JOURNAL OF VIROLOGY, May 1972, p. 823-828
Vol. 9, No. 5

Gene Order of Encephalomyocarditis Virus as Determined by Studies with Pactamycin

BYRON E. BUTTERWORTH AND ROLAND R. RUECKERT
Department of Biochemistry and Biophysics Laboratory, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 2 February 1972

Previous work has shown that translation of the encephalomyocarditis (EMC) viral ribonucleic acid (RNA) generates at least three primary products, polypeptides A, F, and C. The A and C polypeptides then undergo post-translational cleavages to complete the production of the stable viral polypeptides (δ, β, γ, α, G, I, F, H, and E). In this communication we show that A, F, and C are produced in equimolar amounts giving further support to the theory that the RNA of picornaviruses has only a single site for the initiation of protein synthesis. The biosynthesis of viral proteins in EMC virus-infected HeLa cells was studied in the presence of pactamycin at concentrations which preferentially inhibit the initiation of protein synthesis. The amount of each polypeptide formed during the residual period of protein synthesis observed after the addition of pactamycin was used as a criterion for ordering the genes on the viral RNA. The results obtained indicate that the primary gene products are ordered on the EMC viral RNA 5′ → 3′ A-F-C and that the stable products are ordered δ-β-γ-α-G-I-F-H-E. Moreover, the intermediate chains B and ϵ map in the capsid region, whereas the intermediate chain D maps in the E region. This order is largely consistent with previously established relationships of the viral polypeptides and thus indicates that pactamycin is a valid tool for "genetic" mapping of polycistronic RNA molecules with single initiation sites.

The picornaviral genome theoretically is sufficient to encode a mass of about 270,000 daltons of protein (8). The viral ribonucleic acid (RNA), acting as a messenger RNA (mRNA) in the infected cell (10), directs the synthesis of the virus-specific proteins, some of which mature by proteolytic cleavages into smaller stable products (1, 4, 11). In the case of encephalomyocarditis (EMC) virus three polypeptides, A, F, and C, with a cumulative mass of about 220,000 daltons, appear to be primary products of translation (1). A and C then undergo a series of post-translational cleavages to complete the production of the stable polypeptides (δ, β, γ, α, G, I, F, H, E). Those polypeptides generated by the cleavage of A include all of the capsid chains (δ, β, γ, α), and those generated by the cleavage of C include D and E (1). The stable EMC virus chains have a cumulative mass of about 230,000 daltons which may represent the entire protein coding complement of the virus. Furthermore, these stable viral polypeptides are produced in about equimolar amounts (1), supporting the concept that the picornaviral RNA possesses but a single site for the initiation of protein synthesis (5) and that, once initiated, each ribosome completes translation of the entire protein coding region of the RNA.

Recently the use of pactamycin, a drug believed to interfere specifically with initiation of protein synthesis, has been proposed as a tool for constructing "genetic" maps of polycistronic RNA molecules with single initiation sites (12, 13). In this communication we report the gene order on the EMC RNA of the primary, intermediate, and stable viral proteins as determined by the pactamycin mapping technique. We also describe additional data in support of the single initiation site hypothesis.

MATERIALS AND METHODS

Materials. The source of HeLa cells and EMC virus and the composition of the dialysis buffer, of medium AL, medium AH, and of the scintillation solvents tT21 and B10 have been described (1). The L-[^{3}H]amino acid mixture containing 15 amino acids was purchased from New England Nuclear Corp. Pactamycin was a gift of D.F. Summers. A stock solution of 1×10^{-5} M pactamycin (assuming a molecular weight of 558) in 1 mM acetic acid was stored frozen and used throughout these experiments.

Methods. The procedures for infection, preparation of cell lysates, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, gel fractionation, and measurement of radioactivity have been described (1). Unless otherwide indicated, the tT21 counting system was

used. About 95% of the applied radioactivity is recovered from the gels by this procedure.

RESULTS

Molar proportion of the large polypeptide precursors. If EMC viral protein synthesis is initiated at a single site, it should be possible to demonstrate that the precursor chains, like the end products derived from them, are produced in equimolar proportions. A brief incubation of EMC-infected HeLa cells with radioactive amino acids results in the incorporation of radioactivity into the primary products of EMC virus-directed protein synthesis without allowing sufficient time for the complete breakdown of these precursors into the stable viral polypeptides (1). Figure 1 shows the distribution of label found in the viral polypeptides after a 6-min exposure of EMC virus-infected HeLa cells to a [^{3}H]amino-acid mixture. The molar ratios calculated for these polypeptides are summarized in Table 1. A, F, and C are proposed primary products of protein synthesis (1). The molar ratios of A together with its proposed cleavage products (B plus α), of the stable polypeptide F, and of C together with its proposed cleavage products (D plus E) are 1.09, 1.00, and 1.05, respectively (Table 1). Thus, the production of these polypeptides in equimolar amounts is in agreement with the concept that each ribosome completes translation of the entire EMC viral message. Polypeptides G and I are also present at this time. Their molar ratios are 0.79 and 0.85, respectively.

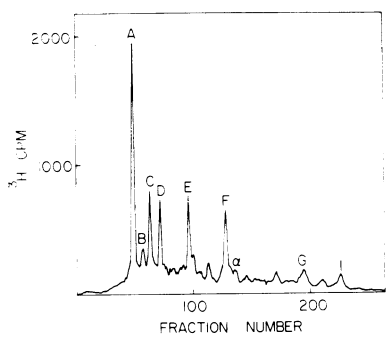

FIG. 1. *Profile of radiolabeled polypeptides after pulsing an encephalomyocarditis (EMC) virus-infected HeLa cell suspension for 6.0 min with a [^{3}H] amino acid mixture. In this and subsequent experiments, HeLa cells were infected with 200 plaque-forming units of EMC virus per cell and cultured at 37 C in medium AL (4 × 10^6 cells/ml) containing 5 μg of actinomycin D per ml as described (1). All manipulations from this point on were carried out in a warm room at 37 C. At 3 hr 46 min postinfection, a 2-ml sample was removed from the infected cell suspension and exposed to 80 μCi of a [^{3}H] amino acid mixture per ml. After a 6.0-min incubation at 37 C, 0.2 ml of 10× solubilizing solution [10% sodium dodecyl sulfate (SDS), 5 M urea, 1% 2-mercaptoethanol] was added, and the sample was heated for 5 min in a boiling-water bath. The solubilized cells were dialyzed overnight against a large volume of dialysis buffer and concentrated about twofold by dialysis against Ficoll. The extract then was subjected to SDS-polyacrylamide gel electrophoresis for 20 hr at 8 ma per gel. In this and subsequent figures, the anode is to the right. Each fraction represents 1 mm of gel.*

Inhibition of protein synthesis by pactamycin. At sufficiently low concentrations pactamycin is reported to inhibit initiation of protein synthesis in cell-free systems from rabbit reticulocytes;

TABLE 1. *Molar ratios of the radiolabeled viral polypeptides after a 6-min exposure to [^{3}H] amino acids*

Polypeptide	Apparent molecular weight[a]	Per cent total viral counts/min[b]	Molar ratio[c]
A	100,000	35.0 (0.8)	0.80
B	90,000	3.6 (0.5)	0.09
C	84,000	10.7 (0.8)	0.29
D	75,000	9.5 (0.4)	0.29
E	56,000	11.5 (0.6)	0.47
F	38,000	16.6 (2.0)	1.00
α	34,000	2.9 (0.7)	0.20
G	16,000	5.5 (1.1)	0.79
I	11,000	4.1 (0.4)	0.85
A + B + α			1.09
C + D + E			1.05

[a] The apparent molecular weight of each component was calculated from its mobility on sodium dodecyl sulfate-polyacrylamide gels relative to that of six standards (1).

[b] Calculated from the average of six independent gels run on three different cell extracts which had been pulse-labeled for 6.0 min. The profiles were similar to those in Fig. 1 and the control profile in Fig. 3. The distribution of ^{3}H was calculated relative to the total radioactivity recovered from all of the viral peaks. The average deviations are indicated in parenthesis. The accuracy of values for chains smaller than F in extracts from experiments using short incorporation periods is subject to some uncertainty because (i) their small size results in less radioactivity incorporated into them relative to an equivalent molar amount of one of the large chains; (ii) at this time the yield of product chains is limited by incomplete cleavage of the precursor chains; (iii) with short pulse periods, there remains a substantial background, presumably due to nascent chains (Fig. 1).

[c] The mass of protein was assumed to be proportional to its radioactivity. The molar ratios were calculated by dividing the mass ratio (column 3) of each chain by its apparent molecular weight and normalizing with respect to the stable primary product F.

higher concentrations of the drug also interfere with translation (9). It also has been reported that pactamycin concentrations as low as 10^{-7} M completely inhibit initiation of protein synthesis in HeLa cells (13). The results presented in Fig. 2 illustrate that exposure of EMC virus-infected HeLa cells to 10^{-7} M pactamycin results in about a 12-min period of decreasing protein synthesis after which no further incorporation of amino acids into protein takes place. This residual synthesis is interpreted as the completion of polypeptide chains by ribosomes already translating the message before the initiation blockade.

Theory of mapping. The transient period of protein synthesis shown in Fig. 2 in which previously initiated ribosomes complete translation of the message defines the period of interest for mapping studies. The number of copies of each polypeptide produced equals the number of ribosomes which translate its gene locus on the RNA. During the "run off" period, those regions nearest the initiation site (assumed to be at or near the 5′ end of the RNA) will be the first to be depleted of ribosomes; hence, polypeptides from this region will be produced in lower amounts than polypeptides whose loci are nearer the 3′ end of the RNA. In theory, the degree of this change from the normal equimolar production of products is dependent on the distance of these loci from the initiation site and thus allows the ordering of the proteins on the RNA.

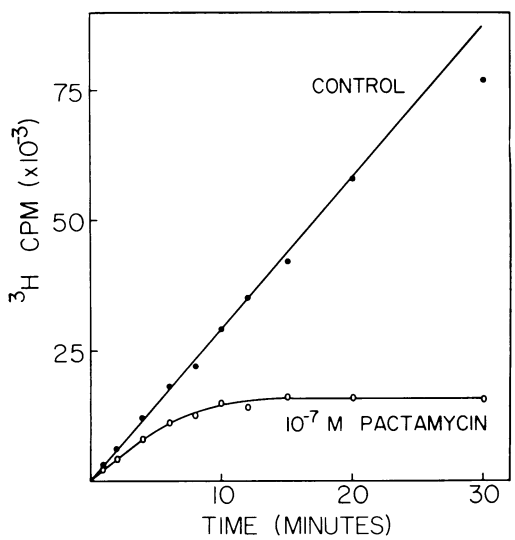

FIG. 2. *Inhibition of virus-specific protein synthesis by pactamycin. At 3 hr 50 min postinfection, duplicate 4-ml portions from an infected cell suspension were each mixed with 1 ml of medium AL containing 25 μCi of a [^{3}H]amino acid mixture and 50 μliters of medium AL (control: ●) or 10^{-5} M pactamycin in 1 mM acetic acid (○), and incubation was continued at 37 C. At the indicated times, 0.3-ml samples were removed into 1 ml of ice cold 10% trichloroacetic acid. After diluting with water to 5% trichloroacetic acid, the samples were heated for 20 min at 90 C and the acid-insoluble material was collected and washed on type HA membrane filters (0.45-μm pores, Millipore Corp.). The filters were allowed to clear overnight in 10 ml of scintillation solvent B10, and radioactivity was measured in a liquid scintillation spectrometer.*

In cases where the action of pactamycin and the incorporation of radioactive amino acids begin simultaneously, the pactamycin-control ratio (the amount of radioactivity incorporated into a polypeptide during the run off period relative to the amount of radioactivity incorporated in the same polypeptide under normal steady-state conditions) is theoretically proportional to the relative map distance of that protein along the mRNA. Assuming that protein synthesis begins at the amino terminal end of the protein and proceeds from the 5′ to the 3′ end of the RNA (3, 7), the smallest pactamycin-control ratio will designate that polypeptide nearest the 5′ end of the genome.

Any delay in the addition of labeled amino acids after inhibition by pactamycin will decrease the resolution of mapping for proteins near the 5′ end of the message because all pactamycin-control ratios will be approaching zero. However, if the delay is not longer than the time required for the last ribosome to complete translation, the relative differences in the pactamycin-control ratios of those polypeptides near the 3′ end will be increased and thus allow some degree of expanded resolution of mapping.

Gene order of the primary products. Figure 3 illustrates that the normal distribution of radioactivity incorporated into the primary products is dramatically changed if the EMC virus-infected cells are first exposed to 10^{-7} M pactamycin and then, after a 6-min delay, are allowed to incorporate radioactive amino acids as the remaining ribosomes run off the EMC viral message. The pactamycin-control ratio for each peak should be an indication of the order of the proteins on the mRNA. The values obtained (Fig. 5) indicate the order of the primary products on the EMC viral RNA to be 5′ → 3′ A-F-C.

Examination of Fig. 3 shows that significant amounts of radioactivity are also found in the cleavage intermediates B and D and in the stable product E. Their map distances are shown in Fig. 5. B maps almost exactly with A. D and E map in the area of C on the 3′ end of the RNA.

Gene order of the stable products. The radiolabeled precursor molecules can be chased into their stable products by incubation of the EMC virus-infected HeLa cells in medium containing unlabeled amino acids (1). Figure 4 illustrates

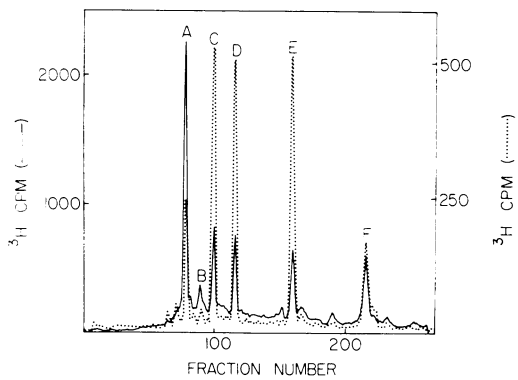

FIG. 3. *Effect of pactamycin on the distribution of radioactivity incorporated into the primary products of encephalomyocarditis-directed protein synthesis. All manipulations were carried out in a warm room at 37 C. At 3 hr 49 min postinfection, an infected cell suspension was exposed to 10^{-7} M pactamycin. Six minutes thereafter, a 2-ml sample was removed, labeled, prepared, and electrophoresed as described in Fig. 1. The control sample was prepared as described in Fig. 1. To improve resolution, the gels were run for 36 hr at 8 ma per gel; material smaller than α migrated from the gel. The two extracts were electrophoresed separately and the electropherograms have been superimposed for comparison. Control, 6-min pulse (solid line); pactamycin, 6-min delay, 6-min pulse (dotted line).*

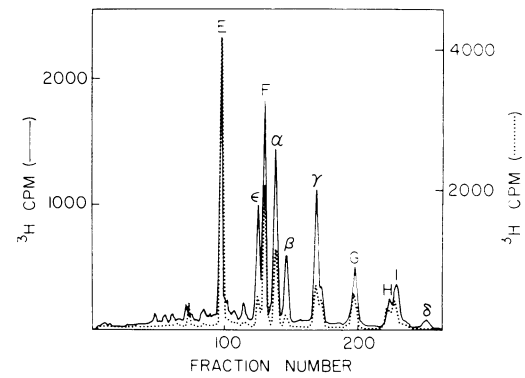

FIG. 4. *Effect of pactamycin on the distribution of radioactivity incorporated into the stable encephalomyocarditis (EMC) virus polypeptides. All manipulations were carried out in a warm room at 37 C. At 3 hr 39 min postinfection, a 2-ml sample from an EMC virus-infected cell suspension was removed and exposed simultaneously to 10^{-7} M pactamycin and 80 μCi of a [^{3}H] amino acid mixture per ml. After a 20.0-min incubation, 50 ml of medium AH containing 10^{-7} M pactamycin was added to the suspension, and the cells were immediately sedimented and suspended in 8 ml of fresh medium AH free of drug and isotope. The total incubation in medium AH was continued for 80 min at 37 C. The cells then were sedimented, suspended in 0.5 ml of water, and solubilized as described in the legend of Fig. 1. An identical control sample was taken simultaneously from the original infected cell suspension and treated in the same way, except that all solutions were free of pactamycin. The extracts were then analyzed directly (dialysis step omitted) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The two samples were electrophoresed separately and the electropherograms have been superimposed here for comparison. Control, 20-min pulse, 80-min chase (solid line); pactamycin, 20-min pulse, 80-min chase (dotted line).*

that the pattern of labeling found in the stable viral polypeptides is altered extensively if, during the labeling period, the cells are also exposed to 10^{-7} M pactamycin. Treating the data as previously described, the gene sequence obtained for the stable viral polypeptides is 5′ → 3′ δ-β-γ-α-G-I-F-H-E (Fig. 5). The precursor chain ε is still present at this time and maps to the left of γ.

Duplicate experiments, similar to the one described in Fig. 4 in which there was a 6-min delay after exposing the infected cells to 10^{-7} M pactamycin followed by a 14-min pulse and an 80-min chase, gave an expanded map for those proteins near the 3′ end of the EMC viral RNA and confirmed the order G-I-F-H-E for those proteins to the right of the capsid proteins.

DISCUSSION

The observation that polypeptides A, F, and C are produced in approximately equimolar amounts (1.09, 1.00, and 1.05, respectively, after correcting for post-translational cleavages; see Table 1) is consistent with the concept that each ribosome completes translation of the entire protein coding region of the viral RNA and reinforces the hypothesis that A, F, and C are primary products in the translation of the EMC viral genome (1). The order given by the pactamycin mapping technique for these primary products is 5′ → 3′ A-F-C (Fig. 5). The pactamycin mapping technique can only be valid if the polycistronic RNA possesses a single initiation site. Hence, the fact that a reasonable linear gene order was obtained is additional support for the single initiation site hypothesis (5). Although the hypothesis that EMC viral RNA has only a single major initiation site cannot yet be considered proven, less likely alternatives involving multiple initiation sites could explain the data only if each cistron were expressed at a rate proportional to its molecular weight and if, in addition, the expression of each cistron were differentially affected by pactamycin in such a way as to produce a reasonable genetic map.

Although the cumulative mass (220,000 daltons) of the A, F, and C chains is close to the theoretical coding capacity (270,000 daltons) of

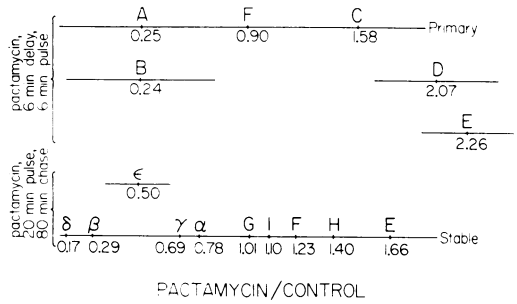

FIG. 5. *Gene sequence of encephalomyocarditis polypeptides as determined by the pactamycin mapping technique. The amount of radioactivity in each peak was calculated as the per cent relative to the total radioactivity recovered from all viral peaks on a gel. The ratio of this percentage in the pactamycin-treated extract relative to the value of its corresponding peak from the control extract then was calculated. This pactamycin-control ratio is related to the relative position of the polypeptide on the viral mRNA. In the above graph, the viral polypeptides have been ordered from left to right according to increasing values of the pactamycin-control ratio so that the 5′ end of the RNA is to the left. Each value is the average from two independent experiments that gave similar results. The average deviation from the mean values shown above is 0.04. The maximum deviation (polypeptide E) is 0.11.*

the genome, the possible existence of additional small primary products is not yet precluded. Indeed, the molar ratios of I and G are sufficiently close to those of A, F, and C after a short pulse (Table 1) to suggest that one, or both, may be stable primary products. The kinetic behavior of I and G in pulse-chase experiments (Fig. 3 of reference 1) is also compatible with this possibility. Further work will be required to settle this point definitively because the accuracy of the molar ratio values for these small chains is uncertain (see footnote to Table 1).

The precursor chains A, B, and ϵ as well as the stable capsid chains (δ, β, γ, α) all map in the same region on the 5′ end of the viral mRNA (Fig. 5). This is consistent with the finding that A is related to B and is the precursor of the capsid chains (1). Furthermore, the gene order determined by the use of pactamycin suggests that the order of the capsid chains within A is δ-β-γ-α, starting with δ at the amino terminal end of the sequence. The ϵ chain maps to the left of γ in agreement with the notion that ϵ is the precursor of the δ and β chains (1).

There are a number of similarities between our results with EMC virus and those reported earlier for poliovirus. The precursor of the polioviral capsid proteins has been designated both NCVP1 and NCVP1a (12, 13). Taber et al., who first used the pactamycin mapping technique, reported the order NCVP1-NCVPX-NCVP2 for three products of polioviral-directed protein synthesis (13). These polypeptides are probably analogous to the EMC viral polypeptides A, F, and D, respectively. The conclusion that the coat protein cistron is nearest the 5′ end of the polio mRMA has been confirmed (12). Other studies using the pactamycin system indicate the order of the capsid proteins on the polioviral RNA to be VP4-VP2-VP3-VP1 (6). These chains are analogous to the EMC viral polypeptides δ, β, γ, and α, respectively (8). These results suggest that all picornaviruses may share the same gene order.

In the noncapsid region of the gene (Fig. 5) C, D, and E all map in the same region on the 3′ end of the viral mRNA; this supports our earlier conclusion, based on pulse-chase experiments and cyanogen bromide mapping of the isolated polypeptides, that D is a cleavage product of C and that E, in turn, is a cleavage product of D (1). The pactamycin-derived gene sequence further suggests that D and H are the cleavage products of C, and their sizes are consistent with this. The observation that the sum of the molar ratios of C, D, and E (Table 1) nearly equals the molar ratio for the stable primary product F gives additional support to the proposed C $\rightarrow$ D $\rightarrow$ E precursor-product relationship. The cleavage of D to produce E appears to be slower than the cleavage of the capsid precursor (1); therefore, such large amounts of both D and E present following a short pulse were unexpected (Table 1). One way of reconciling these observations is to assume that, although the C chain can be translated in the intact form, a fraction of the C chains may be cleaved while still in the nascent form to generate that fraction of its smaller product chains as if they were primary products.

On the whole, the pactamycin map correlates well with previously established relationships of the viral polypeptides and with the proposed model for the biosynthesis of EMC virus proteins (1). However, some discrepancies remain to be resolved. For example, although B is related in sequence to A (1) and maps with it (Fig. 5), the relationship of B to A remains unclear. One possibility is that B is a cleavage product of A. According to this model, the A chain (100,000 daltons, Table 1) is cleaved to form B (90,000 daltons) plus an equivalent amount of a small fragment of about 10,000 daltons. The most obvious candidates for such a small fragment are the stable chains G or I which map close to the capsid region. However, for this to be true, the relative molar amount of these polypeptides (G or I) must equal the sum of the molar amounts of B plus α (0.09 + 0.20 = 0.29). The observed

yields of the G and I chains, about 0.8 each, are too high to satisfy this model. Assuming then that neither G nor I are part of A leaves open the possibility that the missing fragment is degraded in the cell or remains undetected, e. g., the molar ratio of I is a bit high (Table 1 of reference 1) suggesting that it may contain two comigrating polypeptides. An alternative explanation for the origin of B is that it arises by translation of a coinfecting mutant virus with a deletion in the A chain cistron. The DI mutant of poliovirus might represent such a possibility (2). However, this deletion hypothesis seems unlikely in the case of EMC virus because a clonal isolate produces the same polypeptide profile as does the wild type (S. McGregor, *personal communication*). Other possibilities are that B is generated by an infrequent but specific error in initiation or "termination" during the translation of A or that the A chain may be subject to a minor alternative cleavage sequence (e. g., premature cleavage of the δ chain).

A second unresolved problem is that the cleavage of C followed by the cleavage of D should generate two stable polypeptides in addition to E. However, only H and E map to the right of the stable primary product F, again leaving the additional cleavage product missing. In this case, the missing polypeptide may be mapping incorrectly. A simple way of explaining how a single polypeptide might map incorrectly would be to assume that it is on the 3′ end of the mRNA and that translation over this region is incomplete. Such a situation might arise (i) if termination were inhibited in pactamycin-treated cells, thus causing an accumulation of ribosomes at the 3′ end of the message, or (ii) if ribosomes were released prematurely from this region of the message in the presence of pactamycin. In either case, the amount of this polypeptide produced would be decreased, causing it to map to the left of its real position. If this effect were confined to a short region near the 3′ end of the mRNA, this polypeptide would be the only one affected. Based on size, the best candidate for the additional polypeptide generated by the cleavage of D to produce E is G. Assigning G to the 3′ end of the mRNA would indeed place it next to E and account for the cleavage products of C. Making this one change would give the gene order δ-β-γ-α-I-F-H-E-G which is more nearly consistent with the proposed model for the biosynthesis of the EMC viral proteins (1).

Although some details of EMC viral protein biosynthesis remain to be clarified, the major result is that the gene sequence obtained by the pactamycin mapping technique is largely consistent with the cleavage map obtained by independent studies (1). This is an encouraging indication that the pactamycin mapping technique is a valid tool for mapping polycistronic RNA molecules with single initiation sites.

ACKNOWLEDGMENTS

B.E.B. was supported by Public Health Service predoctoral training grant 5-T01-GM-00236 from the National Institute of General Medical Sciences. R.R.R. is a Public Health Service Career Development awardee (2KO3-CA11797, from the National Cancer Institute). This work was supported by grant VC-26A from the American Cancer Society.

LITERATURE CITED

1. Butterworth, B. E., L. Hall, C. M. Stoltzfus, and R. R. Rueckert. 1971. Virus-specific proteins synthesized in encephalomyocarditis virus-infected HeLa cells. Proc. Nat. Acad. Sci. U.S.A. **68**:3083–3087.
2. Cole, C. N., D. Smoler, E. Wimmer, and D. Baltimore. 1971. Defective interfering particles of poliovirus. I. Isolation and peptide properties. J. Virol. **7**:478–485.
3. Dintzis, H. M., 1961. Assembly of the peptide chains of hemoglobin. Proc. Nat. Acad. Sci. U.S.A. **47**:247–261.
4. Jacobson, M. F., J. Asso, and D. Baltimore. 1970. Further evidence on the formation of poliovirus proteins. J. Mol. Biol. **49**:657–669.
5. Jacobson, M. F., and D. Baltimore. 1968. Polypeptide cleavages in the formation of poliovirus proteins. Proc. Nat. Acad. Sci. U.S.A. **61**:77–84.
6. Rekosh, D. 1972. The gene order of the poliovirus capsid proteins. J. Virol. **9**:479–487.
7. Robinson, W. E., R. H. Frist, and P. Kaesberg. 1969. Genetic coding oligonucleotide coding for first six amino acid residues of the coat protein of R17 bacteriophage. Science **166**:1291–1293.
8. Rueckert, R. R. 1971. Picornaviral architecture, p. 255–306. *In* K. Maramorousch and E. Kurstak (ed.), Comparative virology. Academic Press Inc., New York.
9. Stewart-Blair, M. L., I. S. Yanowitz, and I. H. Goldberg. 1971. Inhibition of synthesis of new globin chains in reticulocyte lysates by pactamycin. Biochemistry **10**:4198–4206.
10. Summers, D. F., and L. Levintow. 1965. Constitution and function of polyribosomes of poliovirus-infected HeLa cells. Virology **27**:44–53.
11. Summers, D. F., and J. V. Maizel, Jr. 1968. Evidence for large precursor proteins in poliovirus synthesis. Proc. Nat. Acad. Sci. U.S.A. **59**:966–971.
12. Summers, D. F., and J. V. Maizel, Jr. 1971. Determination of the gene sequence of poliovirus with pactamycin. Proc. Nat. Acad. Sci. U.S.A. **68**:2852–2856.
13. Taber, R., D. Rekosh, and D. Baltimore. 1971. Effect of pactamycin on synthesis of poliovirus proteins a method for genetic mapping. J. Virol. **8**:395–401.