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Picornaviral Gene Order: Comparison of a Rhinovirus with a Cardiovirus

CHARLES McLEAN AND ROLAND R. RUECKERT

Biophysics Laboratory and Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706

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We describe conditions for unmasking virus-specific protein synthesis in cells infected with rhinoviruses 1A and 2. The gene order and cleavage pattern of rhinovirus 1A show many similarities to those of encephalomyocarditis virus and of poliovirus.

The rhinoviruses resemble other picornaviruses, including poliovirus and encephalomyocarditis (EMC) virus, in size and composition (5); they are distinguished, however, from the other picornaviruses by their high buoyant density in cesium chloride solution and by their acid lability. Thus the taxonomic relationship of rhinoviruses to other picornaviruses is still unclear. We show here that at least one rhinovirus (rhinovirus 1A) resembles poliovirus and EMC virus not only in size and composition, but also in location and order of capsid genes and probably several noncapsid genes as well.

Evidence that rhinoviruses 1A and 2, like poliovirus and EMC virus, are able to redirect protein synthesis from that of the host cell to that of the virus is presented in Fig. 1. The electrophoretic profiles of proteins synthesized in HeLa cells 4 to 4.5 hr after infection with human rhinovirus 1A (hrv-1A) (Fig. 1A) or with human rhinovirus 2 (hrv-2) (Fig. 1B) are reminiscent of that observed in EMC virus-infected cells (Fig. 1C, see also reference 1). We interpret the peaks in these profiles, which show characteristic differences as well as similarities, to represent polypeptides encoded by the respective viral genomes. In support of this interpretation, a profile similar to that shown in Fig. 1A was observed 3 hr postinfection with rhinovirus 1A; at 2 hr, however, the profile still resembled that of uninfected (control) cells.

To facilitate comparison of peaks in these profiles, lysates from HeLa cells infected with each of the rhinoviruses were subjected to co-electrophoresis with a lysate from ^{14}C-labeled EMC virus-infected HeLa cells (panel C). Each rhinoviral peak is identified by its apparent molecular weight ($\times 10^{-3}$), relative to the reported (1) sizes of the EMC-encoded chains identified in panel C.

The capsid chains (α, β, γ, δ, ϵ) of hrv-1A (Fig. 1A) and of hrv-2 (Fig. 1B) were identified in separate co-electrophoresis experiments with the corresponding, purified ^{14}C-labeled virions (3). Although the β and δ chains, which are assumed to be cleavage products of the ϵ chain (3), were not evident after a 30-min pulse, they did appear after longer labeling periods. The large hrv-1A chains, 92, 84, and 76, which are similar in size to EMC chains B, C, and D, behaved in pulse-chase experiments (to be reported later in detail) like precursors; little label was ever observed in the minor hrv-1A peak 101 which co-migrates with the EMC chain A. The same pulse-chase experiments showed that chains 39, 38, 35, 30, 25, and 8 were derived from larger precursors, whereas chains 55 and 47 were both primary stable products. The small amount of label in peaks 14 and 13, which could be resolved under other conditions into at least three peaks, precluded reliable interpretation of their kinetic behavior. Peaks 129 and 120 were not reproducibly observed.

To further characterize peaks in this profile we have examined the effect of the drug pactamycin on their synthesis. This drug has been used previously to establish the gene orders of poliovirus and of EMC virus (2, 4, 7, 8). The method is based upon (i) the hypothesis that the picornaviral genome contains only one ribosomal initiation site, and (ii) the observation that, under suitable conditions, pactamycin interferes specifically with initiation of protein synthesis without preventing transation (6). A pactamycin concentration is chosen which gives complete and rapid, but not immediate, cessation of incorporation of labeled amino acids into the culture to be studied. The period of decreasing incorporation (the "run-off" time) is thought to be the time during

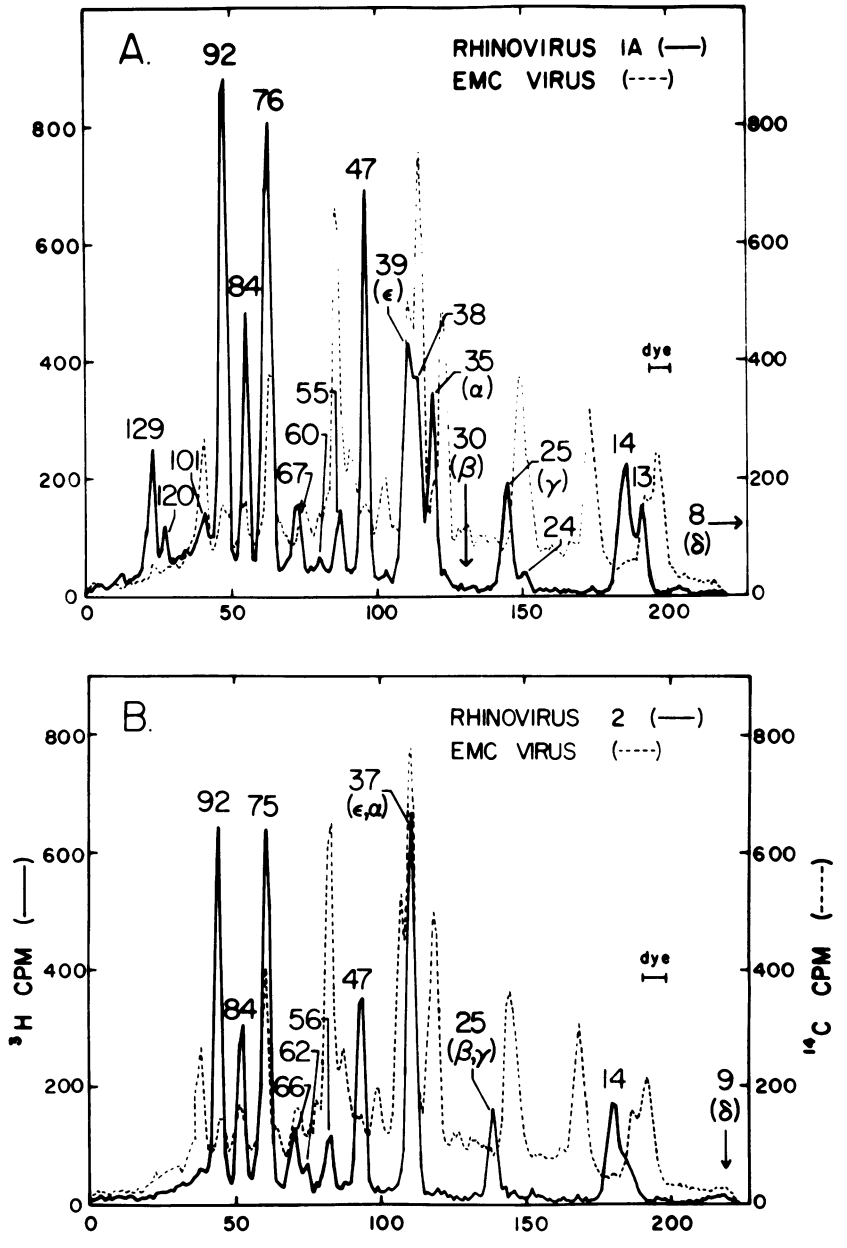
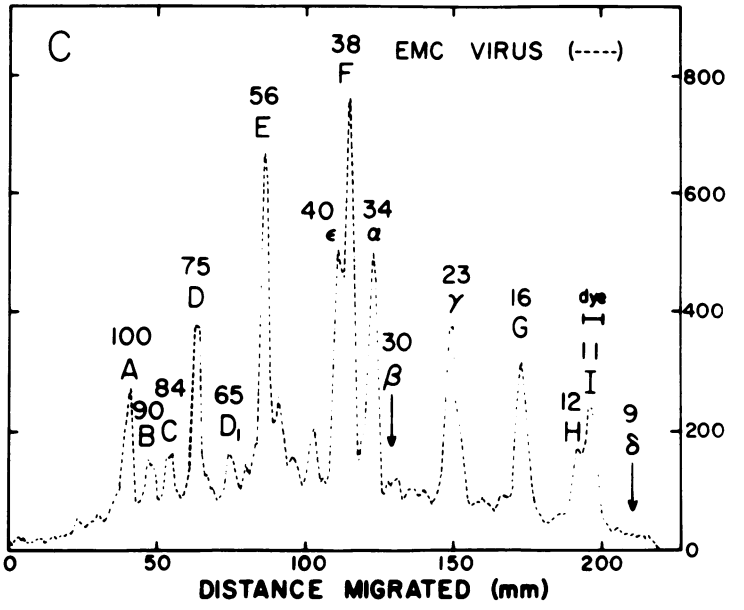


FIG. 1. *Electropherograms of whole cell lysates of rhinovirus-infected H-HeLa cells. Suspensions of H-HeLa cells (4 × 10⁷ cells per ml) in medium P-5 (3) were infected with rhinovirus 1A (panel A) or rhinovirus 2 (panel B) with about 200 plaque-forming units per cell. After 30 min of attachment at room temperature, the cultures were pelleted, resuspended to 4 × 10⁶ cells per ml (at time zero) in medium AL (containing 5.5 μg of actinomycin D per ml), and incubated at 35 C. ³H-amino acid mixture was added (to 20 μCi/ml) at 4 hr postinfection. Thirty minutes later, whole cell lysates were prepared (1). For electrophoresis, samples were mixed with similarly prepared ¹⁴C-labeled EMC-infected HeLa cell lysate. Electrophoresis (from left to right) was on 10% polyacrylamide gels (0.6 × 25 cm) containing 0.1% sodium dodecyl sulfate at 8 ma per gel for 17.5 hr, when the bromophenol blue (dye) marker had moved about 20 cm. Buffer and gel compositions and fractionation and determination of radioactivity in the gels were as previously described (3). The profile of the ¹⁴C-EMC lysate from panel A is redrawn and labeled with EMC nomenclature and apparent molecular weights (× 10⁻³) in panel C.*



which ribosomes initiated before addition of the drug complete their passage down the ribonucleic acid. The rationale of the method is that, after blockage of initiation, genes far from the initiation site are translated more frequently during the ribosome run-off period than those close to the initiation site. Thus, the fraction of a particular polypeptide made in the presence of the drug relative to that made in its absence (pactamycin to control ratio) is theoretically proportional to the distance of the corresponding cistron from the initiation site.

In the case of EMC virus (2), the capsid-related precursor chain A, together with its derivative capsid polypeptides (α, β, γ, δ; and ϵ, which is a precursor of the β and δ), maps nearest the ribosomal initiation site, whereas the noncapsid precursor chain C, together with its derivatives, D and E, map furthest from the initiation site. The stable primary noncapsid product, F, maps between A and C. Poliovirus yields a similar map (4, 7, 8).

A similar pattern emerges from the pactamycin map of hrv-1A (Fig. 2). Thus, the rhinoviral capsid chains (δ, β, γ, α) (3) map nearest the initiation site and in the same order as that of EMC virus and poliovirus. Moreover, chain 92, which is about the 96,000 dalton size predicted (3) for an uncleaved capsid precursor chain, also maps in the capsid region; it is the only obvious candidate for a capsid precursor. Although the δ chain carried too little label in the pactamycin-treated sample to provide a reliable ratio, its apparent position at the extreme left of the map is consistent with the data and with the analogous EMC capsid order. Peak 39, which co-migrates with the virion ϵ chain, maps further to the right than expected for a δ-β precursor; this behavior suggests that peak 39 contains an unidentified co-migrating polypeptide in addition to the ϵ chain.

The two large precursor chains, 84 and 76, map to the far right of the genome; thus they are analogous to the EMC viral noncapsid precursor chains C and D, not only in size and kinetic behavior, but also in mapping position. On the basis of its kinetic behavior, we have tentatively identified peak 38 as a homologue of the stable product E, which is derived by cleavage of the EMC viral noncapsid precursor chain C. Since it maps somewhat to the left of its expected position, peak 38 may also contain, in addition to the E homologue, an unidentified co-migrating polypeptide.

Finally, EMC virus produces only one large, stable, primary chain, F (molecular weight 38,000), while hrv-1A produces two, 55 and 47; both of these stable products, however, like F, map near the middle of the genome. Since comparison of many unrelated picornaviruses has heretofore shown similarities in size and number of capsid chains (5), we regard the existence of an apparent difference in size and number of noncapsid hrv-1A homologues as tentative pending further investigation.

While a number of differences are evident in the apparent size of some of the homologous capsid and noncapsid chains, we wish to emphasize here the basic similarities with EMC

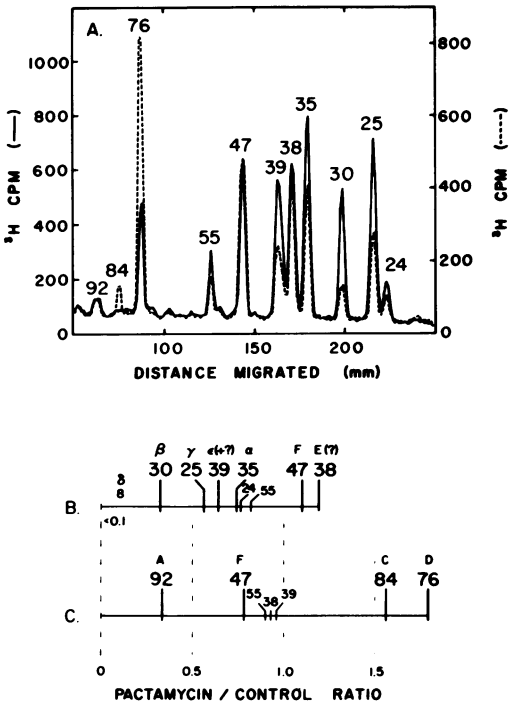


FIG. 2. *Pactamycin map of polypeptides from H-HeLa cells infected with rhinovirus 1A. Cells were infected as described in Fig. 1. A, Electrophoretic profile of lysates from control (——) and pactamycin-treated (-----) cells pulsed for 19 min and chased for 104 min. At 4 hr postinfection, ^{3}H-amino acid mixture (to 100 μCi/ml) was added to 2 ml of the infected culture (control). At the same time, ^{3}H-amino acid mixture and pactamycin (to 10^{-7} M) were added to another 2-ml sample. Under these conditions, the ribosome run-off time was about 14 min. After labeling for 19 min at 35 C, each sample was diluted 20-fold with medium AH(1); the cells were pelleted, resuspended in unlabeled medium AL (1), and incubated for an additional 104 min at 35 C. Both samples were then pelleted and disrupted, and samples corresponding to about 0.8 × 10^6 cells were subjected to electrophoresis. The gels shown were run in parallel for 30.5 hr at 8 ma per gel; the electrophoretic profiles are superimposed for comparison. B, Pactamycin map of stable products, calculated from data in Fig. 2A (19-min pulse, 104-min chase). The percent of total recovered radioactivity was calculated for each peak on a gel. The ratio of this percentage in the pactamycin-treated lysate to that of the corresponding peak in the control lysate (the pactamycin to control ratio) was calculated for each component as described previously (2). The map indicates the apparent order of the rhinovirus 1A gene products; proposed EMC correlates are designated by letters above. The initiation site is represented by zero, and the direction of translation is from left to right. The ratios shown are averages from two experiments. Exceptions are peak 8 and peaks 39 and 38, which were not resolved in the first experiment; in the experiment shown in Fig. 2A, they were resolved (with the loss of 8) by a longer electrophoresis period. The positions of components indicated by small numerals represent ratios calculated from peaks containing less than 5% of the total recovered label; the reliability of these values is uncertain. C, Pactamycin map of early gene products from rhinovirus 1A (6-min delay, 8-min pulse). At 4 hr postinfection, pactamycin was added (to 10^{-7} M) to 2 ml of the infected culture. ^{3}H-amino acid mixture (to 100 μCi/ml) was added 6 min after pactamycin, and incorporation was terminated 8 min later. Another 2-ml sample was pulsed with ^{3}H-amino acids for 8 min, beginning at 4 hr and 6 min postinfection. Both samples were disrupted, dialyzed, and subjected to electrophoresis as described previously (2). Pactamycin to control ratios were calculated as described above; the values reported are averages from two experiments.*

virus and poliovirus. First, the fact that a reasonable linear gene order was obtained at all by the pactamycin technique supports the concept of a single initiation site. Second, all of the results are interpretable within the framework of a common pattern—initiation site: 100,000 dalton capsid precursor: stable noncapsid chain(s): 75,000 to 85,000 dalton noncapsid precursor. The results reinforce the notion that rhinoviruses, enteroviruses, and cardioviruses are related to a common ancestral prototype.

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