Biochemical Mapping of the Foot-and-Mouth Disease Virus Genome

By D. V. SANGAR, D. N. BLACK, D. J. ROWLANDS AND F. BROWN

Animal Virus Research Institute, Pirbright, Surrey

(Accepted 7 December 1976)

SUMMARY

Four primary cleavage products, mol. wt. 10^3 × 100, 88, 56 and 52 (P100, P88, P56 and P52 respectively) are present in BHK 21 cells infected with foot-and-mouth disease virus (FMDV). However, no precursor polyprotein equal to the sum of their mol. wt. was detected, even when amino acid analogues and proteolytic enzyme inhibitors were used. Three of the primary products were shown to cleave to smaller polypeptides, including the capsid polypeptides of the virus. Polypeptide P88, which was shown to be the precursor of the capsid polypeptides, is translated from the gene located at the 5'-end of the genome. The order of the structural polypeptides, determined by the use of emetine, is VP_{1}, VP_{2}, VP_{3}, VP_{4}.

The order of the remaining primary cleavage products is P52, P56 and P100. P56 is a stable product, identical with the virus infection associated (VIA) antigen found in virus harvests. The function of the other two products P52 and P100 is not known.

FMDV thus differs from other picornaviruses in that there is an extra primary cleavage product, apparently resulting from translation of more of the virus genome.

INTRODUCTION

The biosynthesis of picornavirus proteins proceeds by uninterrupted translation of the genome and the product is processed by a series of proteolytic cleavages (Holland & Kiehn, 1968; Jacobson & Baltimore, 1968; Summers & Maizel, 1968). The first series of cleavages occurs on the nascent protein so that the complete polyprotein is usually found only in abnormal conditions such as when the virus is grown in the presence of amino acid analogues (Jacobson, Asso & Baltimore, 1970) or when appropriate ts mutants are grown at a restrictive temperature (Garfinkle & Tershak, 1971). However, traces of the complete polyprotein have been observed after giving a short pulse of radioactivity to cells infected with Coxsackie B1 virus in normal culture conditions (Kiehn & Holland, 1970). The primary cleavages appear to be mediated by host enzyme(s) while the subsequent cleavages probably involve virus specified enzymes (Korant, 1972). Three primary cleavage products have been described in several picornavirus infected cell systems (Taber, Rekosh & Baltimore, 1971; Butterworth & Rueckert, 1972a).

A feature of this mechanism is that there is a single ribosome binding site located near the 5'-end of the virus RNA. This has facilitated biochemical mapping of the gene products, either by pulsing with radioactivity at intervals after the inhibition of ribosome initiation with pactamycin (Summers & Maizel, 1971; Taber et al. 1971; Butterworth & Rueckert,
1972a), or by reversing the inhibition of initiation which occurs under hypertonic conditions (Saborio, Pong & Koch, 1974). Using these methods it has been shown that the primary cleavage product (mol. wt. about 90 to 100 x 10^3) coded for by the 5'-end of the genome of polio and encephalomyocarditis (EMC) viruses is the precursor of the structural poly

peptides (Taber et al. 1971; Butterworth & Rueckert, 1972a). The central region of the genome codes for a smaller primary cleavage product, mol. wt. about 30 x 10^3, which is not processed further, and the 3'-end of the message codes for a product of mol. wt. about 90 x 10^3. This latter product is cleaved into a series of smaller polypeptides, one of which has been found in highly purified preparations of polio and EMC virus RNA polymerases (Lundquist, Ehrenfeld & Maizel, 1974; Traub et al. 1976).

In earlier studies with FMDV, Black (1975) showed that the RNA is translated into three large polypeptides which are subsequently cleaved. In the present study using the discontinuous SDS polyacrylamide gel method (Laemmli, 1970) we have now resolved four primary cleavage products in FMDV infected cells, compared with the three found with other picornaviruses, and report on their position on the virus genome and their final cleavage products.

**METHODS**

**Preparation of labelled virus and virus associated particles.** Virus of type A (subtype 10) or type O (subtype 6) was grown in BHK 21 cell monolayers in the presence of methionine-free Eagle's medium containing 5 μCi/ml 35S-methionine. The medium was harvested when the cells had left the glass. With a high multiplicity of infection this occurred 4 to 5 h after infection. Virus particles of type O were prepared by the method described by Brown & Cartwright (1963) with the modification that 1 % SDS was used instead of DOC to disperse the pellet before sucrose gradient centrifugation. Virus and procapsid particles of type A virus were prepared as described by Rowlands, Sangar & Brown (1975). The virus infection associated (VIA) antigen was prepared by immuno-precipitation from the 100 000 g supernatants obtained during pelleting the virus (Sangar et al. 1976).

**Labelling of virus induced polypeptides.** Monolayers of BHK 21 cells grown in 4 oz bottles were infected with virus at a multiplicity of 100 and incubated in Earle's saline for labelling with 14C-protein hydrolysate or methionine-free Eagle's medium for labelling with 35S-methionine. In chase experiments the 35S-methionine-containing medium was replaced with Eagle's medium supplemented with 1 mM-methionine. The 35S-methionine (sp. act. 460 Ci/mmol) was obtained from the Radiochemical Centre, Amersham.

Attempts to demonstrate a polyprotein precursor in virus infected cells were made using amino acid analogues and the protease inhibitors N-α-p-tosyl-L-lysine chloromethylketone HCl (TLCK), L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK), phenyl methyl sulphonyl fluoride, zinc acetate and Trasylol (Aprotinin, Bayer Ltd). The amino acid analogues were used at the following concentrations in Earle's saline: p-fluorophenylalanine (31 mM), canavanine (36 mM), ethionine (22 mM), azetidine-2-carboxylic acid (16 mM) at 115 min post infection. Ten min. later 14C-leucine was added and the cells were solubilized after a further 30 min at 37 °C. TLCK (0.1 mM), TPCK (0.1 mM), phenyl methyl sulphonyl fluoride (5 mM), zinc acetate (0.1 mM) and Trasylol (10 000 units/ml) were used in methionine-free Eagle's medium and 35S-methionine added 10 min later.

**Polyacrylamide gel electrophoresis.** Infected cell monolayers were lysed by replacing the medium with 1 ml disrupting solution consisting of 1 M-urea, 2 % SDS and 2 % β-mercaptoethanol. The lysates were heated at 100 °C for 15 min and then dialysed against 0.5 M-urea, 0.1 % SDS, 0.1 % β-mercaptoethanol, 0.01 M-phosphate buffer, pH 7.2.
Fig. 1. Polyacrylamide gel electrophoresis of the polypeptides induced in BHK 21 cells infected with FMDV. The cells were pulsed for 5 min with $^{35}$S-methionine at 120 min p.i. and extracts prepared (a) immediately or (b) 30 min after chasing with excess unlabelled methionine. The extracts were electrophoresed on discontinuous polyacrylamide gels containing 0.1% SDS using a 4% stacking gel and a 10% resolving gel. Autoradiographs of the gels were analysed with a microdensitometer. Insert is of actual autoradiographs of the gels: (c) 5 min pulse; (d) 5 min pulse plus 30 min chase showing more clearly the disappearance of P56b during the chase period.
D. V. SANGAR AND OTHERS

Purified virus components were heated with 0·5 M-urea, 1 % SDS and 1 % β-mercapto-ethanol at 100 °C for 15 min and then dialysed as above. All samples were made to 10 % sucrose and 1 drop of 1 % phenol red solution was added before loading them on to the polyacrylamide gels. Samples were electrophoresed at 5 mA/gel for 16 h on continuous phosphate buffer gels as described by Cartwright, Talbot & Brown (1970), or at 3 mA/gel for 6 h on discontinuous gels, using 10 % or 7·5 % gels (Laemmli, 1970). In experiments to detect a polyprotein a stacking gel of 2 % polyacrylamide-0·5 % agarose was used.

After electrophoresis the gels were either cut transversely into 1 mm segments, which were then digested and counted (Talbot & Brown, 1972), or sliced longitudinally and the dried slices exposed to X-Omat film for appropriate times (Rowlands, 1975). In the second method the autoradiographs were analysed in a Joyce–Loebl microdensitometer.

The mol. wt. of the FMDV induced polypeptides were estimated by co-electrophoresis in the same gel with EMC virus induced polypeptides, using the mol. wt. given by Butterworth & Rueckert (1972a). The EMC polypeptides were kindly provided by Dr Rueckert.

RESULTS

Most of the experiments described in this paper have been done with virus of serotype A (sub-type 10). Since the structural polypeptides of this virus do not separate well in polyacrylamide gel electrophoresis, however, virus of serotype O (sub-type 6), with which good separations can be obtained, has been used to elucidate the order of the structural polypeptides.

For ease of reference, the virus induced polypeptides are identified by a number representing their apparent mol. wt. x 10^-3, e.g. P88 is a polypeptide of mol. wt. 88 x 10^3.

Induced polypeptides in virus infected cells

In all experiments cells were labelled with radioactive amino acids 2 h after infection with a high multiplicity of virus (approx. 100 p.f.u./cell). At this time, host cell protein synthesis was almost entirely replaced by the synthesis of virus-specified polypeptides (Black, 1975). After a 5 min pulse of 35S-methionine most of the radioactivity was associated with 4 polypeptides P100, P88, P56 and P52 (Fig. 1a). During a subsequent chase period of 30 min, P56 appeared to be stable but P100, P88 and P52 were replaced by polypeptides with lower mol. wt., viz. P72, P38, P34, P28, P26 and P20 (Fig. 1b). When the chase was extended beyond 30 min, P72 and P38 were also cleaved but no new polypeptides could be detected. In many pulse labelling experiments P56 could be resolved into 2 polypeptides P56a and P56b (Fig. 1a and Fig. 2). When samples were prepared and analysed on gels at intervals during a chase period following a 5 min pulse with 35S-methionine, the faster migrating polypeptide 56b disappeared and there was a corresponding increase in the radioactivity in 56a and radioactivity in the combined peak 56a+b remained constant throughout the chase period. The half life of polypeptide 56b was about 15 min and by 30 min the predominant polypeptide in this region of the gels was P56a (Fig. 1b).

Attempts to demonstrate a polyprotein in virus infected cells

Earlier attempts by Black (1975) to demonstrate a polyprotein in FMDV-infected cells, either under normal culture conditions or in the presence of amino acid analogues or TLCK were unsuccessful. In the present study we have used amino acid analogues at ten times the concentration used by Black (1975) with FMDV-infected BHK cells and by
Fig. 2. Polyacrylamide gel electrophoresis of polypeptides induced by FMDV in the presence of Trasylol. The resolving gels contained 7.5% polyacrylamide and the stacking gels consisted of 2% polyacrylamide and 0.5% agarose. (a) Cells receiving a 5 min pulse of ^35S-methionine at 120 min p.i.; (b) cells treated with Trasylol at 100 min p.i., ^35S-methionine was added 20 min later and the extract prepared after a further 30 min.
Fig. 3. Polyacrylamide gel electrophoresis of polypeptides induced by FMDV in the presence of iodoacetamide. The compound (2.5 × 10⁻⁴ M) was added 105 min p.i. and the cultures pulsed for 10 min with ³⁵S-methionine 15 min later. Conditions of electrophoresis were as in Fig. 1.

Table 1. Molar ratios of the primary cleavage products in cells infected with FMDV in the presence of iodoacetamide and ¹⁴C-amino acids

<table>
<thead>
<tr>
<th>Polypeptide*</th>
<th>Molar ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁₀₀</td>
<td>1.00</td>
</tr>
<tr>
<td>P₈₈</td>
<td>1.22 ± 0.20</td>
</tr>
<tr>
<td>P₅₆a+b</td>
<td>0.90 ± 0.10</td>
</tr>
<tr>
<td>P₅₂</td>
<td>1.10 ± 0.20</td>
</tr>
</tbody>
</table>

* The amount of each polypeptide was estimated by weighing the areas under the peaks of microdensitometer tracings made on cardboard.
† Each value is the mean of three determinations, normalized with respect to P₁₀₀.

Jacobson et al. (1970) with poliovirus infected HeLa cells. Even in the presence of 31 mM-p-fluorophenylalanine, 36 mM-canavanine, 22 mM-ethionine and 16 mM-azetidine 2-carboxylic acid, however, no evidence for the presence of a polyprotein was obtained. The use of the proteolytic enzyme inhibitors TLCK (0.1 mM), TPCK (0.1 mM), phenyl-methyl sulphonyl fluoride (5 mM), zinc acetate (0.1 mM) or Trasylol (10000 units/ml) a naturally occurring peptide which inhibits proteases, also failed to reveal the presence of a polyprotein but a polypeptide P₁₂₂ was observed in infected cells treated with zinc acetate or ‘Trasylol’ (Fig. 2).

Molar ratios of the primary cleavage products

The largest virus-specific polypeptides normally found in picornavirus infected cells have been termed the primary products (Butterworth & Rueckert, 1972a) and are considered to represent host enzyme mediated cleavage products of the nascent polypeptide chain.
Mapping the FMDV genome

Fig. 4. Effect of pactamycin on the polyacrylamide gel electrophoresis patterns of FMDV-induced proteins. (a) Control cells after a 5 min pulse of 35S-methionine at 120 min p.i. (b) Pactamycin (10^{-5} M) was added at 115 min p.i. and the cells pulsed for 5 min with 35S-methionine at 120 min p.i. Conditions for electrophoresis were as in Fig. 1.

(Butterworth & Rueckert, 1972b). The primary products have been shown to occur in equimolar amounts in EMC virus infected cells (Butterworth & Rueckert, 1972a). However, estimation of the molarity of the 4 primary cleavage products found by pulse labelling of FMDV-infected cells was hampered by the extent to which secondary cleavages occurred even in pulse periods as short as 5 min. Iodoacetamide has been shown to inhibit the secondary cleavages of poliovirus induced polypeptides (Korant, 1973) and was found to have a similar effect on the cleavage of FMDV induced polypeptides. In the presence of 2.5 \times 10^{-4} M-iodoacetamide, the only polypeptides extensively labelled during a 10 min pulse with 14C-amino acids were the 4 putative primary cleavage products P100, P88, P56 and P52 (Fig. 3). The molar ratios of these 4 products were close to unity (Table 1) providing evidence that they are the primary cleavage products.
Mapping of the virus genome

The presence of a single ribosome binding site on a polycistronic RNA allows elucidation of the order in which the primary translation products are coded for on the genome. By inhibiting the initiation of translation with pactamycin or high concentrations of salt, followed by pulsing with radioactive amino acids at various intervals afterwards, the order in which the polypeptide products are depleted of radioactivity can be determined and consequently their order on the genome can be ascertained (Butterworth & Rueckert, 1972a; Saborio, Pong & Koch, 1974). The results of such experiments with FMDV are shown in Fig. 4. Infected cells were pulsed with radioactivity for 5 min either in the absence of, or 5 min after, the addition of pactamycin. The polypeptide most affected by the pactamycin treatment was clearly P88, showing that this was at the 5'-end of the genome. There was also a slight reduction in the amount of radioactivity incorporated into P52. The order of P56 and P100 was difficult to determine by this method because pactamycin appeared to affect elongation as well as initiation but in some experiments there was some indication that P100 was located at the 3'-end.

An alternative mapping procedure was used in order to clarify the order of the polypeptides coded for by the 3'-end of the genome. Saborio, Pong & Koch (1974) found that the initiation of protein synthesis on poliovirus polyribosomes in HeLa cells was inhibited by concentrations of NaCl greater than 175 mM above isotonic. They found that there was little effect on elongation until the excess concentration of NaCl exceeded 250 mM when both initiation and elongation were inhibited. The virus messengers could, therefore, be cleared of ribosomes by exposing the cells to concentrations of NaCl between 175 and 250 mM above isotonic, thus preparing them for the synchronization of initiation when they were returned to isotonic conditions. With FMDV infected cells, however, separation of the inhibitory effects of high salt on initiation and elongation was not achieved over a wide range (50 to 400 mM excess). Thus, when 35S-methionine was added at the same time as the cells were restored to isotonic conditions and a cell extract prepared 3 min later, radioactivity was found in all the primary cleavage products, irrespective of the excess level of NaCl that had been used. However, a higher level of radioactivity was found in P88, indicating that initiation was inhibited to a greater extent than elongation and that the 5'-end was preferentially labelled when isotonic conditions were restored.

To facilitate the mapping of the genome after reversal of high salt inhibition, pactamycin was added 2 min after restoring the cells to isotonic conditions. This allowed a group of ribosomes to attach during the 2 min period and then move along the messenger RNA. Radioactive amino acids added at different times after the pactamycin would be incorporated mainly into polypeptides coded for by restricted regions of the genome, thus allowing the mapping of the genome even in the presence of residual background synthesis.

Infected cells were treated with 250 mM excess NaCl for 45 min and then returned to isotonic conditions. Two min later pactamycin was added and this was followed by a pulse of 35S-methionine immediately or 3 or 6 min afterwards. P88 was labelled preferentially only when the 35S-methionine was added at the same time as the pactamycin. P52 and P56 were labelled preferentially when the 35S-methionine was added 3 min after the pactamycin and P100 was labelled to the greatest extent when the 35S-methionine was added 6 min after the pactamycin. Since P52 was labelled to a greater extent than P56 when the 35S-methionine was added 3 min after the pactamycin and to a lesser extent when it was added at 6 min (Fig. 5), the order of the primary cleavage products is P88, P52, P56, P100.
Fig. 5. Effect of pactamycin and NaCl on the polyacrylamide gel electrophoresis patterns of FMDV-induced proteins. Cells were exposed to 250 mM excess NaCl for 45 min commencing at 120 min p.i. and then returned to isotonic medium. Two min later 10^{-7} M-pactamycin was added. Replicate cultures were pulsed with ^{35}S-methionine for 5 min at (a) the time of addition, (b) 3 min and (c) 6 min after addition of pactamycin. Conditions for electrophoresis were as in Fig. 1.
Fig. 6. Effect of pactamycin and NaCl on the polyacrylamide gel electrophoresis patterns of FMDV-induced proteins. The experimental conditions were the same as in Fig. 5, except that a chase period of 120 min with excess unlabelled methionine was included after each pulse period before preparing the cell extracts.
Mapping the FMDV genome

![Diagram of FMDV genome cleavage pathways]

Fig. 7. Proposed cleavage pathways of FMDV-induced polypeptides.

Table 2. Molar ratios of the primary cleavage products and their derivatives in cells infected with FMDV in the presence of \(^{14}\)C-amino acids*

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>P100</td>
<td>0.38</td>
</tr>
<tr>
<td>P80 + 78 + 76 + 72</td>
<td>0.58</td>
</tr>
<tr>
<td>P88</td>
<td>0.52</td>
</tr>
<tr>
<td>P38</td>
<td>0.54</td>
</tr>
<tr>
<td>P56a + b</td>
<td>1.00</td>
</tr>
<tr>
<td>P52</td>
<td>0.55</td>
</tr>
<tr>
<td>P34</td>
<td>0.62</td>
</tr>
<tr>
<td>§P100 + 80 + 78 + 76 + 72</td>
<td>0.96</td>
</tr>
<tr>
<td>P88 + 38</td>
<td>1.06</td>
</tr>
<tr>
<td>P56a + b</td>
<td>1.00</td>
</tr>
<tr>
<td>P52 + 34</td>
<td>1.17</td>
</tr>
</tbody>
</table>

* Cells were labelled with \(^{14}\)C-amino acids for 5 min at 120 min p.i.
† The amount of each polypeptide was estimated as described in Table 1.
‡ Each value was normalized with respect to P56a + b.
§ The molar ratios for the primary products are corrected in each case by summation of the molar ratios of the uncleaved precursor and one of the products for each cleavage event (Butterworth & Rueckert, 1972b).

Precursor-product relationships

These relationships were investigated by using 120 min chases after the pulse period described in the previous section. Polypeptides P38, P28 and P26 could be detected in substantial amounts only when there was efficient labelling of P88 in the pulse experiments. Similarly, the incorporation of radioactivity into P34 and P72 corresponded to the labelling of P52 and P100, respectively, in the pulse experiments (Fig. 6). The level of radioactivity associated with P20 when the label was added 6 min after the pactamycin was lower than would be expected if it had been derived from P100 but higher than if it had been derived from P52. This may suggest that P20 is a mixture of polypeptides derived from P52 and P100. The proposed map and precursor-product relationships are shown in Fig. 7.
Estimation of the molar ratios of the primary cleavage products in the absence of cleavage inhibitors

By elucidating the cleavage pathways of the primary cleavage products, the molar ratios of the primary cleavage products in infected cells in the absence of iodoacetamide were calculated. The relative amounts of the virus induced polypeptides synthesized during a 5 min pulse with 14C-amino acids (protein hydrolysate) were estimated. The molar ratios calculated for the smaller polypeptides were added to those of their primary cleavage product precursors. The adjusted molar ratios of the primary cleavage products were again close to unity (Table 2). This is further evidence for the presence of 4 primary cleavage products and for the authenticity of the map shown in Fig. 7.
Mapping the FMDV genome

Fig. 9. Co-electrophoresis in polyacrylamide gels of \(^{35}\)S-methionine labelled VIA antigen with FMDV-induced polypeptides labelled in a 5 min pulse of \(^{3}\)H-methionine. Conditions for electrophoresis were as in Fig. 1, except that a 7.5\% resolving gel was used: \(\bullet - - \bullet\), \(^{3}\)H; \(\bigcirc - - - \bigcirc\), \(^{35}\)S.

Identification of the virus induced polypeptides

Six polypeptides have been identified in the antigenic particles found in FMDV harvests. The infective virus particles are composed principally of VP\(_3\), VP\(_2\), VP\(_3\) and VP\(_4\) although small amounts of VP\(_{0}\) and a polypeptide with mol. wt. \(52 \times 10^3\) are also present (Sangar et al. 1976). Black (1975) showed that VP\(_2\) and VP\(_4\) are not present in BHK cells infected with the virus, suggesting that the cleavage of VP\(_{0}\) to VP\(_3\) and VP\(_4\) is one of the final events in the maturation of virus particles. The empty procapsids, which may be intermediates in the morphogenesis of virus particles, are composed mainly of VP\(_{0}\), VP\(_1\) and VP\(_{2}\). The VIA antigen, which is found in appreciable quantities in virus harvests, is regarded by Polatnick et al. (1967) as an inactive form of the virus RNA polymerase and migrates in polyacrylamide gels as a single band with an estimated mol. wt. of about \(52 \times 10^3\) (Sangar et al. 1976).

By using purified preparations of \(^{35}\)S-methionine virus particles, procapsids and the VIA antigen in co-electrophoresis experiments with the induced polypeptides extracted from \(^{3}\)H-methionine labelled infected cells, it was shown that P38 coincided with VP\(_{0}\), P28 with VP\(_1\) and P26 with VP\(_3\) (Fig. 8). Similarly, VIA antigen coincided with P56 (Fig. 9) and is probably P56a since P56b is unstable.

Order of the structural polypeptides

The order of the structural polypeptides on the genome was determined by the use of emetine (Rekosh, 1972). This alkaloid freezes the ribosomes on the messenger RNA so that by adding radioactive amino acids shortly before the emetine, those polypeptide
chains released by primary cleavages before protein synthesis is inhibited will only be labelled in a short segment on the 5'-side of the cleavage site. The proportion of the primary cleavage products which are labelled will increase with the interval between the addition of labelled amino acids and the emetine. The order of the polypeptides on the structural precursor polypeptide can then be determined by measuring the specific activities of the individual polypeptides in the virus particles purified from these cultures.

As indicated above, virus of serotype O, in which all the structural polypeptides can be resolved clearly by polyacrylamide gel electrophoresis, was used in experiments to determine the order of the structural polypeptides. These experiments showed that the order on the genome is 5'-VP4, VP3, VP2, VP1 (Fig. 10), the same order as that reported for polio virus and EMC virus (Butterworth & Rueckert, 1972a; Rekosh, 1972).

**DISCUSSION**

In common with other members of the picornavirus group, the genome of FMDV is translated into large polypeptides which are subsequently cleaved to smaller products (Black, 1975). We have found four primary cleavage products after pulsing infected cells briefly with radioactive amino acids or by labelling in the presence of iodoacetamide to inhibit secondary cleavages. Three of the primary cleavage products are unstable and are cleaved to smaller polypeptides while the fourth is stable. Our attempts to demonstrate a polyprotein equivalent to the total uncleaved translation product of the FMDV genome have been unsuccessful but indirect evidence suggests that, as with other picornaviruses, protein synthesis starts at a single initiation site near the 5'-end of the RNA and continues...
through to a termination site near the 3'-end. This evidence is firstly that the primary cleavage products are found in equimolar amounts despite their widely different mol. wt. and secondly that the position of the primary cleavage products along the genome can be determined by biochemical mapping techniques. This evidence, although compelling, is circumstantial and we are currently analysing the tryptic peptide profiles of the induced polypeptides to provide direct evidence for the presence of 4 primary cleavage products. The recent report by Celma & Ehrenfeld (1975) suggesting that poliovirus RNA contains two initiation sites for protein synthesis is based on in vitro analyses and the relevance, if any, of their finding to the in vivo situation is unclear. We have found no evidence for amplification of any of the protein products by premature termination of translation as has been described for the structural polypeptides of Mengo virus (Lucas-Lenard, 1974; Paucha, Sechafer & Colter, 1974).

The presence of four primary cleavage products in FMDV infected cells is in contrast to the three that have been reported for polio and EMC virus (Taber et al. 1971; Butterworth & Rueckert, 1972a). Butterworth & Rueckert (1972a) were uncertain whether polypeptides G and I induced by EMC virus were primary cleavage products. However, both are small polypeptides and together would only increase the mol. wt. of the primary translation product by about 25,000. Recently, Beckman, Caliguiri & Lilly (1976) have suggested that poliovirus induced polypeptides 3b and 5a may be derived from separate translation units. This would increase the mol. wt. of the primary translation products by about 100,000 and raise serious doubts on the accuracy of the published mol. wt. of poliovirus RNA. The total mol. wt. of the primary cleavage products in FMDV infected cells is about $2.9 \times 10^5$. As the mol. wt. of FMDV-RNA is $2.8 \times 10^6$ (Wild & Brown, 1970), similar to the mol. wt. of other picornavirus RNAs (see Newman, Rowlands & Brown, 1973, for references), it would appear that a greater proportion of FMDV-RNA is translated into protein. In fact, the mol. wt. of the RNA quoted above would only be just sufficient to code for the total protein.

The positions of the virus induced polypeptides on the virus genome have been determined. Polypeptide P88, the precursor of the structural proteins, is located at the 5'-end as is the equivalent polypeptide in all the picornaviruses so far examined. In contrast to other picornaviruses, however, P88 is not the largest of the primary cleavage products in FMDV infected cells. Also, it is smaller than the equivalent structural precursor polypeptide found in other picornavirus infected cells, as would be expected from the fact that the sum of the mol. wt. of the structural polypeptides of FMDV is less than that for other picornaviruses (D. J. Rowlands, unpublished data).

The smallest primary product P52, which is located to the 3'-side of the structural precursor polypeptide, is labile and has no counterpart in other picornavirus infected cell systems. The 3'-end of the genome codes for two primary products P56 and P100, probably in that order, although some difficulty was experienced in allocating their relative positions. The stability of P56 during a chase period suggests that this polypeptide may be analogous to NCVPX in poliovirus infected cells and polypeptide F in EMC virus infected cells. In each of these systems the stable primary product has been shown to map in the middle of the genome (Butterworth, 1973).

We have found, in agreement with Van de Woude & Ascione (1974), that P56 co-migrates with VIA, the virus infection associated antigen found in virus harvests. Indirect evidence suggests that this protein may be an inactive form of the virus RNA polymerase (Polatnick et al. 1967), but the protein associated with the most highly purified picornavirus polymerase preparations so far reported are derived from the extreme 3'-end of the genome.
(Lundquist et al. 1974). However, genetic evidence suggests that poliovirus codes for two polymerase activities, one synthesizing single-stranded RNA and one double-stranded RNA (Cooper, Stanček & Summers, 1970), in which case it is possible that polypeptides coded for by both the central region of the genome and the 3′-end may be involved in RNA polymerase activity.

In pulse labelled samples, P56 was found to resolve into two polypeptides P56a and P56b. The sum of the molarities of these two polypeptides was unity. During a chase period, P56b disappeared while P56a increased by an equivalent amount. The rate of disappearance of P56b was similar to that of the FMDV polymerase in virus infected cells (D. N. Black, D. V. Sangar & D. J. Rowlands, unpublished results). This suggests the intriguing possibility that P56b is the active polymerase and is modified in some way to form P56a which is the inactivated enzyme. We are currently investigating the role that protein modification might play in the relationship between P56a and P56b.

The strategy of FMDV infection thus appears to be similar to that of other picornaviruses with the initial production of large precursor proteins which are then cleaved to yield smaller polypeptides. The spatial organization of the genome is also broadly analogous to the other systems. A striking difference is the presence of four primary products in FMDV and only three in other picornaviruses. For two reasons this difference cannot be explained simply by the presence of an extra cleavage site so that two of the FMDV primary cleavage products would be equivalent to a single primary cleavage product of other picornaviruses: (1) the position of the ‘extra’ FMDV polypeptide on the genome is between the structural precursor polypeptide and P56, which from map position and stability is analogous to the polypeptide immediately on the 3′-side of the structural precursor polypeptide in EMC and polio viruses; (2) the total mol. wt. of the FMDV primary translation products is greater than that reported for poliovirus and EMC virus by about $50 \times 10^3$, i.e. the mol. wt. of the ‘extra’ product. As we can only ascribe functions with certainty to the structural polypeptides and possibly P56, it is not possible at this stage in our studies to speculate on the biological significance of the extra product found in FMDV infected cells.

We wish to thank Upjohn Co. Ltd, Crawley, Sussex, for a gift of pactamycin.

REFERENCES


Mapping the FMDV genome


(Received 16 October 1976)