EFFECT OF ELEVATED INCUBATION TEMPERATURE ON NON-STRUCTURAL ARBOVIRUS PROTEINS

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The study of the replication of the arbovirus A group of animal viruses may help to solve some of the problems of transcription and translation of genetic information in animal cells, just as the study of the replication of small RNA bacteriophages promises to do in bacteria; indeed, the replication patterns of arboviruses and of the small RNA bacteriophages have some characteristics in common. Arboviruses, which grow rapidly in the cytoplasm of most animal cells, contain a single stranded RNA with a molecular weight of about 2×10^6 Daltons (Wecker, 1959). The RNA of the arboviruses is produced on a double-stranded template (Friedman et al, 1966); a replicative intermediate (RI) form is involved in the viral RNA replication mechanism (Friedman, 1968a). The RI structure also participates, in some manner, in arbovirus protein synthesis (Friedman, 1968b).

Recent studies on protein synthesis by the group A arbovirus, Semliki Forest virus (SFV), have suggested that the genome of the virus directs the synthesis of five proteins (Friedman, 1968c). These include (Fig. 1) three structural proteins (proteins 1,2,&3) and two non-structural proteins (proteins A & B). In cells infected in the presence of guanidine, washed after 4 hrs to reverse the guanidine inhibition of virus replication, and then incubated with ^{14}C amino acids 1-2 hrs after guanidine reversal, these are the only five proteins present in the cytoplasm in significant amounts on analysis by polyacrylamide gel electrophoresis. When the proteins are labeled 0-1 hrs after guanidine reversal, an additional protein, protein C', is also seen. Results suggest that protein C' is cellular, not viral, in origin (Friedman, 1968c).

When infected cells are incubated at 42°, a temperature at which SFV does not replicate (Ruiz-Gomez and Isaacs, 1963), analysis of the proteins labeled from 0-1 hrs after guanidine reversal showed a decrease in concentration of proteins A & B (Friedman, 1968c). The following experiment was performed in order to determine whether proteins A & B were stable at the 42° incubation temperature.

Methods

Chick embryo fibroblast monolayers containing about 5×10^6 cells (Taylor, 1965) were treated for 1 hr with actinomycin D (2 μg/ml) and then infected with SFV at a multiplicity of 200 in the presence of guanidine (3 mg/ml). After 4 hrs, the cells were washed and incubated for 1 hr at 37° in the presence of 10uC/ml of a purified ^{14}C amino acid mixture in otherwise amino acid free medium. The monolayers were again washed and incubated ("chased") for an additional 1 hr at either 37° or 42° in the presence of an excess of ^{12}C amino acids, Eagle's medium with a four-fold increase in the usual concentration of all amino acids. Proteins were then extracted from cell cytoplasm, solubilized, and 10 ul of this extract containing about 15,000 counts per min of ^{14}C was analyzed by polyacrylamide gel electrophoresis (Summers et al, 1965). The gels were sliced, fixed, stained, and dehydrated (Fairbanks et al, 1965). Autoradiography was performed on the dehydrated gels by exposing films to gels for 7 days. Scans on the autoradiograms were performed in a Joyce-Loebl microdensitometer (Nathans et al, 1966).

Results and Discussion

The results (Fig. 1) show that the previously seen (Friedman, 1968c) pattern of viral proteins is stable to a 1 hr chase with ^{12}C amino acids at 37° (Fig. 1A); that is, before the chase, the pattern was similar to that seen in Fig. 1A. Moreover, in the cells incubated at 42° during the chase, proteins A & B are decreased in amount (Fig. 1B).

Therefore, proteins A and B may be produced at 42°; their rate of destruction in the cell at this temperature would then exceed their rate of synthesis.

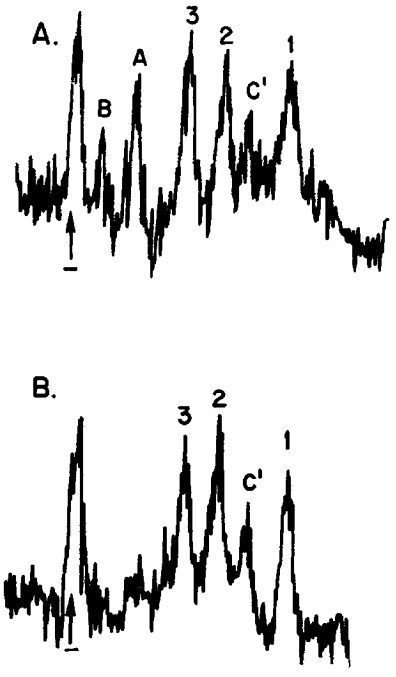

Figure 1. Polyacrylamide gel electrophoresis patterns of viral proteins from infected chick cells. Microdensitometer scans from radioautograms are shown. Viral proteins were labeled by incubating infected CEF with 10 uC/ml of ^{14}C amino acids for 1 hr at 37° after washing out guanidine. The cells were then incubated for an additional hour with ^{12}C amino acids at A) 37° or B) 42°. Proteins were extracted from the cytoplasm and analyzed as described in the text. Arrows indicate the cathodal end of the gel.

On the other hand, it is possible that these proteins are not produced at 42° and that they normally have a rapid turnover rate. There is some evidence that viral RNA polymerase must be continually generated during the course of infection (Levintow et al, 1962). In either case, the destruction of these proteins, possibly by cellular proteolytic enzymes, must reduce them to fragments small enough to be lost in the dialysis step in the processing of cell extracts for electrophoresis.

The general methods employed in this study may be useful in the analysis of selected temperature sensitive mutants of arboviruses (Burge and

Pfefferkorn, 1966). Through study of such mutants, the nature of the functional proteins of arboviruses may be elucidated.

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