5′-Terminal and Internal Methylated Nucleotide Sequences in HeLa Cell mRNA†

Cha Mer Wei, Alan Gershowitz, and Bernard Moss*

ABSTRACT: The 5′-terminal oligonucleotides $m^7G(5')ppp(5')NmpNp$ and $m^7G(5')ppp(5')NmpNmpNp$ were isolated by DEAE-cellulose column chromatography after enzymatic digestion of ^{32}P- or methyl-^3H-labeled poly(A)$^+$ HeLa cell mRNA. The recovery of such oligonucleotides indicated that a high percentage of mRNA has blocked termini. The dimethylated nucleoside, $N^6,O^{2'}$-dimethyladenosine (m^6Am), as well as the four common 2′-*O*-methylribonucleosides (Gm, Am, Um, Cm) were present in the second position linked through the triphosphate bridge to 7-methylguanosine (m^7G) whereas little m^6Am was in the third position. The only internal methylated nucleoside, N^6-methyladenosine (m^6A), was found exclusively as m^6ApC and Apm^6ApC after digestion with RNase A, T_1, and alkaline phosphatase. Digestion with RNase A and alkaline phosphatase revealed that guanosine also precedes m^6ApC but that pyrimidines are present in much smaller amounts or absent from this position. These results imply a considerable sequence specificity since there are thousands of different mRNA species in HeLa cells. Our studies are consistent with the following model of HeLa cell mRNA in which Nm may be m^6Am, Gm, Cm, Um, or Am and one or more m^6A residues are present at an unspecified internal location: $m^7G(5')ppp(5')Nm$-(Nm)···(G or A)-m^6A-C··· $(A)_{100-200}A$.

Methylated nucleosides, long thought to be unique to rRNAs and tRNAs, were recently found in cellular (Perry and Kelley, 1974; Desrosiers et al., 1974) and viral (Furuichi, 1974; Wei and Moss, 1974; Shatkin, 1974; Rhodes et al., 1974) mRNAs. Further analysis of the viral mRNAs indicated that the methylated nucleosides were at the 5′-termini in sequences of the type $m^7G(5')ppp(5')Nm$ (Wei and Moss, 1975; Furuichi et al., 1975a; Furuichi and Miura, 1975; Urishibara et al., 1975; Abraham et al., 1975). Two types of 5′-terminal sequences, $m^7G(5')ppp(5')Nm$ and $m^7G(5')ppp(5')NmpNm$, as well as internal N^6-methyladenosine (m^6A)[1] residues, were found in HeLa cell (Wei et al., 1975a; Furuichi et al., 1975b) and mouse myeloma cell (Adams and Cory, 1975) mRNAs. Results consistent with these types of structures were also obtained for L cell mRNA (Perry et al., 1975) and a related sequence $m_3^{2,2,7}G(5')pp(5')AmUm$ was determined for nuclear U-2 RNA of Novikoff hepatoma cells (Shibata et al., 1975). We now provide additional information regarding the fraction of HeLa cell mRNA containing modified 5′-terminal sequences, the composition and arrangement of methylated nucleosides at the 5′-terminus, and the first evidence that the internal m^6A residues are present in a specific sequence.

Materials and Methods

Isolation of Polyadenylated mRNA. HeLa S3 cells, at a concentration of 2.5×10^6 cells/ml, were labeled with 10 mCi of [*methyl*-^3H]methionine for 4 h as previously described (Wei et al., 1975a). For ^{32}P-labeling, cells were suspended at 3×10^5/ml in medium lacking phosphate. After 7 h carrier-free $^{32}P_i$ (5 μCi/ml) was added and the incubation at 37° was continued for 24 h. Polyadenylated RNA was isolated by sodium dodecyl sulfate, chloroform, phenol extraction, ethanol precipitation, and poly(U)-Sepharose chromatography as described (Wei et al., 1975a). Further purification was achieved by rechromatography on poly(U)-Sepharose after heating the RNA in 10 mM Tris-HCl (pH 7.6) and 0.1% sodium dodecyl sulfate for 2 min at 60°.

Enzymatic and Chemical Treatment. Digestion with a combination of RNase A (100 μg/ml), T_1 (20 μg/ml), and T_2 (10 units/ml) in 0.1 M ammonium acetate (pH 4.5) was for 9 h at 37°. Combined digestion with RNase A (10 μg/ml) and T_1 (20 μg/ml) in 50 mM Tris-HCl (pH 7.5) was for 4 h at 37°. Digestion with RNase A alone at 100 μg/ml in 50 mM Tris-HCl (pH 7.5) was for 1 h at 37°. Nuclease P_1 was used at 0.5 mg/ml in 10 mM sodium acetate (pH 6.0) for 1 hr at 37° and nucleotide pyrophosphatase (1 unit/ml) in 20 mM Tris-HCl (pH 7.6) and 1 mM $MgCl_2$ for 30 min at 37°. Alkaline phosphatase and/or snake venom phosphodiesterase digestion was carried out as described (Wei et al., 1975a).

Electrophoresis and Chromatography. DEAE-cellulose chromatography in 7 M urea at pH 7.6 with oligonucleotide markers was described previously (Wei et al., 1975a). Pooled fractions were desalted on 0.7 cm × 5 cm DEAE-cellulose columns equilibrated either with 10 mM ammonium acetate or 10 mM triethylammonium bicarbonate (pH 8.5) and eluted either with 1 M ammonium acetate or 2 M triethylammonium bicarbonate and then repeatedly dried.

Paper electrophoresis was performed on 40-cm sheets of Whatman 3 MM paper in 50 mM ammonium formate (pH 3.5) and 1 mM EDTA buffer on a cold plate apparatus at 1800 V for 3 h. Whatman No. 1 paper was used for ascending paper chromatography in solvent 1 consisting of 1-butanol–concentrated NH_3–H_2O (86:5:14) and solvent 2 consisting of ethyl acetate–2-propanol–7.5 M NH_4OH–H_2O (3:2:2:1).

† From the Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014. *Received August 11, 1975.*

[1] Abbreviations used are: m^6A, N^6-methyladenosine; m^7G, 7-methylguanosine; Gm, 2′-*O*-methylguanosine; Am, 2′-*O*-methyladenosine; Cm, 2′-*O*-methylcytosine; Um, 2′-*O*-methyluridine, Nm, any 2′-*O*-methylribonucleoside; m^6Am, $N^6,O^{2'}$-dimethyladenosine; $m_3^{2,2,7}G$, $N^2,N^2,7$-trimethylguanosine; iA, isopentenyladenosine.

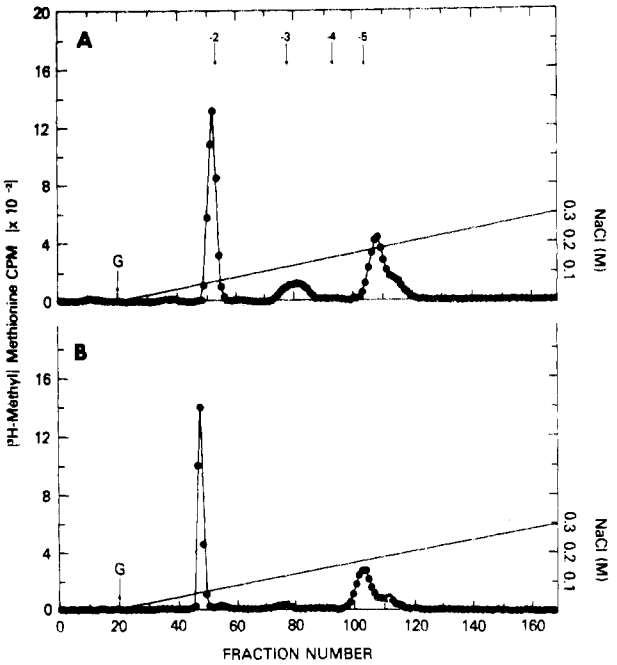

FIGURE 1: DEAE-cellulose column chromatography of ribonuclease digest of HeLa cell mRNA. Methyl-^{3}H-labeled cytoplasmic poly(A)$^+$ RNA isolated by one cycle (A) or two cycles (B) of poly(U)-Sepharose chromatography was digested with a combination of ribonucleases T_2, T_1, and A and chromatographed on DEAE-cellulose. G, start of gradient.

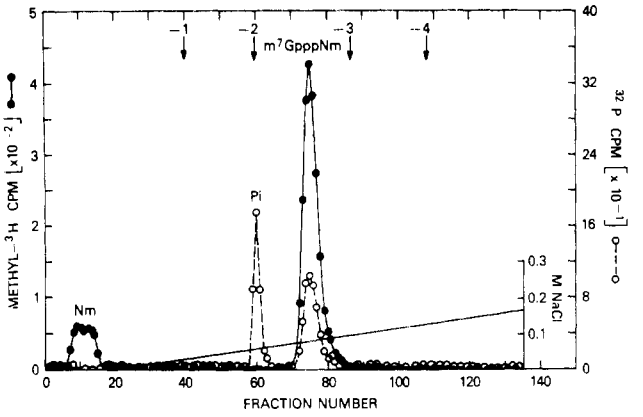

FIGURE 2: DEAE-cellulose column chromatography of nuclease P_1 and alkaline phosphatase digest of 5′-terminal oligonucleotides. Methyl-^{3}H- and ^{32}P-labeled poly(A)$^+$ RNA was mixed and digested with a combination of ribonucleases T_2, T_1, and A. The 5′-terminal oligonucleotide isolated by DEAE-cellulose column chromatography as in Figure 1 was digested with a combination of nuclease P_1 and alkaline phosphatase and rechromatographed on a DEAE-cellulose column.

Materials. Alkaline phosphatase, snake venom phosphodiesterase, and ribonucleases were purchased from Worthington Biochemical Corp.; nucleotide pyrophosphatase was from Sigma Chemical Corp. Penicillium nuclease P_1 was a gift of A. Kuninaka. 2′-*O*-Methylribonucleosides and pm^{7}G were obtained from P-L Biochemicals or Ash Stevens, Inc. Gm synthesized by R. Robins was a gift of M. Sporn. Base methylated derivatives were purchased from Sigma Chemical Corp. or Cyclo Chemical Corp. [*methyl*-^{3}H]Methionine was purchased from Schwarz/Mann; [^{32}P]phosphate was from New England Nuclear Corp. Dinucleotides were purchased from Miles Laboratories and trinucleotides were a gift of D. Hatfield.

Results

When methyl-^{3}H-labeled cytoplasmic poly(A)-containing HeLa cell RNA that was selected by adsorption to poly(U)-Sepharose was completely digested with a mixture of RNases T_2, T_1, and A, three peaks were resolved by DEAE-cellulose chromatography in 7 M urea at neutral pH (Figure 1A). Previous experiments (Wei et al., 1975a) demonstrated that the first peak, eluting with the −2 charge oligonucleotide marker, was m^{6}Ap; that the second peak eluting with a −3 charge consisted of dinucleotides containing all four common 2′-*O*-methylribonucleosides; and that the third peak eluting with a −5 to −6 charge was a mixture of 5′-terminal oligonucleotides with the structures m^{7}G(5′)ppp(5′)NmpNp and m^{7}G(5′)ppp(5′)NmpNmpNp. The dinucleotide peak appears to be derived from small amounts of highly labeled rRNA since it was almost completely absent from poly(A)$^+$ RNA that has been heated at 60° and repurified by poly(U)-Sepharose chromatography (Figure 1B). After the latter treatment 42–53% of the methyl-labeled material was recovered as m^{6}Ap and 44–59% as 5′-terminal oligonucleotides.

Similar experiments were carried out with ^{32}P-labeled poly(A)$^+$ RNA to estimate the fraction of molecules that have blocked 5′-termini. Analysis as in Figure 1 indicated that most of the label was in the −2 charge (mononucleotide) peak, that only slightly above baseline amounts eluted with a −4 charge (? pNp derived from unblocked termini containing a single phosphate) and that 0.19–0.25% eluted in the −5 to −6 charge position expected for m^{7}G blocked 5′-terminal sequences as well as ppNp and pppNp derived from unblocked termini with two or three 5′-terminal phosphates. Further experiments, however, were consistent with nearly equimolar amounts of m^{7}GpppNmpNp and m^{7}GpppNmpNmpNp and little or no unblocked termini. All fractions eluting in the −5 to −6 region were pooled, desalted, and then digested with a combination of P_1 nuclease, an enzyme that degrades RNA to 5′-nucleotides and is not inhibited by 2′-*O*-methyl substitution (Fujimoto et al., 1974), and alkaline phosphatase. The digestion products, Nm (no charge), P_i (−2 charge), and m^{7}GpppNm (−2.6 charge), were resolved by DEAE-cellulose chromatography (Figure 2). Approximately 17% of the ^{3}H-labeled material was in Nm and 83% in m^{7}GpppGm consistent with the digestion of similar amounts of m^{7}GpppNmpNp and m^{7}GpppNmpNmpNp. Moreover, 44% of the ^{32}P-label was in P_i and 56% in m^{7}GpppNm almost exactly the theoretical values (45% in P_i and 55% in m^{7}GpppNm) expected for equimolar amounts of m^{7}GpppNmpNp and m^{7}GpppNmpNmpNp. Therefore, sequences such as ppNp or pppNp which would be converted entirely to P_i and N must be relatively minor or absent. Furthermore, since 0.19–0.25% of the total ^{32}P in poly(A)$^+$ RNA was recovered as m^{7}GpppNmpNp and m^{7}GpppNmpNmpNp, we can estimate from the higher value that there is one 5′-terminal oligonucleotide per 2200 total nucleotides.

Distribution of Methylated Nucleosides Adjacent to 5′-Terminus. Our previous study (Wei et al., 1975a) indicated that the 5′-terminal oligonucleotides contained m^{7}G and an incompletely resolved mixture of 2′-*O*-methylribonucleosides. Further analysis was carried out by digestion of the −5 to −6 charge peak (Figure 1) with nuclease P_1 and nucleotide pyrophosphatase, an enzyme that digests m^{7}GpppNm to yield pm^{7}G, pNm, and P_i. On electrophoresis at pH 3.5, the methyl-labeled products comigrated with

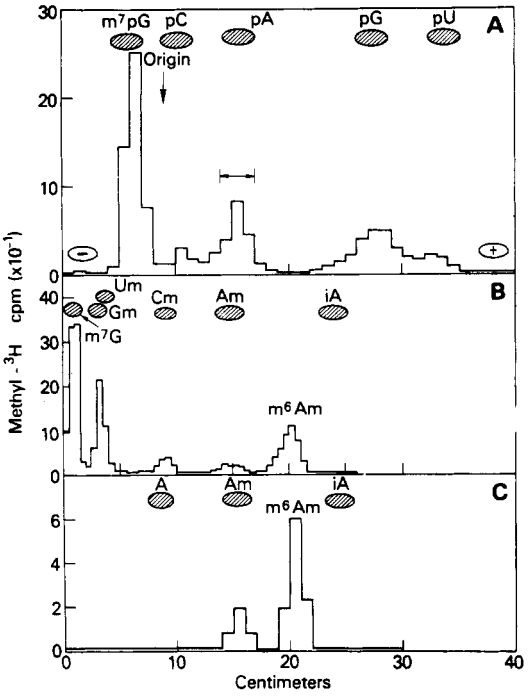

FIGURE 3: Analysis of methylated ribonucleotides and ribonucleosides obtained by enzyme digestion of 5′-terminal oligonucleotides of HeLa cell mRNA. (A) Paper electrophoresis of methylated ribonucleotides. Methyl-^{3}H-labeled 5′-terminal oligonucleotides isolated by ribonuclease digestion and DEAE-cellulose chromatography of HeLa cell mRNA were digested by nuclease P_1 and nucleotide pyrophosphatase. The nucleotides were analyzed by paper electrophoresis at pH 3.5 for 3 h. (B) Paper chromatography of methylated ribonucleosides. Methyl-^{3}H-labeled 5′-terminal oligonucleotides of HeLa cell mRNA were digested by a combination of alkaline phosphatase and venom phosphodiesterase. The nucleosides were analyzed by ascending paper chromatography in solvent 1. (C) Paper chromatography of methylated adenosine derivatives. The fractions migrating with pA in Figure 3A were pooled and digested with alkaline phosphatase. The digest was analyzed by ascending paper chromatography in solvent 1.

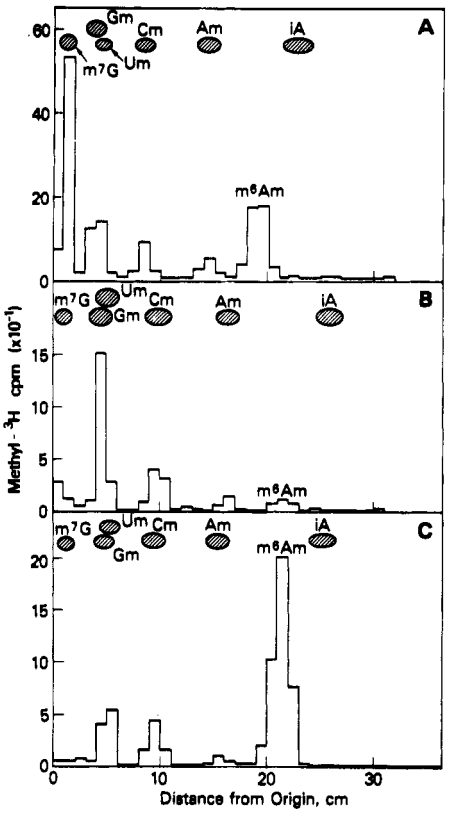

FIGURE 4: Distribution of nucleosides in 5′-terminal oligonucleotides of HeLa cell mRNA. Methyl-^{3}H-labeled 5′-terminal oligonucleotides were isolated as described in Figure 1. The labeled m^{7}GpppNmpNp and m^{7}GpppNmpNmpNp were digested with nuclease P_1 and alkaline phosphatase to give m^{7}GpppNm and Nm which were separated on a DEAE-cellulose column. (A) Paper chromatography of methylated ribonucleosides in m^{7}GpppNm. Methyl-^{3}H-labeled m^{7}GpppNm was digested by a combination of venom phosphodiesterase and alkaline phosphatase and analyzed by ascending paper chromatography in solvent 1. (B) Paper chromatography of methylated ribonucleosides in Nm. Methyl-^{3}H-labeled Nm was analyzed by ascending paper chromatography in solvent 1. (C) Paper chromatography of methylated ribonucleosides in NmpN. The m^{7}G residues were removed by periodate oxidation and β elimination from a mixture of m^{7}GpppNmpNp and m^{7}GpppNmpNmpNp. Following alkaline phosphatase treatment, NmpN was separated from NmpNmpN by DEAE-cellulose chromatography and then digested with snake venom phosphodiesterase and alkaline phosphatase. The nucleosides were analyzed by ascending paper chromatography in solvent 1.

pm^{7}G, pC, pA, pG, and pU (Figure 3A) suggesting that methyl derivatives of all four nucleotides were present. (Unmethylated nucleotides were used as markers since their electrophoretic mobilities are unaltered by methylation at the 2′ position). After complete digestion with snake venom phosphodiesterase and alkaline phosphatase, nucleoside products cochromatographed with m^{7}G, Gm + Um, Cm, and Am (Figure 3B). A major component, however, migrated ahead of Am and was identified elsewhere as the novel dimethyladenosine derivative m^{6}Am (Wei et al., 1975b). A mixture of m^{6}Am and Am was obtained by alkaline phosphatase digestion of material migrating with pA on electrophoresis (Figure 3C). The identities of Am and Cm separated by paper chromatography in solvent 1 were confirmed by elution and rechromatography with standards in solvent 2. Um and Gm, which were not separated in solvent 1, were eluted and resolved by thin-layer electrophoresis in 1 M formic acid.

Periodate oxidation and β elimination previously established that m^{7}G was at the 5′-terminus of HeLa cell poly(A)$^+$ RNA (Wei et al., 1975a). To determine which methylated ribonucleosides are next to m^{7}G and which are in the third position, methyl-^{3}H-labeled m^{7}GpppNmpNp and m^{7}GpppNmpNmpNp were digested with P_1 nuclease and alkaline phosphatase. The labeled products, m^{7}GpppNm and Nm, were separated by DEAE-cellulose chromatography. Analysis of m^{7}GpppNm indicated that it contained 28% m^{6}Am, 39% Gm + Um (31% Gm and 8% Um by electrophoresis), 19% Cm, and 14% Am in the second position (Figure 4A). In calculating the above ratios, we took into account the fact that m^{6}Am has twice the specific radioactivity of singly methylated ribonucleosides. Analysis of the nucleosides released from the third position by P_1 nuclease and alkaline phosphatase revealed a quite different composition: 64% Gm + Um, 25% Cm, 7% Am, and only 4% m^{6}Am (Figure 4B). Thus the novel nucleoside m^{6}Am is located primarily adjacent to the m^{7}G residue.

In the previous experiment, m^{7}GpppNm derived from a combination of type I (m^{7}GpppNmpNp) and type II (m^{7}GpppNmpNmpNp) termini was analyzed. Further studies indicated that m^{6}Am was most abundant in those sequences containing only a single 2′-*O*-methylribonucleoside (type I). This was determined by analysis of NmpN isolated as previously described (Wei et al., 1975a) from the type I structure after removal of m^{7}G by periodate oxidation and β elimination followed by alkaline phosphatase

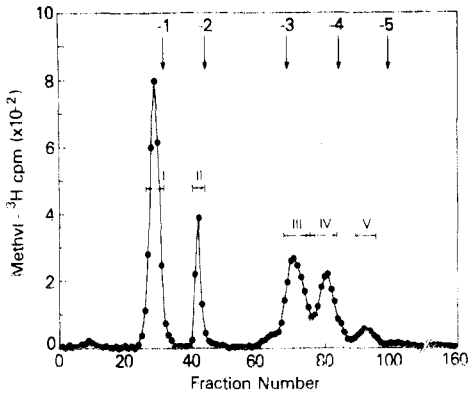

FIGURE 5: DEAE-cellulose column chromatography of combined RNase T_1, A, and alkaline phosphatase digest of HeLa cell mRNAs. Methyl-^{3}H-labeled HeLa cell mRNAs after two cycles of poly(U)-Sepharose chromatography which included a 60° heat step were digested with a combination of RNase T_1, A, and alkaline phosphatase. The digest was chromatographed on a DEAE-cellulose column.

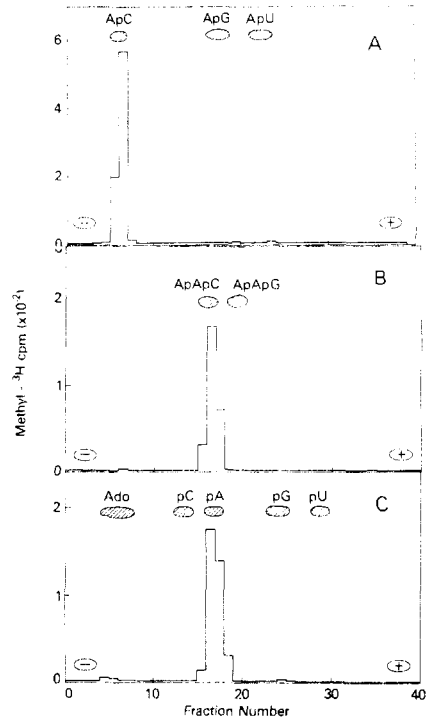

FIGURE 6: Identification of oligonucleotides containing m^6A. (A) Peak I of Figure 5 was desalted and analyzed by paper electrophoresis at pH 3.5 for 4.5 h. The positions of ApC, ApU, and ApG markers are indicated. (B) Peak II of Figure 5 was desalted and analyzed by electrophoresis at pH 3.5 for 4 h. The positions of ApApC and ApApG are indicated. ApApU would be expected to move ahead of ApApG. (C) Peak II of Figure 5 was desalted and digested with nuclease P_1. The digest was analyzed by paper electrophoresis at pH 3.5 for 4 h.

treatment and DEAE-cellulose chromatography (Figure 4C).

Isolation of Oligonucleotides Containing m^6A. Previous experiments (Wei et al., 1975a) showed that m^6Ap was released by alkali or complete RNase digestion of HeLa cell mRNA. This result was consistent with the presence of m^6A either at unphosphorylated 5′-termini or in internal positions. To distinguish between these possibilities, methyl-^{3}H-labeled poly(A)$^+$ RNA was digested with P_1 nuclease. If m^6A were at unphosphorylated 5′ ends then it should be released as a nucleoside whereas pm^6A would be released if it were located at internal positions. Analysis by thin-layer chromatography (not shown) indicated that only 0.6% of the methyl label was in m^6A after P_1 nuclease digestion indicating that m^6A was located internally.

Since m^6A is in an internal position, we considered that it might form part of a unique sequence. To evaluate this possibility, methyl-^{3}H-labeled poly(A)$^+$ RNA was digested with RNase A and T_1. This combination of enzymes should cleave adjacent to guanosine, cytosine, and uridine, but not adenosine or 2′-*O*-methylribonucleosides, to give nucleotides and oligonucleotides with a 3′-terminal phosphate. After treatment with alkaline phosphatase, the digest was chromatographed on DEAE-cellulose in 7 M urea (Figure 5). Five peaks were resolved. To locate the peaks containing m^6A, a sample from each was digested with RNase T_2 and alkaline phosphatase to release m^6A and then analyzed by thin-layer chromatography. The results indicated that m^6A was exclusively in the −1 and −2 charge peaks; peaks III–V contained the 5′-terminal oligonucleotides. In view of the enzymes used for digestion, the −1 charge peak could consist of the following dinucleotides: m^6ApG, m^6ApC, or m^6ApU. Since the dinucleotides ApG, ApC, and ApU can be separated by electrophoresis at pH 3.5 and since methylation at the N^6 position of adenosine does not appreciably alter the charge (e.g., m^6Ap and Ap comigrate), the unmethylated dinucleotides can serve as markers for m^6ApG, m^6ApC, and m^6ApU. We found that the methyl-labeled dinucleotide (Figure 5, −1 charge peak) migrated with ApC during electrophoresis (Figure 6A) indicating that it was exclusively m^6ApC. A similar identification was made by thin-layer chromatography on polyethylenimine (PEI) cellulose (not shown).

Considering once again the enzymes used for digestion, the −2 charge peak of Figure 5 could be a mixture of nine different trinucleotides with the basic sequences ApApG, ApApC, or ApApU with methyl groups on the first, second, or both adenosine residues. Nevertheless, only a single methyl-labeled component migrating with the ApApC marker was detected indicating that it was either Ap-m^6ApC, m^6ApApC, or m^6Apm^6ApC and excluding the other six possibilities (Figure 6B). To determine the position of the m^6A residue, the trinucleotide was digested with P_1 nuclease. Nearly all of the radioactive label was in pm^6A and only trace amounts in m^6A indicating that the only possible trinucleotide sequence is Apm^6ApC (Figure 6C). Thus, only two types of sequences m^6ApC and Apm^6ApC were obtained by complete digestion of poly(A)$^+$ RNA with RNase A and T_1.

The m^6ApC released by RNase A and T_1 must have been preceded by either a pyrimidine or a guanosine residue. To distinguish between these possibilities, the labeled poly(A)$^+$ RNA was digested with RNase A alone followed by alkaline phosphatase treatment. Analysis by DEAE-cellulose chromatography as in Figure 5 indicated that only 2% of the methyl label was in the dinucleoside monophosphate peak and that therefore pyrimidines preceded m^6A only infrequently or perhaps not at all.

Discussion

Previous studies (Wei et al. 1975a; Furuichi et al. 1975b) established that HeLa cell mRNA contains 5′-terminal sequences of the type $m^7G(5')ppp(5')Nm$ and $m^7G(5')ppp(5')NmpNm$. Analysis of ^{32}P-labeled poly(A)$^+$ RNA indicates that approximately one 5′-terminal oligonucleotide was recovered per 2200 nucleotides. This should be

considered a minimal number since the RNA is selected by its 3′-poly(A) end and any nick would result in a loss of the 5′-nucleotide. Nevertheless, if this number is compared with the average length of HeLa cell mRNA estimated as 2000 (Klein et al., 1974; Bishop et al., 1974) to 3000 (Molloy et al., 1974) nucleotides, it indicates that a high percentage of molecules have blocked 5′-termini. This conclusion is also consistent with the estimation of 2 methyl groups per 1000 nucleotides or 4–5 methyl groups per 2200 nucleotides (Wei et al., 1975a).

Nucleoside analysis of the 5′-terminal structures indicated that m^6Am, Am, Gm, Cm, and Um were next to m^7G. Although m^6Am was one of the most abundant nucleosides in the latter position, it was found infrequently in the third position of sequences of the type $m^7GpppNmpNm$. In general the distribution of methylated nucleosides at the 5′-terminus that we obtained was similar to that reported for HeLa cell mRNA by Furuichi et al. (1975b) except that they did not distinguish between Am and m^6Am. m^6Am, although unrecognized until recently, appears to be common to many mRNAs since it has also been identified as a major methylated component of mouse L cell and adenovirus mRNAs as well as HeLa cell mRNAs (Wei et al., 1975b). A dimethyladenosine derivative not yet identified has also been found in vesicular stomatitis virus mRNA synthesized in vivo (Moyer et al., 1975). Although apparently not present in tRNA or rRNA, a single m^6Am residue is located in an internal position of low molecular weight nuclear U-2 RNA (Shibata et al., 1975).

All of the viral mRNAs examined thus far have derivatives of adenosine or guanosine next to m^7G possibly reflecting the preferential initiation of transcription with a purine. The finding of cytosine and uridine adjacent to m^7G in HeLa cell mRNA may signify that these ends were derived by cleavage of higher molecular weight precursors or that cellular RNA polymerases may also initiate with a pyrimidine in vivo. Enzymes that carry out the 5′-terminal modification have not been isolated from cells and the mechanisms involved are not known. Thus far enzymes of this type have only been isolated from vaccinia virus (Ensinger et al., 1975; Martin et al., 1975; Martin and Moss, 1975). In vitro the viral guanylyltransferase catalyzes the transfer of a GMP residue from GTP to mRNA containing a 5′-terminal diphosphate. This can be followed by methylation of the terminal guanosine residue in the 7 position by a methyltransferase which is associated with the guanylyltransferase. The methylation of the penultimate nucleoside in the 2′-O position is then catalyzed by a second methyltransferase. Such a mechanism is appropriate for modification of original 5′-termini containing a di- or triphosphate and not for processed ends containing only one or no phosphates.

m^6A is the only methylated nucleoside detected in an internal position in HeLa cell mRNA. We usually find approximately half of the methyl-^3H label of poly(A)$^+$ RNA in m^6A, consistent with 2 to 3 residues per 5′-oligonucleotide. This number would be an overestimate, however, if some 5′-termini are lost because of degradation. Significantly the m^6A residues are preceded by adenosine or guanosine and followed exclusively by cytosine, a result that implies a remarkable degree of sequence specificity since there are thousands of different mRNAs in HeLa cells (Bishop et al., 1974). The location of the m^6A residue in coding or noncoding regions of the RNA still remains to be determined. In summary, our studies are consistent with the following model of HeLa cell mRNA:

$m^7G(5')ppp(5')Nm\text{-}(Nm)\cdots(G \text{ or } A)\text{-}m^6A\text{-}C\cdots(A)_{100\text{-}200}A$

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