Acta virol. **26**: 427—431, 1982

RNA SYNTHESIS IN THE L CELL-SV5 SYSTEM

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Received July 15, 1981, revised May 15, 1982

Summary. — Comparative analysis of the ribonucleoprotein RNA synthesis was performed in two persistently infected L cell systems. In the first (LSV5-I), cells were infected with the cloned standard SV5 virus, in the second (LSV5-II), infecting virus had been enriched with defective interfering particles (DIP). The LSV5(I) system in its 40th-42nd passages was similar to LSV5(II) at the 2nd-3rd passage levels. There was shown that the ribonucleoprotein ^{3}H-RNA synthesized falls into two classes: the minor corresponding to 50S viral RNA and the major revealing predominantly low molecular RNA. The decrease of the synthesis of the heavy viral RNA fraction and the prevalence of the low molecular RNA promoted the limitation of infection, the survival of cells and prolonged the carrier state. The possible correlation between low molecular RNA synthesis and DIP formation in the L cell-SV5 system is discussed.

Key words: *parainfluenza virus SV5*; *persistent infection*; *defective interfering particles*; *viral RNA synthesis*

Introduction

In stable mouse L cell line persistently infected with the parainfluenza virus SV5 the same virusspecific components — ribonucleoprotein (RNP) and virions — have been produced by early passages than in acute infection. In the course of late passages, the virus replication was sharply inhibited; the analysis of ^{3}H-uridine incorporation into the RNP RNA and into its light fraction indicated that the 50S RNA synthesis declined, while the slower sedimenting heterogeneous RNA fraction prevailed (Azadova, Zhdanov, 1980).

This paper presents the results of ^{3}H-labelled RNA synthesis in two persistently infected L cell systems. The results suggest a relationship between the accumulation of low molecular virus-specific RNA and DIP formation in LSV5 cells.

Materials and Methods

Cells and viruses. Stable mouse cells (line L-1210), were obtained from the Cell Culture Laboratory of D. I. Ivanovsky Institute of Virology. The cells were cultured in Eagle's medium supplemented with 10% or 5% of foetal calf serum (FTS). Simian parainfluenza virus SV5

(strain 12181) was passaged in 8-day-old chick embryos. The virus was collected after 5 days incubation at 37 °C. The titres were determined in haemagglutination reaction using 0.5% chicken or guinea-pig erythrocytes. The standard virus SV5 was obtained by 4-fold passaging at high dilution in chick embryos. The virus obtained had an infectious titre of $10^{6.5-7.0}$ EID_{50}/ml and its haemagglutination activity was 64—128 haemagglutination units (HA units) per ml.

The SV5 virus population enriched with DIP was obtained by 5 to 6-fold passaging of the undiluted virus in chick embryos. This virus revealed an infectious titre of $10^{4.0}$ EID_{50}/ml but a high HA activity — 1024 HA units/ml. After infection of the cell culture with this virus, low-molecular RNA synthesis dominated.

Infectivity determination. Titration of intracellular and extracellular viruses was carried out in L cell monolayers grown in 50 ml bottles. Each bottle was inoculated with 0.5 ml of 10-fold dilution of the tested sample. After adsorption the virus was removed, the monolayer was washed with Hanks' medium and medium Eagle with 2% FCS was added. The results were red daily till the 4th-5th day. Alternatively, chick embryos were inoculated with 0.2 ml 10-fold dilution of each tested sample.

Persistently infected cells. LSV5 (I) cells were prepared with the standard infectious SV5 virus; for the LSV5 (II) cell system the SV5 virus population was enriched with DIP.

Sedimentation analysis of RNP ^3H-RNA. Cell monolayers of both types were labelled with ^3H-uridine in the presence of 1.5 μg/m actinomycin D for 12—14 hr; thereafter, cells were washed with ice-cold 0.01 mol/l Tris-HCl buffer pH 7.4 supplemented with 0.1 mol/l NaCl and 0.005 mol/l EDTA scraped off into the same buffer and homogenized in a Down's homogenizer. Nuclei were precipitated by centrifugation at 3.000×g for 30 min and then the cytoplasmic extract was layered over a continuous saccharose gradient (20-60-80% mass to volume) in Tris-HCl buffer. The centrifugation was carried out in SW 27.1 rotor at 23.000 rev/min for 3 hr at 4 °C. Fractions with the density vcorresponding to RNP were collected, precipitated and resuspended in Tris-HCl buffer. To isolate the ^3H-labelled RNA, 0.3 ml aliquotes of the corresponding RNP samples were supplemented with 60-70 μl of 10% SDS incubated at 60 °C for 2 min and put on the top of a 5—20% saccharose gradient in Tris-HCl. Centrifugation was performed in SW 50 Beckmann rotor at 25 °C and 35,000 rev/min for 150 min. Ribosomes coming from chick embryo fibroblasts marked the positions of 28S and 18S RNAs.

Reagents. Actinomycin D (Calbiochem, U.S.A.); saccharose, DNase free (Sigma, U.S.A.); SDS (Serva, F.R.G.); dextran sulphate (Pharmacia, Sweden).

Results

The influence of SV5 DIP on the course of infection

As shown on Fig. 1, destruction of the cell monolayer was observed 4-5 days after infection of L cells with the standard SV5 virus. The infectious titre has been determined in chick embryos as described above. When the

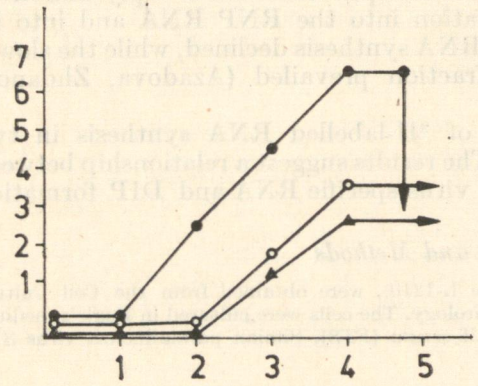

Fig. 1.

Replication of SV5 in L cells

L cells were infected with standard virus (●——● 3—5 $TCID_{50}$/cell), with DIP (○——○ 0.3—0.5 $TCID_{50}$/cell) or subsequently with standard virus and DIP (▲——▲) 0.3—0.5 $TCID_{50}$/cell. At daily intervals the virus yield was determined as described in Materials and Methods.

cells were inoculated with the SV5 virus enriched with DIP, the cytopathic effect was insignificant and it was possible to transfer the cells within a week. Further challenge of cells either with the standard SV5 or with SV5 DIP, in contrast to inoculation of DIP only, enhanced the interference and decreased the virus yield in the absence of any cytopathic changes.

Comparative analysis of RNA synthesis in persistent, acute and mixed infections

To establish the LSV5 (I) persistent system, the standard SV5 was used in the course of 40-42 passages. As mentioned, no infectious virus was produced

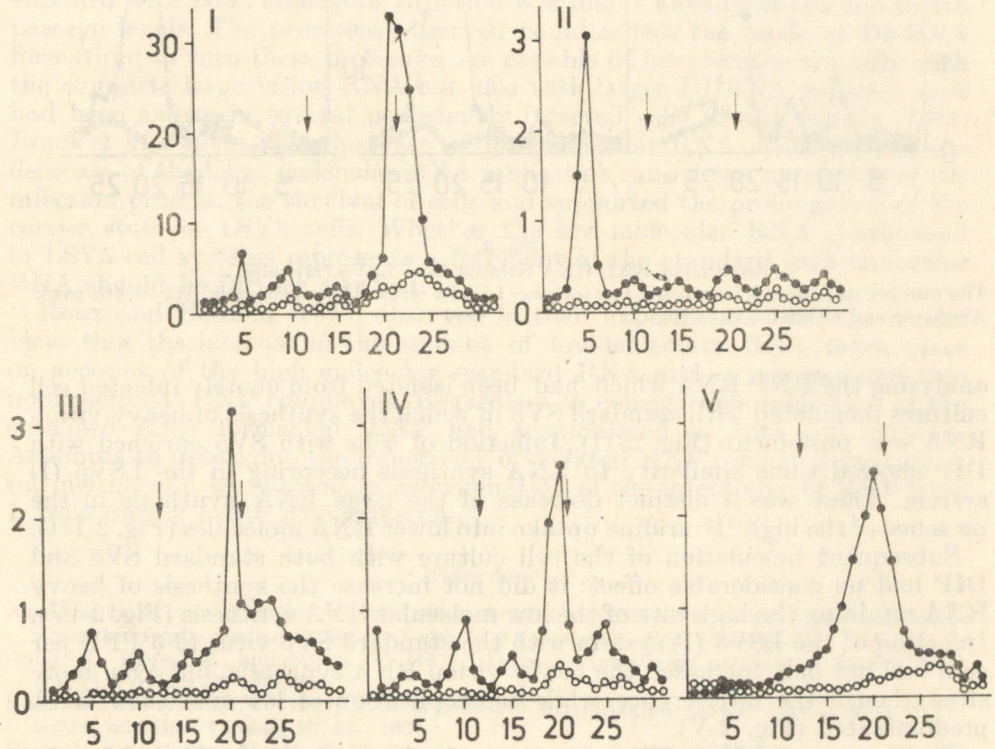

Fig. 2.
Comparative analysis of RNP RNA isolated from L cells infected with SV5 (●) and from uninfected controls (○)
Cells were labelled with ^{3}H-uridine in the presence of actinomycin D (see Materials and Methods). Abscissa: fraction number; ordinate: distribution of radioactivity (counts/min $\times 10^{-3}$).
I — LSV5 (I) cells persistently infected with the standard virus (42nd passage); II — III — LSV5 (II) cells infected with SV5 (3 — 5 $TCID_{50}$/cell) and with SV5 DIP (0.4—0.6 $TCID_{50}$/cell), respectively; IV — subsequent infection with the standard virus and DIP (3 — 5 and 0.4 — 0.6 $TCID_{50}$/cell, respectively); V — LSV5 (I) cells subsequently infected with the standard SV5 virus (3 — 5 $TCID_{50}$/cell).

in these cells, but massive accumulation of RNP had occurred (Azadova, Zhdanov, 1980). The analysis of RNA isolated at the 42nd passage level from the LSV5 (I) derived RNP in SDS-saccharose gradient showed a substantial decrease of heavy RNA synthesis, while the low molecular RNA synthesis predominated (Fig. 2-I). This difference became more apparent

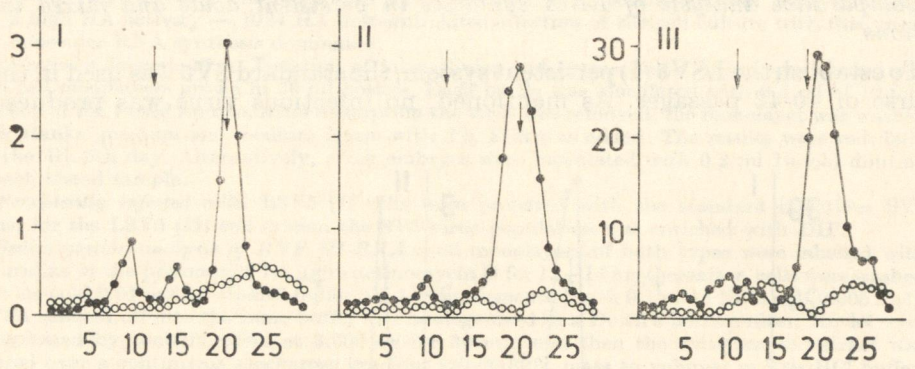

Fig. 3.
Analysis of RNP RNA isolated from LSV5 (II) cells
The number of passages is indicated as follows: I — 1st week, II — 2nd week, III — 4th week. Abscissae and ordinates as in Fig. 2.

analysing the RNP RNA which had been isolated from acutely infected cell cultures inoculated with standard SV5 in which the synthesis of heavy virion RNA was prominent (Fig. 2-II). Infection of cells with SV5 enriched with DIP showed some similarity to RNA synthesis occcuring in the LSV5 (I) system. There was a distinct decrease of the large RNA synthesis in the presence of the high ^{3}H-uridine uptake into lower RNA molecules (Fig. 2-III).

Subsequent inoculation of the cell culture with both standard SV5 and DIP had no considerable effect: it did not increase the synthesis of heavy RNA retaining the high rate of the low molecular RNA synthesis (Fig. 2-IV). Infection of the LSV5 (I) system with the standard SV5 virus (3-5 PFU per cell) had not only increased the heavy virion RNA synthesis, but even more strengthened the interference, while the replication of low molecular RNA predominated (Fig. 2-V).

The persistent LSV5 (II) system was obtained after infection of L cells with DIP of SV5 as described. In the LSV5 (II) system the carrier state developed quickly: there was no cytopathic effect, immunofluorescence showed the presence of virus-specific antigens in 50—60% of cells, the cells could be passaged by intervals of 4-5 days. The analysis of RNP RNA derived from LSV5 (II) system in SDS-saccharose gradient has shown that the synthesis of light RNA predominated already within the first passage in the presence of relatively high ^{3}H-uridine incorporation into the heavy RNA fraction (Fig. 3-I). At this passage level the replication of RNA did not substantially

differ from that of the LSV5 (I) system in the 42nd passage (compare Fig. 2-I). By 2nd passage of the LSV5 (II) cells, the heavy RNA synthesis has considerably decreased, while the low-molecular RNA synthesis prevailed (Fig. 3-II). Similar processes were seen in the 4th passage (Fig. 3-III).

Discussion

The analysis of RNP RNA synthesis was performed in two carrier cultures infected with SV5. In LSV5 (I) cells, infected with the standard cloned virus, a distinct decrease of the SDS virion RNA occurred at the 40th-42nd passages along with the increased low-molecular RNA synthesis. In the LSV5 (II) cell system, in which the carrier state had been established by means of SV5 enriched with DIP, analogous situation was found already at the 2nd to 4th passage levels. The processes observed could reflect the mode of DI-RNA formation; in turn these molecules are capable of interference not only with the complete large virion RNA but also with larger DI-RNA genomes as it had been shown in several persistently infected cells (Guild, Stollar, 1977; Roux *et al.*, 1980). The increase of low-molecular RNA synthesis and the decrease of the large molecular RNA replication caused the restriction of the infection process, the survival of cells and supported the prolongation of the carrier state in LSV5 cells. Whether the low-molecular RNA synthesized in LSV5 cell systems represents a fragment of the standard high-molecular RNA should be further clarified.

Roux and Holland (1979) observed in their experiments with the Sendai virus that the increase in the amount of low-molecular RNA takes place on account of the high-molecular standard RNA and is incorporated into nonfunctional RNP complexes. Regardless to considerable inhibition of the standard RNA formation, RNP had accumulated in both cell systems. Attempts to determine the infectivity of isolated RNP remained unsuccessful; neither did we succeed in determination of the infectivity of RNP from L cells during acute SV5 infection.

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