**In Vitro Transcription of Vesicular Stomatitis Virus**

INCORPORATION OF DEOXYGUANOSINE AND DEOXYCYTIDINE, AND FORMATION OF DEOXYGUANOSINE CAPS*

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Detergent-disrupted vesicular stomatitis virus carried out *in vitro* transcription at a reduced rate in the presence of deoxyguanosine triphosphate. The transcripts annealed completely to excess vesicular stomatitis virus genomic RNA and consisted of the short leader RNA and even polyadenyalted messenger RNA. Incubation with RNase T1, showed that the transcripts were resistant to cleavage. Nearest-neighbor analysis demonstrated that approximately two-thirds of the guanosine residues had been replaced by deoxyguanosine. The hybrid “deoxyguanosine-RNAs” carried cap structures which contained deoxyguanosine instead of guanosine. Competition experiments using both guanosine- and deoxyguanosine triphosphates indicated that GpppA and dGpppA cap structures were synthesized in approximately equal amounts at a ratio of 20 μM guanosine triphosphate to 50 μM deoxyguanosine triphosphate. Deoxyguanosine triphosphate was accepted by the polymerases of various strains and serotypes of vesicular stomatitis virus, demonstrating that its incorporation was a common characteristic. Deoxycytidine triphosphate could also substitute for cytidine triphosphate but to a lesser degree. Deoxyadenosine-, deoxyuridine-, and thymidine triphosphates were not or were very poorly accepted even at concentrations of 2 mM.

Vesicular stomatitis virus is a negative strand RNA virus. Its genome codes for 5 structural proteins (N, NS, M, G, and L) including its own RNA-dependent RNA polymerase. Replication and transcription of the virus take place in the cytoplasm of the host cell without any known involvement of the nucleus (for review, see Ref. 1). Unlike RNA tumor viruses associated with the polymerase of a Newcastle's disease virus mutant, suggesting a mutation in the viral polymerase. However, Bishop and Roy (5) demonstrated that the VSV polymerase does not incorporate combined deoxyribonucleotides during an *in vitro* transcription reaction. In this communication we describe the specific incorporation of deoxyguanosine and deoxycytidine during the *in vitro* transcription by wild type VSV. We show that the guanosine residues in the transcription products can be replaced by deoxyguanosine, including the guanosine residue in the cap structure.

**Materials and Methods**

**Virus—**Throughout this study, VSV Mudd-Summers isolate was used. Propagation and purification of the virus particles have been described earlier (6, 7).

**In Vitro Transcription—**The standard transcription assay was essentially as described earlier (8) and contained 100 mM Tris-HCl, pH 8, 100 mM NaCl, 5 mM MgCl2, 5 mM dithiothreitol, 0.05% Nonidet P-40, 50 μM ATP, 20 μM UTP, 20 μM CTP, 20 μM GTP and 5 μl of virus (approximately 12 mg of protein/ml)/100 μl of reaction mix. Radiolabeled α-[32P]ATP (specific activity, 1 Ci/mmol), α-[32P]GTP (specific activity, 5 Ci/mmol), or α-[32P]UTP (specific activity, 5 Ci/mmol) were added as indicated in the text. Reactions were terminated by adding 0.2% sodium dodecyl sulfate and 50 μg of carrier tRNA. The unincorporated isotopes were removed by gel filtration with Sephadex G-50. After adjusting to 0.4 M NaCl, the transcripts were precipitated with ethanol, washed with 80% ethanol, dried, and stored in water at −20 °C.

**Enzymatic Digestions—**Complete RNase A, T1, or T2 digest were obtained after heating for 1 min at 100 °C in 20 mM Na citrate buffer, pH 5, 5 mM EDTA and incubating for 1 h at 50 °C in the presence of 4 ng of RNase A/μg of RNA, 1 × 10−2 units of RNase T1/μg of RNA, or 1 × 10−7 units RNase T2/μg of RNA. Complete nuclease P, digestion was carried out for 1 h at 50 °C in 10 mM Na acetate, pH 6, using 20 ng of nuclease P/μg of RNA. After adjusting to pH 8 using 100 mM Tris-HCl, pH 8, the terminal phosphates were removed in some cases using 10 units/ml of calf intestinal phosphatase during 30 min at 37 °C. dGpppA was digested with SVPD for 30 min at 37 °C in 10 mM Tris-HCl, pH 8, 20 mM MgCl2, 2 mM NH4 acetate, using either 0.1 units of SVPD/μl or 1 unit of SVPD/μl.

**Periodisation and β-Elimination—**The procedure for the removal of the adenosine in the deoxyguanosine-containing cap core dGpppA was essentially as described by Neu and Heppel (9). The cap core was periodinated using sodium metaperiodate. β-Elimination was carried out for 90 min at 45 °C in the presence of excess lysine.

**Isolation of Cap Cores—**dGpppA was generated using complete nuclease P, and phosphatase digestion. The digest was subjected to ionophoresis on DE81 paper. The spot corresponding to dGpppA was cut out, washed with methanol and water, and eluted with 0.5 M triethylamine bicarbonate. The triethylamine bicarbonate was removed by lyophilization.

**Gel Electrophoresis—**The transcripts or oligonucleotides were separated on 20% polyacrylamide - 7 μm urchin gels containing Tris-borate buffer, pH 8.3 (10). Samples were applied in 4 μm urchin together with the dyes xylene cyanol FF and bromphenol blue.

**Ionophoresis—**Mononucleotides and cap structures were analyzed either on DE81 paper using 7% formic acid, pH 1.7 (11), on DE81 paper and Whatman No. 3MM paper using 0.5% pyridine, 5% acetic acid, pH 3.5 (11), or on polyethylenimine thin layer sheets using 0.1 M K2HPO4/KH2PO4, pH 7.5, 7 μm urea (12). Ionophoresis was carried out at 25 V/cm.

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The abbreviations used are: VSV, vesicular stomatitis virus, SVPD, snake venom phosphodiesterase.
RESULTS

Incorporation of dGTP—While screening for inhibitors of cap formation, we found that normal caps were not synthesized when dGTP was used in the transcription reaction with VSV. However, a new nuclease P1, and phosphatase-resistant oligonucleotide was detected in digests of the transcripts, which we suspected to be a deoxyguanosine containing cap core structure (see below). Since the capping activity in VSV particles is tightly associated with the transcription complex and might actually be an active part of the viral polymerase, we investigated if the transcriptase itself could utilize dGTP during elongation of the transcripts.

Transcription of VSV was carried out in vitro using α-[32P]ATP as the source of label. GTP or dGTP or both were added to the mix while keeping the concentration of the other nucleotide triphosphates constant. Excess α-[32P]ATP was removed by gel filtration over Sephadex G50 and the flowthrough fractions were collected, precipitated with ethanol, and applied to a 20% polyacrylamide gel (Fig. 1A). Both leader RNA (8) and high molecular weight messenger RNAs were transcribed in the presence of 20 μM GTP, the approximate Kₜₐₜ value for GTP (13), as shown in the control reaction (lane 1). The pattern of the transcripts was not altered when increasing amounts of dGTP ranging from 20 μM to 2 mM were added while the concentration of GTP was kept at 20 μM (lanes 2-7). The total amount of incorporated α-[32P]ATP decreased by approximately 30% compared to the control reaction without dGTP. When GTP was omitted from the reaction and replaced by 2 mM dGTP, the pattern also remained identical (lane 8).

If deoxyguanosine actually replaced guanosine during elongation, the resulting “deoxyguanosine-RNA” should be RNase T₁-resistant. This enzyme is unable to cleave at deoxyguanosines because the 2’3’ cyclic intermediate of the reaction involving the 2’-hydroxyl residue of the ribose cannot be formed. Portions of the RNA in Fig. 1A, lanes 1-8, were completely digested with an excess of RNase T₁ and the resulting oligonucleotides were separated on a 20% polyacrylamide gel (Fig. 1B). Besides the poly(A) of the messenger RNAs which remained at the top of the polyacrylamide gel, a characteristic pattern of T₁-oligonucleotides was generated from the RNAs of a normal transcription reaction (lane 1). The largest T₁-oligonucleotides were 28 and 29 nucleotides long and were derived from leader RNA (14), and from positions 125-153 of the 5’ end of N messenger RNA (15). Adding dGTP to the normal transcription mix resulted in an increase in RNase T₁ resistance of the transcripts as demonstrated by the formation of larger T₁ oligonucleotides (lanes 2-7). This increase depended on the concentration of added dGTP which apparently competed with GTP during elongation. When GTP was replaced by 2 mM dGTP (lane 8), most of the oligonucleotides were larger than 28 nucleotides long and as large as poly(A). Low amounts of remaining small T₁ oligonucleotides co-migrating with those in lane 1 suggest that the dGTP contained small amounts of GTP although high pressure liquid chromatography-purified dGTP was used.

Complete RNase T₁ digestion of the RNAs from the control reaction (Fig. 1A, lane 1) and separation of the mononucleotides by ionophoresis on polyethyleneimine thin layer sheets revealed the expected transfer of the label from the labeled nucleotide pA to its neighboring nucleotides Up, Cp, Ap, and Gp (Fig. 2, lane 1). Nearest neighbor analysis of the transcripts from the reaction which contained only dGTP (Fig. 1A, lane 8) revealed that the label was transferred to Up, Cp, and Ap but only to a small amount to Gp (Fig. 2, lane 2). Quantitation of the radioactivity in the spots demonstrates that approximately 67% of the guanosine residues had been replaced by

![Fig. 1. Competition between GTP and dGTP during VSV transcription in vitro. Transcription was carried out by detergent-disrupted virus in vitro as described under “Materials and Methods” with various concentrations of GTP and dGTP. The transcripts were labeled with α-[32P]ATP. A, transcripts from the reactions containing 20 μM GTP (lanes 1-7) and no dGTP (lane 1); 20 μM dGTP (lane 2); 50 μM dGTP (lane 3); 200 μM dGTP (lane 4); 500 μM dGTP (lane 5); 1 mM dGTP (lane 6); 2 mM dGTP (lane 7); and 2 mM dGTP without GTP present (lane 8). B, complete RNase T₁ digestion products of the transcripts from A. The numbering system is identical to that in A. The transcripts and the T₁ oligonucleotides were separated on 20% polyacrylamide gels. XC, xylene cyanol dye, BPB, bromphenol blue dye marker.](http://www.jbc.org/)

![Fig. 2. Nearest neighbor analysis of the transcripts labeled with either α-[32P]ATP, α-[32P]dGTP or α-[32P]dCTP. Transcripts labeled with α-[32P]ATP as shown in Fig. 1A, lanes 1 and 8, were digested using RNase T₁ and were separated by ionophoresis on polyethyleneimine thin layer sheets. Digestion products from transcripts synthesized in a normal transcription reaction without dGTP present (lane 1) and those synthesized in the presence of 2 mM dGTP in the absence of GTP (lane 2). RNase T₁ and nuclease P1, digestion products of transcripts labeled with either α-[32P]dGTP (lanes 3 and 4) or α-[32P]dCTP (lanes 5 and 6).](http://www.jbc.org/)
deoxyguanosine (Table I). The decrease of Gp by 12% and the increase of Ap by 10% compared to the control reaction (Up and Cp remained the same) were presumably caused by the co-migration of the RNase T2-resistant product dGpAp with Ap.

In addition, the transcripts could be labeled with α-[32P]dGTP. The RNAs annealed specifically to VSV genomic RNA (Table II) indicating that they were transcribed from the VSV template. The small amount (6%) of self-annealing without added VSV genomic RNA was due to the template which was co-purified with the transcripts. When these transcripts were digested with nuclease P1, all of the label, except a small amount of inorganic phosphate, co-migrated with the UV marker 5' dGMP (Fig. 2, lane 4). Digestion with RNase T2, however, gave a result which was surprising at first. Most of the label was transferred to the ribonucleotides Up and Ap and only a minor portion to Cp and Gp (Fig. 2, lane 3). While the absence of Gp was anticipated, the low amount of Cp was not. Recent sequence analyses of VSV messages explain this result. The dimer CpG is underrepresented in the N, NS, M, and G messages of VSV when compared to ApG, UpG, or GpG (15, 16). For instance, within the transcripts from position 1 to 250 of the genome, only 3 CpG compared to 8 UpG, 9 GpG, and 21 ApG exist. The quantitation of the radioactivity of the spots Up, Ap, and Gp (Fig. 2, lane 3) was in agreement with this ratio (data not shown).

We have recently shown that the viral polymerase polyadenylates VSV messages by repeated transcription at a stretch of 7 uridine residues (17-19) positioned at the end of each gene (15, 18). Transcripts labeled with α-[32P]dGTP in the presence of 20 μM GTP were poly(A)-selected to approximately the same extent as transcripts labeled with α-[32P]ATP, synthesized in the absence of dGTP. In both cases, 36-44% of the total radioactivity bound specifically to oligo(dT)-cellulose, demonstrating that dGTP was incorporated into polyadenylated transcripts.

α-[32P]dGTP was incorporated not only by the RNA-dependent RNA polymerase of VSV Mudd-Summers strain but also by the San Juan and HR strains of VSV Indiana serotype, as well as by VSV New Jersey serotype (data not shown). This demonstrates that the utilization of dGTP was not limited to a specific mutant as was reported for the incorporation of TTP (4).

The data taken together show that dGTP can replace GTP during VSV transcription. The transcription products are almost identical to those made in the presence of GTP, based on the size as determined on 20% polyacrylamide gels. However, we would like to point out that analyses on 12% polyacrylamide gels indicated some increased premature termination of transcription.

**Synthesis of dGpppA Cap Cores**—Since most of the guanosine residues in the RNA transcripts could be replaced by deoxyguanosine, we investigated whether the guanosine in the cap core, GpppA, could be substituted by its deoxy counterpart. Portions of the transcripts synthesized in the presence of α-[32P]ATP and various amounts of dGTP were digested with nuclease P1 and calf intestinal phosphatase. The products were analyzed by ionophoresis on DE81 paper at pH 1.7 (Fig. 3). Lane 1 shows the nuclease P1, and phosphatase-resistant cap core GpppA as identified by a UV marker. Inorganic

### Table I

**Nearest neighbor analyses of α-[32P]ATP-labeled transcripts**

Transcripts were labeled with α-[32P]ATP in the presence of 20 μM GTP (column 1) or 2 mM dGTP (column 2). After RNase T2 digestion, the products were separated on polyethyleneimine thin layer sheets as shown in Fig. 2, *lanes 1 and 2*. The spots corresponding to the UV markers Up, Cp, Ap, and Gp were excised and the radioactivity (counts/min) in each determined. The per cent radioactivity relative to the total radioactivity of the four mononucleotides are indicated in parentheses.

<table>
<thead>
<tr>
<th>20 μM GTP</th>
<th>2 mM dGTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpm (%)</td>
<td>cpm (%)</td>
</tr>
<tr>
<td>Up</td>
<td>1259 (13)</td>
</tr>
<tr>
<td>Cp</td>
<td>1802 (19)</td>
</tr>
<tr>
<td>Ap</td>
<td>4833 (50)</td>
</tr>
<tr>
<td>Gp</td>
<td>1775 (18)</td>
</tr>
<tr>
<td>Total</td>
<td>9729 (100)</td>
</tr>
</tbody>
</table>

### Table II

**Annealing of α-[32P]dGTP labeled transcripts to VSV genomic RNA**

Transcripts labeled with α-[32P]dGTP were incubated at 50 °C in 400 μl of 0.4 M NaCl, 10 mM NaH2PO4/Na2HPO4, pH 6.8, 50 μg of tRNA in the presence or absence of 0.6 pmol of 42 S VSV genomic RNA. At the indicated times, 90-μl samples were removed and 5 μl of a mixture of RNase A (500 μg/ml) and RNase T2 (100 units/ml) was added. After incubation for 45 min at 37 °C, the RNAs were trichloroacetic acid-precipitated and the radioactivity (counts/min) of the RNA-resistant transcripts was determined on glass fiber filters. The per cent of RNase resistance is indicated in parentheses.

<table>
<thead>
<tr>
<th>Time of incubation (h)</th>
<th>RNase digestion</th>
<th>With VSV genomic RNA</th>
<th>Without VSV genomic RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8725 (100)</td>
<td>6179 (100)</td>
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</tr>
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<td>684 (8)</td>
<td>545 (9)</td>
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<td>1</td>
<td>7801 (91)</td>
<td>862 (14)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6758 (77)</td>
<td>920 (15)</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 3. Competition between GTP and dGTP during cap formation.** The transcripts were labeled with α-[32P]ATP and are identical to those shown in Fig. 1A. The transcription reactions contained GTP and dGTP in the following concentrations: 20 μM GTP (lane 1); 20 μM GTP, 20 μM dGTP (lane 2); 20 μM GTP, 50 μM dGTP (lane 3); 20 μM GTP, 200 μM dGTP (lane 4); 20 μM GTP, 500 μM dGTP (lane 5); 20 μM GTP, 1 mM dGTP (lane 6); 20 μM GTP, 2 mM dGTP (lane 7); 2 mM dGTP (lane 8). The transcripts were completely digested with nuclease P1, phosphatase-treated, and separated by ionophoresis on DE81 paper at pH 1.7.
phosphate migrated at the top of the chromatogram. The ratio of GpppA to internal $^{32}$P label was approximately 1:250 for the control reaction in the absence of dGTP. Addition of various amounts of dGTP to the control reaction gave rise to a new spot which clearly migrated slightly ahead of GpppA. The darkness of this spot, the putative dGpppA, increased with the concentration of dGTP in the transcription reaction.

It almost completely replaced GpppA at 500 $\mu$M dGTP (lane 5). Approximately equal amounts of GpppA and dGpppA were found at 20 $\mu$M GTP and 50 $\mu$M dGTP (lane 3). In the absence of GTP, only the putative dGpppA spot was synthesized (lane 8). When $\alpha$[$^{32}$P]dGTP was the source of label during transcription, nuclease P$_{1}$ and phosphatase treatment of the RNAs resulted in a compound which co-migrated with this spot in four different chromatography systems (Fig. 4, lanes 1–8), strongly suggesting that it contained deoxyguanosine and adenosine.

dGpppA, labeled in the $\alpha$-position of the deoxyguanosine, was isolated from the DE81 paper and subjected to snake venom phosphodiesterase digestion (Fig. 5). Lane 1 shows the migration of untreated dGpppA on a polyethyleneimine thin layer sheet in a phosphate-urea solvent system at pH 7.5. In this system, dGpppA migrates slightly ahead of GpppA, similarly dpG and dppG migrate ahead of their ribose counterparts. During the isolation of dGpppA, a slight breakdown occurs resulting in small amounts of dpG and dppG. Lane 2 again demonstrates that dGpppA is nuclease P$_{1}$- and phosphatase-resistant. It runs slightly slower because of interference due to the excess amount of protein added to this reaction. When incubated with two different concentrations of snake venom phosphodiesterase (lanes 3 and 4), the dGpppA was completely digested into dppG and small amounts of dpG and inorganic phosphate, suggesting a phosphatase-resistant diester linkage of the G and A residues as expected for a cap structure. Breakage of the diester bond made the phosphates completely accessible to phosphatase (lane 5). Identification of the digestion products was also obtained by ionophoresis on DE81 paper at pH 1.7 which gave identical results (data not shown).

If the proposed deoxyguanosine-cap core contains three phosphates, periodination and subsequent $\beta$-elimination using, e.g. lysine (9), would specifically remove only the adenine ribonucleoside, leaving the deoxyguanosine covalently linked to the triphosphate. Only the ribonucleoside and not the deoxyribonucleoside is susceptible to periodination. In addition, the resulting dGTP would be labeled independently of whether the label was introduced into the cap core by $\alpha$[$^{32}$P]ATP or by $\alpha$[$^{32}$P]dGTP. When labeled with $\alpha$[$^{32}$P]ATP, the radioactive phosphate residue would be transferred from the $\alpha$-position of the adenosine residue to the $\gamma$-position of dGTP. As shown in Fig. 5, lanes 6 and 7, periodination and $\beta$-elimination of the proposed dGpppA gave rise to a product which co-migrated with the UV marker dGTP or GTP which ran almost identically in this system. Important is that the same product was obtained whether the cap was labeled with either $\alpha$[$^{32}$P]dGTP (lane 6) or $\alpha$[$^{32}$P]ATP (lane 7). On the other hand, the same treatment of normal GpppA cap core, isolated from $\alpha$[$^{32}$P]ATP-labeled transcripts, did not generate GTP (lane 8). In this case, both adenosine as well as guanosine residues were removed, resulting in the release of triphosphate, which only slightly migrated off the origin.

In summary, the new oligonucleotide was generated in the presence of dGTP at the expense of GpppA. It could be labeled with $\alpha$[$^{32}$P]ATP as well as $\alpha$[$^{32}$P]dGTP. The A and G residues were linked in a nuclease P$_{1}$- and phosphatase-resist-
ant fashion such as an internal polyphosphate linkage. This polyphosphate bond was susceptible to snake venom phosphodiesterase, giving rise to dppG, which was sensitive to phosphatase. Periodation and β-elimination of either α-[32P]ATP or α-[32P]dGTP labeled deoxyguanosine-caps resulted in dGTP. The data taken together clearly demonstrate that the cap contained three phosphates and was indeed dGpppA.

Incorporation of dCTP—The replacement of guanosine residues by deoxyguanosine during VSV transcription raised the question if other deoxynucleotides could be utilized by the viral polymerase.

Transcription was carried out in the presence of 1 mM concentration of either dCTP (Fig. 6, lane 3), dUTP (lane 5), or TTP (lane 7). The products were labeled with α-[32P]ATP and separated on a 20% polyacrylamide gel next to a control reaction containing only ribonucleotide triphosphates (lane 1). In contrast to the reaction in which GTP was replaced by dGTP, dUTP, and TTP supported the synthesis of only short (approximately 18-nucleotide long) transcription products with small amounts of larger products migrating above the xylene cyanol dye. The majority of the label migrated with the xylene cyanol dye and presumably corresponds to the xylene cyanol dye and presumably corresponds to the xylene cyanol dye and presumably corresponds to the xylene cyanol dye and presumably corresponds to the xylene cyanol dye and presumably corresponds to the xylene cyanol dye and presumably corresponds to the xylene cyanol dye and presumably corresponds to the xylene cyanol dye.

The transcription products made in the presence of dCTP (Fig. 6, lane 3) were large and almost identical to those of the control reaction including leader RNA (lane 1). The same pattern was obtained using α-[32P]dCTP as the source of label (data not shown). Complete degradation with pancreatic RNase gave rise to new RNase A oligonucleotides (lane 4) which were significantly larger than those generated from the normal transcription products (lane 2). This indicates that dCTP incorporation increased the pancreatic RNase resistance of the transcripts. The amount of poly(A) which remained at the top of the polyacrylamide gel decreased when compared to the control reaction, suggesting a possible premature termination of the RNAs before poly(A) was added by the viral polymerase (17–19). As expected, the RNase A-oligonucleotides of the dUTP and TTP reactions (lanes 6 and 8) were short and showed a less complex pattern when compared to those in lanes 2 and 4.

To demonstrate that dCTP was indeed incorporated, the transcripts were labeled with 200 μM α-[32P]dCTP. In Fig. 2, lanes 5 and 6 show a nearest neighbor analysis of the transcripts. As expected, when the RNAs were digested with nuclease P1, almost all of the label migrated with dPC (lane 6) which clearly migrated faster than PC. Digestion with RNase T1 yielded Up, Ap, Gp, and Cp (lane 5). It is clear that the label was transferred from the deoxyribose of the dCp to the ribose of the other nucleotides, which ran slightly slower than their deoxy counterparts. However, the fact that Cp could also be labeled indicates a contamination of the dCTP with CTP. We have used various preparations of dCTP from different sources and observed approximately the same amount of contamination. Nevertheless, it was possible to transfer the label from deoxyctydine to ribonucleotides, demonstrating dCTP incorporation during VSV transcription. The abundance of Cp, however, emphasizes that the vast majority of cytosine residues in the RNAs still contained a ribose instead of a deoxyribose.

**DISCUSSION**

In the previous section, we have demonstrated that dGTP and dCTP could partially replace GTP, and to some extent CTP, respectively, during the *in vitro* transcription of VSV, while dATP, dUTP, and TTP were not accepted or very poorly accepted by the viral RNA-dependent RNA polymerase. The reason for the selectivity of the polymerase for guanosine and cytosine is unknown but might reflect base-pairing to the template before covalent linkage to the previous nucleotide. The hydrogen bonding in the case of G-C base pairs would be stronger than for A-T base pairs and might favor the utilization of guanosine and cytosine. The lack of significant TTP incorporation is in agreement with Moreau et al. (4). The fact that dATP is not incorporated might underlie the special role of ATP during the initiation of transcription (13). Obviously the VSV polymerase is not a reverse transcriptase. However, it can partially reverse transcribe *in vitro* only when deoxyguanosine and deoxycytidine are considered. We have not discussed the efficiency of transcription when dGTP or dCTP were present because of ribonucleotide contamination in the preparations of dGTP and dCTP. Slight contamination with ribonucleotides could almost saturate the polymerase when high concentrations of deoxynucleotides were used, since the $K_m$ values for GTP and CTP are both low, on the order of 20 to 30 μM (13). Therefore, it was not possible to determine the $K_m$ value for dCTP, but it is probably 50 times higher than for CTP. CTP was clearly the favored substrate. In fact, dCTP actually inhibited total incorporation in competition with the ribonucleotide (data not shown) as did dGTP. Both stimulated transcription when the corresponding ribonucleotide was omitted from the reaction. In the case of dGTP, we estimate a $K_m$ value of approximately 200 to 500 μM as it is for ATP (13). At a ratio of 20 μM GTP versus 20 μM dGTP (Fig. 1B, lane 2), only a small amount of dGTP...
was incorporated as indicated by larger Tₗ oligonucleotides when compared to those of the control reaction without dGTP (lane 1). Like CTP, GTP was clearly preferred during elongation over its deoxynucleotide-triphosphate counterpart.

This was not the case during cap formation. GTP and dGTP were almost equally well incorporated into the cap structure of the messages in vitro (Fig. 3), demonstrating that the guanyltransferase which appears associated with the transcription complex discriminates against the base rather than the sugar residue of the nucleotide triphosphate. The incorporation of dGTP into caps has also been reported for the vaccinia virus capping enzyme (20). However, a quantitative comparison of the use of both GTP and dGTP in vitro has not been described.

The in vitro transcription of “deoxyguanosine-RNA” by the VSV polymerase might be a useful tool in studies on the mechanism of VSV transcription and especially the capping reaction. It is not likely to be a biologically important reaction, since VSV transcribes and replicates in the cytoplasm of the cell in the presence of ribonucleotides and in the absence of high concentrations of deoxynucleotide triphosphates. Therefore, the chance for incorporation of dGTP or dCTP seems minute even during cap formation. Analysis of VSV genomic RNA, with a detectability of approximately 1/10,000 nucleotides, did not reveal any deoxyguanosine incorporation.² It will be interesting to see if other negative strand RNA viruses with a nuclear involvement during transcription (such as influenza) (21, 22) can incorporate deoxynucleotides at a low frequency.

² M. Schubert and R. Lazzarini, unpublished observation.

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In vitro transcription of vesicular stomatitis virus. Incorporation of deoxyguanosine and deoxycytidine, and formation of deoxyguanosine caps.

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