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RNA SEQUENCES OF OPPOSITE POLARITIES TRANSCRIBED FROM A LARGE PART OF VACCINIA VIRUS GENOME ARE ACCUMULATED IN THE CYTOPLASM

V. I. CHERNOS, N. L. VARICH, T. P. ANTONOVA, I. V. SYCHOVA, N. V. KAVERIN

The Moscow Research Institute of Virus Preparations, U.S.S.R. Ministry of Public Health, 109088 Moscow, U.S.S.R.

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Summary. — Labelled vaccinia virus DNA was used in saturation-hybridization experiments with RNA extracted from virus-infected cells. An excess of "late" cytoplasmic RNA converted 45 % of DNA into DNA-RNA hybrids, whereas 17 % of DNA could be converted into hybrids by RNA extracted from purified nuclei. RNA-RNA hybrids obtained from cytoplasmic RNA by self-annealing and ribonuclease treatment, were melted and used in hybridization with DNA: 36 % of DNA was hybridized at the maximal concentration used.

Key words: vaccinia virus; hybridization; transcription

The transcription of both viral DNA strands in cell nucleus with the subsequent retention of antimessenger sequences in the nucleus and a selective transport of mRNA to cytoplasm has been described for several DNA-containing viruses (Alony, 1972; Kozak and Roizman, 1975; Acheson 1976; Flint, 1977). Poxviruses are generally regarded as "cytoplasmic" viruses; however, synthesis of viral RNA in the nucleus has been reported (Gafford and Randall 1975, 1976). Recently, data were presented suggesting that the RNA sequences transcribed from a part of viral DNA are accumulated in the nucleus (Bolden *et al.*, 1979).

In previous experiments (Varich *et al.*, 1979), we used hybridization of labelled vaccinia virus DNA to an excess of RNA-RNA hybrids in order to evaluate the fraction of the viral genome transcribed "symmetrically", i.e. with the formation of RNA strands of both polarities. RNA preparations extracted from whole cells were used. In the present report we describe an attempt to characterize in this way cytoplasmic RNA in order to check whether antimessenger sequences are accumulated in the cytoplasm of infected cells and to estimate the fraction of DNA giving rise to such sequences.

The procedures of vaccinia virus (strain MM) propagation, purification and labelling, as well as cultivation and infection of chick embryo cells, RNA extraction, RNA-DNA and RNA-RNA annealing have been described (Kaverin *et al.*, 1975; Varich *et al.*, 1979). The ultrasonic treatment of the virus prior to inoculation was omitted since Boone and Moss (1978) reported that such treat-

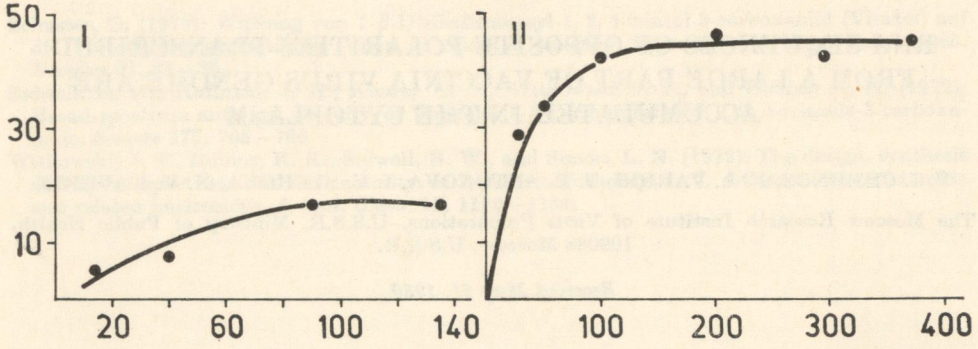

Fig. 1.

Hybridization of vaccinia virus ^{3}H-DNA with nuclear (I) and cytoplasmic (II) RNA
The cells were infected at a m. o. i. of 10 PFU/cell and incubated ofr 8 hr without inhs bibitors. Abscissa: μg RNA per sample; ordinate: % DNA in the RNA-DNA hybrid.

ment may impair the normal course of early viral transcription. The separation of cell nuclei and cytoplasm was performed as described by Gafford and Randall (1975) (citric acid — triton-X-100 technique). The purity of the nuclei was checked electron microscopically. For the preparation of cytoplasmic extracts, a milder technique (Inglis *et al.*, 1977) was used to avoid the breakage of nuclei and the leakage of the nuclear material into the cytoplasm. The cells were suspended in a hypotonic buffer (0.01 M Tris. HCl, pH 7.4, 0.01 M NaCl, 0.016 M $MgCl_2$, 0.001 M $CaCl_2$), disrupted with 10 strokes in Dounce homogenizer, and nuclei were pelleted together with unbroken cells by low speed centrifugation. This procedure, when applied to $_{3}$H-thymidine-labelled uninfected cells resulted in an almost complete retention of the acid-insoluble label in the crude nuclear pellet. No more than 2 % of the labelled DNA was present in the cytoplasmic extract, the predominant part of it probably residing in mitochondria, since it could be pelleted by centrifugation at 8500 $\times$ g for 10 min. Based on these results the breaking of nuclei during the homogenization was considered to be negligible.

Up to 45 % of labelled viral DNA was converted into DNA-RNA hybrids when annealed to an excess of "late" cytoplasmic RNA, whereas nuclear RNA drove into hybrids not more than 17 % of DNA (Fig. 1). This agrees well with the data recently reported (Bolden *et al.*, 1979). Denatured RNA-RNA hybrids obtained by self-annealing of cytoplasmic RNA were able to convert at least 36 % of DNA into DNA-RNA hybrids (Fig. 2). This value is lower than that obtained with hybrids produced by self-annealing of total RNA up to 68 % at maximal RNA concentration (Varich *et al.*, 1979). However, since the saturation plateau level was be acheived, the maximal hybridization value (36 %) should be considered as the lowest limit of the size of the genome fraction giving rise to transcripts of opposite polarities accumulated in the cytoplasm.

While our work was in progress, a report on self-complementary transcripts in poly(A)-containing cytoplasmic vaccinia RNA was published (Boone *et al.*, 1979). The values obtained by the latter authors are lower than ours. It is possible that a part of the transcripts found in our experiments is not polyadenylated.

DNA-containing viruses with nuclear localization of transcription (pavova, adeno and herpesviruses) are subject to a strict control at the level of RNA transport from nucleus to the sites of protein synthesis. In vaccinia virus-infected cells the presence of self-complementary RNA sequences in the cytoplasm was reported as early as in 1969—1971 (Colby and Duesberg,

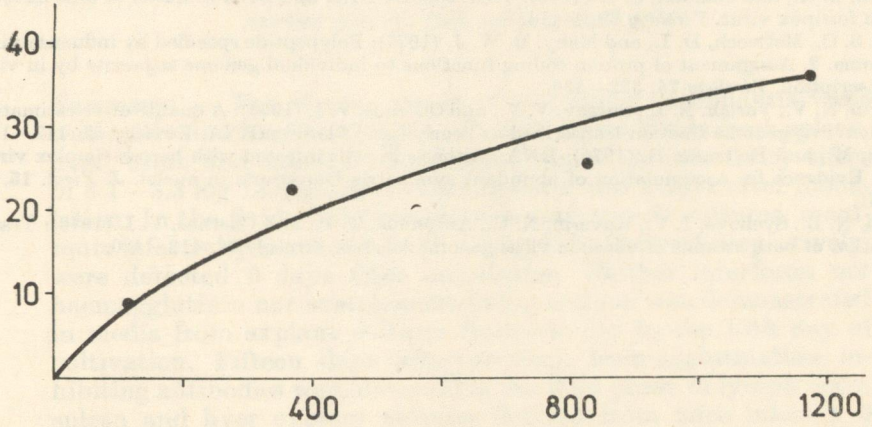

Fig. 2.

Hybridization of vaccinia virus ^{3}H-DNA with melted RNA-RNA hybrids obtained by self-annealing of cytoplasmic RNA

RNA from the cytoplasm of infected cells was self-annealed for 24 hr at 65 °C at a concentration of 5.5 mg/ml and treated with a ribonuclease mixture. Double-stranded hybrids were purified, melted by heating at 115 °C for 5 min and used in DNA-RNA hybridization. The amounts of RNA per sample (μg) indicated on the abscissa refer to the total RNA before ribonuclease treatment and not to the actual amount of RNA-carrier in the sample.
Ordinate: % DNA in the hybrid.

1969; Colby *et al.*, 1971). The present data suggest that the transcripts of both DNA strands corresponding to a large part of vaccinia virus genome accumulate in the cytoplasm. This raises a problem of a special mechanism ensuring the selective translation of the sequences coding for viral proteins and the lack of translation of antimessenger sequences.

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