INHIBITION BY INTERFERON OF SV40 TUMOR ANTIGEN FORMATION IN CELLS INJECTED WITH SV40 cRNA TRANSCRIBED IN VITRO

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1. Introduction

The interferons exert their action on the replication of viruses by inhibition of the viral genome expression. The target viral genome function inhibited was shown for two RNA viruses to be primarily the viral genome transcription (vesicular stomatitis virus [1, 2], reovirus [3]).

In the case of other viruses the viral genome translation is primarily affected by interferon (e.g. arbovirus [4], encephalomyocarditis virus [5], vaccinia virus [6]).

Concerning the DNA-containing simian virus (SV40), it was found that in monkey cells both the viral transcription and viral translation were reduced by the same rate by human interferon [7]. These findings support the hypothesis that the SV40 genome transcription is primarily affected by interferon.

However with polyinosinic:polytidylic acid as an interferon inducing agent we had found that the viral translation (formation of tumor antigen) is inhibited by 99% or higher, whereas the formation of early viral RNA was reduced only to 20% [8, 9]. These findings support the hypothesis that translation rather than transcription of SV40 genome is inhibited by interferon.

In order to get further information whether interferon inhibits SV40 genome expression mainly at the level of transcription or translation SV40 cRNA was synthesized in vitro with *E. coli* RNA polymerase. For translation, this cRNA was injected into monkey kidney cells (TC7) by means of our microinjection technique [10–12].

2. Materials and methods

2.1. *Preparation of human interferon*

Human skin fibroblasts were stimulated with polyinosinic:polycytidylic acid [13]. The crude interferon preparations contained $2–4 \times 10^3$ units ml^{-1} when tested with vesicular stomatitis virus on human skin fibroblasts. In some experiments we used partially purified interferon preparation, from Newcastle disease virus-stimulated human leucocytes, which was a gift from the Behringwerke AG, Marburg.

2.2. *Cell culture and virus*

For all experiments TC7 cells, a subline of the CV1 line of African green monkey kidney cells (kindly supplied by Carel Mulder, Cold Spring Harbor Laboratory), and SV40 strain 777 were used.

2.3. *Detection of SV40 tumor antigen (T-antigen)*

The detection of SV40 T-antigen by immunfluoresence technique and preparation of anti SV40 T-antigen sera in monkeys is described elsewhere [8].

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Table 1
Effect of interferon on the translation of SV40 cRNA injected into TC7 cells

Experiment	Percent of T-antigen positive nuclei
Injection of SV40 cRNA	40.0*
Interferon-pretreatment, injection of SV40 cRNA	0.1*
Injection of SV40 DNA I	59.0*
Interferon-pretreatment, injection of SV40 DNA I	0.2*
Infection with SV40	50.0**
Interferon-pretreatment, infection with SV40	0.5**

* Each measurement is based on a count of 3×10^2 injected cells. The cells were stained for T-antigen 24 hr after injection.

** Precent of T-antigen positive cells of virus infected culture. The cells were stained for T-antigen 24 hr after the infection.

2.4. *Preparation of SV40 cRNA with E. coli RNA-polymerase*

The preparation is described elsewhere [14].

2.5. *Micro-injection of SV40 nucleic acids into TC7 cells*

The procedure is described elsewhere [10–12].

3. Results

3.1. *Formation of SV40 T-antigen in TC7 cells injected with SV40 cRNA*

cRNA obtained by in vitro transcription of SV40 DNA component I was injected into semi-confluent TC7 cells by means of micro-glass capillaries. The micro-injection was performed under a microscope at a 400-fold magnification. The recipient cells were grown on glass coverslides (divided into numbered squares of 1 mm^2). About $5 \times 10^{-12} - 10^{-11}$ ml of cRNA solution (0.1 mg/ml cRNA) were injected into each cell.

As indicated in table 1 40% of the SV40 cRNA-injected cells showed SV40 T-antigen 24 hr after the injection. This SV40 cRNA-induced T-antigen formation is not inhibited by actinomycin D (1 μg/ml). In these experiments where TC7 cells were treated with actinomycin D (a 30 min pretreatment before cRNA injection, during the injection procedure and throughout the following 10 hr of incubation time), the proportion of T-antigen positive cells was in the same range (12% 10 hr after infection) as in cells injected with cRNA but incubated in medium without actinomycin D.

Table 2
SV40 T-antigen formation TC7 cell nuclei

Injection of	Detection of T-antigen
SV40 cRNA, DNase treated	+
SV40 cRNA in actinomycin D treated cells	+
SV40 cRNA, RNase treated	–
SV40 cRNA, alkaline treated	–
RNA from TC7 cells	–

Each measurement is based on a count of 3×10^2 injected cells. The cells were stained for T-antigen 24 hr after injection.

T-antigen formation was not inhibited when the cRNA was treated with pancreatic DNase (Worthington Biochemical, 50 μg/ml, for 3 hr at 25°C, 2 mM $MgCl_2$) before injection. On the other hand, RNase (Worthington Biochemical, 50 μg/ml, for 90 min at 25°C) and alkaline (pH 11.0) treatment destroyed the biological activity of the cRNA (table 2).

The arrangement of T-antigen in the nuclei induced by in vitro cRNA was the same as in SV40 virus infected cells (fig. 1).

3.2. *Inhibition of cRNA-induced T-antigen formation in TC7 cells by human interferon*

To prove whether interferon can prevent SV40 cRNA translation following micro-injection, TC7 cells were treated with human interferon (2000 units/ml) 18 hr before cRNA injection. 24 hr after RNA injection only 0.1% of the cells were positive for SV40 T-antigen while 40% of the control cells (not interferon-treated but cRNA injected) exhibited T-antigen formation.

The rate of reduction of T-antigen positive cells by interferon is in the same range when SV40 DNA (0.1 mg/ml) is injected into TC7 cells instead of virus cRNA (table 1).

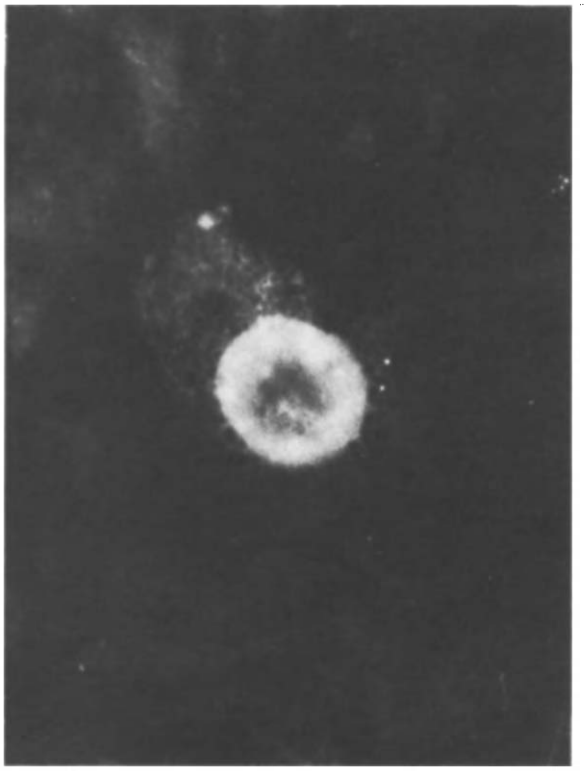

Fig. 1. TC7 cell injected with SV40 cRNA and stained for T-antigen 24 hr after cRNA injection (1:400).

4. Discussion

The supercoiled forms of SV40 and polyoma virus are efficient templates for *E. coli* RNA polymerase. This enzyme transcribes preferentially the minus (early) strand in vitro. In vivo, the early virus messenger RNA is also transcribed from the minus strand while the late messenger RNA is preferentially transcribed from the plus (late) strand [14–18].

Hence it is likely that the in vitro cRNA has messenger qualities for the early virus proteins.

The experiments reported here prove that SV40 T-antigen is virus- but not cell-coded. This was already demonstrated for polyoma virus T-antigen by injection of in vitro cRNA into mouse cells [19].

From the actinomycin D resistance of the cRNA induced T-antigen synthesis it can be concluded that T-antigen formation as well as transport into the cell nuclei is independent from an additional expression of cellular genome functions.

The inhibition of cRNA-induced T-antigen formation by human interferon supports our hypothesis [8, 9] that interferon inhibits primarily the translation of the SV40 genome.

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