Three Strains of European Foot-and-Mouth Disease Virus are Highly Conserved in the 3'-Termini and Highly Variable in the Genes of Two Capsid Proteins

By OTFRIED MARQUARDT
Max-Planck-Institut für Biochemie, Department of Virology, 8033 Martinsried, Federal Republic of Germany

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SUMMARY

Restriction enzyme-generated subgenomic fragments of cloned cDNA prepared from RNA of the strain O1 Kaußbeuren (O₁K) of foot-and-mouth disease virus (FMDV) were compared qualitatively and quantitatively for sequence complementarity with radioactive RNA from strains C Oberbayern (Cobb) and A2 Spain (A₂S) in hybridization experiments on nitrocellulose membranes. Quantitative comparison of nucleic acid sequences neighbouring (Cobb/O₁K) or including (A2S/O₁K) the 3' end of the virus genomes demonstrated more than 80% homology. In contrast, sequences coding for the capsid proteins VP1 (10%, Cobb/O₁K; 16 to 21%, A₂S/O₁K) and VP3 (12%, A₂S/O₁K) were remarkably heterologous. Sequences downstream from the gene for VP1, i.e. those coding for non-structural proteins, showed 23 to 51% homology to both RNAs except for the area coding for protein P56a. Here, the observed homology was 82% to C₀bb and 39 to 46% to A₂S.

INTRODUCTION

The seven serotypes of foot-and-mouth disease virus (FMDV) include more than 60 strains and comprise one of four genera of the picornavirus family (Cooper et al., 1978). The infectious FMDV consists of a single-stranded RNA molecule of about 7800 nucleotides which is encapsidated by multiple copies of the four capsid proteins. The antigenic variation between the serotypes (Wild & Brown, 1967; Wild et al., 1969) is most likely the result of differences in the amino acid sequence of at least one of the capsid proteins. Such differences are recognizable by comparison of the N-terminal amino acid sequences of capsid protein VP1 from four strains (Strohmaier et al., 1978; Bachrach et al., 1979). Since capsid proteins are encoded by the virus RNA, variations in the amino acid sequence must arise from differences in the corresponding nucleic acid sequence.

At the nucleic acid level these genes could not be compared directly until recently and only limited information could be obtained for other subgenomic parts: 38 to 55 residues of the 3' end of five FMDV strains (Porter et al., 1978), 70 to 90 residues of the 5' end of nine strains (Harris, 1980) and 10 to 15 residues on either side of the poly(C) tract of two strains (Harris et al., 1980). These comparative data comprise 1 to 2% of the total RNA and show homology as well as heterology. They do not permit extrapolation to other parts of the RNA. It is therefore not yet known to what extent genes for capsid proteins and especially for non-capsid proteins of different FMDV strains differ from each other and it is of interest to know whether or not different genomic areas show the same degree of heterology. If not, one may assume that areas with a high degree of sequence homology were less susceptible to evolutionary pressure than others with a lower degree of homology. Conservation of a
distinct genomic area may indicate genus specificity whereas divergence of one or more other areas may indicate strain specificity.

Approaches which take advantage of transcription into double-stranded DNA copies of one FMDV strain provide a tool for comparative hybridization studies with RNA from potentially any of the 60 or more other strains. Double-stranded DNA (i) can be compared directly to virus (+) RNA in hybridization experiments, avoiding the necessity of preparing complementary RNA strands, (ii) can be processed by restriction enzymes, allowing the investigation of subgenomic areas of almost any size and location and (iii) can be cloned in Escherichia coli and subsequently obtained cheaply in any quantity desired.

Recent cloning of FMDV strain O1 Kaufbeuren (O1K; Küpper et al., 1981) provides us with such a probe to study the degree of sequence homology to other FMDV strains. The clones cover contiguous 70% of the O1K genome including the 3′ end and capsid protein VP3-specific codons (Kurz et al., 1981). Therefore, genes coding for structural proteins as well as other genes from any other strain can be investigated separately. The data presented in this report were obtained by comparative RNA/DNA hybridization studies using one representative strain of each of the three European serotypes by application of the technique of Southern (1975) or by colony hybridization (Grunstein & Hogness, 1975).

Allowing for complete hybridization (Wetmur, 1975), the degree of homology could be estimated qualitatively by use of virus RNA probes radiolabelled to different specific activities: hybridization detectable with weakly labelled RNA indicated a high degree of homology, whereas hybridization detectable using only highly labelled RNA indicated a low degree of homology. Furthermore, quantification of homology could be obtained by comparing the amount of radiolabelled RNA of the three strains retained by hybridization to O1K-specific DNA immobilized on nitrocellulose membranes. It is thus shown that the sequence homology between O1K and strain C Oberbayern (CObh) or A2 Spain (A2S), respectively, increases from 10 to 20% in the genes coding for the capsid proteins VP1 and VP3 to more than 80% in an area neighbouring or including the 3′ end. This proves differences in conservation of distinct areas of the nucleic acid sequence of three strains of European FMDV during their evolution.

METHODS

End-labelling of virus RNA. RNA from FMDV strains O1K, CObh, and A2S was generously provided by Dr K. Strohmaier, Tübingen. Intact single-stranded 37S RNA was purified as described by Grubman et al. (1979). A 0.3 to 0.4 µg amount of RNA in 10 mM alkaline tris was incubated at 90 °C for different times varying from 15 to 90 min for different preparations (for details see legends to figures). The reaction was done in 70 mM-tris–HCl pH 8, 6 mM-MgCl2, 10 mM-dithiothreitol and 1 µM-[γ-32P]ATP. Four units of polynucleotide kinase (Boehringer, Mannheim) were added and the reaction mixture was incubated at 37 °C for 60 min. Trichloroacetic acid (TCA)-precipitable radioactivity was measured in a liquid scintillation spectrometer. Radioactive RNA was separated from low mol. wt. radioactivity by precipitation with cetyltrimethylammonium bromide (Reitz et al., 1972) and its size was analysed by gel electrophoresis (J. Tu, personal communication) and subsequent autoradiography (data not shown).

FMDV O1K-specific bacterial clones and plasmids. Clones and plasmids have been described previously (Küpper et al., 1981). The size of FMDV O1K-specific inserts and their correlation to the O1K RNA are summarized in Fig. 1. For the purpose of colony hybridization, probes of the strains indicated in Fig. 2 were applied to nitrocellulose membranes (Schleicher & Schüll, 0.45 µm pore size) resting on tetracycline (20 µg/ml)-containing LB plates (1% tryptone, 1% NaCl, 0.5% yeast extract and 1.5% agar)
and incubated overnight at 37 °C. For hybridization experiments, filters were treated following the protocol of Hanahan & Meselson (1980).

Plasmid DNA was isolated following standard procedures (Tanaka & Weisblum, 1975). Plasmids were digested by restriction enzymes as advised by Boehringer, Mannheim (for designation of plasmids and enzymes, see legends to Fig. 3 and 4). Two μg/slot of each digest were applied to agarose gels (1% in 36 mM-tris-30 mM-phosphate buffer pH 7.5 containing 10 mM-EDTA). Electrophoresis was done in a horizontal chamber for 16 h at 100 mA. Gels were stained in 2.5 μg/ml ethidium bromide for 30 rain, destained for 30 rain in H2O and photographed in u.v. light. For hybridization experiments gels were treated according to the protocol of Southern (1975) to transfer the DNA to nitrocellulose membranes.

Hybridization of RNA to membrane-bound DNA. Nitrocellulose filters were incubated overnight at 67 °C in: 5 × SSC (SSC = 150 mM-NaC1, 15 mM-sodium citrate pH 7); 50 mM-potassium phosphate buffer pH 6.5; 0.1% each of bovine serum albumin (BSA), Ficoll, polyvinylpyrrolidone (Denhardt, 1966); 250 μg/ml each of sonicated and denatured calf thymus DNA, polycytidylic acid (Boehringer, Mannheim) and soluble RNA. For hybridization, membranes were immersed in 10 ml of a solution containing the following: 50% formamide; 10% dextran sulphate; 5 × SSC; 20 mM-potassium phosphate pH 6.5; 0.02% each of BSA, Ficoll and polyvinylpyrrolidone; 100 μg/ml soluble RNA. A 0.05 to 1 μg amount of 5′ 32P-labelled RNA (specific activity as indicated in legends to figures) was added to the solution. Hybridization (16 to 24 h at 37 °C) was stopped by transferring the membranes to 2 × SSC buffer (250 ml, prewarmed to 67 °C) containing 10 μg/ml RNase (Boehringer, Mannheim) for 2 to 4 h. The 2 × SSC wash buffer was changed eight times before membranes were air-dried and submitted to autoradiography in a cassette equipped with intensifying screens, at -70 °C for 4 to 24 h. The nitrocellulose membranes were cut into strips corresponding to slots of the gels. Individual strips were fractionated into 5-mm pieces and the radioactivity in each fraction was measured in a liquid scintillation spectrometer.

RESULTS

Detection of highly conserved nucleic acid sequences in FMDV O1K, Cobb and A2S

The first experiment was done to reveal highly conserved coding sequences in the three strains. For this purpose seven E. coli strains harbouring different O1K-specific plasmids, which together represent 70% of the O1K genome (Fig. 1, except for pFMDV 410), and the control strain harbouring the vector plasmid were grown on triplicates of nitrocellulose filters. Colony hybridization experiments were performed with weakly 32P-labelled RNA (specific activity as indicated in legends to figures) from FMDV strains O1K, Cobb or A2S.

The result is shown in Fig. 2. The experiment with O1K clones and O1K RNA revealed hybridization with six of seven clones (Fig. 2b), whereas the negative control did not hybridize. Clone 512, in contrast to results obtained later (Fig. 4b), for unknown reasons revealed no hybridization either, so that 3′ ends cannot be compared in this experiment. In the experiment with O1K clones and either Cobb RNA (Fig. 2c) or A2S RNA (Fig. 2d) only three clones (pFMDV 703, 134 and 217) showed hybridization, proving that FMDV O1K contains nucleic acid sequences neighbouring the 3′ end which are highly homologous to Cobb as well as A2S.

Detection of less-conserved sequences in the three strains

Reported sequence homology of 50% at N-termini of VP1 from four strains by comparing 26 amino acids (Strohmaier et al., 1978; Bachrach et al., 1979) suggests homology within the sequences which code for this protein. In the following experiment attempts were made to
Fig. 1. Correlation of FMDV proteins to the cDNA. The map of FMDV-induced polypeptides (a) (Sangar, 1979) was correlated to the restriction map of cloned cDNA from FMDV OaK (b) (Küpper et al., 1981) using the precise location of the coding sequence of VP1 (Kurz et al., 1981). The length of the cDNA is given in kilobases (kb), starting with the junction of the poly(dT-dA) tract and the FMDV-specific sequences at the 3' end. Cleavage sites for restriction enzymes are indicated: B, BamHI; H, HindIII; E, EcoRI; P, PstI. The length of FMDV-specific DNA was calculated from its mobility in 1% agarose gels, deviation being ± 80 base pairs (bp). The indicated length includes oligo(dG-dC) tracts of about 30 bp (e.g. pFMDV 715; Kurz et al., 1981) which originate from the insertion technique into the cloning vector.

Fig. 2. Results of colony hybridization. (a) E. coli colonies harbouring different plasmids grown on a nitrocellulose filter: 1, pFMDV 1448; 2, pFMDV 1034; 3, pFMDV 715; 4, pFMDV 703; 5, pFMDV 134; 6, pFMDV 217; 7, pFMDV 512; 0, pBR322. Their OaK-specific inserts are presented in Fig. 1. (b to d) Autoradiographs of triplicates of (a) hybridized to (b) OaK RNA (5 × 10^6 ct/min/μg; 40 min alkaline hydrolysis), (c) to Cobb RNA (8 × 10^6 ct/min/2 μg; 15 min alkaline hydrolysis) or (d) to A2S RNA (1.4 × 10^6 ct/min/2 μg; 15 min alkaline hydrolysis).

detect such homology at the nucleic acid level. Plasmids pFMDV 715, 410 and 217 (Fig. 1), representing areas which have been studied in the previous experiment, were chosen for further investigation. They were cleaved with restriction enzymes as indicated in the legend to Fig. 3 and prepared for hybridization according to the Southern (1975) protocol with highly labelled RNA from OaK, A2S or Cobb (for specific activity, see legend to Fig. 3). The six DNA bands with OaK specificity showed hybridization with each of the three RNAs, whereas one (band 5), the plasmid DNA, released from the OaK-specific insert by action of restriction enzyme PstI, never did. If intensities of autoradiographic spots are compared, it can be seen that band 2, slot 1 is less intense in Fig. 3 (c) (A2S) and 3 (d) (Cobb) than in Fig. 3 (b) (OaK). It is concluded that A2S and Cobb contain sequences which are homologous to all of the investigated OaK genome and that the degree of homology differs with distinct subgenomic parts.
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Fig. 3. Hybridization results obtained with three FMDV strains. Digested plasmids pFMDV 715, 410 and 217 (Fig. 1). Slot 1 contains pFMDV 715 DNA digested with restriction enzyme HindIII into two fragments: band 1, total length 5150 bp, O₁K-specific bp 1660; band 2, 1630 bp, O₁K-specific bp 760. Slot 2 contains pFMDV 410 digested with EcoRI: band 3, 4750 bp, O₁K-specific bp 1000; band 4, 1550 bp, O₁K-specific bp 750. Slot 3 contains pFMDV 217 digested with PstI: band 5, 4360 bp, no O₁K-specific bp (internal negative hybridization control); band 6, 1050 O₁K-specific bp, no other; band 7, 230 O₁K-specific bp, no other. All bands except no. 5 contain one oligo(dG-dC) tract of approx. 30 residues (S. Forss, personal communication). Fragment length was calculated from the mobility in the gel, as was done with O₁K-specific inserts (W. Klump, personal communication, data not shown). (a) Photograph of the ethidium bromide stained gel in u.v. light. (b to d) Autoradiographs of duplicates of (a) after hybridization to (b) O₁K RNA (2 × 10⁶ ct/min/0.2 µg; 90 min alkaline hydrolysis), to (c) A₈S RNA (2.5 × 10⁶ ct/min/0.14 µg; 90 min alkaline hydrolysis) and to (d) C₉₃ RNA (1 × 10⁷ ct/min/0.2 µg; 90 min alkaline hydrolysis).

A parallel experiment to those described in the legend to Fig. 3 was done with RNA from a minus-strand virus (Sendai-like strain 6/94, generously provided by Dr W. Neubert, Martinsried, ³²P-labelled to 2.5 × 10⁷ ct/min/0.17 µg). No hybridization was detectable with any of the seven bands (data not shown) which again proves specificity of the results obtained with FMDV RNA.

Quantitative analysis of DNA/RNA hybridization on nitrocellulose membranes

The autoradiographic intensities of bands 1, 3, 4 and 6 in Fig. 3 (b to d) cannot be distinguished optically. In order to measure the degree of homology of these bands, retention
Fig. 4. Calibration curve of hybridization with \( O_{1K} \) DNA and RNA. The retained radioactive RNA (corrected for background) is plotted against the length of \( O_{1K} \)-specific DNA. (a) \( O_{1K} \) RNA retained by bands 1 to 7 (Fig. 3); (b) \( O_{1K} \) RNA; (c) \( A_2S \) RNA retained by ten restriction enzyme-generated \( O_{1K} \)-specific bands (gel pattern not shown) from plasmids pFMDV 1448, 715, 703 and 512 (Fig. 1). Bands 21 (710 \( O_{1K} \)-specific bp) and 22 (980 \( O_{1K} \) bp) derive from pFMDV 1448; bands 29 (530 \( O_{1K} \) bp) and 30 (720 \( O_{1K} \) bp) derive from pFMDV 512 by double-digests with restriction enzymes BamHI and PstI. Bands 23 (760 \( O_{1K} \) bp), 24 (1300 \( O_{1K} \) bp) and 25 (360 \( O_{1K} \) bp) derive from pFMDV 715; bands 26 (460 \( O_{1K} \) bp), 27 (1300 \( O_{1K} \) bp) and 28 (1110 \( O_{1K} \) bp) derive from pFMDV 703 by double-digests with HindIII and EcoRI. Correlation of bands to FMDV \( O_{1K} \)-induced polypeptides is given in Fig. 5. Bands lacking \( O_{1K} \) specificity served as negative hybridization control. With the exception of bands 24 and 27, one oligo(dG-dC) tract of about 30 residues is present per band, but subtracted from the values presented in the panels. Autoradiography patterns of hybridization to \( O_{1K} \) RNA (1.75 \( \times 10^6 \) ct/min/0.16 \( \mu \)g; 90 min alkaline hydrolysis) and \( A_2S \) RNA (6.5 \( \times 10^6 \) ct/min/0.16 \( \mu \)g; 90 min alkaline hydrolysis) are not shown. Filters were fractionated as described. Retention of radioactivity was measured for each fraction. Those containing DNA could be detected by their radioactivity peaks and correlated to the autoradiogram. Corrections have been made for background and difference in specific activity.

Table 1. Quantitative analysis of homology with \( O_{1K} \)-specific DNA and \( A_2S \) or \( C_{obb} \) RNA

<table>
<thead>
<tr>
<th>DNA band*</th>
<th>( A_2S )†</th>
<th>( C_{obb} )†</th>
<th>( O_{1K} )‡</th>
<th>( A_2S/O_{1K} )</th>
<th>( C_{obb}/O_{1K} )</th>
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<tr>
<td>1</td>
<td>162.35</td>
<td>145.52</td>
<td>706.80</td>
<td>23.0</td>
<td>20.6</td>
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<td>2</td>
<td>48.0</td>
<td>26.16</td>
<td>252.80</td>
<td>19.0</td>
<td>10.3</td>
</tr>
<tr>
<td>3</td>
<td>128.47</td>
<td>181.12</td>
<td>393.60</td>
<td>32.6</td>
<td>46.0</td>
</tr>
<tr>
<td>4</td>
<td>114.35</td>
<td>119.20</td>
<td>249.60</td>
<td>45.8</td>
<td>47.8</td>
</tr>
<tr>
<td>6</td>
<td>169.40</td>
<td>361.04</td>
<td>438.40</td>
<td>38.6</td>
<td>82.4</td>
</tr>
<tr>
<td>7</td>
<td>43.76</td>
<td>18.00</td>
<td>89.60</td>
<td>48.8</td>
<td>20.1</td>
</tr>
</tbody>
</table>

* Numbers of DNA bands were designated from Fig. 3.
† Values were corrected for differences in specific activity.
‡ Values were taken from Fig. 4 (a).
§ Percentage homology was calculated as \( 100 \times \frac{\text{ct/min } A_2S \text{ or } C_{obb} \text{ RNA}}{\text{ct/min } O_{1K} \text{ DNA band}} \).
Table 2. Quantitative analysis of homology with OιK-specific DNA and Α2S RNA

<table>
<thead>
<tr>
<th>DNA band*</th>
<th>A2S †</th>
<th>OιK ‡</th>
<th>Homology (%)§</th>
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<tr>
<td>21</td>
<td>6.27</td>
<td>64.60</td>
<td>11.6 ± 6.4</td>
</tr>
<tr>
<td>22</td>
<td>23.10</td>
<td>143.60</td>
<td>16.4 ± 3.3</td>
</tr>
<tr>
<td>23</td>
<td>33.20</td>
<td>165.80</td>
<td>20.7 ± 5.2</td>
</tr>
<tr>
<td>24</td>
<td>79.19</td>
<td>195.40</td>
<td>40.5 ± 7.0</td>
</tr>
<tr>
<td>25</td>
<td>14.40</td>
<td>45.80</td>
<td>32.1 ± 6.4</td>
</tr>
<tr>
<td>26</td>
<td>49.10</td>
<td>100.20</td>
<td>50.9 ± 13.7</td>
</tr>
<tr>
<td>27</td>
<td>104.90</td>
<td>277.60</td>
<td>38.3 ± 6.2</td>
</tr>
<tr>
<td>28</td>
<td>99.70</td>
<td>217.20</td>
<td>46.1 ± 3.8</td>
</tr>
<tr>
<td>29</td>
<td>45.62</td>
<td>103.40</td>
<td>44.6 ± 6.4</td>
</tr>
<tr>
<td>30</td>
<td>68.40</td>
<td>89.60</td>
<td>81.2 ± 27.8</td>
</tr>
</tbody>
</table>

* Numbers of DNA bands and ct/min/DNA on filter fractions were taken from Fig. 4 (b, c).
† Differences between these values and the OιK values of Table 1 are due to continued decay of radioactivity.
§ Percentage homology was calculated as described in Table 1. Deviation was derived from the difference between the experimental and average values in Fig. 4 (b). It is assumed to occur to the same extent with values in Fig. 4 (c). Lower and upper limits of deviation were therefore calculated (average value A2S/experimental value OιK and experimental value A2S/average value OιK).

length of OιK-specific DNA in the bands when OιK RNA had been used as hybridization probe. The ct/min on filter fractions were plotted against the length of OιK-specific DNA/band (Fig. 4a). All six resulting points can be joined by a straight line, showing that radioactive OιK RNA was retained by OιK-specific DNA proportional to its length, independent of experimental variations during blotting or hybridization.

Differences in homology between OιK and A2S or Cobb, respectively, can therefore be examined and further quantified using this kind of analysis. Radioactivity retained by each band in the experiment with OιK RNA is taken as 100% hybridization. Values obtained in the experiments with A2S or Cobb RNA after correcting them for variation in specific radioactivity can now be expressed as percentage hybridization of the result obtained with the corresponding bands in the OιK RNA experiment (Table 1).

It can be seen that homology to band 2, the OιK-specific DNA compound of which represents the major part of the VP1 gene (Kurz et al., 1981), is 19% for A2S and 10% for Cobb, confirming the observation obtained by analysing the autoradiograms (Fig. 3). The highest degree of homology (82%) was observed with band 6 in the experiment with Cobb RNA, confirming the conclusion drawn from Fig. 2 (c). All other bands show less than 50% homology to both RNAs. It is concluded from this experiment that a gradient of decreasing homology from 3' to 5' exists among the coding sequences of the compared FMDV strains and that the amount of homology observed with distinct genomic areas can be expressed as percentage homology to OιK-specific sequences. The results obtained were based on the comparison of subgenomic fragments between 750 and 2800 bp in size. It is therefore of interest to investigate whether the degree of homology changes significantly if the fragment size is reduced. Therefore, pFMDV 1448, 715, 703 and 512 (Fig. 1) which represent the OιK genome from the major part of the gene for the capsid protein VP3 to the 3' end were fragmented by restriction enzyme digestion, so that the OιK-specific compounds of ten resulting fragments ranged in size between 360 and 1300 bp. Following electrophoresis, the bands were transferred to nitrocellulose filters, to which OιK RNA or A2S RNA was hybridized (for details of fragment size, digest and specific activity of RNA, see legend to Fig. 4). Following autoradiography, filters were fractionated as described above and the radioactivity of the fractions was measured.
Results obtained with $O_{iK}$ RNA can again be joined by a straight line (Fig. 4 b) whereas those obtained with $A_{2S}$ RNA cannot (Fig. 4 c). A quantitative analysis was done as outlined in the previous experiment (Table 2). In order to facilitate comparison of results summarized in Tables 1 and 2, they were correlated with the map of FMDV-induced polypeptides (Fig. 5). As can be seen, concordance of data was obtained by two independent hybridization experiments with $A_{2S}$ RNA in the case of bands 2, 22 and 23 (16 to 21% homology), bands 6, 28 and 29 (39 to 46% homology) and bands 3, 25 and 27 (32 to 38% homology). The homology obtained with band 1 which contains the longest $O_{iK}$-specific insert (1630 bp) was lower (23%) than that obtained with its subfragments (41% with band 24, 46% with band 4, 51% with band 26 and 32% with band 25). It should be noted, however, that the homology was the highest with band 26 which is identical to 460 bp in the centre of band 1, and the next highest with band 4 which consists of band 26 and 290 5'-additional base pairs. Such decrease of homology is even more remarkable with band 24 which differs from band 4 in an elongated 5' end (550 bp). Thus, the low homology of band 1 can be explained if a very low homology is assumed for that part of band 24 which does not overlap with band 4 and, at the same time, by considering the low homology observed with band 25.

It is concluded that the values obtained for each fragment represent average homology. That obtained for a large fragment, therefore, may differ from that obtained for its subfragments. It is concluded further that this approach indeed leads to a quantitative analysis of hybridization on the basis of percentage homology. Therefore, the data obtained for bands 21 and 30 which are not controlled by parallel samples are valid. Comparative hybridization data (Fig. 5) can be summarized for $O_{iK}$ and $A_{2S}$ in that they are (i) highly homologous (81%) in a region including the 3' end and (ii) only 23 to 39% homologous in the region coding for the non-structural proteins, if values obtained with long fragments (bands 1A, 1630 bp; 6A, 1050 bp; 27A, 1300 bp) are compared. However, the use of short fragments instead of long ones (bands 7A, 230 bp; 25A, 360 bp; 26A, 460 bp; 29A, 530 bp) in the analysis of homology reveals deviations from the values obtained with the latter. (iii)
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The genes for the two capsid proteins present in the study showed the lowest homology (16 to 21% for VP1 and 12% for VP3).

O1K-specific DNA probes comparable in size to those being used in experiments with A2S were also hybridized to C_obb RNA. Results, essentially confirming those given in Table 1, are omitted from the presentation. The presented comparative data show that the homology of bands 1, 2, 3 and 4 is similar to that obtained with A2S RNA. It is concluded that divergence in homology of A2S or C_obb to O1K occurs mainly with the sequence neighbouring the 3' end.

Estimation of base pairs needed for detection of hybrids

Mismatching in FMDV hybrid molecules is expected to occur with high frequency, since it is reported by Harris et al. (1980) that no long region of RNA hybrids (O-V1/A61) is RNase-resistant and, therefore, no completely conserved sequence longer than the poly(C) tract and the poly(A) tract is detectable. Both tracts are shorter than the shortest fragments used in this study (Fig. 3, band 7). It is therefore of interest to estimate the number of nucleotides necessary for formation of detectable hybrids.

It is shown in Fig. 3 (bands 5) that small oligodeoxyribonucleotides, such as recognition sites for restriction enzymes, do not form hybrids with complementary sequences which may or may not exist in the RNA, due to prehybridization with non-labelled DNA. Detectable RNA/DNA hybridization, therefore, has to comprise more than six nucleotides. On the other hand, hybridization of polycytidylic acid (32P-labelled in a polynucleotide kinase reaction to 5 x 10^5 ct/min/0.05 #g) to oligo(dG-dC) tracts consisting of 30 residues (e.g. pFMDV 715; Kurz et al., 1981) which originate from insertion of cDNA into the plasmids could be detected in an autoradiogram (data not shown). This result could be obtained using a triplicate of the nitrocellulose filters which had been prepared for experiments described in the legend to Fig. 4 and which were prehybridized without non-labelled polycytidylic acid.

It was concluded from the results that hybrids consisting of six base pairs cannot be detected, whereas those consisting of approx. 30 base pairs with 100% complementarity can be detected by this approach. Therefore, the hybrids which gave rise to the results presented in Tables 1 and 2 may also consist of more than 6 to less than 30 base pairs. The varying number of such areas of high complementarity in the bands and the difference in size of the O1K-specific sequences are considered to be responsible for the difference in hybridization which was observed with the individual bands.

DISCUSSION

It is shown in this report that the sequence complementarity between cloned FMDV O1K-specific DNA and radiolabelled O1K plus-strand RNA can be used as a reference in hybridization experiments for comparison of subgenomic parts to plus-strand RNA from other strains. To get optimal resolution, the fragment size can be chosen as small as 230 to 360 bp. Even fragments of 30 bp [which is the size of the oligo(dG-dC) tracts] show hybridization. An analysis using fragments of this size, however, is probably as laborious as nucleic acid sequencing and therefore not advisable.

The approach described here is useful if sequence homologies have to be analysed. Similar studies can be carried out to detect homologous regions in genomes of very long size, e.g. if cloned subgenomic fragments of RNA viruses with a large genome have to be prescreened for nucleic acid sequencing. The comparative data on three strains of European FMDV presented in this report show that evolution of FMDV led to differences in compatibility of mutations with distinct subgenomic areas. The degree of sequence homology decreases from the 3' end to sequences with specificity for most of the non-capsid proteins and further to the two investigated genes for the capsid proteins VP1 and VP3.

Band 30 (Fig. 5) includes the 3' terminal oligo(dT-dA) tract and is supposed to represent
mainly non-coding nucleotides (Kurz et al., 1981) as has been shown with the poliovirus genome (Kitamura & Wimmer, 1980). The high homology obtained with A$_5$S can be compared to the sequence analysis of 38 nucleotides next to the poly(A) tracts of five FMDV strains which indicates 75% homology (Porter et al., 1978). From the basic agreement of values obtained by independent approaches it is assumed that the 3' distal highly conserved sequence comprises more than 38 nucleotides. Thus, the only region of high conservation probably has non-coding character.

The gene for the primary cleavage precursor protein P52 has not yet been located precisely within the genome nor are the functions known to which it gives rise. Although the average homology obtained with a long fraction of this gene is low (20 to 23% with bands 1A and 1C), that obtained with one of the short subfractions is considerably higher (51% with band 26A). Here possibly the sequence coding for one essential part of a virus protein is indicated.

Proteins P56c and P56a originate from the primary cleavage precursor protein P100 after processing. While P56a has been shown to be the virus RNA polymerase (Newman et al., 1979), the functions of P56c are unknown, but speculatively include a proteolytic enzymic activity. The precise location of their coding sequences is unknown. A deviation from the homology obtained with A$_5$S for the longest bands (Fig. 5, bands 27A and 6A, 38% each) can be observed again with their fragments (25A, 7A and 29A). The difference in results obtained with C$_{Obb}$ prohibits a conclusion concerning genus-specific conservation of sequences coding for active sites of enzymes (7A/7C; 6A/6C). If any such conservation exists it probably comprises a very small genomic area.

The gene for VP3 of OIK should mainly be represented by the 710 bp of band 21 of pFMDV 1448 (Fig. 5). The gene for VP1 of OIK has been shown to comprise 639 nucleotides (Kurz et al., 1981). With the exception of 24 nucleotides at the 5' end they are present in the 980 bp of band 22 of pFMDV 1448. Bands 2 and 23, which both originate from pFMDV 715, are identical with respect to their OIK-specific compound (760 bp) which contains 2/3 parts of the 3' VP1-coding sequence (Fig. 5). Conservation of two genes coding for capsid proteins is shown to be below 21%. Since no enzymic activity has been reported to be attributed to the capsid proteins and at least one of them is a target for virus neutralizing antibodies, their genes may mutate at a higher rate than others and, indeed, may have to, for reasons of selective pressure. Thus, the results published on homology of complete RNAs from representatives of European strains to be 44 to 65% (Dietzschold et al., 1971) or 67 to 70% (Robson et al., 1977) have been extended.

Harris et al. (1980) reported on competition hybridization experiments using fragmented RNA from strains O-V1 and A61. The fragments varied in size and contained either the poly(C) tract at the 5' end or the 3' distal poly(A) tract. Comparative data show 65% homology with both kinds of fragments independent of size. These data agree in principle with those presented in this report with respect to average homology of long genomic areas including or neighbouring the 3' end. They are very different, however, in that the decrease of homology at areas 3 to 6 kb away from the 3' end which is demonstrated in this report was not observed. The discrepancy of data possibly originates from the different techniques used for preparation of subgenomic fragments; the sucrose gradients used by Harris et al. (1980) are considered to be less sensitive than restriction enzyme digestion and subsequent electrophoresis.

The data reported here are in agreement with those published by Robson et al. (1980) who compared tryptic peptides obtained from the primary translation products P88, P52 and P100 of two FMDV type A and one type O. The analysis showed that the region of the genome coding for the structural proteins varies more than those coding for P52 or P100. 50% homology is observed from the results of sequencing 26 VP1 N-terminal amino acids of
Four strains (Strohmaier et al., 1978; Bachrach et al., 1979). This reduces to 30% homology if eight N-terminal amino acids are omitted from the comparison in order to make feasible a comparison with the reported hybridization data with band 22 which lacks eight N-terminal codons.

A disagreement has to be noted which may originate for more than one reason. (i) Degeneracy of the genetic code does not reflect the sequence homology at the level of amino acids as compared to that of its nucleic acids. (ii) Distribution of homologies is probably non-random within VP1. The antigenic determinants responsible for virus neutralization are located near the C-terminal end (Strohmaier et al., 1982) which is therefore expected to be less homologous than the N-terminal part. (iii) Homology detectable by the approach used has to comprise more than six nucleotides, as is shown in Fig. 3 (bands 5) and, therefore, the homology of two contiguous codons is not sufficient to be detected.

Experiments of this kind are economical, simple and quick. The techniques described here will therefore be applied further to current studies on nucleic acid homology of FMDV O,K and other picornaviruses such as Mengo, coxsackie B, polio and bovine rhinovirus. While some hybridization was recognized with Mengo RNA and type O genomic areas coding for VP1, VP3 and P52, no such hybridization was detectable with polio RNA.

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REFERENCES


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