JOURNAL OF VIROLOGY, Sept 1976, p. 903-914
Vol. 19, No. 3

Printed in U.S.A.

Evidence of Ambiguous Processing and Selective Degradation in the Noncapsid Proteins of Rhinovirus 1A

C. McLEAN,* T. J. MATTHEWS, AND R. R. RUECKERT

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

Received for publication 16 March 1976

Pulse-chase kinetics and extensive pactamycin mapping studies show that the translation of rhinovirus 1A proceeds in the order: initiate-P_1-S-P_2-terminate, where P_1 is the precursor to the capsid proteins, S is a stable primary gene product, and P_2 is the precursor to a family of noncapsid products. Initial examination of the molar stoichiometry of the families of rhinoviral proteins in infected cells suggested that both the P_1 and P_2 regions were translated more frequently than the S region. However, we show that this apparent asymmetry in translation is an artifact arising from two phenomena: (i) ambiguous cleavage sites which result in two alternative products from the S region, having apparent molecular weights of 47,000 and 38,000, and (ii) several fates for the P_2 precursors, including degradation of 35 to 45% of the P_2 family to small unidentifiable products. Another artifact, a time-dependent shift in the pactamycin mapping position of polypeptide r-39, was traced to a selective inhibition of the rate of cleavage of its precursor (peak 76). The processing rate of the capsid precursor (peak 92) was not retarded by pactamycin.

The picornavirus genome is an mRNA (molecular weight, $\sim 2.5 \times 10^6$) which appears to be translated from a single major initiation site (5, 13, 18, 22). From studies on poliovirus (2, 22), encephalomyocarditis (EMC) virus (5), and rhinovirus 1A (2, 16), it appears that the message is translated in the order P_1-S-P_2, where P_1 is a region encoding the capsid protein, whereas S and P_2 encode the noncapsid proteins. The primary gene products of the P_1 and P_2 regions are precursor polypeptides which undergo further proteolytic cleavages; polypeptides from the middle (S) region are stable. In the case of EMC virus, the three families are produced in equimolar amounts, suggesting a single termination site (3). This termination site must be at or near the opposite end of the molecule, because the polypeptides produced account for the entire 270,000-dalton coding capacity of the genome. Thus the translational pattern of the picornaviral genome can be represented as:

$$i\text{-}P_1\text{-}S\text{-}P_2\text{-}t$$

with a single initiation site, i, at or near the 5' end of the message and a single termination site, t, at or near the opposite end.

However, there are several reports of "asymmetric" translation, in which the amount of capsid protein produced in polio- and mengovirus-infected cells exceeds by two- to threefold the amount of noncapsid protein (14, 19, 20). These observations have been interpreted as evidence for the existence of an "internal" termination site at or near the end of the capsid region. We report here a similar overproduction of capsid protein in rhinovirus 1A-infected cells. In this case, however, we conclude that overproduction of capsid protein is not due to asymmetric translation. Rather, the apparently unequal production of each family of polypeptides is an artifact due to two phenomena: (i) specific degradation of polypeptides in the P_2 family; and (ii) production of two stable (S) polypeptides with apparent molecular weights of 47,000 and 38,000.

MATERIALS AND METHODS

Cells and virus. H-HeLa cells and rhinovirus 1A were as described previously (17). The virus was plaque-purified by four consecutive isolations of single plaques formed on H-HeLa cell monolayers. High-titer stocks of the plaque-purified strain were prepared and titered as described by Medappa et al. (17), except that plaque assays were incubated at 35°C (which gives a titer about twofold higher than that as determined at 37°C). The serotype of the high-titer stock prepared after plaque purification was confirmed as that of rhinovirus 1A by Elliot Dick, Department of Preventive Medicine, University of Wisconsin, Madison.

Media and buffers. Medium AH is medium A (17) containing 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer. Medium AL is medium AH lacking amino acids. Medium P5 is

Eagle minimal medium supplemented with 0.1% bovine serum albumin, 40 mM magnesium chloride, and 75 μg of diethylaminoethyl dextran per ml (17).

Solubilizing solution is 5 M urea, 10% sodium dodecyl sulfate (SDS), and 2% β-mercaptoethanol. Dialysis buffer is 0.01 M sodium phosphate (pH 7.2), 0.5 M urea, 0.1% SDS, 0.1% β-mercaptoethanol, and 1 mM sodium azide. Hypotonic buffer is 10 mM triethanolamine hydrochloride (pH 7.5), 10 mM sodium chloride, and 2 mM magnesium chloride.

Isotopes. The ^{3}H-labeled L-amino acid mixture (catalog no. NET-250) and ^{14}C-labeled L-amino acid mixture (catalog no. NEC-445) each contained 15 amino acids and were from New England Nuclear. The L-[4,5-^{3}H]leucine and [2-^{14}C]uridine were from the Amersham/Searle Corp.

Preparation of whole cell lysates. Whole cell lysates were prepared by mixing 0.9-ml samples of culture with 0.1 ml of solubilizing solution, followed immediately by heating in a boiling-water bath for 5 min. The resulting lysates were dialyzed overnight in the cold room against at least two changes of dialysis buffer. Dialyzed lysates were stored frozen at −70°C.

Electrophoresis. Unless otherwise indicated, polyacrylamide gels were columns (0.6 by 25 cm) of 10% polyacrylamide containing 0.3% (vol/vol) ethylene diacrylate as cross-linker, 0.1% SDS, and 0.1 M sodium phosphate, pH 7.2. The electrophoresis buffer was 0.1 M sodium phosphate, pH 7.2, 0.1% SDS, and 0.1 M sodium 3-mercaptopropionate. Preparation of the gels and of the samples for electrophoresis, the conditions of electrophoresis, fractionation of the gels, and determination of radioactivity in scintillation fluid tT21 were as described by Medappa et al. (17).

Plotting of electropherograms. Electropherograms were plotted using a Hewlett-Packard 9820A programmable calculator equipped with a 9862A calculator plotter, using a program developed by Robert E. Duke of this laboratory.

Subtraction of background. To determine the radiolabel in each protein peak, a uniform background, usually determined from the flat portion of the electropherograms between peaks 24 and 14 (for example, see Fig. 1C), was subtracted from the total counts in each peak on the gel.

Prelabeling of cellular RNA. To label cellular (and therefore ribosomal) RNA, a 300-ml culture of H-HeLa cells in the logarithmic phase of growth was grown under standard growth conditions (17) for 24 h in the presence of 5 μCi of [2-^{14}C]uridine. The cells were then resuspended in fresh medium lacking radiolabel and incubated for an additional 10 h, before being used for infection and subsequent isolation of ribosomes.

Isolation of ribosomes. Infected cells were lysed in hypotonic buffer by Dounce homogenization as described by Penman (21). The nuclei and cell debris were removed by centrifugation at 500 × *g* for 5 min. The resulting cytoplasmic extract was centrifuged at 15,000 × *g* for 15 min to obtain a pellet (P15) and a supernatant (S15). The S15 fraction (1 ml) was sedimented on chilled 12-ml linear 10 to 35% (wt/wt) sucrose gradients in hypotonic buffer. Centrifugation was in an SW41 rotor at 40,000 rpm for 210 min at 4°C. The gradients were fractionated by bottom puncture (17), and the 80S ribosome peak fractions were pooled and stored frozen at −70°C.

RESULTS

Capsid proteins (the P_1 family). Figure 1 shows representative electropherograms of whole cell lysates from a typical pulse-chase experiment initiated 4 h postinfection. The flow of radioactivity from proteins with high molecular weights to those with lower molecular weights is clear; for example, the lysates are rich in the capsid precursor chain 92 after a 3-min chase (Fig. 1A) and low in chain 92 after a 150-min chase (Fig. 1C); during the same time the chains characteristic of mature virions (α, β, γ, δ, ε [39]) increase. Translation of the complete rhinoviral genome requires 10 to 15 min (16); hence, the higher background envelope early in the chase period (Fig. 1A) is due to the presence of nascent chains and perhaps also to some residual host protein synthesis. Note also the presence of peaks 55, 47, 38, 14, and 13 early in the chase period (Fig. 1A).

The relative numbers of molecules in the peaks were calculated from such plots by dividing the radioactive content of each peak by its apparent molecular weight. These values were then normalized to the major stable protein, peak 47, and used to construct the plots of radioactive flow (Fig. 2). Peak 92 has previously been identified (2, 16) as the capsid precursor on the basis of its pactamycin mapping position and its mass. The data in Fig. 2 support this conclusion, for the appearance of the capsid proteins (α, β, γ, δ, ε) parallels the disappearance of peak 92 (compare Fig. 1A, 1D). Peak 101 is probably also a capsid-related protein (2). The minor peaks 67 and 60 exhibit precursor kinetics (Fig. 2C) and probably correspond to intermediates in the cleavage of peak 92; their size suggests that peak 67 corresponds to an uncleaved ε-γ segment and peak 60 to an uncleaved γ-α segment. Consistent with this idea is the finding that peaks 67 and 60 map in the capsid region (2; Fig. 6C). Peak 60 is not always present in detectable amounts.

Stable proteins (the S family). Peaks 47 and 55, which fall in the middle of the pactamycin map (2, 16; Fig. 6A), are the only peaks which behave neither as precursors nor as cleavage products (Fig. 2B). Hence, they are similar in these respects to EMC viral polypeptide F (molecular weight, 38,000) but differ from F in mass.

That peak 55 belongs to the S family is suggested by both pactamycin mapping position (2,

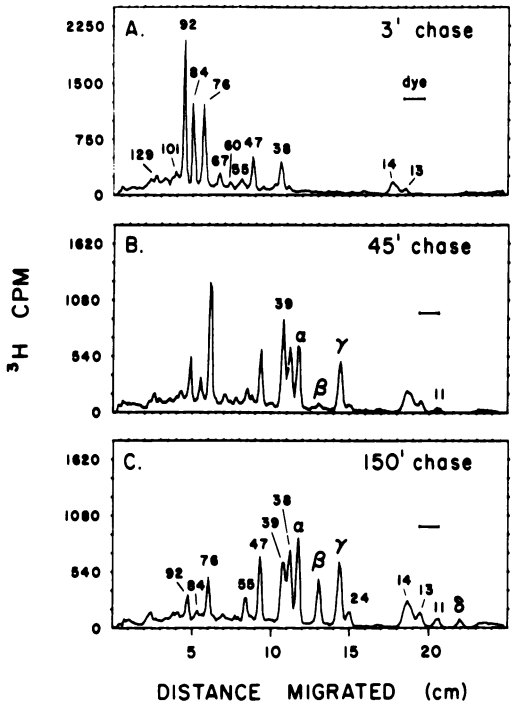

FIG. 1. *Electropherograms of whole cell lysates from pulse-chase of rhinovirus 1A-infected HeLa cells. H-HeLa cells (8 × 10⁷ cells in 2 ml of medium P5) were infected with the plaque-purified strain of rhinovirus 1A at room temperature (multiplicity of infection, ~200 PFU/cell). After 30 min at room temperature, the cells were sedimented and resuspended to a concentration of 4 × 10⁶ cells/ml in medium AL containing 5.5 μg of actinomycin D per ml and incubated at 35°C in a rotary shaker at about 90 rpm (17). At 4 h postinfection a ^{3}H-labeled amino acid mixture was added to the culture (to 300 μCi/ml). Ten minutes later the cells were sedimented, resuspended to 4 × 10⁶ cells/ml in medium AH lacking radiolabel, and reincubated at 35°C. At intervals thereafter, 0.9-ml samples were lysed as described in Materials and Methods. Immediately prior to electrophoresis, samples of the whole cell lysates were made 1% with respect to SDS and β-mercaptoethanol, made 0.005% with respect to bromphenol blue, and heated in a boiling-water bath for 5 min. Electrophoresis on 10% polyacrylamide-SDS gels (17) was for about 18 h at 8 mA/gel. Gel fractionation and determination of radioactivity were as described previously (17). In this and in subsequent figures, electrophoresis was from left to right; the position of the bromphenol blue marker is indicated (–). The peaks are designated by apparent molecular weight (× 10⁻³), except for the capsid proteins which are indicated by their Greek letters (16).*

16; Fig. 6A) and kinetic behavior (Fig. 2B). This predicts sequence homologies between peaks 55 and 47. However, chromatographic profiles of tryptic digests (T. J. Matthews and D. Omilianowski, unpublished observations) show no evidence of sequence homology between peaks 55 and 47 but strong homology between 55 and 76. Thus, peak 55 is apparently not a member of the S family but is a cleavage product of chain 76 as proposed by Butterworth (2).

As noted above, peak 38 also falls in the middle of the pactamycin map (2, 16; Fig. 6A). It is comparable in size to the EMC F chain (molecular weight, 38,000), but its kinetic behavior (Fig. 2B) is not quite that expected of a primary product, in that it increases slightly throughout the chase period. It has been reported previously (16) that this peak may contain two comigrating polypeptides. Since it is present at a relatively high level immediately after a pulse (Fig. 2B), we propose that peak 38 represents a mixture of two polypeptides: a major one which is a stable primary product (38a) translated from the center of the genome, and a minor component (38b) derived by cleavage of a precursor and responsible for the slight increase during the chase. The probable existence of primary product 38a is reinforced by the presence of peaks 38 and 47 in infected cells treated with zinc chloride, which appears to inhibit secondary but not primary cleavages of viral protein (4). Furthermore, tryptic mapping studies (Matthews and Omilianowski, unpublished observations) show significant sequence homologies between peaks 47 and 38, supporting the hypothesis that 47 and 38a are alternative products from the S region.

By extrapolating the kinetic curve for peak 38 (Fig. 2B) to the beginning of the 10-min pulse, when the amount of cleavage product 38b is presumably zero, the proportion of primary product 38a (relative to peak 47) was estimated to be 0.8. Therefore, the relative proportions of 47 and 38a are about 1.0:0.8, suggesting that 47 is produced by about 56% of the translations and 38a by about 44% of the translations. The molar proportion of 38b at any time in the chase was then calculated by subtracting 0.8 from the relative molar proportion of the entire peak 38. In principle, the amounts of 38a and 38b in peak 38 could be computed by a more formal mathematical analysis, but such analysis requires assumptions about constancy of cleavage rates which are as arbitrary as those involved in the extrapolation.

Unstable noncapsid precursor chain and its products (the P_2 family). The P_2 family of EMC virus consists of chain C (molecular weight, 84,000) which is cleaved through an intermediate, D (molecular weight, 75,000), to form a stable chain, E (molecular weight, 56,000) (3). Rhinovirus-infected cells contain a set of polypeptides of similar size – polypeptides 84, 76, and 55, respectively. The kinetic behav-

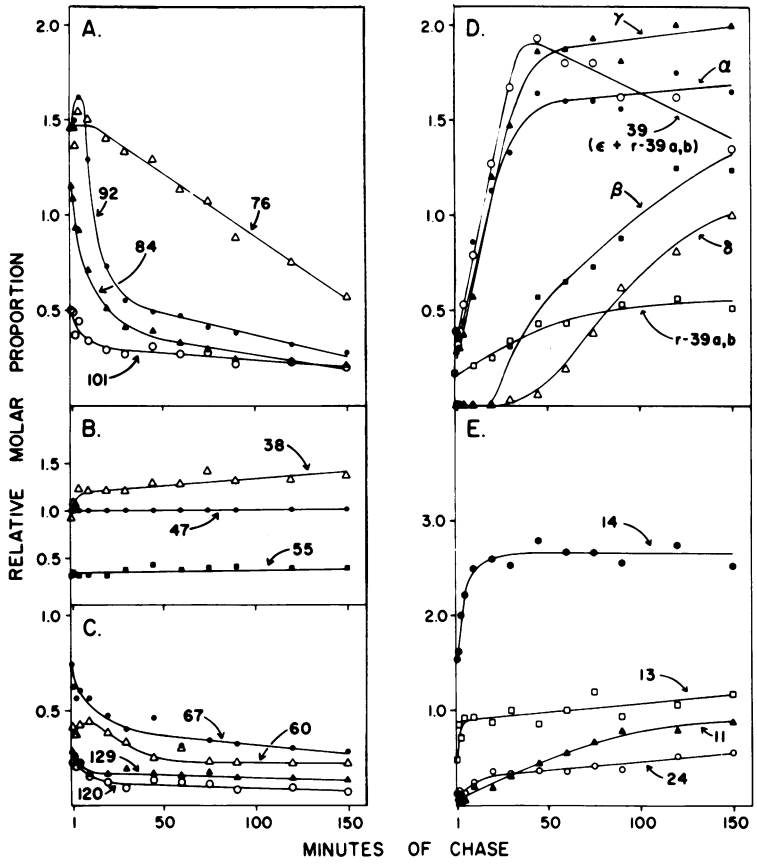

FIG. 2. *Pulse-chase kinetics of rhinovirus 1A proteins. From the electropherogram for each time point in the pulse-chase experiment presented in Fig. 1, the percentage of the total recovered virus-specific radioactivity was calculated for each peak. Each such value was then divided by the appropriate apparent molecular weight to obtain the molar proportion of each component; the molar proportions were normalized relative to peak 47. In most cases electrophoresis was for 18 h. However, the curve for r-39a,b in (D) was obtained by extended electrophoresis (33 h); this procedure resolved peak r-39 from the ε component.*

ior (Fig. 2A) and pactamycin mapping position (2, 16) of rhinoviral peaks 84 and 76 are similar to those of the EMC virus C and D chains. As mentioned above, tryptic mapping studies (Matthews and Omilianowski, unpublished data) show sequence homologies between peaks 55 and 76. It is not clear why rhinoviral peak 55 maps in the S region rather than in the P_2 region; however, it may be related to inaccuracy in the correction for background, since the peak is very small. The reason for the apparent constancy of peak 55 in kinetic experiments will be treated below.

There is also some evidence, from studies of ribosome-bound rhinoviral proteins, that a non-ε component of peak 39 is a cleavage product of peak 76. Ribosomes from HeLa cells infected with rhinovirus 1A carry chain 76 plus a second component, r-39 (Fig. 3). The amount of ribosome-bound chain 76 decreases during a chase, whereas r-39 increases (T. J. Matthews et al., Fed. Proc. 32:461, 1973). Moreover, by electrophoresis in SDS gels containing 8 M urea, r-39 can be separated into two components, r-39a and r-39b (Fig. 4), which are present in equivalent proportions throughout a chase (Fig. 5). This supports the idea that ribosome-bound 76 is cleaved near its middle to form r-39a and r-39b.

Effect of pactamycin on the cleavage of chain 76. To test further the hypothesis that r-39 (r-39a plus r-39b) arises by the cleavage of 76, the pactamycin map position of r-39 was determined. In preliminary studies, irregularities were encountered in the mapping position of r-39; the reason for these irregularities became clear in a systematic study undertaken to relate mapping position to the time of chase (Fig. 6).

This study revealed a time-dependent shift in the position of peaks r-39 and 76 (Fig. 6E); r-39 shifts to the left as 76 moves to the right. No such shift was observed with the capsid proteins (Fig. 6B) or with the S proteins 55, 47, and 38 (Fig. 6A).

These shifts in the mapping positions of peaks 76 and r-39 are related to a specific inhibition of the cleavage of chain 76 (Fig. 7). In the presence of pactamycin this cleavage is reduced to half its normal rate (Fig. 7C); there is a much smaller effect on the cleavage of its precursor, chain 84 (Fig. 7B), and little, if any, effect on the cleavage of the capsid precursor, chain 92 (Fig. 7A).

As a consequence of this inhibition, the amount of chain 76 in pactamycin-treated cells is "abnormally" high, whereas the amount of its cleavage product is proportionately low. This results in elevated values for the pactamycin/control ratio of 76 and reduced values for the ratio for its product. In light of this argument, the shift in map position of 76 and r-39 in opposite directions (Fig. 6E) further supports the proposed precursor-product relationship between 76 and r-39.

Molar stoichiometry of the rhinoviral families. In an earlier paper (16) we proposed that the genome of rhinovirus 1A, like that of EMC virus, is programmed to read "initiate-P_1-S-P_2-terminate." This model requires that each region of the genome be translated with equal frequency. In the case of EMC virus, the model was confirmed by showing equimolar stoichiometry of the capsid family (P_1) and the C-chain family (P_2), relative to the stable primary polypeptide F (S).

As described above, the cleavage of rhinovi-

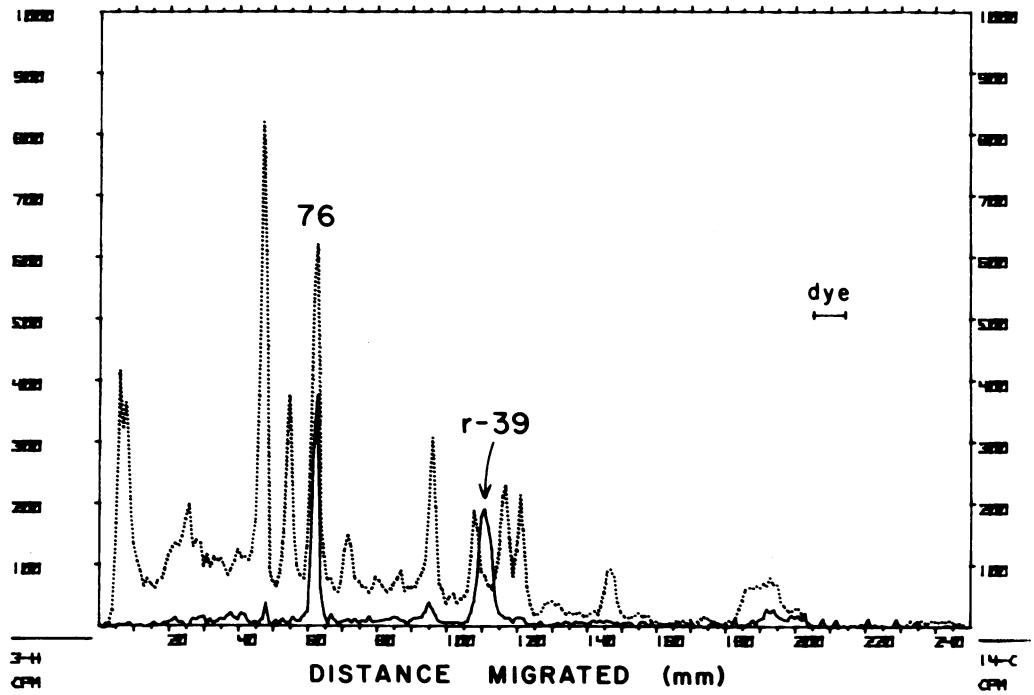

FIG. 3. *Co-electrophoresis of ribosome-bound rhinoviral protein (—) with a rhinovirus-infected whole cell lysate (· · · ·). About 1.8 × 10^8 H-HeLa cells (prelabeled with [^{14}C]uridine as described in Materials and Methods) were infected with rhinovirus 1A (multiplicity of infection, 100 PFU/cell). At 220 min postinfection, a ^{3}H-labeled amino acid mixture (to 50 μCi/ml) was added to the infected culture. After a 40-min labeling period, the cells were transferred to medium AH lacking radiolabel, and incubation was continued at 35°C. Sixty minutes later, the cells were lysed in hypotonic buffer, and ribosomes were isolated as described in Materials and Methods. For electrophoresis, a sample of the isolated ribosomes was mixed with a sample of a whole cell lysate from rhinovirus 1A-infected H-HeLa cells labeled at 4 h postinfection with ^{14}C-labeled amino acid mixture for 30 min (16). The mixture was prepared for and subjected to electrophoresis on a 25-cm 10% polyacrylamide-SDS gel as described in Fig. 1. Electrophoresis was for 19 h at 8 mA/gel. Gel fractionation and determination of radioactivity were as described previously (17). The ^{14}C-label at the top of the gel is due to the [^{14}C]uridine labeled RNA present in the ribosomes.*

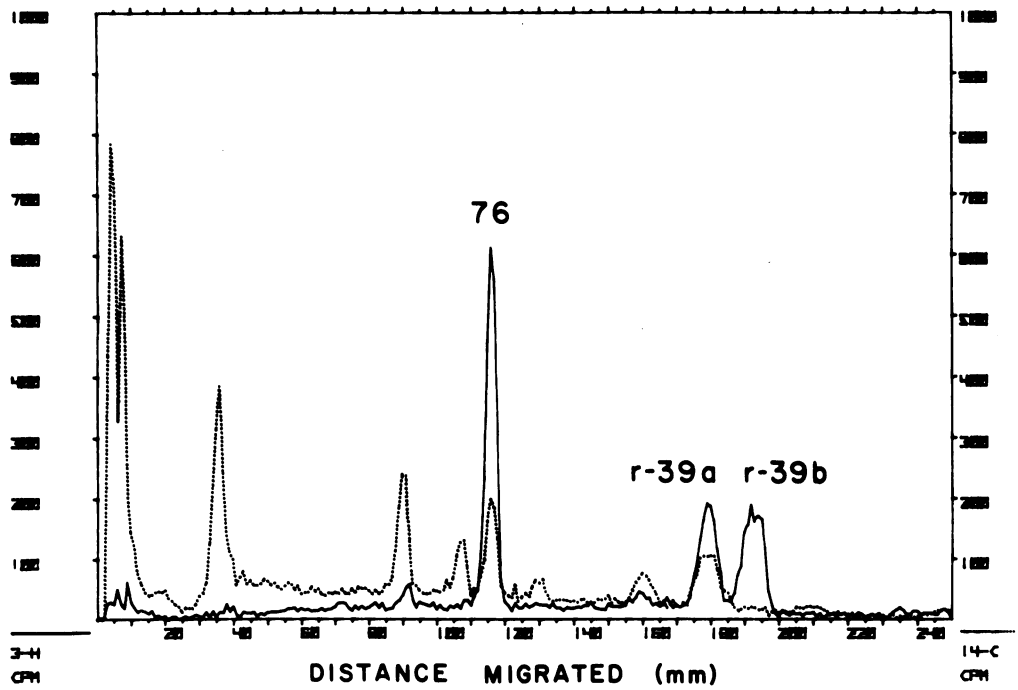

FIG. 4. *Electrophoretic separation of r-39a and r-39b. Ribosomes labeled with ^{3}H-labeled amino acid mixture (–) from rhinovirus-infected cells were the same as described in Fig. 3. The ^{14}C-labeled whole cell lysate marker (· · · ·), the electrophoresis conditions, gel fractionation, and determination of radioactivity were also the same as in Fig. 3, except that 8 M urea was included in the gel and electrophoresis was for 36 h. (In the presence of 8 M urea, rhinoviral polypeptides ϵ, 38, and α migrate as a single peak, with the same mobility as r-39a.)*

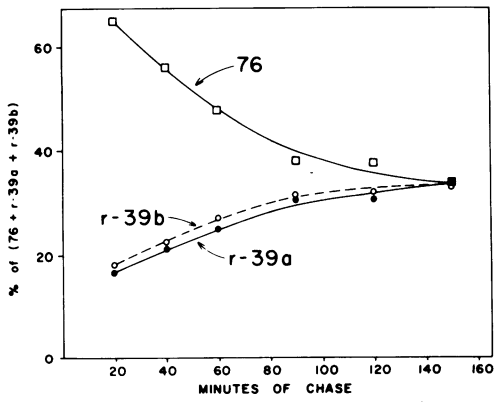

FIG. 5. *Change in the amounts of 76, r-39a, and r-39b on ribosomes isolated from rhinovirus 1A-infected HeLa cells. H-HeLa cells were infected with rhinovirus 1A, pulsed for 40 min with ^{3}H-labeled amino acid mixture, and subjected to a chase in unlabeled medium as described in Fig. 3. At various times during the chase period, samples were taken, and ribosomes were isolated as described in Materials and Methods. Samples of isolated ribosomes were subjected to SDS-polyacrylamide gel electrophoresis in the presence of 8 M urea as in Fig. 4. Gel fractionation and determination of radioactivity were as described previously (17).*

rus 1A polypeptides is somewhat more complicated than that of EMC virus, in that rhinovirus 1A produces two polypeptides (47 and 38a) from the S region, whereas EMC produces only one (chain F); it appears also that the rhinoviral D analogue, chain 76, is cleaved through three different pathways to generate chains 55, 38b, and r-39 (a plus b). To gain perspective on this hypothesis, the molar proportions of the proposed P_1 and P_2 families were normalized to each combination of proposed S proteins (Fig. 8). The amount of P_1 exceeds by more than twofold the amount of chain 47, whereas the amount of P_2 decreases from an excess of nearly fourfold to about twofold during a 150-min chase (Fig. 8A). This nonideal behavior is not appreciably improved by normalizing the chains to the sum of peaks 47 and 55 (Fig. 8B). On the other hand, a relatively good fit is obtained by normalizing to the sum of chains 47 and 38a (Fig. 8C); however, the P_2 family still decreases during the chase.

Degradation of the P_2 family. There are several possible explanations for the decrease in the P_2 family: (i) the cleavage products of 84 and 76 may have been incorrectly or incompletely identified and are therefore counted in

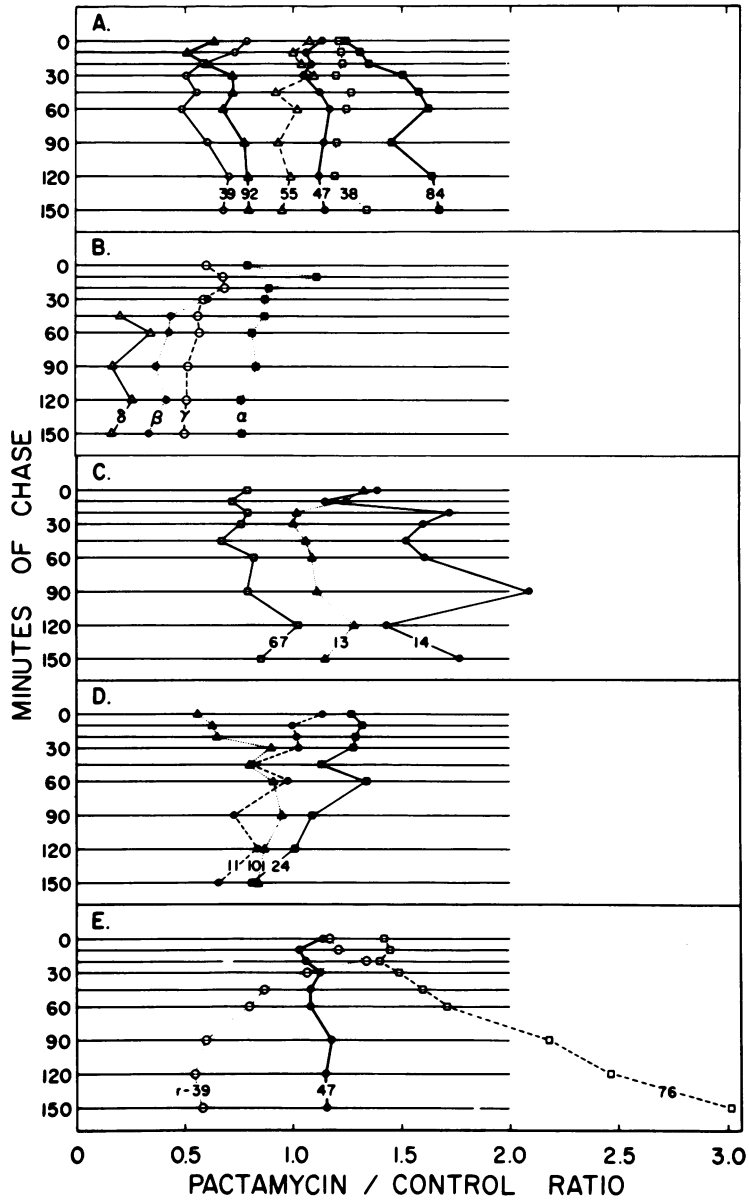

FIG. 6. *Pactamycin mapping positions of rhinovirus 1A gene products after various periods of chase. H-HeLa cells were infected and incubated as described in Fig. 1. At 4 h postinfection a ^{3}H-labeled amino acid mixture (to 200 μCi/ml) was added to 8 ml of the infected cell suspension (control culture). At the same time a ^{3}H-labeled amino acid mixture (to 200 μCi/ml) and pactamycin (to 10^{-7} M) were added to an equivalent 8-ml sample (pactamycin-treated culture). After 15 min, 0.9-ml samples from each labeling culture were lysed and dialyzed overnight as described in Materials and Methods. Immediately after sampling, the remainder of each culture was diluted fivefold with medium AH, the cells were pelleted and resuspended to 3.2 × 10^6 cells/ml in medium AH lacking radiolabel, and incubation was continued at 35°C. At various times (from 10 to 150 min after resuspension) samples were taken simultaneously from each culture, lysed, and dialyzed overnight as above. For each chase time, samples from the whole cell lysates from both the control culture and the pactamycin-treated culture were subjected simultaneously to SDS-gel electrophoresis (17), and pactamycin/control ratios for each component were calculated as described previously (5, 16). The initiation site is represented by zero, and the direction of translation is from left to right. Electrophoresis was at 8 mA/gel for either 18 h (A–D) or 33 h (E).*

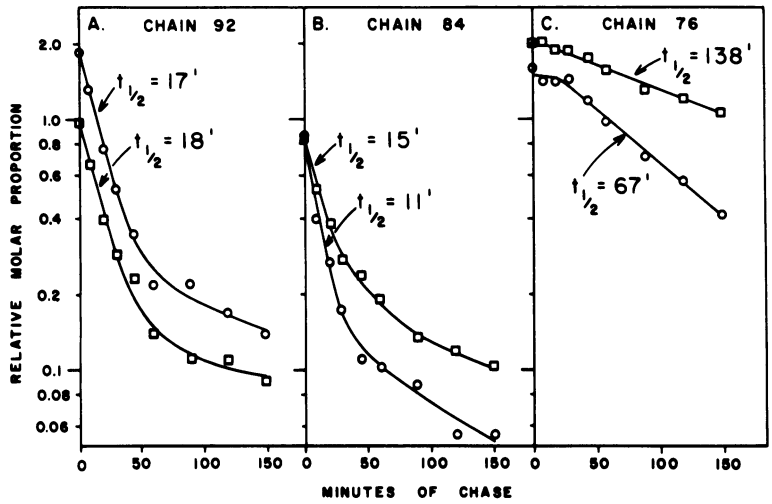

FIG. 7. *Effect of pactamycin on the rate of disappearance of the rhinoviral precursors 92, 84, and 76. Data are from the experiment described in Fig. 6. Log (relative molar proportion) is plotted against time of chase for data from pactamycin-treated cultures (□) and data from control cultures (○).*

the wrong family; (ii) chains 84 and 76 may be degraded; and (iii) the basic assumption that polypeptide 47 is stable may be incorect. With regard to this last possibility, it is important to recall that the other viral polypeptides were normalized to 47; a gradual increase in 47 during a chase would lead to an apparent decrease in a stable family. This idea is not necessarily refuted by the constancy of the capsid family, since a P_2 cleavage product, incorrectly assigned to the capsid family, might fortuitously compensate for an artifactual decrease in the capsid family.

To test these possiblities, the amount of radiolabeled viral protein was normalized to a constant number of cells. This was done by prelabeling host cells for one generation with [^{14}C]uridine; the metabolically unstable label was subsequently chased out by incubating the cells for an additional half generation in medium lacking the radiolabeled precursor. The cells thus tagged were then infected and radiolabeled with [^{3}H]leucine, solubilized, and subjected to electrophoresis in the usual way. Essentially 100% of the cellular ^{14}C-label applied to the gels was reproducibly recovered. Most of this, probably in the form of rRNA, was in the top 1 cm of the gels, whereas about 10% migrated as tRNA, 5S RNA, and 5.8S RNA (Fig. 9). The cellular ^{14}C-labeled RNA remained intact (or at least large) even late into the infection cycle, judging from its failure to move more than a few millimeters into the polyacrylamide gels. By summing the ^{14}C-label on each gel and relating that to the known amount of ^{14}C per cell, it was possible to normalize the ^{3}H-radiolabeled polypeptides to the number of cells. In this way the cellular content of protein radiolabeled from 3.5 to 3.75 h postinfection was examined throughout a 2-h chase. The label in the complete set of stable (S) proteins was constant (Fig. 10); the same was true of the capsid (P_1) polypeptides. In contrast, the label in the P_2 family gradually decreased, so that in 2 h about half of the family was lost, corresponding to the loss of a similar quantity of label in total virus protein. We therefore conclude that the loss of the P_2 family is due to degradation to molecules smaller than 10,000 daltons (which would have migrated from the ends of the gels) and/or to material small enough to be lost during the dialysis step in the preparation of the whole cell lysates. A fortuitous similarity in rates of production and degradation of peak 55 could account for its apparent constancy in the kinetic experiments.

In summary, this experiment establishes two points of major importance: (i) the amount of polypeptide 47 per cell is indeed constant throughout the chase, and (ii) there is a decrease of label in the P_2 family due to metabolic degradation. In addition, the constancy of the P_1 family strengthens the case that the capsid polypeptides have been correctly assigned.

DISCUSSION

Model for rhinoviral translation and processing. Figure 11 summarizes our conclusions

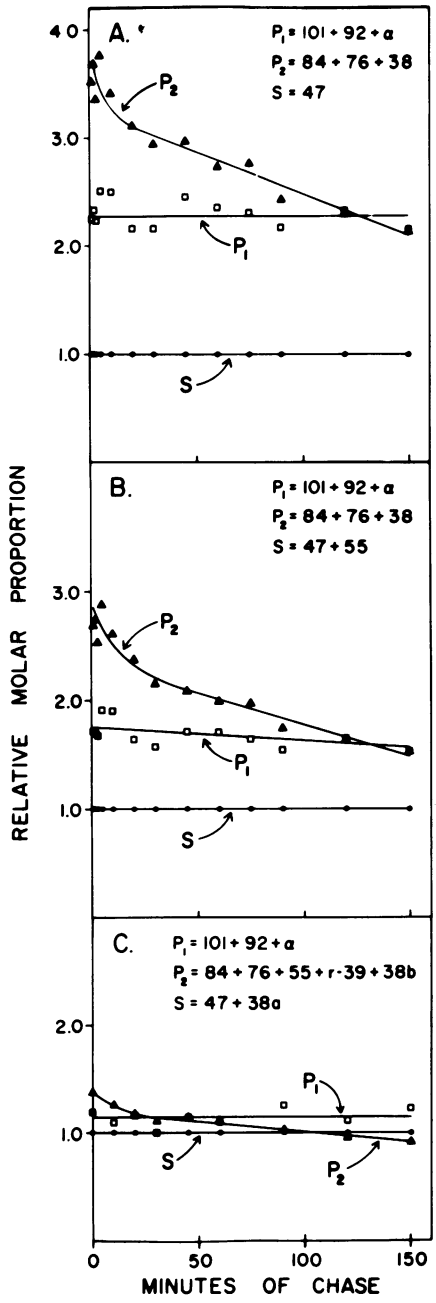

FIG. 8. *Relative molar proportions of proposed families of rhinoviral proteins, based on various assignments to families. From pulse-chase experiments, the molar proportions of the members of each proposed family were summed; since the data being summed are molar proportions rather than units of mass, only one member of the end products of each proposed route of posttranslational cleavage is included. (For example, only one half of the molar proportion of r-39 is included in the P_2 family, and only one (α) of the protomer chains is counted.) For each time point, the sums thus obtained were normalized to a value of 1.0 for each postulated S family. In (A) a single translation product, 47, from the S region is assumed; in (B) two alternative S products, 47 and 55, are assumed; in (C) chains 47 and 38a are assumed to be alternative S products, and it is further assumed that 38a = 0.8 (relative to peak 47), as discussed in the text.*

about the relationships among the proteins produced during rhinovirus 1A infection. Two principal features of the scheme distinguish it from the simpler model for EMC virus translation and processing (3). First, two rhinoviral polypeptides (47 and 38a) are produced from the S region; only one has been identified (2) for EMC virus (chain F) and poliovirus (NCVP X). A second novel feature of this scheme is the identification of more than one cleavage pathway for the P_2 precursor, which generate at least four discrete products (r-39a, r-39b, 38b, and 55). In addition, an appreciable portion of the P_2 family is degraded through unidentified pathways into products too small to be accounted for.

In the cleavage scheme of primary products, chain 76, by virtue of its smaller size, is represented as a cleavage product of 84. However, peak 76 is present in rather high amounts, even at the end of a short pulse (Fig. 2A). This suggests that an appreciable amount of 76 is generated as a primary gene product, as in translational mode "b." A similar situation has been proposed for translational processing of the analogous D chain of EMC virus (6).

Peaks 14 and 13 are both present at relatively high levels immediately after a pulse (Fig. 2E). This suggests that a component of that region of the gels is formed as a primary gene product, possibly as a product of the S region and produced by those translations which also produce 38a, as in mode "b."

Selective effect of pactamycin on the cleavage of chain 76. Pactamycin has been a very useful drug in mapping the translational position of polypeptides on mRNA. However, the rate of cleavage of chain 76 is retarded about 50% by pactamycin. This inhibitory effect results in an erroneous mapping order. Such an effect may be detected by examining mapping order at several times in a pulse-chase experiment.

The reason for the specific effect of pactamycin on the cleavage of 76 is not clear. It is of interest that polypeptide 76 is associated with ribosomes (T. J. Matthews et al., Fed. Proc.

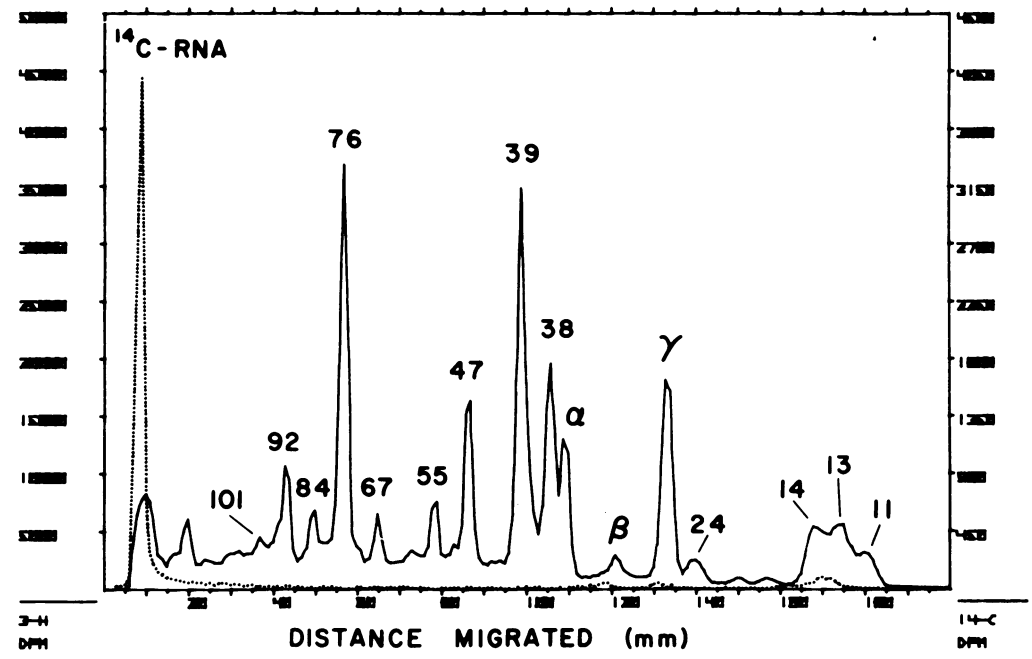

FIG. 9. *Electrophoresis of a whole cell lysate containing rhinovirus 1A polypeptides labeled with [^{3}H]leucine (—) and [^{14}C]uridine-labeled cellular RNA (· · · ·). H-HeLa cells were prelabeled with [^{14}C]uridine as described in Materials and Methods and infected as described in Fig. 3. At 210 min postinfection the virus polypeptides were labeled with 55 μCi of [^{3}H]leucine per ml for 15 min in medium AL. The cells were transferred to medium AH lacking radiolabel and incubated for a 40-min chase period. An SDS-disrupted whole cell lysate was prepared and dialyzed overnight against dialysis buffer (3). A sample of lysate equivalent to about 4 × 10^5 cells was analyzed on a gel (0.6 by 20 cm) as described in Materials and Methods, except that the electrophoresis buffer contained 0.05 M sodium 3-mercaptopropionate. Electrophoresis was for 18 h at 8 mA/gel.*

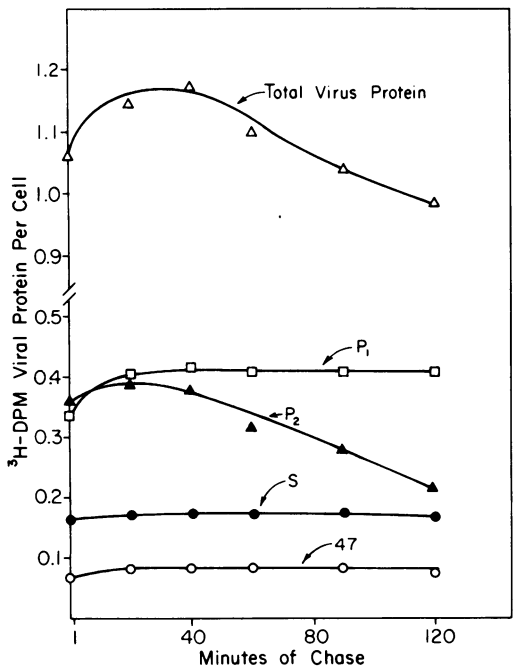

FIG. 10. *Loss of radiolabeled protein in rhinovirus 1A-infected HeLa cells during a long chase, with apparent degradation of the P_2 family of viral polypeptides. H-HeLa cells were prelabeled with [2-^{14}C]uridine as described in Materials and Methods. Immediately before infection, a sample of the cells was removed, washed twice with phosphate-buffered saline, and counted in a scintillation spectrometer to measure the amount of ^{14}C-radiolabel per cell. The cells were infected with rhinovirus 1A, pulse-labeled 15 min with [^{3}H]leucine, and chased as described in Fig. 9. At indicated intervals during the chase, samples were removed, disrupted in SDS, and analyzed on SDS-polyacrylamide gels as described in Fig. 9. The ^{14}C-radiolabel recovered from each gel was divided by the ^{14}C-label per cell to quantify the number of cells analyzed on each gel. The ^{3}H-radiolabeled peaks were normalized to the number of cells analyzed on the corresponding gel. Shown in the figure are the cellular contents throughout the chase period of: (i) △, total ^{3}H-labeled peaks [101, 92, 84, 76, 67, 60, 55, 47, ε, r-39, 38, α, β, γ, 24, 14, 13, and 11]; (ii) □, the P_1 family of polypeptides [101, 92, ε, α, β, and γ]; (iii) ▲, the P_2 family [84, 76, 55, r-39, and 38b]; (iv) ●, the S family [47 and 38a]; (v) ○, the individual polypeptide 47.*

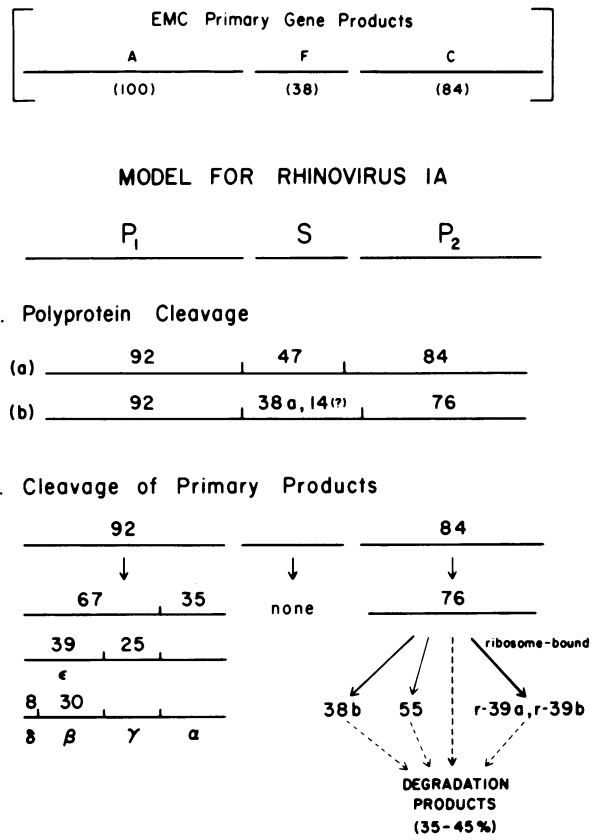

FIG. 11. *Model for the synthesis and processing of rhinoviral protein. Translation begins from an initiation site at or near the 5'-end (left) of the viral RNA and is terminated at a site at or near the opposite end of the molecule. The nascent polyprotein is cleaved to generate the primary capsid protein from the P_1 region and primary noncapsid proteins from the S and P_2 regions. Cleavage mode "a" generates a stable primary S product of molecular weight 47,000, whereas cleavage mode "b" produces a primary S product similar in size (molecular weight, 38,000) to the stable primary S product, F, of EMC virus. The cleavage modes are drawn to account for a similar mass of polyprotein (about 220,000 daltons). The cleavage scheme of the primary product of the rhinoviral P_1 (capsid) family is similar to that of both EMC and poliovirus capsid-related polypeptides. However, the rhinovirus P_2 primary product is cleaved at alternative sites to yield polypeptides of molecular weights 38,000, 55,000, and 39,000, and 35 to 45% of this family is degraded in 2 h to products too small to be detected.*

32:461, 1973); moreover, pactamycin binds to ribosomes (15). This raises the possibility that cleavage of polypeptides of the P_2 family may be connected with some function of the ribosome.

Furthermore, the fact that (with 76) only r-39a and r-39b are found associated with ribosomes, introduces the interesting possibility that the location of a protein within the cell may determine the mode by which it cleaves.

Relevance of the rhinovirus model to other picornaviral systems. The multiple cleavage modes operating in the S region of rhinovirus 1A are reminiscent of "ambiguous" cleavages in the capsid protein of poliovirus and coxsackievirus (8, 9, 11). The term ambiguous cleavage was first used by Cooper et al. (8) to explain the presence of more than four peptides in the capsid protein of poliovirus; it implies the occurrence of cleavages at abnormal sites. An alternative interpretation is that the rhinoviral cleavages are not abnormal but incomplete. We use the term "incomplete" cleavage to imply that all the cleavage sites are normal but are not always cleaved; the epsilon chain found in ME virions is an example (10). According to this idea the complexity in translation of rhinoviral proteins may be attributed not to differences in cleavage sites but merely to differences in the efficiency with which the sites are cleaved.

We think it is unlikely that multiple cleavage modes and degradation of viral protein are

unique to rhinovirus 1A. For example, the large number of unidentified peaks in electrophoretic profiles of poliovirus-infected HeLa cells (2) suggests a similar complexity in cleavages. Degradation in temperature-sensitive mutants of poliovirus has also been described (12); however, this study of rhinovirus 1A is the first report describing degradation confined to a single family of proteins.

It has been reported that mengovirus-infected L cells produce more capsid protein than noncapsid protein (14, 20) and that the ratio of capsid to noncapsid polypeptides progressively increases from early to late log phase in mengovirus-infected L cells and in poliovirus-infected HeLa cells (19). In our studies with rhinovirus 1A, no such change was evident during the period from 4 to 6 h postinfection (data not shown). It was suggested (14, 19, 20) that the enrichment in capsid polypeptides observed in the mengo- and poliovirus systems reflects the operation of a weak termination signal (detachment of ribosomes) which often interrupts translation near the midpoint of the viral mRNA.

An alternative possibility is that a second, internal initiation site exists. Celma and Ehrenfeld (7) report that the mRNA of poliovirus contains two sites capable of initiating protein synthesis in extracts from HeLa cells. Similarly, Abraham and Cooper (1) have suggested on the basis of preliminary tryptic mapping data that poliovirus RNA might be translated in vivo in two "units."

However, the data presented here on the translation of rhinovirus 1A are still compatible with the single initiation site-single termination site model. Multiple cleavage modes and degradation account for "asymmetric" production of rhinoviral proteins: that is, the S and P_2 families were undercounted (i) because only one product from the S region was scored, and (ii) because of degradation of the P_2 family. According to this idea the increasing asymmetry reported for the mengo- and poliovirus systems may be ascribed to deterioration in the precision of the cleavage processing and/or to cleavage by an increasing release of extraneous proteolytic enzymes as the cells progressively deteriorate during infection. Thus, until multiple cleavage modes and degradation of viral proteins are excluded, apparent inequalities in family stoichiometry do not provide compelling evidence for internal initiation or termination sites.

ACKNOWLEDGMENTS

This research was supported in part by grant VC-26E from the American Cancer Society. T. M. was supported by Public Health Service postdoctoral training grant T32-CA09075 from the National Cancer Institute. R. R. R. holds a Faculty Research Award (PRA-106) from the American Cancer Society.

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