Nucleotide Sequence of U-2 Ribonucleic Acid

THE SEQUENCE OF THE 5'-TERMINAL OLIGONUCLEOTIDE*

(Received for publication, October 21, 1974)

TAE SUK RO-CHOI, YONG C. CHOI, DALE HENNING, JAMES MCCLOSKEY,‡ AND HARRIS BUSCH
From the Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77025

The nucleotide sequences were determined for the 5'-oligonucleotides obtained by complete pancreatic RNase digestion (P25) and complete T1 RNase digestion (T27) of U-2 RNA. Complete digestion of oligonucleotide P25 with snake venom phosphodiesterase produced pm32,2,7G, pAm, pUm, and pCp in approximately equimolar ratios. Partial digestion of these oligonucleotides with snake venom phosphodiesterase produced -Urn-C-Gp and pAm-Urn, indicating the sequence of the 3' terminal portion of the 5'-oligonucleotide is pAm-Urn-C-Gp. The 5'-terminal oligonucleotide did not contain a 5'-phosphate and no free nucleoside was released from the 5' end by venom phosphodiesterase digestion. Since free pm3,2,7G was released by digestion with nucleotide pyrophosphatase and limited digestion with snake venom phosphodiesterase, this nucleotide is apparently linked to pAm in a pyrophosphate linkage. Mass spectrometry and thin layer chromatography in borate systems showed the ribose of pm3,2,7G contains no 2'-O-methyl residue. Moreover, the finding that the ribose of m3,2,2,G was oxidized by NaIO4 and reduced by KBH4 in intact U-2 RNA rules out other linkages involving the 2' and 3' positions. Accordingly, it is concluded that the structure of the 5'-terminal pentanucleotide of U-2 RNA is pm3,2,7G

\[ \text{pAm-Urn-C-Gp}. \]

Recent studies from this and other laboratories have demonstrated the existence of several species of low molecular weight RNAs (LMWN RNA or sn RNA) in the eukaryotic cell nucleus (1-3). Of these the 4.5 S RNA,, U-1 RNA, and U-2 RNA have been sequenced (1-3). The sequences of these LMWN RNAs show they have two different types of structures: one like 4.5 S RNA, which has no unusual bases and contains pppGp as the 5'-terminal nucleotide and the other like U-1, U-2 and U-3 RNA which contain modified nucleotides and a highly methylated 5'-terminal oligonucleotide.

The functions of these LMWN RNAs are not known but one, the U-3 RNA, is localized to the nucleolus and its synthesis has been shown to be related temporally to rRNA synthesis (4). Other LMWN RNAs are localized to the chromatin fraction. Their number, which approximates that of the number of functional genes, has suggested the possibility that they may have a unique function in gene activity (1, 2, 5-7). The presence of pppGp at the 5' end of 4.5 S RNA and its unique sequence C-C-U-U-U-U at the 3' terminus indicate that this RNA is a primary transcriptional product like the oop RNA of lambda phage-infected Escherichia coli which also has pppGp at its 5' end and the sequence U-U-U-U-U-A at its 3' end (8, 9).

The U-rich LMWN RNA species, U-1, U-2, and U-3 RNA, contain the residue pm3,2,2,G in their 5'-terminal oligonucleotides. In the present study, this oligonucleotide was sequenced for the U-2 RNA and pm3,2,2,G was found to be linked to the adjacent 2'-O-methylated, alkali-resistant trinucleotide by a 5',5'-pyrophosphate bond.

MATERIALS AND METHODS

Tumor Cells and Preparation of RNA—The Novikoff ascites hepatoma were transplanted 6 days prior to the experiments in male albino rats obtained from the Holtzman Co., Madison, Wis. Purification of the cells and incubation with 3H were carried out as reported earlier (10). In these studies, 10 g of cells were incubated with 500 mCi of 3H in 1 liter of medium for 9 hours. Nuclei were isolated by the citric acid procedure and RNA was extracted by the sodium dodecyl sulfate-phenol procedure (11). The 4 to 8 S RNA was separated from high molecular weight RNA by sucrose density gradient centrifugation and individual LMWN RNA was separated by 10% polyacrylamide gel electrophoresis (1-3).

Isolation of 5'-Terminal Oligonucleotides—Oligonucleotides containing m3,2,2,G from 3H-labeled RNA were isolated by two-dimensional electrophoresis of the complete pancreatic RNase digest of U-2 RNA. The conditions for RNase digestion and electrophoresis were reported earlier (3, 4, 12). For the analysis of RNA by ultraviolet absorbance, approximately 10 mg of U-2 RNA (from Novikoff hepatoma cell nuclei) were collected from approximately 250 rats. This RNA was digested completely with pancreatic and T1 RNases and the 5'-terminal oligonucleotide was purified by column chromatography on DEAE-
Sephadex A-25 at pH 7.4. The 5'-terminal oligonucleotide of U-2 RNA was analyzed by (a) snake venom phosphodiesterase digestion, (b) snake venom phosphodiesterase digestion followed by alkaline phosphatase digestion, and (c) separation of the nucleosides by column chromatography and thin layer chromatography.

**Enzymatic Digestion of 5' Fragments**—Snake venom phosphodiesterase digestion was carried out at 37° for 6 hours in 0.02 M Tris-HCl, pH 7.5, containing 2 mM magnesium acetate with an enzyme to substrate ratio of 1:2. For partial digestion of the fragments with snake venom phosphodiesterase, the incubation was carried out at times ranging from 10 min to 6 hours with an enzyme to substrate ratio of 1:20. The snake venom phosphodiesterase (Worthington) was treated with dilute acetic acid to remove phosphatase activity (13). To obtain nucleosides, the 5'-nucleotides obtained by snake venom phosphodiesterase digestion were digested at 37° for 1 hour with alkaline phosphatase (Worthington) at an enzyme to substrate ratio of 1:2 or 0.02 M Tris-HCl at pH 8.0.

For hydrolysis of the pyrophosphate bonds, the nucleotide pyrophosphatase (Crotalus adamanteus venom type II, Sigma) was used. Hydrolysis of 50 µg of sample was carried out at 37° for 2 min with 0.1 unit of enzyme in 0.01 M Tris-HCl (pH 7.5) containing 10 mM magnesium chloride. The hydrolysate was separated on DEAE-cellulose paper by electrophoresis in 7% formic acid or the pH 3.5 system (3, 11, 12).

**Labeling of 5' and 3' end by KBH₄**—The methods used for the treatment of 4 to 8 S RNA with NaIO₄ and KBH₄ were those reported earlier (11). ³H-labeled 4 to 8 S RNA was separated by preparative gel electrophoresis. U-2 RNA was collected and digested with pancreatic RNase at an enzyme to substrate ratio of 1:20 at 37° for 2 to 3 hours. The digest was separated on DEAE-Sephadex A-25 at pH 7.4 in the presence of 7 M urea. The absorbance at 260 nm was determined with a Zeiss spectrophotometer and the isotope content of 5-µl aliquots was determined using a Packard liquid scintillation counter. The radioactive peak with -6 charge was collected and digested with snake venom phosphodiesterase and alkaline phosphatase.

**Analysis of Nucleosides and Nucleotide Derivatives by Column Chromatography**—The methods used by Vandenheuvel and Randers-Wood (3) were used for thin layer chromatography of the nucleosides and their ³H derivatives. Separations were carried out on cellulose thin layers using solvent systems of acetonitrile-ethyl-acetate-n-butanol-isopropanol-6 N aqueous ammonia (3:2:1:1:2.7, v/v) for the first dimension and t-amyl alcohol-methyl ethyl ketone-acetonitrile-water-formic acid (4:2:1:5:2:15:0.18, v/v) for the second dimension. Fluorescence was detected with the aid of a Kodak X-o-ray film (23). Thin Layer Chromatography of Nucleosides and Nucleotides—Two-dimensional thin layer chromatography was employed to identify and isolate individual nucleosides and nucleotides on cellulose thin layers (Eastman-Kodak, 6664 microcrystalline cellulose) or Avicel (microcrystalline cellulose) thin layers (Analtech, Newark, Del.). To fractionate a mixture of nucleosides and nucleotides, the solvent systems employed were isopropanol acid-NH₄OH-H₂O (7:10:20, v/v) and butanol saturated with 0.15 M NH₄OH (18, 19) or isobutyric acid-acetic acid-water-NH₄OH (5:3:3:3, v/v) and isopropanol-acetic acid-H₂O (70:15:15, v/v) (20).

To determine the presence of 2'-O-methylated nucleosides, a solvent system containing borate was employed. The solvent system for the first dimension (19) was isopropyl alcohol-concentrated HCl-H₂O (70:10:1, v/v, made to 250 ml with water). The second dimension was effected in two steps; for the first, the solvent system was 1-butanol saturated with boric acid (21) which was allowed to migrate 5 to 6 cm above the origin. In the second step (18), isopropyl alcohol-NH₄OH-H₂O (70:10:20, v/v) was allowed to migrate to at least 12 cm above the origin. The nucleosides and nucleotides were eluted with 0.1 n HCl. Mass Spectrometry of Nucleosides—N-O-Bis(trimethylsilyl) tri-fluoroacetamide (BSTFA) containing 1% trimethylcholorosilane was purchased from Regis Chemical Co., Chicago. The nucleoside was trimethylsilylated by dissolving a thoroughly dried sample (10 µg) in BSTFA reagent and heating for 1 hour at 100°. An aliquot of the reaction mixture was injected into the gas chromatographic column (1% OV-17) of an LKB 9000 gas chromatograph-mass spectrometer. The resulting mass spectrum was recorded under the following conditions: ionizing energy, 70 eV; ion source and carrier gas temperature, 250°.

**RESULTS**

**Nucleotide Analyses of 5'-Terminal Fragment of U-2 RNA**

The ³²P-labeled U-2 RNA was completely digested with T₁ RNase, pancreatic RNase, or both. The 5'-terminal fragments T27 and P25 were isolated by the two-dimensional fingerprinting method of Sanger and Brownlee (22). After alkaline phosphatase treatment of T27, snake venom phosphodiesterase produced pG, pC, pUm, pAm, and pm₁³₂³G. The resultant nucleotides were separated by electrophoresis followed by chromatography (Fig. 1). The distribution of radioactivity in the nucleotides (Table I) indicate that pAm, pUm, pm₁³₂³G, and pC are present in approximately 1:1:1:1 molar ratio. Nucleotide analysis by chromatography on a pellicular cation exchange column (Fig. 2) showed that Am, Um, C, and m₁³₂³G were present in a molar ratio of 1.0, 1.3, 1.1 and 0.96.³

**Absence of a 5'-Phosphate from Oligonucleotide P25**

Complete snake venom phosphodiesterase digestion placed pGP at the 3' end of oligonucleotide T27 and pCP at the 3' end of oligonucleotide P25. Chromatography of the pancreatic RNase digestion products of oligonucleotide T27 on DEAE-Sephadex A-25 at pH 7.4 showed that in addition to Gp (the -2 charge peak) a second peak was found with a -6 charge (pentanucleotide region) (Fig. 3a). Treatment of oligonucleotide T27 with both alkaline phosphatase and pancreatic RNase produced inorganic phosphate and a peak in the -4 charge (trimonucleotide) region (Fig. 3b) indicating that one phosphate residue was removed. This result showed that there is no 5'-phosphate group at the 5' end of oligonucleotide T27.

**Internal Nucleotide Sequence of Oligonucleotide T27**

Partial digestion with venom phosphodiesterase of oligonucleotide T27 produced a fragment (mobility 1.7) which contains pUm, pC, and pG (Fig. 4, Slot 3). This fragment was

³The absorbance was recorded at 254 nm and the peaks were cut out and weighed. The correction factors, 0.689 for A, 1.142 for U, 0.643 for G, and 1.552 for C were determined for calculation of molar ratios. These correction factors were used for 2'-O-methylated nucleosides. For m₁³₂³G the correction factor was determined to be 1.03 by absorbance at 254 and 268 nm.
treated with alkaline phosphatase prior to complete digestion with venom phosphodiesterase. Since Gp is the 3' terminus of fragment T27 and Cp is the terminus of fragment P25, the sequence of this fragment of T27 is -Um-C-Gp.

In addition to this fragment, partial digestion with venom phosphodiesterase produced the fragment pAm-Um(mobility 1.47). Complete digestion of this fragment with venom phosphodiesterase produced equimolar amounts of pAm and pUm. Accordingly, the sequence of the 3' end of fragment T27 is pAm-Um-C-Gp.

Chemical Characterization of Nucleoside \( m_3^{2,2,7}G \)

**Thin Layer Chromatography**—Fig. 5 shows the separation of nucleosides obtained from oligonucleotide P25. Of the four nucleosides which were cleanly separated with this procedure, Um and Am were 2'-O-methylated as shown by the fact that their migration was not retarded by the borate in the solvent. The two nucleosides C and \( m_3^{2,2,7}G \) contain no 2'-O-methyl groups inasmuch as they were retarded by the borate. The nucleoside, \( m_3^{2,2,7}G \), was brightly fluorescent under alkaline conditions.

**Table I**

<table>
<thead>
<tr>
<th>5' Fragments from U-2 RNA</th>
<th>Enzymatic treatment</th>
<th>( ^{32}P ) counts per min in nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P1</td>
</tr>
<tr>
<td>5'-Oligonucleotide obtained by T1 RNase digestion</td>
<td>Digested with snake venom enzyme</td>
<td>958</td>
</tr>
<tr>
<td></td>
<td>(1.4)</td>
<td>(0.98)</td>
</tr>
<tr>
<td></td>
<td>Digested with alkaline phosphatase and then with snake venom enzyme</td>
<td>605</td>
</tr>
<tr>
<td></td>
<td>(1.36)</td>
<td>(0.93)</td>
</tr>
<tr>
<td>5'-Oligonucleotide obtained by pancreatic RNase digestion</td>
<td>Digested with snake venom enzyme</td>
<td>872</td>
</tr>
<tr>
<td></td>
<td>(1.3)</td>
<td>(1.0)</td>
</tr>
<tr>
<td></td>
<td>Digested with alkaline phosphatase and then with snake venom enzyme</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>(1.39)</td>
<td>(0.90)</td>
</tr>
</tbody>
</table>
**Ultraviolet Absorption Spectra**—Fig. 6 shows ultraviolet absorption spectra taken of pm\(^{3,2,7}\)G at various pH values. The sharp maximum at 263 nm was observed at pH 2 and 7 but was lost at pH 12; this result corresponds to the earlier result of Saponara and Enger (15) who synthesized pm\(^{3,2,7}\)G. The spectra of naturally occurring pm\(^{3,2,7}\)G was essentially the same as those of synthesized pm\(^{3,2,7}\)G. The disappearance of the maximum peak at 263 nm indicates instability of the 5-membered ring which has been reported to be cleaved slowly at neutral pH and more rapidly at alkaline pH (15).

**Mass Spectrometry**

The nucleoside was trimethylsilylated (Fig. 7) and subjected to gas chromatography-mass spectrometry, resulting in the mass spectrum shown in Fig. 8. In accordance with earlier studies of the mass spectra of silylated nucleosides (23, 24) the molecular ion (M, m/e 629) and M – CH\(_3\) ions (m/e 614) show the molecular weight to be 629. The unusual intensity pattern in which M > M – CH\(_3\), and the high abundance of base + H rearrangement ions (m/e 281), are common features in the spectra of guanosine derivatives (25). The mass of the base fragment (280 a.m.u.) shows by difference (629 to 280) that the ribose moiety in the native nucleoside is not methylated. The mass of the base is consistent with a guanine residue which bears three methyl groups and a carbonyl function at C 8. Earlier work by Von Minden et al. (25) demonstrated that

---

**Fig. 3.** a, Oligonucleotide T27 obtained from \(^{32}P\)-labeled U-2 RNA was digested with pancreatic RNase and chromatographed on DEAE-Sephadex A-25 at pH 7.6 in the presence of 7 M urea. The mononucleotide Gp (–2 charge) was released; the remainder of oligonucleotide P25 was eluted at the pentanucleotide region (–6 charge). b, oligonucleotide T27 was digested with both bacterial alkaline phosphatase and pancreatic RNase. The digestion products were chromatographed on DEAE-Sephadex A-25 at pH 7.6 in the presence of 7 M urea. A peak of inorganic phosphate (–2 charge) and a peak at the trimucleotide (–4 charge) region were observed. This result indicates only the 3' phosphate of oligonucleotide P25 is removed by bacterial alkaline phosphatase.
FIG. 4. Oligonucleotide T27 was digested with 10 μl of alkaline phosphatase (0.1 mg/ml) in 0.002 M Tris-HCl, pH 8.0, for 30 min at 37° and then 10 μl of snake venom phosphodiesterase (0.05 mg/ml) was added. The incubation was carried out for various times (2 min to 1 hour). The products were separated on DEAE-cellulose paper at pH 3.5. Some of the products were eluted from the paper and digested with 10 μl of snake venom phosphodiesterase (1 mg/ml) for 6 hours at 37°. The products were separated on Whatman No. 3MM paper by electrophoresis at pH 3.5. Slot 1, original TA27 which shows all of the components pUm, pG, pAm, PC, and pm32,2,7G. Slot 2, one fragment of oligonucleotide PA25 which contains pU, pG, and pC (N-Urn-C-G). Slot 3, digestion products of oligonucleotide PA25 which are pUm, pAm, pC, and pm32,2,7G. B, blue marker (Xylene cyan01 FF).

7-methylguanosine and other 7-methylpurine nucleosides uniquely undergo conversion to the 7-methyl-8-oxo derivative during trimethylsilylation (Fig. 7). Two of the three methyl groups must therefore be located at N2 and N-7. The third methyl was shown to reside at N2 by detailed analysis of the mass spectrum and most conclusively by comparison with the spectrum of the silyl derivative of authentic N2,N7,7-trimethylguanosine. The abundant ion of m/e 73 represents (CH3)4Si+ and carries no structural information.

Evidence that 2',3'-Hydroxyls of m32,2,7G are Unsubstituted

To label the 5'-nucleoside with KB3H4, a mixture of 4-8 S RNA from Novikoff hepatoma cell nuclei was oxidized with NaIO4 and reduced with KB4H. After the U-2 RNA was purified by preparative gel electrophoresis (11) it was digested with pancreatic RNase and the products were chromatographed on DEAE-Sephadex A-25 at pH 7.4 in the presence of 7 M urea (Fig. 9). Two radioactive peaks were found, one in the nucleoside region, Peak A, and Peak C which contains fragment P25. This oligonucleotide P25 was digested extensively with venom phosphodiesterase and alkaline phosphatase to release the nucleosides which were separated by chromatography on cellulose thin layer (Fig. 10) as well as on Whatman No. 3MM paper chromatography (Fig. 11). In both chromatographic systems, the radioactive spots migrated with the triol derivative of synthetic m32,2,7G. The untreated m32,2,7G migrated differently from the triol in both chromatographic systems (Figs. 10 and 11). These results clearly indicate that the 2' and 3' hydroxyl groups of m32,2,7G

To be published.
were free in intact U-2 RNA. Therefore, the trimethyl G nucleoside monophosphate released by snake venom phosphodiesterase (Figs. 1 and 4) must be the nucleoside 5'-phosphate.

**Failure of Release of a 5'-Terminal Nucleoside from Oligonucleotide P25 by Digestion with Venom Phosphodiesterase**

Snake venom phosphodiesterase digestion alone did not release any free nucleoside from the 5’-terminal oligonucleotide (P25). To test the enzyme, the hydrolysis was carried out simultaneously on a known pentanucleotide (P20), A-A-Gm-A-Up of U-2 RNA. Fig. 12 shows a tracing of the one-dimensional chromatograph of the nucleoside released from P20 which was treated with snake venom phosphodiesterase; the free nucleoside was A.

**Pyrophosphate Linkage of pm$_2^{3',5'}$G with pAm**

Since venom phosphodiesterase also released pAm and no free nucleoside was released by venom phosphodiesterase from the 5’terminal, the linkage between pm$_2^{3',5'}$G and pAm appeared to be different from the usual 3’,5’-phosphodiester linkage. Further evidence for this difference is that the 2’ and 3’ hydroxyls of m$_2^{3',5'}$G are unsubstituted and that the 5’ oligonucleotide P25 obtained by complete pancreatic RNase digestion is resistant to digestion by T$_1$ RNase or alkaline hydrolysis (0.3 N KOH or 0.3 N NaOH for 16 hours at 37°). These nucleotides are linked by a pyrophosphate bond since digestion of oligonucleotide P25 with nucleotide pyrophosphatase at 37° for 2 min released pm$_2^{3',5'}$G and the remaining oligonucleotide contained only pAm, pU, and pCp in a ratio of approximately 1:1:1 is shown by analysis of radioactivity in the spots (Fig. 13).

**DISCUSSION**

The present study indicates the uniqueness of the structure of the 5’ end of U-2 RNA. It was previously reported (1) that the 5’ portion that contains 69 nucleotides was remarkably substituted. It contains 11 ψ residues, 6 alkali stable dinucleotides, 1 alkali-stable trinucleotide, and the unusual base...
FIG. 10. Fluorograph of trialcohol derivatives of m\textsubscript{2,2,7}'G. Radioactive Peak C (Fig. 9) between the tetra- and pentanucleotide region was collected and desalted. The desalted oligonucleotide was then digested with snake venom phosphodiesterase and bacterial alkaline phosphatase. The products were chromatographed on cellulose thin layer using solvent system of Randerath and Randerath (16). The labeled trialcohol derivative of m\textsubscript{1,2}'G migrated differently from the nucleoside m\textsubscript{2,2}'G (left). The radioactive compound released from Peak C (Fig. 9) comigrates with the trialcohol derivative of m\textsubscript{2,2,7}'G (right).

FIG. 11. Fluorograph of one-dimensional chromatography of trialcohol derivatives of nucleosides on Whatman No. 3MM paper using a solvent system containing 1-butanol-isobutyric acid-water-29% NH\textsubscript{2}OH (30:15:10:14:0.86, v/v). A', C', U', G', and m\textsubscript{8,2,7}'G represent trialcohol derivatives of A, C, U, G, and m\textsubscript{2,2,7}'G. (1) trialcohol derivative of m\textsubscript{1,2}'G; (2) m\textsubscript{2,2}'G alone; (3) U-2 RNA, 5'-terminal oligonucleotide, Peak C (Fig. 9) chromatographed with a mixture of unlabeled trialcohol derivatives; (4) same as 3, digested with snake venom phosphodiesterase and alkaline phosphatase, chromatographed only with the nucleoside m\textsubscript{2,2}'G and the trialcohol derivative of m\textsubscript{2,2,7}'G; (5) same as 1.

FIG. 12 (left). One-dimensional thin layer chromatography showing release of A from oligonucleotide P20, A-A-Gm-A-U by complete snake venom phosphodiesterase digestion. Corresponding digestion of oligonucleotide P25 produced no free nucleoside.

FIG. 13 (right). Autoradiograph of snake venom phosphodiesterase digestion products of oligonucleotide obtained before and after treatment of oligonucleotide P25 with nucleotide pyrophosphatase. The digestion products were separated by electrophoresis on Whatman No. 3MM paper at pH 3.5. The oligonucleotide obtained after pyrophosphatase treatment contained pAm, pUm, and pCp, but not pm\textsubscript{2,2,7}'G. B, blue marker (Xylene cyanol FF).

Fig. 12 (left). One-dimensional thin layer chromatography showing release of A from oligonucleotide P20, A-A-Gm-A-U by complete snake venom phosphodiesterase digestion. Corresponding digestion of oligonucleotide P25 produced no free nucleoside.

Fig. 13 (right). Autoradiograph of snake venom phosphodiesterase digestion products of oligonucleotide obtained before and after treatment of oligonucleotide P25 with nucleotide pyrophosphatase. The digestion products were separated by electrophoresis on Whatman No. 3MM paper at pH 3.5. The oligonucleotide obtained after pyrophosphatase treatment contained pAm, pUm, and pCp, but not pm\textsubscript{2,2,7}'G. B, blue marker (Xylene cyanol FF).

m\textsubscript{2,2,7}'G which is linked to 2'-O-methylated adenosine by 5' to 5' pyrophosphate linkage. Partial evidence for the unique sequence of this 5'-terminal oligonucleotide was that complete digestion of oligonucleotide P25 with snake venom phosphodiesterase produced pm\textsubscript{2,2,7}'G, pAm, pUm, and pCp in approximately equimolar ratios and partial digestion of these oligonucleotides with snake venom phosphodiesterase produced -Um-C-Gp and pAm-Um. Thus, the sequence of the 3'-termi-
nal portion of the 5'-oligonucleotide was pAm-Um-C-Gp. Evidence for a free 2'-3'-cis-diol of m$_2$'p$_2$G in the intact U-2 RNA molecule was provided by NaIO$_x$ oxidation and [3H]KBH$_4$ reduction (11). The 5'-oligonucleotide did not contain a 5'-phosphate and no free nucleoside was released from the 5' end by venom phosphodiesterase digestion. Free pm$_3$'p$_2$G was released by digestion with nucleotide pyrophosphatase.

It is not possible at present to define the functional role of this unusual 5'-terminal portion of the sequence of U-2 RNA. In studies currently in progress it has been found that the U-1 and U-2 RNA are in a low molecular weight particle that contains a number of proteins. The modified nucleotides and methylated sugar moieties may serve as binding sites for specific proteins of this particle. On the other hand, they may be binding sites for specific polymerases or subunits that are involved in synthesis of nucleic acids.

Pyrophosphate linkages in RNA molecules have not been described previously although formation of a pyrophosphate linkage has been reported for reactions of polynucleotide ligase which requires DPN or ATP (26, 27). Synthesis of such pyrophosphate linkages in the nucleus may result from the activity of an enzyme previously studied in this and other laboratories (28). Whether this enzyme catalyzes the synthesis of the pyrophosphate linkage of U-2 RNA is not yet known. It is also not clear whether the U-2 RNA is initially synthesized, post-transcriptionally modified, and then linked to pm$_3$'p$_2$G or whether the latter is part of the molecule as synthesized and is methylated when all of the other modifications are made on the molecule.

It will be of interest to determine whether the relative turnover rates of the pm$_3$'p$_2$G are the same as or differ from those of the remainder of the molecule. There are nucleotide pyrophosphatases in cells (29, 30), particularly in the particulate fractions (31) which may affect the pyrophosphate linkage of the pm$_3$'p$_2$G with the remainder of the molecule. Since Fredrickson and Hellung-Larsen (4) suggested that such LMWN RNA molecules are involved in the synthesis of high molecular weight RNA species, it is possible that pyrophosphatases may function in celluar control of such synthetic reactions.

Acknowledgments—The authors are indebted to Dr. Ross E. Nazar who developed the method and Mr. William Spohn for analyses of nucleosides on the Picker analyzer, to Pamela Crain for analyses with the mass spectrometer, to Mrs. Rose K. Busch for thhe tumors employed, and to Charles Taylor for incubations of the cells with 32P-orthophosphate.

REFERENCES


Nucleotide sequence of U-2 ribonucleic acid. The sequence of the 5'-terminal oligonucleotide.
T S Ro-Choi, Y C Choi, D Henning, J McCloskey and H Busch


Access the most updated version of this article at http://www.jbc.org/content/250/10/3921

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/10/3921.full.html#ref-list-1