylation at the 2' position of the terminal guanosine residue is necessary to make the adjacent phosphodiester linkage resistant to alkaline hydrolysis (see Fig. 1), we conclude that the methylations are not mutually exclusive events occurring on different molecules.

Nucleotide composition of the methylated oligonucleotide and origin of the phosphate groups. The next step in the elucidation of the oligonucleotide structure was to determine its nucleotide composition, and the configuration of its phosphate groups. Since the blocking group is methylated and must

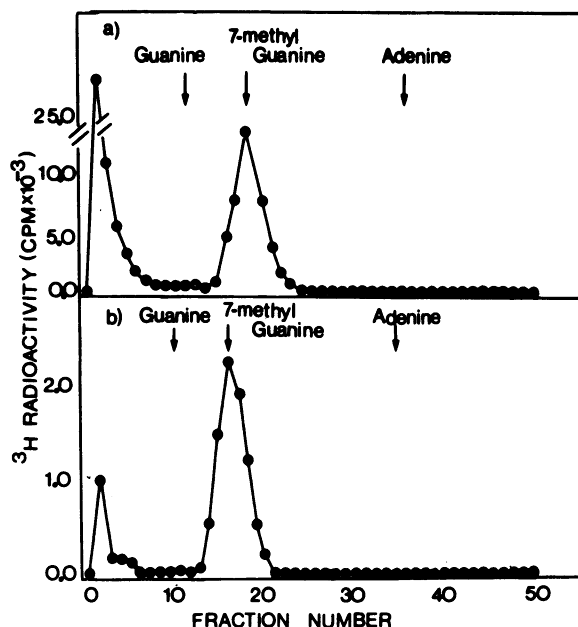

Figure 4. Chromatograpny of 7-methyl guanine on Bio-Rad AG 50W columns. a) total acid hydrolysate of methylated mRNA. b) Base methylated nucleosides isolated from the origin following borate-paper chromatography (see Fig. 3) were hydrolyzed with HCl and the hydrolysate chromatographed.

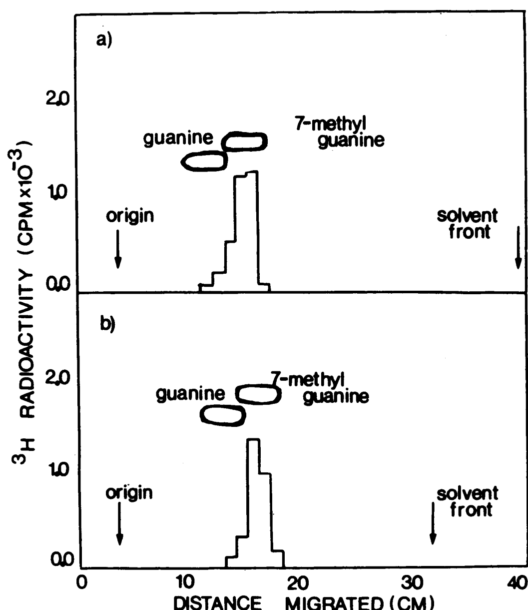

Figure 5. Paper chromatography of 7-methyl guanine. Material recovered from the origin in Fig. 3 was hydrolyzed in 2 N HCl and chromatographed in a) solvent system A or b) solvent system B as described by Iwanami and Brown[13].

have vicinal hydroxyl groups, 7-methyl guanosine was assigned this role. This results in the structure $m^7G^{5'}(p)_n{}^{5'}GmpNp$ with the uncertainty being (i) in the number of phosphates comprising the 5'-5' bridge and (ii) in the identity of the penultimate nucleotide which has been reported as Up in unmethylated mRNA[14] and Cp in both methylated[1] and unmethylated mRNA[16]. To get at this problem, the methylated oligonucleotide was recovered after alkaline hydrolysis of methylated mRNA and the 3' terminal phosphate was removed with alkaline phosphatase. The phosphatase-resistant oligonucleotide was then chromatographed on DEAE-cellulose. When this experiment was carried out using methylated RNA labeled with either [α-^{32}P]CTP or [α-^{32}P]GTP the phosphatase-resistant oligonucleotide eluting between the -3 and -4 charge markers contained both ^{32}P and ^{3}H radioactivity (Figs. 6a,b). In contrast, neither [α-^{32}P]ATP nor [α-^{32}P]UTP contributed their α phosphates to the phosphatase-resistant material. From the radioactivity recovered in the peak fractions and the known specific activities of the radioactive precursors it could be calculated that the molar ratio of methyl : pG : pC in the phosphatase-resistant oligonucleotide is 2:2:1. This means that the phosphatase-resistant oligonucleotide has the general formula $m^7G^{5'}p(p)_n p^{5'}GmpC$, where the phosphates designated $(p)_n$ are derived from either the β or γ positions of GTP.

In order to determine whether any of the phosphates in the 5' terminal oligonucleotide are derived from the gamma position of GTP, mRNA was made in the presence of [γ-^{32}P]GTP and ^{3}H-methyl SAM. When the mRNA product was separated from the radioactive precursors by gel filtration there was no ^{32}P associated with the ^{3}H-methyl mRNA. The extent of methyl labeling indicated that enough high molecular weight RNA had been synthesized so that even low levels of incorporation of [γ-^{32}P]GTP would have been detected. Therefore, methylated mRNA (and the methylated 5' terminal oligonucleotide) contains no γ phosphate from GTP.

It should be pointed out that although the available evidence favors

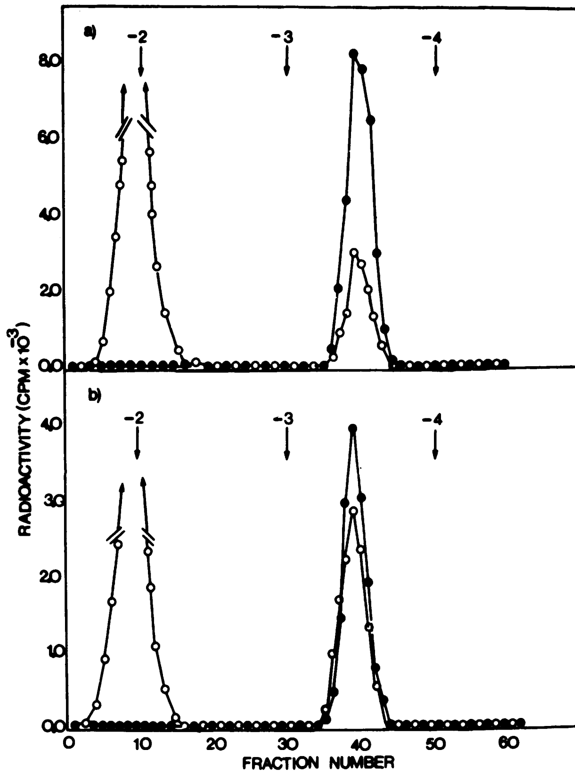

Figure 6. DEAE-cellulose chromatography of the alkaline phosphatase resistant 5' terminal oligonucleotide, a) labeled with ^{3}H-methyl and [α-^{32}P]CTP and b) labeled with ^{3}H-methyl and [α-^{32}P]GTP. The specific activity of both ^{32}P-labeled ribonucleoside triphosphates was 1.85 Ci/mmole. ^{32}P, open circles, ^{3}H, closed circles.

having three phosphates in the bridge[8] the possibility of having four phosphates present has not been rigorously ruled out. To determine the number of β phosphates from GTP which become incorporated into the 5' terminal structure, mRNA was synthesized in the presence of ^{3}H-methyl SAM and [β,γ-^{32}P]GTP. This doubly-labeled mRNA was hydrolyzed in alkali and analyzed by DEAE-cellulose column chromatography. The 5' terminal oligonucleotide recovered from the column contained both ^{3}H and ^{32}P radioactivity in a molar ratio of ^{3}H-methyl: β-phosphate of 1.8. This result supports the view that there are three phosphates in the bridge. However, to avoid possible complications in determining absolute counting efficiencies of different isotopes we also

examined the relative amounts of α and β phosphates from GTP which become incorporated into methylated mRNA. To do this, two samples of mRNA were synthesized under identical conditions in the presence of ^{3}H-methyl SAM. One sample was labeled with [α-^{32}P]GTP and the other with [β,γ-^{32}P]GTP of known specific activities. Both samples were hydrolyzed and analyzed as before.

Table 1. Relative incorporation of [β,γ-^{32}P]GTP and [α-^{32}P]GTP into 5' terminal methylated oligonucleotides.

Experiment	Isotope	Radioactivity (cpm)[a]	$^{32}P/^{3}H$ ratio [b]	Phosphates/oligonucleotide (relative moles)[c]
1	[^{3}H]SAM	8,610	0.65	2.00
	[α-^{32}P]GTP	5,630		
2	[^{3}H]SAM	24,870	0.27	0.83
	[β,γ-^{32}P]GTP	5,900		

[a]Values obtained represent the radioactivities found in the -3 to -4 charge region of a DEAE-cellulose column as in Figure 6a, b.

[b]Corrected for the difference in specific activity between [β,γ-^{32}P]GTP (1.60 Ci/mmole) and [α-^{32}P]GTP (0.90 Ci/mmole).

[c]The $^{32}P/^{3}H$ ratio obtained for [α-^{32}P]GTP was taken as 2.00 and the $^{32}P/^{3}H$ ratio for [β,γ-^{32}P]GTP was normalized accordingly.

Since the ^{3}H-methyl radioactivity measured in this experiment (Table 1) is proportional to the number of oligonucleotides being analyzed and the ^{32}P radioactivities from either [α-^{32}P]GTP or [β,γ-^{32}P]GTP are directly proportional to the actual number of α and β phosphates respectively, the $^{32}P/^{3}H$ ratio in each case yields a value which is in turn proportional to the number of α or β phosphates per oligonucleotide. As shown in Table 1, when the $^{32}P/^{3}H$ ratio for [α-^{32}P]GTP is set at 2.0, we find there is 0.83 or one β phosphate incorporated per oligonucleotide. That is, the phosphatase-resistant oligonucleotide contains twice as much α phosphate from GTP relative to β phosphate. Thus, there are three phosphates in the bridge, two derived from the α position and one from the β position of GTP. These analyses and

the results of the previous section suggest that the structure of the 5' terminal oligonucleotide $m^7G^{5'}ppp^{5'}GmpC$.

Identification of the nucleotide adjacent to the alkali-resistant 5' terminus. When methylated mRNA labeled with [α-^{32}P]UTP and ^{3}H-methyl SAM was hydrolyzed in alkali and the hydrolysate analyzed on DEAE-cellulose columns, both ^{3}H-methyl and ^{32}P radioactivity were found in the 5' terminal structure. Furthermore, the molar ratio of ^{32}P to ^{3}H-methyl in the 5' terminal oligonucleotide was approximately 0.5. When this experiment was repeated with [α-^{32}P]ATP as substrate, no ^{32}P was transferred to the 5' terminal oligonucleotide following alkali hydrolysis. These experiments show that the nucleotide adjacent to $m^7G^{5'}ppp^{5'}GmpC$ at the 5' terminus of methylated mRNA is pU.

DISCUSSION

We have shown that reovirus mRNA synthesized in vitro in the presence of SAM contains the alkali-resistant 5' terminal oligonucleotide $m^7G^{5'}ppp^{5'}GmpCp$. This conclusion is in agreement with that reported recently by Furuichi et al.[8] who arrived at a similar structure using different methodology. This structure is consistent with several observations regarding methylated mRNAs including blocking of the 5' terminal phosphates to alkaline phosphatase and release of the blocking group by $NaIO_4$ oxidation and subsequent β elimination.

Enzymatic hydrolysis of methylated mRNA and subsequent chromatographic analyses (Figs. 3, 4b) showed that the methylated derivatives were 7-methyl guanine and 2'-O-methyl guanosine. Since the blocking group could be removed during the β elimination reaction, 7-methyl guanosine must occupy this position. The alkali stability of the oligonucleotide could be accounted for by the presence of 2'-O-methyl guanosine, the only other methylated species detected. Isotope incorporation studies showed that the 5' terminal oligonucleotide was composed of two moles of pG, two moles of methyl and

one mole of pC. Thus, the methylated species were considered to be fully represented by the mononucleotides m^7pG and pGm, which together with pC, were arranged in an alkali-stable configuration. Incorporation of one β phosphate of GTP further suggested that the oligonucleotide contained one mole of ppG. Finally, α-^{32}P transfer experiments indicated that the terminal phosphomonoester generated during alkaline hydrolysis was derived exclusively from UTP. Thus, methylated reovirus mRNA was shown to possess the 5' terminal sequence $m^7G^{5'}ppp^{5'}GmpCpUp$.

The details of how this unusual structure is formed _in vitro_ remain obscure. Nevertheless one can propose a mechanism to illustrate the source of the phosphates in the bridge structure as follows:

(1) pppG(m) → ppG(m) + Pi

(2) ppG(m) + $(m^7)\overset{**}{p}\overset{*}{p}pG$ ⇄ $(m^7)G^{5'}ppp^{5'}G(m)$ + $\overset{**}{PP}i$

At present all we can say about the methylation event is that it occurs very early on nascent mRNA chains[1,2]. We know equation (1) can occur as a result of the phosphohydrolase which resides in the core[17,18] and also that unmethylated mRNA has ppG at the 5' terminus[14], so in this case at least, the source of the β phosphate is unambiguous. We also know that GTP alone can undergo pyrophosphate exchange when incubated in the presence of viral cores[19] and this feature is taken into account in equation (2). We are presently carrying out experiments which should shed additional light on the mechanism of formation of the capped 5' terminus of reovirus mRNA.

Methylation of mRNA has recently been reported for a number of viral[20-22] and eukaryotic systems[23-25]. The blocking group 7-methyl guanosine, appears to be a constant feature of methylated messenger RNAs as does 2'-O-methylation of the adjacent 5' terminal nucleotide. In addition, all methylated mRNAs so far reported have been shown to contain a 5'-5' bridge consisting of three phosphate groups. An apparent exception is a low molecular weight nuclear

RNA from Novikoff hepatoma cells whose 5' terminus has the structure $m_3^{2,2,7}$ G$^{5'}$pp$^{5'}$ AmpUmp. The function of this RNA species is unknown[26]. Finally, it is interesting to note that in vitro methylation of viral mRNAs seems to occur exclusively at the 5' terminus whereas the methylation pattern in mRNA from eukaryotic cells is more complex. The significance of this difference remains to be determined. Though the function of methylated sequences in mRNA is unknown, its widespread occurrence is suggestive of an important biological role. Methylated sequences may act as recognition signals involved in transcriptional or translational control or in the endonucleolytic processing of mRNA precursors. Recent evidence suggests that the m^{7}G capping group is required for the translation of reovirus and vesicular stomatitis virus mRNAs in vitro[27,28].

It is interesting to note that among the low molecular weight oligonucleotides known to be associated with reovirions, there appears to exist a molecular species with an unusual positively charged group at the 5' terminus[29]. The trinucleotide sequence (p)ppGpCpU has also been reported to be present in this class of oligonucleotides[30,31]. Our finding of a similar sequence at the 5' termini of mRNA lends support to the view that these oligonucleotides arise by multiple reinitiation events during virion assembly[32]. However, this hypothesis does not exclude the possibility that these oligonucleotides might serve a useful biological function during the process of infection. For example, they might confuse the host translational machinery in an as yet, undetermined fashion. It is possible to determine experimentally which species, if any, of these oligonucleotides can interfere with translation in vitro.

Recently it has been shown that m^{7}G$^{5'}$ppp$^{5'}$GmpCp is present at the 5' termini of the plus strands of reovirus genome RNA[33,34]. We have competed out the plus strand of the genome duplex RNA and have arrived at a similar conclusion (Hastings and Millward, unpublished data). This result is

consistent with the precursor-product relationship that exists between reovirus mRNA and the genome RNA[35,36]. It will be interesting to see whether mRNA isolated at late times from reovirus-infected cells contains similarly blocked 5' terminal structures.

ACKNOWLEDGEMENTS

We are indebted to Dr. Ray Lau for his help in preparing 2'-O-methylaed nucleosides and to Mlle Charlotte Legare for expert technical assistance. This work was supported by the Medical Research Council of Canada.

*M. Faust is the holder of a Medical Research Council Studentship (Ottawa).

+Medical Research Council Scholar (Ottawa).

REFERENCES

1. Shatkin, A.J. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3204-3207.
2. Faust, M. and Millward, S. (1974) Nucleic Acids Res. 1, 1739-1752.
3. Furuichi, Y. (1974) Nucleic Acids Res. 1, 809-822.
4. Wei, C.M. and Moss, B. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3014-3018.
5. Rhodes, D.P., Moyer, S.A. and Banerjee, A.K. (1974) Cell 3, 327-333.
6. Desrosiers, R., Friderici, K. and Rottman, F. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3971-3975.
7. Perry, R.P. and Kelley , D.E. (1974) Cell 1, 37-42.
8. Furuichi, Y., Morgan, M., Muthukrishnan, S. and Shatkin, A.J. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 362-366.
9. Nonoyama, M., Millward, S. and Graham, A.F. (1974) Nucleic Acids Res. 1, 373-385.
10. Steinschneider, A. and Fraenkel-Conrat, H. (1966) Biochemistry 5, 2735-2743.
11. Al-Arif, A. and Sporn, M.B. (1972) Analyt. Biochem. 48, 386-393.
12. Singh, H. and Lane, B.G. (1964) Can. J. Biochem. 42, 1011-1021.
13. Iwanami, Y. and Brown, G.M. (1968) Archiv. of Biochem. and Biophys. 124, 472-482.
14. Banerjee, A.K., Ward, R. and Shatkin, A.J. (1971) Nature New Biol. 230, 169-172.
15. Hall, R.H. (1971) The Modified Nucleosides in Nucleic Acids. Columbia University Press.
16. Nichols, J.L., Hay, A.J. and Joklik, W.K. (1972) Nature New Biol. 235, 105-107.
17. Kapular, A.M., Mendelsohn, N., Klett, H. and Acs, G. (1970) Nature 225, 1209-1213.
18. Borsa, J., Grover, J. and Chapman, J.D. (1970) J. Virol. 6, 295-302.
19. Wachsman, J.T., Levin, D.H. and Acs, G. (1970) J. Virol. 6, 563-565.
20. Wei, C.M. and Moss, B. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 318-322.
21. Furuichi, Y. and Miura, K. (1975) Nature 253, 374-375.
22. Abraham, G., Rhodes, D.P., and Banerjee, A.K. (1975) Cell 5, 51-58.
23. Adams, J.M. and Cory, S. (1975) Nature 255, 28-33.
24. Wei, C.M., Gershowitz, A. and Moss, B. (1975) Cell 4, 379-386.
25. Perry, R.P., Kelley , D.E., Friderici, K. and Rottman, F. (1975) Cell 4, 387-394.

26. Ro-Choi, T.S., Reddy, R., Choi, Y.C., Raj, N.B and Hennings, D. (1974) Fed. Proc. 33, 1832.
27. Muthukrishnan, S., Both, G.W., Furuichi, Y. and Shatkin, A.J. (1975) Nature 255, 33-37.
28. Both, G.W., Banerjee, A.K. and Shatkin, A.J. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 1189-1193.
29. Bellamy, A.R. and Hole, L.V. (1970) Virology 40, 808-819.
30. Bellamy, A.R., Hole, L.V. and Baguley, B.C. (1970) Virology 42, 415-420.
31. Stoltzfus, C.M. and Banerjee, A.K. (1972) Archiv. Biochem. and Biophys. 152, 733-743.
32. Bellamy, A.R., Nichols, J.L. and Joklik, W.K. (1972) Nature New Biol. 238, 49-50.
33. Furuichi, M., Muthukrishnan,S. and Shatkin, A.J. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 742-745.
34. Chow, N. and Shatkin, A.J. (1975) J. Virol. 15, 1057-1064.
35. Schonberg, M., Silverstein, S.C., Levin, D.H. and Acs, G. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 505-508.
36. Acs, G., Klett, H., Schonberg, M., Christman, J., Levin, D.H. and Silverstein, S.C. (1971) J. Virol. 8, 684-689.