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REPLICATION OF VEE VIRUS

by

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ENGLISH TITLE: REPLICATION OF VEE VIRUS

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NOTICE

It has been demonstrated earlier [1] that in the cells of the fibroblasts of a chicken embryo which have been infected with Venezuelan Equine Encephalitis virus a double-helix replicative form (RF) RNK develops, becoming converted into a multiple-helix replicative intermediary form (RPF), which ensures the synthesis of daughter RNA and some forms of functional RNA with sedimentation constants from 19 to 38 S. On the model of RNA-containing phages it was determined [2] that the multiple-helix replicative intermediary form associates itself with the fibosomes of the host cells, forming a so-called replicative complex (RK), which ensures simultaneously replication and the synthesis of virus- specific proteins.

In the present communication we show that the replicative intermediary form of VEE functions in cells infected with the virus in the replicative complex form.

The VEE - chicken fibroblasts system described earlier [3] was used. The infecting dose of the virus came to 50-1000 bo. units per cell, actinomycin D (2 µg/ml) were added immediately after contact of the virus with the cells, the newly synthetized virus RNA was labelled with H^3-uridine (2-5 µC/ml), and the proteins with C^{14}-aminoacid hydrolyzate of chlorella (2 µC/ml) at various periods after infection.

Destruction of the cells was carried out in a homogenate buffer (tris HCL 0.05 M pH 7.4: KCl 0.05 M; $MgCl_2$ 0.005 M; saccharose 0.25 M) with the help of a Downs homogenizator. The nuclei and the residue of cytoplasm were precipitated by centrifuging at 3,000 g, the so-called mitochondrial fraction at 15,000 g, and the light polysomes and monosomes were separated from the supernatant at 100,000 g. RNA was separated out by the cold detergent-phenol method [4] from all fractions; in addition, RNA was separated out from the nuclei at 70° [5]. Analysis of the virus-specific polysomes was carried out after centrifuging in a saccharose gradient of 17-40 and 15-60%. The radioactivity count was carried out in a Packard fluid scintillation counter after precipitation of the material with trichloracetic acid in milliporous filters.

In Figure 1 we show the distribution of the labels of the newly synthetized RNA by cell fractions from 3 to 4 hours after infection. As is apparent from these data, the main quantity of the newly synthetized RNA is contained in the nuclei and the mitochondrial fraction, in which connection double-helix RNA (RF) predominates in the nuclei, while in the fraction of the mitochondriae and the heavy polysomes single-helix RNA (replicative intermediary form) predominates. In the infected cells processed with actinomycin D the inclusion of H^3-uridine was insignificant, RNA resistant to ribonucleaze as detected in the double-helix form, also in insignificant quantities (transport RNA).

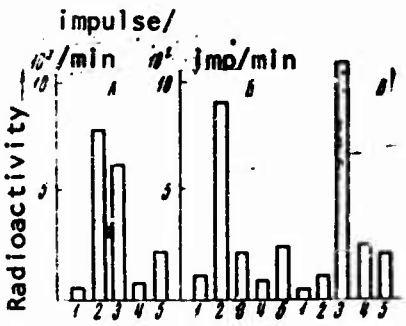

Figure 1. Distribution of Stain (H-Uridine) of Newly Synthetized RNA in Fractions of Cells of Chicken Fibroblasts Infected with VEE. H^3-uridine was added 3 to 4 hours After Infection. A. Total RNA; B. Supernatant after processing 2 M NaCl; C. Precipitate after processing 2 M NaCl. Tests B and C were processed with ribonucleaze (15 µg/ml). 1. RNA from fragments of cells, extracted at 4°; 2. RNA from nuclei, extracted at 70°; 3. RNA from fractions of mitochondriae and heavy polysomes; 4. RNA from fraction of polysomes; 5. RNA from supernatant after precipitation of polysomes.

Upon centrifuging of the cell homogenate in the 17-40% saccharose density gradient after precipitation of the mitochondrial fraction, the label of the newly synthetized RNA of the protein was detected in the polysomes, to begin with in the 160-180 S field, then in later time periods in the 250 S field and in the precipitate (Figure 2). Inasmuch as with this system the heavy polysomes settle on the bottom of the test tube, tests were set up with centrifuging of the homogenate of infected cells in the 15-60% saccharose gradient without preliminary precipitation of the mitochondrial fraction at 15,000 g. In Figure 3 we show the results of one of the tests. As is apparent from these data, the basic label of the newly synthetized RNA and proteins settles in the fraction of the heavy polysomes (the so-called mitochondrial fraction).

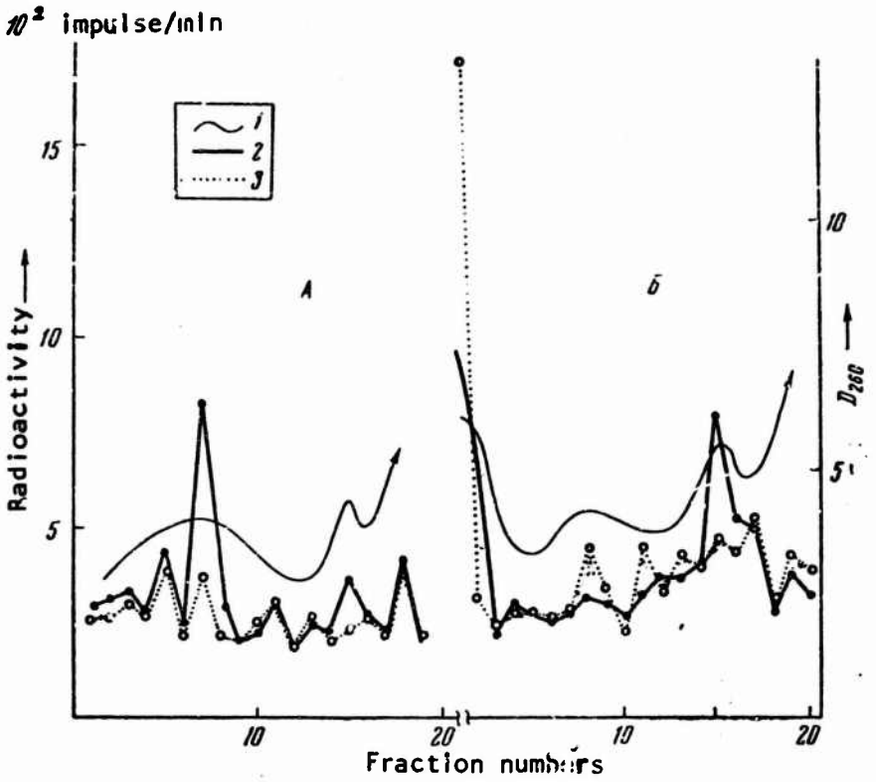

Figure 2. Distribution of Stain of Newly Synthetized RNA (H^3-uridine) and of Protein (C^{14}-aminoacid mixture) upon Polysomes of cells of Chicken Fibroblasts Infected with VEE. A. Isotopes added 1 to 1 1/4 hours after infection; B. 2 1/2 to 2 3/4 hours after infection. Centrifuged in 17-40% Saccharose gradient at 25,000 rpm for 1 1/2 hours. 1. Optical density; 2. Inclusion of H^3; 3-C^{14}. Bottom of gradient to left, top to right.

Since the basic part of the multiple-helix virus RNA (replicative intermediary form) is found in this same fraction, it seemed logical to study its sedimentation properties. In Figure 4 we set for the results of one of the texts. As is apparent from these data, ribonucleaze-resistant RNA separated out from the heavy polysomes is heterogeneous and has constants of sedimentation from 18 to 30 S with a principal peak in the range 25-26 S, i.e., it corresponds completely to the replicative intermediary form of this virus earlier described by us.

Inasmuch as it is associated with ribosomes one may draw the conclusion that the replicative intermediary form in the infected cells functions

in the form of a replicative complex upon which the replication of the virus RNA and the synthesis of the virus-specific proteins run their course simultaneously.

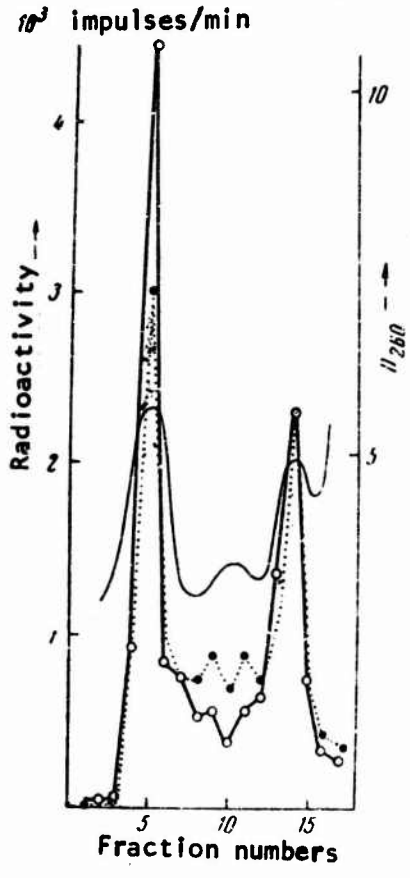

Figure 3. Distribution of Newly Synthetized RNA and of Protein on Polysomes of Cells of Chicken Fibroblasts Infected with VEE. Centrifuged in Saccharose Gradient 15-16% at 25,000 rpm for 2 1/2 hours. Designations same as in Figure 2.

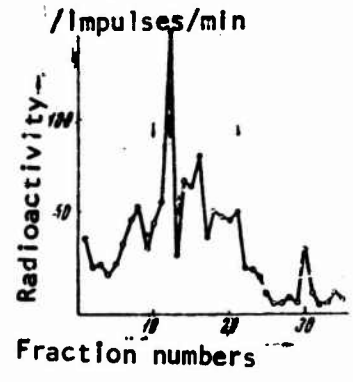

Figure 4. Distribution of Stain of Newly Synthetized RNA of VEE, drawn off from the field of the heavy polysomes and precipitated by 2 M NaCl, in the 5-20% saccharose gradient. Centrifuged at 20,000 rpm for 16 hours, fractions of gradient processed with ribonucleaze (2 µg/ml) after centrifuging. Positions of markers - RNA cell peaks at 28 S and 18 S respectively - are indicated by arrows.

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13. ABSTRACT

The authors report the results of tests which they consider to demonstrate that in cells infected with Venezuelan Equine Encephalitis virus the replicative intermediary form of this virus functions in what they call a replicative-complex form, which ensures simultaneously both replication and the synthesis of virus-specific proteins.

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