Characterization of Foot-and-Mouth Disease Virus Ribonucleic Acid Synthesized *in vitro*

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The present paper deals with the characterization of RNAs synthesized *in vitro* with the help of RNA polymerase (RNA nucleotidyl transferase EC 2.7.7.6) prepared from BHK 21 cells infected with foot-and-mouth disease virus (FMDV), strain A2-SPAIN. Polatnick & Arlinghaus (1967) reported an RNA-dependent RNA-polymerase in BHK cells infected with FMDV. The properties of this enzyme and the *in vitro* synthesized RNA were described by Arlinghaus & Polatnick (1969a, b), Polatnick & Arlinghaus (1967).

The techniques published by Scholtissek (1969) were employed in our investigations. Using hybridization and nearest-neighbour analysis, Scholtissek (1969) was able to show for the fowl plague *in vitro* system, that the newly synthesized RNA consisted mainly of strands complementary to virus RNA. In contrast to these results the present work showed that the *in vitro* product synthesized with the help of a FMDV-induced polymerase consisted mainly of plus-stranded RNA.

The FMDV polymerase was prepared as described by Polatnick & Arlinghaus (1967). The standard incubation mixture for the enzyme assay contained in 1.0 ml.: 2.75 μM-creatine phosphate, 5 μg. creatine phosphokinase, 100 nM of ATP, GTP and CTP, 6 μM-magnesium acetate, 5 μM-mercaptoethanol, 35 μM-tris+HCl (pH 8.o), 1 μg. actinomycin D, 5 μC[3H]ATP and 0.5 ml. of the polymerase preparation of protein content 0.7 mg./ml. Samples were incubated for 25 min. at 37°. The isolation of the RNA synthesized *in vitro* was performed by phenol extraction. 25% of the newly synthesized virus RNA was resistant to the action of RNase. Using precipitation with 1.9 m-LiCl as described by Girard (1969) a partial separation into single and double stranded RNA (DS RNA) was achieved. 90% of the precipitable RNA was RNase-sensitive. Of the non-precipitable RNA, accounting for 12 to 15% of the total yield, about 91% was RNase-resistant.

The heat stability of the *in vitro* synthesized DS RNA recovered from the supernatant after LiCl precipitation was determined (Fig. 1). The technique published by Nayak & Baluda (1969) was followed. The RNA was suspended in a solution containing 1.5 μM-NaCl, 0.15 μM-sodium citrate pH 7.0, heated in sealed ampoules and cooled rapidly in an ice-bath. The RNA solution was adjusted to 0.3M-NaCl and 0.03M-sodium citrate, incubated with 50 μg. of RNase at 23° and thereafter the radioactivity of the insoluble material was determined as described in the text. The melting temperature (Tm) of this RNA was about 92° and coincided with that of the *in vivo* synthesized DS RNA. The melting temperature of the LiCl precipitable RNase resistant *in vitro* RNA was 86°. That the *in vitro* RNA contained mainly virus RNA (plus-stranded RNA) was shown by hybridization experiments.

In our studies the *in vitro* RNA was hybridized with viral RNA and *in vivo* DS RNA, respectively. The DS RNA was prepared from the FMDV-infected cells as described by Colby & Duesberg (1969). Virus RNA was prepared from purified FMDV using the phenol extraction method (Mussgay & Strohmaier, 1958; Brown & Stewart, 1958). For hybridization, 0.3 ml. of the *in vitro* product dissolved to a final concentration of 0.35 mg./ml. in a solution containing 0.3M-NaCl and 0.015M-sodium citrate, pH 7.0, was mixed with unlabelled DS RNA and virus RNA, respectively, and heated to 95° in sealed ampoules for.
Subsequently mixtures were cooled slowly to 68° and kept at this temperature for a further 14 hr. After slow cooling to room temperature the samples were incubated at 23° with 50 μg/ml of RNase and the acid insoluble material was precipitated with 5% (w/v) ice-cold trichloracetic acid (TCA). The precipitates were collected on membrane filters (SM 11305, Sartorius, Membranfilter, G.m.b.H., Göttingen, Germany) and washed five times with TCA. Dried filters were transferred to glass vials containing a toluene scintillator for determination of radioactivity.

![Temperature transition curve of RNase-resistant RNA produced by FMDV induced RNA polymerase](image)

Fig. 1. Temperature transition curve of RNase-resistant RNA produced by FMDV induced RNA polymerase

When the in vitro RNA precipitable by 1.9M-LiCl was hybridized with different amounts of unlabelled in vivo DS RNA (Fig. 2) a plateau was reached at 80 μg. Table 1 shows the results of hybridization using virus RNA and in vivo DS RNA. After hybridization of in vitro RNA with excess (100 μg.) DS RNA isolated from FMDV-infected BHK cells, 90% of the labelled RNA was unaffected by RNase. After hybridization with 100 μg. of virus RNA only 3% was RNase-resistant. The RNA precipitable by LiCl consequently consisted mainly of plus-strands. Of the RNA in the supernatant fluid after LiCl precipitation, 91% was RNase resistant. When this in vitro DS RNA was hybridized with increasing concentrations of virus RNA, the RNase resistance of the preparation was reduced (Fig. 2). Table 1 shows the results of the melting and hybridization experiments using in vitro DS RNA. After melting this DS RNA, as described in the legend to Fig. 1, only 10% of the RNA was RNase-resistant. Annealing and reannealing led to an increase in RNA resistance (44%). After
hybridization of \textit{in vitro} labelled DS RNA with 100 \(\mu\)g. FMDV-RNA, only 0.7\% was RNase-resistant, whereas after hybridization with unlabelled \textit{in vitro} DS RNA about 90\% was recovered. As in both cases an excess of unlabelled RNA was added, it must be concluded

![Graph](image)

**Fig. 2.** Hybridization of RNA synthesized \textit{in vitro} with parental FMDV-RNA and \textit{in vivo} DS RNA. The \textit{in vitro} RNA was isolated by phenol extraction and precipitation with 1.5 M-LiCl. Left ordinate: TCA insoluble radioactivity after hybridization of the precipitable RNase-sensitive \textit{in vitro} RNA with \textit{in vivo} DS RNA (O—O). Right ordinate: TCA insoluble radioactivity after hybridization of the non-precipitable RNase-resistant fraction with parental FMDV RNA (●—●).

**Table 1.** Hybridization of foot-and-mouth disease virus (FMDV) RNA synthesized \textit{in vitro}

| Temperature treatment | \begin{tabular}{c} \textit{In vivo} \end{tabular} | \begin{tabular}{c} \textit{FMDV} \\ RNA \\
added during \\
hybridization \end{tabular} | \begin{tabular}{c} \textit{In vivo} \\
RNA \\
added during \\
hybridization \end{tabular} | \begin{tabular}{c} RNase \\
treatment \end{tabular} | \begin{tabular}{c} Precipitatable \\
Counts/min. \end{tabular} | \begin{tabular}{c} Non-precipitable \\
Counts/min. \end{tabular} |
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</tr>
</thead>
<tbody>
<tr>
<td>23\°</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>10500</td>
<td>5200</td>
<td></td>
</tr>
<tr>
<td>33\°</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>1150</td>
<td>4700</td>
<td></td>
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<tr>
<td>Melting 95\°</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>90</td>
<td>410</td>
<td></td>
</tr>
<tr>
<td>Hybridization</td>
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<td>0</td>
<td>+</td>
<td>910</td>
<td>2340</td>
<td></td>
</tr>
<tr>
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<td>100</td>
<td>+</td>
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<tr>
<td>Hybridization</td>
<td>100</td>
<td>0</td>
<td>+</td>
<td>9200</td>
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The \textit{in vitro} RNA was isolated by phenol extraction and precipitated with 1.5 M-LiCl. The precipitable RNase-sensitive fraction and the non-precipitable RNase-resistant fraction were melted and hybridized with FMDV RNA and \textit{in vivo} DS RNA, respectively.
that the RNA in the supernatant sample consists of double strands of which only the plus-strands are labelled.

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REFERENCES


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