PRIMARY GENE PRODUCTS OF AN ARBOVIRUS

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Summary: Semliki Forest virus (SFV) structural proteins were labeled equally well by a short radioactive amino acid pulse followed by a long or a short chase. The structural proteins of SFV appear to be primary gene products. These results are in marked contrast to findings on protein synthesis directed by poliovirus.

One of the most interesting recent findings in animal virology has been the discovery that poliovirus proteins are not primary translation products of the poliovirus genome (Summers and Maizel, 1968). Apparently large precursor proteins are the primary gene products and these proteins are converted by several cleavages into the structural and functional proteins of the virus. On the basis of these findings it was proposed that this unusual method of protein synthesis may be due to an inability of animal cells to carry out internal initiation of protein synthesis employing a polycistronic messenger RNA such as poliovirus messenger RNA (Jacobson and Baltimore, 1968).

It was of interest to investigate what the primary gene products of a group A arbovirus might be since polioviruses and members of this group have many characteristics in common. The replication mechanism of these virus groups is similar and, based on the size of the polioviruses and group A arbovirus cores, these virus groups probably contain RNA genomes of approximately the same size (Friedman, 1968).

Polyacrylamide gel electrophoresis analysis was employed to study the kinetics of synthesis of Semliki Forest virus (SFV) proteins. It was found that the virus structural proteins were equally labeled by a short radioactive amino acid pulse followed by a long or a short chase. This was true even when the

labeling of the viral proteins was carried out in the presence of amino acid analogues (Jacobson and Baltimore, 1968).

MATERIALS AND METHODS

Chick cell monolayers and SFV pools were prepared as previously described (Friedman et al., 1966). To study early virus protein synthesis actinomycin D treated cells were infected for 4 hrs. in the presence of guanidine . HCl (3 mg/ml), a reversible inhibitor of virus replication. They were then washed and virus replication was permitted to proceed (Friedman, 1968b). In the case of late virus protein synthesis actinomycin D treated cells were infected at a multiplicity of 50:1.

In all experiments cells were washed and labeled for 2 min. with tritiated or ^{14}C-leucine, -isoleucine and -valine in a medium otherwise free of these amino acids. The cells were rapidly washed twice in Eagles medium containing 10 times the normal concentration of all amino acids, with a 50 to 500 fold-excess of leucine, isoleucine, and valine and were then incubated in this medium for 2 or 30 min. The cells were then washed and cytoplasmic extracts were prepared in the presence of 2% mercaptoethanol by the methods of Summers et al (1965). Portions of the extracts containing about 15,000 counts per minute were then analyzed on 7 cm, 5%, 7%, or 10% gels at 5 ma per gel.

After electrophoresis the gels were suspended in 10% trichloroacetic acid for 14 hrs and cut into 1.1 mm segments on a gel slicer. The slices were placed in scintillation vials, solubilized with 30% hydrogen peroxide, and counted in a liquid scintillation spectrometer (Moss and Ingram, 1965).

RESULTS

Virus proteins were labeled with ^{14}C amino acids one hour after the removal of guanidine in order to study early virus protein synthesis. Under these conditions only viral proteins appear to be labeled (Friedman, 1968b). The labeling was followed by chases with ^{12}C amino acids for 2 (Fig. 1A) or 30 (Fig. 1B) minutes. The results of acrylamide gel electrophoresis analysis of

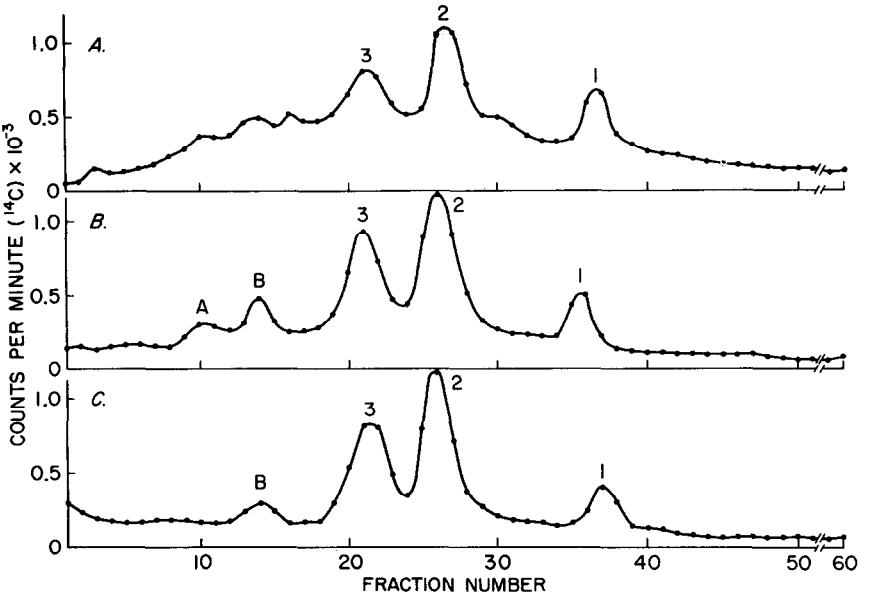

Figure 1. Labeling of early Semliki Forest virus proteins during a pulse and subsequent chase. 1.5 x 10^7 actinomycin D treated cells were infected at a virus:cell multiplicity of 500:1 in the presence of guanidine. After 4 hrs the cells were washed. After 1 additional hr they were incubated for 2 min with ^{14}C amino acid, 10 uC each of ^{14}C-leucine (263 mC/mM), ^{14}C isoleucine (247 mC/mM) and ^{14}C valine (200 mC/mM). The cultures were then washed and chased with large excesses of ^{12}C amino acids and at (A) 2 min or (B) 30 min cytoplasmic extracts were prepared and volumes containing about 15,000 counts per minute were analyzed on 7% acrylamide gel. (C) was prepared from extracts of cells labeled continuously for 30 min with ^{14}C amino acids. The viral proteins are designated 1 (core protein), 2 (envelope protein) or 3; A & B are non-structural viral proteins. The cathode is to the left in both figures.

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the virus proteins produced are shown in Fig. 1 where they are compared to those seen after 30 min of continuous labeling (Fig. 1C). The proteins 1, 2, and 3 (Friedman, 1968b) were labeled after the short chase. Some polydisperse counts were present near the cathodal end of the gel in the short chase experiment (Fig. 1A). No significant quantitative or qualitative changes in proteins 1, 2, or 3 were seen following the 30 min chase (Fig. 1B). In this sample, however, two distinct peaks of non-structural proteins, A & B, were apparent. Analysis of continuously labeled proteins showed 4 peaks, proteins 1, 2 and 3 and non-structural protein B. Protein A was not apparent in this particular experiment but was usually seen in similar experiments employing

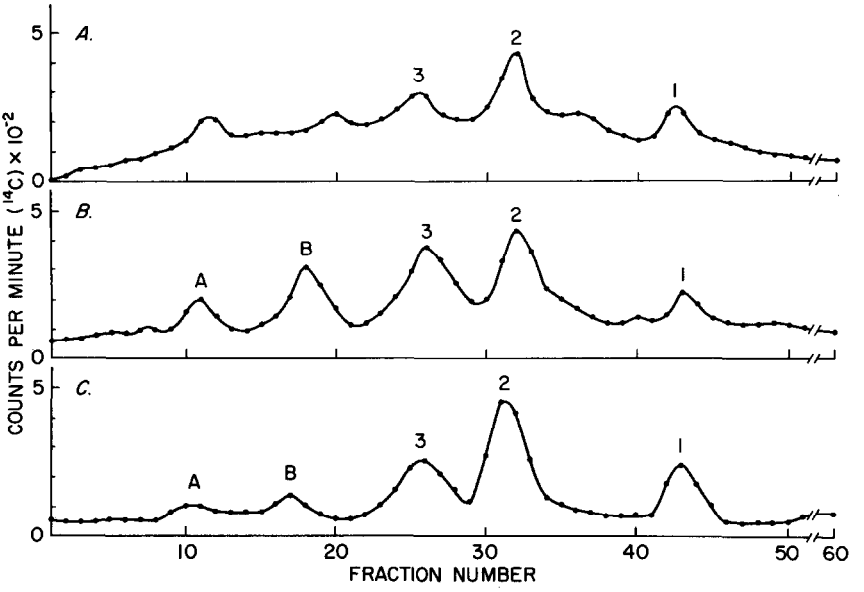

Figure 2. Labeling of early Semliki Forest virus proteins during a pulse in the presence of amino acid analogues and subsequent chase. Cells were treated and extracts prepared and analyzed as described in Fig. 1 except that amino acid analogure were present during the 2 min labeling period. (A) 2 min chase. (B) 30 min chase. (C) Continuous labeling for 30 min. The radioactive amino acids were diluted in Geys salt solution containing fluorophenylalanine (2.5 mM), canavanine (3.3 mM), ethionine (1.8 mM) and azetidine-2-carboxylic acid (5.4 mM). Results similar to those shown were obtained with medium also containing thialysine (2.5 mM) and azatrytophane (1 mM).

7% or 10% gels (Fig. 2C for example). In general, results on 10% gels were like those shown in Fig. 1 except that the peaks were better resolved. Similar results were noted when early virus proteins were labeled with ^{14}C amino acids in the presence of amino acid analogues (Fig. 2A, B and C).

When viral proteins were pulse labeled with ^{3}H amino acids and then chased with cold amino acids at 5 hrs. after infection, again under conditions where almost all of the protein synthesis was virus directed (Friedman, 1968b), similar results in the presence or absence of amino acid analogues were seen.

Additional experiments were performed in an attempt to find precursor proteins of SFV structural proteins. Labeling and chasing at 32° in order to inhibit precursor breakdown and performing the electrophoresis in 5% gels also failed to reveal large viral proteins.

DISCUSSION

The present results differ from those seen in poliovirus infected cells (Summers and Maizel, 1968). In the case of Semliki Forest virus the structural proteins appear to be primary gene products. Under the conditions employed no evidence for a large virus precursor protein was found. Three of the possible reasons for these findings are: 1. A large precursor protein may exist but could not be identified in these experiments because of rapid cleavage This is unlikely as very short pulses were employed. Also experiments employing amino acid analogues failed to elicit any evidence for the existence of this postulated protein. In the case of poliovirus, incorporation of amino acid analogues into the large precursor protein inhibited its subsequent complete cleavage (Jacobson and Baltimore, 1968). 2. The messenger RNA of arboviruses might consist of a number of monocistronic RNA elements. Since the messenger RNA of arboviruses has not yet been identified, this is possible, although no evidence for the existence of small arbovirus RNA's has been found. 3. The messenger RNA of arboviruses is polycistronic and internal initiation of protein synthesis can take place on such an RNA form in animal cells.

The inability to identify the non-structural viral proteins A and B in pulse labeled cells in which labeling was followed by a short, as opposed to a long chase was a consistent finding. It may be that proteins A and B require several minutes for their production or are assembled from some of the polydisperse elements that appeared near the cathodal end of the gel in the 2 min chase experiments several minutes after the synthesis of these elements.

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