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Biochemistry

Transmethylation and transguanylylation in 5′-RNA capping system isolated from rat liver nuclei

(nuclear enzymes/mRNA/methyltransferase/guanylyltransferase)

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ABSTRACT **Rat liver nuclei were isolated and sonicated for extraction in order to study the capping of RNA. The guanosine 7-methyltransferase was purified from the extract by hydroxylapatite column chromatography with stepwise addition of phosphate buffer. It was assayed by using as methyl acceptor synthetic G(5′)ppp(5′)G and *S*-adenosylmethionine as donor. The enzyme appeared in a sharp peak at 160 mM. The same peak fraction was subsequently found to contain the enzyme that guanylylates short synthetic polynucleotides and low molecular weight yeast RNA as acceptors. The two enzymatic activities were separated on Sephadex G-150 chromatography, yielding guanylyltransferase and guanosine 7-methyltransferase with molecular weights of approximately 65,000 and 130,000, respectively. Guanylyltransferase was further purified by CM-Sephadex chromatography, whereby G-7-methyltransferase was completely removed. Dithiothreitol was essential for guanylylation, and 2 mM Mn^{2+} (optimum) was twice as active as 8 mM Mg^{2+} (optimum). The α-^{32}P of [^{32}P]GTP, but not its β- or γ-^{32}P, was incorporated into the cap structure. By using unlabeled GTP with [β-^{32}P]ppGpCpC-poly(A_2,U_2,G) as acceptor, [β'-^{32}P]-GpppG... was formed. Our purified transguanylylation enzyme was found to catalyze a [^{32}P]pyrophosphate exchange with GTP, which may be useful as a rapid assay for transguanylylation.**

An additional role for GTP in protein synthesis has been added through the discovery of its activity in the capping reaction, which results in a blocking of eukaryote mRNA by guanylylation of 5′-terminal phosphoryl groups to form a G(5′)-ppp(5′)NpN... bridge. The primary reaction is followed by methylation at position 7 of the external guanosine and at the 2′-riboses of internal nucleotides. Reviews of various aspects of the modification of the basic mRNA structure have appeared on the mechanism of biosynthesis (1), on the isolation of preformed cap structures from cell nuclei (2), and on the role of RNA processing in eukaryote mRNA production (3). The most thorough studies on the mechanism have been done with virus core-associated or core-derived transguanylylation and transmethylation enzymes (4–6). Cap formation was studied with HeLa cell nuclei by Groner and Hurwitz (7), with L-cell nuclei by Winicov and Perry (8), and with HeLa cell crude nuclear extract by Wei and Moss (9). We chose to use an extract of normal rat liver nuclei as enzyme source in the experiments described here.

MATERIALS AND METHODS

Materials. P-L Biochemicals supplied GpppG, GpppG^m, GpppA, GpppA^m, GpppC, GpppU, their 7-methylguanosine derivatives, and G > p, pG > p, and ppG > p; pppG > p was prepared by incubating ppG > p with pyruvate kinase and phospho*enol*pyruvate and was purified by chromatography on DEAE-Sephadex. *S*-Adenosyl[*methyl*-^{3}H]methionine (11.5 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was obtained from New England Nuclear, and [α-^{32}P]GTP (350 Ci/mmol) and [γ-^{32}P]GTP (17.2 Ci/mmol) were from Amersham/Searle. [β-^{32}P]GTP (10 Ci/mmol) was prepared from $^{32}P_i$ (carrier-free, New England Nuclear) and GDP by exchange reaction with *Escherichia coli* polynucleotide phosphorylase (P-L Biochemicals), followed by phosphorylation with pyruvate kinase (10). *E. coli* alkaline phosphatase (code BAPF), nucleotide pyrophosphatase (type III), and nuclease P1 were obtained from Worthington, Sigma, and Yamasa Shoyu (Chosi, Japan), respectively.

Oligo- and Polynucleotide Synthesis. GpCpC, pGpCpC, ppGpCpC, and pppGpCpC were prepared by a modification of the procedure of Simoncsits *et al.* (11) from (ppp)G > p and CpC by reverse reaction of RNAse T1. ^{32}P was introduced in the following manner: [^{32}P]$\overset{*}{p}$pG > p was prepared from ppG > p and $^{32}P_i$ by exchange reaction with *Micrococcus luteus* primer-dependent polynucleotide phosphorylase, using ApApA as primer (12); after incubation for 6 hr at 37°C, [^{32}P]$\overset{*}{p}$pG > p was purified by DEAE-Sephadex column chromatography and [^{32}P]$\overset{*}{p}$pGpCpC was prepared as described (11). To synthesize [^{32}P]$\overset{*}{p}$pGpCpC-poly(A_2,U_2,G), [^{32}P]$\overset{*}{p}$pGpCpC as a primer and poly(A_2,U_2,G) were added with *M. luteus* polynucleotide phosphorylase according to the method of Both *et al.* (13).

Purification of Yeast Low Molecular Weight RNA. Yeast soluble RNA (type III, lot 15C-8250, Sigma) was passed through a Sephadex G-75 column and the material that eluted at V_e/V_0 = 1.8–2.3 was lyophilized. This RNA fraction of 10–20 nucleotides in chain length was found to be a very active guanylyl acceptor.

Preparation of Nuclei and Crude Nuclear Extract. Nuclei were isolated from 30 livers of Holtzman male rats (140–160 g), and the extract was prepared by sonication according to Roeder and Rutter (14).

Assay for Methyltransferase. Methyl transfer from *S*-adenosyl[*methyl*-^{3}H]methionine with synthetic G$\overset{*}{p}$pppG^m as acceptor was measured as follows. The incubation mixture contained in 10 μl: 50 mM Tris·HCl (pH 7.6), 1 mM $MgCl_2$, 1 mM dithiothreitol, 2 μM *S*-adenosyl[*methyl*-^{3}H]methionine (6000 cpm/pmol), 15% (vol/vol) glycerol, 50 mM $(NH_4)_2SO_4$, 90–180 μM GpppG^m, and the protein fraction to be assayed. After incubation for 40 min at 37°C, the reaction mixture was applied to polyethyleneimine cellulose thin layer chromatography sheets (Brinkmann Instruments), and the sheets were desalted by soaking in absolute methanol and then developed with 0.4 M LiCl together with the m^{7}GpppG^m marker. The chro-

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Abbreviations: m^{7}G, 7-methylguanosine; G^m, 2′-*O*-methylguanosine; AdoMet, *S*-adenosyl$\overset{*}{m}$ethionine; $\overset{*}{p}$, denotes ^{32}P label in that position; G>p, cyclic GMP; $\overset{*}{m}$, denotes ^3H label in methyl group.

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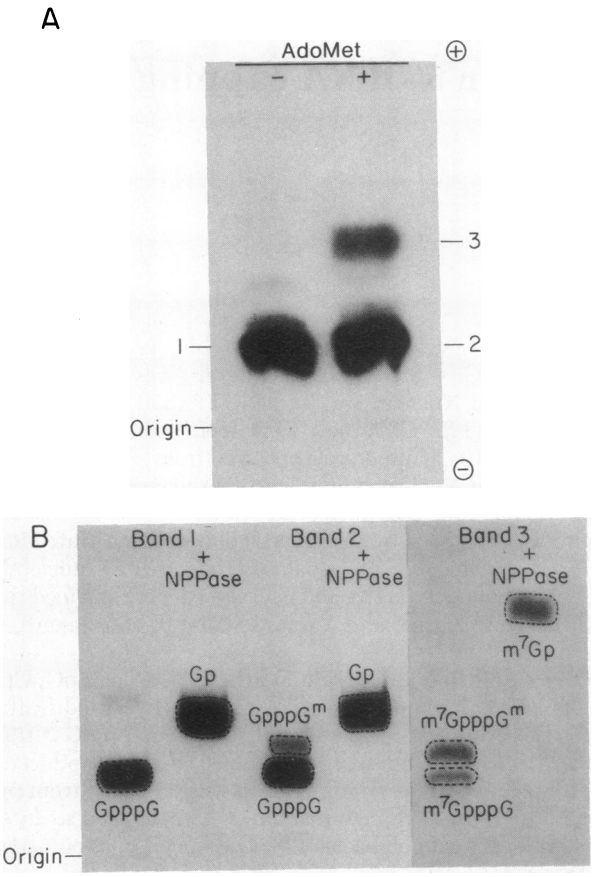

FIG. 1. Cap formation with crude rat liver nuclear extract. Low molecular weight yeast RNA (1.5 A_{260}) was incubated with [α-^{32}P]GTP and rat liver nuclear extract (150 μg protein) in the presence or absence of 0.2 mM AdoMet under standard conditions, except with 2.5 mM $MgCl_2$, 0.5 mM $MnSO_4$, and 15 μM A(5′)pppp(5′)A. AppppA was added to inhibit nonspecific nucleotide pyrophosphatase, a possible contaminant. After incubation for 80 min at 37°C, RNA was extracted and processed. (*A*) Nuclease P1- and alkaline phosphatase-resistant material was subjected to electrophoresis on Whatman DE-81 paper and the electropherogram was then radioautographed. (*B*) Analyses of bands 1, 2, and 3 in *A* by polyethyleneimine cellulose thin layer chromatography. Materials from the three bands were eluted with 1.0 M triethylamine-HCO_3^- buffer (pH 8), lyophilized, and dissolved in a small amount of H_2O. Aliquots of the labeled materials were incubated for 30 min at 37°C with or without 0.03 unit of nucleotide pyrophosphatase (NPPase) in 10 μl of 50 mM Tris·HCl, pH 7/5 mM β-glycerophosphate. The hydrolysate was chromatographed on polyethyleneimine cellulose thin layer plates in 1.0 M LiCl (bands 1 and 2) or 0.4 M LiCl (band 3). The positions of authentic markers under ultraviolet light are indicated by the dotted circles.

matogram was cut into 0.5-cm pieces which were eluted with 0.5 ml of 0.5 M $(NH_4)_2CO_3$, and the overlap with the marker was assayed by scintillation counting in Aquasol (New England Nuclear).

Assay for Guanylyltransferase by Using [α-^{32}P]GTP as Donor in G$\overset{*}{p}$ppN·RNA Synthesis. The standard reaction mixture (0.1 ml) contained: 50 mM Tris·HCl (pH 7.7), 2 mM $MnSO_4$, 0.5 mM $MgCl_2$, 10 mM dithiothreitol, 9% (vol/vol) glycerol, 6 μM [α-^{32}P]GTP (7,000–30,000 cpm/pmol), 0.5–1.5 A_{260} units of purified yeast soluble RNA as acceptor, and enzyme fraction. Incubation was for 60 min at 37°C. RNA was extracted with phenol and passed through a Sephadex G-25 column (0.8 × 20 cm) in 40 mM CH_3COONH_4, and the void volume fractions were lyophilized. The lyophilized sample was digested with nuclease P1 [in 15 μl of 20 mM Na acetate (pH 5.5) containing 10 μg of enzyme for 60 min at 37°C] followed

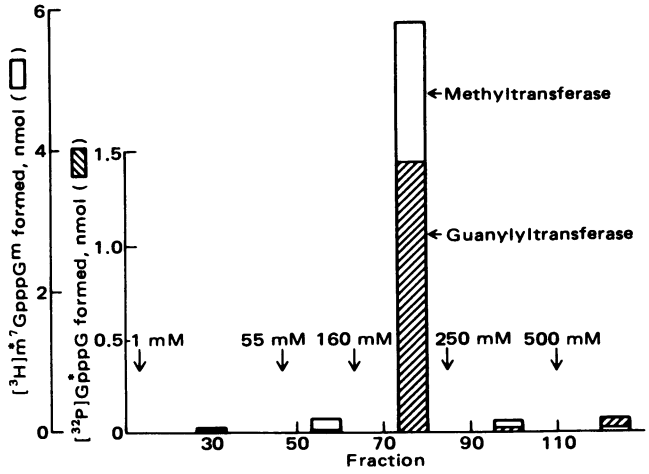

FIG. 2. Separation of methyltransferase and guanylyltransferase on hydroxylapatite column. Rat liver crude nuclear extract (333 mg of protein) from 30 rats was applied to a column of hydroxylapatite (1.0 × 28 cm) and developed stepwise with buffer A (20% glycerol/1 mM dithiothreitol/0.1 mM EDTA/20 mM Tris·HCl, pH 7.7/1 mM $MgCl_2$) containing 1, 55, 160, 250, and 500 mM potassium phosphate buffer, pH 6.8. Each eluate was pooled and concentrated by $(NH_4)_2SO_4$ precipitation. Methyltransferase was assayed with 180 μM GpppG^m as acceptor and [^{3}H]AdoMet as methyl donor. Guanylyltransferase was assayed with 1.0 A_{260} unit of low molecular weight yeast RNA and [α-^{32}P]GTP. Activity is expressed as the total activity present in pooled fractions.

by alkaline phosphatase treatment [in 25 μl of 80 mM Tris·HCl (pH 8.5) containing 5 μg of bacterial alkaline phosphatase for 60 min at 37°C]. Nuclease P1- and alkaline phosphatase-resistant materials were analyzed by paper electrophoresis on a 20-cm-long Whatman DE-81 sheet for 2 hr at 900 volts in pyridine/acetate buffer, pH 3.5. The electropherogram was radioautographed and the radioactivity in the GpppN marker spot was assayed.

RESULTS

Capping Activity and Methyl Transfer in Crude Nuclear Extract. When low molecular weight yeast RNA was incubated with crude nuclear extract and [α-^{32}P]GTP, nuclease P1- and bacterial alkaline phosphatase-resistant ^{32}P-labeled materials appeared in the absence of (band 1) and in the presence of *S*-adenosylmethionine (AdoMet) (band 2 and 3) as shown in Fig. 1*A*. For further identification, extracts of these bands were analyzed by polyethyleneimine cellulose thin layer chromatography, as shown in Fig. 1*B*. The band 1 material comigrated with GpppG marker, and band 2 material gave two spots corresponding to GpppG and GpppG^m; band 3 also gave two spots, m^{7}GpppG and m^{7}GpppG^m. Furthermore, [5′-^{32}P]GMP was formed from bands 1 and 2, and 7-methyl[5′-^{32}P]GMP from band 3 after treatment with nucleotide pyrophosphatase, indicating that the cap structures (m^7)G$\overset{*}{p}$ppG$^{(m)}$ had been formed. There was no significant difference between the amounts of cap structure formed either in the presence or absence of methyl donor. Thus, three enzymatic activities were found to be present: (*i*) incorporation of [α-^{32}P]GMP and methylation from AdoMet to form (*ii*) m^{7}GpppN. . . and (*iii*) m^{7}GpppN^m. . . . In our purified preparation, only the m^{7}G transmethylase has been found and no attempts were made to separate the additional ribose methylase activity from crude extracts.

Chromatographic Purification and Separation of Guanosine 7-Methyl- and Guanylyltransferases. The crude nuclear extract was applied to a hydroxylapatite column, and

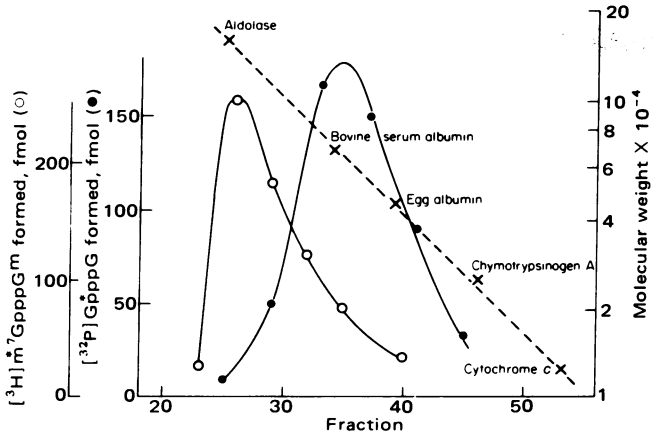

FIG. 3. Separation of enzyme fractions obtained in Fig. 2 by Sephadex G-150 chromatography. A portion of the 160 mM potassium phosphate eluate (11 mg of protein) from the hydroxylapatite column was concentrated by $(NH_4)_2SO_4$ precipitation and chromatographed on a column of Sephadex G-150 (1 × 45 cm) previously equilibrated with buffer A containing 150 mM $(NH_4)_2SO_4$. Fractions (0.75 ml) were collected; 2 and 5 μl were assayed for methyltransferase (○) and guanylyltransferase (●), respectively, as described in Fig. 2. The standard proteins for molecular weight calibration were run under the same conditions.

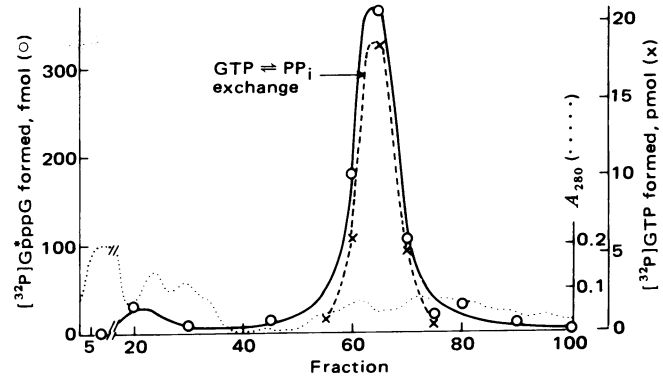

FIG. 4. Purification of guanylyltransferase by CM-Sephadex column chromatography. A portion (3.5 mg protein) of the guanylyltransferase fraction from the Sephadex G-150 column (Fig. 3, fractions 30–44) was diluted with 4 vol of buffer A and applied to a column of CM-Sephadex (0.8 × 22 cm). The proteins were eluted with 100 ml of 50–500 mM KCl gradient in buffer A. Fractions of 1 ml were collected and 30 μl was assayed for guanylyltransferase by using yeast low molecular weight RNA as acceptor (○). In the pyrophosphate exchange experiment (×), fractions of the main peak were incubated for 75 min at 37°C in 100 μl containing 5 mM $MgCl_2$, 0.5 μM $MnSO_4$, 100 μM [^{32}P]PP_i, and 500 μM GTP. Nucleotides were adsorbed and then eluted from charcoal and [^{32}P]GTP was separated by electrophoresis. The [^{32}P]PP_i exchange into [^{32}P]GTP was confirmed by conversion with hexokinase to [^{32}P]GDP and glucose 6-[^{32}P]phosphate, which were separated and assayed for radioactivity; the former gave 1384 cpm and the latter, 1349 cpm.

proteins were successively eluted with 1, 55, 160, 250, and 500 mM potassium phosphate buffer. As shown in Fig. 2, both methylation and guanylylation activities were eluted by addition of 160 mM buffer. This eluate was concentrated and then chromatographed on a Sephadex G-150 column, and methyl- and guanylyltransferases eluted separately as shown in Fig. 3. From the calibration curve with standard proteins, the molecular weights of these fractions were estimated to be approximately 130,000 and 65,000, respectively.

As summarized (15), by using the hydroxylapatite eluate peak at 160 mM potassium phosphate (see Fig. 2), the first experiments on transmethylation were carried out with GpppG, GpppC, GpppA, and GpppU as acceptors. [^{3}H]Methyl was accepted from AdoMet on guanosine at C-7 with greatest abundance when guanosine was in the second position, diminishing in amount from cytosine to uridine and adenosine. Methylation of guanosine at C-7 was unexpectedly stimulated by preparations that were ribose-2′-*O*-methylated on the second guanosine, and particularly on adenosine which was increased 5-fold.

Radioactive Phosphate Incorporation from α-, β-, or γ-^{32}P-Labeled GTP into Various Acceptor RNAs. As shown in Table 1, α-phosphate radioactivity of GTP was found in the cap structure with all three acceptor RNAs. This indicates a GMP transfer by pyrophosphate displacement, and, in experiment 2, that a 5′-diphospho-poly(A) is a good acceptor with our enzyme preparation.

Table 1. Cap synthesis with α-, β-, or γ-phosphate-labeled GTP

Exp.	RNA substrate	[α-^{32}P]GTP, fmol	[β-^{32}P]GTP, fmol	[γ-^{32}P]GTP, fmol
1	Low molecular weight yeast RNA	837	0.7	30
2	$ppA(pA)_n$	113	0.2	1
3	Poly(C)			
	(*a*) Without treatment	96	2	3
	(*b*) Alkaline phosphatase-treated	1		
	(*c*) Alkaline phosphatase-treated/ T4 polynucleotide kinase-treated	2		

Reaction was carried out with 3.5 μg of hydroxylapatite-purified enzyme and with differently labeled GTP under the standard conditions. Incubation was for 90 min. RNAs used were: low molecular weight yeast, 1.5 A_{260} units; $ppA(pA)_n$ (a generous gift from B. Moss, National Institutes of Health), 0.7 A_{260} unit; poly(C) (Miles, No. 11-304, lot no. 48), 1.1 A_{260} units; alkaline phosphatase-treated poly(C), 1.0 A_{260} unit; alkaline phosphatase/T4 polynucleotide kinase-treated poly(C), 1.0 A_{260} unit (650 pmol of terminal [^{32}P]pC). Specific activities and GTP concentrations were: [α-^{32}P]GTP, 1.5 × 10^4 cpm/pmol, 3 μM; [β-^{32}P]GTP, 1.4 × 10^4 cpm/pmol, 2.4 μM; [γ-^{32}P]GTP, 5.4 × 10^4 cpm/pmol, 2.8 μM. Enzyme treatment of poly(C) was carried out as follows: 1.5 mg of poly(C) was dephosphorylated with 4.3 units of alkaline phosphatase for 90 min in a 300-μl reaction mixture containing 5 mM $MgCl_2$ and 0.1 M Tris·HCl, pH 8. After incubation, the mixture was treated twice with an equal volume of phenol, passed through a Sephadex G-25 column, lyophilized, and then dissolved in H_2O. The incubation mixture for T4 polynucleotide kinase contained in 300 μl: 50 mM Tris·HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 50 μM [γ-^{32}P]GTP (specific activity 8000 cpm/pmol), 5 units of T4 polynucleotide kinase, and the above dephosphorylated poly(C). After incubation for 90 min at 37°C, RNA was extracted with phenol, passed through Sephadex G-25, and precipitated twice with ethanol. In experiment 3*c*, both acceptor RNA and donor GTP were labeled with ^{32}P.

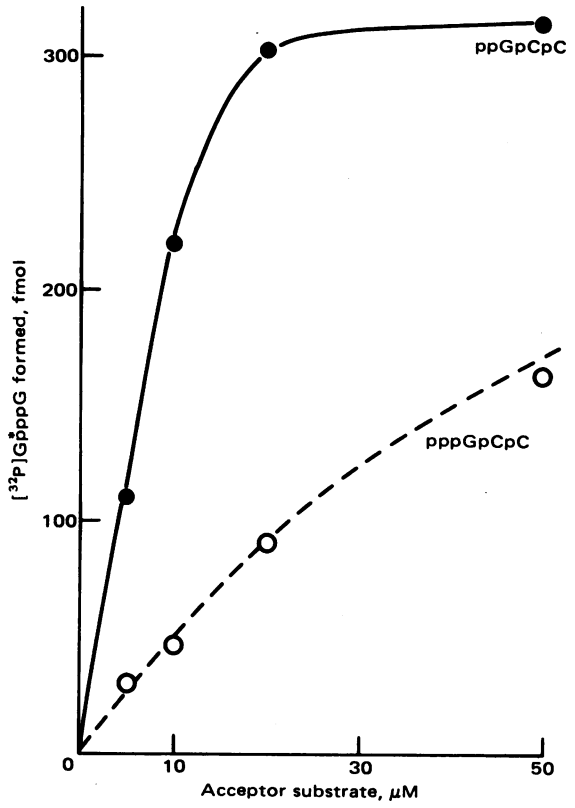

FIG. 5. Cap formation with [α-^{32}P]GTP as donor and various concentrations of ppGpCpC or pppGpCpC as acceptors. Reaction conditions were the same as in Table 1 except that 3 μg of enzyme (Sephadex G-150, fraction 37) and various concentrations of ppGpCpC (●) or pppGpCpC (○) were used and the incubation time was 60 min at 30°C. Reaction mixtures were treated with phenol and the aqueous phase was lyophilized. The lyophilized materials were incubated with alkaline phosphatase and then subjected to electrophoresis on Whatman 3 MM paper in pyridine/acetate buffer, pH 3.5. Oligonucleotide fractions were eluted, hydrolyzed with nuclease P1, and finally applied to polyethyleneimine cellulose thin layer chromatography sheets. The chromatography was carried out with 1 M LiCl, and the ^{32}P in the GpppG spot was assayed.

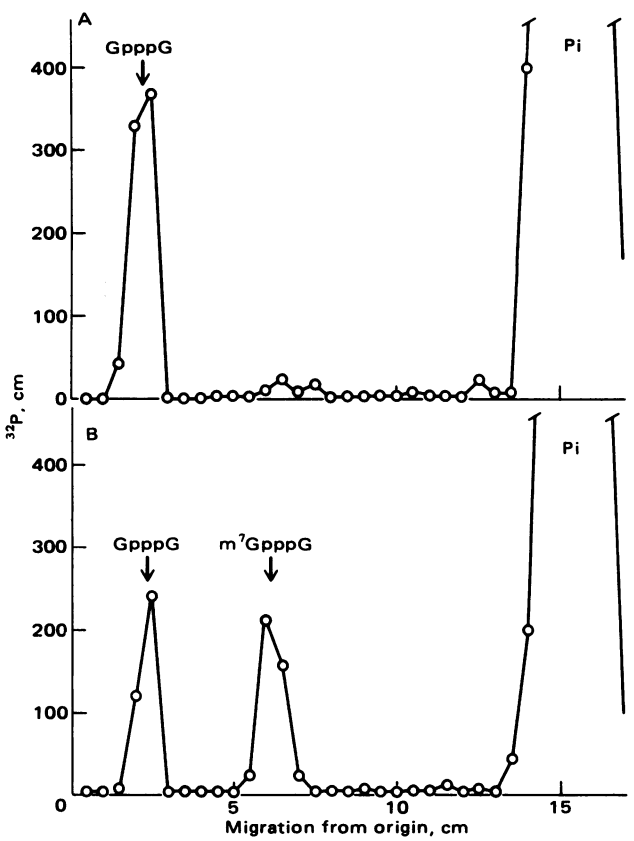

FIG. 6. Sequential formation of [^{32}P]Gp*ppG... and m^7 Gp*ppG... by purified guanylyl- and methyltransferases with [^{32}P]p*pGpCpC-poly(A_2,U_2,G) as acceptor. (*A*) Guanylyltransferase. (*B*) Guanylyltransferase plus methyltransferase. The [^{32}P]p*pG-polynucleotide (700 pmol) was first incubated for 40 min at 37°C with 50 μM GTP and guanylyltransferase (CM-Sephadex-purified enzyme, 1.3 μg, free of methyltransferase) under the standard conditions for guanylyl transfer. After termination of the reaction by the addition of 2.5 mM EDTA, the mixture was further incubated for 40 min with 300 μM AdoMet and methyltransferase (Sephadex G-150, fraction 25, 40 μg) in a final volume of 120 μl. RNA was extracted with phenol, processed with nuclease P1 and bacterial alkaline phosphatase, and analyzed for cap structures by electrophoresis on Whatman DE-81 paper. The mobility of authentic markers is indicated by arrows.

Purification of Guanylyltransferase on CM-Sephadex Column. The guanylyltransferase from Sephadex G-150 was further purified on a CM-Sephadex column as shown in Fig. 4. The transguanylylation activity was eluted at 0.27 M KCl; furthermore, this peak was found to contain the activity that promotes pyrophosphate exchange with GTP. About 350-fold purification and 49% recovery was obtained compared with the crude nuclei extract. At −70°C, this preparation remained active for several months. As mentioned, included in this figure is an experiment showing that this preparation of guanylyltransferase catalyzes a [^{32}P]pyrophosphate exchange with GTP. This indicates a mechanism of GMP transfer from GTP by a pyrophosphate displacement reaction to a diphosphate 5′ terminus of RNA. The perfect overlap of the two assay curves in Fig. 4 indicates that the GTP–PP_i exchange assay can be used for assaying guanylyltransferase.

Comparison of Guanylylation to ppGpCpC and pppGpCpC as Acceptors. As shown in Fig. 5, with ppGpCpC guanylyl transfer reached a plateau at 15 μM, whereas with pppGpCpC only one-third as much was incorporated, increasing to about one-half at 50 μM. The comparably much slower rate of reaction in the latter case could be explained by assuming that 5′-pp-RNA is the acceptor and that it is formed by a relatively slow dephosphorylation reaction possibly through a contaminating phosphohydrolase. Because we have not attempted to verify such a mechanism, no more can be concluded than that, in this system, at limited acceptor concentration and 30°C, the 5′-pp-RNA reacts more rapidly than the 5′-ppp-RNA. It should be mentioned that at 150 μM acceptor concentration and 37°C, in an analogous experiment, nearly equal amounts were guanylylated with di- and triphospho-5′-RNA as acceptor after incubation for the same period. Under these assay conditions, one would have to assume that a fast enough conversion of the triphosphate termini to diphosphate has taken place to reach a similar plateau. As would be predicted from Table 1, GpCpC and pGpCpC were completely inert (data not shown).

Guanylylation onto the diphosphate end of RNA was confirmed by using [β-^{32}P]ppGpCpC-poly(A_2,U_2,G) as acceptor to obtain the formation of [^{32}P]Gp*ppG... (see also Table 1, exp. 2). As shown in Fig. 6*A*, when the β-^{32}P-labeled pp-5′-RNA was incubated with purified guanylyltransferase and GTP and digested with nuclease P1, the ^{32}P-label was found in Gp*ppG. In Fig. 6*B*, efficient methylation on this preformed cap was observed when methyltransferase and AdoMet were added subsequently. The position of ^{32}P in these cap structures was con-

formed by the release of all ^{32}P as inorganic phosphate with nucleotide pyrophosphatase (data not shown).

DISCUSSION

The capping reaction of short-chain yeast RNA or synthetic polynucleotides studied here in sonicates of rat liver nuclei appears to follow the most frequently reported pattern—i.e., $G\overset{\alpha\beta}{p}pp + \overset{\beta'\alpha'}{p}pN... \rightarrow G\overset{\alpha\beta\cdot\alpha'}{ppp}N... + PP_i$ (4, 6, 9). The GpppN... structure was found to be derived from the condensation of the α-phosphate of GTP with terminal 5′-α'-,β'-phosphates of RNA. After prior or simultaneous removal of γ-phosphate, a 5′-ppp-RNA was also capped. This reaction appears to be a pyrophosphate displacement in GTP, and this is confirmed by the fact that transguanylylation activity was found to overlap with a GTP $\rightleftharpoons$ [^{32}P]PP$_i$ exchange reaction (Fig. 4) in the CM-Sephadex chromatogram. Furthermore, such a mechanism was indicated by a slower capping reaction of 5′-ppp-RNA as compared with 5′-pp-RNA at 30°C and at limiting concentrations (Fig. 5). This is tentatively explained by a conversion by contaminating enzyme, or by the intrinsic activity of our guanylyltransferase, of triphospho-termini to yield diphospho-RNA. By use of a highly purified vaccinia virus capping system (5), an exclusive capping of the triphosphate terminal of RNA was observed with liberation of equivalent amounts of P$_i$ and PP$_i$. However, an easy reversal by pyrophosphorolysis was obtained. This indicates a pyrophosphate displacement reaction which might, in this case, be obligatorily linked with RNA phosphohydrolase to remove the terminal γ-phosphate.

In addition to the capping reaction, the methyltransferase was also partially purified. However, the ribose-2′-methyltransferase was lost during the hydroxylapatite column chromatography. In an experiment (Fig. 6) we first capped a short RNA chain, and then methylated it with AdoMet, showing that capping may be followed by guanosine C-7 methylation. Because, in addition to m^{7}GpppN, m^{7}GpppN^m was also formed in the crude extract experiment (Fig. 1), the two methylations must be due here to different enzymes. This was shown by Barbosa and Moss (16) for the vaccinia core enzymes from which they identified and purified the second methyltransferase.

In our experiments, no indication was found for a GppG cap structure which has been reported to be present in Novikoff hepatoma cell nuclei (2) and also as a product with crude extracts of HeLa cell nuclei (7). Furthermore, no indication was found in our system of the unique mechanism of capping indicated in the reaction: $G\overset{\alpha\beta}{p}pp + \overset{\alpha'}{p}N... \rightarrow G\overset{\alpha\beta\alpha'}{ppp}N... + P_i$, which is reported to be the capping mechanism in the vesicular stomatitis virus system (17).

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